UNITED STATES DISTRICT COURT

DISTRICT OF MAINE

MAINE PEOPLE'S ALLIANCE and NATURAL RESOURCES DEFENSE COUNCIL, INC., Plaintiffs v. HOLTRACHEM MANUFACTURING COMPANY, LLC and MALLINCKRODT INC., Defendants	Civil No. 00-69-B-C

Gene Carter, Senior District Judge

ORDER OF NOTICE

The [Revised] Study Plan for Evaluation of the Mercury Contamination of the Penobscot River/Estuary, Maine (hereinafter "Revised Study Plan") has been submitted to the Court. *See* attached Revised Study Plan and Responses to Prioritized list of Mallinckrodt's Issues Discussed at the Proposal Review Meeting in NYC on May 18, 2005. The Court has reviewed the same and herewith provides it to counsel for the parties for their consideration and comment. Any comments on, or objections to, the provisions of the Revised Study Plan shall be submitted to the Court, in writing, on or before August 3, 2005. The Court will consider all such comments and objections in due

course and, thereafter, will take appropriate action to promulgate the final Study Plan.

So **ORDERED**.

/s/ Gene Carter
Gene Carter
Senior United States District Judge

Dated at Portland, Maine this 22nd day of July, 2005.

A Study Plan for Evaluation of the Mercury Contamination of the Penobscot River/Estuary, Maine

The Penobscot River Study Panel

July, 2005

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I. INTRODUCTION

The HoltraChem Manufacturing Facility, located on the Penobscot River, at Orrington, Maine was a chlor-alkali manufacturing plant that was operational between 1967 and 2000. During this time it produced most of the chlorine used by the pulp and paper industry in Maine, and an unknown amount of mercury used in the production of chlorine was released into the environment (Appendix A). The current status of the HoltraChem Manufacturing Facility is that the mercury cells have been removed and an extensive site remediation is ongoing. Mallinckrodt Inc., the original owner and operator of the plant, has hired Camp, Dresser McKee (CDM) to carry out the remediation of the plant site and the southern cove located adjacent to the plant site. This site remediation, which is being required by the Maine Department of Environmental Protection (DEP), involves stabilization of land fills, clean-up of the industrial site, interception of groundwater inputs to the river, and removal of some mercury contaminated river deposits near the plant site. However, the Maine DEP clean-up requirements did not extend to the river and estuary south of the plant site. In April 2000, the Natural Resources Defense Council (NRDC) and Maine People's Alliance brought a suit in Federal District Court against Mallinckrodt Inc., which was designed to force the company to address the downstream mercury contamination.

The Court's Memorandum of Decision and Order dated July 29, 2002 found that "methylmercury downriver of the plant … may present an imminent and substantial endangerment to public health and the environment." The Court's Order required the parties to undertake "genuine, good-faith efforts to agree on a specific plan for an independent study to determine: (1) the extent of the existing harm to the Penobscot River and Bay south of the plant site, (2) the need for a remediation plan, if any, and (3) the elements of, and schedule for, completion of such a remediation plan." The Study Panel has reviewed the suggested study plans submitted by both parties in response to this Order and the responses by each party to the other party's proposed study plan. In addition, the Study Panel has reviewed many technical documents, including those that served as exhibits during the trial and other documents from the scientific literature and relevant state agencies. Based on this review, the Study Panel proposes the following Study Plan.

Our plan addresses items 1 and 2 cited above. That is, the Study Plan is designed to determine the extent of mercury contamination downriver from the HoltraChem plant and whether such contamination endangers human health or the environment. The Study Plan will not address remediation (item 3, above) until it is determined that it is needed. However, the plan is designed to understand the present level of mercury contamination of biota in the river, and to understand the processes that control the bioaccumulation and production of methyl mercury (MeHg). Such information is essential to development of a remediation plan should one be needed.

Some of the possible different hypothetical outcomes of the study are:

- Mercury concentrations in the river system do not pose discernable human health or environmental risks;

- Mercury concentrations in the river system pose unacceptable human health or environmental risks, that such risks are due to historic releases from the Site, and that:
  - "hotspots" of mercury, methyl mercury (MeHg), or mercury methylation have been identified that should be remediated to address the health or environmental effects,
    or
  - the diffuse nature of the contamination suggests that remediation is not feasible;
- Mercury concentrations in the river system pose unacceptable human health or environmental risks, and that such risks are due to ongoing releases from the Site. Opportunities for greater control of Site releases would then be investigated.
- Mercury concentrations in the river system pose unacceptable human health or environmental risks, but it appears that the distribution of mercury in the system is due to regional contamination or contamination from upstream *sources* rather than releases from the HoltraChem Site.

Other possible outcomes exist in addition to those listed above, and indeed some of these possible outcomes are not mutually exclusive (e.g., unacceptable risks may be due to *both* historic and ongoing releases of Hg from the Site). The purpose of identifying a set of hypotheses about mercury in the Penobscot River and Estuary is to identify opportunities for data collection and analysis that would distinguish between these and other possible alternative explanations for the observed patterns.

The following study plan is a document intended to give the reader an understanding of how the Study Panel intends to approach the study of the mercury contamination of the Penobscot River/Estuary. The objectives of the study, as set out by Judge Carter, will not change, but emphasis on the specific tasks outlined below will likely change as data are acquired and analyzed. Depending on prior results, certain tasks may be modified, expanded or terminated. New tasks may be added, if they become necessary.

II. STUDY OBJECTIVES

The overall objective is to determine if the mercury concentrations of biota and human food organisms in the Penobscot River/Estuary are of concern, and whether remediation within the river or additional remediation at the plant site is needed. The study will take a multi-disciplinary, whole-ecosystem approach. The geographic scope of the study will cover the Penobscot River/Estuary from above the Veazie Dam to the middle of the Penobscot Estuary (southern Islesboro Island). *See* Figure 1.

The objectives set out by Judge Carter will be addressed by attempting to answer a series of specific questions. The questions are:

1. What is the source of mercury? (Mass balance studies, cores, mercury isotope studies)
2. How much mercury was lost and how much is retained in different parts of the system? (Mass balance, spatially detailed studies of sediments and water, cores)

3. Are mercury concentrations high enough to be of concern? (Spatially detailed studies of sediment, water and suspended material)
4. Where and when is the contamination most severe? (Spatially detailed studies at one time, especially considering the Bay; temporally detailed studies at a few places; both studies will involve sediments, water and biomonitors)
5. Does methyl mercury get mobilized from the contamination; and if so where and when? (wetland studies, mass balance studies, mercury isotope studies)
6. Does that methyl mercury get into the food web, and which organisms offer the greatest threat for human consumption? (food web studies)
7. Is mercury an ecological threat? (effects studies with key upper trophic level species)

Biogeochemical Objectives

Three general objectives for the biogeochemical studies are:

1) to determine the relative roles of ongoing releases and historical releases from the HoltraChem Site, and other sources including upstream sources to mercury levels in the river and bay.
2) to better define concentrations and fluxes of total mercury (THg) and methyl mercury (MeHg) in the areas up and downstream of the plant site, and
3) to identify sites and factors that are important in controlling the production of MeHg.

This information will be necessary to define the extent and nature of mercury contamination and how it may be contributing currently to MeHg accumulation in biota. It will also be useful in the design of any remediation measures that may be needed.

