Final

Thin Layer Cap Pilot Study Report

Former Custom Plywood Site

Anacortes, Washington

Prepared for
Washington State Department of Ecology

May, 2016
17800-51

Revised by Washington State Department of Ecology

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HARTCROWSER

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This document was originally drafted by Hart Crowser, Inc. for the Department of Ecology in May 2016. Since then, the Department of Ecology revised various portions of the main body texts, as well as Figure 16, which was replaced with two new figures (Figure 16-1: Sediment Dioxin TEQ Variation by Test Plot and Figure 16-2: Sediment Dioxin TEQ (Mean) trend by Deployment Days). The Department of Ecology also reformatted the document, resulting in some differences in formatting from Hart Crowser's original submittal.

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INTRODUCTION

The Washington State Department of Ecology's (Ecology) Toxics Cleanup Program (TCP) proposes to conduct in-water remedial activities at the former Custom Plywood mill site in Anacortes, Washington, on the shore of Fidalgo Bay (Figure 1). Subtidal sediments in the vicinity of Custom Plywood are impacted with polychlorinated dibenzo-p-dioxins and dibenzo furans (PCDD/PCDF), referred to as dioxins throughout this report. One of the proposed cleanup activities includes remediating a large area of contaminated sediment that supports substantial eelgrass habitat. Because protection of eelgrass habitat is of the utmost importance, traditional methods of remediation, such as dredging or placement of a thick cap, are not feasible; therefore, the remediation design must balance the benefits of reducing exposure to contaminated sediment while allowing eelgrass—and its associated marine community—to survive with minimal alteration to its health and productivity. A feasible approach is to implement a "thin" cap that adequately achieves remediation criteria without adversely affecting eelgrass function or productivity.

In order to investigate this approach, a thin layer cap (TLC) pilot study was designed to investigate various capping scenarios and approaches to determine the feasibility and practicability of implementing a thin layer cap to remediate sediment contaminants while preserving both the ecological function and biological productivity of eelgrass habitat at the site. This report documents the approach and results of a small-scale pilot study to determine if a thin layer cap can effectively remediate dioxins which are the contaminant of concern in sediment without adverse impacts on marine habitat. Specifically for eelgrass habitat function, the pilot study investigated the tolerance of eelgrass beds to the placement of a sediment cap with variations on thickness and composition. The pilot study focused on the use of low-impact techniques to disperse sand over the seabed and gradually build up the cap over time. In some areas, a thin layer of activated carbon pellets was dispersed prior to the application of the sand cap to determine whether additional activated carbon provides enhanced adsorption of dioxin. After cap installation, eelgrass habitat metrics and dioxin concentrations were tracked over time to help inform the design of the larger cleanup.

SITE BACKGROUND

The Custom Plywood Site was a lumber and plywood milling operation beginning in about 1900. Through the years, the property changed hands several times, and was rebuilt and expanded until Custom Plywood became an operating entity sometime before 1991. The facility was a sawmill and plywood manufacturing plant until most of the wooden structures in the main plant area were consumed in a fire on November 28, 1992.

The Custom Plywood site has a significant history of chemical handling, use, piping, and distribution, as well as waste materials disposal, which consisted of filling tidelands with wood, ash, bricks, metal, and sediments. Potential contamination sources include releases, spills, or on-site disposal of transformer fluid; wash water and sludge; pollution control sludge; glue wash water sludge; knot filler sludge; boiler ash; scrap steel; barrels and drums; aluminum cans; scrap wood; paper; asbestos pipe coverings; creosote-treated pilings; and transformers with PCB oils. These industrial activities left a legacy of metal, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl compounds (PCBs), petroleum hydrocarbon, and dioxin contamination within the upland soils and intertidal and subtidal areas. The elevated dioxin concentrations in the sediments are likely associated with the combustion of the building materials and wood from a fire that destroyed the facility in 1992.

The Custom Plywood Site is one of several bay-wide, Anacortes-area priority sites for Fidalgo/Padilla Bays being addressed by the Toxics Cleanup Program (TCP) under the Puget Sound Initiative (PSI). The Custom Plywood Site includes property owned by GBH Investments, LLC (GBH) covering approximately 6.6 acres of upland and 34 acres of intertidal and subtidal areas. The site has been under the provisions of the Washington State Model Toxics Control Act (MTCA) cleanup process since the Department of Ecology and the property owner (GBH) entered into an Agreed Order in March 2008. A Phase I interim upland cleanup was completed during the summer of 2011. Contaminated material was removed and disposed off-site, and a 12,000-square-foot wetland mitigation area and vegetation buffer zone were constructed in the upland area. Phase II of the remedial action was driven primarily by contamination left along the shoreline, large accumulations of debris resulting from the 1992 fire, and large derelict structures not consumed by the fire. Phase II interim remedial actions of the intertidal/subtidal area began in July 2013 and included removal of all in-water structures and pilings; excavation, dredging, and disposal of contaminated sediment; construction of protective-in-water features (spit and jetty extension) for the protection of the shoreline and additional habitat benefits; and connection of the previously constructed wetland with Fidalgo Bay (Ecology 2012). The final phase of the project will remediate the low to moderate dioxin contamination found in the subtidal areas of the site.

EELGRASS HABITAT VALUE

Fidalgo Bay supports many important nearshore resources that are both ecologically and economically important to the region. Forage fish, salmonids, and several varieties of shellfish rely on Fidalgo bay during various phases of their life cycles, which is directly related to the use of extensive areas of eelgrass habitat in the bay.

Eelgrass beds are widely recognized throughout the Puget Sound as habitat features of significant ecological importance. Eelgrass and other seagrass species are used as indicators of estuary health since they respond to many natural and human-caused environmental factors. Eelgrass beds play a key role in nearshore ecosystems providing structure and refuge for many species of fish and invertebrates, foraging habitat for migratory waterfowl, and spawning substrate for forage fish like Pacific herring (Dumbauld and Wyllie-Echeverria 2003; Wyllie-Echeverria and Ackerman 2003; Phillips 1984). They also stabilize the sediment (Mumford 2007), trap sediment and detritus, and reduce current speeds, facilitating recruitment of fish and invertebrate larvae. Eelgrass blades also provide

habitat for numerous organisms such as copepods, amphipods, and snails. Eelgrass beds are also important primary producers, fixing carbon that then enters nearshore foodwebs (Thom and Hallum 1990).

Concern over seagrass ecosystem loss has increased in recent years due to the rise in natural and anthropogenic disturbances to the habitat. There are several federal and state regulations protecting seagrass habitat; in Washington State, eelgrass is considered to be a saltwater habitat of special concern and is protected under Washington Administrative Code (WAC) 220-110-250 (3)(a, b). Despite this protection, decreases in eelgrass density and distribution are still being observed throughout the Puget Sound.

