Procedures for the Collection and Preservation of Environmental Samples in the Specimen Bank of the Regional Monitoring Program for Water Quality in the San Francisco Estuary (RMP)

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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 emerging contaminants, and future verification of analytical results if quality assurance issues arise. Several environmental specimen banks exist in the United States (e.g., NOAA Mussel Watch Program, U.S. National Biomonitoring Specimen Bank) and other countries (e.g. Sweden, Canada, Japan, Germany) and are valuable tools for retrospective trend analysis of chemicals using samples that have been archived for decades.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 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 preservation were developed in consultation with representatives from the U.S. National Biomonitoring Specimen Bank, the NOAA Mussel Watch Program, and Environment Canada's Wildlife Specimen Bank.

2. Stability of Tissue and Sediment 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 over time 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., perfluorinated chemicals in Teflon® or plastic additives). Relatively little information on the stability of chemical contaminants under various long-term storage conditions is 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 over the years makes separating out changes in concentration 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 the preservation of samples in a specimen bank, with storage at -150 °C or lower believed to allow sample preservation without any major structural or biochemical changes over several decades (Wise and Koster 1995, McFarland et al. 1996). Preservation 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 PAHs, 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). Though 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 available.

The temperature of conventional, mechanical freezers is -20 °C, thus relatively more information is available regarding storage of environmental samples at this temperature. For trace metals, studies suggest that concentrations in sediment and tissue are not affected (McFarland et al. 1995, Wise and Koster 1995). While there is uncertainty in the effect of -20 °C storage on concentrations of PCBs and chlorinated pesticides over time, particularly in 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 -80 °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 kept at -150 °C appeared the same as they did just after homogenization. Because of moisture loss, chemical concentrations of samples kept 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 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

For the preservation of samples in long-term storage, a temperature of -80 °C or lower and the use of liquid nitrogen (-196 °C) or liquid nitrogen vapor freezers (-150 to -190 °C) are

recommended (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 power failures, which may be 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). 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 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 (i.e., 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. Backup storage is also recommended in case of freezer failure (ISBER 2008). Security systems and continuous, electronic monitoring of storage temperature are used by many specimen banks internationally and are recommended for use with all freezer types.

3. Collection, Prioritization, and Storage of Samples for the RMP Specimen Bank

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 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. Chemical contaminant monitoring of small fish and tributary surface water loading was added to S&T monitoring in 2007. Monitoring of benthos was included in 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 advances in understanding of the Estuary. 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. Both S&T and Pilot and Special Studies will include an archiving plan as part of their project workplan.

3.2 Considerations in 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 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.

Sample Types

Samples that accumulate the chemicals of interest and that are representative of general conditions in the San Francisco Estuary should be collected for the Specimen Bank. For the RMP, the archived sample needs to 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 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.

Sample Volume

A sufficient amount of each sample should be archived to allow multiple chemical analyses both in terms of contaminants and the ability to conduct repeat 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. Sub-samples containing 5-20 g of sediment or 15-20 g of tissue are sufficient to obtain low detection limits in most chemical analyses.

Long-term Archive Sample

At least one sub-sample of each sample homogenate should be specifically collected and labeled for the purpose of long-term archiving. While the other sub-samples would be available for emerging contaminant studies, time trend analyses, and re-analysis due to quality assurance issues, one sample should be kept for more permanent, long-term storage and only accessed if deemed necessary. Labeling as a separate long-term archive sample will insure that at least one sub-sample is preserved indefinitely.

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 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 3). The RMP S&T program has typically focused on the analysis of trace metals and the following persistent organic chemical contaminants: polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), organochlorine, organophosphate and pyrethroid pesticides, chlorinated dioxins and furans, and perfluorinated compounds. It is anticipated that future analyses of RMP Specimen Bank samples will also focus on persistent organic chemicals since these chemicals have generally been suggested to pose the greatest risk of adverse effects in aquatic foodwebs. However, the identity of future target analytes are 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 over long periods will therefore be used for samples collected for the RMP Specimen Bank.

