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Modeling and Decision Support Tools Based on the Effects to Sediment Geochemistry and Microbial Populations on Contaminant Reactions in Sediments

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Abstract

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Keywords: PCBs, polychlorinated biphenyls, microbial transformation, reductive dechlorination, contaminated sediment

Executive Summary

The proper management of polychlorinated biphenyl (PCB)-contaminated sediment has proven to be a wide-spread, complex, and costly issue. PCBs are a primary contaminant driving risk at many Department of Defense facilities. There is a need for sound science and effective tools to characterize and manage these sites and their associated risks. The focus of this project was on the role of site-specific differences in geochemistry and microbial populations in the transformation of polychlorinated biphenyls (PCBs) in sediments and on the development of advanced modeling tools to enhance our ability to predict dechlorination and evaluate the likelihood of natural attenuation at specific sites.

Laboratory analysis of sediment samples and microcosm-based PCB dechlorination experimentation were used to enhance our understanding of microbial populations that are capable of dechlorinating PCBs. Surficial sediments from the Grasse River and the Hudson River were collected, analyzed for congener-specific PCB concentrations, critical PCB transformation markers (e.g., molar dechlorination product ratio (MDPR), chlorines per biphenyl (CPB), homolog concentrations) and for bacterial species of relevance to reductive dechlorination. An intact core from the Grasse River was also analyzed for these criteria.

Total genomic deoxyribonucleic acid (DNA) extracted from the Grasse River sediment was eight times higher than that extracted from the Hudson River sediment, but PCB degrading organisms were more abundant in the Hudson River sediment. *Dehalococoides* and *Chloroflexi* groups were detected in Hudson and Grasse River sediments, and sulfate reducing bacteria were more abundant in the Grasse River sediment. With regards to PCB concentrations, the Hudson River sample was slightly higher in total PCB concentration (6.51 mg/kg sediment) compared with the Grasse River sample (1.35 mg/kg sediment). The number of chlorines per biphenyl was slightly lower in the Hudson River (2.47) than the Grasse River (3.71).

In the intact core from the Grasse River *Bacteria*, *Archaea*, sulfate reducing bacteria, *Chloroflexi* group, *Dehalococcoides* group, and known PCB degraders (*o-17* and DF-1 strains) were detected in all segments of the core by polymerase chain reaction (PCR) assays. Also, selective enrichment of *Dehalococcoides* and *o-17/DF-1* groups was observed in the core with depth, and older sediments had more dechlorination-related bacteria. Grasse River sediment *Chloroflexi* clones were more similar to each other than to known putative dechlorinating *Chloroflexi*.

Multiple methods were used to assess microbial reductive dechlorination in sediment samples. Assessment of changes in total PCBs, PCB homolog concentrations or percentages, MDPR, CPB, specific congener markers and tracker pairs were all evaluated in this work, with particular emphasis on analysis of an intact core from the Grasse River. MDPR was higher in deep undisturbed sediments, and MDPR correlated with the concentration of *Dehalococcoides* but not with concentrations of *Chloroflexi*. Similarly, chlorine content of the PCBs was lower in the deep core than in the surficial sediments and was also lower than the content expected based on the source Aroclor for the site. For the core segments deeper than 190 cm, congeners with fewer than three chlorines account for over 75% of the total PCB weight, a significantly higher percentage than in the original Aroclor and in more recent segments of the core. The percent weight increases in lesser-chlorinated homologs suggests anaerobic microbial dechlorination of

highly chlorinated PCB homologs took place at some time in the past. All three methods suggest that weathering of the original source contamination has occurred, and this was further indicated using multivariate principal component analysis. The results of these multiple methods of analysis all support the conclusion that transformation has taken place in the sediment core analyzed. The transformation of PCBs in the core is consistent with reductive dechlorination although alternative explanations may be possible. In conjunction with the molecular microbial diversity analyses that suggest the presence of PCB degraders at all depths in the core, we conclude that microbial transformation is the likely explanation for congener profile changes with depth in the core studied. Thus, we conclude that for the Grasse River, monitored natural attenuation has significant potential as a remediation strategy.

A series of microcosm-based transformation experiments were carried out to further characterize transformation of specific PCB congeners in Grasse and Hudson River sediment. PCB dechlorination was observed in all treatments of the microcosms except the Hudson River sediment amended with iron and the sterile controls. For the treatments spiked with two different PCB mixtures, the shortest lag time (3 weeks) was found in Grasse Sediment, followed by Hudson Sediment (3-6 weeks) and Grasse Sediment with ferric iron (6-9 weeks). When sulfate was added to both sediment types, an approximately 18-week lag time and slow dechlorination rate were observed, which is expected as the presence of sulfate as a more favorable electron acceptor than PCBs. During 36 weeks' incubation, the average rate of dechlorination was: Grasse Sediment > Hudson Sediment> Grasse Sediment amended with ferric iron>Hudson Sediment amended with sulfate> Grasse Sediment amended with sulfate>Hudson Sediment amended with ferric iron. By the end of 36 weeks, the total PCB amount decreased by 30-35% in Grasse Sediments, followed by 25-30% in Hudson Sediments and 20-25% in ferric iron amended Grasse Sediments. Greater than 10% total PCBs amount was reduced in sulfate amended Hudson Sediments, while less total PCBs reduction was detected in sulfate amended Grasse Sediments.

Previous studies have found the chlorines removed in sulfate amended microcosms were flanked *para*- and/or doubly flanked *meta*- chlorines (May et al. 1992; Rhee et al. 1993b; Cho and Oh 2005). In the present study, flanked *para* and *para*-flanked *meta* dechlorinations were observed. *Ortho*-flanked *meta* dechlorination was partially inhibited. When sulfate level dropped, *ortho*-flanked *meta* dechlorination was also found in both sediment types and PCB Mixtures but not as prevalent as flanked *para* and *para*-flanked *meta* dechlorinations. Total PCB analysis showed that the addition of ferric iron significantly decreased both the rate and extent of dechlorination based on total PCB amount. However, when further study based on individual PCB congeners were conducted, more complicated effects were observed. By the end of 36 weeks, the residual PCB 170 in the iron amended microcosms was 50% less than that in the microcosms without iron. This is the first report that the addition of a favorable electron acceptor is able to accelerate the dechlorination of highly chlorinated PCB congeners. In addition, the accumulation of PCB 32 (26-4-CB) was found. PCB 32 was dechlorinated to PCB 10 (26-CB) in the microcosms without iron amendment. This reveals the lack of the capability of removing unflanked *para* chlorines in ferric iron amended microcosms.

Bacterial diversity changed in the microcosms in response to the specific PCBs in the spiked mixture and in response to the different amendments. This, and the other observations noted above have led us to conclude that sediment biogeochemistry, including initial PCB distributions, alternative electron acceptors (sulfate and iron), and microbial community all affect

the transformation of PCBs in sediments. Extensive sediment characterization can aid in determining the suitability of monitored natural attenuation as a remedy at a specific field site. Sediment amendments may be useful to seed microbial populations or change pathways and end points for reductive dechlorination.

The development of advanced modeling tools was used to enhance prediction of dechlorination and evaluate the likelihood of natural attenuation at specific sites. Classification trees were developed to predict PCB pathways that are likely to be observed when specific processes are active in contaminated sediments. A Bayesian Monte Carlo method was used to identify the occurrence of one or more of the eight known dechlorination processes. The model, termed the Dechlorination Process Estimator (DPE), uses a discrete chemical reaction equation for each of the 209 congeners such that mass from parent congeners is transferred to child congeners across 840 dechlorination pathways. The DPE's ability to identify uncertain parameters, in particular occurring dechlorination processes, was tested with sixteen synthetic dechlorination scenarios, predicting pathways to add to the eight established processes. Utilization of new modeling tools developed on this project will enable prediction of PCB end points and the effect of amendments on the rate, extent and end point of reductive dechlorination. The DPE model demonstrates the capability to simulate large, complex and incompletely understood chemical transformations in a statistically rigorous manner, and the application of the DPE to a laboratory experiment suggests that the DPE is suitable for the identification of process occurrence. Thus, carefully designed microbial experiments, paired with statistically valid models accounting for uncertainty, can provide unique insights into potential remediation strategies.

In conclusion, sediment biogeochemistry, including initial PCB distributions, alternative electron acceptors (sulfate and iron), and microbial community all affect the transformation of PCBs in sediments. Extensive sediment characterization can aid in determining the suitability of monitored natural attention as a remedy at a specific field site. Sediment amendments may be useful to seed microbial populations or change pathways and end points for reductive dechlorination. Utilization of new modeling tools developed on this project will enable prediction of PCB end points and the effect of amendments on the rate, extent and end point of reductive dechlorination. Carefully designed microbial experiments, paired with statistically valid models accounting for uncertainty, can provide unique insights into potential remediation strategies.

List of Acronyms

Alcoa	Aluminum Company of America
ARC	*Archaea*
BAC	*Bacteria*
BLAST	Basic Local Alignment Search Tool
BMC	Bayes Monte Carlo
BBN	Bayesian Belief Network
BMI	benthic macroinvertebrates
CPB	chlorines per biphenyl
CHL	*Chloroflexi*
CTDPG	classification tree dechlorination process generalizations
CTDPGs	*classification tree dechlorination process generalizations*
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CDFs	confined disposal facilities
DPE	Dechlorination Process Estimator
DHC	*Dehalococcoides group*
DGGE	denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DoD	Department of Defense
DF-1	Double-Flanked-1
ER	Environmental Restoration
XML	extensible markup language
GC/ECD	gas chromatograph/electron capture detector
GC	gas chromatography
GE	General Electric Company
GUI	graphical user interface
G	Grasse River sediment
H	Hudson River sediment
ICP-MS	inductively coupled plasma mass spectrometry
C	Laboratory Control
MBT	Methanobacteriales
MMB	Methanomicrobiales
Mst	Methanosaeta
Msc	Methanosarcina
µECD	micro-electron capture detector
MNA	Monitored natural attenuation
MNR	monitored natural recovery
MCDA	Multi-criteria decision analysis
NTCRA	non-time critical removal action
NEA	Northeast Analytical, Inc.

o-17	*ortho-17*
PDG	PCB Declorinator Group with o-17 and DF-1
PMI	Phytophilous macroinvertebrates
PBDEs	polybrominated diphenyl ethers
PCB	polychlorinated biphenyl
PCBs	polychlorinated biphenyls
PCR	Polymerase Chain Reactions
PCA	principal component analysis
QEA	Quantitative Environmental Analysis, Inc.
Q-PCR	quantitative polymerase chain reaction
ROD	Record of Decision
RAMM	reduced anaerobic mineral medium
rRNA	ribonucleic acid
SEM	Simultaneously Extractable Metals
SERDP	Strategic Environmental Research and Development Program
SRB	sulfate reducing bacteria
TBA	tetrabutylammonium
UML	unified modeling language
USEPA	United States Environmental Protection Agency
USA	United States of America

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1 Objectives

The focus of this project was on the role of site-specific differences in geochemistry and microbial populations in the transformation of polychlorinated biphenyls (PCBs) in sediments. Laboratory analysis and experimentation was used to enhance our understanding of microbial populations that are capable of dechlorinating PCBs, and the development of advanced modeling tools enhanced our ability to predict dechlorination and evaluate the likelihood of natural attenuation at specific sites.

Technical objectives included:

1. Use molecular microbiological tools to evaluate population differences and identify organisms associated with PCB dechlorination in river sediments. Evaluate changes in microbial populations over a forty-year time period using intact sediment core samples from PCB contaminated rivers. This objective was explored as part of two tasks, Task A-1 and Task A-2, with Task A-1 focusing on the laboratory analysis, and Task A-2 focusing on data analysis and microbial community identification. Further work related to this objective in laboratory microcosms was completed as Task C.
2. Develop new statistical methods to analyze distributions of PCB congeners in weathered sediments. Integrate advances in Bayesian analysis that allow us to estimate the probability that specific processes and pathways for microbially-mediated reductive dechlorination of PCBs are occurring in particular field data. This objective was explored in Task B.
3. Link the model for environmental conditions, congener distributions and bacterial population dynamics to decision-support tools to enable evaluation of site-specific likely outcomes in order to evaluate remediation plans. This objective was to be explored as part of Task D. (*Note: this objective and associated task were removed after the In Progress Review in early 2008 that reduced scope. Work completed on the task prior to that time is presented in this report.*)

2 Background

The proper management of PCB-contaminated sediment has proven to be a wide-spread, complex, and costly issue. PCBs are a primary contaminant driving risk at many Department of Defense (DoD) facilities. There is a need for sound science and effective tools to characterize and manage these sites and their associated risks.

Dredging is a commonly employed technique to remediate PCB-contaminated sediments. It provides rapid PCB mass removal from the local environment and is a frequently prescribed management technology in Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Record of Decision (ROD)s (U.S. Environmental Protection Agency and Wisconsin Department of Natural Resources 2003; USEPA 2003). Potential disadvantages of dredging include (1) high costs, (2) the generation of large volumes of wastewater requiring treatment, (3) residual contamination from contaminated sediment that is inaccessible to the dredge or spills during dredging (Palermo et al. 1990), (4) difficulty in siting new disposal facilities for dredged materials, (5) increased exposure pathways during handling, transport, and disposal, and (6) loss of habitat (Reible et al. 2003). Because dredged sediments that are sent to landfills or to near-shore or offshore confined disposal facilities (CDFs) are typically not treated, they create new potential human exposure pathways (U.S. Navy Naval Facilities Engineering Command Tech Data Sheet 2002). Furthermore, environmentally sensitive areas or areas without a good disposal site for dredged sediments may eliminate dredging as a viable alternative. There are few other reliable alternatives for treating PCB-contaminated sediments.

Monitored natural attenuation (MNA), sediment capping, and cap-and-treat approaches are potentially viable and attractive treatment alternatives. MNA relies on the deposition of clean sediment and the biodegradation of contaminants in buried sediment to reduce, stabilize, and isolate contaminated sediment from the bioactive zone (USEPA 1998). Cap-and-treat technologies will apply a cap to physically isolate contaminants in buried sediments from the overlying bioactive zone. The effectiveness of these approaches relies on the rate of attenuation of PCBs in the underlying sediment (Murphy et al. 2006). *The decision to use less costly alternatives in place of dredging will ultimately rely on the ability to adequately characterize the natural or enhanced biodegradation potential and rate at a specific site.* Spatial and temporal variability, inadequate understanding of the factors influencing the rate and extent of PCB dechlorination, and the lack of time and resources for extensive characterization at all sites introduces significant uncertainty in assessing the potential for biodegradation and thus the viability of MNA as a remedial solution.

The current study focused on improving the understanding of the role of sediment differences (especially their microbial population and geochemistry) on PCB dechlorination pathways and processes.

2.1 Fundamentals of PCBs

Polychlorinated biphenyls (PCBs) are composed of two linked benzene rings with 1-10 chlorine atom substitutes at 10 possible attachment points. PCB mixtures contain up to 209 different molecular arrangements of the attached chlorine. These are referred to as PCB congeners. There are several naming conventions for the PCB congeners; some examples are shown in **Figure 1**.

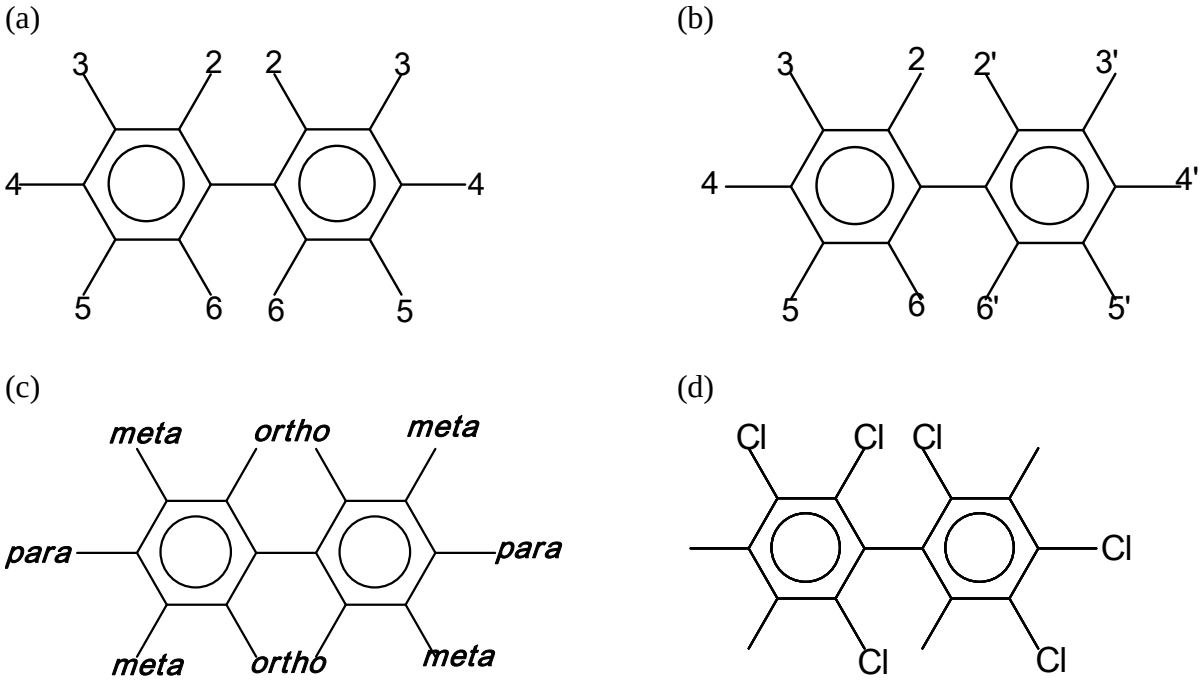


Figure 1. Examples of the Nomenclature used for Polychlorinated Biphenyls (PCBs). (a) On each ring, the position of hydrogen that can be replaced by chlorine is assigned a number from 2 to 6. Position 1 represents the location of the phenyl to phenyl bond; (b) Instead of numbering the positions on both right from 1 to 6, the positions on one right are named 1' to 6'; (c) The positions are labeled ortho, meta, and para based on their position in relation to the phenyl to phenyl bond. As shown, there are four ortho-positions, four meta-positions, and two para-positions available for chlorine substitution; (d) This particular PCB congener could be designated as PCB 149 using the International Union of Pure and Applied Chemistry (IUPAC) naming system, and could also be called 236-245, or 2,2',3,4',5',6, or 236-245-hexachlorobipenyl or 236-245-CB or 2,2',3,4',5,6-hexachlorobipenyl or 2,2',3,4',5,6-CB.

The empirical formula for the PCB congeners is $C_{12}H_{10-n}Cl_n$ where n is from 1 to 10. The 10 possible chlorine attachment positions are divided into three groups: *ortho*, *meta* and *para* positions. Multiple PCB congener naming systems have been developed and applied in PCB studies, and the differences in congener naming systems has led to difficulties in comparisons of PCB data collected from a variety of sources. Mills and colleagues summarized many of the existing naming systems and provided a good reference for data comparison (Mills et al. 2007). In this document, number assignment to congener structure follows the original work of Ballschmiter and Zell (Ballschmiter

and Zell 1980) with corrections to congener numbers 199-201 by Schulte and Malisch (Schulte and Malisch 1983) and corrections to numbers 107-109 by Guitart et al. (Guitart et al. 1993). Congeners having the same number of attached chlorines share the same molecular formula and are members of the same homolog group. Chlorine atoms are classified as unflanked, singly flanked or doubly flanked based on the positions of neighboring chlorines attached to the same ring. Unflanked, singly flanked and doubly flanked chlorines have zero, one and two chlorine neighbors respectively.

PCBs were first synthesized in Germany in 1881. Due to their chemical stability, heat-resistance, non-flammability, low water solubility and insulation, PCBs have been produced and widely used in industry as dielectric fluids, stabilizing additives since 1929 (Brown et al. 1987b; Brown et al. 1987a). Approximately half of the 209 PCB congeners were produced in commercial PCB mixtures including Aroclor and Pyroclor (Monsanto, USA), Fenclor (Caffaro, Italy), Clopen (Bayer, Germany) and Kanechlor (Kanegafuchi, Japan). The Monsanto Aroclor mixtures were synthesized in a batch process by heating biphenyl and adding anhydrous chlorine in the presence of ferric chloride. The average degree of chlorination of the batch was controlled by the reaction time to yield the desired physical and chemical properties. The total percent weight chlorine is indicated in the last two digits of the Aroclor designation, except in the case of Aroclor 1016, which is 41% chlorine by weight (Frame et al. 1996a).

As a result of the growing awareness regarding PCB biological toxicity and its adverse effect on the environment (Quensen et al. 1998), industrial PCB production was banned in United States of America (USA) in 1978. The Stockholm Convention on Persistent Organic Pollutants seeks to eliminate the use of PCBs by 2025 worldwide (UNEP 2004). However, it is estimated that approximately 1.3 million tons of PCBs were produced globally and more than 50% of the PCBs were manufactured in the USA (Breivik et al. 2002). An estimated 80,000 tons of PCBs had already been released into the environment prior to limiting the use of PCBs to closed systems (Durfee et al. 1976).

As a result of atmospheric long range transport and deposition, PCBs are widespread. In the United States, approximately one third of the total PCB products were directly discharged into the environment (Hutzinger and Veerkamp 1981). In the Hudson River, approximately 1.3 million pounds of PCBs were released by the capacitor manufacturing plants of the General Electric Company (GE) from 1947 to 1977 (USEPA 2009). Similarly, in the Grasse River, PCBs were discharged by the Aluminum Company of America (Alcoa) while operating an aluminum smelting and fabricating facility in that area.

The properties that made PCBs useful for industrial purposes also cause them to persistent in the environment. PCBs tend to accumulate in organic phases, especially in soil and sediment organic matter, which represent dominant environmental organic compartments (Schwarzenbach et al. 1993; Connolly et al. 2000; Meijer et al. 2002; Jonsson et al. 2003). PCBs also accumulate preferentially in the tissues of animals and plants (e.g., Secord et al. 1999; Muir et al. 2000; Stapleton et al. 2001).

PCBs can be found in virtually all environmental compartments (USNRC 2001). Air, water, and soil/sediment transport processes have spread PCBs from local sites of contamination across the global environment, and PCBs have been found in remote areas (e.g., Muir et al. 2000; Kalantzi et

al. 2001). Local and regional environmental conditions often lead to the deposition of released PCBs close to source areas (Axelman and Broman 1999; Green et al. 2000; Meijer et al. 2002). Based on evaluation of PCB burial rates in continental shelf sediments compared to an estimated global PCB inventory, it is estimated that global residence times of certain highly-chlorinated PCB congeners is on the order of 100 years (Jonsson et al. 2003).

Soils and sediments play an important role in local, regional, and global scale environmental transfers and cycling of PCBs (e.g., Gobas et al. 1995; Connolly et al. 2000; Meijer et al. 2002; Jonsson et al. 2003). Sediments often are the primary source of PCBs to aquatic ecosystems. PCBs in near-surface sediments are those most available for release and recycling in the environment (Connolly et al. 2000; Jonsson et al. 2003).

2.2 Microbial Transformation of PCBs

Although PCBs are regarded as persistent organic pollutants, which are not easily metabolized by organisms, PCBs in the environment have been observed to biodegrade slowly but significantly. They can be transformed in the environment by microbial process under aerobic and anaerobic conditions. Under aerobic conditions, microorganisms add O_2 to the biphenyl ring via a dioxygenase enzyme, dehydrogenate to form the catechol and cleave the benzene ring (Abramowicz 1990), resulting in the production of environmentally benign compounds. PCBs can be either the growth substrate of the aerobic degrading microorganisms or the co-substrate of microbes growing on other substrates. Aerobic oxidative degradation is limited in the environment in the following ways: 1) it usually attacks only lower chlorinated PCB congeners (those with 1 to 4 chlorine substituents), and 2) for heavily contaminated sediments in rivers and lakes, only the first few millimeters of the sediment layer are aerobic. Under anaerobic conditions, microbial-catalyzed dechlorination has been found to be prevalent in sediments. Instead of breaking the benzene ring, anaerobic reductive dechlorination replaces the chlorine atoms with hydrogen atoms, with the ultimate end product for all PCB congeners being biphenyl. Unlike aerobic oxidative degradation, which has been well characterized, the mechanism of the multi-step anaerobic reductive dechlorination is not as well understood.

Our understanding of PCB biotransformation through reductive dechlorination has developed over more than 20 years of study (see reviews: Abramowicz 1990; Tiedje et al. 1993). In 1987, Brown et al. were the first to suggest that polychlorinated biphenyls (PCBs) in anaerobic aquatic sediment could be dechlorinated by microorganisms (Brown et al. 1987a). This claim was based on the comparison of congener distributions in Hudson River field samples with distributions in source Aroclor mixtures. While at the time, this work led to significant debate about the potential for microbial dechlorination of PCBs (Brown et al. 1988a; Brown et al. 1988b), subsequent extensive laboratory study has confirmed the potential for reductive dechlorination of PCBs in sediments (see for example: Quensen et al. 1990; Abramowicz 1995; Pagano et al. 1995; Rysavy et al. 2005; Yan et al. 2006c; Yang et al. 2008). Extensive field work has confirmed the process is widespread in different anaerobic environments, including freshwater, estuarine and marines sediments (see for example: Sokol et al. 1994b; Pakdeesusuk et al. 2005). However, field transformations are usually not as extensive as laboratory observations would suggest are possible, and enrichment experiments performed in the laboratory have not been applied in the field to accelerate natural attenuation (Bedard 2008).

Due to limited understanding regarding 1) the microorganisms involved in reductive dechlorination of PCBs in sediments, 2) the susceptibility of individual congeners to specific microorganisms, and 3) the role of sediment biogeochemistry in controlling the rate and extent of PCB transformation, the use of natural attenuation of PCBs as a sediment remediation strategy has been limited.

2.2.1 PCB Dechlorinating Microorganisms

After the confirmation of PCB anaerobic reductive dechlorination in the environment, isolating and identifying PCB dechlorinating microorganisms was a focus of much interest. In the 1990's, the observation of microorganisms capable of reductively dechlorinating PCBs in PCB-free sediments led to the hypothesis of the existence of common dechlorinating enzyme(s) in the environment (Rhee et al. 1993a). The results of other studies partially supported this hypothesis by demonstrating the capability of dechlorinating a variety of common chlorinated compounds in the presence of some bacterial transition-metal coenzymes such as B_{12} (Gantzer and Wackett 1991; Assafanid et al. 1992). Since PCBs are anthropogenic compounds, the evolution of preexisting microorganisms under the selective pressure of PCB contamination may have resulted in specific dechlorinating enzyme(s) for different PCB congeners (Rhee et al. 1993c; Young et al. 1995; Wiegel and Wu 2000). However, the communities of dechlorinating microorganisms appear to vary from site to site, probably due to the distinct geochemical conditions of each location (Rhee et al. 1993a; Young et al. 1995; Yan et al. 2006a). PCB dechlorination is thus characterized as a complex process involving PCB, and perhaps even congener specific microorganisms, and/or nonspecific dechlorinating microorganisms (Tiedje et al. 1993; Young et al. 1995; Adrian et al. 2009).

Although microorganisms that dechlorinate PCBs are prevalent, identification of these organisms has proven difficult. To date, only three PCB congener-specific and one nonspecific bacterial strain have been successfully identified. The first congener specific organism, *ortho-17* (*o-17*) was identified in a Baltimore Harbor culture by associating the bacterium with the anaerobic dechlorination of 2,3,5,6-tetrachlorobiphenyl (2,3,5,6-CB). This organism was successfully linked with removal of *ortho* chlorines in laboratory experiments using a molecular biological tool (Denaturing Gradient Gel Electrophoresis (DGGE)); however, it was not isolated in pure culture. Amendment with acetate was found to be crucial for this *ortho*-dechlorinating activity to occur (Cutter et al. 1998; Cutter et al. 2001). A year later, the second anaerobic PCB dechlorinator, called Double-Flanked-1 (DF-1), associated with anaerobic dechlorination of doubly-flanked chlorines in the presence of PCB congener 2,3,4,5-tetrachlorobiphenyl (2,3,4,5-CB) was identified using the same technique (Wu et al. 2000; Wu et al. 2002). The third identified PCB dechlorinating bacterium is *Dehalococcoides enthogenes* strain 195, which contains 17 putative dehalogenase gene homologs. Similar to DF-1, *Dehalococcoides enthogenes*, strain 195 reductively dechlorinates doubly flanked chlorines at *meta* and *para* positions (Fennell et al. 2004). Subsequent work linked species in the *Chlorofelexi* phylum (including *Dehalococcoides* spp. and the *o-17*/DF-1 group) to specific congener transformations. The results showed that a *Dehalococcoides* phylotype DEH10 was responsible for the removal of *para*-flanked *meta* chlorine and the removal of doubly flanked *meta* chlorine. Another phylotype, SF1, closely related to the *o-17*/DF-1 group was observed to reductively dechlorinate doubly flanked chlorines. The above findings suggest significant species and congener specificity and the need for a diverse population to achieve extensive dechlorination in the environment (Fagervold et al. 2005). A final recently identified organism shows more broad dechlorination ability; *Dehalococcoides* sp. strain CBDB1 extensively dechlorinates 43 PCB

congeners in Aroclor 1248 and 1260 (Adrian et al. 2009). However, no *ortho* dechlorination was catalyzed by *Dehalococcoides* sp. strain CBDB1.

2.2.2 Factors Affecting PCB Dechlorination

PCB anaerobic dechlorination has been studied for over twenty years. Geochemical factors have a variety of effects on the dechlorination rate, extent, and processes. Common physical and geochemical factors have been investigated, including (1) temperature, (2) pH, (3) available carbon sources, (4) supplemented electron donors (Fe(0), H_2), (5) competing electron acceptors (Fe(III), nitrate, sulfate), (6) PCB congener profile and concentration, (7) redox level and inhibitors. The effects of temperature and pH have been well studied (see review: Wiegel and Wu 2000). Other factors are not as well understood.

PCB reductive dechlorination uses PCBs as electron acceptor, but requires other compounds for carbon source and electron donor. In sediment, there are a variety of organic compounds that could provide carbon and electrons for bacterial growth. The essential carbon source for PCB dechlorinating microorganisms is not known. Some dechlorinating organisms have an obligate need for acetate, while others require formate or H_2-CO_2 (80:20) as electron and carbon sources (Wu et al. 2000; Wu et al. 2002). Organic carbon sources, such as formate, acetate, pyruvate glucose, methanol and acetone have been found to have a significant impact on the dechlorination rate, extent and processes (Wiegel and Wu 2000). Sodium bicarbonate has also been observed to alter the rate and extent of dechlorination as well as the bacterial community structures in Hudson River sediment cultures (Yan et al. 2006b). The effect of H_2 on PCB dechlorination depends on a variety of factors including H_2 partial pressure, the affinities of H_2 utilizers, available carbon sources, electron acceptors and competing electron donors (Wiegel and Wu 2000). Low hydrogen partial pressure may have no effect, but high H_2 partial pressure (0.1 atm (10%) or 0.2 atm (20%)), was found to inhibit some dechlorination reactions and change the pathways and dechlorination products (Sokol et al. 1994a; Wiegel and Wu 2000).

An additional consideration is the effect of iron and sulfate in sediments. Since anaerobic dechlorinating microorganisms use PCBs as terminal electron acceptors and obtain energy for cell synthesis from this thermodynamically favorable dechlorination step (Brown et al. 1987a; Brown et al. 1987b; Quensen et al. 1988; Wiegel and Wu 2000), the presence of alternative electron acceptors may change the dechlorination rate, extent and preferred pathways. The addition of 3-30 mM sulfate, a preferred electron acceptor for sulfate reducing bacteria, was observed to inhibit overall PCB dechlorination in different types of PCB contaminated sediments (May et al. 1992; Morris et al. 1992a; Alder et al. 1993; Rhee et al. 1993b; Rhee et al. 1993c; Young et al. 1995; Wu et al. 2000; Cho and Oh 2005). Fifty millimolar ferric oxyhydroxide (FeOOH) also inhibited PCB anaerobic dechlorination, but the effect was much weaker than 10 mM sulfate and BESA 10 mM (bromoethane sulfonic acid, a methanogensis inhibitor) (Morris et al. 1992b; Morris et al. 1992a).

2.3 PCB Dechlorination Pathways and Processes

A PCB dechlorination pathway is defined in this report as the *loss of a specific chlorine atom on a specific congener (the "parent" congener), leaving behind a different specific congener (the "child" or "daughter" congener).* Starting at congener 209, there are 840 theoretically possible

pathways that could occur in transforming congener 209 (23456-23456) into biphenyl. These 840 pathways are shown in **Figure 2**.

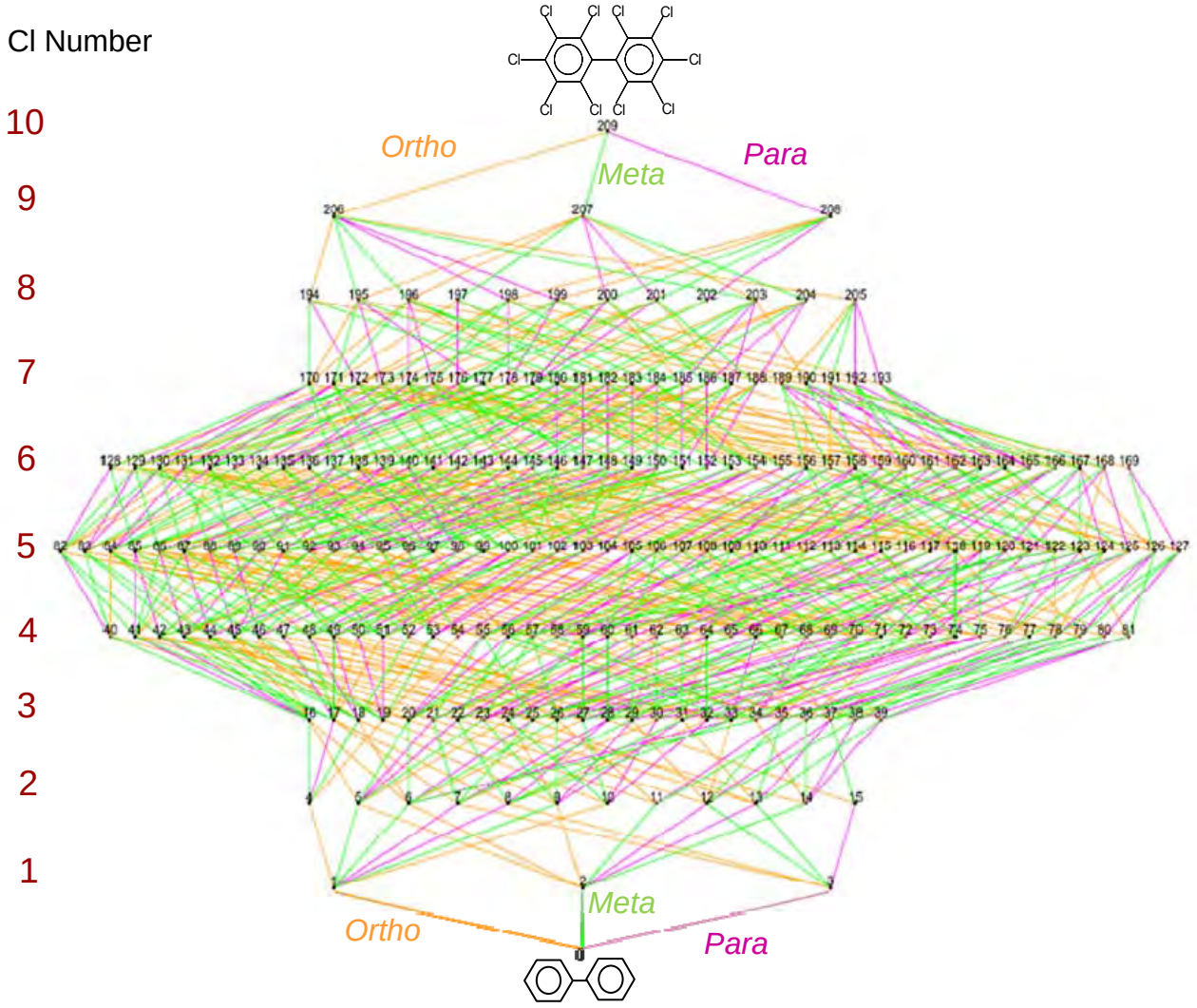


Figure 2. The 840 Theoretically Possible Pathways Between Congener 209 and Biphenyl. This figure (adapted from Karcher 2005), shows each of the individual pathways that could be followed in dechlorinating PCB 209. Notice that once the first chlorine has been removed, that location of the removal further limits the pathway options for the next removal. The orange lines indicate that a chlorine was removed from an ortho-position, the green from a meta-position, and the purple from a para-position.

In this figure, PCB congeners are represented by their congener number (as reported in the original work of Ballschmiter and Zell (1980) with corrections to congener numbers 199-201 by Schulte and Malisch (1983) and corrections to numbers 107-109 by Guitart et al. (1993). Each row of congeners in the diagram represents a homolog group, with congener 209 shown at the top as the only congener with 10 substituted chlorines. The next row down contains three congeners (206, 207 and 208) that each contain 9 chlorines. Each line shows the pathway from one congener (a parent) to another congener (the child) through loss of a single chlorine atom.

With 840 possible pathways, the number of possible combinations of pathways to get from congener 209 to biphenyl is quite large, and different sediments show different reactivity to different congeners (Wiegel and Wu 2000; Cho and Oh 2005; Wu et al. 1997a; Wu et al. 1997b). In most cases, *meta* and/or *para* chlorines are relatively easily transformed and *ortho* chlorines remain as dechlorination products. However, *ortho* dechlorination, though not common, has been observed (Van Dort and Bedard 1991; Berkaw et al. 1996; Wu et al. 1996; Wu et al. 1997b; Cutter et al. 1998; Kuipers et al. 1999).

Many different pathway combinations proceed under different conditions and lead to a variety of PCB congener distribution patterns in environmental samples (Brown et al. 1987a; Brown et al. 1987b). This high degree of variability, along with differences in congener patterns of the original PCB mixtures that were discharged into the environment, and the analytical challenges associated with quantifying all 209 congeners have led to significant confusion in interpreting reports on the nature and extent of PCB dechlorination in the environment.

In order to establish a protocol for characterizing and reporting PCB dechlorination, the concept of PCB Dechlorination Processes was developed (Brown et al. 1987a; Brown et al. 1987b; Brown and Wagner 1990). A process is defined as a specific subset of pathways, contributing to a certain PCB congener distribution pattern in dechlorinated sediment. Beginning in 1995, eight PCB dechlorination processes were identified: Process H, H', M, N, Q, P, LP and T. A small number of pathways were *explicitly reported* as belonging in each process; having been observed in laboratory experiments. However, the sets of *explicitly reported* pathways in each process are incomplete. Due to analytical limitations, some pathways that might be occurring could not be observed in laboratory studies. These pathways may be inferred through structural similarities, but they are not explicitly included in dechlorination processes.

Dechlorination process *generalizations* were extrapolated from explicitly reported pathways (Quensen et al. 1990; Young et al. 1995; Bedard et al. 1997; Van Dort et al. 1997; Wu et al. 1997b; Bedard et al. 2005). Other patterns were believed to result from the combinations of the eight main processes (Young et al. 1995). The characteristics of the eight published processes are presented in Table 1.

Table 1. Characteristics of PCB Dechlorination Processes

Process	Number of explicitly reported pathways	Targeted chlorine atoms in the dechlorination process generalization	Observed at sites
H	21	Flanked *para* (34-, 245-, 345-, 2345-) and doubly flanked *meta* (234-, 2346-) for tetra-, penta, hexa and hepta homologs	Hudson River
H'	20	Flanked *para* (34-, 245-, 2345-) and *ortho* flanked *meta* (23-, 234-) for tri, tetra and penta homologs	New Bedford Hudson River
M	16	Flanked *meta* (23-, 34-, 234-, 236-) and unflanked *meta* (3-, 25-) for di, tri and tetra homologs	Silver River Hudson River
N	29	Flanked *meta* (234-, 236-, 245-, 2345-, 2346-, 23456-) for penta, hexa, hepta, octa, nona homologs	Silver River Hudson River Woods Pond
Q	20	Flanked *para* (34-, 245-), unflanked *para* (4-, 24-, 246-), flanked *meta* (23-) for di, tri and tetra homologs	Hudson River
P	26	Flanked *para* (34-, 234-, 245-, 2345-, 23456-) for tetra, penta and hexa homologs	Woods Pond
LP	31	Flanked *para* (34-, 245-) and unflanked *para* (4-, 24-, 246-), sometimes flanked *meta* (23-, 234-, 235-)	Housatonic River
T	7	Flanked *meta* (2345-) for hepta- and octa homologs	Woods Pond

Note: Modified table from cited references (Young et al. 1995; Wiegel and Wu 2000; Hughes et al. 2010).

The transformation reactions possible in sediment systems have not been fully characterized (Bedard and Quensen 1995; Bedard and May 1996; Bedard et al. 1997; Bedard 2003; Bedard et al. 2005; Wu et al. 1997b). Because the dechlorination process generalizations shown in Table 1 are based on incomplete sets of explicitly reported pathways, they may include inaccuracies. As part of Objective *2*, a statistically rigorous and reproducible method was used to identify these missing pathways. Details are presented in the methods and results sections below.

3 Materials and Methods

The overall approach of this work is to couple molecular microbial analyses of field sediments with Bayesian-based models to develop a decision support tool for managers of PCB-contaminated field sites. **Figure 3** shows the overall approach to the project. Different sediment conditions (microbial populations and geochemical conditions) and different congener distributions interact to complicate our understanding of this system. Our approach is to evaluate differences in microbial diversity and community structure (left side of the figure) and develop novel modeling techniques to handle the different congener distributions (right side of figure). Results developed in these parallel research activities will feed into a decision support model for site specific PCB remediation.

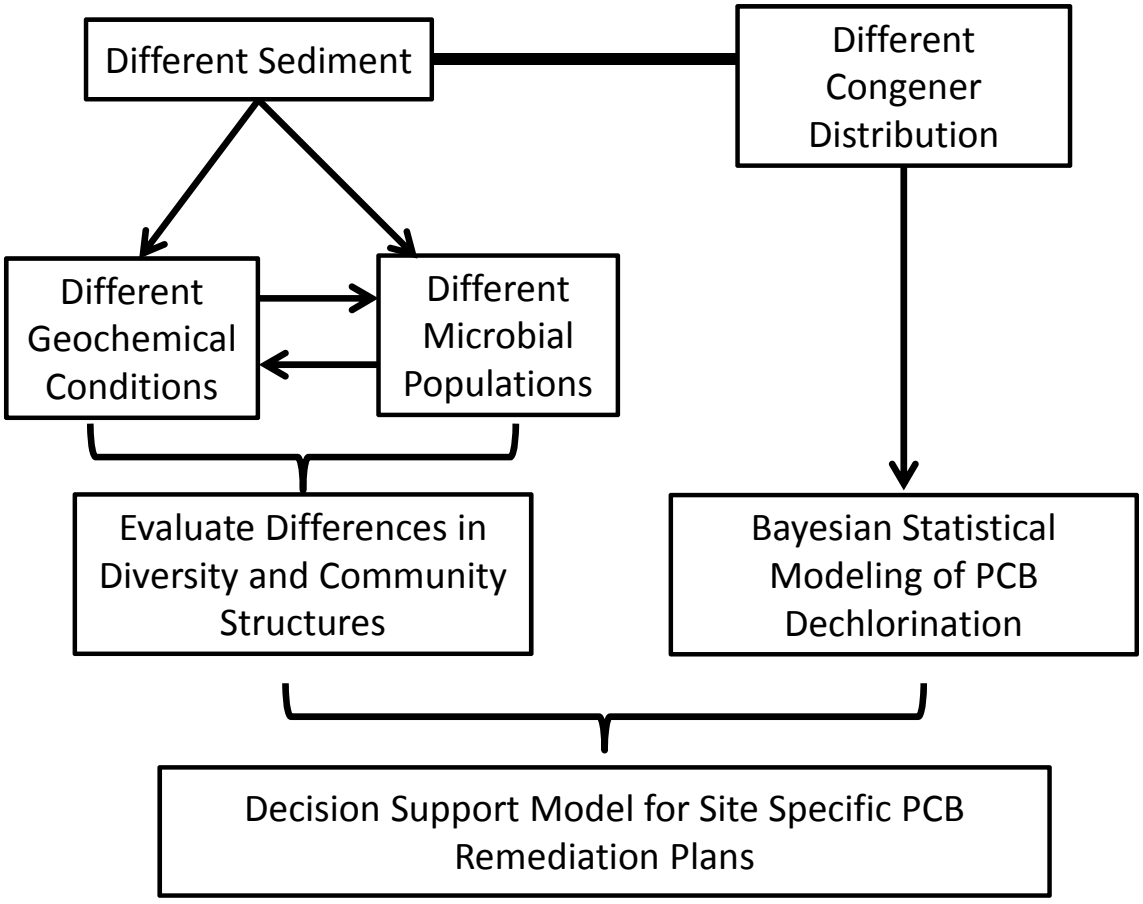


Figure 3. Experimental Approach ER-1495.

Most of the specific methods for sediment analysis, microbial diversity evaluation, and statistical analysis used in this work have been described in the literature, and thus are not extensively reprised here. Only our novel applications are described in detail.

3.1 Site Selection and Initial Characterization

In order to focus on evaluation of differences in sediment biogeochemistry and microbial populations, work initially focused on characterization of several sediment systems known to show different types and extent of reductive-dechlorination activity. This preliminary analysis was designed to identify sediment systems with clear differences in geochemical conditions known to be relevant for PCB dechlorination. Sediment samples from two rivers: the Grasse River and the Hudson River were evaluated as part of this project.

The specific sediment sampling methods are presented here. Characterization results are presented in later sections.

Sediment samples were collected in 2006 and 2007 for the present study. Table 2 provides a list of samples and their locations.

Table 2. Sediment Sample Used in this Study

	Date Sampled	Hudson[1]	Grasse[1]
Grab (aka bulk) sediment sample	2006 and 2009	Moreau, NY N:1609914.52 E: 733570.10	Massena, NY N: 2232531.17 E: 410169.23
Core 7M	8/7/2006		N: 2227150.0 E:397698.5
Core 32S	8/7/2006		N:2231770.1 E:407629.7
Core 23N	8/7/2006		N:2230601.8 E:404663.4
Core 30S	8/7/2006		N:2232260.7 E:407121.8
Core 18M	8/7/2006		N:2230252.6 E:402307.2
Core CMU-01	10/2006		
Core CMU-02	10/2006		
Core CMU-03	10/2006		
Core CMU-04	10/2006		
Core T46.5	9/26/2007		N:2234177.3 E:414324.7
Core T18	9/26/2007		N:2230218.3 E: 402270.2
Core T44	9/26/2007		N:2233613.4 E:413156.5
Core T28	9/26/2007		N:2232105.4 E:406401.0

(1) Coordinates based on North American Datum (NAD) 1983, in the New York East State Plane Zone

3.1.1 The Grasse River

The Grasse River has been the site of extensive research on PCB transformation. Located near Massena, New York, and the site of an Alcoa aluminum smelting facility, the Grasse River was used for historic disposal of PCB contaminated wastes. Alcoa has undertaken an extensive experimental evaluation process as part of the site characterization and feasibility studies of this river. This prior work suggests Grasse River sediments contain PCB-dechlorinating organisms, and thus, they represent a suitable target for our characterization and use for further research.

The Grasse River sediment samples were collected in 2006. For bulk surface (the top 4 inches of sediment) samples, 30 gallons of sediment including a bit of water above was collected using a petite ponar dredge sampler from the Grasse River (N: 2232531.1744 ft; E: 410169.2369 ft, by North American Datum of 1983 [NAD83]) near Outfall 001 of the Alcoa facility, Massena, New York. This location was selected in order to collect samples with total PCBs of approximately 5-10 ppm. The grab sediment was mixed, divided in ten 3-gallon buckets and saturated with river water, then transported to Carnegie Mellon University on ice, where it was stored at 4^{o}C.

For sediment core samples, 5 cores of 4 inches of diameter were taken using manual collection techniques and Lexan tubing from locations in the river that prior coring indicated had intact sediments. Each core was segmented as follows: 0-1 cm, 1-2 cm, 2-3 cm, 3-4 cm, 4-5 cm, 5-10 cm, and every 5cm thereafter to the bottom of the core. Each segment was put in a glass jar and transported to Carnegie Mellon University. All the samples were stored at 4 ^{o}C until evaluation. The Grasse River cores were taken on August 7, 2006. Core 18M, discussed in detail below, was taken at Northing 2230252.6 and Easting 402307.2 in a water depth of 13.0 feet.

3.1.2 The Hudson River

The Hudson River has been extensively studied as it is a well-known site where use and disposal of PCBs has affected sediment. Prior research suggests the sediment will contain PCB-degrading microorganisms. The Hudson River grab sediment collection was performed under similar procedure to the Grasse Sediments at northing 1609931.99 ft and easting 733579.49 ft (by NAD83). Core sampling was also performed in the Hudson River; however, these cores were not intact and further characterization was not completed.

3.2 Core Characterization

Sediment cores were dated with Cs-137 by Mass Spec Services (Orangeburg, NY). This allowed evaluation to determine if the cores were intact (results described below). Only Grasse River core 18M was intact, and all further core characterization was completed only on this core.

Total organic carbon (TOC) was measured in our laboratory using a solids TOC analyzer (O-I-Analytical, College Station, TX). Porosity was estimated by air-drying 5 gram of wet sediment and assuming a solids density of 2.5 g/ml. Inorganic anions, sulfate, sulfide, chloride, bromide, fluoride, nitrite, nitrate and phosphate were determined by ion chromatography following United States Environmental Protection Agency (USEPA) method SW-846 9056A. Samples were analyzed using a Dionex ion chromatograph equipped with a Dionex AG9-HC anion precolumn, a Dionex AS9-HC separator column and a Dionex ED40 electrochemical conductivity detector. Heavy metals in

selected core segments were evaluated by TestAmerica Laboratories, Inc. using inductively coupled plasma mass spectrometry (ICP-MS).

3.3 Grab Sample Geochemical Analysis

Grab sediment samples from the Grasse and Hudson Rivers were analyzed by the Severn Trent Laboratories, Inc. (Pittsburgh, PA) and the Huffman Laboratories, Inc. (Golden, CO). Total organic carbon (TOC) was measured at Carnegie Mellon University using a solids TOC analyzer (O-I-Analytical, College Station, TX). Porosity for each core segment was calculated by air-drying 5-8 gram of wet sediment and assuming a solids density of 2.5 g/ml. Duplicate analysis of TOC and porosity were conducted on all samples. Inorganic anions were determined by ion chromatography using United States Environmental Protection Agency (USEPA) method SW-846 9056A.

3.4 Congener-Specific PCB Analysis of Sediments

In all samples, our objective was to evaluate the full congener profile to the extent possible with standard analytical methods. Standard methods were chosen to ensure consistency with prior work.

3.4.1 Extraction of PCBs from the Grab and Core 18M Sediment Samples

PCB extraction was modified from the method described elsewhere (Quensen et al. 1988; Rysavy et al. 2005; Yan et al. 2006a; Yan et al. 2006c). PCBs were extracted from 5 gram air-dried sediment of 20 segments in the intact core and surface grab sediment sample by an automated soxhlet extraction apparatus (FOSS soxtec 2050 extractor, FOSS, Eden Prairie, MN) following USEPA Method 3541. Briefly, 5 g dry sediment spiked with 0.5 µg PCB 209 (2,2',3,3'4,4'5,5'6,6'-CB) was extracted with 70 ml Acetone/Hexane (1:1) for 2 h. Each extract was concentrated down to 2 ml using a nitrogen evaporator (The Meyer N-EVAP; Associates, Inc., Berlin, MA). Then, two clean-up procedures were applied to avoid analytical interferences: tetrabutylammonium (TBA) sulfite procedure was used according to EPA Method 3660B for the removal of sulfur; Florisil cleanup was conducted according to EPA Method 3620B. In brief, approximately 1 ml of TBA pretreated extract was added to a 13 mm i.d. glass column packed with 7 g 60/100-mesh pesticide grade Florisil (Fisher Scientific, Fair Lawn, NJ) and 2 g anhydrous Na_2SO_4. The column was eluted with 35-40 ml of n-hexnae and the elutant was concentrated to 5.0 ml for gas chromatography (GC) injection. A procedural blank was performed with every eight sediment samples in the same manner.

3.4.2 Congener Specific PCB Analysis with the GC-µECD Method

Individual PCB congeners were analyzed by Hewlett Packard gas chromatograph (Model 6890) equipped with a micro-electron capture detector (µECD) and a 30 m DB-XLB capillary column (0.18 mm diameter and 0.18 µm film thickness; Agilent Technologies, Palo Alto, CA) in splitless mode. All PCB commercially available mixtures and 209 individual congeners were purchased from AccuStandard (AccuStandard, Inc.; New Haven, CT). The GC temperature program started at 50 °C, held for 1 minute, followed by an increase of 12 °C /min to 150 °C, 0.4 °C /min to 220 °C and 2 °C /min to 260 °C. The carrier gas was helium; the injector and detector temperatures were held at 275 °C and 300 °C, respectively. PCB205 was used as an internal standard and a five-point external standard curve was created for each congener (concentration ranged from 8 ng/m to 1 µg/ml). Detection limits for most PCB congeners were below 1 ng/g dry weight, except for

monochlorobiphenyls which were 4 ng/g. A When necessary, X10 and X50 dilutions were made. This dilute-to-match procedure was applied to ensure all PCB congeners fell within their external standard range. Individual PCB concentrations were obtained based on the response factors derived from their most close standards with the equation below.

$$Cx = Cn \times \frac{Ax}{An} \times \frac{Vx}{Mx} \times Fx$$

in which,

Cn=known PCB standard concentration (ng/ml hexane)

An=peak area of known PCB standard

Ax=peak area detected in samples.

Cx=PCB concentration in samples (ng/g dry sediment)

Fx=sample dilution factor

Vx=sample volume prepared for GC injection (ml)

Mx=dry sediment mass used for PCB analysis (g)

GC-μECD chromatograms for a master mixture (containing 209 congeners) showed 126 peaks without coelution and 37 peaks with coelution of 2 or more congeners. For the present study, 30 out of 37 coeluting peaks with slightly different retention times were split by the shoulder detection function of ChemStation software (Agilent Technologies, Palo Alto, CA). For the rest of the coeluting peaks, 2 of them were not detected in Core 18M and 5 peaks were assigned to one of the corresponding co-eluters. For most of the co-eluters, the response factors of GC signals for coeluting congeners were very similar to each other. Therefore, the error for the estimation of total PCB concentrations due to misassignment of these peaks is not expected to be greater than 5%.

Separate analysis conducted by our team enables quantification of the introduced uncertainty due to peak-splitting (Hughes et al. 2010; Hughes 2010). The reported PCB concentrations were not corrected for blank or recovery or reported with ranges related to peak-splitting uncertainty. Total PCB recoveries, in this study, ranged from 92.6% to 104.0%.

3.5 Molecular Microbiological Analyses

The focus of Objective 1 of this project was to evaluate the microbial diversity generally and to target putative PCB dechlorinating organisms from field and laboratory samples. Our approach was to use multiple methods for analysis to understand the microbial diversity and community structure. **Figure 4** shows the overall approach on the right (recall **Figure 3** from *Section* 3), while on the left it shows the components of the evaluation of bacterial diversity. DNA from sediment samples from the grab samples and sections of core 18M are amplified by PCR, evaluated through DGGE, sequencing, and quantitative PCR. DNA from specific microcosm studies is similarly analyzed. This approach provides qualitative and quantitative characterization of the microbial community.

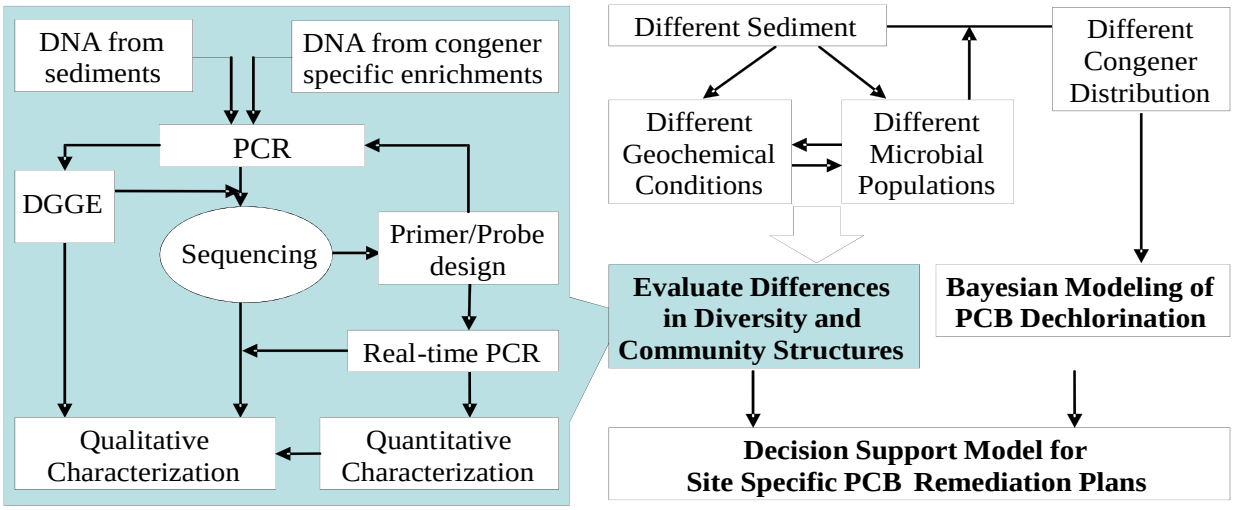


Figure 4. Expanded Research Approach for Evaluation of Differences in Diversity and Community Structures.

3.5.1 Genomic DNA Extraction

Total genomic Deoxyribonucleic acid (DNA) was extracted using a PowerSoil DNA Purification Kit (MoBio, Carlsbad, CA). Three 0.25 – 0.3 g of wet sediment from each core segment were used for the extraction. The concentration of the genomic DNA solutions was measured by a fluorometric method using a PicoGreen Double-stranded DNA Quantification Reagent (Invitrogen, Carlsbad, CA) and Bass-o-Matic 5000 fluorometer (Bio-Rad, Hercules, CA).

3.5.2 Microbial Community Profiles by PCR-DGGE Analyses

Eight core segments from core 18M and surficial sediments from Grasse River and Hudson River were analyzed using denaturing gradient gel electrophoresis (DGGE). Polymerase Chain Reactions (PCR) were performed using 4 group-specific primer sets (seen in Table 3).

Table 3. Group-Specific Primer Sets for PCR-DGGE Analysis of Sediment Samples

Target Group	Forward Primer	Reverse Primer	Amplicon size	References
Bacteria (BAC) *Chloroflexi* (CHL) *Dehalococcoides* (DHC) *o-17*/DF-1(pcb)	BAC341F-[GC clamp] CHL348F-[GC clamp] DHC1F-[GC clamp] BAC908F-[GC clamp]	BAC534R Dehal884R DHC259R Dehal1265R[1]	234 bp 577 bp 299 bp 398 bp	(1) (2) (3) (4)
GC-Clamp	CGCCCGCCGCGCGCGGCGGGCGGG GCGGGGGCACGGGGGG		40 bp	(5)

[1] 5'-CCTATTGCTACCTGCTGTACCAC-3' modified from the original primer

(1) Muyzer et al. 1993
(2) Fagervold et al. 2005
(3) Hendrickson et al. 2002; Duhamel et al. 2004;
(4) Watts et al. 2005; Relman 1993
(5) Muyzer et al. 1993

For *Bacteria*, primer set BAC341F and BAC534R is specific to variable 16S rDNA V3 region (Muyzer et al. 1993). For dechlorination related *Chloroflexi* group, forward primer is phylum *Chloroflexi* specific, but reverse primer targets to putative PCB dechlorinators including *Dehalococcoides* and DF1/*o-17* (Fagervold et al. 2005). Primer set DHC1F and DHC259R is specific to *Dehalococcoides* (Hendrickson et al. 2002; Duhamel et al. 2004), while another primer set with universal primer BAC908F and specific reverse primer Dehal1265R targets DF1/*o-17* and some related PCB dechlorinators (Watts et al. 2005; Relman 1993). Amplification reactions were performed in a total volume of 100 µl. The reaction mixtures contained 10 µl of 10X reaction buffer, 2 mM $MgCl_2$ (1.5 mM $MgCl_2$ for CHL), 0.2 mM of each dNTP, 300 nM of each primer, 2.5 units of Taq DNA polymerase (Invitrogen, Carlsbad, CA), and 15-70 ng of template sediment genomic DNA. The following thermal cycling program was used for the amplification: initial denaturation at 95 ^{o}C for 3 min; 35 cycles of denaturation at 94^{o}C for 1 min, annealing at 55 ^{o}C for 45 s, and extension at 72 ^{o}C for 45 s; and a final extension at 72 ^{o}C for 7 min.

DGGE was performed with a DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA). PCR products (20 µl) were loaded onto 8% (wt/vol) polyacrylamide gels containing a specified linear denaturing gradient formed with 7 M urea and 40% (vol/vol) deionized formamide. Electrophoresis was performed in 1X TAE buffer (40 mM Tris base, 20 mM glacial acetic acid, 1 mM EDTA) at 70 V and 60°C for 15 hours. After electrophoresis, the gels were stained for 20 min in 1X TAE buffer containing 1X SYBR Gold and visualized under UV light in the Gel Doc XR (Bio-Rad, Hercules, CA).

Raw DGGE gel images were analyzed with GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium) to perform Shannon-weaver diversity index analysis and cluster analysis for each target group following the software manual instructions. Relative band surface was used for comparative quantification, and the unweighted-pair group method with Pearson correlation was selected for the comparison setting.

3.5.3 Clone Library for Bacteria in Core Segments

Bacterial clone libraries for two segments of core 18M (100-105cm and 210-215cm) and the surfical sediment samples from the Hudson and Grasse rivers were constructed with the TOPO TA Cloning Kit (Invitrogen Carlsbad, CA) according to the manufacturer's instructions. Primers for the bacterial clone library were Bac27F (Lane 1991) and Bac805R (Yu et al. 2005). Target DNA fragments were amplified with 15-70 ng template genomic DNA under the same conditions described above (Seen in PCR conditions for DGGE), except for a 10 min final extension. For each library, clones containing no contamination were selected for sequencing and sent to SeqWright (Houston, TX) with the M13 forward as a sequencing primer. Sequences of bacterial 16S ribosomal ribonucleic acid (rRNA) gene fragments from SeqWright were trimmed and converted to the FASTA file format using a Chromas (Technelysium Pty Ltd, Australia). The identification and classification of each clone were assigned using the Classifier through the Ribosomal Database Project (RDP) II (http://rdp.cme.msu.edu/). Highly similar sequences (strains) to each clone were searched using MegaBlast through the National Center for Biotechnology Information (NCBI)-BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). A program ClustalX was used for multiple alignment and drawing trees (Thompson et al. 1997), which were manipulated with a TreeView program (Page 1996).

3.5.4 Quantitative Polymerase Chain Reaction (Q-PCR)

Target 16S rRNA genes (copies/g dry wt) in 13 segments and surficial sediments were determined by SYBR Green-based quantitative polymerase chain reaction (Q-PCR) assays using seven group specific primer sets (Table 4).

Table 4. Primer Sets and their Target Groups for Q-PCR Reactions

Target group	Forward primer	Reverse primer	Amplicon size (Estimation)	References
Bacteria (BAC)	BAC338F	BAC534R	197 bp	(1)
Chloroflexi (CHL)	CHL348F	Dehal884R	537 bp	(2)
Dehalococcoides (DHC)	DHC1200F	DHC1271R	72 bp	(3)
o-17/DF-1 (pcb)	BAC1114F	Dehal1265R[1]	152 bp	(4)

[1]:5'-CCTATTGCTACCTGCTGTACCAC-3' modified from the original primer

(1) Muyzer et al. 1993
(2) Fagervold et al. 2005
(3) He et al. 2003
(4) Watts et al. 2005

The amount of sediment genomic DNA used for Q-PCR assays was optimized by serially diluting original DNA extracts to avoid the adverse effects by environmental autofluorescence and inhibitors typically found in sediment. External standard curves were constructed with pure 16S rDNA templates extracted from the corresponding bacterial clones. In brief, amplification reactions were performed with a Power SYBR Green PCR Master Mix in a total volume of 25 µl contained 12.5 µl of Power SYBR Green PCR Master Mix (2X), 300 nM of each primer, 1-7 ng of template genomic DNA from the sediment samples and pure 16S rDNA standards ranged from 101 to 108 copies per well on a ABI 7500 Real-Time PCR System (Applied Biosystems Inc. Foster City, CA). The

thermal cycling parameters consisted of an initial hold at 95°C for 10 min and 40 cycles of 95°C for 15 sec, and 60°C for 1 min, followed by final extension at 60°C for 7min. A thermal cycling step for the dissociation (melting) curve analysis was added at the end of the amplification step and included 95°C for 15 sec, annealing and extension at 60°C for 1 min, and a final denaturation at 95°C for 15 sec.

3.6 Computational and Statistical Analyses Methods

Relationships among data analyzed for the core and grab samples followed standard methods. Pearson correlation analysis between total PCBs and TOC, student T-test, and Principal Component Analysis (PCA) of PCB homolog distributions were performed using Minitab 15 (Minitab Inc, State College, PA). In addition to these data analysis methods, a large part of the project focused on the development of new data analysis techniques for the PCB field. **Figure 5** presents the approach taken for this part of the research. The left side shows the overall approach for ER-1495 (recall, **Figure 3** above), while the right side shows the detail of the activities related to Bayesian modeling methods.

