Procedures for the Collection and Storage of Environmental Samples in the RMP Specimen Bank

by

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REGIONAL MONITORING PROGRAM
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1. Introduction

The Regional Monitoring Program for Water Quality in the San Francisco Estuary (RMP) has been archiving wildlife and sediment samples in a specimen bank since its inception in 1993. The purpose of the RMP Specimen Bank is to serve as a repository for sample material that can be used to assess the quality of the San Francisco Estuary through retrospective chemical analyses. Proper maintenance allows samples in the RMP Specimen Bank to be used for the identification of changes in the accumulation of known or as yet unidentified chemical contaminants over time, investigation of contaminants of emerging concern (CECs), and verification of analytical results if quality assurance issues arise. Several environmental specimen banks (ESB's) exist in the United States (e.g., U.S. National Institute of Standards and Technology's (NIST) Marine Environmental Specimen Bank and the Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry Specimen Packaging, Inventory and Repository (CDC/ATSDR/CASPIR) as well as other countries (e.g. Sweden, Canada, Japan, and Germany) and are valuable tools for retrospective trend analysis of chemicals using samples that have been archived for decades. By providing sample material from years past, the RMP Specimen Bank complements on-going RMP Status and Trends monitoring that is used to assess chemical contaminants in the San Francisco Estuary ecosystem.

This document contains the rationale and procedures for the collection and storage of samples for the RMP Specimen Bank. These procedures are intended to maintain sample integrity by preventing changes in the chemical concentration over time due to sample handling and storage conditions and were developed based on knowledge of the stability of chemical contaminants and environmental samples in long-term freezer storage. Information on the RMP's Specimen Bank inventory, procedures for obtaining samples in the RMP Specimen Bank, and considerations for the future of long-term sample storage are also provided. Methods for sample storage were developed in consultation with representatives from NIST's National Biomonitoring Specimen Bank and Marine Environmental Specimen Bank, the National Oceanic and Atmospheric Administration's (NOAA) Mussel Watch Program, and Environment Canada's Wildlife Specimen Bank.

2. Stability of Environmental Samples in Long-Term Freezer Storage

Analysis of archived material can be a valuable tool for investigating concentrations of chemicals in years past; however, its value is dependent on adherence to procedures that preserve the integrity of chemicals and the environmental sample matrix in storage over time. The storage temperature selected must not subject the samples to microbial degradation, oxidation, or volatilization and the sample containers used must not adsorb the target analytes or contaminate the sample due to diffusion of chemicals from the container (e.g., additives in plastic or perfluorinated chemicals in Teflon). Relatively little information on the stability of chemical contaminants in long-term storage is publicly available, however, presumably because (1) the re-analysis of samples is not generally recognized as a priority and is therefore cost prohibitive, and (2) improvements in analytical methods make differentiating between changes in concentration over time from changes due to the use of different analytical methods very difficult.

Consequently, few studies have been conducted and much of the information that is gathered does not get published (McFarland et al. 1995).

2.1 Sample Stability at Low Temperatures

Storage at -80 °C or lower is recommended for samples in an environmental specimen bank, with storage at -150 °C or lower believed to provide sample preservation without any major structural or biochemical changes over several decades (Wise and Koster 1995, McFarland et al. 1996). Storage at -80 °C or lower is generally recognized to be particularly important for samples that may be analyzed for less persistent (i.e., reactive) chemicals, which are more readily degraded by microbes or susceptible to enzymatic breakdown. Previous studies have indicated that polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and chlorinated pesticides are stable in tissues stored at -80, -120, or -150 °C for six to ten years (Lauenstein 1995; Wise and Koster 1995; Schantz et al 2000). Additionally, routine re-analysis of the certified compounds in NIST environmental standard reference materials (SRMs) (e.g. marine sediments and tissues) has thus far demonstrated that significant degradation of PAHs, PCBs, and organochlorine pesticides does not occur when stored at the recommended temperature for ~20 years (NIST, personal communication). Though published information is not available regarding the chemical stability of samples in storage at these ultra-low temperatures over longer time periods, it is widely believed that temperatures ≤ -80 °C are well below those expected to result in significant degradation of the sample by microbial or enzymatic activity. Information on the stability of trace metals and other chemical contaminants in storage at -80 °C is not publicly available.

More information is available regarding long-term storage of environmental samples in conventional, mechanical freezers (storage temperature of -20 °C). For trace metals, studies suggest that concentrations in sediment and tissue are not affected (McFarland et al. 1995, Wise and Koster 1995). While the effects of long-term storage at -20 °C on concentrations of PCBs and chlorinated pesticides are uncertain, particularly for tissues (McFarland et al 1995), studies have shown that tissue concentrations remain stable at this temperature for up to fifteen years (Lauenstein 1995; Wise and Koster 1995). A major data gap appears to be the stability of chemicals in storage at -20 °C for longer than fifteen years. Information on the stability of other chemical contaminants in storage at -20 °C is not available.

