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Microbial degradation of halogenated organics using microbes associated with marine algae

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MICROBIAL DEGRADATION OF HALOGENATED ORGANICS USING MICROBES ASSOCIATED WITH MARINE ALGAE

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ABSTRACT

Microbial Degradation of Halogenated Organics
Using Microbes Associated with Marine Algae

by
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This study evaluated the microbial degradation of the following chlorinated compounds: Trichloroethylene (TCE), 1,2-Dibromo-3-chloropropane(DBCP), lindane and Pentachlorophenol. The source of the microbial consortia used in this study was from an unknown species of red alga, *Laurencia sp.* and another red alga species *Plocamium cartilagineum*. The consortia consisted of eight isolated strains from *Laurencia sp.* and seven isolated strains from *L. cartilagineum*. The bioreactors consisted of 40 mL glass vials sealed with screwed caps and septa. The microbes were subjected to two different concentrations of the above chlorinated compounds as a sole carbon source in an artificial sea water solution. The bioreactors were extracted and analyzed by Gas Chromatography over a three week period. Degradation was thought to be observed for DBCP only. The chromatographic results indicated a loss of the parent substrate, and some newly formed peaks were observed at earlier elution times. An attempt was made without success to repeat the results. The second set of experiments contained a lower initial biomass, which is thought to be the reason for the lack of degradation. It is not known whether the cells were able to metabolize the substrate directly or cometabolized DBCP using the biomass as the primary substrate, or whether enzymes released when the cell lysed were responsible for DBCP degradation.

KEYWORDS: Chlorinated Hydrocarbons, Biodegradation, Marine Algae

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Chapter 1. Introduction

1.1. Background

Chlorinated hydrocarbon compounds have widespread use in a myriad of man-made products. Many pesticides, wood preservatives and solvents are constructed from chlorinated phenolic and aliphatic compounds. In 1990, there were over 600 pesticides registered for use in the U.S. alone. Millions of pounds are distributed globally each year, with a world market estimated at greater than 20 billion dollars (Racke and Coats, 1990). Many of these compounds have entered the biosphere either through their intended use, improper disposal or spillage. The use of organochlorine pesticides has increased greatly since the 1940's to meet growing food demand and to reduce the spread of insect transmitted diseases. Initially, these compounds were considered a boon to the fields of agricultural and medical entomology. However, chlorinated hydrocarbon compounds can be highly recalcitrant to chemical decomposition or biodegradation. These xenobiotics accumulate in lower organisms and are transferred up the food chain. This process, known as biomagnification, can potentially have adverse effects on the ecosystem.

In recent years, the use of certain chlorinated hydrocarbon pesticides, such as DDT, have been banned in some developed countries because of concern over their recalcitrant and carcinogenic nature. However, organochlorine pesticides are still used in less developed countries due to their relatively low cost and efficiency. Run-off from agricultural zones contains significant quantities of pesticides which pose an environmental threat to surface and ground water supplies. Therefore, it reasonable to assume that cost-effective

bioremediation schemes will be necessary, at least for the near future. This research will attempt to locate a relatively new and novel source of microbes that are potentially capable of degrading several chlorinated hydrocarbons.

Suitable microbes for bioremediation schemes often are found at previously contaminated sites. Naturally occurring mutants that are able to use the xenobiotics as a carbon and/or energy source can be cultured from the contamination zone. Analog substrates, compounds that are chemically similar but usually less toxic, can also be employed to identify suitable microbes. Microbes able to degrade one compound may be applicable to more toxic, structurally similar substrates. By combining the two previous concepts, naturally occurring mutants and analog substrates, it is hypothesized that a source of microbes capable of organochlorine degradation will be located. Naturally occurring halogenated compounds are quite prevalent in marine environments. In some cases, the naturally occurring compounds resemble some of the common pesticides and solvents. Macroalgae, marine acorn worms, and mollusks all produce halogenated compounds, and they have been doing so for a considerably longer time period than anthropogenic sources of chlorinated hydrocarbons have been in existence (Gribble, 1992). Higa and Sakemi (1983) reported on a species of acorn worm that produced approximately 43 kg/day of chlorinated phenolic compounds. However, there did not seem to be a build up of these compounds in the vicinity of the sources. It is reasonable to assume that a symbiotic relationship may exist between the producing host and microbes that may have developed the ability to digest the secreted halogenated compounds.

1.2. Research Focus

The objectives of this research are to study the effectiveness of marine-based microbes in degrading halogenated aliphatic and phenolic compounds. The following compounds: 1,2,3,4,5,6-hexachlorocyclohexane (lindane), 1,2-dibromo-3-chloropropane (DBCP), 1,4-bromochlorobutane (1,4-BCB), pentachlorophenol (PCP) and trichloroethylene (TCE) were selected based upon their detrimental environmental effects, and the wide range of chemical structures.

Chapter 2. Literature Review

2.1. Introduction

Microbial degradation, or biodegradation, refers to the process of microorganisms decomposing or transforming organic compounds. Microorganisms have the ability to consume organic material and use the energy to produce biomass in aerobic and anaerobic environments. They may also use organic materials as secondary substrates which, contribute negligibly to the energy and carbon needs of the cell. Many of the pesticides and solvents used today are organic in nature, but they incorporate copious amounts of chlorine or bromine into their structures. The additional halogen makes them recalcitrant to microbial degradation.

The Agrochemical Handbook (Hartley and Kidd, 1983) was reviewed for some of the more common organochlorine pesticides. Aldrin, toxaphene, chlordane, dibromochloropropane, dichloropropene, dieldrin, dienchlor, endrin, heptachlor, and lindane have all been identified as chlorinated aliphatic or terpenoid pesticides. Alexander (1984) reported that some of these compounds had remarkable persistence in soils, up to 14 years in the cases of toxaphene, lindane, and DDT. Several of the these compounds are restricted in the U.S., but they are extensively used in other countries. In the Netherlands there are over 100 sites severely contaminated by lindane alone (Bachman *et al.* 1988).

2.2. Biodegradation of Anthropogenic Chlorinated Compounds

Microbial degradation of anthropogenic hydrocarbons has been intensely studied for over 25 years. Hill and McCarty (1967) were among the first to investigate the biological degradation of chlorinated hydrocarbons. These investigators reported degradation of chlorinated pesticides, such as lindane and DDT using activated sludge from a waste water treatment facility under anaerobic conditions.

More recently, pesticide degradation has been studied using microbes cultured from soils exposed to pesticides for a prolonged period of time. The literature survey unveiled a plethora of studies on the degradation of pesticides. The majority of the literature reported on the ability to degrade anaerobically the organochlorine pesticides. In fact, a common remediation method for lindane is to flood the field for several weeks and let the indigenous microbes anaerobically degrade pesticide residues (Castro and Yoshida); (Matsumura, 1982); (Mirsatari *et al.*, 1987). The lindane levels were generally reduced to acceptable levels within two months.

Maule *et al.* (1987) performed laboratory studies on the anaerobic degradation of organochlorine pesticides using a consortium of microbes. The microbes were isolated from soil samples, sheep rumen and chicken litter. They achieved partial dechlorination of the substrate, but they were unsuccessful at obtaining complete mineralization. They also found that a consortium of microbes performed better than isolates, indicating potential cometabolism or synergism among the microorganisms.

Finally, the most directly applicable study to the proposed research was done by Patil *et al.* (1972). They investigated the anaerobic degradation of organochlorine pesticides using microorganisms obtained from various marine sources in oceanic conditions, including bottom sediments, surface films and algae. Microbes isolated from ecosystems that had been previously exposed to anthropogenic contaminates were moderately successful at transforming all the studied pesticides to a less toxic compound, but they were unable to achieve complete mineralization of the substrates. Microbes isolated from the open sea, where there had been little or no exposure to pollution, were unable to achieve any metabolism. These citations indicate that microbes may be able to adapt to anthropogenic substrates via naturally occurring mutation in order to incorporate available carbon sources into their metabolic pathways.

2.3. Biodegradation of Naturally Occurring Halogenated Compounds

Certain marine organisms are known to produce halogenated organic compounds. Higa and Sakemi (1983) reported the finding of a species of Acorn worm, *Ptychodera flava*, in Kohama Bay, Okinawa, which excreted copious amounts of halogenated organic compounds. The majority of the compounds consisted of highly brominated phenolic compounds. They estimated 43 kg of halogenated metabolites were excreted daily over a 1 square kilometer area, but noted there was no build up of toxicity in the vicinity. No microbial assays were undertaken in this study, but the quantities of compounds being excreted indicates that some form of degradation might be involved.

In a similar study, King (1988) observed the presence of 2,4-dibromophenol (DBP) in the burrow micro environment of the hemichordate *Saccoglossus kowalewskii*, near

Lowes Cove, Maine. Slurries were prepared using sediments from an inter-tidal mud flat around the burrows of the worm. The slurries were incubated and subjected to concentrations of DBP comparable to those found in the burrow linings in both aerobic and anaerobic environments. The degradation DBP was severely inhibited in aerobic slurry cultures. However, the anoxic slurries showed no signs of inhibition. The original concentrations of DBP were completely dehalogenated within 72 hours. King also noted that residual phenol degradation was dependent on sulfate reducing bacteria that converted the phenol to CO_2 and H_2S. In addition to DBP, anoxic sediments also degraded similar man-made halogenated phenols, 2,4-dichlorophenol and 2,4,6-tribromophenol, at comparable rates.

2.4. Algal Sources of Microbes

Macro algae, such as kelp, are mainly composed of cellulose, lignin and gelatinous materials, but many include some halogenated compounds (Wright, *et al.* 1991). A comprehensive review of naturally occurring organohalogen compounds is presented by Gribble (1992). The article covers both terrestrial and aquatic products. To date almost 1500 different halogenated organic compounds have been isolated from marine organisms. Red algae, particularly species of *Laurencia* and *Plocamium* are rich in halogenated compounds. The types of compounds include simple halogenated alkanes, alkenes, ketones and terpenes (both linear and cyclic). Some of these compounds are shown in Figure 2.4.1. Fuller *et al.* (1992) reported on a cyclic halogenated monoterpenes that closely resembles lindane. The naturally occurring halogenated compounds (Figure 2.4.1) show a number of structural similarities to some biocides and solvents. In some cases, the

natural compounds incorporate bromine into their structures, whereas the pesticides are predominately chlorine.

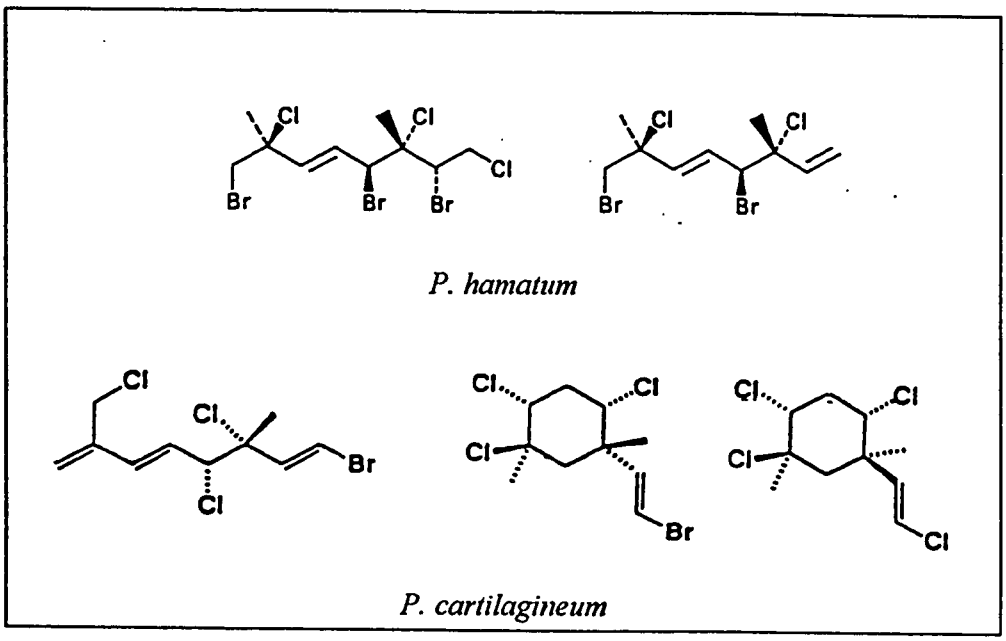


Figure 2.4.1. Naturally Occurring Halogenated Compounds from *Plocamium* Red Algae

Reiper-Kirchner (1989) showed that algae degraded more rapidly in the vicinity of kelp beds than in the open sea. The investigator established that the microbial count was significantly higher near the kelp beds. Hollohan *et al.* (1986) also studied the biodegradation of kelp. Not only were significant numbers of microbial colonies found to be associated with the kelp bed during the degradation process, but the microbes were reported to adapt to the different types of substrates produced during the degradation process.

2.5. Analog Substrates

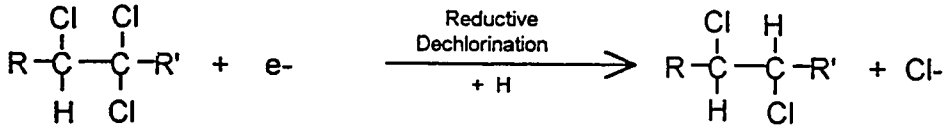
The use of an analog substrate of the target compound is often a common approach to isolate microbes that will degrade a recalcitrant substrate. Walker *et al.* (1976) used this approach to degrade heavy South Louisiana crude oil. These investigators used sediments from two locations in Chesapeake Bay in an attempt to biodegrade crude oil. One location, Baltimore Harbor, had been repeatedly exposed to light petroleum products, and the other location, Eastern Bay, was still relatively pristine. In general, the lighter petroleum products degrade more easily than the larger molecules. The sediments from Baltimore Harbor were able to degrade a significant portion of the various petroleum components in the heavy crude oil. Little degradation was achieved with the Eastern Bay sediments, potentially indicating the limited applicability of analog substrates.

Focht and Alexander (1970) successfully used an analog substrate to locate a suitable microbial degrader of DDT. Diphenylmethane is structurally similar to DDT, but it is not chlorinated and is less toxic. A bacterium was isolated from sewage effluent using diphenylmethane as the sole carbon source in the growth medium. The carbon source was then switched to DDT, which resulted in partial degradation of the more toxic substrate. In a similar study, Furukawa and Matsumura (1976) used biphenyl as the sole carbon source to isolate a bacterium from sewage effluent that would degrade chlorinated biphenyl compounds.

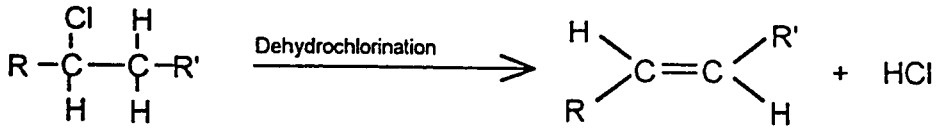
2.6. Metabolic Pathways

Much of the recent research in microbial degradation investigates the metabolic pathways in the degradation process. Lal and Saxena (1982) and Neilson (1990) have presented comprehensive reviews that cover the metabolic pathways for aerobic and anaerobic degradation of the major organochlorine pesticides. The major degradation reactions of organochlorine insecticides are reductive dehalogenation, dehydrochlorination and oxidation.

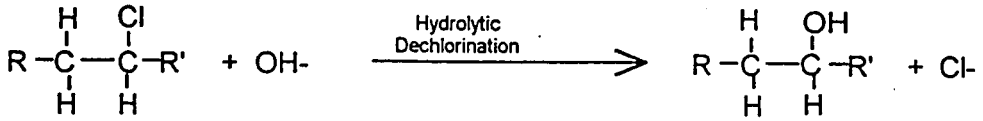
Reductive dechlorination is a nucleophilic substitution of the halogen atom with a hydrogen atom, as shown below. This pathway requires anaerobic conditions and reduced organic compounds that serve as electron donors. In addition, this pathway is more prevalent with increasing halogen content.



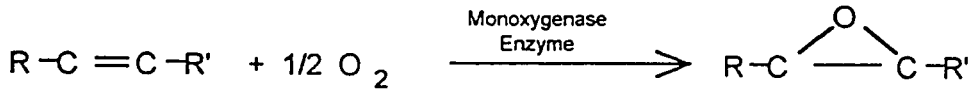
Dehydrochlorination eliminates HCl from two adjacent carbon atoms, thereby forming a double bond between them, as shown below. This reaction generally occurs anaerobically, but, at least in principal, may be facilitated by aerobic bacteria. This pathway is not encountered often, but is found in the microbial degradation of DDT (Lal and Saxena, 1982).



Oxidation reactions can be divided into two major classes. First, hydrolytic substitution, which replaces one or more halogens with hydroxyl groups, In this pathway, nucleophilic substitution of the halogen with a hydroxyl group generally requires aerobic conditions, as shown below. This pathway is more prevalent with decreasing halogen content.



The second class of oxidation reactions uses the monooxygenase pathway. This pathway adds an oxygen atom across two adjacent unsaturated carbons creating an epoxide, shown below, which are highly unstable and abiotically transform into the corresponding aldehyde or diol compounds.



2.6.1. TCE Metabolic Pathways

TCE degradation has been studied under anaerobic and aerobic conditions. Anaerobic degradation of TCE using methanogenic bacteria was studied by Vogel and McCarty (1985). Acetate was used as the primary substrate, and the TCE was cometabolized to CO_2 and intermediates. The authors suggested a reductive dechlorination as shown in

Figure 2.6.1.1. The major intermediates were dichloroethylene (DCE) and vinyl chloride (VC), carcinogenic in their own right.

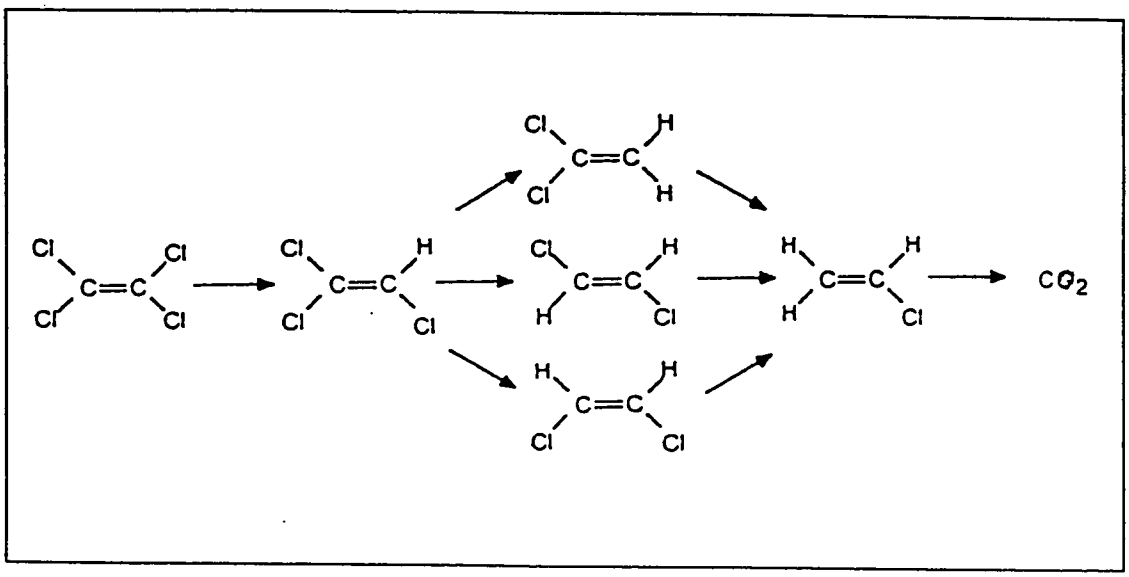


Figure 2.6.1.1. Anaerobic TCE Degradation Pathway (Vogel and McCarty, 1985)

Because VC accumulates during the anaerobic metabolism of TCE, Fiermans *et al.* (1988) studied the aerobic pathways for TCE degradation to avoid the formation of the potent carcinogenic. TCE was not utilized as a sole carbon source, and degradation required propane, methane or methanol as the primary carbon source. The major end products were CO_2 and hydrochloric acid. Little *et al.* (1988) degraded TCE using heterotrophic bacteria with methane as an energy and carbon source. Little suggested that the first step in the degradation process was via a methane monooxygenase reaction of the TCE resulting in the formation of TCE epoxide. The epoxide is unstable and transforms to dichloroacetic acid and TCE diol. Heterotrophic bacteria in the mixed culture were them able to complete the mineralization, as illustrated in Figure 2.6.1.2.

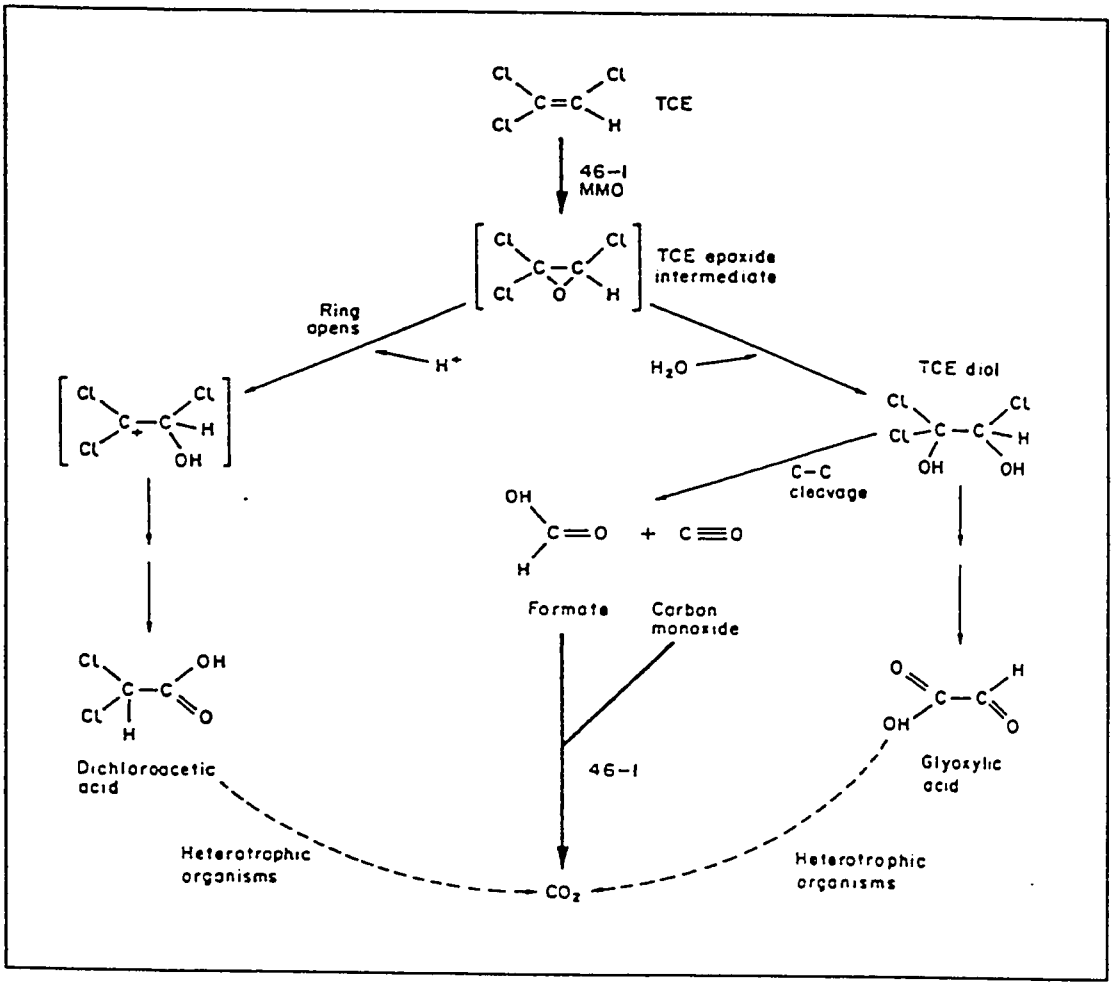


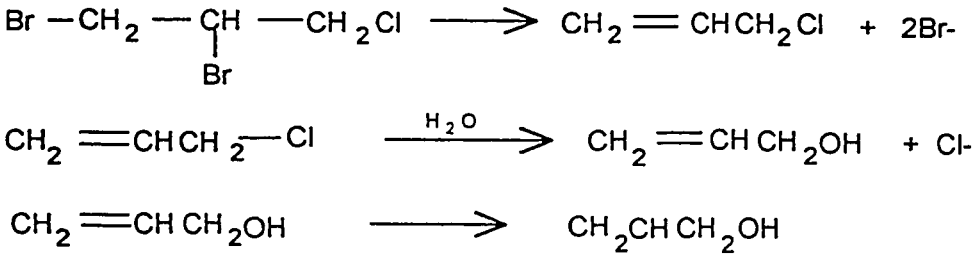
Figure 2.6.1.2. TCE Degradation via Methane Monooxygenase (Little *et al.* 1988)

Aromatic pathways for TCE degradation were first studied by Nelson et al. (1987). They noted that oxygen and an unidentified component in the TCE contaminated water were essential for TCE oxidation. The study demonstrated that phenol was the unidentified component. Subsequent studies have shown that toluene and cresol can also stimulate TCE oxidizing activity (Wackett and Gibson, 1988); (Fan and Scow 1993). All the investigators suggest a variation of a dioxygenase or monooxygenase reaction.

Perchloroethylene (PCE) is not degraded in any of the oxidative pathways, and TCE is degraded slowly in aerobic environments. It appears the most efficient degradation scheme for TCE and PCE begins anaerobically followed by aerobic degradation.

2.6.2. DBCP Metabolic Pathways

DBCP degradation via reductive dehalogenation proceeds by removing the bromine atoms and forming the allyl chloride, which is further hydrolyzed to the allyl alcohol and finally n-propanol as shown in the following reactions (Castro and Belser, 1968).



Rasche *et al.* (1990) used nitrifying bacteria capable of oxidizing ammonia to metabolize DBCP via an ammonia monooxygenase reaction. The investigators did not report on the intermediate products. Several investigators have studied the oxidation of haloalkanes similar to DBCP. Stucki *et al.* (1983) utilized 1,2-dichloroethane as the sole carbon source. A hydrolytic dehalogenase was proposed as the first metabolic step, transforming the halogenated organic to the corresponding alcohol. Jannsen *et al.* (1985) confirmed the results of Stucki and also provided details of the subsequent steps to complete mineralization. The subsequent steps converted the alcohol to the carboxylic acid via the aldehyde. Janssen *et al.* (1987) expanded their findings to longer alkanes with terminal halogens, including 1,2-dichloropropane and 1-chlorobutane.

2.6.3. Lindane Metabolic Pathways

Lindane is degraded under anaerobic environments via reductive dehalogenation (Heritage and Mac Rae, 1977). The first intermediate is 3,4,5,6-tetrachloro-1-cyclohexene. Subsequent dehalogenation leads to *para*-chlorophenol, which can only be metabolized through aerobic pathways. The detailed pathway is shown in Figure 2.6.3.1. Bachmann *et al.* (1988) were able to degrade the alpha isomer aerobically; however, it is the gamma isomer that is much more toxic and distributed as the pesticide.

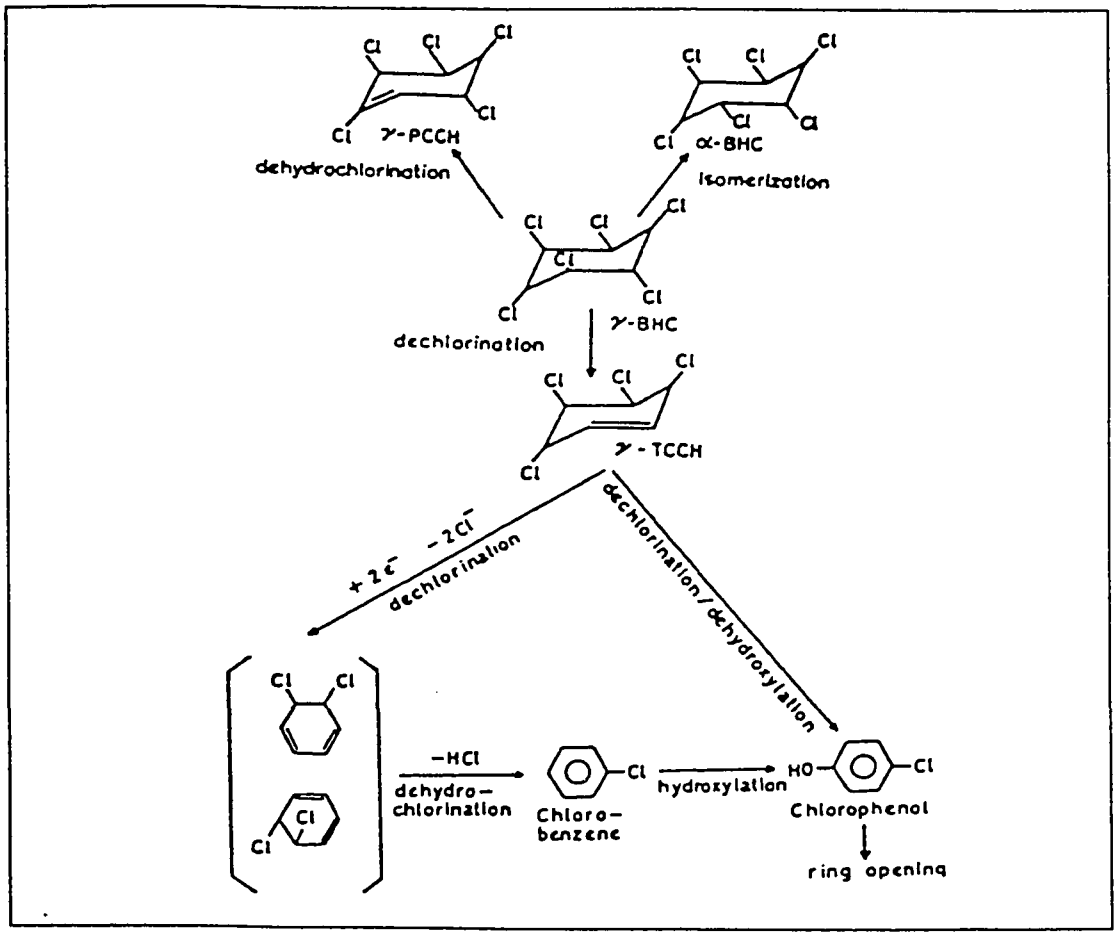


Figure 2.6.3.1. Anaerobic Lindane Pathway (Lal and Saxena, 1988)

2.6.4. PCP Metabolic Pathways

PCP has been successfully degraded in both aerobic and anaerobic environments. Radehaus and Schmidt (1992) aerobically mineralized high concentrations of PCP as the sole carbon source in an aqueous medium. Apajalahti and Salkinoja-Salonen (1986) suggest that aerobic degradation follows a hydroxylase pathway with successive hydrolytic substitutions, as shown in Figure 2.6.4.1. This may lead to ring opening and complete mineralization via heterotrophic microbes.

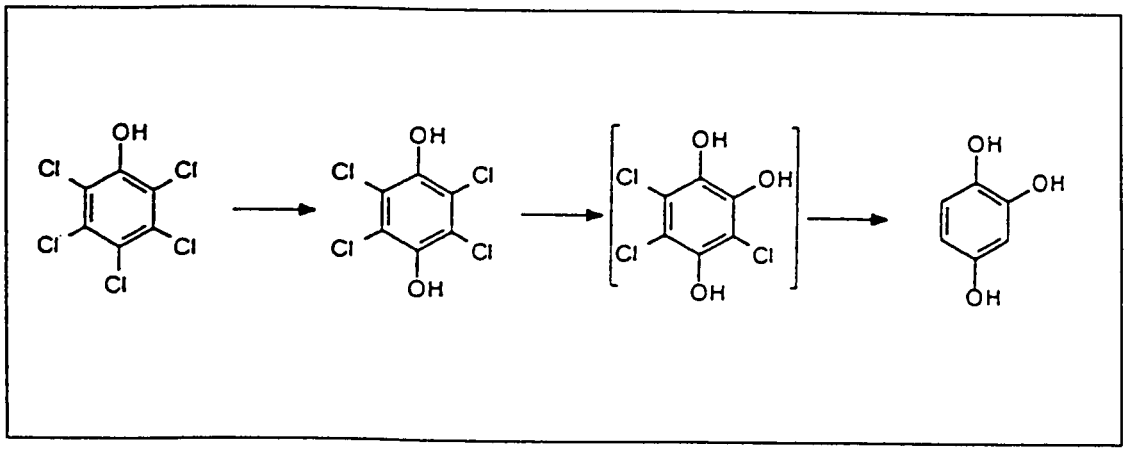


Figure 2.6.4.1. Aerobic PCP Pathway (Apajalahti and Salkinoja-Salonen, 1986)

Anaerobic degradation via reductive dechlorination was proposed by Mikesell and Boyd (1986). This pathway can lead to dichlorophenol as shown in Figure 2.6.4.2. Complete mineralization is not possible under anaerobic conditions, for there are no known anaerobic mechanisms leading to ring cleavage. Krumme and Boyd (1986) used an anaerobic uplow reactor followed by an aerobic trickling reactor to achieve complete degradation.

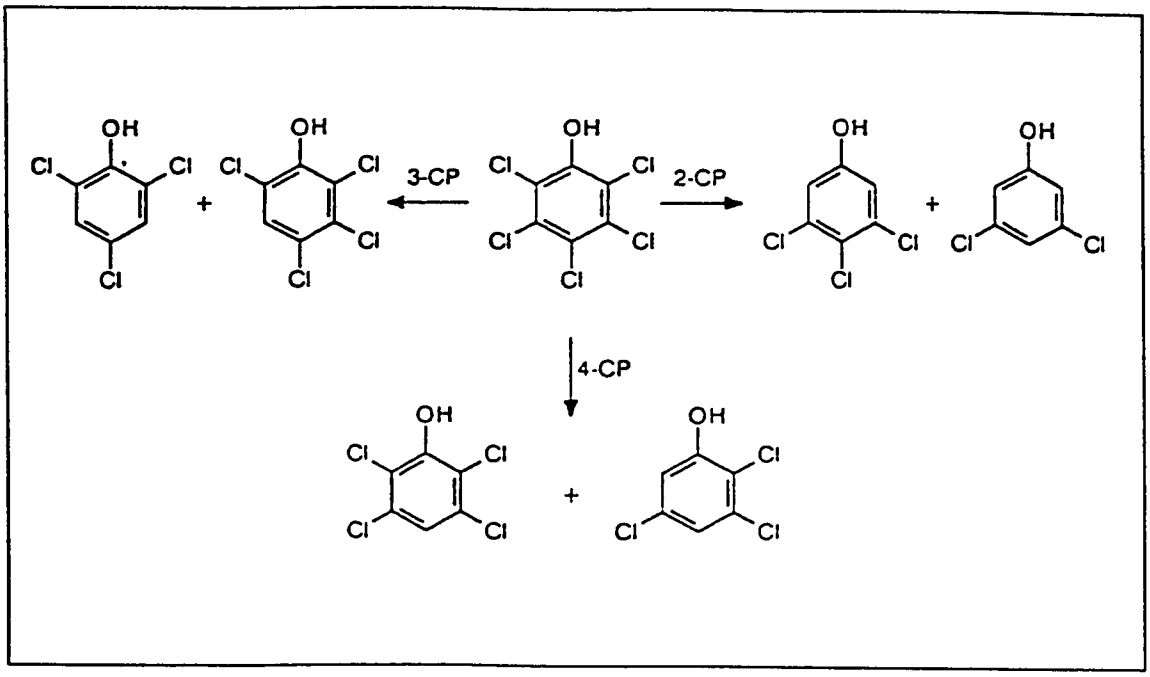


Figure 2.6.4.2 Anaerobic PCP Pathway (Mikesell and Boyd, 1986)

2.7 Proposed Study

The compounds selected for degradation, TCE, DBCP, lindane and PCP, have all been degraded under both reducing and oxidizing conditions. Naturally occurring halogenated compounds resembling the above anthropogenic compounds are produced by two genera of red algae, *Laurencia* and *Plocamium*. It is assumed that there are microbes associated with these alga that are capable of degrading the naturally occurring halogenated compounds. The proposed experimentation will investigate the ability of a microbial consortium derived from *Laurencia* and *Plocamium* to degrade man-made analog compounds.

Chapter 3. Materials and Methods

3.1 Introduction

These experiments will use a consortium of microbes derived from two genera of red algae to degrade the following man-made halogenated compounds: TCE, DBCP, PCP, lindane. 1,4-bromochlorobutane was also added to the list of compounds to be degraded based upon some apparent degradation to DBCP. These compounds were selected based upon their structural similarities to those produced by the red alga genera, *Plocamium* and *Laurencia*. The microbes will be subjected to two different concentrations of each of the aforementioned compounds in a facultative environment to determine their ability to degrade the toxic substrates as sole carbon sources. The concentrations were 10 mg/L and 100 mg/L for TCE, DBCP, and PCP. The lindane concentrations were 250 µg/L and 500 µg/L due to solubility limitations. The concentration used for the 1,4-BCB was 100 mg/L. It has been well documented that many chlorinated hydrocarbon pesticides can be degraded anaerobically and aerobically using terrestrial microbes that have survived repeated or prolonged exposure to the pesticides. This study will be relatively novel in that marine microbes not previously exposed to these anthropogenic compounds will be used for degradation.

3.2 Hypothesis

The use of microbes from the algal sources will be able to partially degrade one or more of the selected compounds.

3.3 Experimental Apparatus

The degradation experiments for this research will be performed in small batch reactors. Each batch reactor used in this experiment consisted of a 40 mL glass vial fitted with a screwed cap and a Teflon lined septum. All of the batch reactors contained 25 mL of artificial sea water solution, and enough of the chlorinated substrate to bring the concentration to the values mentioned in Section 3.1. A small amount (1-2 mL) of washed cell suspension was added to the inoculated vials, and an equal volume of artificial sea water was added to the control vials to equalize the volumes in the reactors.

The vials were placed in a constant temperature shaker water bath to provide agitation and temperature control. The ambient temperature of the marine environment was maintained by recirculating the shaker bath water through a chiller unit. The water from the chiller unit was maintained at 10°C. The temperature in the shaker bath was maintained at 12.5° C with the use of a thermostat and heater in the bath. The vials were placed vertically in the bath and shaken at 60 rpm. Figure 3.3.1 illustrates the experimental apparatus and batch reactor.

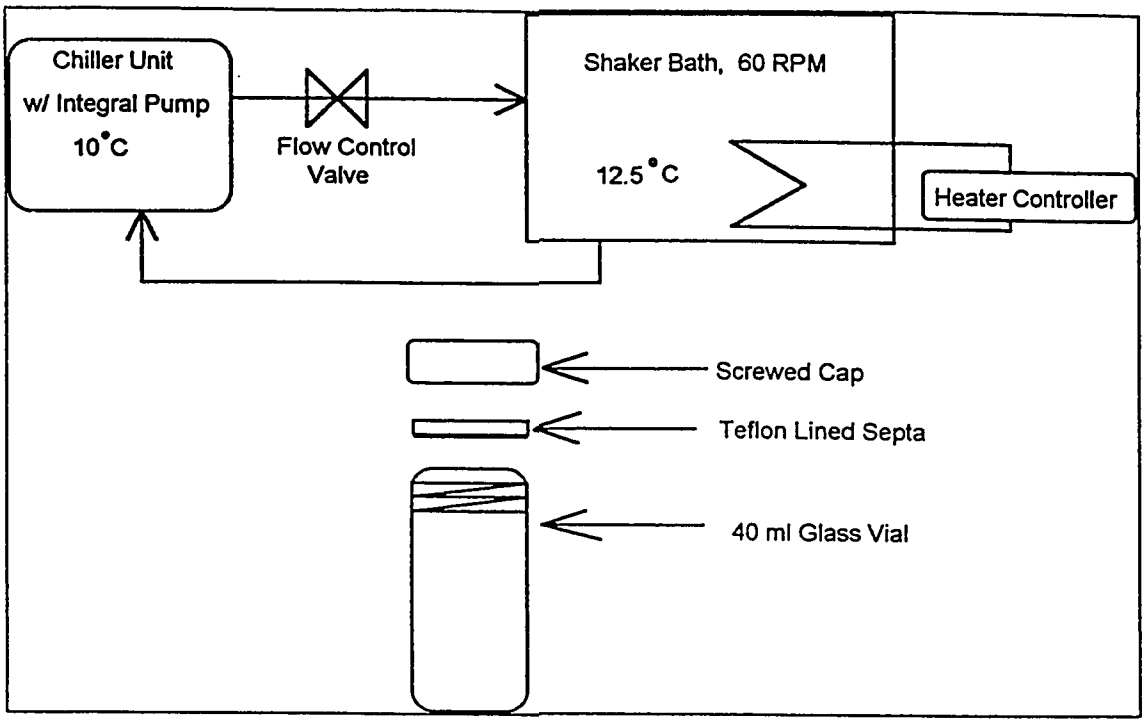


Figure 3.3.1. Experimental Apparatus and Batch Reactor

3.4 Materials

The artificial sea water (ASW) composition has been described by Gerhardt *et al.* (1981). In 1,000 mL of distilled water the following quantities of salts were dissolved: 27.5 grams NaCl, 5.0 grams $MgCl_2$, 2.0 grams $MgSO_4$, 0.5 grams $CaCl_2$, 1.0 gram KCl, 0.001 grams $FeSO_4$, 1.0 gram $(NH_4)_2HPO_4$ and 1.0 gram K_2HPO_4. The artificial sea water solution was autoclaved at 121°C to achieve sterility. The $CaCl_2$ and $FeSO_4$ were filter sterilized separately and added after cooling to prevent the formation of precipitants. The pH was adjusted to 7.8 and allowed to re-oxygenate by diffusion of air through sterile cotton stoppers.

The agar used to culture and isolate the microbes, termed complete medium, contained ASW fortified with tryptone, glucose and yeast extract (1.0 gram tryptone, 0.5 grams glucose, 1.0 gram yeast extract and 15 grams agar, per liter of ASW).

The compounds to be degraded were obtained from Pfaltz & Bauer and supplied as technical grade, greater then 97% pure. The extractant used, pentane and benzene, were obtained from Fisher Scientific as HPLC grade.

3.5 Microbe Isolation and Cell Suspension Procedures

Small amounts of *Plocamium cartilagineum* and *Laurencia sp.* were collected from inter-tidal waters off the northern California coast near Davenport Landing on January 30, 1994. The temperature in the tidal pools ranged from 9°C to 13°C. The pH was between 7.6 to 7.9, and the dissolved oxygen ranged from 4.5 to 8.0 mg/L.