Mercury Bioaccumulation Objectives

Three general objectives for the bioaccumulation studies are:

1) to determine if concentrations in biota in lower river and bay are elevated in comparison to areas upstream of the plant, and to reference system(s),
2) to determine if these biota concentrations are being influenced by ongoing discharges, and /or historical mercury deposits,
3) to determine if differences in bioavailability of mercury to the biota are important in determining the mercury concentrations in the food chain at downstream sites. (Note: “bioavailable mercury”, here and throughout this study plan, is defined as that mercury than can be taken into an organism from aqueous or particulate sources. It is distinct from mercury that is in a refractory form that is unavailable for biological uptake.)

The third objective is needed because there may be sites where bioaccumulation is higher than would be predicted if you assume a direct relationship between mercury in biota and mercury in water and sediments (i.e. the mercury is more bioavailable). These sites might be important candidates for remediation.

Toxic Effects and Human Health Risks Objectives

The two general objectives are:

1) to determine if mercury concentrations in selected aquatic biota are high enough to pose a risk to these biota, and

2) to determine if mercury concentrations in aquatic biota pose a risk to human and wildlife consumers of these biota.

III. OVERVIEW OF THE STUDY PLAN

The Study Panel was directed by Judge Carter to design a study to assess the mercury contamination of the Penobscot River that was based on the best possible science. However, Judge Carter also was clear that our proposal should be limited to proven established methodologies and approaches to address the problem, and at a reasonable cost. That is, we were not to propose to do highly innovative ground-breaking research on mercury cycling in the environment.

With these directives in mind, we designed a Study Plan, which covers three main areas: (1) the transport, storage and production of MeHg in the ecosystem, (2) the bioaccumulation of mercury by food chain organisms and the current concentrations of mercury in these biota, (3) an assessment of the possible risks of mercury contamination to food-chain organisms and to top wildlife and human consumers.

A. General Description of the Plan.

The proposed Study Plan has a duration of three years, plus some time for preliminary study to assist detailed design, and one year of data synthesis and write-up. This time period will likely be sufficient to capture a reasonably wide range of natural variability. This is important because production and bioaccumulation of MeHg are known to have significant inter-annual variations, which may be caused by environmental factors such as ambient temperature, rainfall, and incident solar radiation. These variations may be particularly important for a river/estuarine ecosystem, because downstream river flow of freshwater affects mercury transport as well as estuarine salinities, which are known to affect the biogeochemical cycling of mercury.

Prior to the beginning of the 3 year field program, the Study Panel proposes to do preliminary work on the river and estuary. The purpose of this preliminary work is to test proposed investigative approaches, to locate wetlands and other features of particular interest for the study, to consult with naturalists who have local knowledge of the river and bay to determine which biota will be used for the study, and to do preliminary sampling surveys of the river and bay for the purpose of locating sampling sites that will be monitored on a regular basis for the duration of the study. The Study Panel is of the opinion that this is not only the best way scientifically to begin the study but is also the most cost effective approach in the long run.

The first section of the Study Plan is designed to supplement the rather sparse existing data with additional measurements of THg and MeHg concentrations in water and sediments. Existing data are insufficient in their geographic extent and do not allow for an understanding of mercury sources and removal processes. These supplemental measurements will provide more complete data on the distribution of THg and MeHg at various locations in the river. They will also provide information on the fluxes and mass balance of mercury in the system. That is, these measurements will determine the rate at which mercury flows past the plant from upstream sources, the rate at which mercury moves from the plant site into the river, and the distribution and mobility of mercury in different ecosystem “compartments.” An understanding of the relative contributions from

historical versus ongoing releases is necessary if any remediation is to be considered. Historical releases are discussed in Appendix A.

This first section of the Study Plan also addresses mercury methylation and methyl mercury hotspots. It is the Study Panel's view, based on experience with mercury in other aquatic systems, that methylation is more likely to occur in marshes and the soft sediments along the river than in the well-scoured river bottom. Because MeHg is generally more toxic than inorganic mercury and because it also contributes more to overall tissue body burden as it moves up the aquatic food chain, it is important to understand the quantities, sources and environmental factors controlling production of MeHg in the river and bay.

Finally, this first section addresses the mechanisms and locations whereby mercury is being removed from the river. While it is likely that much of the mercury is transported to the ocean, mercury that is deposited into sediments may also be removed from the system by burial below the active zone of methylation.

The second section of the Study Plan concerns the bioaccumulation of mercury in biota. Mercury (especially MeHg) is known to display unusually high biomagnification in aquatic food webs (more than any other metal), due to its high assimilation efficiency in animals from food and its unusually long retention in organisms once accumulated from food. Consequently, biomagnification (in which the contaminant's concentration in biological tissue increases with each trophic level) becomes particularly evident in ecosystems with long food chains and long-lived predators.

From first principles, it is necessary to evaluate the bioaccumulation of any contaminant, including mercury, in aquatic organisms in order to evaluate the potential impacts of that contaminant, either on populations or communities in the ecosystem, or on human consumers of food from that ecosystem. That is, organisms, including people, only respond to contaminants that are in them; contaminants not associated with organisms have no potential to cause toxic effects. Thus, in the context of understanding the impacts of mercury contamination in the Penobscot, it is critical to assess the extent of bioaccumulation of mercury in diverse components of the ecosystem. The Study Plan proposes focusing on (1) organisms at the base of the food web; (2) select benthic organisms that directly consume sediments for food; (3) organisms that are consumed by man (especially those at the top of food chain); (4) other upper level predators, birds and mammals; and (5) organisms that can be used as bioindicators of the spatial and temporal distributions of mercury in this ecosystem.

The third section of the Study Plan is to characterize the extent to which mercury (inorganic or MeHg) in the river poses risks to health of humans or other organisms. Our approach will be to where possible use exposure or concentration limits established by federal and state agencies as the basis for making such judgments. Ecological effects will be evaluated using physiological endpoints, laboratory toxicity tests, and comparison to a reference system (if a suitable one can be found). For human health, we will use the EPA's reference dose (RfD) for MeHg. This factor is based on the most recent human health studies, and is consistent with the recommendations of a National Academy of Sciences review of MeHg toxicity. In addition to comparing concentrations or exposures with these limits, an evaluation will be made for instances where guidance levels are exceeded, to determine whether the high concentrations appear to be a local problem

specifically connected to inorganic or MeHg in the Penobscot or a regional atmospheric deposition problem.

Appendix B provides a brief description of the proposed organization and management of the study. As described in the Appendix, the first step in implementing the plan is for the Study Panel to identify and recommend a Consultant to be appointed by Judge Carter.

B. Phasing.

Prior to the commencement of the project, the preliminary work described in the beginning of section III, A will be completed.

During the first year of the study, the whole ecosystem will be monitored in the river and the estuary to determine if concentrations of mercury in fish and wildlife are high enough to be of concern. In order to assess these mercury data properly, other necessary mercury and methyl mercury flux and ancillary data will also be collected as shown in Table I. During the first year, studies of one or more reference systems that have experienced no past or present discharges of industrial mercury will be completed (see section VI).

The wildlife data will be assessed by comparison to mercury concentrations available in the peer reviewed scientific literature, by comparison to mercury concentrations of the wildlife in the reference system(s), and by consultation with experts in the field of wildlife mercury toxicity. The risk to human consumers of fish and shellfish will be assessed by comparison of mercury concentrations in Penobscot fish and shellfish to the Maine DEP and EPA action levels for mercury.

