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BIOMONITORING WORK PLAN

SITE 8 - NITROGLYCERIN PLANT OFFICE

FINAL DOCUMENT

Indian Head Naval Ordnance Station
Indian Head, Maryland

Submitted to:

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BIOMONITORING WORK PLAN
INDIAN HEAD NAVAL ORDNANCE STATION

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BIOMONITORING WORK PLAN
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1.0 INTRODUCTION

The Chesapeake Division (CHESDIV) of Naval Facilities Engineering command has requested the support of the Department of Energy (DOE) in conducting Site Characterization, Feasibility Study, and Remedial Design activities at Indian Head Naval Ordnance Station (NOS), Indian Head, Maryland. In particular, assistance has been sought to develop a solution to the contamination problem associated with releases of mercury to the environment from the Nitroglycerin Plant Office (Building 766) at NOS. Martin Marietta Energy Systems, Inc., operators of Hazardous Waste Remedial Action Planning, Support Contractors Office, Oak Ridge, Tennessee, has been assigned responsibility for managing this effort for the Navy under interagency agreement (1791-1791-A1) with the DOE. ABB Environmental Services, Inc. (ABB-ES), formerly E.C. Jordan Co. (Jordan), has assisted in conducting studies focused on the mercury contamination problem associated with historical releases from the Nitroglycerin Building.

As a component of this program, ABB-ES has been specifically tasked to develop and implement a biomonitoring program for Site 8. As defined by the Confirmation Study (CHESDIV, 1985), the site consists of a stream, cattail marsh and tidal pond downgradient of the Nitroglycerin Building. The main objectives of this task are to determine the biological consequences of the current contamination problem, and to document potential ecological effects and long-term recovery associated with the remedial actions taken at the site.

As a first step in meeting these objectives, this Biomonitoring Work Plan describes in detail how these goals will be achieved. In developing this work plan guidance as provided in the United States Environmental Protection Agency (USEPA) "Ecological Assessment of Hazardous Waste Sites: A Field and Laboratory Reference" (USEPA, 1989), "Protocol for Bioassessment of Hazardous Waste Sites (USEPA, 1983), and "Biological Monitoring of Toxic Trace Metals, Volume 1" (Jerkins, 1980) manuals were used. Additionally, numerous papers from the scientific literature were reviewed in developing the work plan.

This introduction briefly describes relevant background information, the fate and transport of mercury at Site 8, and potential effects associated with ecological exposure to mercury at the site. This information was used in focusing the design of the biomonitoring program. An overview of program objectives and the recommended approaches to meeting these goals is presented in Section 2. General issues concerning sample design are discussed in Section 3 and a detailed description of the specific

biomonitoring activities is presented in Section 4. Meeting the goals of this work plan will depend upon a close adherence to scheduling requirements as presented in Section 5.

1.1 Background

Over a period of approximately 20 years, mercury was released in small amounts from sink and floor drains in the laboratory of the Nitroglycerin Plant Office into a storm drain outside the building. The storm drain system discharges into a small stream that flows approximately 0.25 miles across Navy property through a cattail marsh and tidally influenced pond before emptying into Mattawoman Creek. The location of this stream and tidal pond system is presented in Figure 1. Previous investigations (CHESDIV, 1985; Jordan, 1987; Jordan, 1988) have determined that sediment and surface water of the stream, marsh, and pond contain elevated concentrations of mercury. It is believed that between 200 and 500 pounds of mercury were released to the environment (CHESDIV, 1985; Jordan, 1989).

Because the toxicological effects of mercury exposure are highly dependent upon the particular form of mercury (USEPA, 1985), a speciation study was conducted in 1990 to determine the relative abundance of the various forms of mercury in surface water and sediment at the site. This study confirmed the conclusions of earlier studies regarding the extent and magnitude of mercury contamination in soil and sediment. In addition, methylmercury, as well as other forms of mercury which can readily be methylated (e.g., elemental, bound), were detected in sediment throughout the site. As a result of these findings, it was recommended that a biological assessment and monitoring program be designed and implemented to evaluate the effects of mercury on the environment at Site 8 (Jordan, 1990).

The United States Fish and Wildlife Service (USFWS) has conducted a biomonitoring program in the adjacent stretch of Mattawoman Creek since 1987 (USFWS, 1991). This program was designed to evaluate the uptake of mercury by recreationally and commercially important aquatic species which occur in the creek and which may have been exposed to mercury contamination migrating from NOS. Results to date indicate that tissues of channel catfish (*Ictalurus punctatus*) collected in the vicinity of Marsh Island (near the Site 8 outlet) have statistically significant higher mercury concentrations as compared to samples collected from an upstream reference location on Mattawoman Creek. Tissue concentrations of largemouth bass (*Micropterus salmoides*) and bluegill sunfish (*Lepomis macrochirus*) have not been found to be significantly different between sampling locations (USFWS, 1991), however. The USFWS has recently questioned the adequacy of their original control site due to concerns about other potential sources of contamination or anthropogenic impacts. Moreover, the study was not designed to evaluate the consequences of potential chronic impacts to these organisms as a result of mercury exposure (USFWS, 1991). Consequently, the results of this study must be