EELGRASS HABITAT VS. REMEDIATION

The governmental mandate to preserve eelgrass is challenged by the need to remediate contaminated sediment in areas where critical eelgrass beds are found. During the final phase of site cleanup, subtidal areas with dioxin concentrations ranging from 10–25 parts per trillion (ppt) would require remedial action (see the Cleanup Action Plan for more specifics on cleanup action levels; Hart Crowser 2013). Based on background sampling for dioxin contamination and eelgrass coverage associated with the Custom Plywood Remedial Cleanup, there would be approximately eight acres of eelgrass habitat at the site that would require remedial action under the overall cleanup. The third and final phase of the cleanup action targets the subtidal area of the site (the remaining unremediated contamination), which includes areas of eelgrass.

Unlike other forms of contamination (e.g. petroleum), dioxins seem to have little effect on eelgrass function and productivity. This has the unfortunate consequence of uptake and transfer of the dioxins into the food chain. Shellfish and fish that use this habitat can and do incorporate this class of contaminants into their flesh (Boese et al. 1995; Landrum 1989; Lee et al. 1994; Meador et al. 2010), and these contaminants have specifically been measured in shellfish at this site (SAIC, 2010). This creates an exposure pathway where dioxins can enter the foodweb and accumulate in shellfish and fish harvested in Fidalgo bay for consumption. This is especially true of those that subsistence harvest in the area. This creates a situation where remediation is warranted, but a prescriptive remediation action may have very negative effects on overall nearshore productivity and function.

Traditional methods of dredging and/or the placement of a thick-layer of capped material on the contaminated sediment are not feasible cleanup methods within eelgrass habitat areas. Therefore, the sediment remediation approach within this area needs to reduce exposure to contaminated sediments while allowing existing eelgrass to remain. This pilot study was designed to inform remediation clean up decisions by evaluating the tolerance of eelgrass burial to a thin cap of sand in order to provide information to complete the final phase of cleanup for the project.

In addition to the traditional capping approach, the study wanted to investigate whether an amendment to the capping material could increase cap effectiveness. In some areas, a thin layer of activated carbon pellets was dispersed into the seabed before the sand cap was placed to determine whether additional carbon provides enhanced adsorption of dioxins. The potential benefits of carbon

in the thin layer cap include reduced volume of cap material to achieve the same level of protectiveness, and increased adsorption capacity of organic pollutants relative to conventional cap material. The additional adsorptive capacity provided by the carbon may decrease mobility of organic pollutants. When organic compounds like dioxins come into contact with the carbon surface, they strongly adsorb to the carbon and become significantly less bioavailable (Ghosh et al. 2011).

PROJECT PLANNING

Permitting Process

This pilot study was authorized without the carbon amendment under the US Army Corps of Engineers (USACE) Nationwide Permit 18 (NWS-2010-288) and was contingent on implementation of the requirements and/or agreements set forth in the Biological Evaluation, Eelgrass Remediation Thin Layer Cap Pilot Study Custom Plywood Interim Remedial Action dated August 5, 2011, and the special conditions specified in the USACE authorization letter dated October 18, 2011. Special conditions included an in-water work period of July 16 through January 31 and pre-construction forage fish spawning surveys.

Since the original permit was issued, new information gathered over the winter in 2011/2012 identified a need to increase cap effectiveness. In addition, all Nationwide Permits were reissued with new stipulations and conditions (corrected March 19, 2012). A reverification process was initiated on June 29, 2012, to reauthorize these activities (including the additional carbon amendment activities) under the Nationwide Permit 18.

Site Selection

The reference and test plots were located in a 70- by 100-foot area within the larger 13-acre area of eelgrass beds, within the Custom Plywood remediation area (Figures 2 and 3). Their locations were based on the 2011 macrovegetation survey data of moderately dense eelgrass and 2012 sediment chemistry data. In addition to evaluating the 2012 sediment chemistry data, Hart Crowser collected additional sediment samples in May 2013 in order more specifically characterize the area where the test plots would be specially placed. The target elevation for the study was –4 feet mean lower low water (MLLW), although elevations in the area range from about –3 to –5 feet MLLW. This small study area was deliberately chosen to minimize the potential for project impacts to measurably affect the larger eelgrass bed or threatened and endangered species that may use Fidalgo Bay eelgrass habitat. In addition, consideration was given to the natural patchiness and great annual variations in eelgrass bed density and distribution to minimize the possible loss of eelgrass in the Custom Plywood remediation area. Test plots were placed in the middle of a 6.5-acre bed, providing untreated buffers of eelgrass around each test plot, possibly allowing more natural recruitment of eelgrass seeds and plants into these test plots.

IMPLEMENTATION AND DESIGN

The pilot study was conducted as a limited-scale, semi-factorial exposure experiment using the Nationwide Permit 18, allowing for a maximum placement of 25 cubic yards of capping material. The

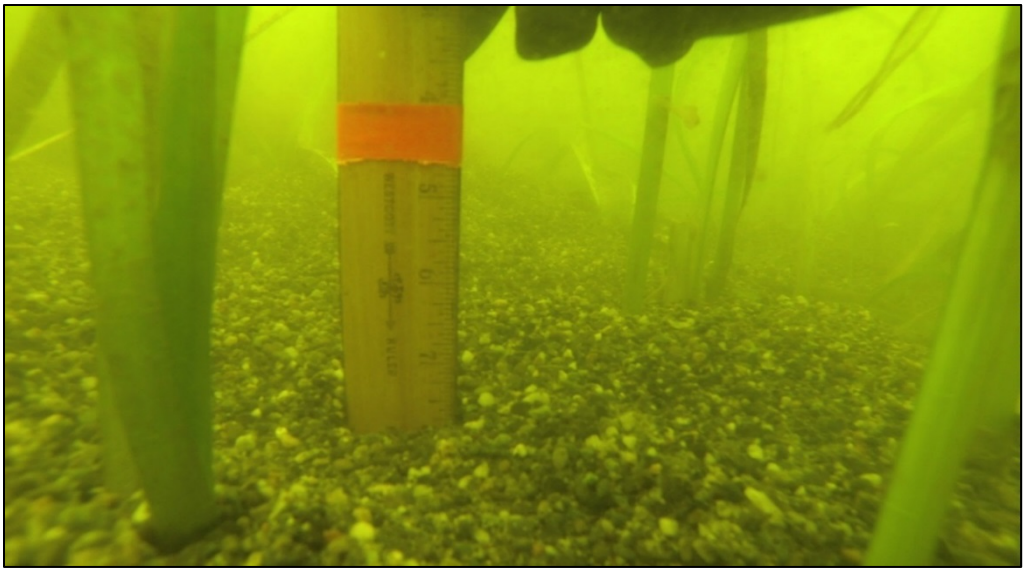
pilot study began with the application of the carbon and sand cap material which was conducted July 13–23, 2013. The four plots were labeled and constructed as follows: 4-inch sand only; 8-inch sand only; 4-inch sand plus 0.25-inch carbon; and 8-inch sand plus 0.25-inch carbon. All pilot study test plots are listed and described in Table 1 and shown in detail on Figure 3.