The algae samples were streaked onto complete medium in Petri dishes at the collection site. Following a five day incubation period at 17°C, the colonies were restreaked to assure to obtain isolated strains. Seven isolates were obtained by the Biology Department from the *P. cartilagineum* (P1, P3 and P6-P11) and eight from *Laurencia sp.* (L1-L5) (Dr. Grilione, personal communication). The morphology of the isolates has not yet been determined, and some isolates may be duplicates. The isolated strains were then transferred to capped slants containing the complete medium and stored at approximately 5°C until required for use, which was approximately 3 to 5 months.

The cell suspension used for inoculation were prepared as follows: Isolates from the stored slants were streaked on complete medium agar (30 Petri dishes) and incubated approximately five days at 15°C. The growth was washed from the agar surfaces with sterile ASW into centrifuge tubes and spun at 900 rpm for approximately 15 minutes. Some strains were more prolific than others; however, sufficient growth was obtained from all isolates. The centrifuge tubes were decanted, refilled with ASW, agitated and centrifuged again. After decanting the second centrifugation, the cell pellets were washed into a single volumetric flask with a final volume of 50 mL. In order to determine the biomass concentration, a known portion of the suspension was poured through a previously weighed filter paper and oven dried. The dried biomass and filter paper was weighed again to determine a cell concentration of approximately 1.4 grams biomass per liter.

3.6 Preparation of Batch Reactors

The DBCP and TCE experiments had 2 mL of cell suspension added to each of the inoculated vials. Due to emulsifications formed when extracting the inoculated vials, the PCP and lindane inoculated vials had only 1 mL of cell suspension added. The control vials of the DBCP and TCE experiments had 2 mL of additional ASW added to each, and the control vials for the PCP and lindane experiments had 1 mL of additional ASW added to equalize the volumes. A final pH of 7.8 was used for all the vials.

TCE and DBCP were added directly to vials already containing the 25 mL aliquot of ASW and the 2 mL washed cell suspension. Because TCE and DBCP are relatively volatile at room temperature, the vials containing the already inoculated ASW and the chlorinated

compounds were all cooled to 5°C for approximately one hour in a refrigerator to prevent volatilization of the hydrocarbon. The vials were removed from the refrigerator, and the TCE and DBCP were added directly to the vials using a Hamilton # 7101 syringe and immediately capped. The degradation experiments for DBCP and TCE were each performed at concentrations of 10 mg/L and 100 mg/L. The solubility limit of TCE and DBCP in water is approximately 1000 mg/L at room temperature.

PCP and lindane are in the crystalline form at room temperature, and volatilization is not significant. The appropriate amount of solid PCP and lindane were added to a 1 liter volumetric flask and then diluted with 1 liter of sterile ASW. Twenty five mL aliquots of the toxified solutions were pipetted into the empty vials and immediately capped. The vials were then re-opened to add 1 mL of ASW for the controls or 1 mL of washed cell suspension for the inoculated vials. Two concentrations were used for both the lindane and PCP degradation experiments. The PCP experiments were performed at 10 mg/L and 100 mg/L. The maximum solubility of PCP is function of pH and temperature. At higher pH values, the hydrogen associated with the phenol group dissociates, and the PCP forms the sodium salt, thereby increasing its ability to dissolve into aqueous solutions. At a pH of 7.8 and room temperature the maximum solubility limit is approximately 1000 mg/L. The concentrations of lindane used in the experiments were 250 μg/L and 500 μg/L. The maximum solubility of lindane in water at room temperature is approximately 500 μg/L.

It appeared that DBCP degradation was occurring based upon the results from the first series of experiments. A second series of experiments was carried out to validate the results. In an attempt to elucidate the pathway, the second series of experiments included a set of reactors containing 1,4-bromochlorobutane (1-4-BCB) as the sole carbon source.

The concentration used was 100 mg/L for both compounds. The procedure for adding the sole carbon source was altered slightly. Instead of adding the substrate to each vial individually, a 1.0 liter batch was prepared adding the appropriate amount of substrate to 5°C ASW in a volumetric flask. The mixture was then re-chilled, and 25 mL samples were pipetted into the vials already containing the washed cell suspension in the case of the inoculated vials or ASW in the case of the controls. This procedure reduces the variability of the substrate concentration in the reactors. Only 1 mL of cell suspension or 1 mL of ASW was used for the second set of degradation experiments. The cell suspension used in this series did not contain isolate, P11. In addition, only half the number of Petri dishes (15) was used to harvest the cells for the suspension. However, the measured biomass density was approximately equal.

3.7 Analytical Procedures

Data was taken at three intervals: initially (time equal to zero), at 1 week and at 3 weeks. For Experiments 1 through 8, one control vial (A) and one inoculated vial (A) were analyzed at time equal to zero. In the subsequent intervals, one control vial (A) and two inoculated vials (A and B) were designated for extraction and analysis each substrate and concentration. In second series of experiments, Experiments 9 and 10, two vials (A and B) for the control and inoculated vials were designated for extraction and analysis for all sampling intervals. Several first week vials were re-extracted later to check for cometabolism, but no definitive results were obtained. Table 3.7.1 lists the experimental matrix.

Table 3.7.1. Experimental Parameters and Sampling Schedule

Exp. # 1	TCE, 10 mg/L				**Exp. # 2**	TCE, 100 mg/L			
Time, Weeks	Control Vials		Inoculated Vials		Time, Weeks	Control Vials		Inoculated Vials	
0	A		A		0	A		A	
1	A		A	B	1	A		A	**
3	A		A	B	3	A		A	B
Exp. # 3	DBCP, 10 mg/L				**Exp. # 4**	DBCP, 100 mg/L			
Time, Weeks	Control Vials		Inoculated Vials		Time, Weeks	Control Vials		Inoculated Vials	
0	A		A		0	A		A	
1	A		A	B	1	A		A	B
3	A		A	B	3	A		A	B
Exp. # 5	PCP, 10 mg/L				**Exp. # 6**	PCP, 100 mg/L			
Time, Weeks	Control Vials		Inoculated Vials		Time, Weeks	Control Vials		Inoculated Vials	
0	A		A		0	A		A	
1	A		A	B	1	A		A	B
3	A		A	B	3	A		A	B
Exp. # 7	Lindane, 250 µg/L				**Exp. # 8**	Lindane, 500 µg/L			
Time, Weeks	Control Vials		Inoculated Vials		Time, Weeks	Control Vials		Inoculated Vials	
0	A		A		0	A		A	
1	A		A	B	1	A		A	B
3	A		A	B	3	A		A	B

Table 3.7.1 Experimental Parameter and Sampling Schedule, continued

Exp. # 9	1,4-BCB, 10 mg/L				**Exp. # 10**	DBCP, 100 mg/L, Set # 2			
Time, Weeks	Control Vials		Inoculated Vials		Time, Weeks	Control Vials		Inoculated Vials	
0	A	B	A	B	0	A	B	A	B
1	A	B	A	B	1	A	B	A	B
3	A	B	A	B	3	A	B	A	B

**Note: The Week 1, Inoculate B vial for the 100 mg/L TCE experiment was accidentally broken prior to analysis.

To analyze the substrate and degradation product concentrations at the end of a given interval, the vials were extracted using an organic solvent. Gas Liquid Chromatography (GLC) was then performed on the organic phase to quantify the substrate and degradation product concentrations. Keith (1976) recommends pentane for extraction of lighter organics at an aqueous/organic ratio of 10:1. Afghan and Chau (1989) recommend using benzene to extract PCP and lindane, with an aqueous/organic ratio of 10:1. In general, the proper extractant resembles the compounds of interest in structure and molecular weight, and they have minimal solubility in water. The extraction of PCP was enhanced by acidifying the aqueous solution with 200 μL of 0.5 N HCl to a pH of approximately 2. Acidifying the solutions below the pa value of PCP forces more of the PCP into the organic phase, since the PCP molecule becomes less hydrophilic when it is not in the sodium salt form.

The vials were cooled to 5°C prior to removing 2.5 mL of reactor contents and replacing them with 2.5 mL of the appropriate extractant. Syringes were used for transferring solution and solvent through the septum to avoid head space losses that would occur if the vials were opened. Cooling lowered the head space pressure and allow easier injection of the extractant. The vials were agitated manually then placed upright on a shaker table at approximately 100 rpm for 10 to 20 minutes. After cooling the vials again to 5°C, 1.0 μL samples for GLC analysis were drawn directly from the vials through the septum and injected in the gas chromatograph.

Internal standards were employed for quantification of the substrate concentrations. Internal standards are used to normalize fluctuations in GLC injection volume. A minimum of 3 GC injections were done for each extracted vial. The internal standards were in the extractant prior to extraction, and some partitioning does occur. Ideally, a known quantity of the internal standard is added to the organic phase after it has been separated from the aqueous phase, but this was not feasible given the experimental conditions. The internal standard is expected to be consistent when it partitions between the organic and aqueous phases, and it can be used as repeatable standard against which the compounds of interest can be measured. The peak area of the compound is divided by the peak area of the standard. Hexadecane at a concentration of 90 mg/L in the extractant was used for the benzene extraction. 1,3,5-trichlorobenzene (TCB) at a concentration of 100 mg/L and an impurity in the pentane, C5-I, were used as internal standards for the TCE, DBCP and 1,4-BCB analyses. The same lot of pentane was used for duration of the experiments, and the concentration of C5-I was consistent. Benzene was also used as an internal standard for the Week 3 analysis of TCE and the DBCP experiments, but the

information was superfluous and not used. Table 3.7.2 summarizes the extractant and the internal standards used in the experiments.

Table 3.7.2. Extract and Internal Standard Parameters

Substrate	Extractant	Internal Standard
Trichloroethylene 1,2-Dibromo-3-chloropropane 1,4-Bromochlorobutane	Pentane	Pentane Impurity, C5-I 1,3,5-Trichlorobenzene Benzene (N/A)
Pentachlorophenol Lindane	Benzene	Hexadecane

Emulsions formed in the organic phase when extracting the inoculated vials due to the presence of the cellular material, especially in Experiments 1 through 8. The emulsions made it difficult to obtain repeatable injection volumes. The emulsified phase contained air, cellular material and micro-bubbles of the aqueous phase. Great care had to be taken when withdrawing the injection sample to minimize induced experimental error. By slowly rotating the vial the emulsion could be broken down somewhat. A centrifuge was used in Experiments 9 and 10 which produced excellent phase separation.

A Model 5890A Hewlett-Packard Gas Chromatograph (GC) equipped with a flame ionization detector (FID) was used to monitor substrate concentration during the course of the experiment. The chromatograph was linked to a Model 3396A Hewlett-Packard integrator. Separation was affected on an Alltech Econo Cap 30 m x 0.53 mm x 1.2

micron film thickness column coated with SE-54. Hydrogen at a linear velocity of approximately 80 cm/sec was be used as a carrier gas. Nitrogen was supplied as makeup gas to the FID at approximately 20 mL/min. Hydrogen gas was supplied to the FID at 30 mL/min and air was supplied at 400 mL/min. A Cyclo-View® direct injection liner supplied by Restek was used in the inlet. Table 3.4.3 summarizes the GC parameters used for these experiments. Table 3.7.3 shows the injector and detector temperatures, the initial column temperature, the time the initial temperature was maintained, the ramp rate, the final temperature and the elution time of the compound of interest. The column is baked out after the temperature ramp on each injection to remove any high boiling material, such as cellular material or column oxidation products. During bake out, the GC oven was ramped at 25°C/min to 250°C and was held for 2 minutes for TCE, DBCP and 1,4-BCB. The final bake out temperature was 290°C for PCP and lindane.

Table 3.7.3. Gas Chromatogragh Parameters

Compound	Injector Temp, °C	Detector Temp, °C	Initial Temp, °C	Initial Time, minutes	Ramp Rate, °C/min	Final Temp, °C	Elution Time, minutes
TCE	150	250	35	3.0	5	50	3.9
DBCP	200	250	35	1.0	8	135	10.6
1,4-BCB	200	250	35	1.0	8	135	8.8
PCP	200	300	50	3.0	10	180	11.2
Lindane	200	300	50	3.0	10	180	11.1

3.8. Analysis of Results

Quantification of the degradation was done by plotting the ratio of substrate peak area divided by the internal standard area versus time. The plots of the control vials were compared to those of the inoculated vials. Accelerated degradation rates in the inoculated vials when measured against the control, indicate potential biodegradation. In addition, degradation products may be of lower molecular weight and are expected to be more volatile. Therefore, a gradual increase in the amount of compounds with shorter retention times may also indicate potential degradation. A qualitative analysis was used to search for peaks earlier than the parent compound that increases with time. Bidleman and Williams (1978) used this technique to monitor the degradation of toxaphene. Degradation products with elution times greater than the parent compound, a single hydrolytic substitution for example, were difficult to distinguish because of the multitude of peaks at elution times beyond that of the parent compounds. These later peaks were caused by a combination of cell material and column oxidation.

Chapter 4. Experimental Results and Discussion

4.1. Introduction

Both concentrations of TCE and the first series of DBCP degradation experiments (Exp. #1 through #4) were performed between July 8 and August 3, 1994. The PCP and lindane degradation experiments (Exp. #5 through #8) were carried out between July 27 and August 19, 1994. And finally, the 1,4-BCB and the second series of DBCP degradation experiments (Exp. #9 and #10) occurred between August 27 and September 21, 1994. The results, grouped by substrate, are presented and discussed in this chapter.

4.2. TCE Degradation

Results from the 10 mg/L and 100 mg/L TCE degradation experiments (Exp. #1 and #2) are reported in this section. The peak area of the TCE was divided by the peak area of a contaminant in the pentane extractant, C5-I. The same pentane stock was used throughout the course of the experiments, and the concentration of the contaminant was constant. The concentration of TCE steadily decreased with time in both the control and the inoculated systems. The TCE concentration in the inoculated vials actually appeared to be higher than that of the controls, due to the emulsification in the organic phase. TCE adsorbed to the cells, which were concentrated in the extractant. When cells are drawn into the injection sample, the TCE concentration is elevated relative to a cell free sample. It was difficult to obtain an injection sample that was free of cellular material, air and small amounts of the aqueous phase, and therefore, the injection volume varied considerably. In many cases, the GC run could not be used because either the TCE or pentane C5-I peaks

were below the detection limits. Therefore, the uncertainty in the data was relatively large. No error bars are shown for data with uncertainty less than 5%. Circled data points indicate only one valid injection was obtained, and error bars can not be applied.

A typical chromatogram of a 10 mg/L TCE inoculated vial in the third week is shown in Figure 4.2.1. Benzene was added as an additional internal standard in the Week 3 analyses, which results in a peak at 3.072 minutes. The TCE peak is at 3.917 minutes, and the C5-I peak appears at 3.645 minutes. However, the benzene was not used in the analysis, as it represented superfluous data.

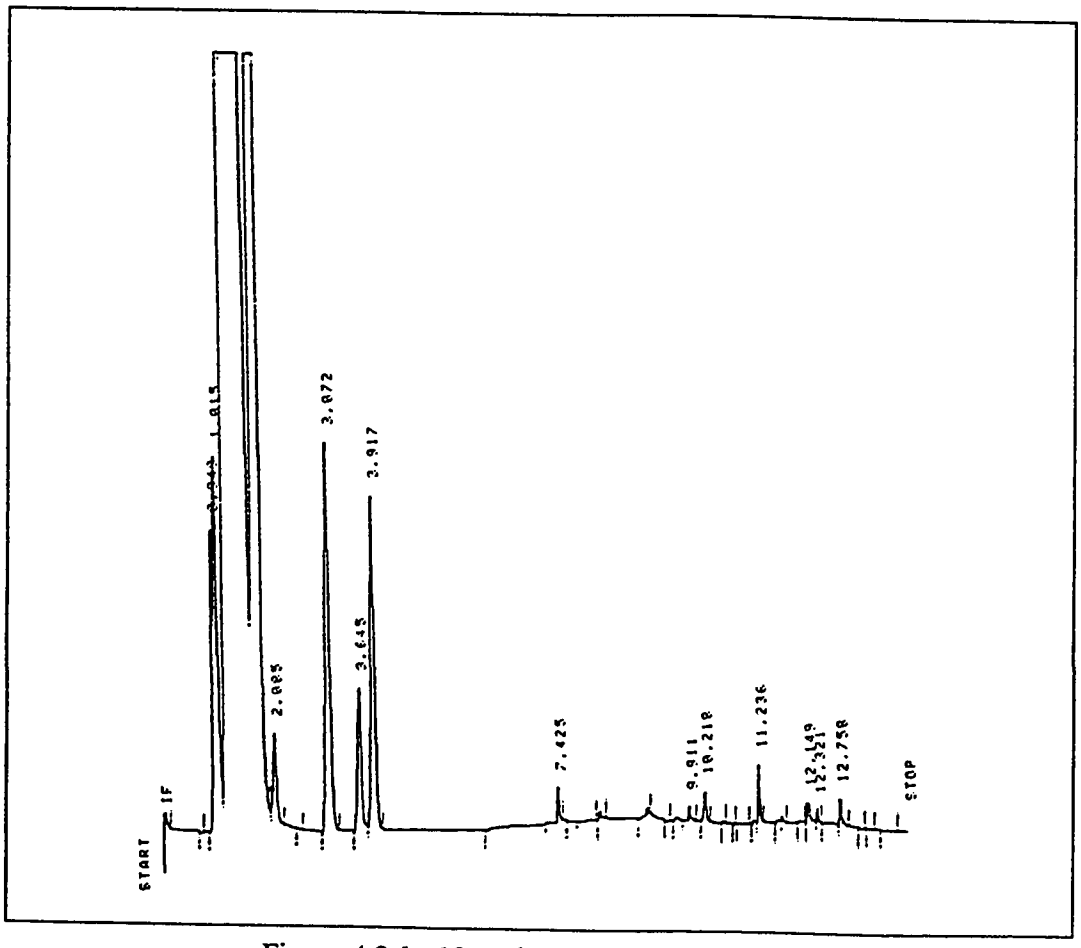
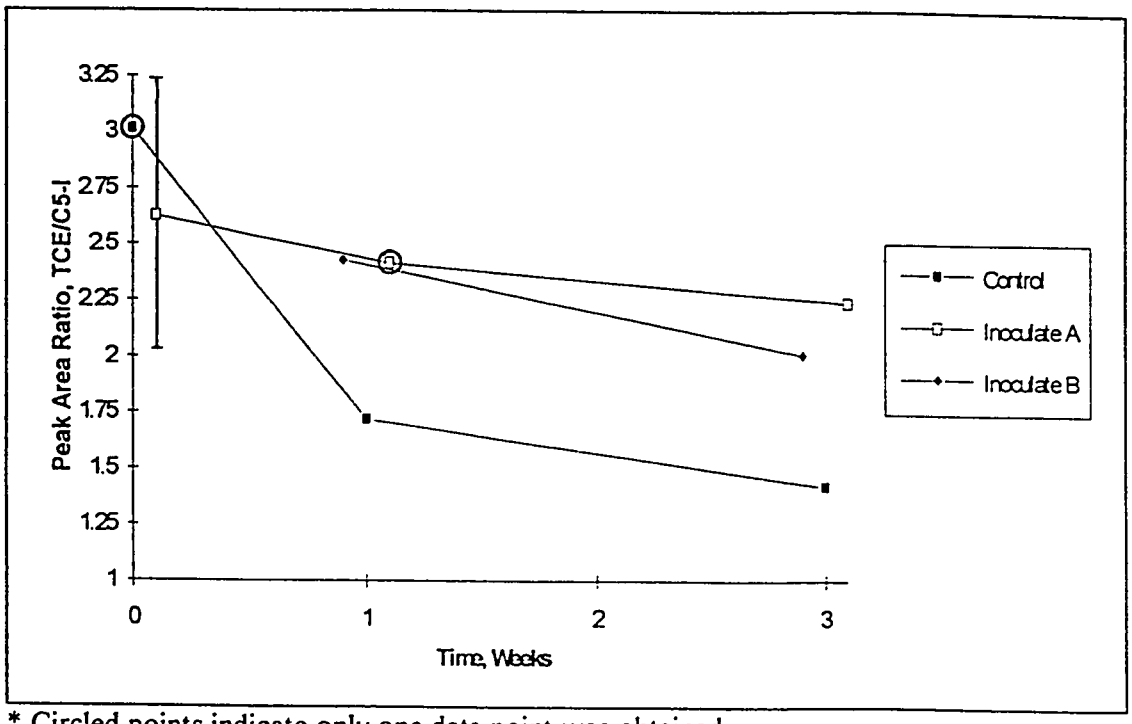


Figure 4.2.1. 10 mg/L TCE Chromatogram

Figure 4.2.2 is a graphical representation the average peak area ratio and percentage error versus time for the 10 mg/L TCE degradation experiment. The concentration of the control and inoculated systems appear to decrease steadily. However, the experimental errors in the initial data points preclude any definitive determination as to whether the degradation in the inoculated system was biologically enhanced.



* Circled points indicate only one data point was obtained

Figure 4.2.2. 10 mg/L TCE Degradation versus Time

A typical chromatogram of a 100 mg/L TCE inoculated vial from Week 3 is shown in Figure 4.2.3. The TCE peak is at 3.856 minutes, and the C5-I peak appears at 3.584 minutes. A full set of chromatograms for time equal zero and three weeks are contained in Appendix B (Figures B.1.1 through Figure B.2.4) for all the TCE degradation experiments.

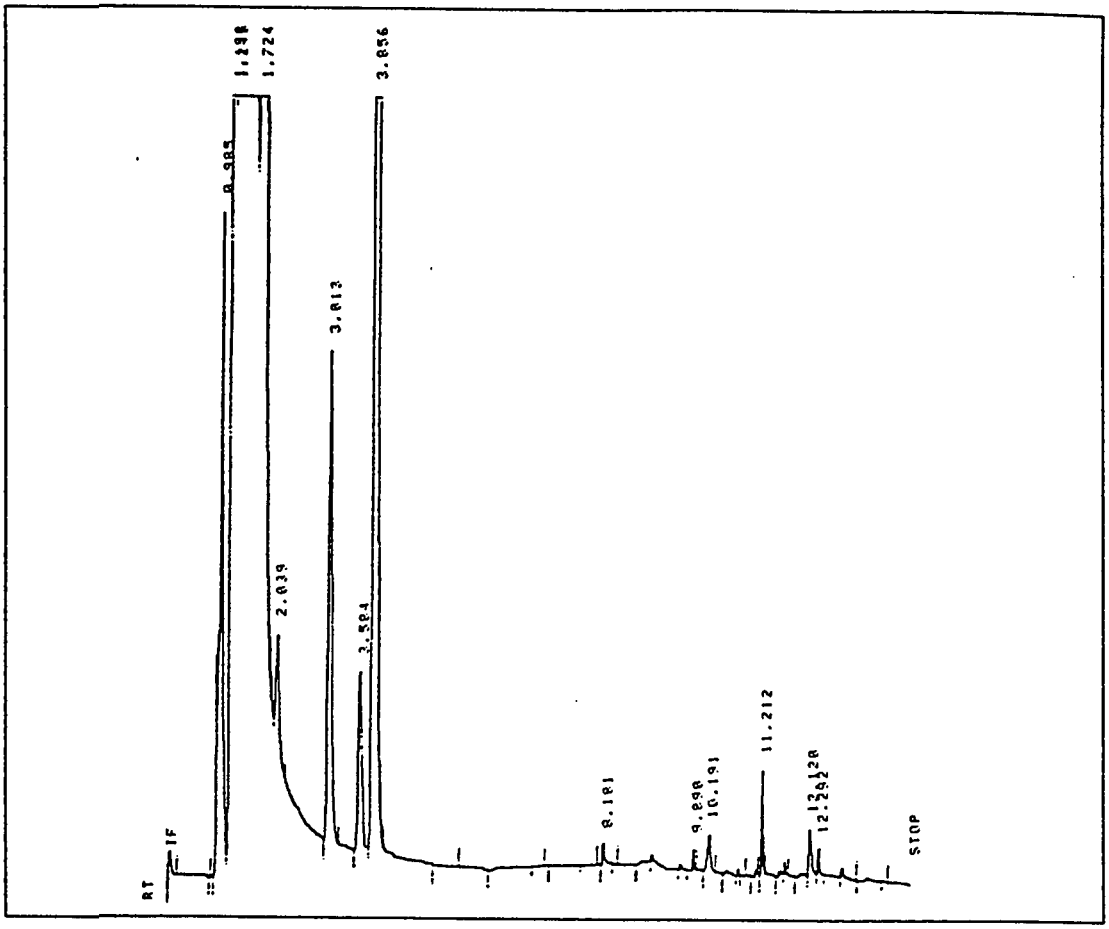
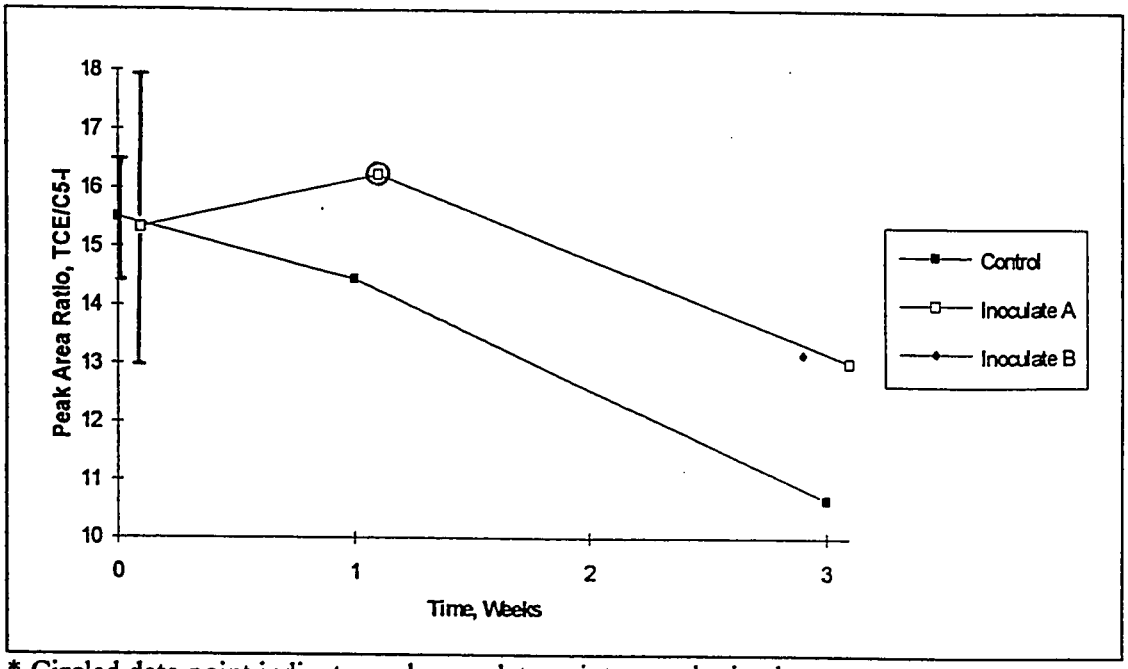


Figure 4.2.3. 100 mg/L TCE Chromatogram

Figure 4.2.4 is a graphical representation the average peak area ratio and percentage error versus time for the 100 mg/L TCE degradation experiment. The concentration of the control and inoculated systems appear to steadily decrease. However, the experimental errors in the initial data points preclude any definitive determination as to whether the degradation in the inoculated system was biologically enhanced. The Week 1 inoculated vial was broken, and an analysis was not performed.



* Circled data point indicates only one data point was obtained

Figure 4.2.4. 100 mg/L TCE Degradation versus Time

The volatility of TCE made it difficult to get repeatable results. Once the vial septum was pierced, the TCE concentration decreased from one injection to the next. Meaningful results from TCE degradation experiments are difficult to obtain from a batch reactor. A better experimental design would incorporate a plug flow reactor, where one would sample at different reactor lengths. Inspection of the chromatograms in Figures 4.2.1 and 4.2.3, does not reveal any obvious degradation product. The 10 mg/L and the 100 mg/L TCE degradation experiments (Figure 4.2.2 and 4.2.4) showed steady degradation in both the control and inoculated systems, but degradation in the inoculated systems did not appear accelerated when compared to the control.

To date, there have been no known cases of TCE being used as a sole carbon source in an aerobic system. TCE is a relatively small molecule with very few hydrogen-carbon or

carbon-carbon bonds that microbes can use as an energy source. TCE can be aerobically cometabolized, but no other substrate was available in these experiments. Reductive dechlorination can occur in an anaerobic environment as long as there is an oxidizable species available. Oxidants include both reduced organic matter or a reduced inorganic species such as ammonia. The graphical data are inconclusive and do not support either of these mechanisms. The decrease in concentration is due to abiotic degradation, as well as volatility and absorption losses.

4.3. DBCP Degradation, Series # 1

Figure 4.3.1 shows a typical Week 3 chromatogram for a 10 mg/L DBCP inoculated vial. Examination of the chromatogram reveals dual peaks at approximately 7 minutes that are not present in the control chromatograms. The DBCP peak appears at 10.601 minutes, and the C5-I peak appears at 2.988 minutes. The small peak that appears immediately prior to DBCP is thought to be water.

Figure 4.3.2 is a graphical representation of the average peak area ratio versus time for the 10 mg/mL DBCP degradation experiment. As with TCE, the peak area of DBCP was divided by the pentane impurity area. Error bars are not shown for experimental uncertainty less than 5%. The DBCP concentration in the inoculated and control systems steadily decreased with time. However, the degradation rate in the inoculated system appears higher than abiotic degradation rate in the control.

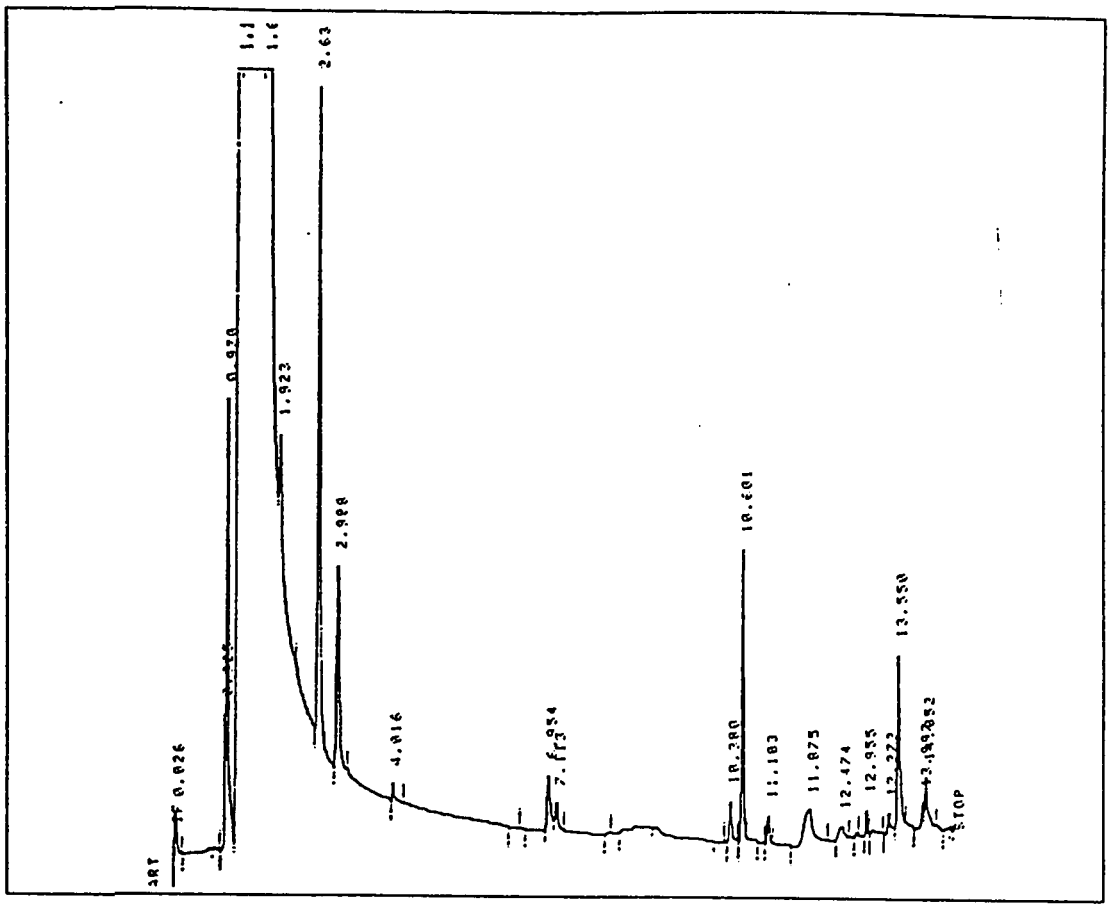


Figure 4.3.1. 10 mg/L DBCP Chromatogram

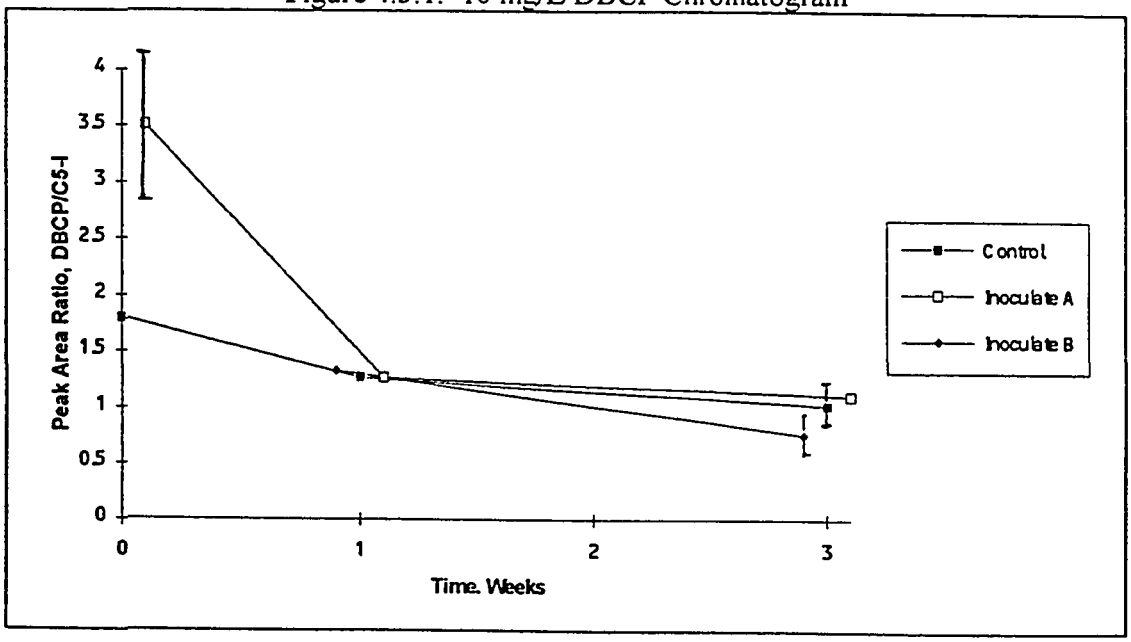


Figure 4.3.2. 10 mg/L DBCP Degradation versus Time, Series # 1

Figure 4.3.3 shows a Week 3 chromatogram of a 100 mg/L DBCP inoculated vial. Examination of the chromatogram reveals dual peaks around 7 minutes similar to the 10 mg/L DBCP experiments. The C5-I and DBCP peaks appear at 2.991 and 10.601 minutes respectively. A full set of chromatograms for all the DBCP Series #1 experiments from time equal zero and Week 3 is contained in Appendix C (Figures C.1.1 to C.2.4).

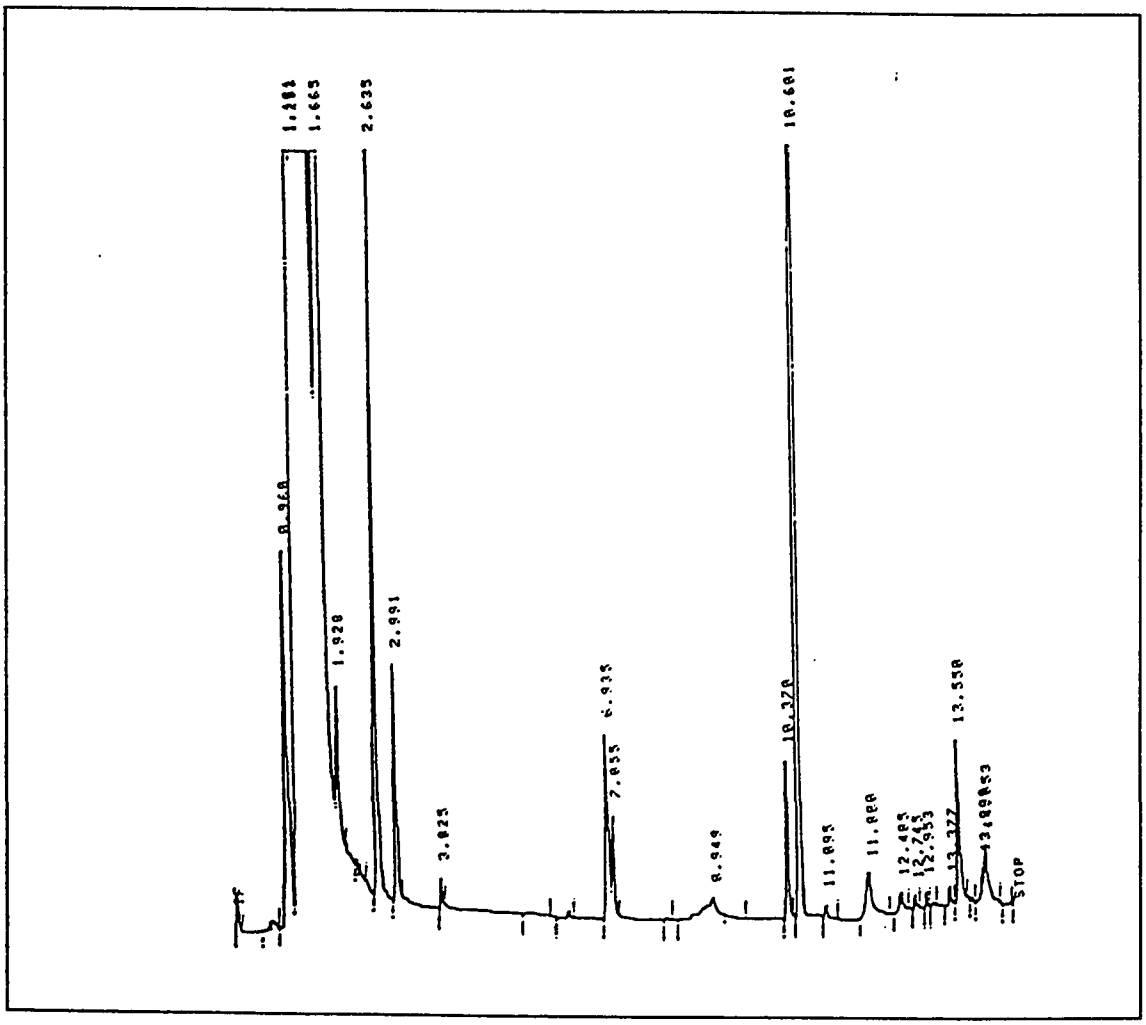
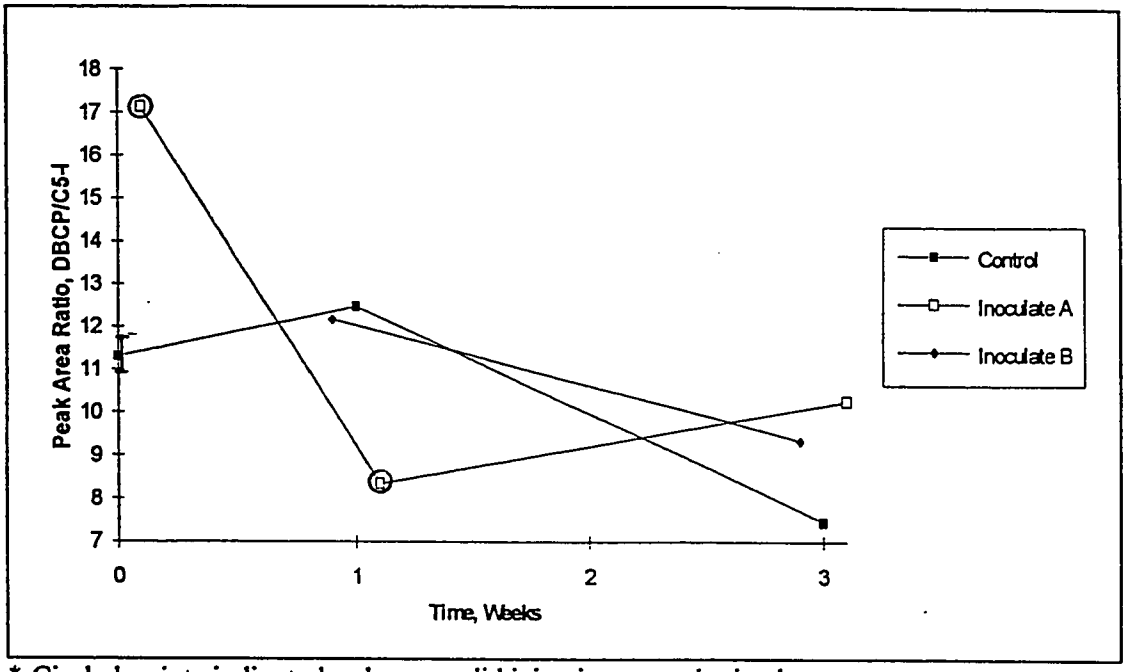


Figure 4.3.3.100 mg/L DBCP Chromatogram

The results from the 100 mg/L DBCP degradation experiments are presented in Figure 4.3.3. The DBCP concentration in both the control and the inoculated systems steadily decreased with time, but the degradation rate appears to be higher in the inoculated systems similar to the 10 mg/L DBCP experiment.



* Circled points indicated only one valid injection was obtained

Figure 4.2.4. 100 mg/L DBCP Degradation versus Time, Series # 1

The 10 mg/L DBCP experiment, Figure 4.3.2, shows a higher degradation rate in the inoculated systems. The first data point is elevated due to a concentrating effect of the cells, as discussed in section 4.2. If the only degradation in the inoculated systems were abiotic, then the rate should be approximately equal in both systems, although the inoculated systems would consistently appear higher due to the concentrating effect. Inspection of Figures 4.3.1 and 4.3.3 shows two peaks at approximately 7 minutes that are not present in the control chromatographs. This corresponds to an elution temperature of

approximately 83°C. It is possible that these peaks are due to cellular material and not substrate degradation products. Assuming cell degradation rates were similar in the TCE and DBCP experiments, one would expect to find similar peaks at an elution temperature of 83°C or 7.3 minutes in the TCE chromatograms. The absence of these peaks in the TCE experiments suggests that the new peaks in the DBCP chromatograms are degradation products. This evidence alone can not preclude these peaks are not cellular products, but there is a high probability they are degradation products.