If after the first year of study it is concluded that the concentrations of mercury in wildlife and/or in biota that could be consumed by humans are high enough to be of concern, the following studies will be phased in during the second year of the study, as shown in Table I: the biota toxicity studies (see section VII, with the exception of the phytoplankton toxicity studies, which are more cost effective to do in year 1), studies of stable isotopic ratios of mercury at the HoltraChem site and in the river/estuary (IV,C,2,b), the aerial deposition study(IV,B), mercury bioaccumulation in sediments (IV, D),C,N isotope ratios in food chain organisms (V, A), studies of human health risk assessment (VIII).

During the 4th year of the study when research activities are anticipated to be completed, data analyses will be completed and a final report of the study will be prepared by the Project Leader in consultation with the Study Panel.

IV. PROPOSED BIOGEOCHEMICAL STUDIES

To account for temporal changes in river flows, tides, water chemistry, and biological activities, field sampling will take place at a number of locations and at regularly scheduled intervals over a three-year period. It is anticipated that over this time period there will be a natural range of river flows and tides that will enable us to study the dynamics of mercury and MeHg in the ecosystem under a wide range of natural conditions. The number of sampling locations will be chosen to be appropriate to the heterogeneity of biological habitat and mercury distribution within the system.

A. Site Description

The study area will include the Penobscot River from above the Veazie Dam to southern Islesboro Island in mid Penobscot Bay. Previous studies have shown that there has been some upstream tidal movement of mercury from the HoltraChem site, but the extent of this upstream movement needs to be better defined and understood. The upstream limit of tidal influence is the Veazie Dam Fig 1. Therefore, three sampling sites will be established above the dam to assess mercury concentrations in the river at locations that do not have any water borne influence from the plant. Two sampling sites will be established between the dam and the HoltraChem site to assess upstream movement of mercury from the plant towards the dam. About 15 additional sites will be established below the plant and out into the bay as far as southern Islesboro Island.

GPS coordinates will be recorded for all of these sites to enable reoccupation at regular time intervals throughout the study so that co-located biological and geochemical samples (surface water and sediments) can be taken.

There is also a possibility that air emissions of mercury from the HoltraChem plant may have impacted the river above the Veazie Dam. High concentrations of mercury in loons nesting in neighboring lakes, which have no water connection to the plant site, are suggestive of this. Prevailing winds at Bangor, during summer months, are from the south, and the Veazie Dam is north of the plant.

Some information concerning regional atmospheric mercury deposition is available from the National Mercury Deposition Network. This data collection system has four active monitoring stations in Maine. These stations measure and report wet deposition of mercury on a weekly basis. The monitoring station closest to the HoltraChem site is at Acadia National Park. This station (McFarland Hill) began operating in late September, 1995. Analysis of data from the Mercury Deposition Network will contribute to the overall understanding of the mercury mass balance of the system. A preliminary study of the possible importance of this air deposition from the HoltraChem plant is proposed.

For the lower river south of the Veazie Dam, a GPS based map of the bathymetry and bottom type of the river system will be produced using appropriate methods. This map will assist with sample site location and mass balance calculations. The NOAA map of river bathymetry, which was produced for navigation purposes, has been found to be of insufficient accuracy for our purposes.

The river bathymetry will enable us to calculate river volumes at different tide stages. This information is necessary for making estimates of mass transport of mercury through certain reaches of the river, as described below. In addition to bathymetry, the mapping will delineate types of bottom sediment. Sites of soft sediment deposition will be of particular interest because they may be hotspots of mercury methylation and locations of benthic activity, but other types of river sediment will be sampled with less intensity in case they contain unexpected deposits of mercury.

Wetlands are known to be important sites of mercury methylation, so the extent of fringe wetlands and their connectivity to the lower Penobscot River and estuary will also be determined, along with their major vegetation types, and put onto the GIS map. GPS coordinates of each sampling site will be obtained, so that all of the mercury and ancillary data can be visualized on a series of GIS maps.

Before the three- year project begins, preliminary work will be done to focus the study on representative critical habitats. Examples of the preliminary work are searches of maps to identify prospective wetlands for study, production of the GPS based bathymetric map that will identify areas of soft sediments where methylation may occur, a survey of concentrations of methyl mercury and total mercury in water and sediments to identify hot spots of methyl mercury production in the river and bay.

B. Impact of Aerial Deposition of Mercury Originating from the HoltraChem Plant on Concentrations of Mercury in the River (biogeochemical objectives 1, 2,)

Questions: Since the HoltraChem plant began operation in 1967, has there been air transport of mercury from the plant and deposition to the Penobscot River above Veazie Dam in an amount that could affect the river downstream of the HoltraChem plant site? Does some of the mercury that is transported down the river have its origin as historical air emissions from the HoltraChem plant? Is it correct to assume that the area above the Veazie Dam is a proper reference site with respect to determining downstream mercury concentrations and fluxes?

Rationale: It is well known that, while mercury cells are in operation at chlor-alkali plants, there is substantial loss of mercury to the atmosphere in the form of elemental and reactive gaseous mercury species. Past emissions of mercury to the atmosphere from the HoltraChem plant were not directly measured, but it has been estimated that during the early years of plant operation about 90 pounds of mercury per day was "lost" from the inventory that did not leave via the plant outfall. To put this loss number in perspective, the present atmospheric deposition of mercury to the entire State of Maine is estimated to be about 3 pounds per day. Studies by CDM have estimated that it is likely that emission rates from the plant have been much reduced in later years as compared to the early years.

Some of the mercury emitted into the atmosphere may have been deposited as dry mercury deposition to vegetation and soil humus in the vicinity (i.e. within several miles) in all directions from the plant site. Because the prevailing wind direction in Bangor is from the south, much of this atmospherically deposited mercury may be north of the Veazie dam, but there may have been substantial deposition in other wind directions as well. Plant-derived mercury that has been atmospherically deposited to the river basin will join the pool of other atmospherically deposited mercury and mercury weathered from crustal material, and it will subsequently be transported down the river with mercury from the other two sources.

An important overall goal of our study (see IV, C, 4) is to compare mass fluxes of mercury over the Veazie dam to fluxes through reaches of the river below the HoltraChem plant. In order to interpret these mass flux data correctly, it is important to know if some of the mercury entering the lower Penobscot River over the Veazie dam originated from atmospherically deposited mercury emitted by the plant. In addition to the mercury deposited north of the plant, there may also have been plant-emitted mercury deposited atmospherically south of the plant, and this mercury may also be being transported downstream and impacting the ecosystem south of the plant.

The goal of this task is to determine if there has been significant aerial deposition of mercury above the Veazie Dam originating from the HoltraChem plant. One of the charges of the Study Panel is to determine what part of the mercury in biota in the lower

river and bay can be attributed to activities of HoltraChem. One of the methods of answering this question is by upstream/downstream comparisons. If the sampling sites immediately above the Veazie Dam have been contaminated by past aerial deposition of mercury the plant, this would impair the use of these upstream sites as a reference for the downstream sites. The proposed tree ring study is semi-quantitative, and if significant aerial deposition was found, further studies would be needed to determine the extent of the aerial contamination, and the upstream reference sites might need to be moved further upstream.

The purpose of this study is not to determine if aerial deposition, if found, could be ameliorated. It is the opinion of the Study Panel that (a) this issue is not within the Study Panel's scope, and (b) this amelioration would be too extensive to be practical.