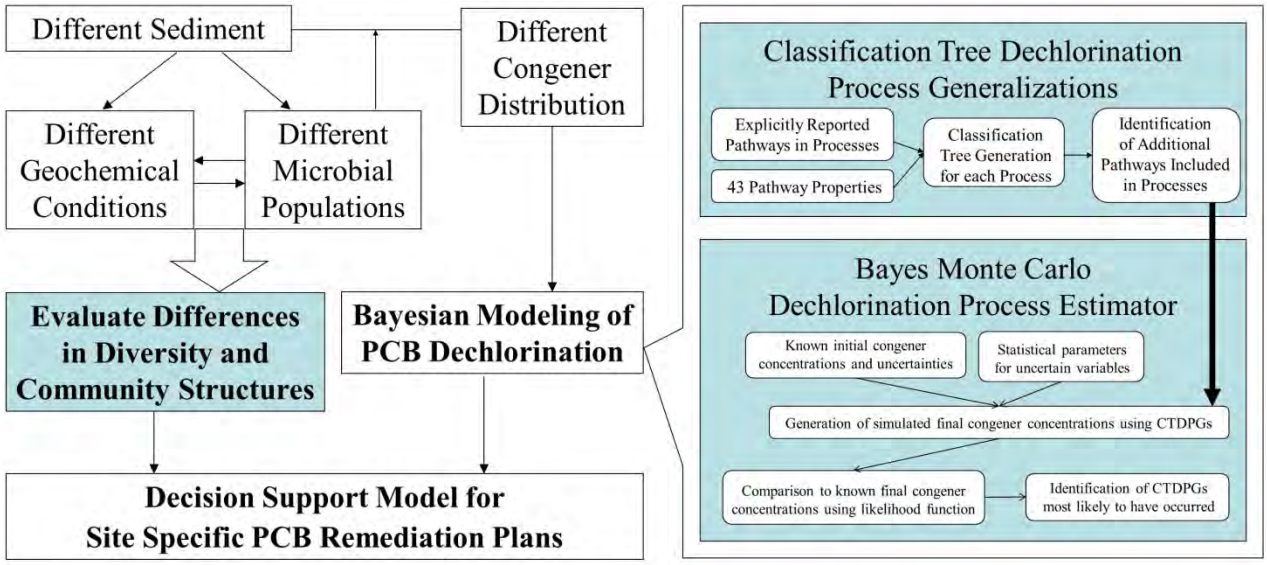


Figure 5. Research Approach for Statistical Methods.

3.6.1 Molar Dechlorination Product Ratio (MDPR)

In order to address the question of whether PCB sediments (in the Hudson River) have been subjected to dechlorination, the USEPA developed the metric Molar Dechlorination Product Ratio (MDPR). MDPR is defined as the sum of the molar concentrations of five congeners (PCB 1 (2-), PCB 4 (2-2), PCB 8 (2-4), PCB 10 (26-) and PCB 19 (26-2)) divided by the by the total molar concentration of PCBs in the sample (USEPA 1997). The selected congeners represent the presumed end products of dechlorination in the Hudson River, under the assumption that *ortho* dechlorination has not occurred. Although it is not an endpoint of *para*/*meta* dechlorination, PCB 8 was included in the methodology because it accumulated in Hudson sediments during development of the ratio.

3.6.2 Identification of Missing Pathways in Eight Processes

The primary objective of this portion of the project was to identify pathways missing from sets of explicitly reported pathways in each dechlorination processes presented in Table 1. Missing pathways in are largely due to analytical limitations. They were statistically identified based on their similarity to pathways explicitly reported to belong in each dechlorination process. The methods for identifying these pathways are presented in detail in Hughes et al. 2010, and thus are not recapitulated here. A brief summary is provided.

Identification of missing pathways was accomplished through the application of *classification trees*. Briefly, a classification tree was constructed for each of the eight dechlorination processes using the C4.5 algorithm with standard pruning (Quinlan 1993; Witten and Frank 2005). The data set investigated consisted of the 840 possible dechlorination pathways and corresponding properties of each pathway's parent congener, in addition to the information regarding which pathways are explicitly reported in a given process. Forty-three properties of the parent congener and three attributes encompassing a change in structure between the parent and daughter congeners of a pathway were considered.

The classification trees were generated using only the 108 pathways explicitly reported in one or more processes and their respective properties. Each classification tree represents common properties of the pathways in the given dechlorination process. These common properties can be statistically interpreted as criteria that dechlorination pathways must meet in order to be included in the given dechlorination process. These criteria were then applied to pathways not explicitly reported in the given process. Pathways that were not explicitly reported in a given process but meet the classification tree's criteria for belonging in the given process, are identified as missing pathways from that dechlorination process. This set of pathways predicted to belong in a dechlorination process by the classification tree (missing pathways), in addition to the explicitly reported pathways constitute *classification tree dechlorination process generalizations* (CTDPGs).

3.6.3 Bayes Monte Carlo (BMC) Method for Process Occurrence

The Bayes Monte Carlo (BMC) method (Dilks et al. 1992) has been applied to a wide variety of environmental problems (Sohn et al. 2002; Lo et al. 2005; Schenker et al. 2009; Schoen et al. 2010). It is a technique for decreasing the uncertainty of a calculation's input parameters through a comparison of calculated output values to observed values (Sohn et al. 2000).

In this work, the BMC method is used to identify the occurrence of one or more of the eight known dechlorination processes. The model, termed the Dechlorination Process Estimator (DPE), uses a discrete chemical reaction equation for each of the 209 congeners such that mass from parent congeners is transferred to child congeners across 840 dechlorination pathways. The chemical reaction equation is as follows:

$$C_{f,a} = M^{R} C_{i,a}$$

Where *C* is an array of normalized biphenyl and congener masses with *a* equal to 1, ..., 210 (where biphenyl is assigned number 1 and congener 1 is assigned number 2, etc.). Subscripts *i* and *f* on variable *C* represent the initial and final biphenyl and congener distributions, respectively. Variable *R* represents the number of dechlorination steps. It represents the potential transfer of mass from all

parent congeners to all child congeners, such that ten steps are required to transfer mass in the most chlorinated congener, 23456-23456, to biphenyl. Variable *M* is a 210 by 210 matrix; it describes the percentage of mass remaining or transferred from a parent congener to one or more daughter congeners or biphenyl in a single dechlorination step. Together, variables *M* and *R* represent the dechlorination rates of each congener. The above chemical reaction equation assumes *measured* initial and final concentrations or relative masses are available for each congener. Individual uncertainties can be assigned to each measured congener concentration to account for congener-specific co-elutions or volatilizations. Through the Monte Carlo method, the chemical reaction equation is calculated 10,000 times, where each calculation is referred to as a simulation. In each simulation, samples are drawn for unknown variables (including the number of dechlorination steps and the percentage of mass in each parent congener transferred to each daughter congener in each dechlorination step) such that a distribution of values is generated for each congener in the calculated final congener distribution, $C_{f,a}$.

Variables *M* and *R* are considered uncertain and are therefore assigned prior probability distributions. *R* is represented by a geometric distribution with parameter 0.25, such that its mean value is four. Values in *M* are first determined according to the uncertain occurrence of the eight *classification tree dechlorination process generalizations* (CTDPGs). The occurrence of each CTDPG is sampled from a Bernoulli distribution with parameter 0.5. Thus, CTDPGs can occur individually or simultaneously. Pathways in the occurring CTDPG(s) are then sampled from a uniform distribution with a range of zero to one. These values are contained in matrix *M* and change for each Monte Carlo simulation. Prior to inclusion in the chemical reaction equation, the values representing mass removal from a congener via dechlorination and mass remaining with a congener across a dechlorination step are normalized to one, so that mass is conserved in the system of 209 congeners and biphenyl.

Given sampled values in *M* and *R*, the calculated final congener distributions are compared to the measured congener distribution using Bayesian statistics. A likelihood function is used to weight the final calculated congener distributions such that calculated distributions more similar to the measured final congener distribution receive greater weight. A normal likelihood function represents uncertainty associated with quantification of the congeners:

$$f(x|\mu,\sigma) = \frac{1}{\sqrt{2\pi\sigma^2}} exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right)$$

where x is the natural log of the observed congener relative mass, μ is the natural log of the congener relative mass calculated by the DPE and σ is the standard deviation of the natural log of the observed congener relative mass. Weights of each simulation generated by the likelihood function are then normalized across the simulations. These normalized weights are used to generate weighted averages of the uncertain variables in the form of posterior distributions (considered to be updates of the prior distributions). The most significant update is to variables for dechlorination process occurrence, which indicate whether one or more (or no) dechlorination process occurred between the initial and final measured congener distributions.

Next, the DPE's ability to identify uncertain parameters, in particular occurring dechlorination processes, was tested with sixteen synthetic dechlorination scenarios (see results below). The use of

synthetic data in place of an observed final congener distribution ensured that the true values of the uncertain parameters were known. The generation and archiving of greater than 1200 variables in each MC simulation was found to be too computationally intensive for available standalone workstations. Therefore, the DPE was run in parallel on the Pople supercomputer, a 768 core machine, at the Pittsburgh Supercomputing Center.

3.6.4 Decision Support Modeling Methods

Although Objective 3 (and related Task D) was cancelled in the middle of the project, initial results were generated through scoping activities that took place in the early part of the project.

Multi-criteria decision analysis (MCDA) methods and tools can provide a systematic approach to integrating competing criteria into decisions. A detailed analysis of the theoretical foundations of these decision methods and their comparative strengths and weaknesses is presented in Belton and Steward 2002, while reviews of MCDA applications in various environmental areas is presented by Kiker et al. 2005a. The common purpose of MCDA methods is to evaluate and choose among alternative courses of action based on multiple criteria using systematic analysis that overcomes the limitations of unstructured individual or group decision-making. While the basic organization of criteria and alternatives is similar in most MCDA approaches, the methods differ in their synthesis of the information and strategy in ranking the alternatives by different means. The novel approach taken to integrate MCDA with biogeochemical fate and transport models is described in the results section below.

3.7 Microcosm Study Methods

The objective of these PCB microcosm experiments was to: 1) verify the dechlorination processes that were previously observed in PCB contaminated sites, 2) to evaluate the effect of different geochemical conditions on extent of dechlorination, and 3) determine if critical microbial dechlorinating species are present in the sediment samples.

3.7.1 Selection of PCB Congeners

In 2004, Karcher et al. used the Aroclor distribution patterns published by Frame, Cochran and Bowadt (1996a) to identify several groupings of congeners that were correlated in the original Aroclor mixtures. Using these congeners, the changes in relative proportions of pairs of the correlated congeners (referred to as *tracker pairs*) found in sediment samples collected from the Hudson River (USEPA 1995) were examined. Some pairs were found to exhibit statistically significant shifts from their Aroclor relative proportions. To maximize the likelihood of identifying dechlorination pathways in the field samples and to evaluate the utility of tracker pairs as indicators of reductive dechlorination, 13 congeners were selected for among the list of correlated congeners to be spiked in to our microcosms. The congeners were chosen based on the following criteria: (1) the congener pairs showed interesting changes in prior work with Grasse and Hudson River samples; (2) the tracker pairs appear as parent congeners in explicitly reported dechlorination processes or in the classification tree dechlorination process generalizations (CTDPG) (as discussed below); (3) the tracker pairs do not co-elute in the gas chromatograph/electron capture detector (GC/ECD) method used in our laboratory; (4) the first generation daughters do not overlap with other selected congeners; and (5) the tracker pairs have the fewest number of co-eluting daughter products; (6) the spiked congeners have a high Aroclor percentage contribution and the daughter

products have a low Aroclor percentage contribution (cutoff = 1.00%); and (7) each mixture includes a dioxin-like congener or a congener associated with a non-carcinogenic risk.

The above criteria exclude a large number of PCB congeners. Following these criteria, two combinations of PCBs were developed and used to spike the microcosms. Each mixture contained 9 PCB congeners. The first set, referred to as *Mixture 1*, contained PCB 5, 12, 64, 71, 105, 114, 149, 153, and 170. The second set, referred to as *Mixture 2* contained PCB 5, 12, 64, 71, 82, 97, 99, 144, and170. All the selected PCB source congeners, their likely first generation product congeners, co-eluters in the system and the processes of corresponding dechlorination pathways are shown in **Figure 6** and **Figure 7**. The characteristics of the congeners selected to be spiked into the microcosms are shown in **Table 5**.

Table 5. Summary of Criteria for Selecting Congeners

	# of Pathways		Aroclor Percentage Contribution		Health Risk
Congener	**High Priority**	**Original**	**Parent**	**Daughter**	**Dioxin-like**
PCB 5	0	2	Low	Low	No
PCB 12	1	2	Low	Low	No
PCB 64	0	4	High	High	No
PCB 71	1	3	High	High	No
PCB 105	1	5	High	High	Yes
PCB 114	0	5	Low	Low	Yes
PCB 149	0	6	High	High	No
PCB 153	0	3	High	High	No
PCB 170	0	7	Low	Low	No
PCB 82	0	5	High	Low	No
PCB 97	3	5	High	High	No
PCB 99	1	5	High	High	No
PCB144	0	6	Low	High	No

The Aroclor percentage contribution was calculated by averaging the results from Aroclors 1016, 1242, 1248, and 1254 as found in the literature (Frame et al. 1996b). Some of the lower chlorinated Aroclors were omitted as it is believed that the higher chlorinated mixtures were introduced into the Hudson and Grasse Rivers. In **Table 5,** and Aroclor percentage of High indicates that, in at least one of the Aroclors, the congener contributes greater than 1.0% to the Aroclor mass. Conversely, a low percentage indicates that the congener did not constitute greater than 1.0% mass in any Aroclor.

3.7.2 Sediment Characterization for Microcosm Preparation

The Hudson River and the Grasse River sediments (described in *Section 3.1* above) were homogenized and sieved with autoclaved 48 mesh and 60 mesh metal sieves respectively under a stream of nitrogen. A 48 mesh metal sieve was applied for the Hudson sediment due to its small silt plus clay percentage. The sieved wet sediment was stored at 4ºC under nitrogen until use.

Sediment PCBs were determined via the same method described *Section 3.4* above. Both the Hudson and the Grasse River sediment samples have a similar total PCB level (1-2 mg/kg dry weight). Since the background PCBs account for less than 1% of the total PCBs in the PCB spiked sediments (250 mg/kg dry weight was spiked), individual PCB analysis is unlikely to be affected by the background concentrations. Sediment moisture and total organic carbon (TOC) were measured prior to microcosm setup. To determine the sediment moisture, approximately triplicate 10 g homogenized wet sediment subsamples were weighed out in aluminum weighing dishes. The sediments were air-dried for 5-7 days until constant weights were achieved. Traditionally, wet sediment is dried at 105 ºC. Herein, air-drying at room temperature was used to minimize the change of sediment properties. TOC was analyzed as described above. The results show that the average dry/wet ratios are 0.68 for the Hudson sediment and 0.35 for the Grasse sediment. The Grasse sediment has much higher total organic carbon content (5.73%) than the Hudson sediment (1.26%). The two sediment systems are different in sulfate and Fe concentrations. Sulfate and Fe (III) are more favorable electron acceptors that may inhibit PCB dechlorination. Therefore, the effect of sulfate and FeOOH are examined in the present study. To determine the concentrations of sulfate and Fe to be added to the sediments, previous studies were reviewed and compared (Morris et al. 1992a; Cho and Oh 2005), and 16 mmole/kg slurry (= 80 mmole/kg dry weight) of sodium sulfate (Na_2SO_4) and 40 mmole/kg slurry (= 200 mmole/kg dry weight) of iron gel (FeOOH) were used as amendments.

3.7.3 Preparation of PCB Spiked Sediments

The PCB spiked sediments were prepared in several steps. First, the specific PCB mixture (1 or 2) was prepared and its concentrations confirmed. Next, the PCB mixture was spiked onto dried sediment, and the concentrations were again confirmed. Then, microbial media was prepared. Finally, the microcosms were assembled with the proper spiked sediment, seed sediment, microbial media and chemical amendments following the plan in Table 6. Details of each step in this process are provided in the following sections.

3.7.3.1 Prepare the PCB Mixture

Neat PCB standards (>99%) were purchased from AccuStandard Inc. (New Haven, CT, USA). The specific amount of each congener selected for the microcosm was weighed into a 20 ml glass vial; a total of 50 ppm (μg/g slurry) total PCBs were spiked. For PCB *Mixture 1*, tracker pairs 5/12, 64/71, 105/114 each contribute to 20% of the total PCB by weight and 149/153/170 contributes 40% of the total PCBs. For PCB *Mixture 2*, the abundance of each tracker pair 5/12, 64/71, 144/170, is 20% and 82/97/99 contributes 40%. These distributions were determined by examining the PCB congener distributions in the 17 commercial Aroclor lots (Frame et al. 1996a).

For each mixture (1 and 2), the congeners were dissolved in pure hexane and transferred to a 1 L volumetric flask. The vials were further rinsed 5-7 times with approximately 5 ml of hexane to ensure that all the PCBs were transferred to the volumetric flask. After bringing the volume to 1 L, the volumetric flask was sonicated for 10 min to mix the solution. In order to confirm the initial PCB concentrations in the spike, 0.2 ml of the solution was withdrawn and diluted with 9.8 ml hexane in a 12 ml amber glass vial, and full congener PCB analysis was performed as described in *Section 3.4.2* above. The measured concentrations were very close to the concentrations calculated from the weighed PCBs when the peak areas fell within the linear range of the standard curves. In the three dilutions, X50 was relatively concentrated where the GC response factors decreased.

Therefore, the measured concentrations based on the response factors derived from relatively low concentrations of individual standards were underestimated. Thus, 0.02% of the total solution volume is negligible and the total PCB mixture solution is still regarded as 1.0 L.

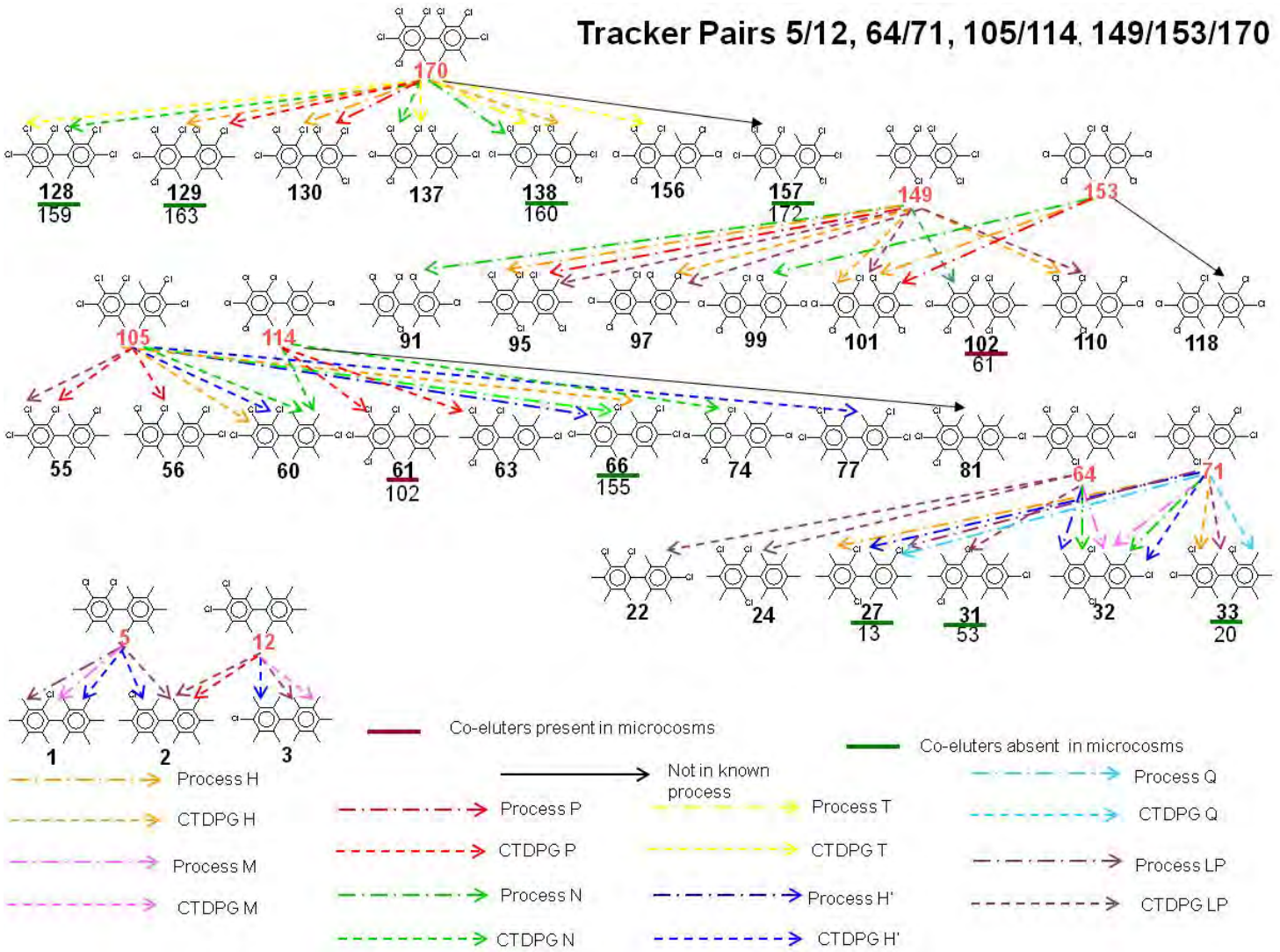


Figure 6. Selected PCB Congeners and Dechlorination Pathways for Mixture 1.
Generalized established dechlorination pathways and processes, and additional CTDPG identified pathways and processes along with their likely first generation products.

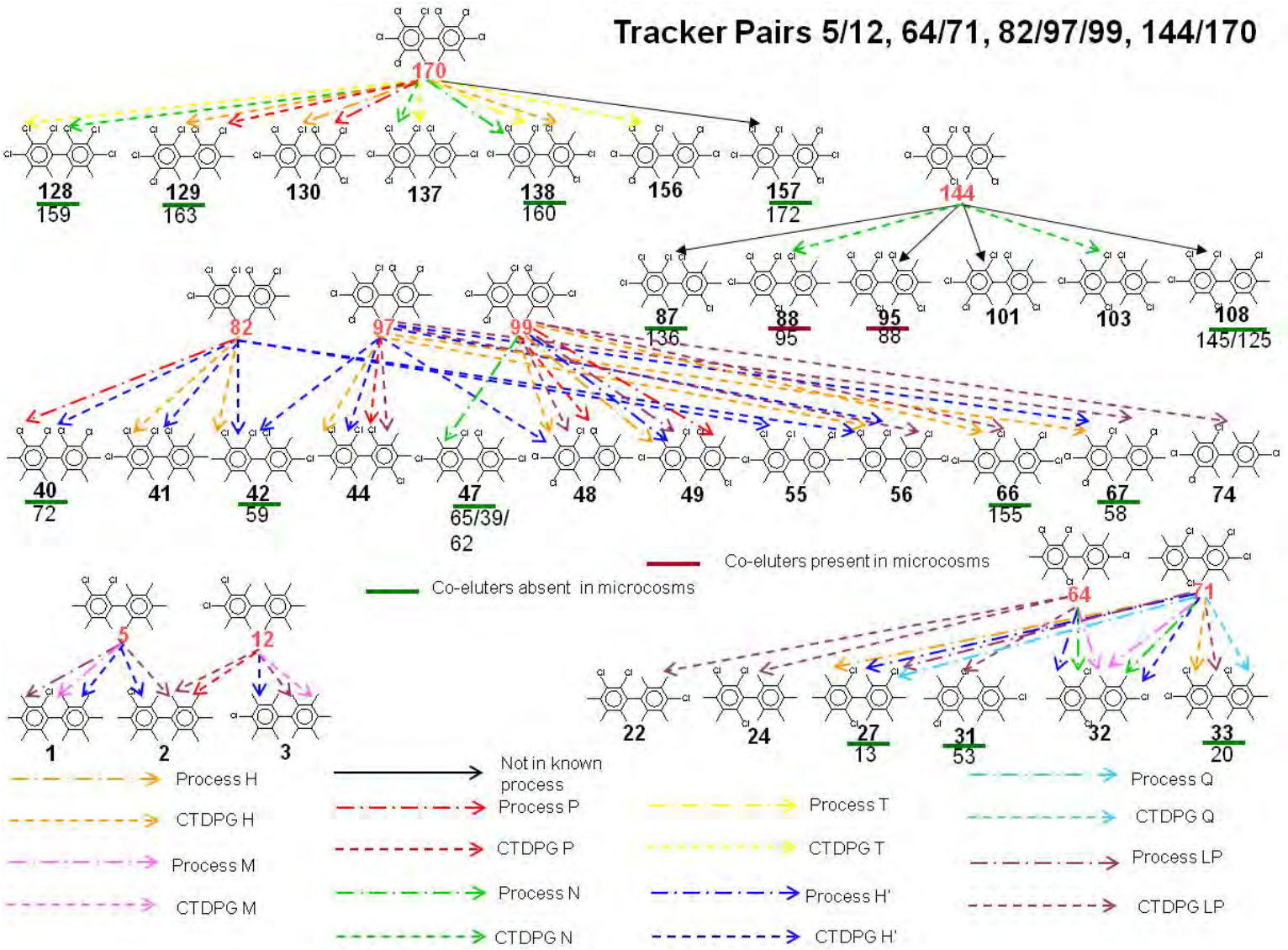


Figure 7. Selected PCB Congeners and Dechlorination Pathways for Mixture 2.
Generalized established dechlorination pathways and processes, and additional CTDPG identified pathways and processes along with their likely first generation products.

Table 6. Experimental Setup in Microcosm Study

	PCB Mixture 1 (5/12; 64/71; 105/114; 149/153/170)			**PCB Mixture 2 (5/12; 64/71; 82/97/99; 144/170)**			**No PCB**
	TP	TP & Na_2SO_4	TP & FeOOH	TP	TP & Na_2SO4	TP &FeOOH	
Hudson	Set 1 H-1-01(36)	Set 2 H-1-S-01(36)	Set 3 H-1-Fe-01(36)	Set 7 H-2-01(36)	Set 8 H-2-S-01(36)	Set 9 H-2-Fe-01(36)	Live Control 1 H-01(36)
Grasse	Set 4 G-1-01(36)	Set 5 G-1-S-01(36)	Set 6 G-1-Fe-01(36)	Set 10 G-2-01(33)	Set 11 G-2-S-01(36)	Set 12 G-2-Fe-01(36)	Live Control 2 G-01(36)
Hudson (Killed)	Killed Control 1 H-1-K-01(20)			Killed Control 2 H-2-K-01(20)			
Grasse (Killed)	Killed Control 3 G-1-K-01(20)			Killed Control 4 G-2-K-01(20)			
Hudson (Substrate)	PCB-spiked Sediment Control 1 H-1-A-01 (06)			PCB-spiked Sediment Control 2 H-2-A-01 (06)			
Grasse (Substrate)	PCB-spiked Sediment Control 1 G-1-A-01 (06)			PCB-spiked Sediment Control 1 G-2-A-01 (06)			
Hudson (River Water)	Long Term Treatment 1 H-1-W-01(06)			Long Term Treatment 2 H-2-W-01(06)			
Grasse (River Water)	Long Term Treatment 3 G-1-W-01(06)			Long Term Treatment 4 G-2-W-01(06)			

Note that each set represents different experimental conditions.

TP: Track Pair; H: Hudson Sediment; G: Grasse sediment; 1: PCB *Mixture 1* containing PCB tracker pairs 5/12, 64/71, 105/114, 149/153/170; 2: PCB *Mixture 2* containing PCB tracker pairs 5/12, 64/71, 82/97/99 and 144/170; S: sulfate amendment; Fe: FeOOH amendment; K: killed control; A: PCB-spiked, autocalved wet inoculum sediment control; W: long term control using river water. Number in parenthesis is the number of replicate bottles prepared for that set.

3.7.3.2 Prepare the Spiked Sediment

In order to maintain the natural sediment geochemical conditions, the dried sediments were not autoclaved. Rather, 468 g air-dried sediment and tracker pair mixture combination was weighed out, and 500 ml of the PCB mixture solution was poured over the 468 g sediment in an organic-free 2 L glass beaker. Sediment was covered by the solution. The beaker was placed in a laminar flow hood and the spiked sediment was mixed with an autoclaved stainless steel spoon periodically until hexane fully evaporated. In addition to confirming the original solution provided the desired PCB congener concentrations (described above), the spiked sediment samples for each sediment type and each spiked PCB mixtures were analyzed in duplicate to determine the true PCB amount in the spiked sediments. This was important because solvent evaporation might result in loss of lower chlorinated PCBs, leading to lower than planned concentrations of these congeners in the microcosm. Based on the previous GC results of PCB solutions, X400 dilutions were performed the spiked sediment PCB analysis. Very few losses were observed, confirming the spiking technique and the analytical methods are acceptable. PCB recoveries were higher than 95%. The high recoveries for lower chlorinated PCBs indicate that evaporative losses during hexane evaporation were negligible.

3.7.3.3 Prepare the Microbial Media

Modified reduced anaerobic mineral medium (RAMM) was prepared as described by others (Shelton and Tiedje 1984; Yan et al. 2006b), except that 2 mM L-Cysteine·HCl was used as the reducing agent and 1% (vol/vol) Wolfe's vitamin solution was added to the medium. The pH of the medium was adjust to 7.0 using 1 M NaOH and autoclaved for 20 min at 121ºC under a nitrogen atmosphere.

3.7.3.4 Assemble the Microcosms

Microcosms were prepared in a gas atmosphere of 0.9% H_2 in O_2-free N_2 gas. Unless stated otherwise, 3 g dry sediment substrate, 3 g fresh sediment inoculum (dry weight basis), and a certain amount of freshly prepared RAMM medium with or without amendments were added to a 50 ml serum bottle to achieve a total weight of 30 g. All bottles were sealed with Teflon-lined gray butyl rubber septa (The West Pharmaceutical Co. PA) and crimped with aluminum crimp caps. Live control vials received 3 g fresh sediment inoculum (dry weight basis) and 3 g dry sediment. Duplicate killed controls were prepared by autoclaving the serum bottles for 45 min at 121ºC on three consecutive days. To set up PCB spiked sediment controls, serum bottles containing 3 g fresh sediment inoculum (dry weight basis), RAMM medium were pre-autoclaved three times at 121 ºC for 15 min. 3 g dry sediment substrate was added to the autoclaved bottles and resealed. Long-term testing microcosms consist of medium 3 g dry sediment substrate, 3 g fresh sediment inoculum (dry weight basis) and river water. After mixing on a rotating mixer (40 r.p.m) for 24 hr in dark, triplicate bottles for each treatment were sampled and the rest of microcosms were incubated at ambient temperature in drawers.

The treatments used in this study are tabulated and presented in Table 6. For most treatments, 36 identical bottles were set up to ensure at least 12 sampling points (triplicate bottles were sampled at each date point). To differentiate the treatments, the Hudson microcosms were crimped with blue

crimp caps and labeled with blue marker, while the Grasse microcosms were sealed with grey crimps and labeled with black marker as shown in **Figure 8**.

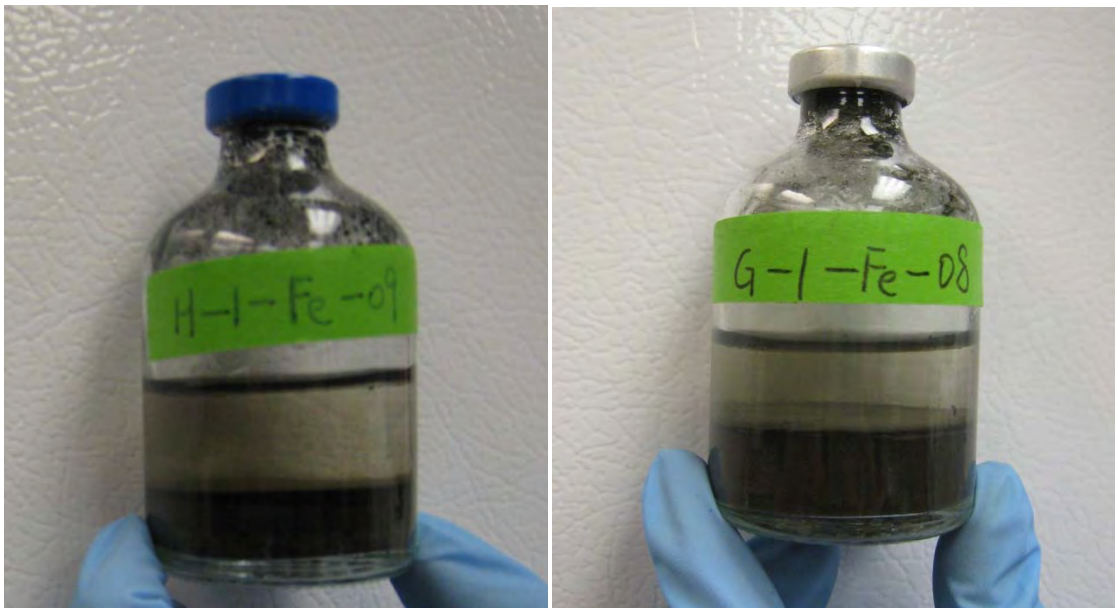


Figure 8. Photos of the Microcosm Bottles with Containing River Sediment. Hudson River microcosms were capped in blue and labeled using a blue marker (left) and Grasse River samples were capped in grey and labeled using a black marker (right).

Most previous studies used nondestructive sampling method to reduce the number of microcosms in their experiment; each microcosm was opened and recapped several times during incubation. The impact of microcosm size the headspace content on dechlorination is unclear. Therefore, destructive sampling was used in the current study with each data point involving the sacrifice of three replicate bottles. The first nine samplings were performed at three-week intervals until 24 weeks, following by two samplings at six-week intervals and a final sampling at a fifteen- week interval (in total, 51 weeks' incubation).

3.7.4 Microcosm Sampling and Analysis

Prior to slurry withdrawal, a microcosm headspace sample (200 µl) was analyzed for gas composition (hydrogen, methane, carbon dioxide, trace oxygen, etc.) utilizing a gas chromatograph (Agilent 6850 series II) equipped with a thermal conductivity detector. Gas components were separated by packed column (30 in Hayesep D, 30 in x 0.125 in). High purity nitrogen at a flow rate of 20 ml min^{-1} was used as a carrier gas. Oven and detector temperatures were held at 50 ºC and 155 ºC.

After mixing the sediment slurry thoroughly, microcosm bottles were opened and sampled under a stream of nitrogen. A 5 ml of volume of sediment slurry was withdrawn from the bottles using autoclaved pipettes and the exact weight was determined. Exactly 2 g sediment slurry was taken from the 5 ml subsample for PCB extraction. Also, 0.5 ml of slurry was placed in a sterile 1.5 ml microcentrifuge tube and centrifuged at 16,000g for 8 min. The sediment pellet was stored at -80 ºC until DNA extraction.

3.7.4.1 Iron and Sulfide Analysis

The reduction of Fe (III) to Fe (II) in the anaerobic microcosms was determined using a ferrozine method described elsewhere (Sorensen 1982). Briefly, a subsample of 200 µl of sediment slurry was weighed and transferred into a 1.5 ml microcentrifuge tube containing 800 µl of 0.5 M HCl. 100 µl of acid treated sample was added to 4.9 ml ferrozine solution and filtered. The absorption was measured at 562 nm. As sulfate reducing bacteria are capable of reducing sulfate and other oxidized sulfur compound to sulfide, sulfide concentrations were measured in sulfate amended treatments following the spectrometric method developed by Cline (1969).

3.7.4.2 PCB Analysis

PCB analysis methods were similar to those described in *Section 3.4.2*. Slight modifications to extraction volumes were used due to the smaller size of the samples.

3.7.5 DNA Extraction and Microbial Community Analysis

Microbial community analysis methods are the same as those used in the analysis of the grab and core samples (described in *Section 3.5* above). Briefly, total genomic DNA was extracted using a PowerSoil DNA Purification Kit (MoBio, Carlsbad, CA). Target 16S rRNA genes (copies/g dry wt) were determined by SYBR Green-based Q-PCR assays using seven group specific primer sets (see Table 4).

4 Results and Discussion

As discussed above, the three objectives were pursued through four tasks. The results of these tasks are presented and discussed in this section.

4.1 Task A-1. Grab Sample Sediment Characterization

The initial stages of the present work focused on characterizing the grab samples taken from the sediment systems (Grasse and Hudson). Sediment collected from these systems have been reported to dechlorinate PCBs, but to different extents. These sediments form the foundation of the microcosm studies, and thus their characterization is important to understand the conditions chosen for the microcosms.

4.1.1 Geochemical Characterization

Extensive geochemical characterization of these samples was undertaken using external laboratories. Sediment and porewater were analyzed by the Severn Trent Laboratories, Inc. (Pittsburgh, PA) and the Huffman Laboratories, Inc. (Golden, CO) (see **Table 7)** and Northeast Analytical, Inc. (now Pace Analytical, Inc.) (see **Table 8**, **Table 9**, **Figure 9**, and **Figure 10**).

These results showed higher sulfate in the Hudson than the Grasse River and much higher iron in the Grasse sediment and porewater than the Hudson. Both sediment and porewater contain some metals in very high concentrations (including iron and manganese) that could inhibit microbial activity toward PCBs. However, the dechlorinating activity in sediments from Hudson and Grasse Rivers has been shown in microcosm enrichments in our previous work and the molecular microbiological analyses described in this report suggest adequate organisms with PCB-dechlorinating ability for our studies.

Table 7. Geochemical Analysis of Grab Sediments (Hudson and Grasse River)

Matrix	Parameter	Hudson (Grab)	Grasse (Grab)	Analytical method
Sediment	**Simultaneously Extractable Metals [SEM]**	(nmole/g)	(nmole/g)	EPA SEM
	Cadmium	0.44	3.3	
	Copper	89	230	
	Mercury	0.013	0.024	
	Nickel	63	140	
	Lead	65	73	
	Zinc	800	2100	
	ICP-MS [6020]	(mg/kg)	(mg/kg)	SW846 6020
	Aluminum	2350	10100	
	Cobalt	1.8	7.9	
	Copper	4.1	18.8	
	Iron	5310	18000	
	Manganese	40.5	505	

	Nickel	3.1	14.2	
	Inorganic Analysis Acid volatile sulfide (umoles/g) Total phosphorus (mg/kg) Total residue as % solids (%)	 ND 559 74.3	 6.4 2400 33.2	 EPA AVS MCAWW 365.1 MCAWW160.3 Mod
Pore Water	**ICP-MS [6020]** Aluminum Cobalt Copper Iron Manganese Nickel	(mg/kg) 190 1.2 15.5 13700 3380 6.3	(mg/kg) 80900 39.7 232 129000 5720 104	 SW846 6020
	Inorganic Analysis Alkalinity (mg/L) Specific conductance (umhos/cm) Total Hardness (mg/L) Nitrate as N (mg/L) Sulfate (mg/L) Nitrogen, ammonia (mg/L) pH Sulfide (mg/L) Total phosphorus (mg/L)	 245 636 290 ND 85.8 3.4 7.0 ND 0.039	 115 270 160 0.014 6.5 3.6 6.7 20.0 6.1	 MCAWW 310.1 MCAWW 120.1 MCAWW 130.2 MCAWW 300.0A MCAWW 300.0A MCAWW 350.1 MCAWW 150.1 MCAWW 376.1 MCAWW 365.1
	Total Organic Carbon (mg/L) Total hydrophobics Total hydrophilics	11.1 8.5 2.6	9.4 5.8 3.6	 TOC SM 5310C

In addition to geochemical condition analysis, we also evaluated the PCB congener distribution in the grab sediment samples that were used as the basis of the microcosm studies. Samples from each river were sent to Northeast Analytical (NEA) Inc. (now Pace Analytical, Inc.). **Table 8** and Table 9 provide a summary of PCB analyses.

Table 8. Summary of Grab Sediment PCB Analyses (Hudson and Grasse River)

	Hudson	Grasse
Location	Moreau, NY N:1609914.52 E: 733570.10	Massena, NY N: 2232531.17 E: 410169.23
PCB concentration	6.51 mg/kg Sediment	1.35 mg/kg Sediment
PCB homolog distribution [Top 4 homolog] (Weight %)	12.48 (Mono) 34.92 (Di) 36.06 (Tri) 13.54 (Tetra)	15.76 (Di) 27.27 (Tri) 23.23 (Tetra) 12.19 (Penta)
Cl distribution in byphenyl residue (average number of Cl/Biphenyl)	1.44 (*Ortho*) 0.45 (*Meta*) 0.58 (*Para*) 2.47 (Total)	1.81 (*Ortho*) 1.12 (*Meta*) 0.78 (*Para*) 3.71 (Total)

In the Grasse River, four homologs including di-, tri-, tetra-, and penta-chlorinated biphenyl were over 10 weight percent (15.8, 27.3, 23.2, and 12.2 %, respectively). The average number of chlorine per biphenyl residue was 3.71 with 1.81 chlorines per biphenyl residue at the *ortho* position. The total PCB concentration in the sample was 1.35 mg/kg dry sediment. This was a lower total PCBs than expected for the location of the sample, likely due to redistribution of sediment in the Grasse River following an ice jam event in March 2003 (see http://www.thegrasseriver.com/Ice_2003_Event.htm).

In the Hudson River, four homologs including mono-, di-, tri-, and tetra-chlorinated biphenyl were over 10 weight percent (12.48, 24.92, 36.06, and 13.54%, respectively). The average number of chlorine per biphenyl residue was 2.47 with 1.44 chlorines per biphenyl residue at the *ortho* position. The total PCB concentration in the sample was 6.51 mg/kg dry sediment. This was higher total PCBs than expected, as we targeted a location expected to be 3-5 mg/kg. About 70% (weight) of congeners in Hudson sediment have only two or three chlorines per biphenyl residue and no congener has more than 6 chlorines. For the Grasse sediment, tri or tetra chlorinated congeners were about 50%. About 32% of congeners have 5 to 8 chlorines per byphenyl. Total number of chlorine per biphenyl was 2.47 for Hudson and 3.71 for Grasse sediment. The results of congener specific PCB analysis suggest that the anaerobic PCB dechlorination occurred more extensively in Hudson sediment.

The Grasse River samples were below 3 ppm (the target selected for sampling), thus we sent an additional sample for congener analysis to evaluate the heterogenity in the sample. Table 9 shows a comparison of the duplicate Grasse River analyses.

Table 9. Comparison of Grasse River Replicate Bulk Sediment Samples

	Sample 1	Sample 2
PCB concentration	1.85 mg/kg Sediment	1.35 mg/kg Sediment
PCB homolog distribution [Top 4 homolog] (Weight %)	16.10 (Di) 27.26 (Tri) 22.24 (Tetra) 12.44 (Penta)	15.76 (Di) 27.27 (Tri) 23.23 (Tetra) 12.19 (Penta)
Cl distribution in byphenyl residue (average number of Cl/Biphenyl)	1.79 (*Ortho*) 1.06 (*Meta*) 0.77 (*Para*) 3.61 (Total)	1.81 (*Ortho*) 1.12 (*Meta*) 0.78 (*Para*) 3.71 (Total)

Figure 9 shows a comparison of these samples by homolog groups. These results indicated that the sample showed acceptable homogeneity, despite being lower than originally desired.

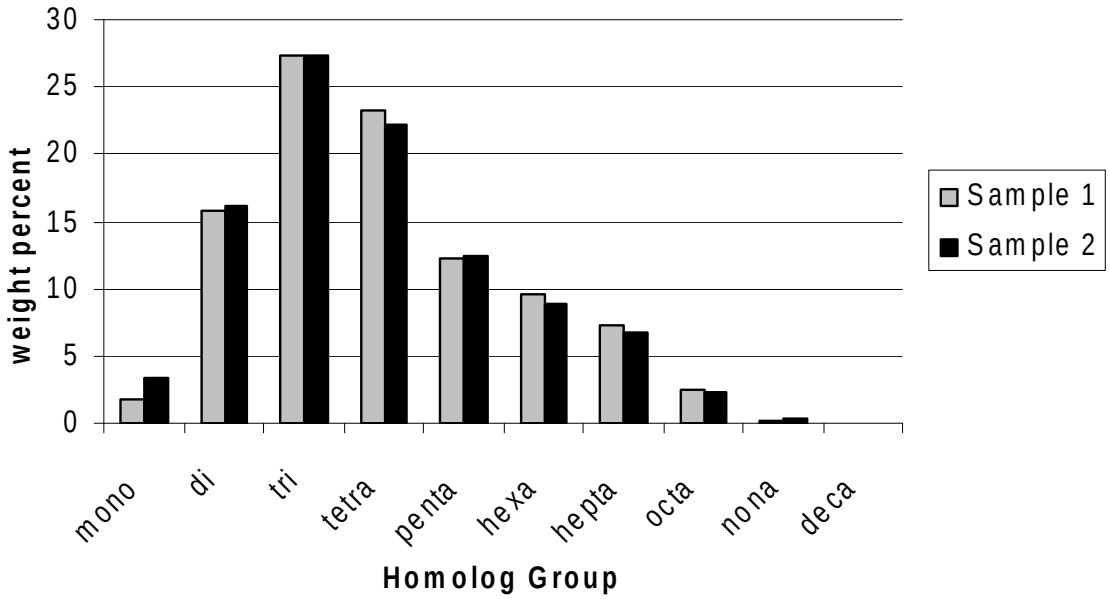


Figure 9. Grasse River Replicate Bulk Sediment Comparison by Homolog.
Figure shows the results of PCB congener analysis by homolog of the Grasse River duplicate sample. This information is comparison of these samples by homolog group and is provided in Table 9**.**

To better understand the comparison of grab sediments and Core 18M segments by depth, Table 10, Table 11, and **Table 12** provide the data of PCB homolog concentration, total PCB concentration, PCB homolog distribution and %Cl in each homolog

Figure 10 shows the results of the full congener analysis for Grasse and Hudson River grab samples that were used for seed for the microcosm experiments. As mentioned above, these samples were sent to external analysis, and NEA, Inc. reported the complete 209 congener analysis. The method used by NEA Analytical, Inc. at the time did not individually quantify all 209 congeners, but rather, quantified a sub-set and uses a computational method to divide co-eluting peaks and provide estimated values for all congeners.

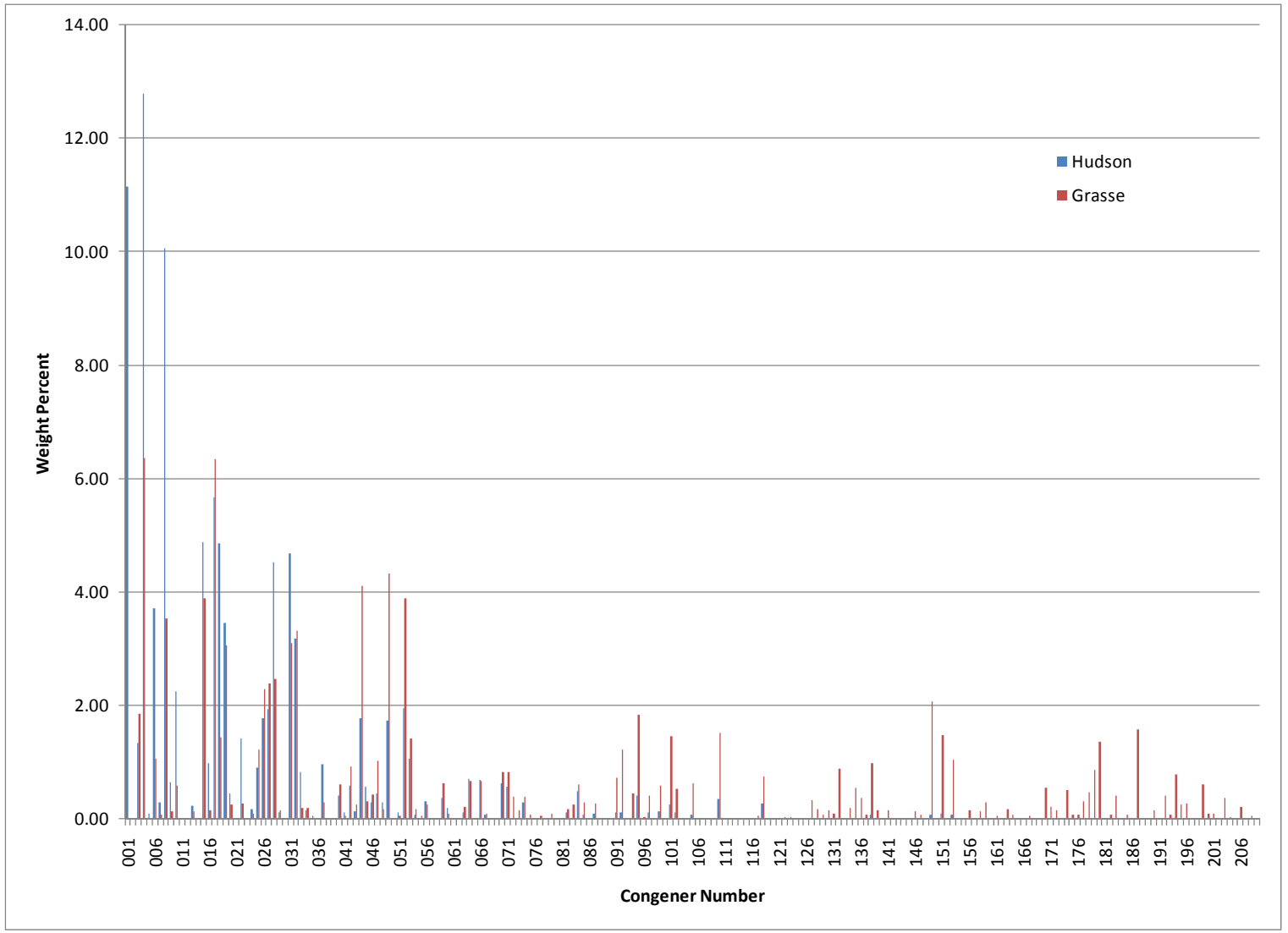


Figure 10. Results of Full Congener Analysis for Grasse and Hudson Grab Samples.
Congener number is shown in the y-axis and the weight percent of each the congener is shown on the x-axis.

Table 10. PCB Homolog Concentrations (ng/g dry weight) in Core 18M

Depth (cm)	Mono	Di	Tri	Tetra	Penta	Hexa	Hepta	Octa	Nona	Total PCBs
Grab(0-5 cm)	0.06	0.30	0.50	0.41	0.23	0.17	0.13	0.04	0.01	***1.8***
20-25	0.13	0.79	1.53	0.85	0.48	0.40	0.33	0.12	0.01	***4.6***
50-55	0.74	1.93	3.89	2.26	1.54	1.30	1.26	0.50	0.05	***13.5***
75-80	1.23	6.34	8.69	4.37	2.37	1.62	1.38	0.55	0.05	***26.6***
100-105	0.94	6.78	2.89	1.58	0.79	0.51	0.35	0.13	0.02	***14.0***
140-145	0.84	3.54	4.49	3.37	1.21	0.62	0.41	0.14	0.01	***14.6***
145-150	0.75	2.85	4.69	2.93	1.41	0.68	0.38	0.12	0.01	***13.8***
150-155	1.55	32.78	28.35	13.88	7.78	1.98	1.30	0.46	0.09	***88.2***
155-160	1.60	6.75	4.51	2.02	0.84	0.44	0.31	0.10	0.01	***16.6***
160-165	0.81	2.36	2.89	2.85	1.37	0.68	0.42	0.13	0.01	***11.5***
165-170	0.63	1.83	4.13	3.02	1.15	0.59	0.37	0.11	0.01	***11.8***
170-175	0.28	2.58	6.52	7.55	2.81	1.51	0.90	0.33	0.04	***22.5***
175-180	0.82	4.60	11.32	7.64	2.99	1.71	1.26	0.38	0.02	***30.7***
180-185	2.37	6.66	13.23	8.86	3.15	1.61	1.26	0.38	0.02	***37.5***
185-190	3.99	22.82	29.15	17.31	5.60	2.21	1.15	0.42	0.05	***82.7***
190-195	10.96	76.24	61.90	32.30	9.37	3.79	1.88	0.81	0.16	***197.4***
195-200	14.00	191.46	118.27	39.46	12.21	5.94	3.35	1.46	0.19	***386.3***
200-205	98.37	381.77	234.26	81.25	20.03	11.00	6.88	2.93	0.32	***836.8***
205-210	88.66	307.06	212.10	71.35	18.79	12.20	8.41	3.31	0.33	***722.2***
210-215	6.02	142.56	69.14	30.16	10.54	7.88	6.23	2.31	0.33	***275.1***
215-220	20.16	61.59	45.36	19.65	9.81	8.82	7.12	2.97	0.33	***175.8***

Table 11. PCB Homolog Percent Distribution (% of total PCB) in Core 18M and Three Commercial Aroclors

Depth (cm)	Mono	Di	Tri	Tetra	Penta	Hexa	Hepta	Octa	Nona
Grab (0-5)	3.42	16.10	27.26	22.24	12.44	8.96	6.83	2.38	0.37
20-25	2.83	17.04	32.94	18.43	10.37	8.69	7.02	2.49	0.19
50-55	5.48	14.31	28.90	16.76	11.42	9.64	9.35	3.73	0.40
75-80	4.62	23.85	32.67	16.43	8.90	6.07	5.18	2.08	0.20
100-105	6.71	48.38	20.65	11.31	5.65	3.64	2.52	0.96	0.17
140-145	5.77	24.20	30.73	23.02	8.25	4.23	2.79	0.94	0.07
145-150	5.41	20.63	33.99	21.20	10.20	4.90	2.73	0.87	0.06
150-155	1.76	37.18	32.15	15.74	8.82	2.25	1.48	0.52	0.10
155-160	9.67	40.74	27.21	12.18	5.07	2.63	1.87	0.57	0.05
160-165	7.03	20.49	25.04	24.72	11.90	5.92	3.64	1.16	0.09
165-170	5.36	15.48	34.86	25.52	9.74	4.95	3.12	0.91	0.06
170-175	1.25	11.46	28.96	33.51	12.48	6.72	4.02	1.46	0.16
175-180	2.65	14.98	36.83	24.85	9.74	5.57	4.09	1.23	0.06
180-185	6.31	17.74	35.27	23.60	8.40	4.28	3.35	1.01	0.05
185-190	4.83	27.60	35.25	20.93	6.78	2.67	1.39	0.50	0.06
190-195	5.55	38.62	31.36	16.36	4.74	1.92	0.95	0.41	0.08
195-200	3.62	49.56	30.61	10.21	3.16	1.54	0.87	0.38	0.05
200-205	11.76	45.62	27.99	9.71	2.39	1.31	0.82	0.35	0.04
205-210	12.28	45.70	26.18	9.88	2.60	1.69	1.16	0.46	0.05
210-215	2.19	51.81	25.13	10.96	3.83	2.86	2.26	0.84	0.12
215-220	11.47	35.03	25.80	11.18	5.58	5.02	4.05	1.69	0.19
Aroclor 1260	0.02	0.10	0.19	0.46	8.59	43.36	38.54	8.27	0.70
Aroclor 1242	0.75	15.04	44.89	32.53	6.44	0.32	0.00	0.00	0.00
Aroclor 1248	0.05	1.00	21.63	55.62	19.79	1.81	0.48	0.00	0.00

Table 12. PCB Concentration in Core 18M Shown as Percent Chlorine (%Cl) of Each PCB Homolog Group

Depth (cm)	Mono	Di	Tri	Tetra	Penta	Hexa	Hepta	Octa	Nona	Total %Cl
Grab(0-5)	0.64	5.12	11.26	10.80	6.76	5.28	4.29	1.57	0.25	***46.0***
20-25	0.53	5.42	13.60	8.95	5.63	5.12	4.41	1.65	0.13	***45.4***
50-55	1.03	4.55	11.94	8.14	6.20	5.68	5.87	2.46	0.27	***46.1***
75-80	0.87	7.58	13.49	7.98	4.83	3.58	3.25	1.37	0.14	***43.1***
100-105	1.26	15.38	8.53	5.50	3.07	2.15	1.58	0.63	0.12	***38.2***
140-145	1.08	7.69	12.69	11.18	4.48	2.49	1.75	0.62	0.05	***42.0***
145-150	1.02	6.56	14.04	10.30	5.54	2.89	1.72	0.57	0.04	***42.7***
150-155	0.33	11.82	13.28	7.64	4.79	1.32	0.93	0.34	0.07	***40.5***
155-160	1.82	12.95	11.24	5.92	2.76	1.55	1.17	0.38	0.04	***37.8***
160-165	1.32	6.51	10.34	12.00	6.46	3.49	2.29	0.77	0.06	***43.2***
165-170	1.01	4.92	14.40	12.39	5.29	2.92	1.96	0.60	0.04	***43.5***
170-175	0.23	3.64	11.96	16.27	6.78	3.96	2.52	0.89	0.11	***46.4***
175-180	0.50	4.76	15.21	12.07	5.29	3.28	2.57	0.81	0.04	***44.5***
180-185	1.19	5.64	14.56	11.46	4.56	2.52	2.10	0.67	0.03	***42.7***
185-190	0.91	8.77	14.56	10.17	3.68	1.57	0.87	0.33	0.04	***40.9***
190-195	1.04	12.27	12.95	7.95	2.58	1.13	0.60	0.27	0.06	***38.8***
195-200	0.68	15.75	12.64	4.96	1.72	0.91	0.54	0.25	0.03	***37.5***
200-205	2.21	14.50	11.56	4.72	1.30	0.77	0.52	0.23	0.03	***35.8***
205-210	2.31	13.51	12.13	4.80	1.41	1.00	0.73	0.30	0.03	***36.2***
210-215	0.41	16.47	10.38	5.32	2.08	1.69	1.42	0.55	0.08	***38.4***
215-220	2.15	11.13	10.65	5.43	3.03	2.96	2.54	1.11	0.13	***39.1***
Arolcor 1260	0.00	0.03	0.08	0.23	4.66	25.56	24.19	5.46	0.48	***60.7***
Arolcor 1242	0.14	4.78	18.54	15.80	3.50	0.19	0.00	0.00	0.00	***42.9***
Aroclor 1248	0.01	0.32	8.93	27.01	10.74	1.07	0.30	0.00	0.00	***48.4***

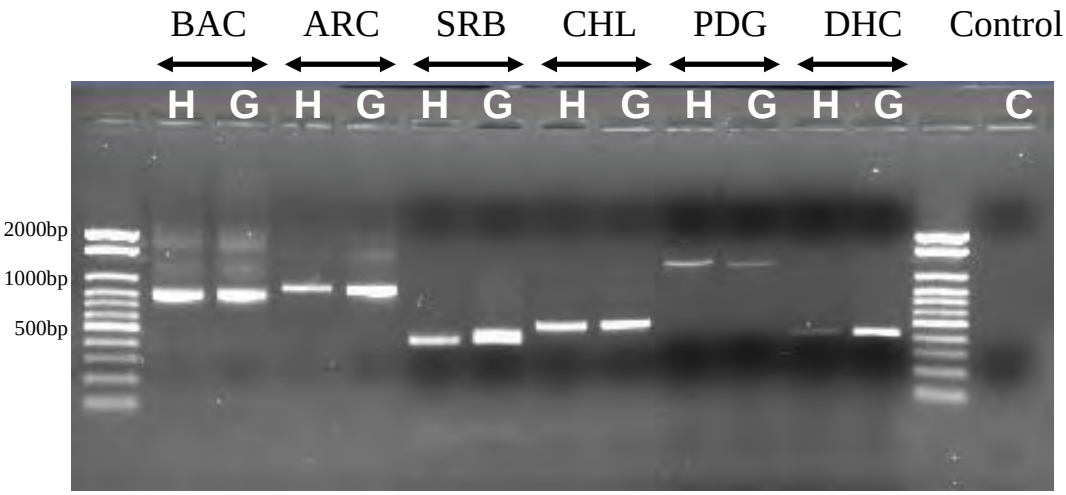
4.1.2 Microbial Analysis of Hudson and Grasse Sediment

For the total genomic DNA extraction, the DNA yield from Grasse sediment was 8 times higher than that of Hudson sediment. Grasse sediment looked silty and was composed of smaller particles, which would provide much higher surface area for bacteria, and also provide more adsorption or physical entrapment of organic materials without washout. This agreed well with the geochemical analysis results that the organic carbon content in Grasse sediment was much higher than that in Hudson sediment. **Figure 11** shows PCR products for different microbial groups in the two sediments. The abbreviations used in the figures are provided here for convenience:

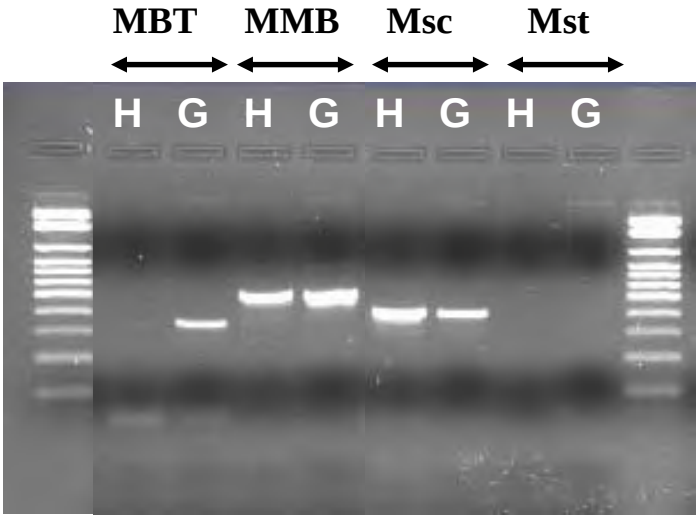
- Hudson River sediment (H)
- Grasse River sediment (G)
- Laboratory Control (C)
- Bacteria (BAC)
- Archaea (ARC)
- Sulfate reducing bacteria (SRB)
- Chloroflexi (CHL)
- PCB Declorinator Group with o-17 and DF-1 (PDG)
- Dehalococcoides (DHC)
- Methanobacteriales (MBT)
- Methanomicrobiales (MMB)
- Methanosarcina (Msc)
- Methanosaeta (Mst)

For the microbial group-specific PCR detection, most microbial groups were more abundant in Grasse sediment. Higher SRB in Grasse sediment agreed with higher sulfide concentration in Grasse sediment. On the other hand, the PCB dechlorinator group (*o-17* & DF-1) and Methanosarcinales group were more abundant in Hudson sediment. These corresponded to the results of PCB congener analysis and to the result of the clone library comparison for *Archaea*, respectively. *Dehalococoides* group and *Chloroflexi* group microorganisms were also detected in both sediments. It should be noted that the estimation of microbial abundances by the PCR detection is just an approximation and may be biased due to inhibitors in samples, the nature of the PCR reaction, and other conditions. Also, a comparison of band intensities on gels among different groups (that is, using different primer sets) may not be meaningful because of the difference in the amplification efficiencies with different primers.

Figure 12 show results of DGGE for Hudson and Grasse samples for *Bacteria*, *Archaea*, *Chloroflexi* and *Dehalococcoides*. Differences in the number of bands, location and intensity are all indicated, suggesting populations of these groups differ in these two sediments.



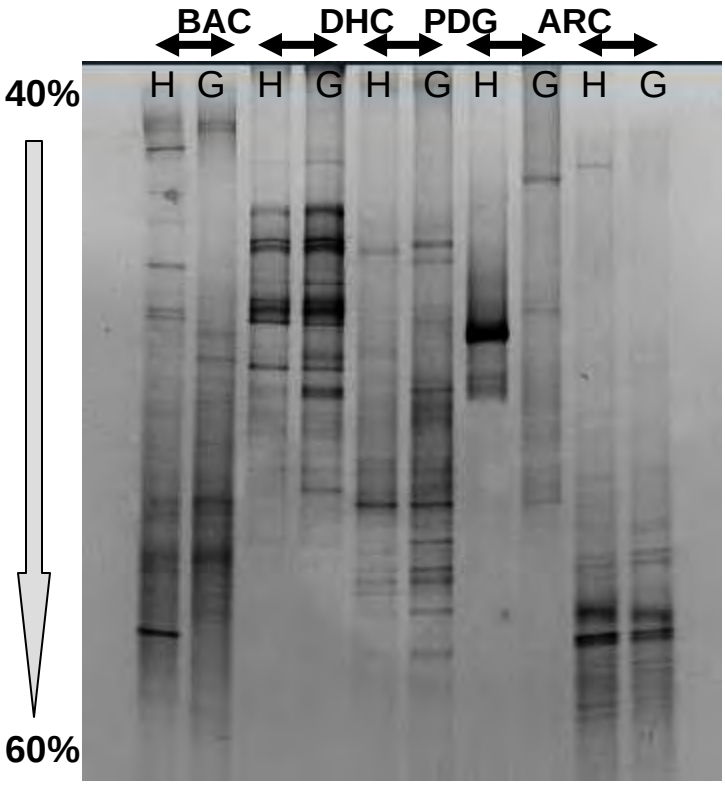
1.0% gel, 2-12ul loading



1.5% gel, 5ul loading

H: Hudson sediment
G: Grasse sediment
C: Control (negative)
BAC: *Bacteria*
ARC: *Archaea*
SRB: Sulfate reducing bacteria
CHL: *Chloroflexi*
PDG: *o-17* and DF-1
DHC: *Dehalococcoides*
MBT: Methanobacteriales
MMB: Methanomicrobiales
Msc: Methanosarcina
Mst: Methanosaeta

Figure 11. PCR gels from DNA extracted from Hudson and Grasse River Sediments.



BAC: *Bacteria*
ARC: *Archaea*
PDG: *o-17* and DF-1
DHC: *Dehalococcoides*

8% Acryamid gel
40-60% of denaturing gradient
5min at 170V
12hrs at 70V
Staining: EtBr

Figure 12. DGGE Results for the Hudson River and the Grasse River Sediments.