If biological degradation is occurring, aerobic and anaerobic mechanisms can be proposed. In an aerobic environment, a hydrolytic substitution on one of the terminal halogens would have resulted in a chlorinated alcohol, which would have a higher boiling point. Due to steric hindrance, it is not likely the substitution would occur on the central bromine. However, the alcohol could be chemically unstable and degrade to the aldehyde (1,2-dibromopropionaldehyde or 1-chloro-2-bromopropionaldehyde), which have boiling points less than the original substrate. In an anaerobic environment, a reductive dechlorination also produces products (2,3-dibromopropane or 2-bromo-3-chloropropane) with reduced boiling points. If the microbes could operate on either bromine or chlorine, then one could expect two different degradation products; a de-brominated and a de-chlorinated compound. This offers a potential explanation for the dual peaks. The possible degradation products described for the aerobic pathway were not available in their pure form, and therefore could not be analyzed for their elution times. The potential anaerobic degradation products were available, and the elution time for both compounds was approximately 6.2 minutes. This does not preclude a reductive dehalogenation pathway because the compound may have undergone abiotic transformation after the initial biodegradation.

The 100 mg/L DBCP experiment, Figure 4.3.2, has similar results to those above. The inoculated systems appear to have an accelerated rate of degradation. Inspection of the chromatograms Figure 4.3.4 also show peaks at elution times near 7 minutes. Note that these peaks are larger in area than the corresponding peaks in the 10 mg/L DBCP experiments, lending some credibility to the fact that degradation products are from DBCP degradation and not cellular material because the quantity of biomass was equal in both experiments. It is not known which mechanism is responsible, or whether the microbes are able to utilize the substrate as a sole carbon source. Potentially, the degradation could be caused by enzymes released from the cells upon lysing. However, there is a high probability the degradation is biological and not abiotic.

4.4. Lindane Degradation

Figure 4.4.1 shows a typical Week 3 chromatogram for a 250 μg/L lindane inoculated vial. Hexadecane was used as the internal standard for the lindane degradation experiments. The elution times for hexadecane and lindane are 6.225 and 8.283 minutes respectively. Inspection of the chromatogram reveals no obvious degradation peaks.

A graphical representation of the average peak area ratio versus time is presented in Figure 4.4.2. The peak area of lindane is divides by the peak area of hexadecane. Figure 4.4.2 shows a decrease of concentration degradation in the control and inoculated systems. It appears that the inoculated system has a higher degradation rate, but the experimental error of more than 22% decreases the validity of the data point.

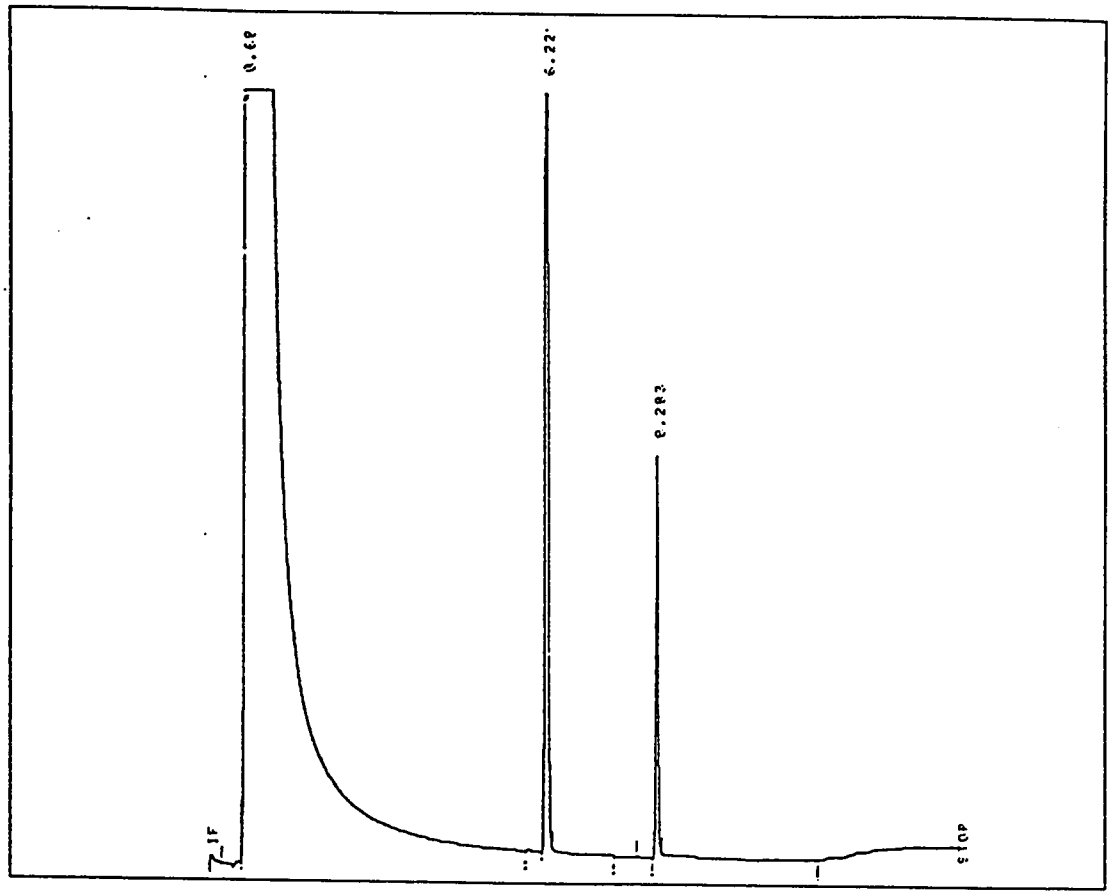


Figure 4.4.1. 250 µg/L Lindane Chromatogram

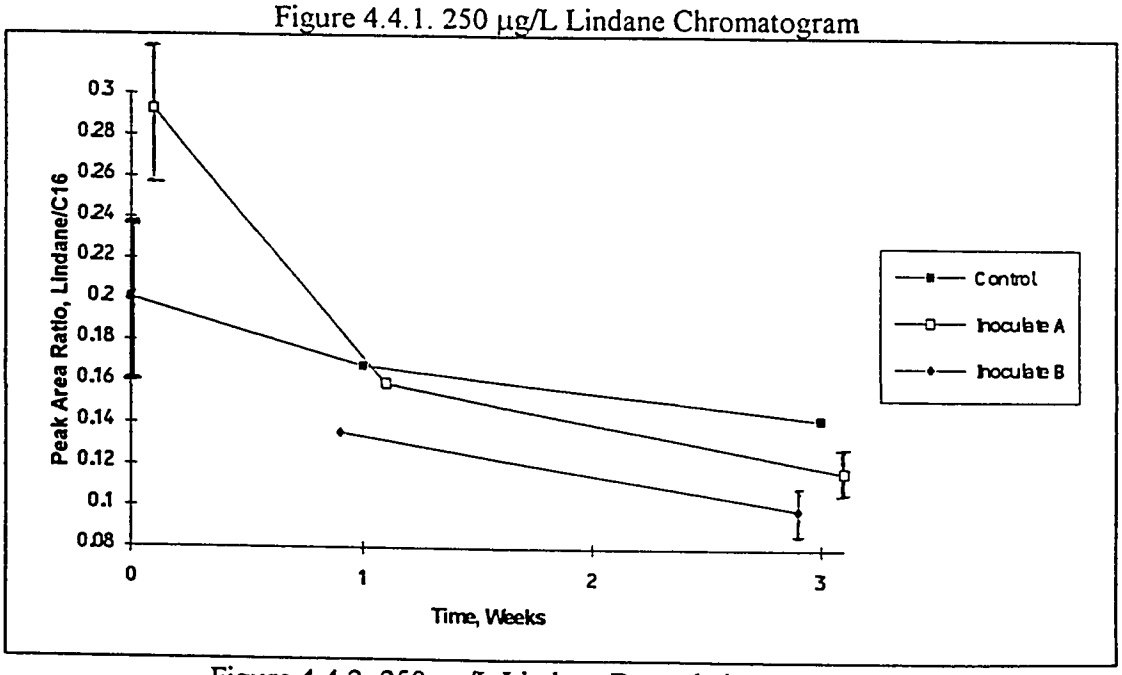


Figure 4.4.2. 250 µg/L Lindane Degradation versus Time

A typical Week 3 chromatogram of a 500 µg/L inoculated vial Week 3 is shown in Figure 4.4.3. The elution times for hexadecane and lindane are 6.162 and 8.218 minutes respectively. No degradation products are evident. A full set of chromatograms of all the lindane experiments from time equal zero and Week 3 is contained in Appendix D (Figures D.1.1 through Figure D.2.4).

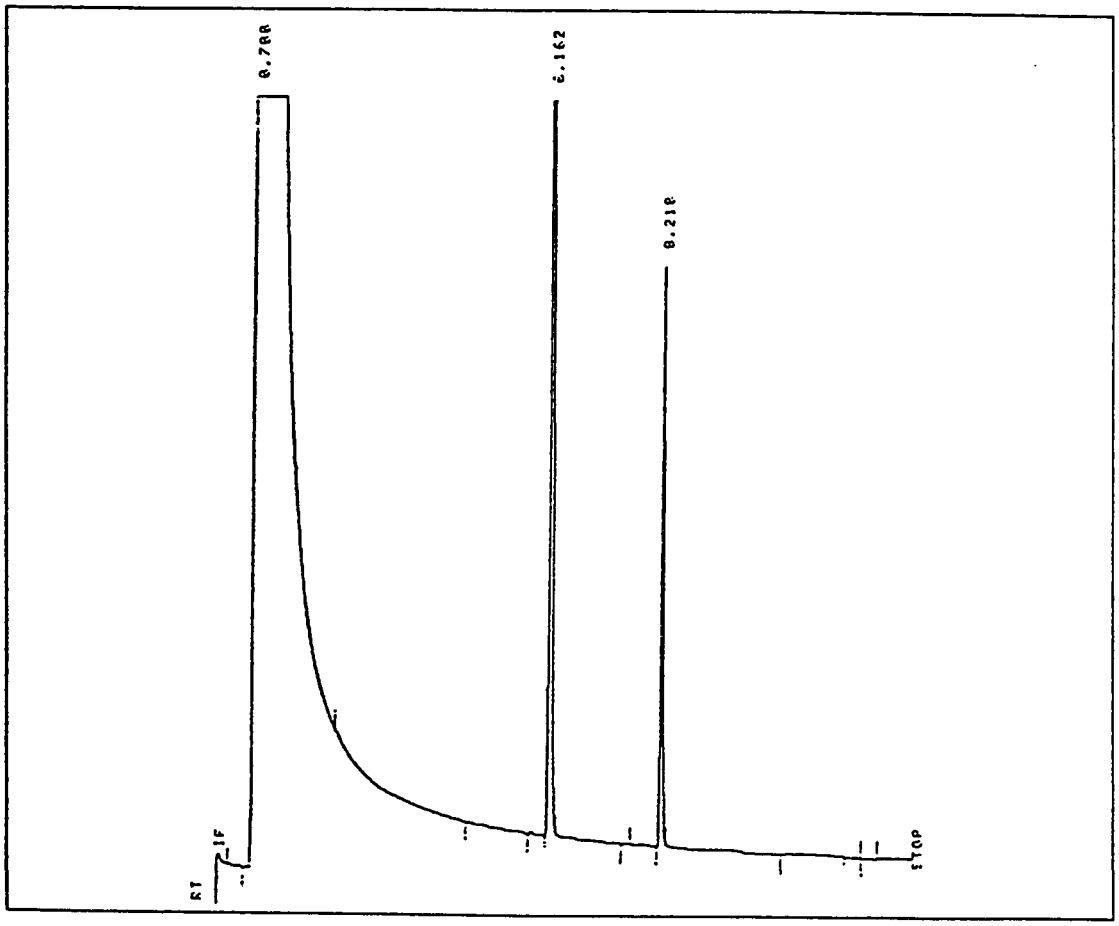


Figure 4.4.3. 500 µg/L Lindane Chromatogram

Figure 4.4.4 is a graphical representation of lindane degradation versus time. No clear trends are evident from this data.

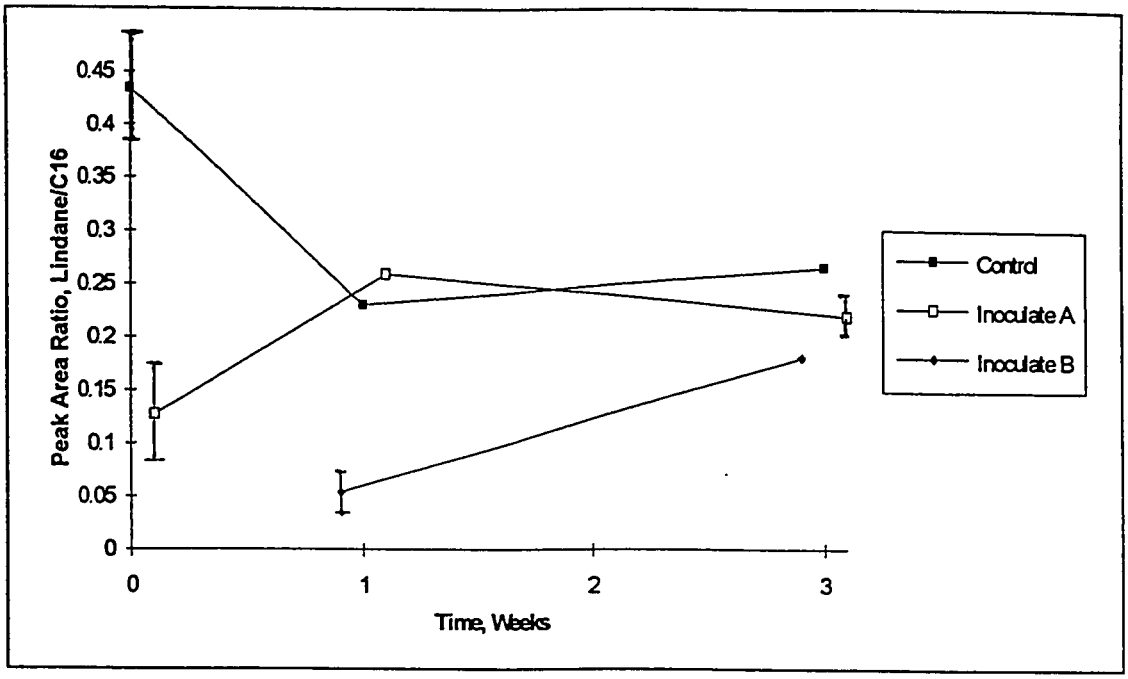


Figure 4.4.4. 500 μg/L Lindane Degradation versus Time

The lindane degradation experiments show no clear trends, and the chromatographs do not show any degradation product peaks. One of the most common degradation pathways for lindane is trans-isomerization. The resulting product would have a very slightly different elution time, which was not evident.

4.5. PCP Degradation

Figure 4.5.1 shows a typical Week 3 chromatogram of a 10 mg/L PCP inoculated vial. Hexadecane was used as the internal standard for these experiments. The elution times for hexadecane and PCP are 6.199 and 8.180 minutes respectively. Examination of the chromatogram shows no obvious degradation peaks. The FID is relatively insensitive to

PCP at low concentrations, due to the paucity of non-ionizing bonds, and relatively large experimental errors are induced. C-Cl bonds produced few ions when combusted.

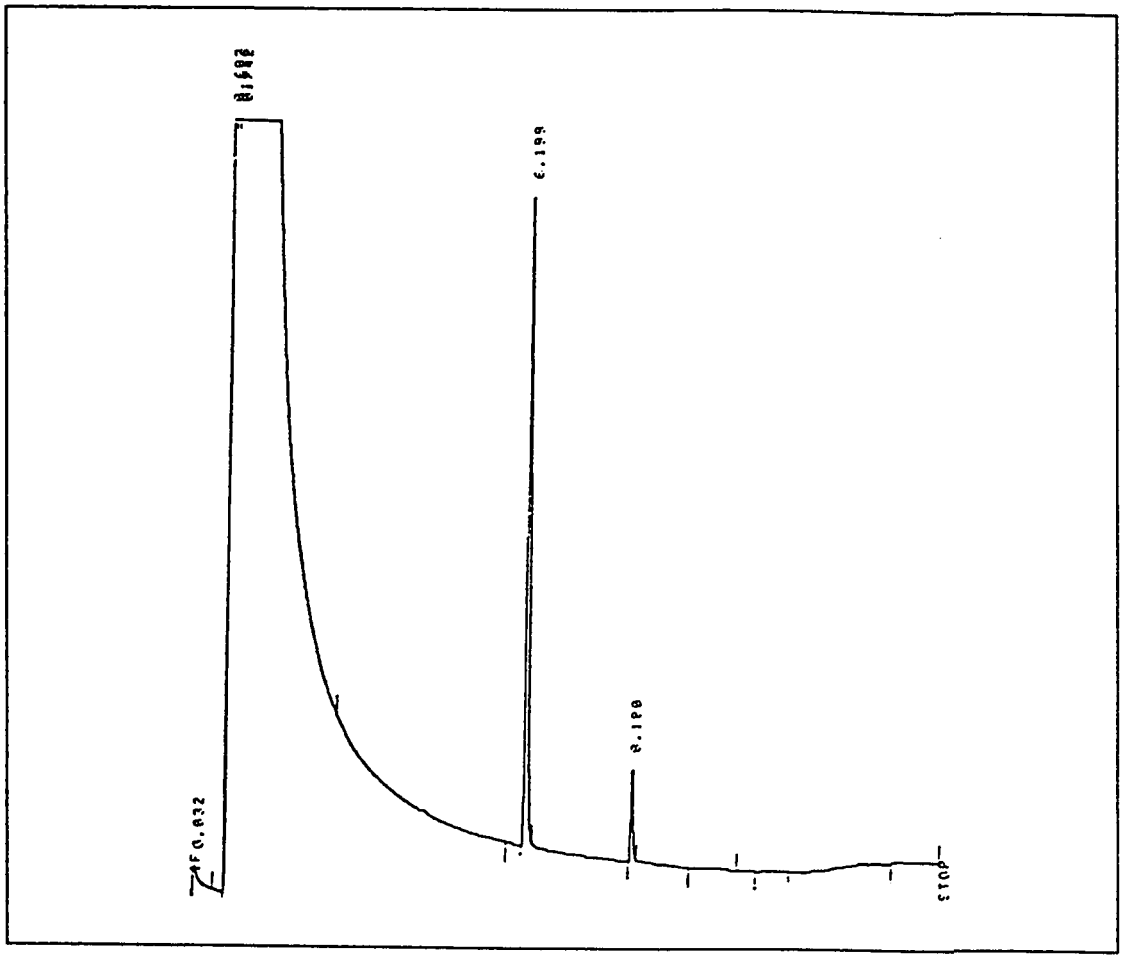
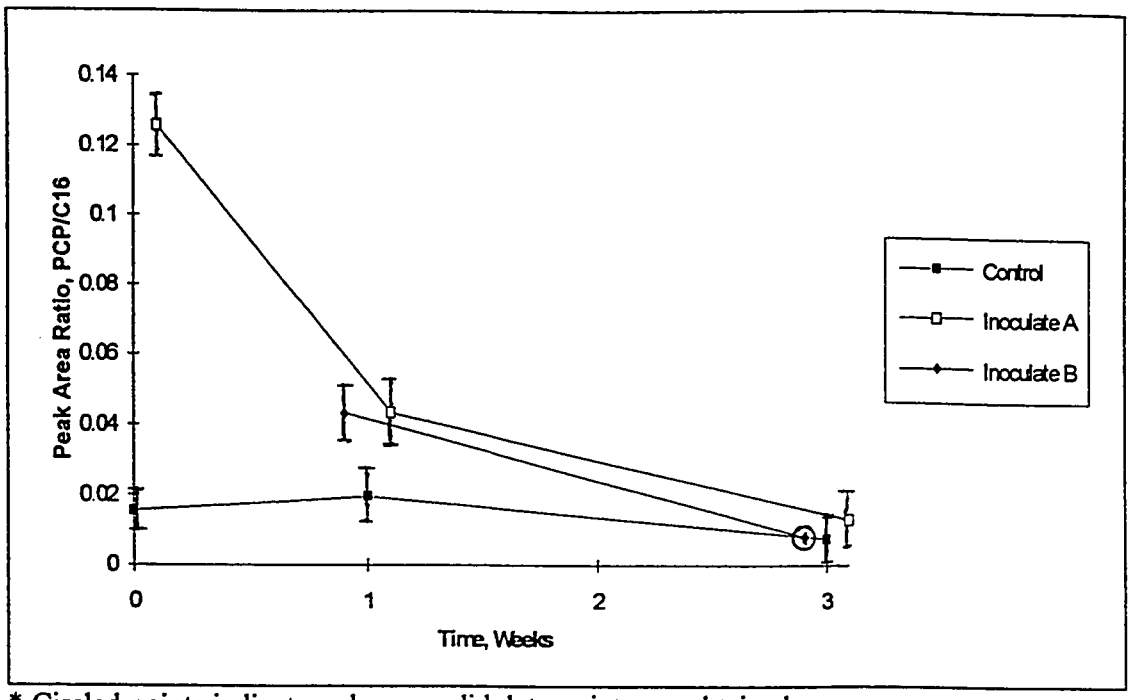


Figure 4.5.1. 10 mg/L PCP Chromatogram

Figure 4.5.2 is a graphical representation of the peak area ratio versus time for the 10 mg/L PCP experiments. The inoculated systems appear to have a higher degradation rate when compared to the controls.



* Circled points indicate only one valid date point was obtained

Figure 4.5.2. 10 mg/L PCP Degradation versus Time

A typical Week 3 chromatogram of a 100 mg/L PCP inoculated vial Figure 4.5.3. Again, inspection of the chromatogram reveals no degradation peaks. A full set of chromatograms from time equal zero and three weeks are contained in Appendix E for all the PCP experiments (Figures E.1.1 through Figure E.2.4).

A graphical representation of the peak area ratio versus time for the 100 mg/L PCP degradation experiments is presented Figure 4.5.4. There appears to be less scatter than the 10 mg/L PCP experiments because the increased concentration is reduces the uncertainty of the FID signal. However the degradation rate appears equal in the control inoculated systems.

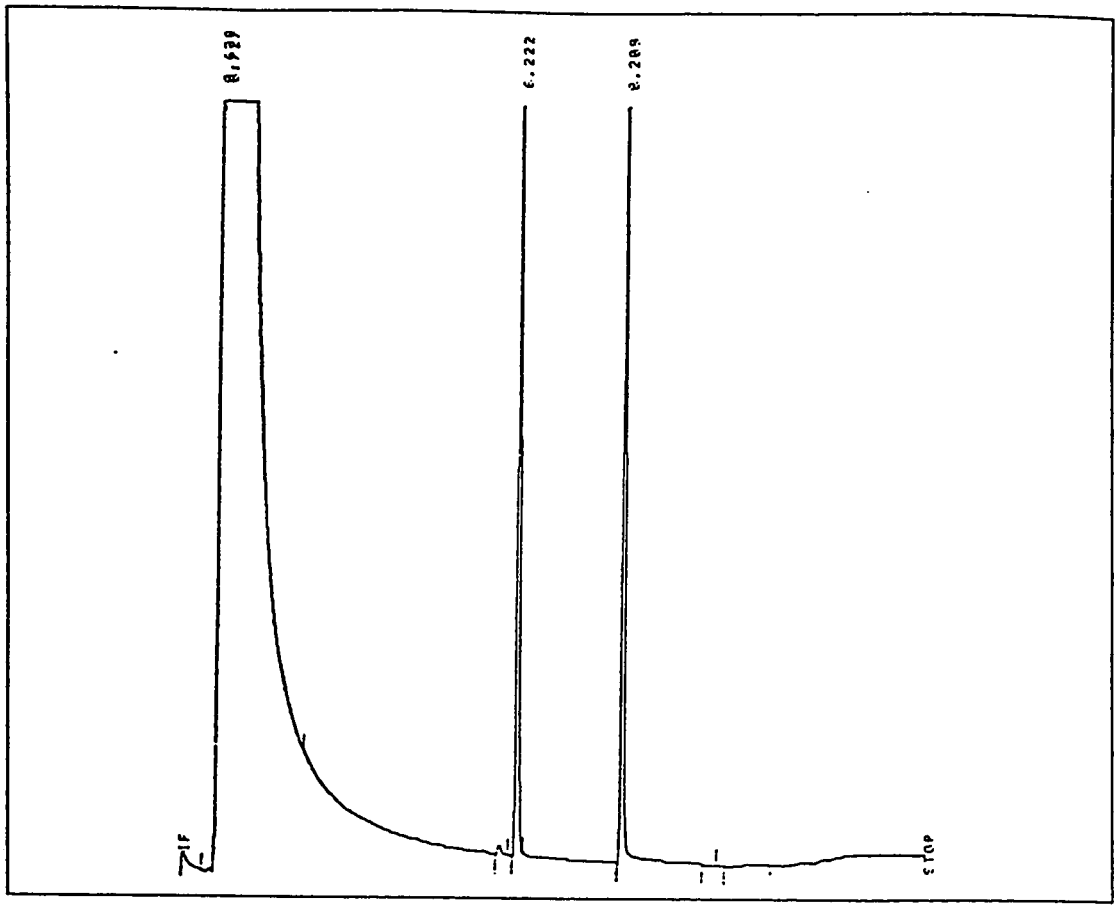


Figure 4.5.3. 100 mg/L PCP Chromatogram

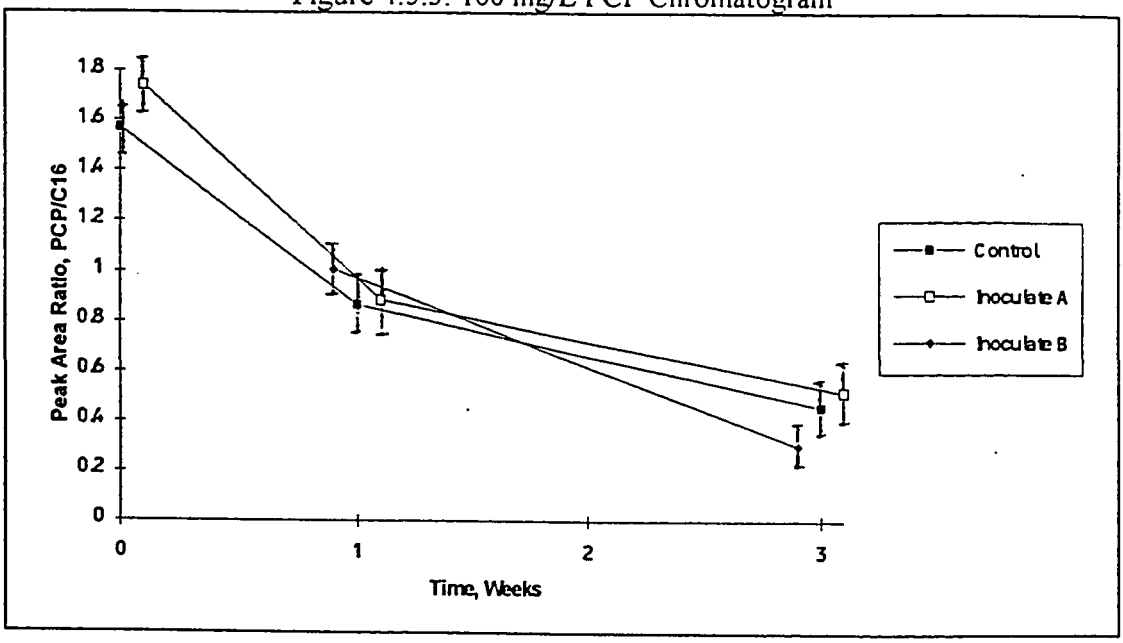


Figure 4.5.4. 100 mg/L PCP Degradation versus Time

The 10 mg/L PCP degradation, Figure 4.5.2, shows accelerated degradation initially followed by the same rate as the control. There is no evidence of degradation products in the chromatograms. However, given the high rate of initial degradation in the inoculate vial, biologically enhanced degradation can not be precluded. In a separate experiment by other investigators (Dr. Grilione, personal communication), it appeared the cells remained viable in PCP spiked solutions. No conclusions can be drawn from the 100 mg/l PCP degradation data, Figure 4.5.4. This high concentration could well be toxic to the microbes. There are many examples of PCP degradation as a sole carbon source, and further studies at 10 mg/L or lower may be warranted.

4.6 DBCP Degradation, Series # 2

The results of the second series of 100 mg/L DBCP degradation experiments are presented in this section. In this set of experiments, the pentane extractant was spiked with 1,3,5-trichlororobenzene, TCB, to a concentration of 100 mg/L. This set of experiments also has the advantage of the use of a centrifuge to separate the emulsion formed when extracting the inoculated systems. This proved to dramatically reduce the experimental error. A typical Week 3 chromatogram of a 100 mg/L PCP inoculated vial from Week 3 is shown in Figure 4.6.1. The elution time DBCP and TCB are 10.570 and 11.555 minutes respectively. A full set of chromatograms from time equal zero and three weeks are contained in Appendix C (Figure C.3.1 through Figure C.3.4) for the control and inoculated vials. Degradation peaks were not observed unlike the first series of DBCP degradation experiments. This suggests the chance of repeatability in these experiments is low. Figure 4.6.2 presents the results from the experiment using TCB as

the internal standard. The experimental errors for this experiment were all less than 2%, and thus, the error bars are not shown.

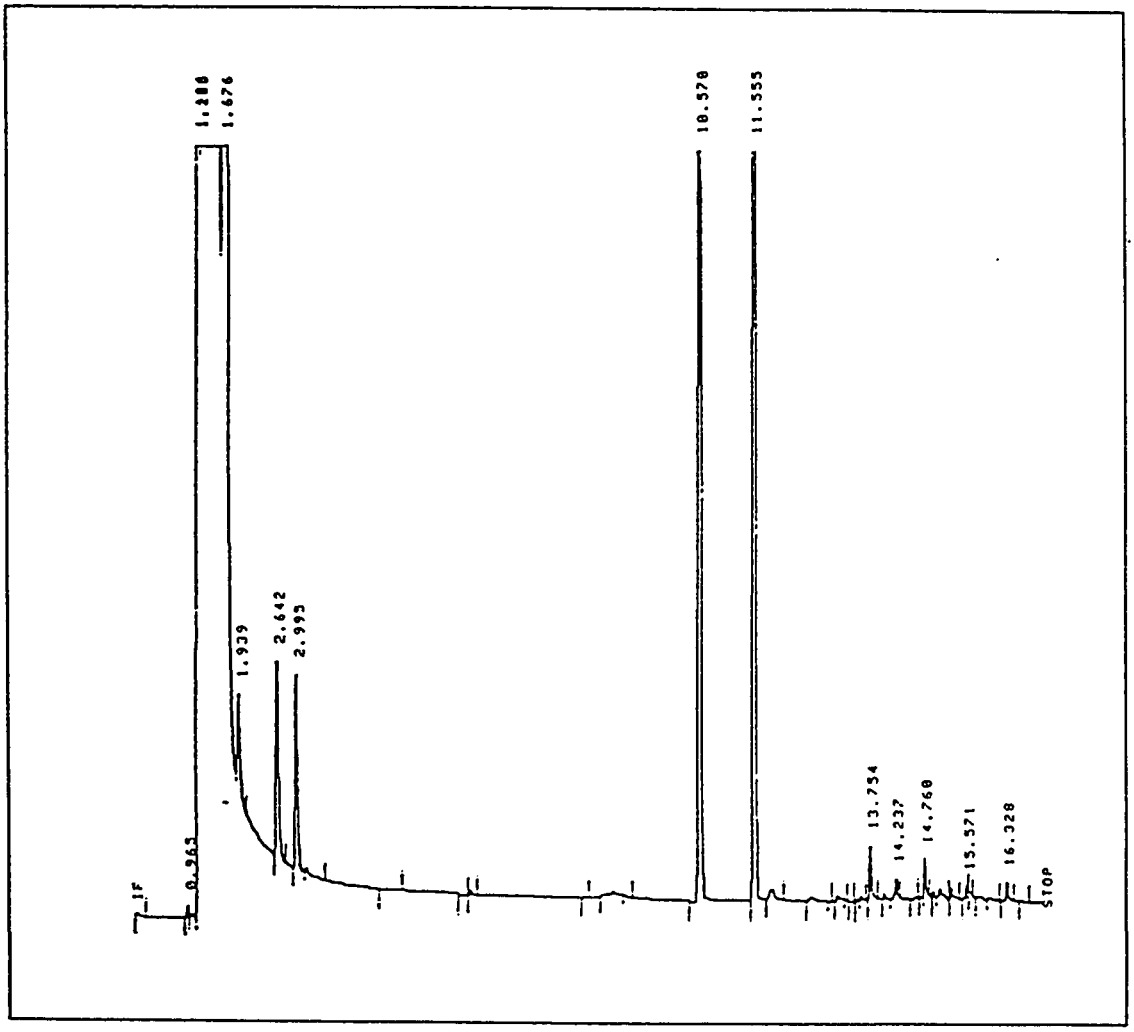


Figure 4.6.1. 100 mg/L DBCP Chromatogram, Series # 2

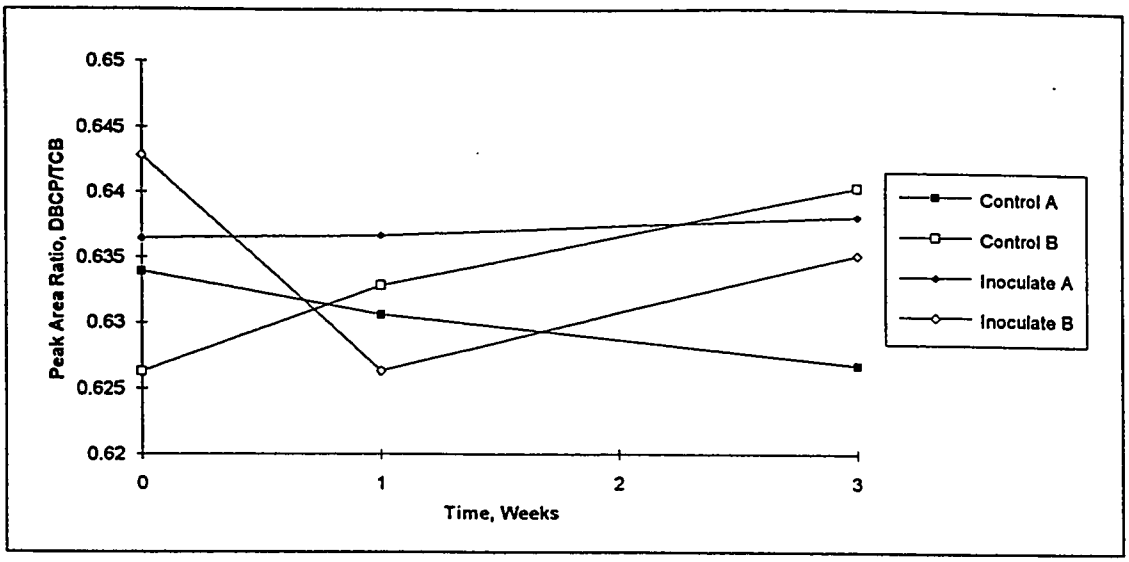


Figure 4.6.2. 100 mg/L DBCP Degradation versus Time, Series # 2

The second series of DBCP degradation experiments did not reproduce the findings from the first series. There may be two explanations for this. First, the isolate, P11, was not included in the consortia used for the second series since the Petri dish containing it appeared contaminated. The periphery of the colonies contained a dark brown ring that was morphologically different. In addition, the quantity of biomass introduced to the inoculated vials was less, although when the suspension density was approximately equal when measured. Only half the number of dishes was used to prepare the cells for the suspension, and only 1 mL was used for the inoculation volume. Although isolate P11 was not included in the consortia, it is not likely this was responsible for the lack of degradation. Most probably, it was due to the smaller quantity of cellular material introduced to the inoculated systems. It is possible that the degradation seen in the first series of DBCP experiments was due to enzymes released when the cells lysed, and the microbes were not able to digest the DBCP as a sole carbon source. Due to a greater

quantity of cells in the first series, more enzymes were released, and the degradation products were more evident.

4.7. 1,4-BCB Degradation

A typical Week 3 chromatogram of a 100 mg/L 1,4-BCB inoculate vial is shown in Figure 4.7.1. A full set of chromatograms from time equal zero and three weeks are contained in Appendix F for the control and inoculated systems. The 1,4-BCB contained impurities at elution times of 7 and 10.4 minutes. The impurity at 7 minutes 1,4-BCB-I, was also monitored for potential degradation. TCB was again used as the internal standard. A graphical representation of the 100 mg/L 1,4-BCB degradation experiments is presented in Figure 4.7.2. The concentration of 1,4-BCB steadily decreased approximately 16% relative to the internal standard. The concentration of 1,4-BCB-I, was also plotted, and the results are shown Figure 4.7.3. The concentration of the impurity steadily increased approximately 38%. The maximum chromatographic error in all results reported in this section are less than 2%, and thus, the error bars are not shown.

The 1,4-DBCP degradation experiments, Figure 4.7.2 indicated a decrease in concentration of the parent substrate in the control and inoculated system at an almost identical rate. Concurrent with decrease of 1,4-BCB, was an increase in the impurity that was being monitored. Again, the impurity concentration increased at the same rate in both the control and the inoculated systems. Inspection of the chromatograms in Appendix F indicated no biologically induced degradation. Clearly, the 1,4-BCB was abiotically degrading to the impurity that was being monitored.

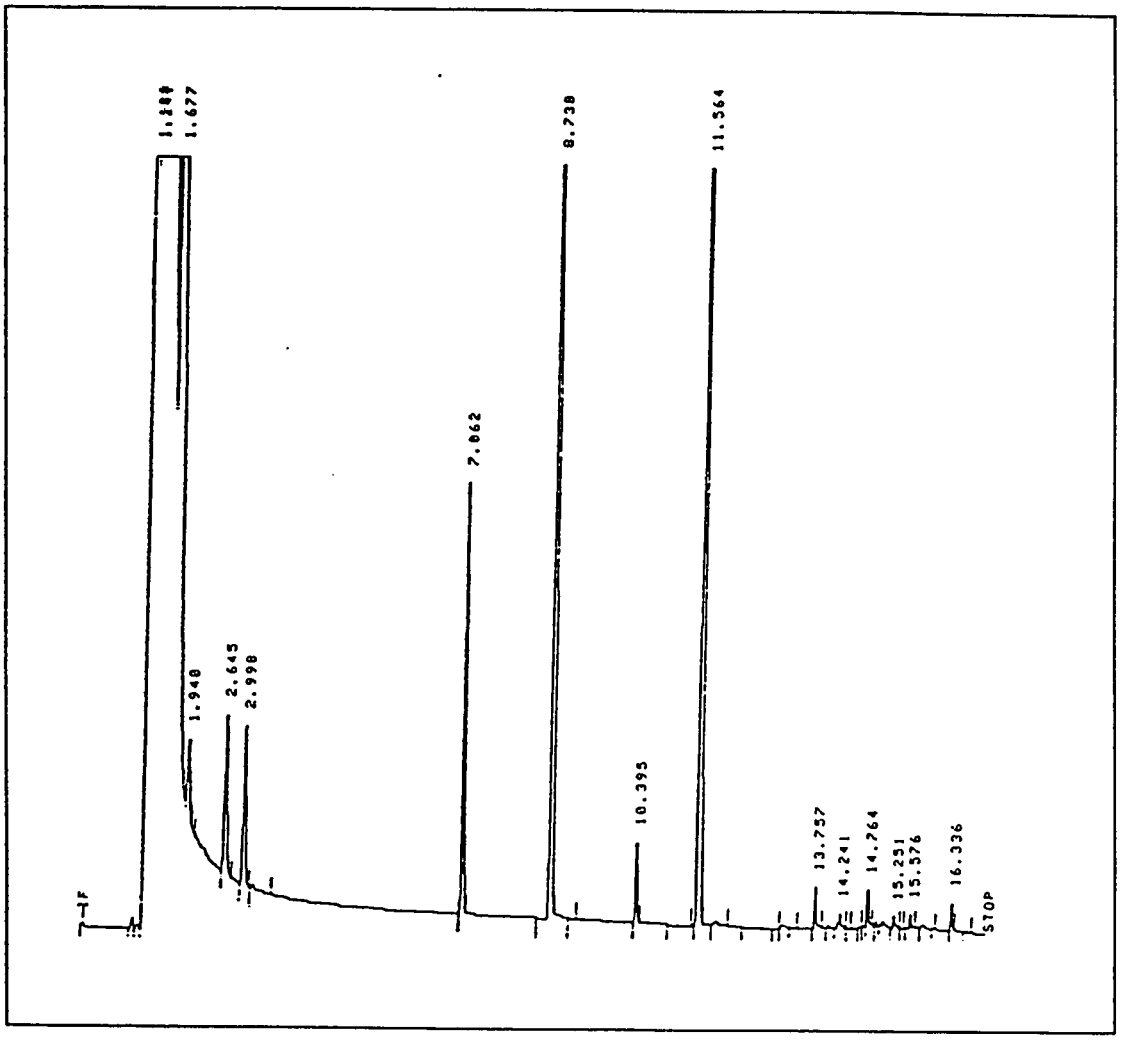


Figure 4.7.1 100 mg/L 1,4-BCB Chromatogram

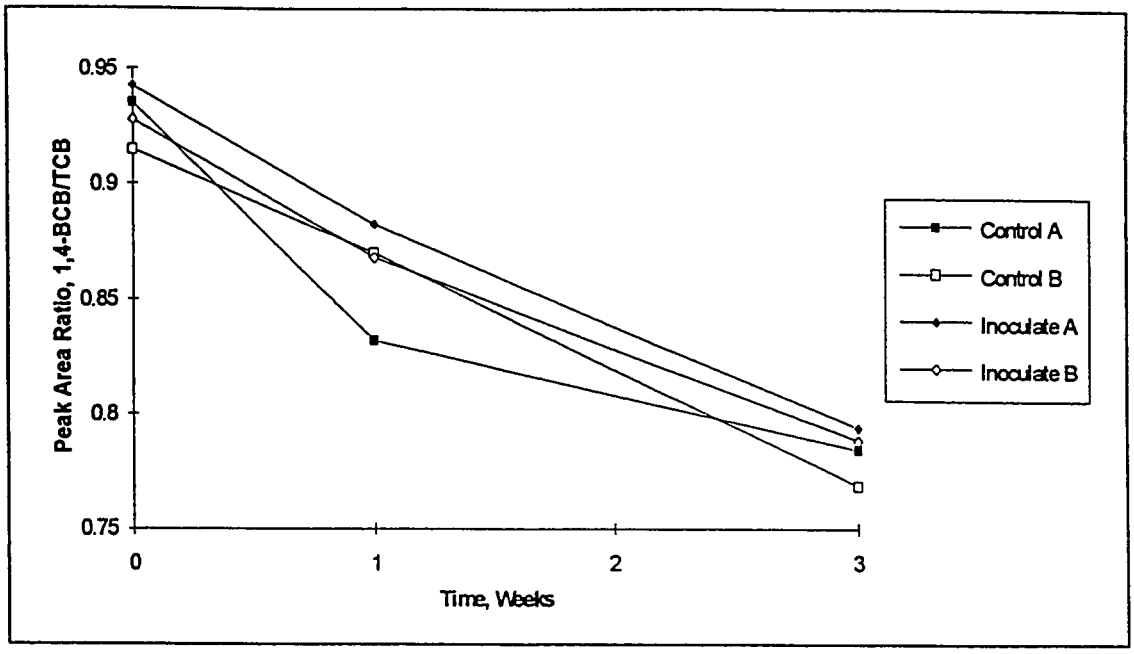


Figure 4.7.2. 1,4-BCB Degradation versus Time

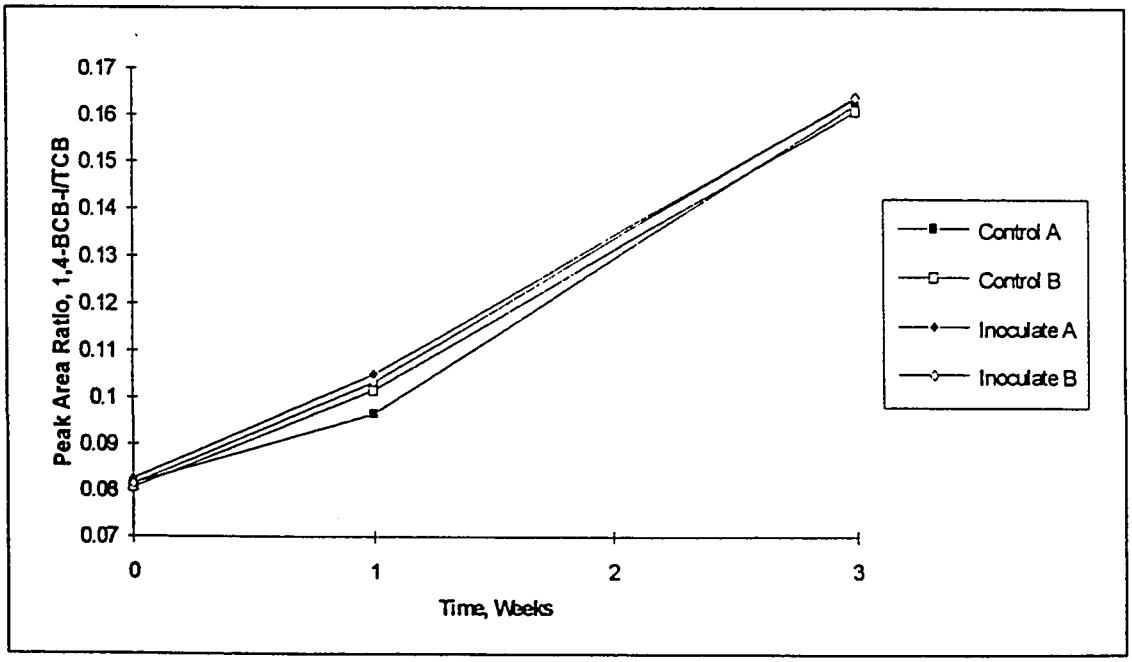


Figure 4.7.3. 1,4-BCB-I Degradation versus Time

4.8 Errors

There are essentially two main sources of errors associated with these experiments. The first being the error introduced due to the emulsification of the inoculated vials. The emulsification contained air, cellular material, small amounts of the aqueous phase and the organic phase. Therefore, in any given injection the ratios of all the components could vary. This induces error into the ratio of the substrate and internal standard, which can be seen in the error bars associated with the graphs presented in the previous sections. A minimum of three samples were injected from each vial, but in some cases only one valid injection was obtained.