Study plan: To determine the pattern and geographic extent of air deposition of mercury to the river basin from the HoltraChem plant, and to determine if these emission rates have varied with time, we propose to do a simple a cost effective tree ring study in the vicinity of the plant. Other studies, particularly in the vicinity of a chlor-alkali plant in Squamish BC (R. Turner, pers.com.), have demonstrated that trees absorb mercury that has been emitted to atmosphere, and store some of this mercury in their annual growth rings. Trees closer to the source have higher accumulation rates, and accumulation rates within trees vary over time according to the changes in mercury emission rates from the chlor-alkali plant. We propose to carry out a tree ring study by coring living trees at increasing distance and in several directions from the HoltraChem plant. It is anticipated that these data will give us an indication of the chronology, and the relative strength of air emissions from the plant, as well as the direction from the plant that most of the mercury was transported and then deposited.

C. Mercury in Water

1. Measurement of mercury concentrations in surface waters (biogeochemical objectives 1, 2 & bioaccumulation objectives 2, 3)

Questions: In general, do mercury and MeHg concentrations in river water at the co-located biological and geochemical sampling sites differ above and below the HoltraChem plant? What are the long-term mercury concentrations in surface waters at the co-located sampling sites, and how do these concentrations compare to the sites above the Veazie Dam?

Rationale: Reliable measurements of THg and MeHg concentrations in water are fundamental to achieving all of the objectives of this proposal. Concentrations of MeHg in water and on suspended particles are important factors controlling mercury bioaccumulation by lower members of the food web (e.g., algae, bacteria, other protists), but very few measurements of MeHg have been done. Furthermore, accurate measurements of mercury concentration in water are also essential for achieving all of the biogeochemical objectives presented in this part of the proposal and for some of the bioaccumulation objectives in the following sections. Therefore, these sampling sites for mercury in water will be co-located with sites where concentrations of mercury in sediments and biota are being measured on a periodic basis (e.g., see sections IV,E,1 V,C & V,F. These sites will primarily be located along the margins of the river and bay in areas of soft sediments or wetlands.

Study plan: A routine (monthly) sampling schedule will be set up at about 15-20 sampling sites beginning with the 3 sites above the Veazie Dam and extending to mid Penobscot Bay. At these sites, samples will be taken for dissolved and particulate total and MeHg. The sampling sites will be co-located with sites chosen for biological and sediment sampling.

Water samples for THg and MeHg concentrations will be taken using ultra clean techniques, and the samples will be analyzed by accredited laboratories using established methods (EPA 1630 and 1631).

Because the study is taking place in a tidal river situation, where currents can be strong, there is frequent resuspension of surface sediments resulting in variable concentrations of suspended particulate matter. Therefore, in addition to determination of THg and MeHg concentrations, emphasis will be placed on filtration of samples to determine mercury concentrations on particulate matter and to determine concentrations of dissolved mercury as defined by a membrane filter size of 0.4 μm.

To assist in the interpretation of the mercury data and to understand better the factors controlling the bioaccumulation of mercury by food chain organisms, ancillary water chemistry parameters will be obtained at the same times and locations as the mercury samples. They will include total suspended solids (TSS), transparency, pH, dissolved organic carbon, chlorophyll, salinity, and temperature.

2. Studies of the impact of past and present mercury discharges from the plant on mercury concentrations in river sediments and water (biogeochemical objective 1, 2 bioaccumulation objectives 2, 3).

Question: Are present and past mercury discharges affecting mercury concentrations in the water column of the Penobscot River/Estuary?

Two types of investigations are proposed.

a) *Rationale:* Concentration of mercury on suspended particulate matter at different locations in a river can be used as an indicator of the extent and source of mercury contamination of the river. This approach was used in a previous study of the Penobscot River conducted on behalf of Mallinckrodt, where it was found that mercury concentrations on particulate matter were similar at sampling sites above and below the plant site. Because concentrations in the vicinity of the plant were not higher than at the upstream site, it was concluded that historical and/or ongoing inputs of mercury from the plant must not be having a significant impact. However, a shortcoming of this study was that the upstream sampling site was not beyond the limit of tidal influence. Thus mercury concentrations in particulate matter at this upstream site could have been influenced by past or ongoing mercury discharges and so would not serve as a good reference point.

Study plan: To address this question, it is proposed that comparisons be made of concentrations of mercury on suspended particulates at locations downstream of the HoltraChem plant to particulate mercury concentrations at the three sites just above the Veazie Dam. This study would utilize the same samples that are obtained for the mercury mass flux study (task IV,C,4), and so the particulate study will be done at no incremental cost to the overall project.

The initial river sampling in the fall of 2004 showed that particulate mercury concentrations were elevated at the site downstream of HoltraChem as compared to the site upstream of the Veazie Dam. We propose to repeat this study, sampling immediately

upstream of the Veazie dam, downstream from the HoltraChem site, and at the other sites farther down the river where mass flux studies are being done (see IV,C,4*)* With this approach, it will be possible to determine the contribution of present and past mercury discharges to particulate mercury concentrations in the Penobscot.

b) Rationale: The stable isotope signature of mercury in the Penobscot River may be useful in identifying its sources. New stable isotopic methods have been perfected in the laboratory of Dr. Holger Hintelmann (Trent University, Peterborough, Ontario), and he has agreed to work with us to apply these methods to the Penobscot River. Dr. Hintelmann uses a multi-detector mass spectrometer, which is capable of separating the various stable mercury isotopes at environmental concentrations. (These methods have now been published in the peer-reviewed scientific literature.) Working at other locations he has found that the isotopic signature of mercury at point sources (e.g. mines and industrial sites) is heavier than mercury that has been in the natural environment for periods of time. We hypothesize that the isotopic signature of the mercury at the HoltraChem plant will be different (likely heavier) than the mercury in sediments at the three sampling sites above Veazie Dam, and that the mercury in sediments below the dam will be intermediate in mass, with the ratio being more similar to the plant site or upstream signatures depending on the relative strength of the sources.

Study plan: Isotopic signatures will be determined for mercury samples obtained from the ground water and soils of the HoltraChem plant. These signatures will be compared to mercury in 1 meter (m) long core profiles taken from the three upstream sites and from several sites located just below the HoltraChem site and in increasing distance from the plant. This study will not be pursed if early results are not promising.

3. Studies of present mercury inputs from the HoltraChem plant (biogeochemical objective 1, 2 & bioaccumulation objectives 2, 3).

Question: Are there significant ongoing inputs of mercury from the HoltraChem plant?

Rationale: Previous studies of mercury contamination in the vicinity of several chlor-alkali plants located at estuarine and freshwater locations have found that concentrations of mercury in biota continue to be elevated above expected background levels for decades after the plants have ceased operation. While these concentrations are very likely lower than they were when the plant was operational, they may still above acceptable levels and they show no sign of decreasing with time. One possible explanation for these continued high concentrations is methylation and bioaccumulation of mercury historically deposited in the sediments of the receiving waters during the time of plant operation. However, detailed investigations of deactivated chlor-alkali plants have demonstrated that ongoing, comparatively small inputs of mercury from the plant site are often responsible for the continued high concentrations of mercury in biota. Usually this mercury is entering in ground water, and after leaching from soils of high mercury concentration near the plant, it may arrive in the receiving waters with a chemical speciation that is particularly suitable for mercury methylating bacteria (e.g., $HgCl_2$).