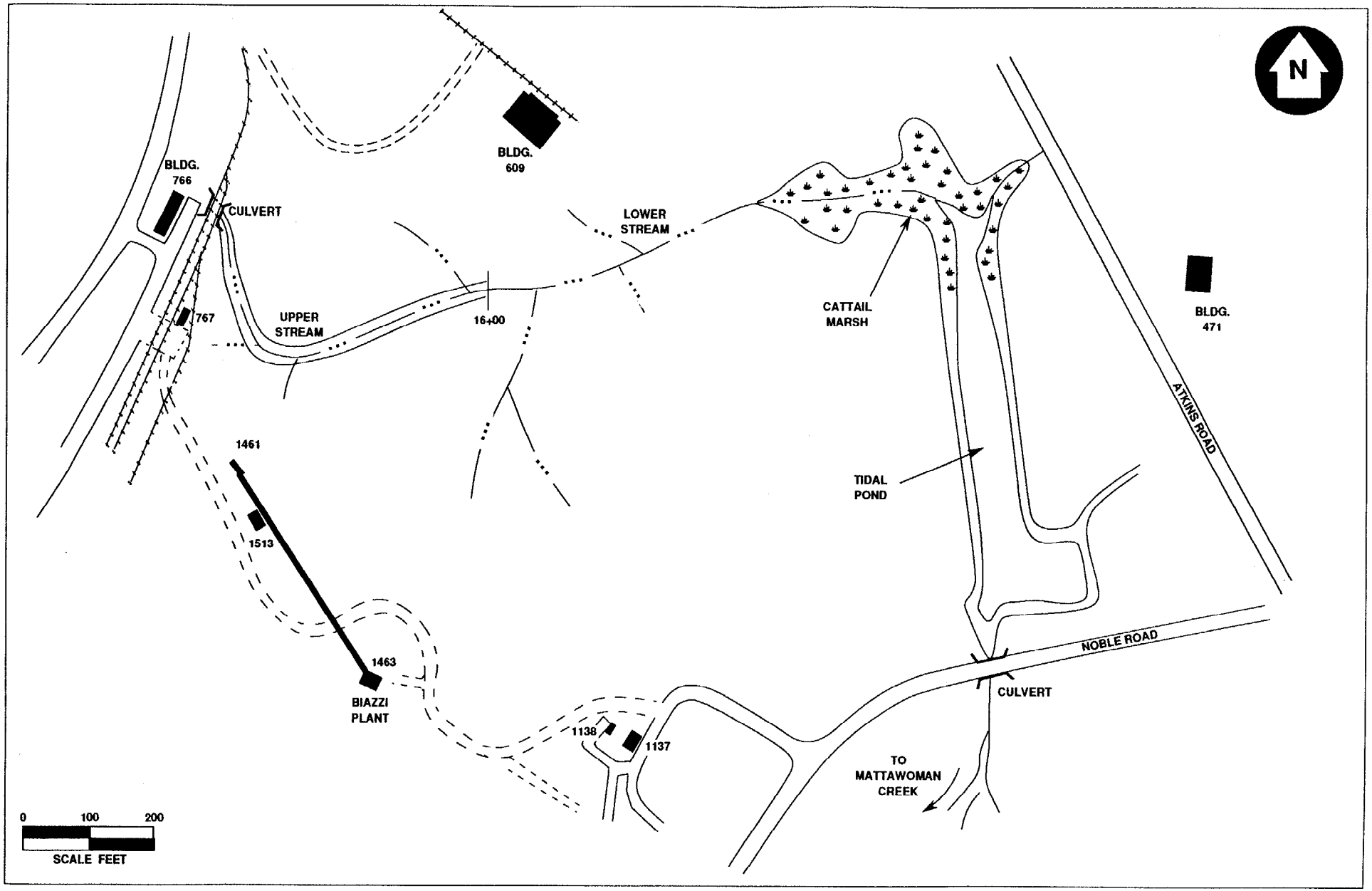

ABB Environmental Services Inc.

ASEA BROWN BOVERI

FIGURE 1
SITE MAP
NAVAL ORDNANCE STATION
INDIAN HEAD, MARYLAND

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considered tentative and may not completely address the full impact of contaminant migration into Mattawoman Creek.

1.2 Summary of Fate and Transport of Mercury at Site 8

The ultimate fate, and particular forms of mercury, is an extremely complex process (Moore and Ramamoorthy, 1984; NRC, 1978; Fitzgerald, 1979; Ridley et al., 1977). Elemental mercury may become methylated under certain environmental conditions, and inorganic mercury can be methylated both by sediment-dwelling microorganisms and abiotically. There is also evidence that the methylation process can occur in fish tissue as well (Moore and Ramamoorthy, 1984). Organic mercurials, including monomethylmercury and dimethylmercury, are more toxic primarily because of their enhanced bioavailability and capacity to move into the biological food chain and affect biological tissue toxicologically (Eisler, 1987).

Studies indicate that absorption of methylmercury by aquatic organisms is enhanced by increased salinity and at pH levels below 7.0 (Major and Rosenblatt, 1991). Based on preliminary water quality measurements, rates of conversion to methylmercury are expected to be greatest in the tidal pond habitat at the site. However, concentrations of total mercury are generally lower in the tidal pond as compared to the stream and tidal marsh areas.

1.3 Ecological Impacts Associated With Exposure to Mercury

Ecological impacts associated with mercury contamination of aquatic habitats have been well studied (reviews in Sorensen, 1991; Moore and Ramamoorthy, 1984). Mercury is one of the most toxic inorganic compounds with many aquatic organisms deleteriously affected at exposure concentrations in the parts per billion range. In aquatic organisms, at comparatively low concentrations, mercury can adversely affect reproduction, growth, behavior, metabolism, blood chemistry, osmoregulation, and oxygen exchange (Eisler, 1987). Mercury is also a mutagen, teratogen, and carcinogen, and causes embryocidal, cytochemical, and histopathological effects (Eisler, 1987).

Exposure pathways by which aquatic and terrestrial organisms may be exposed to mercury at Site 8 were described in the ecological risk assessment as presented in the NIRP Feasibility Study and Remedial Design report (Jordan, 1988). Aquatic organisms may be exposed to contaminants in surface water and sediment through direct contact and incidental sediment ingestion. In addition, the consumption of contaminated prey items may represent a significant exposure route to higher trophic organisms such as fish. Dietary exposure may similarly be important for terrestrial organisms such as the blue heron (*Ardea herodias*), whose diet is comprised largely of aquatic organisms. In the forested areas surrounding the stream and pond, animals may be exposed to mercury by ingesting contaminated biota or through the incidental ingestion of soil or sediment.

In an assessment of the potential impacts to ecological receptors at Site 8, it was concluded that little or no risk is expected at NOS due to direct contact with surface water (Jordan, 1987). Exposure to contaminants in soils through direct contact is also limited because animal fur will impede absorption of mercury across the skin. In addition, the limited areal extent of mercury contamination in soil, as well as the relatively low concentrations detected, would suggest that potential risk to terrestrial wildlife is low (Jordan, 1987). Direct contact with sediment, however, may pose a hazard to aquatic organisms. In addition, risks to organisms that feed on aquatic organisms (e.g., fish, and some birds and mammals) are potentially high because of the tendency of mercury to accumulate in biological tissue.