The other error is in the injection volume. A total of five injections were done on both the control and an inoculated vial for the 100 mg/L PCP degradation experiment. The peak area of the internal standard, hexadecane, was compared. The total area of the chromatogram can not be used for comparison because the integrator employed for these experiments must be tuned to accurately detect and integrate peaks associated with the contaminants of concern, and it is not able to simultaneously integrate the very large and broad solvent peak. Table 4.8.1 presents the error associated with repeated injections of the same control, where no emulsification occurred, and the emulsified, inoculated vials. Centrifugation was extremely effective in reducing the emulsification and injection volume error. The injection volume errors associated with the centrifuged samples are presented in Table 4.8.2, using the TCB internal standard.

The error introduced due to the emulsification of the inoculated systems proved significant in many cases. When drawing an injection sample from an emulsified sample, an attempt

was made to draw from an area of the vial that was predominantly organic phase. However, the sample inevitably contained small amount of cellular material and even some of the aqueous phase. Figure 4.8.1 showed the injection volume error to be over 28% in the case of the inoculated PCP experiments. Centrifugation proved to be invaluable in reducing the injection and emulsification error and should be employed for all future experimentation.

A minor source of error in Experiments 1 though 4 was introduced since the substrate was added individually to each vial, as opposed to all the other experiments where a single batch of ASW containing halogenated substrate was prepared. Inspection of the graphical results shows that in most cases this was not significant, but future studies should employ the use of batched solutions.

Table 4.8.1. Injection Error without Centrifugation

Peak Area, Hexadecane		
Run #	Control	Inoculate
1	992944	593927
2	1017512	598438
3	995915	458036
4	994721	924984
5	956411	544261
Average	991500	623929
Standard Deviation	220022	177518
% Error	2.22%	28.45%

Table 4.8.2. Injection Error with Centrifugation

Peak Area, 1,3,5-TCB		
Run #	Control	Inoculate
1	1499699	1392508
2	15450009	1503831
3	1526798	1424846
Average	1523835	1440395
Standard Deviation	227800	57267
% Error	1.50%	3.98%

Chapter 5. Conclusion

The microbes associated with *P. cartilagineum* and *Laurencia sp.* appear to have an effect in enhancing the degradation DBCP when added to the system in sufficient quantity. It is not clear whether DBCP is metabolized as a sole carbon source, or if the cells lysed and released enzymes that degraded the DBCP. It is also feasible that some strains in the consortia were digesting others, and cometabolizing the DBCP. The structure of DBCP resembles some of the branches of the compounds shown in Figure 2.4.1. Microbes are able to attack terminal halogens away from structures with significant steric hindrance, and the microbes are unable to attack the halogens associated with lindane. Certainly, one would expect degradation of 1,4-BCB given the proper initial cell concentration. It also appears that low concentrations of PCP may also undergo biologically enhance degradation in spite of the steric hindrance, although definitive results were not obtained in this study.

Future studies should deduce whether the microbes are able to survive on DBCP as a sole substrate, or if enzymes from lysed cells were responsible for the apparent degradation. This could be accomplished by disrupting the cells prior to experiments and checking for degradation. Useful information could be obtained from culturing the inoculated systems after exposure to DBCP. This information could potentially indicate which microbial strains are able to survive or metabolize DBCP. The inoculation size appears critical for degradation and needs further evaluation. Using the viable count method is a more reliable method of microbe enumeration. If the results from the first series of DBCP experiments could be reproduced, it is critical to determine the degradation products. A more definitive mechanism can be proposed once the degradation products are known.

Simple enrichment studies should be performed using PCP in low concentrations as a substrate. This would involve subjecting a consortium of cells to an aqueous solution with PCP as a sole carbon source and determining if the cells remain viable for an extended period of time.

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Appendix A. Reduced Chromatography Data

Table A.1. 10 mg/L TCE Degradation Data

	Peak Area Ratio, TCE/C5-I		
Time, Weeks	Control	Inoculate A	Inoculate B
0	3.013	2.62 ± 25.5%	N/A
1	1.719 ± 0.64%	2.414	2.428 ± 2.53%
3	1.421 ± 5.23%	2.414 ± 1.78%	2.010±4.59%

Table A.2. 100 mg/L TCE Degradation Data

	Peak Area Ratio, TCE/ C5-I		
Time, Weeks	Control	Inoculate A	Inoculate B
0	15.495 ± 6.07%	15.319 ± 17.26%	N/A
1	14.432 ± 0.42%	16.2133	N/A
3	10.662 ± 0.05%	13.017 ± 5.84%	13.163 ± 4.04%

Table A.3. 10 mg/L DBCP Degradation Data, Series # 1

	Peak Area Ratio, DBCP/ C5-I		
Time, Weeks	Control	Inoculate A	Inoculate B
0	1.797 ± 5.10%	3.502 ± 20.03%	N/A
1	1.269 ± 1.53%	1.267 ± 3.55%	1.328 ± 3.81
3	1.022 ± 13.70%	1.110 ± 6.25%	0.75 ± 22.77%

Table A.4. 100 mg/L DBCP Degradation Data, Series # 1

	Peak Area Ratio, DBCP/ C5-I		
Time, Weeks	Control	Inoculate A	Inoculate B
0	11.302 ± 5.95%	17.133	N/A
1	12.454 ± 0.47%	8.342	12.16± 0.93%
3	7.460 ± 3.37%	10.254 ± 2.50%	9.336 ± 5.02%

Table A.5. 250 µg/L Lindane Degradation Data

	Peak Area Ratio, Lindane/ C16		
Time, Weeks	Control	Inoculate A	Inoculate B
0	0.201 ± 24.27%	0.292 ± 11.37%	N/A
1	0.168 ± 0.61%	0.159 ± 3.33%	0.136 ± 5.723%
3	0.143 ± 1.20%	0.145 ± 6.28%	.097 ± 5.61%

Table A.6. 500 µg/L Lindane Degradation Data

	Peak Area Ratio, Lindane/ C16		
Time, Weeks	Control	Inoculate A	Inoculate B
0	0.435 ± 11.90%	0.127 ± 33.34%	N/A
1	0.230 ± 7.20%	0.258 ± 5.26%	0.054 ± 17.84%
3	0.266 ± 1.63%	0.219 ± 1.74%	0.180 ± 6.58%

Table 4.7. 10 mg/L PCP Degradation Data

	Peak Area Ratio, PCP/ C16		
Time, Weeks	Control	Inoculate A	Inoculate B
0	0.0156 ± 4.54%	0.126 ± 9.43%	N/A
1	0.0195 ± 65.40%	0.0434 ± 45.23%	0.0432 ± 63.09%
3	0.0075 ± 20.09%	0.0129 ± 46.45%	0.0081

Table A.8. 100 mg/L PCP Degradation Data

	Peak Area Ratio, PCP/ C16		
Time, Weeks	Control	Inoculate A	Inoculate B
0	1.571 ± 13.14%	1.174 ± 10.40%	N/A
1	0.865 ± 20.21%	0.884 ± 15.29%	1.006 ± 13.58%
3	0.458 ± 8.20%	0.516 ± 11.36%	0.303 ± 10.55%

Table A.9. 100 mg/L DBCP Degradation Data, Series # 2

	Peak Area Ratio, DBCP/TCB			
Time, weeks	Control A	Control B	Inoculate B	Inoculate B
0	0.634 ± 1.40%	0.626 ± 0.93%	0.636 ± 0.53%	0.643 ± 0.47%
1	0.631 ± 0.50%	0.623 ± 0.19%	0.637 ± 0.23%	0.626 ± 0.94%
3	0.621 ± 1.54%	0.640 ± 0.25%	0.638 ± 0.31%	0.635 ± 0.63%

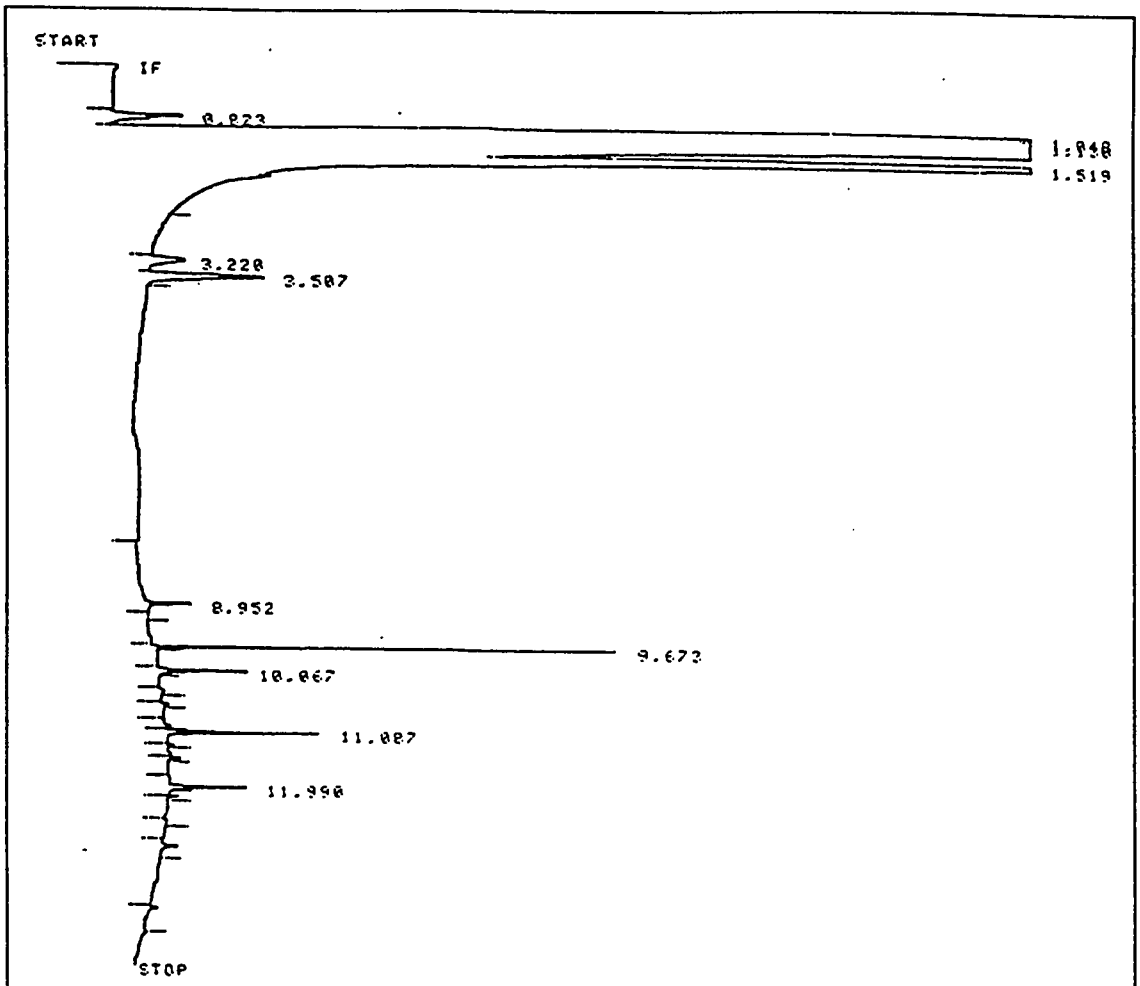
Table A.10. 1,4-BCB Degradation Data

	Peak Area Ratio ± % Error, 1,4-BCB/TCB			
Time, weeks	Control A	Control B	Inoculate A	Inoculate B
0	0.935 ± 0.44%	0.915 ± 0.26%	0.942 ± 0.10%	0.928 ± 0.37%
1	0.832 ± 0.67%	0.870 ± 0.25%	0.882 ± 1.95%	0.868 ± 1.38%
3	0.784 ± 0.74%	0.769 ±1.25%	0.794 ± 0.52%	0.788 ± 0.12%

Table A.11. 1,4-BCB-I Degradation Data

	Peak Area Ratio, 1,4-BCB-I/TCB			
Time, weeks	Control A	Control B	Inoculate A	Inoculate B
0	0.0816±0.64%	0.0807±0.06%	0.0826±0.24%	0.0815±0.45%
1	0.0965±0.38%	0.1015±0.51%	0.1050±0.89%	0.1033±0.06%
3	0.1622±0.36%	0.1608±0.72%	0.1636±0.60%	0.1637±0.42%

Appendix B: TCE Sample Chromatograms



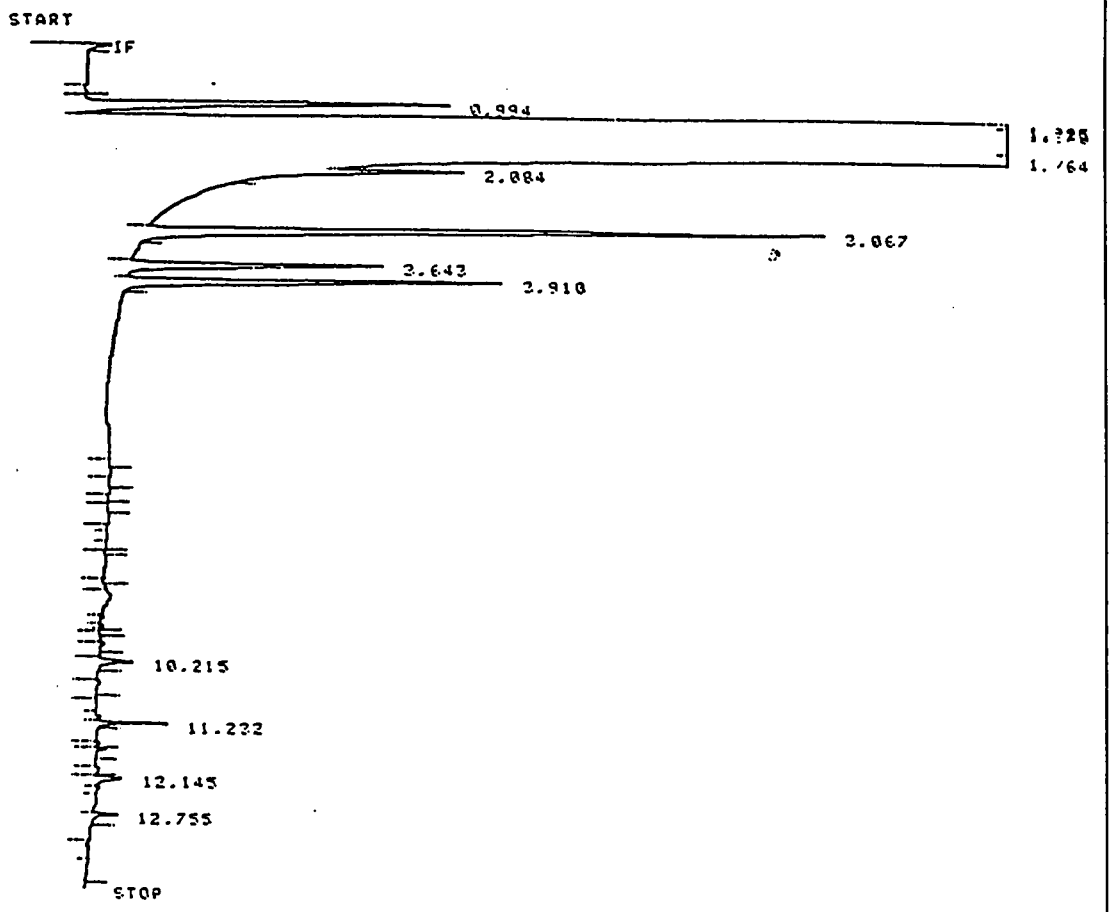
RUN# 106 JUL 8, 1994 14:10:24

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.823	24127	BP	.070	.00436
1.040	1800249	PH	.040	.32556
1.130	550452400	SHB	.080	99.54579
1.519	544201	TBP	.080	.09842
3.220	14807	BV	.099	.00268
3.587	44618	VB	.090	.00807
9.673	38292	PB	.020	.00692
10.067	14151	BB	.036	.00256
11.087	19420	BB	.030	.00351
11.990	12171	PB	.037	.00220

TOTAL AREA=5.529€E+08

Figure B.1.1. 10 mg/L TCE, Control, Time = 0



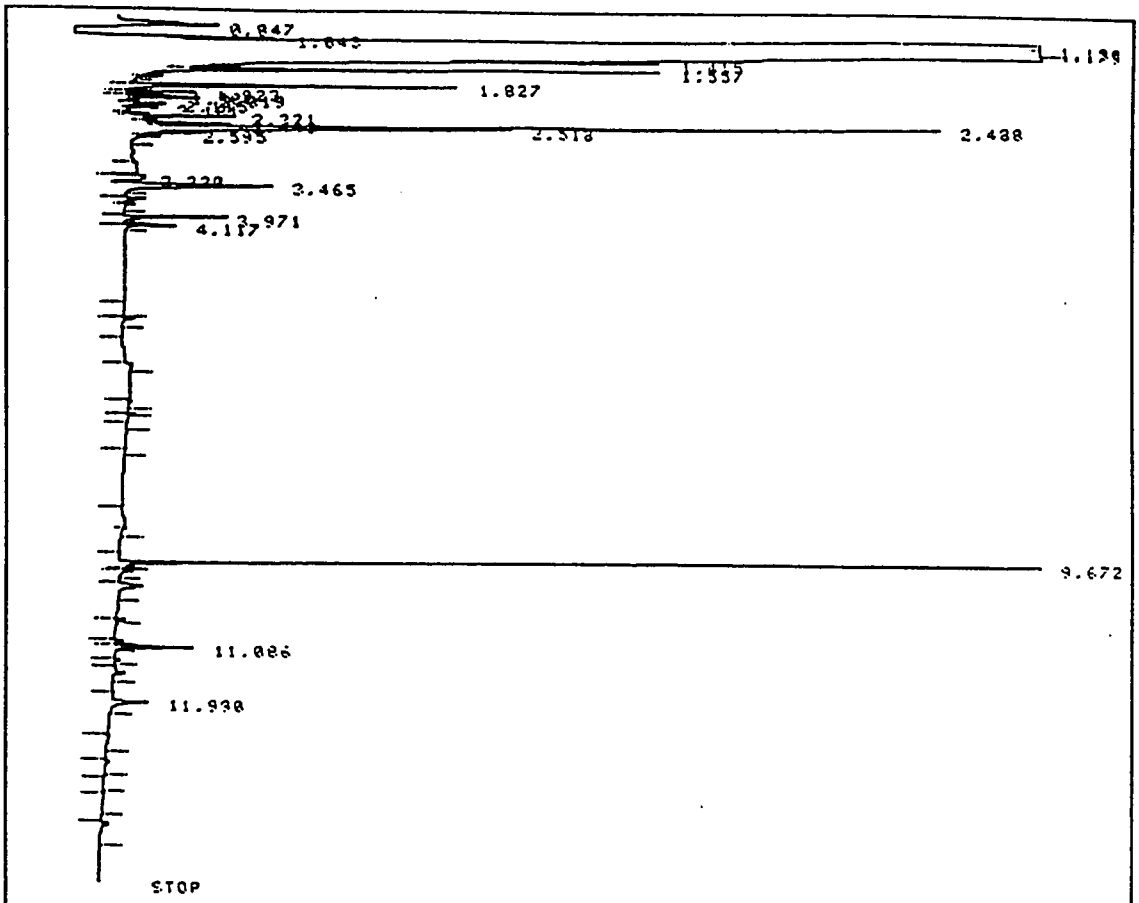
RUN# 213 AUG 2, 1994 22:56:24

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.994	124802	BP	.081	.00371
1.225	14085376	PM	.035	.41917
1.270	3.343E+09	SMB	.079	99.47363
1.764	3016174	TBP	.069	.08976
2.084	34278	TPV	.056	.00102
3.067	221780	PB	.079	.00660
3.643	80485	BV	.076	.00240
2.910	114449	VB	.073	.00341
11.232	11130	VB	.037	.00033

TOTAL AREA=3.3603E+09
MUL FACTOR=1.0000E+00

Figure B.1.2. 10 mg/L TCE, Control, Time = 3 Weeks



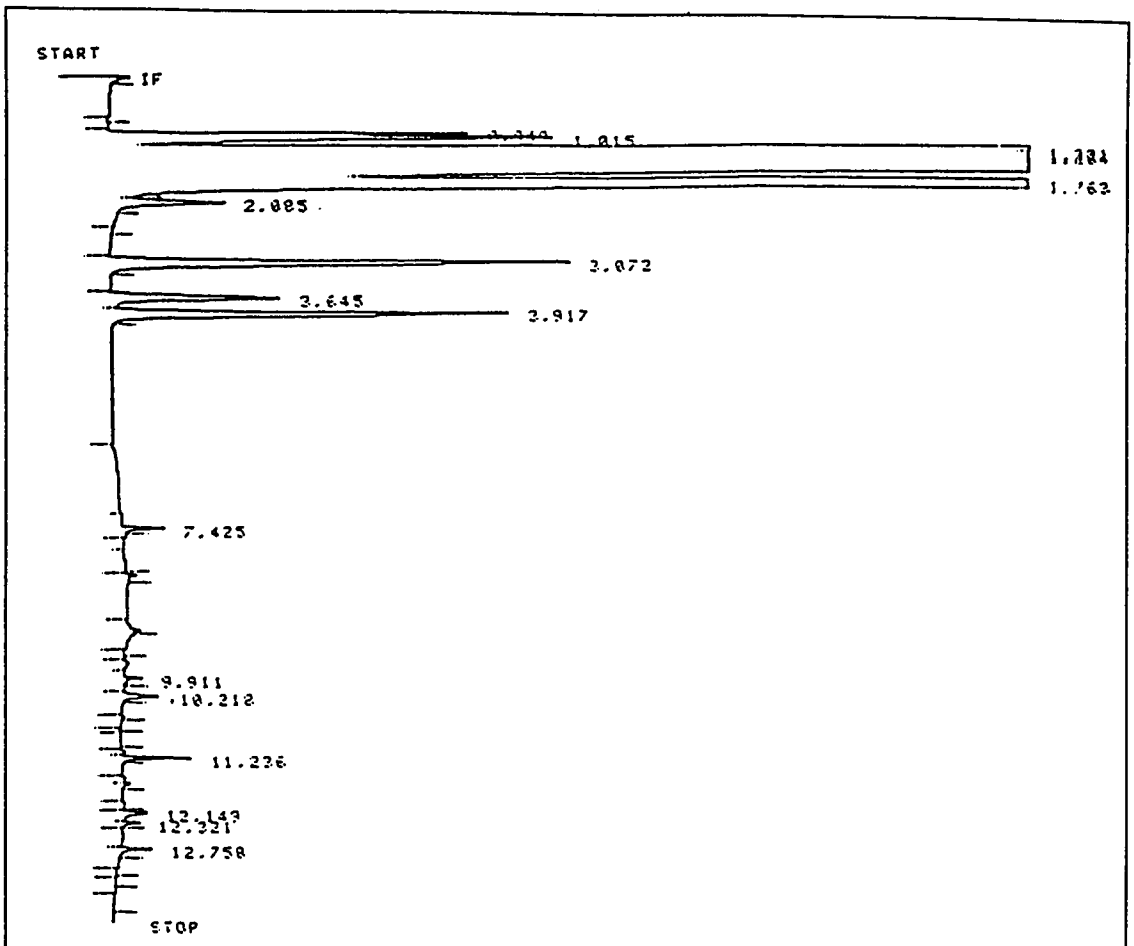
RUN# 100 JUL 8, 1994 15:06:45

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.847	86540	BP	.121	.39188
1.130	2162746	PV	.023	9.79264
1.179	19390784	VB	.038	87.80797
1.557	47152	BP	.023	.21352
1.827	36812	BB	.027	.16670
2.321	13220	VV	.029	.05986
2.443	17175	VV	.038	.07777
2.488	74151	VV	.021	.33578
2.518	48036	VV	.020	.21752
3.220	13060	PP	.086	.05914
3.465	40453	PB	.062	.18318
3.971	12712	PB	.028	.05756
9.672	129283	PB	.020	.58544
11.026	11056	BB	.033	.05007

TOTAL AREA=2.2083E+07

Figure B.1.3. 10 mg/L TCE, Inoculated, Time = 0



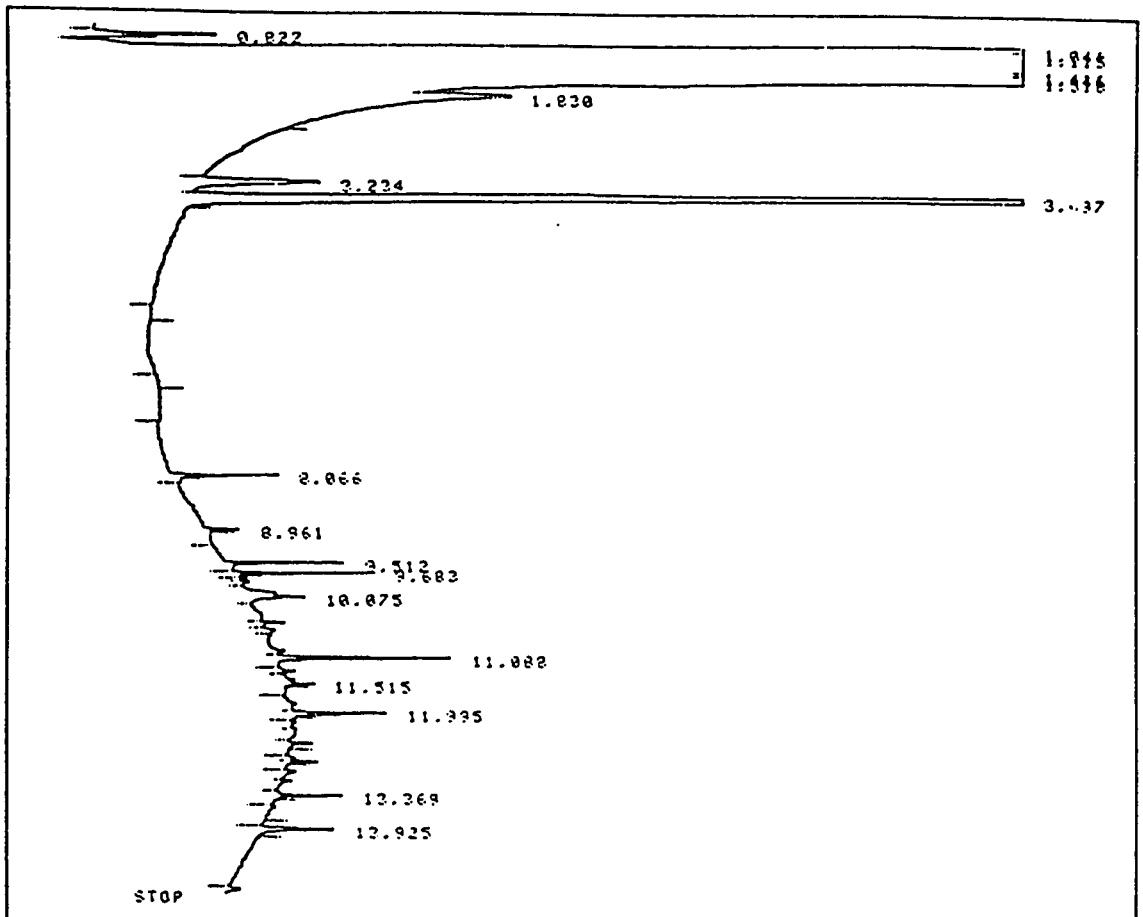
RUN# 315 AUG 2. 1994 23:41:53

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.949	55695	BV	.037	.00214
1.015	153402	VV	.082	.00589
1.221	9016435	VH	.040	.34681
1.284	2.594E+09	SHB	.021	99.53901
1.763	2372525	TBB	.081	.09105
2.085	29965	BB	.072	.00115
3.072	172958	PB	.089	.00664
3.645	62245	BV	.083	.00242
3.917	140198	VB	.084	.00538
11.236	10718	VB	.026	.00041

TOTAL AREA=2.6058E+09
MUL FACTOR=1.0000E+00

Figure B.1.4. 10 mg/L TCE, Inoculated, Time = 3 Weeks

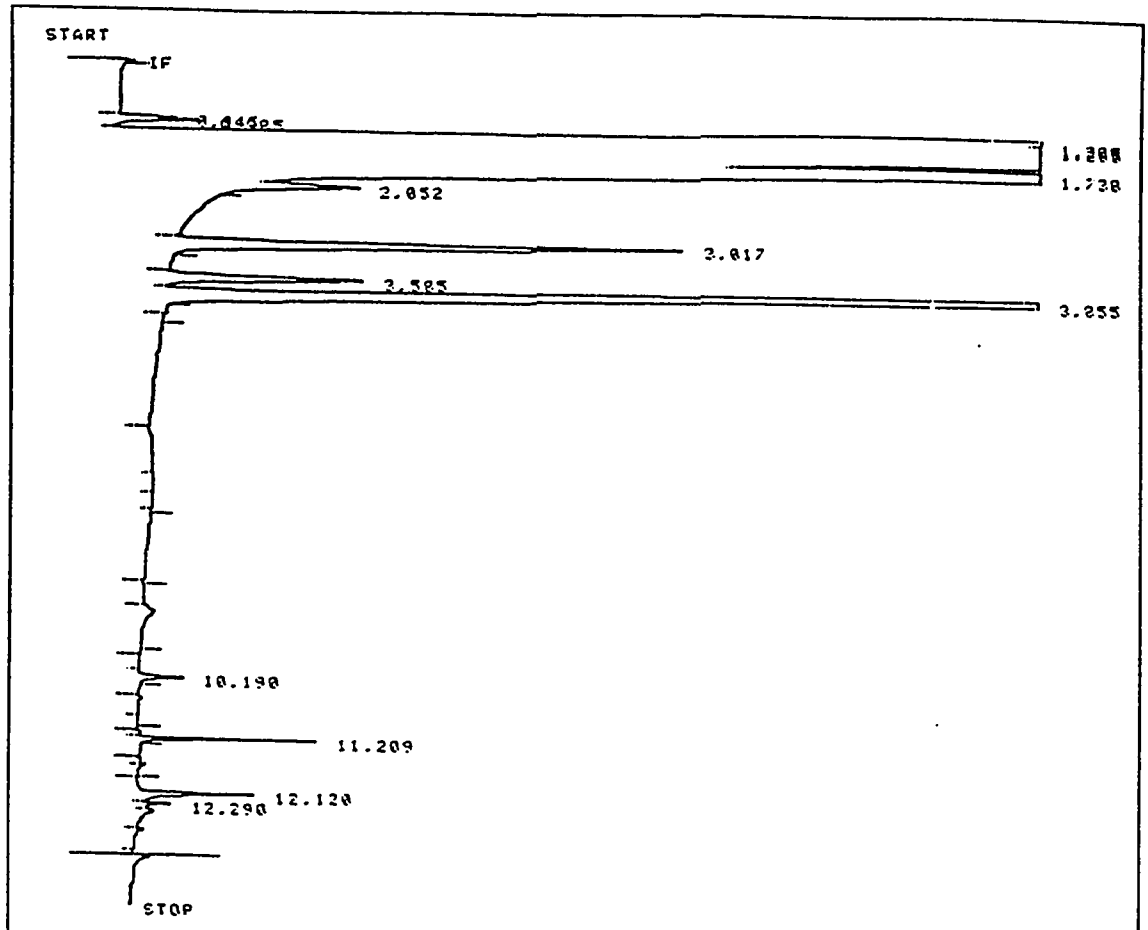


RUN# 119 JUL 8, 1994 20:27:25

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.822	21585	BV	.038	.00139
1.841	7107946	PM	.050	.45864
1.115	1.540E+09	SHB	.079	99.39013
1.411	54077	TBV	.018	.00249
1.512	1589768	TVP	.076	.10258
1.830	48631	TPB	.102	.00314
3.234	42620	BP	.085	.00275
3.437	632949	PB	.079	.04084
9.512	10028	BB	.021	.00065
9.683	12060	BB	.021	.00078
10.075	19176	PP	.081	.00124
11.022	27303	VB	.037	.00176
11.995	17035	VB	.042	.00110
13.369	10579	PB	.037	.00068
13.925	14700	BB	.047	.00095

Figure B.2.1. 100 mg/L TCE, Control, Time = 0



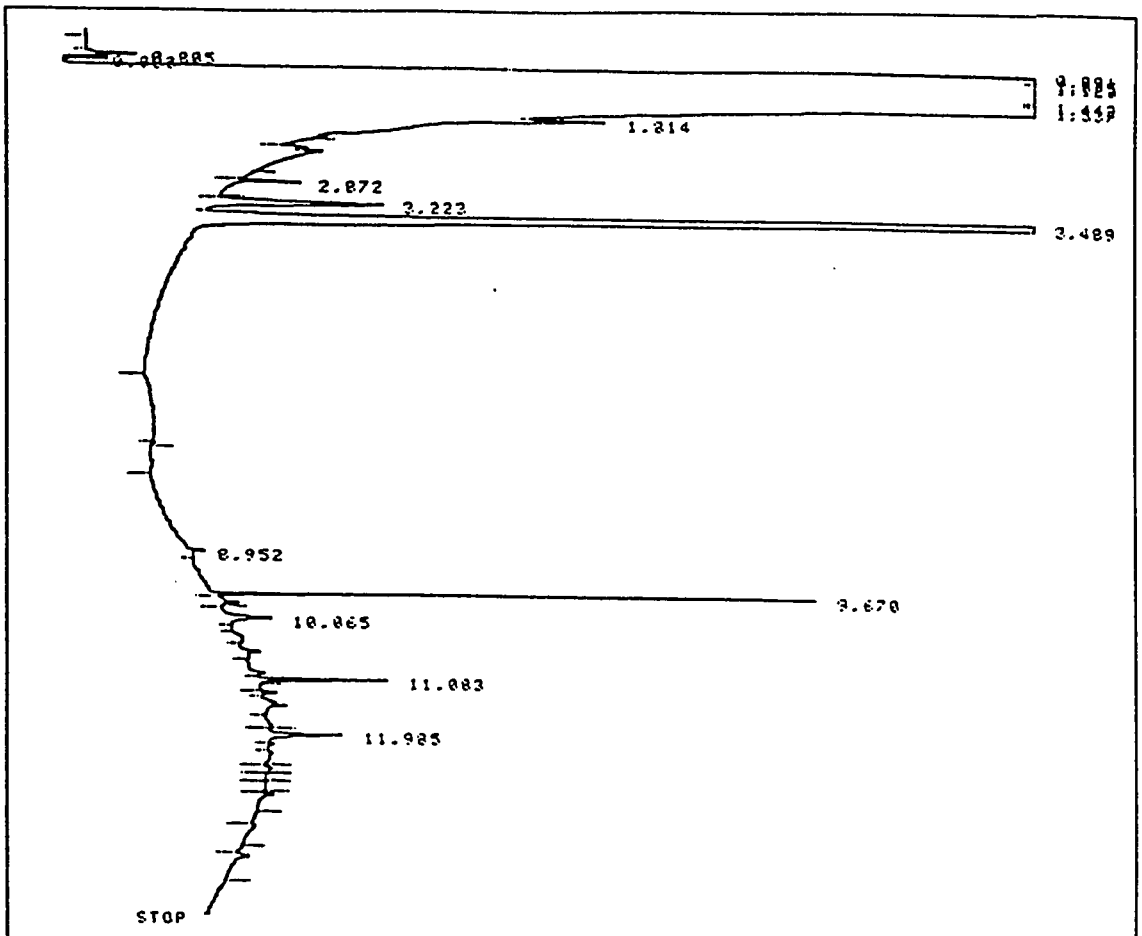
RUN# 308 AUC 2. 1994 12:04:27

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.925	16621	VP	.047	.00053
1.205	10750496	PH	.042	.34028
1.265	3.145E+09	SH2	.090	99.53226
1.738	2225605	TBP	.099	.08944
2.052	33299	TPV	.076	.00105
2.017	208781	BB	.098	.00661
2.585	75454	BP	.092	.00239
3.255	204246	PB	.090	.02546
10.190	10162	PB	.054	.00032
11.209	24196	VB	.032	.00077
12.120	30592	BV	.062	.00097

TOTAL AREA=3.1592E+09
MUL FACTOR=1.0000E+00

Figure B.2.2. 100 mg/L TCE, Control, Time = 3 Weeks

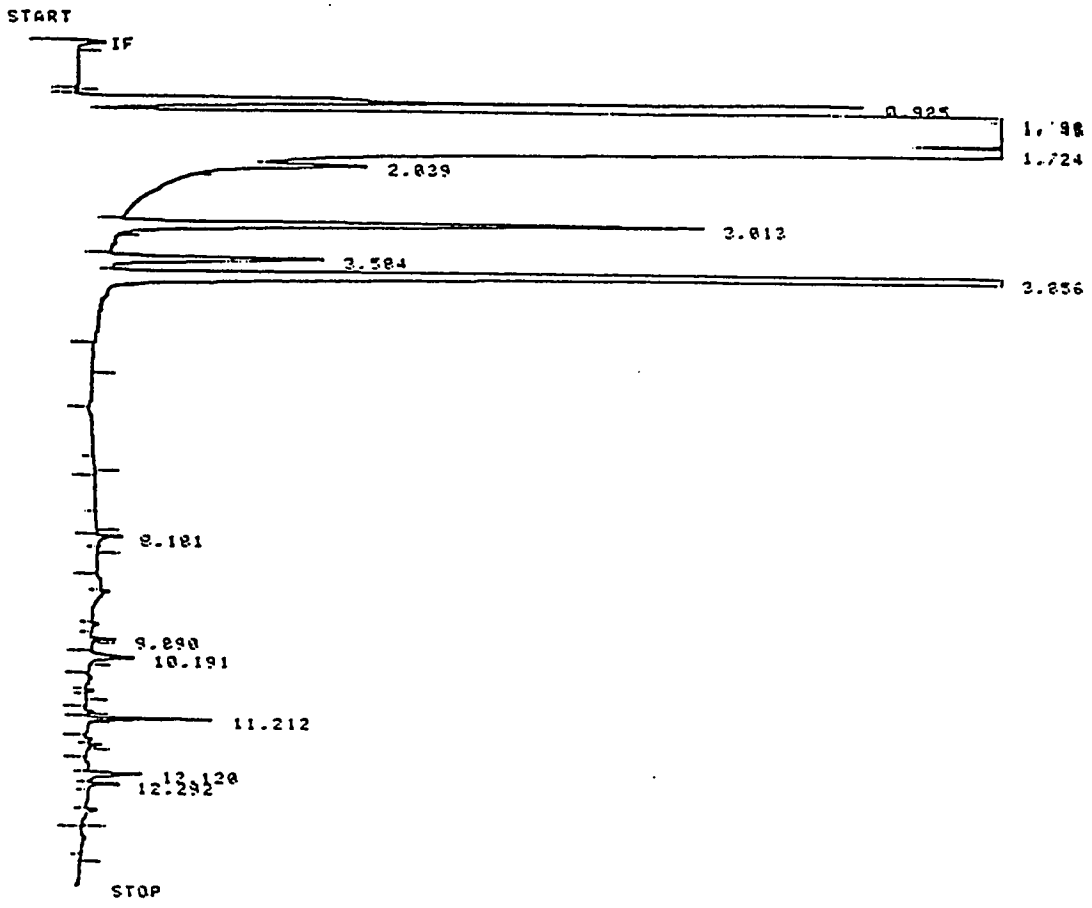


RUN# 102 JUL 8, 1994 12:36:43

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.805	33913	VV	.087	.00192
.823	10474	VP	.028	.00052
.991	98777	PV	.021	.00490
1.075	25976064	VH	.024	1.28939
1.122	1.985E+09	SHB	.060	98.54099
1.442	13943	TBV	.030	.00069
1.527	2206581	TVB	.055	.10953
1.814	24343	TBP	.050	.00121
2.223	52368	BP	.075	.00260
2.489	892050	PB	.066	.04428
9.670	38881	PB	.015	.00193
10.065	10736	PV	.060	.00053
11.083	18555	VB	.035	.00092
11.985	12961	PV	.043	.00064

Figure B.2.3. 100 mg/L TCE, Inoculated, Time = 0



RUN# 389 AUC 2, 1994 21:26:31

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.985	220624	BV	.066	.00725
1.193	12388392	VH	.039	.40723
1.245	3.025E+09	SHB	.076	99.45354
1.724	2712464	TBP	.074	.08916
2.039	31508	TPV	.062	.00104
3.013	201561	BB	.082	.00662
3.584	71448	PV	.090	.00235
3.856	956115	VB	.077	.03142
10.191	13649	BB	.072	.00045
11.212	17213	BB	.032	.00057
12.120	14100	VV	.052	.00046

TOTAL AREA=3.0421E+09

Figure B.2.4. 100 mg/L TCE, Inoculated, Time = 3 Weeks

Appendix C: DBCP Sample Chromatograms

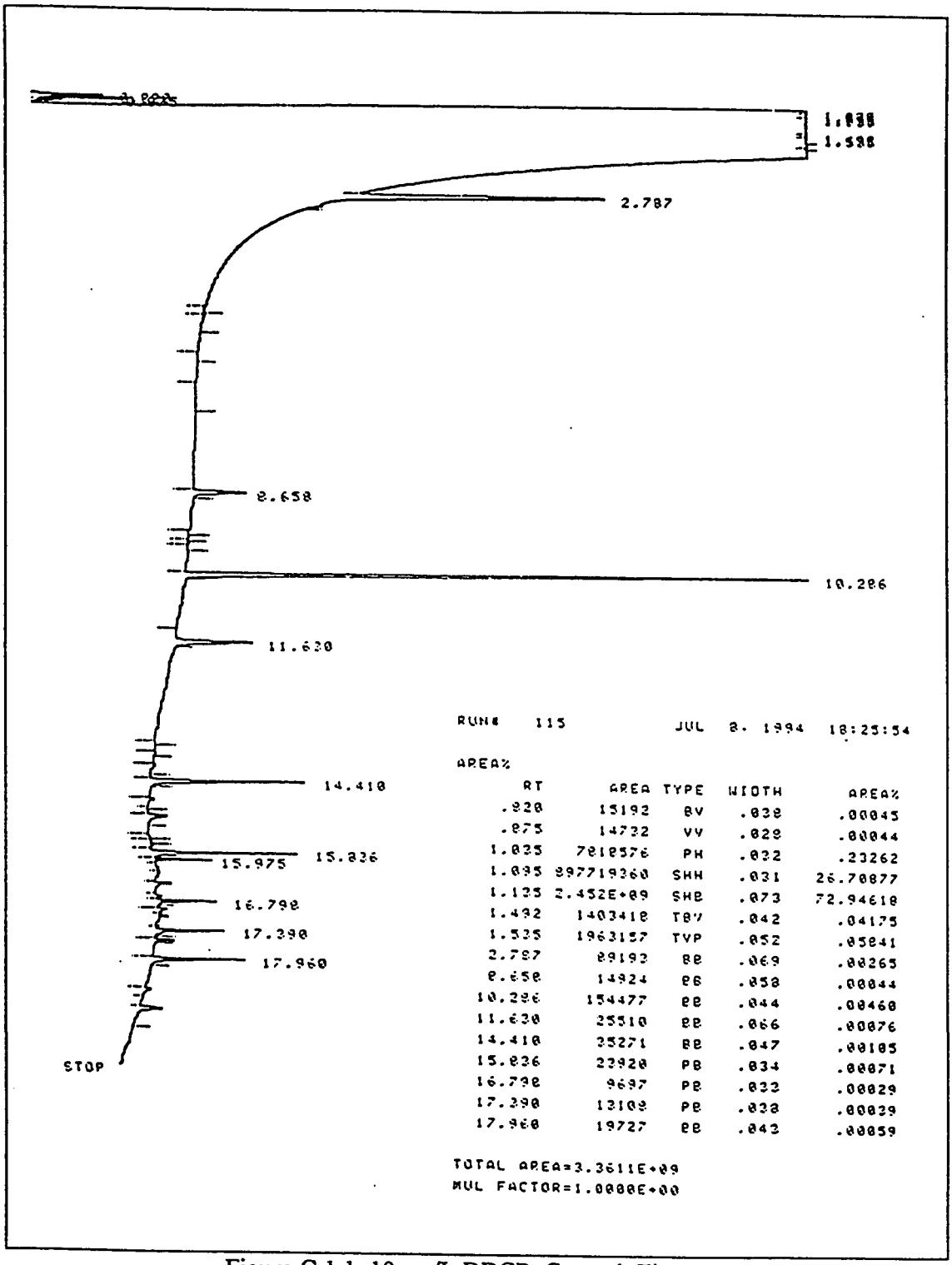
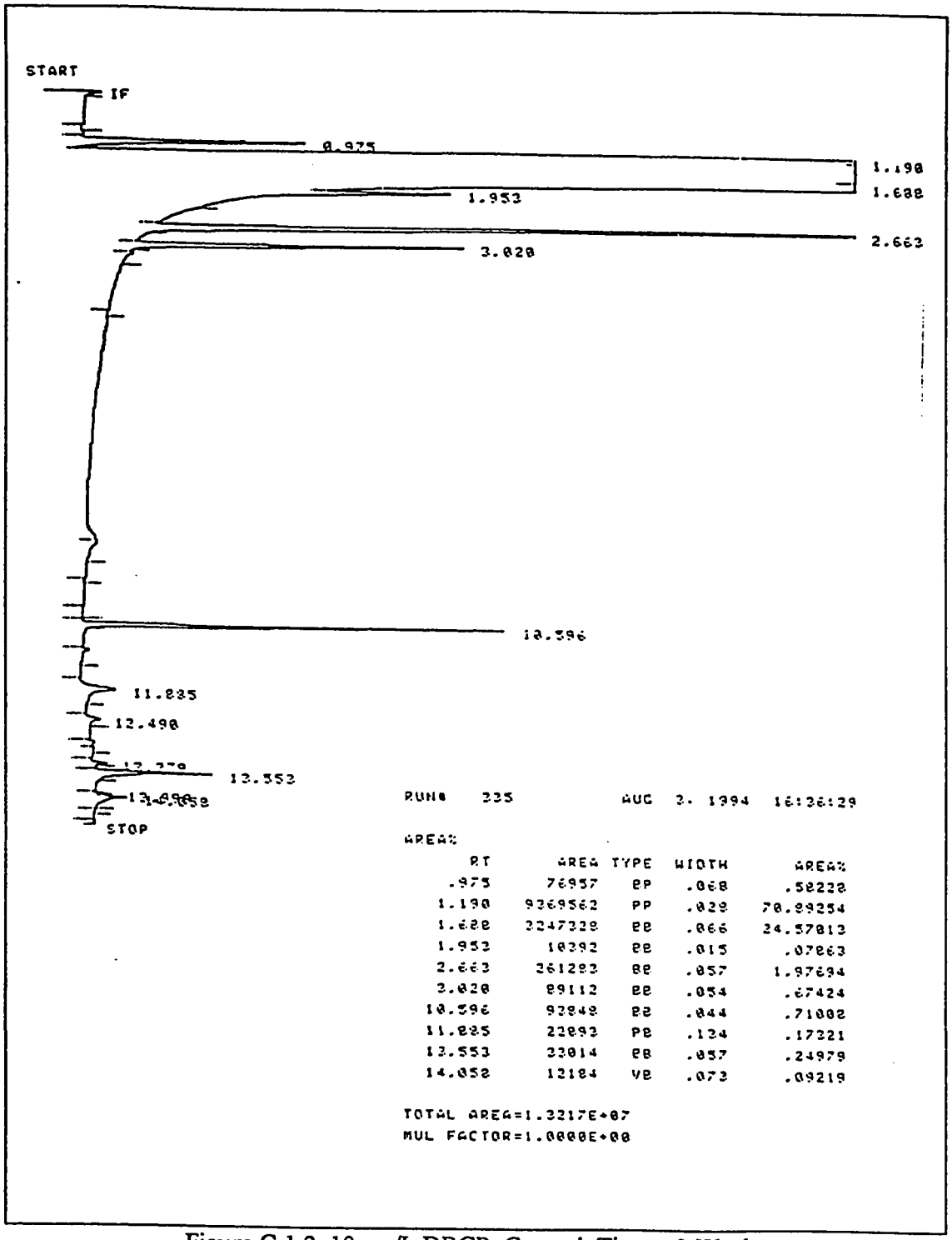


Figure C.1.1. 10 mg/L DBCP, Control, Time = 0



RUN# 235 AUG 3, 1994 16:36:29

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.975	76957	BP	.068	.58228
1.190	9369562	PP	.029	70.89254
1.688	3247328	BB	.066	24.57013
1.953	10392	BB	.015	.07863
2.663	261283	BB	.057	1.97694
3.020	89112	BB	.054	.67424
10.396	93848	BB	.044	.71002
11.885	22893	PB	.124	.17321
13.553	33014	BB	.057	.24979
14.052	12184	VB	.073	.09219

TOTAL AREA=1.3217E+07
MUL FACTOR=1.0000E+00

Figure C.1.2. 10 mg/L DBCP, Control, Time = 3 Weeks

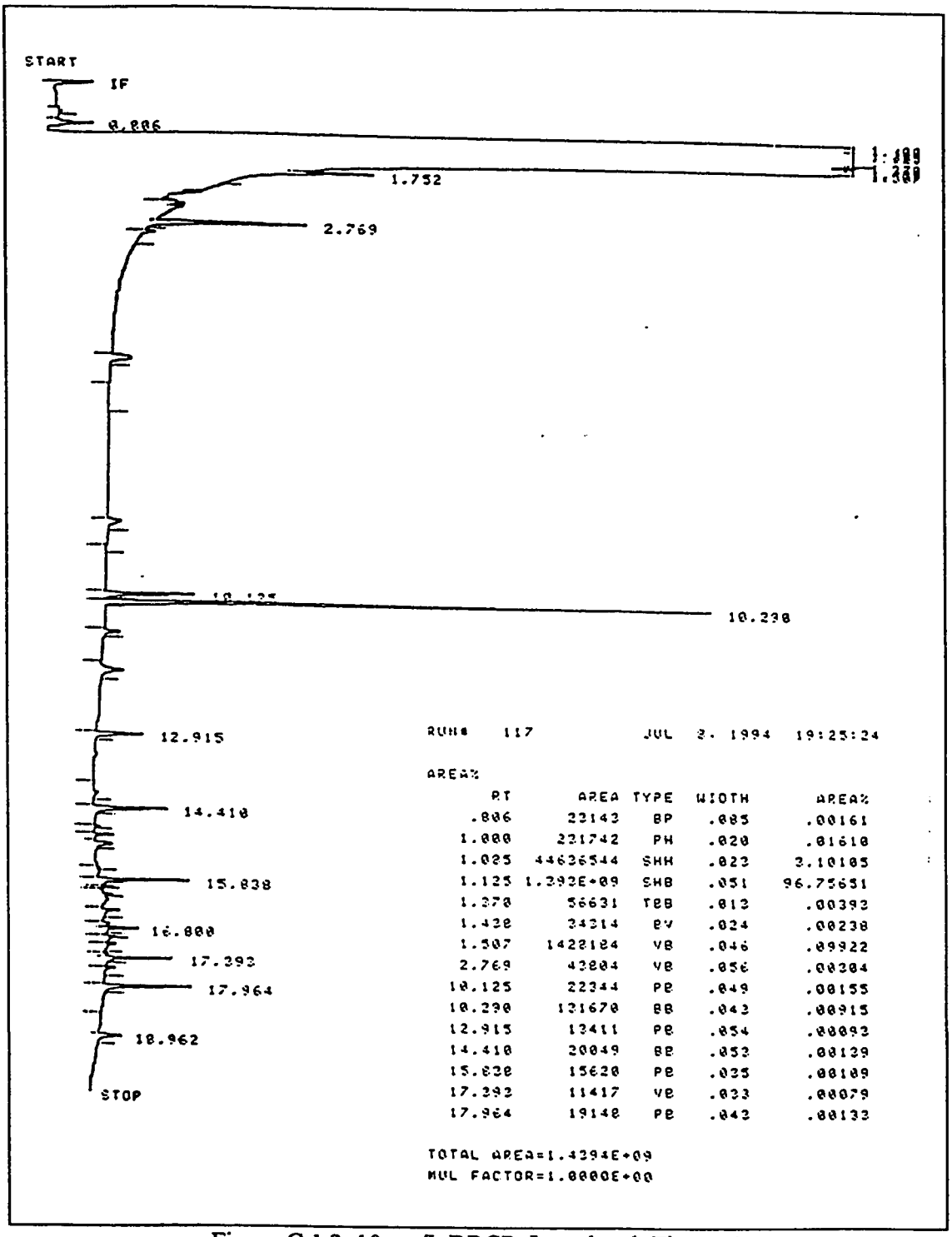


Figure C.1.3. 10 mg/L DBCP, Inoculated, Time = 0

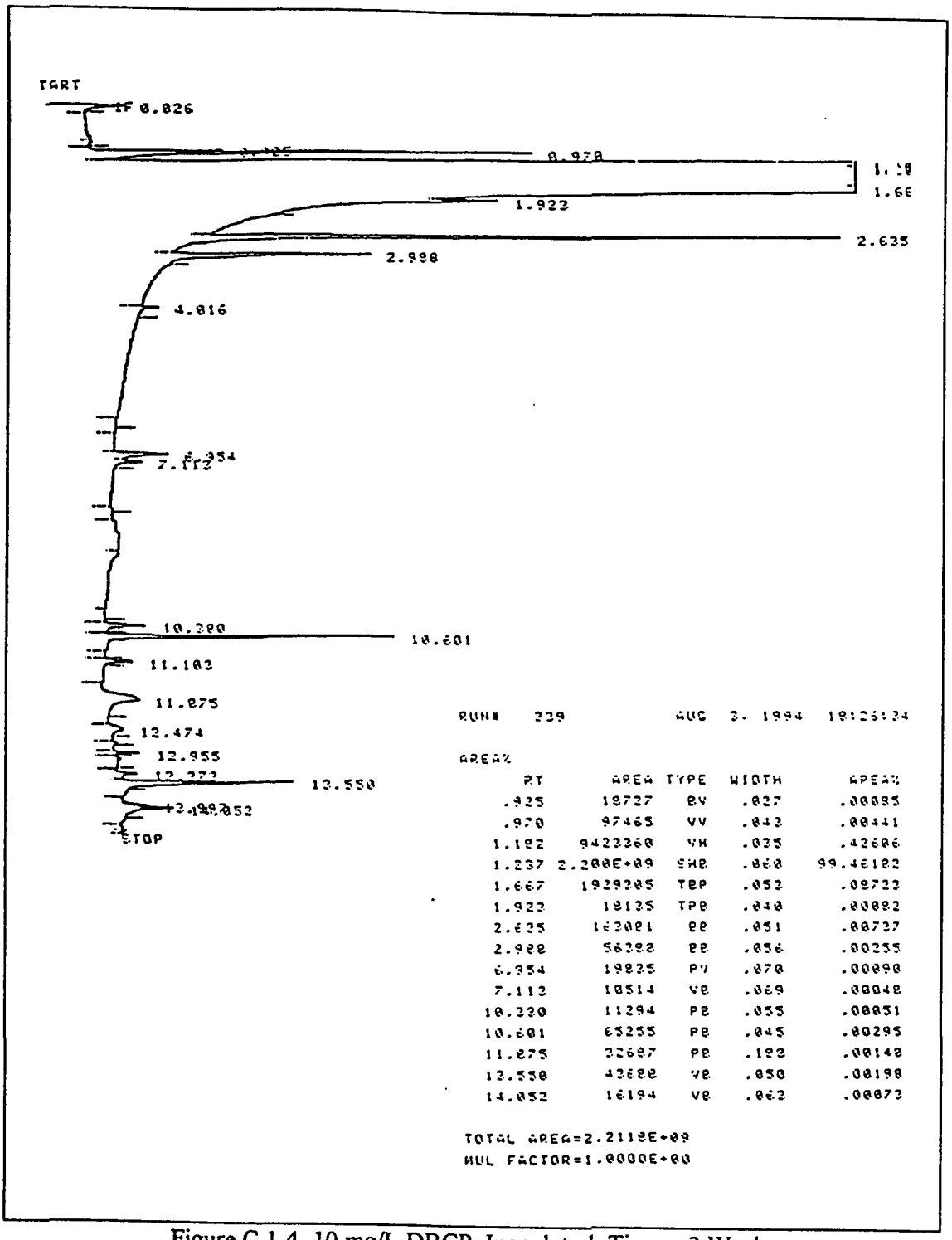


Figure C.1.4. 10 mg/L DBCP, Inoculated, Time = 3 Weeks

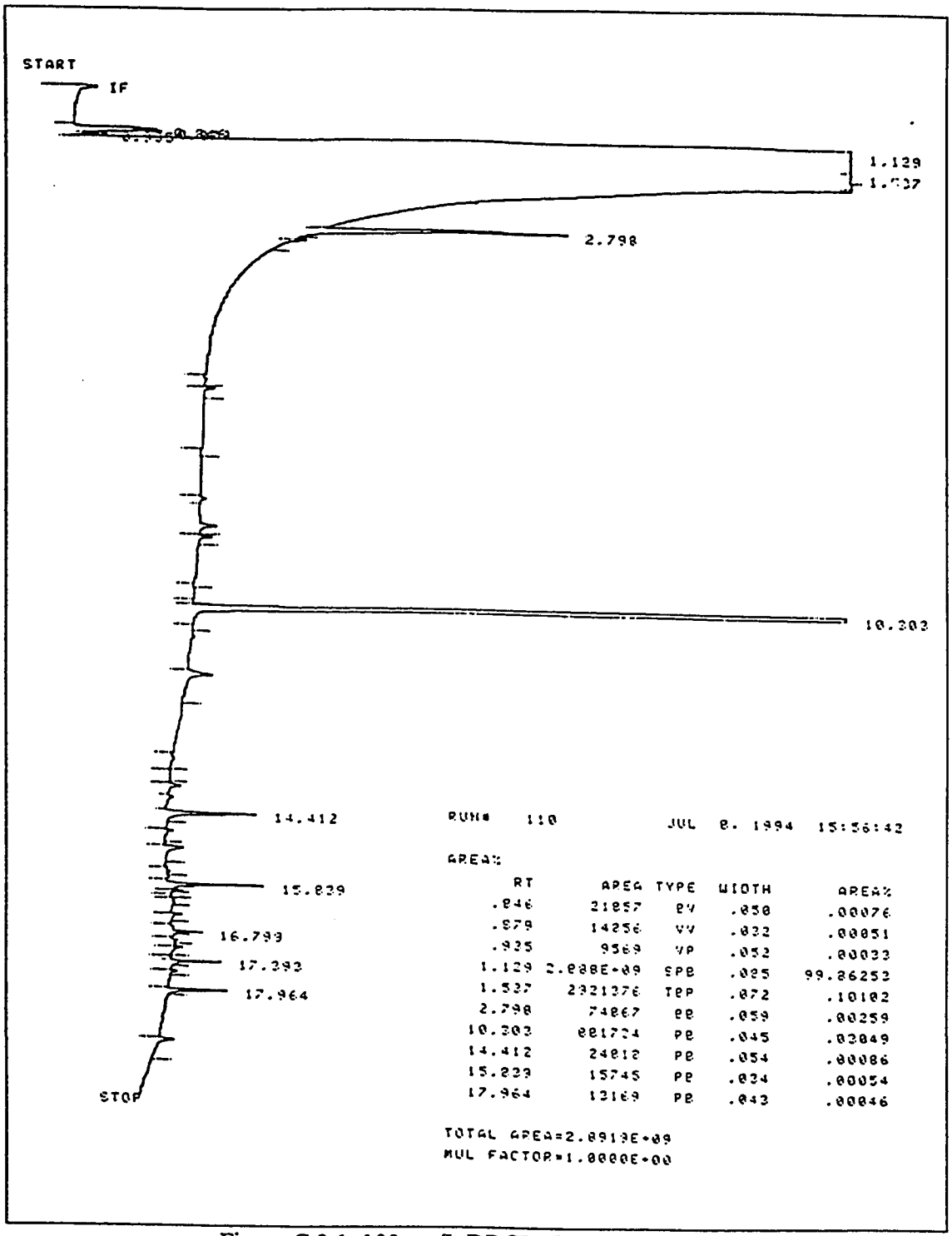
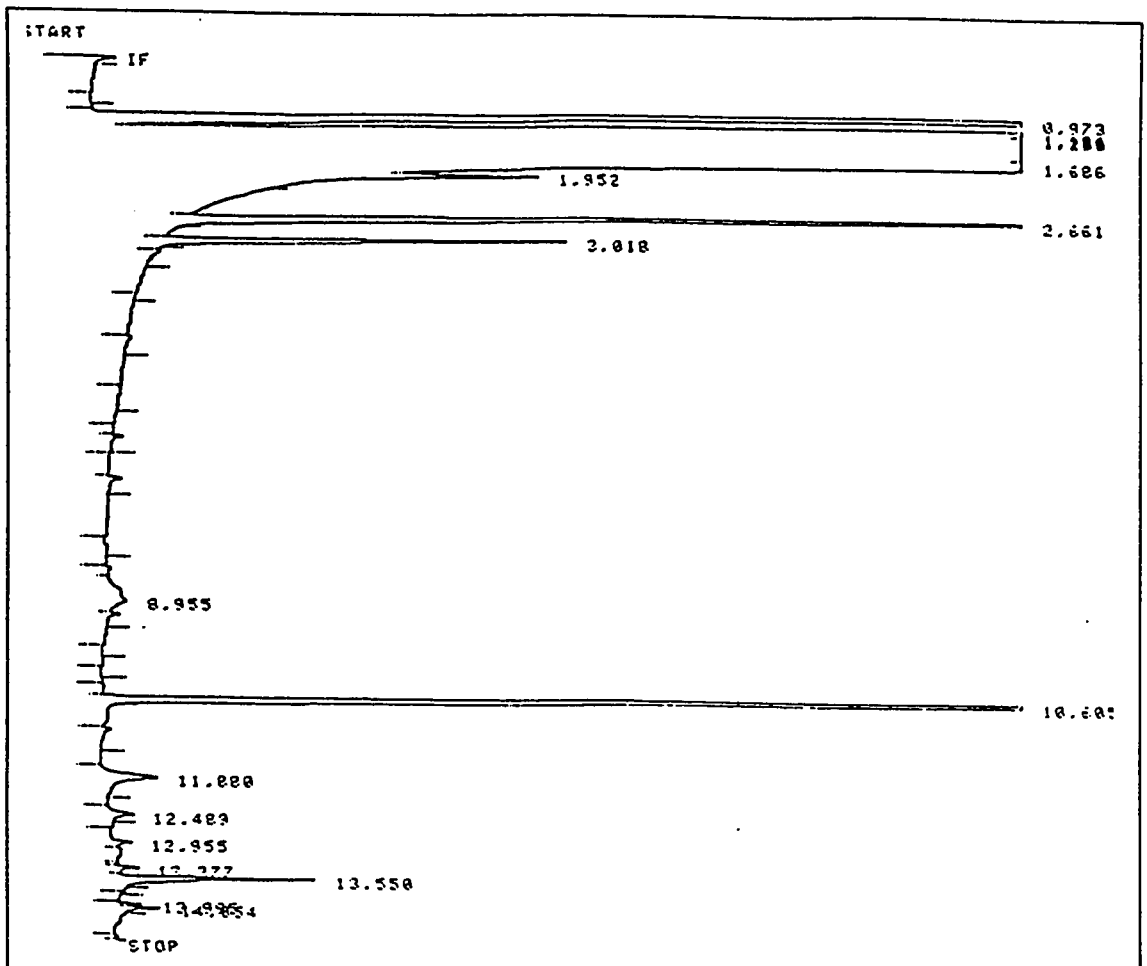


Figure C.2.1. 100 mg/L DBCP, Control, Time = 0



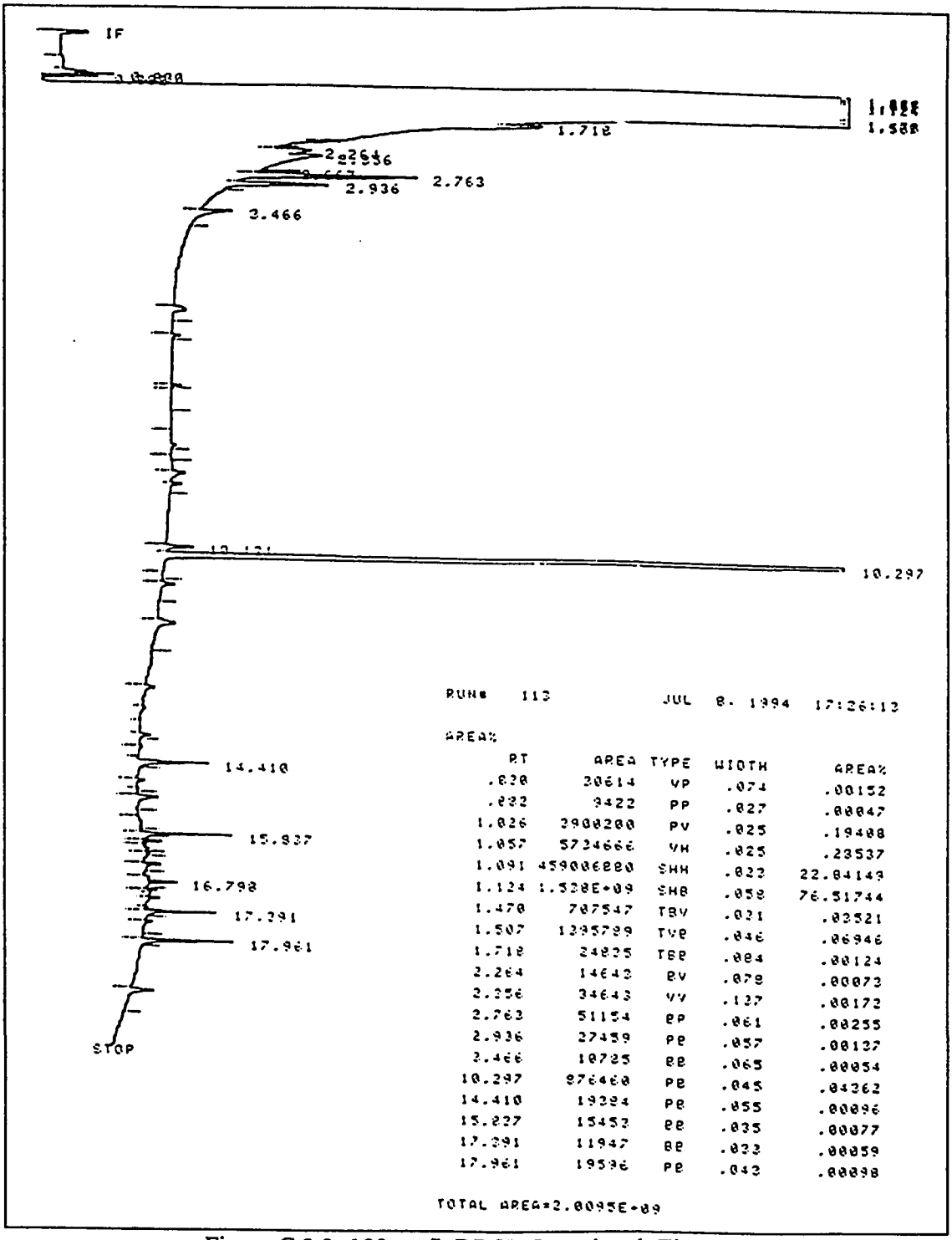
RUN# 326 AUC 3. 1994 13:12:31

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.973	685431	BV	.057	.01846
1.186	15679272	VH	.040	.42235
1.232	3.691E+09	SHB	.081	99.43559
1.686	3342574	TBP	.071	.09020
1.952	36520	TPV	.053	.00098
2.661	271704	BB	.061	.00732
3.018	96058	PB	.057	.00259
8.955	29274	VV	.322	.00079
10.685	733650	PB	.045	.01976
11.880	31132	BB	.128	.00084
13.550	43904	VB	.054	.00118

TOTAL AREA=3.7124E+09

Figure C.2.2. 100 mg/L DBCP, Control, Time = 3 Weeks



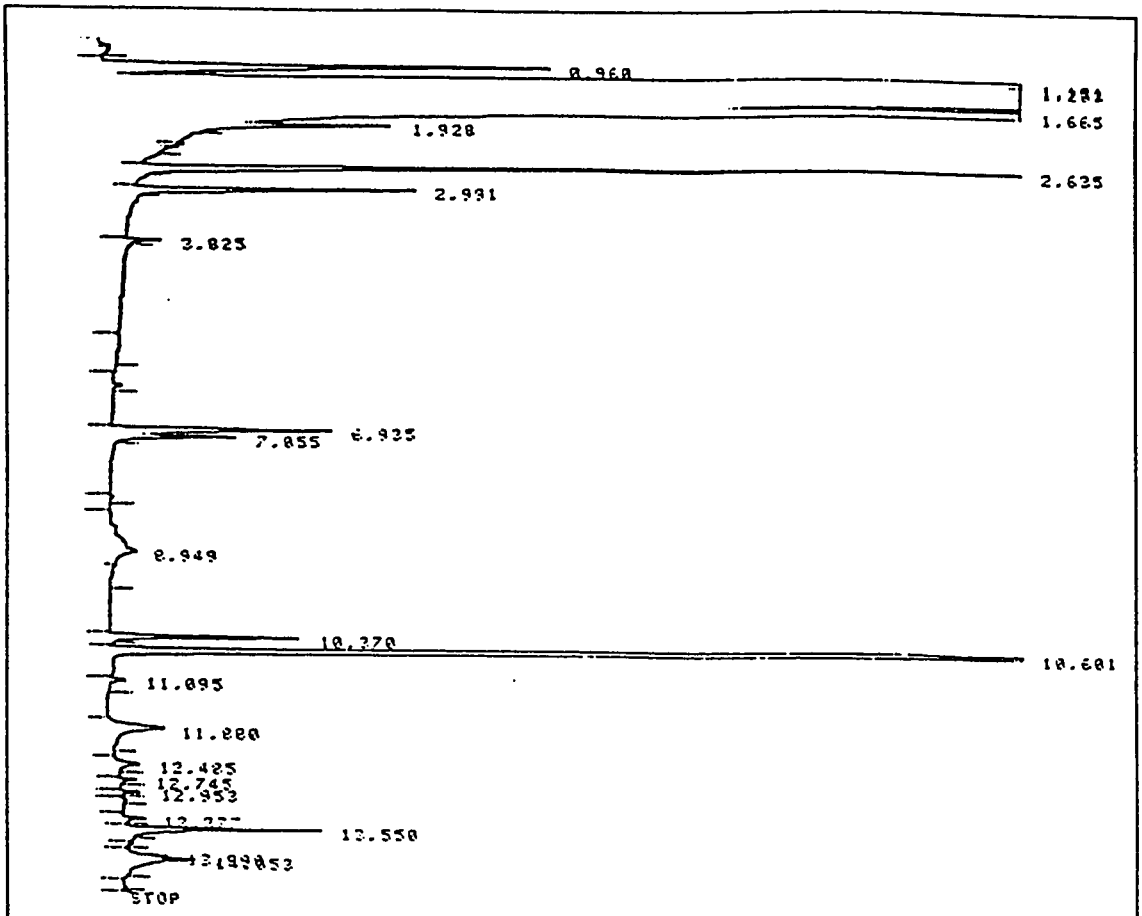
RUN# 113 JUL 8, 1994 17:26:13

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.030	20614	VP	.074	.00152
.032	9422	PP	.027	.00047
1.026	3900200	PV	.025	.19408
1.057	5734666	VH	.025	.23537
1.091	459006880	SHH	.023	22.84143
1.124	1.538E+09	SHB	.058	76.51744
1.470	707547	TBV	.031	.03521
1.507	1395799	TVB	.046	.06946
1.718	24835	TBB	.004	.00124
2.264	14643	BV	.079	.00073
2.356	34643	VV	.127	.00172
2.763	51154	BP	.061	.00255
2.936	27459	PB	.057	.00137
3.466	10725	BB	.065	.00054
10.297	876460	PB	.045	.04362
14.410	19394	PB	.055	.00096
15.837	15453	BB	.035	.00077
17.391	11947	BB	.033	.00059
17.961	19596	PB	.043	.00098

TOTAL AREA=2.0095E+09

Figure C.2.3. 100 mg/L DBCP, Inoculated, Time = 0

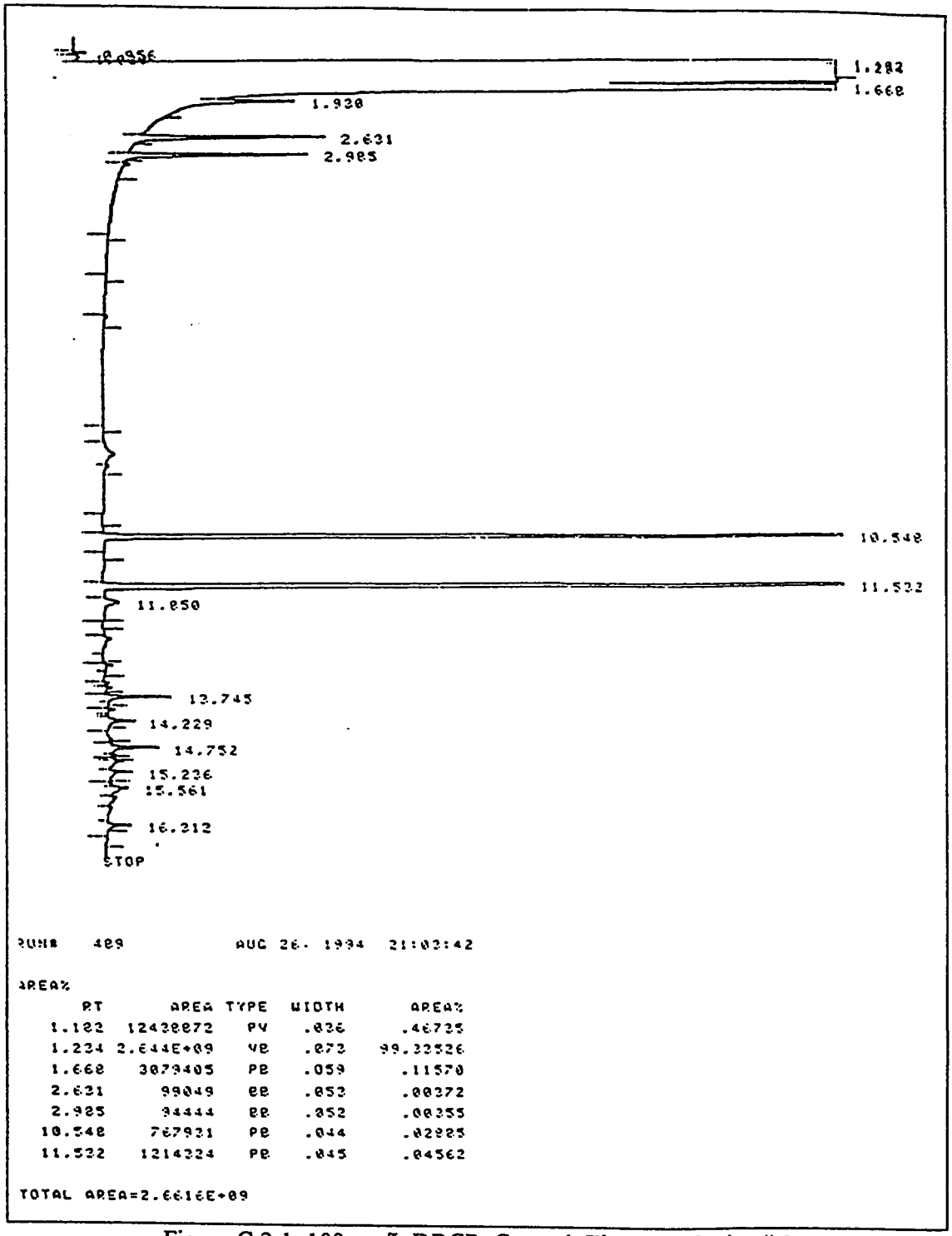


RUN# 328 AUC 3. 1994 12:57:48

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.960	158629	PV	.084	.00643
1.121	10979824	VH	.032	.44483
1.233	2.454E+09	SHB	.059	99.41008
1.665	2261064	TBP	.051	.09160
1.928	27968	TPV	.044	.00113
2.635	186729	PB	.049	.00757
2.991	60932	PB	.052	.00247
6.935	57781	BV	.062	.00234
7.055	27026	VB	.052	.00109
8.949	33646	PV	.302	.00136
10.270	39284	PB	.050	.00159
10.601	636277	PB	.044	.02578
11.880	29842	BB	.120	.00121
13.550	39687	PB	.043	.00160
14.053	25995	VB	.089	.00105

Figure C.2.4. 100 mg/L DBCP, Inoculated, Time = 3 Weeks



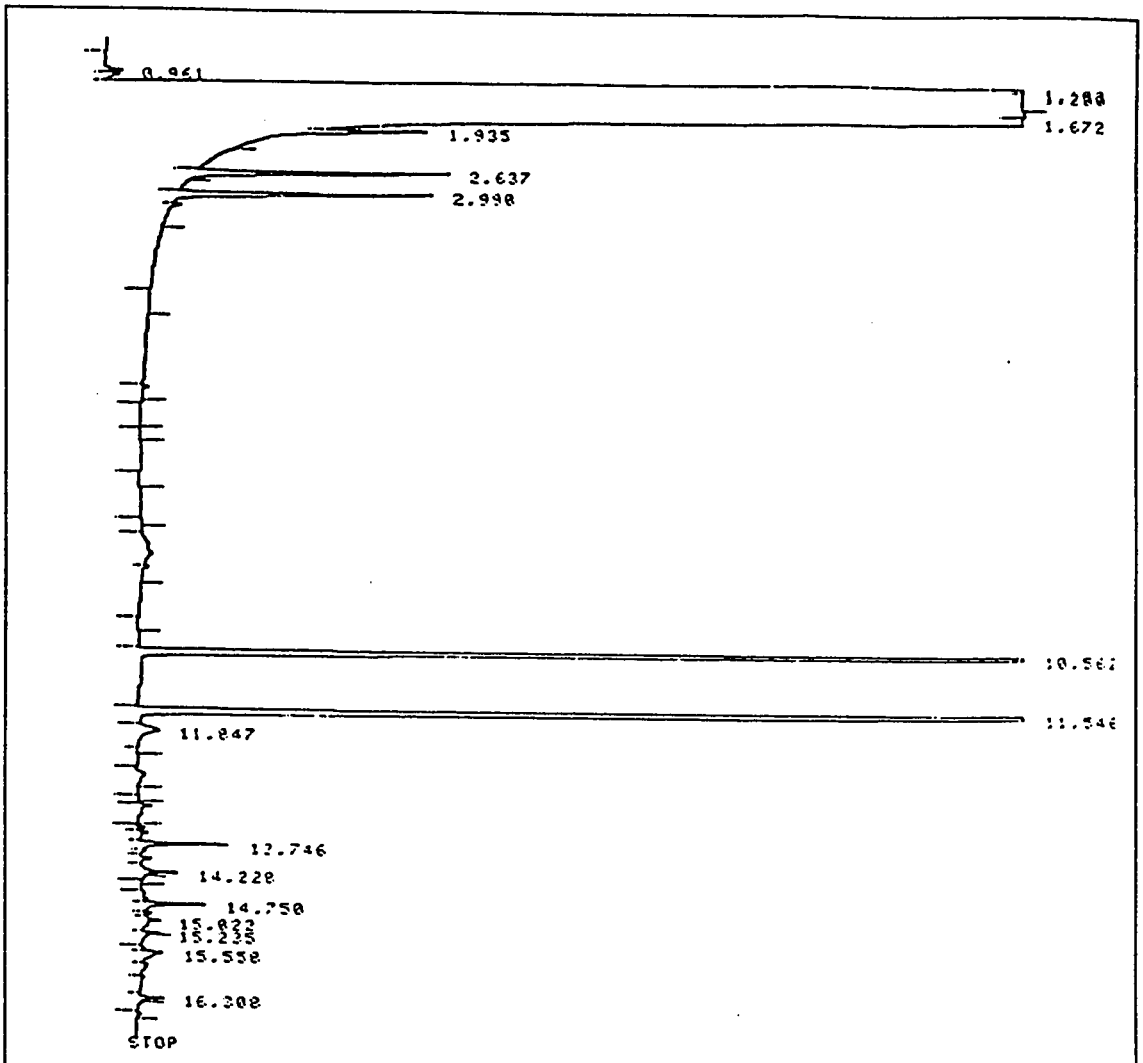
RUN# 489 AUG 26, 1994 21:03:42

AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.182	12438872	PV	.036	.46735
1.234	2.644E+09	VB	.073	99.33526
1.668	3079405	PB	.059	.11570
2.631	99049	BB	.053	.00372
2.985	94444	BB	.052	.00355
10.548	767931	PB	.044	.02885
11.532	1214324	PB	.045	.04562