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 Water

RMP Surface Water Sampling

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.

Water Sample Collection, Prioritization, and Storage Procedures

Water samples for the RMP Specimen Bank are not collected because of the instability of organic 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.

3.4 Sediment

RMP Sediment Sampling

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 will alternate between the summer and rainy season each year beginning in 2010. Sediment will be sampled during the summer of 2009 and winter sediment sampling will begin in 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. Additional coring work is anticipated in future years.

Sediment Sample Collection, Prioritization, and Storage Procedures

Table 1 provides a summary of the collection and storage of sediment samples for the RMP Specimen Bank. Sediment samples for the Specimen Bank are sub-samples of the sample

composite collected for chemical analysis as part of S&T monitoring. Following RMP methods (David et al. 2001), sediment is collected, homogenized into a single composite at each site, and allocated into samples for chemical analysis and the specimen bank.

From the historic sites, a total of four samples are collected from each composite for the specimen bank: two large volume samples (250 ml containers, one glass and one high density polypropylene (HDPE) or polypropylene (PP)) for time trends, emerging contaminants, and QA/QC purposes, and two smaller volume samples (125 ml containers, one Teflon® and one HDPE or PP) for the long-term archive. From the random sites, only two large volume samples (250 ml containers, one glass and one HDPE or PP) for time trends, emerging contaminants, and QA/QC purposes are collected. Samples for the long-term archive are not collected from random sites because these samples are less useful for long-term trend analysis. Samples stored in glass or Teflon® are intended for the analysis of persistent organic chemicals, while samples stored in HDPE or PP are intended for analysis of trace metals or chemicals that may be present in Teflon®, such as perfluorinated chemicals (PFCs). The large volume samples are stored at -18 °C since concentrations of persistent organic chemicals are expected to be stable at this temperature for at least 10 to15 years (Section 2.1) and the cost associated with storage is substantially less compared to low temperature storage (- 80 °C). Samples collected for the long-term archive are stored at -80 °C and can therefore be used for the analysis of less persistent, more reactive chemicals in the short-term or chemicals that may not be stable in long-term storage at -18 °C. Storage of sediment samples from the same site in separate freezers also provides added security in case of freezer failure.

Samples collected from the historic sites will be kept in storage for 40 years to support time trend analysis. After 40 years, only samples that were collected every fifth year will be kept and the remaining will be discarded. Samples collected from random sites will be stored for only five years since these samples are not useful for long-term trend analysis but are valuable for recent accumulation of yet unidentified chemical contaminants among specific regions of the Estuary. Five years is also a sufficient length of time for resolution of quality assurance issues associated with routine S&T chemical analysis. Extracts of the sediment samples are also retained by the analytical laboratories for one year to address any quality issues that may arise.

3.5 Bivalves

RMP Bivalve Sampling

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 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.

Bivalve Sample Collection, Prioritization, and Storage Procedures

Table 2 provides a summary of the collection and storage of bivalve samples for the RMP 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. 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.

A total of nine aliquots are collected from each bivalve composite for the Specimen Bank: five small volume aliquots (15-20 g) and two large volume aliquots (50 g each) for time trends, emerging contaminants, and QA/QC purposes, and two aliquots for the long-term archive (50 g each). The small volume aliquots are stored in glass at -18 °C and are intended for analysis of persistent organic chemicals and those not expected to degrade at this temperature in the short-term (three to five years). A 50 g aliquot is stored in HDPE or PP at -18 °C and is intended for analysis of trace metals or chemicals that may be present in Teflon®, such as perfluorinated chemicals (PFCs). Small volume aliquots are collected for organic chemical but not metals analysis because organic chemicals are the chemicals most frequently analyzed in emerging contaminant studies and the availability of small aliquots prevents compromising the bulk sample as a result of several freeze-thaw, sub-sampling procedures. Another 50 g aliquot for time trends, emerging contaminants, and QA/QC purposes, is stored in Teflon® at -80 °C and is intended for the analysis of persistent organic chemicals when the 15 g aliquots are no longer available, or for chemicals that are suspected to be less persistent or susceptible to degradation in long-term storage at -18 °C. Two 50 g aliquots are collected for the long-term archive and are kept at -80 °C. One aliquot is stored in Teflon® for the analysis of organic chemicals and the other is stored in HDPE or PP for the analysis of perfluorinated chemicals (PFCs) or other chemicals that may be present in Teflon®. The long-term archive aliquot will only be accessed when the other samples are depleted. Storage of bivalve sample aliquots in both -18 and -80 °C freezers provides security in case of freezer failure. Bivalve sample aliquots from all sites will be kept in storage for 40 years to support time trend analysis. After 40 years, only sample aliquots collected every fifth year will be kept and the remaining will be discarded due to storage costs/space and the decreased probability of use. Extracts of the bivalve samples are also retained by the analytical laboratories for one year to address any quality assurance issues that may arise.

3.6 Sport Fish

RMP Sport Fish Sampling

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. Because they are the targets

most directly linked with Bay impairment in the methyl mercury and PCB TMDLs, sport fish 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 emerging contaminants in the Bay.

Sport Fish Sample Collection, Prioritization, and Storage Procedures

Table 2 provides a summary of the collection and storage of sport fish sample aliquots for the RMP 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. Following RMP methods (MLML / MPSL 2001), fish are homogenized and each composite is aliquoted into samples for chemical analysis and the Specimen Bank.

Similar to bivalves and bird eggs, a total of nine aliquots are collected from each sport fish composite for the Specimen Bank. The volume of tissue aliquots per composite, sample containers, storage temperatures, and hold times for sport fish samples are identical to those for bivalve and bird eggs. Extracts of the sport fish samples are also retained by the analytical laboratories for one year to address any quality assurance issues that may arise.

3.7 Bird Eggs

RMP Bird Egg Sampling

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, piscivorous bird eggs 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 emerging contaminants. 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. The number of sample collection sites will be determined in early 2009.

Bird Egg Sample Collection, Prioritization, and Storage Procedures

Table 2 provides a summary of the collection and storage of bird egg samples for the RMP Specimen Bank. Because cormorants occupy a high trophic position, concentrations are relatively high compared to organisms lower in the food web, and a large amount of sample material can easily be collected, all cormorant egg samples from the S&T monitoring sites are

kept for the RMP Specimen Bank. Tern egg samples are not collected for the Specimen Bank because their relatively small size makes collection of a sufficient amount of material for archiving difficult. Eggs (seven eggs for each composite) are homogenized and each composite is aliquoted for chemical analysis and the Specimen Bank.

Similar to bivalves and sport fish, a total of nine aliquots are collected from each egg composite for the Specimen Bank. The volume of tissue aliquots per composite, sample containers, storage temperatures, and hold times for egg samples are identical to those for bivalve and sport fish. Extracts of the bird egg samples are also retained by the analytical laboratories for one year to address any quality assurance issues that may arise.

3.8. Prioritization for Preservation When Limited Tissue is Available for Banking

The collection of 300 g of bivalve, sport fish, and bird egg tissue per sample is ideal for RMP archiving needs, though this amount of material may not always be available. Table 3 outlines the priorities for allocating the available tissue for the Specimen Bank. Samples stored at -18 °C in glass and HDPE or PP for organics and trace metals analyses, respectively, are the highest priority since they have the highest probability of use. Once these have been allocated, the remaining sample material should be distributed equally among the sub-samples stored at -80 °C as indicated.

3.9 Monitoring Chemical Degradation

Regardless of the temperature and methods selected for long-term storage of samples in the Specimen Bank, 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 standard reference materials (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 the chemical concentration in the samples.