Table 1 – Custom Plywood TLC Plot Designation and Description

Plot Name	Plot Description
HC-1	8-inch sand only
HC-2	Carbon only
HC-3	4-inch sand plus 0.25-inch carbon
HC-4	8-inch sand plus 0.25-inch carbon
HC-5	4-inch sand only
HC-Control	Control
HC-Reference	Reference

The cap material was a combination of ASTM C33 Washed Sand, which was sourced from Concrete Nor'west, located in Oak Harbor, Washington and Sedimite™, which is an agglomerate comprised of activated carbon, a weighting agent, and an inert binder. The cap materials were deployed using a spinning disc delivery mechanism (Photograph 1). This low-impact method avoided the detrimental effects associated with conventional methods that could adversely impact the viability of eelgrass (i.e., smothering, burial, or creation of a reducing environment). The cap material was delivered in 2-inch layers until final thickness was achieved. Final thickness ranged from 4 to 8 inches on four test plots. The cap was verified by a diver to confirm that appropriate cap thickness was achieved (Photograph 2). This application required multiple placement events over the course of a single week in July 2013. Photograph 3 shows the TLC plots after cap placement was complete. The center of two plots is marked with a buoy.

Photograph 1 – Application of capping material



Photograph 2 – Assessment of deployed cap material at 4-inch test plot

Photograph 3 – View of TLC Plots from boat with buoy marking center point

Based on the above thicknesses and the limitation of 25 cubic yards of cap material, the areal extent of the four pilot plots was approximately 2,000 total square feet. The cap thickness was verified by divers after installation using sediment stakes, rulers and hand probing (Photograph 2). Cap height varied at the edges of each test plot due to current and tidal effects; therefore, each plot size was effectively 12 by 16 feet.

RESPONSE VARIABLES

The goals of this study were to evaluate: (1) the burial tolerance and recovery of eelgrass; (2) effectiveness of cap at reducing bioavailability; and (3) the effectiveness of a sand cap at remediating dioxin in the native sediment. In order to accomplish this, we measured dioxin concentrations in the cap material, bent-nose clam (*Macoma nasuta)* tissue, passive sampling device (PSDs) concentrations, and above- and belowground eelgrass biomass and individual shoot biomass.

Sediments

To evaluate the concentrations of dioxin in the sediment/cap, we collected approximately the top 10 centimeters (cm; 4 inches) via divers using a hand coring device as shown in Photograph 4. Samples collected from test plots HC-2, HC-3, and HC-5 with thin caps (4 inches or less) contained a mixture of capping material and underlying sediment. We collected samples in late August 2013 to measure baseline cap/sediment dioxin concentrations. Collection took place 30 days post-capping to allow the cap material to settle. We then sampled the sediment/cap 28 days later (September 2013), and then 60 days (October 2013) after baseline measurements. Sediment from the control and reference plots were also sampled at 90 (November 2013) and 120 (December 2013) days after baseline measurements. This coincided with other response variable sampling.

Photograph 4 – Example of core collection for belowground eelgrass biomass

Eelgrass

Eelgrass health was determined through several indicators measured after the capping material was installed at treatment plots and a control plot located in the contamination plume. A reference eelgrass bed was also examined for the same indicators to take into account stochastic changes in regional eelgrass health that could confound exposure study results. The indicators of eelgrass health that were measured during the pilot study were:

- Shoot biomass (gram dry weight);
- Shoot density; and
- Belowground biomass (gram dry weight)

Divers assessed each test, control, and reference plot for total area using presence/absence notations along transects within each plot. Divers counted shoot density within a minimum of three randomly

placed, 0.25-square-meter (m^2) quadrats in each test and reference plot. Divers collected five shoots from each test, control, and reference plot to assess biomass. Shoots were collected by hand and placed in a plastic bag to be processed for dry weight. The sample bags were transferred to the boat and stored in a cooler until they were later processed in the lab. All shoots were rinsed to remove sediment before being stored. An 8-inch core sample was also collected for belowground eelgrass biomass analysis (Photograph 4).

We collected these samples immediately after cap placement in July 2013, and then again following the settlement of the cap in late August, September, and October 2013. Additional eelgrass sampling was performed in August 2014 and August 2015 to examine interannual variability.

Bent-nose Clam

The bent-nose clam (*Macoma nasuta)* was selected as our test organism to evaluate bioaccumulation (Figure 3). This is a naturally occurring clam in Fidalgo Bay and an appropriate indicator of shellfish uptake of dioxins in these contaminated eelgrass beds. The bioaccumulation of dioxin in clam tissue was measured in two ways: standard 28-day bioaccumulation assays, and long-term bioaccumulation tests over 60, 90 and 120 days (Boese et al. 1995). Following cap placement, we installed 12-inch-diameter, mesh-covered, PVC cages into each test plot and placed 12 bent-nose clams in each. Cages were designed to exclude predators while allowing porewater movement and particle accumulation form the water column. Each PVC cage was designated for a different bioaccumulation study duration. There were several plots that included only a single, short-term bioaccumulation cage installed over a 28-day deployment (HC-1, HC-4, and HC-5). Long-term bioaccumulation studies (60-, 90-, and 120-day deployments) were conducted in test plots HC-2, HC-3, HC-control and HC-Reference. See Figure 3 for details on each test plot. The placement of clams in July 2013 was for an initial 28-day assessment of bioaccumulation and these clams were collected by a diver in August. During the August collection, new clams were placed into cages designated for an additional 28-day study, and the long-term bioaccumulation tests. The 28-day samples were collected in September, 60-day samples in October, 90-day samples in November, and 120-day samples in December.

Prior to deployment the clams were depurated and weighed to determine mass of clams (composite). Each clam was then marked (Photograph 4) and deposited into the PVC cage for the study duration. In addition to marking each cage with the designated study length, we used a lettering system to identify study duration for the clams to ensure that no errors were made. This also allowed us to identify native versus non-native clams collected within in cage. Photograph 5 presents clams designated for the HC-3 plot (“B” identifies that they were for the 28-day study duration).