TOTAL AREA=2.6616E+09

Figure C.3.1. 100 mg/L DBCP, Control, Time = 0, Series # 2



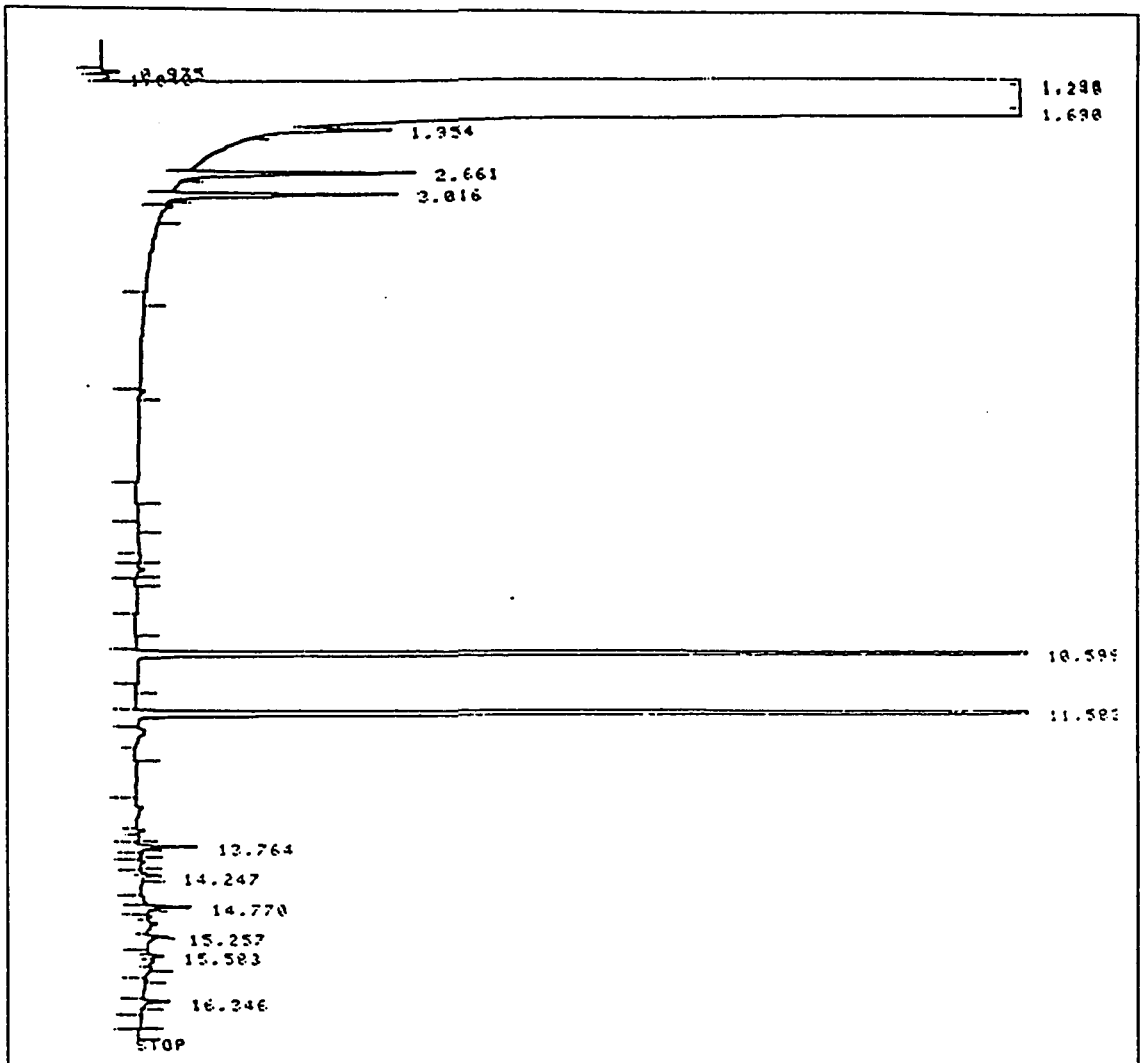
RUN# 552 SEP 20, 1994 16:43:57

AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.103	14105400	PV	.034	.44275
1.220	3.166E+09	VB	.078	99.36723
1.672	3417248	PB	.060	.10726
2.637	114456	PB	.055	.00359
2.998	112719	BV	.054	.00354
10.562	929385	PB	.044	.02914
11.546	1482685	PB	.045	.04654

TOTAL AREA=3.1859E+09

Figure C.3.2. 100 mg/L DBCP, Control, Time = 3 Weeks, Series # 2



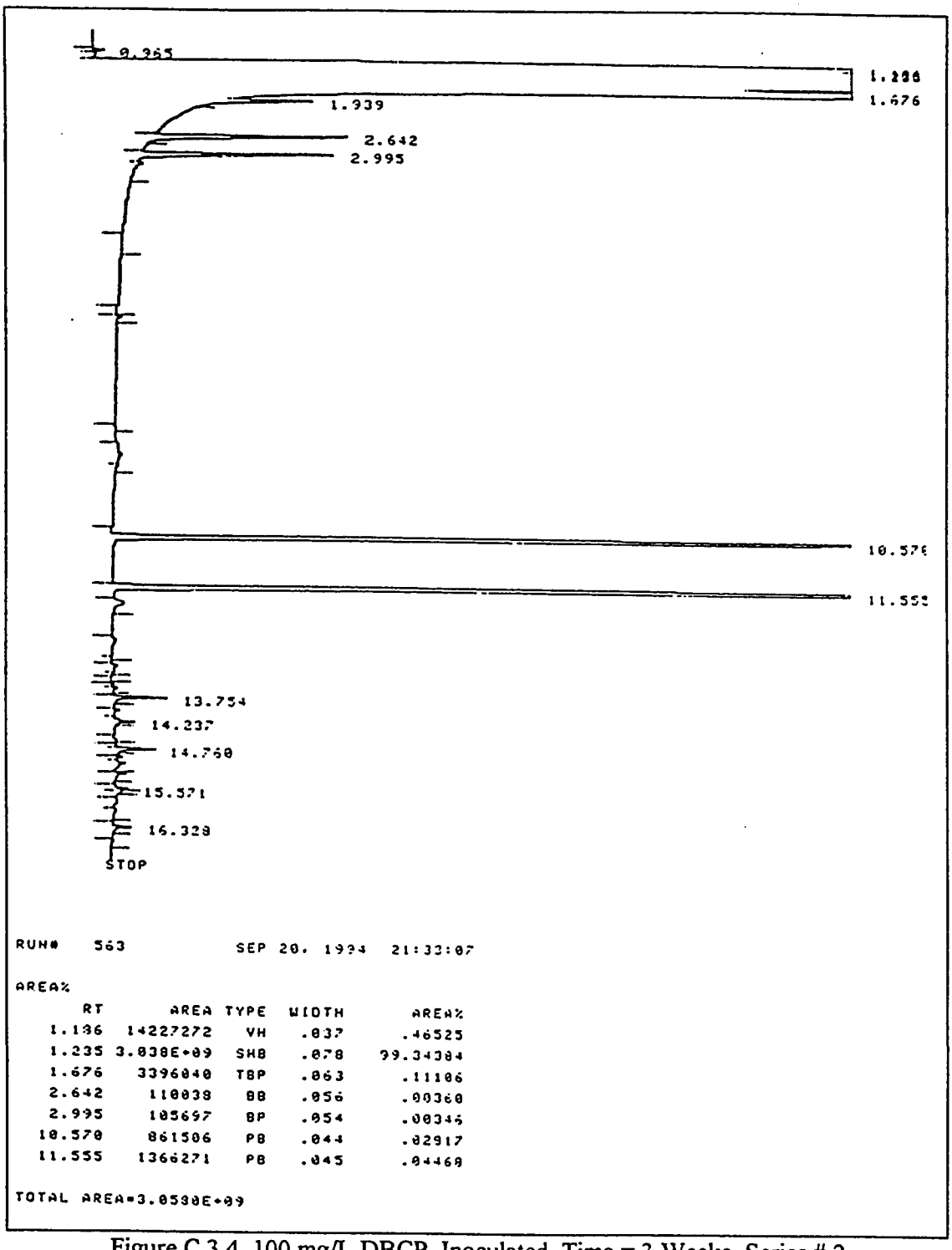
RUN# 499 AUG 27, 1994 14:32:26

AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.198	13717656	VH	.036	.46870
1.248	2.907E+09	SHB	.072	99.33683
1.698	3288750	TBP	.062	.11237
2.661	107244	BB	.056	.00366
3.016	103121	BB	.054	.00353
10.599	859361	PB	.044	.02938
11.583	1333864	PB	.045	.04557

TOTAL AREA=2.926BE+09

Figure C.3.3. 100 mg/L DBCP, Inoculated, Time = 0, Series # 2



RUN# 563 SEP 20, 1994 21:33:07

AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.186	14227272	VH	.037	.46525
1.235	3.038E+09	SHB	.078	99.34384
1.676	3396040	TBP	.063	.11106
2.642	110038	BB	.056	.00360
2.995	105697	BP	.054	.00346
10.570	861506	PB	.044	.02917
11.555	1366271	PB	.045	.04468

TOTAL AREA=3.0580E+09

Figure C.3.4. 100 mg/L DBCP, Inoculated, Time = 3 Weeks, Series # 2

Appendix D: Lindane Sample Chromatograms

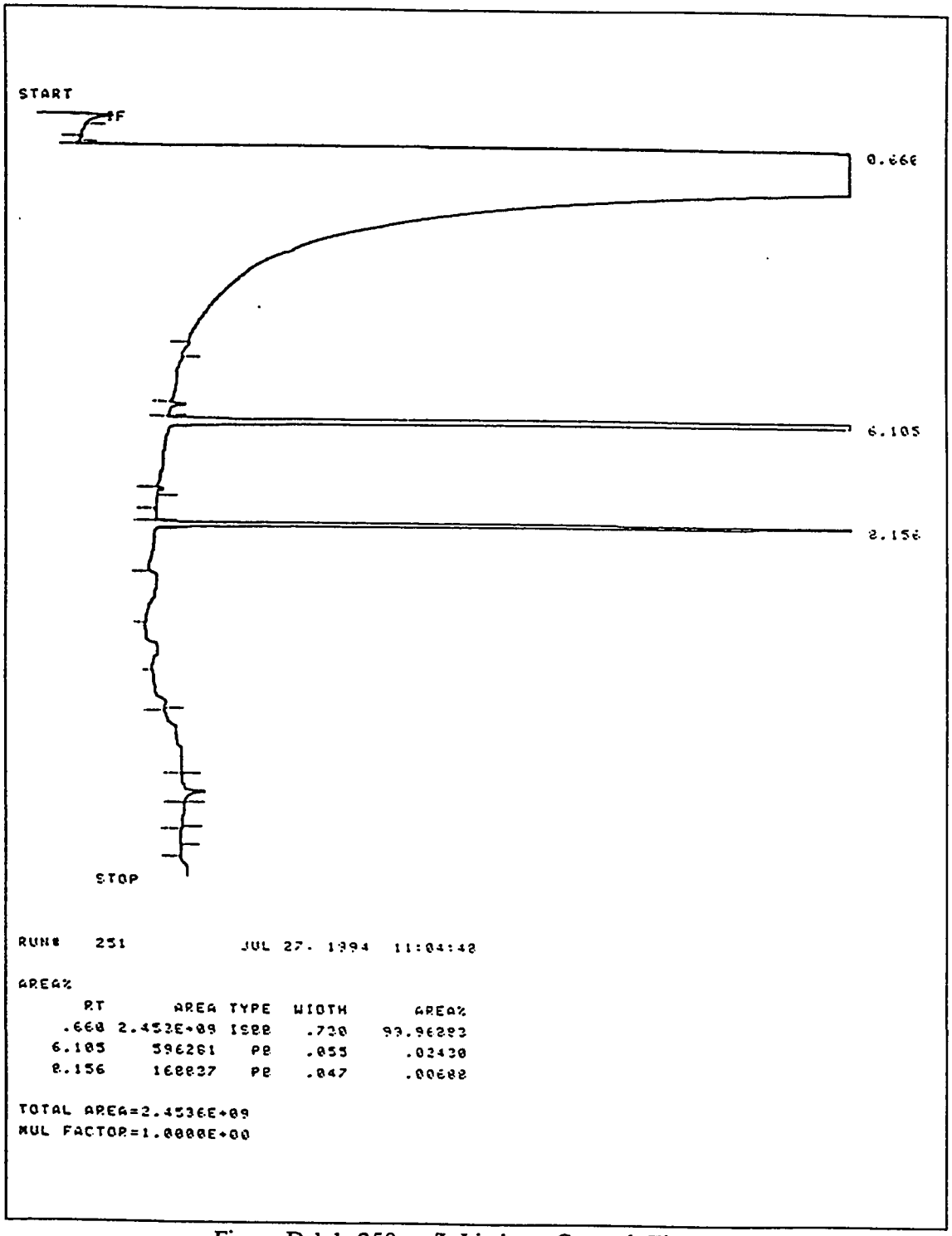
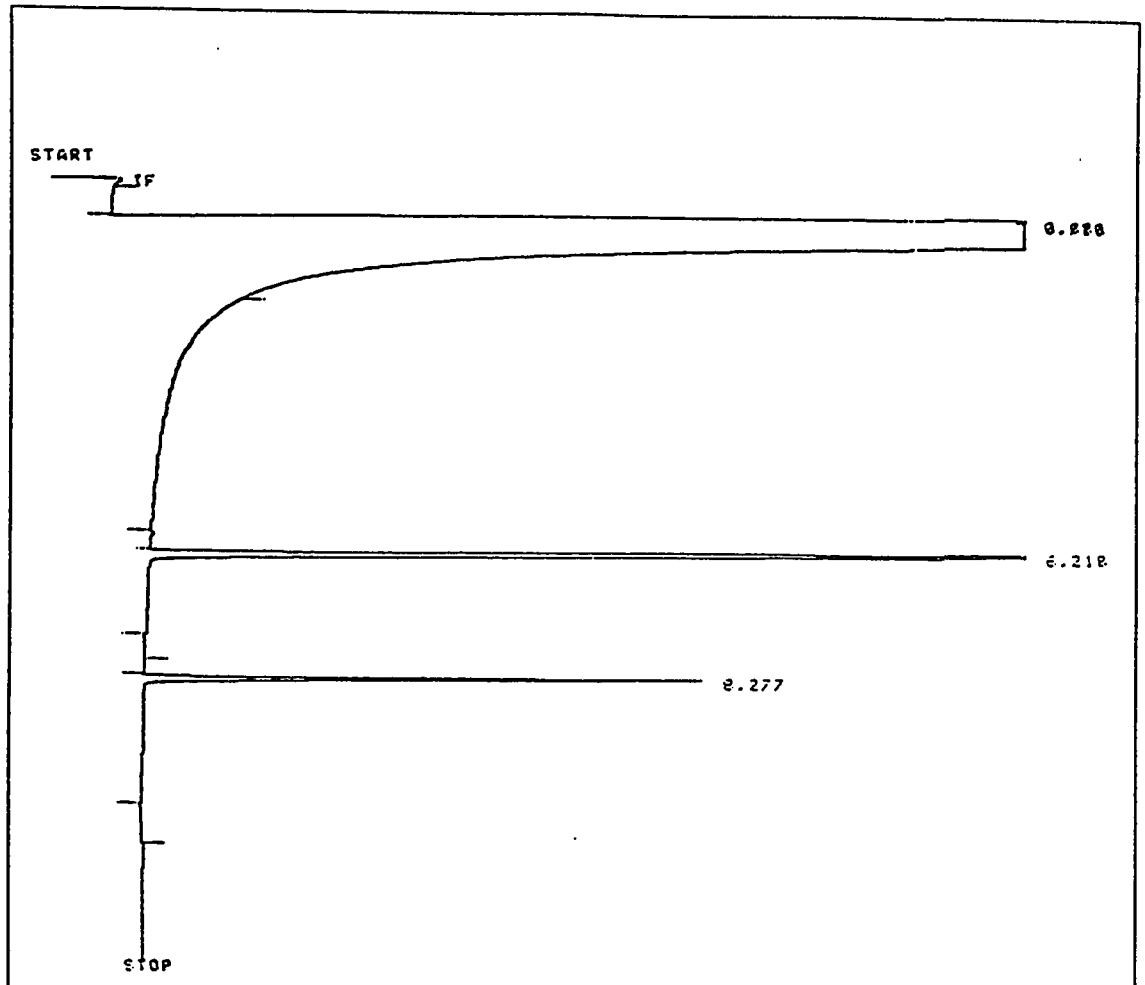


Figure D.1.1. 250 µg/L Lindane, Control, Time = 0



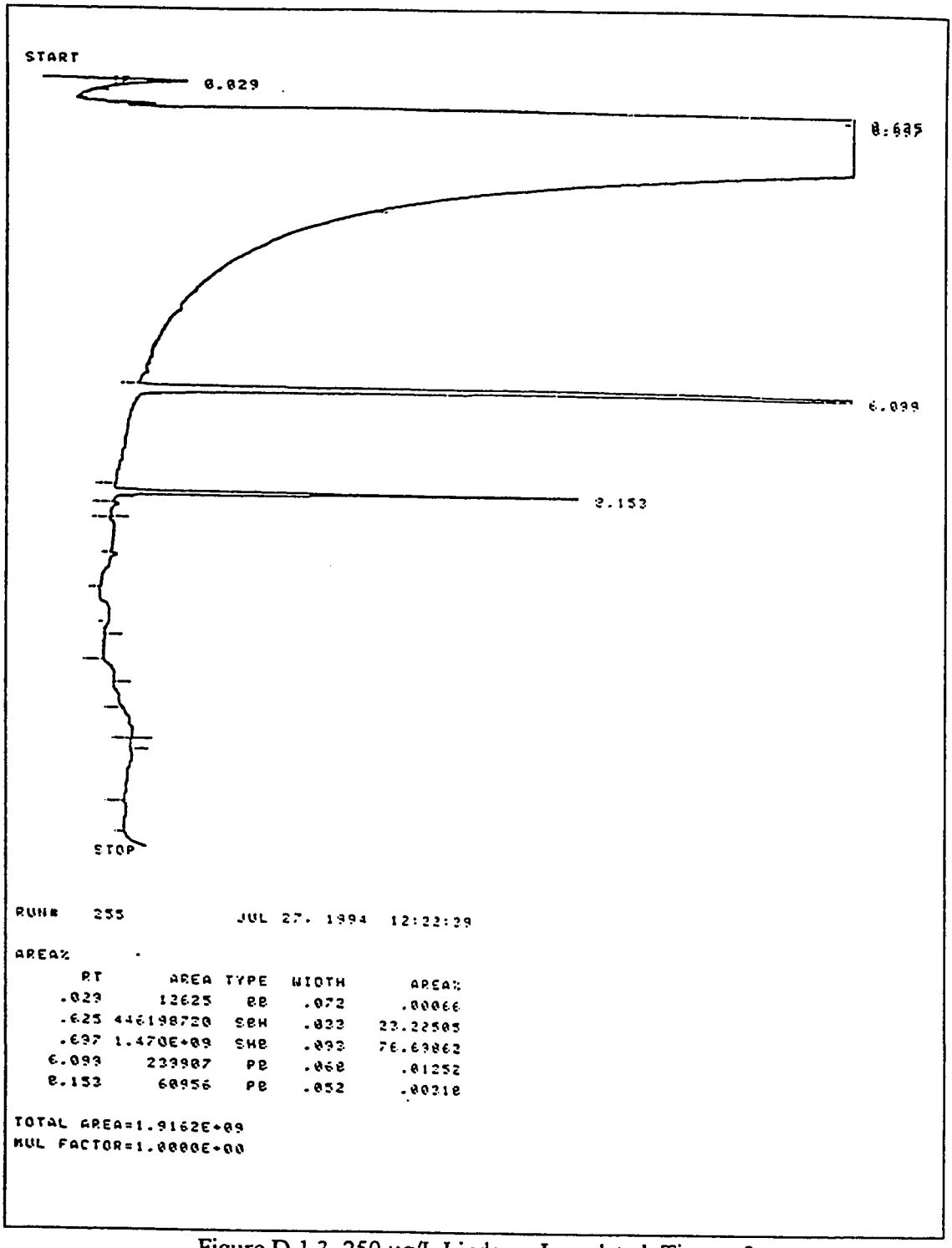
RUN# 422 AUG 19, 1994 09:55:02

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.698	5240842	PM	.024	.10253
.718	5.112E+09	SMB	.085	99.87837
6.218	754002	PB	.055	.01473
8.277	226742	PB	.048	.00443

TOTAL AREA=5.1195E+09
MUL FACTOR=1.0000E+00

Figure D.1.2. 250 µg/L Lindane, Control, Time = 3 Weeks



RUN# 255 JUL 27, 1994 12:22:39

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.029	12625	BB	.072	.00066
.625	446198720	SBH	.033	23.22505
.697	1.470E+09	SHB	.093	76.69862
6.093	239987	PB	.068	.01252
8.153	60956	PB	.052	.00318

TOTAL AREA=1.9162E+09
MUL FACTOR=1.0000E+00

Figure D.1.3. 250 µg/L Lindane, Inoculated, Time = 0

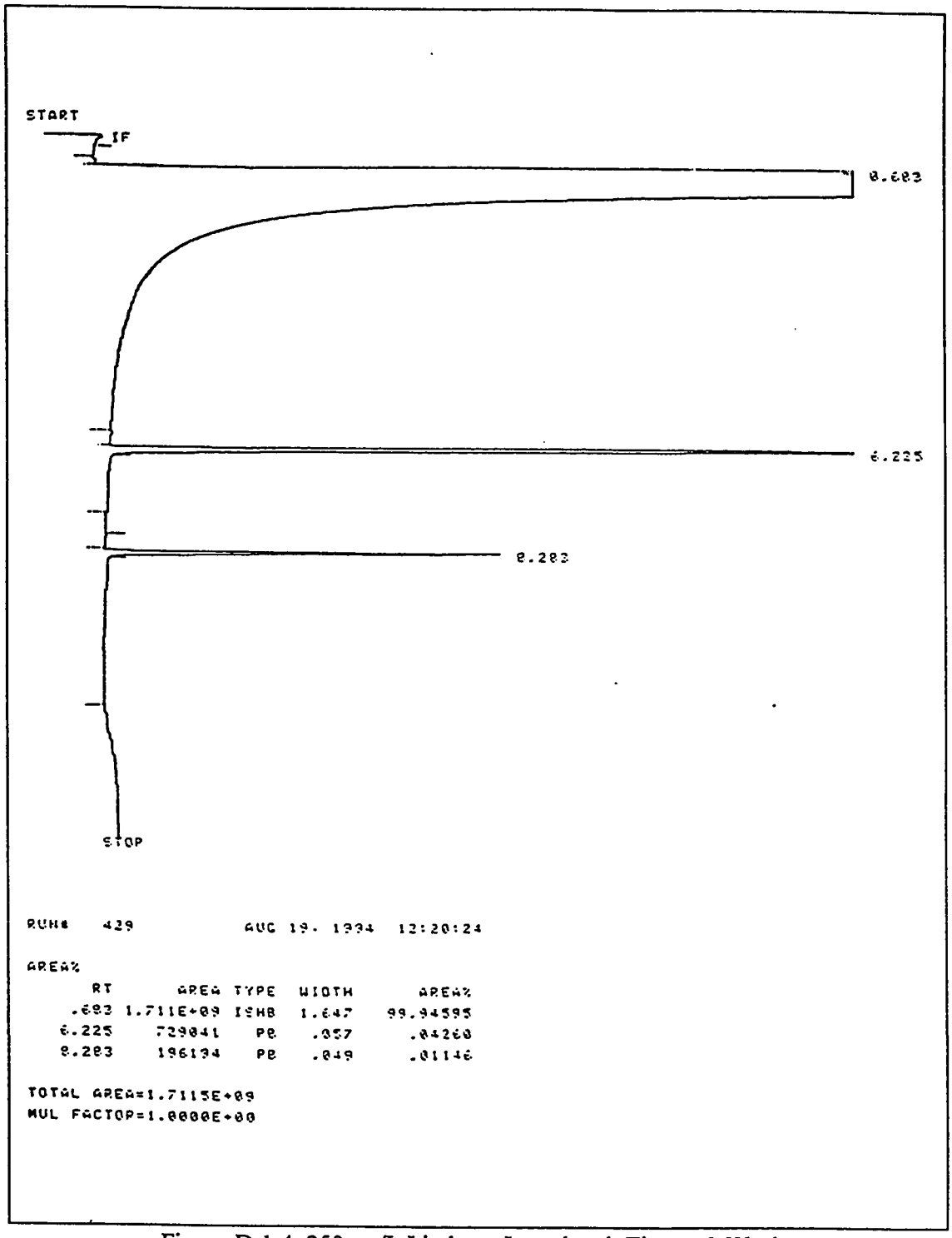
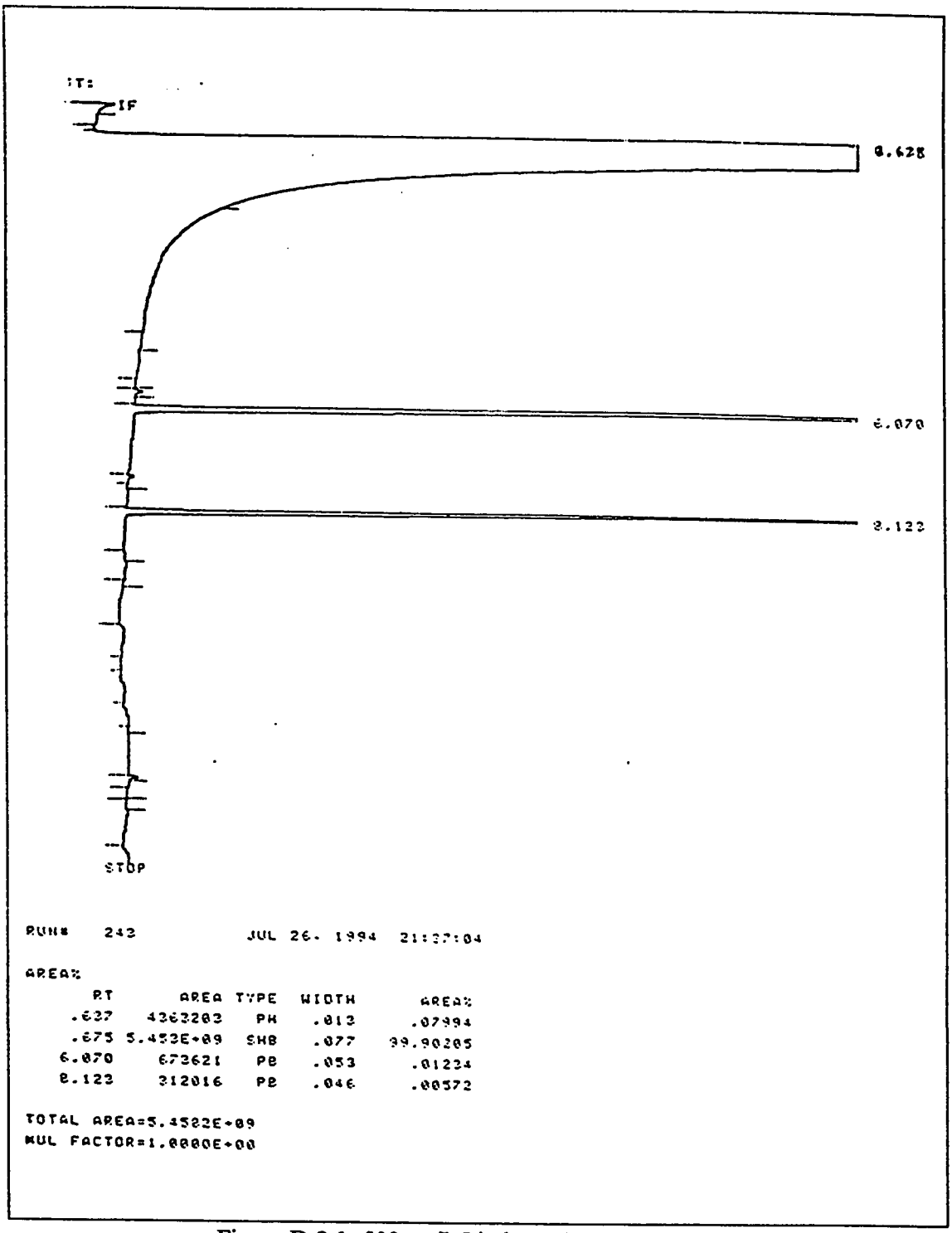


Figure D.1.4. 250 µg/L Lindane, Inoculated, Time = 3 Weeks



RUN# 242 JUL 26, 1994 21:37:04

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.627	4363203	PH	.013	.07994
.675	5.453E+09	SHB	.077	99.90205
6.070	673621	PB	.053	.01234
8.122	312016	PB	.046	.00572

TOTAL AREA=5.4582E+09
MUL FACTOR=1.0000E+00

Figure D.2.1. 500 µg/L Lindane, Control, Time = 0

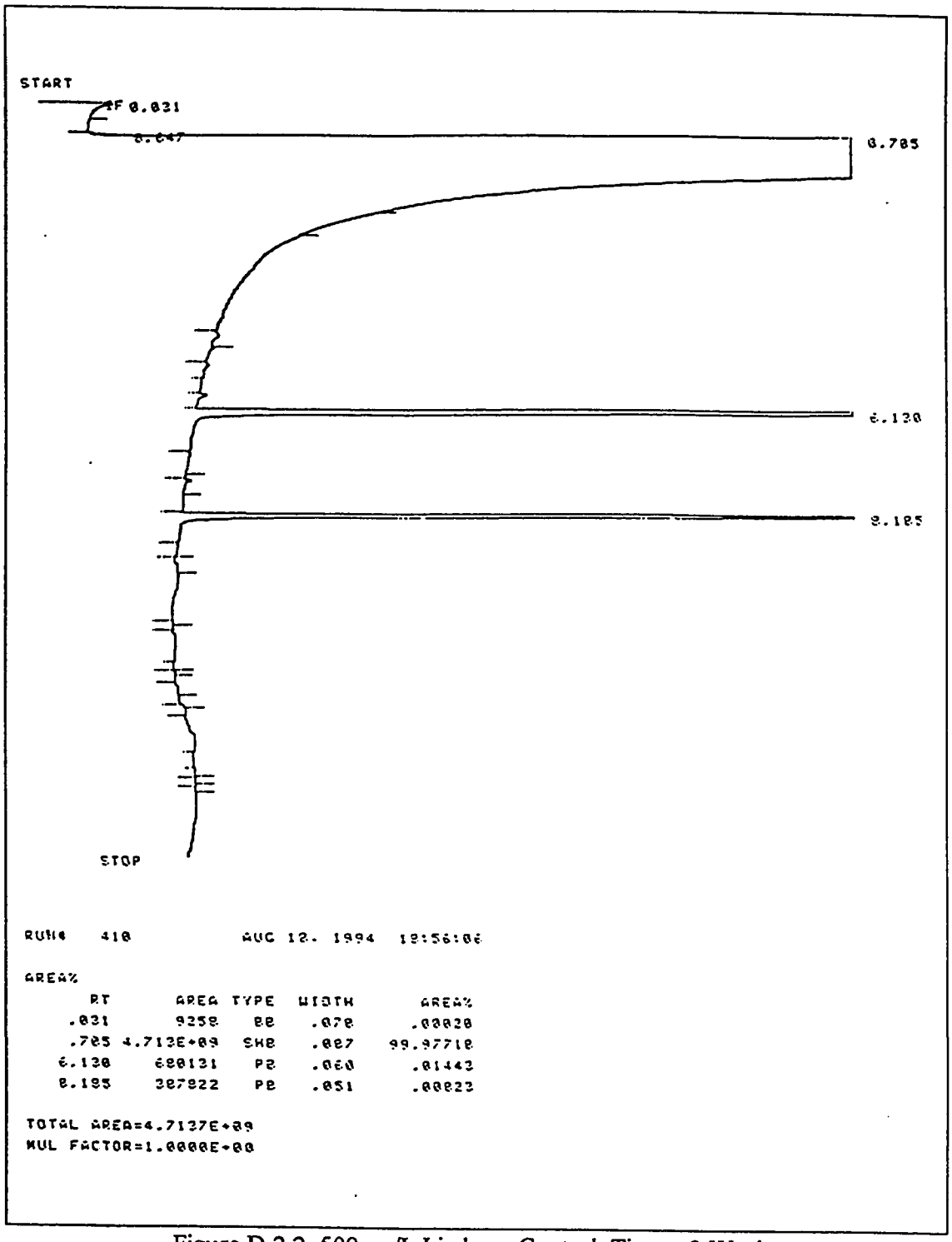
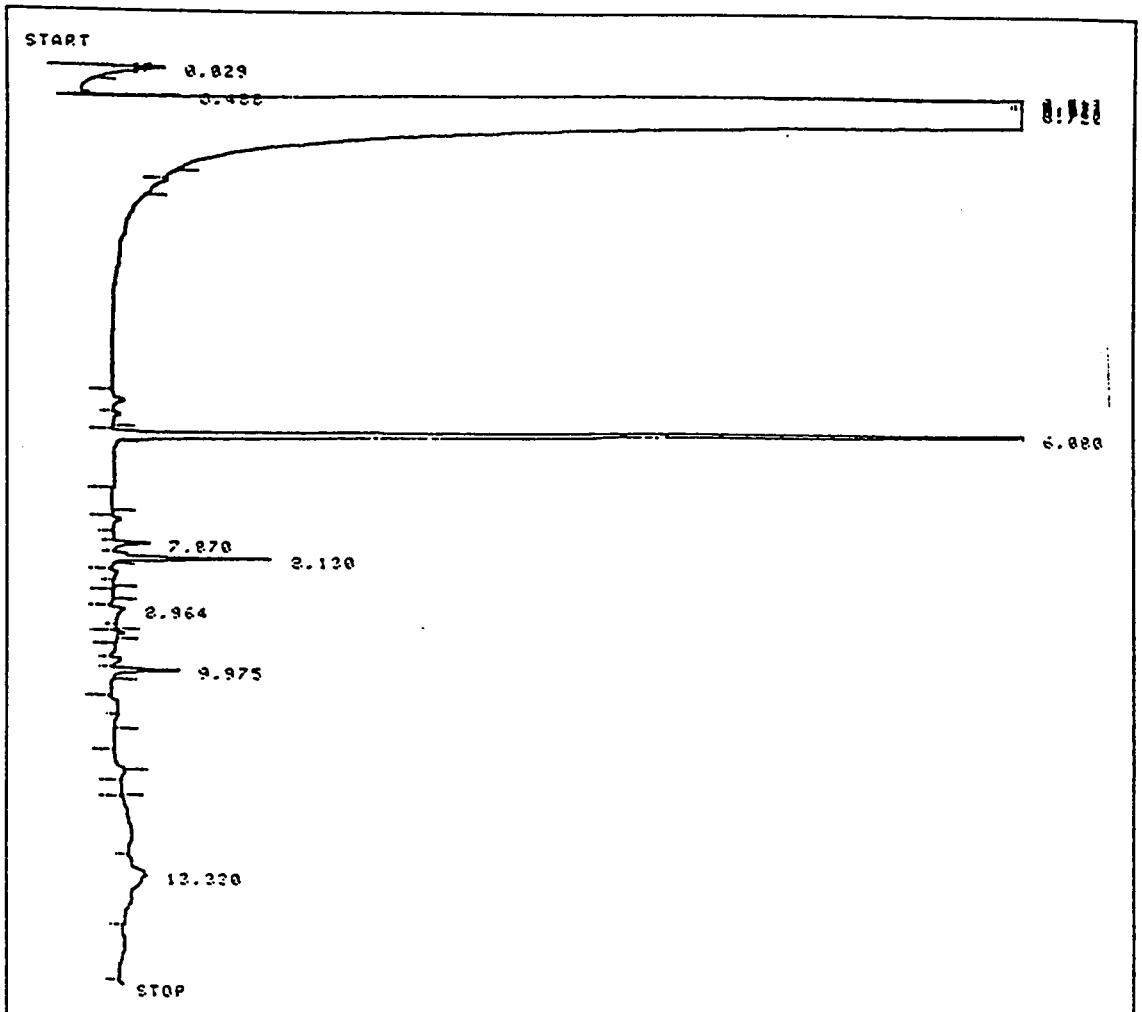


Figure D.2.2. 500 µg/L Lindane, Control, Time = 3 Weeks



RUN# 245 JUL 26, 1994 22:24:23

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.829	11390	BB	.061	.00254
.517	197387	PV	.016	.04393
.572	5881011	VV	.016	1.30943
.624	32478336	VH	.020	7.22145
.720	409990400	SHB	.067	91.28621
6.000	459442	BB	.053	.10230
7.870	9202	VV	.050	.00205
8.130	36192	VB	.054	.00806
9.975	14482	PB	.051	.00322
13.330	48864	VV	.474	.01088

TOTAL AREA=4.4913E+08

Figure D.2.3. 500 µg/L Lindane, Inoculated, Time = 0

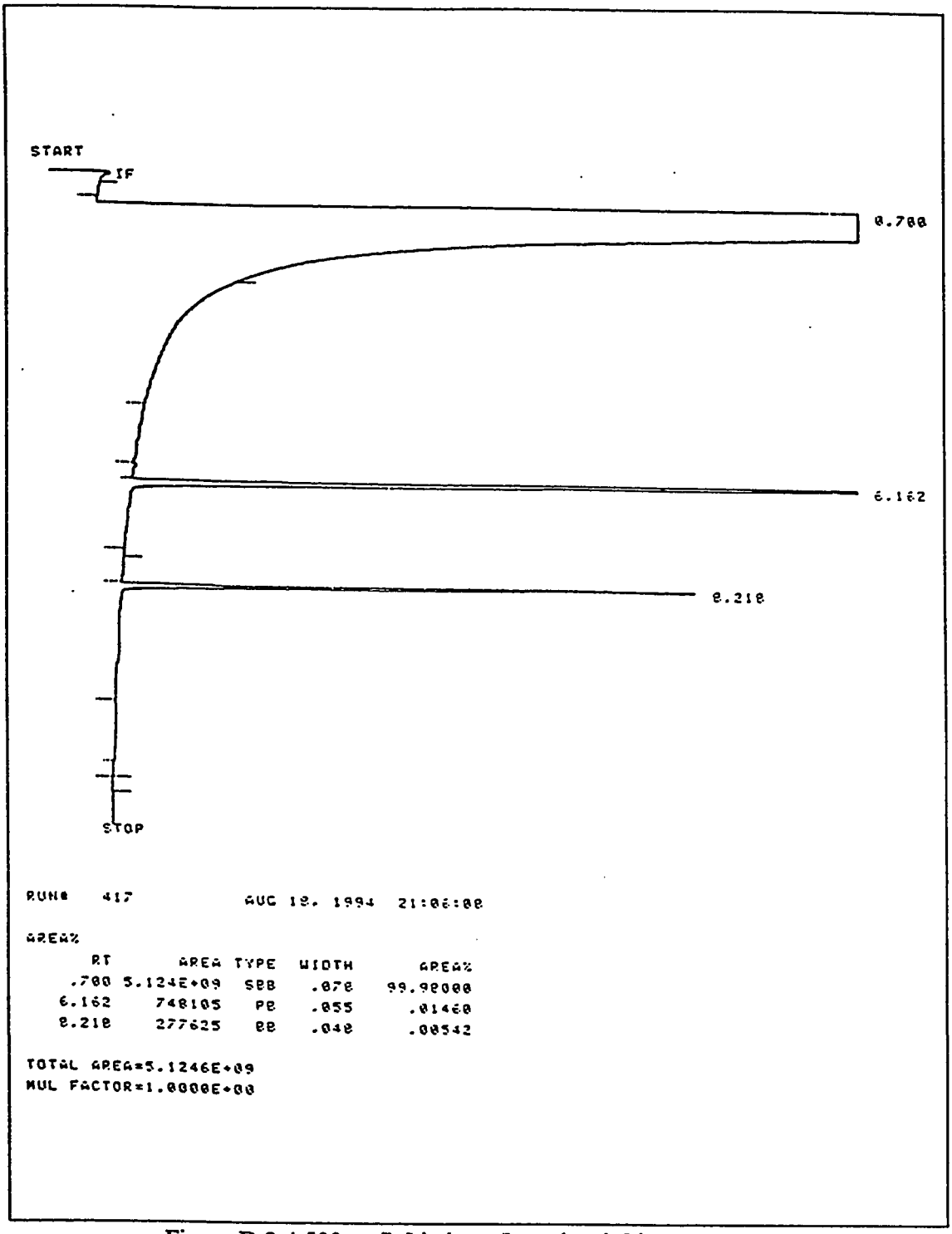


Figure D.2.4 500 µg/L Lindane, Inoculated, Time = 3 Weeks

Appendix E: PCP Sample Chromatograms

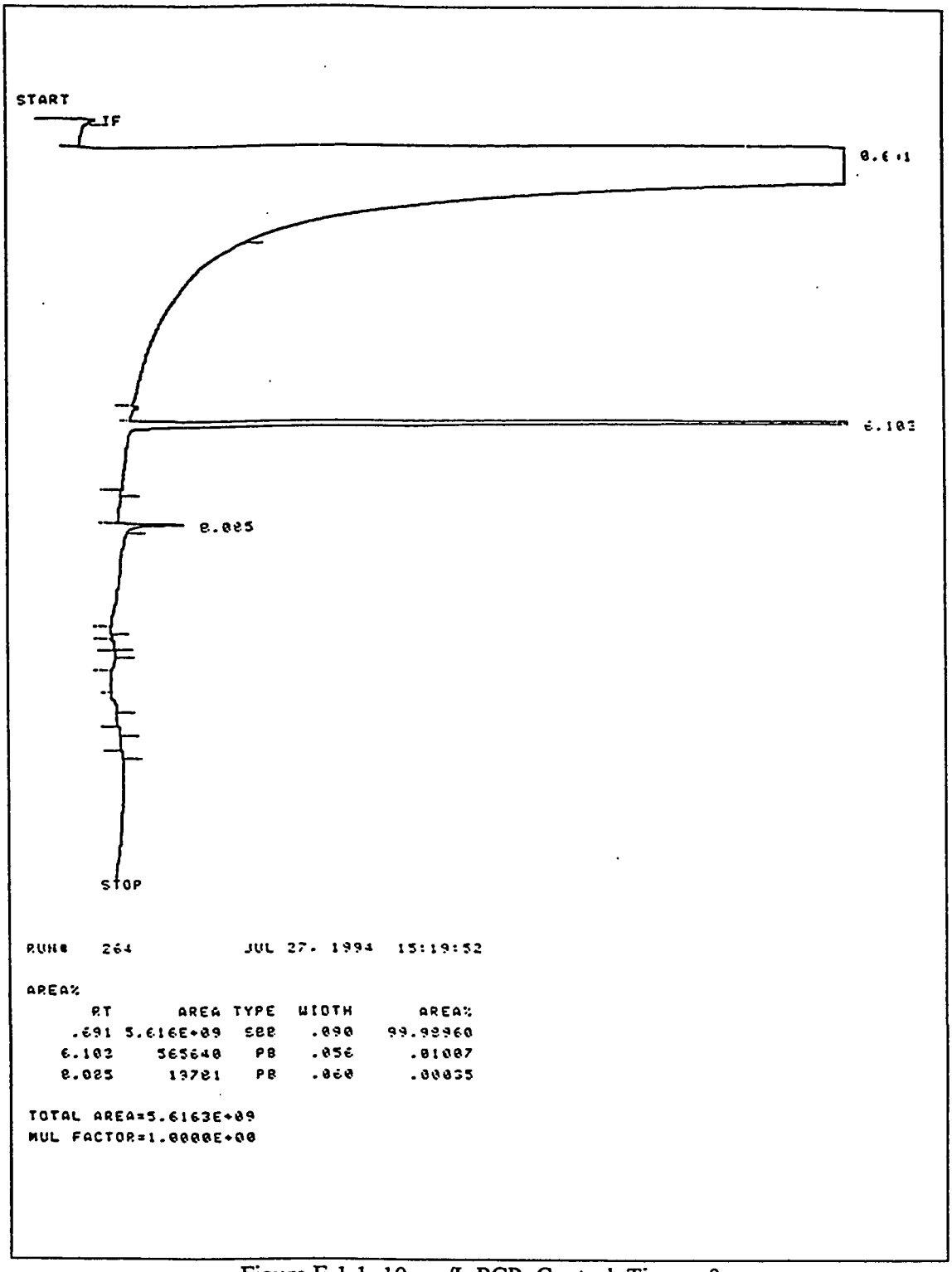


Figure E.1.1. 10 mg/L PCP, Control, Time = 0

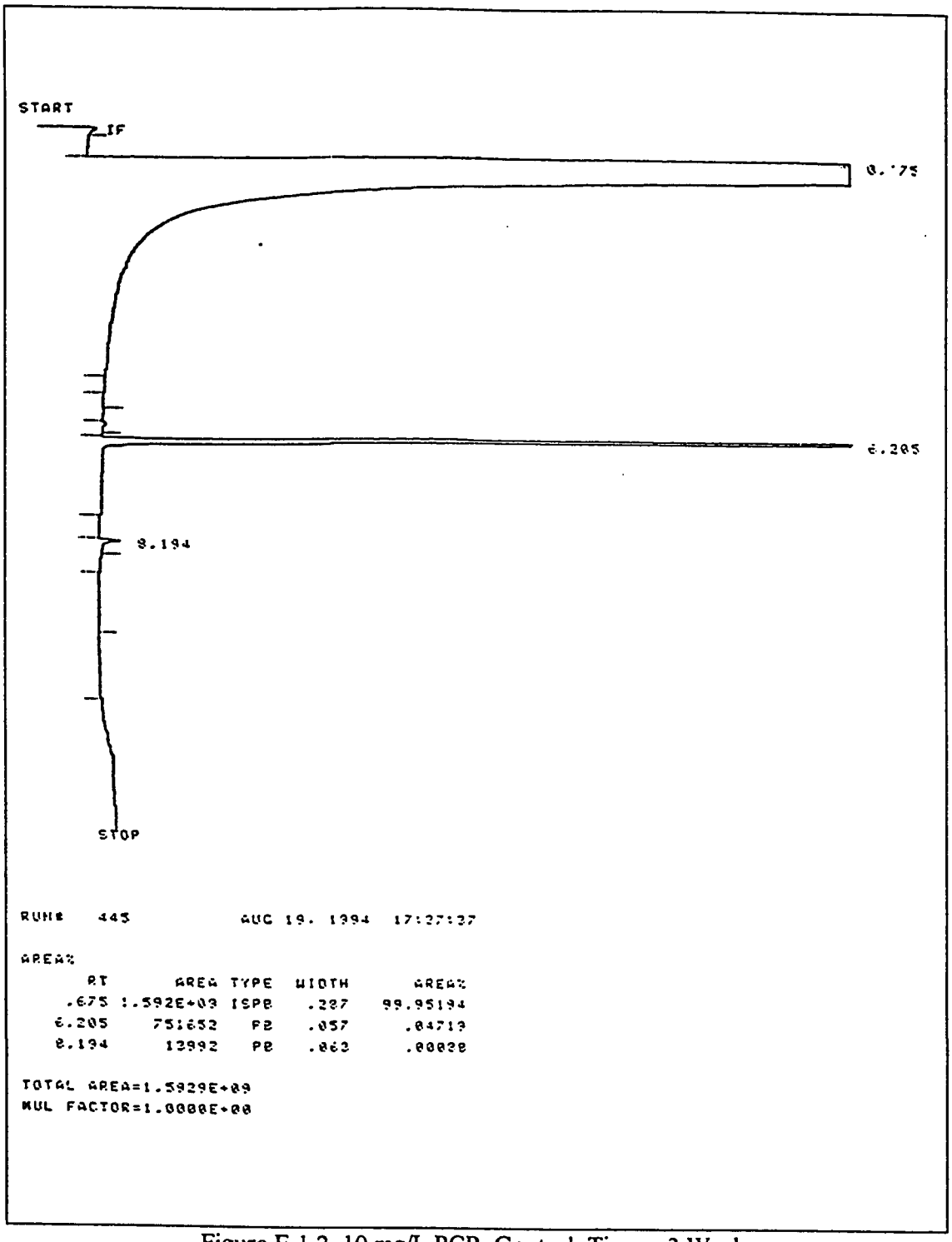


Figure E.1.2. 10 mg/L PCP, Control, Time = 3 Weeks

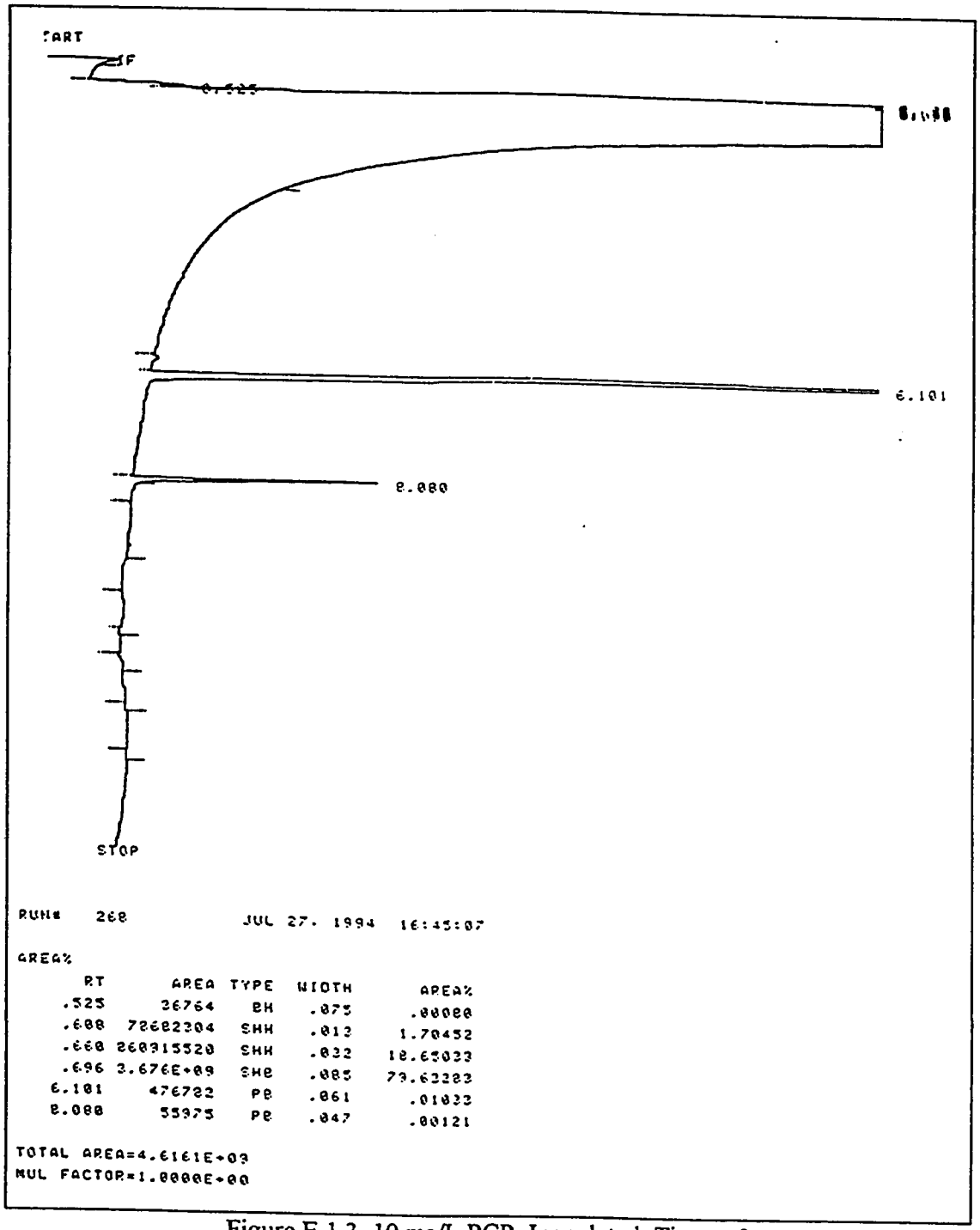
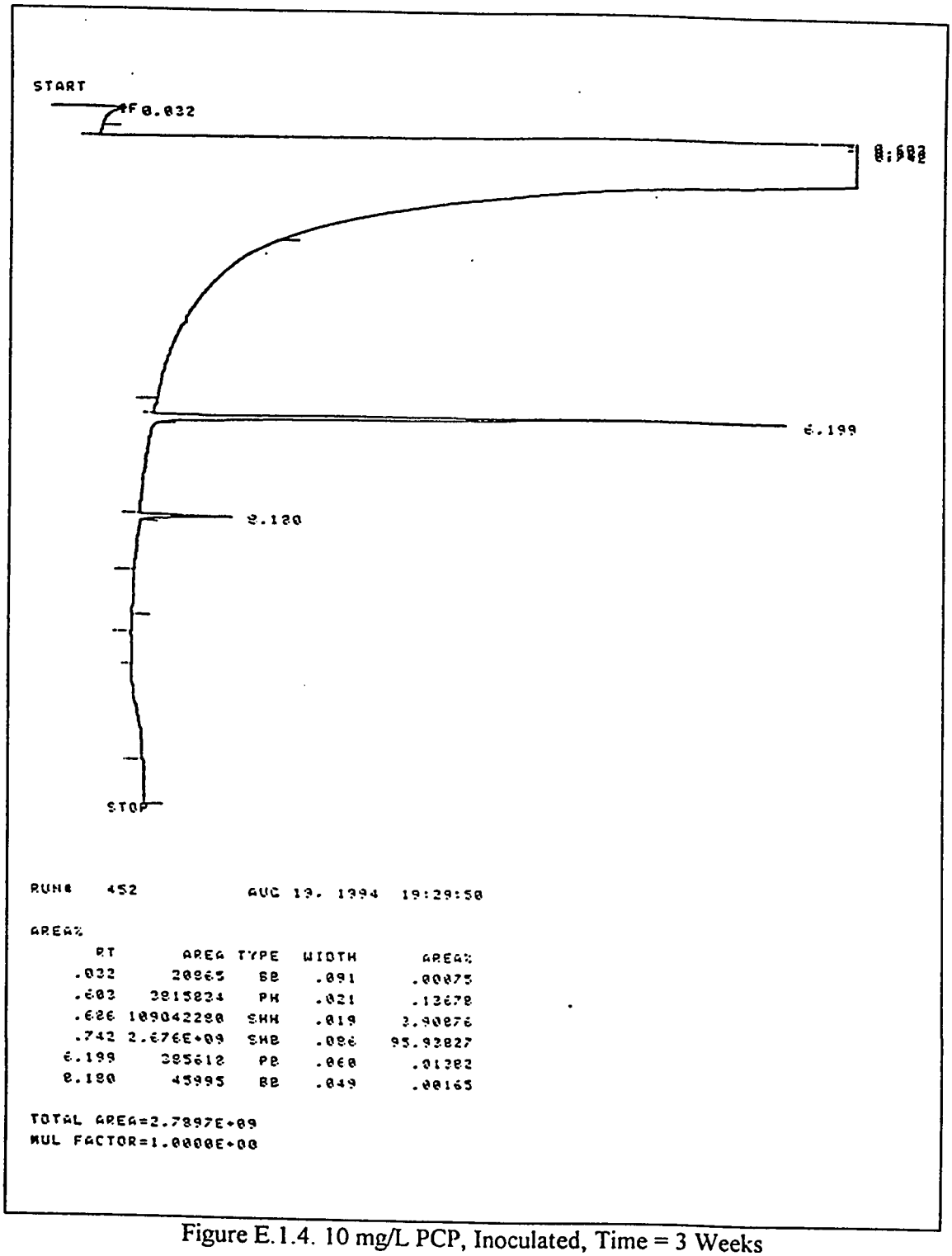


Figure E.1.3. 10 mg/L PCP, Inoculated, Time = 0



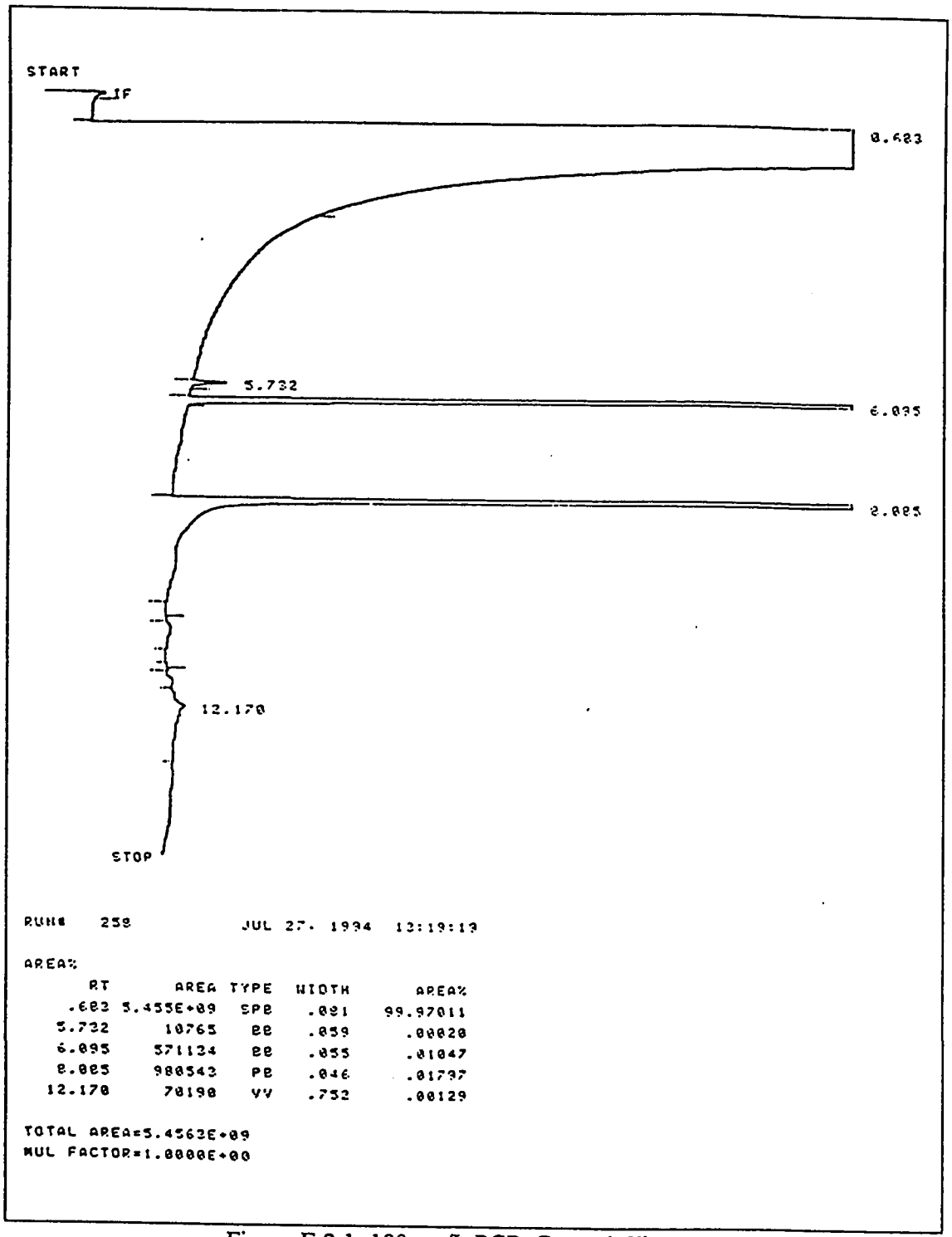
RUN# 452 AUG 19, 1994 19:29:50

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.032	20965	BB	.091	.00075
.602	3815824	PM	.021	.13678
.626	109042280	SHH	.019	3.90876
.742	2.676E+09	SHB	.096	95.93827
6.199	385612	PB	.060	.01382
8.120	45995	BB	.049	.00165

TOTAL AREA=2.7897E+09
MUL FACTOR=1.0000E+00

Figure E.1.4. 10 mg/L PCP, Inoculated, Time = 3 Weeks



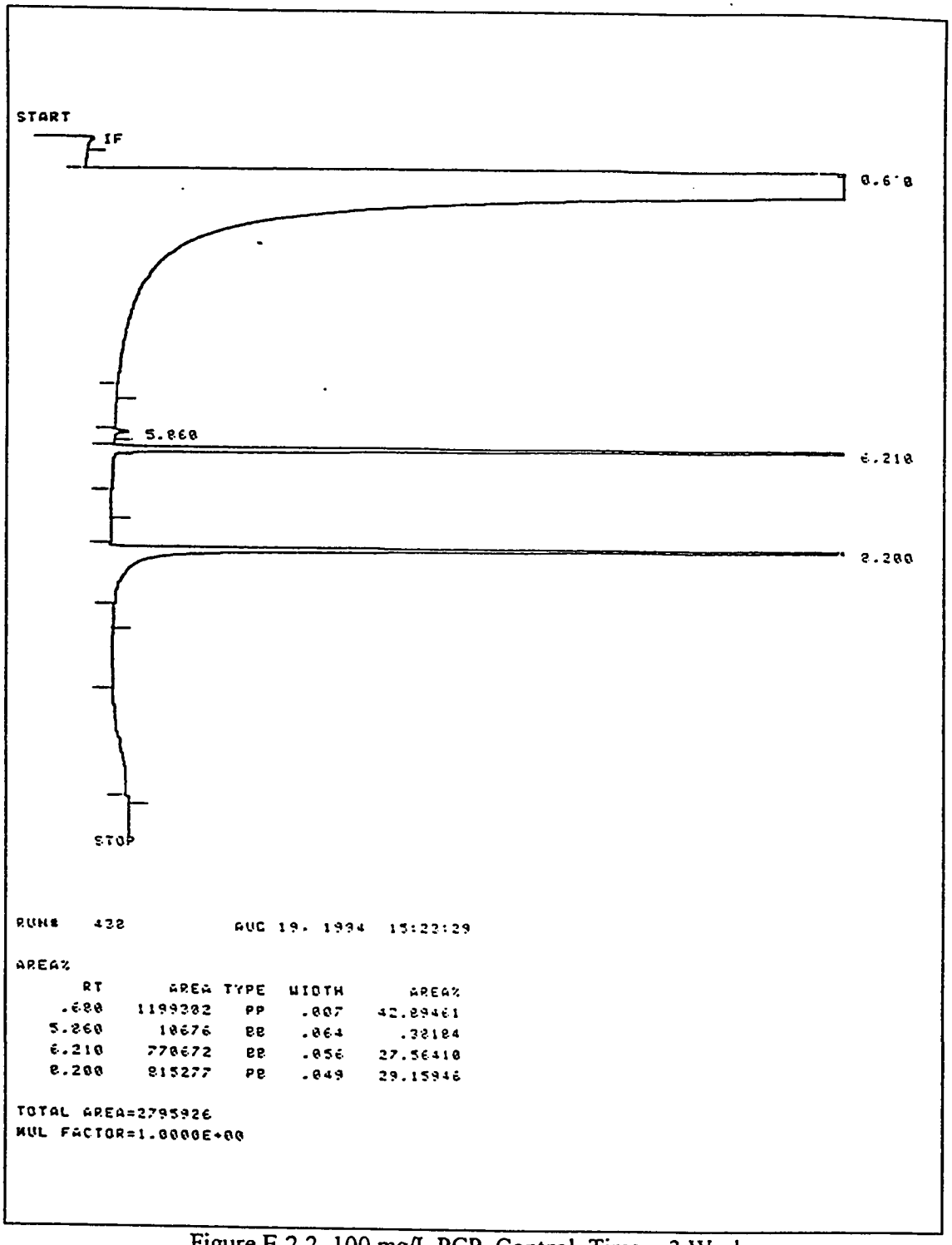
RUN# 258 JUL 27, 1994 12:19:19

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.683	5.455E+09	SPB	.081	99.97011
5.732	10765	BB	.059	.00020
6.095	571134	BB	.055	.01047
8.085	980543	PB	.046	.01797
12.170	70190	VV	.732	.00129

TOTAL AREA=5.4563E+09
MUL FACTOR=1.0000E+00

Figure E.2.1. 100 mg/L PCP, Control, Time = 0



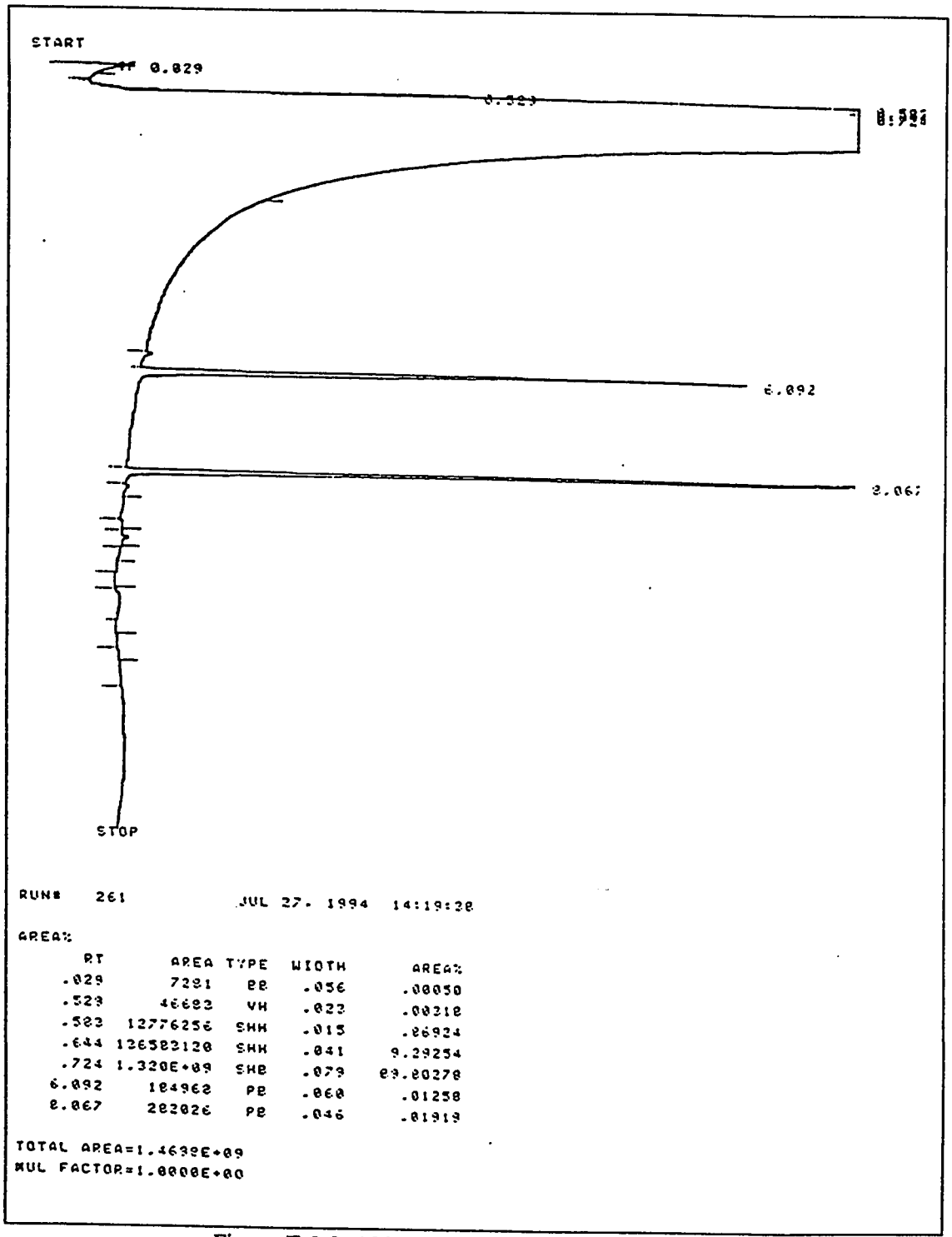
RUN# 432 AUG 19, 1994 15:22:29

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.680	1199302	PP	.007	42.89461
5.860	10676	BB	.064	.38184
6.210	770672	BB	.056	27.56410
8.200	815277	PB	.049	29.15946

TOTAL AREA=2795926
MUL FACTOR=1.0000E+00

Figure E.2.2. 100 mg/L PCP, Control, Time = 3 Weeks



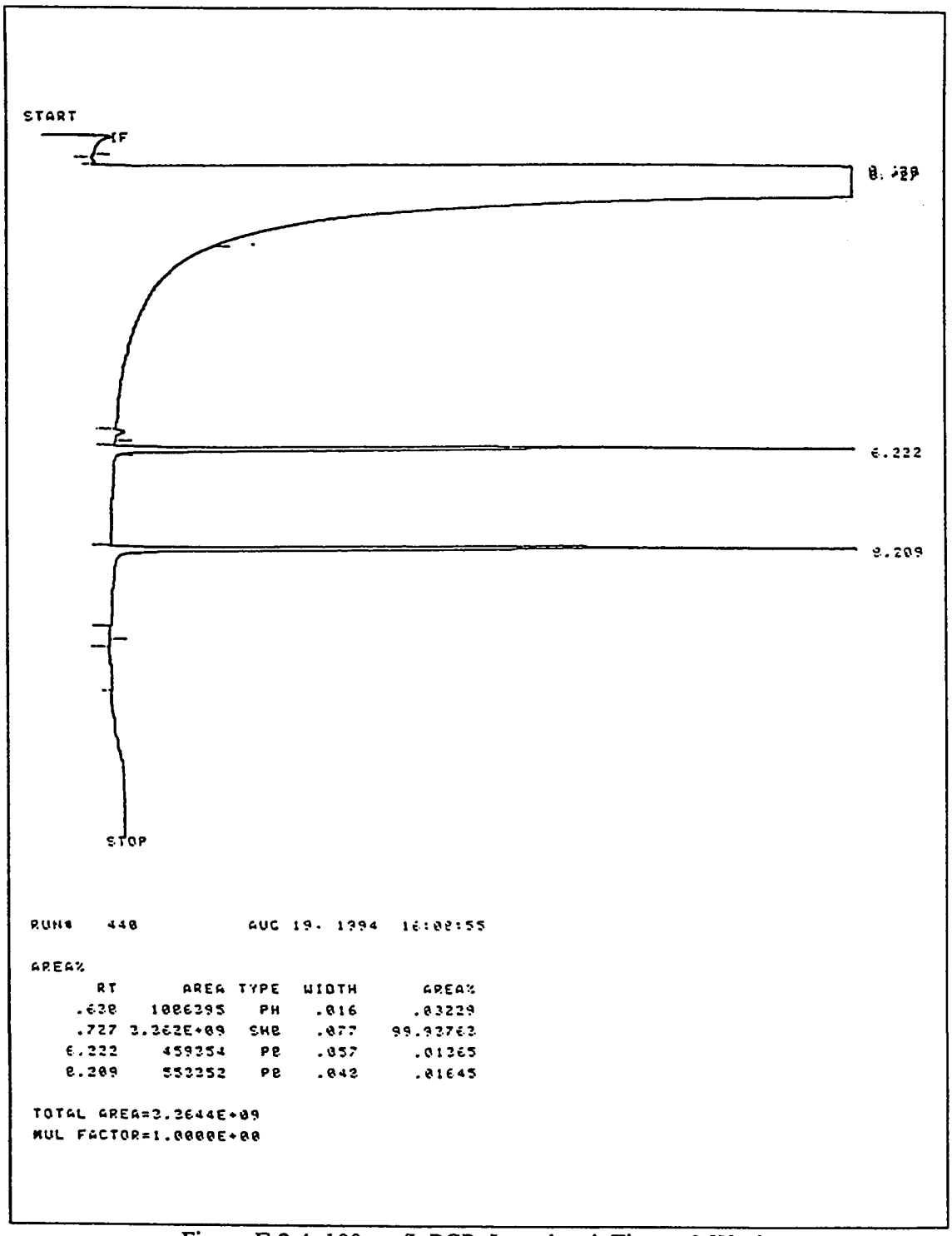
RUN# 261 JUL 27, 1994 14:19:38

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.029	7281	BB	.056	.00050
.523	46683	VH	.023	.00318
.583	12776256	SHH	.015	.86924
.644	1365823120	SHH	.041	9.29254
.724	1.320E+09	SHB	.079	89.80278
6.092	184962	PB	.060	.01258
8.067	28202E	PB	.046	.01919

TOTAL AREA=1.4699E+09
MUL FACTOR=1.0000E+00

Figure E.2.3. 100 mg/L PCP, Inoculated, Time = 0



RUN# 448 AUG 19, 1994 16:02:55

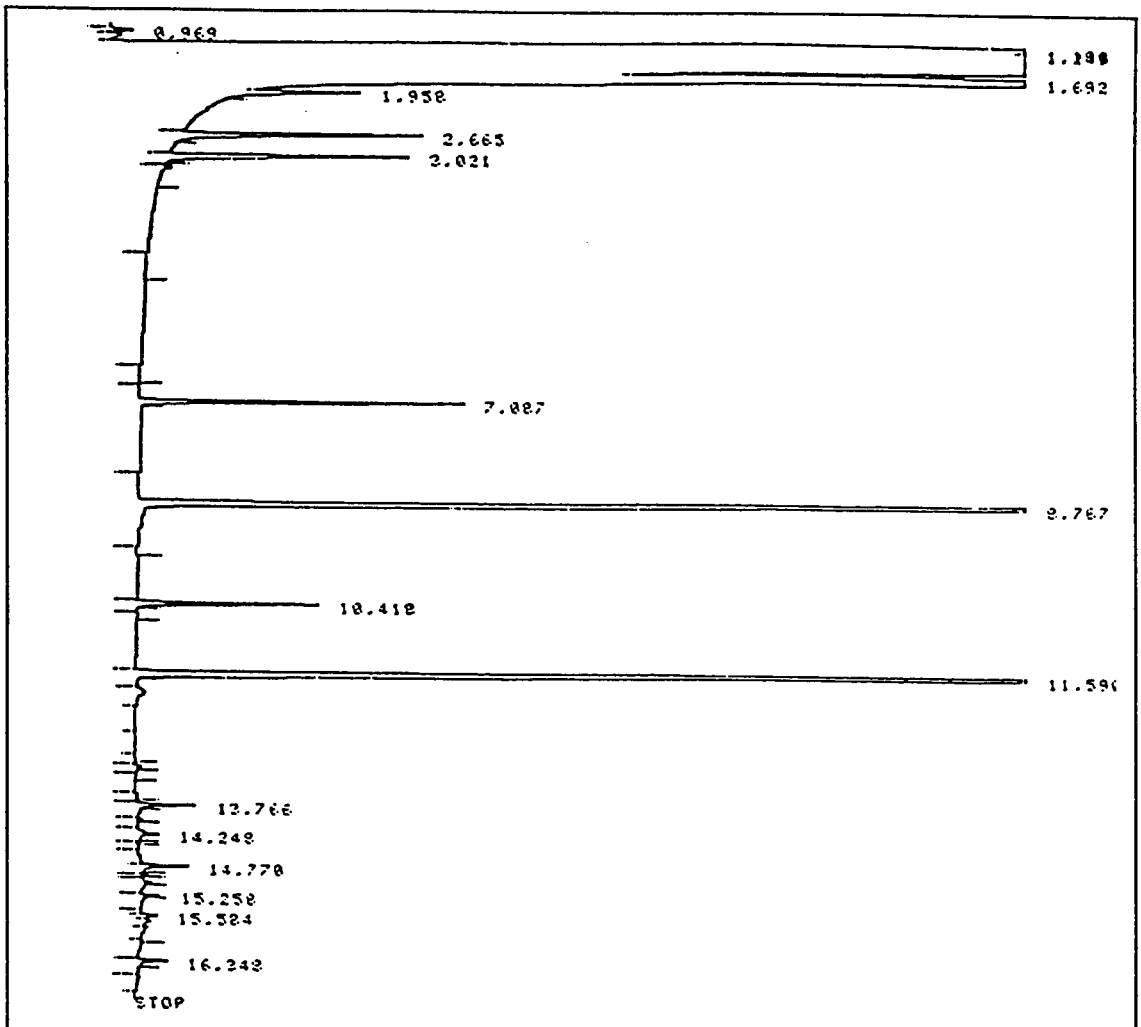
AREA%

RT	AREA	TYPE	WIDTH	AREA%
.630	1006295	PH	.016	.03229
.727	3.362E+09	SHB	.077	99.93763
6.222	459354	PB	.057	.01365
8.209	553252	PB	.042	.01645

TOTAL AREA=3.3644E+09
MUL FACTOR=1.0000E+00

Figure E.2.4. 100 mg/L PCP, Inoculated, Time = 3 Weeks

Appendix F: 1,4-BCB Sample Chromatograms



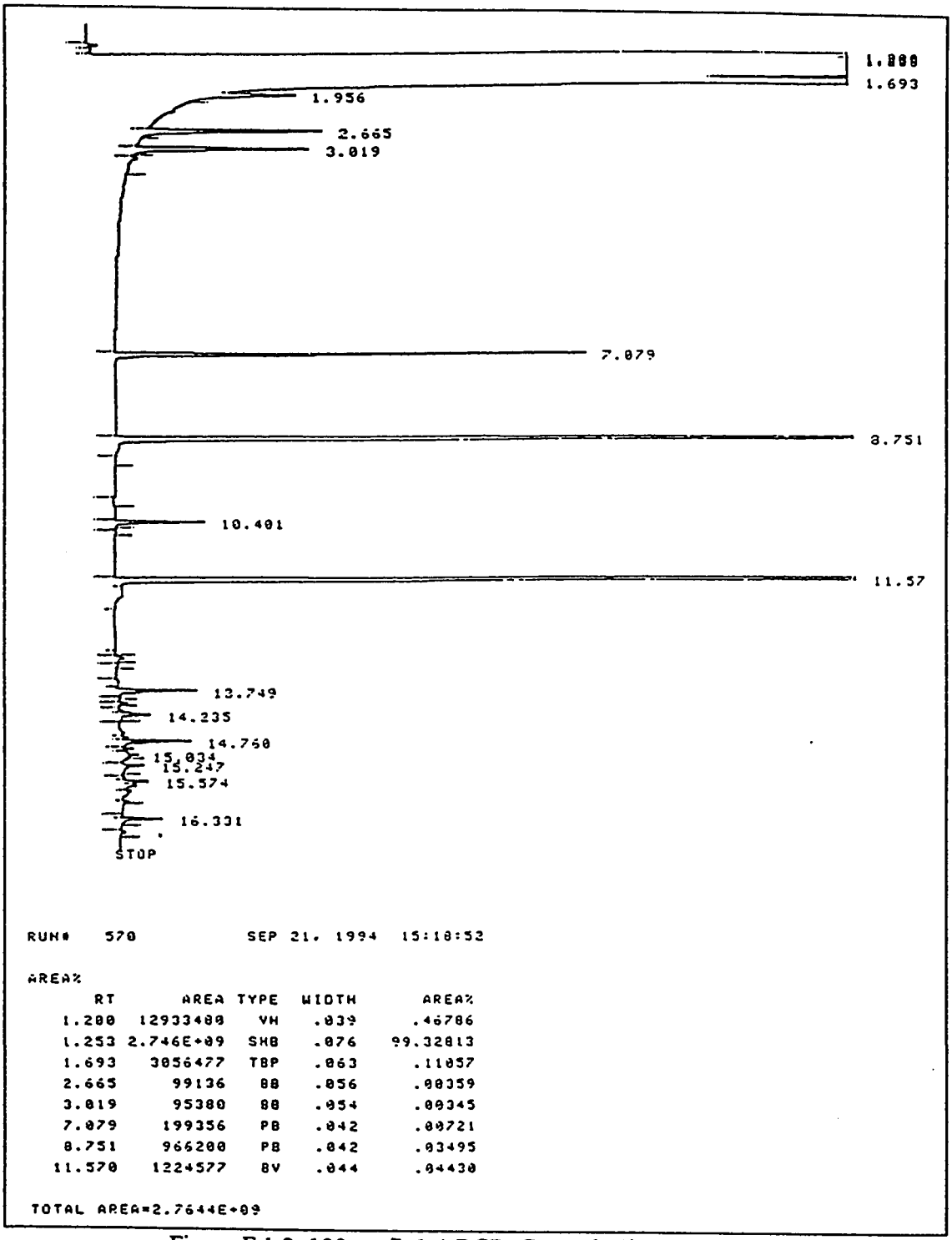
RUN# 504 AUG 27, 1994 16:42:20

AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.198	14343288	VH	.026	.49204
1.249	2.894E+09	SHB	.076	99.28355
1.692	3467718	TBP	.061	.11896
2.665	109304	PB	.053	.00375
3.021	105826	PB	.052	.00363
7.007	112564	PB	.041	.00386
8.767	1292804	PB	.042	.04435
10.418	66631	PB	.042	.00229
11.590	1389564	PB	.045	.04767

TOTAL AREA=2.9150E+09

Figure F.1.1. 100 mg/L 1,4-BCB, Control, Time = 0



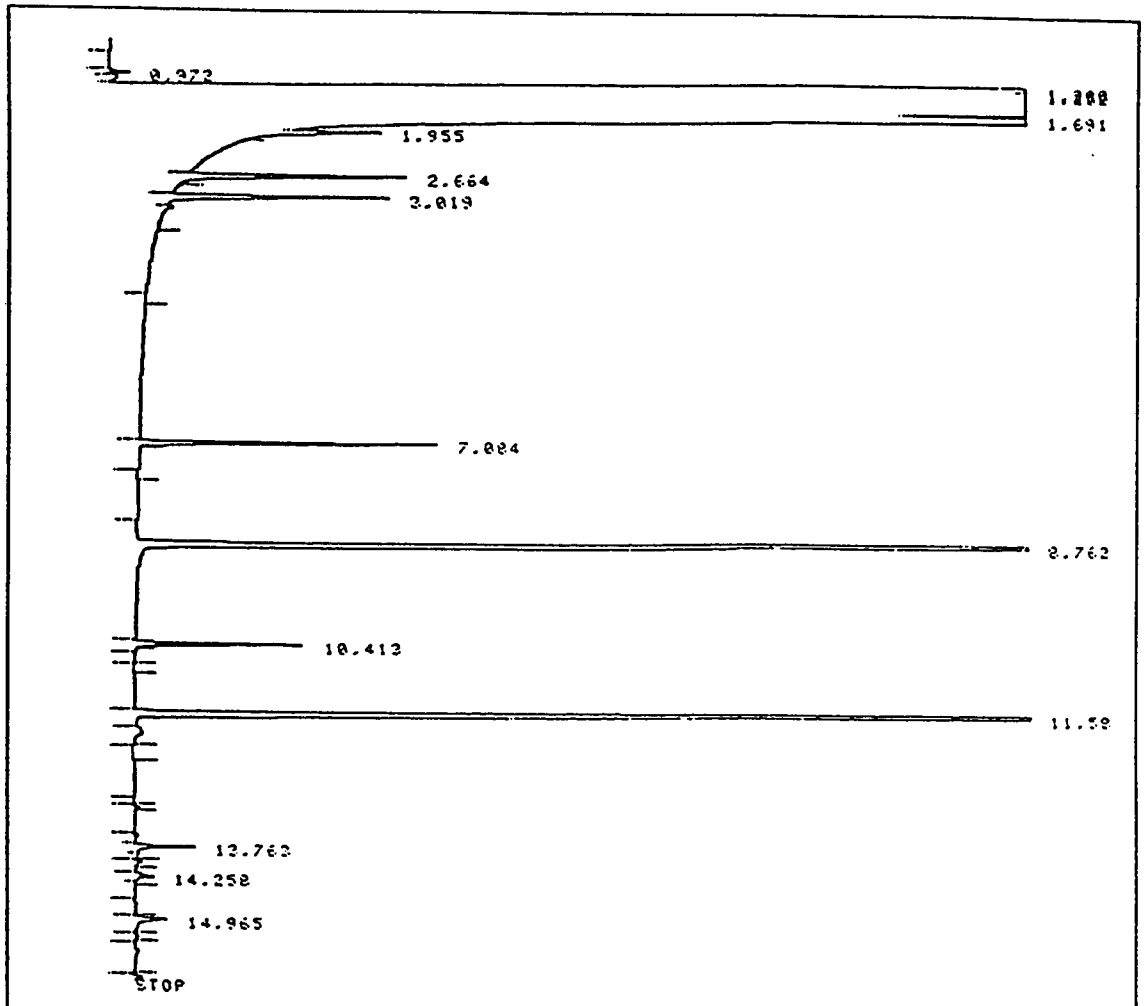
RUN# 570 SEP 21, 1994 15:18:52

AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.200	12933480	VH	.039	.46786
1.253	2.746E+09	SHB	.076	99.32813
1.693	3056477	TBP	.063	.11057
2.665	99136	BB	.056	.00359
3.019	95380	BB	.054	.00345
7.079	199356	PB	.042	.00721
8.751	966200	PB	.042	.03495
11.570	1224577	BV	.044	.04430