In addition to the potential for chemical degradation, the sample matrix itself has the potential to be compromised in long-term storage at -20 °C. Moisture migration, as evidenced by the formation of ice crystals in the sample container and tissue desiccation, has been reported to occur in samples kept at this temperature (Lauenstein 1995; Wise and Koster 1995). In contrast, tissue samples stored at -150 °C appeared the same as they did just after homogenization. Because of moisture loss, chemical concentrations of samples stored at -20 °C can only be reported on a dry weight basis, a practice consistent with NOAA Mussel Watch Program methods. Tissue color changes, changes in tissue sample consistency, and decreased lipid content have also been reported to occur at -25 °C (Wise and Koster 1995; McFarland et al. 1996); bacterial action has been strongly suspected to contribute to tissue color changes but it has not been demonstrated or reported (B. Porter, NIST, personal communication). Samples wrapped in aluminum foil have also been reported to have problems, with foil degradation resulting in the sample becoming inseparable from the foil over time and coating of the sample with aluminum oxide powder (Lauenstein 1995; G. Lauenstein, NOAA, personal communication).

2.2 Low Temperature Freezers

Temperatures ≤ -80 °C and the use of liquid nitrogen (-196 °C) or liquid nitrogen vapor freezers (-150 to -190 °C) are recommended for the preservation of samples in long-term storage (McFarland et al. 1995; Wise and Koster 1995; ISBER 2008). While more than necessary for achieving -80 °C, liquid nitrogen freezers require less maintenance and are lower in cost compared to ultra-cold (- 80 °C) mechanical freezers. Unlike mechanical freezers, liquid nitrogen freezers are unaffected by short-term power failures, which is an important consideration for sample storage in seismically active regions such as the San Francisco Bay Area. Additionally, liquid nitrogen vapor freezers are recommended over freezers that store samples in the liquid phase of nitrogen because storage in the vapor phase is sufficient to maintain the desired temperature and avoids the safety hazards associated with liquid nitrogen storage (ISBER 2008) as well as the potential for cross-contamination of samples if the storage containers are not closed tightly. Disadvantages of liquid nitrogen freezers include the need to renew liquid nitrogen approximately once per month and the costs and availability of liquid nitrogen. An additional consideration is that glass containers cannot be used for storage of samples in low temperature freezers because they shatter, and therefore Teflon or cryogenic plastic containers must be used.

The alternative to liquid nitrogen freezers are mechanical freezers that are generally maintained at -20, -40, or -80 °C. Theses compression-type freezers are not ideal for long-term sample storage due to high maintenance costs (e.g., replacement of failed compressors), low efficiency, and the potential to be affected by power failure, necessitating a backup power system and an emergency response plan. There is also a larger fluctuation in temperature when the freezer door is opened which can cause damage to the sample over time.

Regardless of the type of freezer that is utilized, backup storage is recommended in case of freezer failure (ISBER 2008). Security systems and continuous, electronic monitoring of storage temperature are also recommended and are used by most specimen banks internationally.

3. Prioritization, Storage, and Collection of RMP Samples

3.1 RMP Objectives and Sampling Components

The overarching goal of the RMP is to collect data and communicate information about water quality in the San Francisco Estuary to support management decisions. The RMP seeks to fulfill this goal by answering the following questions:

1. Are pollutant concentrations in the Estuary at levels of concern and are associated impacts evident?
2. What are the concentrations and masses of pollutants in the Estuary and its segments?

3. What are the sources, pathways, loadings and processes leading to pollutant-related impacts in the Estuary?

4. Are the concentrations, masses, and associated impacts of pollutants in the Estuary increasing or decreasing?

5. What are the projected concentrations, masses, and associated impacts of pollutants in the Estuary?

To answer these questions, a large number of samples are collected by the RMP each year as part of Status and Trends monitoring (S&T) and Pilot and Special Studies. The primary purpose of S&T monitoring is to build a long-term dataset to characterize the Estuary and develop an understanding of estuarine chemical processes on regional, spatial, and temporal scales. S&T monitoring has primarily included chemical monitoring of water and sediment annually, native and deployed bivalves, sport fish and bird eggs every two or three years, toxicity monitoring, and hydrographic and sediment transport studies. Monitoring of benthic community structure was added to the program in 2008. A variety of short-term Pilot and Special Studies are also conducted each year to allow the RMP to adapt in response to changes in the regulatory landscape, and to address specific data gaps identified as part of longer term strategies for contaminants (e.g., mercury uptake into the food web and contaminant loads from small tributaries, etc.). Pilot and Special Studies are designed to answer specific management questions or to test the efficacy of new monitoring approaches or methodologies on a small scale for possible inclusion in the S&T program. In addition to S&T, Pilot and Special Studies may also include an archiving plan as part of their project workplan.

3.2 Considerations for the Design of the RMP Specimen Bank

The intended uses of the Specimen Bank (Section 1), known stability of environmental samples in long-term freezer storage (Section 2), and the following considerations adapted from the NIST National Biomonitoring Specimen Bank Program (Wise and Koster 1995) were used to develop proposed procedures for the collection, prioritization, and storage of S&T monitoring samples for the RMP Specimen Bank.

3.2.1 Sample Types

Samples collected for the RMP Specimen Bank should accumulate the chemicals of interest, be representative of general conditions in the San Francisco Estuary, and have the potential to characterize both inorganic and organic chemical contaminants. Tissue samples from apex predators are likely the most beneficial because chemical concentrations are typically elevated for bioaccumulative compounds. In addition, apex predators are generally longer lived and forage over relatively broad areas, therefore integrating chemical concentrations observed on a regional scale in the Estuary. Because the RMP Pilot and Special Studies are of limited duration (typically not more than 1-2 years) and the samples collected as part of these studies may not meet the intended uses of the Specimen Bank, the decision to archive samples from Pilot and Special Studies in the RMP Specimen Bank will be made on a project-specific basis.

3.2.2 Sample Volume

A sufficient amount of each sample should be archived to allow for multiple chemical analyses. A number of sub-samples, rather than one or two large volume samples, should be available to prevent subjecting the samples to several freeze-thaw cycles during the sub-sampling process. Repeated freeze-thaw and handling may cause chemical degradation or volatilization or result in contamination of the sample. Sub-samples containing 5 to 20 grams (g) of sediment or 15 to 20 g wet weight of tissue are sufficient to obtain low method detection limits in most chemical analyses.

3.2.3 Target Analytes

The appropriate storage conditions for samples in the RMP Specimen Bank are dependent on the target analytes of interest in future chemical analyses. While very persistent chemicals are not expected to degrade when stored at -20°C, these conditions are not appropriate for the preservation of more reactive chemicals, especially in tissue, and thus low temperature storage is generally recommended (Section 2). The RMP S&T program has typically focused on the analysis of trace metals and the following persistent organic chemical contaminants: PCBs, PAHs, polybrominated diphenyl ethers (PBDEs), organochlorine, organophosphate and pyrethroid pesticides, chlorinated dioxins and furans, and perfluorinated compounds (PFCs). It is anticipated that future analyses of RMP Specimen Bank samples will also include persistent organic chemicals since many of these chemicals have the potential to cause adverse effects in aquatic foodwebs. However, the identity of future target analytes is unknown and may include less persistent chemicals (e.g., pharmaceuticals and personal care products, pesticides) and other specialty chemicals (e.g., nanoparticles) that require lower temperature storage conditions to maintain chemical integrity over long periods. Storage conditions that will insure the integrity of both persistent and reactive chemicals for decades will therefore be used for samples collected for the RMP Specimen Bank.

3.2.4 Storage Costs

Though the RMP collects a large number of samples each year for chemical analysis, minimizing the number of samples kept in the Specimen Bank is desirable because of the costs associated with the maintenance of samples in low temperature storage. As stated previously, the intended uses of samples in the Specimen Bank are for identification of time trends, investigation of yet unidentified chemical contaminants, and addressing quality assurance issues. Not every sample needs to be archived indefinitely given that the function of the Specimen Bank is not to provide samples that represent comprehensive coverage of the Estuary. Prioritization of samples is thus required to insure that the samples maintained in long-term freezer storage fulfill the intended uses of the Specimen Bank while also minimizing the amount of storage space required. Representative samples have been selected for archiving based on the anticipated future use of the samples, with consideration of storage constraints.

3.3 RMP Sample Storage: Rationale and Freezer Locations

RMP samples are stored in both a short-term and long-term archive. Samples in the short-term archive are stored at -18 °C and are intended for use in the identification of short-term time trends (i.e. < 5-10 years), the investigation of yet unidentified chemical contaminants, and addressing quality assurance issues that may arise during the routine analyses of samples for S&T monitoring. These samples are intended for the analysis of chemicals which are not expected to degrade in five years of storage at -18 °C. The short-term archive is located in a commercial freezer facility near SFEI (Schaefer's Meats & Cold Storage, 1110 98th Ave., Oakland, CA) that continuously monitors temperature electronically and maintains hard copies of temperature log records. The facility is not equipped with a backup generator; however, in the event of power failure the facility contingency plan is to keep the freezer closed, providing maintenance of low temperatures for several days.

Samples in the long-term archive are stored at -150 °C in liquid nitrogen (LN_2) vapor freezers and are primarily intended for use in the identification of time trends occurring over decadal time frames (i.e. > 10 years). Samples stored in LN_2 vapor freezers are not expected to degrade over time and are thus reliable for chemical contaminant studies occurring well into the future. The long-term archive was established in 2010 and is located in the Marine Environmental Specimen Bank (Marine ESB), operated by NIST at the Hollings Marine Laboratory in Charleston, SC. The Marine ESB is characterized by having well developed banking protocols and standard operating procedures (SOPs), computerized sample tracking (chain-of-custody) systems, maintenance of many forms of data associated with original specimens, and large investments in state-of-the-art facilities and equipment required to store specimens over long periods of time. The Marine ESB emphasizes cryogenic storage using LN_2 vapor storage freezers, security systems, and electronic monitoring of storage conditions 24 hours a day, 365 days a year. The Marine ESB also maintains high efficiency particulate air (HEPA)-filtered clean air laboratories for cleaning storage containers, preparing banked specimens for analysis, and processing and storing samples. Additional details about the Marine ESB facility are described in Pugh et al. 2007.

3.4 Sample Storage Containers

A number of small volume sub-samples, rather than one or two large volume samples, are collected for storage in the Specimen Bank to avoid subjecting the samples to several freeze-thaw cycles. Each sub-sample contains a sufficient amount of material for most chemical analysis, and when needed, can be removed from the freezer and sent to the appropriate laboratory without the need to sub-sample. For most samples, two or three small volume sub-samples for each type of analysis (i.e. organics, trace metals, PFCs) are collected. A larger number of sub-samples (typically three or four) are collected for organic contaminant analyses since these have historically generated greater interest for study, particularly for investigations of CECs.

Samples for the short-term archive are stored in either glass jars with Teflon-lined lids for non-fluorinated organic chemical and trace metal analysis or in polyethylene (PE) or polypropylene (PP) for fluorinated chemical (i.e. PFCs) or trace metals analysis. Samples for the long-term archive are stored in either Teflon vials for non-fluorinated organic chemical and trace metal analysis or PP cryovials for fluorinated chemical analysis. Glass and PE/PP containers are the least expensive containers and thus are used when possible; however, only Teflon and PP cryovials are able to withstand LN_2 temperatures for long periods without shattering and are therefore used for storing samples in the long-term archive.

Teflon and cryo-containers used for the storage of samples in the long-term archive are pre-cleaned by NIST Marine ESB personnel using established protocols (Pugh et al. 2007) and shipped to SFEI contract laboratories or designated field personnel for use. For storage of samples in the short-term archive, glass and plastic containers are pre-cleaned using appropriate acids or solvents by SFEI contract laboratories or purchased pre-cleaned commercially (e.g. from Fisher or ESS Vial). For containers purchased 'pre-cleaned' from ESS Vial or other companies, a minimum of two per shipment will not be opened and kept in storage with the other samples in case container contamination issues arise.

3.5 Water Samples

Surface water samples have been collected for chemical contaminant analysis as part of S&T monitoring each summer since 1993. Seasonal sampling was also conducted during the first few years of the RMP. In addition to random sites throughout the Estuary, five historical water sites are sampled to maintain a time series at fixed locations for long-term trend analyses. The total number of surface water sites monitored each year has varied due to management priorities, statistical power, and fiscal considerations.

Unlike other sample matrices, water samples are not collected for the RMP Specimen Bank because of the instability of organic chemical and trace metal contaminants in this matrix over time. Additionally, achieving detection limits appropriate for the RMP requires the collection of large volume water samples (ranging from 4 to100 L samples), which substantially increases the time required for sample collection and costs when duplicate water samples are collected. The refrigerator/freezer storage space required for these high volume whole water samples is also not practical. Though water samples are not archived, extracts of the water samples are retained by the analytical laboratories for one year to address any quality assurance issues that may arise.

3.6 Sediment Samples

Surface sediment samples have been collected for chemical contaminant analyses as part of S&T monitoring each summer since 1993. The program has largely focused on summer sampling for water and sediment because inter-annual variation due to natural variables, primarily freshwater inflow, is reduced during summer. However, significant toxicity is observed in the rainy season (winter/spring months) in sediments. To better understand sediment toxicity and the variability that may be observed in the rainy season, sediment sampling in each year began to alternate between the summer/dry and winter/rainy season in 2010, with wet season sediment sampling occurring in January 2010. In addition to random sites throughout the Estuary, seven historical sediment sites are sampled to maintain a time series at fixed locations for long-term trend analyses. The total number of surface sediment sites monitored each year as part of S&T monitoring has varied due to management priorities, statistical power, and fiscal considerations. Sediment cores were also collected from various locations throughout the Estuary in 2007 as part of an RMP Special Study investigating historic deposition of PCBs and mercury. Additional coring work is anticipated in future years.

Table 1 provides a summary of the collection and storage of sediment samples for the RMP Specimen Bank. Following RMP methods (David et al. 2001), site sediment is homogenized into a single composite and allocated into sub-samples for both chemical analysis as part of S&T monitoring and the Specimen Bank. Sediment samples for the long-term archive are not collected from random sites because these samples are less useful for long-term trend analysis. Though it is the goal to archive only the small volume sample needed for a single chemical analysis (to avoid repeated freeze thaw cycles), larger PE jars (250 ml) are used for the short-term archive because they are the smallest pre-cleaned jars available and it was determined that space is not an issue at the Oakland freezer facility. Despite the larger sample volume available, the PE jars in short-term storage should be used for a single analysis and not be subjected to repeated freeze thaw cycles, similar to other archived samples.

3.7 Bivalve Samples

Bivalves are collected for chemical analyses every two years as part of S&T monitoring and have been collected since 1993. Prior to 2006, bivalves were collected annually. Mussels *(Mytilus californianus)* are collected from a reference site (Bodega Bay) and deployed at nine fixed stations throughout the Estuary for 90-100 days in the dry season to monitor the bioaccumulation of several chemical contaminants. Resident clams *(Corbicula fluminea)* have also been collected since 1999 from two fixed sites in the northern portion of the Estuary at the mouth of the Sacramento and San Joaquin Rivers. The RMP deployed bivalve monitoring component continues the long-term database started by the State Mussel Watch Program in 1976.

Table 2 provides a summary of the collection and storage of bivalve samples for the RMP Specimen Bank. Following collection of whole bivalves using RMP methods (David et al. 2001), bivalves are separated for shipment to either an organic or inorganic analytical laboratory for homogenization and chemical analysis. At each laboratory, bivalve samples from each site are homogenized in a single composite and aliquoted into samples for chemical analysis and the Specimen Bank. Bivalve samples from all S&T monitoring sites are kept for the Specimen Bank each year to maintain the long-term trend database and to provide information on chemical accumulation at the lower end of the foodweb, which may differ from accumulation in higher trophic level organisms due to differences in metabolic capabilities. Homogenized tissue from each composite, rather than whole bivalves, are stored in the Specimen Bank to facilitate the use of these samples for verification of analytical results if quality assurance issues arise (i.e. so that the samples are from the same composite) and to minimize the freezer storage space needed.

3.8 Sport Fish Samples

Sport fish have been collected for chemical contaminant analyses every three years since 1994 as part of S&T monitoring, which targets species that are frequently caught and consumed by Bay anglers at five popular fishing areas in the Bay. Sport fish are the targets most directly linked with Bay impairment in the methyl mercury and PCB TMDLs and are key indicators of Bay water quality. Contaminant concentrations in sport fish are compared to screening values for protection of human health and provide information for determining fish consumption advisories. They are also valuable for time trend analysis of foodweb contamination and investigation of CECs in the Bay.

Table 2 provides a summary of the collection and storage of sport fish samples for the RMP Specimen Bank. Following RMP methods (MLML/MPSL 2001), fish are homogenized and each composite is aliquoted into sub-samples for chemical analysis and the Specimen Bank. Sample aliquots from all S&T monitoring sites are kept for the RMP Specimen Bank each year because of the value of sport fish to management decisions and the potential risk to human and wildlife consumers of fish. However, the number of samples collected for the archives varies by species, with the largest number of samples collected from the white croaker and shiner surfperch composites. These species generally represent worst-case scenario concentrations among Bay sport fish because they typically contain the highest concentrations of chemical contaminants, in part because of their high lipid content. Additionally, white croaker are good indicators of regional foodweb contamination, while shiner surfperch are more indicative of local contaminant sources due to their smaller home range. For these reasons, these species are often the most desirable for assessments of chemical contamination in the Bay foodweb. Shiner surfperch are useful for investigating spatial trends of chemical uptake in the Bay and thus a larger number of samples are collected for this species. For the anchovy samples, only one sample per analysis is collected for the archives due to the low frequency of consumption of this species by anglers. Samples of only one species (i.e. white croaker) in the long-term archive were deemed sufficient for future investigations of fluorinated chemical analysis.

3.9 Bird Egg Samples

Eggs of double-crested cormorants have been monitored for chemical contaminants by the RMP since 2002. Eggs are collected from three sites spatially distributed throughout the Bay, with collections currently occurring on a three-year cycle as part of S&T monitoring. As with sport fish, eggs of piscivorous birds are a powerful monitoring tool because their high position in the food web allows them to be used for time trend analysis of foodweb contamination and the investigation of CECs. The inclusion of avian egg targets in the San Francisco Bay mercury and PCB TMDLs also makes egg monitoring valuable for evaluating impairment. Lastly, because of their relatively wide foraging ranges, cormorants are valuable indicators of regional contamination in the Bay. In addition to cormorants, the RMP will begin to monitor tern eggs on a triennial basis beginning in 2009. Terns were selected in addition to cormorants because they are indicators of shallow water habitats on the margins of the Bay and have lower effects thresholds.

Table 2 provides a summary of the collection and storage of bird egg samples for the RMP Specimen Bank. All cormorant egg samples from the S&T monitoring sites are kept for the RMP Specimen Bank because concentrations are relatively high compared to organisms lower in the food web and a large amount of sample material can easily be collected. Eggs (seven eggs for each composite) are homogenized and each composite is aliquoted for chemical analysis and the Specimen Bank.

3.10 Sample Hold Times

All tissue and sediment samples added to the short-term archive (-18 °C) will be kept for 15 years and discarded thereafter. This becomes effective beginning with samples added to the short-term archive in 2010 (i.e. samples added to the short-term archive in 2010 will be discarded in 2025). All samples that were added to -18 °C storage prior to 2010 will be kept indefinitely since these do not have replicate samples in the long-term archive (-150 °C). Samples that are visibly degraded (e.g. whole mussels wrapped in aluminum foil and placed in plastic bags) or that have excessive ice buildup on them will be discarded during the 2010 sample inspection. Additionally, extracts of all samples are retained by the analytical laboratories for one year to address any quality issues that may arise.

All tissue and sediment samples added to the long-term archive (-150 °C) will be kept for 40 years. After 40 years, only sediment samples collected every third year and tissue samples collected every sixth year will be kept. After these time limits, the samples will be discarded due to storage costs and the decreased probability of use.

3.11 Prioritization for Banking When Limited Sample Mass is Available

Tables 1-4 list the tissue mass or sediment volume needed from each sample site composite for samples added to the RMP Specimen Bank. However, due to logistical or other issues, this amount of material may not always be available. When limited sample mass is available, the sample containers for the archives should be filled in the following order, using the same sample masses/volume indicated in Tables 1-4 for each container: Teflon vials (long-term archive), PP cryovials (long-term archive), glass jars (short-term archive), PP/PE jars (short-term archive). Samples stored in the long-term archive are the highest priority since these may be used for chemical analysis at any time in the future for a wide variety of chemical contaminants, and are therefore the most valuable to the RMP.

4. Monitoring Chemical Degradation

Regardless of the temperature and methods selected for long-term storage of samples in the Specimen Bank, where possible, it is important to monitor chemical concentrations over time to assess the effects of the preservation procedure on sample integrity. This can be accomplished by the re-analysis of certified or standard reference materials (CRMs or SRMs) or another representative sample on a regular time interval. However, when determining if samples have been comprised due to chemical degradation, it is important to consider that differences in the analytical methods used may also affect comparison of the results.

For the following reasons, samples in the RMP Specimen Bank will <u>not</u> be re-analyzed over time for the purpose of monitoring chemical degradation:

- For chemical contaminants the RMP currently analyzes on a routine basis (e.g. legacy persistent organic pollutants (POPs), PBDEs), NIST already monitors these as part of their assessment of the integrity of chemicals in their biological and marine sediment SRMs at the recommended storage temperature (-80 °C for tissue, < 30 °C for sediment).

Thus far they have been able to conclude that significant degradation does not occur for at least 20 years for the legacy POPs and for at least 10 years for PBDEs. NIST will continue to re-analyze these compounds, and likely new analytes, as they become certified for the purpose of monitoring chemical degradation in the SRMs over time.

- For contaminants of emerging concern (chemicals not currently analyzed as part of RMP Status and Trends monitoring), because the RMP is not currently analyzing these compounds, baseline concentration values will not be available to which future analytical results would be compared. Thus, for now, storage of samples for the purpose of monitoring chemical degradation for compounds the RMP is not currently analyzing is not useful. However, if in the future the RMP decides to routinely analyze a chemical not currently re-analyzed over time by NIST, storage and re-analysis of samples for the purpose of monitoring chemical degradation will be considered.

To minimize the potential for degradation to the greatest extent possible, samples in the RMP Specimen Bank to be used for the future analysis of chemical contaminant time trends are stored in the Marine ESB, operated by NIST, at ultra low temperatures (LN_2 vapor freezers, -150 °C).

5. Special Storage Procedures for Analysis of Perfluorinated Chemicals

Perfluorinated chemicals (PFCs) are used in the manufacture of fluoropolymers such as poly-tetrafluoroethylene (PTFE or Teflon), therefore contact with these materials should be avoided if samples will be analyzed for PFCs. Research is ongoing in various laboratories to determine the appropriate handling procedures and storage containers for analysis of PFCs in environmental samples. Current knowledge suggests that glass jars covered with aluminum foil to prevent contact of the sample with a Teflon-lined lid, polyethylene (PE) containers, or polypropylene (PP) containers can be used and will not result in PFC contamination. The only samples that can therefore be used for the analysis of PFCs in the RMP Specimen Bank are those that are stored in PE or PP, which are available in storage at both -18 and -150 °C.

6. Inventory Databases

Inventory databases for RMP samples in both the short-term and long-term archives are maintained at SFEI. Each inventory includes basic information for each sample (e.g. site information, species, date collected), sample storage location (some are temporarily stored at the analytical laboratory or at the sample processing lab), the approximate mass or volume of sample available for chemical analyses, and details on how the storage containers were cleaned. Applied Marine Sciences (AMS) is contracted to maintain the inventory for samples kept in the short-term archive in Oakland and provides updates on at least a yearly basis to the RMP Data Manager. NIST personnel are responsible for maintaining the inventory database for RMP samples stored in the long-term archive at the Marine ESB and also provide an updated copy of their database to SFEI at least once a year. Both databases are maintained by the RMP Data Manager and located on the SFEI shared drive (S:\Research\RMP\Archived_Samples).

7. Procedures for Accessing Samples in the RMP Specimen Bank

7.1 Sample Access

Samples for the RMP Specimen Bank have been collected since 1994 and are available to the scientific community for research and evaluations consistent with the goals of the RMP. Requests for samples in the Specimen Bank are reviewed on an individual basis and have been accommodated for most of the samples collected from the inception of the RMP. However, past utilization of samples, changes to the program over the years, and loss of samples (or loss of sample integrity) due to container breakage has resulted in an incomplete sample set. Once a request is approved, SFEI and Applied Marine Sciences (AMS) personnel work with the researcher to obtain the samples of interest. Researchers should be aware that due to the restraints imposed by the request review process, freezer operators, and potential scheduling constraints of AMS personnel, sampling requests should be presented at least one month prior to the date the sample is needed. A protocol for accessing and sampling archived sediments from the RMP is also available on the SFEI website (http://www.sfei.org/rmp/documentation/archive.html).

7.2 Sub-Sampling Procedure

For sediment and bivalve samples collected prior to 2010 and sport fish and bird egg samples collected prior to 2009, sample composites in the Specimen Bank (short-term archive) will need to be sub-sampled for use. Samples collected for the Specimen Bank beginning in 2009-2010 are already stored as small sub-samples so that the sub-sampling procedure is avoided.

The integrity of samples in the RMP Specimen Bank during sub-sampling will be maintained using clean techniques. Sub-sampling will be conducted by AMS or other personnel designated by the RMP Manager in a clean analytical laboratory. Once the requested samples are identified and removed from the freezer, they will be allowed to thaw. Containers of thawed samples will then be opened and thoroughly mixed with a pre-cleaned stainless steel or Teflon implement (no plastic) for samples specified for organics analysis or a PP or titanium implement (no stainless steel or Teflon) for samples specified for trace metals or perfluorinated chemical analysis. Once mixed, the sub-sample will be removed and placed in containers provided by the requester. Depending on anticipated use of the sample mass remaining, the sample container will either be re-sealed and the sample re-frozen or the remaining sample mass will be aliquoted into smaller sub-samples to avoid repeated refreezing and thawing. Implements used for sub-sampling will be cleaned between samples using the same RMP protocol used to clean sampling implements when samples are originally collected (Liquinox®, hydrochloric acid, and methanol, with de-ionized water rinses). The remaining samples will then be returned to the commercial freezer space. Chain of custody forms will be created to track sub-samples, and a brief report will be produced for SFEI by AMS or other designated personnel describing the samples that have been sub-sampled, the requesting organization or individual, and the intended analyses to be conducted on the sub-sampled material. Eventual reporting of results of these analyses will be determined by SFEI and the applying organization or individual.

7.3 Costs

For each sample set obtained, a minimum fee will be charged to cover costs associated with access to commercial freezer storage, dry ice to re-freeze samples, and labor costs for AMS or SFEI personnel to conduct the sub-sampling. At the writing of this report, this fee is approximately $250.

8. Considerations for the Future of Long-Term Sample Storage

Several matters associated with the increase in the number of samples stored in the RMP Specimen Bank, as well as sample quality, will need to be addressed in the future. Issues of concern include freezer space management, associated costs, and the stability of chemicals and samples in low temperature storage over the long-term.

8.1 Freezer Space

As of February 2010, ~5,000 samples were stored in the RMP Specimen Bank at the Oakland freezer facility (-18 °C). An inspection of all samples in the Oakland freezer facility will occur in 2010, during which a small fraction of samples will likely be discarded because of sample degradation or re-located to a more appropriate facility (i.e. Arctic samples from Bob Risebrough's previous studies). Storage of samples at NIST in the Marine ESB will begin in 2010, with the addition of ~700 samples. Every three years ~850 and 600 samples will be added to the short-term (Oakland) and long-term (NIST) archives, respectively, thus the volume of freezer space needed to store samples will steadily increase over time. Space availability at the Oakland storage facility is not expected to be an issue due to its large storage capacity. Though the sample storage containers used in the long-term archives are substantially smaller than those in the short-term archive, NIST has indicated that storage space in the Marine ESB is also not likely to be an issue given our proposed rate of sample addition. The NIST facility was recently constructed (2000) and can accommodate a large number of LN_2 freezers.

8.2 Costs

As the number of samples in storage increases over time, costs associated with the maintenance of these samples will also increase. The cost to store the current amount of samples in the Oakland freezer facility is ~$1,600 per month. Given the anticipated rate of sample addition to the short-term archives at this facility, costs are expected to increase by ~$1,000 every year. The cost is not likely to change substantially over the next few years, however, because the additional space needed to store the samples added over the next three years will likely equal the space that will become available once the samples that are deemed no longer useful to the RMP are removed in 2010. The Oakland freezer facility charges per pallet, not per sample, in storage.

The cost of RMP sample storage in the long-term archive at the NIST Marine ESB includes sample/freezer maintenance, sample container cleaning, and database maintenance by NIST personnel. The cost for Specimen Bank development and storage of 2009 and 2010 samples is $18,500, which includes a one time set up fee. In subsequent years the annual cost will range from $5,000 (sediment only years) to $10,000 (bird egg and sport fish monitoring years). Storage costs are not expected to increase substantially over time, if at all, though the terms of the collaboration with NIST past 2015 have not yet been established.

As part of the collaboration with NIST, SFEI has agreed to supply NIST with additional specimens, when requested, for the Marine ESB during the sample collection for SFEI monitoring programs. Increases in sample collection costs are not anticipated for sediments due to the ease of collection of large amounts of sediment using current RMP methods during the S&T collections. For bivalves and sport fish, there may be increases in costs to cover additional field sampling and sample processing. Collecting additional bird egg samples may not be possible due to permit restrictions but it may be possible to share unused material from the S&T monitoring in some cases.

Costs associated with the additional storage containers needed for samples in the Specimen Bank will increase compared to previous years. Additional storage container costs for the short-term archive will increase by a small amount ($100-$600 per matrix) while container costs for the long-term archive will increase by ~$20 per sample (~$500-$3,500 per matrix). Small increases in costs associated with pre-cleaning sample containers for the short-term archive are also expected but will be low (< $500 per matrix). Costs associated with the increased amount of laboratory processing time to do the aliquoting are expected and will be dependent on the laboratory and the matrix. For example, aliquoting subsamples of the 2010 bivalve composites in the laboratory will cost ~$4,000, while there will be no additional charge for aliquoting subsamples of the 2010 sediment samples on the boat during the sediment cruise. Charges for shipping samples and sample containers to and from NIST (for the long-term archive) and to AMS (for the short-term archive) via Federal Express are expected to increase costs by ~$500-$2,000 per year depending on the number of matrices sampled each year.

8.3 Long-term Sample Stability

The stability of chemicals and samples in low temperature storage is largely uncertain for samples stored for more than 20 years (see section 2.1). Storage conditions for samples in the RMP Specimen Bank have therefore been determined based on information available to date and best professional judgment. As more information becomes available, procedures for the long-term preservation of RMP samples will be modified, if necessary, based on this information.

9. References

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Table 1. Sediment samples collected for the RMP Specimen Bank

Samples	# of containers	Sediment per container (ml)	Container	Storage Purpose	Storage Temperature	Volume needed for each composite (ml)
Historic sites (n = 7 composites)	3	45-50	60 ml glass jar[a]	Time trends, CECs, QA/QC	-18 °C	100
	2	200	250 ml PE jar[b]	Time trends, CECs, QA/QC	-18 °C	
	3	18-20	22 ml Teflon vial[c]	Long-term time trends	-150 °C	
	5	4	5 ml PP cryovial[c]	Long-term time trends	-150 °C	
Random sites (n = 40 composites in summer/dry season or 20 composites in winter/wet season)	3	45-50	60 ml glass jar[a]	Time trends, CECs, QA/QC	-18 °C	75
	2	200	250 ml PE jar[b]	Time trends, CECs, QA/QC	-18 °C	

Table 2. Bivalve samples collected for the RMP Specimen Bank

Samples	# of containers	Tissue mass per container (g wet wt)	Container	Storage Purpose	Storage Temperature	Mass needed for each composite (g wet wt)
All sites (n = 11 composites)	3	15	60 ml glass jar[a]	Time trends, CECs, QA/QC	-18 °C	135
	2	15	30 ml PP jar[d]	Time trends, CECs, QA/QC	-18 °C	
	3	15	22 ml Teflon vial[c]	Long-term time trends	-150 °C	
	5	3	5 ml PP cryovial[c]	Long-term time trends	-150 °C	

PE = polyethylene; PP = polypropylene; CECs = contaminants of emerging concern; QA/QC = quality assurance/quality control

a = Pre-cleaned/PC class jars, Teflon-lined lid, supplied by ESS Vial (Oakland, CA)
b = Pre-cleaned by a commercial supplier (e.g. I-Chem)
c = Pre-cleaned by NIST
d = Pre-cleaned by AXYS Analytical, linerless lid, supplied by Fisher Scientific

Table 3. Sport fish samples collected for the RMP Specimen Bank

Samples	# of containers	Tissue mass per container (g wet wt)	Container	Storage Purpose	Storage Temperature	Mass needed for each composite (g wet wt)
Baseline for all samples except white croaker, shiner surfperch, and northern anchovy	3	15	60 ml glass jar[a]	Time trends, CECs, QA/QC	-18 °C	120
	2	15	30 ml PP jar[b]	Time trends, CECs, QA/QC	-18 °C	
	3	15	22 ml Teflon vial[c]	Long-term time trends	-150 °C	
White croaker	3	15	60 ml glass jar[a]	Time trends, CECs, QA/QC	-18 °C	135
	2	15	30 ml PP jar[b]	Time trends, CECs, QA/QC	-18 °C	
	3	15	22 ml Teflon vial[c]	Long-term time trends	-150 °C	
	5	3	5 ml PP cryovial[c]	Long-term time trends	-150 °C	
Shiner surfperch	4	15	60 ml glass jar[a]	Time trends, CECs, QA/QC	-18 °C	135
	2	15	30 ml PP jar[b]	Time trends, CECs, QA/QC	-18 °C	
	3	15	22 ml Teflon vial[c]	Long-term time trends	-150 °C	
Northern anchovy (30-45g tissue)	1	10-15	60 ml glass jar[a]	Time trends, CECs, QA/QC	-18 °C	30-45
	1	10-15	30 ml PP jar[b]	Time trends, CECs, QA/QC	-18 °C	
	1	10-15	22 ml Teflon vial[c]	Long-term time trends	-150 °C	
Northern anchovy (20-30g tissue)	1	10-15	60 ml glass jar[a]	Time trends, CECs, QA/QC	-18 °C	20-30
	1	10-15	22 ml Teflon vial[c]	Long-term time trends	-150 °C	
Northern anchovy (10-20g tissue)	1	10-15	22 ml Teflon vial[c]	Long-term time trends	-150 °C	10-20

PE = polyethylene; PP = polypropylene; CECs = contaminants of emerging concern; QA/QC = quality assurance/quality control

a = Pre-cleaned/PC class jars, Teflon-lined lid, supplied by ESS Vial (Oakland, CA)
b = Pre-cleaned by Moss Landing Marine Labs, linerless lid, supplied by Fisher Scientific
c = Pre-cleaned by NIST

Table 4. Bird Egg samples collected for the RMP Specimen Bank

Samples	# of containers	Tissue mass per container (g wet wt)	Container	Storage Purpose	Storage Temperature	Mass needed for each composite (g wet wt)
All sites (n = 9 composites)	4	15	60 ml glass jar[a]	Time trends, CECs, QA/QC	-18 °C	150
	2	15	30 ml PP jar[b]	Time trends, CECs, QA/QC	-18 °C	
	3	15	22 ml Teflon vial[c]	Long-term time trends	-150 °C	
	5	3	5 ml PP cryovial[c]	Long-term time trends	-150 °C	

PE = polyethylene; PP = polypropylene; CECs = contaminants of emerging concern; QA/QC = quality assurance/quality control

a = Pre-cleaned/PC class jars, Teflon-lined lid, supplied by ESS Vial (Oakland, CA)
b = Pre-cleaned by AXYS Analytical or other designated laboratory, linerless lid, supplied by Fisher Scientific
c = Pre-cleaned by NIST