Photograph 5 – Bent-nose clam prior to deployment

PSDs

Passive sampling devices (PSDs) contained two sampling strips of pre-cleaned polyoxymethylene approximately 2 inches wide and 4 inches long mounted in a frame and deployed adjacent to each PVC clam cage (Figure 4). PSDs were deployed at each test plot with one sampling strip (1 gram in weight) positioned within the first 5 cm of the sediment and the other sampling strip positioned in the water column 10 cm above the sediment. The set below the sediment surface was designed to mimic bioaccumulation similar to that of the exposed clams. The second set, deployed above the sediment surface, was designed to monitor ambient water conditions. The PSD frames were placed adjacent to each PVC cage and were sampled at the same intervals as the clams in the 28-day (August and September), 60-day (October), 90-day (November), and 120-day (December) studies.

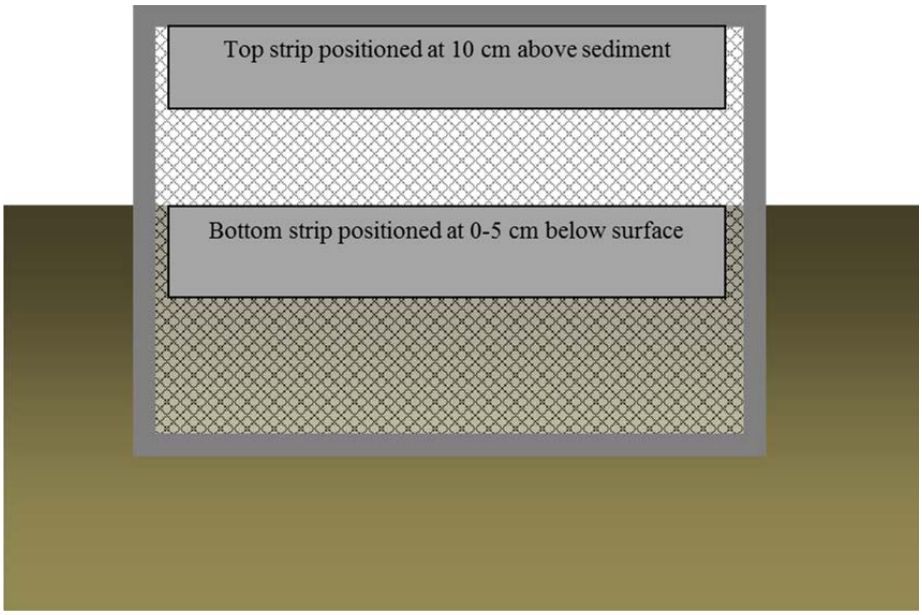


Figure 4 – Example of installed PSD

CHEMICAL ANALYSES

Sediment, clam, and porewater passive sampling device (PSD) samples were submitted to Analytical Resources, Inc. Laboratory (ARI) for chemical analysis. Samples were analyzed for 2,3,7,8-substituted polychlorinated dioxins and furans (PCDD/PCDF) using EPA Method 1613B. An additional low-level standard (in addition to the standards specified by the analytical method) was used to establish the instrument calibration curve in order to lower the practical quantitation limit (PQL) and increase analytical precision.

Laboratory results underwent in-depth data validation by a Hart Crowser chemist not directly associated with the project. In general, data were acceptable with only minor qualifications due to matrix interferences and low-level (less than the practical quantitation limit) blank contamination. Data validation memoranda and laboratory reports are included in Appendix A.

Sediment

In addition to dioxin analysis, sediment total organic carbon (TOC) was analyzed using the Ecology modification of EPA Method 9060. TOC results were used to organic carbon normalize sediment dioxin results and to calculate biota-sediment accumulation factors (BSAF).

Sediment analytical results are presented in the Results section below.

Clam Tissue

Clam lipid content was determined using the Bligh-Dyer Method so that lipid normalized dioxin concentrations and BSAF could be calculated. In addition, the following physical measurements were obtained:

- Mass of clams (composite) after depuration prior to deployment;
- Mass of clams (composite) after depuration after deployment; and
- Mass of shucked clams (composite) prior to homogenization.

Clam tissue analytical results are presented in the Results section below.

Passive Sampling Devices

In addition to dioxin analysis, ARI performed a recovery study to determine analytical method extraction efficiency and to establish laboratory quality control criteria (laboratory control sample recovery limits) prior to field deployment of the PSDs. Ten PSD samples were spiked with known amounts of PCDD/PCDFs and the samples were then extracted and analyzed. Laboratory recovery limits were established as the average percent recovery ± 3 standard deviations. Recovery limits were comparable to labeled isotope compound recoveries specified in the analytical method. Recovery limits are show in Table 2; passive sampling device analytical results are presented in the Results section below.

Table 2 – Laboratory Recovery Limits for Dioxin Congeners

Dioxin Congener	Percent Recovery Limits	
2,3,7,8-TCDD	92.9	108.2
1,2,3,7,8-PeCDD	90.5	107.9
1,2,3,4,7,8-HxCDD	87.3	112.7
1,2,3,6,7,8-HxCDD	85.8	112.5
1,2,3,7,8,9-HxCDD	88.4	116.8
1,2,3,4,6,7,8-HpCDD	86.7	107.9
OCDD	88.3	108.8
2,3,7,8-TCDF	93.2	113.9
1,2,3,7,8-PeCDF	97.5	124.7
2,3,4,7,8-PeCDF	92.1	113.1
1,2,3,6,7,8-HxCDF	82.8	113.6
1,2,3,7,8,9-HxCDF	87.9	119.3
1,2,3,4,7,8-HxCDF	88.1	108.3
2,3,4,6,7,8-HxCDF	76.0	96.0
1,2,3,4,6,7,8-HpCDF	93.6	127.6
1,2,3,4,7,8,9-HpCDF	73.8	99.2
OCDF	73.6	105.2

After sample extraction the mass of each PSD was determined by the laboratory so that dioxin (PCDD/PCDF) concentrations could be expressed on a mass basis rather than a total concentration per PSD.

EELGRASS LABORATORY PROCEDURES

Processing of aboveground eelgrass samples began by thawing the frozen sample and then placing the eelgrass shoots in a large bowl or bucket of water. Five shoots were then removed for further processing. The blade of each shoot was individually cleaned and any epifauna or algae was removed. The shoot was then cut 2 millimeters (mm) above the last belowground root node to remove any belowground material which was sampled and processed separately. The length and width of the blades for each shoot were then measured and recorded. If any blades were broken or damaged, they were measured to the point at which the blade was still intact and a note was made on the datasheet. The width of each blade was measured at the midpoint between the tip and the leaf base. After measuring, the samples were patted dry, weighed using a tared beaker, and recorded. The shoots were then placed in a labeled paper bag and dried at 65° C for at least 48 hours. Once dry, the dried shoots were removed from the bag and reweighed in a tared beaker and recorded.