Clone libraries were constructed for each river for *Bacteria (BAC)*, *Archaea (ARC)*, *Chloroflexi (CHL)*, and DF-1/*o-17* groups. Bacterial and archaeal clone libraries for both river sediments were constructed (91 – 95 clones) to investigate differences in overall community structures and diversity. **Figure 13** shows bacterial community structures by clone library analysis results for the two sediments. **Figure 14** shows archaeal community structures by clone library analysis results for the two sediments. The pie charts show major 6-8 groups with higher number of clones, not all groups. Both sediments contained one clone belonging to the *Chloroflexi* group that includes known dechlorinating species; this was unexpected as in a clone library of approximately 90, a low occurrence bacterial species such as a *Chloroflexi* would not be expected. The Hudson River bacterial population is dominated by Bacteroidetes (50 of 91 clones). Sequences of about 25 clones among them were very similar (appr. 99%). These are likely from the same species and would therefore be expected to form a distinct intense band on the DGGE gel. The Grasse River bacterial population is more diverse, showing a slight dominance of unclassified bacteria (31 of 94 clones) which don't belong to any known phylum of bacteria. When comparing the Hudson and Grasse bacterial clone libraries, no significant differences were found at the domain level. At the Phylum level, *Bacteroidetes* were significantly different between Hudson and Grasse sediment systems ($p<0.01$). At class level *Betaprotebacteria* were distinct in the two sediment systems, especially for order *Rhodocyclales* ($p<0.001$).

The Hudson River archael population is dominated by *Thermoprota* (41 of 93 clones), while the Grasse River archael population is again more diverse, showing a slight dominance of

unclassified euryarchaeota (28 of 95 clones). Methanogen compositions of the archaeal library were a substantial component in both sediments, 19% for Hudson and 31% for Grasse. But *Methanosarcinales* were slightly higher in Hudson sediment.

While the bacterial and archaeal clone libraries provided some general information, clone libraries for the DF-1/*o-17* group were more specific. **Figure 15** shows a phylogenetic tree with 50 clones from the two clone libraries for Hudson and Grasse grab sediments with some reference strains of known as PCB dechlorinators (Watts et al. 2005). Only 3 clones from Hudson sediment were included in this analysis because the other 45 clones did not belong to the DF-1/ *o-17* group. This likely was caused by the low specificity of the primer sets; only the reverse primer has specificity to DF-1/ *o-17* group.

Among the 47 clones for Grasse sediment, 33 clones showed the highest identity (over 97% except 2 clones) with JWBH clones from the study using the same primer set (Watts et al. 2005). Nine clones have the highest identity of 91 – 94% with PCB dechlorinating bacteria clones deposited by the Sowers' group (Watts et al. 2005). Two clones have the highest identity of 89 and 90% with environmental clones belonging to *Chloroflexi* group. This result agreed well with the DGGE result (**Figure 12**) showing 2 major bands in the Grasse sediment lane. Three clones of the Hudson clone library were somewhat different with clones of the Grasse library and were more similar to *Dehalomonas* and *Dehalococcoides* groups.

Figure 16 and **Figure 17** show phylogenetic trees for the *Chloroflexi* group with 92 and 90 clones from the two clone libraries for Hudson and Grasse sediments, respectively, with 9 reference strains known as possible dechlorinators. Each clone sequence was compared with Genebank entries (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) to obtain the most similar reference sequences, and the clones were classified by the sources of their reference sequences found in other studies related to dechlorination. The dominant sequence sources included PCB dechlorinating enrichment culture (Watts et al. 2005), Chesapeake Bay Watershed (unpublished study on Specific PCR amplified 16S rRNA gene detection of bacteria linked to the reductive dechlorination of polychlorinated biphenyls in microcosms and Chesapeake Bay Watershed sediments (Watts et al. 2005)), chlorinated ethene-contaminated aquifer (unpublished study on Field-Scale Quantification of *Dehalococcoides* sp. in a Chlorinated Ethene-Contaminated Aquifer (Burgmann et al. 2008)), dibenzofuran contaminated river (unpublished study on Microbial Functional Diversity Shifts in Passaic River Sediments During the Biodegradation of Dibenzofuran (Ni Chadhain S.M. 2009)), monochlorobenzene contaminated groundwater (Alfreider et al. 2002). In **Figure 16** and **Figure 17**, the classification trees demonstrate that many of the *Chloroflexi* clones in both Hudson and Grasse grab sediments had similar sequences to those found in these previous studies on dechlorination.

Clones from the Grasse sediment were more closely related to each other and indicated more distinctive clades than those obtained from the Hudson, corresponding with the DGGE profile of *Chloroflexi* group. Some clones in the two libraries were more closely related to *D. ethenogenes* 195, *D. chlorocoercia* DF-1, and *o-17* among the reference strains. This indicates that the possibility of the detection of PCB dechlorinators in the grab sediment samples would be significant. A combined tree of both clone libraries was also constructed to compare the two libraries (**Figure 18**). In general, many of the clones from the two libraries were closely related

to each other. However, several smaller clades contained only one shared representative. *Chlorolfexi* community structures were further analyzed using a statistical comparison of gel images; cluster analysis shows that Hudson and Grasse *Chloroflexi* communities were 81.6% similar with each other.

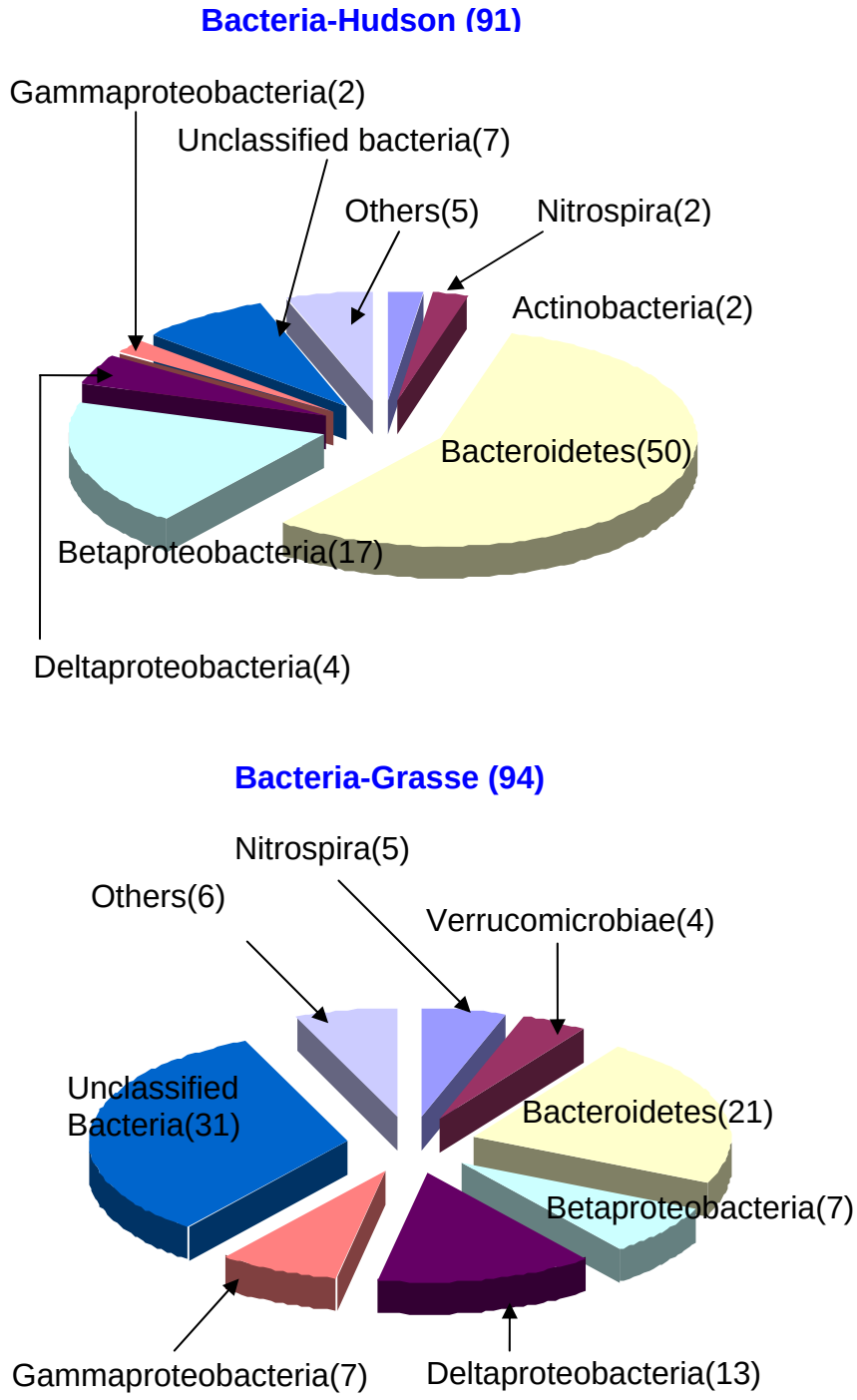


Figure 13. Bacterial Clone Library Results for the Sediment Samples: Hudson (top) and Grasse (bottom).

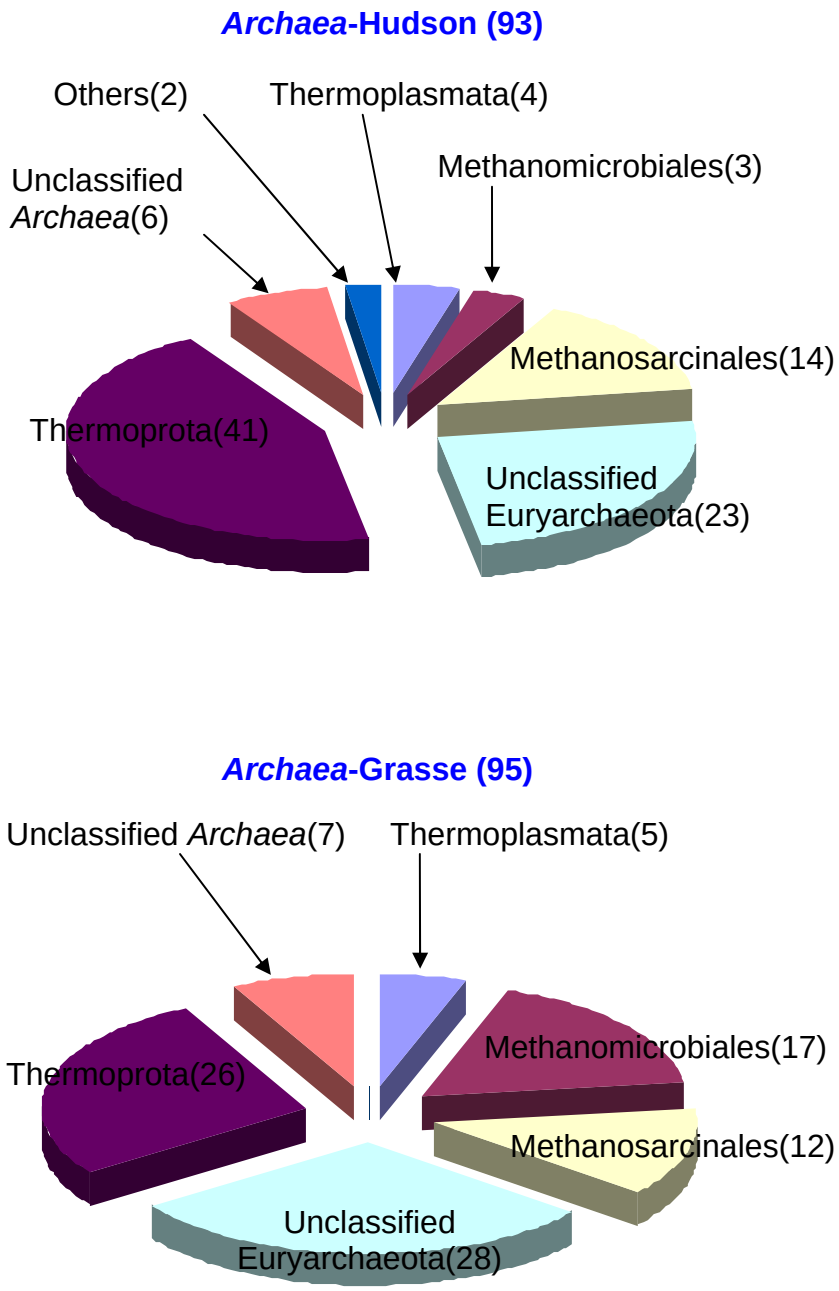


Figure 14. Archaeal Clone Library Results for the Sediment Samples: Hudson (top) and Grasse (bottom)

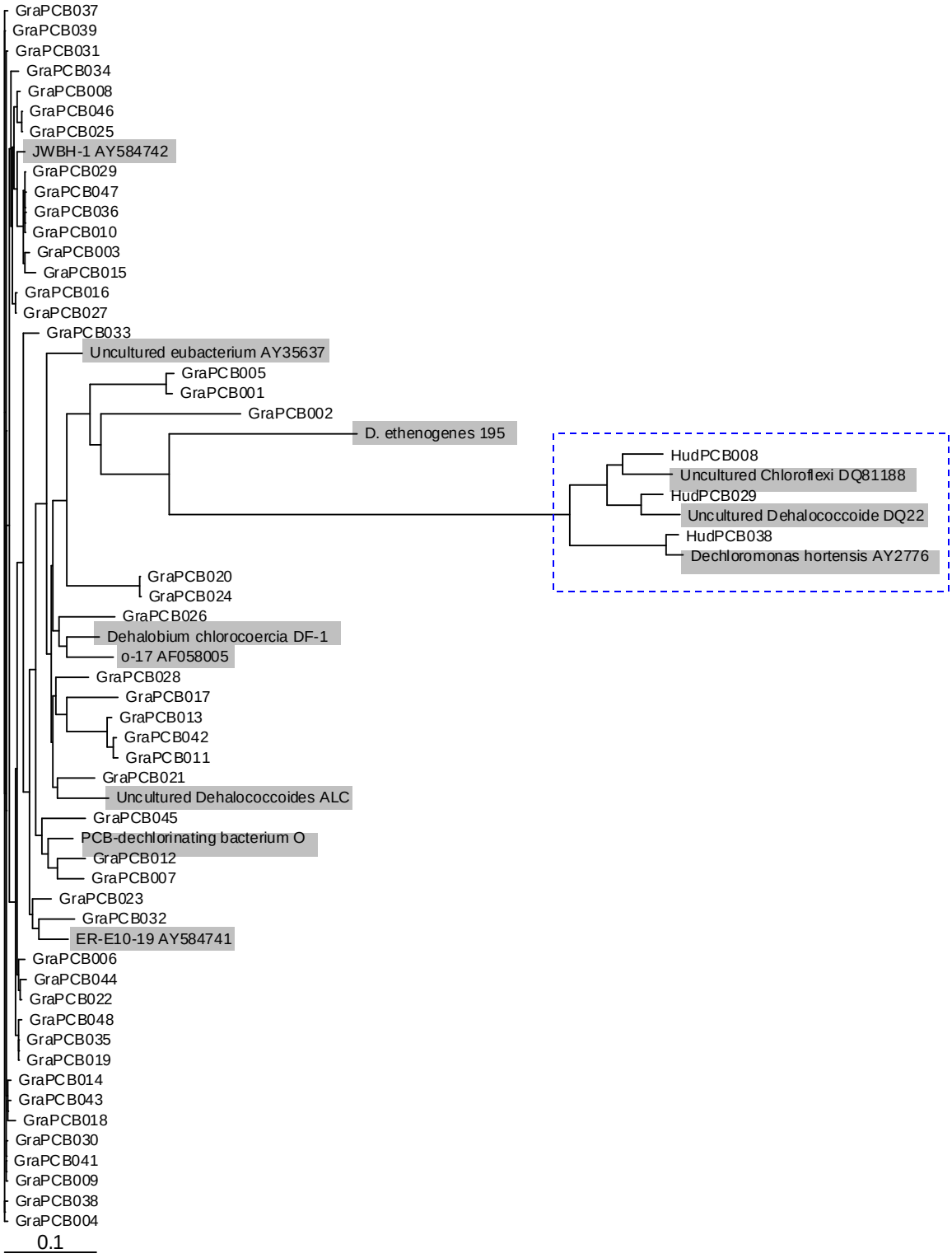


Figure 15. A Phylogenetic Tree from Two Clone Libraries (DF-1/ o-17). Results from Hudson and Grasse grab sediments targeting the DF-1/ o-17 group. Known dechlorinators are indicated by shading. Clones for Hudson sediment are in the dotted box.

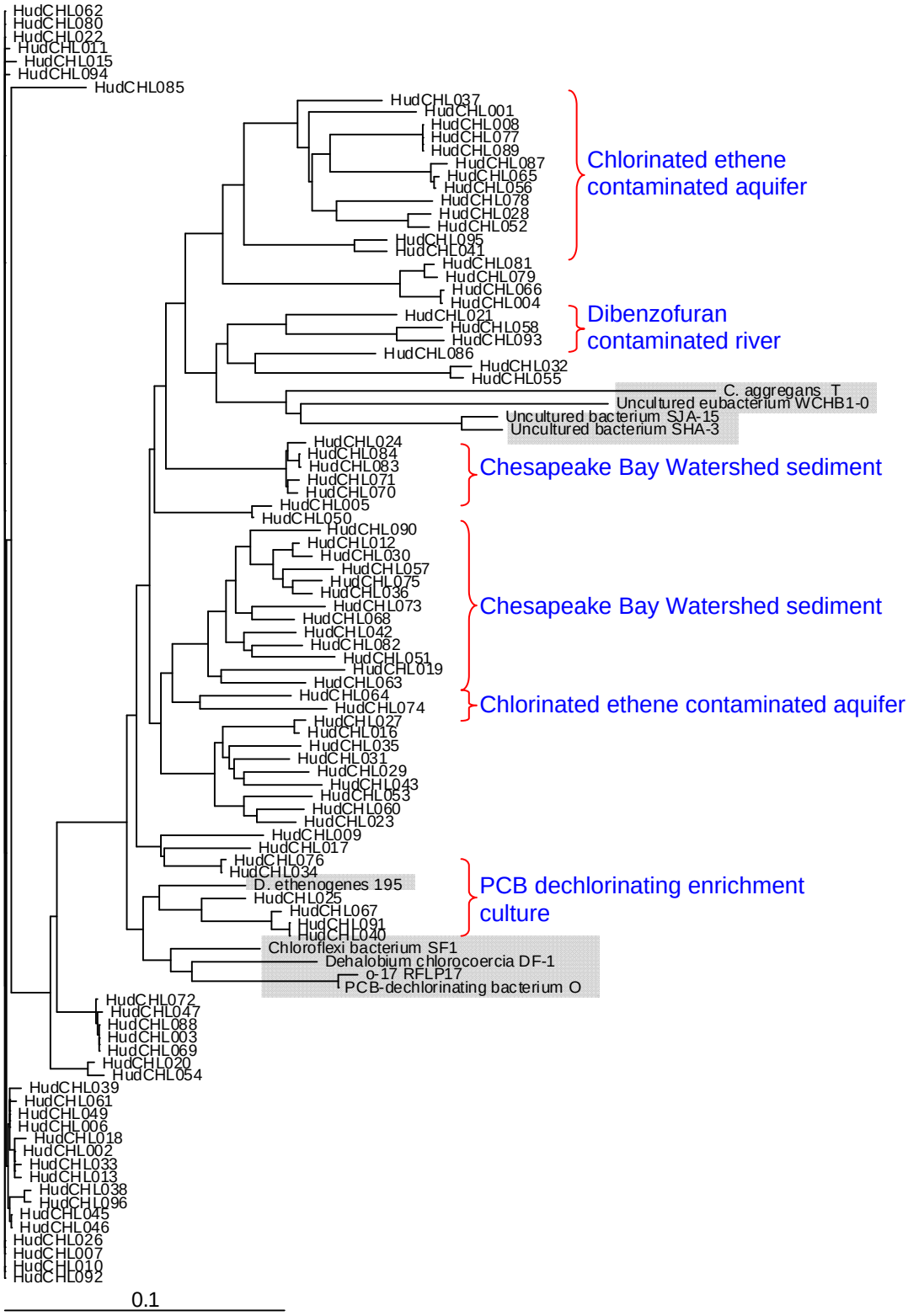


Figure 16. A Phylogenetic Tree from Two Clone Libraries (Hudson - Chloroflexi). Results of Hudson River grab sample of Chloroflexi group. Known dechlorinators are indicated by shading. Most related sources from GenBank are noted to the right.

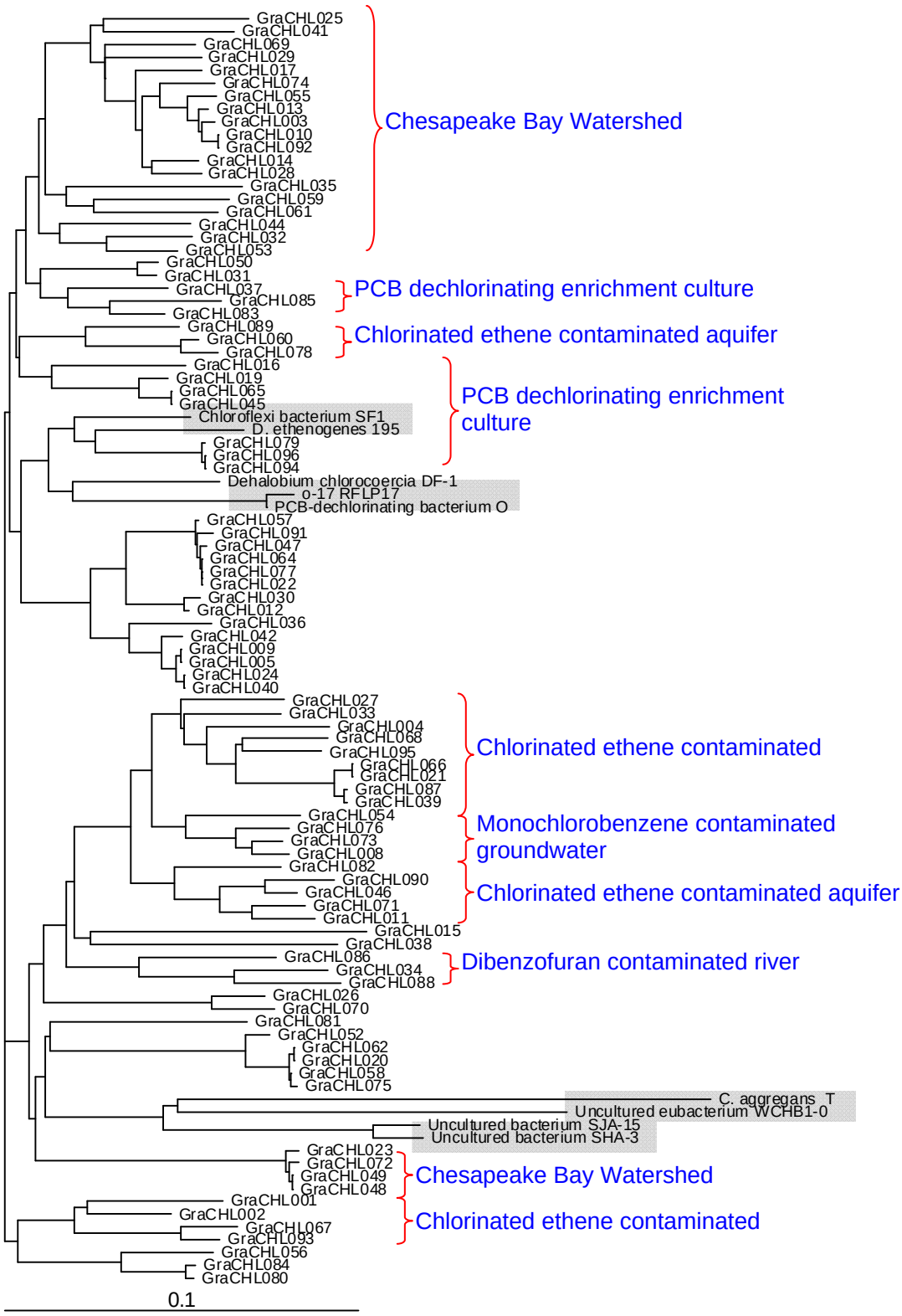


Figure 17. A Phylogenetic Tree from Two Clone Libraries (Grasse - Chloroflexi). Results of Grasse River grab sample of Chloroflexi group. Known dechlorinators are indicated by shading. Most related sources from GenBank are noted to the right.

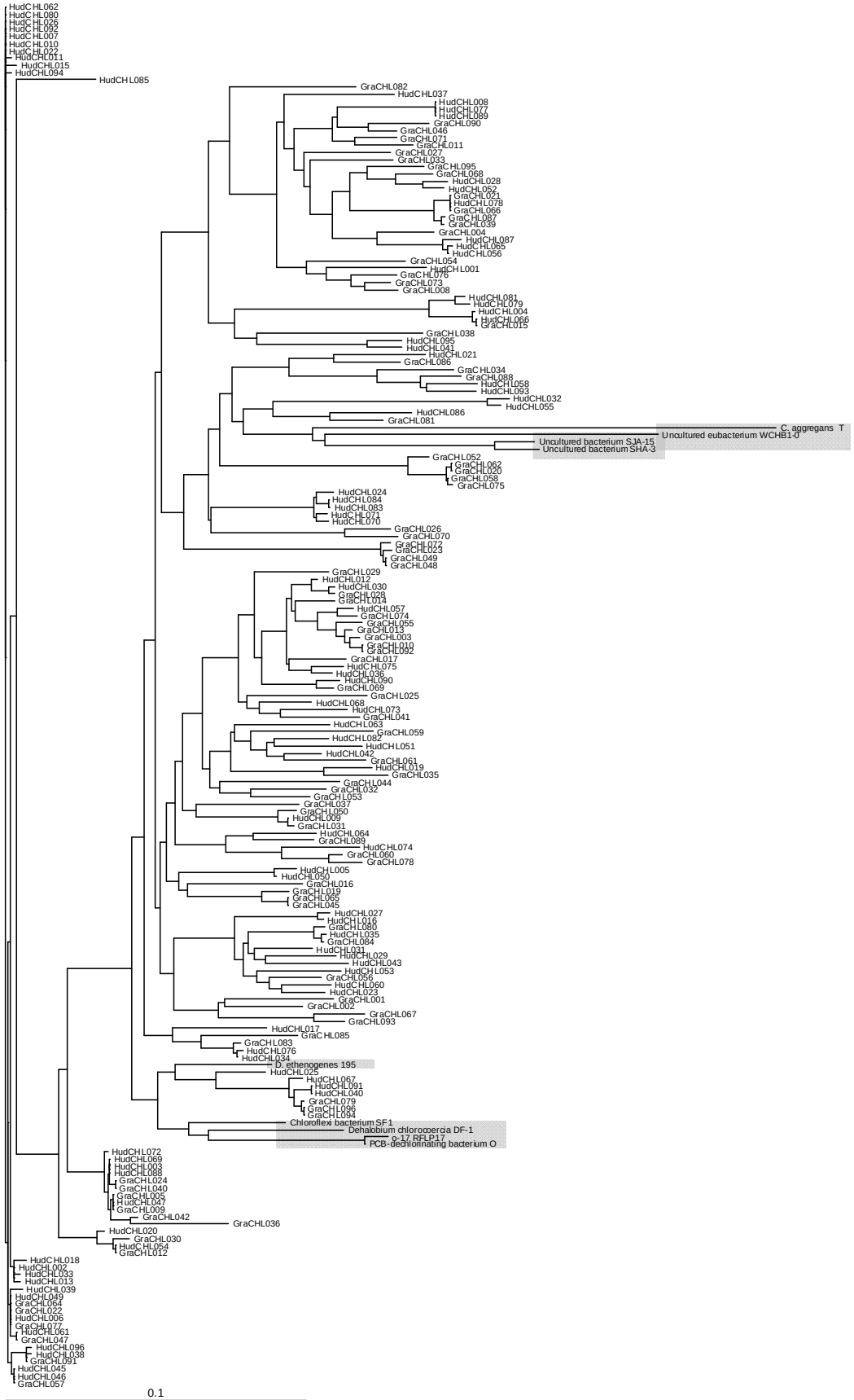


Figure 18. A phylogenetic Tree from Two Clone Libraries (Chloroflexi). Results of Hudson and Grasse River grab samples of Chloroflexi group. Known dechlorinators are indicated by shading.

Sources of the most similar (published) sequence for each clone was searched using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) program through the website (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and the number of clones corresponding to each source was determined with the percentage of sequence similarity (Table 13 and Table 14). Among the most similar sequences to each clones, 43 (46.7 %) and 67 (74.4 %) clones of Hudson and Grasse libraries, respectively, were the most similar to a corresponding published sequence for which the isolation source is likely to have dechlorinating activity. This agrees with the observation that many *Chloroflexi* bacteria are known as dechlorinators. The source with the closest matches among the isolation sources was the PCB-spiked and enriched Ohio River sediment; 17 clones (18.5 %) for Hudson and 23 clones (25.5%) for Grasse libraries. The number of clones with higher similarity than 97 % to the most similar sequence were 4 and 8 for Hudson and Grasse libraries, respectively.

Table 13. Sources of the Most Similar Sequence and Number of Clones for the *Chloroflexi* Clones (Total 92) from Hudson River Sediment

Source of the most similar sequence	>97%	>95%	>93%	>90%	>87%	Sum
Chesapeake Bay Watershed sediment (PCB spiked) Ohio River sediment	3	5	4	5		**17**
PCB dechlorinating enrichment culture *Dehalococcoides* enrichment cultures			5	2	1	8
Chlorinated ethene contaminated aquifer		2	5	6		13
Dibenzofuran contaminated river sediment	1	1		1		3
Monochlorobenzene contaminated groundwater			1	1		2
Sum of clones (%)	**4 (4.3)**	8 (8.7)	15 (16.3)	15 (16.3)	1 (1.1)	**43 (46.7)**

Table 14. Sources of the Most Similar Sequence and Number of Clones for the *Chloroflexi* Clones (Total 90) from Grasse River Sediment

Source of the most similar sequence	>97%	>95%	>93%	>90%	>87%	Sum
Chesapeake Bay Watershed sediment (PCB spiked) Ohio River sediment	5	5	5	3	5	**23**
PCB dechlorinating enrichment culture *Dehalococcoides* enrichment cultures			11	5	1	17
Chlorinated ethene contaminated aquifer		1	14	6		21
Dibenzofuran contaminated river sediment		1		1		2
Monochlorobenzene contaminated groundwater	3	1				4
Sum of clones (%)	**8 (8.9)**	8 (8.9)	30 (33.3)	15 (16.7)	6 (6.7)	**67 (74.4)**

4.2 Task A-2. Characterization of Sediment Cores

Sediment cores have been used to evaluate PCB-contaminated rivers and lakes for several decades (see for example: Imamoglu et al. 2004; Bzdusek et al. 2006; Iozza et al. 2008). Most of this work has focused on total PCB levels and PCB congener profiles and has been used for source identification and differentiation. We completed a comprehensive core characterization, including geochemistry and microbial analysis.

4.2.1 Cesium Results

The Cs-137 was analyzed for dating of selected core samples from Grasse River by Mass Spec Services (Orangeburg, NY). For each core, five segments from about 20, 40, 60, 80 % of the total core length, and the second to the last segment of the core were analyzed for dating. **Figure 19** shows the Cs data for the first four cores from the Grasse River; no distinct Cs-137 peak is seen in any of these cores. This was unexpected as these locations had intact cores taken in 1997 and thus were expected to be in areas of the river with stable deposition.
Figure 20 shows the results for core 18M. Panel a (left) is the original analysis and panel b (right) shows the results with three additional points sent for analysis. A clear Cs-137 peak is seen, indicating no mixing along the vertical axis.

Comparison with prior data 1997 cores taken at these locations by Alcoa Inc indicates significant changes to river conditions during the past decade. While these five locations showed intact Cs peaks and predictable PCB concentration peaks in 1997, the coring completed in the summer of 2006 found a much deeper core in location 18M but shallower cores in the other locations. A review of river conditions indicated that an ice jam that occurred in 2003 led to significant scour and mixed the sediment in many locations of the river (see further information at http://www.thegrasseriver.com/Ice_2003_Event.htm).

Unfortunately, the Hudson River coring also resulted in cores that did not show the characteristic Cs-137 peak associated with intact deposition. **Figure 21** shows these results. Again, the locations were chosen based on prior intact cores; it is not clear why these samples showed completely mixed sediments.

Due to the paucity of intact cores, in the summer of 2007, our collaborators at Alcoa, Inc., again provided four cores, including one from 18M and three from locations they believed to have been depositional during the ice jam event. **Figure 22** shows the Cs-137 for these cores. Again, only the core from location 18M showed intact sediments.

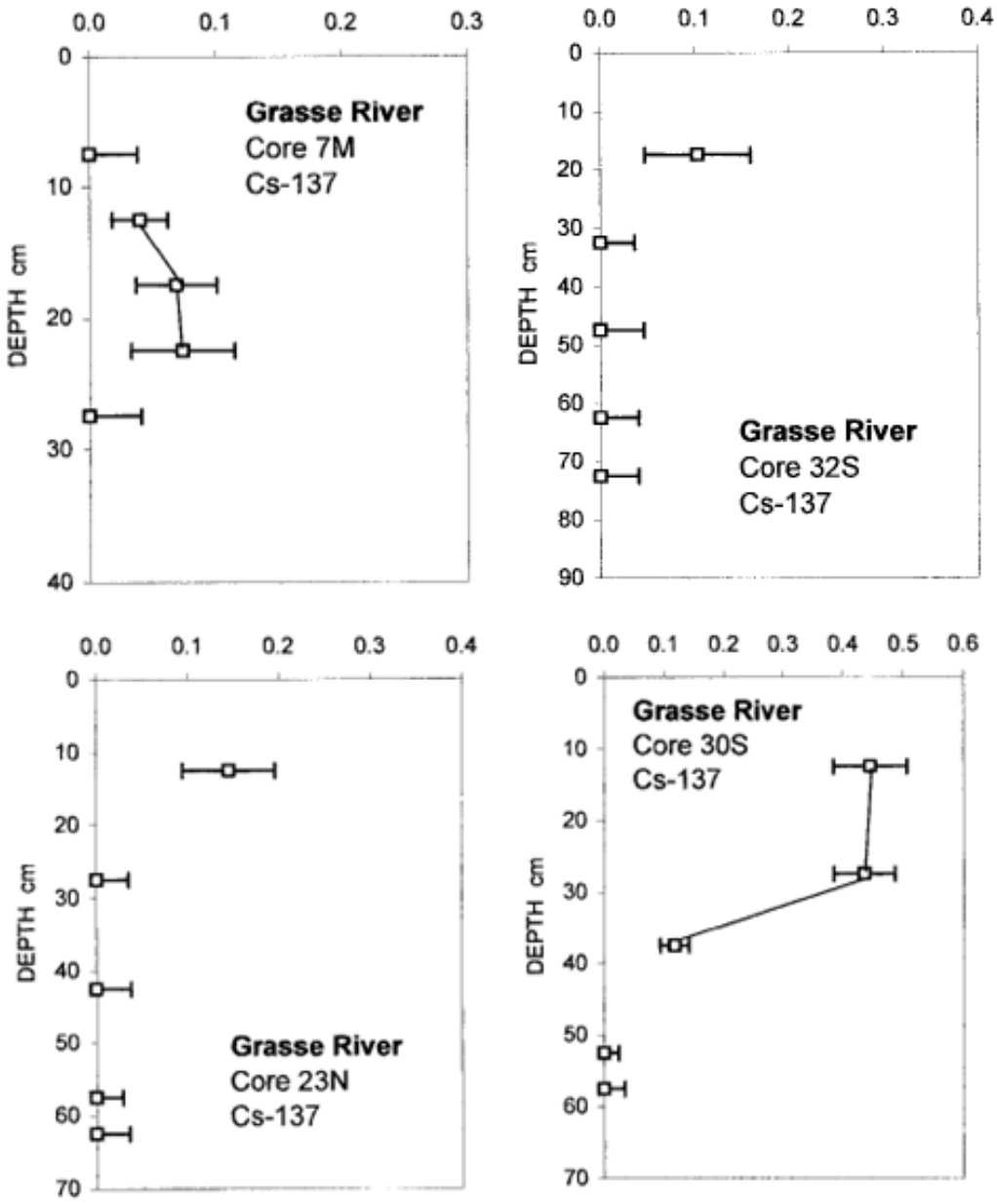


Figure 19. Grasse River Core Cs-137 data for cores 7M, 32S, 23N, 30S. Samples taken in 2006 as described in Section 3.1.1.

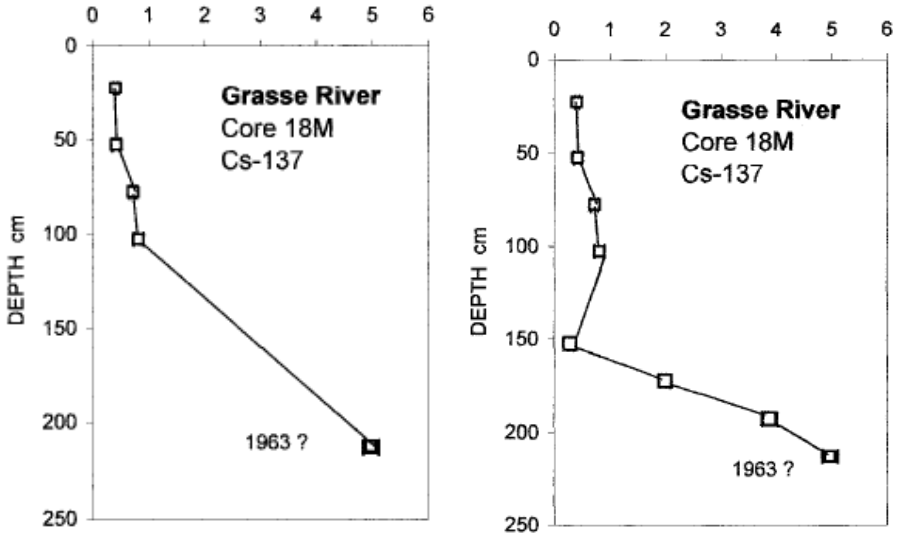


Figure 20. Grasse River Core Cs-137 Data for core 18M.
Panel a shows original results; Panel b includes additional three data points

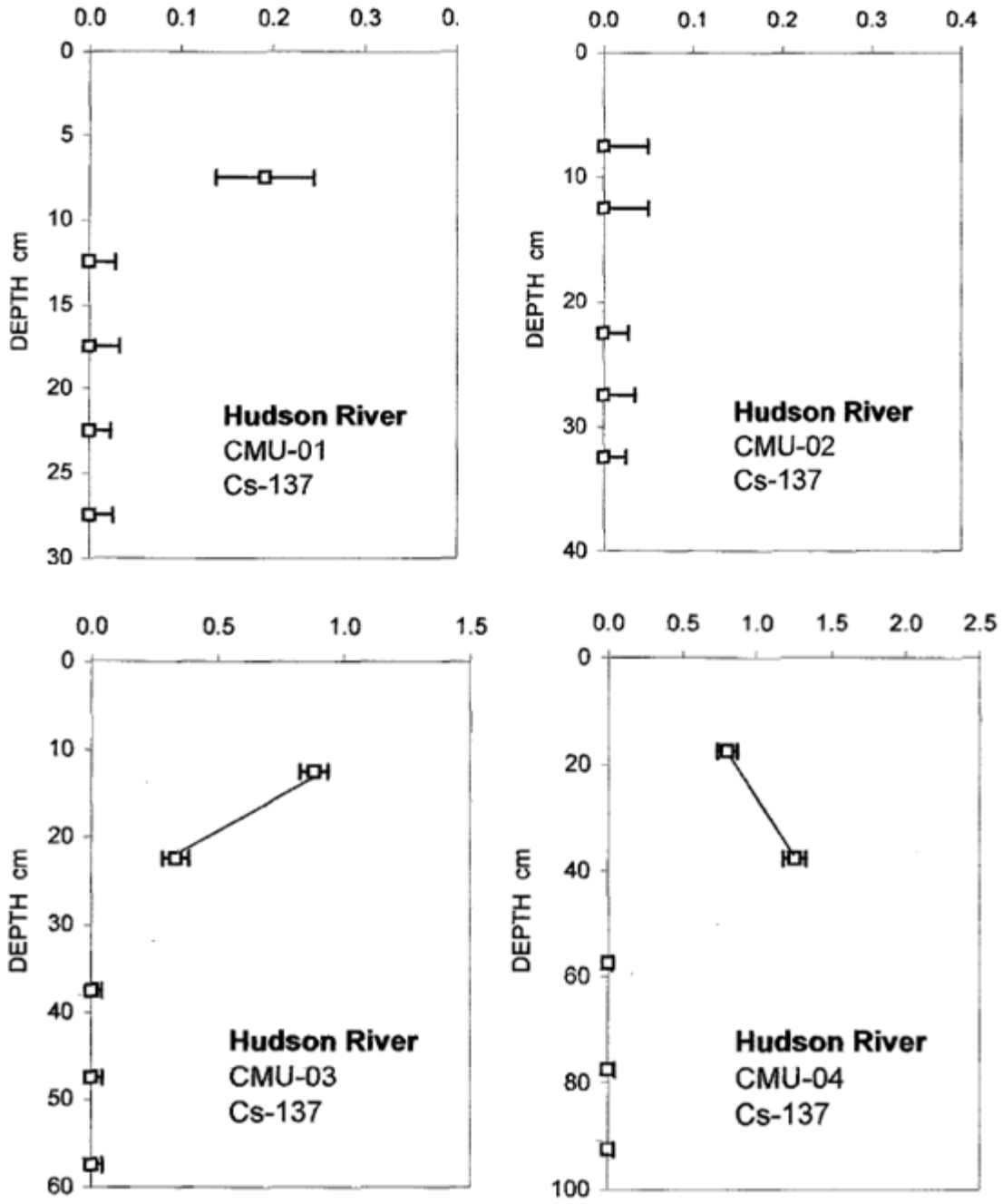


Figure 21. Hudson River Core Cs-137 Data.
Cores sampled at locations described in Section 3.1.2.

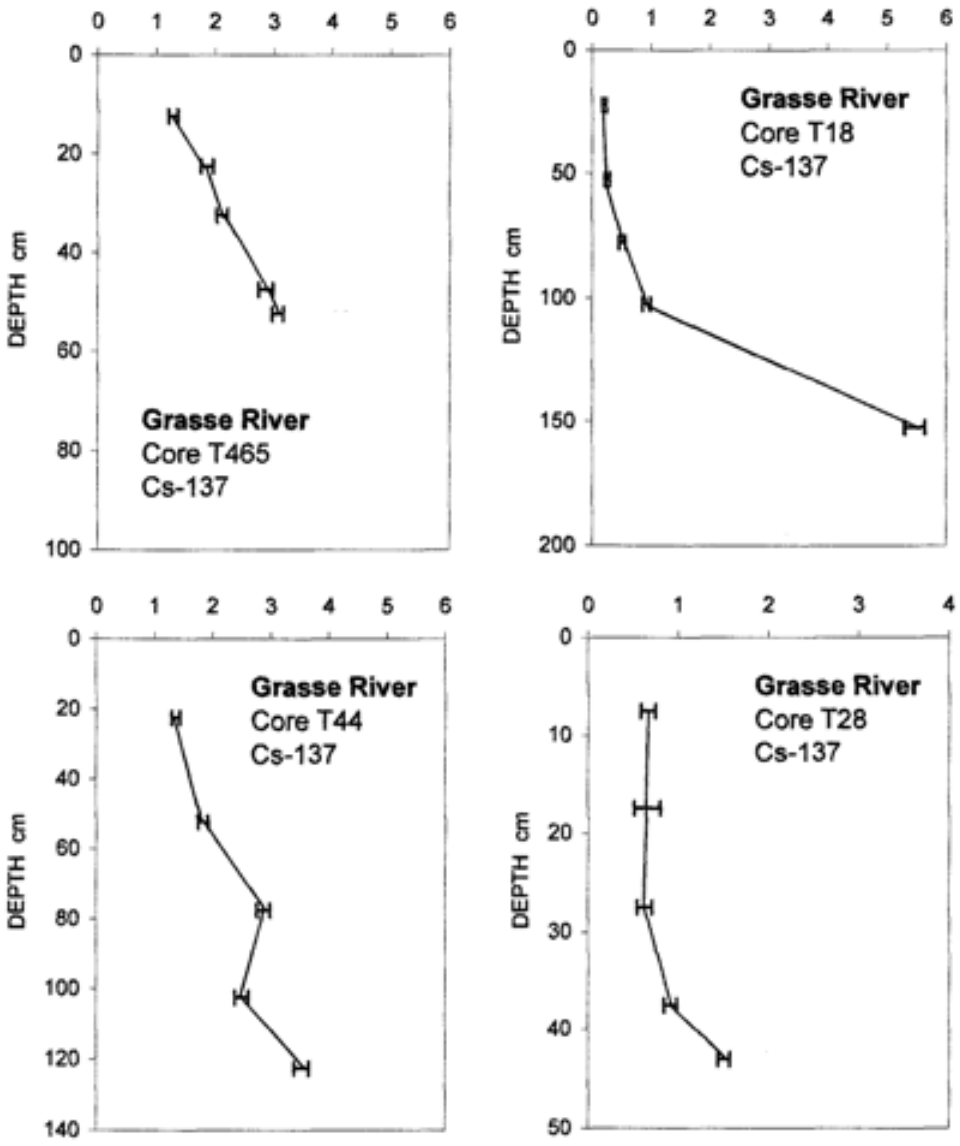


Figure 22. Grasse River Core Cs-137 Data for 2007 Cores.

Subsequent analysis focused on the 18M core from 2006 as this presented the only intact core. The core indicated sediment at this location was twice as deep in this 2006 sampling as when previously sampled in 1997, suggesting the top half was mixed and recent deposition associated with the ice jam event. Thus, while we consider population diversity throughout the core, conclusions regarding the potential for long term in situ reductive dechlorination cannot be made from the upper part of the core. This is confirmed by the 137Cs data, which show values below 2 pCi/g until 150cm down the core (**Figure 20** above).

4.2.2 TOC, Geochemical Characteristics and Total PCBs

TOC and total PCBs are consistent with recent significant deposition on top of intact sediments. TOC varied from 1.2% to 12.4% (**Figure 23**), with a peak near the bottom of core 18M. Similarly, total PCBs (**Figure 23**) show low values in the surficial sediments (1.85 mg/kg dry sediment) and the mixed sediments to 125 cm (under 30 mg/kg) due to their recent depositional nature. Below 140 cm, PCB values ranged from 11.5-836.8 mg/kg dry sediment, with highest levels deeper in the core.

Interestingly, segment 150-155cm contained anomalously high PCB (88.2 mg/kg dry wt) compared with segments above and below. The segment 150-155 likely represented the surface sediment prior to 2003 (suggested by the depth of the 1997 core compared with the 2007 core). These would be expected to be lower in PCBs than deeper sediments (deposited earlier) and shallower sediments (deposited during the ice jam scour and deposit). Since this represented surficial sediment prior to 2003, the only likely reason for this high value was deposition of more contaminated sediments. Discussions with engineers at Alcoa suggest that this area was subject to some deposition during the non-time critical removal action (NTCRA) in 1995. Alcoa reported the post-NTCRA concentrations of 75±40 mg/kg to 108±106 mg/kg in two areas of the river. Thus, 88.2 mg/kg PCBs at segment 150-155 cm is consistent with PCB concentrations observed after the NTCRA in 1995. Further, the NTCRA resulted in a thin layer of sand below the surface layer of loose silt Alcoa 1999. The texture analysis of 2006 Core 18M showed a distinct thin layer of coarser sand in 145-155cm, while the texture above and below was silty. These multiple threads of information suggest that the deposition of more contaminated sand in this location was from NTCRA in 1995 (Alcoa 1999).

Analysis of the core includes all sediment segments; however, conclusions should be made only on results for core segments below the re-depositional layer, and results for 150-155 cm segment must always be considered within the context of its anomalous characteristics.

Even though the total PCBs varied significantly down the sediment core, a positive correlation (Pearson Correlation r=0.614, p=0.003) was found between TOC and total PCBs, consistent with previous work. Anion analysis in the core showed that sulfate and nitrate were 1 to 3 orders of magnitude higher than other anions. Surficial sediment contained less sulfate and nitrate, but more phosphate in general (**Table 15**). No obvious trends of anion change are observed with depth (analysis not shown). The levels of eight metal elements, aluminum (Al), calcium (Ca), Chromium (Cr), Copper (Cu), Iron (Fe), magnesium (Mg), manganese (Mn) and zinc (Zn). **Table 16** shows high aluminum and iron in all the examined segments due to the historical discharges by Alcoa.

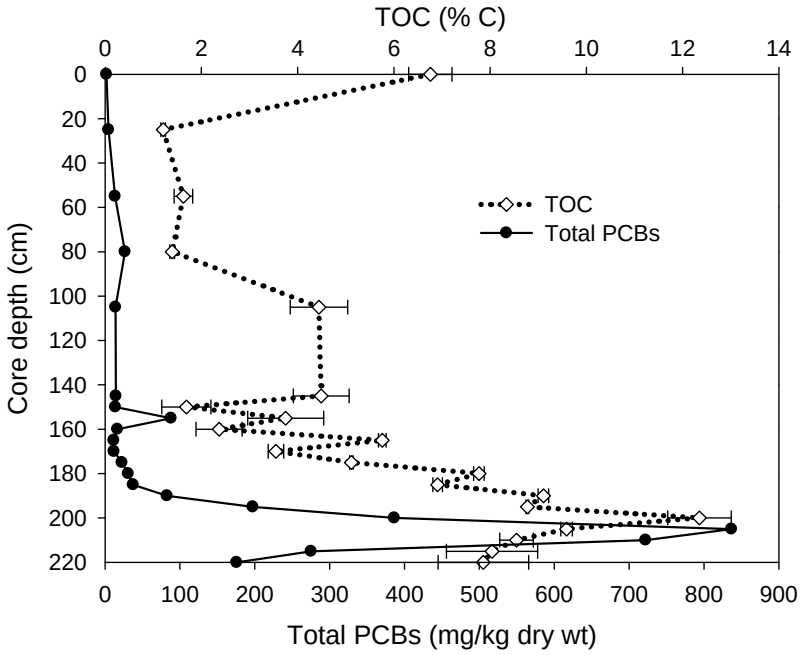


Figure 23. Core Physical and Geochemical Characteristics. Total PCBs and TOC in Grasse River Sediment Core 18M. Error bars represent one standard deviation.

Table 15. Concentrations of Anions in Core 18M (in ppm, mg/kg dry weight)

Depth (cm)	Nitrate	Sulfate	Chloride	Nitrite	Bromide	Fluoride	Phosphate
0-5	14.8	6.2	20.6	1.1	1.7	1.1	16.3
20-25	23.5	1103.3	11.5	0.3	0.6	0.6	8.1
50-55	201.9	3644.0	39.9	0.6	0.3	0.6	4.3
75-80	163.5	2532.3	28.9	0.3	0.4	1.1	2.7
100-105	662.5	1258.3	15.0	ND	0.4	0.8	2.3
140-145	1617.5	2287.1	19.0	0.2	0.6	2.5	3.1
145-150	260.2	201.9	4.2	0.1	0.3	1.2	1.3
150-155	5.4	508.6	24.6	0.2	0.1	2.1	1.4
155-160	1646.1	1081.0	14.3	ND	0.3	1.7	2.1
165-170	936.4	1160.7	12.1	0.3	0.3	0.7	1.1
170-175	2062.1	2107.4	30.6	0.9	0.2	1.1	1.0
175-180	745.9	1579.8	20.7	0.0	0.4	0.7	1.3
180-185	1615.6	1575.1	20.0	ND	0.6	1.6	2.6
190-195	2076.5	1583.7	35.9	0.4	0.4	0.4	1.0
195-200	542.5	2012.9	24.4	0.2	0.4	0.9	1.0
200-205	10.2	760.1	26.9	ND	1.4	0.5	1.5
210-215	1168.2	1632.3	28.6	ND	0.5	1.0	2.3
215-220	236.9	1543.5	15.1	1.2	0.4	1.0	2.1

Table 16. Concentrations of Eight Metals in Core 18M (in mmole/kg dry weight)

Depth (cm)	Al	Ca	Cr	Cu	Fe	Mg	Mn	Zn
160-165	448.1	267.5	0.5	0.9	342.9	303.7	7.5	3.8
165-170	300.0	205.5	0.3	0.7	239.3	230.5	5.3	2.5
175-180	566.7	262.5	0.5	1.8	414.3	314.4	12.0	4.7
185-190	611.1	297.5	0.6	3.1	439.3	332.9	20.4	5.1
195-200	614.8	270.0	0.6	3.3	430.4	319.8	15.4	5.6
200-205	718.5	285.0	0.7	4.9	473.2	344.0	21.1	6.0
205-210	733.3	327.5	0.7	4.4	501.8	398.8	19.5	5.7

In addition to bulk geochemistry and total PCBs, each core segment was evaluated for full congener distribution. In the next section, these data and their implications for reductive dechlorination are examined.

4.2.3 Evidence of Reductive Dechlorination in Sediment Cores

The extensive full congener analysis in the sediment core enabled consideration of multiple methods to assess whether reductive dechlorination may have taken place in the sediments represented by the intact core. While there are other processes that can alter congener distributions, our evaluation focused on consideration of reductive dechlorination. A variety of surrogates have been used to evaluate reductive dechlorination extent or potential in sediments.

The MDPR is the molar fraction sum of four exclusively *ortho*-substituted PCB congeners (2-CB, 2,2'-CB, 2,6-CB, 2,2',6-CB) and one PCB congener with an *ortho* and a *para* chlorine (2,4'-CB). Generally, *ortho* dechlorination does not occur in many sediment systems and the end-products consist of mainly *ortho*-substituted PCBs. Thus, MDPR is a surrogate for how extensive dechlorination has been in a sediment. While PCB 8 (2,4'-CB), a *para*-substituted PCB congener is not an expected endpoint of reductive dechorination, it appears to accumulate in Hudson River sediment, suggesting it is resistant to dechlorination for other reasons. The use of MDPR in other sediment systems (other than the Hudson River) has not be extensively studied, and the initial evaluation here focused on consideration of the role of PCB8 or MDPR as a surrogate for dechlorination extent in Grasse River sediments.

To address the question of whether PCB 8 is a dechlorination end-product in the Grasse River, the MDPR was evaluated with and without incorporating PCB 8. **Figure 24** (panel a) shows MDPR for the core segments, with PCB8 shown in black and the balance of the components of MDPR in grey. MDPR values varied along the sediment core. Relatively high MDPR values (greater than 0.30) were found in the upper part (20-105 cm), in the middle part (150-160 cm) and in the deep undisturbed segments (190-220 cm). The high MDPRs in the upper and middle segments are likely redepositional material from the NTCRA and the ice jam. The high MDPR in deeper (older) sediment is associated with increases in all the constituents of MDPR – especially PCB8,where PCB 8 contributes to greater than 21% of the overall MDPR. In contrast, PCB 8 accounts for 4% to 18% of the MDPRs in the upper and middle segments. The elevated molar percent of PCB 8 suggests that the dechlorination of unflanked *para* is not preferred in Core 18M, and PCB 8 can be regarded as an end-product in the system.

In the area of the 18M core, the original source of PCB contamination is believed to have been predominantly Aroclor 1248 (Alcoa 1999). The MDPR for commercial Aroclor 1248 is <0.01, significantly lower than the values observed in the core, which indicates the sediment is enriched in the MDPR congeners compared with the original discharged concentrations. The high MDPR values (>0.35) in 190-220 cm indicate that extensive dechlorination has occurred in the undisturbed deeper sediment segments. Since dechlorination produces lighter molecules that are more soluble, volatile, and of potentially greater susceptibility to aerobic degradation processes, estimating the extent of dechlorination based on the current concentrations of lightly chlorinated congeners probably represents an *underestimation* of the actual degree of dechlorination.

Another way to assess changes in congeners in the sediment is to consider the chlorine content of the PCBs. The chlorine content of an Aroclor 1248 source was 48%. The deep undisturbed sediments all have chlorine contents significantly below 48% (**Figure 24** panel a right axis). Only the upper segments and middle segment 170-175 cm have chlorine contents similar to Aroclor 1248. This suggests less extensive dechlorination in these sediments. Use of the chlorine content of current PCBs is a conservative measure as fully dechlorinated PCBs would no longer be quantified in total PCB.

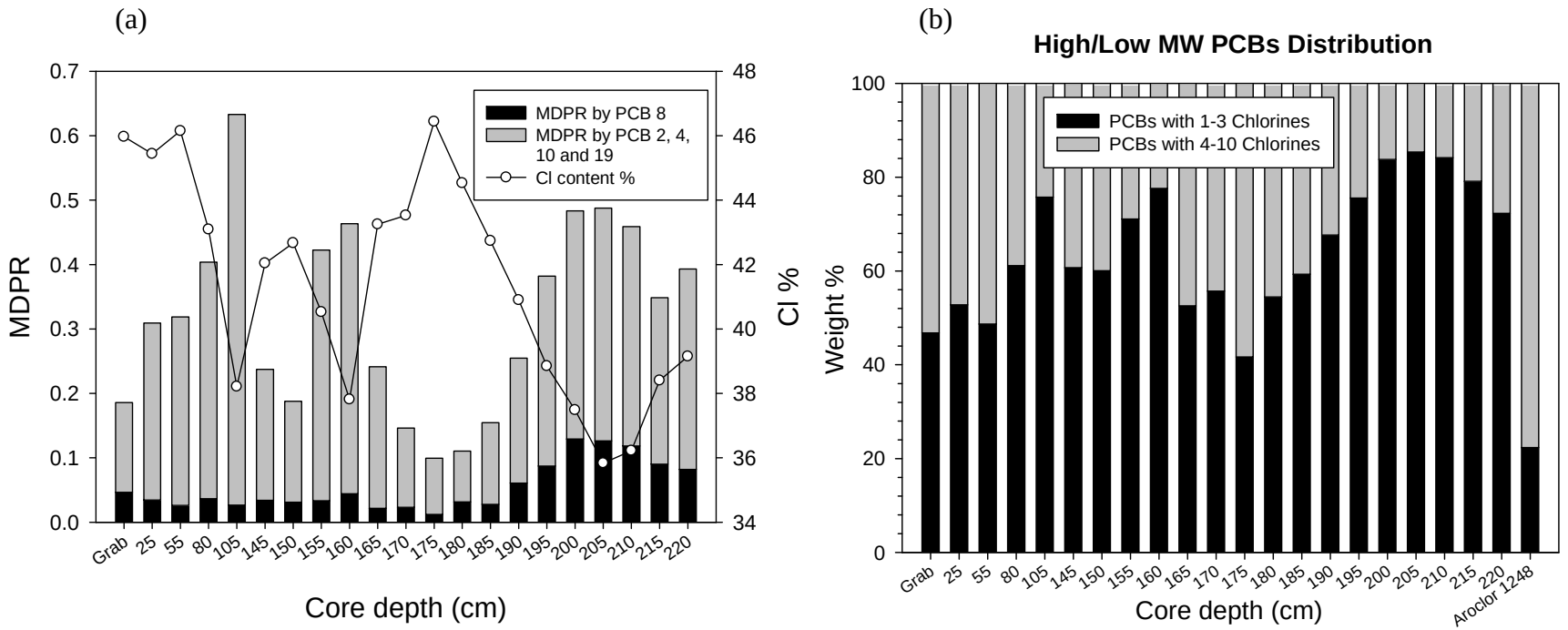


Figure 24. Degree of Dechlorination in Different Core Depth by MDPR and %Cl.
(a) Total chlorine percentage and MDPR for the grab sediment and the core segments from Grasse River; (b) PCB distribution for the grab sediment and the core segments from the Grasse River. PCB homologs are grouped into those with four or more chlorines (grey) and those with three or fewer chlorines (black). The putative source Aroclor (1248) is shown for comparison. X axis is depth in the core from the surface. G: Grasse River surficial grab sample, 25: 20-25 cm segment, 55: 50-55 cm, 80: 75-80 cm, 105: 100-105 cm, 145: 140-145 cm, 150: 145-150 cm, 155: 150-155 cm, 160: 155-160 cm, 165: 160-165 cm, 170: 165-170 cm, 175: 170-175 cm, 180: 175-180 cm, 185: 180-185 cm, 190: 185-190 cm; 195: 190-195 cm, 200: 195-200 cm, 205: 200-205 cm, 210: 205-210 cm, 215: 210-215 cm, 220: 215-220 cm.

Similarly, grouped PCB homolog distributions visually represents the degree of dechlorination with depth. **Figure 24** panel b shows the homologs with 4 or more chlorines and the homologs with 3 or fewer chlorines separately. Significant changes to homolog groups are seen in the core. For the core segments deeper than 190 cm, congeners with fewer than three chlorines account for over 75% of the total PCB weight, a significantly higher percentage than in the original Aroclor 1248 (22.4%) The percent weight increases in lesser-chlorinated homologs, likely represent the effect of anaerobic microbial dechlorination of highly chlorinated PCB homologs. When considering the full homolog distribution (**Figure 25**), the percent weight of dichlorobiphenyl increases while trichlorobiphenyl remains relatively stable with depth.

When considering concentration rather than percent weight (see Table 10), the homolog groups show similar trends to the total PCBs, with higher concentrations of each homolog detected in deeper sediments showing higher total PCBs. However, less chlorinated homologs, mono-, di-, and tri- chlorobipenyls increased more dramatically than highly chlorinated homologs with 7, 8 or 9 chorines. For example, considering mass based concentrations, monochlorobipenyls were three orders of magnitude higher in deeper sediments than in upper sediments, whereas, nonachlorobipenyls increased only 50 times from the top to the bottom of the Core 18M. Total PCBs increased by one order of magnitude (see **Figure 23** and Table 10).

Complete PCB homolog percentage compositions for the grab sample, each core segment, and three commercial PCB mixtures, Aroclor 1242, Aroclor 1248 and Aroclor 1260, were evaluated with multivariate principal component analysis (PCA). These particular Aroclors were selected for comparison due to the uncertainty in knowledge of the source Aroclor in the Grasse River. The source is reported by Alcoa to have been predominately Aroclor 1248 (Alcoa 1999). However, a separate analysis based on current congener levels suggested an 80% Aroclor 1242 to 20% Aroclor 1260 mixture (Ortiz et al. 2004).

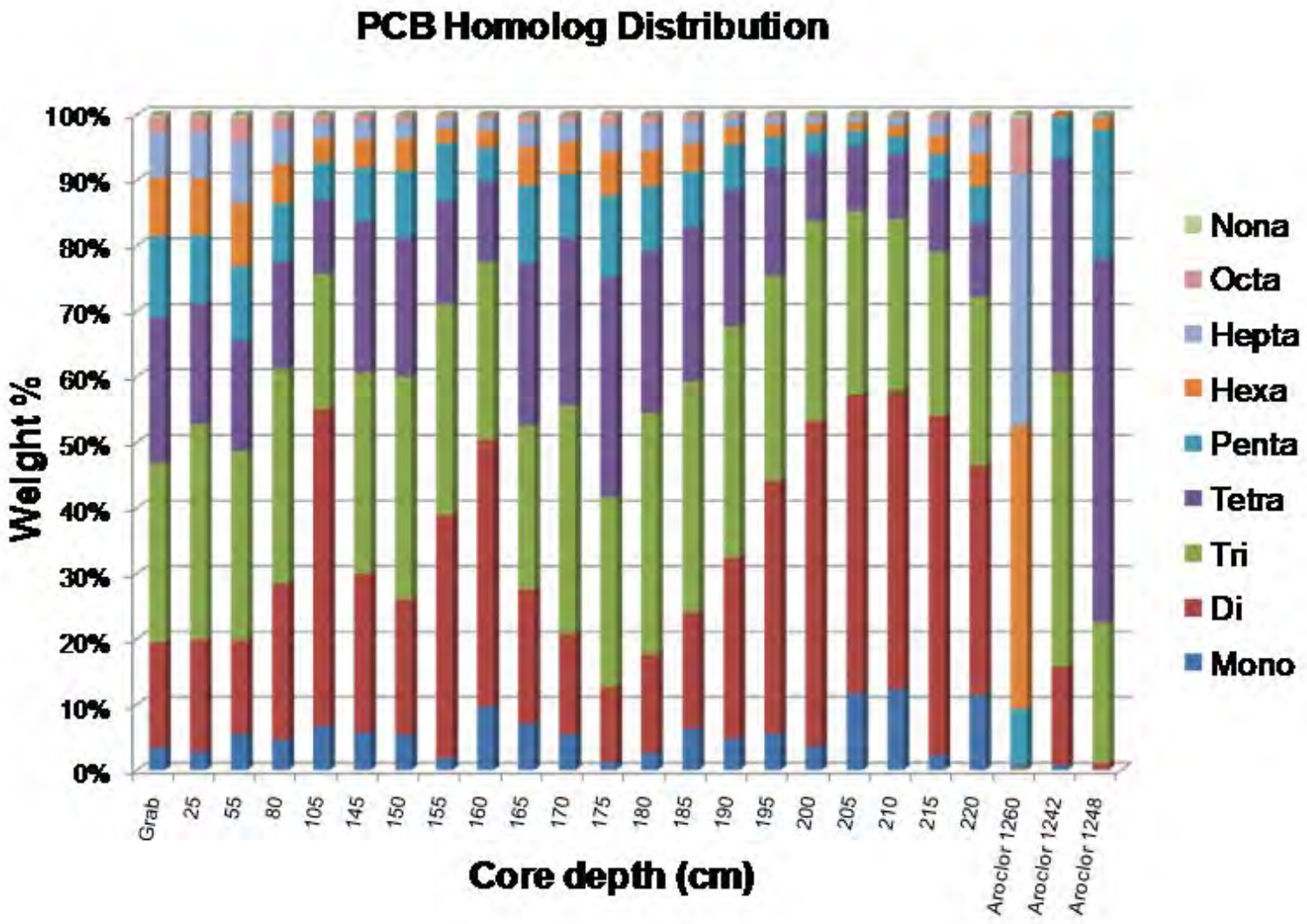


Figure 25. PCB Homolog Distribution with Core Depth for Core 18M.

Shown in **Figure 26**, the first two components explained 55.8% and 34.7% of the total variance respectively. All segments showed significant difference from Aroclor 1248 (where tetrachlorobiphenyls and pentachlorobiphenyls are dominant) and Acoclor 1260 (where hexachlorobiphenols and heptachlorobiphenyls are most abundant). However, the surficial sediment from the grab sample, upper core sediments 20-25 cm, 50-55 cm, 75-80 cm and core sediments from 140-150cm and 160-190 cm were more similar to Aroclor 1242 (where trichlorobiphenyls are the most abundant homolog), which indicates relatively low level of dechlorination in these sediments. The homolog distributions of deeper sediments from 190-220 cm, and sediments from 100-105 cm, 150-160 cm (where dichlorobiphenyls are the most prevalent PCB congeners) were distinct from the commercial Aroclor mixtures and other sediment segments and were generally clustered into two groups along the first component, where variable dichlorobiphenyls was most responsible for discriminating the two groups from right to left in **Figure 26**.

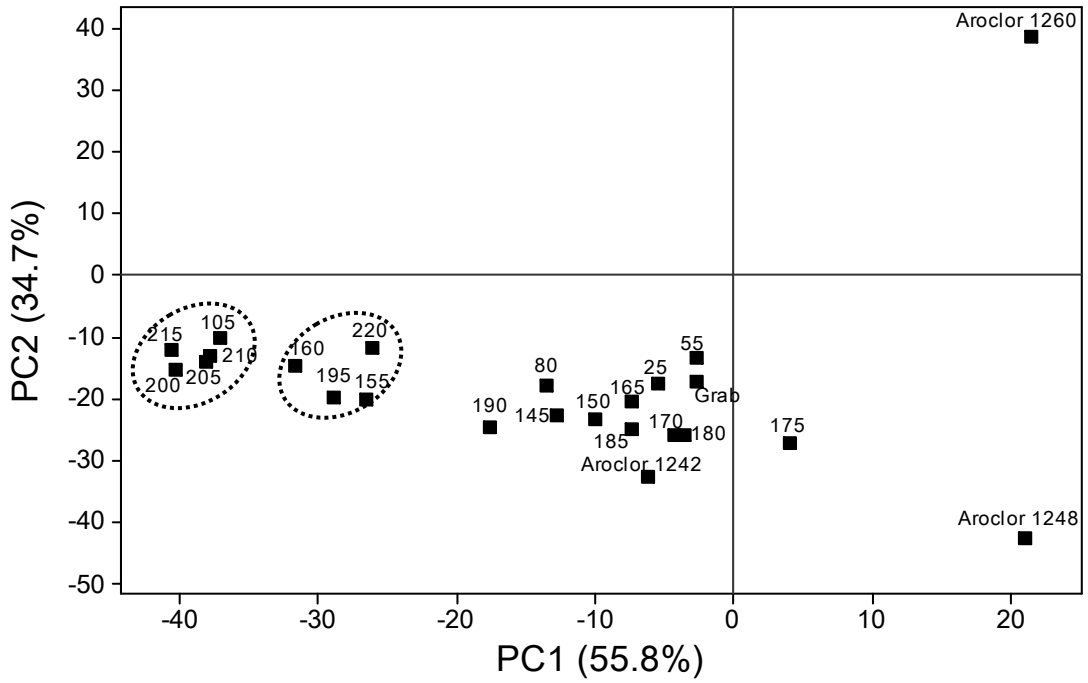


Figure 26. Results of PCA Comparing Aroclors with Congener Patterns in Sediments.

In addition to these classical analyses of PCB reductive dechlorination in historical sediments from intact cores, we also applied a novel analysis that was developed in our research group, assessing the changes in relative proportions of congeners that are correlated in the Aroclors (Karcher et al. 2004; Karcher 2005). These correlated congeners were studied in the core segments and significant divergence from and "Aroclor-like" proportion of pairs of congeners was assessed. For example, two tracker pairs are shown in **Figure 27**. The ratio of tracker pair 110/97 (shown on the left) was relatively stable and close to the Aroclor line in the upper sediments and increased dramatically in the older undisturbed sediments (lower than 160 cm). Similarly, the ratio of tracker pair 153/135 in the core segments was also significantly different from the ratio in Aroclors (**Figure 27** right). The difference between the Aroclor ratio and

present ratios is a strong evidence of dechlorination. In addition, some or all of the congeners among PCB 110, 97, 153, 135 and their parents and daughter congeners are expected to be among the most active targets in the dechlorination process. Thus, this finding helps us to use tracker pairs rather than individual PCB congeners to construct sediment microcosms to study the mechanisms in the complex reductive dechlorinating reactions.

A comprehensive analysis of all tracker pairs down the core was undertaken through related research funding and is beyond the scope of the present report. **Figure 28** provides a snapshot of the analysis, showing 35 tracker pairs and four sediment segments in the deep core. Plotted as open circles are the normalized mean value of the ratio for each tracker pair for the Aroclors. Bars are 1 standard deviation above and below this value. Data from the core segments are shown as solid squares (150-155cm), open squares (170-175cm), open triangles (190-195) and + signs (210-215). The results show that for many tracker pairs, values in the core segments are quite far from the relationships that would have held in the original Aroclors and in some cases movement away from the Aroclor relationship increases with depth (for example, tracker pair 49/44 and to some extent tracker pairs 177/170, 177/174 and 179/174).

Assessment of reductive dechlorination through homolog or congener-specific changes, changes in MDPR or CPB or percent chlorine are always subject to uncertainty as additional physical and chemical processes may alter these factors. The analysis in this section suggest that in situ transformations of congeners took place in the sediments in the core from 18M. The presence of microbial species known to dechlorinate PCBs and species related to known dechlorinators supports the hypothesis that the observed transformations were facilitated by microbial activity. However, the current work, while suggestive, is far from conclusive.

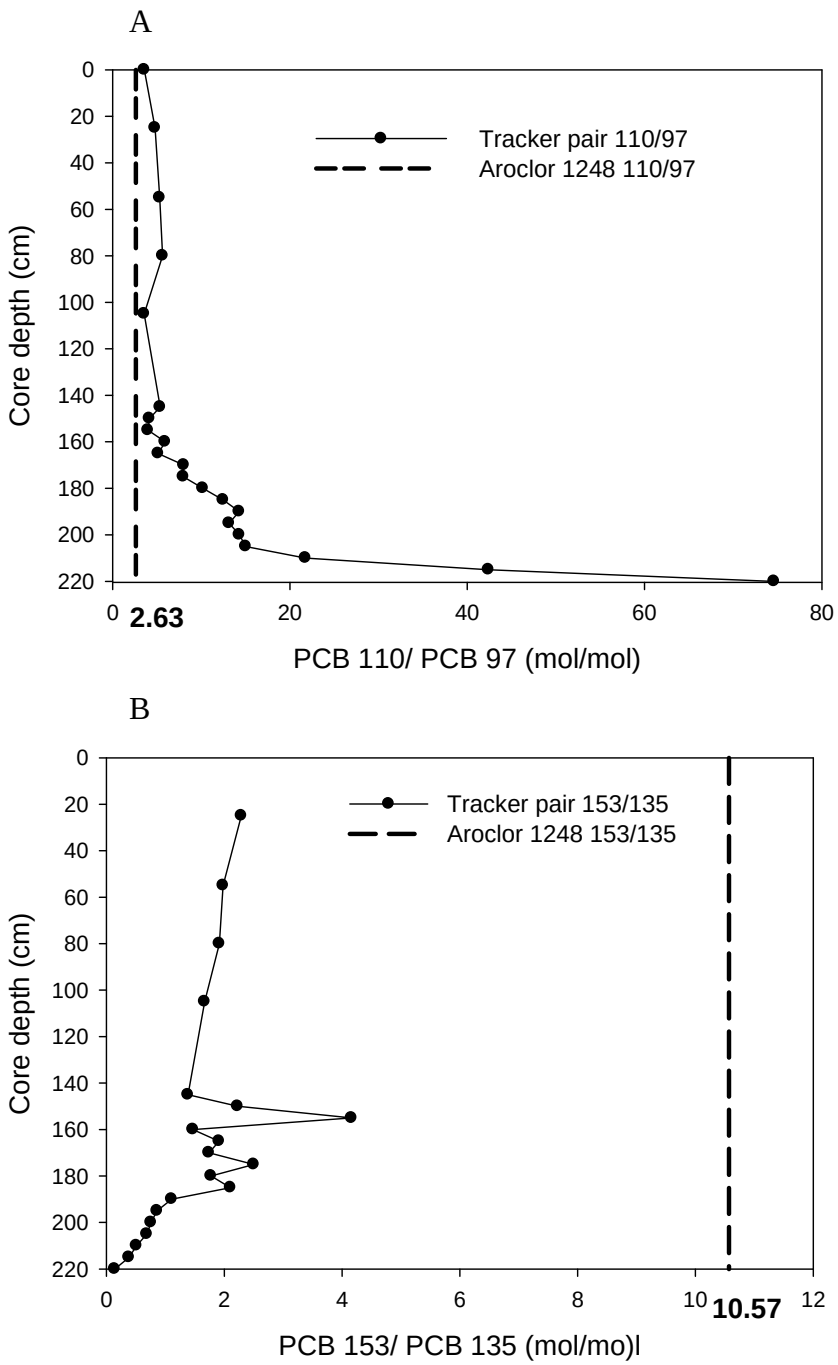


Figure 27. Tracker Pair Ratios Along the Sediment Core.
A) PCB 110/ PCB 97. The Aroclor line (2.63) represents PCB 110/ PCB 97 ratio in original Arcolor mixtures. B) PCB 153/ PCB 135. The Aroclor line (10.57) represents PCB 153/ PCB 135 ratio in original Arcolor mixtures.

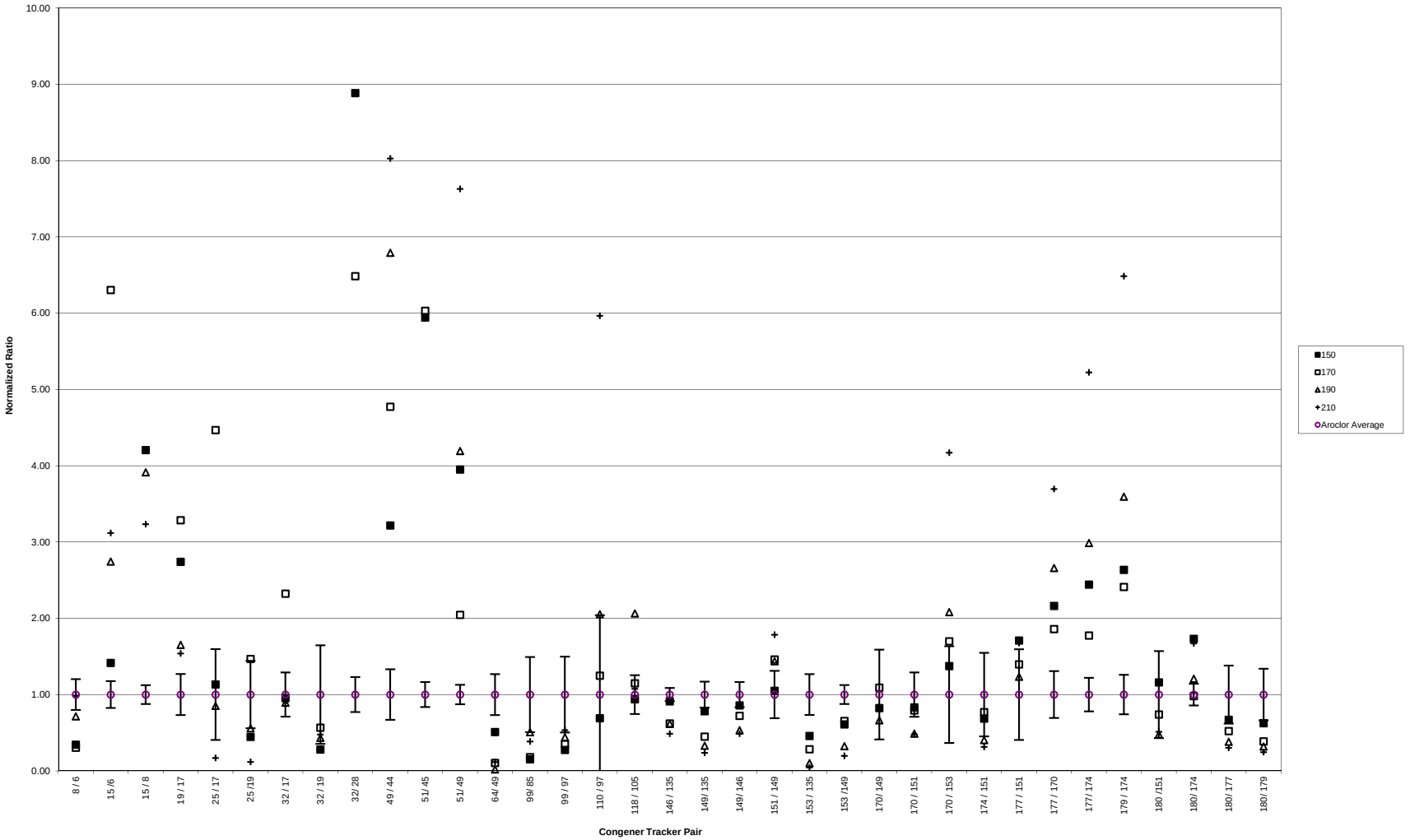


Figure 28. Tracker Pair Ratios in the Core Segments Normalized with Aroclor Ratio.
The Aroclor average represents the ratio of these congeners found to be constant through all the commercial Aroclors and any mixture of Aroclors. Bars are 1 standard deviation above and below the mean value of the ratio for the Aroclors. Solid squares represent the top of the intact core (150-155cm segment), open squares (170-175cm), open triangles (190-195cm), and + (210-215 cm).

4.2.4 Changes in Congener Distributions in Cores from 1997 to 2006

Access to the results from the 1997 coring in the Grasse River (also at location 18M) provided two congener-specific analyses a decade apart.[1] While there is uncertainty regarding the alignment of the cores (**Figure 29** represents a simple alignment from the bottom of each core), we evaluated changed in tracker pairs with depth in each core *separately* to consider whether similar changed down the core were observed in 1997 and 2006.

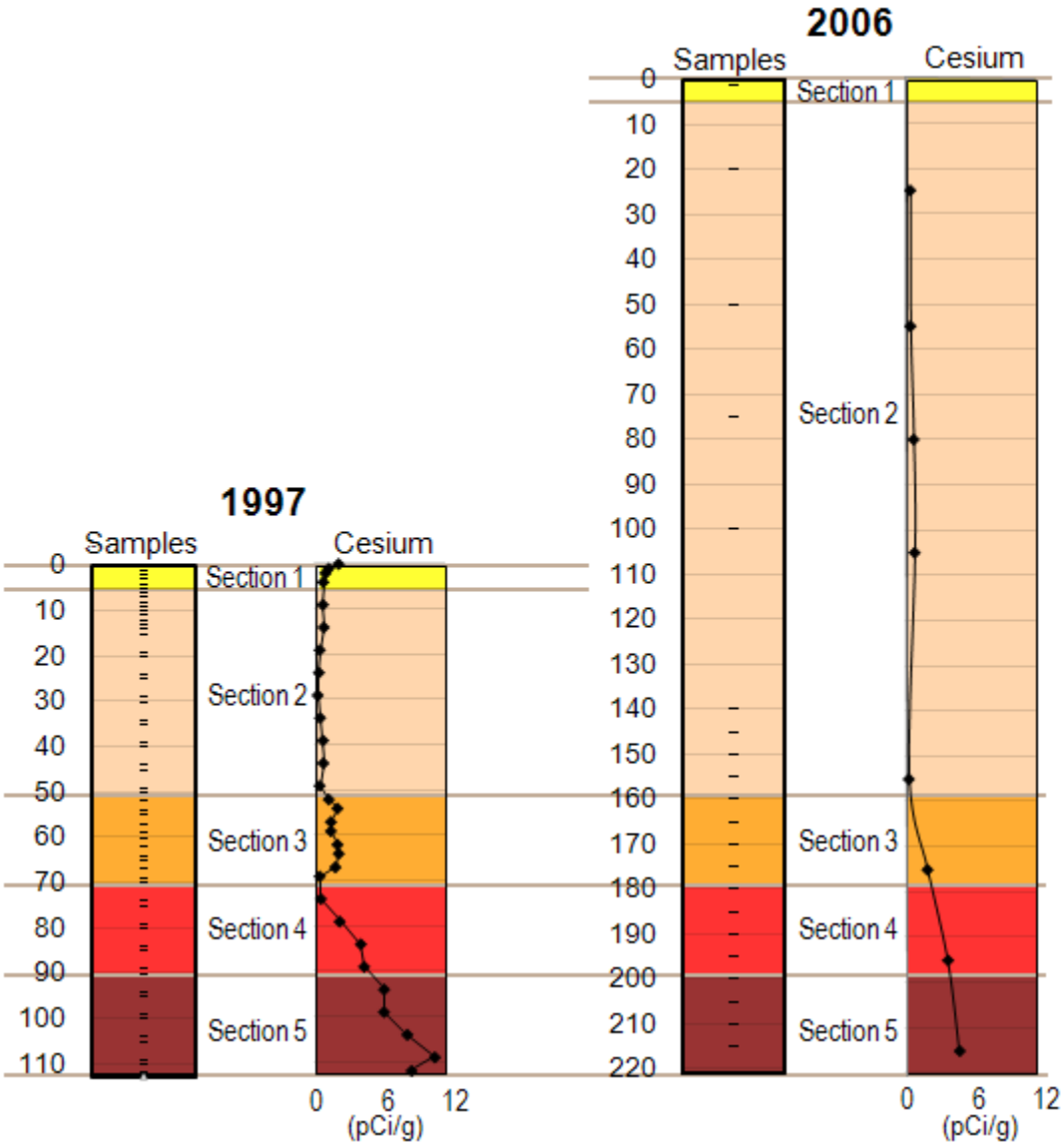


Figure 29. Sectioning of the Grasse River 18M Cores Collected in 1997 and 2006.

[1] Material on the decadal core analysis is contained in a paper under preparation for submission in early 2011.

The 18M core from the 1997 sample is shown on the left and the 18M core from 2006 is shown on the right. Again, it is important to note that the cores are aligned from the bottom up (in 1997, sampler refusal was at 113 centimeters; in 2006, sampler refusal was at 221 centimeters). There are two representations of each core in **Figure 29**, the left side showing the depths from which samples were collected and analyzed for PCBs, and the right side showing the Cesium 137 profiles. In all representations, the depths are shown down the core. For cesium profiles, the *x*-axis goes from 0 to 12 picocuries/dry gram of sediment (pCi/g dry). The top left of the figure shows the sectioning in tabular form.

The cores were sectioned so that sufficient data would be available in each section for the statistical analysis. The sectioning is not meant to imply a one-to-one correspondence between the cores, rather they cores are divided into three deep layers, one near surface layer and one surficial layer. The near surface layer in 2006 is extensive due to the redepositional nature of those sediments. Although laboratory analysis for PCBs was performed on the data from Sections 1 and 2, these results cannot be used to offer insight in the depth study since Section 1 and Section 2 of the 2006 18M core are recently deposited sediments, not part of the intact core.

There are 198 pair of correlated congeners (tracker pairs) for which PCB analysis was available for the cores from location 18M in the Grasse 1997 (G97) core and the Grasse 2006 (G06) core. These are considered by analyzing the relative change in tracker pair ratios within each core separately (intra-core analysis).

Raw data from each of the cores were processed according to the procedures detailed in Karcher, Small and VanBriesen (2004). After processing, 171 of the 198 tracker pair were identified as having three or more usable field sampling points in all three sections for sampling dates 1997 and 2006 (six sections in total). In this study, we were interested in determining if the samples in each section of each core showed a trend either towards or away from the correlated relationship observed in the Aroclors. To determine this, for each of the three sections for each sampling date (six in total), the average distance of the field sample points from the Aroclor correlation relationship (the residual) was calculated, and these averages were compared. Of the 171, in 1997, the average residual of all three sections was on the same side of the Aroclor correlation in 150 pair; in 2006, 143 pair met the criteria. In 1997, 82 pair show a trend in the core (the residual of Section 3, 4, and 5 are either always moving away from or towards the Aroclor relationship), and 114 show a trend in the 2006 core.

While the cores were sampled from the same GPS location using the same methodology and the cores are chronologically similar as shown by their Cs profiles, comparisons between cores taken nearly a decade apart are difficult at best. The only inter-core comparison considered is when a trend in tracker pair was observed in the same section of the two cores (where sections are defined as 20 cm units, not individual core segments). There were 56 pair where a trend was found in both cores; in 41 pair, both cores show a trend away from the Aroclor relationship and 3 a trend towards. In the remaining 12 pair, the trend is in the opposite direction when comparing the two cores (**Table 17**).

Table 17. Congeners Pairs that Overlap Between the G97 and G06 in Core 18M

X	Y	G97	G06
8	4	Away	Towards
9	6	Away	Towards
15	4	Towards	Away
15	8	Away	Towards
18	17	Away	Away
19	18	Away	Away
20	17	Away	Away
20	19	Away	Away
22	17	Away	Away
22	19	Away	Away
25	16	Away	Towards
26	16	Away	Away
26	22	Away	Away
27	20	Away	Away
31	22	Away	Away
32	17	Towards	Towards
32	18	Away	Away
32	22	Away	Away
32	28	Away	Away
49	44	Away	Away
59	45	Away	Away
71	49	Away	Towards
77	56	Away	Away
83	82	Away	Away
85	82	Away	Away
91	84	Towards	Away
97	82	Away	Away
97	83	Away	Away
99	82	Away	Away
99	83	Away	Away
99	85	Away	Away
99	97	Away	Away

X	Y	G97	G06
110	87	Away	Away
110	97	Away	Away
117	83	Away	Towards
117	87	Away	Away
117	97	Away	Away
130	128	Towards	Away
134	132	Towards	Towards
138	132	Away	Away
141	136	Away	Away
158	156	Away	Away
163	136	Away	Away
163	141	Away	Away
163	149	Towards	Away
163	153	Away	Away
164	132	Away	Away
170	151	Away	Away
172	171	Away	Towards
174	151	Away	Away
177	171	Away	Away
180	151	Away	Away
180	174	Away	Away
183	172	Towards	Towards
183	179	Away	Away
190	183	Towards	Away

An example of a tracker pair (congener 8 and 15) is shown in **Figure 30**. Looking at the left panel, it can be seen that the average of the residuals of Section 5 (dark brown square) is the farthest away from the Aroclor correlation relationship (thin blue line cutting diagonally across the upper left quadrant of the plot). The Section 4 average is next farthest (dark orange square), and the Section 3 average is the closest (light orange square). This figure shows a trend away from Aroclor with increasing depth. In the right panel of the figure, the Section 5 residual is closest to the Aroclor line, followed by the Section 4 average, then the Section 3 average. This is an example of moving towards the Aroclor relationship with increasing depth. This tracker pair is an example of one where the trend in the 2006 core is different from what was observed in the 1997 core. It should be noted that, while the trend towards/away from the Aroclor line is in opposite directions as a function of depth, in both 1997 and 2006 there was more congener 15 relative to congener 8 (all the points are to the right of the Aroclor-line).

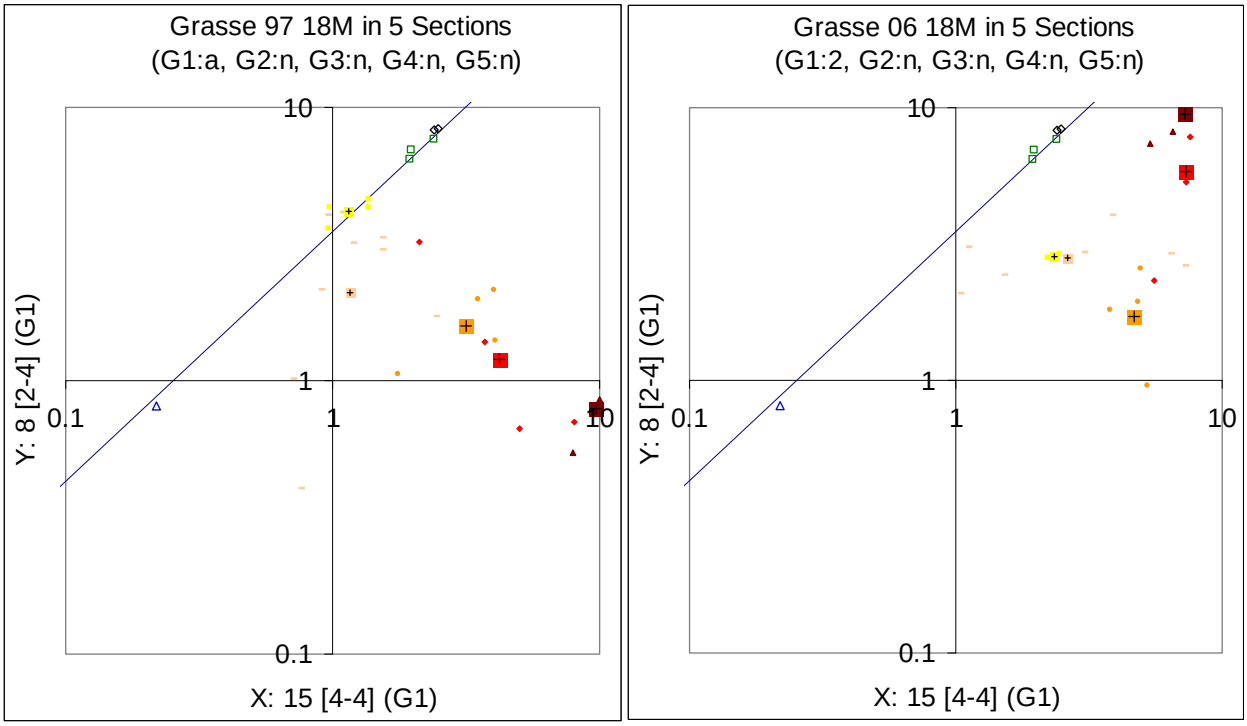


Figure 30. Comparing Grasse 1997 and 2006 18M for Pair 15-8.
In the left panel, a trend away from the Aroclor relative proportions (shown with the blue line) is shown to increase with depth (section 3, then 4, then 5). In the right panel, the relative proportion of congener 15 is higher than would be expected in the Aroclors (as is also the case in the left panel), but the trend with depth is not evident.

Excluding the possible influence of redepositional material from the NTCRA and the ice jam, if reductive dechlorination is an ongoing process in this system, deeper sediment core segments would be expected to have undergone more extensive dechlorination, due to the longer time in the environment. The results in this section, evaluation the changes in congener concentrations from Aroclor-like ratios in the 18M core from 1997 and the one from 2006 support the conclusion that PCBs in Grasse River sediments have undergone transformation for decades. While alternative explanations for these transformations could be posited, reductive dechlorination by microbial species is a plausible conclusion.

4.2.5 Microbiological Analysis of Grasse River Core 18M.

In order to further evaluate the support for the conclusion that reductive dechlorination was an active transformational process in the Grasse River, we considered the microbial community in the 18M core. The concentrations and yields of the DNA extraction for each core segment are summarized in Table 18.

Table 18. Yields of Triplicate DNA Extractions from Core and Grab Sediment Samples

Source	Segment or Sediment	Yield (µg/g wet sediment)	
		Avg.	StDev.
Core 18M (Grasse)	20-25 cm	**8.54**	1.13
	50-55 cm	**6.01**	1.64
	75-80 cm	**5.16**	1.10
	100-105 cm	**9.67**	1.09
	145-150 cm	**0.55**	0.14
	150-155 cm	**1.48**	0.49
	155-160 cm	**5.84**	0.41
	165-170 cm	**6.11**	0.82
	170-175 cm	**7.67**	0.44
	180-185 cm	**7.60**	1.42
	190-195 cm	**7.95**	0.87
	200-205 cm	**7.82**	0.89
	210-215 cm	**6.92**	0.38
Bulk (Grab) Sediment	Hudson	**3.98**	0.84
	Grasse	**17.40**	0.84

The Cs-137 concentrations and DNA extraction yields of the 14 segments were plotted with the depth of core 18M (**Figure 31**). The DNA yields from 8 segments were in a range of 5 -10 µg/g. However, the core segment of 140-145 and 150-155 cm showed only 0.55 and 1.5 µg/g of DNA yield respectively, probably due to its matrix containing lots of wood fragments and coarse sands, reducing the amount of organic carbon in the sample. DNA yields for the core were less than a half of the DNA yield in surface (grab) sediment from Grasse River. Surface sediment is known to have more microorganisms than subsurface sediment due to the growth of aerobic bacteria and the higher organic content.

In the deeper sediment core (below 155 cm), the DNA yields ranged from 5.8 to 8.0 mg/kg (wet weight). In the upper part of the core (20-105cm) DNA levels were similar, ranging from 5.2-9.7 mg/kg (wet weight). TOC and PCB levels vary in the core, and are highly correlated ($R=0.617$; $p<0.01$) as is expected since PCBs sorb strongly to sediment organic carbon. However, no correlation was found between DNA yields and either TOC or PCB concentration. This is not unexpected since PCB degraders represent a very small fraction of the DNA extracted.

Total genomic DNA including both extracellular and intercellular DNA was extracted in this study. Extracellular DNA doesn't represent the gene information of live microbial communities in sediment (Pietramellara et al. 2009), and thus, is a potential confounder for the analysis. However, the persistence of extracellular DNA in aged sediments is not expected to be long. The degradation rate of extracellular DNA is controlled by a variety of environmental factors, such as temperature, pH, salinity, redox potential and organic carbon content (Corinaldesi et al.

2008). Knowledge on extracellular DNA in freshwater sediment cores is limited. However, studies of extracellular DNA in marine sediment system have been conducted by many researchers. They report that, although extracellular DNA accounts for a majority of total DNA in many sediment types (Dell'Anno and Corinaldesi 2004; Corinaldesi et al. 2005), the turnover times of extracellular DNA content were much shorter than the age of the sediment (Dell'Anno and Corinaldesi 2004). This suggests that the detected extracellular DNA does not represent the residual extracellular DNA present when the sediment was deposited at the surface. Rather, extracellular DNA detected in deep sediments is related to organisms currently or recently present in those sediments, not organisms present decades previously.

Further, in general the origins of extracellular DNA are cell lysis and cell extrusion (Pietramellara et al. 2009). In sediment, extracellular DNA is mainly from bacterial extrusion, and bacterially extruded DNA is different from intercellular 16S rDNA in sequences (Whitchurch et al. 2002; Corinaldesi et al. 2005). Previous studies have found that, in sediment, extracellular DNA didn't contain amplifiable 16S rRNA genes or contained much less 16S rRNA gene copies than intercellular DNA (Corinaldesi et al. 2008).

In this project, we used 16S rRNA gene clone library and Q-PCR to study the bacterial communities. These methods focus on amplification of the 16S region of the DNA. Since extracellular DNA is less likely to contain amplifiable 16S rRNA genes, these methods reduce the potential for interference from extracellular DNA in the present work.

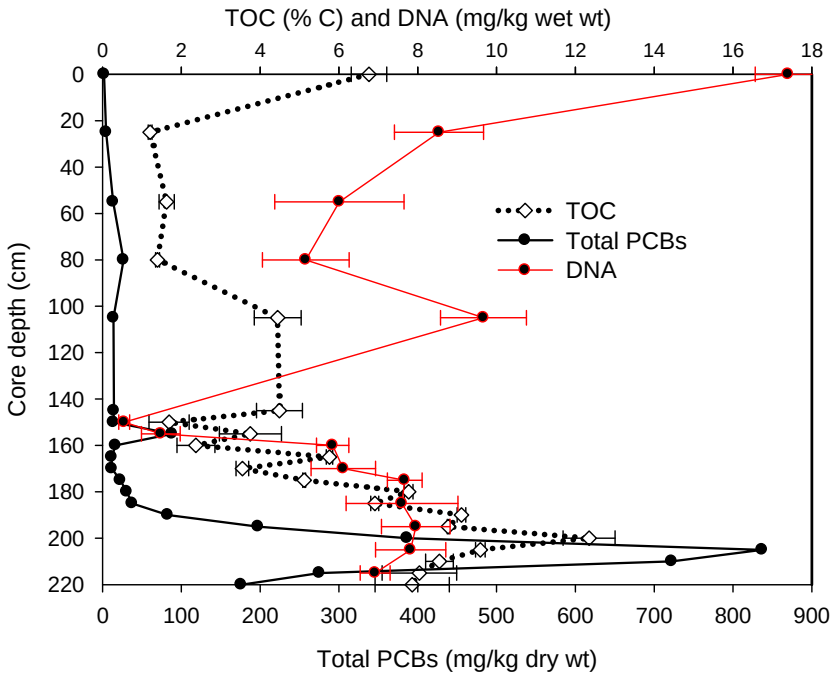


Figure 31. DNA Yields, TOC (%) and total PCBs concentrations in Core 18M.

4.2.5.1 PCR Detection of 6 Microbial Groups from the 8 Segments of Core 18M

All six microbial groups, *Bacteria* (BAC), *Archaea* (ARC), sulfate reducing bacteria (SRB), *Chloroflexi* group (CHL), *Dehalococcoides* group (DHC), and PCB degraders (*o-17* and DF-1 strains) were detected in all segments of the core by PCR assays **Figure 32**. The band intensities (brightness) did not show notable differences under these PCR conditions except lower intensities of some bands for the 150-155 cm segment due to a lower DNA concentration. From the PCR detection of *Chloroflexi*, *Dehalococcoides*, and DF-1/*o-17* groups, it appears that the dechlorination occurs over decades and does not just take place only in the first few years after deposition. This is further confirmed by the results from DGGE analyses and clone libraries described below. To the best of our knowledge, this is the first indirect validation of existence of 16S rRNA genes of dechlorinating bacteria with depth (or age) more than 2m-depth (or 50 year-old sediment core).

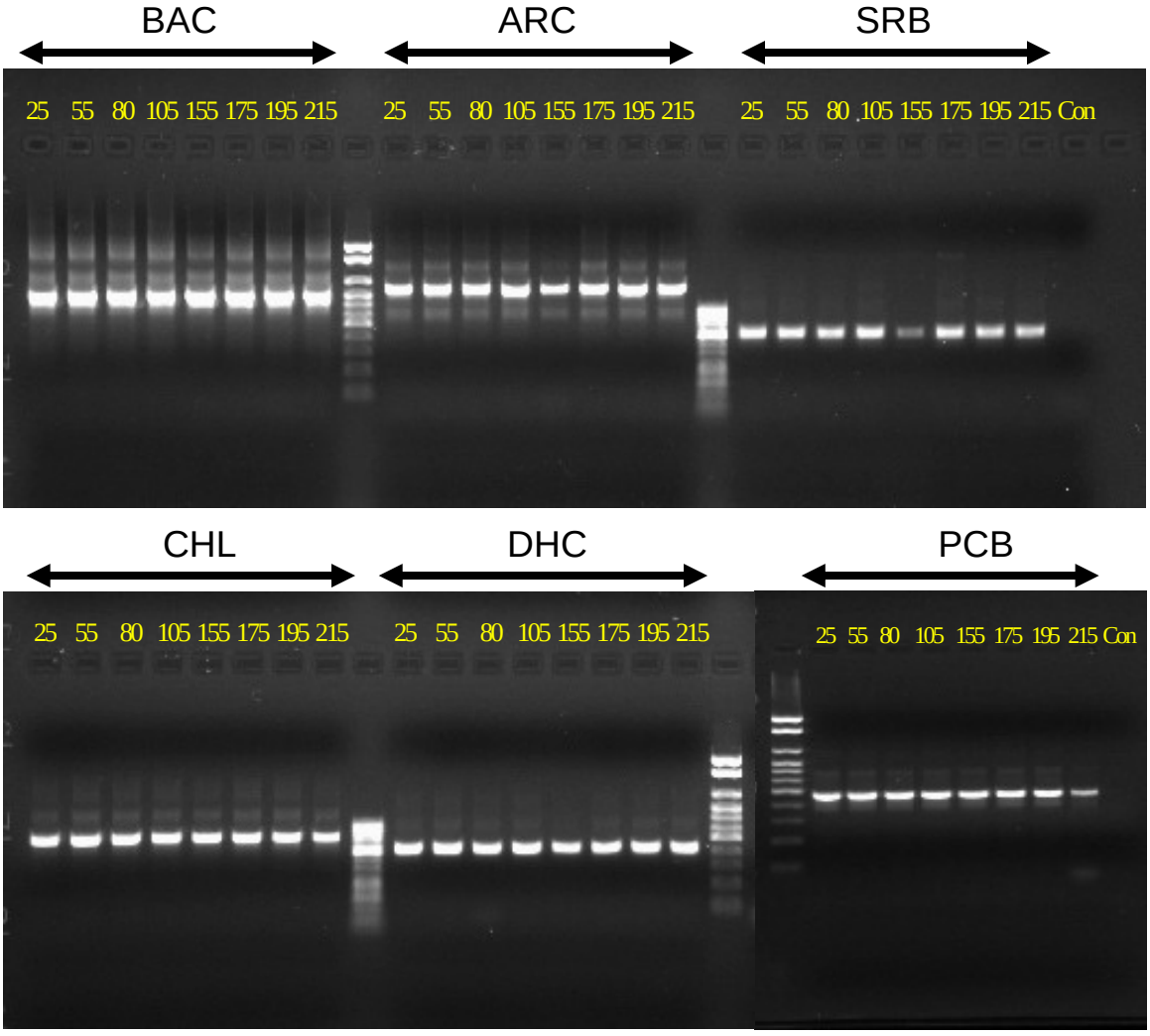


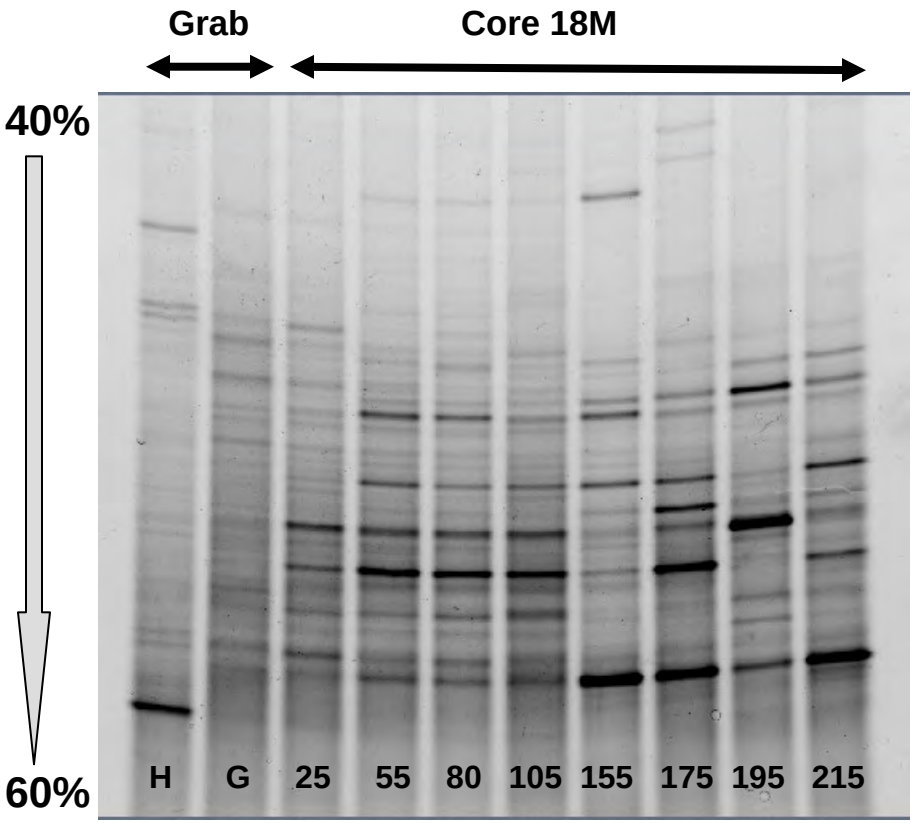
Figure 32. PCR Detections with 6 Group-Specific Primer Sets for the 8 Segments. Segments from the core 18M. 25: 20-25 cm segment, 55: 50-55 cm, 80: 75-80 cm, 105: 100-105 cm, 155: 150-155 cm, 175: 170-175 cm, 195: 190-195 cm, 215: 210-215 cm, Con: negative control.

4.2.5.2 Group-Specific Microbial Community Profiles by DGGE Analyses

Band patterns on DGGE gels represent the community profiles of specific microbial groups. The band patterns among core segments and between the Grasse River and the Hudson River were distinct in the *Bacteria* (**Figure 33**), *Dehalococcoides* (**Figure 34**), and *o-17*/DF-1 (**Figure 35**) groups, but similar in the *Chloroflexi* group (**Figure 36**).

Based on cluster analysis, Hudson River surficial sediment bacteria community and *o-17*/DF-1 community diverged from the entire Grasse River sediment Core 18M, which indicates the effect of site specific geochemical properties on microorganisms and the possible different PCB dechlorination processes in the two rivers. In general, the band patterns between upper (newer) segments representing deposition during the ice jam (20-25 cm, 50-55 cm, 75-80 cm, and 100-105 cm) and most of the middle to deeper segments from 150-155 cm, 170-175 cm, 190-195 cm, and 210-215 cm are different for *Bacteria* and *Dehalococcoides* groups. For *Dehalococcoides* and *o-17*/DF-1 groups, the community in segment 170-175 cm was quite different from other two deeper segments, which might be linked to the low dechlorinating activity observed in this segment. The deeper segments show much darker bands, which may indicate populations present throughout the core were selectively enriched in the deeper segments. This may be due to higher concentrations of PCBs, more highly chlorinated congeners, or longer time for enrichment. Further, bacteria diversity is reduced in all core segments compared with the surficial grab samples from the Grasse River where more aerobic species contribute to the overall bacterial community.

A



B

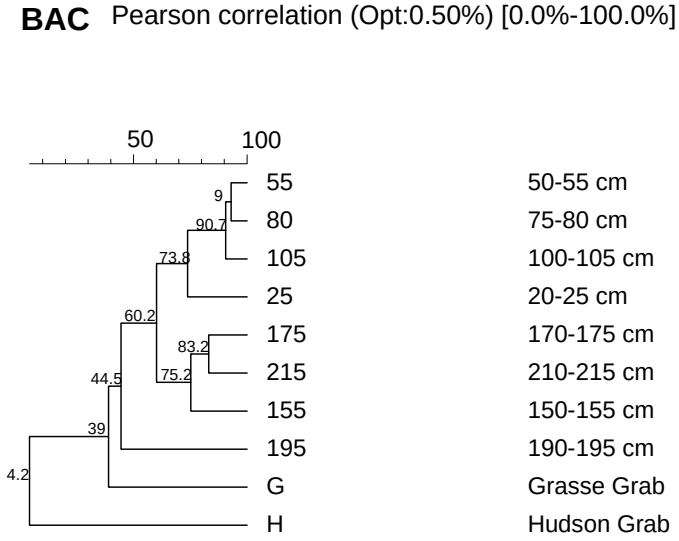


Figure 33. Profile of Bacteria (BAC) by DGGE and Cluster Analysis.
A) DGGE profile of Bacteria (BAC) group for grab and core 18M sediments. The gradient of denaturants was from 40 to 60%. B) Cluster analysis for bacteria in the sediment core. Similarity values are shown at branch nodes.

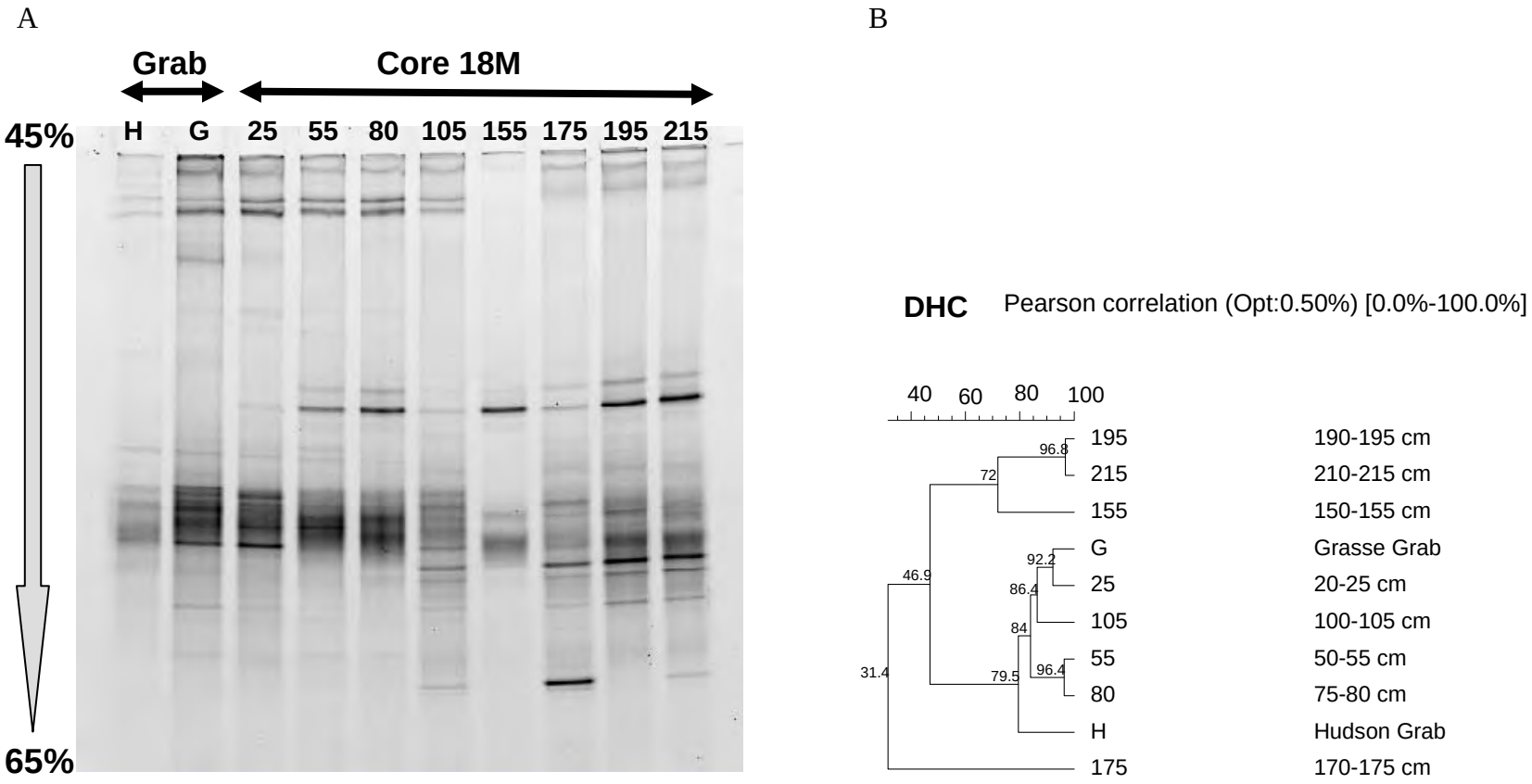


Figure 34. Profiles of Dehalococcoides (DHC) by DGGE and Cluster Analysis.
A) DGGE profile of Dehalococcoides (DHC) group for grab and core 18M sediments. The gradient of denaturants was from 45 to 65%. B) Cluster analysis for Dehalococcoides (DHC) group in the sediment core. Similarity values are shown at branch nodes.

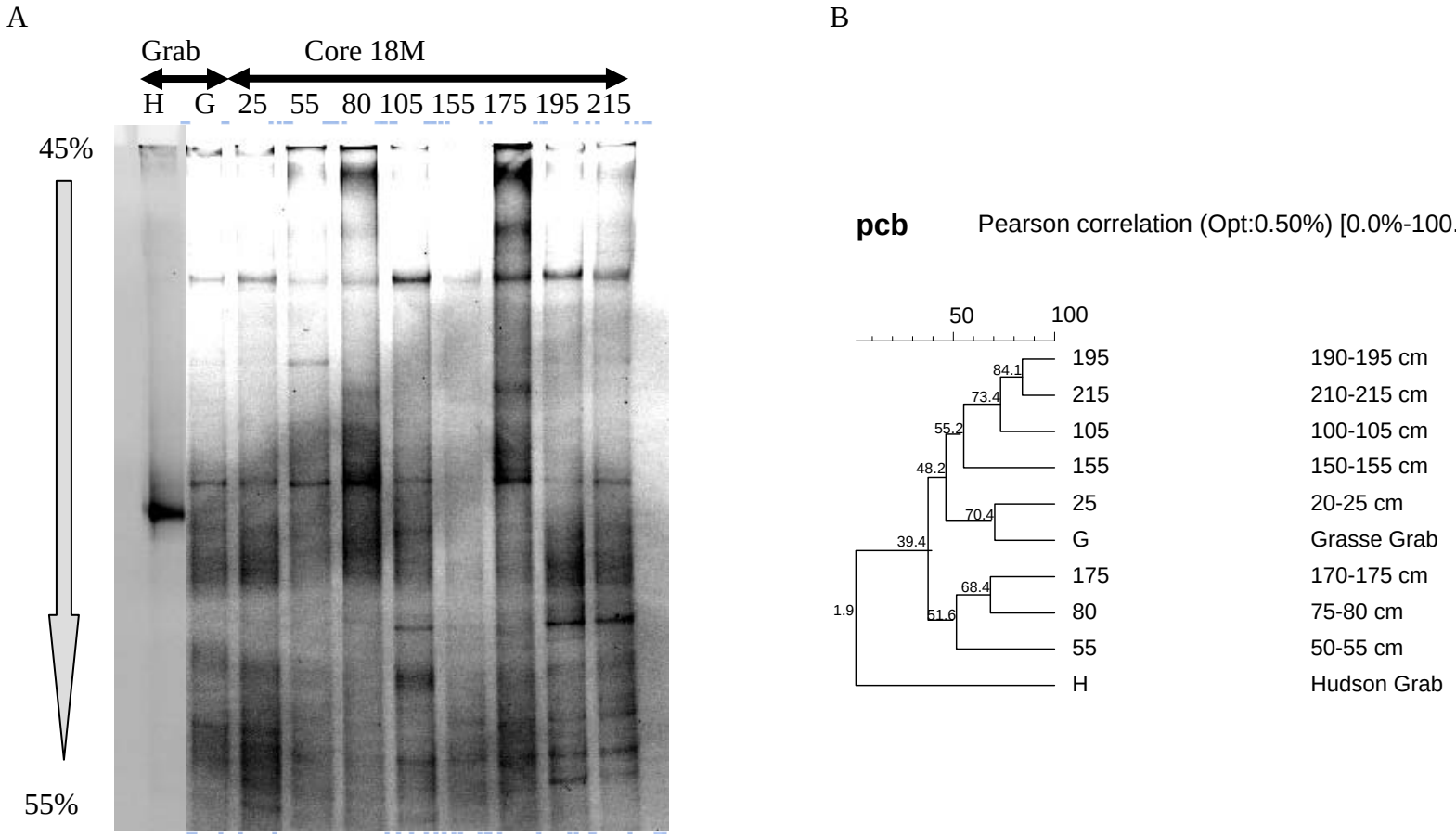
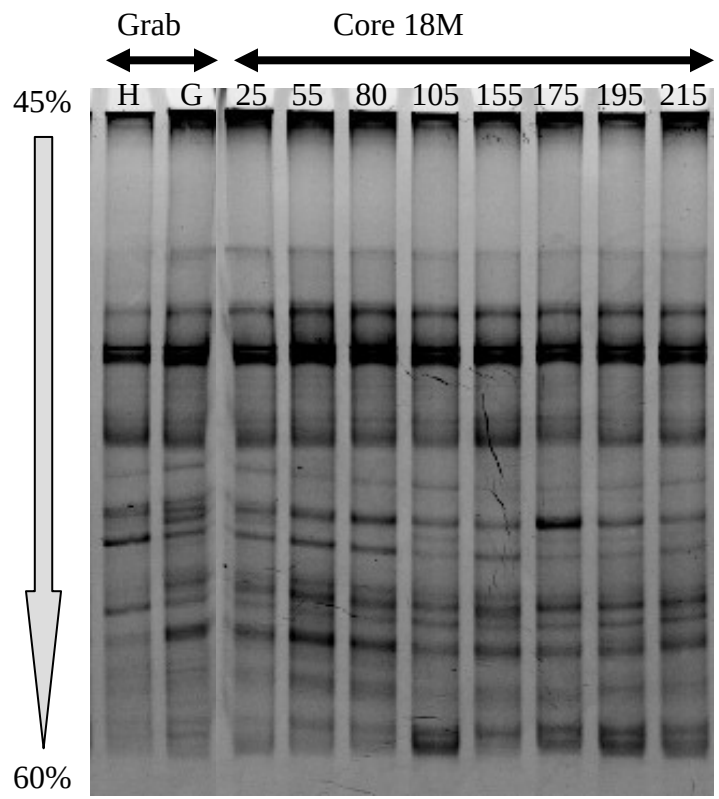


Figure 35. Profiles of o-17/DF-1 by DGGE and Cluster Analysis.
A) DGGE profile of o-17/DF-1 (PCB) group for grab and core 18M sediments. The gradient of denaturants was from 45 to 55%. B) Cluster analysis for o-17/DF-1 (PCB) group in the sediment core. Similarity values are shown at branch nodes.

A



B

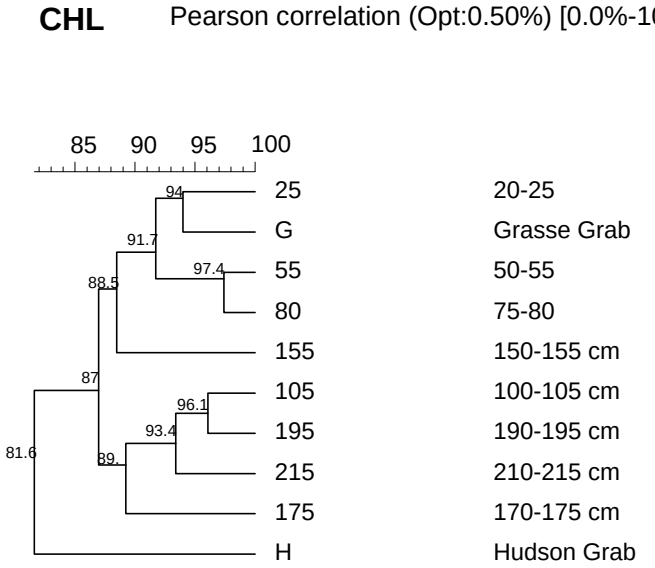


Figure 36. Profiles of Chloroflexi by DGGE and Cluster Analysis.
A) DGGE profile of Chloroflexi (CHL) group for grab and core 18M sediments. The gradient of denaturants was from 45 to 60%. B) Cluster analysis for Chloroflexi (CHL) group in the sediment core. Similarity values are shown at branch nodes.

4.2.5.3 Bacterial Clone Libraries from Two segments of Core 18M and the Grasse River Surficial Sample

As described above, the DGGE results indicate two main different profile patterns in the upper and deeper sediments. The distinct banding patterns are expected since the upper sediments represent recent deposition and the lower sediments represent intact sediment. Congener-specific analysis showed the upper core segment 100-105 cm to have higher MDPR value and a lower total PCB content (14.0 mg/kg dry wt) than intact segments. To better understand the microbial dechlorination potential, segments 100-105 cm, 210-215 cm and surfical sediment were selected for constructing bacterial clone libraries.

The phylum-level community compositions of the two core segments were compared with libraries created from surficial sediment samples from the Grasse River (**Figure 37**). The core segment samples have much higher percentages of *β-proteobacteria* and lower percentages of *Bacteroidetes* than the near surface grab sediment samples. For these sediments (i.e., grab sample from Grasse River and two core sediment samples), the *β-proteobacteria* percentage dramatically increased with depth, while percentages of *Bacteroidetes*, *γ-proteobacteria*, δ-*proteobacteria* and other bacteria decreased with depth.

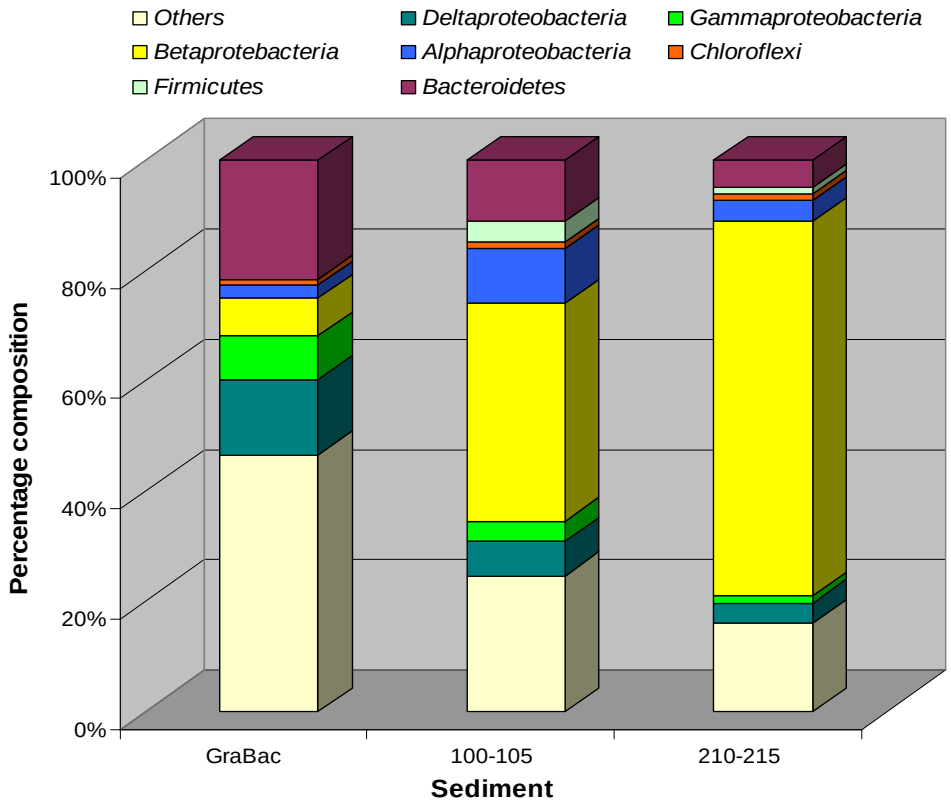


Figure 37. Bacterial Community Compositions.
Results for the surficial grab sediment sample and two core segments. GraBac: Grasse River surficial grab sample; 100-105: segment of 100-105 cm; 210-215: segment of 210-215 cm.

The most similar sequence for each clone of the two core segments and the surficial sediment along with its isolation source was retrieved from the NCBI sequence database (**Table 19**). Approximately 26% for 100-105 cm and 36% for 210-215 cm of clones were genus level similar (> 97%) to clones from soils and sediments with observed dechlorinating activity, while only 2.2% these types of organisms were found in grab sediment. This result shows an evidence of enrichment of dechlorination-related bacteria along the depth of the core, suggesting that PCB dechlorination becomes an increasingly important component of microbial metabolic activity in deeper (older) sediments.

Table 19. Result of the Search for the Most Similar Sequence

	Grasse River Surficial Sediment (88 clones)				
	>99%	>98%	>97%	>95%	Sum
Chlorinated-solvent contaminated site or Microcosm		1	1	2	4
Sum		1 1.1%	1 1.1%	2	4 4.5%
Isolation Source of the most similar sequences	100-105 cm (81 clones)				
	>99%	>98%	>97%	>95%	Sum
Microcosm (PCB)		1	4	1	6
PCE contaminated river sediment	7	4	3	5	19
Microcosm (Dioxin)				3	3
Chlorinated compound contaminated soil	1		1		2
Sum	8 9.9%	5 6.2%	8 9.9%	9	30 37%
	210-215 cm (81 clones)				
	>99%	>98%	>97%	>95%	Sum
Microcosm (PCB)		1	3		4
PCE contaminated river sediment	1	2	3		6
Microcosm (Dioxin)				1	1
Chlorinated compound contaminated soil	18	1			19
Sum	19 23.5%	4 4.9%	6 7.4%	1	30 37%

Note: Result of the Search for the Most Similar Sequence **related to dechlorinating activity to each clone of the bacterial clone libraries for the core and surficial sediments.**

Notably, each clone library contained one species in the group *Chloroflexi*. The *Chloroflexi* clones in the three Grasse River sediment samples we considered were more related to each other (group III shown on **Figure 38**) than to putative dechlorinating *Chloroflexi* in groups I (*Dehalococcoides*) or II (DF-1 and *o-17*).

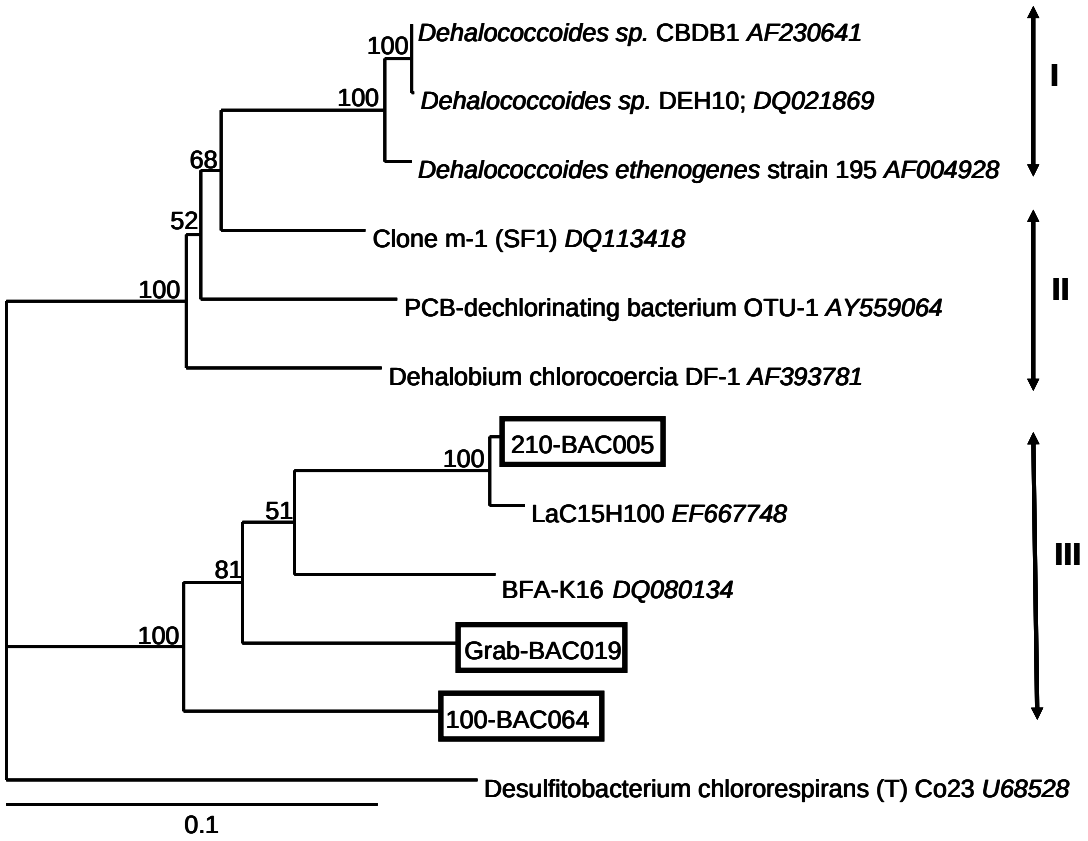


Figure 38. Phylogenetic Tree Constructed by Maximum Parsimony Analysis.
Tree shows the relationship of 3 representative clones Gra-BAC019, 100-BAC064, 210-BAC005 associated with a Grass River sediment grab and 18M core sediment samples in 100-105 cm and 210-215 cm segments to 16S rRNA gene sequences of previously described bacteria. Bootstrap values at branch nodes indicated the percentage (After 1000 bootstrap replicates, bootstraps >50% are shown). The scale bar indicates 10% estimated sequence divergence. The outgroup is Desulfitobacterium chlororespirans (T) Co23 (U68528) I: Dehalococcoides strains; II: o-17/DF1 clade; III: Chloroflexi clones in the present study and their closest clones obtained in other dechlorination studies. Group I and Group II cover the putative dechlorinating Chloroflexi. Group I has typical Dehalococcoides strains CBDB1, DEH10 and D. ethenogenes strain 195. Group II represents o-17/DF1 clade (Clone m-1 sequence is partially identical to SF1 and OTU-1 is greater than 99% identical to o-17) (Bedard 2008).

4.2.5.4 Quantitative PCR for Group Specific Enumeration

Many earlier investigations have shown that dechlorination activities are related to the concentration of putative dechlorinating microorganisms. Thus, enumeration of bacterial

populations associated with PCB dechlorination is essential to evaluate the potential of dechlorination in the fields. Q-PCR is a sensitive method that could quantify target genes as low as 2 copies per microliter. Here, the 16S rRNA gene (rDNA) copy amounts per gram of dry sediment for *Bacteria* (BAC), *Chloroflexi* (CHL), *Dehalococcoides* (DHC), and *o-17*/DF-1 group (pcb) in core 18M sediment samples are shown in **Figure 39**. Populations of likely dechlorinating organisms are found throughout the core. For all groups, coarser sand segments (145-155 cm) contained smaller populations than more clayey segments. The populations of two sulfate reducing bacteria *Desulfobacterales* (DSB), *Desulfuromonales* (DSM) were 1 to 2 orders of magnitude higher than *Chloroflexi* , while *Desulfovibrionales* (DSV) were comparable to *Chloroflexi* (Data not shown).

Chloroflexi specific 16s rRNA genes were from $1.7\times10^7 \pm 2.1\times10^6$ to $2.9\times10^8 \pm 4.3\times10^7$ copies/g dry wt in the core. In the surficial sediment sample, gene copy number determined in the present study ($2.1\times10^8 \pm 2.6\times10^7$ copies/g dry wt) is very close to the previous study ($3.0\times10^8 \pm 4.0\times10^7$ copies/g dry wt) in the same river but at different sampling sites (Kjellerup et al. 2008). Unlike *Bacteria*, the trend of *Chloroflexi* (16S rRNA gene copies was not clear (**Figure 39b**)). Furthermore, *Chloroflexi* populations were not correlated with degree of chlorination, total PCB content or TOC in the core, which indicates that *Chloroflexi* populations in this core were not limited by the amount or the composition of their putative target electron acceptors-PCBs or electron donors-TOC. Therefore, no conclusion could be drawn directly from the enumeration of *Chloroflexi* to the potential dechlorination activities.

Seen in **Figure 39c** and **Figure 24a**, the trend of *Dehalococcoides* 16S rRNA gene copies along the entire sediment core were consistent with the changes in MDPR. Excluding the anomalous segment 150-155 cm (PCBs are much higher than other neighbor segments), significant Pearson Correlation was obtained between the MDPRs and log DHC copies in the core (r=0.595, p=0.041). More interestingly, MDPRs were well correlated with log DHC copies (r=0861, p=0.001) from segments with similar particle contents (segments except for 145-150 cm and 150-155 cm). Segments with higher MDPR (indicating greater dechlorination) contained higher numbers of *Dehalococcoides* genes than segments with lower MDPRs in this sediment core. As an example, segment 100-105 cm had a higher MDPR value and contained 2.5 times the number of *Dehalococcoides* but much lower *o-17*/DF-1 or *Chloroflexi* than deeper segment 210-215 cm. This correlation may suggest that the population of *Dehalococcoides* plays an important role on the dechlorination degree in this sediment core.

Bacteria gene copies were approximately two orders of magnitude higher than *Chloroflexi* genes and *Chloroflexi* gene copies were 1-2 orders higher than *Dehalococcoides* (ranged from $2.3\times10^4 \pm 1.5\times10^3$ to $4.7\times10^6 \pm 2.1\times10^5$copies/g dry wt in the core and $7.5\times10^6 \pm 1.4\times10^5$copies/g dry wt in surficial sediments), and *o-17*/DF-1 group (ranged from $6.9\times10^4 \pm 5.0\times10^3$ to $9.6\times10^6 \pm 5.6\times10^5$copies/g dry wt in the core and $1.1\times10^7 \pm 8.3\times10^5$copies/g dry wt in surficial sediments), which indicates that *Dehalococcoides*, and *o-17*/DF-1 group were not the dominant populations in the *Chloroflexi* communities. The results are consistent with the clone library results, where only 1 *Chloroflexi* clone not closely related to *Dehalococcoides*, and *o-17*/DF-1 strains was detected in each library. However, *Chloroflexi* primer set applied in this study was designed to target both *Dehalococcoides*, and *o-17*/DF-1 strains, the discrepancy might be attributed to primer efficiency and more probably primer specificity. Further studies on suitable primers for dechlorinating microorganism are necessary.

Kjellerup et al. 2008 report no detection of organisms related to *o-17*/DF-1 in their Grasse River surficial sediment sample, although they did observe other organisms related to *Dehalococcoides*. We observe both groups through the core samples, with the *o-17*/DF-1 group outnumbering the *Dehalococcoides* in most segments. This difference may be due to differences in total PCBs (much higher than in Kjellerup's sample), differences in populations at different locations in the river, or due to selective enrichment of the *o-17*/DF-1 organisms in the sediment core with high concentration of *ortho*-substituted PCBs in the present work.

In the entire sediment core, *Dehalococcoides* populations and were significantly correlated to both total PCBs and TOC, while *o*-17/DF-1 populations were correlated with TOC, which suggests that enrichment is closely linked to available substrates (see 20). The findings were derived from Pearson correlation analysis among log copy numbers of bacterial groups and geochemical properties in Core 18 M (**Table 20**). Very interestingly, all bacterial groups quantified in the present study were significant correlated with each other ($p<0.005$), indicating possible consortium-effects in dechlorination. Bacterial groups were not correlated with inorganic properties, including sulfate and nitrate.

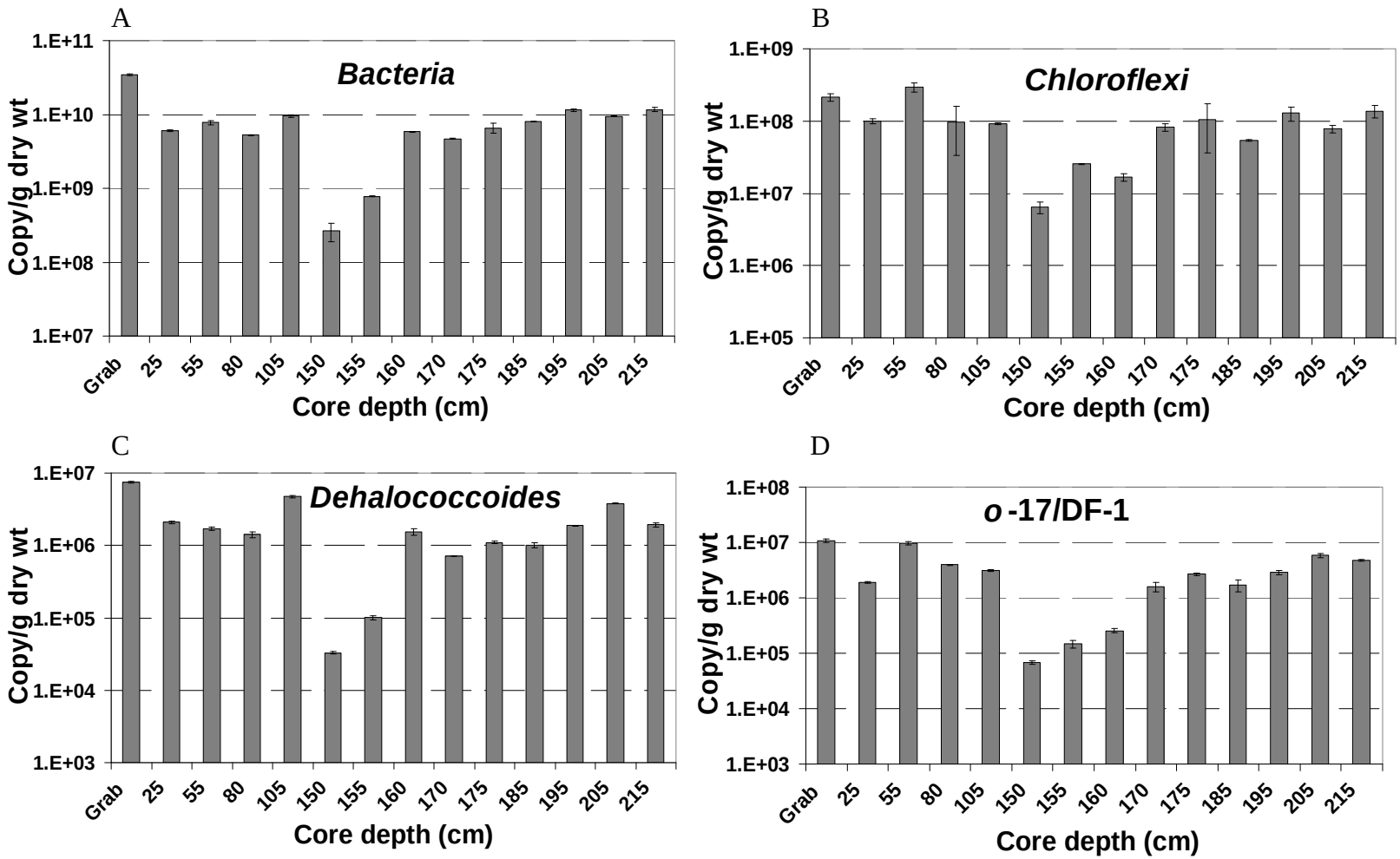
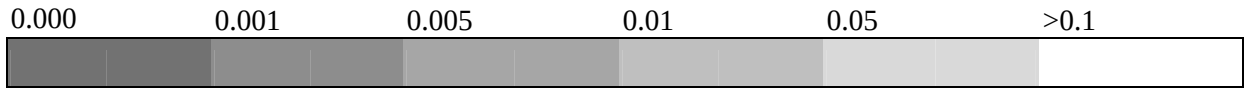


Figure 39. Quantitative Assessment of Bacterial Populations in Core 18M. Populations of A) Bacteria, B) Chloroflexi, C) Dehalococcoides, D) o-17/DF-1 related populations.

Table 20. Pearson Correlations Among Bacterial Groups and Geochemical Properties (18M)

	BAC	CHL	DSB	DSM	DSV	DHC	PCB	Cl^-	NO_2^-	Br^-	NO_3^-	SO_4^{2-}	F^-	PO_4^{3-}	PCBs	TOC
CHL	0.813															
DSB	0.913	0.783														
DSM	0.972	0.769	0.934													
DSV	0.953	0.767	0.930	0.910												
DHC	0.960	0.765	0..913	0.979	0.915											
pcb	0.870	0.947	0.886	0.863	0.857	0.850										
Cl^-	0.425	0.647	0.344	0.321	0.458	0.341	0.586									
NO_2^-	0.364	0.527	0.431	0.364	0.306	0.267	0.436	0.330								
Br^-	0.552	0.333	0.769	0.621	0.584	0.559	0.505	0.007	0.319							
NO_3^-	0.225	-0.009	-0.036	0.113	0.263	0.085	-0.029	0.155	-0.025	-0.324						
SO_4^{2-}	0.245	0.519	0.045	0.210	0.158	0.222	0.459	0.624	0.090	-0.407	0.254					
F^-	-0.407	-0.570	-0.516	-0.484	-0.412	-0.467	-0.611	-0.241	-0.111	-0.136	0.206	-0.096				
PO_4^{3-}	0.468	0.404	0.583	0.529	0.339	0.449	0.401	-0.095	0.620	0.701	-0.385	-0.291	-0.023			
PCBs	0.171	0.083	0.346	0.194	0.348	0.232	0.250	0.292	-0.287	0.426	-0.173	-0.113	-0.317	-0.240		
TOC	0.500	0.302	0.569	0.404	0.689	0.399	0.405	0.317	-0.095	0.353	0.198	-0.075	-0.238	-0.119	0.617	
MDPR	0.089	-0.018	0.077	0.108	0.125	0.284	0..030	0.137	-0.393	-0.005	-0.239	0.009	-0.088	-0.177	0.474	0.126

Significance level



4.3 Task B. PCB Model Development

The field of PCB modeling has extensive prior work, but critical challenges remain. Some of these are best addressed through analysis of sediment samples (such as those described above) and some are best addressed through laboratory experiments (such as those described below). However, many of the challenges in modeling PCBs will be addressed through development of new data management and modeling techniques. Development of these critical new techniques has been the focus of Task B.

4.3.1 Classification Trees for the Identification of Missing Pathways in Dechlorination Processes

The application of classification trees to sets of explicitly reported pathways in dechlorination processes aimed to identify pathways missing from these processes due to analytical limitations. The classification trees for Processes H, H', LP, M, N, P, Q and T appear in **Figure 40** through **Figure 47**, respectively (figures reproduced from Hughes et al. 2010). The numbers in these figures are arranged by homolog and correspond to congener structures assigned in the original work of Ballschmiter and Zell (1980) with corrections to congener numbers 199-201 by Schulte and Malisch (1983) and corrections to numbers 107-109 by Guitart et al. (1993). Nodes of the tree appear in three shapes. Rectangles and the numbers below them depict criteria (for binomial criteria values of zero and one are negative and positive responses, respectively). For example, in CTDPG H (**Figure 40**) the root node criterion is greater than zero flanked *para* chlorine atoms on the parent congener of the dechlorination pathway. If this is true for a given pathway, then one proceeds to the criteria down and to the right. If this is false, then one moves down and to the left, where an oval node indicates that the given dechlorination pathway does not belong in CTDPG H. A pathway is considered to belong in CTDPG H if its ability to meet criteria leads it to a diamond shaped node.

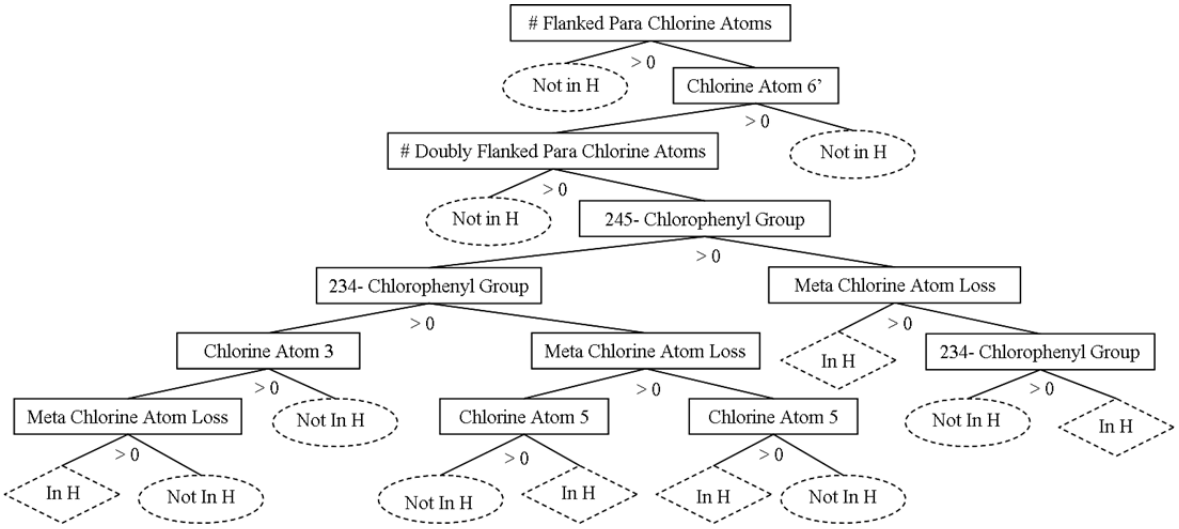


Figure 40. Process CTDPG H (Hughes et al. 2010).

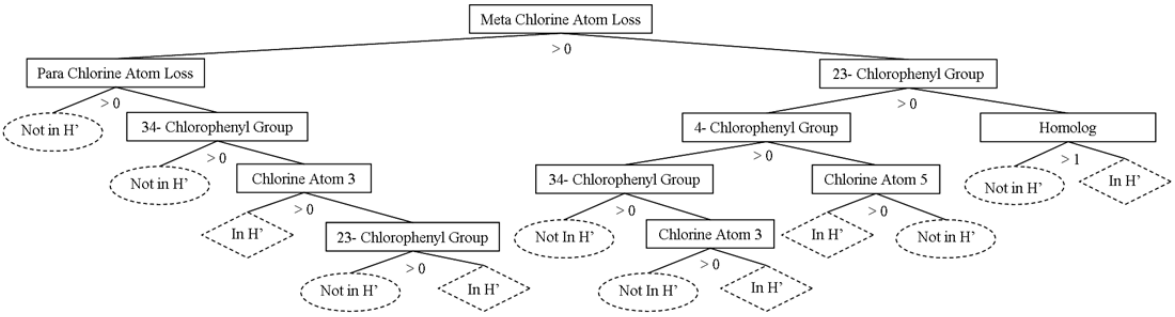


Figure 41. Process CTDPG H' (Hughes et al. 2010).

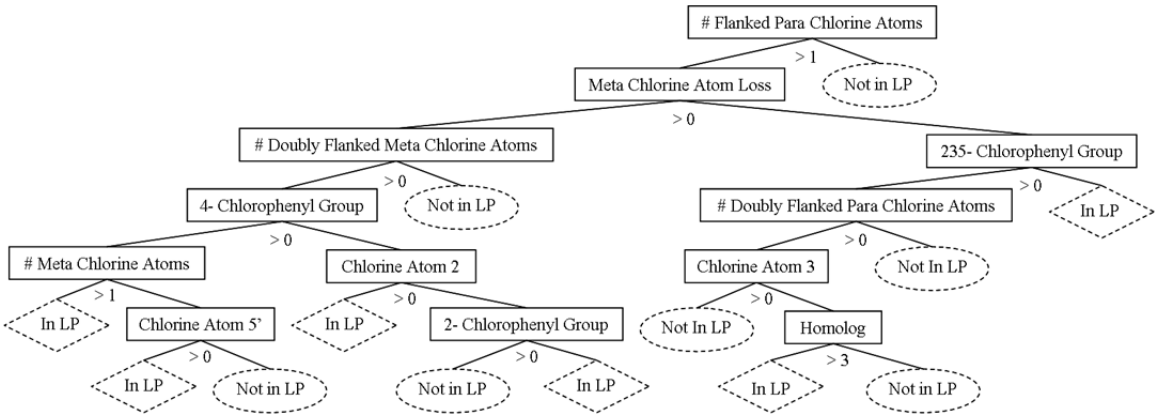


Figure 42. Process CTDPG LP (Hughes et al. 2010).

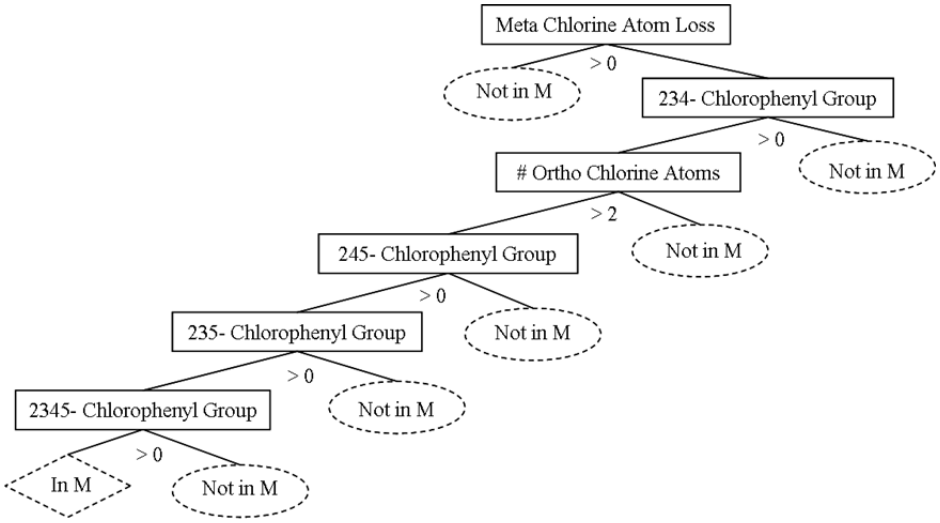


Figure 43. Process CTDPG M (Hughes et al. 2010).

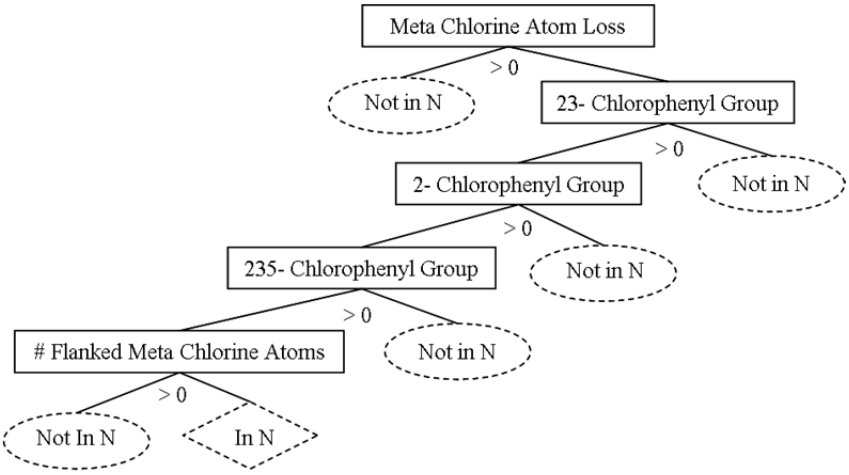


Figure 44. Process CTDPG N (Hughes et al. 2010).

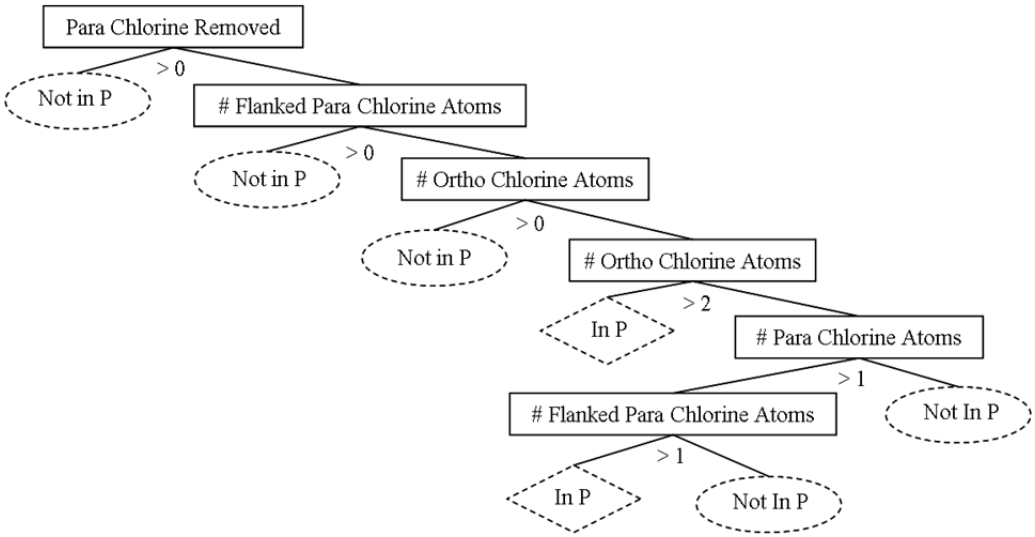


Figure 45. Process CTDPG P (Hughes et al. 2010).

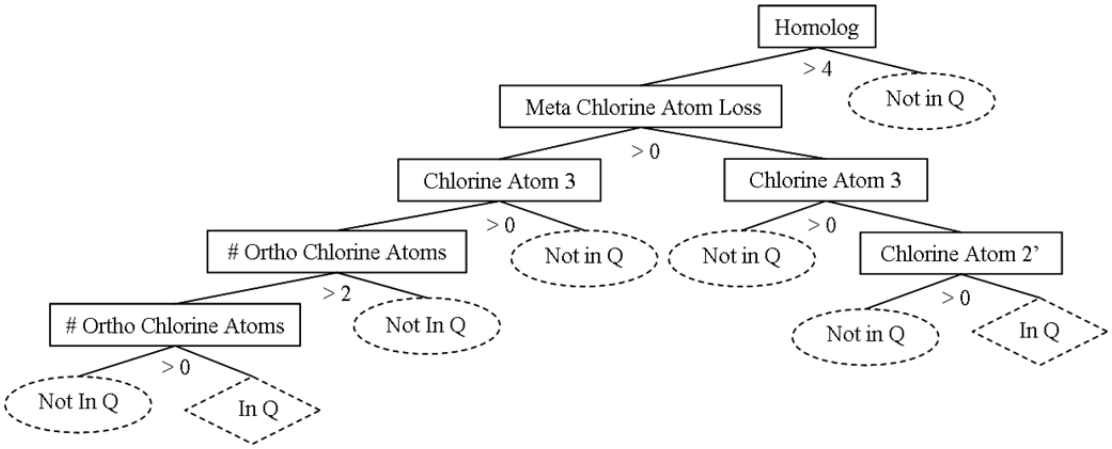


Figure 46. Process CTDPG Q (Hughes et al. 2010).

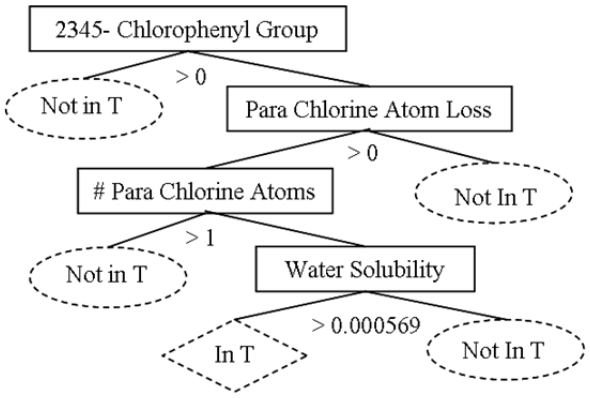


Figure 47. Process CTDPG T (Hughes et al. 2010).

The ability of each of the eight trees to identify missing pathways was determined using ten-fold cross validation. The most important validation measurement was the true classification rate, which imparts the rate at which the tree is able to correctly classify explicitly reported pathways. True classification rates of one indicate perfect performance. Four of the eight classification trees (Processes H, M P and T) achieved perfect true classification rates (**Figure 48**). True positive rates for processes H, H', LP and Q classification trees were greater than 0.86. Additionally, classification trees should have a small size, defined by the number of nodes in the tree. **Figure 48** illustrates adequately small tree sizes achieved by the eight classification trees.

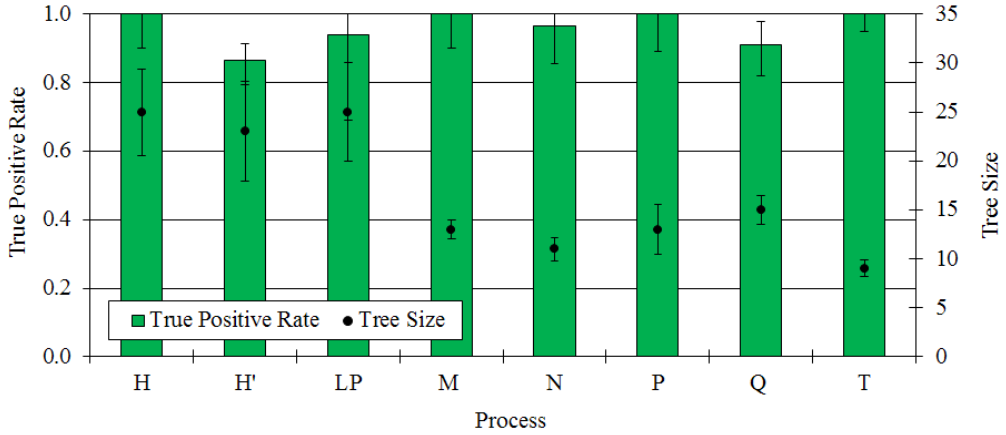


Figure 48. Classification Tree Results for Eight Dechlorination Processes.
True positive rates and tree sizes of classification trees are presented in the figure for the eight dechlorination processes listed along the x-axis.

CTDPGs include the explicitly reported pathways, as well as pathways predicted to belong in the dechlorination process by the classification tree. Pathways predicted to belong in each of the processes by the classification trees are illustrated in **Figure 49** through **Figure 56** (figures reproduced from Hughes et al. 2010). **Table 21** displays the number of pathways explicitly reported and included in each CTDPG. On average, the number of pathways in the CTDPG is five times greater than the number of explicitly reported pathways.

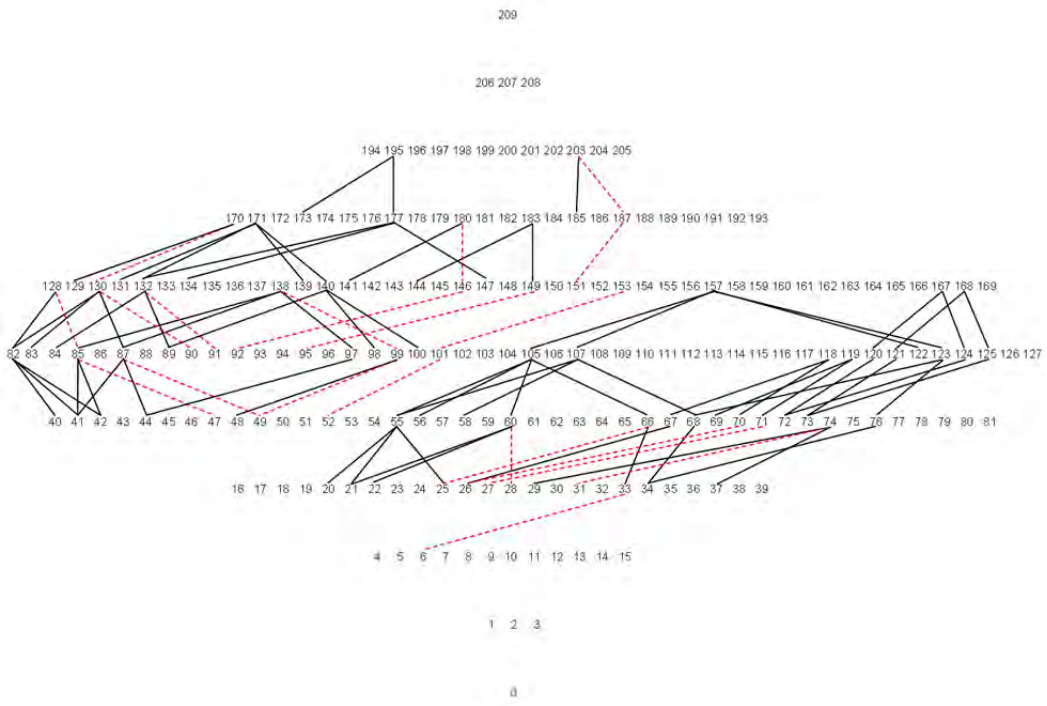


Figure 49. Explicitly reported pathways, Process H.
The DP (dashed lines) and pathways added to dechlorination Process H through the CT analysis (solid lines) (Hughes et al. 2010).

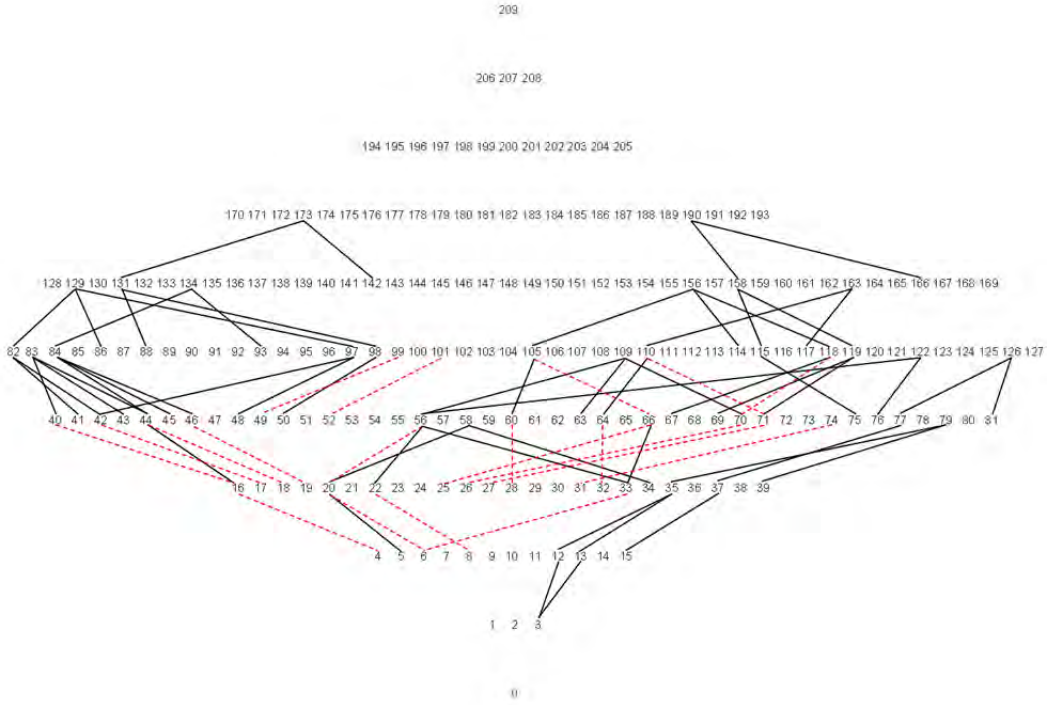


Figure 50. Explicitly reported pathways, Process H'.
The DP (dashed lines) and pathways added to dechlorination Process H' through the CT analysis (solid lines) (Hughes et al. 2010).

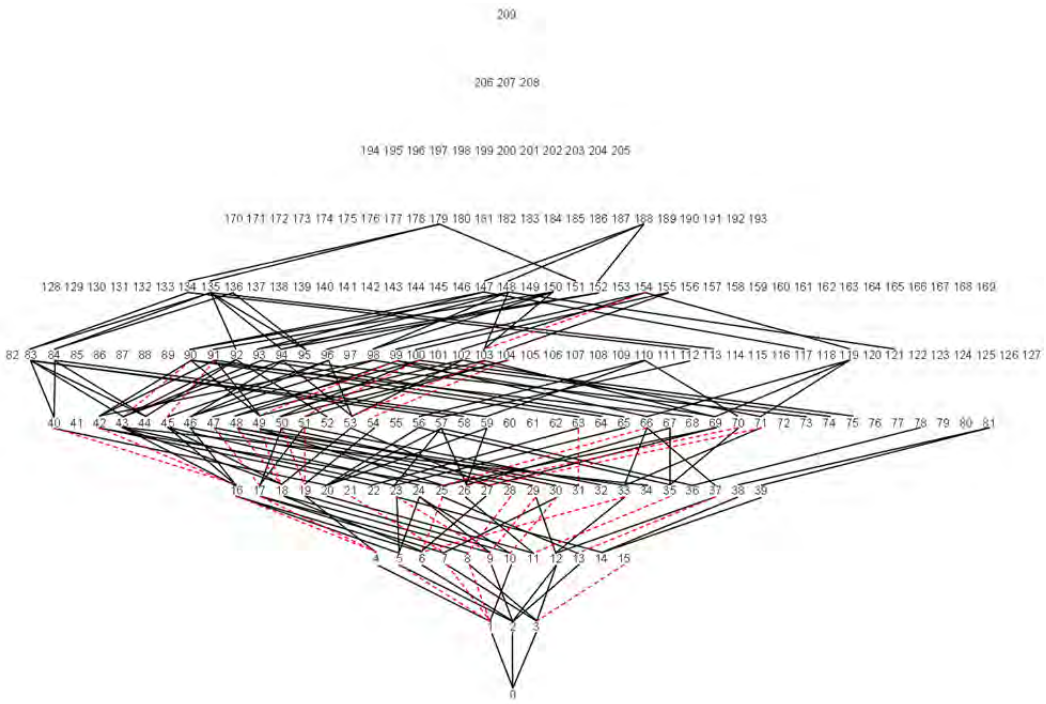


Figure 51. Explicitly reported pathways, Process LP.
The DP (dashed lines) and pathways added to dechlorination Process LP through the CT analysis (solid lines) (Hughes et al. 2010).

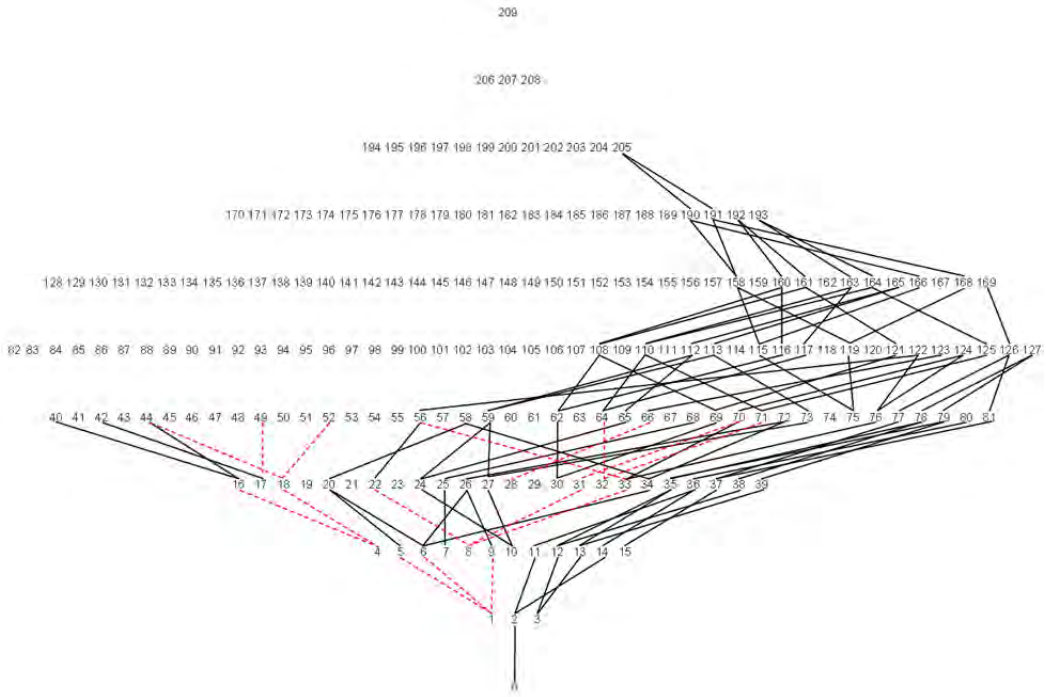


Figure 52. Explicitly reported pathways, Process M.
The DP (dashed lines) and pathways added to dechlorination Process M through the CT analysis (solid lines) (Hughes et al. 2010).

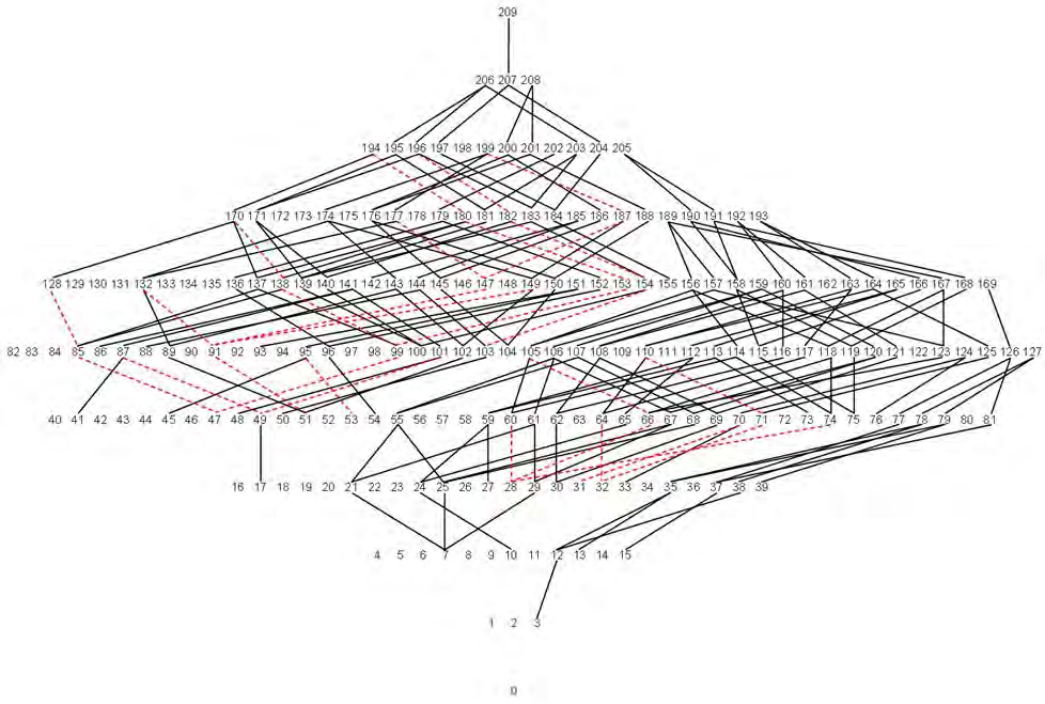


Figure 53. Explicitly reported pathways, Process N.
The DP (dashed lines) and pathways added to dechlorination Process N through the CT analysis (solid lines) (Hughes et al. 2010).

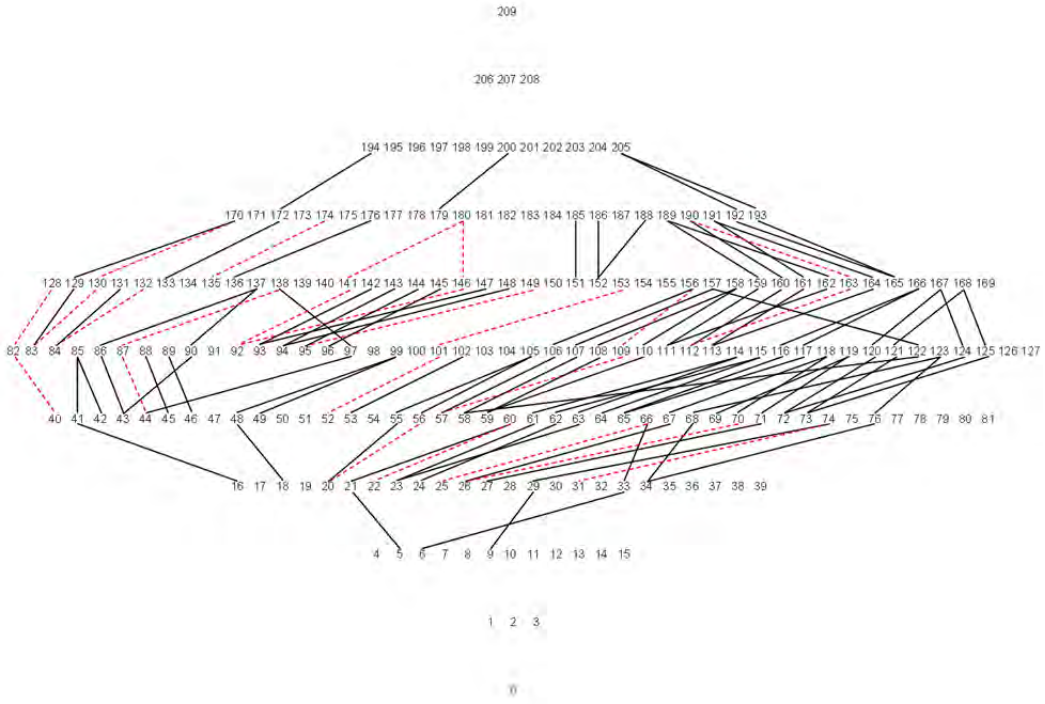


Figure 54. Explicitly reported pathways, Process P.
The DP (dashed lines) and pathways added to dechlorination Process P through the CT analysis (solid lines) (Hughes et al. 2010).

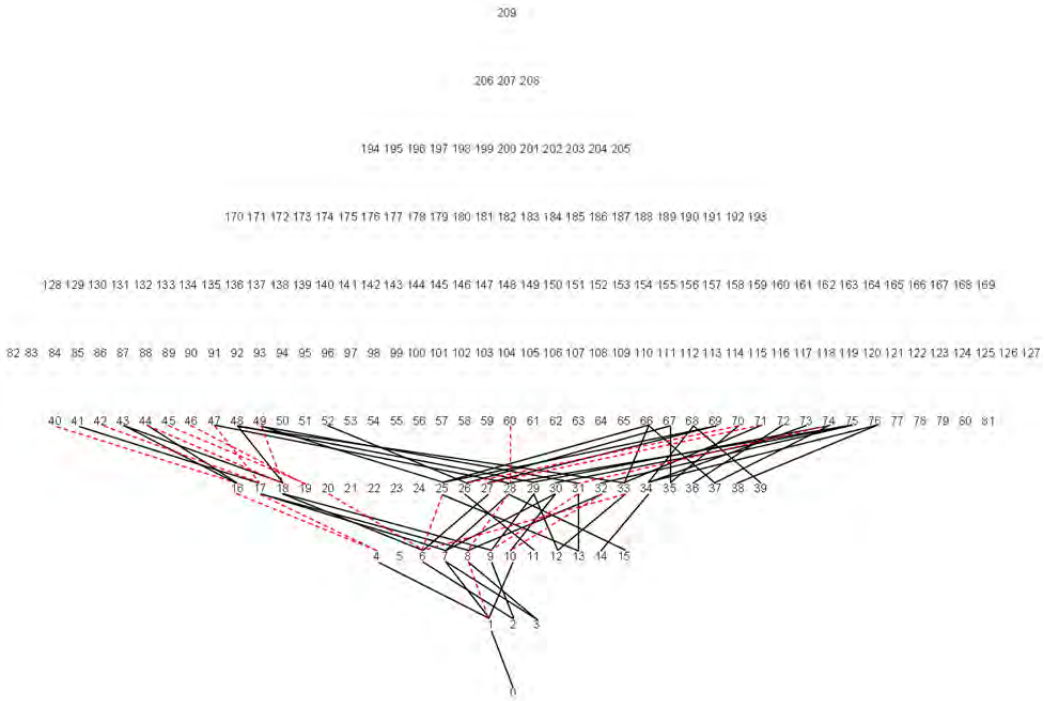


Figure 55. Explicitly reported pathways, Process Q.
The DP (dashed lines) and pathways added to dechlorination Process Q through the CT analysis (solid lines) (Hughes et al. 2010).

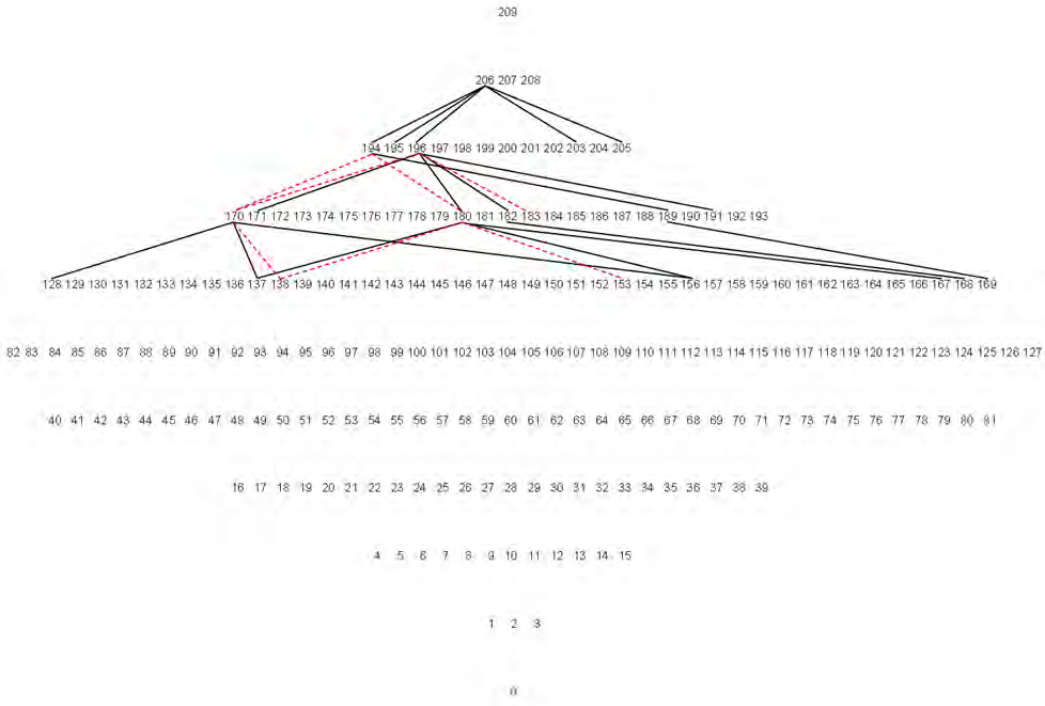


Figure 56. Explicitly reported pathways, Process T.
The DP (dashed lines) and pathways added to dechlorination Process T through the CT analysis (solid lines) (Hughes et al. 2010).

Table 21. Comparison of the Number of Pathways that were Explicitly Reported, and Appear in CTDPG

Dechlorination Process	H	H'	LP	M	N	P	Q	T	All
Explicitly Reported	22	22	33	17	29	28	22	6	108
CTDPG	91	76	199	108	204	117	76	25	594

Also note that the creation of classification trees (and subsequent results) shown for Processes M and Q excluded and included homolog-like attributes, respectively. Because the true homolog range of these Processes was limited by a paucity of dechlorination studies, two classification trees were created for Processes M and Q. The first tree was created using all forty-six properties of the pathway or parent congener in the pathway. The second tree did not include pathways directly correlated with homolog, such as vapor pressure, and water solubility.

For Process M, the classification tree in which homolog-like attributes were excluded performed better (e.g. in terms of true positive rate and tree size) than the classification tree created using homolog-like attributes. Therefore, the former classification tree was adopted and it was concluded that the homolog range of Process M likely ranges from homolog eight down to biphenyl. Alternately, the classification tree created with all forty-six properties outperformed the classification tree created without homolog-like properties for Process Q. Thus, the homolog range for this process is most likely no greater than four, as reported based on a small number of laboratory experiments and environmental observations.

Finally, the classification tree method was applied to a recent report of dechlorination by *Dehalococcoides sp*. Strain CBDB1. The study's authors reported observed dechlorination patterns that "matches PCB dechlorination Process H" (Adrian et al. 2009). However, a classification tree that was created using the 35 reported pathways (**Figure 57**) did not closely resemble the aforementioned classification tree for Process H (figure reproduced from Hughes et al. 2010). Furthermore, only 25 of these 35 reported pathways are included in CTDPG H, which has 91 dechlorination pathways. Thus, the classification tree method suggests that strain CBDB1 is likely not *the* microorganism responsible for Process H. Rather, CBDB1 is more likely closely related to one or more of the responsible microorganisms and/or shares some dechlorination pathways with the responsible microorganism(s) (Hughes et al. 2010).

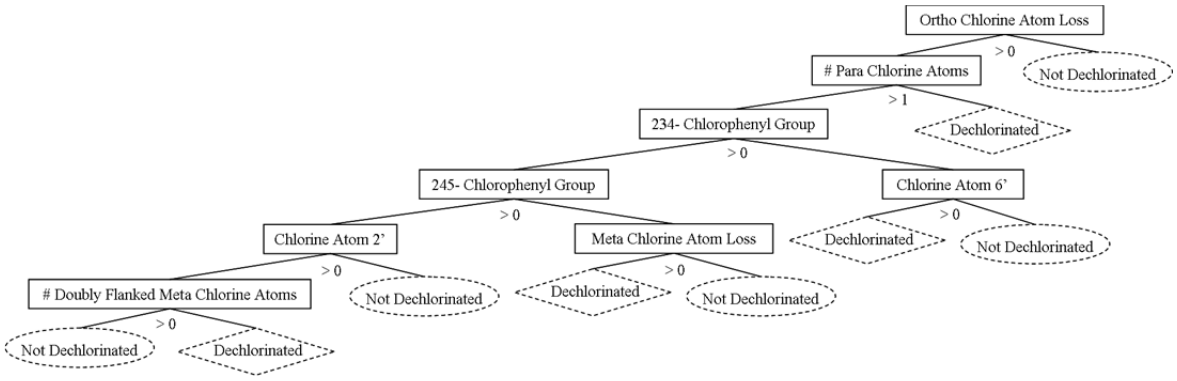


Figure 57. Classification tree for pathways degraded by microorganism CBDB1. The classification tree was created using all forty-six properties of the pathway or parent congener in the pathway and pathways reported to be dechlorinated by Dehalococcoides sp. Strain CBDB1 (Hughes et al. 2010).

4.3.1.1 Bayes Monte Carlo (BMC) for Process Occurrence

The DPE described in the methods section aims to statistically identify the occurrence of CTDPGs, whose generation was discussed in the previous section. [2] Four important modeling consequences of applying the BMC approach to PCB dechlorination are (1) quantification of expert knowledge in Bayesian prior distributions, (2) an initial congener distribution that is not limited to an Aroclor, (3) all pathways in an active dechlorination process are not assumed to be occurring, and (4) all 840 dechlorination pathways and all congeners are simulated.

Statistically rigorous identification of occurring dechlorination processes will enable comparisons of PCB dechlorination occurring across sediments and laboratory treatments. Because one or a group of microorganisms is believed to be responsible for each dechlorination process, such comparisons may aid in the identification of the responsible microorganisms. Further, dechlorination reported in the literature (e.g., Bedard et al. 1996; Fagervold et al. 2007) often similar to, but never exactly like the explicitly reported pathways in dechlorination processes. Thereby indicating the importance of sediment conditions in directing microbially mediated dechlorination (likely via multiple dehalogenases) and suggesting the need for a dechlorination model that is capable of accounting for these variations in pathway occurrence within dechlorination processes.

Note that the DPE is designed for applications to controlled laboratory experiments, in which the initial and final congener concentrations of all 209 congeners are known or can be reasonably represented by a statistical distribution and dechlorination, rather than transport, is the only major process that can explain differences in congener concentrations over time.

The DPE was validated using sixteen *synthetically generated* initial and final congener distributions in which the occurring dechlorination process was known. Synthetic scenarios have

[2] The BMC model work forms the basis for a paper planned for submission in 2011.

initial congener distributions of Aroclor 1242 or 1260. Aroclors were then dechlorinated according to one, two or all CTDPGs simultaneously occurring. Synthetic scenarios are listed in the first column of **Table 21**. The first five characters of each scenario represent the initial Aroclor distribution. The following characters describe which dechlorination process is occurring. For example, A1242P describes a scenario in which Aroclor 1242 was synthetically dechlorinated by pathways in CTDPG P, which transferred random percent weights of the parent congener to the daughter congener.

Generally, successful applications of the BMC method can be indicated in two ways. For scenarios in which none of the posterior calculated congener distributions is exactly or nearly the same as the measured final congener distribution, then it is preferable for several of the Monte Carlo simulations to receive significant weight (here we arbitrarily focus on weights greater than 0.01). This is the case for scenario A1242P, as shown in **Figure 58**. However, if one or more calculated posterior final congener distribution exactly or very closely matches the measured congener final distribution, then a single highly weighted simulation or many similarly weighted scenarios is acceptable. The latter is true for scenario A1242T, which had 85 simulations receiving a weight greater than 0.01.

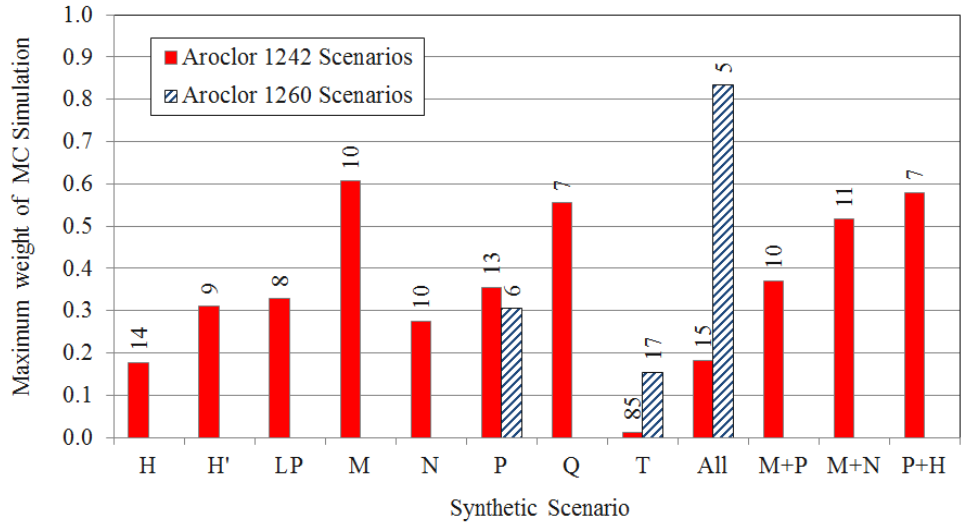


Figure 58. MC Simulation Statistics for Synthetic Scenarios.
Scenarios describing the dechlorination of Aroclor 1242 and 1260 are shown. Numbers above the bars represent the number of simulations receiving a weight greater than 0.01 in the given scenario (Hughes et al. 2011).

Instances in which the DPE identifies the occurrence of only the known dechlorination process(es) indicate perfect model performance (represented by a value of one).

The sixteen synthetic scenarios and the DPE's ability to identify the known occurring process(es) are shown in Error! Reference source not found.. In this table, synthetic **validation scenarios are given in the first column and posterior mean and standard deviations generated by the DPE for each CTDPG are given across the first row.** Missing values in the table are 0.0000 ± 0.0000 reflect perfect identification of a non-occurring CTDPG. Bold values reflect perfect identification of an occurring CTDPG and shading indicates perfect or near perfect CTDPG occurrence identification. Italicized values do not trend correctly.

The DPE performed perfectly in four of the sixteen instances. Near perfect performance was achieved in five of the remaining 14 scenarios (note that the reported posterior probabilities are shifts from uninformed prior probabilities of 0.5 for the occurrence of each process). Adequate performance was achieved by all but two of the scenarios: A1242M+P and A1260All (in which all processes occurred simultaneously). The roots of process misidentification in these scenarios are twofold. First, the number of congeners dechlorinated by pathways in the occurring process is small or equal to zero. For example, the DPE correctly identifies the "no dechlorination" state for scenario A1242T because congeners present in Aroclor 1242 are not dechlorinated by Process T. The second cause of process misidentification is a large number of overlapping pathways in processes. For example, Processes H and P share greater than half of their pathways with each other. For A1260All, Process H' has the greatest number of overlapping pathways with the eight other processes. Thus, DPE results must be interpreted in light of shared pathways in processes as well as pathways overlapping with congeners in the initial congener distribution.

An additional investigation into the effect of the number of pathways included in the synthetic dechlorination was carried out. As expected, decreases in the number of pathways occurring in a process reduced the models ability to correctly identify only the occurring process (Error! Reference source not found., scenario A1242P50 in which half of the pathways in the process were randomly allowed to occur).

Table 22. Posterior probabilities of calculated dechlorination process occurrence for scenarios

	CTDPG H	CTDPG H'	CTDPG LP	CTDPG M	CTDPG N	CTDPG P	CTDPG Q	CTDPG T	No Dech.
Scenario A1242H	1.0000 ± 0.0000	0.0000 ± 0.0001			0.0000 ± 0.0006				
Scenario A1242H'	0.0000 ± 0.0004	1.0000 ± 0.0000	0.0000 ± 0.0002	0.0000 ± 0.0001	0.0000 ± 0.0002				
Scenario A1242LP			1.0000 ± 0.0000						
Scenario A1242M		0.0000 ± 0.0005		1.0000 ± 0.0000	0.0000 ± 0.0002				
Scenario A1242N	0.0002 ± 0.0141	0.0000 ± 0.0002			1.0000 ± 0.0000	0.0000 ± 0.0009			
Scenario A1242P	0.1230 ± 0.3285	0.0001 ± 0.0078		0.0000 ± 0.0001	0.0000 ± 0.0039	1.0000 ± 0.0002			
Scenario A1242Q							1.0000 ± 0.0000		
Scenario A1242T	0.0000 ± 0.0045	0.0027 ± 0.0518	0.0000 ± 0.0001	0.0011 ± 0.0328	0.0002 ± 0.0147	0.0000 ± 0.0006			0.9962 ± 0.0619
Scenario A1242M+P	0.7203 ± 0.4489	0.0572 ± 0.2322		1.0000 ± 0.0000	0.1669 ± 0.3729	1.0000 ± 0.0069			
Scenario A1242M+N	0.1218 ± 0.3271	0.0001 ± 0.0078		1.0000 ± 0.0001	1.0000 ± 0.0005	0.0033 ± 0.0571			
Scenario A1242P+H	0.2188 ± 0.4135	0.0000 ± 0.0020			0.0000 ± 0.0017	1.0000 ± 0.0002			
Scenario A1242All	0.6892 ± 0.4628	0.5722 ± 0.4948	1.0000 ± 0.0005	0.7306 ± 0.4436	0.9913 ± 0.0931	0.9504 ± 0.2171	0.8723 ± 0.3338		
Scenario A1242P50	0.3074 ± 0.4614	0.0801 ± 0.2714	0.0000 ± 0.0017	0.0080 ± 0.0893	0.0293 ± 0.1687	0.9997 ± 0.0183	0.0000 ± 0.0003		0.0000 ± 0.0015
Scenario A1260P						1.0000 ± 0.0000			
Scenario A1260T								1.0000 ± 0.0000	
Scenario A1260All	0.9964 ± 0.0600	0.0278 ± 0.1644	1.0000 ± 0.0008	0.9590 ± 0.1983	1.0000 ± 0.0000	1.0000 ± 0.0000	0.8991 ± 0.3012	1.0000 ± 0.0004	
Grasse River	0.0000 ± 0.0045	0.0001 ± 0.0113	0.0000 ± 0.0051	0.0084 ± 0.0912	1.0000 ± 0.0000	1.0000 ± 0.0000	1.0000 ± 0.0023	0.9916 ± 0.0911	

In the synthetic scenarios the value of parameter *R* in the chemical reaction equation was assumed to be 5. Validation of the model by the synthetic scenarios produced values of *R* near the assumed value (**Figure 59**). Small deviations of the posterior value from the assumed value are acceptable, as these differences are accounted for by negatively correlated shifts of values in *M*.

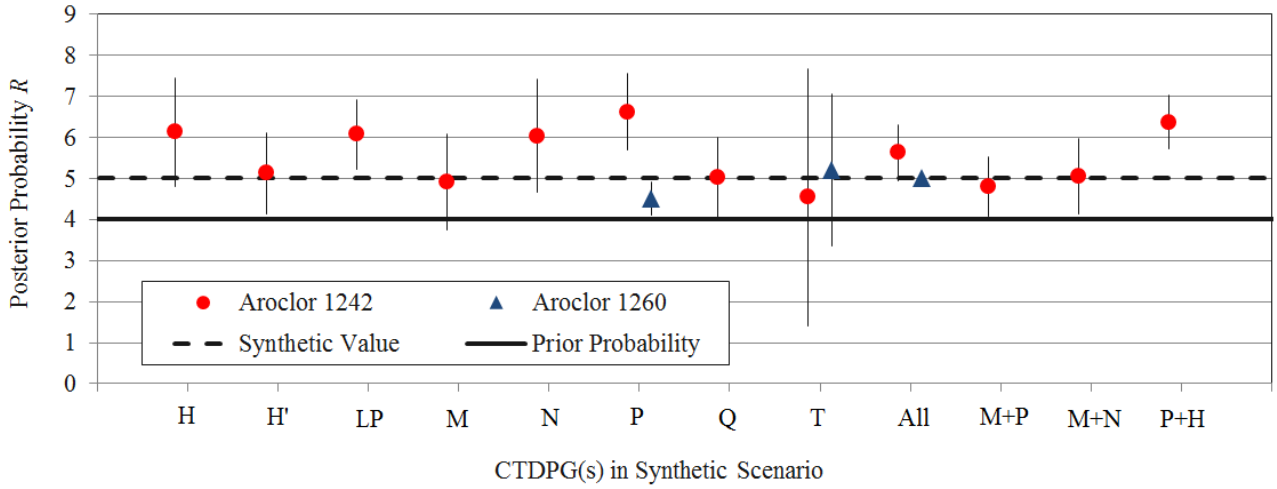


Figure 59. Calculated Posterior Statistics for Variable R.
Mean and standard deviations of variable R for each of synthetic scenarios (Hughes et al. 2011).

A preliminary application of the DPE to a laboratory experiment in which Grasse River sediment was spiked with Aroclor 1242 was conducted. It has been suggested that processes N, P Q and T (or an unidentified process sharing many pathways with these processes) had taken place across the twelve week study (Minkley et al. 1999). Pathways in CTDPGs N, P, Q and T as well as the initial and final congener distributions in this experiment are illustrated in **Figure 60**.

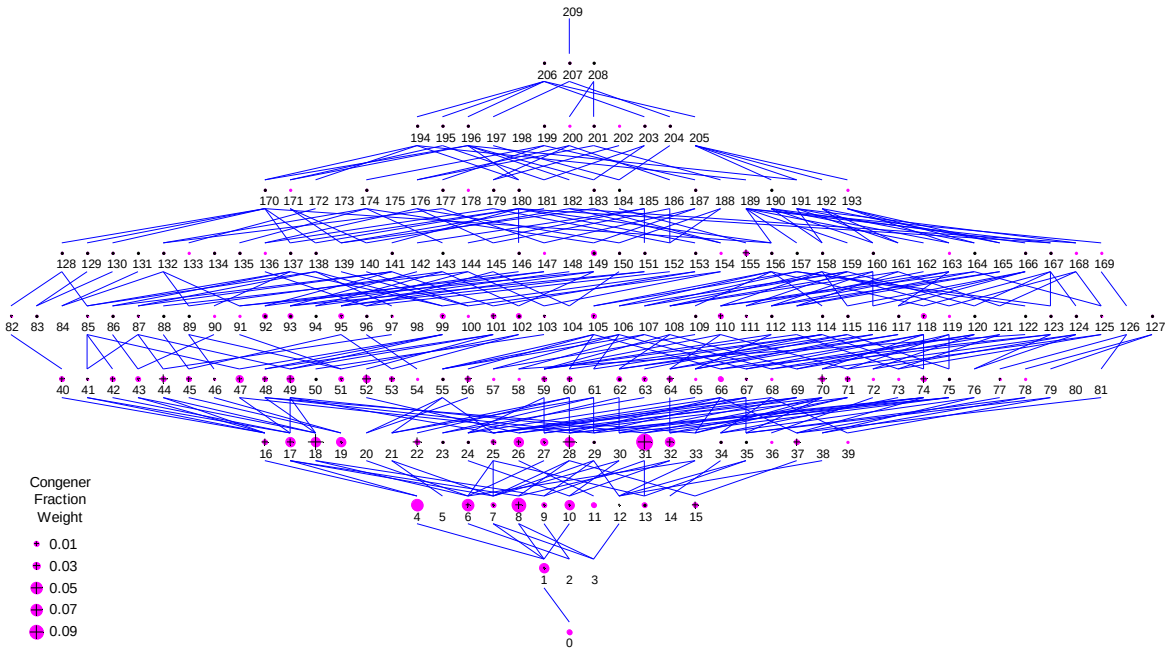


Figure 60. Diagram of the Observed Congener Fraction Weights.
Initial fraction weights are shown using black crosses and observed final congener fraction weights are shown using pink circles. All pathways in the CTDPGs identified to be occurring (N, P, Q, T) in the Grasse River experiment by the DPE are included. Note that the rows are arranged by homolog and correspond to congener structures assigned in the original work of Ballschmiter and Zell (Ballschmiter and Zell 1980) with corrections to congener numbers 199-201 by Schulte and Malisch (Schulte and Malisch 1983) and corrections to numbers 107-109 by Guitart et al. (Guitart et al. 1993).

While Process T was estimated to be occurring by the DPE, it is not considered to have been a significantly occurring process in the experiment; as the number of congeners present in the initial distribution that could have been dechlorinated by pathways in Process T was very small. Significant pathways, defined as those estimated to be transferring greater than 0.0001 fraction weight across the experiment are illustrated in **Figure 61**. Notably, the DPE estimates that mass is being transferred from 2-chlorobiphenyl (labeled congener one in the figure) to bipenyl. This pathway represents complete destruction of PCBs and consequently risk to human health.

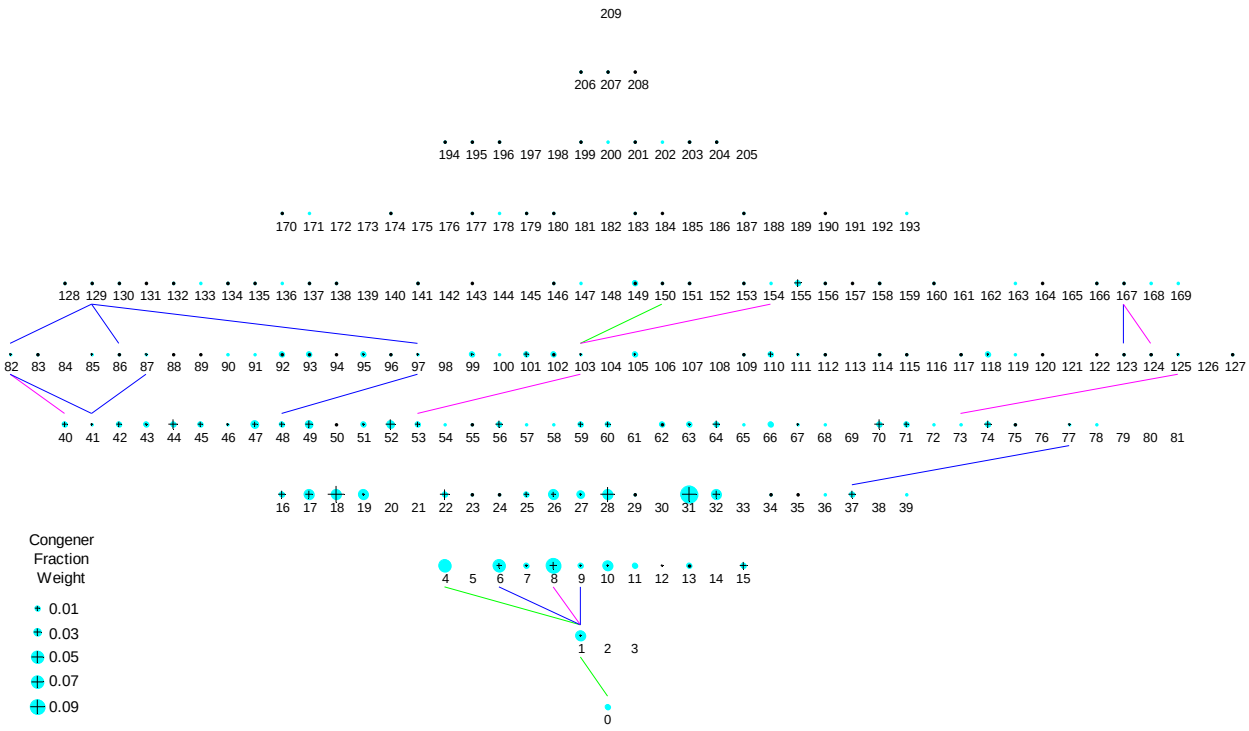


Figure 61. Diagram illustrating the most significant pathways (Grasse River). Most significant is defined as greater than 0.001 fraction weight transferred. Illustrated are observed initial (black crosses) and observed final congener fraction weights (cyan circles) and pathways (lines colored according to the position of the chlorine removed across the pathway: removal of a para chlorine in pink, meta chlorine in blue and ortho chlorine in green).

On a whole, the BMC model performs quite well despite minor indications that additional MC simulations are needed. The biggest hurdle to reducing the number of such simulations is the common analytical challenge of co-eluters, which limits the number of quantified congener concentrations. Missing congener concentrations is likely to increase an already substantial amount of uncertainty. Assessment of the occurrence of pathways whose parent and/or daughter congeners are not measured will also pose a problem. Subsequently, the authors stress the quantification of as many congeners as possible and caution that the method's success may lie in the researcher's ability to do so.

The value of applying this model to laboratory experiments rests in its ability to investigate a data set, which is typically examined at a congener level, at the pathway level. In the case of PCBs, the standard question of which congener is losing mass becomes which chlorine atom on a particular atom congener is being removed? This deeper level of dechlorination data has great potential to correlate to biogeochemical conditions, where congener concentrations have produced mixed results (e.g. Wu et al. 1997a; Wu et al. 1997b; Kim and Rhee 2001; Kuzyk et al. 2009). Further, the BMC model can glean additional information from past and future costly and time-consuming laboratory experiments while providing a better understanding for the drivers of biotransformation of PCBs in sediments. Notably, the dechlorination *process* estimator has laid substantial groundwork for a dechlorination *pathway* estimator. The pathway estimator will generate substantially more specific and valuable information by identifying the likelihoods of occurrence of each of the 840 dechlorination pathways.

Finally, this approach can be applied to other chemical systems, such as polybrominated diphenyl ethers (PBDEs), which also consist of 209 congeners. As with PCBs, degradation pathways are extrapolated from shifts in congener mass (He et al. 2006; Robrock et al. 2008; Tokarz et al. 2008). To the best of the authors' knowledge, no mechanistic PBDE debromination model has been published to date. Consequently, the BMC method demonstrated can significantly contribution to the modeling of closed complex chemical systems, such as PCB and PBDE congener distributions.

4.4 Task C. Microcosm Study

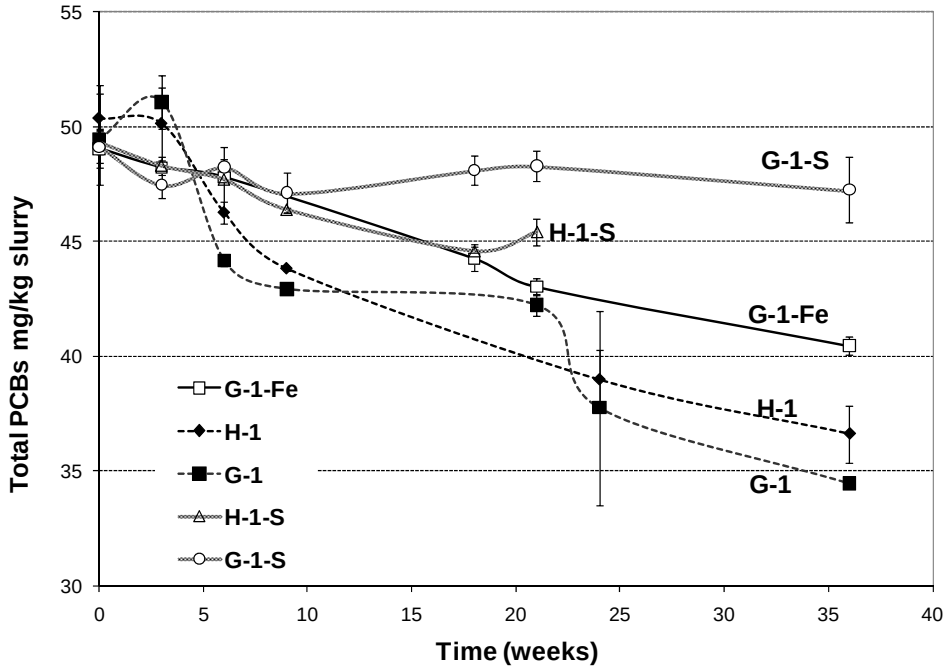
4.4.1 PCB Dechlorination in Microcosms Spiked with Two PCB Mixtures

PCB Dechlorination was observed in all treatments except the Hudson River sediment amended with 40 mmole/kg slurry ferric oxyhydroxide (FeOOH) and sterile controls (data now shown). Seen in **Figure 62**, for the treatments spiked with each of the PCB mixtures, the shortest lag time (3 weeks) was found in Grasse Sediment, followed by Hudson Sediment (3-6 weeks) and Grasse Sediment with ferric iron (6-9 weeks). When sulfate was added to both sediment types, an approximately 18-week lag time and slow dechlorination rate were observed, which is expected as the presence of sulfate as a more favorable electron acceptor than PCBs. During 36 weeks' incubation, the average rate of dechlorination was: Grasse Sediment > Hudson Sediment> Grasse Sediment amended with ferric iron>Hudson Sediment amended with sulfate> Grasse Sediment amended with sulfate>Hudson Sediment amended with ferric iron.

By the end of 36 weeks, the total PCB amount decreased by 30-35% in Grasse Sediments, followed by 25-30% in Hudson Sediments and 20-25% in ferric iron amended Grasse Sediments. Greater than 10% total PCBs amount was reduced in sulfate amended Hudson Sediments, while less total PCBs reduction was detected in sulfate amended Grasse Sediments.

As mentioned before in method section, to investigate the influence of PCB composition on reductive dechlorination, we choose two PCB Mixtures containing 9 PCB congeners in each mixture. PCB 5 (23-CB), PCB 12 (34-CB), PCB 64 (236-4-CB), PCB 71 (26-34-CB) and PCB 170 (2345-234-CB) are present in both mixtures. PCB 105 (234-34-CB), PCB 114 (2345-4), PCB 149 (236-245-CB) and PCB 153 (245-245-CB) are in PCB Mixture 1 while PCB 82 (234-23-CB), PCB 97 (245-23-CB), PCB 99 (245-24-CB) and PCB 144 (2346-25-CB) are included in PCB Mixture 2. The two mixtures contain very similar levels of total PCBs, not only in total PCB amount, but also in mole concentrations and chlorine contents. Seen in **Figure 62**, all the treatments spiked with PCB Mixture 1 dechlorinated slightly slower than the treatments spiked with PCB Mixture 2. This result suggests that PCB composition is an important factor in controlling PCB dechlorination activity.

A



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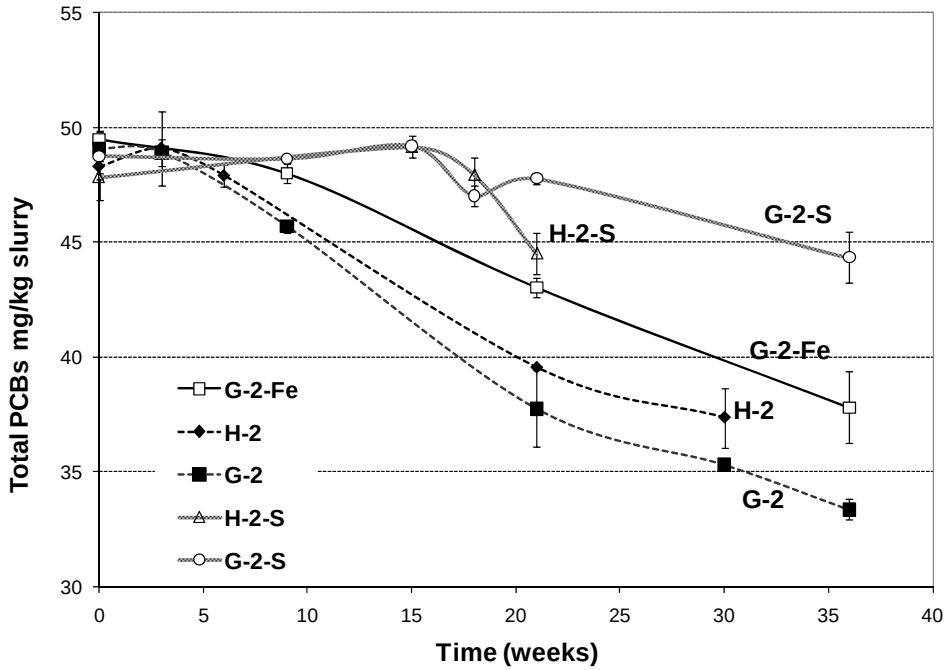

Figure 62. Dechlorination of spiked PCBs over time.
A) treatments spiked with PCB Mixture 1; B) treatments spiked with PCB Mixture 2. All data points averaged triplicate microcosms. Error bars represent one standard deviation.

4.4.1.1 Shifts of Tracker Pair Ratios in Microcosms Spiked with PCB Mixture 1.

Here, we used the data from the first four sampling points to study the change of PCB tracker pairs in both the Hudson and the Grasse microcosms spiked with PCB Mixture 1. No change of highly chlorinated tracker pairs (149/153/170) was detected due to their longer lag time or/and their slow dechlorination rate. Interestingly, 5/12 ratio did not shift in the Hudson sediment, which indicates a similar first order dechlorination rate constant for both congeners. In the Grasse sediment microcosms, 5/12 ratio dropped dramatically after 6 weeks of incubation, then increased rapidly after 3 more weeks (**Figure 63**). The ratios of tracker pairs 64/71 and 105/114 in this system decreased versus incubation time for both sediment types, though a rapid decrease was observed in the Grasse sediment

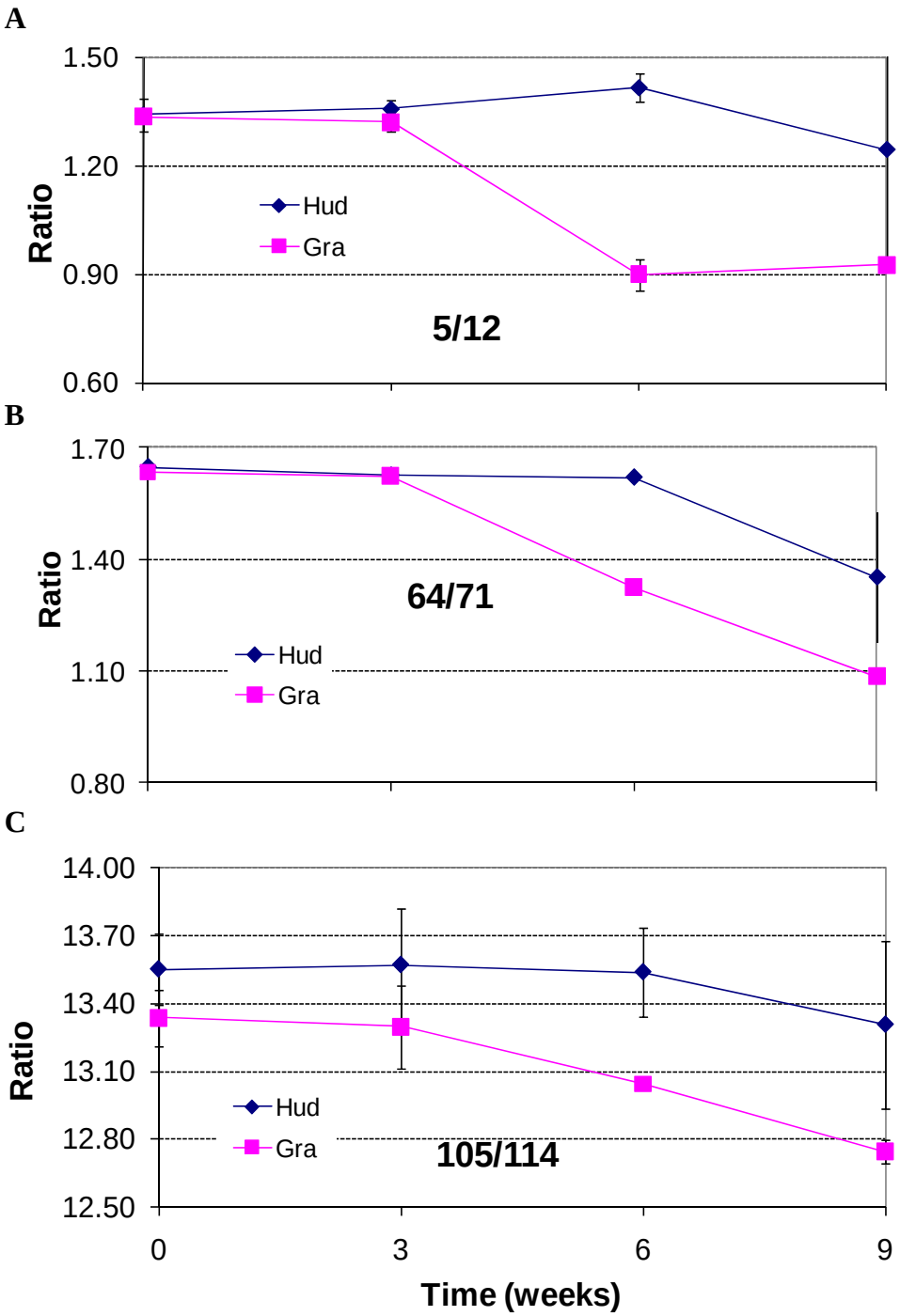

Figure 63. The Changes of Tracker Pair Ratios in Sediment Microcosms. Microcosms are spiked with PCB Mixture 1. A) tracker pair 5/12; B) tracker pair 64/71; C) tracker pair 105/115;

4.4.1.2 Pathways Occurred in Microcosms Spiked with PCB Mixture 1 (No Sulfate or Iron Amendment)

By analyzing the first generation dechlorination products of Hudson and Grasse sediment spiked with PCB Mixture 1, 13 first generation products were found in at least one of the two sediments and six or more observed pathways were not included in the published processes (seen in Error!

Reference source not found.). The result partially supports our Process classification tree work in adding more reasonable pathways into originally published Processes.

Table 23. Detected First Generation Products and their Corresponding Parents in Sediment Microcosms Spiked with PCB Mixture 1

Source Congener		Product Congener
5	→	1
12	→ *	3
71	→	27
64/71	→	32
71	→ *	33
105	→ *	55
105	→ *	56
114	→ *	63
149	→	91
149	→	95
149/153	→	101
170	→ *	129
170	→	130

* represents pathway absent in originally published Processes.

4.4.1.2.1 Target Chlorines in Microcosms with Sulfate Amendments

Previous studies have found the chlorines removed in sulfate amended microcosms were flanked *para*- and/or doubly flanked *meta*- chlorines (May et al. 1992; Rhee et al. 1993b; Cho and Oh 2005). In the present study, flanked *para* and *para*-flanked *meta* dechlorinations were observed. *Ortho*-flanked *meta* dechlorination was partially inhibited. When sulfate level dropped, *ortho*-flanked *meta* dechlorination was also found in both sediment types and PCB Mixtures but not as prevalent as flanked *para* and *para*-flanked *meta* dechlorinations. These results again coincided with the augmented Processes.

4.4.1.2.2 Altered Dechlorination Preference in the Grasse Microcosms with Iron Amendments

Total PCB analysis showed that the addition of ferric iron significantly decreased both the rate and extent of dechlorination based on total PCB amount. However, when further study based on individual PCB congeners were conducted, more complicated effects were observed. By the end of 36 weeks, the residual PCB 170 in the iron amended microcosms was 50% less than that in the microcosms without iron. This is the first report that the addition of a favorable electron acceptor is able to accelerate the dechlorination of highly chlorinated PCB congeners. In addition, the accumulation of PCB 32 (26-4-CB) was found. PCB 32 was dechlorinated to PCB 10 (26-CB) in the microcosms without iron amendment. This reveals the lack of the capability of removing unflanked *para* chlorines in ferric iron amended microcosms.

4.4.1.2.3 Evidence of Ferric Iron Reduction in Microcosms Spiked with PCBs

PCB characterization result showed that ferric iron slowed down dechlorination in Grasse sediment spiked with PCBs and totally inhibited dechlorination in Hudson sediment with PCBs. Ferric iron is regarded as an alternative electron acceptor of reductive reaction in the

microcosms. Theoretically, ferric iron reduction is more thermodynamically favorable than PCB reduction. Therefore, ferric iron is reduced to ferrous iron prior to PCB reductive dechlorination. To better understand the reduction reactions in our microcosms, ferrous iron concentration were tracked over time.

In the present study, 40 mmole/kg slurry ferric oxyhydroxide was amended to both the Hudson and the Grasse sediment microcosms. In other words, when iron reduction is complete, the concentration differences between FeOOH amended and no FeOOH amended groups are expected to be 40 mmole/kg slurry. Seen in **Figure 64**, in the Grasse microcosms, the 40 mmole/kg slurry differences were observed after 15 weeks. However, significant PCB dechlorination had taken place in Week 12 and Week 9 for microcosms spiked with PCB Mixture 1 and PCB Mixture 2, respectively. In other words, PCB dechlorination starts before ferric iron is depleted. In contrast, very slow Fe (II) increase was found in the Hudson microcosms amended with FeOOH during 36 weeks' incubation. It is suggested that Fe reduction rate was very low. At the last sampling point (Week 36), the Fe (II) increased to around 40 mmloe/kg slurry. Considering the background Fe (II) level, only 60% amended FeOOH was reduced. PCB analysis showed that no dechlorination in the Hudson microcosms with FeOOH after 36 weeks' incubation. pH measurement suggested that the increase of pH in the Hudson microcosms with the addition of FeOOH might be responsible for the low reduction rate of ferric iron. The initial pH was 7.1-7.4, while the pH after 15 weeks incubation was between 7.6 to 7.9.

PCB spiked microcosms amended with sodium sulfate showed similar *final* Fe (II) concentrations with the unspiked microcosms. However, time 0 point data, which were collected after 24 hours mixing, showed that the rate of ferric iron reduction was slowed down when a more favorable electron acceptor sulfate was present. Additionally, the fluctuation of Fe (II) concentration in the Grasse River microcosms amended with sulfate might be a result of the interaction between the two electron acceptors, Fe (III) and sulfate, in the same systems.

As mentioned in the initial characterization above, the Hudson and the Grasse River are different in their properties. On one hand, the Grasse sediment has very high Fe contents, while the Hudson sediment has relatively low Fe. It is very likely that the Grasse sediment contains more abundant and acclimated iron reducing bacteria than the Hudson sediment. With a low level of background iron and lack of necessary species for Fe (III) reduction, the Hudson sediment showed slow iron reduction. On the other hand, the buffering capacity of the Hudson sediment is much lower than that of the Grasse sediment. The reduction reaction of ferric iron increased the pH in the Hudson microcosms amended with FeOOH, which is likely to have adverse effects on the mechanism of iron reducing bacteria *and* PCB dechlorinators.

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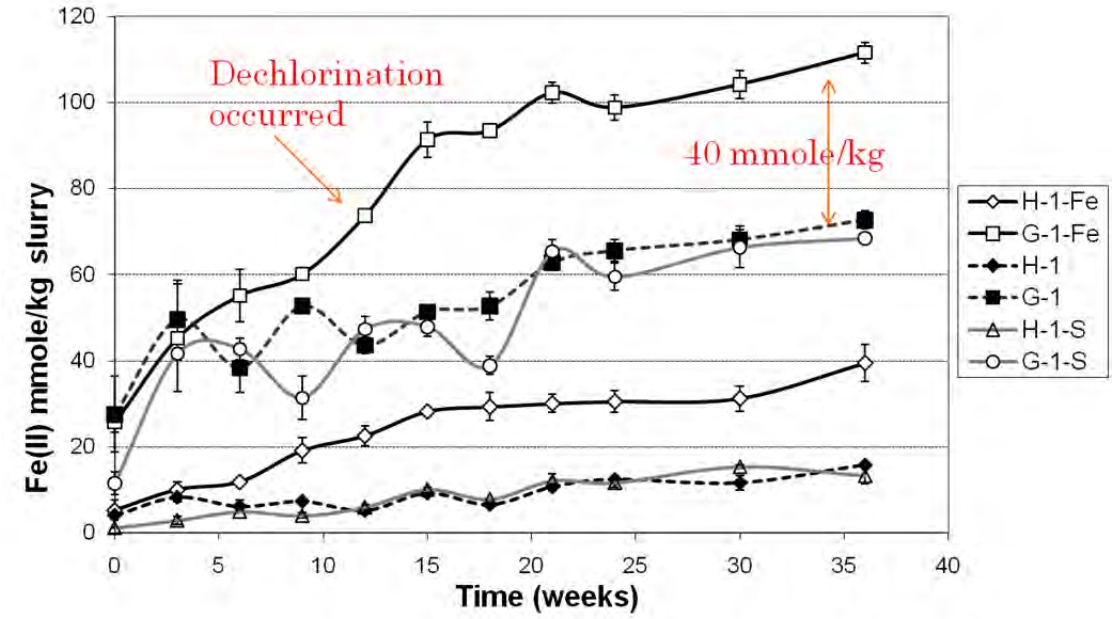

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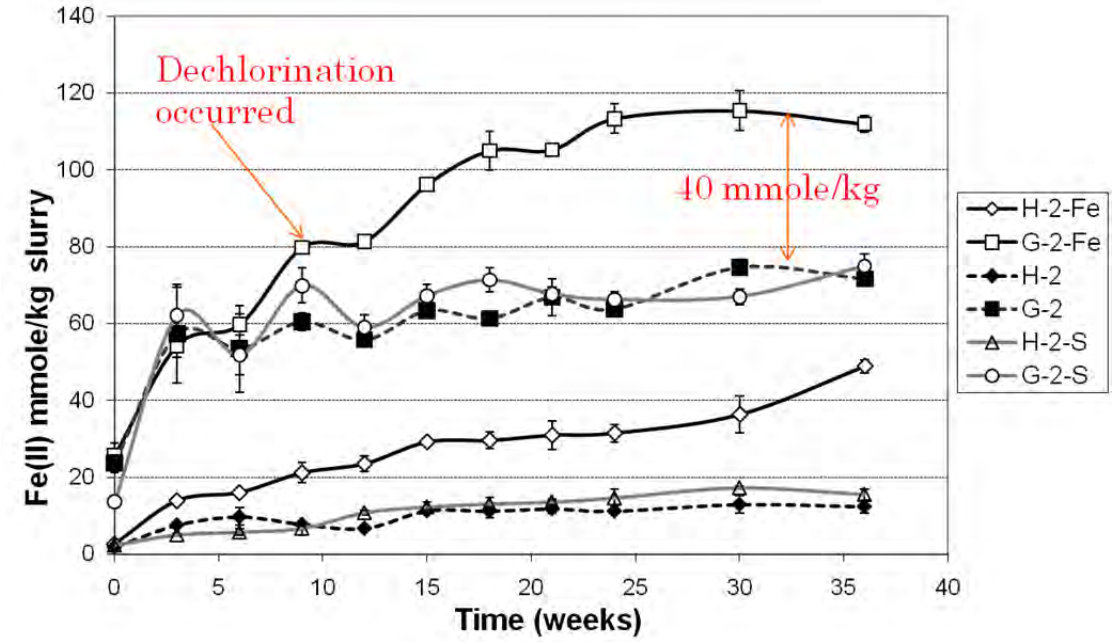

Figure 64. The Change of Fe (II) Concentrations During 36 Weeks' Incubation Time. A: Fe(II) in treatments spiked with PCB Mixture 1; B: Fe(II) in treatments spiked with PCB Mixture 2. All data points averaged triplicate microcosms. Error bars represent standard deviation. All data points averaged triplicate microcosms. Error bars represent standard deviation.

4.4.1.2.4 Evidence of Methanogenesis

Methane was detectable in all treatments and the "No PCB" live controls. Generally, the addition of sulfate or ferric iron reduced the production of methane. The treatments using the Grasse sediment generated more methane than those using the Hudson sediment. However, the

production of methane varied among the different sediment types and treatments. Shown in **Figure 65** (Panel A), only a trace amount of methane was detected in the PCB spiked Hudson sediments amended with ferric iron, which is another indicator of low microbial activity in these microcosms. The effect of PCB composition on methane production was different in different sediment types. By the end of 51 weeks, for the Hudson sediment, the microcosms spiked with PCB Mixture 1 produced 4.6 mmole/kg slurry of methane, which is 1.0 mmole/kg higher than that of the Hudson sediment "No PCB" control. On the contrary, the microcosms spiked with PCB Mixture 2 produced much less methane (3.0 mmole/kg) than the Hudson sediment "No PCB" control. Alternatively, for the Grasse sediment, PCB Mixture 1 spiked microcosms and PCB Mixture 2 spiked microcosms produced 14.4 mmole/kg and 15.3 mmole/kg of methane respectively. These values are greater than methane produced by Grasse sediment "No PCB" control (12.6 mmole/kg). Interestingly, PCB Mixture 2, which slightly inhibited methane production in the Hudson sediment, strongly *increased* the production of methane when adding into the Grasse sediment. The varied methane production suggests that the effect of PCB composition is different in different sediments. The low levels of methane in sulfate amended microcosms and iron amended Hudson sediment microcosms is very likely due to the outcompeted bacteria utilizing sulfate or iron as their terminal electron acceptors instead of carbon dioxide. In addition, the production of methane in sulfate amended microcosms and iron amended Hudson sediment microcosms ceased after six weeks' incubation. That is to say, methanogenesis activity was entirely inhibited after six weeks. However, PCB dechlorination occurred in sulfate amended microcosms 9 weeks later. This result suggests that methanogenesis is not essential for PCB dechlorination.

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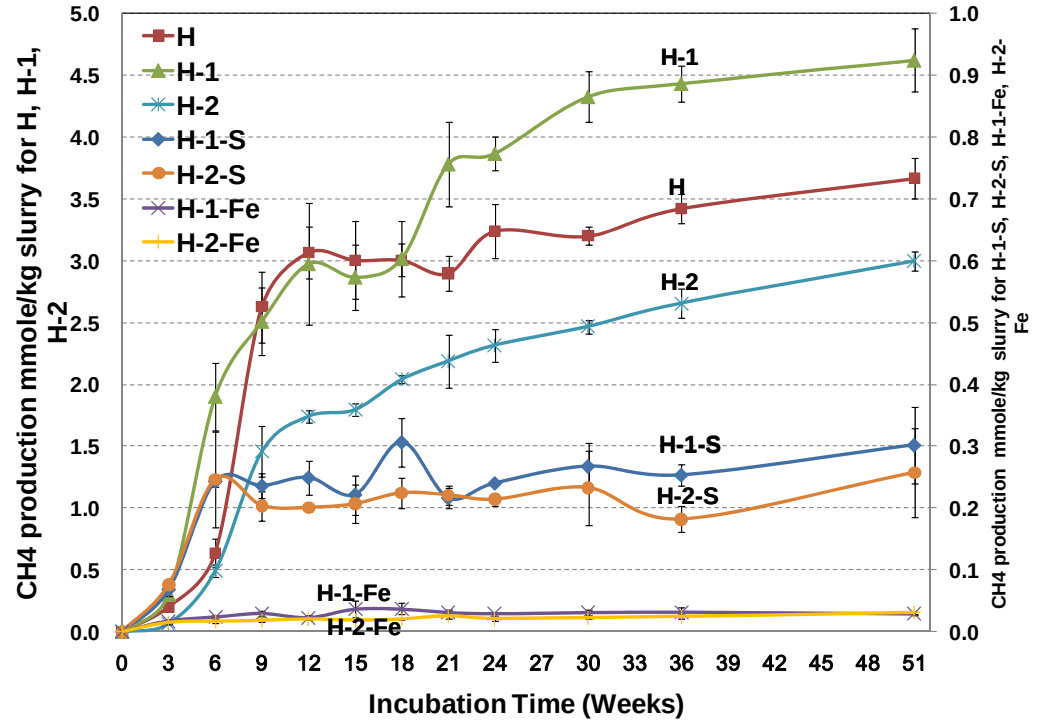

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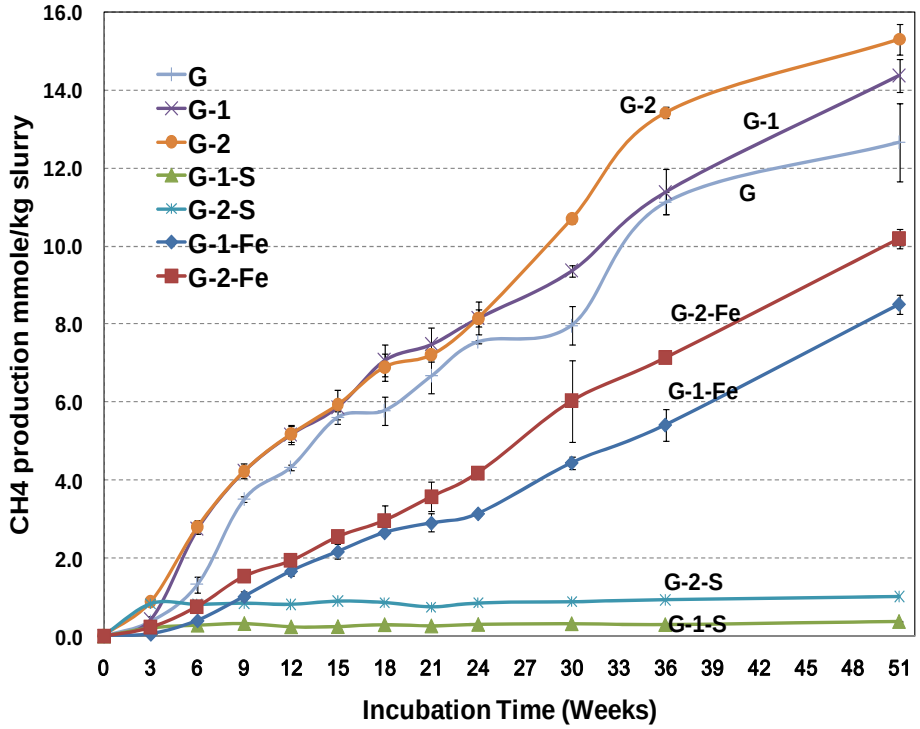

Figure 65. Methane Production in all Treatments Over Time.
A) methane in the Hudson River microcosms; B) methane in the Grasse River microcosms. All data points averaged triplicate microcosms. Error bars represent one standard deviation.

4.4.1.2.5 Quantification of Putative Dechlorinating Microorganisms

16S rRNA gene copies of *Dehalococcoides* (DHC) were determined by a Q-PCR assay. Two primer sets were selected for this study. Primer set DHC1200F and DHC1271R is specific for *Dehalococcoides* group. Shown in **Figure 66**, during the 51 weeks' incubation, the increase of *Dehalococcoides* (DHC) 16S rRNA genes was observed in H-1, G-1, H-2, G-2, G-1-Fe and G-2-Fe groups, where PCB dechlorination activities had been confirmed by PCB congener-specific analysis. *Dehalococcoides* (DHC) 16S rRNA genes remained at a low level in PCB spiked Hudson sediment microcosms amended with ferric iron (H-1-Fe, H-2-Fe), where no dechlorination was observed.

Naturally, Hudson sediment and Grasse sediment contain different levels of *Dehalococcoides*. At time zero, the *Dehalococcoides* 16S rRNA gene copies in the Grasse River sediment were 10 to 20-fold higher than that in the Hudson River sediment (**Figure 66**). This difference may explain the longer lag time observed in Hudson microcosms. Seen in H-1 and H-2 groups, comparing to time zero, one order of magnitude increase of *Dehalococcoides* 16S rRNA genes were detected at Week 6 and Week 9, respectively. Week 6 and Week 9 were also the first time point that significant dechlorination was found in group H-1 and group H-2. This finding is an evidence of the link of *Dehalococcoides* growth to PCB reductive dechlorination. We have found that the PCB spiked Grasse sediment microcosms dechlorinated more extensively than the PCB spiked Hudson sediment microcosms. However, after 9 weeks' incubation, the Hudson sediment microcosms (H-1, H-2) showed higher numbers of *Dehalococcoides* 16S rRNA genes than the Grasse Sediment microcosms (G-1, G-2). This result suggests different *Dehalococcoides* species in the two sediment systems. More interestingly, the addition of ferric iron increases the number of *Dehalococcoides* 16S rRNA genes in the PCB spiked Grasse River sediment microcosms (G-1-Fe, G-2-Fe) (**Figure 66).** This may explain the more rapid dechlorination of highly chlorinated PCB compounds in the iron amended Grasse sediment microcosms.

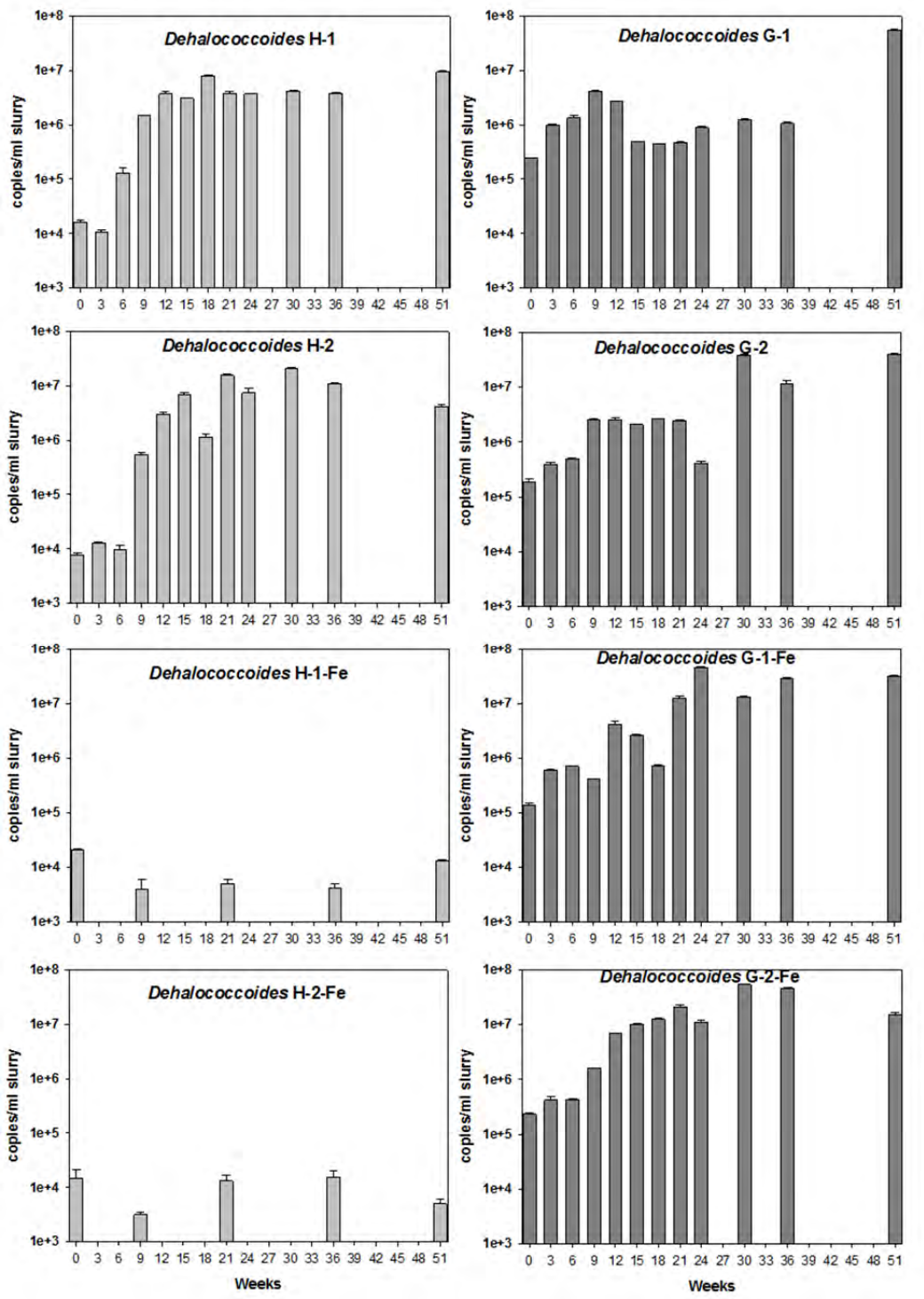

Figure 66. Quantitative Assessment Dehalococcoides 16S rRNA Genes. Assessed in PCB Mixture 1 spiked Hudson Sediment microcosms (H-1), PCB Mixture 1 spiked Grasse Sediment (G-1), PCB Mixture 2 spiked Hudson Sediment (H-2), PCB Mixture 2 spiked Grasse Sediment (G-2), PCB Mixture 1 spiked Hudson Sediment with ferric iron amendment (H-1-Fe), PCB Mixture 1 spiked Grasse Sediment with ferric iron amendment (G-1-Fe); CB Mixture 2 spiked Hudson Sediment with ferric iron amendment (H-2-Fe), and PCB Mixture 2 spiked Grasse Sediment with ferric iron amendment (G-2-Fe).

4.5 Task D. Decision Support Model

Work on this task was halted after the 2007 In-Progress-Review. A summary of results for 2006-2007 are presented here. A white paper summarizing the potential of this method to link biogeochemical models with decision support models was requested in 2007 and prepared by the research team. It is included, with only minor changes, as Appendix C.

The overall objective was to link the model for environmental conditions, congener distributions and bacterial population dynamics to decision-support tools to enable evaluation of site-specific likely outcomes in order to evaluate remediation plans. The two modeling efforts were conducted in parallel. However the biogeochemical model was needed to inform the decision support model, and thus a linkage between the two was originally envisioned as shown in **Figure 67**.

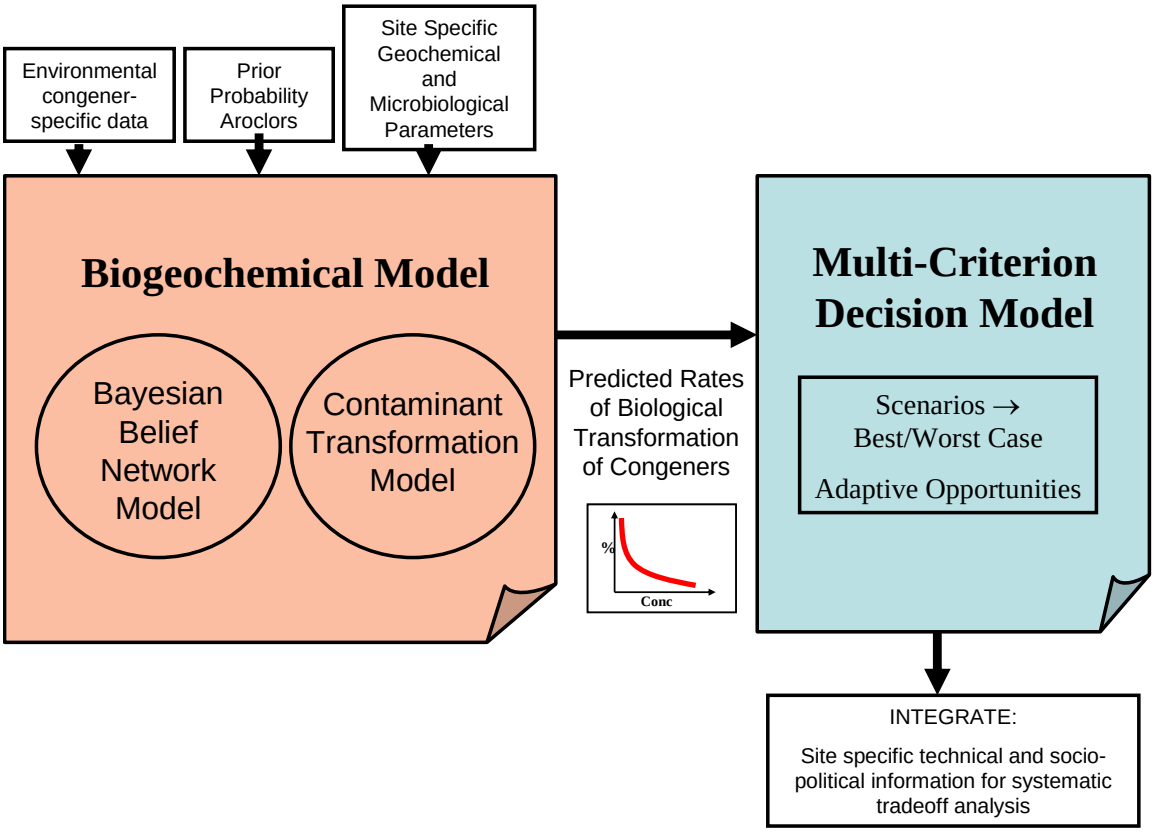

Figure 67. Information Exchange Among the Models.
Models include a Bayesian Belief Network (BBN) Model, the contaminant transformation model, and the multi-criterion decision model.

Figure 67 provides the conceptual model integration, with the biogeochemical model predicting the rates of biological transformations at the site on a congener-specific basis. The multi-criterion decision model uses this information along with additional site information (sociopolitical aspects, ecosystem specifics, other abiotic processes) to develop scenarios and enable decision-makers to explore different remediation options.

Thus, we envisioned that the QnD system would expand the unit-scale information simulated by the biogeochemical model by providing specific river reaches as well as additional simulated

items (such as ecosystem or human-management responses) that stem from the input of various scenario/planning designs. The QnD platform can provide a game-style simulation in which stakeholders can role-play possible futures to develop rules of thumb, further learning and adaptive opportunities to feed back into various management or policy efforts being made at a particular site.

The QnD system is a flexible object-orientated modeling platform developed by Greg Kiker (Kiker et al. 2005b; Kiker and Linkov 2005). Platform use is divided into three layers, each geared toward a specific user. The base layer, written in JAVA, contains instructional code for platform performance. It is meant to be accessed on a limited basis by programmers. In the second layer, modelers write in extensible markup language (XML) code to produce a model in the QnD platform. The XML is structured in unified modeling language (UML), a standard object-orientated modeling approach. In this language, objects are represented by components, some of which may be location specific. Components can be described by site and time specific data or predicted stochastically. These objects interact through processes that are quantified by equations defined by the modeler and use data assigned to components. Finally, the modeler specifies what is seen by stakeholders in the third layer, a graphical user interface (GUI). Here, stakeholders selectively interact with the QnD computing engine to discover the effects of remedial decisions on congener concentrations.

The design of the Grasse River environment within QnD reflects management decisions at the site, which are primarily based on PCB concentrations in fish. Thus, the movement of PCB congeners up the food chain and into fish will be the model's endpoint. Further, the design is highly dependent upon modeling work completed by QEA. In an effort to utilize the extensive Grasse River data set and avoid recreating their comprehensive hydrodynamic, sediment transport, PCB fate and bioaccumulation models, QEA offered to share their work. Thus, the design focused on model components.

In the "fish-centric" model, a representative species of fish must be chosen or possibly three species, the largemouth bass, brown bullhead, and pumpkinseed, to be modeled, as was done in the bioaccumulation QEA model. The aquatic environment occupied by fish is separated into two zones; a shallow zone and a deep zone. The shallow zone describes areas where fish spend the majority of their time, and therefore accumulate the majority of PCBs from. These zones were identified during fish studies funded by Alcoa, as demonstrated in **Figure 68**.

PCB accumulation in fish was planned to be modeled as a percent intake of objects in the deep and shallow zones. However, unlike QEA models, QnD will predict congener-specific uptakes. Congener mass was intended to be modeled as data belonging to components. PCB uptake in fish was intended to be modeled as a function of food intake and water movement across the gill. PCB loss occurs across the gill and through growth dilution. The food web was intended to be simulated by two groups of macroinvertebrates with different PCB sources. Phytophilous macroinvertebrates (PMI) primarily accumulate PCBs from the water column, while benthic macroinvertebrates (BMI) primarily accumulate PCBs from the sediment. Thus, processes will be written to describe the uptake of PCBs from the water and sediment to macroinvertebrates and subsequent uptake of macroinvertebrates by fish, in addition to direct PCB gains and losses between fish and water.

Grasse River sediment within QnD is divided into aerobic and anaerobic zones. PCB congener mass was intended to be modeled separately within each sediment zone based on results of grab samples and core analyses. For example, QEA has recorded location-specific total PCB sediment surface concentrations, as depicted in **Figure 69** (Alcoa 2001). Processes were to be written to describe the transfer of PCBs between the sediment zones, between the aerobic zone and water, and congener weathering within the sediment zones. This weathering will be based on results from Task B. In Task B, a BBN will predict congener-specific rates of weathering in aerobic and anaerobic sediment using Grasse River grab and core samples, respectively. Rates of degradation pathways not modeled in the BBN were to be estimated based on the rates of similar degradation pathways or congeners.

In summary, the components of the preliminary Grasse River representation in QnD are the air, shallow and deep aquatic zones, aerobic sediment, anaerobic sediment, fish, PMI and BMI. These components, and their object-oriented counterparts, are depicted in **Figure 70**. Data describing components, including congener mass, must be linked to time and location specific electronic files provided by QEA or Alcoa when possible. In cases where data are unavailable, stochastic predictions could be made based on available data. Time steps were expected to be annual. However, significant uncertainty was expected, as some data for the site were collected every ten years or in some cases only once.

Two sets of processes will be layered on top of the aforementioned environmental processes. The first set of processes will describe the effects of three management options, dredging, cap-and-treat and monitored natural recovery (MNR), have on sediment and fish PCB concentrations. Further, remediation processes can be automated in terms of policies. For example, stakeholders may impose a policy in which sediment with a concentration greater than 1000 ppm is always dredged. The second set of processes will quantify the effects of scenarios, such as an ice jam, on management methods and the Grasse River environment. The occurrence of such scenarios is likely to be modeled stochastically.

Stakeholders will be shown select pieces of the Grasse River computational engine through QnD's GUI. A preliminary version of the GUI appears in **Figure 71**. The diagram depicts the Grasse River divided into 8 reaches. The color of each reach will correspond to the selected radio button on the right side of the screen. The cursor can be used to select management methods, using the three sliders at the top of the GUI, for several or one river reach per time step. The single and double arrows at the upper right hand corner of the GUI advance the model by annual and decadal time steps, respectively. By clicking the white flag, a stakeholder can reset the model time, currently set for January 1, 1990. The warning lights across the top of the GUI will indicate if greater than $50 million is spent to remediate the site, the stakeholder's management popularity index and if the average total PCB concentration in fish is greater than 1 ppm. The tabs will display anaerobic sediment homolog, *ortho, meta, para* and dioxin-like congener concentrations in fish, and total PCB mass distribution in model components over time. Through this interface, stakeholders will be encouraged to explore the effects of remediation policies and ad hoc site management methods on PCB concentrations in sediment and fish, cost, and their popularity in the face of natural events such as ice jams and floods. Further, the QnD model will produce PCB concentration results in congeners-specific and total PCB terms. Thus, the QnD model is expected to illuminate differences in management decisions that are total PCB-based versus congener-based.

The design of the Grasse River environment in QnD, and subsequently the GUI, is expected to change as data and QEA models are acquired. While it appears that the greatest challenge will be the efficient programming of congener weathering and storage of congener mass, other challenges that were expected to be addressed, included the following: congener transfer between components, quantification of the effects of an ice jam, dredging and cap-and-treat remediation methods, and quantification of the management popularity index. Additionally, the QnD model was expected to be altered to reflect the vision stakeholders, such as Alcoa, have of the Grasse River and the management decisions they face. When the components, data and processes were finalized, an uncertainty and decision analysis was expected to be performed.

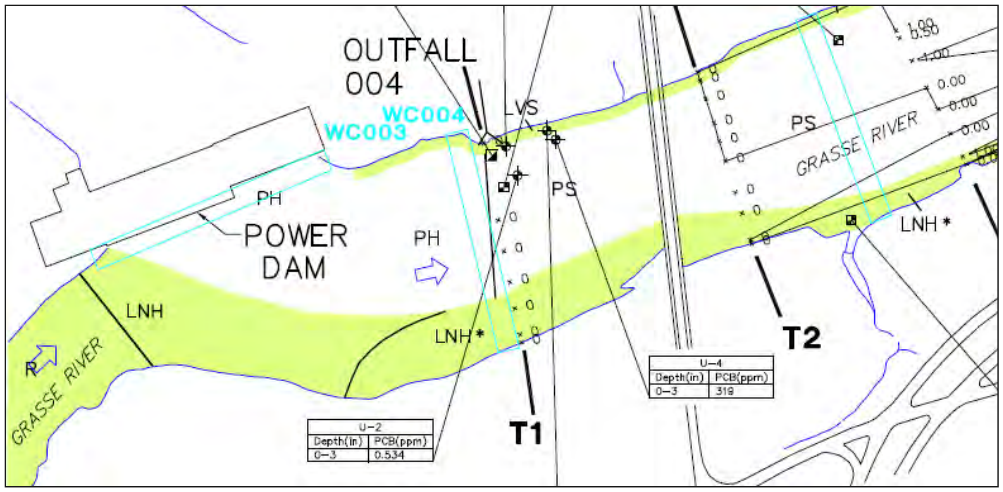

Figure 68. Alcoa Fish Habitats in the Grasse River.
A study completed by Alcoa identified the locations of aquatic fish habitats in the Grasse River (fish habitats appear in green) (Alcoa 2001).

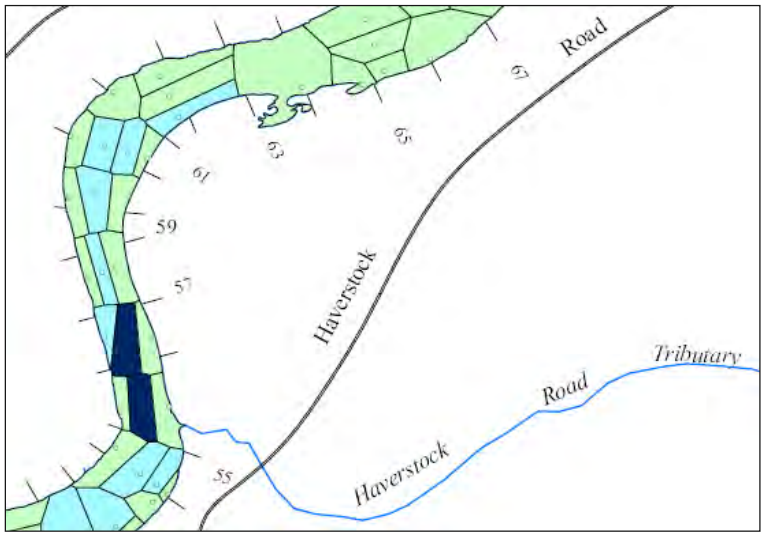

Figure 69. QEA Location-Specific Total-PCB Concentration (Grasse River).
Example of QEA location-specific total-PCB surface concentration data for the Grasse River (colors correspond to ranges of PCB concentrations). (Alcoa 2001).

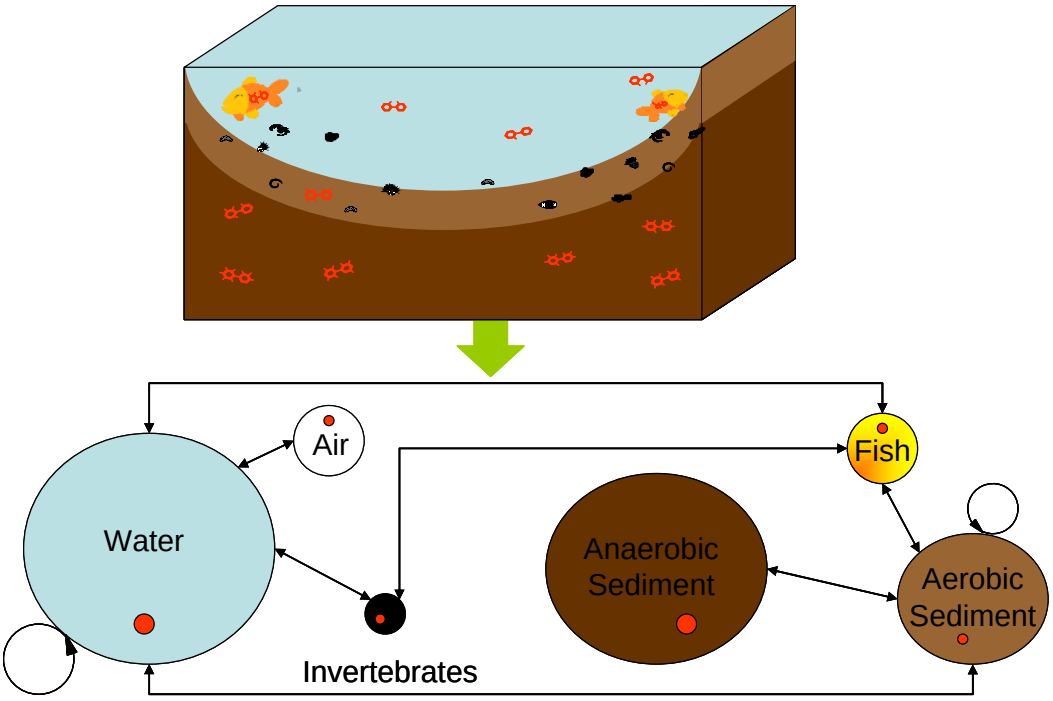

Figure 70. QnD Modeling of Grasse River.
Representation of the Grasse River and the components it is converted into for QnD modeling. Red circles represent congener mass data and arrows represent proposed processes.

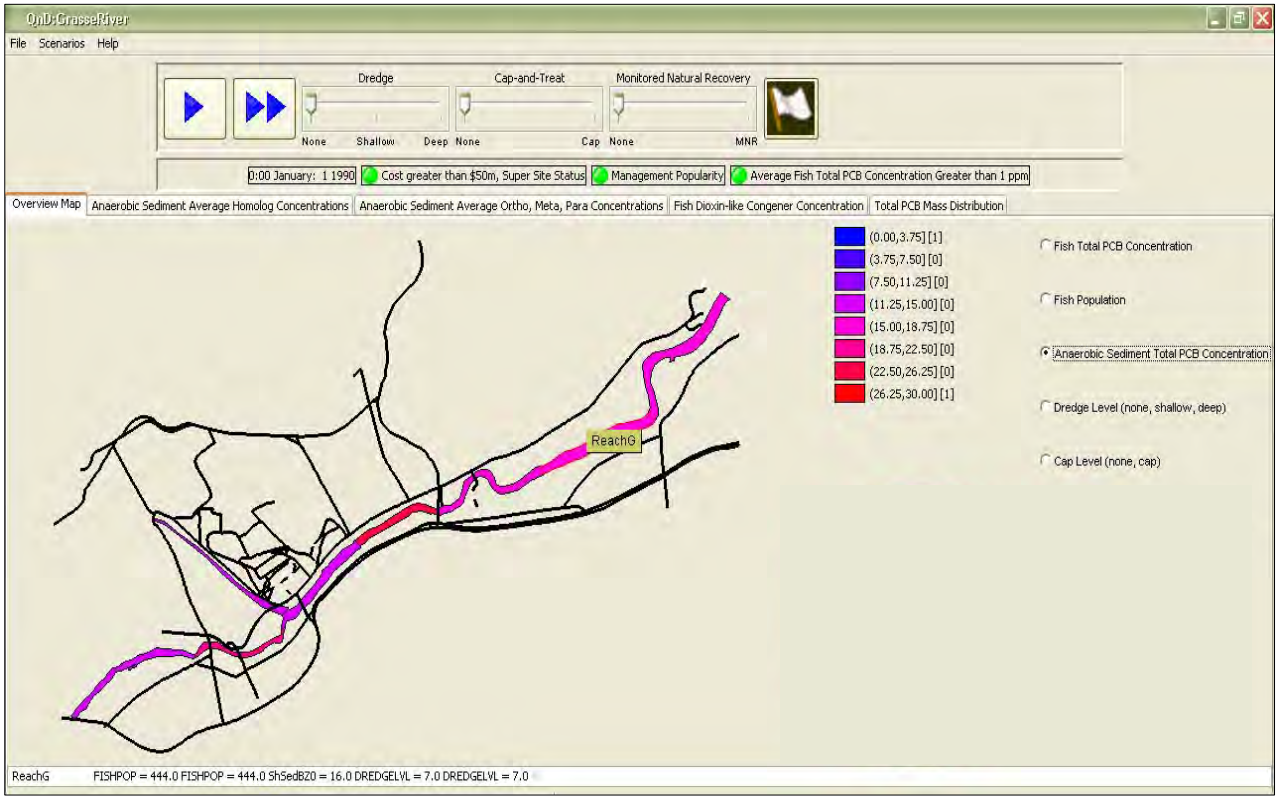

Figure 71. A preliminary GUI for 8 reaches of the Grasse River in QnD.

5 Conclusions and Implications for Future Research

Table 24 provides a summary of the results and conclusion of each task in this study. As shown in the table, several critical conclusions are apparent. First, sediment biogeochemistry, including initial PCB distributions, alternative electron acceptors (sulfate and iron), and microbial community all affect the transformation of PCBs in sediments. Extensive sediment characterization can aid in determining the suitability of monitored natural attention as a remedy at a specific field site. Sediment amendments may be useful to seed microbial populations or change pathways and end points for reductive dechlorination. Utilization of new modeling tools developed on this project will enable prediction of PCB end points and the effect of amendments on the rate, extent and end point of reductive dechlorination. Carefully designed microbial experiments, paired with statistically valid models accounting for uncertainty, can provide unique insights into potential remediation strategies.

Table 24. Summary of Results and Conclusion for Each Task

Task A-1 Grab Sample Detailed Sediment Characterization

- The Hudson River has higher sulfate than the Grasse River.
- The Grasse River has much higher iron than the Hudson River.
- The Hudson River and Grasse River have comparable amounts of organic carbon.
- For our selected sites, the Hudson River sample was slightly higher in total PCB concentration (6.51 mg/kg sediment) compared with the Grasse River sample (1.35 mg/kg sediment).
- The number of chlorines per biphenyl was slightly lower in the Hudson River (2.47) than the Grasse River (3.71).
- Total genomic DNA extracted from the Grasse River sediment was eight times higher than that extracted from the Hudson River sediment.
- Sulfate reducing bacteria were more abundant in the Grasse River sediment.
- PCB degrading organisms were more abundant in the Hudson River sediment.
- *Dehalococoides* and *Chloroflexi* groups were detected in Hudson *and* Grasse River sediments.
- All microbial populations show differences between the Hudson and Grasse Rivers using diversity indicators (e.g., DGGE).

Conclusion: Microbial diversity indicators are useful as tools to evaluate differences in microbial populations that result from differences in sediment geochemistry, whether in native sediment samples or in amended microcosms.

Task A-2 Characterization of Sediment Cores

- Only one core (18M) was intact from 10 taken in the Grasse River and 4 taken in the Hudson River.
- MDPR is greater in deep undisturbed sediments. PCB 8 is an observed end-product in the Grasse River.
- Percent chlorine is lower in deep undisturbed sediments.
- Congeners with fewer than three chlorines account for 75% of the total PCBs in deep undisturbed sediments.
- Congener distributions in core segments show significant differences from Aroclor 1248 and Aroclor 1260 (putative discharge congeners for the Grasse River).
- Congener distributions in core segments show significant differences from tracker pair relationship that represent Aroclor mixtures. Deeper segments show shifts further from tracker relationships than shallower segments.

- *Bacteria* (BAC), *Archaea* (ARC), sulfate reducing bacteria (SRB), *Chloroflexi* group (CHL), *Dehalococcoides* group (DHC), and PCB degraders (*o-17* and DF-1 strains) were detected in all segments of the core by PCR assays.
- Selective enrichment of *Dehalococcoides* and *o-17/DF-1* groups was observed in the core with depth. Older sediments had more dechlorination-related bacteria.
- Grasse River sediment *Chloroflexi* clones were more similar to each other than to known putative dechlorinating *Chloroflexi*.
- MDPR was correlated with concentration of *Dehalococcoides* but not with concentration of *Chloroflexi* in the core.

Conclusion: PCBs in Grasse River sediments have undergone reductive dechlorination for decades and the Grasse River contains abundant PCB degrading populations throughout the sediment. Core analysis is a useful technique for evaluation of MNA potential.

Task B. PCB Model Development

- Classification trees built for PCB dechlorination processes enable evaluation of unobserved pathways. High true classification rates confirm the suitability of the classification trees. The trees identify five times more pathways as belonging to Processes than previously explicitly reported pathways.
- Dechlorination scenarios highlight the utility of the expanded Process definitions to assist with prediction of congener end point in sediments undergoing reductive dechlorination.
- The DPE model demonstrates the capability to simulate large, complex and incompletely understood chemical transformations in a statistically rigorous manner.
- Application of the DPE to a laboratory experiment suggests that the DPE is suitable for the identification of process occurrence.

Conclusion: CTDPGs reflect the capability of microbe(s) that have not been identified or are not well understood and can provide insight into congener endpoints in laboratory experiments and in the field, particularly in sediments undergoing monitored natural attenuation. The dechlorination process estimator will enable more accurate identification of processes occurring in experimental systems.

Task C. Microcosm Study

- PCB dechlorination was observed in all treatments except the Hudson River sediment amended with iron.
- Pathways not previously reported but predicted by CTDPGs were observed in the microcosms.
- Sulfate-amended microcosms showed flanked *para* and *para*-flanked *meta* dechlorination. *Ortho*-flanked *meta* dechlorinaton was inhibited until sulfate concentrations declined.
- Ferric iron addition significantly decreased rate and extent of dechlorination. The effect was greater in the Hudson River sediment than in the Grasse River sediment.
- Specific congener effects of amendments were observed: PCB 170 was transformed to a greater extent in iron amended microcosms. PCB 32 persisted in iron amended sediments, while it was transformed in microcosms without iron amendment.
- PCB dechlorination was observed concurrent with iron reduction in Grasse River sediments; however, no dechlorination was observed in iron amended Hudson River sediments.
- Bacterial diversity changed in the microcosms in response to the specific PCBs in the spiked mixture and in response to the different amendments.

Conclusion: PCB dechlorination is significantly affected by sediment biogeochemistry (including sulfate and iron concentrations) as well as by initial sediment microbial community (Grasse has more dechlorinators than Hudson). Initial PCB congeners, sediment biogeochemistry and microbial community all affect rate, extent and nature of PCB dechlorination in ways that can be quantified through careful experimentation.

The technical objectives of the current study were met. Task A met the objective of using molecular microbiological tools to evaluate population differences and identify organisms associated with PCB dechlorination in river sediments. Although difficulties with collection of intact cores limited the breath of our analysis of the changes associated with long time periods in PCB contaminated rivers, the core study highlighted critical features of deep sediments that suggest long-term PCB dechlorination in buried sediments is viable. Task B met the objective to develop new statistical methods to analyze distributions of PCB congeners in weathered sediments. We integrated advances in Bayesian analysis that allowed us to estimate the probability that specific processes for microbially-mediated reductive dechlorination of PCBs are occurring in particular situations. The method is more applicable to experimental conditions where congeners can be controlled, but its application to field sites is a logical next step for research. The objective to link environmental conditions, congener distributions and bacterial population dynamics to decision support tools was only partly met as this objective and its associated tasks were removed during a scope reduction in 2008. Task C explored the role of environmental conditions, congener distributions and bacterial population dynamics in PCB transformations through an extensive microcosm study. Parallel work to integrate these findings into a decision-support tool (Task D) was halted in 2008; however, the results of the microcosm study suggest a role for integration of key features of sediment systems within decision models. A logical next step would be to integrate the statistical models from Task B with the knowledge gained in Tasks A and C to create a decision model that could be used to predict the suitability of monitored natural attenuation at specific PCB-contaminated sites.

Direct implementation of the results will require the integration that was the final planned (but not completed) component for this work. The present study significantly increased our understanding of features controlling the transformation of PCBs in sediment systems. Further, it improved our understanding of how to identify critical system features through targeted experimental and modeling analysis work. Extending the present work to additional sediment sites and coupling the new knowledge with a decision support model are critical next steps to enable implementation at field scale.

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Final Report

ER 1495

Modeling and decision support tools based on the effects of sediment geochemistry and microbial populations on contaminant reactions in sediments

Appendix A

Version 2.0

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Appendix A

Supporting Data

Table A-1. Grasse River Core Sampling 2006

Sample Location	Sample Date	Collection Time	Northing [1]	Easting [1]	Water Depth (ft)	Probe Depth (ft)	Penetration Depth (ft)	Recovery Depth (ft)	Description	
7M	8/7/06	1300	2227150.0	397698.5	14.1	1.7	1.3	1.05	0-1.50 ft	Gray brown fine sand, trace silt, trace organics
18M	8/7/06	1220	2230252.6	402307.2	13.0	7.0	7.8	7.25	0-0.64 ft	Dark gray brown organics (vegetation, wood pulp), little silt
									0.64-0.85 ft	Dark gray brown fine sand, trace silt, trace organics (wood pulp)
									0.85-0.95 ft	Dark gray brown organics, trace silt, trace fine sand
									0.95-1.15 ft	Gray brown fine sand, trace silt, trace organics (wood pulp)
									1.15-2.53 ft	Dark brown organics (wood pulp), trace silt, trace fine sand
									2.53-3.30 ft	Gray brown fine sand, trace silt, trace organics (wood pulp)
									3.30-4.75 ft	Dark brown silt, trace fine sand, trace organics (vegetation, roots)
									4.75-4.95 ft	Dark gray brown fine sand, trace coarse sand, trace silt
									4.95-5.01 ft	Dark gray brown coarse sand, fine gravel, trace fine to medium sand
									5.01-5.14 ft	Light gray brown fine to medium sand, trace coarse sand, trace fine gravel, trace silt
									5.14-7.25 ft	Dark brown silt, trace organics (vegetation)
23N	8/7/06	1145	2230601.8	404663.4	5.0	2.8	2.8	2.3	0-0.2 ft	Dark brown very loose silt, trace organics (vegetation), trace very fine sand
									0.2-0.8 ft	Dark gray brown clay, little silt, trace fine sand, trace organics (vegetation)
									0.8-1.4 ft	Dark brown organic silty gray clay, trace organics (vegetation)
									1.4-2.3 ft	Gray clay, little dark brown high degraded natural organics, trace fine sand
30S	8/7/06	1445	2232260.7	407121.8	1.6	2.7	2.5	2.1	0-0.6 ft	Dark gray brown fine sand, trace silt, trace organics (vegetation)
									0.6-0.8 ft	Gray coarse gravel, little fine sand, trace silt
									0.8-1.55 ft	Dark gray brown fine sand, little silt, trace fine gravel, trace organics (vegetation)
									1.55-2.1 ft	Dark brown highly degraded organic silt, trace fine sand, trace organics (wood)
									0-0.52 ft	Dark gray fine sand, trace silt, trace fine to medium gravel, trace organics (vegetation, roots)
									0.52-2.6 ft	Dark gray brown organic silt, little highly degraded organics, trace fine sand
32S	8/7/06	1505	2231770.1	407629.7	1.0	2.6	2.6	2.6	0-0.52 ft	Dark gray fine sand, trace silt, trace fine to medium gravel, trace organics (vegetation, roots)
									0.52-2.6 ft	Dark gray brown organic silt, little highly degraded organics, trace fine sand

Table A-2. Grasse River Core Sampling 2007

Sample Location	Sample Date	Collection Time	Northing [1]	Easting [1]	Water Depth (ft)	Probe Depth (ft)	Penetration Depth (ft)	Recovery Depth (ft)	Description	
T46.5M	9/26/2007	0822	2234177.3	414324.7	17.7	2.5	2.2	1.9	0-1.9 ft	Dk brown soft silt, little organics (roots, leaves)
T18M	9/26/2007	0945	2230218.3	402270.2	12.9	6.0	5.4	5.2	0-2.7 ft	Dk grey brown silt, some organics (wood, wood pulp), little fine sand
									2.7-5.2 ft	Dk grey brown soft silt, little organics (wood, wood pulp)
T44M	9/26/2007	1230	2233613.4	413156.5	15.0	5.0	5.0	4.2	0-4.2 ft	Dk brown soft silt, little organics (roots, wood pulp)
T28M	9/26/2007	1025	2232105.4	406401.0	25.7	3.4	2.1	1.5	1-1.5 ft	Dk brown-black soft silt, little fine sand, trace organics (roots, wood pulp); strong odor

Table A-3. Individual PCB recoveries in Analytical Method

IUPAC#	Structure	Arithmetic Mean	Standard Deviation
1	2-	107.7	7.3
2	3-	107.7	9.5
3	4-	92.3	0.0
4/10	2-2 + 10	86.5	1.0
5	23-	121.8	4.1
6	2-3	106.4	12.7
7	24-	85.6	5.5
8	2-4	104.0	10.1
9	25-	114.5	1.7
10/4	26- + 4	86.5	1.0
11/18	3-3 + 18	94.4	12.5
12	34-	98.3	0.6
13/27	3-4 + 27	94.0	5.8
14	35-	88.4	6.4
15	4-4	104.1	4.5
16	23-2	97.3	5.2
17	24-2	89.6	0.8
18/11	25-2 + 11	94.4	12.5
19	26-2	96.5	5.9
20/33	23-3 + 33	81.3	2.6
21	234-	82.0	0.6
22	23-4	86.6	3.4
23	235-	86.7	1.2
24	236-	99.3	4.6
25	24-3	96.8	7.5
26/50	25-3 +50	86.6	0.0
27/13	26-3	94.0	5.8
28	24-4	93.2	4.1
29	245-	90.4	7.1
30	246-	89.9	0.1
31/53	25-4	85.0	5.2
32	26-4	84.2	8.0
33/20	34-2	81.3	2.6
34	35-2	95.5	6.3
35	34-3	86.9	3.4
36/69	35-3	92.1	9.2
37	34-4	85.3	1.0
38/75	345-	81.0	3.4
39/47/62/65	35-4	94.6	6.3
40/72	23-23	81.7	5.3
41	234-2	85.9	1.0

IUPAC#	Structure	Arithmetic Mean	Standard Deviation
42/59	23-24	87.7	4.7
43	235-2	81.6	2.9
44	23-25	87.5	4.1
45	236-2	85.4	1.3
46	23-26	86.3	1.6
47/39/62/65	24-24	94.6	6.3
48	245-2	86.8	5.8
49	24-25	90.5	2.7
50/26	246-2	86.6	0.0
51	24-26	86.6	2.0
52	25-25	81.2	3.5
53/31	25-26	85.0	5.2
54	26-26	97.3	2.9
55	234-3	84.0	3.2
56	23-34	83.8	1.4
57/94	235-3	87.5	0.0
58/67	23-35	88.3	1.2
59/42	236-3	87.7	4.7
60	234-4	88.3	8.4
61/102	2345-	99.9	12.2
62/39/47/65	2346-	94.6	6.3
63	235-4	84.7	4.3
64	236-4	98.6	6.9
65/39/47/62	2356-	94.6	6.3
66	24-34	88.2	6.4
67/58	245-3	88.3	1.2
68	24-35	88.2	5.5
69/36	246-3	92.1	9.2
70	25-34	94.8	0.7
71	26-34	88.4	4.7
72/40	25-35	81.7	5.3
73	26-35	86.5	4.3
74	245-4	94.5	9.4
75/38	246-4	81.0	3.4
76/93	345-2	84.7	1.3
77	34-34	83.8	0.6
78	345-3	96.8	7.7
79	34-35	87.3	7.9
80	35-35	88.8	5.2
81	345-4	86.5	3.0
82	234-23	85.2	2.6
83/119	235-23	86.2	4.2
84/89	236-23	86.8	3.2

IUPAC#	Structure	Arithmetic Mean	Standard Deviation
85	234-24	87.2	8.1
86/112	2345-2	84.8	0.4
87/136	234-25	79.6	1.9
88	2346-2	80.8	5.1
89/84	234-26	86.8	3.2
90	235-24	82.9	7.6
91	236-24	86.7	2.3
92	235-25	85.8	0.7
93/76	2356-2	84.7	1.3
94/57	235-26	87.5	0.0
95	236-25	90.6	0.2
96	236-26	85.6	2.5
97	245-23	86.0	2.4
98	246-23	89.2	0.2
99	245-24	88.2	5.4
100	246-24	91.4	5.3
101	245-25	93.9	3.4
102/61	245-26	99.9	12.2
103	246-25	88.8	5.3
104	246-26	92.9	7.0
105	234-34	86.9	2.1
106/142	2345-3	78.6	1.8
107	234-35	83.3	0.5
108/125	2346-3	81.5	4.2
109/134	235-34	88.8	1.0
110	236-34	86.0	2.4
111	235-35	85.1	6.2
112/86	2356-3	84.8	0.4
113	236-35	87.4	2.8
114	2345-4	92.3	3.4
115	2346-4	79.8	3.3
116	23456-	84.3	1.7
117	2356-4	88.6	7.7
118	245-34	81.1	0.3
119/83	246-34	86.2	4.2
120	245-35	83.9	1.4
121	246-35	80.7	3.0
122/184	345-23	89.2	3.8
123	345-24	93.9	4.5
124/140	345-25	88.8	1.1
125/108	345-26	81.5	4.2
126	345-34	78.5	4.4
127	345-35	81.5	1.0

IUPAC#	Structure	Arithmetic Mean	Standard Deviation
128/159	234-234	84.8	2.3
129/163	2345-23	85.2	3.7
130	234-235	81.6	2.8
131/188	2346-23	86.4	5.4
132/161	234-236	85.5	1.3
133	235-235	84.5	0.3
134/109	2356-23	88.8	1.0
135	235-236	93.3	4.3
136/87	236-236	79.6	1.9
137	2345-24	88.0	0.2
138/160	234-245	86.5	2.8
139/143	2346-24	89.4	2.5
140	234-246	88.8	1.1
141/176	2345-25	89.4	1.1
142/106	23456-2	78.6	1.8
143/139	2345-26	89.4	2.5
144	2346-25	84.3	0.7
145	2346-26	90.3	0.9
146	235-245	83.8	0.9
147	2356-24	87.3	1.5
148	235-246	84.1	3.0
149	236-245	87.8	4.5
150	236-246	88.6	1.5
151	2356-25	89.1	3.1
152	2356-26	90.1	4.5
153	245-245	90.1	0.2
154	245-246	82.8	5.4
155	246-246	86.1	8.2
156	2345-34	82.4	1.9
157/172	234-345	84.4	2.5
158/178	2346-34	84.2	2.6
159/128	2345-35	84.8	2.3
160/138	23456-3	86.5	2.8
161/132	2346-35	85.5	1.3
162	235-345	89.1	1.5
163/129	2356-34	85.2	3.7
164	236-345	91.7	7.0
165	2356-35	79.3	2.5
166	23456-4	85.9	6.4
167/202	245-345	86.6	2.6
168	246-345	91.7	3.7
169/203	345-345	83.9	3.3
170	2345-234	83.8	0.3

IUPAC#	Structure	Arithmetic Mean	Standard Deviation
171/201/204	2346-234	88.6	2.3
172/157	2345-235	84.4	2.5
173	23456-23	87.0	2.3
174	2345-236	85.9	1.3
175/182	2346-235	87.3	1.6
176/141	2346-236	89.4	1.1
177	2356-234	83.9	6.4
178/158	2356-235	84.2	2.6
179	2356-236	88.9	4.9
180	2345-245	84.7	2.1
181	23456-24	83.1	4.1
182/175	2345-246	87.3	1.6
183	2346-245	85.9	1.4
184/122	2346-246	89.2	3.8
185	23456-25	84.1	0.5
186	23456-26	89.5	6.9
187	2356-245	84.6	0.7
188/131	2356-246	86.4	5.4
189	2345-345	92.2	4.9
190	23456-34	87.3	2.5
191	2346-345	83.4	5.6
192	23456-35	83.5	3.8
193	2356-345	83.3	1.1
194	2345-2345	91.5	0.8
195/207	23456-234	93.2	2.3
196	2345-2346	92.2	1.3
197	2346-2346	85.9	1.5
198	23456-235	88.4	1.4
199	2345-2356	87.8	3.5
200	23456-236	87.4	1.1
201/170/204	2346-2356	88.6	2.3
202/167	2356-2356	86.6	2.6
203/169	23456-245	83.9	3.3
204/170/201	23456-246	88.6	2.3
205	23456-345	93.1	4.3
206	23456-2345	91.1	2.8
207/195	23456-2346	93.2	2.3
208	23456-2356	89.2	2.9
209	23456-23456	91.4	1.4

Table A-4. PCB Concentrations.
Concentrations calculated from neat PCBs dissolved in 1 L hexane and PCB concentrations measured by GC-ECD with X50, X200 and X500 dilutions. Confirmation of concentrations in PCB mixtures created for spiking.

PCB Mixture 1

IUPAC#	mg/L	x50	x200	x500
5	54.01	51.41	53.85	54.03
12	39.59	35.86	39.94	42.6
64	59.06	55.59	58.05	58.43
71	34.54	32.33	34.16	35.03
105	87.61	80.13	83.99	88.24
114	5.99	5.98	6.65	7.04
149	72.82	65.95	72.85	74.71
153	79.75	78.95	79.43	80.39
170	34.73	34.14	35.52	37.41
Total	468.09	440.34	464.44	477.88

PCB Mixture 2

IUPAC#	mg/L	x50	x200	x500
5	54.01	50.33	54.36	54.07
12	39.59	35.21	40.07	39.67
64	59.06	54.75	58.47	58.91
71	34.54	31.73	34.16	34.13
82	33.04	29.33	32.12	33.28
97	63.46	57.89	64.37	62.66
99	90.70	80.05	83.57	90.28
144	11.70	11.18	11.4	11.82
170	81.90	81.1	82.73	84.12
Total	468.00	431.57	461.25	468.94

Table A- 5. Tracker Pair Abundances and concentrations in microcosms.
A: PCB Mixture 1, B: PCB Mixture 2. (50 ppm in total; 250 ug/g dry sediment)

Tracker Pairs	Model	Slope (m)	b (intercept)	R-square	Abundance (X%)	Abundance (Y%)	Ratio	Final Cx ppm	Final Cy ppm
5/12 (Y/X X+Y=20)	Log-Log Linear Model	0.77	0.06	1.00	12.10	7.90	0.66		
5/12 (Y/X X+Y=20)	Linear Model	1.23	0.04	0.99	8.90	11.10	1.24	4.46	5.54
5/12 (Y/X X+Y=20)	Linear Model (intercept=0)	1.36	0.00	0.95	8.46	11.54	1.36	4.23	5.77
64/71 (Y/X X+Y=20)	Log-Log Linear Model	1.07	0.27	0.97	6.41	13.87	2.12		
64/71 (Y/X X+Y=20)	Linear Model	1.69	0.02	0.99	7.42	12.58	1.70	3.71	6.29
64/71 (Y/X X+Y=20)	Linear Model (intercept=0)	1.71	0.00	0.99	7.38	12.62	1.71	3.69	6.31
105/114 (Y/X X+Y=20)	Log-Log Linear Model	0.95	1.07	0.81	1.60	18.40	11.47		
105/114 (Y/X X+Y=20)	Linear Model	14.73	-0.02	0.99	1.27	18.73	14.72	0.64	9.36
105/114 (Y/X X+Y=20)	Linear Model (intercept=0)	14.68	0.00	0.99	1.28	18.72	14.68	0.64	9.36
149+153+170 =40%									
149/153 (Y/X)	Log-Log Linear Model	0.97	-0.04	1.00	18.75	15.73	0.84		
149/153 (Y/X)	Linear Model	0.92	0.06	0.99	16.85	15.58	0.92	8.43	7.79
149/153 (Y/X)	Linear Model (intercept=0)	0.91	0.00	0.99	17.03	15.55	0.91	8.52	7.78
149/170 (Y/X)	Log-Log Linear Model l	1.14	0.35	0.91	5.52	15.73	2.85		
149/170 (Y/X)	Linear Model	2.02	0.27	0.96	7.57	15.58	2.06	3.79	7.79
149/170 (Y/X)	Linear Model (intercept=0)	2.10	0.00	0.86	7.42	15.55	2.10	3.71	7.78

Tracker Pairs	Model	Slope (m)	b (intercept)	R-square	Abundance (X%)	Abundance (Y%)	Ratio	Final Cx ppm	Final Cy ppm
5/12 (Y/X X+Y=20)	Log-Log Linear Model	0.77	0.06	1.00	12.10	7.90	0.66		
5/12 (Y/X X+Y=20)	Linear Model	1.23	0.04	0.99	8.90	11.10	1.24	4.46	5.54
5/12 (Y/X X+Y=20)	Linear Model (intercept=0)	1.36	0.00	0.95	8.46	11.54	1.36	4.23	5.77
64/71 (Y/X X+Y=20)	Log-Log Linear Model	1.07	0.27	0.97	6.41	13.87	2.12		
64/71 (Y/X X+Y=20)	Linear Model	1.69	0.02	0.99	7.42	12.58	1.70	3.71	6.29
64/71 (Y/X X+Y=20)	Linear Model (intercept=0)	1.71	0.00	0.99	7.38	12.62	1.71	3.69	6.31
PCB 82/97/99 =40%									
82/97 (Y/X)	Log-Log Linear Model	0.70	-0.27	0.91	7.16	2.13	0.30		
82/97 (Y/X)	Linear Model	0.49	0.05	0.97	13.59	6.73	0.50	6.79	3.36
82/97 (Y/X)	Linear Model (intercept=0)	0.52	0.00	0.95	13.57	7.05	0.52	6.78	3.53
82/99 (Y/X)	Log-Log Linear Model	0.57	-0.35	0.91	30.72	2.13	0.07		
82/99 (Y/X)	Linear Model	0.34	0.07	0.97	19.70	6.73	0.34	9.85	3.36
82/99 (Y/X)	Linear Model (intercept=0)	0.36	0.00	0.95	19.38	7.05	0.36	9.69	3.53
144/170 (Y/X X+Y=20)	Log-Log Linear Model	1.05	-0.80	0.97	16.90	3.10	0.18		
144/170 (Y/X X+Y=20)	Linear Model	0.14	0.02	0.96	17.56	2.44	0.14	8.78	1.22
144/170 (Y/X X+Y=20)	Linear Model (intercept=0)	0.14	0.00	0.96	17.50	2.50	0.14	8.75	1.25

Table A-6. Calculated and true PCB concentrations.
Confirmation of initial spiked concentrations in sediment microcosms in spiked Hudson and Grasse River sediments.

Sediments spiked with PCB Mixture1

IUPAC#	mg/kg SED	Hud A	Hud B	Gra A	Gra B
5	57.7	55.1	55.44	56.43	56.82
12	42.3	40.3	40.95	41.89	42.12
64	63.1	60.04	60.41	63.53	62.61
71	36.9	36.03	36.21	36.48	36.15
105	93.6	88.45	89.7	93.72	92.28
114	6.4	6.64	6.66	7.12	7.05
149	77.8	75.29	75.08	77.49	76.65
153	85.2	84.37	85.28	86.09	85.05
170	37.1	36.45	37.12	39.1	39.09
Total	500.1	482.7	486.9	501.9	497.8

Sediments spiked with PCB Mixture 2

IUPAC#	mg/kg SED	Hud A	Hud B	Gra A	Gra B
5	57.7	51	55.1	55.9	57
12	42.3	39.5	41.1	42	42.4
64	63.1	62.5	61.9	63.3	62.7
71	36.9	37.4	36.7	38.2	37.9
82	35.3	35.1	32.6	33.8	33.3
97	67.8	66.8	62.1	64.2	63.4
99	96.9	98.4	94.2	97.2	95.8
144	12.5	13.7	12.6	13.1	12.8
170	87.5	95.8	88.4	93.8	91.5
Total	500	500.2	484.7	501.5	496.8

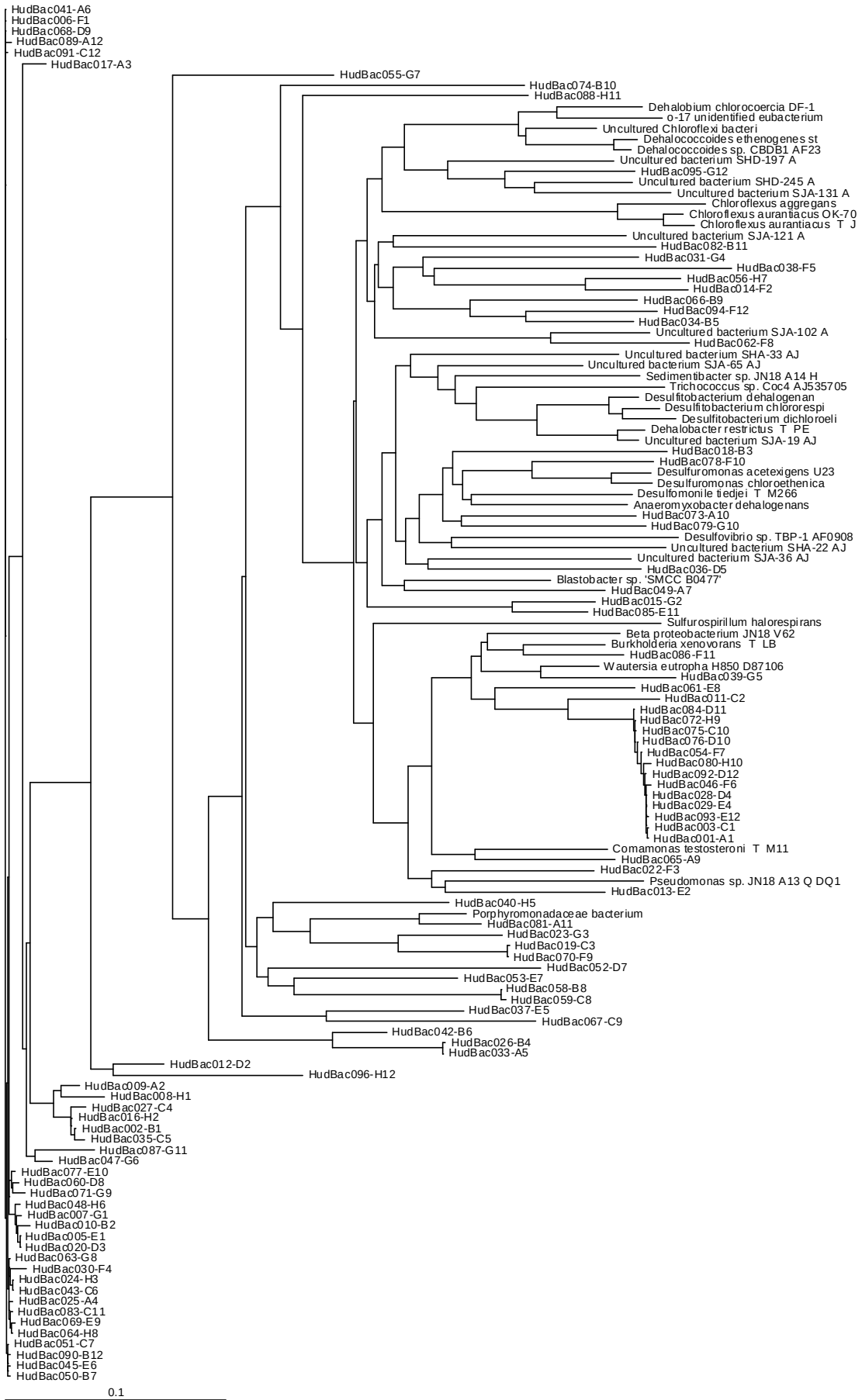

Figure A-1. A phylogenetic tree of Bacteria – Hudson: 91 clones + 37 references strains (dechlorinators)

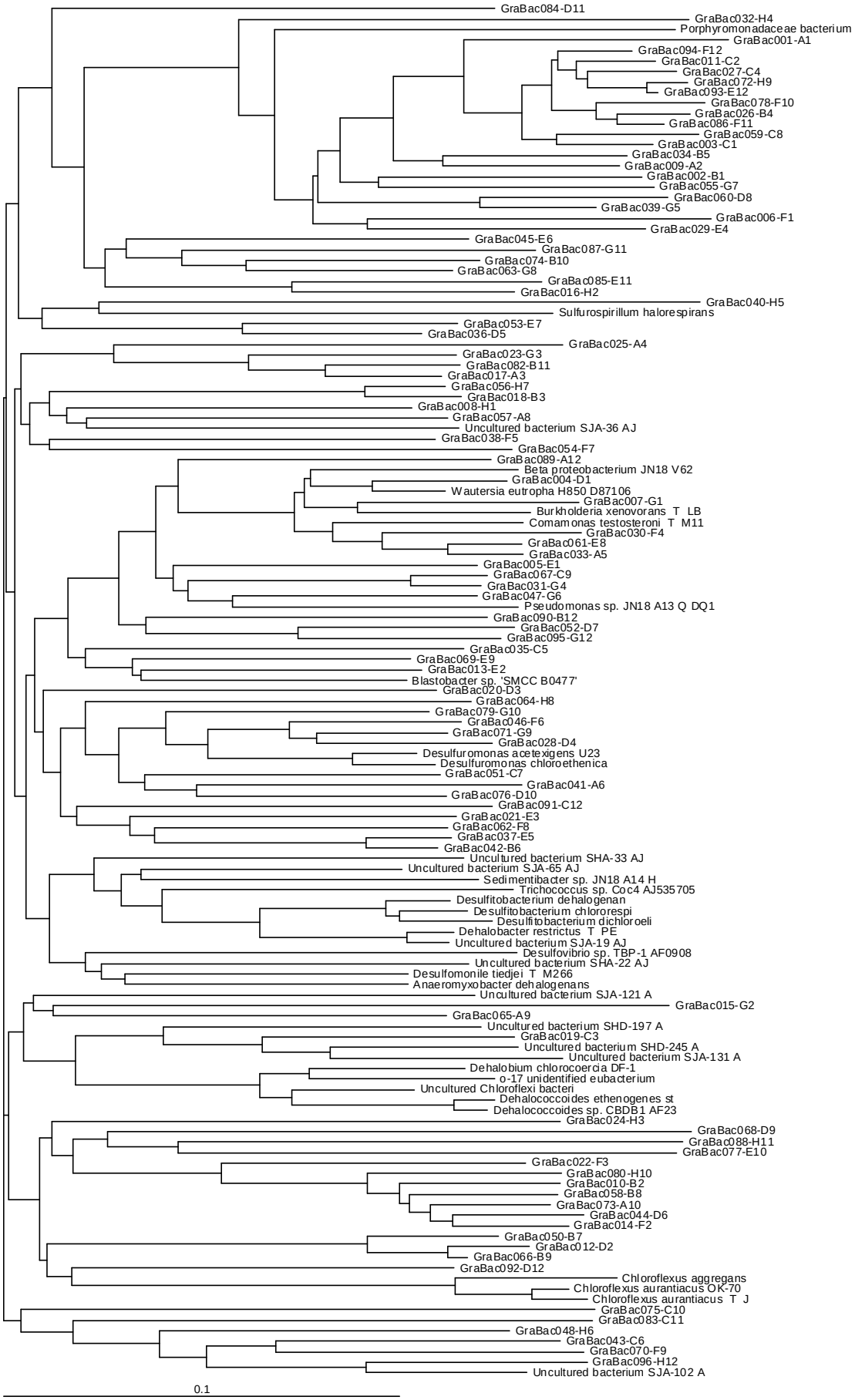

Figure A-2. A phylogenetic tree of Bacteria – Grasse: 94 clones + 37 references strains (dechlorinators)

Figure A-3. A phylogenetic tree of Archaea – Hudson: 93 clones + 14 references strains

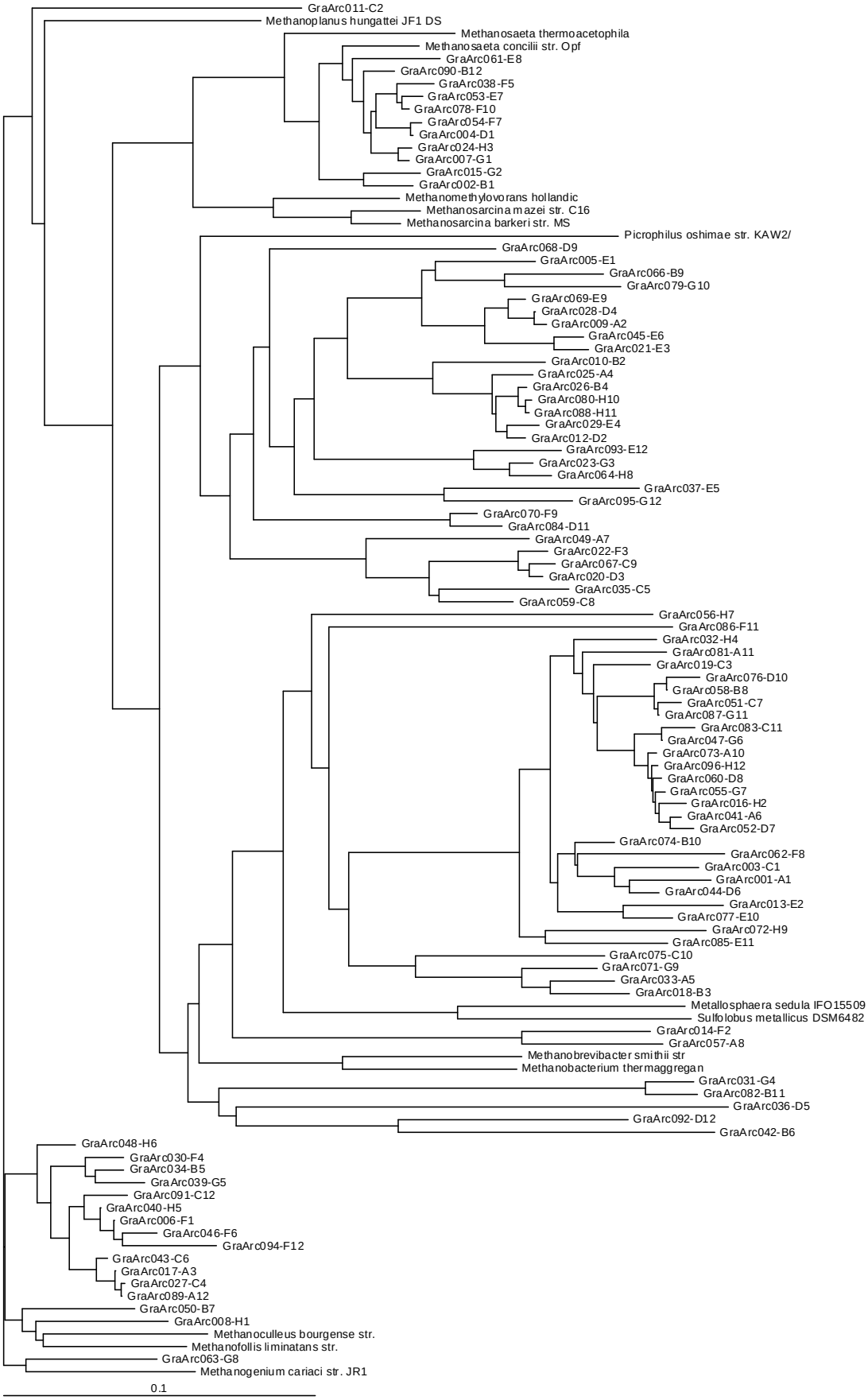

Figure A-4. A phylogenetic tree of Archaea – Grasse: 95 clones + 14 references strains

no rank Root (91/94/9.99E-1) (Hudson clone number/Grasse clone number/significance value)
» » domain Bacteria (91/94/9.99E-1)
» » » phylum Spirochaetes (1/2/6.5E-1)
» » » » class Spirochaetes (1/2/6.5E-1)
» » » » » order Spirochaetales (1/2/6.5E-1)
» » » phylum Chloroflexi (1/1/9.76E-1)
» » » » class Anaerolineae (1/1/9.76E-1)
» » » » » order Anaerolinaeles (1/1/9.76E-1)
» » » phylum Nitrospira (2/5/3.11E-1)
» » » » class Nitrospira (2/5/3.11E-1)
» » » » » order Nitrospirales (2/5/3.11E-1)
» » » phylum Lentisphaerae (1/0/4.84E-1)
» » » » class Lentisphaerae (1/0/4.84E-1)
» » » » » order Victivallales (1/0/4.84E-1)
» » » phylum Verrucomicrobia (2/3/7.18E-1)
» » » » class Verrucomicrobiae (2/3/7.18E-1)
» » » » » order Verrucomicrobiales (2/3/7.18E-1)
» » » phylum Actinobacteria (2/0/2.38E-1)
» » » » class Actinobacteria (2/0/2.38E-1)
» » » » » subclass Coriobacteridae (2/0/2.38E-1)
» » » phylum Proteobacteria (26/30/6.24E-1)
» » » » class Alphaproteobacteria (2/2/9.7E-1)
» » » » » order Rhizobiales (0/1/5.16E-1)
» » » » » order Rhodospirillales (1/0/4.84E-1)
» » » » » unclassified Alphaproteobacteria (1/1/NA)
» » » » class Deltaproteobacteria (5/13/7.43E-2)
» » » » » order Desulfobacterales (0/2/2.62E-1)
» » » » » order Desulfuromonales (1/4/2.34E-1)
» » » » » order Syntrophobacterales (1/4/2.34E-1)
» » » » » order Myxococcales (1/1/9.76E-1)
» » » » » unclassified Deltaproteobacteria (2/2/NA)
» » » » class Betaproteobacteria (17/6/1.14E-2)
» » » » » order Burkholderiales (0/3/1.33E-1)
» » » » » order Hydrogenophilales (1/0/4.84E-1)
» » » » » order Rhodocyclales (15/0/2.35E-5)
» » » » » unclassified Betaproteobacteria (1/3/NA)
» » » » class Gammaproteobacteria (2/7/1.21E-1)
» » » » » order Xanthomonadales (0/1/5.16E-1)
» » » » » order Methylococcales (1/2/6.5E-1)
» » » » » unclassified Gammaproteobacteria (1/4/NA)
» » » » unclassified Proteobacteria (0/2/NA)
» » » phylum Bacteroidetes (50/20/2.6E-6)
» » » » class Sphingobacteria (1/1/9.76E-1)
» » » » » order Sphingobacteriales (1/1/9.76E-1)
» » » » class Bacteroidetes (3/3/9.65E-1)
» » » » » order Bacteroidales (3/3/9.65E-1)

Figure A-5.Comparsion of Hudson and Grasse River bacterial clone libraries. Numbers in parentheses indicate number of clones from the Hudson Library, number of clones from the Grasse Library and percent homology. Significant differences were highlighted when $p<0.05$.

Full Congener Analysis for Core Segments

Table A-7. Individual PCB concentrations in 20 analyzed Core 18M segments (mg/kg dry wt)

IUPAC#	Structure	Co-eluters	20-25	50-55	75-80	100-105	140-145	145-150	155-160	160-165	165-170	170-175	175-180	180-185	185-190	190-195	195-200	200-205	205-210	210-215	215-220
1	**2-**		0.13	0.45	0.82	0.59	0.13	0.18	0.56	0.26	0.17	0.09	0.12	0.70	1.27	4.56	6.44	46.52	38.33	1.83	8.03
2	**3-**		0.00	0.00	0.00	0.04	0.46	0.24	0.26	0.27	0.21	0.19	0.32	0.42	0.33	0.25	0.25	0.79	0.63	0.17	0.20
3	**4-**		0.00	0.29	0.41	0.31	0.26	0.33	0.78	0.28	0.25	0.00	0.37	1.25	2.39	6.15	7.31	51.06	49.70	4.02	11.93
4	**2-2**	**10**	0.40	0.96	4.10	5.12	1.97	1.28	5.08	1.61	0.81	1.00	1.49	2.72	10.37	40.85	103.23	196.91	155.93	53.92	34.65
5	**23-**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.02	0.01	0.01	0.00
6	**2-3**		0.03	0.06	0.18	0.11	0.15	0.15	0.30	0.08	0.20	0.13	0.75	0.91	1.83	4.23	4.80	6.55	6.83	4.48	3.77
7	**24-**		0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.05	0.09	0.30	0.40	0.05	0.07
8	**2-4**		0.13	0.28	0.81	0.34	0.43	0.36	0.67	0.21	0.23	0.21	0.79	0.87	4.38	15.46	45.98	99.52	80.03	22.50	12.92
9	**25-**		0.00	0.01	0.03	0.01	0.01	0.02	0.02	0.00	0.01	0.01	0.02	0.03	0.07	0.25	0.36	0.62	0.64	0.29	0.46
10	**26-**	**4**	0.12	0.48	0.92	0.99	0.02	0.01	0.02	0.01	0.01	0.03	0.01	0.02	0.10	0.43	0.85	2.22	1.61	0.43	0.28
11	**3-3**	**18**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	**34-**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.00
13	**3-4**	**27**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	**35-**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	**4-4**		0.11	0.14	0.30	0.21	0.95	1.01	0.65	0.44	0.57	1.19	1.52	2.09	6.05	14.94	36.15	75.65	61.61	17.96	9.43
16	**23-2**		0.00	0.00	0.03	0.00	0.06	0.02	0.03	0.02	0.02	0.05	0.05	0.05	0.10	0.20	0.21	0.15	0.14	0.10	0.10
17	**24-2**		0.15	0.28	0.92	0.31	0.99	1.08	1.00	0.59	0.63	0.66	1.45	1.75	5.97	18.88	40.47	87.97	80.62	27.95	14.71
18	**25-2**	**11**	0.06	0.16	0.44	0.15	0.37	0.18	0.30	0.22	0.56	0.73	1.78	2.16	2.37	2.22	2.64	3.47	3.38	2.59	3.06
19	**26-2**		0.41	1.36	2.56	1.06	0.46	0.37	0.69	0.22	0.22	0.48	0.41	0.54	2.31	6.67	16.25	36.02	32.17	9.22	5.31
20	**23-3**	**33**	0.00	0.00	0.03	0.01	0.05	0.01	0.03	0.04	0.01	0.01	0.04	0.03	0.04	0.09	0.10	0.23	0.14	0.09	0.08
21	**234-**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22	**23-4**		0.01	0.02	0.07	0.02	0.08	0.04	0.05	0.04	0.02	0.02	0.04	0.09	0.17	0.14	0.12	0.16	0.14	0.09	0.09
23	**235-**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	**236-**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00
25	**24-3**		0.04	0.04	0.15	0.04	0.19	0.31	0.13	0.14	0.30	0.41	0.97	0.79	1.28	2.15	2.24	2.81	2.84	0.56	0.56
26	**25-3**	**50**	0.16	0.36	0.90	0.23	0.47	0.53	0.46	0.31	0.64	1.36	2.05	2.45	4.29	5.99	7.01	8.89	6.20	3.78	3.03
27	**26-3**	**13**	0.20	0.55	0.95	0.36	0.33	0.38	0.38	0.16	0.16	0.37	0.38	0.59	2.26	7.53	14.52	28.67	22.99	42.64	3.57
28	**24-4**		0.05	0.06	0.20	0.10	0.34	0.35	0.20	0.30	0.40	0.60	0.81	0.51	0.82	0.96	1.73	4.80	4.32	0.69	0.65
29	**245-**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	**246-**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.02	0.00	0.01

31	**25-4**	**53**	0.13	0.25	0.67	0.22	0.51	0.68	0.53	0.43	0.71	0.78	2.28	2.87	5.48	6.09	11.86	14.56	12.10	7.47	6.00
32	**26-4**		0.29	0.77	1.69	0.33	0.57	0.69	0.67	0.36	0.44	1.02	0.99	1.30	3.83	10.53	20.55	45.64	45.73	15.97	7.79
33	**34-2**	**20**	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.03	0.00	0.00	0.00	0.02
34	**35-2**		0.01	0.04	0.07	0.02	0.02	0.03	0.02	0.01	0.02	0.02	0.05	0.06	0.15	0.38	0.48	0.81	1.25	0.58	0.32
35	**34-3**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.00
36	**35-3**	**69**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37	**34-4**		0.01	0.01	0.00	0.02	0.06	0.03	0.02	0.03	0.01	0.02	0.03	0.04	0.07	0.05	0.03	0.04	0.04	0.04	0.04
38	**345-**	**75**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39	**35-4**	**47&62&65**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40	**23-23**	**72**	0.01	0.03	0.02	0.01	0.06	0.01	0.01	0.01	0.02	0.11	0.07	0.11	0.20	0.42	0.26	1.04	0.92	0.12	0.14
41	**234-2**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
42	**23-24**	**59**	0.02	0.05	0.11	0.04	0.10	0.08	0.07	0.10	0.08	0.25	0.24	0.39	0.77	1.20	1.45	1.46	1.54	0.68	0.71
43	**235-2**		0.02	0.04	0.07	0.01	0.02	0.03	0.03	0.03	0.03	0.07	0.07	0.06	0.13	0.12	0.22	0.96	0.95	0.21	0.20
44	**23-25**		0.03	0.09	0.22	0.09	0.23	0.10	0.14	0.23	0.19	0.52	0.50	0.37	0.73	1.44	1.90	2.47	1.43	0.91	1.08
45	**236-2**		0.02	0.05	0.09	0.03	0.05	0.04	0.04	0.04	0.05	0.14	0.13	0.16	0.29	0.57	0.69	0.75	0.69	0.48	0.30
46	**23-26**		0.01	0.02	0.04	0.01	0.03	0.02	0.02	0.02	0.05	0.13	0.14	0.17	0.22	0.20	0.18	0.19	0.14	0.09	0.07
47	**24-24**	**39&62&65**	0.19	0.43	0.67	0.21	0.47	0.64	0.32	0.42	0.41	0.86	0.91	1.09	2.85	6.59	11.87	26.54	24.78	8.29	3.91
48	**245-2**		0.00	0.02	0.02	0.02	0.03	0.01	0.01	0.03	0.05	0.01	0.07	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
49	**24-25**		0.13	0.30	0.66	0.20	0.45	0.47	0.31	0.41	0.61	1.53	1.65	2.05	4.07	6.68	7.48	10.53	8.34	5.01	3.42
50	**246-2**	**26**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
51	**24-26**		0.05	0.12	0.25	0.06	0.16	0.20	0.13	0.13	0.13	0.25	0.27	0.32	0.90	2.20	4.26	9.69	9.81	3.20	1.62
52	**25-25**		0.13	0.34	0.68	0.24	0.49	0.41	0.33	0.43	0.68	1.79	1.83	2.18	4.07	5.99	6.38	8.21	5.90	4.61	4.12
53	**25-26**	**31**	0.06	0.16	0.40	0.11	0.36	0.23	0.00	0.00	0.00	0.43	0.00	0.00	0.00	3.24	0.00	11.62	9.24	1.99	0.00
54	**26-26**		0.01	0.05	0.09	0.02	0.01	0.02	0.02	0.01	0.01	0.02	0.01	0.02	0.06	0.18	0.55	1.20	1.18	0.44	0.24
55	**234-3**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
56	**23-34**		0.00	0.01	0.02	0.04	0.05	0.02	0.02	0.07	0.01	0.01	0.01	0.01	0.02	0.00	0.03	0.02	0.01	0.00	0.01
57	**235-3**	**94**	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.02	0.04	0.05	0.05	0.09	0.17	0.25	0.00	0.00	0.10	0.15
58	**23-35**	**67**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
59	**236-3**	**42**	0.01	0.02	0.05	0.02	0.03	0.03	0.02	0.02	0.02	0.00	0.05	0.08	0.16	0.22	0.31	0.68	0.71	0.79	0.15
60	**234-4**	**60**	0.01	0.01	0.02	0.02	0.03	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.02	0.02
61	**2345-**	**102**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.00
62	**2346-**	**62&39&65**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
63	**235-4**		0.01	0.03	0.05	0.01	0.02	0.04	0.02	0.03	0.03	0.04	0.08	0.07	0.13	0.24	0.31	0.69	0.71	0.14	0.15
64	**236-4**		0.01	0.05	0.13	0.05	0.12	0.04	0.08	0.11	0.05	0.10	0.09	0.15	0.20	0.08	0.16	0.21	0.30	0.38	0.46
65	**2356-**	**39&47&62**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
66	**24-34**		0.01	0.06	0.08	0.10	0.13	0.06	0.07	0.14	0.04	0.06	0.05	0.05	0.06	0.08	0.06	0.09	0.06	0.08	0.06

67	**245-3**	**58**	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.04	0.06	0.10	0.15	0.08	0.04	0.02
68	**24-35**		0.01	0.03	0.04	0.01	0.02	0.02	0.02	0.02	0.02	0.04	0.05	0.07	0.15	0.29	0.40	0.66	0.81	0.36	0.21
69	**246-3**	**36**	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.13	0.00	0.25	0.04	0.03
70	**25-34**		0.01	0.06	0.07	0.09	0.15	0.06	0.08	0.16	0.05	0.08	0.05	0.05	0.05	0.06	0.05	0.08	0.08	0.06	0.06
71	**26-34**		0.04	0.10	0.25	0.06	0.18	0.21	0.12	0.21	0.33	0.75	0.91	0.94	1.39	1.17	1.13	2.20	1.59	1.09	1.84
72	**25-35**	**40**	0.01	0.04	0.08	0.02	0.01	0.04	0.03	0.04	0.07	0.13	0.20	0.22	0.40	0.40	0.56	0.12	0.10	0.45	0.28
73	**26-35**		0.01	0.03	0.06	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.03	0.03	0.09	0.18	0.29	0.65	0.67	0.25	0.17
74	**245-4**		0.02	0.06	0.09	0.06	0.10	0.06	0.06	0.09	0.03	0.03	0.05	0.05	0.08	0.06	0.08	0.15	0.11	0.07	0.04
75	**246-4**	**38**	0.01	0.02	0.03	0.01	0.01	0.03	0.01	0.02	0.02	0.04	0.05	0.05	0.11	0.29	0.33	0.81	0.85	0.19	0.12
76	**345-2**	**93**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
77	**34-34**		0.00	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.03
78	**345-3**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
79	**34-35**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.04	0.03	0.02	0.00
80	**35-35**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
81	**345-4**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
82	**234-23**		0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.03	0.02	0.02	0.02	0.01	0.02	0.03	0.03	0.01	0.01
83	**235-23**	**119**	0.01	0.04	0.05	0.02	0.03	0.05	0.03	0.05	0.05	0.06	0.15	0.15	0.29	0.40	0.78	0.82	0.70	0.33	0.59
84	**236-23**	**89**	0.02	0.07	0.11	0.04	0.08	0.09	0.05	0.08	0.10	0.24	0.27	0.36	0.68	1.04	1.05	1.04	0.80	0.71	0.55
85	**234-24**		0.01	0.03	0.04	0.02	0.03	0.02	0.02	0.04	0.02	0.06	0.05	0.04	0.04	0.04	0.05	0.07	0.06	0.03	0.01
86	**2345-2**	**112**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.00	0.05	0.00	0.00	0.00	0.02
87	**234-25**	**136**	0.01	0.03	0.06	0.02	0.03	0.04	0.03	0.04	0.03	0.06	0.07	0.05	0.03	0.04	0.05	0.11	0.11	0.09	0.07
88	**2346-2**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
89	**234-26**	**84**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
90	**235-24**		0.02	0.06	0.10	0.02	0.04	0.05	0.03	0.03	0.04	0.10	0.10	0.12	0.22	0.45	0.55	1.15	1.33	0.47	0.45
91	**236-24**		0.04	0.11	0.16	0.04	0.08	0.11	0.06	0.08	0.09	0.23	0.24	0.30	0.61	1.04	1.24	1.74	1.59	1.12	0.82
92	**235-25**		0.04	0.13	0.18	0.05	0.07	0.10	0.05	0.08	0.09	0.23	0.26	0.23	0.44	0.88	1.09	1.98	2.27	1.11	1.42
93	**2356-2**	**76**	0.00	0.02	0.03	0.01	0.00	0.01	0.01	0.01	0.01	0.02	0.03	0.02	0.05	0.09	0.13	0.29	0.47	0.15	0.12
94	**235-26**	**57**	0.02	0.07	0.11	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.03	0.03	0.05	0.10	0.13	1.07	1.16	0.19	0.09
95	**236-25**		0.05	0.14	0.33	0.00	0.18	0.24	0.13	0.20	0.20	0.00	0.53	0.54	1.12	0.00	3.27	4.29	3.40	0.00	1.96
96	**236-26**		0.00	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.06	0.12	0.13	0.19	0.19	0.13	0.09
97	**245-23**		0.01	0.04	0.05	0.03	0.04	0.04	0.02	0.05	0.02	0.06	0.05	0.05	0.07	0.08	0.10	0.15	0.10	0.04	0.03
98	**246-23**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.04	0.05	0.08	0.06	0.04	0.02
99	**245-24**		0.03	0.09	0.14	0.05	0.06	0.08	0.04	0.07	0.04	0.10	0.11	0.10	0.14	0.18	0.23	0.42	0.29	0.12	0.05
100	**246-24**		0.01	0.02	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.05	0.11	0.18	0.40	0.40	0.16	0.11
101	**245-25**		0.04	0.12	0.15	0.07	0.08	0.11	0.05	0.10	0.06	0.12	0.14	0.10	0.15	0.20	0.31	0.46	0.24	0.13	0.06
102	**245-26**	**61**	0.02	0.08	0.11	0.03	0.04	0.05	0.03	0.05	0.05	0.09	0.13	0.14	0.26	0.35	0.53	1.03	1.24	0.52	0.71

103	**246-25**		0.01	0.02	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.03	0.03	0.06	0.14	0.20	0.46	0.41	0.25	0.20
104	**246-26**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
105	**234-34**		0.01	0.04	0.05	0.03	0.03	0.04	0.03	0.05	0.03	0.06	0.05	0.05	0.04	0.03	0.04	0.06	0.05	0.03	0.02
106	**2345-3**	**142**	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
107	**234-35**		0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.06	0.07	0.02	0.01
108	**2346-3**	**125**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
109	**235-34**	**134**	0.00	0.01	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.13	0.11	0.00	0.00
110	**236-34**		0.07	0.20	0.29	0.10	0.19	0.16	0.14	0.23	0.19	0.45	0.50	0.57	0.93	1.03	1.38	2.20	2.21	1.68	2.01
111	**235-35**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00
112	**2356-3**	**86**	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.03	0.00	0.09	0.10	0.03	0.00
113	**236-35**		0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.06	0.13	0.26	0.28	0.19	0.17
114	**2345-4**		0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
115	**2346-4**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
116	**23456-**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
117	**2356-4**		0.01	0.03	0.04	0.01	0.02	0.02	0.01	0.02	0.02	0.04	0.04	0.05	0.11	0.20	0.33	0.57	0.54	0.19	0.13
118	**245-34**		0.03	0.11	0.15	0.07	0.10	0.12	0.07	0.12	0.06	0.11	0.13	0.13	0.14	0.11	0.13	0.22	0.09	0.05	0.04
119	**246-34**	**83**	0.01	0.02	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.19	0.00	0.41	0.35	0.14	0.00
120	**245-35**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.02	0.02	0.04	0.04	0.02	0.01
121	**246-35**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.08	0.03	0.03
122	**345-23**	**184**	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
123	**345-24**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
124	**345-25**	**140**	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00
125	**345-26**	**108**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
126	**345-34**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.00	0.00
127	**345-35**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
128	**234-234**	**159**	0.01	0.02	0.02	0.01	0.01	0.02	0.01	0.02	0.02	0.04	0.04	0.04	0.04	0.04	0.08	0.12	0.10	0.03	0.02
129	**2345-23**	**163**	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.05	0.00
130	**234-235**		0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.03	0.03	0.07	0.11	0.23	0.27	0.15	0.16
131	**2346-23**	**188**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.01	0.01
132	**234-236**	**161**	0.02	0.07	0.08	0.03	0.04	0.04	0.03	0.05	0.04	0.11	0.12	0.12	0.17	0.32	0.55	0.91	0.98	0.60	0.64
133	**235-235**		0.01	0.02	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.03	0.07	0.11	0.27	0.34	0.23	0.34
134	**2356-23**	**109**	0.01	0.02	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.04	0.05	0.04	0.07	0.15	0.18	0.16	0.13	0.16	0.13
135	**235-236**		0.02	0.09	0.11	0.03	0.05	0.04	0.03	0.04	0.04	0.08	0.12	0.10	0.17	0.35	0.57	1.14	1.36	1.03	1.26
136	**236-236**	**87**	0.03	0.06	0.06	0.04	0.04	0.03	0.02	0.04	0.02	0.09	0.05	0.04	0.15	0.19	0.27	0.36	0.36	0.29	0.32
137	**2345-24**		0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.02	0.03	0.05	0.05	0.02	0.01
138	**234-245**	**160**	0.04	0.10	0.12	0.03	0.05	0.07	0.04	0.07	0.06	0.13	0.16	0.16	0.15	0.16	0.30	0.52	0.46	0.18	0.11

139	**2346-24**	**143**	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.04	0.05	0.02	0.02
140	**234-246**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.08	0.10	0.05	0.09
141	**2345-25**	**176**	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.01	0.03	0.04	0.04	0.03	0.02	0.08	0.05	0.06	0.04	0.16
142	**23456-2**	**106**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
143	**2345-26**	**139**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
144	**2346-25**		0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.04	0.05	0.05	0.03
145	**2346-26**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
146	**235-245**		0.02	0.07	0.09	0.03	0.03	0.03	0.02	0.03	0.02	0.06	0.08	0.08	0.11	0.23	0.39	0.80	0.91	0.57	0.56
147	**2356-24**		0.01	0.03	0.04	0.01	0.03	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.04	0.06	0.09	0.18	0.21	0.10	0.10
148	**235-246**		0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.08	0.09	0.06	0.09
149	**236-245**		0.07	0.25	0.33	0.09	0.14	0.14	0.09	0.14	0.12	0.31	0.38	0.34	0.52	0.86	1.26	2.28	2.60	1.82	2.09
150	**236-246**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02
151	**2356-25**		0.03	0.11	0.15	0.04	0.04	0.04	0.03	0.04	0.04	0.11	0.13	0.11	0.16	0.32	0.45	0.89	1.14	0.84	0.88
152	**2356-26**		0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.07	0.14	0.05	0.05
153	**245-245**		0.05	0.18	0.22	0.06	0.07	0.09	0.05	0.08	0.06	0.21	0.21	0.21	0.19	0.30	0.43	0.79	0.70	0.39	0.18
154	**245-246**		0.01	0.04	0.05	0.02	0.02	0.01	0.01	0.01	0.01	0.03	0.03	0.03	0.05	0.10	0.17	0.40	0.47	0.24	0.39
155	**246-246**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
156	**2345-34**		0.00	0.02	0.02	0.00	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.05	0.08	0.05	0.02	0.01
157	**234-345**	**172**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.05	0.00
158	**2346-34**	**178**	0.01	0.02	0.02	0.00	0.01	0.01	0.01	0.01	0.01	0.03	0.03	0.03	0.03	0.02	0.10	0.15	0.14	0.06	0.25
159	**2345-35**	**128**	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00
160	**23456-3**	**138**	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00
161	**2346-35**	**132**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
162	**235-345**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
163	**2356-34**	**129**	0.03	0.09	0.11	0.03	0.04	0.05	0.03	0.05	0.04	0.09	0.13	0.13	0.17	0.29	0.55	1.10	1.24	0.56	0.76
164	**236-345**		0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.01
165	**2356-35**		0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.05	0.14	0.16	0.09	0.13
166	**23456-4**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
167	**245-345**	**202**	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.05	0.00
168	**246-345**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
169	**345-345**	**203**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
170	**2345-234**		0.03	0.12	0.12	0.03	0.03	0.04	0.03	0.04	0.03	0.09	0.11	0.11	0.10	0.16	0.29	0.54	0.58	0.43	0.37
171	**2346-234**	**201&204**	0.01	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04	0.04	0.04	0.06	0.15	0.21	0.26	0.21	0.31
172	**2345-235**	**157**	0.01	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.03	0.03	0.04	0.09	0.20	0.22	0.10	0.16
173	**23456-23**	**173**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01
174	**2345-236**		0.03	0.10	0.11	0.03	0.03	0.03	0.02	0.03	0.03	0.10	0.12	0.12	0.08	0.13	0.22	0.39	0.43	0.26	0.22

175	**2346-235**	**182**	0.00	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.09	0.00
176	**2346-236**	**141**	0.01	0.04	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.04	0.00	0.14	0.16	0.14	0.00
177	**2356-234**		0.03	0.10	0.11	0.03	0.03	0.03	0.02	0.03	0.03	0.08	0.09	0.09	0.10	0.20	0.33	0.70	0.88	0.71	0.86
178	**2356-235**	**158**	0.02	0.06	0.08	0.02	0.02	0.01	0.01	0.02	0.01	0.04	0.04	0.04	0.05	0.12	0.15	0.40	0.52	0.48	0.37
179	**2356-236**		0.02	0.08	0.10	0.03	0.03	0.02	0.02	0.03	0.03	0.07	0.08	0.08	0.08	0.14	0.22	0.48	0.62	0.57	0.65
180	**2345-245**		0.07	0.30	0.30	0.07	0.09	0.10	0.07	0.10	0.08	0.20	0.31	0.31	0.27	0.35	0.74	1.40	1.58	1.06	1.12
181	**23456-24**		0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01
182	**2345-246**	**175**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.04	0.09	0.12	0.00	0.13
183	**2346-245**		0.02	0.07	0.08	0.02	0.02	0.02	0.02	0.03	0.02	0.06	0.08	0.08	0.07	0.10	0.19	0.38	0.51	0.43	0.48
184	**2346-246**	**122**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
185	**23456-25**		0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.07	0.08	0.06	0.07
186	**23456-26**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.01	0.00
187	**2356-245**		0.06	0.24	0.29	0.08	0.10	0.07	0.06	0.09	0.08	0.16	0.26	0.26	0.27	0.41	0.77	1.59	2.11	1.47	2.15
188	**2356-246**	**131**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
189	**2345-345**		0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.02	0.02
190	**23456-34**		0.01	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.03	0.02	0.04	0.07	0.16	0.17	0.14	0.14
191	**2346-345**		0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.03	0.00	0.01
192	**23456-35**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
193	**2356-345**		0.00	0.02	0.02	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.02	0.04	0.07	0.08	0.06	0.04
194	**2345-2345**		0.03	0.13	0.13	0.03	0.03	0.03	0.02	0.03	0.03	0.07	0.09	0.09	0.10	0.21	0.38	0.73	0.76	0.58	0.68
195	**23456-234**	**207**	0.02	0.06	0.07	0.01	0.02	0.01	0.01	0.02	0.01	0.03	0.04	0.04	0.05	0.08	0.16	0.36	0.37	0.22	0.35
196	**2345-2346**		0.01	0.06	0.06	0.02	0.01	0.01	0.01	0.02	0.01	0.03	0.04	0.04	0.04	0.09	0.15	0.30	0.35	0.27	0.32
197	**2346-2346**		0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.01	0.03	0.04	0.03	0.04
198	**23456-235**		0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.02	0.04	0.04	0.04	0.04
199	**2345-2356**		0.03	0.12	0.13	0.03	0.03	0.03	0.02	0.04	0.03	0.07	0.11	0.11	0.12	0.21	0.39	0.73	0.88	0.58	0.78
200	**23456-236**		0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.03	0.02	0.05	0.06	0.06	0.07
201	**2346-2356**	**171&204**	0.01	0.02	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.07	0.09	0.08	0.00
202	**2356-2356**	**167**	0.00	0.02	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.03	0.04	0.04	0.11	0.21	0.24	0.08	0.22
203	**23456-245**	**169**	0.02	0.07	0.07	0.02	0.02	0.02	0.01	0.02	0.02	0.04	0.06	0.06	0.06	0.11	0.22	0.41	0.49	0.37	0.47
204	**23456-246**	**171&201**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
205	**23456-345**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
206	**23456-2345**		0.01	0.04	0.04	0.02	0.01	0.01	0.01	0.01	0.01	0.03	0.02	0.02	0.04	0.12	0.16	0.27	0.28	0.21	0.28
207	**23456-2346**	**195**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.08	0.00
208	**23456-2356**		0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.05	0.05	0.04	0.06
209	**23456-23456**		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table A-8. Examples of congener-specific PCB analysis in H-1 microcosms (μg/kg slurry). Total PCBs (ppm) were listed in the last row.

IUPAC#	Structure	Co-eluters	H-1-01	H-1-02	H-1-03	H-1-04	H-1-05	H-1-06	H-1-07	H-1-08	H-1-09	H-1-10	H-1-11	H-1-12	H-1-25	H-1-26	H-1-27	H-1-31	H-1-32	H-1-33
1	2-		0.0	0.0	0.0	0.0	0.0	0.0	360.8	548.8	683.1	1926.	3201	3234	4302.	4869.	4182	4784	5166	5070
2	3-		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	4-		0.0	0.0	0.0	0.0	0.0	0.0	37.8	267.7	238.1	927.0	1643	1606	1610	1945	1700	1573	2006	1763
4	2-2	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2343	3431	658.3	3894	2970	3397
5	23-		5281	5428	5668	5401	4931	5089	4822	3860	4278	2416	507.7	714.9	180.6	160.7	279.2	140.0	141.9	208.7
6	2-3		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	47.2	206.3	76.6	248.7	397.3	310.1	1713	808.2	643.3
7	24-		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.4	708.2	730.2	203.0	31.8	384.1	526.9
8	2-4		0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.9	23.5	77.3	76.5	72.1	116.4	180.0	422.7	566.6	112.4	197.3
9	25-		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	26-	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	138.0	692.8	189.1	3347	1842	417.8
11	3-3	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	34-		4089	3932	4210	4004	3692	3772	3474	2732	3173	1503	539.5	604.7	217.7	239.8	334.2	274.7	232.5	293.9
13	3-4	27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	35-		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	4-4		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	23-2		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	24-2		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	239.9	97.4	162.6	568.4	155.7	140.0
18	25-2	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	115.6	112.1	205.7	272.2	432.7	123.3
19	26-2		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	119.6	251.7	74.2	2148	1052	865.2
20	23-3	33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	234-		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	23-4		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.8	9.8	0.0	0.0	45.3	0.0	0.0	0.0
23	235-		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.7	14.1	0.0
24	236-		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	24-3		0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.4	0.0	175.4	702.9	592.5	3603	2850	3028	907.0	2637	2396
26	25-3	50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	26-3	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	130.7	28.5	16.8	1082	1380	654.7	853.1	1372	2301
28	24-4		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	28.5	35.1	26.5	90.5	82.7	293.9	223.6	110.1	65.3
29	245-		0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.4	11.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	246-		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	25-4	53	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	26-4		0.0	0.0	0.0	0.0	0.0	0.0	5.0	74.1	54.9	422.0	1301	1019	3691	3640.	4027	2037	3330	4010

33	**34-2**	**20**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.9	83.0	373.1	214.9	17.9	38.0	131.9	0.0	0.0	0.0
34	**35-2**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35	**34-3**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
36	**35-3**	**69**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
37	**34-4**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
38	**345-**	**75**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39	**35-4**	**47&62&65**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40	**23-23**	**72**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
41	**234-2**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42	**23-24**	**59**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.8	31.4
43	**235-2**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.6	203.4	0.0	0.0	299.8	170.0
44	**23-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
45	**236-2**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	56.5
46	**23-26**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
47	**24-24**	**39&62&65**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36.8	20.5	487.2	2611.	428.7	29.5
48	**245-2**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
49	**24-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	55.4	28.4	248.2	553.4	288.7	34.5
50	**246-2**	**26**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
51	**24-26**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	68.2	88.4	1075	2374	2914	115.6
52	**25-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.9	0.0	71.3	442.3	217.8	559.4	512.0	1244	313.4	582.0	535.3
53	**25-26**	**31**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	149.3	215.1	71.1	267.6	332.2	781.8
54	**26-26**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
55	**234-3**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.9	7.4	72.4	119.9	61.6	13.1	37.7	16.9	0.0	0.0	32.0
56	**23-34**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	88.1	66.4	144.7	368.2	309.2	0.0	0.0	0.0	9.5	0.0	12.5
57	**235-3**	**94**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
58	**23-35**	**67**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
59	**236-3**	**42**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.5	16.7	16.2	0.0	0.0	0.0
60	**234-4**	**60**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0	7.4	19.0	16.4	0.0	0.0	0.0	0.0	0.0	0.0
61	**2345-**	**102**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
62	**2346-**	**62&39&65**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
63	**235-4**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.3	0.0	22.9	78.0	61.8	311.2	334.6	269.2	157.8	287.7	353.6
64	**236-4**		6369	5999	6147	6318	6002	5837	5566	5530	5446	4913	4193	4487	964.8	797.1	1297	290.5	428.2	779.8
65	**2356-**	**39&47&62**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
66	**24-34**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.0	7.7	42.6	76.4	113.5	49.1	0.0	167.3	126.0	16.8	0.0
67	**245-3**	**58**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

68	**24-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
69	**246-3**	**36**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
70	**25-34**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
71	**26-34**		3864	3646.	3733.	3898	3698	3597	3416	3440	3356	3170	3475	3443	1847	1558	1919	273.4	767.7	262.1
72	**25-35**	**40**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
73	**26-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
74	**245-4**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
75	**246-4**	**38**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
76	**345-2**	**93**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
77	**34-34**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
78	**345-3**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
79	**34-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
80	**35-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
81	**345-4**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
82	**234-23**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
83	**235-23**	**119**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	78.1	667.5	41.9	0.0	201.5	624.1
84	**236-23**	**89**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85	**234-24**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
86	**2345-2**	**112**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
87	**234-25**	**136**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
88	**2346-2**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
89	**234-26**	**84**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
90	**235-24**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1417	61.5	864.5	206.2	309.8	72.8
91	**236-24**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	41.8	45.8	65.4	0.0	29.9	36.6
92	**235-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.6	0.0	0.0	0.0	22.0	24.5	0.0	0.0	0.0	24.5
93	**2356-2**	**76**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
94	**235-26**	**57**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
95	**236-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.3	26.6	24.5	37.1	54.7	28.2	0.0	0.0	802.9
96	**236-26**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
97	**245-23**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
98	**246-23**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
99	**245-24**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	26.9	11.9	28.2	0.0	74.7	52.3	24.4	17.4
100	**246-24**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
101	**245-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	38.0	0.0	81.0	208.3	212.2	142.8	63.0	297.1	0.0	0.0	98.2
102	**245-26**	**61**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.7	14.1	887.0	1120	356.7	44.2	234.0	234.1
103	**246-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

104	**246-26**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
105	**234-34**		9747	8895	9548	9626	9307	9094	8716	8785	8544	7926	6579	7029	1803	1344	2345	1124	1223	1391
106	**2345-3**	**142**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
107	**234-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
108	**2346-3**	**125**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
109	**235-34**	**134**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110	**236-34**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.0	7.2	0.0	8.0	9.5	0.0	0.0	0.0	0.0	0.0	0.0
111	**235-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
112	**2356-3**	**86**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
113	**236-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
114	**2345-4**		711.4	657.0	712.1	723.2	690.8	677.1	634.0	650.6	639.3	583.7	510.6	522.3	143.6	102.4	196.8	87.4	102.2	125.8
115	**2346-4**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
116	**23456-**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
117	**2356-4**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
118	**245-34**		0.0	0.0	0.0	0.0	0.0	0.0	29.2	21.2	22.7	20.8	20.4	19.1	0.0	0.0	0.0	0.0	0.0	0.0
119	**246-34**	**83**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
120	**245-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
121	**246-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
122	**345-23**	**184**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
123	**345-24**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
124	**345-25**	**140**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
125	**345-26**	**108**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
126	**345-34**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
127	**345-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
128	**234-234**	**159**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.4	9.1	8.5	0.0	0.0	0.0	0.0	0.0	0.0
129	**2345-23**	**163**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.2	6.9	0.0	37.6	0.0	0.0	0.0	30.3
130	**234-235**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.7	60.6	25.0	79.9	545.2	134.8	13.8	137.0	755.0
131	**2346-23**	**188**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
132	**234-236**	**161**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
133	**235-235**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
134	**2356-23**	**109**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
135	**235-236**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
136	**236-236**	**87**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
137	**2345-24**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.1	0.0	24.2	24.9	15.2	0.0
138	**234-245**	**160**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
139	**2346-24**	**143**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

140	**234-246**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
141	**2345-25**	**176**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
142	**23456-2**	**106**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
143	**2345-26**	**139**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
144	**2346-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
145	**2346-26**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
146	**235-245**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	55.1	61.1	58.5	58.7	0.0	0.0	0.0	0.0	0.0
147	**2356-24**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
148	**235-246**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
149	**236-245**		8151	7820	8133	8357	8100	7845	7498	7615	7295	7298	7485	7408	6234	5754	5830	1089	2104	4444
150	**236-246**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
151	**2356-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
152	**2356-26**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
153	**245-245**		9071	8523	8951	9388	9178	8892	8348	8468	8097	8030	7645	7804	2745	1938	4219	1527	1635	2083.
154	**245-246**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
155	**246-246**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
156	**2345-34**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
157	**234-345**	**172**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	30.8	0.0	31.0	29.5	0.0	0.0	0.0	0.0	0.0	0.0
158	**2346-34**	**178**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
159	**2345-35**	**128**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
160	**23456-3**	**138**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
161	**2346-35**	**132**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
162	**235-345**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
163	**2356-34**	**129**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
164	**236-345**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
165	**2356-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
166	**23456-4**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
167	**245-345**	**202**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
168	**246-345**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
169	**345-345**	**203**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
170	**2345-234**		4007	3748	4035	4196	4088	3980	3842	3937	3717	3661	3689	3655	1573	1586	2294	1052	1152	1640
171	**2346-234**	**201&204**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
172	**2345-235**	**157**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
173	**23456-23**	**173**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
174	**2345-236**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
175	**2346-235**	**182**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

176	**2346-236**	**141**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
177	**2356-234**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
178	**2356-235**	**158**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
179	**2356-236**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
180	**2345-245**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
181	**23456-24**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
182	**2345-246**	**175**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
183	**2346-245**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
184	**2346-246**	**122**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
185	**23456-25**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
186	**23456-26**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
187	**2356-245**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
188	**2356-246**	**131**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
189	**2345-345**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
190	**23456-34**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
191	**2346-345**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
192	**23456-35**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
193	**2356-345**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
194	**2345-2345**		0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
195	**23456-234**	**207**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
196	**2345-2346**		0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
197	**2346-2346**		0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
198	**23456-235**		0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
199	**2345-2356**		0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
200	**23456-236**		0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
201	**2346-2356**	**171&204**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
202	**2356-2356**	**167**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
203	**23456-245**	**169**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
204	**23456-246**	**171&201**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
205	**23456-345**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
206	**23456-2345**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
207	**23456-2346**	**195**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
208	**23456-2356**		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
209 (IS)	**23456-23456**		503.1	490.2	504.2	490.1	461.2	481.9	491.9	521.3	527.4	491.7	500.1	502.1	462.6	497.9	487.1	519.7	497.7	505.7
Total	**ppm**		51.8	49.1	51.6	52.4	50.1	49.3	46.9	46.2	45.6	42.5	41.1	41.0	34.3	33.9	36.8	31.3	31.6	33.4

Final Report

ER 1495

Modeling and decision support tools based on the effects of sediment geochemistry and microbial populations on contaminant reactions in sediments

Appendix B

Version 2.0

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September 22, 2011

Appendix B

List of Scientific / Technical Publications

Amanda S. Hughes, Jeanne M. VanBriesen and Mitchell J. Small**, "**Bayesian Modeling of PCB Dechlorination in Sediment for Remediation Decision Support," The Society of Environmental Toxicology and Chemistry (SETAC) North America 28th Annual Meeting. Milwaukee, Wisconsin, November, 2007 (Poster presentation).

Amanda S. Hughes, Jeanne M. VanBriesen and Mitchell J. Small, **"**Bayesian Modeling of PCB Dechlorination in Sediment for Remediation Decision Support," The Technology, Management and Policy Graduate Consortium 2007. Pittsburgh, Pa, June 2007 (Poster presentation).

Amanda S. Hughes, Jeanne M. VanBriesen and Mitchell J. Small, **"**Bayesian Modeling of PCB Dechlorination in Sediment for Remediation Decision Support,**"** Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 4-6, 2007 (Poster presentation).

Youngseob Yu, Jeanne M. VanBriesen, Edwin Minkley, William Brown "Investigation of Microbial Community Structures and PCB Dechlorination Patterns in River Sediments," SERDP and ESTCP's Partners in Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 4-6, 2007 (Poster presentation).

Youngeseob Yu, Jeanne M. VanBriesen, W.E. Brown, E.J. Minkley, Jr., "Microbial diversity and community profiles in PCB contaminated sediments from Hudson and Grasse Rivers," American Society for Microbiology (ASM), 107th General Meeting, Toronto, Canada, 2007. (Poster Presentation).

Xu, Y., Yu, Y., Minkley, E.J., Gregory, K.B., VanBriesen, J.M Bacterial communities in core sediments. Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 2-5, 2008 (Poster Abstract).

Hughes, A. VanBriesen, J.M., Small, M.J. Dechlorination Pattern Augmentation and Bayesian Modeling of Polychlorinated Biphenyl (PCB) Dechlorination in Sediment, Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 2-5, 2008 (Poster Abstract).

VanBriesen, J.M., Small, M., Lowry, G., Gregory, K., Minkley, E. Karcher, S, Congener tracker pair analysis for evaluation of reductive dechlorination of polychlorinated biphneyls (PCBs) in river sediments, Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 2-5, 2008 (Poster Abstract).

Hughes, A., J.M. VanBriesen, M.J. Small. “Objective Identification of Structural Properties Associated with Polychlorinated Biphenyl Dechlorination Processes,” Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 1-3, 2009.

Hughes, A., J.M. VanBriesen, M.J. Small. “Bayes Monte Carlo Model to Identify the Most Likely Polychlorinated Biphenyl Dechlorination Pathways and Processes,” Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 1-3, 2009.

Thompson, S.L., Xu, Y., Gregory, K., and VanBriesen, J.M. “Bacterial Population Shifts Related to Anaerobic Reductive Dechlorination of PCB Tracker Pairs in Hudson and Grasse River Sediment,” Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 1-3, 2009.

Xu, Y., Thompson, S.L., Gregory, K. and VanBriesen, J.M. “Reductive dechlorination of polychlorinated biphenyls (PCBs) Tracker Pairs in Hudson and Grasse River Sediment Microcosms,” Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 1-3, 2009.

Mitchell, A., Hughes, A., Karcher, S.C., Small, M.J. , and VanBriesen, J.M. “PCB analytical method uncertainty and evaluation of PCB transformations in natural systems,” Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 1-3, 2009.

VanBriesen, J.M., Small, M., Karcher, S. “Evaluation of reductive dechlorination of polychlorinated biphenyls (PCBs) in Sediment Core Samples using Tracker Pairs,” Environmental Technology Technical Symposium & Workshop, Washington, D.C., December 1-3, 2009.

Hughes, A. S., Vanbriesen, J. M., and Small, M. J. (2010). "Identification of Structural Properties Associated with Polychlorinated Biphenyl Dechlorination Processes." Environ. Sci. Technol., 44(8), 2842-2848.

Amanda S. Hughes, Jeanne M. VanBriesen and Mitchell J. Small, **“**Simulation of PCB Biotransformation,” The United States Army Corps Engineering Research and Development Center. Vicksburg, MI, June 2010 (Invited talk).

Final Report

ER 1495

Modeling and decision support tools based on the effects of sediment geochemistry and microbial populations on contaminant reactions in sediments

Appendix C

Version 2.0

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List of Acronyms

BBN	Bayesian Belief Network
CMB	chemical mass balance
CPB	chlorines per biphenyl
CPT	conditional probability tables
DoD	Department of Defense
ERDC	Engineer Research and Development Center
XML	extensible markup language
FA	factor analysis
GIS	geographic information system
IUPAC	International Union of Pure and Applied Chemistry
MCC	Monsanto Chemical Company
MCDA	Multi-criteria decision analysis
PCBs	polychlorinated biphenyls
PVA	polytopic vector analysis
PMF	positive matrix parameterization
QnD	Questions and Decisions
TEF	toxic equivalence factors
TEQ	toxic equivalency
USEPA	United States Environmental Protection Agency

Appendix C

Modeling and Decision Support Tools for Contaminated Sediments: linking biogeochemical models with decision-support tools

J.M. VanBriesen, M.J. Small, G. Lowry, E. Minkley, W. Brown, G. Kiker, T. Bridges

Executive Summary

A Bayesian statistical approach will be used to implement an evaluation of alternative biogeochemical models with different reaction pathways. A sequence of advanced optimization and statistical methods will be used to estimate the parameters of the alternative models, characterize the uncertainty in model inputs and predictions, and evaluate the added explanatory power and predictive capability associated with the successive refinements in model structure. The focus of the model is on biogeochemical reactions in the sediment; physical processes including transport will not be included. This focus is predicated on the importance of biological transformation in affecting long term persistence of polychlorinated biphenyls (PCBs); only biological processes result in mass reduction. Thus, the determination of the potential for biological transformation is a critical issue for decision making related to PCB-contaminated sediment.

An easy-to-use decision tool will be developed that accepts (as input) information on sediment geochemical conditions and contaminant concentrations, including specific chemical and biological measurements, then predict the likelihood that natural degradation will occur, to different extents and within specific time periods. These tools will be interfaced with multi-criteria decision models under development at the United States Army Engineer Research and Development Center (ERDC) in Vicksburg, MS, so that the predicted extent and time frame of natural degradation can be considered along with other decision criteria (such as costs, ecological impact, and public acceptance) in the evaluation of monitored natural (or enhanced) attenuation options relative to other alternatives for cleanup. It is anticipated that the model will be designed to accept easily measured characteristics of field sites as input. The model will not *a priori* predict outcomes based on the analysis of a set of sites, but rather enable evaluation of each site using its own observable characteristics.

The coupled biogeochemical dynamics model and decision-support model will be evaluated vis-à-vis two test sites. The model structure will thus be developed with the information from two sites, but this structure will be generalizable to other sites. The information from the test sites does not define or parameterize the model and thus does not limit the usability of the developed model.

1 Introduction

The proper management of PCB-contaminated sediment has proven to be a wide-spread, complex, and costly issue. PCBs are a primary contaminant driving risk at many Department of Defense (DoD) facilities. There is a need for sound science and effective tools to characterize and manage these sites in ways that reduce risk to human health and the environment and gain regulatory acceptance. The primary goal of this research is development of a model that incorporates (1) congener-specific biodegradation pathways, (2) geochemically-modified microbial population parameters, and (3) uncertainty analysis to evaluate the **likelihood of natural attenuation and/or the success of a planned bioremediation intervention.** This model will be linked to a decision-support tool to enable improved decision making by site managers.

2 Background

2.1 Polychlorinated biphenyl contamination in sediment

The term PCB encompasses 209 anthropogenic molecules called congeners. Congeners are composed of a biphenyl ring with 10 possible attachment sites for chlorine atoms. Multiple naming systems exist for congeners, and the absolute and relative positions of chlorine atoms on the biphenyl ring. The International Union of Pure and Applied Chemistry (IUPAC) system is widely used and is used in this paper (Guitart, Puig and Gomezcatalan 1993). Generally, higher IUPAC numbers correspond to more highly-chlorinated congeners. Chlorines attach to biphenyl rings at the ortho, meta and para positions.[1] A chlorine atom located next to another chlorine atom is termed flanked. **Figure C-1** shows PCB structure, IUPAC24, and locations of ortho, meta and para positions.

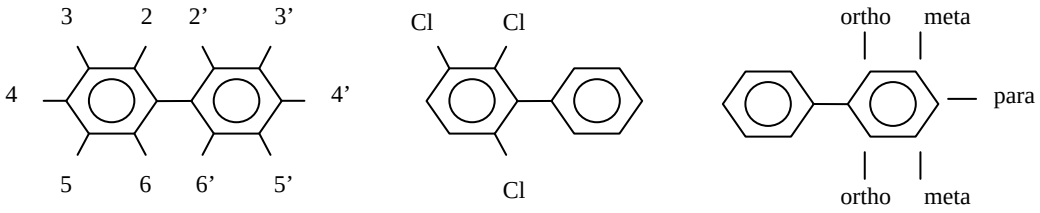

Figure C-1. Congener structure with ten possible chlorine attachment sites, IUPAC24, which has two ortho chlorines, one flanked by a meta chlorine, and ortho, meta and para positions.

[1] Meta and para chlorines appear to be more susceptible to degradation, as ortho chlorines are most abundant in environmental samples Quensen, J. F., M. A. Mousa, et al. (1998). "Reduction of Aryl Hydrocarbon Receptor-Mediated Activity of Polychlorinated Biphenyl Mixtures Due to Anaerobic Microbial Dechlorination." Environmental Toxicology and Chemistry **17**(5): 806-813..

In the United States, combinations of 60 to 90 congeners were manufactured and sold by the Monsanto Chemical Company (MCC) under the trademark Aroclor® (Wiegel and Wu 2000).[2] Between 1929 and 1977, MCC manufactured more than 1.5 billion pounds of Aroclors USEPA 2006. They are identified by four digits, the final two indicating chlorine percent weight, with the exception of Aroclor 1016, which is 41% weight chlorine (USEPA 2006). Aroclors were valued for their low flammability and thermal and chemical stability, and were used in capacitors, transformers, fire retardants and plasticizers (Hutzinger, Safe and Zitko 1974).

Over the years, PCBs have been released to the environment in significant quantities in PCB-bearing liquids, via spills, leaks, and wastewater discharges. It has been estimated by the US Environmental Protection Agency (EPA) that US production of PCBs from 1929 to 1976 was 700,000 tons, of which 625,000 tons were used domestically, with about half of that amount disposed before the ban in 1976 (USNRC 2001). A 1975 estimate of PCBs mobile in the environment was 75,000 tons (Durfee, Contos, Whitmore, Barden, Hackman and Westin 1976; USNRC 1979). There have been many new discoveries of PCB contamination and continued releases of PCBs to the environment since that time. Between 1989 and 2001, for example, there were 2,611 reported spills of PCBs greater than 1 pound reported to the United States Environmental Protection Agency (USEPA) National Response Center (USNRC 2001).

The low reactivity properties that made PCBs very useful for industrial purposes also make them very persistent in the environment. PCBs tend to accumulate in organic phases, especially in soil and sediment organic matter which represent dominant environmental organic compartments (Schwarzenbach, Gschwend and Imboden 1993; Connolly, Zahakos, Benaman, Ziegler, Rhea and Russell 2000; Meijer, Steinnes, Ockenden and Jones 2002; Jonsson, Gustafsson, Axelman and Sundberg 2003). PCBs also accumulate preferentially in the tissues of animals and plants (e.g., Secord, McCarty, Echols, Meadows, Gale and Tillitt 1999; Muir, Rigit, Cleeman, Skaare, Kleivane, Nakata, Dietz, Severinsen and Tanabe 2000; Stapleton, Masterson, Skubinna, Ostrom, Ostrom and Baker 2001).

Air, water, and soil/sediment transport processes have distributed PCBs from local sites of contamination across the global environment, including the most remote areas of earth (e.g., Muir, Rigit et al. 2000; Kalantzi, Alcock, Johnson, Santillo, Stringer, Thomas and Jones 2001). PCBs can be found in virtually all environmental compartments (USNRC 2001). Local and regional environmental conditions often lead to the deposition of released PCBs close to source areas (Axelman and Broman 1999; Green, DePinto, Sweet and Hornbuckle 2000; Meijer, Steinnes et al. 2002). Jonsson et al. (Jonsson, Gustafsson et al. 2003) estimated the global residence time of selected highly-chlorinated PCB congeners as on the order of 100 years, based on evaluation of PCB burial rates in continental shelf sediments compared to an estimated global PCB inventory.

Soils and sediments have an important role in local, regional, and global scale environmental transfers and cycling of PCBs (e.g., Gobas, Z'Graggen and Zhang 1995; Connolly, Zahakos et al. 2000; Meijer, Steinnes et al. 2002; Jonsson, Gustafsson et al. 2003). Sediments often are the primary source of PCBs to aquatic ecosystems. PCBs in near-surface sediments are those most

[2] Trademark omitted for the remainder of paper.

available for release and recycling in the environment (Connolly, Zahakos et al. 2000; Jonsson, Gustafsson et al. 2003).

2.2 Biological Transformation of PCBs

The first report of biodegradation of PCBs appeared in 1973 (Ahmed and Focht 1973)(Ahmed and Focht 1973) and involved aerobic degradation producing different product mixes, and suggesting multiple metabolic pathways. Laboratory studies over the following 25 years resulted in additional isolates, more metabolic pathways, and an expanded understanding of the fundamental processes for aerobic PCB degradation (Masse, Messier, Peloquin, Ayotte and Sylvestre 1984). A more recent discovery was a bacterial mode for anaerobic transformation of PCBs. In the mid 1980s, deep sediment analysis of PCB congener distribution led to the discovery that in addition to physical weathering and chemical redistribution of congeners, anaerobic biological processes were altering PCBs in the deep sediments. Specifically, some highly chlorinated congeners were being dechlorinated (Brown, Bedard, Brennan, Carnahan, Feng and Wagner 1987; Brown, Bush, Rhee and Shane 1988) to produce lightly chlorinated forms. PCB removal appears to be controlled by bacterial consortia effects (Pettigrew, Breen, Corcoran and Sayler 1990).

Our understanding of PCB biotransformation and biodegradation has developed during 25 years of study (see reviews of biotransformation of PCBs (Bedard 1990; Young, Bedard and Quensen 1995; Unterman 1996); however, complex interactions, feedbacks and process dynamics are not well understood. Complete mineralization of lightly chlorinated PCBs can be achieved by many aerobic organisms (Furukawa 1982; Bedard 1990; Furukawa 1994; Abramowicz 1990). Anaerobic dechlorination is a reductive process that results in more lightly chlorinated mono-, di- and tri-chlorobiphenyls or completely dechlorinated biphenyl (Brown, Bedard et al. 1987; Brown, Bush et al. 1988; Natarajan, Wu, Nye, Wang, Bhatnagar and Jain 1996). Coplanar PCBs appear to be more easily fully dechlorinated (Mousa, Ganey, Quensen, Madhukar, Chou, Giesy, Fischer and Boyd 1998); however dechlorinating organisms show specific congener preferences (Rhee, Sokol, Bethoney and Bush 1993; Sokol, Kwon, Bethoney and Rhee 1994) and different sediment systems appear to have different populations of dechlorinating organisms (Young, Bedard et al. 1995; Quensen, Boyd and Tiedje 1990; Sokol, Kwon et al. 1994)(Bedard and Quensen 1995; Quensen, Boyd, and Tiedje 1990; Sokol et al. 1994). In general, dechlorination is preferential for meta and para cholorines (Nies and Vogel 1990; Quensen, Tiedje and Boyd 1988; Quensen, Boyd et al. 1990)(Nies and Vogel 1990; Quensen, Tiedje, and Boyd 1988; Quensen, Boyd, and Tiedje 1990). Limited cases of ortho dechlorination have been reported (Berkaw, Sowers and May 1996; Van Dort and Bedard 1991; Williams 1994). It has been suggested that ortho-chlorinated PCBs can inhibit further dechlorination (Dai, Vaillancourt, Maaroufi, Drouin, Neau, Snieckus, Bolin and Eltis 2002). Biological mineralization of biphenyl or monochlorinated biphenyls in anaerobic systems has been suggested but not verified (Rhee, Sokol, Bush and Bethoney 1993). In general, the biphenyl ring structure is not degraded and a separate carbon and electron donor source is needed (a co-contaminant (Alder, Häggblom, Oppenheimer and Young 1993) or soil organic carbon (Rhee, Bush, Bethoney, Denucci, Oh and Sokol 1993b)). Partial dechlorination results in a different set of PCB congeners rather than removal of the contaminant (Liu, Sokol, Kwon, Bethoney and Rhee 1996). Lower chlorinated products are more soluble and more mobile in the sediments and the water column (Gevao,

Hamilton-Taylor, Murdoch, Johnes, Kelly and Tabner 1997), but have a lower toxicity (Mousa, Ganey et al. 1998; Safe 1993; Silberhorn, Glauert and Robertson 1990) and a smaller likelihood of bioaccumulating in the food chain.

Reductive dechlorination of PCBs is generally associated with their use as electron acceptors and thus is strongly dependent upon the redox state of the sediments. PCB dechlorination requires a low redox potential, and the presence of more energetically favorable electron acceptors (e.g., O_2, NO_3^-, SO_4^{2-}) inhibits dechlorination in some systems (Alder, Häggblom et al. 1993)(Alder et al. 1993); although in other experiments amendment with $FeSO_4$ stimulated nearly complete meta plus para dechlorination (Zwiernik, Quensen and Boyd 1998; May, Boyle, Price and Blake 1992; Morris, Mohn, Quensen, Tiedje and Boyd 1992; Rhee, Bush, Bethoney, Denucci, Oh and Sokol 1993a). The specificity of microbial dechlorination shows wide variability, meaning that control of the loss of a specific chlorine from a specific congener depends on several interacting conditions: (1) the population of dechlorinating organisms and the population and activity of non-dechlorinating organisms in the system (2) the position (ortho, meta, or para) of the chlorine on the ring, the presence and location of other chlorines on the same ring and the opposite ring, and (3) other environmental conditions in the system such as the presence of carbon sources, electron donors, other electron acceptors, temperature, pH, salinity, etc. (Abramowicz 1993; Rhee, Bush et al. 1993a; Wu, Bedard and Wiegel 1996; McCue, Gauger, Holsen, Kelly and Cha 1996). PCB dechlorination in a system may be extensive, resulting in removal of all para and meta chlorines and leaving only lightly chlorinated ortho compounds. Alternatively, PCB dechlorination may involve only a few congeners resulting in minor changes to the distribution and little change to the overall chlorine content of the contaminants. These variations are not well understood and may be based on biological populations of degraders, presence or absence of suitable carbon sources or electron donors, physico-chemical conditions in the sediments, or presence of toxic or sequestering co-contaminants (Sokol, Kwon et al. 1994; Zwiernik, Quensen and Boyd 1999). A concentration dependent total PCB or congener-specific threshold for dechlorination may exist (Abramowicz 1990; Quensen, Tiedje et al. 1988; Sokol, Bethoney and Rhee 1998); however, different patterns of congener reactivity complicate the development of a modeling relationship (Young, Bedard et al. 1995).

2.3 Biogeochemical Models for PCB Transformation in Sediments

Among the most broadly applicable PCB fate and transport models are chemical mass balance (CMB)- based multimedia (Mackay and Diamond 1989; Warren and Mackay 2004). Multimedia CMB models divide a contaminated environment into compartments such as theatmosphere, water and sediment. Movement of PCBs among compartments occurs according to user-specified environmental and chemical parameters. Estimates for these parameters can introduce significant uncertainty into the model; sometimes enough uncertainty to limit the predictive value of the model (Webster, Mackay, Di Guardo, Kane and Woodfine 2004). In 1992, Bayesian methods were applied to a CMB fate and transport model to better predict these parameters (Wolpert, Steinberg and Reckhow 1993; Steinberg, Reckhow and Wolpert 1996). Bayesian parameter estimation methods are used to represent uncertain variables with a distribution of likely values and allow prior knowledge, from literature or expert elicitation, to be updated at any time with newly acquired knowledge. These CMB models are further limited by their ability to track only one PCB mixture or congener at a time. Modeling a single congener is

inappropriate, as sites are contaminated by multiple congeners. Modeling an Aroclor oversimplifies the dechlorination of a parent congener to a daughter congener and is accomplished by weight-averaging the half-lives of the congeners that compose the Aroclor.[3]

We considered this type of approach by evaluating a Level I Mackay model for PCB fate and transport. The Level I Mackay Model is a closed system in which equilibrium is attained but steady state is not. Equilibrium is defined as the point at which the fugacities, *F*, of the PCB in all compartments are equal. Fugacity is measured in units of pressure and described as the tendency of a chemical to escape its current phase. Fugacity is a useful property because it can be interpreted as the driving force for mass transfer between phases (Warren and Mackay 2004).

Within the Mackay Model, the environment is broken down into seven compartments: air, aerosols, soil, sediment, water, suspended particles and fish. PCBs divide themselves between the compartments according to six partition coefficients: air-water, K_{aw}, soil-water, K_d, sediment-water, K_{sed}, suspended particles-water, K_{sp}, fish-water, K_f and aerosol-water, K_{aero}. **Figure C-2** illustrates these compartments and partition coefficients.

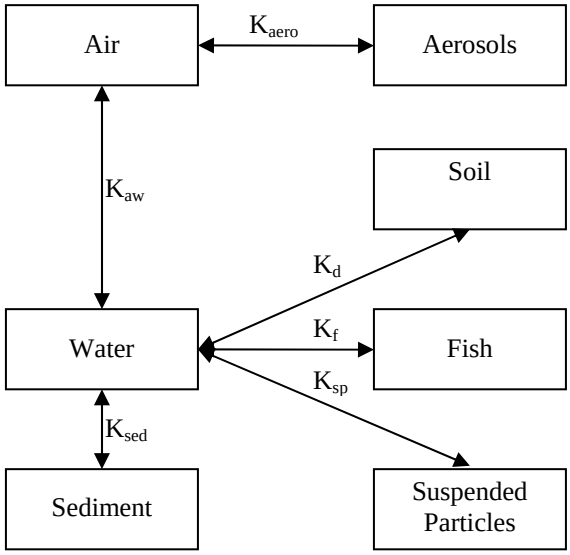

Figure C-2. Environmental compartments and partition coefficients in the Level I Mackay Model.

In our evaluation, a comparison of Aroclor 1242 and Aroclor 1254 outputs show that persistent organic pollutants act differently in the same environment. A greater percentage of PCBs partition to the air compartment in the Aroclor 1242 model because it is composed of more lightly chlorinated congeners. In both models, low vapor pressures and high octanol-water partition coefficients drive congeners towards organic materials. While the soil-water partition coefficient is less than the sediment-water partition coefficient and the sediment has a greater fraction of organic carbon than the soil, volumetric differences between the soil and the sediment result in the majority of PCBs partitioning to the soil compartment. Furthermore, it was demonstrated that significant inconsistencies result from analyses performed on a congener basis

[3] While some CMB models were published before congener-specific analyses were possible, later models have continued to group PCB congeners and dechlorination pathways.

versus those performed on an Aroclor basis for Aroclors 1242 and 1254. It is believed that while a mass balance model can be used to model congener fate-and-transport, reasonably accurate dechlorination simulation will require the addition of poorly understood weathering processes. Such data are not available on a congener level. Thus, we considered other modeling frameworks that were congener specific.

The first models to track individual congener degradation combined receptor models with least-squares analysis (Jarman, Johnson, Bacon, Davis, Risebrough and Ramer 1997; Rachdawong and Christensen 1997; Rachdawong, Christensen and Chi 1998; Imamoglu, Li, Christensen and McMullin 2004; Li, Mgonella, Bzdusek and Christensen 2005; Magar, Johnson, Brenner, Quensen, Foote, Durell, Ickes and Peven-McCarthy 2005; Ogura, Gamo, Masunaga and Nakanishi 2005; Bzdusek, Lu and Christensen 2006; Bzdusek, Christensen, Lee, Pakdeesusuk and Freedman 2006; Bzdusek and Christensen 2006). Receptor methods applied to PCBs are polytopic vector analysis (PVA), factor analysis (FA) and positive matrix parameterization (PMF). Receptor models are used to reduce the dimensions of a problem.[4] In the case of PCB models, a congener-specific observation is reduced to two or three congener distributions representing dechlorinated Aroclors discharged to the site. These distributions become input into a least-squares analysis yielding the most likely dechlorination process.

Recently, a statistical method was developed to predict dominant patterns of chlorine atom removal (Karcher, Small and VanBriesen 2004; Karcher 2005). The statistical analysis of natural dechlorination in situ method exploits tracker pairs, which are select pairs of congeners that maintain the same relative concentrations across all Aroclor mixtures. Use of tracker pairs reduces uncertainty resulting from laboratory measurements and bias associated with identifying the site's original Aroclor contamination. Examination of tracker pairs in the environment reveals that most concentration ratios differ from original Aroclor-like ratios.

2.4 Multi-Criteria Decision Analysis

Multi-criteria decision analysis (MCDA) methods and tools can provide a systematic approach for integrating risk levels, uncertainty and valuation for differing criteria. A detailed analysis of the theoretical foundations of these decision methods and their comparative strengths and weaknesses is presented in Belton and Steward (Belton and Steward 2002) while reviews of MCDA applications in various environmental areas is presented by Kiker *et al.* (Kiker, Bridges, Linkov, Varghese and Seager 2005). The common purpose of MCDA methods is to evaluate and choose among alternatives based on multiple criteria using systematic analysis that overcomes the limitations of unstructured individual or group decision-making. While the basic organization of criteria and alternatives is similar in most MCDA approaches, the methods differ in their synthesis of the information and strategy in ranking the alternatives by different means.

[4] PVA was the first receptor model applied to PCBs . FA, unlike PVA, assumes that sources are not necessarily orthogonal, or independent . The most recent receptor method applied to PCB degradation is PMF . It allows weighting of individual data points such that relatively large or small concentrations have larger uncertainties so these outliers do not overly influence results.

3 Model Development

The overall objective is to link the model for environmental conditions, congener distributions and bacterial population dynamics to decision-support tools to enable evaluation of site-specific likely outcomes in order to evaluate remediation plans. At present, the two modeling efforts have been proceeding in parallel. However it is necessary for the biogeochemical model to inform the decision support model, and thus a linkage between the two is envisioned. **Figure C-3** provides the conceptual model of this integration, with the biogeochemical model predicting the rates of biological transformations at the site on a congener-specific basis. The multi-criterion decision model uses this information along with additional site information (sociopolitical aspects, ecosystem specifics, other abiotic processes) to develop scenarios and enable decision-makers to explore different remediation options.

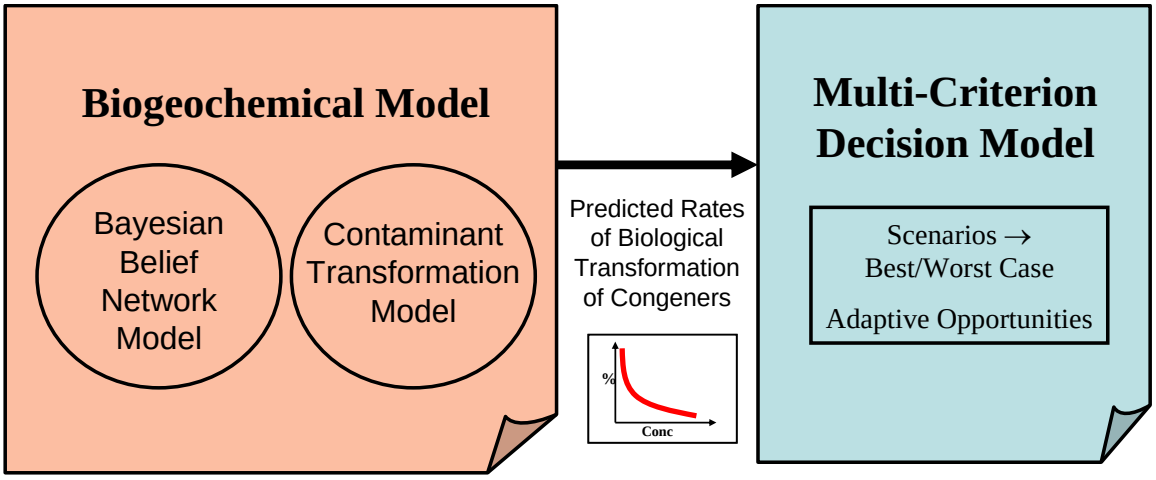

Figure C-3. Conceptual model of integration of biogeochemical model and multi-criterion decision model.

In the following sections, we will first summarize the parallel development work in detail and then provide additional information on the way in which we anticipate the two models being linked.

3.1 Biogeochemical Model Development

The many limitations inherent in traditional fate and transport modeling convinced us to pursue an alternative modeling framework for the biogeochemical model. We selected development of a Bayesian Belief Network (BBN). This decision-support tool will inform a contaminant transformation model, which will then interface with the decision support model.

Like Bayesian parameter estimation, BBNs combine prior knowledge and empirical evidence. Both methods are rooted in Bayes rule:

$$\Pr(A|B) = \frac{\Pr(B|A)\Pr(A)}{\Pr(B)}$$

The equation yields the posterior probability, Pr($A|B$), that event A will occur given some knowledge of event B. This probability is considered to be more informed than the prior probability of event A occurring, Pr(A). Pr(B) relates the probability of event B occurring. The conditional probability, Pr($B|A$), assumes knowledge of the occurrence of event B given event A (Mackay 1995).

A BBN links probabilities of multiple events together. The dechlorination BBN links the events leading to an observed distribution of congeners together based on congener-specific dechlorination pathways. It is capable of revealing the likelihoods of dechlorination, dechlorination processes and discharged Aroclors, and the most influential known biogeochemical factor at a site. Knowledge of the most likely Aroclors discharged to the site can aid in the determination of responsible polluters. Knowledge of the occurrence of dechlorination, the dominant dechlorination processes, and the most influential known biogeochemical factor(s) at a site can influence remedial design.

The BBN structure reflects PCB discharge in Aroclor form and congener-specific dechlorination governed by biogeochemical factors (**Figure C-4**). Variables are represented by nodes containing all the possible states of that variable. For example, the "Discharged Aroclors" node represents the likelihood that each Aroclor was discharged to a site. States of the node are 8 Aroclors (1221, 1232, 1016, 1242, 1248, 1254, 1260, and 1262) with quantified congener compositions and a "No Discharge" category (Frame, Cochran and Bowadt 1996). Similarly, the node titled "Discharged Congeners" contains 210 states, one for each congener and a "No Discharge" state. Variables contained in the "Biogeochemical Factors" node will be determined from laboratory experiments conducted as part of Task A and from published literature. These factors influence the likelihood of dechlorination processes. Biogeochemical factors being considered include total PCB concentration, the presence of particular dechlorinating organisms, and sediment pH. The probabilities of states within a node sum to 1.

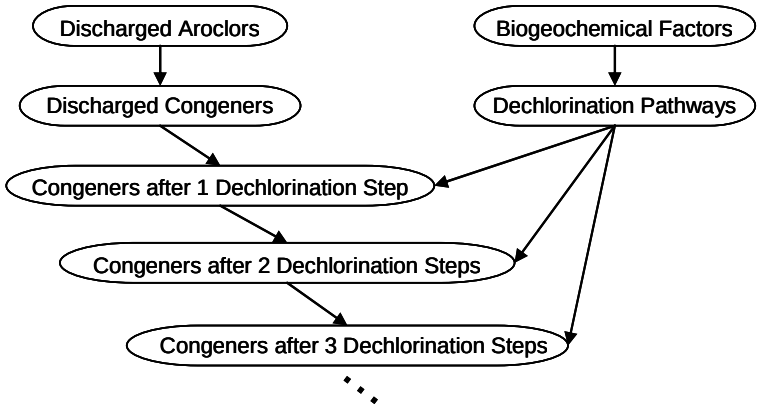

Figure C-4. BBN framework for the anaerobic reductive dechlorination of PCBs in aquatic sediment. It was created in BBN software, Netica® Norsys Software Corp. 1998.

Nodes are connected by arrows representing conditional probability tables (CPT). CPTs quantify the probabilities of each state within a child node occurring given all possible combinations of states in higher connected nodes. Probabilities flow through CPTs in the causative direction

(with the arrows) and in the diagnostic direction (against the arrows) (Pearl 1988). Thus, the CPT relating the "Discharged Aroclors" node to the "Discharged Congeners" node contains the percent weight distributions for each Aroclor. The "Dechlorination Pathways" node, which contains a state for each dechlorination pathway and a "No Dechlorination" state, is connected to dechlorinated congener nodes by a single CPT. This CPT contains probabilities for the presence of every congener and a "None" state given all combinations of congeners and dechlorination pathways.

Application of a BBN requires quantification of prior probabilities for discharged congeners and geochemical conditions in the sediment. Prior probabilities are subjective but have less impact on posterior probabilities as the BBN is updated. Updates are of two forms: observations used to update prior probabilities or CPTs updated through a process called learning (Pearl 1988; Jensen 2001). They do not replace old knowledge, but are instead combined with existing information. For example, updates to dechlorinated congener nodes with congener-specific Hudson River samples will cause probabilities to propagate throughout the BNN; thereby updating probabilities and increasing the certainties of the states of probabilities in all nodes within the network. Thus, with increasing information about the state in the Hudson River system, the model becomes less dependent on prior probabilities.

The extent of dechlorination of the weight of a congener is dependent upon two conditions that are in turn a function of the bioavailability of PCBs and biogeochemical factors. First, dechlorination extent depends on the fraction of a congener present at a site that loses a chlorine atom. This loss is referred to as a dechlorination step. It is modeled by adding the lost percentage of the percent weight of a parent congener to the percent weight of its daughter congener. The second condition affecting the extent of dechlorination is the number of dechlorination steps. Because the impacts of bioavailability and biogeochemical parameters on PCB dechlorination are not yet quantified, the model makes assumptions for the percent weight of a parent congener transferred to a daughter congener and the number of dechlorination steps that have occurred at a site. These values are parametrically estimated by comparing the average number of chlorines per biphenyl (CPB) in an observed congener distribution to those predicted by the final dechlorinated congeners node in the BBN given site-specific prior probabilities for Aroclor discharge. The number of dechlorination steps and the percentage of percent weight transferred during a dechlorination step are varied. This analysis will provide the modeler with several sets of dechlorination steps and corresponding percentages of percent weights transferred. The choice among them is left to the modeler, as dechlorination is not sufficiently quantified to indicate the appropriate set of values. It is recommended that a conservative percentage of weight transferred and number of dechlorination steps are chosen.

The output of the BBN will be of two forms; posterior probabilities and predictions. Posterior probabilities are the result of site-specific updates to the network. The probabilities of each Aroclor having been discharged to the site, the likelihood of individual pathways and processes, and the probabilities of each biogeochemical factor being present at the site, and their respective uncertainties will be quantified. Predictions are made from information contained in the network. First, the change in risk of each dioxin-like congener will be predicted via toxic equivalence factors (TEF), then the change in total risk, measured in terms of toxic equivalency (TEQ), will be tracked through dechlorination steps. Second, uncertainties associated with

biogeochemical factors will be compared to indicate the factor for which further efforts and funding could be targeted to reduce uncertainty in test results indicating the presence of the factor at the site. From these results, posterior probabilities of dechlorination processes and the presence of biogeochemical factors will provide input for a small scale multi-component model (CCBATCH (VanBriesen and Rittmann 2000b; VanBriesen and Rittmann 2000a; Rittmann and VanBriesen 1996) focused on predicting the effects of biological processes on congener distribution.[5]

CCBATCH is a major advancement over previously applied models that attempt to link biological reactions with geochemistry because it comprehensively couples microbially catalyzed reactions to aqueous geochemistry through a full biological stoichiometry and inclusion of feedback effects between physical processes, geochemistry and biological processes. The stoichiometric implementation also allows CCBATCH to track explicitly the formation and subsequent degradation of intermediates in the biological reactions, which is critical for reductive dechlorination of PCBs where the transformation of one congener begets another. Multiple degradation pathways can be simulated to represent the behavior of real systems with complex, interacting biotic and abiotic reactions. Further, CCBATCH is specifically designed to handle systems containing multiple organic substrates and multiple bacterial species involved in different kinds of reactions or different steps in a single reaction pathway. CCBATCH is also designed to handle interactions between chemicals and their physical environment that may reduce their bioavailability. **Figure C-5** shows the fundamental structure of CCBATCH. It is designed to receive information on congener-specific rates of transformation and use this in conjunction with total mass of each congener at the site to predict concentration profiles for each congener over time.

[5] It is also possible to pass the results of the BBN model directly to QnD without the CCBATCH interface. Depending upon the complexity of the relationships among congeners determined in the BBN and the level of detail needed in the mass balance modeling, we will decide on one of these approaches.

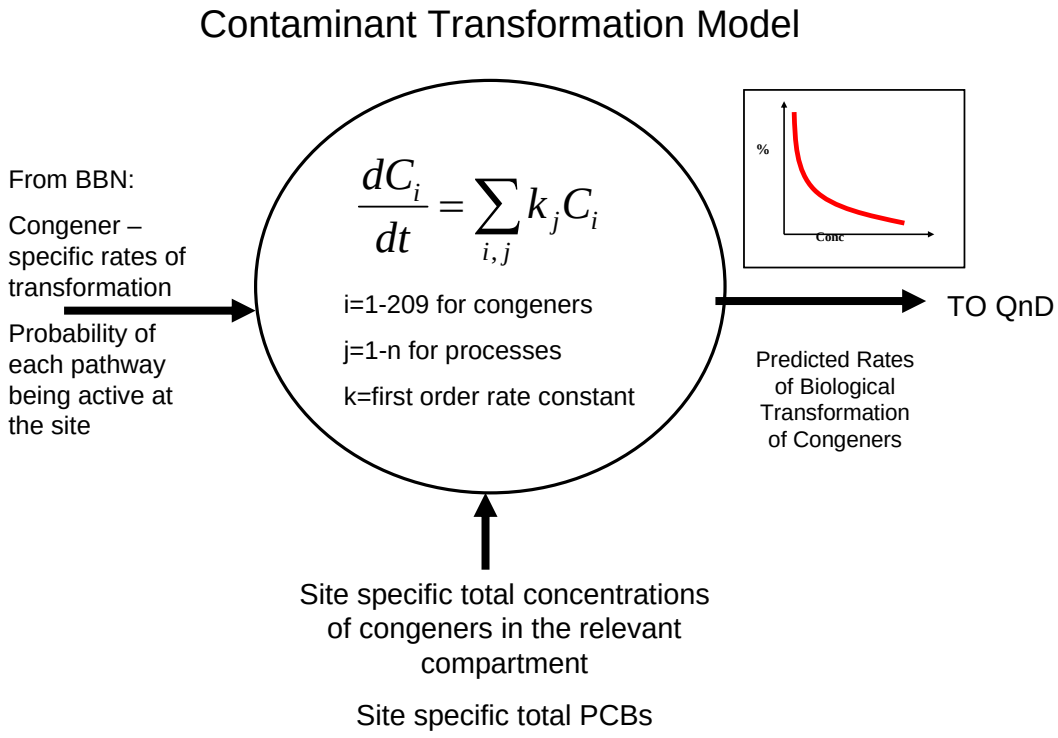

Figure C-5. Schematic representation of the Contaminant Transformation Model (CCBATCH).

Control of the rate and extent of biodegradation will be modeled with a kinetic formulation and a stoichiometric representation including intermediate formation. Information on the appropriate rate will be determined from the BBN results or from estimates based on field data. Microorganisms utilize nutrients (C, N, P, O, and H), electrons, and energy to build new cells. The rate at which cells grow is controlled by inherent characteristics of the cells and by system-dependent factors, such as the amount of available substrates and the total number of cells present in the system. These same factors may affect pathways of dechlorination, but this will be accounted for in the BBN formulation. Here, in the biogeochemical model, these factors will affect the growth rate of cells and the rate of substrate utilization. Substrate utilization consumes and produces other general chemical species (e.g., oxygen, acidic hydrogen, ammonium, and carbonate species) and direct biodegradation intermediates (e.g., 4-chlorobenzoate). The stoichiometry between substrate utilization and production and consumption of other chemicals of interest is based on balanced chemical equations representing the biological reactions (VanBriesen and Rittmann 2000a; VanBriesen 2001; VanBriesen 2002; Yuan and VanBriesen 2002; Xiao and VanBriesen 2006). One or more chemical forms of the substrate (e.g., different congeners of PCBs) may be biologically available to the microorganisms, and biodegradation kinetics can depend strongly on these differences (Lauff, Steele, Coogan and Breitfeller 1990; Palumbo, Lee and Boerman 1994; Bolton, Girvin, Plymale, Harvey and Workman 1996).

It is important to realize that CCBATCH is NOT designed to take as input the probability of various reaction pathways (except as a binary Yes/No) or to handle uncertainty. It is a mechanistic model, driven by mass balance assumptions to provide a deterministic prediction.

However, the BBN is capable of much more in terms of predicting the probability and providing a bound on uncertainty related to site processes. If these aspects are needed to support the decision-making at the site, we will alter the current plans and instead of using CCBATCH to predict concentrations at the site, we will hand the probability distributions directly to the decision support model. QnD (as described below) does not currently have the capability to take as input this type of probability information. However, this aspect is in development, and we anticipate that the model will have this ability before the end of 2007.

3.2 Decision Support System Model Development

The Questions and Decisions™ (QnD™) model system was created to provide an effective and efficient, open-source, decision education tool. QnD incorporates ecosystem, management, economics and socio-political issues into a user-friendly model/scenario framework (Kiker et al. 2006, Kiker and Linkov, 2006). The QnD model utilizes a basic finite difference approach with simple Euler numerical integration of various rate transformation and mass-balance transfer equations (Keen and Spain, 1992) as defined by the input files. For our purposes the input files will be either the contaminant concentrations over time as generated by the biogeochemical model OR the probability of various processes and the associated probability of their rates. As noted above, QnD can take the rates as input currently and will be able to take the probabilities soon.

The model is written in object-oriented Java and can be deployed as a stand-alone program or as a web-accessed tool. The QnD model links the spatial components within geographic information system (GIS) (ArcInfo Shape) files to the abiotic (climatic), biotic and chemical/contaminant interactions that exist in a watershed. The model can be constructed using any combination of detailed technical data or estimated interactions of the ecosystem elements. The model development is iterative and can be initiated quickly through conversations with users or stakeholders. Model alterations and/or more detailed processes can be added throughout the model development process. QnD can both provide rigorous modelling to mimic system elements obtained from scientific data and create a “cartoon” style depiction of the system to promote learning and discussion among decision participants.

The QnD system has two primary parts: the game view and the simulation engine as shown in **Figure C-6**. The game view has several types of outputs that can be configured by the user via e**x**tensible **m**arkup **l**anguage (XML) file inputs. By presenting selectable outputs, QnD allows users to choose how they want to see their output, including the following output options:

- GIS Maps that are updated on each time step
- Warning lights that change at user-selected critical levels
- Mouse-activated charts and text for individual spatial areas (pie charts and text line descriptions)
- Time-series charts (listed on several tabbed pages)
- Text output files (in comma separated format)

The simulation engine of QnD is made of objects linked together into simple or complex designs, determined by the needs of decision participants. The most elemental objects of QnD are

Components, Processes and Data as shown in **Figure C-6**. A Component is an object that is of interest to the user, such as a specific congener or biological entity (*i.e.*, fish, benthic invertebrates). Processes are the actions that involve Components and their Data. Data are the descriptive objects assigned to various Components such as K_{ow} as a data object modifying a specific congener component. Components objects are situated into the virtual QnD landscape and can interact with each other over space and time. Within the QnD object framework, both simple and complex designs are possible. In more complex designs, building block components and processes designed as clusters of subcomponents or sub-processes. For clarification, a **"C"** prefixes Components, a **"P"** prefixes Processes, and a **"D"** prefixes Data objects.

Upon startup, specialized internal QnD objects read the relevant XML input files and create all the engine parts (Components, Processes and Data) as well as the game view (maps, charts and management options) required for the simulation. Users can manipulate the game view in the following ways:

- Set some management options (using the slider bars)
- View the map page and switch between maps
- View the various Chart pages
- Simulate time steps at user-defined levels
- Reset the game to the startup conditions

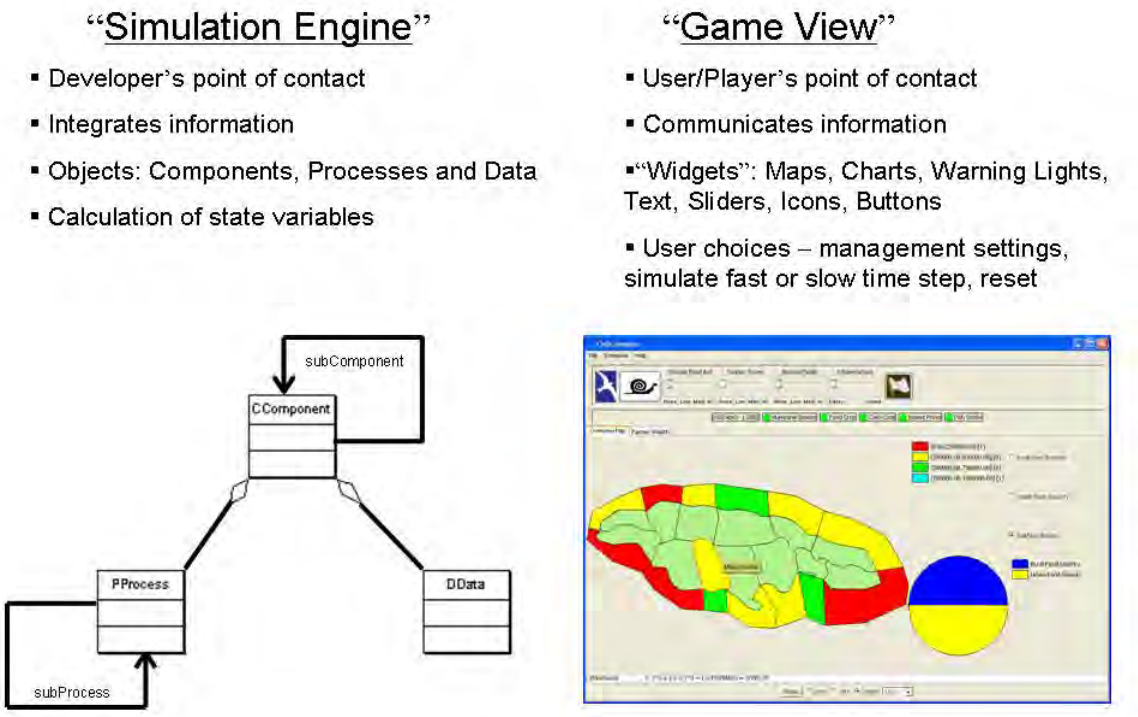

Figure C-6. QnD model structure (after Kiker et al., 2006).

The user may explore the system outputs, choose new management options and continue with the simulation. Certain end points can be created to show various ramifications of management

actions including such socio-economic aspects as financial status, public acceptability or overall ecosystem health.

3.2.1 QnD Component Objects

The relationships among the most fundamental building block components in QnD include CSpatialUnits, CHabitats and CLocalComponents are described in the object design in **Figure C-7**. A CSpatialUnit is the basic spatial unit of the QnD system and can represent either terrestrial or aquatic spaces. CSpatialUnits can be linked to one another and have a specific location as seen in the river reaches in **Figure C-5**. A CSpatialUnit can have either zero or any number of other CSpatialUnits connected to them. In addition, these connections can be labeled with useful words to group similar types of connections. For example, a riverine description may be "UPSTREAM" to describe all connections that move against a prevailing current. CHabitats exist within CSpatialUnits and are not spatially defined but can be described with any number of data objects. Within the example appearing in **Figure C-5**, the river reach represented by SpatialUnit2 has two constituent habitats. A CHabitat can hold any number of CLocalComponents representing various organisms or chemicals present in the system. In this example, Habitat A can be used to hold CLocalComponents of interest (congeners 1 through 209) as well as various biological components (fish).

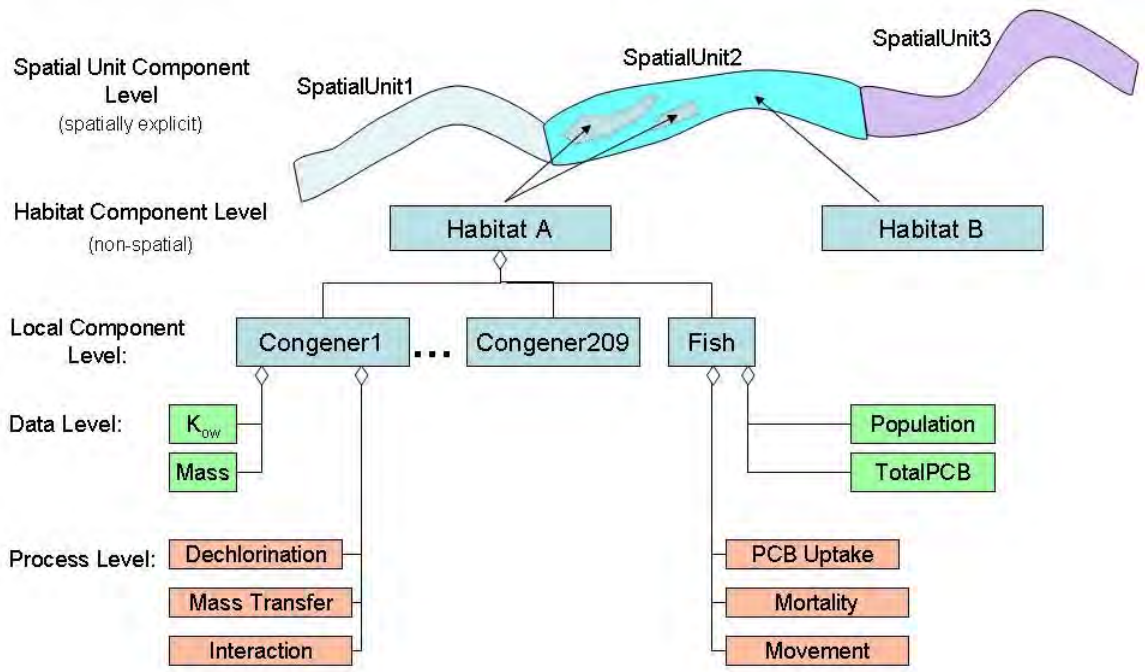

Figure C-7. QnD design example showing the relationships among Component, Process and Data objects.

3.2.2 QnD Data Objects

DData objects are used to describe various attributes of any component object (CSpatialUnits, CHabitats, or CLocalComponents) and store all the relevant information for a simulation. **Figure C-7** provides an example of a Data layer that modifies the Congener1 CLocalComponent object within Habitat A. The DData objects may be used to store input parameter objects such as

K_{ow} or state variables such as mass. All DData objects are created in the XML input files and represent a composite variable with several attributes that allow for various manipulations by PProcess objects. In addition, DData objects can be linked directly with time series input files to reflect changing values over time. Other DData options include linkage to a variety of distributions (Uniform, Gaussian, Poisson, Gamma, Exponential, Chi Square and Beta) through the use of library-derived generators.

3.2.3 QnD Process Objects

Processes provide various calculations within QnD by manipulating the values of various DData objects. Process objects use DData objects as inputs, provide a calculation or series of calculations and then write the resulting products into output DData objects. Processes are modular, as such, they can used be individually or with constituent sub-processes to create more complex calculations. **Figure C-5** shows a sample design to implement the elements of the biogeochemical model described in Section 3.1. Each CLocalComponent representing a specific congener (such as Congener1) may have specific Process objects to represent various dechlorination features calculated by the BBN/CCBATCH model. The Process objects can be designed to simply relay previous BBN/CCBATCH simulation results into the QnD object structure or they can be used to create entirely new algorithms to integrate additional biological or socio-economic features into QnD calculations. For example, the Movement Process object attached to the Fish CLocalComponent may be used to simulate elementary metapopulation responses to various congener biogeochemical reactions.

3.3 Link Between biogeochemical model and decision support model

Figure C-8 provides an overall conceptual figure of the QnD model with integration of the components and processes that will be generated by the BBN and the biogeochemical model. Two basic options exist for integration including external or internal linkages. Each QnD model can be a mixture of internal and external linkages. *External* linkages include the incorporation of model results or additional information to drive some of the basic inputs to QnD's simulation engine components. These external linked sources can be incorporated via time series files or stochastic relationships and can be linked to global or local objects as required. The primary design intent of external linkages is to provide exogenous inputs or influences upon the system. Internal linkages will be used if the BBN will pass probability distributions directly to QnD.

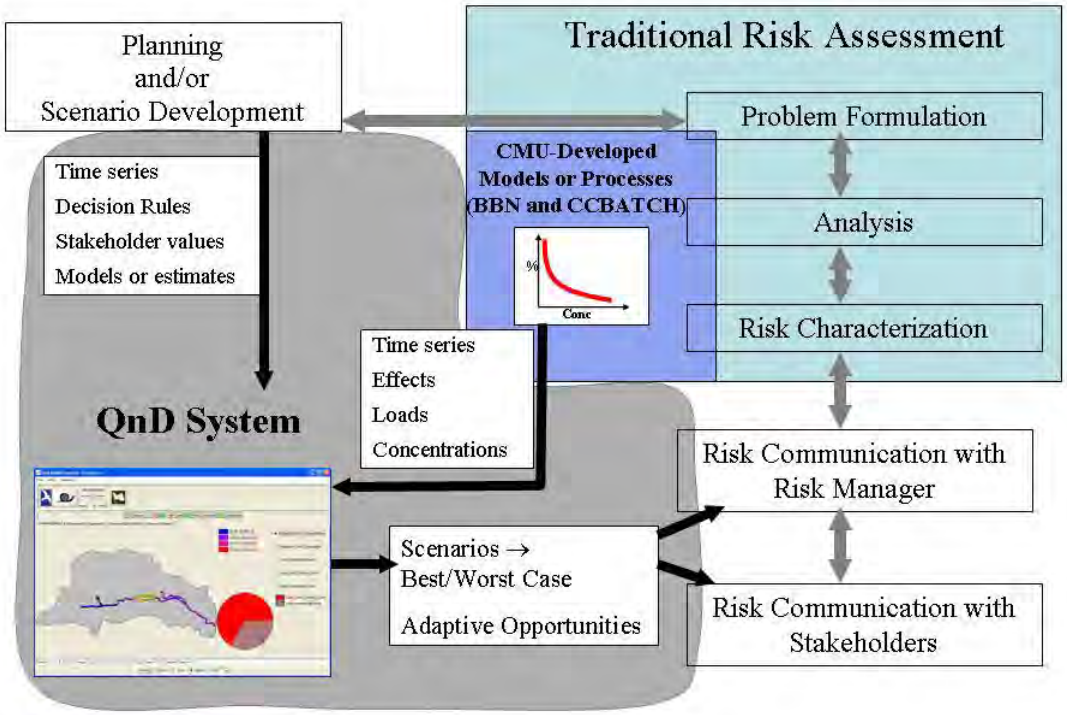

Figure C-8. Integration of scenarios, QnD and traditional risk analysis (after Kiker and Linkov, 2006).

The decision support tool will provide decision makers with a novel way to approach and draw initial conclusions regarding the application of remedial technologies, especially monitored natural recovery. It will be developed based on data from two sites, which serve as case examples, and will not eliminate the need to gather information from new sites at which it will be applied. Rather, the tool structure will be developed and evaluated with the case example data. The decision support tool will be linked to a congener-specific BBN that drives a simplified mass-balance based biogeochemical transformation model.

The transition from the modeling effort to the formation of the decision support tool is dependent upon the information passed between the BBN and the decision support tool. **Figure C-9** shows the anticipated interchange of information among the different components of the model.

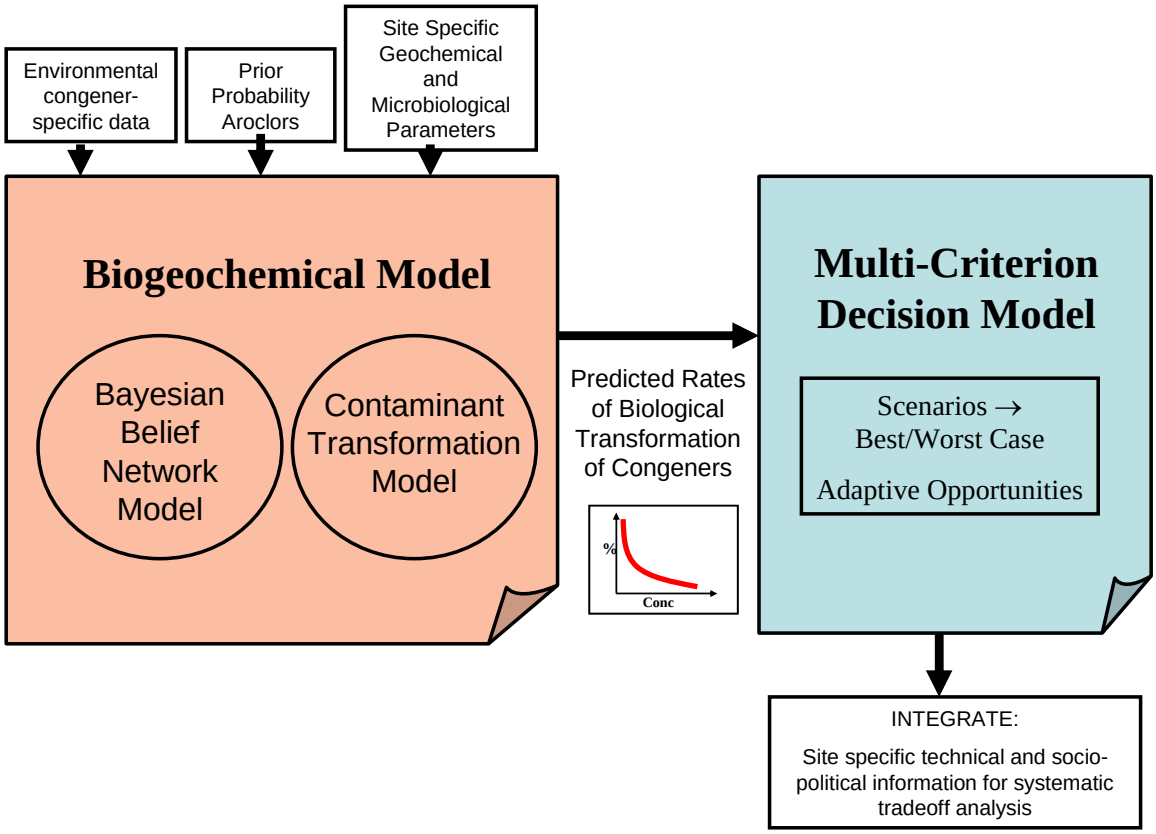

Figure C-9. Information exchange among the Bayesian belief network model, the contaminant transformation model, and the multi-criterion decision model.

Thus, we envision that the QnD system will expand the unit-scale information simulated by the biogeochemical model by providing specific river reaches as well as additional simulated items (such as ecosystem or human-management responses) that stem from the input of various scenario/planning designs. The QnD platform can provide a game-style simulation in which stakeholders can role-play possible futures to develop rules of thumb, further learning and adaptive opportunities to feed back into various management or policy efforts being made at a particular site.

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