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BENCH-SCALE TESTING OF PHOTOLYSIS, CHEMICAL OXIDATION AND BIODEGRADATION OF PCB CONTAMINATED SOILS AND PHOTOLYSIS OF TCDD CONTAMINATED SOILS.

by

IT Corporation
Knoxville, Tennessee 37923

Cooperative Agreement No. CR816817-020-0

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FOREWORD

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. As the enforcer of national environmental laws, the EPA strives to balance human activities and the ability of natural systems to support and nurture life. A key part of the EPA's effort is its research into our environmental problems to find new and innovative solutions.

The Risk Reduction Engineering Laboratory (RREL) is responsible for planning, implementing, and managing research, development, and demonstration programs to provide an authoritative, defensible engineering basis in support of the policies, programs, and regulations of the EPA with respect to drinking water, wastewater, pesticides, toxic substances, solid and hazardous wastes, and superfund-related activities. This Publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

Now in its sixth year, the Superfund Innovative Technology Evaluation (SITE) Program is part of EPA's research into cleanup methods for hazardous waste sites around the nation. Through cooperative agreements with developers, alternate or innovative technologies are refined at the bench- and pilot-scale level and then demonstrated at actual sites. EPA collects and evaluates extensive performance data on each technology to use in remediation decision-making for hazardous waste sites.

This report documents the results of bench-scale testing of UV photolysis, chemical oxidation and biological treatment on soils contaminated with toxic compounds.

E. Timothy Oppelt, Director
Risk Reduction Engineering Laboratory

ABSTRACT

This report presents the results of bench-scale testing on degradation of 2,3,7,8-TCDD using W photolysis, and PCB degradation using UV photolysis, chemical oxidation and biological treatment. Bench-scale tests were conducted to investigate the feasibility of a two-phase detoxification process that would have application to the treatment of soils contaminated with polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The first step in the process was to degrade the contaminants by using ultraviolet *(UV)* radiation facilitated by the addition of a surfactant to mobilize the contaminants. As an alternative, an advanced oxidation process using iron (Fe) catalyzed hydrogen peroxide (Fenton's Reagent) was also tested. Biological degradation, the second step, was then used to further degrade organic contaminants and detoxify the soil.

UV photolysis tests were conducted independently using a medium pressure mercury (Hg) lamp, a 10 hertz (Hz) pulsed Hg lamp and sunlight. Results from *UV* testing on a TCDD soil (200-300 parts per billion) indicated that there was no apparent destruction of the dioxin on the soil. Surface soil contaminated with about 10,000 parts per million (ppm) PCBs and a pit soil containing about 200 ppm PCBs were tested under similar conditions. The PCB reductions spanned the range up to a maximum of 69 percent. Batch experiments using the Fenton's Reagent alternative to degrade PCBs gave similar results with reaction times of over 100 hours.

Biological treatment on surfactant/UV-treated and untreated soil was evaluated in two bioslurry treatment experiments. The bioslurry experiments evaluated PCB degradation on surfactant/UV-treated and untreated soils using cultures, with and without PCB degradation inducer chemical addition. The inducers used were biphenyl and 4-bromobiphenyl. Bioslurry treatment did not provide significantly different results for the UV-treated surface soil versus the untreated soil. Percent reductions of PCBs were highest for an untreated soil containing 350 ppm PCBs which gave 70, 20 and 30 percent reduction of the di, tri and tetra-PCBs, respectively. In the enhanced bioslurry experiment using inducers, the addition of 1,000 ppm biphenyl stimulated greater reduction in PCB concentrations on the same soil.

This report is submitted in fulfillment of cooperative agreement number CR816817-02-0 by IT Corporation under partial sponsorship of the USEPA. This report covers the period from September 1990 to July 1993, with the completion of work in July 1993.

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IT Corporation would also like to acknowledge the University of Tennessee Center for Environmental Biotechnology (CEB) and General Electric Company (GE) for their contribution to this project. Both institutions lent valuable support in obtaining and identifying bacterial cultures for the biological treatment of PCBs.

SECTION 1

EXECUTIVE SUMMARY

The tests reported herein were conducted to investigate the feasibility of a two-phase detoxification process that would have application to the treatment of soils contaminated with polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The first step in the conceived process was to degrade or chemically alter the organic contaminants by using ultraviolet (UV) radiation. The source of UV radiation may be either artificial UV light or natural sunlight, but generally photolytic processes are more rapid with artificial UV light. Alternatively, advanced oxidation processes, such as iron catalyzed hydrogen peroxide (Fenton's Reagent), may be used to initiate contaminant degradation. Both photolysis and chemical oxidation were expected to be facilitated by the application of a surfactant solution to the soil to mobilize the contaminants and provide a medium for degradation reactions. These reactions were expected to convert the contaminants to more easily biodegradable compounds. Biological degradation, the second step in the process, would then be used to further oxidize organic contaminants and detoxify the soil. Biodegradation is typically enhanced by the addition of microorganisms and nutrients to the soil and may be further enhanced by the addition of biodegradation inducers, such as biphenyl or 4-bromobiphenyl.

This report presents the results of bench-scale testing on degradation of 2,3,7,8-TCDD using UV photolysis, and PCB degradation using both UV photolysis and chemical oxidation. Biological treatment was also performed on both untreated and post UV photolyzed PCB contaminated soils.

UV PHOTOLYSIS PERFORMANCE

UV photolysis testing was performed on three soils; one containing 2,3,7,8-TCDD contamination and two containing PCB contamination. The tests were conducted independently using a medium pressure mercury (Hg) lamp, a 10 hertz (Hz) pulsed Hg lamp and sunlight, employing different surfactants and surfactant application procedures.

Testing was performed with a cornposited TCDD soil from the Vertac site in Jacksonville, Arkansas using two surfactant levels, 2.5 percent and 5 percent by weight of the dry soil. TCDD concentrations on the soil ranged from about 200 to 300 parts per billion. The soil was mixed and sprayed at l/2 hour intervals with either surfactant solution or water to a total irradiation time of 48 hours. Results from these tests indicated that there was no apparent destruction of the dioxin on the soil in any of the tests.

Surface soil from a Texas Eastern Gas Pipeline site in Danville, Kentucky contaminated with about 10,000 parts per million (ppm) PCBs (Aroclor 1248) and a pit soil from the same site containing 150 ppm PCBs were tested. Testing conditions differed from that above by using different surfactants, application procedures, soil mixing intervals and lamp to soil distances. The test results showed minimal reduction of PCBs, ranging from none detected to a maximum of 69 percent. In two tests, in which soil temperatures were elevated to over 100°C, loss of 32 to 44 percent of the PCBs due to volatilization was observed. Typically, in tests in which soil temperatures were limited to 50°C or less, reductions of soil PCBs were in the range of 15 to 35 percent. Best results were obtained using a 2-3 percent surfactant spray loading on soil ground to particle sizes less than 63 microns with a minimum bed depth (l/4 inch) and lamp to soil distance (4 inches). PCB reductions in these tests ranged from 23 to 69 percent with 6 hours or longer of *UV* exposure. Decreases in concentration at temperatures of 50°C or less occurred for tri- through hepta-PCB homologs while the di-PCB congener group (homolog) displayed an increase in concentration because of di-PCB by-product generation. Generation of some specific Tri- and tetra-PCB by-products was also detected. These results indicated degradation of higher chlorinated PCBs to lower chlorinated di-, tri- and tetra-PCBs.

CHEMICAL, OXIDATION PERFORMANCE

Five batch experiments using Fenton's Reagent (H_2O_2/Fe) were performed at ambient temperature. All five used the same surface soil used in the *UV* photolysis testing. This soil provided samples for treatment which ranged from 6,000-10,000 ppm PCBs (Aroclor 1248). Conditions were established to provide the best opportunity for observing an effect due to treatment. Each experimental mixture was pH adjusted to between 2 and 4 and continuously stirred. Hydrogen peroxide concentration was monitored throughout the experiments as loss, primarily through decomposition, was continuous. Additions of hydrogen peroxide were made as necessary to maintain a concentration (1 to 2 percent). Reagent to soil ratios were high, usually 8:l to lO:l, and iron concentrations were varied between experiments, up to 2.5 percent of the soil, to investigate the effect.

Results from these tests showed minimal reduction of PCBs on the highly-contaminated surface soil tested. The PCB concentration reductions ranged from none detected to a maximum of 54 percent in reaction times of well over 100 hours. Highest reductions were observed with higher iron to soil ratios along with higher concentrations of hydrogen peroxide. Where reductions in concentration were noted, the loss of PCBs were observed more from the lower chlorinated congeners, di and tri-PCBs, and trended less, progressing through the higher chlorinated congeners, tetra through Observed

reduction in PCB concentrations are suspected to have been primarily due to volatilization from solution by gas purging. Oxygen was continually generated in solution from hydrogen peroxide decomposition.

BIOLOGICAL TREATMENT PERFORMANCE

Bioslurry experiments evaluated the biological reduction of PCB congeners in surfactant/UV-treated and untreated soils. Experiments were also conducted to evaluate the impact of PCB-biodegradation inducers: biphenyl and 4-bromobiphenyl, on congener removal.

The bioslurry experiments were conducted under aerobic conditions at 25°C using PCB-degrading organisms from two sources. PCB-degrading organisms were isolated from an impacted New England Superfund Site soil. In addition, known-PCB degrading microorganisms were obtained from General Electric Company (GE). Soils employed were untreated surface soil from the *UV* photolysis testing, surfactant/UV-treated surface soil, and New England Superfund Site soil. In separate tests, each soil was treated with bacterial cultures.

Bioslurry treatment did not provide significantly different results for the *UV* treated surface soil versus the untreated soil. This was not surprising since *UV* treatment was not successful in significantly degrading the higher chlorine level PCB congeners. Percent reductions of PCBs were highest for an untreated New England Superfund Site soil which had a significantly lower concentration of PCB contamination than either the *UV* treated or untreated PCB surface soil from Danville, Kentucky. The culture isolated from the New England soil gave 70, 20 and 30 percent reduction of the di, tri and tetra-PCBs, respectively in the New England soil. PCB reductions lessened with increasing level of chlorination with no significant reduction noted for penta, hexa or hepta-PCBs. Similar results were obtained with inducer additions to the soils. Biphenyl addition gave even greater reduction in PCB concentrations for the New England site soil with reductions of 82, 54, 63 and 16 percent for di, tri, tetra and penta-PCBs, respectively.

LIST ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

2,3-dhb	--	2,3_dihydroxybiphenyl
BAC	--	Biotechnology Application Center
cc	--	cubic centimeter
CEB	--	Center for Environmental Biotechnology
CFU/mL	--	colony-forming units per milliliter
CFU/g	--	colony-forming units per gram
cm	--	centimeter
DCMA	--	Dry Color Manufacturers' Association
DOC	--	dissolved organic carbon
DOT	--	Department of Transportation
ECD	--	electron capture detector
EPA	--	Environmental Protection Agency
g	--	gram
GC/ECD	--	gas chromatography with electron capture detection
GC	--	Gas Chromatograph
GC/FID	--	gas chromatography with flame ionization detection
GE	--	General Electric Company
hr	--	hour
HZ	--	hertz
IR	--	infrared
IT	--	IT Corporation
KD	--	Kuderna Danish
L	--	liter
µL	--	microliter
mg O_2/kg-hr	--	milligram oxygen per kilogram-hour
mg	--	milligram
mg/kg	--	milligram per kilogram
mg/L	--	milligram per liter
mL/min	--	milliliters per minute
mL	--	milliliter
N	--	Normal
ng	--	nanogram
nm	--	nanometer
PCB	--	polychlorinated biphenyl
ppb	--	parts per billion
PPE	--	personal protective equipment
ppm	--	parts per million
QA	--	quality assurance
QC	--	quality control
RCRA	--	Resource Conservation and Recovery Act
RPD	--	relative percent difference
rpm	--	revolutions per minute
RREL	--	Risk Reduction Engineering Laboratory
RSD	--	relative standard deviation
SARA	--	Superfund Amendments and Reauthorization Act

SD	--	standard deviation
SITE	--	Superfund Innovative Technology Evaluation
TCDD	--	2,3,7,8-tetrachlorodibenzo-p-dioxin
TDL	--	Technology Development Laboratory
TSCA	--	Toxic Substances Control Act
uv	--	ultraviolet
v/v	--	volume to volume
°C/min	--	degrees Celsius per minute

SYMBOLS

Fe	--	iron
$FeSO_4$	--	iron (II) sulfate
H_2O_2	--	hydrogen peroxide
H_2O_2/Fe	--	Fenton's Reagent
H_2SO_4	--	sulfuric acid
HCl	--	hydrochloric acid
Hg	--	mercury
$KMnO_4$	--	potassium permanganate
NaOH	--	sodium hydroxide
$O_2/kg-hr$	--	oxygen per kilogram-hour
To	--	study initiation
T_2	--	study at 2 weeks
T_4	--	study at 4 weeks
T_{final}	--	study final

SECTION 2

INTRODUCTION

The Superfund Amendments and Reauthorization Act of 1986 (SARA) directed the Environmental Protection Agency (EPA) to establish an "Alternative or Innovative Treatment Technology Research and Demonstration Program." In response, the EPA's Office of Solid Waste and Emergency Response and the Office of Research and Development established a formal program called the Superfund Innovative Technology Evaluation (SITE) Program, to accelerate the development and use of innovative cleanup technologies at hazardous waste sites across the country.

The SITE program comprises the following five component programs:

- Demonstration Program
- Emerging Technologies Program
- Measurement and Monitoring Technologies Development Program
- Innovative Technologies Program
- Technology Transfer Program

This report is sponsored by the SITE Emerging Technologies Program. Before a technology can be accepted into the Emerging Technologies Program, sufficient data must be available to validate its basic concepts. The technology is then subjected to a combination of bench- and pilot-scale testing in an attempt to apply the concept under controlled conditions.

The tests reported herein were conducted to investigate the feasibility of a two-phase detoxification process that would have application to the treatment of soils contaminated with polychlorinated biphenyls (PCBs) and 2,3,7,8Tetrachlorodibenzo-p-dioxin (TCDD). The first step in the process was to partially degrade or chemically alter the organic contaminants by using ultraviolet (UV) radiation. Typically the rate of photolytic degradation is faster with artificial UV light than with natural sunlight, but both sources of UV radiation were proposed. As an alternative, an advanced oxidation process, iron catalyzed hydrogen peroxide (Fenton's Reagent), was investigated as a means to provide initial contaminant degradation. Both photolysis and chemical oxidation were expected to be facilitated by the addition of a surfactant solution to the soil to mobilize the contaminants and provide a medium for degradation reactions. Both processes, UV-photolysis and chemical oxidation, were expected to convert the contaminants to more easily biodegradable compounds. Biological degradation, the second step in the overall process, was then envisioned as a final step to further oxidize organic contaminants and detoxify the soil.

Biodegradation is typically enhanced by the addition of microorganisms and nutrients to the UV treated soil and can be further enhanced by the addition of biodegradation inducers.

This two-phase treatment was conceptualized as a potential in-situ process for shallow contamination on soils. More probable, however, was the use of the technology for ex-situ, on-site treatment of excavated soils in a specially constructed shallow treatment basin, which would meet the requirements of the Resource Conservation and Recovery Act (RCRA). The process may have required longer treatment times than other technologies, but was anticipated to have a trade off in economy. The only residue generated from this combination of technologies would be soil contaminated with surfactants and the end metabolites of the biodegradation processes. The end metabolites depend on the original contaminants. The surfactants are common materials used in agricultural formulations.

This report presents the results of bench-scale testing on degradation of 2,3,7,8-TCDD using *UV* photolysis, and PCB degradation using *UV* photolysis and chemical oxidation (Fenton's Reagent). Biochemical treatment testing was also performed on soil contaminated with PCBs both untreated and after **surfactant/UV** photolysis treatment. Soil contaminated with TCDD was not subjected to chemical oxidation or biodegradation testing.

Chemical oxidation was proposed as an alternate means to partially degrade or chemically alter PCB contaminants to more easily biodegradable products after tests showed little PCB degradation from *UV* photolysis treatment. Chemical oxidation testing using Fenton's Reagent was performed on the same PCB contaminated soil used in the *UV* photolysis tests to compare these two technologies.

The work presented in this report is divided into three parts based on the technology employed; *UV* photolysis, chemical oxidation and biological treatment.

Testing in this program involved TCDD soils regulated by the RCRA and PCB soils regulated by the Toxic Substances Control Act (TSCA). The TDL is authorized to perform treatability studies on RCRA hazardous wastes under the treatability exemptions of Tennessee Department of Environment and Conservation, Division of Solid Waste Management (TN Rule Chapter 1200-1-11-.02(1)(d) 6.). A TSCA bench scale permit for treatability testing of PCB contaminated soil was obtained from EPA Region IV Toxic Substances Control Branch on September 4, 1990.

SECTION 3

UV PHOTOLYSIS

INTRODUCTION

Earlier work performed by IT Corporation (IT) showed a practical rate of photolytic destruction of PCBs and TCDD (Exner, et. al., 1984) on soil when the soil surface was treated with a surfactant solution and irradiated by UV light. The reactions were aided by the presence of a surfactant, which ideally is transparent to the UV radiation in the region of activity (generally 254 nanometers) and which has increased solubility for the contaminants being destroyed. Conceptually, the irradiation process can be performed on excavated soils or in situ using enhanced radiation from lamps or natural sunlight. The process usually involves the continued application of the solubilizing aid (surfactant) and continued exposure of fresh surface to the irradiation source. The solubility aid helps to transfer the contaminant from the pores of the soil to the soil surface where the reactions can take place. The surfactant or solubilizing aid may also act as a medium for the degradation process by providing labile protons to allow the reaction to proceed more easily. Because the presence of UV light is usually accompanied by significant amounts of infrared (IR) radiation or heat, the solubilizing aid needs to be continually or periodically refreshed to provide a continued reaction medium.

The testing in this study was performed on three soils, one contaminated with 2,3,7,8-TCDD and two from a site contaminated with PCBs. The tests, conducted independently, used a medium pressure Hg lamp, a pulsed Hg UV lamp, and sunlight as the sources of W radiation. Different surfactants were tested and different surfactant application procedures were tried to establish the procedures that would allow the *UV* reaction to proceed. The objective of the tests were to preliminarily investigate the feasibility of the technology for application to soils contaminated with TCDD or PCBs. The treatment was monitored primarily by the disappearance of contaminant with qualitative notation of any by-product production.

EXPERIMENTAL PROCEDURES

Site Sampling

IT personnel traveled to the Vertac site in Jacksonville, Arkansas to obtain soil samples contaminated with TCDD. The soils from several areas within the site were sampled using a shovel. 5-gallon pails lined with plastic were filled and sealed (GG3866 and GG3867). The pails were then packaged in boxes and shipped to the Technology Development Laboratory (TDL)

located in Knoxville, Tennessee. Workers handling the unpackaged soils were outfitted with Level C personal protective equipment (PPE). This level includes a full plastic coated Tyvek suit, nitrile gloves with PVC under gloves, PVC boots and air purifying respirators.

To obtain PCB contaminated soil, IT personnel traveled to a Texas Eastern Gas Pipeline site in Armaugh, Pennsylvania. The soils were again packaged in 5-gallon pails lined with plastic bags. The samples were transported back to the TDL in a truck by IT personnel. These soils were found to be unsuitable for use as noted below. A second sampling trip to a Texas Eastern site in Danville, Kentucky by IT personnel was completed on April 1, 1991. The samples were returned to the laboratory on the same day. All shipping and transportation activities were in compliance with applicable Department of Transportation (DOT) regulations.

Sample Preparation

Samples containing TCDD (GG3866 and GG3867) were received at the TDL on December 21, 1990 and logged into the sample receiving system. The samples were held in Sample Receiving until January 8, 1991. They were opened and the contents spread into large aluminum baking pans to air dry. During the air drying, the soils were crushed and screened to less than l/8 inch (0.125 inch) particle size. The final weight of the dried and screened material was approximately 25 kilograms. Testing of these soils proceeded under IT's Treatability Exemption. At the conclusion of testing, the TCDD soil was packaged and returned to the site for disposal.

The first set of PCB contaminated soil samples (Armaugh, Pennsylvania) were received on February 8, 1991 and logged into the sample tracking system. The samples were air dried on February 14, 1991. The soil was a very sticky clay-type material and dried to a hard cake that dusted badly when it was crushed and sieved. The soil was also expected to become sticky and form lumps when the surfactant solution was added during the experiments. Based on this, IT decided that the soil was not suitable for testing and another sample site was chosen. This soil was packaged and returned to the site for disposal.

Samples from a second site (Danville, Kentucky) were received on April 1, 1991. These samples consisted of soils from four locations on site and were very different in nature, ranging from pure gravel to topsoil. The PCB concentration ranged from 100 parts per million (ppm) to greater than 10,000 ppm of Aroclor 1248 in the different samples. Two of the soils which had moderate PCB concentrations and were available in larger quantities were selected for testing. These two soils were identified as surface soil (GG4202), containing 1700 ppm PCBs

(Aroclor 1248) by analysis, and pit soil (GG4199), containing 150 ppm PCBs. They were processed by air drying and screening to less than l/8 inch (0.125 inch) particle size prior to UV testing.

Initial testing began using the 1,700 ppm surface soil which had a high humic content. In subsequent analyses the concentration of PCBs in the surface soil was found to be greater than 10,000 ppm instead of the expected 1,700 ppm. This deviation from the expected value may have been a result of the initial sampling or the preparation of the soil to exclude debris and stones.

For the fine ground soil testing (small particle size soil), the GG4202 surface soil was ground in a standard kitchen blender in a fume hood until the soil passed a 230 mesh sieve (particle size less than 63 microns).

Bench-Scale Testing

Testing with the cornposited TCDD soil began on February 21, 1991.. Analysis of the composite soil gave a 2,3,7,8-TCDD concentration of 271 parts per billion (ppb) (nanogram/gram). The initial tests used a 7-inch by 11-inch Pyrex baking dish filled to a 1 inch depth of soil. Two surfactant levels, 2.5 percent and 5 percent by weight surfactant as a percentage of the dry soil, were used in testing. The surfactant was applied 'by spraying approximately one half of the target concentration on the soil initially and then the remainder was applied during irradiation by periodic spraying and mixing steps. The surfactant solution was 8 percent of a 1:l mixture of nonionic surfactants: Hyonic NP-90® (Diamond Shamrock Corporation) and Adsee 799® (Witco Chemical Corporation), in deionized water. A more dilute solution (less than 2 percent) was used for periodic spraying during the tests. Hyonic NP-90® is a polyethoxylated nonyl phenol and Adsee 799® is a polyoxyalkyl fatty acid ester. It was found that when the surfactant level approached 3.5 percent of the soil, it became very sticky and lumped badly when it was stirred. In the higher surfactant concentration tests, the surfactant spraying to reach 5 percent loading was discontinued and the irradiation continued until the soil dried sufficiently to be worked. At this point, spraying with water only was continued to the end of the test.

Tests with a 450 Watt Hanovia medium pressure Hg lamp and a 10 Hz pulsed Hg lamp operating at 450 Watts total power were carried out with the lamps approximately 10 inches above the soil and a parabolic reflector above the lamp. The soils were mixed and sprayed at l/2 hour intervals with either surfactant solution or water to a total irradiation time of 48 hours. Most of the samples were sent to the IT laboratory in St. Louis for TCDD analysis by Region VII TCDD Rapid Turnaround method. One set of

duplicate samples was analyzed by the Dioxin Analysis Group at the TDL using SW-846 Method 8280.

Testing of PCB contaminated soils followed the same general procedure as described above for the TCDD soil. Lamp to soil distance, surfactant and surfactant application procedure, as well as soil mixing/overturning interval and soil particle size were all variables that were adjusted to optimize degradation of PCBs.

Bench-Scale Sunlight Testing

UV irradiation by sunlight of TCDD and PCB contaminated soil was performed during the months of July and August 1991. The surfactant mix used initially in the TCDD experiments was discontinued during the test after surfactant extraction tests showed Hyonic NP-90@ to be superior for PCB extraction. The soils were raked daily and sprayed at the beginning of the day with the surfactant solution. Subsequent sprayings during the day used water only to try to maintain a moist surface. The evaporation rate was very high and it was difficult to keep the soil moist with only one spraying. If the surface became dry, the extraction rate of the surfactant became negligible.

Three trays with cornposited PCB soil and three trays with cornposited TCDD soil were weighed out on June 25, 1991. The trays were 7 x 11 glass Pyrex oven baking dishes. Each tray contained approximately 1 kilogram of soil. One sample in the PCB set and one in the TCDD set were preloaded with 2.5 percent of a surfactant mixture using a 25 percent concentrated surfactant solution. Another sample in each set was preloaded to 1.2 percent surfactant concentration using 12.5 percent concentrate. The samples were loaded to a total of 8 to 10 percent moisture content. The third sample in each set was sprayed with water only. Triplicate aliquots were removed from each tray for starting analyses for Duplicates were removed from the TCDD samples for analysis.

The sprayed samples were positioned in a metal tray on the roof of the TDL building for sunlight exposure. During the evening and when raining, the tray was covered. For the first month of exposure the sample was weighed after it was removed from the sunlight and reweighed after spraying with surfactant solution. Surfactant was sprayed only for the first month when the total surfactant had reached 5 percent loading for the high loading and 2.2 percent for the lower loading test.

During testing the soil became very sticky and produced balls of material. The top surface caked as the moisture dried out during the daytime exposure. Samples were removed for analyses after 0, 40 and 197 hours of exposure. After 1 month the soil was broken up using a blender to totally remix the soil.

During the second month, testing continued using a solution of the Hyonic NP-90@ surfactant. The surface was sprayed with a dilute 0.2 percent solution twice per day. The soil was turned over using a stainless spatula before stirring.

The UV intensity at the surface of each tray was monitored for the first month along with a position just outside the tray. The readings ranged from a high of about 360 microwatts/cm* to below .04 microwatts/cm*. Temperature readings ranged from about 26°C at the start of the testing to a high of 41°C on July 2, 1991. Temperatures usually were in the low 30°C range during the month of July. The readings from the radiometer were also sent to a recorder for continuous monitoring. The readout peaks were about 345 microwatts/cm*. The exposure varied greatly during the day as the sun rose and moved in and out of clouds. Radiometer readings were made with a radiometer specific to 254 nanometers wavelength ultraviolet light.

MATERIALS AND METHODS

Eauinment

The irradiation of PCBs and/or TCDD contaminated soils was carried out using glass or incoloy metal trays and stainless implements (spatulas). The application of water or surfactant solution to the soil was done using a commercially available plastic spray bottle purchased at a local department store. These spray bottles are typically used for the application of aqueous solutions in the home (window spray, insecticide or fungicide solution spray). The spray nozzles were adjusted to give a fine mist when spraying to provide the best distribution possible. For the preliminary application, when the greatest amount of moisture was added, the soil was sprayed incrementally and mixed with a stainless spatula until the moisture was uniformly distributed.

The incoloy metal tray was approximately 3 inches by 6 inches by l/2 inch deep. The glass trays were approximately 7 by 11 by 1 l/2 inch Pyrex baking dishes purchased from a laboratory supply house and identical to the baking dishes available in local department stores. The mixing tools were stainless steel spatulas.

The soils were weighed on a 12-kilogram capacity, digital top-loading balance (Sartorius Model 1200LC) inside the laboratory for the determination of moisture weight addition. For the sunlight experiments on the roof, a lo-kilogram capacity, Ohaus, top-loading balance was used. The balance was housed inside a plastic cabinet with a hinged door to allow weighings. The trays containing contaminated soils for the sunlight experiments on the roof of the laboratory were positioned inside a secondary galvanized tray located on several concrete blocks.

A lid of plywood with a 2 x 4 inch drip edge was fabricated to position over the galvanized tray with the drip edge downwards during the periods when sunlight was not available (rain or evenings). The lid was secured to keep it from being blown off.

Chemical Reasents

The commercial surfactants used in the UV photolysis tests were the following:

Adsee 799® - Witco Chemical Corp., polyoxyalkylated fatty acid ester
Hyonic NP-90® - Diamond Shamrock Corp., polyethoxylated (9) nonylphenol

In addition, two other nonionic commercial surfactants were used in the surfactant extraction tests.

Brij 30® - Supplied by Aldrich Chemical Co., polyoxyethylene (4) lauryl ether
Brij 35® - Supplied by Aldrich Chemical Co., polyoxyethylene (23) lauryl ether

Analysis of 2,3.7.8-TCDD

Dioxin analyses were performed by two different laboratories using different analytical techniques. The samples sent to the IT St. Louis Laboratory were analyzed by USEPA Region VII Rapid Turnaround Method for TCDD. The dioxin levels contained in the soil samples being analyzed were much higher than normally analyzed by this technique and the soil was also somewhat heterogeneous. The extraction and spiking technique were modified after consultation with the laboratory to better suit the sample needs. Copies of the analytical reports are included in Appendix A.

Samples submitted for analysis at the TDL were extracted and analyzed using SW-846 Method 8280. The preliminary soil analysis to establish starting concentration was done at the TDL. One set of duplicate samples for one of the *UV* experiments was analyzed at the TDL for verification of the IT St. Louis Laboratory method. Agreement between the two laboratories was within reasonable expectations given the differences in methodology. Copies of the analytical reports are included in Appendix A.

Analysis of PCBs

The soils were extracted by sonication (SW-846 Method 3550) or Soxhlet extraction (SW-846 Method 3540) using a mixture of methylene chloride and acetone with subsequent solvent exchange to hexane. Samples were then analyzed by gas chromatography with electron capture detection (GC/ECD).

The analysis and quantification of PCBs was performed by one of two methods, EPA SW-846 Method 8080 or a PCB homolog procedure, which is a modified version of the Dry Color Manufacturers' Association (DCMA) PCB Method, June 1981. Untreated samples were analyzed and quantified for Aroclor 1248 or Aroclor 1260 using GC/ECD methods consistent with SW-846 Method 8080. Treated samples containing altered PCB patterns were analyzed by a GC/ECD, semi-specific PCB homolog method (DCMA). The DCMA method divides the PCB chromatographic elution window into semi-specific homolog windows. Individual peaks are quantified versus the appropriate homolog standard based on the homolog window in which it elutes. Homolog totals are obtained by summing the individual PCB peak amounts for each homolog window. The total PCB concentration is then calculated from the sum of the individual homolog totals. A copy of the laboratory standard operating procedure for this analysis is included in Appendix B. Analytical methodology for PCB analysis allows for a variability of a minimum of plus or minus 15 percent (plus or minus 25 percent for DCMA method). A statistical determination of the limit of significance for whether there was a difference between starting and final PCB concentrations on soils was not determined because it was beyond the scope of the preliminary work being performed. In addition to insufficient data, the use of different methodologies complicates the process of determining a limit of significance for the percent PCB reduction data based on the difference of starting and final PCB concentrations. A PCB reduction of less than 15 percent is clearly not considered significant based on the minimum variability allowed by the methodology. This, however, is not intended to signify that 15 percent is the limit of data significance and that any PCB reduction greater than 15 percent is necessarily statistically significant.

QUALITY ASSURANCE/QUALITY CONTROL

Because of the nature of the samples under investigation, many of the samples were taken in duplicate and often the samples were analyzed in duplicate to compensate for the variability within the sample matrix. The variability was a result of the particle size distribution with a significant quantity of small gravel-like material within the soil. This gravel material tends to hold a very low quantity of the contaminant under investigation. If an aliquot is removed which contains no stones, the analytical result will be disproportionately higher and the results will be biased. When a large enough aliquot is taken for the analysis, this bias is either removed or lessened but the ability to spike the sample at the high levels contained in the sample becomes impossible.

RESULTS AND DISCUSSION

TCDD Photolysis

The photolysis of the 271 parts per billion TCDD contaminated soil using UV lamps under the conditions tested was not successful in destroying TCDD to a detectable degree. The lack of destruction may have been a result of many factors, such as soil depth, surfactant type, lamp distance, soil particle size, etc. Some of these factors were evaluated using the PCB contaminated soils. The conditions tested for TCDD destruction were the two UV lamp types and two surfactant concentrations for each UV source. The results for the 48 hour tests, shown in Table 1 shows no significant difference between the final TCDD concentration in any of the tests and the starting TCDD concentration (271 ppb).

TABLE 1. SUMMARY OF TCDD UV-PHOTOLYSIS TESTING

Test.	Lamp Type	Suffactant (% of Dry Soil)	Final TCDD Cont. (ppb)	Percent TCDD Reduction'
1	Medium Pressure Hg	2.5	245	10
2	Medium Pressure Hg	5	356	0
3	Pulsed Hg- 10 Hz	2.5	250	8
4	Pulsed Hg- 10 Hz	5	244	10

• Initial soil concentration - 271 ppb TCDD.
Surfactant - Hyonic NP-90® and Adsee 799® in 1: 1 ratio.
Soil bed depth - 1 inch.
Lamp to soil distance - 10 inches.

The soil samples fram the TCDD sunlight tests were not analyzed for TCDD destruction because of the lack of effect in the TCDD *UV* lamp tests and the PCB sunlight tests.

PCB Photolysis Using UVLamlPs

Following these initial TCDD experiments, the PCB soils were tested under similar conditions. Since PCB analytical results were available with a faster turnaround time than TCDD analyses, the experimental conditions could be adapted to suit the needs of the experiments.

The initial PCB irradiation experiments used the highly contaminated (Approximately 10,000 ppm Aroclor 1248) surface soil

from Danville, Kentucky. In the first experiment (Test #l), the pulsed lamp was used with a lower surfactant concentration and spraying at l/2 hour intervals to a total irradiation time of 12 hours. The surfactant was the same nonionic mix used in the TCDD tests. There was no perceptible change in the PCB concentration.

A second experiment to test the effect of stirring the soil more frequently was carried out with stirring and spraying at 2 minute intervals to a total irradiation time of 12 hours. These test conditions were also used in testing the Hanovia lamp for 7 hours of irradiation (Test #3). No change in the PCB concentration was detected in these tests, again using the high PCB concentration soil.

A fourth experiment using the Hanovia lamp with air cooling instead of water cooling in the lamp well appeared to produce a slight change in the PCB concentration.

A fifth experiment, again using air cooling but with the lamp at 3.5 inches from the soil surface produced about a 50 percent loss in the PCB concentration after 3.5 hours. However, the temperature of the soil was significantly higher than in previous tests (approximately 105°C) because of the lack of well cooling water and the short distance between the lamp and soil. In addition, the loss of PCBs was highest for the lighter chlorinated PCB congeners suggesting loss due to volatilization at the higher temperature. The same loss was then duplicated in a separate test (Test #6) by heating the soil in an oven at 140°C for 4 hours, with spraying and stirring at l/2 hour intervals. This temperature was chosen because the bottom of the glass tray reached temperatures in this region during the fifth irradiation test. Results from these first six tests are summarized in Table 2.

TABLE 2. SUMMARY OF UV PHOTOLYSIS TESTING ON PCB SURFACE SOIL

Test	Lamp Type	Soil Depth (in)	Lamp/Soil Distance (in)	Time (Hours)	Temp. (°C)	Initial PCB Conc (ppm)	Final PCB Conc (ppm)	Percent PCB Reduction
1	Pulsed	1	10	12	25	13,200	14,100	0
2	Pulsed	0.25	10	12	28	7,240	7,950	0
3	Cont.	0.25	10	7	28	7,430	6,960	6
4	Cont.	0.25	10	7	40	8,440	5,680	33
5	Cont.	0.25	3.5	3.5	105	6,020[a]	4,080	32
6	Oven[b]	0.25	NA	4	140	8,300	4,690	44

Initial soil was PCB surface soil.
Pulsed - mercury lamp pulsed at 10 Hz (70 Watts/inch for 6 inch lamp).
Cont. - Hanovia 450 Watt medium pressure continuous mercury lamp.
NA - Not applicable.
Surfactant - Hyonic NP-90® and Adsee 799® in 1 :I ratio at 2 percent of the soil.
[a] Starting soil was residue from previous treatment experiment.
[b] Soil was heated in oven at 140° C, no irradiation.

At this point in the testing, a radiometer was used to check the distribution of light intensity on the soil at various distances from the lamp. It was found that the intensity was fairly uniform across the tray at 9-10 inches from the lamp using the parabolic reflector. The edges fell off rapidly as the tray was raised closer to the reflector since the edges of the tray fell outside the reflector.

Based on the results of the UV distribution measurements and in an effort to observe smaller absolute changes in PCB concentration, it was decided to test a less contaminated starting soil, in a smaller tray closer to the lamp. For this test, the PCB pit soil from Danville, Kentucky with a PCB concentration of approximately 150 ppm PCBs (Aroclor 1248) was used. A shallow soil bed with frequent 10 minute raking intervals was also used. This test used the Hanovia lamp in the water-cooled light well. Additional air cooling above the soil reduced the effect of heat generated by the lamp at this close distance to the soil. A reduction in the PCB concentration of 18 percent was achieved (Test #7).

A further test (Test #8) used the same soil spiked with additional Aroclor 1260 to test the hypothesis that spiked contaminants could be more easily photolyzed than weathered

contaminants because they would be easily extracted from the soil by the surfactants. This suspicion appeared to be confirmed although the rate of destruction of Aroclor 1260 was not as high as expected and not that much greater than the destruction of Aroclor 1248 in the test.

The ninth test repeated the eighth test using the pulsed lamp instead of the Hanovia lamp. Both lamps performed about the same in terms of PCB (Aroclor 1260) reduction. Results from these tests are summarized in Table 3.

TABLE 3. SUMMARY OF *UV* PHOTOLYSIS TESTING ON PCB PIT SOIL USING MIXED SURFACTANT

Test	Lamp Type	Soil Depth (in.)	Lamp/Soil Distance (in.)	Time (Hours)	Temp. (°C)	initial PCB Cone (ppm)	Final PCB Cone (ppm)	Percent PCB Reduction
7	Cont.	0.25	3.5	10	50	194	159	18
8	Cont.	0.25	3.5	10	58	104'	24'	77
9	Pulsed	0.25	3.5	10	35	121'	45'	63

Initial soil was PCB pit soil.
Cont. - Hanovia 450 Watt medium pressure continuous mercury lamp.
Pulsed - mercury lamp pulsed at 10 Hz (70 Watts/inch for 6 inch lamp).
Surfactant - Hyonic NP-90® and Adsee 799® in 1 :I ratio at 2 percent of the soil,
' Concentration of Aroclor 1260 spiked onto soil.

The efficiency of the surfactant solution to extract the contaminants from the soil was becoming suspect due to the low destruction rates observed. To test the ability of the surfactant to remove the PCBs from the soil, several different surfactants were evaluated by shaking 2 grams of soil in 20 milliliters of a 3 percent surfactant solution for a total of 60 minutes on a platform shaker and then analyzing the supernatant solution for PCBs. It was found that the Adsee 799® surfactant being used in the test program was hindering the extraction efficiency of the Hyonic NP-90® surfactant. The PCB extraction screening tests are summarized in Table 4.

TABLE 4. SUMMARY OF SURFACTANT PCB EXTRACTION SCREENING TESTS

Surfactant.	Surfactant Type	PCB Conc.b (ppm)	Percent Extracted'
Brij 30®	ethoxy alkyl alcohol	11.9	79
Brij 35®	ethoxy alkyl alcohol	5.4	36
Brij 30® + Brij 35®d	nonionic mix	4.2	28
Adsee 799Q	oxyalkylated fatty acid ester	2.3	15
Hyonic NP-90®	ethoxylated nonyl phenol	11.1	74
Adsee 799® + Hyonic NP-90®d	nonionic mix	2.5	17
SDS'	anionic	8.3	55
Hyonic NP-90®f	ethoxylated nonyl phenol	8.3	55
None (Water)	NA	0.4	3

Brij 30®/Brij 35® - Aldrich Chemical Co., polyethoxylated alkyl alcohols.
Adsee 799® - Witco Chemical Corp., polyoxyalkylated fatty acid ester.
Hyonic NP-90® - Diamond Shamrock Corp., polyethoxylated nonylphenol.
NA - Not applicable.
[a] Total surfactant concentration is 3 percent by weight in water.
[b] PCB concentration in the aqueous surfactant solution.
[c] Extraction based on 150 ppm PCB in 2 grams of soil in 20 mL of extraction solution.
[d] Mixtures are 1 :I by weight, total is percent.
[e] Sodium Dodecyl Sulfate.
[f] Single extraction, all others are averages of duplicate extractions.

Another *UV* test (Test #10) was then performed using the nonionic Hyonic NP-90® surfactant only on the PCB pit soil. This test used a depth of soil of about l/2 inch in the large tray at a distance of 10 inches from the lamp, (water-cooled Hanovia lamp), with 10 minute raking intervals for a total irradiation time of 16 hours. A reduction in PCB concentration of approximately 30 percent was achieved on the weathered, contaminated soil. Two more tests were then performed using the same conditions, but using the pulsed *UV* lamp instead of the Hanovia continuous lamp. One had a total irradiation time of 16 hours (Test X11) and the other was twelve hours (Test X12). Results were not quite as good with the pulsed lamp, but were considered to be within experimental variability to the results from Test #10 with the continuous Hanovia lamp. Results from these tests are summarized in Table 5.

TABLE 5. SUMMARY OF UV PHOTOLYSIS TESTING ON PCB PIT SOIL USING SINGLE SURFACTANT

Test	Lamp Type	Soil Depth (in.)	Lamp/Soil Distance (in.)	Time (Hours)	Temp. (°C)	Initial PCB Cone (ppm)	Final PCB Cone (ppm)	Percent PCB Reduction
10	Cont.	0.5	9	16	30	140	98	30
11	Pulsed	0.5	9	16	28	157	137	13
12	Pulsed	0.5	9	12	28	170	131	23

Initial soil was PCB pit soil.
Cont. - Hanovia 450 Watt medium pressure continuous mercury lamp.
Pulsed - mercury lamp pulsed at 10 Hz (70 Watts/inch for 6 inch lamp).
Surfactant - Hyonic NP-90® at 2 percent of the soil.

PCB Photolysis Using Sunlight Exposure

Tests were conducted as described in the Experimental Section using three different concentrations of surfactant. The nonionic mix of surfactants was used throughout the first half of testing and then was changed to the use of Hyonic NP-90® alone after the results of surfactant PCB extraction tests were realized. These tests showed no significant change in PCB concentration after 197 hours (25 days) of sunlight exposure. Because of the summertime conditions the soil surface dried rapidly and this is considered partially responsible for the lack of PCB degradation. Results from these tests are summarized in Table 6.

TABLE 6. SUMMARY OF UV PHOTOLYSIS TESTING ON PCB PIT SOIL USING SOLAR IRRADIATION

Test	Lamp Type	Soil Depth (in.)	Surfactant Conc (%)	Time (Days)	Temp. (°C)	Initial PCB Conc (ppm)	Final PCB Conc (ppm)	Percent PCB Reduction
13	Solar	1	4.5	25	26 - 41	132	156	0
14	Solar	1	2	25	26 - 41	159	143	10
15	Solar	1	0	25	26 - 41	171	157	8

Initial soil was PCB pit soil.
Surfactant - Hyonic NP-90® and Adsee 799®.

Samples from the second month of sunlight testing were not analyzed for PCB degradation because of the lack of effect shown during the first month of testing.

Soil Particle Size Testing

Following the poor results of the UV testing on the screened and dried soils (less than 1/8 inch), the effect of particle size was tested by grinding the clayey PCB contaminated surface soil to pass a 230 mesh screen (particle size less than 63 microns). This ground soil was used as the basis for an additional ten experiments. The medium pressure Hanovia lamp was again used in a water cooled quartz light well. Exposure times ranged from 3-20 hours, and surfactant concentrations were also varied. The distance of the lamp to the soil and the cooling water rate were kept constant to maintain a maximum measured soil surface temperature of 54°C. All of the surfactant (Hyonic NP-90®) was applied at the beginning of each experiment, the soil moistened periodically with water only and tilled or raked periodically during the UV exposure. Results from these tests are summarized in Table 7.

TABLE 7. SUMMARY OF UV PHOTOLYSIS TESTING ON FINE GROUND PCB SURFACE SOIL

Test	Surfactant Conc.(% of Dry Soil)	Time (Hours)	Initial PCB Conc (ppm)	Final PCB Conc (ppm)	Percent PCB Reduction
16	2.0	6	10,970	3,380	69
17	2.5	3	10,970	7,100	35
18	0	3	10,970	12,860	0
19	2.8	10	10,970	8,500	23
20	2.1	3.7	10,970	8,930	19
21,	2.8	3	10,970	12,180	0
22	2.3	3	10,970	9,525	13
23	2.0	20	7,324	3,537	52
24	2.3	10	6,753	4,566	32
25	2.0	20	8,572	5,925	31

Initial soil ground to < 230 mesh.
Hanovia 450 watt medium pressure continuous mercury lamp.
Soil depth - 0.25 inch.
Lamp/soil distance - 4 inches.
Surfactant - Hyonic NP-90®.
Temperature - Approximately 50°C.

The results of Tests #16 through #22 are from analyses of a single sample of treated soil. Tests #23, 24, and 25 had samples removed and analyses performed as a function of treatment time. The PCB concentration of soil moistened with water only (no surfactant) and irradiated with the UV lamp was unchanged. The PCB concentration of nearly all soils to which surfactant was applied and then irradiated showed some decrease. Figure 1 shows the kinetics for total PCB reaction in the 20 hour UV photolysis Test #25.

A more detailed look at the effect of UV photolysis on PCB chlorine level group (homolog) concentration is presented in Table 8. This data shows the change in each PCB homolog concentration (di through heptachlorobiphenyl) after UV treatment. Congeners with three or more chlorine atoms (tri through hepta-PCBs) showed a relatively consistent reduction in concentration, whereas there was an increase in dichlorobiphenyl concentration. The di-PCB fraction of the total PCB

concentration increased from 24 percent to 42 percent as a result of irradiation. The presence of monochlorobiphenyls (single chlorine atom substituent) was not detected in any of the samples. Figure 2 shows PCB chromatograms of untreated soil versus soil irradiated for 20 hours with *UV* light (chromatogram scales have been adjusted based on sample weights and dilution volumes used in the analysis to present relative response equal to relative concentration). In the *UV* treated soil, higher chlorinated PCBs appearing later in the chromatographic analysis are smaller and some of the peaks in the di and tri-PCB elution window are larger and a few new peaks are seen in the di-, tri- and tetra-PCB windows. These data are consistent with degradation of higher chlorinated PCBs to lower chlorinated (di, tri and tetra) PCBs.

Summaries of analytical data for Tests #l through **16** and 23, 24 and 25 are included in Appendix C.

TABLE 8. *UV* PHOTOLYSIS - 20 HOUR TEST #25
PCB CONCENTRATION RESULTS - SOXHLET EXTRACTION

	Starting Soil (ppm)	20 Hour UV Treated Soil (ppm)	Percent Change in PCB Conc.
Dichlorobiphenyls	2,027	2,465	22
Trichlorobiphenyls	1,134	556	-51
Tetrachlorobiphenyls	2,370	1,390	-41
Pentachlorobiphenyls	1,806	826	-54
Hexachlorobiphenyls	1,112	624	-44
Heptachlorobiphenyls	109	53	-52
Total PCB concentration DCMA	8,570	5,925	-31

Figure **1**

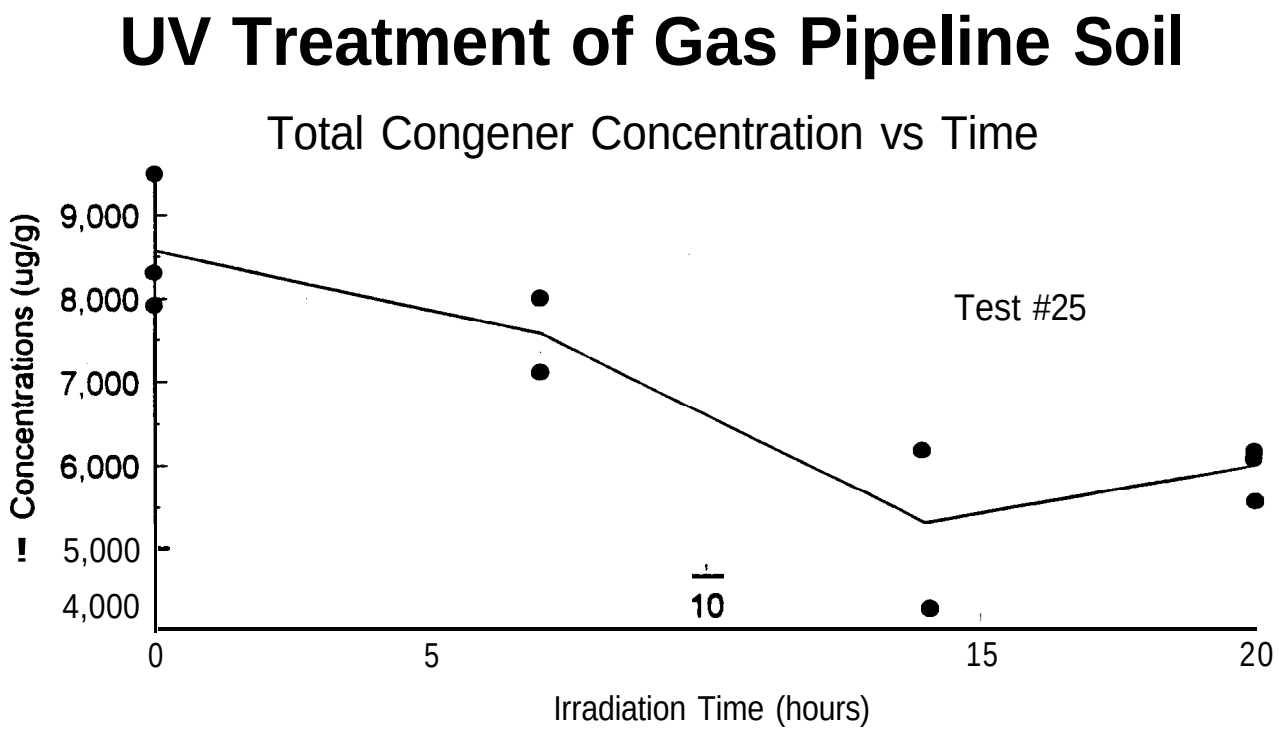

Figure 1. W Treatment of Gas Pipeline Soil

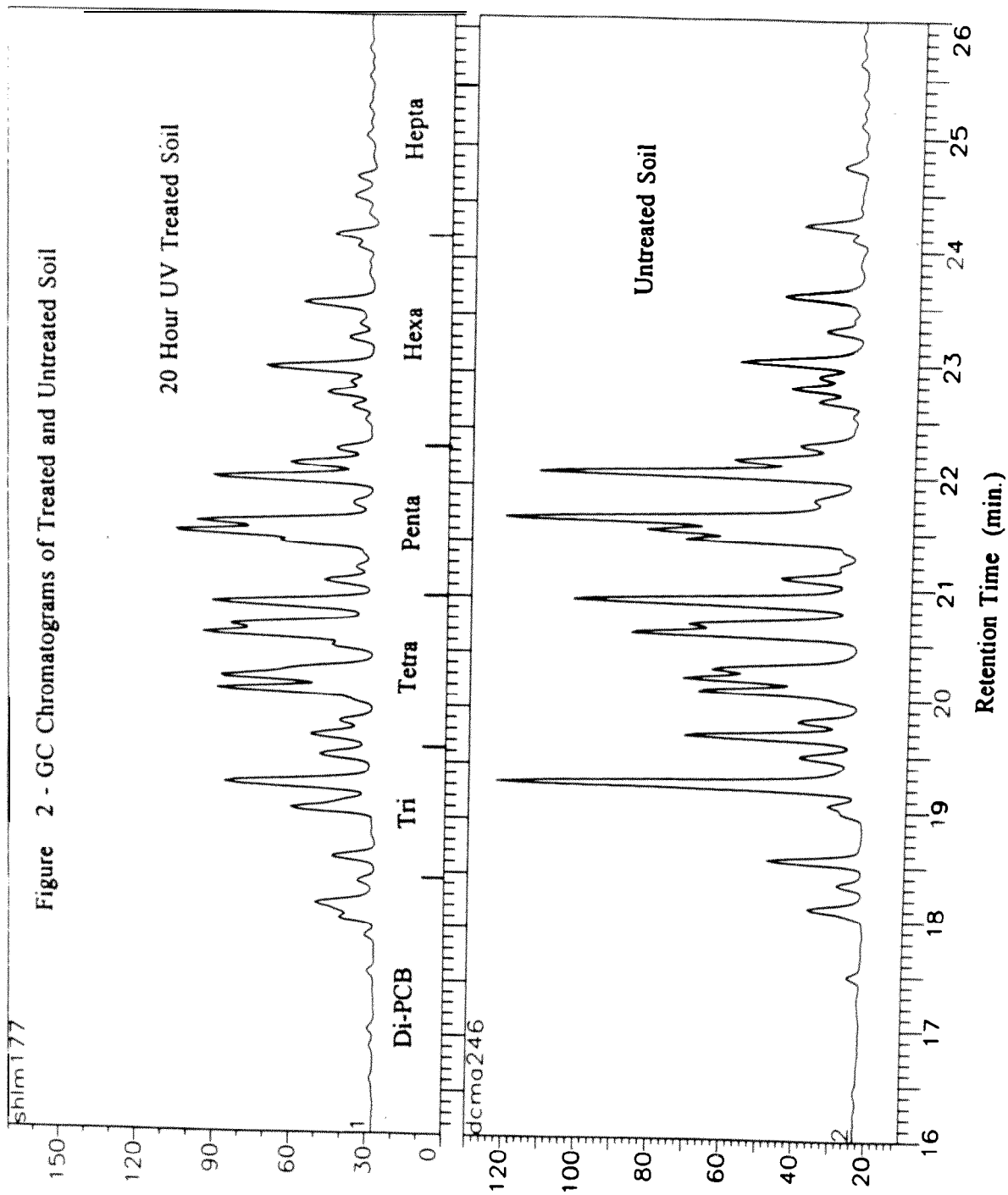

Figure 2. GC Chromatograms of Treated and Untreated Soil.

CONCLUSIONS AND RECOMMENDATIONS

UV photolysis tests using high intensity UV lamps on **TCDD** contaminated soil with surfactant application gave no detectable change in contaminant concentration for the soil.

In tests using high intensity UV lamps on two different soils with surfactant applications, PCB'reductions ranged up to a maximum of 69 percent. In general, changes that were detected in soil PCB concentrations were obtained using UV lamps and were less than 50 percent, typically **15** to **35** percent. Best‘results were obtained using a 2-3 percent (Hyonic **NP-90®)** surfactant spray loading on fine ground soil (<230 mesh) with a minimum bed depth of l/4 inch and a lamp to soil distance of 4 inches. In these tests, with ***UV*** exposure times of six hours or longer, PCB reductions were consistently in the range of 23 to 69 percent. Loss of PCBs occurred for the higher chlorine level (tri through hepta) PCBs. The loss of these PCBs was coupled with generation of by-products in the di-, tri- and tetra-PCB gas chromatographic elution windows. It was concluded from this data that degradation of higher chlorinated PCBs to lower chlorinated PCBs was occurring to a detectable degree.

Photolysis tests using sunlight exposure on PCB contaminated soil with surfactant application gave no detectable change in contaminant concentration for the soil. This was not surprising as the results from high intensity UV lamp testing did not show significant effectiveness.

The photolysis of TCDD or PCB contaminated soils using in-situ or ex-situ configurations appears to be a process with numerous variables which contribute to its success or failure. Some of these variables appear to be more significant than others but the net effect makes the process very difficult to predict. Because many of the variables are dependent, the scope of testing required becomes enormous. The variability of the analyses resulting from the heterogeneity of the soil make interpretation of results difficult. If the variables are to be properly tested, the individual experiments need multiple replicates that use the entire sample of each condition tested to remove the variability introduced by subsampling for analysis. The criterion of success or failure for each variable tested depends on the ability of the analyst to rely on the data produced from the experiment. The method of sample extraction has a significant effect on the final analytical result. The experimenter must rely on the analysis of multiple replicates to interpret data by applying statistical methods.

The process should be tested using the above procedures after a surfactant or solubilizing aid has been carefully selected for the soil under consideration. Since this is one of

the major variables, the selection becomes critical to the success or failure of the program to follow.

In addition, examination of different soil types should be performed as results from these tests were much less successful than results obtained from previous work using similar conditions, indicating that soil type is a major variable. The soil used in these tests had a higher humic content than the sandy soils used in earlier successful testing.

Fine ground surface soil, both untreated and from the 20 hour W photolysis tests were supplied for biological treatment. The soil residue from the 20 hour UV photolysis tests consistently showed the highest effect from irradiation as given by the reduction in PCB concentration.

SECTION 4

CHEMICAL OXIDATION OF PCBS

INTRODUCTION

Chemical oxidation by Fenton's Reagent has been used to destroy organic compounds such as formaldehyde (Murphy, et al., 1989), azo dyes (Kitao, Kiso and Yahashi, 1982) and chlorinated phenols (Barbeni, et al., 1987) in groundwater and wastewater. The reaction is ideally performed at a pH of 2-4 using hydrogen peroxide as the oxidant in the presence of a ferrous salt. Ferrous ions catalyze the decomposition of hydrogen peroxide. In the process of decomposition, the reactive hydroxyl radical is produced and it is capable of oxidizing organic contaminants. However, if the desired oxidation reaction is slow, significant amounts of hydrogen peroxide can be consumed in unproductive decomposition instead of participating in the desired process. Reaction conditions must be established to provide useful rates of contaminant oxidation with efficient use of hydrogen peroxide reagent.

Performing this reaction on soil contamination requires making a slurry with the soil and the aqueous reagent. Testing was performed in small batch systems of various sizes under ambient conditions with concentrations of hydrogen peroxide and PCBs monitored as a function of time. The tests were performed on the ground PCB surface soil from Danville, Kentucky (GG4202) which was used in the UV photolysis testing. The PCB concentration of this soil was determined to be approximately 10,000 ppm Aroclor 1248.

The objective of the tests was to preliminarily investigate the feasibility of applying the technology to soils contaminated with PCBs. This process was investigated as an alternative to W photolysis to provide initial contaminant degradation to more easily biodegradable compounds. Conditions were established to provide the best opportunity for observing an effect due to treatment; reagent to soil ratios were high, pH maintained in the range of 2-4 and hydrogen peroxide concentrations were maximized by periodic replenishment. Iron concentrations were adjusted from test to test to determine optimum concentration for maximum PCB degradation.

EXPERIMENTAL PROCEDURES

Overview

Five experiments with Fenton's Reagent were performed at ambient temperature. All five used the clay/humic, surface soil GG4202, from the Danville, Kentucky site. This soil had been

air-dried in a hood and screened to remove gravel and debris. Each experiment was conducted in batch mode in covered glass vessels with vents for gas escape. In each case, soil and reagents were added to the reaction vessel and conditions were established at the beginning of the experiment. Periodic adjustments were made in pH and hydrogen peroxide concentration as noted for each experiment. In each experiment the reagent/soil mixture was continuously stirred except Experiment 1 which was stirred only during initial reagent additions and at the 24-hour sample time. Table D-l in Appendix D is a summary of soil, water, pH, iron sulfate and hydrogen peroxide initial conditions for the tests. Further experimental procedural details are presented elsewhere; however, important points concerning these tests are the following:

Only Experiment 1 was not stirred continuously.

Experiment 2, Flask 2 was a control: no iron sulfate was added to this flask.

Experiments 4 and 5 were considerably larger scale and periodic samples were taken for PCB analysis.

Feed Soil Preparation

The soil used for all experiments was from the air-dried PCB surface soil sample (GG4202). The soil was ground in a standard kitchen blender and sieved. Particles not passing the sieve were reground in the blender. Fenton's Reagent Experiments 1, 2, and 3 used soil passing 230 mesh standard U.S. Sieve. Fenton's Reagent Experiments 4 and 5 used soil passing 100 mesh but retained by 200 mesh.

Sampling

Experiment 1 was sampled for PCB at 24 hours and at the end of the'experiment at 92 hours. Each jar/flask was stirred for 15 minutes and sampled using a 60 cubic centimeter (cc) polypropylene syringe. The residual hydrogen peroxide in the sample was neutralized with sodium bisulfite. After settling, any aqueous supernatant was removed and the residual wet solids dried at 48°C for 20 hours. Samples were extracted by Soxhlet extraction and analyzed by GC/ECD.

Experiment 2 was periodically sampled for hydrogen peroxide and pH. Typically, pH was measured and stirring was stopped long enough for a 5- or lo-milliliter (mL) supernatant sample to be pipetted off for potassium permanganate titration. The solids were rinsed from the flask into tared wide-mouth jars and allowed to settle. The reaction flasks were rinsed (3 times) with water, followed by methylene chloride (3 times). The rinsewater, the original supernatant, and the supernatant from the transferred

solids were combined and extracted with methylene chloride. These extracts were combined with the sonication extracts of the solids and, following solvent exchange, analyzed as a single sample by GC/ECD.

Experiment 3 was sampled similar to Experiment 2 with the following changes. The excess hydrogen peroxide was quenched using sodium bisulfite after titration. The remaining solids after liquid removal were air dried before extraction. In addition, the extracts for the liquid and solids were analyzed separately.

Experiment 4 and 5 samples were withdrawn from below the surface of the stirred mixture using a 60 cubic centimeter (cc) polypropylene syringe fitted with a short length of Teflon™ tubing. The sample was then transferred to a clear glass jar with a Teflon™ lined cap and allowed to settle for at least 45 minutes. Aliquots of the supernatant were then removed and immediately titrated for residual hydrogen peroxide. The remaining supernatant was then carefully removed from the jar and replaced into the reaction flask. The wet solids samples were then weighed, quenched with sodium bisulfite and then reweighed. The inside of each sample jar was then rinsed with a small amount of deionized water and the samples were allowed to air dry in a fume hood.

MATERIALS AND METHODS

Equipment

Experiment 1 was performed in 250-mL and 500-mL straight-sided glass jars, Experiment 2 used two 250-mL Phillips' flasks and Experiment 3 used two 125-mL Erlenmeyer flasks. The mixtures were stirred with Teflon™-coated magnetic stir bars.

Experiments 4 and 5 were larger scale. Experiment 4 was done in a straight-sided a-liter (L) Pyrex jar. The soil-water mixture was agitated with a two-bladed approximately 30° pitch polypropylene covered steel stirrer driven by a variable speed lab motor. Stirrer speed requirement was determined by prior testing of a small amount of sand in water.

The Experiment 5 slurry was reacted in a baffled 4-L reaction pot/kettle. Stirring was provided by a stainless steel three-bladed turbine propeller driven by a variable speed electric motor. As in Experiment 4 a good mixing speed was determined using a clean sand-in-water mixture.

Chemical Reasents

Iron (II) Sulfate, $FeSO_4 \cdot 7H_2O$ - Alfa, ACS reagent
Hydrogen Peroxide - Aldrich, ACS, 30 percent weight, stabilized
Potassium Permanganate - Mallinkrodt, volumetric solution; 1.00 ± .005N
Sulfuric Acid - Mallinkrodt, 95.0-98.0 percent, AR
PCB Aroclor 1248 standard - Chem Service, **Fll0**
Sodium Bisulfite - Mallinkrodt, AR, granular
Sodium Oxalate - Mallinkrodt, AR
Surfactant -Stepan Co, Bio-Soft S-100, Dodecylbenzene sulfonic acid

pH Measurement

The pH was measured using a calibrated pH meter and a combination pH probe. Measurements were made directly in the reaction vessel contents while they were being mixed.

Hydrogen Peroxide

Hydrogen peroxide concentrations were measured by titration of a 2.0 - 10 mL sample aliquot diluted with 25 percent sulfuric acid solution using a potassium permanganate standard solution. The sample density was taken as 1.0 gm/mL and the peroxide calculated from:

$$weight\ \%\ H_2O_2 = \frac{(mL\ KMnO_4)\ (N\ KMnO_4)\ (1.7)}{mL\ of\ sample}$$

PCB Analysis

Sample Preparation--
The air-dried soil samples were crushed to a dust in their sample jars with a clean stainless steel spatula and mixed thoroughly. For Experiment 4, the sample jars were not scraped, but methylene chloride was added after solids removal to extract any residual PCBs on the walls of the sample container. This extract was analyzed separately to determine loss from PCBs adhering to the sample jar surface.

Samples were extracted by one of two methods: sonication or Soxhlet. Some samples from Experiments 3 and 4 were extracted by both methods.

Sample Extraction - Sonication--
The sonication extraction procedure was based on EPA Method 3550 (SW-846). Crushed dried soil sample aliquots weighing 2.0 to 2.5 grams were mixed with 2-3 grams of oven-treated sodium sulfate in a 20 mL glass vial and extracted with lo-12 mLs of 1:l volume to volume (v/v) acetone to methylene chloride solvent by

sonication. After sonication, the extract was gravity filtered through a bed of sodium sulfate and collected directly into a 50 mL volumetric flask. This process/cycle was repeated three times for each sample. Sample extracts were solvent exchanged to hexane by Kuderna Danish (KD) evaporators for analysis by GC/ECD, or they were diluted with methylene chloride for analysis by gas chromatography with flame ionization detection. (GC/FID).

Sample Extraction - Soxhlet--

The Soxhlet extraction procedure was based on EPA Method 3540A (SW-846). Soxhlet extractions were done on two scales: the procedure described in Method 3540A and a micro procedure essentially identical except that the entire setup is proportionally smaller (2 gram sample size). Sample extracts were solvent exchanged to hexane by KD evaporators if they were to be analyzed by GC/ECD, or they were diluted with methylene chloride for GC/FID analysis.

Instrumental Analysis--

The analysis of PCBs was performed by one of two methods: EPA Method 8080 (SW-846) or a DCMA (PCB homolog) procedure, using either GC/ECD or GC/FID instrumentation. Samples were initially analyzed by GC/ECD for its selectivity and sensitivity, but because of the high concentrations of PCBs in the samples, ease of sample preparation, and extended linear range, analyses were switched to GC/FID instrumentation. The analytical methods were applied in the same manner for either instrument. Samples from Experiment 3 were analyzed by both GC/ECD and GC/FID. Untreated samples were analyzed and quantified for Aroclor 1248 using methods consistent with SW-846 Method 8080. Treated samples were analyzed and quantified by the DCMA semi-specific PCB homolog method.

QUALITY ASSURANCE

Potassium Permancfanate

The potassium permanganate ($KMnO_4$) titrant solution was prepared from a 1.00 f.005 Normal (N) standard by diluting an aliquot 1:20 with deionized water. The titrant concentration was verified by titration of an accurately weighed sample of sodium oxalate in 12.5 percent sulfuric acid and found to be 0.052 N.

PCB Analysis

The air-dried soil samples from Experiment 4 were oven, dried at 106°C after aliquots had been taken for Soxhlet extraction. The percent moisture ranged from 1.4 to 2.8 percent; the average was 2.2 percent. These values were low and consequently the analytical PCB results were not corrected for this amount of moisture in Experiments 4 and 5.

To check for the possibility of PCB adhering to the sample jar walls, the crushed soil was removed from each Experiment 4 sample jar and the jars themselves filled with methylene chloride. Insignificant amounts of PCB were found in these jar soak extracts. PCB loss from adhering to sample jar surface was found to be less than 2 percent in all cases and was not considered significant.

Analyses were performed on three samples (feed and flask samples from Experiment 3) by both GC/FID and GC/ECD instrumentation to evaluate differences. The relative percent differences (RPD) in results from analyses by both instruments were 3, 15 and 31 percent. The difference between instrumental methods was not considered significant, since data from each experiment was obtained by one method or the other and results from GC/ECD analyses were not compared with results from GC/FID analyses, or vice versa.

Analyses were also performed to determine if PCB recovery was complete after three sonication extraction cycles of a sample to assure PCB loss was not occurring from incomplete extraction. An additional two sonication extraction cycles were performed on a sample after the extraction procedure using three cycles had been performed. The fourth and fifth extraction cycle extracts were analyzed separately and found to contain only two percent of the PCBs extracted by the first three cycles. The three extraction cycles were considered sufficient since 98 percent of the PCBs recoverable by sonication were being extracted. Further analysis details are supplied in a summary in Appendix E.

A difference was noted during these tests, however, between sonication and Soxhlet extraction efficiency. In Experiment 4, samples were analyzed by both procedures and PCB recovery by sonication ranged from 43 to 74 percent of the PCBs recovered by Soxhlet extraction. The average ratio of PCBs recovered by sonication versus Soxhlet extraction was 58 percent with a relative standard deviation (RSD) of 15 percent. The sonication extraction results were consistently lower than those obtained by Soxhlet extraction and although the difference was significant, the loss of PCBs could be monitored by either method as long as data from one method of extraction was not used with data from the other. The results from Experiment 4 showed that the same conclusions would be reached using data from either PCB extraction method as long as the data was distinguished by the extraction method used.

A check on the reproducibility of the micro Soxhlet extractions for PCB was performed by triplicate extractions of samples from Experiment 5. This was conducted to evaluate variability which may have been introduced because of the small sample sizes (2 grams) used in the micro procedure. Nine sample sets were extracted in triplicate and one in duplicate. The

highest RSD or RPD of any set was 4.7 percent; the average RSD/RPD of all nine sets was 3.0 percent. Detailed individual and sample set values are presented in a summary in Appendix E.

An unknown PCB quality control (QC) sample was analyzed by both the TDL and the Biotechnology Application Center (BAC) as an independent QC check on PCB calibration. The sample was prepared from an independent source of Aroclor 1248 and provided to both labs as a QC sample. The percent recoveries reported by both laboratories engaged in work for this project were well within the expected ±25 percent for demonstration of analytical control. In addition, the interlab agreement was excellent. There was less than 3 percent RPD between the two laboratories' results. Further analysis details are supplied in a summary in Appendix E.

Finally, two micro-Soxhlet sample extracts from Experiment 4 were spiked with an equivalent amount of PCBs from a known standard to check for interferences and extraneous peaks. Both spikes were prepared by adding 2.0 milliliters (mL) of an Aroclor 1248 standard (at about the same concentration as the extracts) to 2.0 mLs of the sample extract. PCB recoveries for the spike samples were 90 and 102 percent, showing excellent PCB accountability. Further analysis details are supplied in a summary in Appendix E.

RESULTS AND DISCUSSION

Experiment 1 - 24 and 92 Hours, No Mixing

The first experiment consisted of two batch reactions with the mixtures stirred only during the initial reagent additions and during the 24 and 92 hour sample times. Table 9 summarizes the conditions used. The two reactions differed primarily in the ratio of reagent to soil. Flask 85 (GG4202-1018-85) had a water to soil ratio of 0.8 and Flask 86 had a ratio of 3.1. All subsequent tests used higher ratios, in the range of 8 to 10. After 9'2 hours, dried soils were Soxhlet extracted and aqueous phases were separatory funnel extracted. Analyses by GC/ECD of the aqueous and solid phase extracts indicated no significant change from the starting 'PCB concentration. At least 95 percent of the PCBs remained at the end of the tests for both reactions. As shown in Table 9 no significant change in PCB concentration was found in the samples from this experiment. Further detail on these analyses is included in Table D-2 in Appendix D.

Fifteen vials were established for each treatment. Three vials from each treatment set (15 total vials) were sacrificed at five time points. The time points were study initiation, 24 hours (hr), 48 hr, 94 hr, and 140 hr. Vials were extracted by sonication with 2 mL of pentane (Aldrich Chemical Co., Milwaukee, Wisconsin) for one minute in a Bransonic 220 Sonicator Bath.

Pentane extracts were analyzed by a Hewlett Packard 5890A Gas Chromatograph (GC) with an automatic sampler, ECD, splitless injector, and Supelco SPB-1 capillary column [75 meter by 0.75 millimeter (internal diameter)].

Nitrogen was used as the carrier and make-up gas. The carrier gas flow was 2 milliliters per minute (mL/min) at 40°C. The make-up gas was introduced at 60 mL/min. During sample analysis, the GC oven initial temperature was 45°C. This was held for one minute, raised to 150°C at a rate of 10°C/min and then to 300°C at a rate of 3°C/min. The 300°C temperature was held for 5 minutes.

Bioslurry Evaluation

Three PCB-contaminated soils were evaluated for biological reduction of PCB congeners. Soils employed *were* identified as untreated soil (Sample ID No. GG4202-1018-61), surfactant/UV-treated soil (Sample ID No. GG4202-1018-96A), and New Englanq Superfund Site soil.

The following treatments were prepared:

- Treatment Bl - surfactant/UV-treated soil, PAS medium, BAC 17 culture
- Treatment B2 - surfactant/UV-treated soil, PAS medium, H850 culture
- Treatment B3 - surfactant/UV-treated soil, PAS medium, Hydrochloric acid (Killed control)
- Treatment B4 - Untreated soil, PAS medium, BAC 17 culture
- Treatment B5 - Untreated soil, PAS medium, H850 culture
- Treatment 86 - Untreated soil, PAS medium, Hydrochloric acid (Killed control)
- Treatment B7 - New England soil, PAS medium, BAC 17 culture
- Treatment B8 - New England soil, PAS medium, H850 culture

TABLE 9. FENTON'S REAGENT EXPERIMENT 1
SUMMARY **OF PCB RESULTS**

Series:	GG4202-1018-85	GG4202-1018-86
Feed Soil PCB, ppm	**10,930**	**10,930**
24 Hour'		
Soil PCB, ppm	**11,210**	**10,940**
92 Hour'		
Soil PCB, ppm	**11,360**	**9,710**
Decanted aq. (43 mL) PCB, ppb	**---**	**779**

• **Duplicate samples, dried at 48°C for 20 hr,**

The flask mixtures were not pH adjusted after reagent addition, since the pH was less than 4. Additional observations and comments:

1. The stirring mixtures fizzed slightly during H_2O_2 addition.
2. After initial stirring stopped, the 85 (1018-85) series (the thicker mixture) formed a stable foam within 10 minutes which filled the 250 mL jar.
3. Similarly, the 86 (1018-86) series formed a thinner (more gas) foam layer which was easily reincorporated into the mixture.
4. The analytical results were corrected for solids added and for residual moisture remaining after drying at **48°C.**
5. Residual moisture was calculated based on known solid weights and ranged from 2 percent to 14 percent. All samples appeared dry and crumbled easily.
6. The **"single shot"** addition of H_2O_2 and not stirring the mixture may have been too restrictive.
7. The ratio of available H_2O_2 to total oxidizable components may have been too low.

To address these possible restrictions a second experiment was designed.

Experiment 2 - 162 Hours, Continuously Stirred

Experiment 2 used a higher ratio of reagent to soil (9.7), a higher concentration of iron (2.5 percent of the soil in Flask I), and incorporated a control reaction which had no added iron (Flask 2). See Table D-l for reagent details.

Table D-3 in Appendix D shows the hydrogen peroxide analysis and pH data collected during the experiment.

Both flasks were continuously stirred for 162 hours except for short periodic intervals when the aqueous supernatants were sampled for hydrogen peroxide titrations. At these times, supplemental hydrogen peroxide was added if low. Ferrous sulfate solution was also added to Flask 1 to compensate for losses caused by the removal of aliquots for hydrogen peroxide analysis.

The effects of the iron sulfate and hydrogen peroxide additions upon pH and temperature were significant. The initial iron sulfate addition in Flask 1 dropped the pH from 6.3 to 5.0. The hydrogen peroxide drove the pH further to 2.8 with subsequent foaming and increase in temperature. In contrast, the Flask 2 pH went from 6.3 to 6.7 upon addition of the same amount of hydrogen peroxide with less foaming and no significant temperature change.

Table 10 shows the total congener concentration for each chlorine level (homolog totals) plus the total PCB results given by the DCMA analysis for Flask 1 and Flask 2 at the end of the test. These values include the aqueous phase and flask rinse extracts. The starting soil was also analyzed by sonication extraction and the concentration was 7,325 ppm PCBs. Percent reductions in PCB concentrations given by Flask 1 versus the control, Flask 2, are also presented. The treatment decreased the total PCB concentration by 45 percent of the control concentration.

TABLE 10. FENTON'S REAGENT EXPERIMENT 2
PCB CONCENTRATION RESULTS - SONICATION EXTRACTS

	Starting Soil	Flask 1 (H_2O_2 and Fe) (ppml	Flask 2 (Control, no Fe) (ppm)	Percent PCB Reduction (vs. Control)
Dichlorobiphenyls	—	125	1,272	90
Trichlorobiphenyls	—	169	879	81
Tetrachlorobiphenyls	—	946	2,125	55
Pentachlorobiphenyls	—	1,265	1,594	0
Hexachlorobiphenyls	—	1,166	896	0
Heptachlorobiphenyls	—	86	77	0
Total PCB concentration (ppm) DCMA, GC/ECD	7,325	3,760	6,840	45

In contrast to the trend of PCB loss observed from UV irradiation, PCB loss decreased with increasing chlorination level in the same manner as the oven heated sample described earlier (*UV* photolysis testing). That is, higher percentage losses were observed for lighter chlorinated congeners, di, tri and tetrachlorobiphenyls with smaller losses observed for penta, hexa and heptachlorobiphenyls. Also, as shown in Figure 3, the GC chromatogram of the treated soil shows no new peaks from.by-product generation or alteration of the PCB pattern (chromatogram scales have been adjusted based on sample weights and dilution volumes used in the analysis to present relative response equal to relative concentration). What is shown is a decrease in the pattern trending from late to early elution. These results are consistent with loss of PCBs through volatilization, although reaction with hydrogen peroxide cannot be ruled out.

Based on the observations of this experiment a third flask experiment was designed with the objective of determining the effect of iron levels in the reaction system.

Experiment 3 - 118 Hours, Iron Effect

Experiment 3 was designed to verify the PCB reduction seen in Experiment 2 and to investigate the effect of iron concentration in the reaction mixture. Flask 3A contained the equivalent of 100 ppm iron while Flask 3B contained the equivalent of 450 ppm iron. Both iron levels were considerably

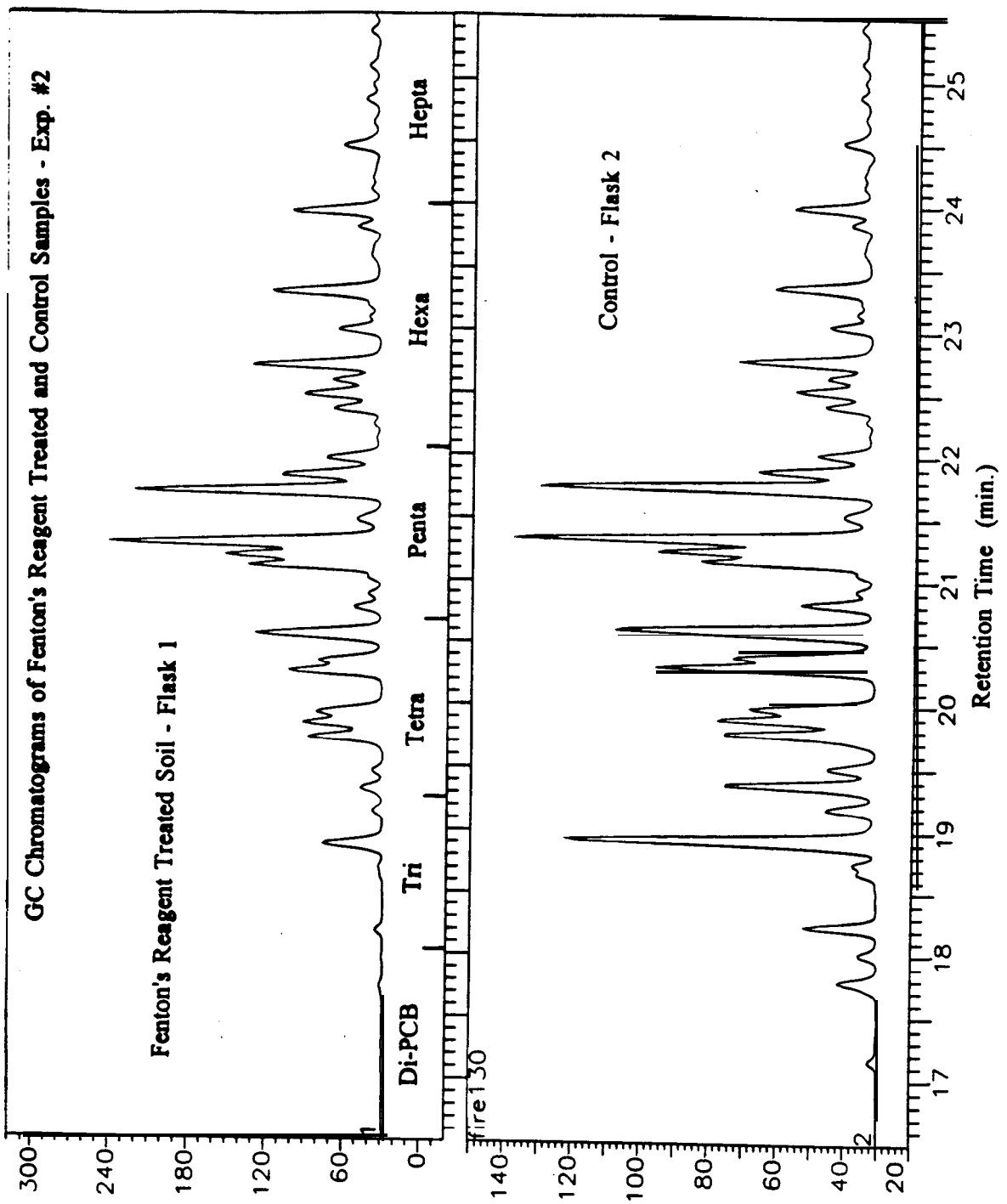

Figure 3. GC Chromatograms of Fenton's Reagent Treated and Control Samples.

lower than the Experiment 2 Flask 1 concentration of 2,200 ppm iron.

Table D-4 in Appendix D shows the hydrogen peroxide analysis and pH data collected during.the experiment.

In this experiment the pH was adjusted to 2.2-2.5 with concentrated sulfuric acid after the iron sulfate addition but before the hydrogen peroxide addition. The temperature rose only slightly in the high iron flask, 3B, after hydrogen peroxide addition, but produced a foam for nearly two hours. Flask 3A foamed only slightly with no perceptible temperature change. Both flasks were periodically sampled for hydrogen peroxide. The volume removed was made back up with deionized water or 23 percent hydrogen peroxide solution, as appropriate.

As detailed in the sampling section, the soil extracts and flask rinses - aqueous phase extracts were analyzed separately to check for loss of PCB through the intermediate sampling of the supernatant for hydrogen peroxide determination.

Table 11 summarizes the results of GC/ECD analysis of the sonicated soil extracts (feed and treated soils) and the flask solvent rinse - aqueous phase extracts at the completion of 118 hours of reaction.

TABLE 11. FENTON'S REAGENT EXPERIMENT 3
PCB CONCENTRATION RESULTS - SONICATION EXTRACTS

	Feed	Flask 3A (118 hours)	Flask 3B (1 18 hours)
Percent of total PCB from flask rinse and 'aqueous extract	---	4.1	0.2
Total PCB - soil basis (ppm 1	6,833	3,171	3,762
Percent reduction of PCBs	---	54	45

The low values for the flask rinses and aqueous phase extracts indicates minimal losses through supernatant sampling or reaction vessel holdup/wall adhesion, although a clear thin hydrophobic film was noted on the flask walls during and at the conclusion of the experiment.

Significant amounts of PCB have either been reacted or lost, with little significant difference noted for the different amounts of iron used in the tests for Experiments 2 and 3. The

trend of PCB loss as a function of homolog group was also consistent with results from Experiment 2.

Experiment 4 - 2 Liter Reactor, 850 Hours

The fourth experiment was designed to allow multiple soil samples to be taken over time and to duplicate the previous results on a larger scale. The equipment and sampling procedures are described in detail in the experimental procedures section. Experiments 4 and 5 used soil which had been freshly ground to between 100 and 200 U.S. sieve mesh.

During startup, initial addition of hydrogen peroxide caused foaming and loss of solution into a containment tray. Addition of sulfuric acid reduced the foaming and allowed replacement of the overflow solution. The exterior of the reaction flask was rinsed and this rinsate was added to the flask. It was estimated that less than 0.3 percent of the soil was lost in the entire episode.

During the reaction, small amounts (less than 2 mL) of 50 percent sodium hydroxide or 25 percent sulfuric acid were periodically added to maintain the reactor pH between 3 to 3.5. As before, periodic supplemental additions of 30 percent hydrogen peroxide were also made.

A few sample aliquots were initially extracted by sonication and soon after fresh aliquots of the same samples were Soxhlet extracted. All extracts were analyzed by GC/FID.

Table D-5 in Appendix D details the hydrogen peroxide analysis and pH data collected during the experiment and the results of PCB analyses that were performed.

The results of the PCB analyses are graphed in Figure 4. After 845 hours the PCB reduction in the flask was 34 percent. This reduction is somewhat less, but consistent with the results from Experiments 2 and 3. The reaction time was much longer than that for Experiments 2 and 3; however, the decrease in PCB concentration appears to have occurred in the first 100 hours.

Considerable scatter is evident in the PCB analysis data. The initial discontinuity in the data from 0-50 hours is most likely due to the reactor overflow episode; however subsequent anomalies cannot be fully explained. Some of the PCB variation in the sample analyses may be a result of particulate size segregation during sampling.

As noted earlier, the sample jars were tested for residual PCB after the soils had been sampled and removed, and the residual PCB in the sample jar was less than 0.7 percent of the total PCB present in any sample jar.

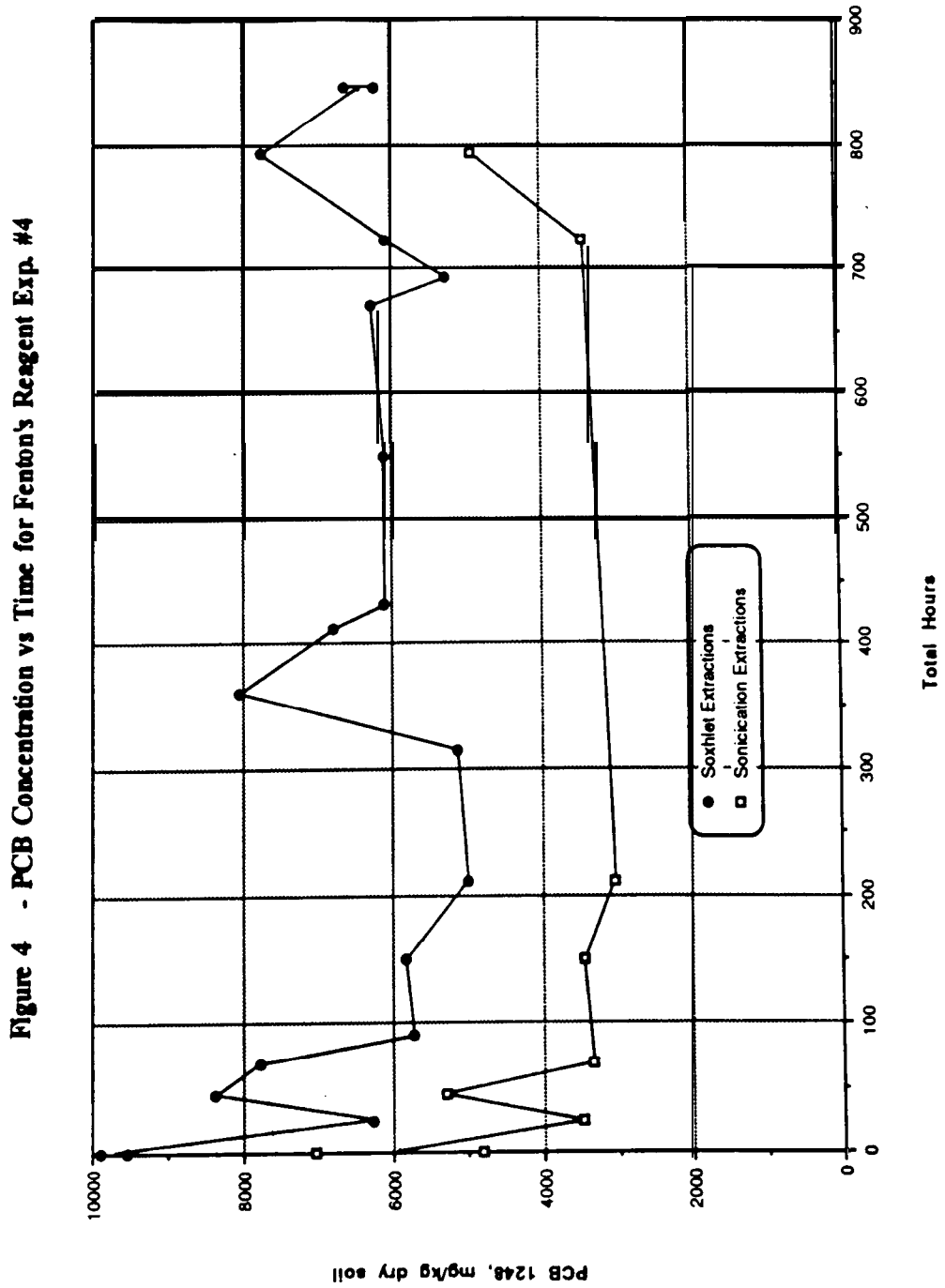

Figure 4. PCB Concentration vs Time for Fenton's Reagent Experiment #4.

The PCB reduction and trend in the data is similar for the sonication and Soxhlet extraction data. The consistent difference between the Soxhlet and sonication values may represent more tightly adsorbed or shielded PCB in the clay-type soil which is not recovered during sonication extraction.

Experiment 5, the final Fenton's Reagent experiment, was planned to address the variation seen in Experiment 4 and to test a surfactant enhancement.

Experiment 5 - 2 Liter Reactor-Surfactant Addition, 184 Hours

Experiment 5 differed from Experiment 4 in several aspects. The all-glass reactor was a four baffled type with a 4-liter capacity to allow for foaming and had improved mixing to keep larger particles suspended.

The major differences in the experimental procedure included the adjustment to pH 2.95 before any hydrogen peroxide addition, the removal of triplicate sample aliquots at each sampling interval, and finally, the addition of a surfactant at the midpoint of the test. These changes resulted in an experimental startup without incident.

Table D-6 in Appendix D details the hydrogen peroxide analysis and pH data collected during the experiment and the results of PCB analyses that were performed. All samples from Experiment 5 were Soxhlet extracted.

With a starting concentration of 9,400 ppm PCBs and a final concentration of 8,048 ppm PCBs after 185 hours, the results indicate destruction of PCBs to be 14 percent. This may have been due to a lower average hydrogen peroxide concentration 'for this test, as well as a low iron concentration, .09 percent of the soil. Hydroxide radicals from hydrogen peroxide reaction with iron are responsible for advanced oxidation reactions. This experiment had the lowest combination of iron and hydrogen peroxide and based on this, should be expected to provide less effectiveness for PCB reaction. This combination also provides the lowest amount of hydrogen peroxide decomposition and oxygen generation which would produce less purging of PCBs from the reaction mixture.

After 117 hours of treatment, a solution of Bio-Soft S-100 surfactant was added to the reaction flask to bring the solution concentrationto 100 ppm to see if surfactant addition wou1.d aid PCB degradation. Bio-Soft S-100 is an anionic surfactant, dodecylbenzene sulfonate, which biodegrades and is relatively stable in oxidative systems. The PCB concentration was not detectably affected by the surfactant addition.

Summary of Chemical Oxidation Testing Results

Table 12 presents a summary of testing results along with key experimental parameters. These data indicate that PCB reductions due to chemical degradation required the presence of iron, but was not strongly affected by the iron concentration. Most important was maintaining a high concentration of hydrogen peroxide in the presence of the iron. This is not easy, however, because this condition is coincident with a high rate of hydrogen peroxide decomposition. In order to be effective, efficient use of the reactive intermediates must be achieved. The other observation from the data is that relatively long reaction times (100 hours) under these conditions appear to be necessary in order to achieve a detectable change.

TABLE 12. SUMMARY OF CHEMICAL OXIDATION (FENTON'S REAGENT) TESTING

Experiment	Test/ Flask	Soil (g)	Water/ Soil Ratio	pH	Fe (% of Soil)	H_2O_2 Conc. (%) Average*	Time (Hours)	Percent Reduction of PCB Conc.
1[b]	85	50	0.8	3.6	0.5	2.5'	92	0
	86	50	3.1	3.3	0.5	0.7'	92	4
2	1	10	9.7	2.8	2.5	.07	162	45[d]
	2'	10	9.4	6.7-4.5	0	1.4	162	7
3	3A	8.0	8.4	2.5	0.1	1.8	118	54
	3B	8.1	9.5	2.2	0.5	0.87	118	45
4	1	170	10.1	3.1	.09	1.6	845'	34
5'	1	196	8.0	2.9	.09	0.88	184	14

* Time weighted average.
[b] Reaction mixtures were not continuously stirred.
[c] Hydrogen peroxide added at beginning of experiment and was not monitored or adjusted thereafter.
[d] As compared to control: Flask 2.
[e] Control reaction.
[f] No further decrease in PCB concentration observed after 211 hours.
[g] Surfactant addition (100 ppm Bio-Soft S-l 00) was made at 117 hours of experiment,

CONCLUSIONS AND RECOMMENDATIONS

Under controlled conditions and using relatively high reaction medium to soil ratios, PCB concentration reductions of up to 55 percent were achieved in reaction times on the order of 100 hours. These reactions were conducted at ambient temperature, but some heat was generated by decomposition of hydrogen peroxide which was most rapid at the beginning of the tests and when supplemental additions of hydrogen peroxide.were made.

PCB loss was not strongly affected by iron concentration in the range of 0.09 percent to 2.5 percent; however, the presence of iron (lowest concentration used in the tests was 0.09 percent of the soil) was required for meaningful effectiveness.

The most important parameter for PCB reduction was maintaining optimum hydrogen peroxide concentration (2 percent in the reaction solution) in the presence of iron at concentrations above 0.1 percent. These attempts are thwarted by high rates of hydrogen peroxide decomposition under these conditions.

The use of an alkyl benzenesulfonic acid at 100 ppm in the reaction solution had no detectable impact on the rate of PCB loss.

The loss of PCBs occurs predominantly through loss of lighter chlorinated, more volatile PCBs in a smooth trend to heavier chlorinated PCBs without generation of by-product peaks or PCB pattern alteration, as seen in the *UV* photolysis tests. This behavior suggests PCB loss is occurring via volatilization. Volatilization may occur during gas purging (foaming) of the solution from generation of oxygen by hydrogen peroxide decomposition. This process could be verified by conducting experiments in reaction vessels vented through activated carbon traps. Analysis of the carbon traps for PCBs would quantify loss through volatilization.

In order for Fenton's Reagent to be of significant use, the rate of reaction must be increased. The use of a solubilizing aid or surfactant to increase the solubility of PCBs has potential. The test described herein used a surfactant, which is relatively stable to oxidative systems, at low concentration. To further evaluate the impact of surfactant addition on this reaction, tests should be performed with higher concentrations of surfactants, 0.2 percent to 1 percent.

In addition, if the PCB reaction rate is limited by PCB solubility (mass transfer into solution), the rate of reaction would be more or less independent of soil PCB content. Moderate to low PCB concentration soils (100-500 ppm) would be detoxified at a faster rate than the high PCB content soil used in these

tests. Tests should be conducted on a lower PCB concentration soil, such as the PCB pit soil from Danville, Kentucky, to evaluate the effect on PCB reaction rate.

SECTION 5

BIOLOGICAL TREATMENT

INTRODUCTION

The primary objective of this investigation was to evaluate the effect of surfactant/W-treatment on aerobic, polychlorinated biphenyl (PCB) biodegradation. Aerobic biodegradation of the lower chlorinated PCB (1 - 3 chlorines) has been well-documented (Ahmed and Focht, 1973; Furukawa and Matsumura, 1976; Furukawa et ***al.,*** 1978; Shiaris and Sayler, 1982; Masse et ***al.,*** 1984; Brunner et ***al.,*** 1985; Sylvestre et ***al.,*** 1985; Barton and Crawford, 1988; Adrians et ***al.,*** 1989; Pettigrew et ***al.,*** 1990). However, the more highly chlorinated congeners are generally resistant to microbial attack although, there have been reports of microbial degradation of the highly chlorinated PCB congeners (greater than 4 chlorines) (Furukawa et ***al., 1978;*** Furukawa ***et al.,*** 1979; Bopp, 1986; Bedard et ***al.,*** 1987a; Bedard ***et al.,*** 1987b). In situ stimulation of PCB degradation has been shown for Hudson River sediments (Harkness et ***al.,*** 1993).

Biological degradation of PCB congeners is highly affected by chlorination pattern and the number of chlorines per biphenyl. Congeners chlorinated in the 2,4- and 2,6- positions are resistant to aerobic metabolism (Furukawa et ***al., 1978;*** Bedard and Haberl, 1990) . Further hindering microbial biodegradation of PCB is their hydrophobicity which inhibits their bioavailability. To increase the rate and extent of PCB biodegradation, two conditions are necessary. First, the bioavailability of the PCB should be increased and second, decrease the amount of chlorines per biphenyl ring. This study addresses the bioavailability and microbial attack of PCB after the combined surfactant/UV treatment of highly contaminated PCB soil. The theory behind this approach is that surfactants would render PCB bioavailable and surfactant/UV treatment would affect dechlorination, making the desorbed PCBs more amenable to biological treatment.

The surfactant/W treated soil used in these tests was residual soil from the 20 hour ***UV*** photolysis tests. The original source of this material was surface soil from the highly contaminated Texas Eastern Site in Danville, Kentucky. This material was fine ground prior to ***UV*** photolysis to pass a 230 mesh sieve (particle size less than 63 microns) and had 2 percent by weight of Hyonic **NP-90®** surfactant applied during the test. After ***UV*** photolysis, the PCB concentration was reduced to about half of the starting concentration (starting concentration was approximately 10,000 ppm PCBs: Aroclor 1248). Fine ground, untreated soil from this site was also provided for biological treatment.

EXPERIMENTAL DESIGN AND TEST OBJECTIVES

Physical dechlorination of weathered PCB-contaminated soil to produce material which would facilitate biological transformation of specific congeners was conducted. Materials produced were subjected to bench-scale biotreatability testing. The testing objectives included:

- Isolating PCB-degrading microbial species from environmental soil samples
- Determining the biological reduction of weathered PCB congeners in soil samples
- Determining impact of PCB-biodegradation inducers and growth substrates on congener reduction
- Determining the effectiveness of the combined physical and biological PCB treatment.

All test objectives were met during the course of the investigation.

PCB-contaminated soils treated with Hyonic NP-90® and exposed to UV light at 254 nm were employed during biotreatability testing. The investigation examined the biodegradability of the PCB in the surfactant/UV treated soil, the untreated soil, and a separate PCB contaminated soil known to have biological activity against PCB. The biotreatability laboratory-scale investigation was conducted in four separate phases to achieve the defined testing objectives. The four phases of investigation were:

- Phase 1 - Isolation of PCB-degrading bacterial cultures
- Phase 2 - Rapid PCB Screening Assay
- Phase 3 - Bioslurry evaluation
- Phase 4 - Enhanced bioslurry evaluation.

During Phase 1 testing, PCB-degrading organisms were isolated from impacted sail. In addition, known-PCB degrading microorganisms were obtained from General Electric Company (GE). Phase 2 used a Rapid PCB Screening Assay to further characterize isolates selected during Phase 1. The results of both phases were evaluated and bacterial cultures were selected for further testing.

The ability of selected organisms to biotransform PCB congeners in surfactant/UV-treated and untreated soil was evaluated during two bioslurry treatment experiments. Phase 3 experimentation evaluated the biological reduction of PCB congeners in surfactant/UV-treated and untreated soils. A following bioslurry experiment (Phase 4) evaluated the impact of

PCB-biodegradation inducer and growth substrate addition on congener removal.

All experiments were conducted under aerobic conditions and with adequate replication and experimental control to determine the effect of biological removal. All biological testing was conducted by IT personnel at IT's BAC and the University of Tennessee Center for Environmental Biotechnology (CEB). Both facilities operate under a State of Tennessee exemption for treatability testing.

MATERIALS AND METHODS

Isolation of PCB-Desraders

Isolation of PCB-degrading bacteria from untreated soils and from a New England Superfund Site was attempted. Bacteria demonstrating activity against biphenyl and PCB were found in the New England Superfund Site soil.

Cultures were isolated by mixing one gram of soil with 25 mLs of phosphate-buffered mineral salts medium, referred to as PAS medium (Bedard et ***al.***, 1987). This medium was augmented with biphenyl crystals (Mallinckrodt Inc., Paris, Kentucky) until saturation in the medium was reached. Biphenyl saturation in water at **25°C** is 7 milligrams per liter (mg/L).

The soil slurries were incubated at **25°C** and 200 revolutions per minute (rpm). Following 2 weeks of incubation, the culture was transferred, using sterile technique, to fresh PAS medium containing biphenyl crystals. The culture was incubated for one week. Following the second incubation period, the enrichment was plated on **R2A** agar (Difco Inc., Detroit, Michigan). Once growth appeared, the plates were sprayed with 2,3_dihydroxybiphenyl (2,3-dhb) in ether (0.1 percent weight:volume).

Colonies that turned yellow, indicating cleavage of 2,3-dhb, were restreaked on R2A medium. Several strains turned yellow and three were isolated for further characterization. These cultures were labelled BAC 15, BAC 17, and BAC 19.

Isolates were also characterized by colony hybridization using the bphC gene probe. This probe codes for the 2,3-dhb dioxygenase of ***Pseudomonas pseudoalcaligenes*** KF707. Bacterial colonies were transferred to Biotrans" Nylon Membranes (ICN Biomedical, Costa Mesa, California) and lysed with 0.5 Normal (N) **NaOH** for 5 minutes. Filters were allowed to dry and baked for 1 hour at 80°C. Purified probe was labeled with digoxigenin in a random primed reaction with the Genius DNA Labeling System (Boeringer Mannheim **Biochemicals,** Indianapolis, Indiana) following company protocols. Prehybridization, hybridization and detection of the digoxigenin probe was according to the Genius

DNA Labeling System (Boeringer Mannheim Biochemicals, Indianapolis, Indiana).

Ranid PCB Screening Assav

A Rapid Screening Assay for the determination of bacterial attack of specific PCB congeners has been developed (Bedard et *al.*, 1987). This assay was undertaken to aid in the selection of cultures for additional bioslurry investigations.

All cultures isolated were evaluated in the screening assay. In addition, Alcaligenes eutrophus H850 (H850) obtained from GE was used as the positive control because of its demonstrated activity against PCB (Bedard et *al., 1987). Pseudomonas putida 2440 (2440),* a non-PCB degrader obtained from the University of Tennessee CEB, was used as the negative control for the experiment.

Five bacterial cultures (i.e., BAC 15, BAC 17, BAC 19, H850, and 2440) were grown in PAS medium containing biphenyl and 0.005 percent yeast extract. The cultures were grown to an optical density of 1.0 at 615 nanometer (nm). Cells were harvested by centrifugation and washed twice with potassium phosphate buffer (pH 7.5). Cells were resuspended in potassium phosphate buffer to an optical density of 1.0 at 615 nm; this solution was identified as the culture solution.

Each culture solution (5 mL) was aseptically transferred into 5 sets of fifteen 40-mL glass vials. Each vial was spiked with 10 microliter (µL) of a 7-congener mixture. The congener mixture contained 2,4,4'-trichlorobiphenyl, 2,3,4-trichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, 2,3,5,6-tetrachlorobiphenyl, 2,2',3,3/-tetrachlorobiphenyl, 2,3',4',5-tetrachlorobiphenyl, and 3,3',4,4'-tetrachlorobiphenyl; congeners were obtained from AccuStandard, New Haven, Connecticut. The final concentration of all congeners in the treatment vial was approximately 10 mg/L. Biphenyl was also added at a concentration of approximately 40 mg/L. The following treatments were prepared.

Treatment Rl - BAC 15, 7-congener substrate, biphenyl

Treatment R2 - BAC 17, 7-congener substrate, biphenyl

Treatment R3 - BAC 19, 7-congener substrate, biphenyl

Treatment R4 - H850, 7-congener substrate, biphenyl (positive control)

Treatment R5 - 2440, 7-congener substrate, biphenyl (negative control)

Fifteen vials were established for each treatment. Three vials from each treatment set (15 total vials) were sacrificed at five time points. The time points were study initiation, 24 hours (hr), 48 hr, 94 hr, and 140 hr. Vials were extracted by sonication with 2 mL of pentane (Aldrich Chemical Co., Milwaukee, Wisconsin) for one minute in a Bransonic 220 Sonicator Bath.

Pentane extracts were analyzed by a Hewlett Packard 5890A Gas Chromatograph (GC) with an automatic sampler, ECD, splitless injector, and Supelco SPB-1 capillary Column [75 meter by 0.75 millimeter (internal diameter)].

Nitrogen was used as the carrier and make-up gas. The carrier gas flow was 2 milliliters per minute (mL/min) at 40°C. The make-up gas was introduced at 60 mL/min. During sample analysis, the GC oven initial temperature was 45°C. -This was held for one minute, raised to 150°C at a rate of 10°C/min and then to 300°C at a rate of 3°C/min. The 300°C temperature was held for 5 minutes.

Bioslurry Evaluation

Three PCB-contaminated soils were evaluated for biological reduction of PCB congeners. Soils employed were identified as untreated soil (Sample ID No. GG4202-1018-61), surfactant/UV-treated soil (Sample ID No. GG4202-1018-96A), and New England Superfund Site soil.

The following treatments were prepared:

Treatment Bl - surfactant/UV-treated soil, PAS medium, BAC 17 culture

Treatment B2 - surfactant/UV-treated soil, PAS medium, H850 culture

Treatment B3 - surfactant/UV-treated soil, PAS medium, Hydrochloric acid (Killed control)

Treatment B4 - Untreated soil, PAS medium, BAC 17 culture

Treatment B5 - Untreated soil, PAS medium, H850 culture

Treatment B6 - Untreated soil, PAS medium, Hydrochloric acid (Killed control)

Treatment B7 - New England soil, PAS medium, BAC 17 culture

Treatment B8 - New England soil, PAS medium, H850 culture

Treatment B9 - New England soil, PAS medium, Hydrochloric acid (Killed control)

Treatments B1, B4, and B7 were inoculated with BAC 17. Treatments B2, B5, and B8 were inoculated with H850. The cultures were grown in PAS medium as described in the isolation procedure.

Treatments were prepared using 2 g soil and 8 mL phosphate-buffered mineral salts medium. All treatments were prepared in 40-mL glass vials with a Teflon™-lined septum screw cap. Six vials per treatment were prepared, with duplicates sacrificed at 3 time points (i.e., study initiation, 2 weeks, and 4 weeks).

Microbial densities of the BAC 17 and H850 culture inoculum (optical density of 2.0 at 615 nm) were 6.2 x 10^7 and 9.3 x 10^8 colony-forming units per mL (CFU/mL), respectively. The cultures were added to the treatments at an optical density of 1.0 at 615 nm. The estimated cell concentration added to each vial was 3.1 x 10^7 and 4.7 x 10^8 CFU/mL for BAC 17 and H850, respectively. The main carbon source in all treatments was weathered PCB contamination in the soil.

Treatments B3, B6, and B9 were killed controls established for each soil evaluated. These treatments were maintained identically to all biologically-active treatments. Killed controls were established by the addition of 300 ul of 6 N hydrochloric acid (HCl) (Mallinkrodt Inc., Paris, Kentucky), resulting in a pH less then 1. No bacterial cultures were added to these treatments.

Treatments were shaken at 150 rpm at 25°C in the dark. Duplicate vials were sacrificed at study initiation (T_1), 2 weeks (T_2), and 4 weeks (T_4). Vials were extracted with 5 mL dichloromethane (DCM) (Burdick and Jackson, Muskegon, Kentucky) by sonication (Tekmar 375 watt Ultrasonic Disrupter) and analyzed for specific PCB congeners and total PCB. DCM was used instead of pentane due to the increased extraction efficiency achieved when soil was present.

After sonication, the solvent layer was separated using an IECCentra-4B Centrifuge (International Equipment Company). For improved analysis, the solvent layer was diluted for the surfactant/UV-treated and untreated soils due to the high PCB levels present in the soil (approximately 0.4 to 0.8 percent). The New England soils had PCB levels around 0.03 percent and were not diluted. Individual PCB congeners were analyzed by GC under the same conditions previously described.

To assure aerobic conditions in all treatments, oxygen measurements of vial headspace were made at Day 2, Day 4, Day 7,

and Day 11. Oxygen measurements were made using a modified galvanic cell.

Enhanced Bioslurry Evaluation

An additional study was initiated to look at the effects of specific inducers and growth substrate on the stimulation of PCB degradation. It has been shown in previous studies that the addition of biphenyl, 4-bromobiphenyl (4-BB), 4-chlorobiphenyl, 2-chlorobiphenyl, or other monochlorobiphenyls have induced and enhanced aerobic PCB biodegradation (Bedard et ***al., 1987;*** Furukawa, et ***al.,*** 1990; Layton ***et al.,*** unpublished; Pettigrew ***et al., 1990; Rhee et al.,*** 1989).

The objective of this investigation was to determine the effect of biphenyl and 4-BB (Fluka Ag, Buchf FG) addition on PCB biodegradation. Two PCB-contaminated soils were analyzed in this experiment (i.e., New England Superfund Site soil and the untreated soil).

Inducers (i.e., 4-BB and biphenyl) were dissolved in DCM and added to the treatment vials. The DCM was allowed to evaporate before introduction of soil to the treatment vials. Treatments were established using 2 grams of soil and 8 mL phosphate-buffered mineral salts medium.

Killed controls were established for each soil evaluated by the addition of 300 μL of 6 N HCl. Bacterial culture was also added to the killed controls to account for any PCB adsorption by bacterial cell walls.

Based on positive activity against PCB, BAC 17 was the only culture employed in this investigation. BAC 17 culture inoculum was added to the treatments at an optical density of 0.9 (615 nm). BAC 17 was grown following the procedure previously stated in Section 3.2. The inoculum added to each vial was 9.3 x 10^8 CFU/mL, dry weight of 7 milligram (mg).

Treatments were established in triplicate using 40-mL glass vials. The treatments were:

Treatment El - Untreated soil (Unamended)

Treatment E2 - Untreated soil, BAC 17, and 1,000 mg/L 4-BB

Treatment E3 - Untreated soil, BAC 17, and 1,000 mg/L biphenyl

Treatment E4 - Untreated soil, BAC 17, and hydrochloric acid (Killed)

Treatment E5 - New England soil (Unamended)

Treatment E6 - New England soil, BAC 17, and 1,000 mg/L 4-BB

Treatment E7 - New England soil, BAC 17, and 1,000 mg/L biphenyl

Treatment E8 - New England soil, BAC 17, and hydrochloric acid (Killed).

Six vials were established for Treatments El and E5. Three vials per treatment were sacrificed for initial analyses (T_0). The remaining 3 vials per treatment were analyzed at T_{final}. The initial analysis of Treatment El vials produced To data for untreated soil treatments. The initial analysis of Treatment E5 vials produced To data for New England Superfund Site soil treatments. Treatment vials were incubated at 25°C on a shaker table at 150 rpm in the dark. All remaining vials were sacrificed after one week.

Deviation in the extraction procedure described previously involved the addition of 300 µL of 6 N HCl to every treatment before extraction. This accounted for any differences in the extraction efficiency due to acid addition.

W-Photolysis

The surfactant/UV-treated soils were prepared in the photolytic study described previously. In general, the soils were ground to 200 mesh and treated with the surfactant Hyonic NP-90® (Henkel Co., Ambler, Pennsylvania) to a concentration of 2.1 percent (wt. NP-90 per wet wt. soil). The experimental set-up used a 450-watt Hanovia lamp with a parabolic reflector at a distance of 4 inches. The treated soil temperature did not rise above 52°C; overheating was prevented by cooling the lamp well. The soil was periodically raked and moistened throughout the process. The UV study demonstrated a decrease in the higher chlorinated PCB with a subsequent increase in the dichlorinated-PCB. See Section 3 for results.

Data Handling

Soils were evaluated initially and found to resemble an Aroclor 1248 standard profile. Therefore, soil concentration and percent degradation of PCB were calculated based on Aroclor 1248 equivalent. Equivalent 1248 is defined as the amount of Aroclor 1248 that it would take to produce a peak of the same size observed in the soil sample. Total PCB was determined for each sample by taking the average of the PCB congener 1248 equivalent. Equivalent concentrations were converted to equivalent mass by multiplying the equivalent concentration by the mass of the soil

used during analysis. Equivalent mass removed was determined by comparison of final data with To results.

Peak positions for 32 PCB congeners were established (Table 13) based on the pattern of Aroclor 1248 in commercially-available standards (Ultra Scientific Inc., Kingston, Rhode Island) and by published congener profile of Aroclor 1248 (Bedard et ***al.***, 1987). Percent degradation was calculated for each congener by direct comparison of its 1248 equivalent to that found in the killed controls. Percent degradation was normalized by subtracting the average percent degradation of the internal standard peaks. Internal standards were identified as Peaks 32 and -33. These peaks were chosen as internal standards due to their recalcitrant nature and used to adjust for abiotic loss of contaminant. Bedard et ***al.***, 1987 have shown that ***A. eutrophus*** H850 cannot degrade 2,4,5,3' '4, pentachlorobiphenyl (peak 31), **2,3,4,3',4'-pentachlorobiphenyl/2,3,4,2',3'6'-hexachlorobiphenyl** (peak. 32)' and 2,3,4,2' **,4',5'-hexachlorobiphenyl/2,3,5,6,3',4'-** hexachlorobiphenyl (peak 33). These peaks are traditionally used as internal standards to determine extraction efficiency and to determine biodgradation of other congeners. If the ratio of peak 32 to peak 33 changes then degradation of one of these congeners has occurred and they cannot be used as internal standards. Degradation of these peaks did not occur. Degradation of less than 15 percent was not considered significant based on analytical and instrument variation. Total percent loss was determined by comparison of the total average equivalent 1248 to that of the respective killed control.

Congener groups were also established based on the DCMA method. Retention time windows were determined for the di-PCB, tri-PCB, tetra-PCB, penta-PCB, hexa-PCB, and hepta-PCB. Percent loss of each group was determined by comparison of biologically-active treatments with the killed controls. Reduction was normalized by subtracting the average of internal standard Peaks 31 and 32 loss.

Hewlett Packard 5895A GC Chem Station Software system was used to analyze the data. A complete data package for all analyses conducted during Phases 3 and 4 is included in Appendix F.

RESULTS AND DISCUSSION

Isolation of PCB-Dearaders

Colony morphology of isolates BAC 15, BAC 17, and BAC '19 indicated small, off-white colonies with smooth edges. All isolates grew on biphenyl as the sole carbon and energy source. BAC 15 and 17 turned yellow after exposure to the compound, indicating biodegradation of 2,3-dhb, and hybridized with the

TABLE 13. CONGENER IDENTIFICATION

Peak No.	Congener Identification
1	2,5,2'- trichlorobiphenyl
2	2,4,2'-trichlorobiphenyl/4,4'- dichlorobiphenyl
3	2,3,2'-trichlorobiphenyl/2,6,4'-trichlorobiphenyl
415	2,5,4-trichlorobiphenyl/2,4,4'-trichlorobiphenyl
6	2,3,4-trichlorobiphenyl/2,5,2',6'-tetrachlorobiphenyl
7	2,3,4'-trichlorobiphenyl/2,4,2',6'-tetrachlorobiphenyl
8	2,3,6,2'-tetrachlorobiphenyl
9	2,3,2',6'-tetrachlorobiphenyl
10	2,5,2',5'-tetrachlorobiphenyl
11	2,4,2',5'-tetrachlorobiphenyl
12/13	2,4,3',4'-tetrachlorobiphenyl/2,4,5,2'-tetrachlorobiphenyl
14	2,3,2',5'-tetrachlorobiphenyl
15	3,4,4'-trichlorobiphenyl/2,3,2',4'-tetrachlorobiphenyl
16	2,3,4,2'-tetrachlorobiphenyl/2,3,6,4'-tetrachlorobiphenyl/2,6,3',4'-tetrachlorobiphenyl
17	2,3,2',3'-tetrachlorobiphenyl
18	2,4,5,4'-tetrachlorobiphenyl
19	2,5,3',4'-tetrachlorobiphenyl
20	2,4,2',4'-tetrachlorobiphenyl/2,3,6,2',5'-pentachlorobiphenyl
21	2,3,6,2',4'-pentachlorobiphenyl
22	2,3,3',4'-tetrachlorobiphenyl/2,3,4,4'-tetrachlorobiphenyl
23	2,3,6,2',3'pentachlorobiphenyl/2,3,5,2',5'-pentachlorobiphenyl
24	2,3,5,2',4'pentachlorobiphenyl/2,4,5,2',5'-pentachlorobiphenyl
25	2,4,5,2',4'-pentachlorobiphenyl
26	2,4,5,2',3'-pentachlorobiphenyl/2,3,5,6,2',6'-hexachlorobiphenyl
27	2,3,4,2',5'-pentachlorobiphenyl
28	2,3,4,2',4'-pentachlorobiphenyl
29	2,3,6,3',4'-pentachlorobiphenyl/3,4,3',4'-tetrachlorobiphenyl
30	2,3,4,2',3'-pentachlorobiphenyl
31	2,3,6,2',4',5'-hexachlorobiphenyl/2,4,5,3',4'-pentachlorobiphenyl
32	2,3,4,3',4'-pentachlorobiphenyl/2,3,4,2',3',6'-hexachlorobiphenyl
33	2,3,4,2',4',5'-hexachlorobiphenyl/2,3,5,6,3',4'-hexachlorobiphenyl

bphC gene of P. *pseudoalcaligenes* KF707 (Furukawa et *al.,* 1987). Fatty acid profiles identified the BAC 17 strain as *P. **cepacia*** subgroup B with a similarity index of 0.71 (Appendix G).

BAC 19 did not turn yellow after exposure to 2,3 dhb and was not tested further.

Rapid PCB Screening Assay

Congener percent reduction was determined for each culture evaluated (BAC 15, BAC 17, BAC 19, H850 and 2440) using the Rapid PCB Screening Assay. Data generated during the assay is included in Table 14. The test results were inconclusive, due to the substantial congener reduction exhibited by the negative control (2440). Therefore, growth characterization and hybridization with the *bphC* gene probe was used as criteria for selection of cultures for additional testing. Cultures selected for testing were BAC 17 and H850.

TABLE 14. RAPID PCB SCREENING ASSAY

Congener	Initial Concentration (ng/μl)	Percent Loss per Culture Tested				
		BAC15	BAC17	BAC19	H850	2440
2,4,4′-trichlorobiphenyl	1.20	93	92	93	67	75
3,4,2-trichlorobiphenyl	1.20	96	95	96	93	97
2,5,2′,5′-tetrachlorobiphenyl	1.20	98	98	98	98	86
2,3,5,6- tetrachlorobiphenyl	1.11	38	52	56	58	67
2,3,2′,3′ -tetrachlorobiphenyl	1.06	99	97	98	98	99
2,5,3,4-tetrachlorobiphenyl	1.20	91	92	91	89	83
3,4,3′,4′-tetrachlorobiphenyl	1.42	0.7	13	30	7	56

Bioslurry Evaluation

Three PCB-contaminated soils were evaluated for biological reduction of PCB congeners. Soils employed were identified as untreated soil (Sample ID No. GG4202-1018-61), surfactant/UV-treated soil (Sample ID No. GG4202-1018-96A), and New England Superfund Site soil.

Untreated, surfactant/UV-treated, and New England Superfund Site soils used in the bioslurry evaluation were analyzed for indigenous microbial populations. The microbial density of the untreated, surfactant/UV-treated, and New England soils were 6.9 x 10^5, 1.1 x 10^5, and less than 3.0 x 10' CFU/g, respectively. It

should be noted that, surfactant/UV-treatment reduced the microbial populations rather than sterilized the soil.

Dissolved organic carbon (DOC) was analyzed in the three soils evaluated to estimate the distribution of surfactant. The objective of this testing was to determine if surfactant distribution may have had an effect on the aerobic respiration and PCB removal in the varying treatments. The results indicated that approximately 10 times more DOC was found in the surfactant/UV-treated soil than in the untreated soil; no. appreciable DOC was measured in the New England soils. Surfactant/UV-treated, untreated, and New England Superfund Site soils had DOC concentrations of 2,500, 220, and 9 mg/kg, respectively.

Soil pH was also analyzed. Surfactant/UV-treated, untreated and New England soils demonstrated a slurry pH of 5.5, 6.3, and 7.0, respectively. All pH values were within the range acceptable for biological activity. Elevated DOC concentration and low pH in the surfactant/UV-treated soil was a result of surfactant use during the treatment process.

Initial analysis of the surfactant/UV-treated soil indicated total PCB concentrations of 4,000 mg/kg. This concentration was approximately 50 percent less than the untreated soil total PCB concentration of 8,400 mg/kg. The New England soil had 350 mg/kg total PCB.

Out of the 32 specific congeners monitored, there was minimal specific congener loss in the surfactant/UV-treated soil and untreated soil during this phase of experimentation as shown in Table 15. Percent removal was determined by comparison of biologically-active treatments to their respective control treatments. As expected, BAC 17 preferentially attacked lower chlorinated compounds consisting mostly of trichlorobiphenyls; some reduction in tetrachlorobiphenyls and reduction in one hexachlorobiphenyl congener was also observed (Figure 1). In the surfactant/UV-treated soil Treatment Bl, BAC 17 degraded 25, 22, 21, 22, and 20 percent of Peaks 1, 2, 6, 17, and 31, respectively. In the untreated soil Treatment B4, BAC 17 removed a greater quantity of the lower chlorinated species, demonstrating 58, 77, 27, and 46 percent reduction in Peaks 1, 2, 6, and 17, respectively, This isolate demonstrated increased activity in the New England soil Treatment B7; removal efficiencies ranging from 17 to 73 percent were measured for Peaks 1, 2, 4 through 15, 17, and 23 (Table 15).

H850 demonstrated reduced performance as compared to the BAC 17 culture. Treatment B2 established with surfactant/W-treated soil demonstrated a 24, 18, 18, 18, 15, 15, and 25 percent reduction in Peaks 1, 2, 4 through 7, 18, and 31, respectively. No significant removal was noted in Treatment B5 using untreated

soil. Optimum activity of H850 was illustrated in Treatment B8 which evaluated New England soil contaminant reduction. Peaks 1, 2, 6, 8 through 15, and 23 were preferentially attacked in this treatment, resulting in percent removals ranging from 18 to 40 percent (Table 15).

Both cultures demonstrated unusual degradation of Peak 31 in surfactant/UV-treated soils. In addition it should be noted that, BAC 17 exhibited 20, 46, and 50 percent degradation of Peak 17 in untreated, surfactant/UV-treated, and New England soils, respectively. Reduction of Peak 17, 2,3,2',3'-tetrachlorobiphenyl, was not demonstrated by H850 (Table 15).

Based on the DCMA method of classification of PCB congeners, BAC 17 treatment of New England soil (Treatment B7) illustrated optimal percent reduction as compared to all other treatments. Treatment B7 demonstrated di-, tri-, and tetra-chlorobiphenyl reductions of 70, 20, and 30 percent, respectively (Table 16). Treatment B4, which evaluated contaminant reduction in untreated soils using BAC 17, demonstrated appreciable loss of dichlorinated species at 67 percent. Reduced performance was measured in Treatment Bl using BAC 17 and surfactant/W-treated soils. (Note: Even though specific analysis of congeners indicated no dichlorobiphenyls present, the DCMA classification contains overlap between congener groups. Therefore, some of the trichlorobiphenyls are grouped with the dichlorobiphenyls, some of the tetrachlorobiphenyls are grouped with the trichlorobiphenyls, and so forth.)

TABLE 15. PERCENT SPECIFIC CONGENER PCB DEGRADATION BIOSLURRY EVALUATION

Peak No.	UV-Treated Soil		Untreated Soil		New England Soil	
	Treatment B1 (BAC 17)	Treatment B2 (H850)	Treatment B4 (BAC17)	Treatment B5 (H850)	Treatment B7 (BAC 17)	Treatment B8 (H850)
1	25*	24	58	0	67	39
2	22	18	77	0	73	40
3	0	0	0	0	0	0
4/5	0	18	0	0	17	0
6	21	18	27	0	37	22
7	0	15	0	0	26	0
8	0	0	0	0	31	23
9	0	0	0	0	34	18
10	0	0	0	0	15	23
11	0	0	0	0	34	22
12/13	0	0	0	0	33	21
14	0	0	0	0	47	20
15	0	0	0	0	37	18
16	0	0	0	0	0	0
17	22	0	46	0	50	0
18	0	15	0	0	0	0
19	0	0	0	0	0	0
20	0	0	0	0	0	0
21	0	0	0	0	0	0
22	0	0	0	0	0	0
23	0	0	0	0	24	21
24	0	0	0	0	0	0
25	0	0	0	0	0	0
26	0	0	0	0	0	0
27	0	0	0	0	0	0
28	0	0	0	0	0	0
29	0	0	0	0	0	0
30	0	0	0	0	0	0
31	20	25	0	0	0	0
32	0	0	0	0	0	0
33	0	0	0	0	0	0

* Percent degradation less than 15 percent is not considered significant and is reported as zero.

TABLE 16. PERCENT LOSS OF CONGENER GROUPS - DCMA METHOD BIOSLURRY EVALUATION

Congener Group	UV-Treated Soil		Untreated Soil		New England Soil	
	Treatment B1 (BAC 17)	Treatment B2 (H850)	Treatment B4 (BAC 17)	Treatment B5 (H850)	Treatment B7 (BAC 17)	Treatment B8 (H850)
Dichlorobiphenyl	24	21	67	0	70	40
Trichlorobiphenyl	0*	16	0	0	20	0
Tetrachlorobiphenyl	0	0	0	0	30	0
Pentachlorobiphenyl	0	0	0	0	0	0
Hexachlorobiphenyl	0	0	0	0	0	0
Heptachlorobiphenyl	0	0	0	0	0	0

* Percent degradation less than 15 percent is not considered significant and is reported as zero.

Similar to the results presented in Table 15, PCB removal based on the DCMA method illustrated reduced performance of H850 as compared to BAC 17 (Table 16). H850 preferentially attacked dichlorobiphenyls in surfactant/UV-treated and New England soils, i.e., Treatments B2 and B8. Biological removal of PCB congeners in the untreated soils by H850 was not evident. Organization of congener reduction using the DCMA method demonstrated results similar to those obtained through congener specific analyses.

Respiration is a measurement of oxygen consumption by the bacteria, indicating microbial activity. Oxygen consumption was measured by loss of oxygen in the headspace over time. Oxygen consumption was greater in the treatments containing biologically-active cultures compared to the killed controls, where respiration was insignificant. Treatments B2, B5, and B8 containing H850 demonstrated respiration rates of 2.5, 1.5, and 1.0 milligram oxygen/kilogram-hour (mg O_2/kg-hr) at 48, 96, and 168 hours, respectively. Oxygen consumption remained at 1.0 mg O_2/kg-hr through 264 hours. In Treatments B1, B4, and B7 containing BAC 17 culture, oxygen consumption was 2.4, 1.2, 1.0, and 0.7 mg O_2/kg-hr at 48, 94, 168, and 264 hours, respectively. Oxygen consumption data indicated that the majority of oxygen demand was satisfied during the first 2 days of incubation (Table 17). Respiration rates were similar in all biologically-active treatments, although PCB removal rates varied across treatments. Initial oxygen concentrations in the headspace were considered to be 300 mg/L (atmospheric concentration).

TABLE 27, OXYGEN CONSUMPTION IN TREATMENTS
BIOSLURRY EVALUATION

Time	Oxygen Consumed (mg O_2/kg-hr)	
	H850	BAC 17
48 hours	2.5	2.4
96 hours	1.5	1.2
168 hours	1.0	1.0
264 hours	1.0	0.7

Enhanced Bioslurry Evaluation

The objective of this investigation was to determine the effect of biphenyl and 4-BB (Fluka Ag, Buchf FG) addition on PCB biodegradation. Two PCB-contaminated soils were analyzed in this experiment (i.e., New England Superfund Site soil and untreated soil).

BAC 17 culture was added to all treatments evaluated during the Phase 4 investigation. Unamended controls for New England and untreated soil did not receive bacterial culture; congener removal in these treatments were adjusted for abiotic losses evident in the killed controls, i.e., Treatments E4 and E8. Bacterial culture was added to the killed controls to determine its effect on PCB adsorption.

Treatments established with New England soil were identified as Treatments E5, E6, and E7. Treatment E5 was the unamended control for the experimental set. Treatment E6, which received 4-BB, demonstrated substantial removal of Peaks 2, 4 through 7, 10 through 15, 17, 43, and 22 (Table 18). In comparison to Treatment B7 (Table 15) of the bioslurry investigation, approximately a two-fold increase in congener removal was demonstrated in the majority of higher chlorinated congeners, i.e., Peaks 10 through 15, 17, 19, and 23. Biphenyl addition to Treatment E7 resulted in substantial increases in congener removal efficiency. Removal efficiencies ranging from 28 to 100 percent were noted in Peaks 1 through 17, 19, and 23. Once again this was a notable increase in congener removal as compared to Treatment B7 which did not receive a growth substrate/metabolic inducer. The unamended control (Treatment E5) demonstrated a moderate reduction (i.e., 22 percent) of Peak 17 (Table 18). Increased congener removal in comparison to Treatment 87 is illustrated in Table 19.

TABLE 18. PERCENT SPECIFIC CONGENER PCB DEGRADATION ENHANCED BIOSLURRY EVALUATION

Peak No.	Untreated Soil (Percent Removal)			New England Soil[a,b] (Percent Removal)		
	Treatment E1 (Unamended)	Treatment E2 (4-BB)	Treatment E3 (Biphenyl)	Treatment E5 (Unamended)	Treatment E6 (4-BB)	Treatment E7 (Biphenyl)
1	0	21	28	0	0	59
2	0	38	45	0	66	100
3	0	0	0	0	0	28
415	0	0	0	0	30	46
6	0	0	15	0	34	87
7	0	0	0	0	75	85
8	0	0	0	0	0	73
9	0	0	0	0	0	38
10	0	0	0	0	38	47
11	0	0	0	0	60	65
12/13	0	0	0	0	53	71
14	0	0	0	0	94	98
15	0	0	0	0	66	64
16	0	0	0	0	0	27
17	0	22	31	22	100	9.1
18	0	0	0	0	0	0
19	0	0	0	0	43	39
20	0	0	0	0	0	0
21	0	0	0	0	0	0
22	0	0	0	0	0	0
23	0	21	0	0	43	38
24	0	0	0	0	0	0
25	0	0	0	0	0	0
26	0	0	0	0	0	0
27	0	0	0	0	15	0
28	0	0	0	0	0	0
29	0	0	0	0	0	0
30	0	0	0	0	0	15
31	0	0	0	0	0	0
32	0	0	0	0	0	0
33	0	0	0	0	0	0

[a] Percent degradation less than 15 percent is not considered significant and is not reported.

[b] All treatments evaluated used 8AC 17 culture inoculum.

TABLE 19. COMPARISON OF PERCENT SPECIFIC CONGENER PCB DEGRADATION WITH AND WITHOUT BIPHENYL AUGMENTATION

Peak No.	New England Soil (Percent Removal)		
	Treatment E5 (Unamended)	Treatment B7 (BAC 17)	Treatment E7 (Biphenyl)
1	0	67	59
2	0	73	100
3	0	0	28
4/5	0	17	46
6	0	37	87
7	0	26	85
8	0	31	73
9	0	34	38
10	0	15	47
11	0	34	65
12/13	0	33	71
14	0	47	98
15	0	37	64
16	0	0	27
17	22	50	91
18	0	0	0
19	0	0	39
20	0	0	0
21	0	0	0
22	0	0	0
23	0	24	38
24	0	0	0
25	0	0	0
26	0	0	0
27	0	0	0
28	0	0	0
29	0	0	0
30	0	0	15
31	0	0	0
32	0	0	0
33	0	0	0

The significant benefit of inducer and growth substrate addition, specifically biphenyl, in increasing PCB removal was

not demonstrated in the untreated soil treatments. Treatment E2 received 4-BB as a metabolic inducer. This treatment demonstrated reduced performance in comparison with Treatment B4 (untreated **soil/BAC** 17). Likewise, the addition of biphenyl to Treatment E3 did not significantly improve PCB congener removal over that evident in Treatment B4. The unamended (Treatment El) demonstrated no removal of any PCB congener.

Congener removal was also determined based on the DCMA method. The New England soil Treatment E7 demonstrated a.82, 54, 63, and 16 percent reduction in the di-, tri-, tetra-, and penta-PCB, respectively (Table 20). Treatment E6 showed a 28, 29, and 21 percent reduction in the di-, tetra-, and penta-PCB, respectively. Unamended Treatment E5 showed no significant loss of specific congeners.

TABLE 20. PERCENT LOSS OF CONGENER GROUPS - DCMA METHOD ENHANCED BIOSLURRY EVALUATION

Congener group	Untreated Soil			New England Soil		
	Treatment E1 (Unamended)	Treatment E2 (4-BB)	Treatment E3 (Biphenyl)	Treatment E5 (Unamended)	Treatment E6 (4-BB)	Treatment E7 (Biphenyl)
Dichlorobiphenyl	0	29	37	0	28	82
Trichlorobiphenyl	0	0	0	0	0	54
Tetrachlorobiphenyl	0	0	0	0	29	63
Pentachlorobiphenyl	0	0	0	0	21	16
Hexachlorobiphenyl	0	0	0	0	0	0
Heptachlorobiphenyl	0	0	0	0	0	0

DCMA results illustrated no significant congener loss in untreated soil Treatment El. Treatments E2 and E3 demonstrated 29 and 37 percent reduction of di-PCB, respectively (Table 19). There was no significant:loss of the higher chlorinated PCB in Treatments El, E2, and E3.

Oxygen uptake was measured in all treatments at 48 and 96 hours (Table 21). At 48 hours, BAC 17 demonstrated less than 0.3, 1.4, and 0.6 mg O_2**/kg-hr** in Treatments El, E2, and E3, respectively. After 96 hours, an increase in oxygen consumption of 0.5, 2.1, and 1.8 mg O_2**/kg-hr** was noted in Treatments El; E2, and E3, respectively. At 48 hours, respiration in Treatments E6 and E7 was measured at 2.3 mg and 1.8 mg O_2**/kg-hr,** respectively. The New England soil treatments demonstrated no appreciable oxygen consumption at 96 hours.

TABLE 21. OXYGEN CONSUMPTION
ENHANCED BIOSLURRY EVALUATION

Treatment Identification	Oxygen Consumed (mg O_2/kg-hr)	
	48 hours	96 hours
Treatment El (Untreated/Unamended)	co.3	0.5
Treatment E2 (Untreated/4-BB)	1.4	2.;
Treatment E3 (Untreated/Biphenyll	0.6	1.8
Treatment E4 (Untreated/Killed)	co.3	co.2
Treatment E5 (New England/Unamended)	co.3	co.2
Treatment E6 (New England/4-BB)	2.3	co.2
Treatment E7 (New England/Biphenyl)	1.8	0.3
Treatment E8 (New England/Killed)	<0.3	<0.2

CONCLUSIONS AND RECOMMENDATIONS

Several obstacles exist to biodegradation of complex mixtures of PCBs in soil. First, bioavailability is a significant problem. If bacteria cannot come in contact with the substrate, the substrate cannot be metabolized. PCBs are very hydrophobic and sorb readily to surfaces; therefore, in any biological treatment scenario, desorption of the PCBs is a primary concern.

Second, the highly chlorinated congeners are resistant, generally, to biological degradation. Two enzymes are thought to mediate the initial biotransformations of the lower chlorinated congeners, biphenyl 2,3-dioxygenase and biphenyl 3,4-dioxygenase. Highly chlorinated congeners may cause steric hindrance of these two enzymes inhibiting the initial hydroxylation step (Abramowicz, 1990; Parsons et *al., 1988).* Although certain bacteria have demonstrated an ability to cometabolize the highly chlorinated congeners, extensive aerobic degradation has not been observed in the environment (Bedard et *al.,* 1987a; Bopp, 1986) although this may be due to the lack of bioavailability, cosubstrates, or thermodynamically unfavorable degradative pathways.

Third, the inducers of the biphenyl operon must be present to maintain PCB-degrading activity. Normally, biphenyl and the lower chlorinated congeners will be degraded first. Biphenyl, 2-chlorobiphenyl, 4-chlorobiphenyl and 4-bromobiphenyl have been shown to induce the biphenyl operon (Bedard, 1993; Furukawa, **et** *al.,* 1990; Pettigrew et *al.,* 1990; Rhee et *al.,* 1989; Bedard et *al.,* 1987; Layton et *al.,* unpublished data). This group is also

responsible for induction of the biphenyl operon. When the inducers disappear from the environment, no further PCB degradation would be expected. Addition to a system of non-toxic, degradable inducers of the biphenyl operon may increase the extent of degradation of.the highly chlorinated congeners. Continuous feeding of biphenyl to a PCB-contaminated soil in a batch slurry bioreactor has given preliminary results of 1,000 mg/kg biphenyl to degrade approximately 10 mg/kg PCB (Layton, personal communication).

Phase 4 of this investigation studied the effect of the combined surfactant/UV treatment on biological degradation of weathered PCBs. In the bioslurry evaluation test strain H850 degraded 21 and 16 percent of the di- and tri-chlorobiphenyls (Table 16), respectively, in the treated soil while no significant degradation was observed in the untreated soil. The opposite situation was observed with strain BAC 17. Sixty-seven percent degradation of the dichlorobiphenyl was degraded in the untreated soil versus 24 percent in the treated soil. The treated soil contained 2,480 mg/kg DOC versus 250 mg/kg in the untreated soil. This indicates that high amounts of surfactant were carried through the treatment process and may be inhibitory to bacterial activity or promote non-PCB degrading activity. Likewise, the treated soil had a pH of 5.5 which probably was a result of the surfactant. An additional soil washing step may be necessary to remove/recycle surfactant from the soil and neutralize the pH before biological treatment.

Strain BAC 17 removed approximately 30 percent of the tetrachlorobiphenyls (as defined by the DCMA method) from the New England soil (Table 16). BAC 17 was originally isolated from this soil and was expected to perform well.

Augmentation of biphenyl and 4-BB to the New England soil stimulated biodegradation of the di-, tri-, tetra-, and pentachlorobiphenyls (Tables 18 and 19). Biphenyl was the better growth‘substrate and inducer of PCB degradation than the 4-BB under these conditions. The untreated soil showed stimulation of only the lower chlorinated congeners. Why degradation was not as extensive as in the surfactant/UV-treated soil is not understood. The New England soil with a higher bacterial activity against PCBs, was composed mainly of sands while the GG4202 soils, with a low bacterial activity against PCBs, had a strong clay component. Similar observations were made using a clayey PCB-contaminated soil from a transformer substation. Correlation of PCB-degrading activity with soil type, PCB concentration and composition, biphenyl/PCB concentrations, and bacterial populations needs to be explored.

In addition, if 1,000 mg/kg biphenyl (or, possibly, any other inducer) is required to reduce the total PCB concentration 10 mg/kg as suggested earlier (Layton, personal communication),

the loss of PCBs in the untreated soil would be masked by the analytical variability.

Specific conclusions from this study are:

PCB removal in the surfactant/UV-treated soil was slightly higher when augmented with strain H850.

PCB removal in the untreated and the New England soil was enhanced by augmentation with strain BAC 17.

Biphenyl was more effective at stimulating PCB degradation than 4-BB in the untreated and the New England soil.

Surfactant treatment may have been inhibitory to microbial activity as evidenced by the high DOC and low of the treated soil.

SECTION 6

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APPENDIX A
TCDD ANALYTICAL REPORTS

TCDD UV PHOTOLYSIS SAMPLE ANALYSIS CROSS-REFERENCE FOR KEY SAMPLES

Sample	Sample No.
TCDD Starting Soil	GG3866/67 Composite
Test 1 Final Soil (48 Hours)	684-14-2A, 684-14-2B
Test 2 Final Soil (48 Hours)	684-18-2A
Test 3 Final Soil (48 Hours)	684-36-1A
Test 4 Final Soil (48 Hours	684-39-IA

CENTRAL FILES

Project No. 483000

Sent By ESA Date 4/3/91

Return Copy to Sender AG

INTERNATIONAL TECHNOLOGY CORPORATION

CERTIFICATE OF ANALYSIS

ITAS-Knoxville
304 Directors Drive
Knoxville, TN 37923
Attention: Mr. Ed Alperin

March 28, 1991

ITAS-Knoxville Project Number: Site ETP-03/483000

This is the Certificate of Analysis for the following:

Project Number:	217-92
Date Received by Lab:	February 26, 1991
Number of Samples:	Eighteen (18)
Sample Type:	Eiuhteen (18) Soil

Introduction

On March 26, 1991, eighteen (18) samples were received at ITAS - St. Louis laboratory from ITAS-Knoxville. The list of analytical tests performed, as well as receipt and analysis, can be found in the attached report. We were instructed to only analyze samples below. The samples were labeled as follows:

Soil samples:	1407-001	684-ll-1A
	1407-002	684-ll-4A
	1407-003	684-12-1A
	1407-004	684-13-1A
	1407-005	684-14-1A
	1407-006	684-14-2A
	1407-007	684-15-1A
	1407-008	684-16-1A
	1407-009	684-16-2A
	1407-010	684-17-1A
	1407-011	684-18-1A
	1407-012	684-18-2A
	1407-013	684-19-1A
	1407-014	684-19-4A
	1407-015	684-34-1A
	1407-016	684-34-3A
	1407-017	684-35-1A
	1407-018	684-36-1A

II Methodology Modification

Background

According to Hr. D. Hesse, Organic Technical Director; samples received from Project 217-92 have been very high in dioxin, requiring about a 1:25 dilution prior to GC/MS analysis. In order to maintain the highest accuracy possible, the following dilution procedure was adopted.

Dilution Procedure

1) The sample extraction weight is reduced from 10grams to 5grams. This accounts for a 1:2 dilution.

2) Internal standard and surrogate concentrations are added at 12.5 times their normal levels. 100-ml of extract solvent (acetone and hexanes) are added to the sample then the sample/solvent is shaken as normal.

   The sample is allowed to settle, then 8-mls of the supernatant extract is pipetted off the sample and placed onto the cleanup columns, etc. This accounts for a 12.5 dilution.

This procedure, then, dilutes the sample 25 times, but results in normal concentrations of the internal standard and surrogate standard in the final extract.

Calculations

Calculations are performed using the normal DBASE program, LRGCMS, except that the sample weight is divided by 12.5 prior to entry into the program to account for the 12.5 times greater internal standard concentration. This avoids having to modify the program.

III Reoroducibilitv Problems

IIIa Backsround

Duplicate analyses of sample 1407-006, using the 25-fold dilution procedure, has resulted in poor reproducibility, giving results of 174 and 268 ng/gm.

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CORPORATION

ITAS-Knoxville
March 28, 1991
Project Number: 217-92

IIIb Discussion

Typical reproducibility for the Region 7 methodology is less than 10%. Therefore, we do not suspect that the problem lies in the GC/MS analysis, but rather lies either in the problems associated with obtaining two equivalent aliguots of the sample or with varying extraction abilities.

To test the latter, extraction of the same two aliquots of 1407-006, which had previously given 174 and 268 ng/gm, was continued by sonicating the sample solvent for 10 minutes each. After the sonication (and setting in solvent overnight), the samples had become fine powders, as opposed to the half-pea size lumps previously observed. Concentrations of dioxin were determined at 238 and 356 ng/gm, respectively. The percentage increase was the same for both. This test shows that extraction, cleanup and GC/MS analyses are reproducible (based on the equivalent increases). However, it also shows that ultrasonication for these samples is important.

IIIc Conclusion

The irreproducibility lies in the inability to obtain a homogeneous sample, which we assign to clumpiness of the sample.

Our best alternative is to analyze each sample in total, so that no aliquot discrepancies are possible. However, this would not eliminate sampling errors from occurring at the sample site.

Reviewed and Approved:

Sally A. Lane
Project Manager

ITAS-Knoxville
304 Directors Drive
Knoxville, TN 37923

PROJECT NO.: 217-92

CATEGORY : DIOXIN
METHOD : Region VII
MATRIX : SOIL
SAMPLE DATE: 02/26-03/20/91

REPORT DATE : 03/29/91
DATE RECEIVED : 03/26/91
DATE EXTRACTED: 03/27-28/91

Client ID	LAB ID	PARAMETER	DATE ANALYZED	DETECTION LIMIT NG/GM	CONC NG/GM
684-11-1A	1407-001	TCDD	03/28/91	3.75	200.1
684-11-4A	1407-002	TCDD	03/28/91	3.75	208.4
684-12-1A	1407-003	TCDD	03/28/91	3.75	157.6
684-13-1A	1407-004	TCDD	03/28/91	3.75	133.6
684-14-1A	1407-005	TCDD	03/28/91	3.75	191.4
684-14-2A	1407-006	TCDD	03/28/91	3.75	174.4
684-14-2A	1407-006 DUP	TCDD	03/28/91	3.75	267.7
NA	BLK8694A	TCDD	03/28/91	3.75	ND
NA	BLK8694B	TCDD	03/28/91	3.75	ND
NA	SPK8694	TCDD	03/28/91	NA	101 %
684-15-1A	1407-007	TCDD	03/28/91	3.75	251.9
684-16-1A	1407-008	TCDD	03/28/91	3.75	255.0
684-16-2A	1407-009	TCDD	03/28/91	3.75	258.9
684-17-1A	1407-010	TCDD	03/28/91	3.75	238.4
684-18-1A	1407-011	TCDD	03/28/91	3.75	424.9*
684-18-2A	1407-012	TCDD	03/28/91	3.75	238.9

NOTES: NA=NOT APPLICABLE; ND=NOT DETECTED

ITAS-Knoxville
304 Directors Drive
Knoxville, TN 37923

PROJECT NO.: 217-92

CATEGORY : DIOXIN
METHOD : Region VII
MATRIX : **SOIL**
SAMPLE DATE: 02/26-03/20/91

REPORT DATE : **03/29/91**
DATE RECEIVED : **03/26/91**
DATE EXTRACTED: 03/27-28/91

Client ID	LAB ID	PARAMETER	DATE ANALYZED	DETECTION LIMIT NG/GM	CONC NG/GM
684-ll-1A	**1407-001**	**TCDD**	03/28/91	3.75	200.1
684-ll-4A	**1407-002**	**TCDD**	03/28/91	3.75	208.4
684-12-1A	**1407-003**	**TCDD**	03/28/91	3.75	157.6
684-13-1A	**1407-004**	**TCDD**	03/28/91	3.75	133.6
684-1401A	**1407-005**	**TCDD**	03/28/91	3.75	191.4
684014-2A	**1407-006**	**TCDD**	03/28/91	3.75	174.4
684-14-2A	**1407-006 DUP**	**TCDD**	03/28/91	.75	267.7
NA	BLK8694A	TCDD	03/28/91	3.75	**D**
NA	BLK8694B	TCDD	03/28/91	3.75	**ND**
NA	SPK8694	TCDD	03/28/91	NA	**101 %**
684015-1A	1407-007	TCDD	03/28/91	3.75	**251.9**
684-16-1A	1407-008	TCDD	03/28/91	3.75	**255.0**
684-16-2A	1407-009	TCDD	03/28/91	3.75	**258.9**
684-17-119	1407-010	TCDD	03/28/91	3.75	**238.4**
684-18-1A	1407-011	TCDD	03/28/91	3.75	424.9
6840la-2A	1407-012	TCDD	03/28/91	3.75	**238.9**

NOTES: NA=NOT APPLICABLE; ND=NOT DETECTED

ITAS-Knoxville
304 Directors Drive
Knoxville, TN 37923

PROJECT NO.: 217-92

CATEGORY : DIOXIN
METHOD .Region VII
MATRIX : SOIL
SAMPLE DATE: 02/26-03/20/91

REPORT DATE : 03/29/91
DATE RECEIVED : 03/26/91
DATE EXTRACTED:03/27-28/91

Client ID	LAB ID	PARAMETER	DATE ANALYZED	DETECTION LIMIT NG/GM	CONC NG/GM
684-18-2A	1407-012 DUP	TCDD	03/28/91	3.75	471.5*
684019-1A	1407-013	TCDD	03/28/91	3.75	263.7
684-19-4A	1407-014	TCDD	03/28/91	3.75	249.3
684-34-1A	1407-015	TCDD	03/28/91	3.75	286.8
684-34-3A	1407-016	TCDD	03/28/91	3.75	247.8
684-35-1A	1407-017	TCDD	03/28/91	3.75	218.1
684-36-1A	1407-018	TCDD	03/28/91	3.75	224.3
684-36-1A	1407-018 DUP	TCDD	03/28/91	3.75	274.6
NA	BLK8727A	TCDD	03/28/91	3.75	ND
NA	BLK8728B	TCDD	03/28/91	3.75	ND
NA	SPK8728	TCDD	03/28/91	NA	102 %

*Concentration reported greater than curve level. Sample already diluted 1:25, further dilution would be prohibitive based on cost of standards.

NOTES: NA=NOT APPLICABLE; ND=NOT DETECTED

IT Analytical Services • St. Louis

Page 1 of 2

Login No. 1407

Date 3-26-91

Project No. 217-92

Sample Arrival Date 3-26-91

Expected No. of Samples 18

Expected Matrix SOIL

Client IT-Knoxville

Proj. Mgr. S. LANE

Shipper Fed Ex

Due Date 4-24-91

DISTRIBUTION

AUTO
ICAP
I-PREP
O-PREP
GC
GCMS
RAD
PROJ MGR
OTHER

ORIGINAL Sally

REVISION

STORAGE/LOCATION

[illegible]

*ICAP: AG AL AS B BA BE BI CA CD CO CR CU FE K

LI MG MN MO NA NI P PB SB SE SI SN SR TE

TI TH TL U V ZN ZR

*GFAA: AS SB PB SE TL

Sample No.	1407/001	1407/002	1407/003	1407/004	1407/005	1407/006	1407/007	1407/008	1407/009	1407/010
Samp ID No.	684-	684-	684-	684-	684-	684-	684-	684-	684-	684-
Description	11-1A	11-4A	12-1A	13-1A	14-1A	14-2A	15-1A	16-1A	16-2A	17-1A
Sample Date	2-26-91	→	2-27-91	2-28-91	3-1-91	3-4-91	3-5-91	3-6-91	→	3-7-91
Matrix	Soil	→	→	→	→	→	→	Soil	→	Soil

LIMS ANAL CODE	DESCRIPTION	CONTAINER	PRESERVATIVE	001	002	003	004	005	006	007	008	009	010
Dixin/TCDD7/21	Reg 7	40mL	Cold	X	X	X	X	X	X	X	X	X	X
P-Dixin//Q2	Gen. Dioxin prep	↓		X	X	X	X	X	X	X	X	X	X
Data/Stdrep/12	Std rep	↓		X	X	X	X	X	X	X	X	X	X
PM//01			3-26-91 [illegible]	X	X	X	X	X	X	X	X	X	X

DISPOSAL:

NORMAL

HAZARDOUS

DATE TO DISPOSE

PM SIGNATURE

Comments:

IT Analytical Services - St. Louis

LOGIN NO. ______

Date	3-26-91	Client	IT-Knoxville
Project No.	217-92	Proj. Mgr.	S. LANE
Sample Arrival Date	3-26-92	Shipper	Fed. Ex.
Expected No. of Samples	18	Due Date	4-24-91
Expected Matrix	SOIL		

DISTRIBUTION: AUTO ___ ICAP ___ I-PREP ___ O-PREP ___ GC ___ GCMS ___ RAD ___ PROJ MGR ___ OTHER ___

ORIGINAL ______

REVISION ______

STORAGE/LOCATION ______

*ICAP: AG AL AS B BA BE BI CA CD CO CR CU FE K
LI MG MN MO NA NI P PB SB SE SI SN SR TE
TI TN TL U V ZN ZR

*GFAA: AS SB PB SE TL

Sample No.	1407/011 6-8	1407/012	1407/013	1407/014	1407/015	1407/016	1407/017	1407/018		
Samp ID No.	684-	684-	684-	684-	684-	684-	[illegible]	684-		
Description	18-1A	18-2A	19-1A	19-4A	34-1A	34-3A	[illegible]	[illegible]		
Sample Date	3-11-91	3-12-91	3-13-91	→	3-14-91	3-15-91	[illegible]	[illegible]		
Matrix	Soil	→	→	→	→	→	→	→		

LIMS ANAL CODE	DESCRIPTION	CONTAINER	PRESERVATIVE										
Dixin/TCDD7/01	Reg 7	40ml L.HL	(cil)	X	X	X	X	X	X	X	X		
P-Dixin//LE2	Gen. Dixin prep	↓		X	X	X	X	X	X	X	X		
Data/StdRep/01	StdRep	↓		X	X	X	X	X	X	X	X		
PM//01	PERCENT MOIST		MT	X	X	X	X	X	X	X	X		

DISPOSAL:

NORMAL ______

HAZARDOUS ______

DATE TO DISPOSE ______

PM SIGNATURE ______

Comments: ______

INTERNATIONAL TECHNOLOGY CORPORATION

CHAIN-OF-CUSTODY RECORD

R/A Control No 23385 7

C/C Control No A 93221

PROJECT NAME/NUMBER [illegible]

LAB DESTINATION [illegible]

SAMPLE TEAM MEMBERS A. GROEN

CARRIER/WAYBILL NO FED EX

Sample Number	Sample Location and Description	Date and Time Collected	Sample Type	Container Type	Condition on Receipt (Name and Date)	Disposal Record
684-11-1A	-untreated soil	2/26/91	SOIL	VIAL		
684-11-4A	4 hr treatment	2/26/91	↓	↓		
684-12-1A	6 hr treatment	2/27/91	↓	↓		
684-13-1A	-16 hr treatment	2/28/91	↓	↓		
684-14-1A	30 hr treatment	3/1/91	↓	↓		
684-14-2A	48 hr treatment	3/4/91	↓	↓		

Special Instructions: USE METHOD DEVELOPED FOR PROJECT SAMPLES # 1A & 1A [illegible]

Possible Sample Hazards: ALL SAMPLES CONTAIN TCDD ARE AT HOLDING [illegible] LIMIT - NEED TO BE EXTRACTED IMMEDIATELY IF POSSIBLE LIMIT IS 30 DAY ACCORDING TO [illegible]

SIGNATURES: (Name, Company, Date and Time)

1. Relinquished By: ITAS/TDL [signature] 3/25/91

   Received By: [signature] 3-26-91 8:40AM

2. Relinquished By:

   Received By:

3. Relinquished By

   Received by:

4. Relinquished By,

   Received By

WHITE - To accompany samples

INTERNATIONAL TECHNOLOGY CORPORATION

REQUEST FOR ANALYSIS

RIA Control No 233337
C/C Control No A9322

PROJECT NAME SITE TYPE 3
PROJECT NUMBER 483000
PROFIT CENTER NUMBER 4622
PROJECT MANAGER E.S. ALPERIN
BILL TO

PURCHASE ORDER NO. 4622-0391-036

DATE SAMPLES SHIPPED 3/25/91
LAB DESTINATION ITAS ST LOUIS
LABORATORY CONTACT SHELLY LAMB
SEND LAB REPORT TO E S ALPERIN
301 DIRECTORS
KNOXVILLE 379
DATE REPORT REQUIRED 4/25/91
PROJECT CONTACT ARIE GREEN
PROJECT CONTACT PHONE NO. 615-690-3211 ext 281

Sample No.	Sample Type	Sample Volume	Preservative	Requested Testing Program	Special Instructions
684-11-1A	SOIL	10-30 g	NONE (REF.)	2,3,7,8 TCDD only	USE MODIFIED
684-11-4A					TO METHOD
684-12-1A					DEVELOPED
684-13-1A					EARLIER
684-14-1A					PROT # 217-92
684-14-2A					

TURNAROUND TIME REQUIRED: (Rush must be approved by the Laboratory Project Manager)
Normal X Rush _____ (Subject to rush surcharge)

QC LEVEL (Levels II and III subject to surcharge; project specific requirements must be submitted to lab before beginning work)
I X II _____ III _____ Project Specific

POSSIBLE HAZARD IDENTIFICATION. (Please indicate if sample(s) are hazardous materials and/or suspected to contain non-hazardous substances)
Non-hazard _____ Flammable _____ Skin Irritant _____ Highly Toxic X TCDD Other _____ (Please Specify)

SAMPLE DISPOSAL (Please indicate disposition of sample following analysis. Lab will charge for packing, shipping, archive and storage)
Return to Client X Disposal by Lab _____ Archive _____ (Indicate number of months)

FOR LAB USE ONLY
Received by Mary P L
Date/Time 3-26-5 [illegible]

INTERNATIONAL TECHNOLOGY CORPORATION

CHAIN-OF-CUSTODY RECORD

R. A Control No 233688

C. C. Control No. A 33222

PROJECT NAME/NUMBER SITE ETP03 / 483000

LAB DESTINATION ITAS / ST LOUIS

SAMPLE TEAM MEMBERS A. GROEN

CARRIER/WAYBILL NO [illegible]

Sample Number	Sample Location and Description	Date and Time Collected	Sample Type	Container Type	Condition on Receipt (Name and Date)	Disposal Record No.
684 -15-1A	untreated sample	3/5/91	SOIL	VIAL		
684 -16-1A	4 hr treatment	3/6/91	↓	↓		
684 -16-2A	6½ hr treatment	3/6/91				
684 -17-1A	15 hr treatment	3/7/91				
684 -18-1A	30 hr treatment	3/11/91				
684 -18-2A	48 hr treatment	3/12/91	↓	↓		
					B	

Special Instructions: USE METHOD DEVELOPED FOR PROJECT *(HOLDING TIME [illegible]

Possible Sample Hazards: ALL SAMPLES CONTAIN TCDD — [illegible] 3 DAY FROM SAMPLE

SIGNATURES: (Name, Company, Date and Time)

1. Relinquished By: ITAS/TDL - A Glese 3/25/91

Received By: Mary [illegible] 3-26-91 - 8:40AM

2. Relinquished By:

Received By:

3. Relinquished By

Received by.

4. Relinquished By

Received By.

INTERNATIONAL TECHNOLOGY CORPORATION

REQUEST FOR ANALYSIS

R/A Control No 233999
C/C Control No A93222

PROJECT NAME SITE TYPE 3
PROJECT NUMBER 483000
PROFIT CENTER NUMBER 4622
PROJECT MANAGER E.S ALPERIN
BILL TO

PURCHASE ORDER NO. 4622-6391-036

DATE SAMPLES SHIPPED 3/25/91
LAB DESTINATION ITAC / SAL LOUIS
LABORATORY CONTACT SALLY LANE
SEND LAO REPORT TO E S ALPERIN
201 DIRECTORS BLD
KNOXVILLE 3792
DATE REPORT REQUIRED 4/25/91
PROJECT CONTACT ARIE GROTON
PROJECT CONTACT PHONE NO 615-690-3211 ext 28

Sample No.	Sample Type	Sample Volume	Preservative	Requested Testing Program	Special Instructions
684-15-1A	SOIL	10-30g	REFRIGERATE	2,3,7,8 TCDD ONLY	USE METHOD MODIFICATION AS DEVELOPED EARLIER PICS #217-9
684-16-1A	"	"	"	"	"
684-16-2A	"	"	"	"	"
684-17-1A	"	"	"	"	"
684-18-1A	"	"	"	"	"
684-18-2A	V	V	V	V	

TURNAROUND TIME REQUIRED: (Rush must be approved by the Laboratory Project Manager)
Normal X Rush ____ (Subject to rush surcharge)

QC LEVEL (Levels II and III subject to surcharge; project specific requirements must be submitted to lab before beginning work)
I X II III Project Specific

POSSIBLE HAZARD IDENTIFICATION (Please indicate if sample(s) are hazardous materials and/or are suspected to contain high levels of hazardous substances)
Non-hazard ____ Flammable ____ Skin Irritant ____ Highly Toxic X TCDD Other ____ (Please Specify)

SAMPLE DISPOSAL (Please indicate disposition of sample following analysis; Lab will charge for packing, shipping, and storage)
Return to Client X Disposal by Lab ____ Archive ____ (Indicate number of months)

FOR LAB USE ONLY
Received by Mary P. [illegible] Date/Time [illegible]

INTERNATIONAL TECHNOLOGY CORPORATION

CHAIN-OF-CUSTODY RECORD

R A Control No 233869

C C Control No A 93223

PROJECT NAME/NUMBER [illegible]

LAB DESTINATION [illegible]

SAMPLE TEAM MEMBERS A. GREEN

CARRIER/WAYBILL NO. FED EX

Sample Number	Sample Location and Description	Date and Time Collected	Sample Type	Container Type	Condition on Receipt (Name and Date)	Disposal Record No.
684-19-1A	UNTREATED SOIL	3/13/91	SOIL	VIAL.		
684-19-1A	4 hr treatment	3/13/91	↓	↓		
684-34-1A	6 hr treatment	3/14/91	↓	↓		
684-34-3A	15 hr treatment	3/15/91	↓	↓		
684-35-1A	30 hr treatment	3/18/91	↓	↓		
684-36-1A	48 hr treatment	3/20/91	↓	↓		

Special Instructions: USE METHOD DEVELOPED FOR PROJECT (*HOLDING TIME IS 30 DAYS FROM SAMPLING.)

Possible Sample Hazards: ALL SAMPLES CONTAIN TCDD

SIGNATURES: (Name, Company, Date and Time)

1. Relinquished By: ITAS/TDL A Green 3/25/91

   Received By: [illegible] 3/26/91 8:40 AM

2. Relinquished By:

   Received By:

3. Relinquished By:

   Received by

4. Relinquished By

   Received By

accompany samples

INTERNATIONAL TECHNOLOGY CORPORATION

REQUEST FOR ANALYSIS

R/A Control No 233889
C/C Control No, A93223

PROJECT NAME [illegible]
PROJECT NUMBER 463007
PROFIT CENTER NUMBER 4622
PROJECT MANAGER E.S. ALPERIN
BILL TO

PURCHASEORDER NO. 4622-0391-036

DATE SAMPLE(S) SHIPPED 3/11/91
LAB DESTINATION IT ATS / ST LOUIS
LABORATORY CONTACT SALLY LANE
SEND LABREPORT TO E S ALPERIN
304 DIRECTORS DR
KNOXVILLE 37922
DATE REPORT REQUIRED 4/5/91
PROJECT CONTACT ARIE GREEN
PROJECT CONTACT PHONE NO 615-690-3211 ext 261

Sample No.	Sample Type	Sample Volume	Preservative	Requested Testing Program	Special Instructions
684-19-1A	SOIL	10-30 g	REFRIGERATE	2,3,7,8 TCDD	USE MODIFICATION TO METHOD AS DEVELOPED BY EHRLER (PROTECT #217-92
684-19-4A					
684-34-1A					
684 34 3A					
684 -35-h-					
684 36-1A					

TURNAROUND TIME REQUIRED: (Rush must be approved by the Laboratory Project Manager)
Normal X Rush (Subject to rush surcharge)

QC LEVEL (Levels II and III subject to surcharge project specific requirements must be submitted to lab before beginning work)
I X II III Project Specific

POSSIBLE HAZARD IDENTIFICATION (Please indicate if sample(s) are hazardous materials and/or suspected to contain high levels of hazardous substances)
Non-hazard Flammable ____ Skin Irritant ____ Highly Toxic X TCDD Other ____ (Please Specify)

SAMPLE DISPOSAL (Please indicate disposition of sample following analysis La; will charge for packing shipping archive and disposal)
Return to Client X Disposal by Lab ____ Archive ____ (Indicate number of months)

FOR LAB USE ONLY
Received by [signature] Date/Time 3-26-91 [illegible]

INTERNATIONAL TECHNOLOGY CORPORATION

REQUEST FOR ANALYSIS

R/A Control No 233889
C/C Control No A9322

PROJECT NAME [illegible]
PROJECT NUMBER 483000
PROFIT CENTER NUMBER 4622
PROJECT MANAGER E. S. ALPERIN
BILL TO

PURCHASE ORDER NO. 4622-0391-036

DATE SAMPLES SHIPPED 3/1?/91
LAB DESTINATION ITAS / ST. LOUIS
LABORATORY CONTACT SALLY LANE
SEND LAB REPORT TO E S ALPERIN
364 DIRECTORS DR
KNOXVILLE 3792?
DATE REPORT REQUIRED 4/25/91
PROJECT CONTACT ARIE GREEN
PROJECT CONTACT PHONE NO 615-690-3211 ext 2?

	Sample No.	Sample Type	Sample Volume	Preservative	Requested Testing Program	Special Instructions
684	-19-1A	SOIL	10-30 g	REFRIGERATE	2,3,7,8 TCDD	USE MODIFICATION
684	-19-4A	↓	↓	↓	↓	TO METHOD
684	-34-1A					AS DEVELOPED
684	-34-3A					EARLIER
684	-35-1A					(PROJECT #217-92
684	-36-1A					

TURNAROUND TIME REQUIRED: (Rush must be approved by the Laboratory Project Manager)
Normal X Rush ____ (Subject to rush surcharge)

QC LEVEL I X II III Project Specific
(Levels II and III subject to surcharge project specific requirements must be submitted to lab before beginning work.)

POSSIBLE HAZARD IDENTIFICATION (Please indicate if sample(s) are hazardous materials and/or suspected to contain high levels of hazardous substances)
Non-hazard ____ Flammable ____ Skin Irritant ____ Highly Toxic X TCDD Other (Please Specify)

SAMPLE DISPOSAL (Please indicate disposition of sample following analysis. Lab will charge for packing, shipping, archive and disposal.)
Return to Client X Disposal by Lab ____ Archive ____ (Indicate number of months)

FOR LAB USE ONLY
Received by [signature] Date/Time 3/26/91 [illegible]

11:13:58

ITAS - St. Louis
MasterSample Login: 1407

03/26/91

Account: 10610
Project No.: 217.99
Project: Itas - Knoxville
Description: Monsanto Thermal 482322.38
Facility:
Project Manager: S. Lane

DRAFT / FINAL / NONCONFORMANCE
COPY:
Reviewed By: S. Lane

Sample NO.	Client ID	Key Dter COLL RECV DUE	Shipper	Matrix	Analyses	Class	Preservatives	Container	Storage Sire
1407-001	684-11-1A	02/26 03/26 04/24	I CD-EX	Soil		COMMENTS:			
				Soil	DATA/STDREP/01	S			
				Soil	DIXIN/TCDD7/01	S	COLD	40 ML VIAL	● IWE
				Soil	P-DIXIN//02	S	COLD	co ML VIAL	#109E
1407-002	684-11-4A	02/26 03/26 04/24	FED-EX	Soil		COMMENTS:			
				Soil	DATA/STDREP/01	S			
				Soil	DIXIN/TCDD7/01	S	COLD	40 ML VIAL	#109E
				soil	P-DIXIN//02	S	COLD	40 ML VIAL	● IWE
1407-003	684-12-1A	02/27 03/26 04/24	FED-EX	Soil		COMMENTS:			
				Soil	DATA/STDREP/01	S			
				Soil	DIXIN/TCDD7/01	S	COLD	40 ML VIAL	#109E
				Soil	P-DIXIN//02	S	COLD	CO ML VIAL	#109E
1407-004	684-13-1A	02/28 03/26 04/24	FED-EX	Soil		COMMENTS:			
				Soil	DATA/STDREP/01	S			
				Soil	DIXIN/TCDD7/01	S	COLD	LO ML VIAL	● Im
				Sol I	P-DIXIN//02	S	COLD	40 ML VIAL	#109E
1407-005	684-14-1A	03/01 03/26 04/24	FED-EX	Soil		COMMENTS:			
				Soil	DATA/STDREP/01	S			
				Soil	DIXIN/TCDD7/01	S	COLD	40 MI VIAL	#109E
				Soil	P-DIXIN//02	S	COLD	40 ML VIAL	#109E
1407-006	684-14-2A	03/04 03/26 04/24	FED-EX	Soil		COMMENTS:			
				Soil	DATA/STDREP/01	S			
				Soil	DIXIN/TCDD7/01	S	COLD	LO ML VIAL	#109E
				soil	P-DIXIN//02	S	COLD	CO ML VIAL	#109E
1407-007	684-15-1A	03/05 03/26 04/24	FED-EX	Soil		COMMENTS:			
				Soil	DATA/STDREP/01	S			
				Soil	DIXIN/TCDD7/01	S	COLD	CO ML VIAL	#109E
				Soil	P-DIXIN//02	S	COLD	40 ML VIAL	● 109E
1407-008	684-16-1A	03/06 03/26 04/24	FED-EX	Soil		COMMENTS:			
				Soil	DATA/STDREP/01	S			
				Soil	DIXIN/TCDD7/01	S	COLD	CO ML VIAL	● IWE
				Soil	P-DIXIN//02	S	COLD	40 ML VIAL	#109E
1407-009	684-16-2A	03/06 03/26 04124	FED-EX	Soil		COMMENTS:			
				soil	DATA/STDREP/01	S			
				soil	DIXIN/TCDD7/01	S	COLD	CO ML VIAL	● IWE
				Soil	P-DIXIN//02	S	COLD	40 ML VIAL	#109E
1407-010	684-17-1A	03/07 03/26 04/24	FED-EX	Soil		COMMENTS:			
				Soil	DATA/STDREP/01	S			
				Soil	DIXIN/TCDD7/01	S	COLD	LO ML VIAL	#109E
				Soil	P-DIXIN//02	S	COLD	LO ML VIAL	#109E
1407-011	684-18-1A		FED-EX	Soil		COMMENTS:			