Belowground eelgrass samples were defrosted using a warm water bath and once thawed, the sample was emptied into a mixing bowl in order to stir and loosen the sediment. Afterwards the sediment sample was then sieved using a 0.5-mm screen. The sample was rinsed until only roots and rhizomes, infauna, and larger wood debris was left. All roots and rhizomes were removed from the sieve with forceps and kept in a second tray filled with water. The remaining sieved material was placed in a glass tray to be stained with Rose-Bengal dye solution overnight for infauna analysis by mixing several drops of the dye into the remaining belowground material. Any intact, recognizable infauna was collected from the stained belowground material and was placed into a jar for preservation with 70 percent ethanol until covering the sample. The infauna samples were then archived for future identification. The roots and rhizomes that were removed from the sieve were then cleaned of any additional debris. Any decayed root matter was rejected from further processing and discarded. If any aboveground eelgrass was attached to the root material, it was clipped and removed. Cleaned and clipped belowground material was then patted dry and transferred and wrapped in a pre-labeled and pre-weighed coffee filter. After taring the scale, the weight was recorded in grams and then placed in an empty glass beaker for containment during the drying process in the oven. The filter-wrapped samples were then dried at 65° C for at least 48 hours. The dried samples were then reweighed and recorded.

DATA ANALYSIS

Eelgrass

Eelgrass data were analyzed using standard parametric techniques such as analysis of variance (ANOVA) followed by multiple comparison techniques. Data sets that did not meet the assumptions of normalcy and equal variance were log transformed using standard techniques. The requirements of data normality and equal variance required by ANOVA were tested with the Chi squared procedure

(test for normality) and Bartlett's test (test for equal variance). In some cases, even after log-transforming the data, normality and/or homoscedasticity was not reached. ANOVA is considered a robust technique against violations of these assumptions, particularly with balanced designs with high data replication (Zar 1999). To further increase test robustness, when log transformations failed to achieve normality and/or homoscedasticity, we reduced the significance level (α value in the test) from 0.05 to 0.01 to minimize the chances of committing Type I error (Underwood 1997). All analyses were performed using Sigma Stat (SPSS product).

Analysis for areal biomass was derived from areal shoot counts and individual shoot biomass data. This was accomplished by applying mean shoot weight to raw shoot counts for each treatment. To analyze these derived numbers, error propagation (Stutes et al. 2007), which is a modification of the techniques used in Bevington 1969, was implemented to account for the error associated with the mean shoot biomass. By implementing this technique, parametric techniques, such as ANOVA, could be used with robust datasets based on shoot count data.

Relative changes in areal biomass were evaluated standardizing experimental treatment results to control and/or reference estimates. This procedure is useful for eliminating the seasonal signal out of temporal datasets. This type of standardization was attempted only if reference and control biomass estimates were not significantly different from each other. If no significant difference was found, reference and control estimates were pooled and the average value was used to standardize the individual experimental plot estimate (i.e. each replicate) into a percentage difference. Then all the estimates were analyzed using standard ANOVA procedures after the error of the pooled estimate was propagated (Stutes et al. 2007).

Sediment

Dioxin toxic equivalents (TEQ) were calculated by summing the products of each detected dioxin congener concentration times its toxicity equivalence factor (TEF). Zero was substituted for non-detected congeners. (Van den Berg et al. 2006). Summary statistics for these TEQs were developed using ProUCL Software. These data, along with their 95 percent confidence intervals, are presented in the Results section below.

Clam Tissue

Dioxin toxic equivalents (TEQ) were calculated by multiplying each detected dioxin congener concentration by its toxicity equivalence factor (TEF) and summing the results. Zero was substituted for non-detected congeners. Summary statistics for these TEQs were developed using ProUCL Software. These data, along with their 95 percent confidence intervals, are presented in the Results section below.

Passive Sampling Devices

Dioxin TEQs were calculated by summing the products of each detected dioxin congener concentration times its TEF. Zero was substituted for non-detected congeners. Summary statistics for these TEQs were developed using ProUCL Software. These data, along with their 95 percent confidence intervals, are presented in the Results section below.

RESULTS

Eelgrass

Belowground Biomass

Belowground biomass in this study ranged from near zero to as high as 86 grams dry weight per square meter (g DW/m^2). There were changes in belowground biomass suggesting that eelgrass accumulated belowground biomass throughout the year in preparation for winter (in reference samples), but that trend is not as apparent in experimental plots. There did not appear to be differences due to treatment effects and showed no distinct trends (Figure 5).

Statistical analysis confirmed these findings with little indication of trends over time or between treatments. When looking at the overall magnitude of belowground biomass present, it was clear that there may have been an issue with sampling technique. Typically, rhizome biomass should show a marked increase in biomass from spring to late summer, doubling in some populations (Jacobs 1979). We did not see this relative change over the course of the study. In fact, when examining reference and control plots alone, a decrease in biomass was observed year over year.

While this could be an argument for the effects of sampling repeatedly within the sample plots, it should be noted that during sampling it was difficult to sample to sufficient depth to capture rhizome material (especially in the 8-inch experiment plots). When processing, it was also difficult to distinguish live from dead material. This led to high data variability in a set with low sample size to begin with (one sample per plot per sampling event). Thus, conclusions based on this data set should be interpreted cautiously.

Shoot Biomass

Due to the nature of the sampling design for shoots and by proxy aboveground biomass, the dataset proved to be quite robust proving to meet the assumptions of ANOVA testing. This was key to the power of each test and in validating the results of the analyses.

When examining individual shoot biomass, there was a strong seasonal trend in 2013 with higher shoot biomass in summer months with reference and control samples averaging above 1.0 g DW/shoot (Figures 6 through 10). In fall and leading into winter, individual shoot biomass averaged less than 1.0 g DW/shoot. When comparing year-over-year differences (August only), shoot biomass was observed to be higher in 2013 than 2014 on average (Figures 7 and 10). The one exception is the HC-4 treatment which showed significantly higher biomass per shoot in 2014 when compared to 2013 (1.67 g DW/shoot as compared to 1.42g DW/shoot). Within a given month treatments were not significantly different from each other or from reference or control save three exceptions. HC-4 was significantly higher than all other samples (treatments, control, and reference) in September 2013 and August 2014 while HC-3 was significantly lower than all other samples in July 2013.

Aboveground Biomass

Reference v/s Control

Aboveground biomass is an areal estimate derived from shoot density and shoot biomass. When examining reference and control plots, there was a strong seasonal trend of higher eelgrass biomass in summer tapering off toward fall months. In 2013, summer average aboveground biomass peaked in reference and control areas with values over 45 g DW/m^2 and reduced to approximately 18 g DW/m^2 (Table 3; Figure 11). Comparing year-to-year differences in August, there was over twice as much aboveground biomass in 2013 compared to 2014 (40.7g DW/m^2versus 16.3 g DW/m^2, respectively).

Table 3 – Aboveground Biomass: Summary of Areal Biomass Estimates (in g DW/m^2 ± standard error)

Test Plot	July 2013	August 2013	September 2013	October 2013	August 2014
HC-1	22.9 ± 1.9	5.1 ± 1.4	4.0 ± 3.1	0.8 ± 0.8	5.3 ± 0.8
HC-2	20.6 ± 10.7	33.0 ± 3.2	16.9 ± 2.4	24.6 ± 3.1	16.0 ± 3.0
HC-3	12.4 ± 1.0	24.5 ± 2.5	25.7 ± 6.4	32.6 ± 2.4	NA
HC-4	60.2 ± 12.7	22.2 ± 1.8	20.2 ± 6.7	9.4 ± 3.6	35.7 ± 5.9
HC-5	38.5 ± 4.9	19.4 ± 2.3	28.8 ± 3.0	14.5 ± 4.0	18.8 ± 2.9
HC-Control	29.4 ± 1.9	45.6 ± 3.1	21.2 ± 1.1	18.7 ± 1.5	15.9 ± 1.8
HC-Reference	46.5 ± 6.7	35.8 ± 3.3	29.5 ± 4.8	18.0 ± 1.1	16.7 ± 1.9

Statistically, there are no significant differences in areal biomass between reference and control sites. This suggests that dioxin contamination at intermediate to low levels has little effect on eelgrass productivity. When comparing for effects across months, there are highly significant differences between months, mostly driven by August 2013 biomass estimates. July and September estimates are not different from one another and are slightly lower (although significantly) than August 2013 estimates. All three of these estimates are significantly higher that October suggesting a strong season pattern. What is unexpected is that August 2014 is not statistically different from the October 2013 estimate, this suggests that there is quite a bit of year-to-year variation in biomass which is mostly due to changes in density.

Experimental Results

In July 2013, one month after capping material had been placed, aboveground biomass ranged from 12.4 g DW/$m^2$to 60.2g DW/m^2 (Table 3). The only significant difference during this sampling period was between HC-3 and HC-4 (the 4-inch and 8-inch carbon-amended treatments; Figure 12). Rather than showing the long-term effects of the capping material on sediment chemistry and eelgrass health, these differences are indicative of the initial effects of depositing cap material (which may potentially smother eelgrass through burial). None of the treatments were statistically different from the reference or control estimates.

In August 2013, differences among treatments are much more pronounced (Figure 13). The carbon-only treatment has the highest areal biomass estimate (approximately 33 g DW/m^2) but is not

statistically different from either of the other carbon-amended treatments (HC-3 and HC-4). All three of the carbon-amended treatments have statistically higher biomass estimates than the non-carbon-amended treatments (HC-1 and HC-5). Also, within the non-carbon-amended treatments, there was significantly less eelgrass aboveground biomass in the 8-inch treatment when compared to the 4-inch treatment. In addition to this, the carbon amended treatments were statistically similar to the reference estimates while the non-carbon amended treatments were significantly lower than the reference estimates.

In September 2013, a general decrease in overall biomass across all treatments was oserved. Reference and control plots are not significantly different from each other averaging 25.4 g DW/m^2. In the experimental plots, only HC-1 is significantly distinct from the other plots, with nearly 80 percent less areal biomass than the other experimental plots (Figure 14). HC-1 is also significantly lower than the reference and control. The other experimental plots were not significantly different from either the control or reference estimates.

In October 2013, a slight reduction in overall biomass when compared to September's areal biomass estimates was observed. Reference and control plots were not significantly different from each other, averaging 18.4 g DW/m^2. In the experimental plots, there are significant differences between the highest biomass estimates found in HC-3 (32.6 g DW/m^2) and the lowest in found again in HC-1 (0.8 g DW/m^2) (Figure 15). Plots HC-2, HC-4, and HC-5 were not significantly different from each other nor were they significantly different from the control or reference estimates.

In August 2014, there was a laboratory error that resulted in the loss of biomass samples for HC-3. For those remaining samples, Hart Crowser see reduced biomass in 2014 when compared to the same time period in 2013. In reference (18.0 g DW/m^2) and control plots (18.7 g DW/m^2) there is approximately a 53 percent reduction in biomass from year to year. Again, reference and control plots were not significantly different from each other or from experimental plots HC-2 and HC-5. HC-4 was significantly higher than all the experimental plots as well as the reference and control plots with an areal biomass estimate of 35.7 g DW/m^2. HC-1 continued to be significantly lower that all other experimental plots with an estimate of 5.3 g DW/m^2.

Sediment

Sediment chemistry results and summary statistics are presented in Tables 4 and 5, respectively. Table 4 also presents the additional sediment data that was collected in May of 2013, prior to the deployment of the cap and establishment of the test plots. These sample names begin with "SS-". With the exception of HC-1 (8-inch sand) and the reference (area background) test plots, sediment/cap dioxin concentrations exhibit a wide range of variability. Test plot variability is presented graphically in sediment dioxin TEQ boxplots (Figure 16). This variability is likely a result of heterogeneous underlying sediment and that samples collected from areas with 4 inches or less of capping material likely contained a mixture of cap material and underlying sediment. Variability associated with test plot HC-4 (8 inches of sand with carbon) may be a reflection of varying amounts of carbon incorporated into the samples.

Clams

Clams obtained from Discovery Bay were deployed at four test plots (HC-Control, HC-Reference, HC-2, and HC-3) for up to 120 days. Clam tissue chemistry results and summary statistics are presented in Tables 6 and 7, respectively. Boxplots showing clam tissue dioxin TEQs as a function of deployment days for the HC-Control, HC-Reference, HC-2, and HC-3 test plots are presented in Figures 17 through 20.

Composite clam tissue dioxin concentrations were evaluated separately for each test plot as a function of the length of deployment (0, 28, 60, 90, and 120 days). All tissue dioxin concentrations were below the laboratory practical quantitation limit (PQL) so there is uncertainty in the numerical values - these values are estimates. In addition, since only a single sample was obtained for the 60-, 90-, and 120-day deployments, no evaluation of sample variability could be performed.

Due to the limited number of clam tissue measurement per deployment time frame, and because tissue concentration were below the estimated PQL throughout the 120 days of measurement, we could not determine whether equilibrium had been reached within clam tissue. For this study, we assumed equilibrium was achieved within 28 days. Clam tissue data collected over 120 days from HC-control, HC-2, and HC-3 indicate that equilibrium may have been reached within 28 days. However, it is important to note that higher molecular weight hydrophobic organic contaminants often take longer than 28 days to reach equilibrium within passive sampling devices used as surrogates to measure body burden in benthic invertebrates such as clams (USEPA, 2012). Several general observations can still be made in spite of these caveats.

- Dioxin concentrations in clams deployed at the reference test plot (average sediment dioxin TEQ = 3.35 nanograms per kilogram [ng/kg]) are relatively constant and do not appear to increase over an initial concentrations of approximately 0.05 ng/kg.
- HC-Control plot (average sediment dioxin TEQ = 50.37 ng/kg) clam tissue dioxin TEQ concentrations ranged from approximately 0.25 to 0.85 ng/kg between 28 days and 120 days.
- Test plot HC-2 (average sediment dioxin TEQ = 46.08 ng/kg) clam tissue dioxin TEQ concentration ranged from approximately 0.55 to 0.7 ng/kg between 28 days and 90 days. The tissue dioxin TEQ concentration for the 120 day deployment was 0.1 ng/kg.
- Test plot HC-3 (average sediment dioxin TEQ = 17.95 ng/kg) clam tissue TEQ concentrations ranged from approximately 0.08 to 0.45 ng/kg between 28 days and 120 days.

A boxplot showing clam tissue dioxin TEQs by test plot for samples deployed 28 days or longer is presented in Figure 21.

Bioaccumulation factors (BAFs) as well as biota-sediment accumulation factors (BSAF), were assumed to reach equilibrium (within the limits of uncertainty) within 28 days. Figures 22 and 23 present BAFs and BSAFs, respectively, for each test plot as a function of deployment time.

Since dioxin concentrations were assumed to equilibrate within 28 days, average BAFs expressed in terms of dioxin TEQs were calculated using all 28 day and longer deployment data for each test plot by dividing the average tissue TEQ concentration by the average test plot sediment TEQ using the following equation:

BAF = tissue dioxin concentration (TEQ)/sediment dioxin concentration (TEQ)

BAFs and BSAFs were calculated on a total TEQ basis rather than for each individual congener since a number of congeners were either not detected or were present at concentrations below the PQL. In addition, detected congeners varied from sample to sample.

Average BSAFs, essentially a tissue lipid and sediment total organic carbon normalized BAF, were also calculated using the following equation:

BSAF = (tissue dioxin TEQ /% lipids)/(sediment concentration TEQ /%TOC)

Results are summarized in Table 8.

Table 8 – Clam and Sediment TEQs, BAFs, and BSAFs

Test Plot	Average Tissue TEQ	Average Sediment TEQ	Average BAF	Average BSAF
HC-2	0.508 ng/kg	46.1 ng/kg	0.011	0.111
HC-3	0.249 ng/kg	17.95 ng/kg	0.014	0.098
HC-Control	0.539 ng/kg	50.37 ng/kg	0.011	0.047
HC-Reference	0.067 ng/kg	3.35 ng/kg	0.020	0.122

Figures 24 and 25 present calculated BAFs and BSAFs, respectively, for all exposure times 28 days or longer by test plot.

It should be noted that tissue dioxin TEQ concentrations were all below the estimated practical quantitation limit of approximately 2.5 ng/kg. Therefore there is uncertainty in the tissue concentrations and the calculated BAFs. However, calculated BAFs are approximately the same across the test plots. The fact that BAFs are less than 1 implies that dioxins are more strongly bound to the sediment and are not bioavailable to a significant degree. Tissue dioxin concentrations are strongly correlated with sediment concentrations (Figure 26) and it appears that, at the Custom Plywood site, addition of organic carbon (test plots HC-2 and HC-3) has no effect on tissue dioxin concentrations.

Since tissue dioxin TEQ concentrations were all below the estimated practical quantitation limit, calculated BSAFs are also considered estimates with an unknown uncertainty. BSAFs, similarly to BAFs, are approximately the same (within limits of uncertainty) at all four test plots. Variability is likely a result of uncertainty due to tissue concentrations below the PQL, heterogeneous underlying sediment, and that samples collected from areas with 4 inches or less of capping material contained a mixture of cap material (sand and carbon) and underlying sediment. As found for BAFs, the fact that BSAFs are

less than 1 also implies that dioxins are more strongly bound to the sediment and are not bioavailable to a significant degree.

Passive Sampling Devices

PSD chemistry results are shown in Table 9. PSDs placed above the sediment surface have "-A" following the sample name, and those placed below the sediment surface have "-B" following the sample name. Table 10 includes summary statistics for PSDs inserted below the sediment surface. Boxplots showing below sediment surface PSD dioxin TEQs as a function of deployment days (0, 28, 60, 90, and 120 days) for the HC-Control, HC-Reference, HC-2, and HC-3 test plots are presented in Figures 27 through 30. The 0-day samples were PSD blank samples that were analyzed prior to deployment. All PSD dioxin concentrations were below the laboratory practical quantitation limit (PQL) so there is uncertainty in the numerical values. In addition, since only a single sample was obtained for the 60-, 90-, and 120-day deployments; no evaluation of sample variability could be performed.

DISCUSSION

Eelgrass Tolerance to Burial

One of the goals of this study was to determine if a prescriptive thin layer cap would be tolerated by eelgrass habitat. In this study, we attempted to examine various eelgrass metrics (belowground biomass, individual shoot biomass, and aboveground biomass) that would be indicators of eelgrass health and productivity. While differences among treatments were noted in various analyses it was clear that seasonal variation was a large driver of system variability. As a result, patterns derived from individual analyses performed each month or over the entire data set were hard to discern. In order to better understand our results, we standardized the results to reference and/or control results in order to transform the data into terms of relative difference. This allows data pooling across all time frames and effectively subtracts the seasonal signal (Stutes et al. 2007). Based on this approach, an estimate of zero means no difference from the control estimate, a positive number indicates an estimate higher than control, and a negative number indicates and estimate lower than control. This is a suitable approach since we were able to demonstrate a seasonal signal in the reference plots and the comparison of reference to control estimates showed no significant differences over the course of the project (e.g., Figure 11). By using this approach, the data was reanalyzed for differences among treatments for each of the eelgrass metrics.

Belowground Biomass

There was very little that could be discerned from the belowground biomass data and statistical analysis showed high variability and very little power within the raw dataset. In an effort to reduce this variability, we attempted to standardize the experimental plot data against reference and control values (to reduce the variability associated with season) and then analyze for differences among the treatments. Analyzing these results showed no treatment effects (Figure 31). There might have been a peripheral effect in the carbon treatments (reduction in belowground biomass compared to the control/reference plots), but that difference was not statistically significant and any inference from the test is mostly dismissed due to the continued low power of the dataset. Based on the variability of

each treatment, the standardized estimates were not significantly different from zero. This should not be interpreted as the absence of effects due to experimental manipulations, but as a lack of sensitivity to detect a measureable difference if one had occurred. This, coupled with the assertion that sampling was not penetrating deep enough every time to capture all the rhizome and root material, especially in the 8-inch plots, means that the data is highly suspect.

Shoot Biomass

Shoot biomass is potentially an important metric to examine since it is essentially a proxy for shoot morphology, integrating number of leaves and leave surface area into one metric. The data collected created a robust dataset that was capable of detecting even small changes in shoot biomass (as exhibited in July and September 2013 and again in August 2014). In reality, although some significant difference were noted, the relative magnitude of those changes were not very large. In July 2013, the differences in shoot biomass were coupled with changes in shoot density which led to similar, significant changes in aboveground biomass estimates. (Recall that July 2013 is a measure of acute effects of laying the cap as oppose to true effects of the cap on eelgrass dynamics that would develop in subsequent months.) Later, contrary to the coupled effect, the September 2013 results showed a different story. The HC-4 treatment had significantly higher biomass per shoot when compared to the other experimental treatments, but did not show that same difference when translated to the areal biomass estimate. In this case, shoot density was the overwhelming driver of the biomass estimate. This was the case for most of the differences in areal biomass estimates: the absolute magnitude of the difference in density was far more predictive of areal biomass than individual shoot biomass. As a result, the importance of the effect of burial on shoot biomass (or shoot morphology) is negligible, with small differences detected between treatments and time of year having little effect on areal biomass.

Aboveground Biomass

This metric, by far, exhibited the largest changes within treatment and across months. Standardization to remove seasonal variability was essential for providing information on whether experiment effects occurred. After standardization, the clear effect or difference between certain treatments and surprising lack of effect between other treatments (Figure 32) was obvious (the zero line indicates no difference from control). After statistical analysis, it appears on HC-1 (the 8-inch, sand-only treatment) is significantly different from the other treatments with nearly an 80 percent reduction in areal biomass over the course of the study. This is not surprising since HC-1 was significantly lower in aboveground biomass over most of the survey period. The other treatments are not significantly different from each other and not significantly different from zero. This means that over the course of the study, plots HC-2 through HC-5 were not significantly different from the control despite different degrees of cap thickness and carbon incorporation (Figure 32). This is extremely surprising considering that HC-4 has the same cap thickness as HC-1 (8 inches), with carbon incorporation being the only difference between the two treatments.

Summary and Synthesis

During the early stages of this study, these basic questions served as the focus of our efforts which would in turn help guide future remedial alternative design at this site:

- Can you effectively cap contaminated sediments in eelgrass habitat with little to no effect on the eelgrass?
- Can you remediate intermediate levels of dioxin contamination through thin layer capping?
- Can you reduce uptake by marine organisms through thin layer capping?

When examining the effects of the cap treatments on eelgrass habitat, the data clearly show that the application of 4 inches of cap material had little if any effect on eelgrass areal aboveground biomass regardless of whether or not carbon was used. In the 8-inch treatments, there was a significant reduction in eelgrass biomass in the sand-only treatments. While eelgrass areal aboveground biomass in the 8-inch plus carbon treatment did not seem to be reduced relative to the control/reference estimates; the mechanism behind this is unknown.

Due to the combination of sample variability, pretreatment dioxin concentrations at or near detection limits, and a relatively small sample size, we were unable to statistically confirm if remediation has been achieved or if performance was better in any one treatment relative to another treatment. Though our bulk sediment results were statistically inconclusive, we can suggest with some level of certainty that there was a trend of reduced dioxin sediment concentrations with the application of 4 or 8 inches of clean sand and/or carbon-amended sand (Figure 16). Average concentration for the control and for the carbon-only treatment were at or above the estimated practical quantitation limit while all other treatments were below it. This infers that the installation of a cap tended to isolate the existing contamination. Nothing presented in the sediment data collected during this study suggests that a thicker cap would be more effective or that activated carbon is necessary to achieve remediation goals.

When looking at uptake either by clams or tissue mimics (PSDs), generalizations based on the data are limited. All tissue and PSD dioxin concentration data were below the estimated practical quantitation limit established by the analytical methodology and data precision. This made it difficult to determine whether equilibrium of contaminants in clam tissue and PSDs to the surrounding environment was in fact reached after 28 days. A lower quantitation limit, greater replication, and potentially a longer deployment timeframe are necessary to verify equilibrium timeframes for clams and PSDs, and to statistically evaluate the effect of each treatment on tissue concentrations.

Both BAF and BSAF for the clam tissue are essentially the same regardless of treatment and are less than 1, which implies that dioxins are more strongly bound to the sediment and are not readily bioavailable. The addition of carbon had no effect on reducing bioavailability, which suggests that organic carbon may already be abundant and not limiting in the system. Based on these findings, the addition of extra carbon to enhance cap effectiveness is not warranted. There also does not seem to be a discernable difference in cap effectiveness when comparing the different thicknesses.

Conclusions and Recommendations

Based on the data, application of **a maximum of 4-inch sand-only cap** appears to be an effective remedial alternative at the Custom Plywood site. However, due to permitting limitations (i.e. the limitation of placing only 25 cubic yards of fill), the scope of the pilot study encompassed a very limited surface area. While the eelgrass biomass data obtained from the pilot study is statistically solid, the results should be considered very specific to the site (Custom Plywood) and to the eelgrass population that was tested. One should be cautious in scaling up using these results. Despite this study's limitation, we do recommend implementing a preferred capping thickness based on these results, though caution should be used in scaling up to the larger cleanup area. A recommended next step would be to implement the remedy in phases. The initial phase would apply the preferred thickness over a much larger intermediate area (e.g. 1 acre) to verify that the results are consistent over different ecological scales.

Regardless of the results of this study, we suggest additional site specific investigations in order to determine potential variation in eelgrass habitat and response to remediation. As we have seen in the overall cleanup of Fidalgo Bay, remedial actions and habitat enhancements across cleanup sites can vary greatly in performance with minor site specific differences.

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