For the past several years, CDM has been doing an intensive study of mercury losses from the HoltraChem site to the Penobscot River via stream and outfall flow. Monitoring of these fluxes will continue for the foreseeable future by CDM. Current estimates are that about 3 lbs of mercury are lost from the site annually. Some of this loss occurs at discreet discharge points (outfalls and streams), which are easily measured, but CDM estimates that most of this loss (> 70%) is by ground water discharge. Much of this ground water discharge may be stopped when a groundwater "pump and treat" program commences.

Study plan: While there have been extensive efforts by CDM personnel to identify and understand ground water flows at the site, accurate estimation of mass flux of dissolved chemicals by ground water flow is notoriously difficult. Therefore, in addition to the ongoing CDM study, we propose to carry out a near-bottom river sampling program in the vicinity of the HoltraChem plant to try to detect any residual or unidentified inputs of ground water mercury to the Penobscot River. Sampling will take place four times a year during low tide. The study will concentrate on measurements of dissolved total mercury, because this is the form of mercury usually found in ground water. This near-bottom sampling will be done at 50-meter intervals over a river distance of 0.5 km above and below the plant site. Concentration of dissolved mercury in these samples will be compared to samples taken at three sites on the opposite side of the river. If a hotspot of dissolved mercury concentration is found, sampling intervals would be decreased in an effort to pinpoint the site of ground water discharge. If ongoing inputs are found, this would be an indication for further remediation, because the speciation of these types of inputs is often high in $HgCl_2$, which is very available to methylating bacteria.

In addition to the dissolved total mercury samples discussed above, there will be selected sampling of dissolved MeHg concentrations to determine if the plant site is a source of MeHg to the river.

To the extent that surface sheet flow occurs during heavy rainstorms, the mercury transported to the river would not be measured in the monitoring of outflows and of the creek described above. Therefore, we propose to do episodic high-frequency storm water sampling at the site for concentrations and flux of mercury to the river

For all of these studies of ongoing inputs, we propose that they not be pursued after year one of the study if ongoing inputs are not found to be significant. However, it should be noted that even small masses of ongoing inputs may be in a form that is very bioavailable to mercury methylators (i.e. $HgCl_2$). If this is the case, these small amounts may be very significant in supporting ongoing mercury methylation in the ecosystem.

4. <u>Mass flux studies of mercury in surface waters (biogeochemical objectives 1-3 & bioaccumulation objectives 2, 3).</u>

Questions: Is the vicinity of the HoltraChem plant presently a source of mercury and MeHg to the downstream river? Are downriver wetlands or sediments significant sources of mercury and MeHg to the river and to Penobscot Bay?

Rationale: The extent of net downstream movement of mercury from the vicinity of the plant site to upstream and downstream areas is unknown, and one of the charges to the Study Panel is to look at impacts of the HoltraChem plant site on the downstream river and estuary. To understand the net downstream movement of mercury, an evaluation of the mass flux of mercury into and out of the study area as well as among

compartments within the ecosystem is needed. This information could also be useful in the possible future development and calibration of a mechanistic model to assess factors controlling MeHg production, transport and bioaccumulation. Such a model would be useful for testing of proposed amelioration measures, and it is also needed to predict natural attenuation of mercury in the system after inputs of mercury from the plant site have been stopped.

Study plan: The geographic extent of the mass flux study would extend from the Veazie Dam to the mouth of the river near Fort Point Cove (Fig. 1). Net mass flux of THg, MeHg and particulate mercury species will be estimated at four points along the river: 1) at the Veazie dam, 2) at a suitable point one mile south of the HoltraChem site, 3) above Frankfort Flats, and 4) where the mouth of the river begins to widen above Fort Point Cove. Transport into the study area will also be estimated by monitoring representative tributaries.

Mass flux of mercury downriver will be estimated as the product of mercury concentration and net volume of water that flows past a sampling point. Over a three-year period flux estimates will be made monthly by sampling four times over each tidal cycle over a 2 day period (i.e. four complete tidal cycles in all). This regular sampling schedule may be interrupted by river inaccessibility during winter.

In addition to the regular monthly sampling, mass flux will also be determined during selective high flow events on the river when mercury transport is expected to be high. Some of these events during spring snow melt are predictable and will be sampled. Other events due to heavy rainfall will be sampled when possible.

Compared to the three downstream sites, estimation of mass flux of mercury over the Veazie Dam will be relatively simple, because the river is not tidal at this point. Each month, mercury concentrations will be measured twice daily, for two days, in the river water as it flows over the dam. River flow is continuously monitored at Eddington, which is near the Veazie Dam. Estimation of mercury flux at this sampling point will give us an estimate of mass inputs of mercury to the lower Penobscot River. It is assumed that this mercury has originated either from weathering in the upstream catchments or (mostly) from atmospheric deposition.

At the three downstream locations, measurement of the net fluxes will be more difficult because these locations are tidal and there is a possibility of salt wedges. When top to bottom salinities differ by more that 2‰, four sampling sites will be established across the river at each of the downstream locations. Four times over a tidal cycle, two samples will be taken near the top and bottom of the fresh and saltwater wedges, and fresh and saltwater samples will be separately composited. Net river flows over a tidal cycle will be estimated by continuously monitoring river stage at each of the three downstream sampling locations and volume of flow will be calculated from river stage and the bathymetric map of the river channel. Ancillary measurements taken to assist in the interpretation of the data will include depth profiles of current velocity and direction, temperature, and salinity.

To close the mass balance, at each of the four sampling sites, estimates of flux of elemental mercury (Hg^0) across the air-water interface will be made using Fick's law and measurements of water temperature, wind speed and the dissolved Hg^0 concentration.

If early in the study the above approach does not appear to be useful for the measurement of net flux of mercury down the river, this method will be stopped and alternative methods of measuring flux will be sought.

D. Studies of Rates of Mercury Accumulation in Depositional Sediments (biogeochemical objective 1, 2)

Questions: What has been the increase in mercury deposition rate to sediments resulting from industrial discharges of mercury to the Penobscot River? Have past discharges of mercury from the HoltraChem plant been buried in sediments below the depth of MeHg production, or is this mercury still being methylated? Are surficial sediments still receiving elevated inputs of mercury compared to pre-industrial accumulation rates.

Rationale: If the sediments in some depositional areas of the Penobscot River and Bay have been undisturbed since the time prior to the industrial use of, and if these sediments could be successfully dated, we would expect that close interval depth profiles of THg concentrations in the river sediments would show a peak during the time of operation of the HoltraChem plant, which would be followed by a decline in concentrations in shallower sediments after closure of the plant. Undisturbed mercury depth profiles will likely also be found in Penobscot Bay sediments, and possibly in sediments if some wetlands along the margins of the river.

Understanding the stratigraphy of mercury in depositional sediments is important in several respects. First, this coring approach can be used to estimate mercury deposition rates prior to the industrial use of mercury in the river basin and these early rates can be compared to rates which occurred during the peak operation of the HoltraChem plant and to more recent rates since discharge rates have been reduced. Second, because most MeHg production is likely to occur in surficial sediments, an understanding of accumulation rates of surface sediment is needed to make estimates of rates of natural attenuation, after any ongoing point source inputs are eliminated. Third, measurements of depth profiles of MeHg concentration are useful in delineation of sites of MeHg production. Often peaks in MeHg concentration occur within 4-5 cm of the sediment-water interface and this is the zone of mercury methylation. In ecosystems that are microbially active, peak MeHg concentrations may be even closer to the sediment-water interface and this situation promotes the flux of mercury across the sediment-water interface.

(It should be noted that in turbulent areas, where sediments are often reworked, both historical mercury and recent mercury could be at the surface of the sediments. In this case, both the new and old mercury could be contributing to ongoing methylation. This type of disturbed site may also be found for the studies described in Section IV, E, 1 where distributions of mercury and MeHg in surface sediments will be studied)

Study plan: To carry out the mercury accumulation studies, cores will be obtained from about 15 depositional sites located between the HoltraChem facility and mid Penobscot Bay. Identification of the other sites awaits site characterization. These sites should be co-located with sites where biological sampling is taking place.

Cores of up to 1 m in length will be sliced at 1 cm intervals, and the core slices will be immediately frozen. Initially, every 3rd slice will be dated using ^{210}Pb and ^{137}Cs to determine if this is a site of long term deposition. For sites that are found to be

depositional, core slices will be analyzed for THg, MeHg concentration, and for wet and dry weight, grain size, organic carbon concentration, and concentrations of acid volatile sulfide. The core slices will be frozen immediately upon slicing in the field to preserve in situ concentrations of MeHg.

E. Studies of Net Mercury Methylation (biogeochemical objective 3 & bioaccumulation objectives 2, 3)

Question: Where are the active sites of mercury methylation in the Penobscot River/Estuary ecosystem? Does the mercury being methylated at these sites originate form the HoltraChem plant? Is mercury that has originated form the plant being methylated in the river and bay?

A clear understanding of microbial mercury methylation activity in the ecosystem is central to the study. Therefore, emphasis will be placed on locating sites of methylation, quantifying their strength, and studying factors that control rates of MeHg production. Certain species of bacteria, particularly sulfate-reducing bacteria, are known to convert inorganic mercury to MeHg under prevailing environmental conditions. Other bacterial species demethylate mercury, so the concentration of mercury in the aquatic environment is the net result of these two opposing processes. Net methylation rates vary from site to site because of geochemical factors that control the activity of the bacteria and/or the bioavailability of the inorganic mercury (HgII) or MeHg to the two groups of organisms. The following sections outline a series of studies that are intended to identify sites of mercury methylation in the system, to determine the strength of these sources, and to investigate factors that control rate of MeHg production. Understanding the methylation process in the ecosystem offers opportunities for the design of mitigative measures at hotspots of MeHg production.

1. % MeHg in surface sediments

Questions: In depositional and non-depositional sediments in the river, wetlands, and bay, how do methylation rates vary at different locations and at different times of the year? Do methylation rates correlate with any of the ancillary measurements? Are there "hotspots" of MeHg production that could be good candidates for remediation?

Rationale: Several studies have shown that a good indicator of the intensity of net mercury methylation is the percentage of total mercury that is MeHg (%MeHg). Sediments with high rates of MeHg production have 5% or greater of their total mercury present as MeHg. In mercury contaminated systems, such as the Penobscot River, it is not advisable to do these measurements in the immediate vicinity of the point source (e.g., Southern Cove) because of very high concentrations of inorganic mercury that may be toxic to the methylators, so these measurements will be done downriver where THg concentrations are lower and more uniform.

Study plan: Sampling sites will be chosen by making use of available mapping data and the GPS/sonar based bathymetric map that will be produced as part of this project, and from the survey of MeHg concentrations in surface water described above. Likely study sites will be in areas of soft depositional sediment in the river, wetlands or bay. These may be sites where the sediments are periodically worked or sites where there is long term undisturbed sediment accumulation. Three core samples will be obtained from each site. The top 7 cm of each sample will be sliced into 3 sections (0-2, 2-4, 4-7

cm) and frozen for subsequent analysis. Ancillary measurements, which may affect the activity of the methylating bacteria and/or the bioavailability of Hg(II), will include analyses of organic carbon content, dissolved sulfide concentration, grain size and porosity, overall microbial activity, and pH.

There is evidence in the scientific literature that periodic exposure of sediments to the atmosphere is stimulatory to mercury methylation, likely because of oxidation/reduction processes that affect the bioavailability of inorganic mercury or sulfur to the methylators. In locations where tidal mudflats are exposed, two sampling sites will be chosen – one sub tidal and a second that is tidally inundated. The MeHg content in surface sediment at these two sites and ancillary data will be collected as described above.

During the first year of the study, in order to identify hotspots of methylation and their seasonality, a survey at about 15-20 sites will be performed at biweekly intervals between May 1 and Sept 1. This sampling time is chosen because rates of MeHg production tend to peak in summer. In some estuaries (e.g. Lavaca Bay) a peak in methylation occurred during spring, but in other cases (e.g. Venice Lagoon) methylation rates do not peak until mid summer. Based on the first year of sampling, a subset of 4 wetlands and possibly two or three other sites will be chosen, and the % MeHg will be followed at these locations at 3 week intervals over the next 2-year period. If the pattern of methylation is similar during first two years of the study, measurement of the full seasonal pattern would not be repeated in the third year of the study.

2. Measurement of net MeHg flux from wetlands

Question: Are wetlands important sources of MeHg to rest of the ecosystem?

Rationale: We hypothesize that riparian wetlands will be important sites of mercury methylation. This has been demonstrated in numerous other river systems. It also known that MeHg concentrations in water overlying wetlands quickly equilibrates with the MeHg in wetland sediments, and so the MeHg can be transported from wetlands to the main stem of the river or into the bay.

Study plan: In addition to studying MeHg production in wetlands by looking at % MeHg in sediments (section IV, E, 1) we propose to study the transport of MeHg from wetlands. Two or three representative wetlands will be chosen for these studies using data from the previously described studies of MeHg in sediment and concentrations of MeHg in surface waters. In addition to being sites of high MeHg, these wetlands must also be partially disconnected hydrologically from the main river channel, so that a mass balance approach could be used to study net rates of MeHg flux to the river. During years 2 and 3 of the study, at monthly intervals, inflowing and out flowing water from these wetlands will be analyzed for MeHg concentration. These data plus flow measurements will be used to estimate net flux of MeHg to the river. These wetlands will also be sampled as often as practicable during periods of high flow following summer rain events.

If suitable wetlands can not be found for measuring MeHg flux as described above, other methods such as in situ flux chambers and/or placement of caged bivalves in wetland areas will be attempted to obtain some measurement of MeHg flux from wetlands sediments.

3. Isotopic studies of MeHg:

Question: Is mercury that has been released from the HoltraChem plant being methylated in the river sediments and wetlands?

Rationale: If we are able to trace the inorganic mercury that has been released from the plant as described in section IV,C,3, we then propose to determine if this mercury is being methylated in the ecosystem. Dr. Holger Hintelmann has recently also perfected methods for measuring the isotopic signature of MeHg.

Study plan: Samples of river and wetland sediments will be obtained from sites where inorganic mercury originating from the plant has been found. The top 5 cm of sediment will be sampled because this is where methylation is most likely to occur. Isotopic ratios of inorganic and MeHg will be determined by the mass spectrometer methods recently developed in Dr. Hintelmann's lab. This study will only be pursued if early results prove promising.

V. PROPOSED BIOACCUMULATION STUDIES

We will study concentrations of mercury in key organisms (objective 1), and also the bioaccumulation process itself using appropriate field and laboratory data (objectives 2, 3).

To assist us in selection of study organisms to be used, a targeted biological survey for species of interest will be carried out at selected sites by local naturalists. The purpose of the targeted survey is to locate specific fish, bivalve, benthic, bird and mammal species that will be useful for the bioaccumulation, bioindicator and toxicity studies outlined below. The survey will also determine if sufficient numbers of the targeted species are present for the study. It is recognized that there are anadromous and catadromous fish species in the river and that some of these species will be missed by a single targeted survey. The survey will likely take place in the late summer because this is when the young of some fish species such as stickleback, which may be a good indicator organism, will have been resident in the river throughout the summer months when most mercury is methylated and bioaccumulated.

For the selected organisms, concentrations of mercury will be determined on a seasonal basis (plankton, benthos, bivalves and fish) at about fifteen to twenty sites throughout the system. Emphasis will be placed on establishing concentrations in biota above the Veazie dam, where there is not influence of HoltraChem, and comparing these concentrations to the downstream sites. Mercury concentrations in water and sediments and ancillary data will be collected at these sites as described above. Bioaccumulation factors will be calculated from these data, as well as from appropriate laboratory studies.

We anticipate that, as with most other aquatic ecosystems, there will be two distinct food webs operating in the Penobscot River and Bay, namely benthic and pelagic. The benthic food web will likely be most important in the river while both the benthic and pelagic food webs will be important in the bay. However, both should be assessed in each case. Each food web may encounter different forms and different concentrations of Hg, but both are important to the healthy functioning of the ecosystem and both may serve as conduits of bioavailable contaminants to higher organisms, including man. It is anticipated that emphasis will be placed on wetland areas in or near the river where

methylation rates are expected to be high, and wetlands in or near the bay. The bay is the source of most harvested seafood and will ultimately receive most of the mercury from the river. Overall, it is planned to sample spatially intensively, but to sample temporally intensively in only a few locations. The temporally intensive sampling would probably occur in select bay and wetlands locations.

A. Studies of Stable Isotope Ratios of Food Chain Organisms (bioaccumulation objectives 2, 3)

Questions: What is the relative position in the food web of the various biota sampled? What is the relative importance of the benthic versus pelagic food chains to top predators?

Rationale: We need to understand the ecological interactions of select components of the food web because Hg accumulates in animals primarily through their diet and it is critical to understand the sources of food for the animals. This can be accomplished with a field program using concurrent measurements of stable isotopes of carbon and nitrogen and mercury concentrations in tissues of organisms from different trophic levels. Since the different isotopes of a given element react at different rates, the ratios of the heavy to light isotopes of an element in a sample can often be an indication of the processes involved in its creation. In ecological studies, the fractionation of the stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provide powerful tools to elucidate trophic transfer processes. Because $\delta^{13}C$ values tend to vary at the base of the food chain but then remain relatively constant through trophic transfer to higher trophic levels, $\delta^{13}C$ is a good tracer of carbon source. In contrast, $\delta^{15}N$ becomes more and more fractionated through each trophic transfer, so that consumers in a food chain become enriched in $\delta^{15}N$ relative to their food. This fractionation allows for the assessment of relative trophic position of an organism. When used together, $\delta^{13}C$ and $\delta^{15}N$ ratios can provide a realistic measure of trophic position, and they provide potential to capture complex trophic interactions (e.g., omnivory).

Study plan: During the second year of the study, if concentrations of mercury in wildlife or in fish or shellfish for human consumption are high enough to be of concern, a one time isotopic study of the food web will be carried out at the sampling locations along the Penobscot River and Estuary. This study will include phytoplankton, zooplankton, benthic animals (molluscs and polychaetes), forage and predatory fish, as well as biota from the bay that are presently or potentially being consumed by humans (lobster, crab, fish, bivalves). The improved understanding of the transfer of mercury from benthic and water column bases of the food chain will be fundamental to interpreting the data described in the following sections.

Throughout the study, samples will be taken of fish gut contents when fish are caught for mercury analyses. This sampling will provide short-term information on types of fish food, which can be compared to the longer term record provided by C/N isotope studies.

B. Mercury Accumulation by Plankton (bioaccumulation objectives 1, 2, 3)

Questions: What are the mercury concentrations in plankton (both phytoplankton and zooplankton)? Are there environmental factors at particular sites in the ecosystem

that affect mercury bioaccumulation rates? Do the mercury concentrations in plankton vary seasonally?

Rationale: In taking a whole-ecosystem approach, we will consider the bioaccumulation of mercury in phytoplankton and zooplankton as they represent the initial bioaccumulators from dissolved and particulate sources that can eventually lead to elevated food chain concentrations. Phytoplankton cells serve as one of the bases of most aquatic food webs. They are known to concentrate many metals, including inorganic and MeHg, very substantially out of ambient water. This bioconcentration can be very pronounced, and in the case of inorganic and MeHg concentration factors can exceed 100,000; that is, the phytoplankton cells are 100,000 more enriched in mercury than the water surrounding them. This bioconcentration step is by far the greatest for any trophic level in the food web. The bioconcentration of mercury, like other metals, is dependent somewhat on the species composition of the phytoplankton community and on several physical/chemical characteristics of the surrounding water. Among the latter, it is anticipated that the high dissolved organic carbon (DOC) concentrations in the Penobscot River (7-15 mg/l) may influence the bioavailability of mercury for resident organisms, since mercury is known to complex with dissolved organic matter. In most circumstances, organically complexed metal is less bioavailable than non-complexed metal, however there are some cases in which organic complexation is known to enhance metal (including mercury) uptake from the dissolved phase.

Once mercury is concentrated by phytoplankton, these cells essentially serve as mercury-enriched particles for herbivores that consume them. Key herbivores to consider would be 1) pelagic zooplankton (e.g., copepods) (which would serve as conduits of mercury from the base of the food chain to fish) and 2) suspension-feeding bivalve molluscs like mussels. Mussels are considered as a "special case" below. Further, modeling efforts to evaluate the food web build-up of mercury require knowledge of phytoplankton mercury concentrations.

Another reason for studying mercury concentrations in phytoplankton is that phytoplankton cells can be very sensitive to mercury, but sensitivity to mercury and other metals can vary greatly among algal types. Therefore, toxic effects of mercury on these organisms could result in significant shifts in the species composition of the algal community, which would likely have important consequences for the grazer community since herbivores are often very specific in what foods they can ingest or digest. Once associated with phytoplankton cells, mercury can also be transported vertically in the water column as uneaten cells sink toward the bottom and ultimately decompose. The phytoplankton therefore can influence the residence times of mercury in different "compartments" of the river ecosystem.

Study plan: Because the river ecosystem is likely dominated by benthic food webs, initial focus on mercury uptake in the plankton will be with estuarine/marine species representative of the bay. If chlorophyll measurements in the river clearly indicate a healthy phytoplankton standing crop, at least at certain times of year, however, studies will also explore the extent to which mercury bioaccumulates in riverine phytoplankton communities following the protocol outlined for the estuarine cells. The extent to which mercury in the Penobscot Estuary is available for uptake by phytoplankton should be assessed at the ten-fifteen different locations at varying locations within the bay. This can be done in two ways, and both should be conducted, as

neither approach will give the "whole story" regarding mercury concentrations in phytoplankton or bioavailability for these cells. (1) One approach is to estimate mercury concentrations in phytoplankton cells. Because it is not possible to separate suspended phytoplankton cells from other suspended particles, which may actually greatly outnumber phytoplankton cells in surface waters, analysis of mercury in suspended particulate matter will not yield unambiguous data. Because particulate matter from surface waters of the river will be analyzed for mercury (along with measurements of dissolved mercury), chlorophyll concentrations in suspended particles should also be concurrently analyzed so that an estimate of the algal biomass can be made. These measurements will allow us to calculate the concentration factors of inorganic and MeHg in the suspended particles on a dry weight basis (i.e., Kd values). Seasonal and spatial patterns of Kd values should be analyzed in the context of the measured chlorophyll measurements.

(2) In addition, the bioavailability of mercury from water at these sites should also be determined for select algal species in laboratory experiments. To accomplish this, water from the same sites should be filtered through 0.4 μm cartridge filters to remove all particles larger than bacteria. Aliquots of each parcel of filtered water (i.e., from each site) should be analyzed for inorganic MeHg. The filtered water should then be inoculated with monocultures of one species of diatoms, one of green algae, and one species to be selected based on the prevailing algal species in the estuary, to measure the bioavailability of the existing mercury (inorganic and methyl forms) from the dissolved phase under the prevailing DOC concentrations. Inocula can be obtained from national algal culture collections, such as at the University of Texas or at the Bigelow Laboratory for Ocean Sciences in Maine. Nutrients should be added to allow for cell growth, but organic chelators like EDTA should be avoided. Cells should be allowed to grow for 4-5 days, after which they will be harvested by filtration onto polycarbonate membrane filters and analyzed for inorganic and MeHg. The mercury concentrations associated with filter digests should be compared with those from uninoculated control "cultures." Three replicate cultures should be assessed for each algal species (plus three controls). Bioconcentration factors (on dry weight bases) should be determined with the monocultures and compared among species and between monocultures and natural particle assemblages. Differences between the Kd values in the cultures and the particle assemblages, if they occur, are likely attributable to differences between algal and abiotic particle Kd values.

Zooplankton are one of the primary consumers of phytoplankton, and they further bioconcentrate the MeHg present in phytoplankton in their bodies. Because zooplankton are an important food source of forage fish, they are also an important conduit of mercury up the food chain. Concentrations of inorganic and MeHg in zooplankton will be determined on the same schedule and at the same sites as for phytoplankton. Sufficient biomass for mercury analyses will be obtained by tows of clean 73 μm nytex nets. Seasonal trends in mercury concentration will be examined and compared to changes in environmental factors such as temperature, pH and DOC concentrations. Zooplankton are known to bioaccumulate mercury from both dissolved and dietary sources. An attempt should be made to combine dissolved and particulate mercury concentrations with known (from the literature) uptake and loss parameters from these sources using an established biokinetic model to evaluate the relative importance of these sources of

mercury for the resident zooplankton. Zooplankton mercury concentrations can also be used for modeling assessments of mercury transfer up to fish.

C. Evaluation of Mercury in Deposit-Feeding Benthic Organisms (bioaccumulation objectives 1, 3)

Questions: What are the mercury concentrations of deposit-feeding benthic organisms? Are there environmental factors at particular sites in the ecosystem that affect mercury bioaccumulation rates? Do these concentrations vary seasonally?

Rationale: Sediments can be highly enriched in mercury (and other particle-rich contaminants) and have often been thought of as the final repository of such contaminants in aquatic systems. However, in recent years there is a growing body of evidence to show that metals, including mercury, that are bound to sediments can be assimilated in animals that consume these sediments as a food source. These organisms can in turn be consumed by other animals higher in the food chain and thus they may mobilize the mercury in sediments in a form that can appear in the tissues of other animals that may be eventually consumed by man. For example, worms can assimilate mercury out of ingested sediments and are consumed by bottom feeding fish such as flounder and stickleback. Thus, it is proposed that the mercury and MeHg concentrations in select deposit-feeding invertebrates (worms, clams) be measured at the established sampling locations within the river and the estuary. Secondly, measurements of the assimilation efficiency of mercury from contaminated sediments in deposit-feeding invertebrates should be assessed using established experimental protocols. Assimilation efficiencies of ingested mercury, combined with known feeding rates, will provide critical information in interpreting the extent to which these animals can serve to mobilize sediment-bound mercury as well as how likely they will be as biological sources of mercury to predators.

Study plan: Infaunal clams and polychaete worms, and amphipods if available, should be sampled at the same riverine and estuarine sites where other geochemical and biological sampling is being done. The animals should be depurated for at least 12 hours after collection to ensure that undigested sediment in their guts are released, after which they should be analyzed for inorganic and MeHg. The tissue concentrations in these animals should be compared to the mercury concentrations in the sediments from which they were collected. The polychaetes in each sample will be divided into functional feeding groups (carnivores, omnivores, and surface deposit/suspension feeders), because they feed at different levels of the food chain, and thus have different mercury concentrations. This approach was used for the Lavaca Bay mercury study where the polychaetes proved to be very useful bioindicator organisms. If present, the clams will be separated into suspension feeders, and those feeding beneath the sediment surface.

D. Studies of Mercury Accumulation by Aquatic Biota at Higher Trophic Levels (bioaccumulation objective 1)

Question: What are the mercury concentrations in biota at higher trophic levels? Are mercury concentrations in fish high enough to warrant toxicity testing?

Rationale: Although the greatest concentration "step" (by far) for mercury into aquatic organisms occurs from water into phytoplankton (and probably other protists), the concentration of this metal actually continues to increase with each step in the food chain, a process known as biomagnification. It is generally thought that, in contrast to hydrophobic organic contaminants, most metals do not display biomagnification in food chains, but mercury is a notable exception, again mostly attributable to the unusual behavior of MeHg. Of all the metals, mercury is probably most affected by trophic transfer in aquatic food chains. This is because both inorganic mercury and most especially MeHg are assimilated very appreciably out of food, and because MeHg displays such long biological retention in animals. Indeed, the loss rate of MeHg from large fish is nearly zero. As a consequence, the overall tissue burden of mercury, primarily in the form of MeHg, increases with the trophic position and age of the fish, since uptake through consumption of food can greatly exceed loss. Therefore, health advisories for consumption of seafood due to mercury contamination are primarily for large predatory fish.

Consumption of aquatic animals, such as seafood, is known to be the dominant source of mercury and MeHg for man. Earlier studies have documented in some cases severe poisoning of humans, but much more commonly mercury is at concentrations in seafood that can lead to sub lethal effects that are nevertheless worrisome. Man consumes a variety of invertebrates (crabs, bivalve molluscs, lobsters) and fish that may present high concentrations of mercury. Given the importance of trophic transfer as a mechanism that can dominate the delivery and final concentrations of mercury and especially MeHg in aquatic food chains, attention should be focused on the mercury concentrations in aquatic species that are consumed or potentially consumed by man as seafood.

Study plan: It is anticipated that most of the ongoing and potentially important fisheries and important wildlife will be located in the bay, and most of our sampling emphasis will be located in the bay (with the exception of eels). We recommend analysis of eels [*Anguilla anguilla*], striped bass [*Morone saxatilis*]), lobster (*Homarus americanus*), mussels, quahogs and crab. Every attempt should be made to collect 15-20 individuals per species for each sample location and sample time, and when possible the biota will be aged because mercury concentrations often increase with age.

Eels are harvested commercially in the river, but primarily for export. Lobster, crabs, mussles, and some fish (e.g., striped bass) caught either commercially or for sport should be analyzed. The striped bass data need to be interpreted with caution, since it is recognized that these fish may accumulate dietary MeHg from sources well away from the Penobscot estuary. (The stable isotope study will assist with these interpretations.) