Final Synthesis Report for Factors Controlling DDE Dechlorination Rates on the Palos Verdes Shelf: A Field and Laboratory Investigation

Submitted to the U.S. Environmental Protection Agency
by

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1. Executive Summary. (Eganhouse, Orem, Reinhard)

This project was organized into separate field and laboratory studies aimed at answering "18 questions" in the original Scope of Work (*cf*., section 2 of this report, **Background**, for explanation). Because of some early results, certain questions became irrelevant and were, therefore, not pursued. In other cases, there simply was not enough time to complete the originally planned studies. On the other hand, additional work, not identified in any of the original "18 questions", was carried out for purposes of addressing specific issues of concern to the USEPA (United States Environmental Protection Agency). Examples of the latter include: 1) the analysis of an expanded list of sediment cores for DDX (DDX refers to the ten DDT-related compounds of interest in this study; *cf*., Eganhouse *et al*., [1]) and selected PCB congeners to facilitate estimation of site-specific reductive dechlorination (RDC) and total loss rates, 2) analysis of gravity and box cores for trace elements to allow stratigraphic alignment, and 3) determination of the extent of mineralization of *p,p*'-DDE (1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene) in microcosm experiments.

In this **Executive Summary**, we offer a brief recapitulation of what was learned about the factors controlling reductive dechlorination of *p,p*'-DDE in Palos Verdes Shelf (PVS) sediments using the "18 questions" as a structural guide. The summary is written in narrative form, but references to specific sections (corresponding to the "18 questions") are identified parenthetically in the text so that the reader can explore the expanded answers that appear later in the report.

A significant component of this study was an effort to better understand the character of PVS sediments, including the chemistry of pore-water and solid phases as well as microbiology. Prior to this investigation, studies concerning pore-water chemistry and microbiology on the PVS were extremely limited or absent (*e.g*., [2-4]) despite their obvious relevance to natural attenuation of *p,p*'-DDE *via* reductive dechlorination. The main purpose of the field characterization work, then, was to establish the biogeochemical conditions under which RDC of *p,p*'-DDE is taking place and to consider what might be controlling RDC rates.

Based on pore-water chemistry of the upper 50 cm of the sediment column, three major biogeochemical redox zones can be delineated. In these zones, the dominant terminal electron accepting processes (TEAPs), all operating under anoxic conditions, are manganese reduction, iron reduction and sulfate reduction (*cf*., sections **3.1.**, **3.4.**). No evidence of methanogenesis was observed in the cores. The thicknesses of these zones at three locations on the PVS (Los Angeles County Sanitation Districts [or LACSD] stations 3C, 6C, and 8C; Figure 1) differ, redox zone thickness increasing with distance from the outfall system. These differences are probably attributable to variations in the rates of microbial degradation processes, which in turn are driven by the availability of degradable organic matter (substrate). Dissolved hydrogen, which was originally hypothesized to be a possible control on RDC rates, was observed at all three sites at all sampled depths. Lowest concentrations were found at station 3C, with increasing concentrations in proximity to the outfalls (*i.e*., stations 6C and 8C).

Dissolved organic carbon (DOC) concentrations in sediments on the PVS varied from about 5 to 125 mg/L; higher concentrations were found in the upper 10 cm at station 3C, but no consistent trends were noted at stations 6C and 8C (section **3.5.**). The elevated DOC concentrations in the upper portion of the sediment column at station 3C may be associated with generation of volatile fatty acids in excess of their microbial utilization. The character of the

DOC, as revealed by excitation emission matrix spectroscopy (EEMS), was dominated by humic substances in the upper portion of the sediment column (≤ 10 cm) and by proteinaceous material at greater depth. Volatile fatty acids are not observed in the EEMS spectra.

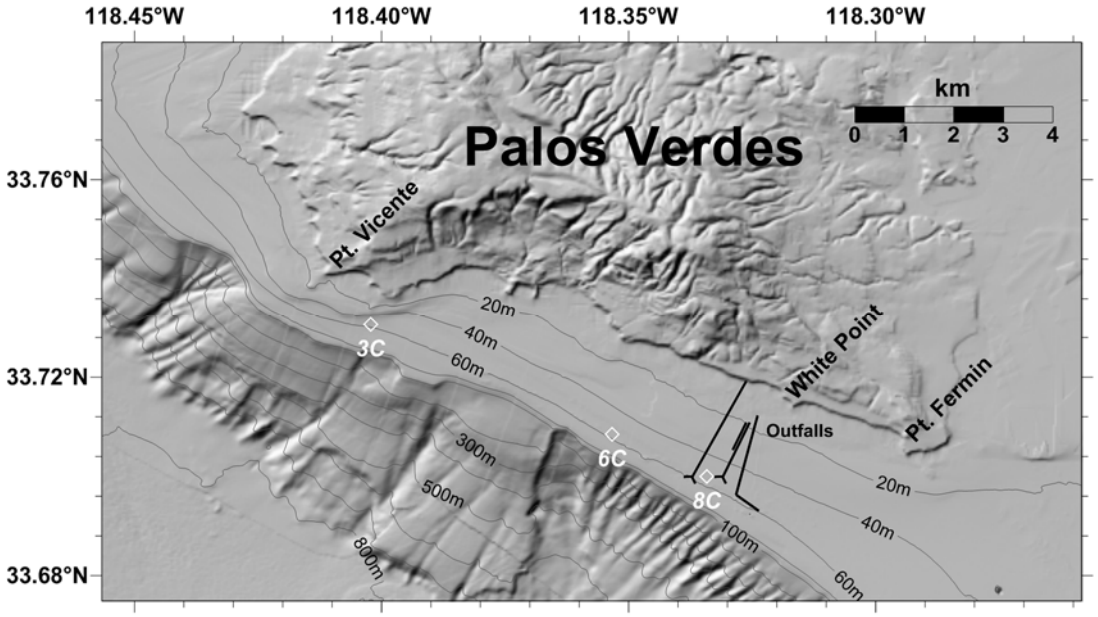

Figure 1. Palos Verdes Shelf study site showing three coring stations.

It was of interest to know how much of the *p,p*'-DDE is present in association with colloidal/dissolved organic matter *versus* freely-dissolved *p,p*'-DDE. Calculations of the fraction associated with colloidal/dissolved organic matter (f_{DOC}) were made based on measurement of *p,p*'-DDE concentrations in pore water collected from station 6C using whole-core squeezing (*i.e.*, freely-dissolved + colloidal/dissolved organic matter-associated) and analysis by solid-phase microextraction (SPME: freely-dissolved). The results indicate that in the upper portion of the sediment column (< 10-15 cm), the majority of the *p,p*'-DDE is associated with colloidal/dissolved organic matter (section **3.6.**). At greater depth, freely-dissolved *p,p*'-DDE predominates. It is possible that this pattern reflects the fact that the humic-rich DOC in the upper portion of the sediment column has a greater proportion of hydrophobic moieties, which tend to sorb *p,p*'-DDE, than the proteinaceous DOC in the lower portion of the sediment column (section **3.5.**).

The current paradigm is that uptake and metabolism of organic substances, such as *p,p*'-DDE, only occurs when those substances are in the freely-dissolved state. The fraction of *p,p*'-DDE that is *bioavailable*, however, includes not only the standing stock of freely-dissolved *p,p*'-DDE in the pore water but also that which is potentially available through the process of desorption from the solid phase (or from dissolved organic matter). This is the so-called *bioaccessible* fraction (section **3.7.**). Desorption occurs when freely-dissolved *p,p*'-DDE concentrations are reduced due to microbial transformation and the system readjusts to establish equilibrium.

In an effort to understand what controls the sorption of *p,p*'-DDE in PVS sediments, we used a triple-domain sorption model (section **3.8.**). The purpose was to evaluate the relative importance of the major organic geochemical phases in PVS sediments (amorphous organic

matter, black carbon, and hydrocarbons) as sorbents. The results showed that hydrocarbon residues, derived primarily from oil refinery wastes introduced to and discharged from the Joint Water Pollution Control Plant (JWPCP), are largely responsible for sorption of *p,p*'-DDE in PVS sediments. Based on the near-constant (first order) rate of RDC of *p,p*'-DDE over the last three decades, it appears that the reservoir of *bioaccessible p,p'*-DDE has not yet been depleted to the point of limiting the rate of RDC. In other words, sequestration, if it is occurring, has had little effect on the bioavailability of *p,p*'-DDE to the microorganisms responsible for RDC (section **4.8.**).

One of the hypotheses posed in this study was that slow desorption kinetics was limiting the rate of RDC (section **4.9.**). Although direct measurement of the rates of desorption of *p,p*'-DDE from PVS sediments could not be completed, we compiled information from the literature on the desorption kinetics of comparable systems for this compound. The results showed that the *rapidly desorbed* fraction of *p,p*'-DDE (related to the *bioaccessible* fraction) is typically released with rate constants in the range of about 0.1- 1.0 h^{-1} (876-8,760 yr^{-1}; [5]). *P,p*'-DDE released in *slowly* and *very slowly* desorbed fractions (operationally defined using polyphasic models; ***e.g.***, [6]) exhibit desorption rate constants that are approximately two to three orders of magnitude lower [5-7]. When these rate constants are compared with the best available estimates of the rate of RDC (derived from analyses of sediment cores from stations 3C and 6C, namely, 0.044 ± 0.004 and 0.008 ± 0.002 yr^{-1}, respectively), it is clear that desorption kinetics does not limit RDC in PVS sediments. Rather, it is the metabolic activities (and factors that affect those activities) that control *p,p*'-DDE RDC rates.

What about the microorganisms responsible for RDC of *p,p*'-DDE? As noted above, the presence of early diagenetic reaction products (NO_3^-, Mn^{2+}, Fe^{2+}, SO_4^{2-}, H_2S, H_2) in pore water clearly indicates that manganese-, iron- and sulfate-reducing as well as fermentative bacteria are present and active in PVS sediments. As noted earlier, methane was not detected in sediments at the time of collection. However, in microcosm experiments production of methane following depletion of sulfate signaled the presence in PVS sediments of dormant methanogens that can be reactivated (section **3.2.**).

An attempt was made to identify the bacteria (section **3.3.**) responsible for dechlorination of *p,p*'-DDE. The basic hypotheses were that dehalogenation occurred *via* organohalide-respiring bacteria and that these bacteria belonged to one or several of the genera, *Dehalococcoides (Dhc)*, *Desulfitobacterium*, *Dehalogenimonas*, *Dehalobium*, *Dehalobacter*, or *Sulfurospirillum.* Bacteria from the genus *Dhc* have been shown to dechlorinate chlorinated ethylenes to ethane and ethene *via* dehalorespiration. *Dhc* respiration involves using chlorinated ethenes and other chloro-organics as the electron acceptor and hydrogen as the electron donor.

To assess whether low hydrogen concentrations could be a limiting factor, hydrogen was quantified in native PVS sediments and in microcosm experiments as the putative electron donor (section **4.1.**). Initially, the microbial communities of PVS sediment samples were characterized based on sequencing the genes encoding 16S ribosomal RNA. Results showed that Proteobacteria represented 30-65% of the total, whereas *Dehalococcoidetes* accounted for 0.04-4.76% (two sections: 0-2 cm, ~25 cm, respectively). The class identified as most likely responsible for the reductive dechlorination of DDE was *Dehalococcoidetes*. Generally, the relative *Dhc* abundance increased with depth and with *p,p*'-DDE concentrations, suggesting that *Dhc* may play a role in the RDC of *p,p*'-DDE. However, the putative organohalide-respiring *Dehalococcoidetes* strains detected were not in the same monophyletic clade as the previously

characterized organohalide-respiring strains. Therefore, evidence that *Dhc* is responsible for RDC of DDE in PVS sediments is circumstantial. Efforts to verify the presence of dehalogenase enzymes were unsuccessful.

RDC of *p,p*'-DDE in the microcosms was observed in samples from all depths under sulfate reducing and methanogenic conditions, even at hydrogen concentrations ranging from 0.005 to 0.015 ppm. Fe- and Mn-reducing conditions were not tested. Comparing field with laboratory data suggests that RDC of *p,p*'-DDE under sulfate reducing conditions is not limited by hydrogen (section **4.1.**).

Growth of *Dhc* coupled to reduction of *p,p*'-DDE and oxidation of hydrogen could not be established. Growth of sulfate reducers (with resultant depletion of sulfate) and production of hydrogen were stimulated by the addition of electron donors/precursors (section **4.3.**). This resulted in promotion of methane production and accelerated RDC of *p,p*'-DDE (and –DDMU; 1-chloro-4-[2-chloro-1-(4-chlorophenyl)ethenyl]benzene). Nevertheless, RDC of *p,p*'-DDE was observed even without introduction of electron donors although the origin of the native substrate(s) was not determined (section **4.5.**). Addition of sulfate reduced the RDC rate, but this cannot be attributed to the lowering of hydrogen concentration because the RDC of *p,p*'-DDE occurred in the absence of added substrate (section **4.6.**). Hydrogen sulfide produced during sulfate reduction does not appear to inhibit RDC of *p,p*'-DDE (section **4.7.**).

Finally, knowing when site remediation will reach the desired endpoint is important because monitoring is costly and time-consuming. In the case of this project, one of the main goals was to try to improve our ability to characterize the pathway(s) and duration of natural recovery. Microcosm studies with labeled *p,p*'-DDE demonstrated that degradation proceeds through *bis*(4-chlorophenyl)acetic acid (DDA) to CO_2. Under ideal conditions, prediction of the duration of natural recovery requires an understanding of which kinetic model/reaction order (or orders) best describes the microbiologically-driven reactions on the PVS (section **4.10.**).

Taking into account the extreme complexity of a natural system like the PVS, the difficulty and expense of sampling, and the relative paucity of high quality field data, it seems unlikely that any single model could provide an accurate description of the system [8-11] let alone deliver predictive capability for the site as a whole [12]. Nevertheless, it is useful to examine what we can learn from existing information while focusing attention on one component, the rate of attenuation of *p,p*'-DDE by RDC. To this end, there are two types of data that might be considered: 1) field data in the form of chemical analyses (inventories) for *p,p*'-DDE in sediment cores collected over time, and 2) kinetic data produced in laboratory microcosm experiments. Each of these types of data has advantages and limitations, which will now briefly be described.

In the case of field data, attempts have been made to determine the total (*i.e.*, physical + microbial transformation) loss rate of *p,p*'-DDE using a first-order rate model by performing linear regression analysis of LACSD core inventories [13]. The main problem with this approach is that because of natural spatial (vertical and lateral) variability in the distribution of *p,p*'-DDE on the PVS and variations in navigation (positioning) and gravity core recoveries (completeness of capturing the effluent-affected sediments), there is a significant amount of scatter in the data and, hence, uncertainty in the derived rate constants. In the present study, we sought to mitigate these problems through normalization of the *p,p*'-DDE inventories with those of selected PCB congeners. Because there is no evidence that the PCBs are degrading on the PVS [14] and because the PCBs and DDTs have had approximately parallel discharge histories,

normalization with a PCB congener having similar physico-chemical properties can greatly reduce the uncertainty in the rate (*i.e*., slope). That is because the same factors that affect variation in spatial distribution of *p,p*'-DDE will have operated for the selected PCBs. The derived first-order rate constants in this case reflect the RDC, not total loss, rates. The principal downside of this approach is that data for the DDTs and PCBs produced on cores collected and analyzed using exactly the same methods are few in number. Also, the use of whole-core inventories prevents any analysis of depth-dependent RDC kinetics, which are known to vary [15]. The main advantage of this approach is that it relies upon actual field cores that have been collected over time scales (decades) appropriate for accurate estimation of loss/RDC rates (hundredths of yr^{-1}).

In the case of the laboratory microcosm experiments, abundant new kinetic data have now been made available. However, it must be remembered that conditions in the laboratory are, by necessity, very different from those in the field. Among other things, *p,p*'-DDE added to microcosms had little time (on the order of days to weeks) to approach equilibrium. Hence, it seems likely that the *p,p*'-DDE added to the microcosms was more readily available to the microorganisms responsible for reductive dechlorination than native (historical) *p,p*'-DDE, which has been present in PVS sediments for 4-6 decades [16, 17]. No specific experiments were performed under manganese- or iron-reducing conditions (section **3.2.**), which predominate in the upper portion of the sediment column of the PVS because the existence of these redox zones was not anticipated (sections **3.1.**, **3.4.**). Moreover, because the microcosm systems were closed, significant depletion of terminal electron acceptors, notably sulfate, occurred during the course of the experiments (section **4.3.**). This drove biogeochemical processes (including RDC) faster and to endpoints (*e.g*., methanogenesis) that were not observed in the field, at least to the sub-bottom depths sampled. Other variables that may differ in the laboratory from those in the field and which may have some influence on the observed rates include hydrostatic pressure, temperature, and the efficiency of mass transfer (*i.e*., due to mixing in the microcosms *vs*. largely static conditions in the field). In the laboratory portion of this study, better correlations were found when a zero-order rate law was used. If RDC is progressing according to a zero-order rate law (rather than by first-order kinetics), remediation *via* natural recovery will proceed more rapidly than has been predicted from our analysis of the field data. However, for the reasons mentioned earlier, it is unclear whether zero-order kinetics is an artifact of experimental conditions or if it is transferable to the field. Only time (and more high quality data) will tell.

2. Background. (Eganhouse)

This project originated from a presentation made by R. Eganhouse at a USEPA-organized PVSTIEG (**P**alos **V**erdes **S**helf **T**echnical **I**nformation **E**xchange **G**roup) meeting held in Los Angeles on July 19, 2005. The presentation entitled, "Fate and mobility of DDT: What we don't know", offered an overview of what had been learned during (and after) the so-called Montrose investigations (1992-1994) concerning: 1) the chemistry of DDT in pore water of Palos Verdes Shelf sediments, 2) the composition of PVS sedimentary organic matter, and 3) DDE biodegradation rates. The final slide of the presentation offered two hypotheses for consideration by the then USEPA Remedial Project Manager (RPM) for the PVS, Ms. Carmen White:

1. DDE biodegradation rates are variable across the PV shelf.
2. Bioavailability of DDE is different between capped and uncapped sediments.

Later that year, the LACSD, which had for the first time made measurements of *p,p*'-DDMU in sediments collected as part of their biennial benthic survey, discovered that the abundance of *p,p*'-DDMU relative to *p,p*'-DDE had increased significantly since the USGS (U.S. Geological Survey) testing in 1992. A conference call (LACSD, USEPA, and USGS) was held on 9/20/2005 to discuss the new data and to make plans for verification of the results through additional analyses of archived LACSD cores and extracts. A major question at this time was "Why did sediments near LACSD station 3C (about 6 km northwest of the outfalls) appear to be undergoing reductive dechlorination of *p,p*'-DDE so much faster than at other locations nearer the outfalls (*e.g.*, 5C, 6C, 8.5C) and specifically at station 6C (about 2 km northwest of the outfalls)"?

During late 2005 and the first few months of 2006, arrangements were made to initiate an investigation of what was known about the spatial variability in *p,p*'-DDE RDC rates on the PVS. This involved compilation of existing historical core data, computation of hypothetical RDC rates, and chemical analysis of a pair of box cores collected by the USGS near LACSD station 3C in 1992 and 2003. Work Plans for this investigation were submitted by the USGS (R. Eganhouse) to the USEPA (Ms. Carmen White) on August 8, 2005 (compilation, computation) and October 17, 2005 (core analysis).

Meanwhile, the USGS (R. Eganhouse) had offered to provide another tentative Work Plan re: the 'bioavailability' hypothesis. This document, entitled "Application of solid-phase microextraction (SPME) to the direct determination of dissolved DDE concentrations in porewaters of the Palos Verdes Shelf and a study of the factors controlling porewater concentrations of DDE" was delivered to EPA on 11/5/2005 and was reviewed by Dr. R. Burgess (USEPA, Narragansett, RI) on behalf of Ms. Carmen White. Because the above-mentioned study re: spatial variability of *p,p*'-DDE RDC rates on the PVS was already underway, further consideration of the 'bioavailability' study was tentatively placed on hold.

Work on the spatial variability of *p,p*'-DDE RDC rates proceeded through 2006, culminating in a final USGS Open-File Report entitled, "Assessment of 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene (DDE) Transformation Rates on the Palos Verdes Shelf, CA", which was delivered to the USEPA on 9/15/2008 [18]. The study resulted in two journal publications [15, 19] based on the analysis of the 1992 and 2003 box cores for several classes of organic contaminants (DDTs, PCBs, long-chain alkylbenzenes-LCABs). This study provided estimates of first-order *p,p*'-DDE 'transformation' rates at LACSD station 3C (1992-2003). The major findings were that the PCBs and one class of LCABs (*i.e.*, the tetrapropylene-based alkylbenzenes [TABs]) were effectively recalcitrant, whereas reductive dechlorination of *p,p*'-DDE and *p,p*'-DDMU was occurring; highest first-order 'transformation' rates were observed at sub-bottom depths of about 44 g/cm^2 (corresponding to ~41-44 cm below the seafloor) in the 3C cores. The average first-order 'transformation' rate for the period 1992-2003, ~0.050 yr^{-1}, was similar to that derived from linear regression analysis (by C. Sherwood; 0.047 yr^{-1}) of DDE inventories in cores (below 10 g/cm^2) collected and analyzed by the LACSD for the period 1991-2005 [18]. By contrast, the rate was lower than (by factors of 1.4 to 4.5) those obtained in the most comparable microcosm experiments [20, 21].

In the latter half of 2007, the USGS (R. Eganhouse) was approached by the USEPA (Ms. Carmen White) to develop a **Scope of Work** for the 'bioavailability' study, which had been dormant while the project on spatial variability of *p,p*'-DDE RDC rates was underway. As originally conceived, the focus of this new investigation was to extend the earlier research by

examining the *factors* controlling *p,p*'-DDE RDC rates. The principal investigator (R. Eganhouse) assembled a team of researchers from the USGS (Dr. William Orem) and Stanford University (Dr. Martin Reinhard) to undertake a multi-disciplinary study that encompassed both field and laboratory components. The new **Scope of Work**, entitled "Factors Controlling DDE Dechlorination Rates on the Palos Verdes Shelf: A Field and Laboratory Investigation", was delivered to the USEPA on 3/14/2008. This **Scope of Work** was accepted by the USEPA, and a signed IAG (DW-14-95575301-0) was delivered on 6/4/2008 with a period of performance of 4/30/2008 to 12/31/2012.

The **Scope of Work** for the new project did not identify 'deliverables' to be produced for the USEPA. However, on July 20, 2010, after the final sampling cruise had taken place, an informal meeting was held in San Francisco between Ms. Carmen White, R. Eganhouse and M. Reinhard. Among other things, the issue of potential 'products' was discussed. Ms. White asked the team to produce a series of small 'data reports' (or one large comprehensive data report) and a final interpretive report as well as publications in the scientific literature. This agreement was never subsequently formalized or discussed. Because of the unexpectedly long time required to complete the microbiological (Stanford U.-M. Reinhard *et al*.) and organic geochemical (USGS-R. Eganhouse *et al*.) components of the study, individual data reports (with the exception of one created by Dr. W. Orem and submitted to Ms. C. White on 5/6/2011), were <u>not</u> produced. Instead, one large **Draft Data Report** was assembled and submitted to the current RPM at the USEPA, Ms. Judy Huang, on 4/16/2014. In addition, three journal publications related to this project have appeared in the scientific literature [22-24].

Just prior to submission of the **Draft Data Report**, the USGS project coordinator (R. Eganhouse) and the USEPA RPM (Ms. J. Huang) discussed delivery of a final product. That product is the present **Final Synthesis Report**. The purpose of the **Final Synthesis Report** is to "….answer the 18 questions posed in the IAG Statement of work with supporting data (can be by reference to other reports or links to a database.)" The 'supporting data' referred to here are contained within the **Final Data Report** [1]. Both the **Final Data Report** (with the exception of the Stanford University contributions) and the **Final Synthesis Report** (this document) have been reviewed and have received official USGS approval as *Cooperator Publications* submitted to the USEPA.

3. Field Studies.

3.1. Under what field (redox) conditions does p,p'-DDE dechlorination occur? (Orem, Eganhouse)

Parent DDT entering the Los Angeles County sewer system and discharged into PVS waters through the outfall system is believed to have undergone dehydrohalogenation during transport and shortly following sedimentation. This resulted in the presence of predominately *p,p*'-DDE in PVS sediments. *P,p*'-DDE was then transformed sequentially *via* RDC to *p,p*'-DDMU and *p,p*'-DDMU to *p,p*'-DDNU in the anoxic layers of the sediment. The major biogeochemical redox zones in the upper 50 cm of sediment at all three PVS sites (3C, 6C, 8C; Figure 1) are a manganese reduction zone near the surface, an iron reduction zone below this, and a sulfate reduction zone below the zone of iron reduction that extends to the bottom of the cores (about 50 cm below sediment surface). The thickness of each of these redox zones varies from site to site. The existence of these reduction zones was not previously documented but might affect RDC of *p,p*'-DDE and its metabolites. This study was designed to investigate dechlorination in the sulfate reducing zone, and it is possible that other degradation pathways are effective in the Mn- and Fe- reduction zones. There may be a mm-wide oxic zone at the sediment surface that was missed by our 1-cm sediment thickness resolution [4]. No methane was detected in any of these cores, but microbial methanogenesis may be occurring at depths greater than 50 cm. A more complete description of the redox zones in sediments of PVS is presented in the answer to section **3.4**., "What biogeochemical differences exist across the shelf?"

P,p'-DDE would have passed through all three observed redox zones as burial by sedimentation occurred. Currently, the rate of *p,p*'-DDE dechlorination at station 3C is highest in the zone of sulfate reduction, as shown in Figure 2 below. Whether this reflects specific biogeochemical factors related to sulfate reduction, or simply reflects the higher concentrations of *p,p*'-DDE in this zone at the present is not clear.

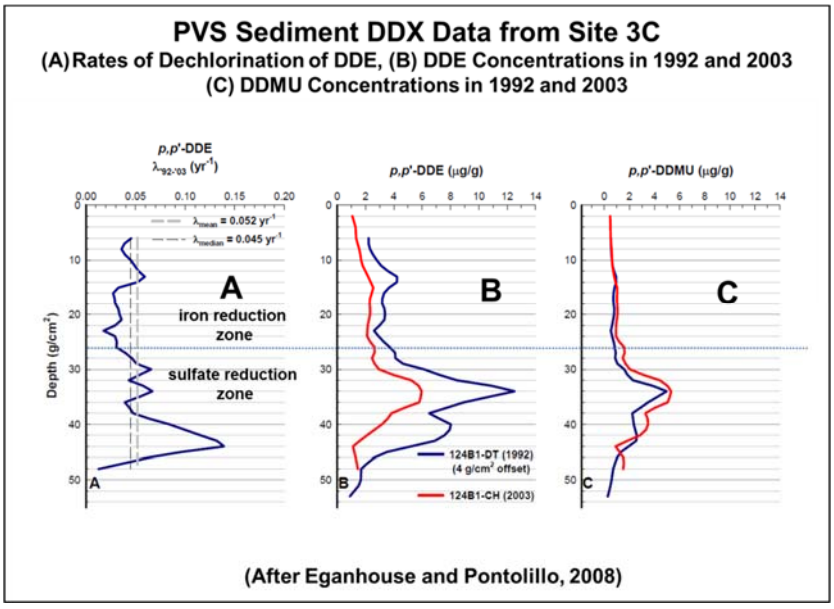

Figure 2. Vertical profiles of A) 1st-order *p,p*'-DDE RDC rate constant, and concentrations of B) *p,p*'-DDE, and C) *p,p*'-DDMU in 1992 and 2003 box cores [15].

3.2. What microbial populations dominate each redox zone? (Reinhard, Orem)

Microbial communities in PVS sediments in samples from cores 147 and 124 (collected at stations 6C and 3C) were broadly characterized based on sequencing of the genes encoding 16S ribosomal RNA. There is a paucity of information on the occurrence of dehalorespiring bacteria in marine sediments, especially DDE dehalorespiring bacteria [25]. Sediment samples were characterized in their native state and after enrichment with DDE. Results shown in Figure 3 indicate the occurrence of Proteobacteria, which include iron and sulfate reducers, and bacteria belonging to the phylum Chloroflexi, which includes the *Dehalococcoidetes*. The latter are a class of obligate organohalide respiring bacteria.

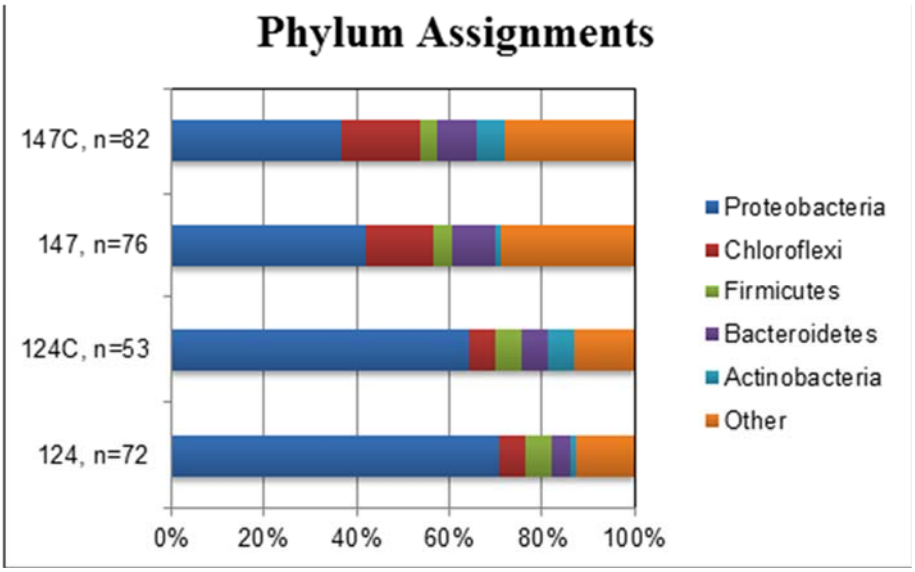

Figure 3. Phylum-level taxonomic assignment for all bacterial 16S rRNA gene sequences in microcosms constructed with sediments from stations 6C and 3C before incubation (124, 147) and after incubation (147C and 124C), respectively.

Initial screening indicated that *Dehalococcoidetes* were most likely responsible for the reductive dechlorination of *p,p*'-DDE. A PCR (polymerase chain reaction) technique was developed and applied to several PVS sediment samples (*cf*., section **3.3.**).

The populations dominating microbial activity are evidenced by the presence or absence of geochemical redox indicators that persist at different zones below the sea floor. The microbial populations dominating at a certain depth reflect the combined effects of the mass flux of soluble electron acceptors (oxygen and sulfate) diffusing from the overlying seawater downwards, and the availability of sediment-bound electron acceptors (iron and manganese oxides) and electron donors (sedimentary organic matter-SOM).

Pore-water analyses for soluble Mn, Fe (II) and S^{2-} indicated the expected sequence of microbial activity: manganese-, iron-, and sulfate-reducing bacteria (MnRB, FeRBA, and SRB, respectively). The depths of the manganese and iron reduction zones were not previously characterized and were deeper than anticipated (section **3.1.**). The sulfate reduction zone extended to a depth of 50 cm, the practical penetration limit of the box corer. Methane was not detected in the pore-water samples even though batch laboratory studies demonstrated that methanogens could readily become active in samples from all depths given the right conditions. Apparently, sulfate was diffusing into field sediments at sufficiently high rates to suppress methanogenesis down to at least 50 cm. Laboratory experiments conducted in batch microcosms (containing a limited supply of sulfate) indicated that methanogenesis was activated only after sulfate was depleted.

3.3. How does microbial community composition vary spatially? (Reinhard)

This question was limited to the characterization of organohalide-respiring bacteria (OHRB) or dehalorespiring bacteria. OHRBs are bacteria capable of utilizing aromatic or aliphatic halogenated compounds as electron acceptors [26]. The most detailed studies on RDC have been conducted with chlorinated ethylenes, especially trichloroethylene (TCE) and its lesser chlorinated analogues. TCE is dechlorinated sequentially to ethene and ethane by strains of *Dehalococcoides* (*Dhc*) *mccartyi* with hydrogen as the electron donor [27]. TCE and DDE share the 1,1-dichlorinated ethene substructure and are, therefore, structural analogs. Thus, TCE was considered a model for the RDC of DDE with the main difference being that DDE is much more hydrophobic due to the two *p*-chlorophenyl groups attached to the 1-carbon. On this basis, we hypothesized that 1) the bacteria dechlorinating DDE were similar to the TCE-respiring bacteria (*i.e.*, *Dhc* or *Dhc*-like organisms) and 2) RDC was dependent on hydrogen as the electron donor. Although RDC of chlorinated ethylenes by *Dhc* has been intensively studied (*e.g.*, [26]), reports of DDE dechlorination by *Dhc* are lacking, perhaps because DDE is much more hydrophobic than the chlorinated ethylenes and difficult to study with pure cultures.

The fraction of the bacterial community that consisted of the *Dehalococcoidetes* relatives (*Dhcetes*) in PVS sediment samples was estimated by quantitative PCR to be between 0.04% and 4.76% (25 cm below sea floor; *cf.*, Table 1). There was a general trend towards increasing relative abundance in samples deeper in the core. The trend suggests that *Dhcetes* are relatively sparse in the Mn-/Fe-reducing zone relative to the sulfate-reducing zone and that, therefore, RDC of DDE should be slower in the shallower depth intervals of the sediment column.

Table 1. Fraction of bacterial community made up by *Dehalococcoidetes* relatives in PVS sediment samples.

Station/core	Year of Sampling	Depth Range (cmbsf)	% *Dhcetes*	Standard Deviation (3 replicates)
3C/124	2010	0-2	0.08%	0.03%
3C/124	2010	10-12	0.12%	0.04%
3C/124	2010	24-26	0.69%	0.33%
3C/124	2009	10	1.92%	1.08%
3C/124	2009	25	4.76%	2.19%
6C/147	2010	0-2	0.28%	0.08%
6C/147	2010	10-12	0.24%	0.12%
6C/147	2010	24-26	0.04%	0.02%
6C/147	2009	10	1.74%	0.63%
6C/147	2009	25	3.27%	1.83%

However, based on analyses of the 16S ribosomal RNA sequences, these putative organohalide-respiring *Dehalococcoidetes* strains were not in the same monophyletic clade as previously characterized organohalide-respiring strains. Hence, it is difficult to say with certainty that they carry out the reductive dehalogenation of DDX compounds in PVS sediments. The increased abundance of the strains in laboratory incubations and their increasing relative abundance in deeper, more anoxic parts of the sediment column suggest that they may play a role in the reductive dehalogenation of DDE. Attempts to PCR-amplify genes encoding reductive dehalogenases, the enzymes responsible for reductive dehalogenation, were unsuccessful (degenerate primer pairs RRF2 and B1R). However, this does not rule out the presence of reductive dehalogenase genes, as many known genes in this class are not able to be amplified by this primer pair.

3.4. What biogeochemical differences exist across the shelf? (Orem, Eganhouse)

Our observations on biogeochemical differences across the shelf are based on a very limited data set; specifically cores collected at three sites along the PVS (stations 8C, 6C, and 3C; Figure 1). Discussion here will be restricted to the major biogeochemical parameters thought to affect DDX dechlorination.

Biogeochemical redox parameters were very similar among the three sites: a manganese reduction zone near the surface, an iron reduction zone below this, and a sulfate reduction zone below the iron reduction zone. Delineation of the zones is based on pore-water measurements of dissolved manganese, dissolved iron (II), dissolved total sulfide, and dissolved methane gas. Van Cappellen and Santschi [4] observed an oxic zone at the sediment surface at station 6C that was 1-2 cm in thickness, based on microelectrode O_2 measurements. We did not observe an oxic layer in sediments from our cores, but our sampling resolution of 1 cm may have missed an oxic layer several mm in thickness.

Although all sites had similar redox zonation patterns, the depths of these zones varied. For example, the manganese reduction zone extends from the sediment/water interface to about 4 cm at stations 8C and 6C, but extends to about 10 cm at station 3C. Similarly, the iron reduction zone is also deepest at 3C (25-30 cm), and shallowest at 8C (10 cm), with 6C intermediate (15-20 cm). Thus, the zone of sulfate reduction starts as shallow as 10 cm at 8C, but not until about 25-30 cm at 3C; again 6C is intermediate. Sulfide concentrations also follow a pattern of being higher at 8C (up to 10,000 ppb), intermediate at 6C (up to 7,000 ppb), and much lower at 3C (up to 120 ppb). Differences in redox zonation across sites may reflect proximity to the White Point wastewater outfalls (8C closest and 3C farthest). Deposition of readily biodegradable organic matter from the outfalls provides substrate that drives anaerobic microbial processes faster at 8C compared to 3C. Van Cappellen and Santschi [4] reached a similar conclusion from studies at 6C and 8C. Terminal electron acceptors such as dissolved manganese and iron are depleted much faster at 8C and the zones of manganese and iron reduction are compressed compared to 3C.

The measurement of dissolved hydrogen (H_2) in sediments is difficult to make due to the extreme volatility of this gas; results should, therefore, be considered semi-quantitative. Hydrogen was hypothesized to be an important control on DDE dechlorination based on lab studies with TCE, a structural analog of DDE [28, 29]. Hydrogen gas was detected at all sites (concentrations ranged from 25 to 375 ppb). Although there is scatter in the data set, overall concentrations of H_2 are lowest at station 3C and highest at 8C; again 6C appears to have intermediate concentrations. There is indication of an increase in H_2 concentration with depth, but data scatter is too great to draw a definitive conclusion. As with the redox zonation, organic matter (content and biodegradability) may be the driver for the observed H_2 pattern. Hydrogen gas is produced by anaerobic fermenting microorganisms from biodegradation of organic matter, and is used by other microorganisms as an energy source. Higher anaerobic microbial activity at 8C may be producing higher concentrations of H_2 compared to 3C.

The overall pattern observed across PVS is: 1) higher H_2 and sulfide concentrations at 8C compared to 3C, 2) shallower manganese and iron reduction zones at 8C, and 3) DDE dechlorination primarily in the sulfate reduction zone at 8C, and in both the iron and sulfate reduction zones at 3C (see Figure 2) and 6C.

3.5. What is the composition of DOC in pore water and how does it vary spatially? (Orem)

The highest concentrations of dissolved organic carbon (DOC) were observed in the upper 10 cm at station 3C (farthest from the outfalls, *cf*., Figure 1), with a concentration of 125 mg/L at 0-2 cm, then decreasing below 15 cm to 6-20 mg/L. Stations 6C and 8C had generally comparable DOC concentrations (range of 2-18 mg/L, but with most values between 5-10 mg/L), somewhat lower than those at 3C especially near the surface. No distinct downcore patterns in DOC were observed at stations 6C or 8C. Van Cappellen and Santschi [4] observed DOC concentrations from 2 to 10 mg/L, generally in the range observed in our study. Vertical changes in DOC concentrations in the upper 15 cm roughly corresponded to changes in volatile fatty acid (VFA) concentrations at all sites; VFA carbon can account for more than 60% of the total DOC here. This suggests that DOC concentrations near the sediment surface are partly controlled by production of VFA from the biodegradation of more complex organic matter. VFAs (primarily acetate) are key intermediates in the pathway leading from complex sedimentary organic matter to methane or CO_2 and are important organic substrates in pore water for iron and sulfate reduction and methanogenesis. The highest VFA concentrations were in the upper 15 cm (up to 115 mg/L), with concentrations below 15 cm generally low (< 8 mg/L) or undetectable (< 0.1 mg/L). High concentrations of VFA in the upper 15 cm may be due to higher rates of organic matter biodegradation and production of VFA, as anaerobic microbial activities and numbers are greater nearer the sediment/surface water interface [30, 31].

The chemical structure of the dissolved organic matter in the pore water was examined by excitation emission matrix spectroscopy (EEMS; Figure 4). This technique measures the fluorescent characteristics of organic matter in water by scanning across both excitation and emission wavelengths generating a resulting spectral pattern. Typical downcore spectra are illustrated here for pore water collected from station 6C (Figure 4). The near-surface (0-2 cm) pore water dissolved organic matter exhibited fluorescence in the range of 350-450 nm. This is characteristic of humic substances. This pattern changed with increasing sub-bottom depth, as the fluorescence from humic substances was replaced by increasing fluorescence by proteinaceous organic matter. This could indicate a transition from deposition and biodegradation of typical nearshore marine organic matter in the surface sediments, to more wastewater-dominated organic matter (proteinaceous) below 10 cm at all three sites. The impact of this change on exchange of DDX between the solid sediment and pore-water phases, and on DDX dechlorination is unclear at this time.

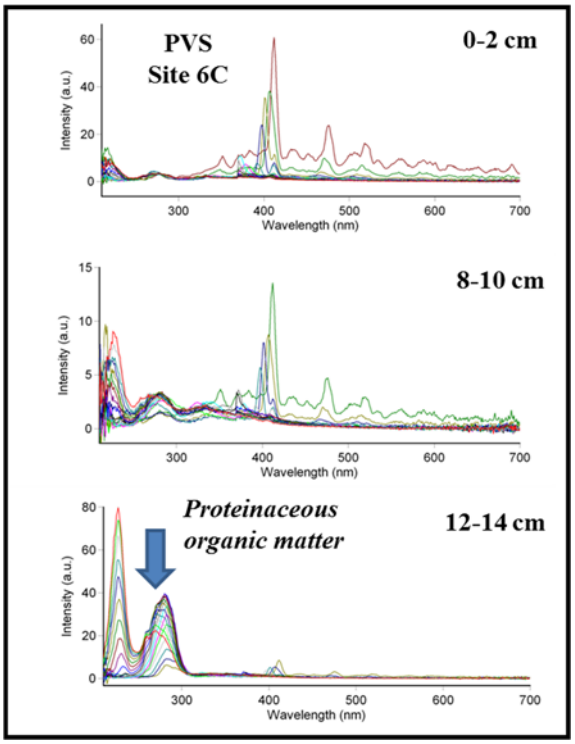

Figure 4. Excitation emission matrix spectra (EEMS) of PVS sediment pore water at site 6C.

3.6. In what forms is p,p'-DDE present in the sediments (dissolved, colloidal)? (Eganhouse, Orem)

This part of the project was not completed. The original intent was to collect pore water by centrifugation and subject the centrate to ultrafiltration and/or ultracentrifugation to isolate and analyze size fractions of the DOM-associated *p,p*'-DDE (DOM includes dissolved + colloidal organic matter passing a filter; *cf*., [32]). The results would then be compared with the freely-dissolved *p,p*'-DDE concentration (C_{free}) as determined by matrix-solid phase microextraction (matrix-SPME). Although absent from the original **Scope of Work**, plans were also made to carry out whole-core squeezing (W-CSq) of a core followed by solvent extraction of the expressed pore water and GC/MS analysis to determine C_{total}, where $C_{total} = C_{free} + C_{DOC}$, but the target core was lost (through leakage), and this work could not be completed.

In the present study, the concentration of DOC was determined in cores collected in 2010 at three stations (3C, 6C, 8C; *cf*., Figure 1). DOC data also exist at stations 6C and 8C for cores collected as part of the Montrose investigations in 1997 [4]. Finally, W-CSq data (C_{total}) for *p,p*'-DDE were acquired for a core collected from station 6C in 1992 (Eganhouse, unpublished). Using these data (station 6C only), we: 1) estimated the DOC-water distribution coefficient (K_{DOC}) of *p,p*'-DDE in PVS sediments, 2) compared these estimates with published K_{DOC} data for *p,p*'-DDE, and 3) estimated the fraction of DOC-associated *p,p*'-DDE (f_{DOC}) in PVS pore water. Log K_{DOC} values for *p,p*'-DDE (N=22) at station 6C were estimated using the following equation:

$$K_{DOC} = \frac{C_{total} - C_{free}}{m_{DOC} \cdot C_{free}} = \frac{C_{DOC}}{m_{DOC} \cdot C_{free}} \quad (1)$$

where m_{DOC} = the concentration of DOC (kg/L).

Log K_{DOC} values estimated using the Van Cappellen and Santschi [4] DOC data ranged from 2.3 to 6.7 L/kg OC with a mean ± 1 std dev of 5.5 ± 0.8 L/kg OC, whereas those using DOC data from the present study (W. Orem) were: log K_{DOC} = 2.8-7.4 L/kg OC with a mean ± 1 std dev of 4.4 ± 1.0 L/kg OC. Eight published log K_{DOC} values for DDE obtained using a variety of methods (SPME, dialysis, linear free energy relationships, reverse-phase chromatography) and different types of dissolved organic matter (soil humic acids, isolates from streams) were found in our literature search [33-36]. Log K_{DOC} values ranged from 4.4 to 6.1 L/kg OC, with a mean ± 1 std dev = 5.0 ± 0.5 L/kg OC. From this, we conclude that our estimates for sediments at station 6C fall within the range of published data. To estimate the fraction of *p,p*'-DDE associated with DOC, f_{DOC}, the following equations were used:

$$f_{DOC} = \frac{C_{total} - C_{free}}{C_{total}} \quad (2)$$

and

$$f_{DOC} = \frac{m_{DOC} \cdot K_{DOC} \cdot C_{free}}{C_{free} + m_{DOC} \cdot K_{DOC} \cdot C_{free}} \quad (3)$$

In the upper few cm of the sediment column near station 6C, estimated f_{DOC} values (equation 2) were near unity (meaning nearly 100% of DDE is associated with DOC), decreasing with depth linearly to zero at 19 cm and increasing with depth to 0.4-0.7 down to 27 cm. Using equation 3, the parameters that yielded results best matching this trend were a log $K_{DOC} \approx 5$ and m_{DOC} data from the present study (W. Orem). Clearly, the magnitude of K_{DOC} and f_{DOC} in PVS sediments remains uncertain, but it appears likely that in the upper 10-15 cm of the sediment column at station 6C, the majority of the *p,p*'-DDE in pore water is bound to DOM.

3.7. Which form or forms of p,p'-DDE are bioavailable? (Eganhouse)

The term 'bioavailability' is widely used in a variety of disciplines, but it has no strict definition. Its meaning depends on the field in which it is being used [37]. The current paradigm is that only the freely-dissolved form of an organic compound is available for microbial uptake and degradation [38]. However, local depletion of a dissolved hydrophobic organic contaminant (HOC), such as *p,p*'-DDE, in pore water will result in its release (desorption) from the solid and DOM phases. Consequently, a distinction can be made between the immediately available contaminant pool (freely dissolved in pore water) and that which is potentially available (weakly sorbed to the sediments and/or DOM) through the process of desorption.

Measures of bioavailability currently include two endpoints: *chemical activity* and *bioaccessibility*. *Chemical activity* reflects the potential of a contaminant to partition into organisms (including microorganisms) at equilibrium. It is commonly measured by 'equilibrium samplers' (such as SPME) as the freely-dissolved concentration (C_{free}). *Bioaccessibility*, on the other hand, refers to the fraction of "weakly or reversibly sorbed" contaminant that can enter the aqueous phase *via* desorption from the solid phase. In essence, it is "…the amount or portion of contaminant that is or can become available within a given time span". It is often measured by partial extraction methods, such as mild solvent or Tenax® extraction, and is therefore operationally defined. It has been suggested that in processes such as biodegradation, bioavailability is more dependent on *bioaccessibility* than *chemical activity* [37].

In the case of PVS sediments, it appears that the majority of the DDE in pore water, at least in the upper sediment column, is present in association with dissolved/colloidal organic matter (see section **3.6.**). According to the current paradigm, this form is not directly available to the microorganisms responsible for reductive dechlorination of DDE. Although no measurements of *bioaccessibility* were made in this project, it can safely be assumed that there is a discrete fraction of the *p,p*'-DDE sorbed onto the sediments, which serves as a bioavailable reservoir. This is evidenced by the ongoing transformation of DDE to DDMU and DDMU to DDNU over several decades now [18]. If *p,p*'-DDE was increasingly becoming sequestered in a phase that was <u>not</u> *bioaccessible*, the rate of natural attenuation could be expected to decrease with time and possibly approach zero [16]. However, the evidence to date, limited though it may be, does not indicate that RDC rates have decreased with time. This suggests that the reservoir of *bioaccessible* (and, hence, bioavailable) DDE has not been depleted to the point that it is limiting the RDC rate.

Based on results of the triple-domain (3 sorbent phases) modeling of sorption described below (see section **3.8.**), we believe the dominant sorbent phase in PVS sediments is residual hydrocarbons ('oil'; [39, 40]). Under the optimized model conditions reported by Koelmans *et al*. [41], the fraction of *p,p*'-DDE associated with residual hydrocarbons in the sediments was calculated to be 96-98% of the total sedimentary *p,p*'-DDE. Although the hydrocarbon phase is very hydrophobic, it represents a partitioning medium that should allow for ready exchange of sorbed constituents, such as *p,p*'-DDE, with the pore water. The hydrocarbons lower the *chemical activity* of *p,p*'-DDE but not its *bioaccessibility*. This may explain why C_{free} concentrations of the DDX are so low in PVS sediments, but natural attenuation appears to be proceeding at constant rates. If this hypothesis is correct, the *bioaccessible* (and, hence, bioavailable) fraction of *p,p*'-DDE in PVS sediments is probably quite high.

3.8. What controls sorption of p,p'-DDE? (Eganhouse)

Sorption of DDX is the main process controlling availability of *p,p*'-DDE (and *p,p*'-DDMU) to the microorganisms responsible for reductive dechlorination. But, what controls sorption? We were interested in learning whether sorption could be attributed primarily to partitioning between pore water, dissolved organic matter and the bulk sedimentary organic matter or if the organic composition of sedimentary organic matter might influence the sorption process.

The original plan was to isolate geochemical fractions (or phases) of the sedimentary organic matter and measure sorption isotherms for *p,p*'-DDE to these phases. However, this could not be completed within the available time. Instead, we conducted a limited organic petrographic investigation of the composition of organic matter in unaltered PVS sediments collected near station 6C in 1992; insoluble organic matter (IOM) was isolated by sequential extraction from the sediments. Microscopic examination revealed the presence of marine kerogen in the form of "…amorphous organic matter (AOM) and/or bituminite and/or alginate…" as well as terrestrial OM in the form of vitrinite and inertinite. Char particles were also observed in the IOM. All of these phases could, in principle, act as carbonaceous geosorbents [17] capable of strongly (and essentially irreversibly) sorbing hydrophobic substances such as *p,p*'-DDE [42].

In the absence of experimental sorption data for potential geosorbent phases, we investigated a model for *p,p*'-DDE sorption to three phases: AOM (as *hydrocarbon-free* organic carbon; OC), hydrocarbons (HCs), and black carbon (BC) [41]. The sediment/pore-water distribution coefficient, K_d, in the triple-domain model is given by:

$$K_d = \frac{C_s}{C_w} = f_{oc}K_{oc} + f_{HC}K_{HC} + f_{BC}K_{BC}C_w^{n_{F,BC}-1} \quad (1)$$

where: K_d is the sediment/pore water distribution coefficient (L/kg), C_s (µg/kg) and C_w (µg/L) are the measured *p,p*'-DDE concentrations in sediment and water, respectively, f_{oc}, f_{HC}, and f_{BC} are the measured OC, HC and BC weight fractions, K_{oc} (L/kg), K_{HC} (L/kg), and K_{BC} ([µg/kg]/[g/L]$^{n_{F,BC}}$) are the calculated sorption constants for these phases, and $n_{F,BC}$ = the Freundlich exponent for nonlinear sorption to BC (~0.7).

K_{oc}, K_{BC} and K_{HC} values were estimated based on regressions by van Noort [43], whereas f_{oc}, f_{HC}, f_{BC}, C_s, and C_w were determined in the USGS/Reston, VA laboratory for subcores 124B1-, 147B4-, and 171B2-WC (stations 3C, 6C and 8C, respectively). Comparison of calculated K_ds using one- (OC), two- (OC+BC or OC+HC) and three- (OC+BC+HC) domain models showed little effect of BC (0.3-0.9% sorbed, 'optimized' parameters) but a very significant effect when the HC phase was included (96.3-97.9% sorbed, 'optimized' parameters). This reflects the unusually high level of contamination by petroleum hydrocarbon wastes in PVS sediments (HC/TOC [w/w] = 0.16 ± 0.05) and its corresponding influence on *p,p*'-DDE sorption. One potential consequence is that biodegradation of residual hydrocarbons may lead to enhanced release of *p,p*'-DDE due to decreased sorptive capacity [41].

In conclusion, residual waste-derived hydrocarbons [39] appear to exert a dominant control on sorption and, therefore, bioavailability of *p,p*'-DDE in PVS sediments.

4. Laboratory studies.

4.1. Is the presence of hydrogen required for the dechlorination of p,p'-DDE and p,p'-DDMU? (Reinhard)

The study of the dechlorination of TCE and other chlorinated hydrocarbons has led to the discovery of novel bacteria that reductively dechlorinate chlorinated compounds utilizing hydrogen as an electron donor. The novel bacteria belong to genus *Dehalococcoides*, which belong to the class *Dehalococcoidetes* [27]. *Dehalococcoides* grow at extremely low aqueous hydrogen concentrations (<1 nM or less than 2 ng/L). However, growth of *Dehalococcoides* coupled to the reduction of DDE and the oxidation of hydrogen has not been demonstrated. Therefore, it is unknown whether the presence of hydrogen is required for DDE dechlorination to occur.

Given the structural similarity between DDE and perchloroethylene, we hypothesized that *Dehalococcoides* bacteria were responsible for dehalogenating DDE in PVS sediments utilizing hydrogen as the electron donor. In microcosms, DDE dechlorination occurred in samples from all sub-bottom depths in all microcosms tested, suggesting that the necessary populations were ubiquitous. DDE and DDMU were dechlorinated while sulfate was present and at hydrogen concentrations ranging from 0.01-0.02 mg/L. Adding organic donors (a mixture containing acetate, propionate, butyrate, pyruvate, lactate, and benzoate) stimulated both dechlorination and the formation of hydrogen. However, the stimulatory effect of hydrogen on DDE dechlorination could not be demonstrated. Taken together, the evidence suggests that in PVS sediments, DDE dechlorination is not limited by hydrogen availability even in the presence of sulfate. This is consistent with field data that show that hydrogen is present at sufficient concentrations to support dechlorination at all locations.

4.2. How is hydrogen formed, and what are the precursors fermented to hydrogen (e.g., short chain fatty acids)? (Reinhard)

This question turned out to be immaterial and was not pursued because the lowest measured hydrogen concentrations (0.01–0.02 mg/L) supported RDC. Thus, a minimum concentration below which RDC stopped because of hydrogen limitation could not be established. Hydrogen gas was detected in all field samples at sufficient levels, either because it was formed locally by fermenting bacteria or diffusing from methanogenic layers at greater depth. As discussed below, added fatty acids were utilized rapidly (likely by SRBs), and addition of fatty acids to microcosms quickly resulted in methanogenic conditions, which, in turn, stimulated DDE dechlorination. The fact that no methane was found in the top 50 cm of the sediments on the PVS is unexplained. Possibly methane formed *in situ* or methane intruding from deeper levels was utilized by methanotrophs. The discrepancy between conditions observed in batch laboratory studies and the field indicates that microcosms that are run for extended periods of time do not simulate field conditions. Therefore, extrapolating kinetic data from microcosm to the field is fraught with uncertainties.

4.3. Can hydrogen formation by fermentation be stimulated by adding fermentation precursors, short chain fatty acids? (Reinhard)

The addition of readily degradable organic carbon (a mixture of pyruvate, lactate and glycerol) to microcosms was previously demonstrated to provide sulfate-reducing organisms with enough electron donors to reduce all of the sulfate in the medium [21]. It was shown that depletion of sulfate led to methane formation and to the acceleration of the RDC of DDE. However, the formation of hydrogen and its effect on RDC were not measured in previous investigations.

We studied hydrogen formation and sulfate depletion in microcosms constructed with sediments from stations 8C and 3C in the presence of a mixture of acetate, propionate, butyrate, pyruvate, lactate and benzoate at initial concentrations of 1.5, 0.86, 0.6, 1.2, 1 and 0.4 mM, respectively. In the augmented microcosms, hydrogen concentration increased to a maximum of approximately 3000 ppm in 13-14 weeks with concurrent sulfate depletion. Controls with DDE (added in acetone) but without the donor mix unexpectedly showed a peak hydrogen concentration of approximately 1300 ppm after about 13 weeks. This was attributed to the addition of 10 μL acetone, which served as a carrier of the highly hydrophobic DDE. The formation of hydrogen from acetone was also shown in separate experiments.

4.4. What bacterial groups are responsible for hydrogen production? (Reinhard)

The presence of low molecular weight (*i.e*., volatile) fatty acids in the sediments suggests fermentative bacteria could be involved in the production of hydrogen.

4.5. Do fatty acid utilizing sulfate reducing bacteria affect the dechlorination of DDE? (Reinhard)

In microcosms, growth of sulfate reducing bacteria (SRB) affected dechlorination indirectly by depleting sulfate and promoting methanogenesis. RDC of DDE was faster under methanogenic conditions, as was observed previously by Quensen *et al*. [21]. SRB growth and sulfate utilization was stimulated by the addition of electron donor (a mixture of acetate, propionate, butyrate, pyruvate, lactate, and benzoate at concentrations ranging from 0.4 to 1.2 mM) to microcosms. Depletion of sulfate promoted methane and hydrogen formation and the transformation of DDE to DDMU and DDMU to DDNU. Although hydrogen concentrations increased after sulfate depletion, a relationship between the rate of RDC and hydrogen concentration could not be established. In the field, sulfate depleted by microbial sulfate reduction is relatively rapidly resupplied from the water column, and sulfate utilization does not proceed to completion. This is in contrast to conditions in microcosms, which are closed systems. The relatively high sulfate concentrations in the field sediments explain the absence of methane in the top 50 cm of the sediment column.

4.6. Does sulfate inhibit dechlorination by depressing the hydrogen threshold? (Reinhard)

The underlying hypothesis for this question was that DDE dechlorinating bacteria require a minimum (threshold) hydrogen concentration for growth. Laboratory experiments demonstrated that sulfate depressed hydrogen concentrations but not to the point of inhibiting the RDC of DDE. DDE transformation proceeded at sulfate concentrations as high as 1000 µg/mL (1 g/L) or higher. Depletion of sulfate (to below approximately 100 mg/L) led to methanogenic conditions and high hydrogen concentrations and to an increase in the RDC rate. RDC was observed at hydrogen concentrations of 0.01-0.02 mg/L, which is significantly above the threshold for sulfate reduction 1.5-4.5 ng/L [44]. Thus, hydrogen was always present in excess. The dependence of RDC of DDE on the presence of hydrogen has not been established, and the value of a hydrogen threshold for DDE dechlorination could not be demonstrated from our data. DDE was dechlorinated in all microcosms tested, even at the lowest hydrogen concentrations measured. If a hydrogen threshold for DDE dechlorination exists, it must be far below these concentrations.

4.7. Is hydrogen sulfide inhibiting DDE dechlorination? (Reinhard, Orem)

Hydrogen sulfide, which is often toxic to bacteria, was hypothesized to be a factor affecting the rate of dechlorination. At station 3C, where RDC rates were faster than at stations 6C and 8C, H_2S concentrations were lower. This is consistent with an inhibitory effect of H_2S. However, laboratory experiments did not indicate that H_2S formed during the reduction of natural levels of sulfate were inhibiting the dechlorination of DDE. Experiments to determine threshold of hydrogen sulfide inhibition on dechlorination were, therefore, not conducted.

4.8. Is there a fraction of p,p'-DDE that resists desorption and is, therefore, not bioavailable? (Eganhouse)

In the **Scope of Work** we hypothesized that the rate of reductive dechlorination was limited by desorption kinetics and that the association of *p,p*'-DDE with different organic geosorbent phases within the sediments (and aging) could result in fractions of *p,p*'-DDE that desorbed at different rates. It was our intent to experimentally determine the rate(s) of desorption of *p,p*'-DDE from PVS sediments and estimate the size of the 'rapidly desorbing' (*i.e.*, '*bioaccessible*') fraction and compare the results with model calculations [45] and possibly observations of kinetics made during the ^{14}C-DDE microcosm experiments.

To accomplish this, we proposed using a solid sorbent (Amberlite® XAD4 or Tenax®) to study the kinetics of desorption according to procedures described by Lamoureux and Brownawell [46] and You *et al*. [32]. In this experiment, the sorbent would have been exposed to PVS sediments and sampled at time intervals followed by extraction of the added sorbent and instrumental analysis of the extract using gas chromatography/mass spectrometry. The data were expected to provide estimates of the desorption rate coefficient(s) and the sizes of the *rapidly* and *slowly* desorbing fractions of *p,p*'-DDE.

Due to unforeseen difficulties encountered during the early stages of the project, primarily related to the heavy burden of method development and changes in cruise scheduling, this component of the study was not attempted. However, as discussed in section **3.8.**, it appears that residual petroleum hydrocarbons are the organic geosorbent phase exerting dominant control on sorption of *p,p*'-DDE in PVS sediments. By comparison, 'black carbon' appears to play an insignificant role in sorption of *p,p*'-DDE on the PVS.

The hydrocarbons, which are primarily derived from refinery wastes present in discharged final JWPCP effluent [39, 40, 47], are likely disseminated within and sorbed to amorphous organic matter and mineral surfaces within the sediments. The strongly hydrophobic character of these hydrocarbons would favor partitioning rather than adsorption of *p,p*'-DDE, resulting in relatively faster release rates from the sediments to the pore water as a result of competition [48]. Insofar as the vast majority of the hydrocarbons in PVS sediments are derived from the JWPCP effluent and the fact that the residual sedimentary hydrocarbons are significantly degraded when compared with those in wastewater effluent [39], release of *p,p*'-DDE during the early stages of diagenesis was likely tied, at least in part, to loss of organic matter, including simple hydrocarbons (such as the *n*-alkanes).

With continued stabilization of the sedimentary organic matter over the last four to five decades, release of *p,p*'-DDE from the dominant hydrophobic organic sorbent phase (hydrocarbons) appears to have continued unabated [49]. If these hydrocarbons were not so prevalent in PVS sediments, *p,p*'-DDE would likely have become progressively sequestered within micropores and/or associated with carbonaceous geosorbents, such as black carbon, thereby reducing its bioavailability. Again, because existing evidence suggests that RDC rates have been constant since ~1981, progressive sequestration does <u>not</u> appear to be occurring to a significant extent. Laboratory kinetic studies indicate that freshly added *p,p*'-DDE is dechlorinated more rapidly than native (historical) *p,p*'-DDE (*p,p*'-DDE present in "aged" sediment), suggesting that the aged *p,p*'-DDE may not be as bioavailable as freshly added *p,p*'-DDE.

4.9. Does desorption of p,p'-DDE limit dechlorination rates? (Eganhouse)

Biotransformation depends on two processes: mass transfer of the contaminant **to** the cell and uptake and metabolism **by** the cell [50]. When mass transfer is fast compared to biodegradation, the transformation rate is at its maximum and the contaminant "…is fully available for biodegradation".

It is generally believed that the bioaccessible portion of sedimentary HOCs is contained within the rapidly-desorbed fraction (F_{rap}). Thus, the desorption rate constant of the rapidly-desorbed fraction (k_{rap}) yields information on the potential mass transfer rate of the *bioaccessible* portion of sedimentary HOCs. Although the desorption rate of *p,p*'-DDE from PVS sediments was not measured directly in this study, evidence from the literature suggests that rate constants for the rapidly- (k_{rap}) and slowly- (k_{slow}) desorbed fractions of HOCs are on the order of 0.1-1 h^{-1} (876-8,760 yr^{-1}) and 10^{-3} h^{-1} (8.8 yr^{-1}), respectively [5]. You *et al*. [6] presented data for *p,p*'-DDE-spiked sediments and found k_{rap} = 0.17-0.57 h^{-1} (1,480-4,980 yr^{-1}), k_{slow} = 0.0014-0.058 h^{-1}(12.2-508 yr^{-1}) and $k_{very\ slow}$ = 0.0003-0.0033 h^{-1}(2.6-28.9 yr^{-1}). Mehler *et al*. [7] separated soils into three size ranges (fine, coarse, and combined), added water, spiked them with *p,p*'-DDE, and measured k_{rap}, k_{slow}, and $k_{very\ slow}$. They obtained rate constants as follows: k_{rap} = 0.29-0.37 hr^{-1} (2,540-3,240 yr^{-1}), k_{slow} = 0.016-0.020 hr^{-1} (5.8-7.3 yr^{-1}), and $k_{very\ slow}$ = 0.00008 hr^{-1} (0.7 yr^{-1}). Finally, You *et al*. [51] conducted a study with matrix-SPME and Tenax® extraction of field-contaminated sediments (PCBs, 18 congeners: 44, 49, 52, 66, 70, 87, 95, 97, 99, 101, 105, 110, 118, 128, 138, 153, 156, 170) and obtained values of k_{rap} = 0.094-0.202 hr^{-1} (824-1,770 yr^{-1}) and k_{slow} = 0.0014-0.0043 hr^{-1} (12.6-37.7 yr^{-1}). [Results for PCB 153, which has physico-chemical properties similar to *p,p*'-DDE, were: k_{rap} = 0.12 hr^{-1} (1,090 yr^{-1}) and k_{slow} = 0.0030 hr^{-1} (26.3 yr^{-1}).] These desorption rates can be directly compared with first-order rates of *p,p*'-DDE loss due to reductive dechlorination for the period 1981-2010.

First-order rates of *p,p*'-DDE loss due to reductive dechlorination as determined from comparison of PVS sediment core data for the period 1981 to 2010 (see section **4.10.**) are as follows: station 3C - 0.044 ± 0.004 yr^{-1}, 6C - 0.008 ± 0.002 yr^{-1}. Obviously, the expected rates of rapid (k_{rap}), slow (k_{slow}) and even very slow ($k_{very\ slow}$) desorption for this compound are orders of magnitude greater than the first-order rates of reductive dechlorination. Hence, it appears that the rate of desorption does <u>not</u> limit dechlorination rates on the PVS. Rather, the metabolic activities of the microorganisms responsible for RDC govern the rate of transformation. This means that the spatial variation in apparent RDC rates is not related to the sorptive properties of the sediments *per se* but the metabolic activities (and numbers) of the microorganisms and factors that affect those activities. Addition of substrate could increase metabolic activity and (and numbers) of dechlorinating microorganisms. This would be expected to increase rates of dechlorination ([21]; this study).

4.10. Is first-order kinetics relevant to model dechlorination reactions? (Eganhouse, Reinhard)

Knowing the appropriate kinetic model for reductive dechlorination of *p,p*'-DDE (and other DDX) on the PVS is critically important for understanding (and predicting) the course of natural attenuation. Kinetic models commonly used for fitting biotransformation data include first-order, zero-order and Monod [52]. The simplest variable-order model would be Michaelis-Menten, which is first order at low substrate concentrations and zero order at high substrate concentration. However, it is unknown *a priori*, whether DDE concentrations are relatively high or low or whether DDE can be considered a substrate. Moreover, it cannot be safely assumed that the reaction order in PVS sediments has been constant over time or that it is spatially uniform [18].

In the absence of evidence to the contrary, a number of previous laboratory and field studies [9, 15, 20, 21, 53] have tacitly assumed that total loss (including that due to RDC + physical loss processes) or transformation of *p,p*'-DDE alone was first order. Others [8, 54] have applied zero-order loss rates based on regression analysis of *p,p*'-DDE inventories *versus* time or have assumed *p,p*'-DDE was inert [11]. However, accurate determination of reaction order, at least in the case of studies on the PVS, has been hampered by a shortage and the inherent scatter of field data. This, in turn, is due to variations in the effectiveness of sampling and natural variability in the spatial distribution of *p,p*'-DDE. When comparing small data sets, the models are difficult to distinguish, but the choice has significant consequences when extrapolating to longer reaction periods.

Eganhouse and Pontolillo [18] attempted to analyze (post-lag period) microcosm data from the publication of Quensen *et al*. [55] (station 3C, methanogenic conditions) using the van't Hoff log-difference method [56]. A slope (*i.e*., order) of 1.49 was obtained. A similar analysis was applied to data of Quensen *et al*. [21], but results were unsatisfactory (order < 1). Using whole-core *p,p*'-DDE inventories for cores (1981-2003) analyzed in the USGS/Reston environmental organic geochemistry laboratory, a slope of 1.09 was determined. Although suggestive of a reaction order of one, the data were too few to draw a definitive conclusion. In short, the existing data do not allow a clear choice between first- or zero-order kinetics.

To mitigate the effect of natural variability in *p,p*'-DDE distributions, whole-core inventories were normalized to that of PCB congeners having similar physico-chemical properties (*i.e.*, 118, 153, 138), and the data were modeled using first-order kinetics. Because PCBs are known to be recalcitrant on the PVS [14], the slope of the regression line for the natural logarithm of the PCB-normalized inventory of *p,p*'-DDE *versus* time yields the first-order RDC transformation rate, k_{RDC}. First-order *p,p*'-DDE RDC rate coefficients were 0.044 ± 0.004 yr^{-1} and 0.008 ± 0.002 yr^{-1} for cores collected at stations 3C and 6C, respectively (mean ± 1 std dev for the three normalization congeners). These data can be compared with first-order <u>total loss</u> rates, k_{total}, developed by Sherwood *et al*. [13] based on linear regression analysis of *p,p*'-DDE inventories in LACSD cores (1985-2007), which were 0.046 ± 0.009 yr^{-1} and 0.023 ± 0.005 yr^{-1} for 3C and 6C cores, respectively. The results suggest that at station 3C, the loss of *p,p*'-DDE is dominated by RDC (~97%), whereas at station 6C, RDC accounts for about 37% of the total loss.

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