2.0 BIOMONITORING OBJECTIVES/APPROACH

The goals of this biomonitoring program include the following objectives:

- determination of the *in situ* toxicity of mercury
- determination of the extent of mercury contamination in the aquatic food chain
- evaluation of the effects of remedial actions in the upper stream, and
- evaluation of the effectiveness of the remedial response.

These goals focus both on the evaluation of current impacts of mercury contamination in these habitats as well as the monitoring and assessment of the long-term ecological responses to remediation of stream sediment. The recommended approach described in this work plan consists of activities including a preliminary survey, the establishment of baseline conditions, selection of appropriate methods for long-term monitoring, and the actual monitoring of representative components of the ecosystem to evaluate remedial effectiveness. These activities are discussed in detail in Section 4.

The proposed suite of studies focuses on different aspects of the potentially impacted ecosystem and works to build an overall picture of changes that may occur as a result of the planned remediation. Ecosystem recovery, not just a reduction in tissue concentrations in a few species, is the ultimate goal of site remediation. Biomonitoring to assess the natural recovery of the ecosystem after the source of contamination has been eliminated and the most contaminated areas have been cleaned up can best be addressed by examining changes at several levels of organization throughout the range of effected habitats.

Table 1 outlines the matrix of biological studies planned for the preliminary survey and describes the ecosystem element, habitat areas involved, and some of the advantages and disadvantages of each study. As noted later in Section 2.3, not all studies initiated during the preliminary survey will necessarily be carried forward to the post remediation biomonitoring. However, a suite of different activities, which address individual,

TABLE 1. MATRIX OF BIOMONITORING ACTIVITIES

ACTIVITY	ECOSYSTEM LEVEL	HABITAT TYPE	ADVANTAGES (a)/DISADVANTAGES (d)
Sediment Bioassay	individual	marsh	a: sensitive measure of potential impacts; direct assessment of toxicity; provides information on a range of organisms/trophic levels. d: may be difficult to extrapolate findings to *in situ* conditions.
Clam Bioaccumulation Study	individual	pond	a: provides a measure of food-web bioavailability/trophic transfer. d: may be influenced by environmental fluctuations unrelated to toxicological impacts (due to duration).
Clam Toxicity Study	individual	pond	a: direct measure of toxicity; integrates both dietary and direct exposures; provides information on several sensitive parameters. d: may be influenced by environmental fluctuations unrelated to toxicological impacts (due to duration).
Rapid Bioassessment Protocol	community	stream	a: quick screening of community-level impacts. d: not particularly sensitive; may not be applicable for particular habitat.
Invertebrate Community Study	community/ population	marsh/ pond	a: provides varied information on community-level response. d: potentially not a sensitive measure of stress; labor intensive.
Artificial Substrate Study	community	stream	a: sampling variability reduced; various aquatic subhabitats can be evaluated depending upon substrate type. d: may be influenced by environmental fluctuations unrelated to toxicological impacts (due to duration).
Fish Tissue Residue Analysis	individual	pond	a: provides a measure of food-web bioavailability/trophic transfer; integrates both dietary and direct exposure pathways. d: may be unresponsive to gradual amelioration of conditions.

population, and community level responses in the different habitats are necessary to provide a reliable and comprehensive picture of ecological response to remediation. The tiered nature of the approach, which eliminates inappropriate studies based on the results of the preliminary survey, will ensure that the biomonitoring effort is cost-effective.

2.1 Preliminary Survey

A preliminary survey will be conducted to identify appropriate test species and reference sites. As a transitional habitat between the unnamed freshwater stream and the oligohaline (i.e., salinity between 0.5 and 5 ppt) Mattawoman Creek (Herricks and Sale, 1981), the tidal pond at Site 8 represents a complex environment where several biologically important physical parameters vary on both a daily and seasonal basis. The first activity of the biomonitoring program will consist of the monitoring of the following water quality parameters: salinity, conductivity, temperature, and dissolved oxygen. In addition, benthic macroinvertebrates will be sampled from the cattail marsh and tidal pond areas, and fish populations will be surveyed. This information will be used to select suitable test species which are able to survive under these particular conditions and occur in high enough densities to allow facile sampling on a regular basis. In addition, analytical fish tissue residue data and macroinvertebrate survey data will be collected to estimate statistical variability for sampling design considerations. Finally, the water quality information and biological characterization of the habitat will be used to select one or more reference locations. These reference locations will be chosen so as to minimize all biologically important differences with Site 8 as determined by preliminary survey activities.

2.2 Establishment of Baseline Conditions

Various activities will be undertaken prior to stream remediation. These include activities focused on determining the direct toxicity of mercury exposure to environmental receptors, on evaluating the potential exposure through the contamination of prey items in the aquatic food web, and the determination of natural variability in the ecosystem. Biomonitoring activities, including sediment bioassays and in situ toxicity tests, will be used to evaluate the toxicological impacts of direct exposure to contaminated media at the site. Bioaccumulation studies and residue analyses of various biological organisms within the aquatic ecosystem at the site will quantify the magnitude of exposure to environmental receptors, and the extent of mercury contamination in the aquatic food chain. For instance, the filter feeding clam, *Rangia cuneata*, is an ideal organism for monitoring the migration of mercury-bound sediment particles in the water column. Cattails may uptake and translocate mercury (Jerkins, 1980), and are consumed by such species as muskrats (*Ondantra zibethicus*). Finally, quantitative analyses of the benthic macroinvertebrate and planktonic community abundance/diversity will provide a

broader perspective of community-level impacts. In concert, the results of these activities will provide an understanding of the impact of mercury contamination in the study area.

2.3 Selection of Methods for Long-Term Biomonitoring

A number of preliminary studies will be conducted concurrently during pre-remediation investigations to select those methods that are most appropriate for routine post-remediation evaluation at the site. Due to the short period of time between project commencement and scheduled remediation activities, it is not feasible to conduct the tests planned during the preliminary survey sequentially.

Based on the performance of the preliminary studies, a subset of methods that will provide an adequate measure of performance will be selected for continuation during the post-remediation studies. The preliminary method screening phase was planned in order to ensure that adequate data will be available to select the subset of specific methods that will be used to effectively evaluate the long-term performance of the planned remediation at this site (ABB-ES, 1991). The rationale and/or specific criteria for eliminating each of the planned preliminary survey methods from the suite of post remediation methods are presented in Table 2. This initial screening and selection of methods provides a balanced approach that addresses both the initial uncertainty about method suitability and the requirement that only a cost-effective subset of studies which provide heuristic data for evaluating remediation performance be continued for long-term evaluation.

2.4 Long-Term Biomonitoring

Based on the results of the pre-remedial phase of this biomonitoring program, a number of the activities described above will be continued for five years following stream remediation. Biological response data collected throughout this period will be statistically compared with results from the baseline period to test the hypothesis that conditions have improved over the intervening years. During this phase of the biomonitoring program, the possible inclusion of other components of the ecosystem (such as terrestrial receptors) will also be evaluated. This would be an important consideration should significant food chain contamination be found during the early phases of this program.

The third objective of the task order is to evaluate the effects of remedial action on the upper section of the stream where remedial activities are planned. As described in the Remedial Action Specification Document (ABB-ES, 1991), the Interim Removal Action for Site 8 will include the diversion of stream waters around an 800-foot section of the upper stream (down to Station 16+00 in Figure 1) and removal of contaminated soil and sediment. To limit the environmental impact of these activities, the stream diversion system will be designed to maintain baseflow to the wetlands downgradient of the section

TABLE 2. DECISION CRITERIA FOR EXCLUDING STUDIES FROM THE LONG-TERM BIOMONITORING PROGRAM

ACTIVITY	EXCLUSION CRITERIA
Sediment Bioassay	• No demonstrable difference in bioassay results between experimental and reference or control sediment. • No correlation between sediment mercury concentrations and biological response variables.
Clam Bioaccumulation Study	• No suitable test organism available for the particular environmental conditions in the Tidal Pond. • Environmental exposure is found to be acutely toxic to the test organisms during the Preliminary Survey.
Clam Toxicity Study	• No suitable test organism available for the particular environmental conditions in the Tidal Pond.
Rapid Bioassessment Protocol	• No demonstrable differences are found in the RBP metrics between sampling locations in the stream below Site 8 and the upstream reference location.
Invertebrate Community Study	• No demonstrable differences in macroinvertebrate community structure detected between samples collected at the site and the reference location. • No correlation between sediment mercury concentrations and macroinvertebrate community structure detected.
Artificial Substrate Study	• No demonstrable differences are found in settling community composition between sampling locations in the stream below Site 8 and the upstream reference location.
Fish Tissue Residue Analysis[1]	• No resident fish species are found during the Preliminary Survey. • Local populations are too sparse to provide sufficient material for analysis.

Notes: [1]Even if these conditions apply, the possibility of testing non-resident organisms using *in situ* cages or tanks will be considered if the clam bioaccumulation studies indicate that aquatic organisms can rapidly accumulate mercury in the Tidal Pond.

of stream to be remediated. In addition, the diversion system will be designed to prevent any erosion of the stream bed at the discharge point, and the existing drainage system will be restored to its original condition following the completion of the remedial actions (ABB-ES, 1991). Consequently, it is not expected that these removal activities will impact downgradient habitat, although existing aquatic and benthic habitats in the affected section of upper stream will be completely destroyed.

To evaluate the effects of remedial actions on the affected section of stream, a complete characterization of the physical and biological components of this habitat prior to remedial action will be conducted. Following the completion of remedial activities, the reestablishment of the macroinvertebrate community will be monitored on a biannual basis. It is expected that recolonization of this habitat will be fairly rapid because the affected stream section is relatively small, and unaffected habitat exists both up- and down-stream to supply organisms for recolonization. At least initially, recolonization rates of macroinvertebrates should be rapid due to the lack of competitive pressures. In addition to these community studies, caged clams will be placed in the lower stream to monitor and assess the effects of potential resuspension of sediment during and after remediation.

To meet the requirements of the final objective of this biomonitoring program (i.e., evaluation of the effectiveness of remediation) it is necessary to establish an adequate representation of baseline conditions at the site. These baseline data are necessary to allow for statistically valid comparisons with post-remedial response. Baseline data which are considered crucial to meeting the goals of this biomonitoring program include the following:

- Estimates of the seasonal variability of the benthic macroinvertebrate and planktonic communities in the various habitats at the site.

- Estimates of the variability in sediment toxicity among the various habitats at the site.

- Estimates of the variability in tissue residue concentrations in organisms representative of different ecological trophic levels.

It is essential that this underlying biological variability be quantified before the post-remediation biomonitoring program is implemented because baseline response data are needed to provide a yardstick against which ecosystem improvement can be statistically measured. Without an understanding of natural spatial and temporal variability in the system, it would be impossible to substantiate conclusions regarding the effectiveness of any potential ecosystem response following remedial activities.

3.0 STATISTICAL CONSIDERATIONS AND EXPERIMENTAL DESIGN

To achieve the objectives of this biomonitoring program, experiments must be designed in such a manner that potential differences in test results can be identified. The main categories of statistical considerations which must be considered in order to meet the stated goals include the location of sample collection sites, experimental procedure specifications, and analytical techniques (USEPA, 1983).

The selection of sampling locations is dependent upon the particular type of biomonitoring activity to be performed, as well as the uniformity of the habitat. At NOS, sampling activities will be stratified (i.e., stream, cattail marsh, and tidal pond) due to the tremendous differences among habitats and in the distribution and species of mercury distributed among these habitats. In addition, certain biomonitoring activities are only suitable for specific habitats. For example, fish are only likely to occur in the tidal pond and sampling of these organisms for mercury tissue residue analysis will be restricted to this area of the site. Within a particular habitat, sampling locations on a gridded map will be established randomly by choosing coordinates from a table of random numbers.

Sample sizes will be calculated based on estimates of sample variances (as determined during the preliminary survey) and for a specified degree of precision (Sokal and Rohlf, 1969; Elliot, 1971). It is recommended that the degree of precision used be decided after estimates of sample variability have been obtained so that an optimum balance between statistical discriminatory power and time/effort be obtained. The suitability of particular biomonitoring activities will also be assessed at this point based on these criteria.

4.0 BIOMONITORING ACTIVITIES

The following describes the various activities which are tentatively recommended to meet the objectives of this biomonitoring program. As discussed previously, the actual test species will depend upon the evaluation of habitat suitability and measurement of physico-chemical parameters.

4.1 Preliminary Survey

A preliminary survey will be conducted to collect the necessary biological and physical information necessary to finalize the sampling design of the various biomonitoring activities described in this section. This information is necessary in order to ensure that statistical considerations relating to sampling design and ultimate hypothesis testing of the results are adequately addressed. In addition, preliminary results will provide the necessary information to select test species which are both numerous enough to be readily collected as well as being appropriate for the particular environmental conditions

at NOS. Finally, several potential biomonitoring activities will be evaluated for suitability during this phase of the biomonitoring program. Those activities that prove most suitable will be carried forward to the next phase. The preliminary survey will consist of the following components:

1) **Measure Biologically Important Physicochemical Parameters.** Various water quality parameters, including salinity, conductivity, temperature, and dissolved oxygen, will be monitored in the stream and tidal pond. In the tidal pond, data will be collected at three approximately equidistant locations in midchannel every three hours for two complete diurnal cycles. Because conditions vary less dramatically in the stream, measurements in this habitat will limited to every six hours. Sample locations along the stream will include one above Building 766, one directly below the storm drain outlet, and one near the beginning of the cattail marsh.

   The sediment substrate will be characterized across the site in terms of percent organic matter, sand or other substrate type.

2) **Biological Survey.** Macroinvertebrates will be sampled from the stream, cattail marsh, and tidal pond using an Ekman grab sampler and dipnets. In the stream, sample locations will include one above Building 766, one directly below the storm drain outlet, and one near the beginning of the cattail marsh. The cattail marsh and tidal pond will be gridded and a randomly chosen sample will be collected from each sample grid. The number of grids will depend upon habitat configuration and sample variability, but will number between 15 and 20. Individuals will be classified typologically in the field and data concerning the relative abundance of species (types) determined. Representative individuals will be sent to a laboratory for taxonomic identification.

   In addition, fish will be sampled in the tidal pond using a combination of minnow traps and seine nets. Individuals will be identified to species and information on the relative abundance and ease of capture noted.

3) **Field Test Method Suitability.** Certain proposed biomonitoring activities described in this section may either not be feasible or will not provide cost-effective information regarding the objectives of the biomonitoring program. These activities include the *in situ* bioaccumulation studies and the use of artificial substrates to evaluate settling community attributes. Consequently, it is proposed that these activities be attempted during the preliminary survey phase to determine field feasibility. Based on results at this time, these activities may be eliminated or modified prior to full implementation.

Although the major focus of the study is to evaluate the temporal change in biological response variables as environmental conditions improve following remediation, adequate reference sites are a necessary component of the planned activities. For instance, data collected from the impacted section of the stream will be compared with upstream locations both to evaluate method suitability and to monitor remedial effectiveness. A reference area, similar in physico-chemical parameters with the tidal pond, will be used to conduct a "transplant control" experiment for possible clam toxicity studies.

The selection of reference sites, proposed as an element of the preliminary study, is problematic however. Three different types of habitats, stream, marsh, and tidal pond, are being investigated and the entire nearby area is known to have other anthropogenic impacts. As noted previously, the U.S. Fish and Wildlife Service has identified potential problems with their initial reference site.

Prior to initiation of the field component of the preliminary study, ABB-ES will examine local maps and literature on local conditions to identify candidate reference sites. State agency staff familiar with the area will also be contacted for information. The State of Maryland has conducted a comprehensive study of coastal plain streams in the state as part of a major investigation concerning the impacts of acid deposition. The physico-chemical data from this study and numerous other studies along this section of the Potomac River Basin will be examined to aid in prescreening potential reference sites.

4.2 Sediment Bioassay

A sediment bioassay will be conducted on sediments collected from the cattail marsh area and the tidal pond. The objective of the sediment bioassay is to assess the toxicity of aquatic sediments to fish and invertebrates. The general steps involved in the sediment bioassay proposed for NOS are described below.

1) **Identify Sampling Stations.** As mentioned above, sediment samples will be collected from the cattail marsh area and the tidal pond. The specific location of sampling stations will be determined during the preliminary survey (see Section 2.1). Sample sites will be selected to encompass the range of aquatic habitats available in the marsh and pond. ABB-ES proposes that six permanent sample stations be used in the sediment bioassay, distributed as follows: cattail marsh - three stations; and tidal pond - three stations. Each station will be staked and its location indicated on a base map.

2) **Collect Sediment Samples.** At each location, five replicate samples will be collected for sediment bioassay and mercury analysis. Replicate samples will be collected randomly from a circular area (2 meter radius) around each sample location stake. Sediments will be collected using an Ekman or Ponar grab

sampler, and placed in a sieve bucket for removal of large macrophytes, rocks, and other debris, as well as for homogenization and draining of the sample. The exact quantity of sample required will be determined based on discussion with the laboratory conducting the analysis; we anticipate that 2 ℓ will be required for the bioassay and 1 ℓ or less required for mercury analysis. Control sediment will consist of potting sand (five replicates). Sediment samples will be stored and shipped on ice (2°C - 4°C).

ABB-ES proposes that one sediment bioassay be conducted prior to remediation, and once each year following remediation for five years. Annual sampling is appropriate because sediment toxicity is less likely to fluctuate seasonally than other biological processes.

3) **Conduct Bioassay Analysis.** The sediment samples will be shipped to a qualified laboratory for the bioassay test. The procedure used will be based on accepted protocols (e.g., LeBlanc and Surprenant, 1985; Dawson et al., 1988). Because the objective of the bioassay is to assess the toxicity of sediments, but not identify a threshold toxicity concentration, sediment dilutions will not be necessary.

   The species used in the sediment bioassay will be determined based on species identified and salinity measurements made during the preliminary survey. The organisms used will be sensitive species (not necessarily resident fauna) as recommended in various guidance documents (USEPA, 1978; USEPA, 1988; Green et al., 1988), and will include a fish, a benthic invertebrate, and a free-swimming or floating invertebrate. The duration of the tests will vary depending on the particular species used. Biological endpoints that will be analyzed will include larval fish growth, water flea reproduction, and mortality in all species (Suter et al., 1987). In addition to the bioassay procedure, mercury analysis will be performed on each replicate sample.

4) **Data Summary and Interpretation.** Bioassay results will be presented as percent survivorship, weight gain, and increase in water flea abundance for each station. The results will be tested for significance using a one-way ANOVA followed by Dunnett's Test (Steel and Torrie, 1980) against both results from control samples as well as among years. The results will be discussed and interpreted in conjunction with the analytical results, and any correlations between mercury concentration and response data quantified (Clark et al., 1987).

4.3 *In Situ* Toxicity and Bioaccumulation Monitoring

The objective of this biomonitoring task is to assess, through *in situ* testing, the toxicity and uptake of mercury from water and via contact with mercury-bound suspended particles at the site by aquatic organisms. The general approach to this task is to place

caged organisms in aquatic habitats at the site and measure growth rates, mortality and tissue concentrations over time. Because of uncertainties concerning habitat suitability for test species, the efficacy of *in situ* monitoring at the NOS will be determined based on the preliminary survey. An overview of procedures that may be appropriate for the site is presented below.

The clam, *Rangia cuneata*, is a brackish water organism that has been used in *in situ* toxicity testing (Versar, 1989) and may be suitable for bioaccumulation monitoring at the site. This species may also be an appropriate one for monitoring any increased mercury loadings due to remediation in the lower stream and tidal pond because it is a suspension feeder and serves as a good indicator of the toxic effects associated with exposure to resuspended sediment during remediation.

The general procedure for *in situ* toxicity testing is as follows. *Rangia* individuals would be collected from an unimpacted area elsewhere in Chesapeake Bay, marked, and measured. Small individuals (15 mm-25 mm) of similar size would be placed in trays (e.g., 30 cm x 25 cm x 10 cm) containing tidal pond sediments coarse-sieved to remove other clams and larger fauna. Clams would be placed in the trays at numbers similar to natural densities (approximately 30 per tray). The trays would be covered with cages (e.g., 6-mm mesh hardware cloth) to exclude predators, and placed in the bottom so that the top of the tray was flush with the sediment surface. Tentatively, 10 trays would be placed at each of four locations in the tidal pond, and two trays retrieved from each location at approximately four-week intervals. Ten additional trays would be placed in a suitable reference location to provide data on whether the stress involved in transplanting organisms was a significant factor. All marked *Rangia* individuals would be removed from the trays and preserved in 70 percent ethanol for subsequent mortality and growth determinations in the laboratory.

The general procedure for the bioaccumulation monitoring is similar to the procedure for evaluating *in situ* toxicity. *Rangia* individuals would be collected from an unimpacted area elsewhere in Chesapeake Bay. Individuals would be placed in trays (e.g., 30 cm x 25 cm x 10 cm) containing tidal pond sediments coarse sieved to remove other clams and larger fauna. Clams would be placed in the trays at numbers similar to natural densities (approximately 30 per tray). The trays would be covered with cages (e.g., 6 mm mesh hardware cloth) to exclude predators, and placed in the bottom so that the top of the tray was flush with the sediment surface. Tentatively, two trays would be placed at each of four locations in the tidal pond, and sufficient organisms for analysis (2 gm-5 gm of tissue) removed every two weeks for two months (during remediation more frequent sampling would probably be appropriate). The organisms collected would be stored on ice (2°C - 4°C) and shipped to a laboratory for mercury analysis.

A freshwater species that may be useful for this type of monitoring activity in the stream is the fingernail clam (*Sphaerium striatinum*). This species has been used for *in situ*

testing of the bioavailability of PCBs as a result of river dredging in Michigan (Rice and White, 1987). The procedure used for this species would be similar to that described above for *Rangia*, except that only two locations in the lower stream are proposed.

The feasibility of conducting *in situ* toxicity and bioaccumulation monitoring will be determined based on the results of the preliminary survey as well as additional literature research. A primary consideration is the viability of *Rangia* in the slightly brackish water of the tidal pond. Additionally, freshwater species endemic to the Chesapeake region may be more suitable than *Sphaerium* for testing in the stream.

4.4 Quantitative Sampling of Benthic Macroinvertebrate/Pelagic Communities

This activity will consist of several different sampling and analytical procedures. Sampling activities will be focused on the benthic communities because macroinvertebrates are good indicators of localized conditions, they are fairly easy to collect in large numbers, and are an important component of aquatic food webs. A combination of qualitative and quantitative techniques will be used to evaluate and monitor biota occurring in sediment. Sediment samples in the cattail marsh area will be quantitatively sampled. Finally in the stream habitat, the pelagic community will be sampled using artificial substrates.

4.4.1 Rapid Bioassessment. The USEPA Rapid Bioassessment Protocol (RBP) (Plafkin et al., 1989) will be used to evaluate the biological condition of the stream flowing from Building 766 into the tidal pond. The specific procedure used will be RBP I, which involves the following general steps:

1) **Identify Sampling Stations.** Permanent sampling stations will be established along the stream between Building 766 and the tidal pond, including stream reaches within and outside of planned remediation areas. The specific location of sampling stations will be determined during the preliminary survey (see Section 3.1). ABB-ES proposes that sampling stations be distributed as follows: two reference stations upstream of Building 766; two stations in the 800-foot long planned remediation area; and two stations below the planned remediation area. Each station will be staked and its location indicated on a base map.

2) **Conduct Rapid Bioassessment.** Procedure RBP I is a screening assessment of biological condition involving systematic documentation of specific visual observations. The procedure is used to discriminate impacted areas from non-impacted areas. RBP involves the evaluation of physical characteristics (e.g., water quality parameters, surrounding land use), a habitat assessment (e.g., substrate characteristics, percent cover), and biological survey at each sampling station. The biological survey component focuses on qualitative sampling of benthic macroinvertebrates (using kick and dip nets), supplemented by field

examination of other aquatic biota (periphyton, macrophytes, slimes, and fish). Invertebrate families/orders and their relative abundance are recorded on a field data sheet.

Based on the observations of physical characteristics, habitat, and biological composition, an assessment of impairment is made. If impairment is detected, the probable cause is recorded on the Impairment Assessment Sheet provided in the protocol. Additionally, the aquatic biota that indicated impairment and other information are recorded.

The RBP will be conducted three times prior to remediation, and twice yearly for five years following remediation. This should provide a reasonable assessment of baseline conditions. The biannual sampling events following remediation will be conducted in spring and summer to provide a measurement of seasonal variability.

3) **Data Summary and Interpretation.** The data from the RBP will be summarized and interpreted in the context of identifying trends in biological impairment both between sampling stations during each sampling event, as well as over time (Plafkin et al., 1989; NYSDEC, 1991). Additionally, the results will be compared with an ecoregional database if available.

4.4.2 Quantitative Macroinvertebrate Sampling. Benthic macroinvertebrates in the cattail marsh and tidal pond will be quantitatively sampled and the results compared with both a reference location and between years. The results of preliminary survey macroinvertebrate sampling activities will be used to locate sampling locations where the benthic community has been most impacted. Further macroinvertebrate sampling will be limited to these locations in order to maximize the detection of long-term improvement and to limit among sample variability (Jackson and Resh, 1989). By reducing sample variability, fewer samples will be required during the semi-annual sampling events. The number of sampling locations will be calculated based on the variability found in the subset of most contaminated samples.

An Ekman dredge will be used to collect a sediment sample at each sampling location and the material sieved to isolate benthic fauna. Collected biological material will be preserved in 70% ethanol and sent to a laboratory for identification. At the same time, sediment samples will be collected and sent to a qualified analytical laboratory for mercury analysis. Data analysis and presentation of the data derived from identified organisms of abundance tables, species richness (= number of taxa), a diversity index (Shannon-Weiner), and an evenness index (Heip) (Boesch, 1977; Cairns and Dickson, 1971; Kaesler and Herricks, 1977; Schaeffer et al., 1985; Godfrey, 1978). In addition, hierarchical classification (e.g., SYSTAT's CLUSTER routine) will be performed and differences between samples and the reference location or between years compared for significance (Nemec and Brinkhurst, 1988; Green, 1980). Finally, the degree of

correlation between macroinvertebrate community data and mercury concentration data will be evaluated.

4.4.3 Artificial Substrate Sampling. In the stream, algae will be sampled using a series of artificial substrates (the particular types of substrate will be determined during the preliminary survey). Samples collected using artificial substrates are often characterized by having greater precision as compared to grab samples. This greater degree of discriminatory power will be useful in the statistical comparison of samples among years following remediation (Dean, 1981; Flannagan and Rosenberg, 1982; Lamberti and Resh, 1985). Percent cover of the substrate will be measured in the field and then the biological material will be scraped and sent to a laboratory for species identification. Data on the diversity and abundance of the settling community components will be compared with a reference location located on the stream above Building 766 and between years.

4.5 Mercury Residue Analyses in Food Web Components

This activity will consist of sample collection and mercury residue analysis in the tissues of various plant, benthic macroinvertebrate, amphibians, and fish species at the study area. These data will provide information necessary to evaluate whether mercury has entered the aquatic food chain in the study area, and whether detected concentrations are sufficiently high as to adversely impact specific receptors. Residue analyses will consist of the following steps.

1) **Identify Sampling Stations.** The residue analysis program will include sampling from the stream, the cattail marsh area, and the tidal pond. The specific location of sampling stations will be determined during the preliminary survey (see Section 3.1). Sample sites will be selected to encompass the range of aquatic habitats available in the stream, marsh, and pond. Each station will be staked and its location indicated on a base map.

   ABB-ES tentatively proposes that 26 samples be collected for mercury residue analysis, distributed as follows: stream - three invertebrate, three amphibian; cattail marsh - three plant, three invertebrate, three amphibian; tidal pond - two invertebrate, three fish, three amphibian.

2) **Collect Tissue Samples.** Biological tissue samples will be collected using a variety of techniques. Large macroinvertebrates (e.g., dragonfly nymphs, megalopteran larvae, snails), cattail tubers, and fish will be collected in the vicinity of each appropriate sample station as to provide sufficient tissue for mercury residue analysis. Macroinvertebrates will be collected using a combination of dipnet and handpicking techniques. The invertebrate samples will be composited because of the need to obtain the recommended sample quantity of 2 gms-5 gms (USEPA,

1981). Cattail tubers will be obtained using a shovel, and leafy and root tissue removed. Amphibians will be collected using a dip net or baited hook, possibly at night. Fish will be caught using a combination of minnow traps, seine nets, and electrofishing. Samples will also be collected from similar habitat at a reference location to be determined during the preliminary survey. All samples will be rinsed clean, wrapped in aluminum foil and stored and shipped on ice (2°C - 4°C).

3) **Conduct Tissue Residue Analysis.** The tissue samples will be shipped to a qualified laboratory for mercury residue analysis. The analytical procedures used will be based on established protocol (e.g., USEPA, 1981).

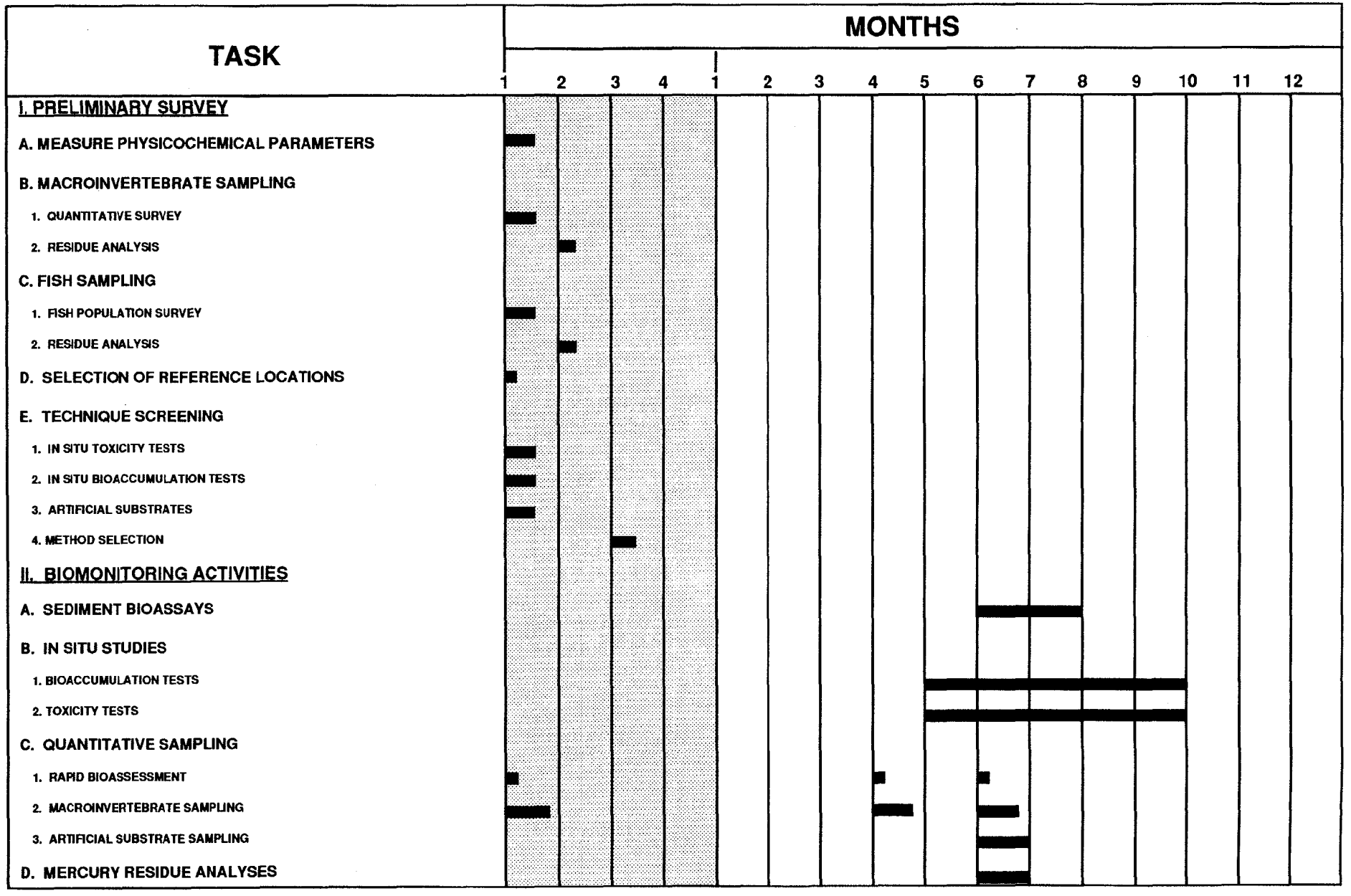
4) **Data Summary and Interpretation.** The analytical results from the samples collected at the site will be compared with similar results from the reference area. In addition results collected during the post-remediation phase of the study will be compared with results from previous years.

If preliminary studies indicate that obtaining adequate resident fish samples is a problem, the use of *in situ* test chambers (Wilde and Parrot, 1984) or on-site tanks will be evaluated. *In situ* test studies have been effectively used to study selected species in the Chesapeake Bay area for some time (Hall, 1988).

Should mercury be consistently detected in biological tissue throughout the study area, the possibility that fish-eating birds are being significantly exposed will be evaluated. The great blue heron (*Ardea herodias*) has been regularly seen feeding in the study area, and may be exposed to mercury if its prey items (i.e., fish and benthic macroinvertebrates) are contaminated. A non-destructive sampling technique that could be implemented is the neutron activation analysis of mercury deposited in feathers (Fimreite, 1979). These data correlate well with previous exposure to mercury, with the highest levels associated with predatory birds (Berg et al., 1966). In addition, small mammals that ingest aquatic invertebrates or wetland plants (e.g., muskrats) may also be exposed to mercury via ingestion. These organisms could be collected and submitted to the analytical laboratory for whole-body analysis (Hoffman et al., 1990; Kendall et al., 1990) should food-web contamination become evident.

5.0 SCHEDULE

The proposed scheduling of biomonitoring activities is presented in Figure 2. Due to uncertainties that will not be resolved until the completion of the preliminary survey, the sequence of activities should be considered tentative at this time. Additionally, certain activities may be excluded from the program based on the screening results from the preliminary survey.



NOTE: ACTIVITIES IN THE SHADED AREA WILL BE CONDUCTED ONLY ONCE.

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ASEA BROWN BOVERI

FIGURE 2
PROPOSED SCHEDULE OF BIOMONITORING ACTIVITIES
NAVAL ORDNANCE STATION
INDIAN HEAD, MARYLAND

Following the preliminary survey and pre-remedial activities, a detailed sampling and analysis plan (SAP) will be written. The SAP will present the biomonitoring activities selected and will provide further details concerning sampling locations, required sample sizes, reference locations, and selected indicator organisms. In addition, analytical protocols for the various tissue analyses and other laboratory analyses, and quality control procedures will be provided. Finally, the SAP will present a schedule for the reports summarizing the interim and final conclusions of this biomonitoring program.

GLOSSARY OF ACRONYMS

ABB-ES	ABB Environmental Services, Inc.
CHESDIV	Chesapeake Division of Naval Facilities Engineering Command
DOE	Department of Energy
NOS	Naval Ordnance Station
RBP	Rapid Bioassessment Protocol
SAP	Sampling and Analysis Plan
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service

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