To monitor chemical degradation of tissue samples in the RMP Specimen Bank, three replicates of National Institute of Standards and Technology (NIST) SRM 1974b, a fresh-frozen mussel (*Mytilus edulis)* tissue homogenate, will be analyzed every four years. The four-year cycle of SRM analysis will coincide with the biennial analysis of chemical contaminants in bivalves as part of RMP S&T monitoring. The SRM will be kept in storage with the other RMP samples at -20 and -80 ° and analyzed for the same target chemicals monitored in that particular year as part of RMP S&T. SRM 1974b was selected because mussels are one of the tissue types most frequently analyzed by the RMP, the mussels were collected from an urbanized estuary (Boston Harbor, MA), and because the concentrations of several contaminants have previously been determined in them. In addition to NIST-certified values for PAHs, PCBs, and chlorinated pesticides, concentrations of PBDEs (Stapleton et al. 2007), organotins (Point et al. 2007), synthetic musks (Peck et al. 2007), and methylmercury (Davis et al. 2007) in SRM 1974b have also been reported. Because SRM 1974b is packaged by NIST in glass jars containing 8-10 g (wet basis) of frozen tissue homogenate, the sample will not be affected by potential

contamination due to sub-sampling or moisture migration as a result of several freeze-thaw cycles prior to chemical analysis. Changes in lipid content can also be assessed concurrently with organic contaminant analyses of the SRM. A sub-set of samples in the Specimen Bank will also be inspected visually on a yearly basis for signs of moisture migration in the samples. NIST sediment SRMs are freeze-dried and therefore are not useful for monitoring chemical degradation in RMP sediment samples in the Specimen Bank, which are stored as whole sediments.

3.10 Storage Freezer Information

Samples in -18 °C storage are 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. <<*Information on -80 °C storage to be determined*>>.

4. 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 the plastic or Teflon®-lined lid, high density polyethylene (HDPE) 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 HDPE or PP, which are available in storage at both -18 and -80 °C. PFCs are persistent chemicals and thus are not expected to degrade in environmental samples in long-term storage at -18 °C. However, if in the future it is determined that there is potential for degradation of PFCs in storage at -18 °C, the long-term archive sample aliquots stored in PP at -80 °C can also be used for PFC analysis. While the primary intention of the long-term archive sample aliquots in storage is not to be made available for PFC analysis, PFCs are a unique class of contaminants that are not expected to be analyzed often. The potential use of the long-term archive sample aliquots for analysis of PFCs or other specialty organic chemicals present in Teflon® is considered an acceptable, low cost alternative to collecting an additional sample specifically for PFC analysis at every RMP S&T sampling site.

5. Specimen Bank Inventory Database

An inventory of the biota and sediment samples in the RMP Specimen Bank is maintained in a database at SFEI (S:\Research\RMP\Archived_Samples). In addition to basic information about each sample (e.g. location, species, date collected), the database includes information on where the sample is currently stored (some are temporarily stored at the analytical laboratory), whether or not the sample has been sub-sampled previously, and the approximate mass of tissue available in each sample for chemical analyses. Applied Marine

Sciences is contracted to maintain the specimen bank sample inventory for the RMP and provides updates on a yearly basis to the RMP Data Manager for maintenance of the inventory database.

6. Procedures for Accessing Samples in the RMP Specimen Bank

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, the commercial freezer operator, and potential scheduling constraints of AMS personnel, sampling requests should be presented at least one month prior to the intended sampling date. 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).

Sub-Sampling Procedure

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. For bivalves, sport fish, or bird eggs, the 50 g sample aliquot for time trends, emerging contaminants, and QA/QC purposes should only be sub-sampled when the 15 g sample aliquots are no longer available. 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 polypropylene 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. The sample container will then be re-sealed, and the sample re-frozen. 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 (Alconox®, 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 which 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.

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, the fee is approximately $250.

7. Considerations for the Future of 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 near future. Issues of concern include freezer space management, maintenance costs, and the stability of chemicals and samples in low temperature storage over the long-term.

Freezer Space

Approximately 3,000 samples were stored in the RMP Specimen Bank as of spring 2008. Because ~250 samples are added to the Specimen Bank every three years, the volume of freezer space needed to store samples will steadily increase over time. Space availability at the commercial storage facility (-20 °C) is not expected to be an issue due to its large storage capacity. << *Concerns for -80 °C storage space will be determined following selection of sample storage location*>>

Maintenance Costs

As the number of samples in storage increases over time, costs associated with the maintenance of these samples will also increase. Costs to consider include freezer maintenance and monthly storage charges. << *Cost details and concerns for -80 °C storage space will be determined following selection of sample storage location*>>

Long-term Sample Stability

The stability of chemicals and samples in low temperature storage is largely uncertain for samples stored for more than 10-15 years (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.

8. References

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Table 1. Collection and storage of RMP sediment samples

Sample	# of containers	Container type	Analysis	Purpose	Hold Temp (°C)
Historic sites	1	250 ml, glass	POPs	Time trends, emerging contaminants, QA/QC	-18
	1	250 ml, HDPE or PP	PFCs, metals	Time trends, emerging contaminants, QA/QC	-18
	1	125 ml, Teflon	POPs	Long-term archive	-80
	1	125 ml, HDPE or PP	PFCs, metals	Long-term archive	-80
Random sites	1	250 ml, glass	POPs	Emerging contaminants, QA/QC	-18
	1	250 ml, HDPE or PP	PFCs, metals	Emerging contaminants, QA/QC	-18
Cores	-	-		Time trends, emerging contaminants, QA/QC	-18

Table 2. Collection and storage of RMP bivalve, sport fish, and bird egg samples

Sample	# of containers	Container type	Analysis	Purpose	Hold Temp (°C)
All sites	5	15 g, glass	POPs	Time trends, emerging contaminants, QA/QC	-18
	1	50 g, HDPE or PP	PFCs, metals	Time trends, emerging contaminants, QA/QC	-18
	1	50 g, Teflon	POPs	Time trends, emerging contaminants, QA/QC	-80
	1	50 g, Teflon	POPs	Long-term archive	-80
	1	50 g, HDPE or PP	PFCs, metals	Long-term archive	-80

Tissue mass (g) per aliquot is wet weight.
HDPE = high density polyethylene; PP = polypropylene
For samples more than forty years old, keep only those collected every five years.

Table 3. Priorities for preservation of RMP samples when less than the preferred mass (300 g) of homogenized bivalve, sport fish, and bird egg tissue is available.

Tissue Mass Available (g)	# of containers	Container Type	Purpose	Hold Temp (°C)
150	5	15 g, glass	Time trends, emerging contaminants, QA/QC	-18
	1	50 g, HDPE or PP	Time trends, emerging contaminants, QA/QC	-18
150-200	5	15 g, glass	Time trends, emerging contaminants, QA/QC	-18
	1	50 g, HDPE or PP	Time trends, emerging contaminants, QA/QC	-18
	1	50 g, Teflon	Long-term archive	-80
200-250	5	15 g, glass	Time trends, emerging contaminants, QA/QC	-18
	1	50 g, HDPE or PP	Time trends, emerging contaminants, QA/QC	-18
	1	50 g, Teflon	Long-term archive	-80
	1	50 g, HDPE or PP	Long-term archive	-80
250-300	5	15 g, glass	Time trends, emerging contaminants, QA/QC	-18
	1	50 g, HDPE or PP	Time trends, emerging contaminants, QA/QC	-18
	1	50 g, Teflon	Long-term archive	-80
	1	50 g, HDPE or PP	Long-term archive	-80
	1	50 g, Teflon	Time trends, emerging contaminants, QA/QC	-80

[a] Tissue mass (g) per aliquot is wet weight. Once the samples for -18 °C storage are aliquoted, the remaining sample material should be distributed evenly among the -80 C samples as indicated.
[b] HDPE = high density polyethylene; PP = polypropylene