TOTAL AREA=2.7644E+09

Figure F.1.2. 100 mg/L 1,4-BCB, Control, Time = 3 Weeks



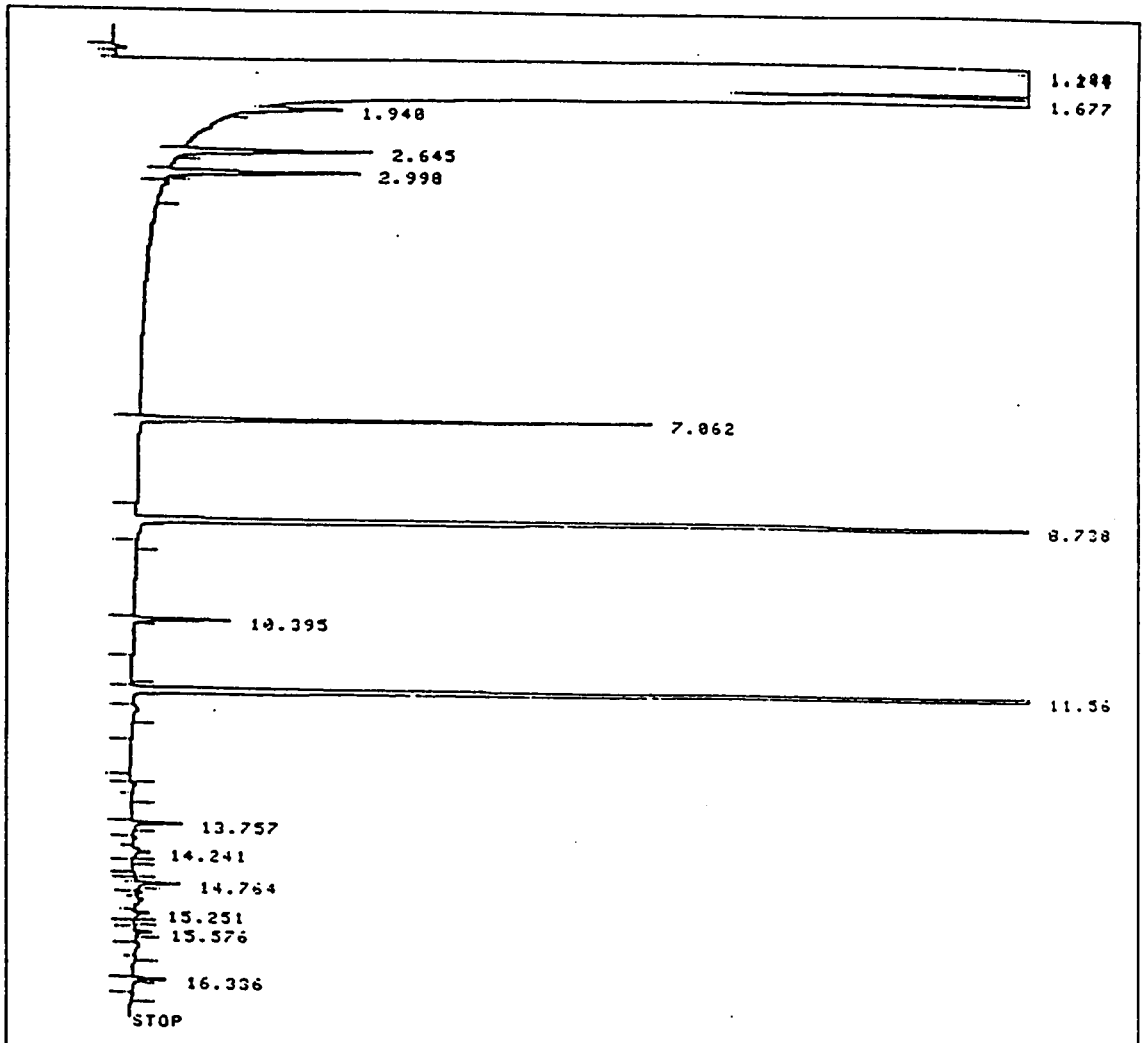
RUN# 506 AUG 27, 1994 17:38:06

AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.200	13037664	VH	.037	.47911
1.252	2.708E+09	SHB	.075	99.30554
1.691	3089890	TBP	.060	.11331
2.664	100210	BB	.054	.00367
3.019	96756	BP	.052	.00355
7.084	104362	BB	.042	.00383
8.762	1189496	PB	.043	.04362
10.413	60876	PB	.043	.00223
11.584	1260911	PB	.045	.04624

TOTAL AREA=2.7269E+09

Figure F.1.3. 100 mg/L 1,4-BCB, Inoculated, Time = 0



RUN# 575 SEP 21, 1994 21:16:36

AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.188	11244376	VH	.040	.43679
1.241	2.558E+09	SHB	.074	99.36266
1.677	2825646	TBP	.065	.10976
2.645	90074	BB	.057	.00350
2.998	87173	BB	.055	.00339
7.062	179306	PB	.042	.00697
8.738	858878	PB	.043	.03336
11.564	1123716	PB	.045	.04365

TOTAL AREA=2.5743E+09

Figure F.1.4. 100 mg/L 1,4-BCB, Inoculated, Time = 3 Weeks

Appendix G. Raw Data

Table G.1. 10 mg/mL Trichloroethylene Raw Data

10 ppm Trichloroethylene														
			Time = 0, 7/8/92					Time = 1 Week			Time =3 Weeks, 8/2/94			
			Area	Ratio				Area	Ratio				Area	Ratio
	TCE	C5-Imp	Pentane	TCE/C5-I		TCE	C5-Imp	Pentane	TCE/C5-I		TCE	C5-Imp	Pentane	TCE/C5-I
Control					Control					Control				
Run #1	40633	Too Small	3.58E+08	N/A	Run #1	143156	82858	3.46E+09	1.727727	Run # 1	114323	84958	3.55E+09	1.345641
Run # 3	44618	14807	5.5E+08	3.013305	Run # 2	135596	78708	3.28E+09	1.722773	Run # 2	114449	80485	3.34E+09	1.421992
					Run # 3	140998	82618	3.39E+09	1.706626	Run # 2	88358	59065	2.55E+09	1.495945
Average	42625.5	14807	4.54E+08	3.0133										
Std Dev	2817.821		1.36E+08		Average	139916.7	81394.67	3.38E+09	1.719042	Average	105710	74836	3.15E+09	1.421193
% Error	6.610645		29.90612		Std Dev	3894.273	2329.814	91270660	0.011034	Std Dev	15027.4	13839.99	5.27E+08	0.075155
					% Error	2.78328	2.862367	2.702447	0.641888	% Error	14.21569	18.49376	16.72977	5.288169
Inoc. A														
					Inoc. A					Innoc A				
Run # 1	30827	9314	3.88E+08	3.309749										
Run # 2	40453	13060	2.12E+07	3.097473	Run # 1	20987	Too small	2.39E+08		Run # 1	140198	63345	2.59E+09	2.213245
Run # 3	63179	29361	1.12E+07	2.1518	Run # 2	74738	30963	1.29E+09	2.413784	Run # 2	169481	74672	3.21E+09	2.269673
					Run # 3	31195	Too small	2.36E+08						
Average	30827	9314	1.62E+07	2.624637						Average	154839.5	69008.5	2.9E+09	2.241459
Std Dev			7045612	0.668692	Average	74738	30963	1.29E+09	2.413784	Std Dev	20706.21	8009.399	4.33E+08	0.0399
% Error			43.56673	25.47751	Std Dev					% Error	13.37269	11.60639	14.92239	1.780111
					% Error									
Extractant														
					Inoc. B					Innoc B				
Run # 138		76058												
Run # 139		76420			Run # 1	97457	41320	1.65E+09	2.358591	Run # 1	55586	26142	1.07E+09	2.12631
Run #283		84411			Run # 2	114000	46062	1.92E+09	2.474925	Run # 2	149685	76424	3.17E+09	1.958612
Run # 157		80921			Run # 3	137909	56264	2.33E+09	2.451106	Run # 3	123296	64319	2.61E+09	1.916945
										Run # 4	147491	72432	3.00E+09	2.036269
Average		79452.5			Average	116455.3	47882	1.96E+09	2.428207					
Stdev		3977.516			Std Dev	20337.47	7636.431	3.42E+08	0.061454	Average	119014.5	59829.25	2.46E+09	2.009534
% Error		5.006156			% Error	17.46375	15.94844	17.4165	2.530847	Std Dev	43943.51	23015.96	9.55E+08	0.092226
										% Error	36.92282	38.46942	38.77142	4.589438

Table G.2. 100 mg/mL Trichloroethylene Raw Data

100 ppm Trichloroethylene														
	Time = 0, 7/8/94					Time = 1 Week, 7/14/94					Time =3 Weeks, 8/2/94			
			Area	Ratios				Area	Ratios				Area	Ratios
	TCE	C5-Imp	Pentane	TCE/C5-I		TCE	C5-Imp	Pentane	TCE/C5-I		TCE	C5-Imp	Pentane	TCE/C5-I
Control					Control					Control				
Run #1	632949	42680	1.54E+09	14.83011	Run #1	941869	64947	2.82E+09	14.50212	Run #1	841254	78857	3.36E+09	10.6681
Run # 2	1240220	76747	3.09E+09	16.15985	Run # 2	849861	59008	2.49E+09	14.40247	Run # 2	804346	75454	3.15E+09	10.66008
Run # 3	48136	Too small	97206976	Too Small	Run # 3	816939	56760	2.40E+09	14.39286	Run # 3	923508	86650	3.66E+09	10.65791
Average	936584.5	59713.5	2.31E+09	15.49498	Average	869556.3	60238.33	2.57E+09	14.43248	Average	856369.3	80320.33	3.39E+09	10.66203
Stdev	429405.4	24089.01	1.1E+09	0.94027	Std Dev	64751.88	4229.897	2.23E+08	0.060495	Std Dev	61002.05	5739.653	2.59E+08	0.005364
% Error	45.84802	40.34097	47.32782	6.068222	% Error	7.446542	7.021936	8.683532	0.419157	% Error	7.123335	7.145952	7.629374	0.050307
Inoc. A					Inoc. A					Inoc. A				
Run # 1	560119	45633	1.29E+09	12.27443	Run # 1	134189	Too small	1.84E+08		Run # 1	153755	10953	4.21E+08	14.03771
Run # 2	892050	52368	1.99E+09	17.03426	Run # 2	98130	Too small	1.39E+08		Run # 2	524233	42964	1.56E+09	12.20168
Run # 3	519839	31225	1.01E+09	16.64817	Run # 3	690250	42573	1.78E+09	16.21333	Run # 3	248073	19128	7.3E+08	12.9691
										Run # 4	173740	13509	4.79E+08	12.86106
Average	657336	43075.33	1.43E+09	15.31895	Average	690250	42573	1.78E+09	16.2133					
Stdev	204263.6	10801.06	5.03E+08	2.643691	Std Dev					Average	274950.3	21638.5	7.98E+08	13.01739
% Error	31.07446	25.07481	35.23728	17.25765	% Error					Std Dev	171071.2	14621.31	5.26E+08	0.760088
										% Error	62.21897	67.57082	65.91267	5.839019
										Inoc. B				
										Run # 1	732344	54047	2.28E+09	13.55013
										Run # 2	956115	71448	3.03E+09	13.38197
										Run #3	368264	29329	1.13E+09	12.55631
										Average	685574.3	51608	2.15E+09	13.1628
										Std Dev	296703.1	21165.16	9.52E+08	0.531927
										% Error	43.27804	41.0114	44.38808	4.041137

Table G.3. 10 mg/mL DBCP, Series # 1 Raw Data

10 ppm DBCP														
	Time = 0, 7/9/94					Time = 1 Week, 7/16/94					Time =3 Weeks, 8/3/94			
	Area			Ratios		Area			Ratios		Area			
	DBCP	C5-Imp	Pentane	DBCP/C5-I		DBCP	C5-Imp	Pentane	DBCP/C5-I		DBCP	C5-Imp	Pentane	DBCP/C5-I
Control					Control					Control				
Run #1	154477	89193	3.35E+09	1.7319408	Run #1	38175	Too small	30644640		Run #1	81133	93381	3.66E+09	0.8688384
Run # 2	154802	83162	3.03E+09	1.8614511	Run # 2	107684	85789	3.47E+09	1.2552192	Run # 2	93848	89112	12616890	1.0531466
					Run # 3	93862	73179	3.02E+09	1.2826357	Run # 3	106832	93427	3.70E+09	1.143481
Average	154639.5	86177.5	3.19E+09	1.796696	Average	100773	79484	3.24E+09	1.2689275	Average	93982.5	93404	3.68E+09	1.021822
Std Dev	229.8097	4264.561	2.28E+08	0.0915776	Std Dev	9773.63	8916.617	3.17E+08	0.0193864	Std Dev	18171.94	32.52691	31819805	0.1399752
% Error	0.14861	4.948578	7.157185	5.0970008	% Error	9.698659	11.21813	9.788529	1.5277794	% Error	19.33545	0.034824	0.864551	13.698591
Innoc. A					Innoc. A					Innoc. A				
Run # 1	131670	43804	1.44E+09	3.0058899	Run # 1	9745	Too small	3.13E+08		Run # 1	77536	75221	3.04E+09	1.030776
Run # 2	168191	42073	1.11E+09	3.9975994	Run # 2	82588	66858	2.73E+09	1.2352748	Run # 2	65255	56388	2.20E+09	1.1572498
					Run # 3	82617	63603	2.62E+09	1.2989482	Run # 3	94846	82955	3.24E+09	1.1433428
Average	149930.5	42938.5	1.28E+09	3.5017446										
Stdev	25824.25	1224.002	2.31E+08	0.7012445	Average	82602.5	65230.5	2.67E+09	1.2671115	Average	79212.33	71521.33	2.83E+09	1.1104562
% Error	17.22415	2.850593	18.07975	20.025576	Std Dev	20.5061	2301.633	73539105	0.0450239	Std Dev	14866.55	13664.44	5.51E+08	0.0693545
					% Error	0.024825	3.528461	2.751182	3.5532702	% Error	18.76798	19.10541	19.50388	6.2455894
					Innoc. B					Innoc. B				
					Run # 1	40371	31760	1.25E+09	1.2711272	Run # 1	61055	96284	3.68E+09	0.6341137
					Run # 2	85871	62786	2.65E+09	1.3676775	Run # 2	36081	35938	1.45E+09	1.0039791
					Run #3	72760	54082	2.26E+09	1.3453644	Run #3	83602	95275	3.68E+09	0.877481
					Average	66334	49542.67	2.05E+09	1.3280564	Average	72328.5	95779.5	3.68E+09	0.7557973
					Std Dev	23420.77	16003.35	7.18E+08	0.0505487	Std Dev	15943.14	713.4707	2121320	0.1720867
					% Error	35.30734	32.30216	35.01084	3.8062134	% Error	22.04268	0.74491	0.057637	22.768892

Table G.4. 100 mg/mL DBCP. Series # 1 Raw Data

100 ppm DBCP Series # 1

	Time = 0, 7/08/94					Time = 1 Week, 7/15/94					Time =3 Weeks, 8/3/94			
		Area		Ratio					Ratios				Area	
	DBCP	C5-Imp	Pentane	DBCP/C5-I		DBCP	C5-Imp	Pentane	DBCP/C5-I		DBCP	C5-Imp	Pentane	DBCP/C5-
Control					Control					Control				
Run #1	881734	74867	2.89E+09	11.777338	Run #1	1080626	87000	3.51E+09	12.420989	Run #1	515251	98223	3.73E+09	5.245727
Run # 2	689699	63709	2.11E+09	10.82577	Run # 2	1092304	87234	3.59E+09	12.52154	Run # 2	733650	96058	3.69E+09	7.637573
					Run # 3	1051132	84632	3.48E+09	12.42003	Run # 3	688458	94544	3.68E+09	7.281879
Average	785716.5	69288	2.5E+09	11.301554	Average	1074687	86288.67	3.52E+09	12.454186	Average	711054	95301	3.68E+09	7.459726
Std Dev	135789.3	7889.897	5.49E+08	0.6728603	Std Dev	21218.72	1439.478	57419509	0.0583319	Std Dev	31955.57	1070.56	9192388	0.251513
% Error	17.28222	11.38711	21.94859	5.9536971	% Error	1.974409	1.668212	1.629384	0.4683718	% Error	4.494113	1.123346	0.249488	3.371618
Innoc. A					Innoc. A					Innoc A				
Run # 1	49490	Too small	1.18E+08	N/A	Run # 2	46683	N/D	1.61E+08	N/D	Run # 1	636277	60933	2.45E+09	10.44224
Run # 2	876460	51154	2.00E+09	17.133753	Run # 3	10982	N/D	26616416	N/D	Run # 2	556980	55910	2.09E+09	9.962082
Run # 3	75327	Too small	1.33E+08	N/A	Run # 4	286092	34296	1.07E+09	8.3418474	Run # 3	648048	62560	2.43E+09	10.35882
Average		51154	2E+09	17.133753	Average	166387.5	34296	6.17E+08	8.3418474	Average	613768.3	59801	2.32E+09	10.25438
Std Dev		N/A	N/A	N/A	Std Dev	169287.7	N/A	6.44E+08	N/A	Std Dev	49531.05	3466.51	2.03E+08	0.256552
% Error		N/A	N/A	N/A	% Error	101.7431	N/A	104.4427	N/A	% Error	8.069992	5.796743	8.72155	2.501878
					Innoc. B					Innoc B				
					Run #1	766668	63453	2.63E+09	12.082455	Run # 1	674308	76523	2.98E+09	8.811834
					Run# 3	757984	61914	2.62E+09	12.24253	Run # 2	321601	33099	1.30E+09	9.716336
					Run # 4	273922	26623	8.32E+08	10.288923	Run # 3	423742	44701	1.64E+09	9.479475
					Average	762326	62683.5	2.62E+09	12.162492	Average	473217	51441	1.97E+09	9.335882
					Std Dev	6140.515	1088.237	6222540	0.1131903	Std Dev	181483.9	22482.92	8.88E+08	0.469036
					% Error	0.805497	1.736083	0.237266	0.9306504	% Error	38.35108	43.70622	45.00339	5.024016

Table G.5. 250 µg/mL Lindane Raw Data

250 PPB Lindane

	Time = 0, 7/27/94					Time = 1 Week,8/4/94					Time =3 Weeks			
			Area				Area					Area		
	Lindane	Hexadec	Benzene	Lind/C16		Lindane	Hexadec	Benzene	Lind/C16		Lindane	Hexadec	Benzene	Lind/C16
Control					**Control**					**Control**				
Run #1	165722	996763	5.34E+09	0.16626	Run #1	102537	914748	1.83E+09	0.112093	Run #1	226742	754002	5.11E+09	0.300718
Run # 2	168873	596281	2.45E+09	0.28321	Run # 2	158320	938706	5.25E+09	0.168658	Run # 2	238158	763981		0.311733
Run # 3	167042	710415	5.54E+09	0.235133	Run # 3	163290	976601	5.39E+09	0.167202	Run # 3	234190	761584		0.307504
										Run # 4	237461	754758		0.314619
Average	166382	853589	5.44E+09	0.200697	Average	160805	957653.5	5.32E+09	0.16793					
Std Dev	933.381	202478.6	1.39E+08	0.0487	Std Dev	3514.321	26795.81	96166522	0.001029	Average	234137.8	758581.3		0.308643
% Error	0.560987	23.72086	2.562311	24.2657	% Error	2.185455	2.79807	1.807981	0.612802	Std Dev	5225.14	4958.514		0.006038
										% error	2.231652	0.653656		1.956188
Innoc. A					**Innoc. A**									
										Innoc. A				
Run # 1	48728	157254	1.28E+09	0.309868	Run # 1	161103	1018423	2.24E+09	0.158189					
Run # 2	106111	338666	3.17E+09	0.31332	Run # 2	120259	961478	1.68E+09	0.125077	Run # 1	72343	325759	2.50E+09	0.222075
Run # 3	60956	239907	1.92E+09	0.254082	Run # 3	153016	988263	5.21E+09	0.154833	Run # 2	177585	748442	4.98E+09	0.237273
					Run # 4	169146	1023727	5.21E+09	0.165226	Run # 3	181321	712767	1.66E+09	0.25439
Average	71931.67	245275.7	2.12E+09	0.292423						Run # 4	196194	729041	1.71E+09	0.269112
Std Dev	30225.01	90825.08	9.63E+08	0.03325	Average	161088.3	1010138	5.21E+09	0.159416					
% Error	42.01906	37.0298	45.36868	11.3704	Std Dev	8065.01	19128.75	2908245	0.005304	Average	185033.3	730083.3	2.78E+09	0.253592
					% Error	5.006576	1.893677	0.0558	3.327007	Std Dev	9844.277	17860.33	1.9E+09	0.015935
										% Error	5.320272	2.446341	68.31117	6.283632
					Innoc. B					**Innoc. B**				
					Run # 1	135355	1004256	2.63E+09	0.134781	Run # 1	141195	716446	1.67E+09	0.197077
					Run # 2	130704	1017936	5.49E+09	0.128401	Run # 2	133233	667376	4.61E+09	0.199637
					Run #3	142655	991673	5.32E+09	0.143853	Run # 3	158913	727137	1.76E+09	0.218546
										Run # 4	91163	417200	3.20E+09	0.218512
					Average	136679.5	1004805	5.41E+09	0.135678					
					Std Dev	8450.633	18570.75	1.18E+08	0.007765	Average	131126	632039.8	3.90E+09	0.208443
					% Error	6.18281	1.848195	2.177661	5.723011	Std Dev	28722.71	145570.9	9.91E+08	0.011693
										% Error	21.90467	23.03192	25.39354	5.609695

Table G.6. 500 µg/mL Lindane Raw Data

500 PPB Lindane			scaling factor, inj		2.160058									
			Scaling factor, %		1.928735									
			Time = 0, 7/26/94				Time = 1 Week, 8/4/94				Time =3 Weeks			
			Area				Area					Area		
	Lindane	Hexadec	Benzene	Total	Lind/C16		Lindane	Hexadec	Benzene	Lind/C16		Lindane	Hexadec	Benzene	Lind/C16
Control						Control					Control				
Run #1	264606	568357	5.37E+09	5.37E+09	0.465563	Run #1	224635	1047232	5.37E+09	0.214504	Run #1	408724	727438	5.06E+09	0.561868
Run # 2	221395	590641	5.82E+09	5.84E+09	0.374839	Run # 2	220855	978400	5.31E+09	0.225731	Run # 2	387822	680131	4.71E+09	0.570217
Run # 3	312016	673621	5.45E+09	5.46E+09	0.463192	Run # 3	247239	992439	5.41E+09	0.249123	Run # 3	437880	753537	5.13E+09	0.5811
											Run # 4	442886	762043	5.11E+09	0.581182
						Average	230909.7	1006024	5.37E+09	0.229786					
Average	266005.7	610873	5.55E+09	5.56E+09	0.434531	Std Dev	18656.31	9927.072	73044130	0.016541	Average	419328	730787.3	5E+09	0.573592
Std Dev	45326.71	55471.86	2.39E+08	2.5E+08	0.051709	% error	8.079482	0.986763	1.361434	7.19824	Std Dev	25847.17	36840.86	1.95E+08	0.00936
%Error	17.03975	9.080752	4.313573	4.506746	11.89995						% error	6.163951	5.041256	3.895828	1.631819
						Innoc. A									
Innoc. A											Innoc. A				
						Run # 1	100393	582977	3.13E+09	0.172207					
Run # 1	34871	219314	2.21E+08	2.23E+08	0.159	Run # 2	217363	873536	4.71E+09	0.248831	Run # 1	289848	619643	4.32E+09	0.467766
Run # 2	36192	459443	4.42E+08	4.49E+08	0.078774	Run # 3	261365	975051	5.25E+09	0.268053	Run # 2	358838	761285		0.471358
Run # 3	25842	271404	3.23E+08	3.29E+08	0.095216						Run # 3	334151	711083	1.93E+09	0.469918
						Average	239364	924293.5	4.98E+09	0.258442	Run # 4	365057	751294		0.485904
Average	31017	365423.5	3.83E+08	3.89E+08	0.127108	Std Dev	31114.11	71781.94	3.84E+08	0.013592					
Std Dev	7318.555	132963.7	84287128	84980093	0.042378	% Error	12.99866	7.766142	7.707698	5.259063	Average	336973.5	710826.3	3.13E+09	0.473737
% Error	23.5953	36.38618	22.01456	21.84354	33.33972						Std Dev	34134.63	64545.01	1.68E+09	0.008245
						Innoc. B					% Error	10.12977	9.08028	53.89851	1.740396
											Innoc. B				
						Run # 1	28573	481805	2.51E+09	0.059304					
						Run # 2	10382	175978	9.68E+08	0.058996	Run # 1	277625	748105	5.12E+09	0.371104
						Run # 3	23279	546654	2.96E+09	0.042585	Run # 2	197025	445226	3.34E+09	0.442528
											Run # 3	287235	705215	4.88E+09	0.407301
						Average	20744.67	401479	2.14E+09	0.053628					
						Std Dev	9356.562	197963.1	1.04E+09	0.009565	Average	282430	726660	5E+09	0.389203
						% Error	45.10346	49.30845	48.68024	17.83641	Std Dev	6795.296	30327.81	1.7E+08	0.025595
											% Error	2.406011	4.17359	3.405871	6.576299

Table G.7. 10 mg/mL PCP Raw Data

10 PPM PCP

			Time = 0, 7/27/94				Time = 1 Week, 8/4/94					Time =3 Weeks			
			Area	Ratios			Area		Ratios			Area			
	PCP	Hexadec	Benzene	PCP/C16		PCP	Hexadec	Benzene	PCP/C16			PCP	Hexadec	Benzene	PCP/C16
Control					Control					Control					
Run #1	19781	565640	5.62E+09	0.034971	Run #1	32638	9.95E+05	1.64E+09	0.032818	Run #1	13992	751652	1.59E+09	0.018615
Run # 2	9643	594406	5.71E+09	0.016223	Run # 2	19360	1.05E+06	5.46E+09	0.018417	Run # 2	10548	754333	5.22E+09	0.013983
Run # 3	10181	687238	5.84E+09	0.014814	Run # 3	7406	1.01E+06	1.99E+09	0.007351					
Run #4	9478	609005	5.57E+09	0.015563	Run #4					Average	12270	752992.5	5.22E+09	0.016299
										Std Dev	2435.276	1895.753		0.003275
Average	9767.333	630216.3	5.71E+09	0.015533	Average	19801.33	1017754	3.03E+09	0.019528	% Error	19.8474	0.251763		20.09414
Std Dev	367.6225	49918.79	1.35E+08	0.000705	Std Dev	12621.79	29678.88	2.11E+09	0.01277					
% Error	3.763796	7.920897	2.37407	4.536916	% Error	63.74211	2.916115	69.62293	65.39077	Innoc. A				
Innoc. A					Innoc. A					Run # 1	13336	710645		0.018766
										Run # 2	26722	719791		0.037125
Run # 1	55975	476782	4.54E+09	0.117402	Run # 1	8247	1.03E+06	2.20E+09	0.008042					
Run # 2	57713	189465	1.73E+09	0.30461	Run # 2	19157	8.98E+05	5.03E+09	0.021322	Average				0.027945
Run # 3	43617	325063	2.39E+09	0.13418	Run # 3	36314	7.26E+05	4.09E+09	0.050029	Std Dev				0.012982
Run # 4	25306	152762	1.03E+09	0.165656	Run # 4	46149	7.84E+05	4.10E+09	0.058884	% Error				46.45316
Average	49796	400922.5	3.47E+09	0.125791	Average	33873.33	802690.7	4.41E+09	0.043411					
Std Dev	8738.426	107281.5	1.52E+09	0.011864	Std Dev	13660.51	87858.43	5.38E+08	0.019636					
% Error	17.54845	26.75867	43.72332	9.431665	% Error	40.32823	10.94549	12.20148	45.23256	Innoc.B				
Extractant					Innoc.B					Run # 1	12898	738999		0.017453
Run # 240		1516182			Run # 1	43895	753650	3.93E+09	0.058243					
Run # 346		1447421			Run # 2	20251	996613	1.67E+09	0.02032	Average				0.017453
					Run # 3	42812	577173	3.33E+09	0.074175	Std Dev				
					Run # 4	21090	1039830	1.67E+09	0.020282	% Error				
					Average	32012	841816.5	2.65E+09	0.043255					
					Std Dev	13107.97	216780.1	1.16E+09	0.027292					
					% Error	40.94706	25.75147	43.78304	63.09438					

Table G. 8. 100 mg/mL PCP Raw Data

100 PPM PCP

	Time = 0, 7/27/94					Time = 1 Week, 8/4/94					Time =3 Weeks			
			Area				Area		Ratio		Area			
	PCP	Hexadec	Benzene	PCP/C16		PCP	Hexadec	Benzene	PCP/C16		PCP	Hexadec	Benzene	PCP/C16
Control					Control					Control				
Run #1	78047	109164	3.21E+08	0.714952	Run #1	4.65E+05	9.93E+05	1.80E+09	0.467881	Run #1	683026	746016		0.915565
Run # 2	980543	571134	5.46E+09	1.716835	Run # 2	9.40E+05	1.02E+06	2.24E+09	0.923686	Run # 2	682820	742663	2.14E+09	0.919421
Run # 3	807824	566897	5.68E+09	1.424993	Run # 3	1.29E+06	9.96E+05	2.03E+09	1.2975	Run # 3	815277	770672		1.057878
					Run # 4	5.58E+05	9.95E+05	2.62E+09	0.56121					
Average	894183.5	569015.5	5.57E+09	1.570914	Run # 5	6.47E+05	9.56E+05	2.63E+09	0.676525	Average	749048.5	756667.5	2.14E+09	0.98865
Std Dev	122130.8	2996.011	1.61E+08	0.206364						Std Dev	76414.69	15295.23		0.081074
% Error	13.65836	0.526525	2.89496	13.13656	Average	8.59E+05	9.91E+05	2.38E+09	8.65E-01	% Error	10.20157	2.021394		8.200511
					Std Dev	207059.3	43204.93	2.78E+08	0.174769					
					% Error	24.09525	4.359116	11.67127	20.21086	Innoc. A				
Innoc. A					Innoc. A					Run # 1	314738	424662	3.23E+09	0.741149
										Run # 2	553352	459354	3.36E+09	1.204631
Run # 1	431819	224765	2.01E+09	1.921202	Run # 1	863106	834335	4.37E+09	1.034484	Run # 3	462212	450749	3.38E+09	1.025431
Run # 2	174443	94015	7.69E+08	1.855481	Run # 2	500699	593927	3.49E+09	0.843031					
Run # 3	282026	184968	1.46E+09	1.524729	Run # 3	770632	996261	5.23E+09	0.773524	Average	507762	455051.5	3.37E+09	1.115031
Run # 4	320331	192614	1.55E+09	1.663072						Std Dev	64445.71	6084.654	8485281	0.126713
					Average	711479	808174.3	4.36E+09	0.88368	% Error	12.69161	1.337135	0.251864	11.36412
Average	302154.8	174090.5	1.45E+09	1.741121	Std Dev	188305.6	202438.7	8.74E+08	0.135145					
Std Dev	106236.6	56099.41	5.12E+08	0.18113	% Error	26.46679	25.0489	20.02916	15.29345					
% Error	35.15967	32.22428	35.40939	10.40309						Innoc.B				
					Innoc.B					Run # 1	428843	708213		0.605528
										Run # 2	505444	718858		0.703121
					Run # 1	513575	598438	3.19E+09	0.858192	Run # 3				
					Run # 2	473260	458036	2.59E+09	1.033238	Run # 4				
					Run # 3	461000	924984	4.99E+09	0.498387					0.654325
					Run # 4	613649	544261	3.07E+09	1.12749	Average				0.069008
										Std Dev				10.5465
					Average	515969.7	642427	3.55E+09	1.006307	% Error				
					Std Dev	69261.18	204064	1.05E+09	0.136654					
					% Error	13.4235	31.76454	29.57881	13.57974					

Table G.9. 100 mg/mL 1,4-BCB Raw Data

100 ppm 1,4-Bromochlorobutane														
		Time = 0					Time = 1					Time = 3		
		Area		Ratios			Area		Ratios			Area		Ratios
	1,3,5 TCB	1,4 BCB	Total	BCB/TCB		1,3,5 TCB	1,4 BCB	Total	BCB/TCB		1,3,5 TCB	1,4 BCB	Total	BCB/TCB
Control A					Control A					Control A				
Run #1	1582797	1481935	3.34E+09	0.936276	Run # 1	1356772	1127290	3.10E+09	0.830862	Run # 1	1166682	916732	2.64E+09	0.78576
Run # 2	1455162	1365228	3.10E+09	0.938197	Run # 2	1439478	1190151	1.58E+07	0.826793	Run # 2	1224577	966200	2.76E+09	0.789007
Run # 3	1389564	1292804	3.10E+09	0.930367	Run # 3	1484084	1243417	1.67E+07	0.837835	Run # 3	1304276	1014464	1.50E+07	0.777799
Average	1475841	1379989	3.18E+09	0.934946	Average	1426778	1186953	1.04E+09	0.83183	Average	1231845	965798.7	1.81E+09	0.784189
Std Dev	98262.22	95425.62	1.42E+08	0.004081	Std Dev	64599.18	58129.53	1.78E+09	0.005584	Std Dev	69084.33	48867.24	1.55E+09	0.005767
% Error	6.658049	6.914955	4.456469	0.436474	% Error	4.527626	4.897375	170.5046	0.67128	% Error	5.6082	5.059775	85.94716	0.735429
Control B					Control B					Control B				
Run #1	1164864	1064735	2.53E+09	0.914042	Run # 1	1631916	1420607	3.57E+09	0.870515	Run # 1	1123716	858878	2.57E+09	0.764319
Run # 2	1406744	1290352	3.00E+09	0.917261	Run # 2	1481845	1285072	3.22E+09	0.867211	Run # 2	1551185	1209236	1.50E+07	0.779556
Run # 3	1426176	1301656	3.06E+09	0.91269	Run # 3	1691201	1473403	3.65E+09	0.871217	Run # 3	1109438	845110	1.30E+07	0.761746
Average	1332595	1218914	2.86E+09	0.914664	Average	1601654	1393027	3.48E+09	0.869648	Average	1261446	971074.7	8.66E+08	0.768541
Std Dev	145583.6	133642.8	2.88E+08	0.002349	Std Dev	107908.9	97147.41	2.32E+08	0.002139	Std Dev	251022.6	206368.6	1.48E+09	0.009626
% Error	10.92482	10.96408	10.05176	0.256766	% Error	6.73734	6.973834	6.664655	0.245992	% Error	19.89958	21.25157	170.405	1.252536
Innoc A					Innoc A					Innoc A				
Run # 1	1416099	1334276	3.02E+09	0.942219	Run #1	1478256	1275863	3.31E+09	0.863087	Run #1	1563870	1247206	3.49E+09	0.797513
Run # 2	1260811	1189496	2.73E+09	0.943437	Run # 2	1490474	1322246	1.69E+07	0.887131	Run # 2	1653844	1305244	3.60E+09	0.789218
Run # 3	1368638	1288827	2.94E+09	0.941686	Run # 3	1475202	1322378	3.21E+09	0.896405	Run # 3	1604174	1274522	1.80E+07	0.794504
Average	1348516	1270866	2.9E+09	0.942447	Average	1481311	1306829	2.18E+09	0.882208	Average	1607296	1275657	2.37E+09	0.793745
Std Dev	79575.51	74042.23	1.52E+08	0.000898	Std Dev	8081.258	26817.42	1.87E+09	0.017196	Std Dev	45068.17	29035.65	2.04E+09	0.004199
% Error	5.900969	5.826122	5.248485	0.09525	% Error	0.545548	2.052099	85.96195	1.949211	% Error	2.803975	2.276133	85.97596	0.528994
Innoc. B					Innoc. B					Innoc. B				
Run # 1	1377412	1274616	1.57E+07	0.92537	Run # 1	1661258	1448062	3.77E+09	0.871666	Run # 1	1480651	1167095	1.49E+07	0.788231
Run # 2	1643587	1528806	1.65E+07	0.930164	Run # 2	1593859	1398460	1.62E+07	0.877405	Run # 2	1455293	1145728	3.10E+09	0.787283
					Run # 3	1424070	1216704	1.62E+07	0.854385	Run # 3	1454146	1147587	1.60E+07	0.789183
Average	1510500	1401711	16074500	0.927767										
Std Dev	188214.1	179739.5	579120.5	0.00339	Average	1559729	1354409	1.27E+09	0.867819	Average	1463363	1153470	1.04E+09	0.788232
% Error	12.46039	12.82286	3.602728	0.36539	Std Dev	122221.8	121807.3	2.17E+09	0.011983	Std Dev	14982.54	11836.15	1.78E+09	0.00095
					% Error	7.836095	8.993394	170.991	1.380772	% Error	1.023843	1.026134	170.641	0.120485

Table G.10. 100 mg/mL DBCP, Series # 2 Raw Data

100 ppm DBCP			Series # 2													
			Time = 0				Time = 1								Time = 3	
		Area		Ratios					Ratios					Area		Ratios
	1,3,5 TCB	DBCP	Total	DBCP/TCB		1,3,5 TCB	DBCP	Total	DBCP/TCB		1,3,5 TCB	DBCP	C5-Imp	Total	DBCP/TCB
Control A					Control A					Control A					
Run #1	1410259	882701	3.08E+09	0.6259141	Run #1	1499699	941281	3.25E+09	0.6276466	Run #1	1515941	925063	116580	1.51E+07	0.6102236
Run # 2	1214324	767931	2.66E+09	0.6323938	Run # 2	1545009	973562	3.33E+09	0.6301335	Run # 2	1482685	928305	112719	3.19E+09	0.6260972
Run # 3	1386349	892109	2.99E+09	0.6434953	Run # 3	1526798	967938	3.27E+09	0.633966	Run # 3	1628398	1021592	123228	1.69E+07	0.6273601
Average	1336977	847580.3	2.91E+09	0.6339344	Average	1523835	960927	3.28E+09	0.630582	Average	1542341	958320	117509		0.621227
Std Dev	106891.5	69138.56	2.22E+08	0.0088912	Std Dev	22799.83	17244.75	46169290	0.0031835	Std Dev	76359.69	54819.13	5315.736		0.0095501
% Error	7.995015	8.157168	7.632509	1.4025494	% Error	1.496213	1.794595	1.406671	0.5048463	% Error	4.950895	5.720337	4.523684		1.5372979
Control B					Control B					Control B					
Run #1	1134699	690921	2.44E+09	0.6089024	Run #1	1629069	1028699	3.44E+09	0.6314644	Run #1	1629087	1041978	123114	1.61E+07	0.6396086
Run # 2	1450795	902639	3.11E+09	0.6221685	Run # 2	1550096	981914	3.31E+09	0.6334537	Run # 2	1480132	950477	113390	3.28E+09	0.6421569
Run # 3	1698618	1070863	1.61E+07	0.6304319	Run # 3	1461747	926108	1.61E+07	0.6335624	Run # 3	1311395	838290	100279	1.40E+07	0.6392353
Average	1428037	888141		0.6263002	Average	1546971	978907	2.25E+09	0.6328268	Average	1473538	943581.7	112261		0.6403336
Std Dev	282647.5	190385.5		0.0058431	Std Dev	83704.77	51361.56	1.94E+09	0.0011812	Std Dev	158948.6	102018.9	11459.29		0.00159
% Error	19.79272	21.4364		0.9329539	% Error	5.410883	5.246827	86.02973	0.1866524	% Error	10.78687	10.81188	10.20772		0.2483124
Innoc A					Innoc A					Innoc A					
Run # 1	1285213	816988	2.82E+09	0.635683	Run # 1	1392508	888102	1.62E+07	0.6377716	Run # 1	1421265	904479	108484	3.18E+09	0.6363901
Run # 2	1257586	800052	2.82E+09	0.6361807	Run # 2	1503831	958177	1.47E+07	0.6371574	Run # 2	1561889	995998	120480	1.65E+07	0.6376881
Run # 3	1442519	924825	1.64E+07	0.6411181	Run # 3	1424846	904799	1.64E+07	0.6350153	Run # 3	1546627	990219	119256	1.76E+07	0.6402442
Run # 4	1301417	823724	1.50E+07	0.6329439											
					Average	1440395	917026	15755667	0.6366481	Average	1509927	963565.3	116073.3		0.6381075
Average	1321684	841397.3	1.42E+09	0.6364814	Std Dev	57267.19	36602.61	921627	0.001447	Std Dev	77161.81	51251.78	6600.987		0.001961
Std Dev	82564.19	56503.02	1.62E+09	0.003403	% Error	3.975798	3.991447	5.849495	0.227283	% Error	5.110301	5.318973	5.686911		0.3073121
% Error	6.246894	6.71538	114.1928	0.5346508											
					Innoc. B					Innoc. B					
Innoc. B					Run # 1	1320072	825627	1.49E+07	0.6254409	Run # 1	1201658	765376	92506	2.67E+09	0.6369333
Run # 1	1333864	859861	2.93E+09	0.6446392	Run # 2	1300224	807322	1.46E+07	0.6209099	Run # 2	1366271	861506	105697	3.06E+09	0.6305528
Run # 2	1133712	724883	2.49E+09	0.639389	Run # 3	1584039	1002120	3.42E+09	0.6326359	Run # 3	1464634	934329	112676	1.60E+07	0.6379266
Run # 3	1169144	753508	2.53E+09	0.6444955											
					Average	1401445	878356.3	1.15E+09	0.6263289	Average	1344188	853737	103626.3		0.6351376
Average	1212240	779417.3	2.65E+09	0.6428412	Std Dev	158442.1	107572.5	1.97E+09	0.0059132	Std Dev	132871.6	84744.01	10243.19		0.0040015
Std Dev	106809	71121.27	2.43E+08	0.0029906	% Error	11.30563	12.24703	170.9828	0.9441087	% Error	9.884896	9.926243	9.884738		0.6300164
% Error	8.810876	9.124928	9.160699	0.4652097											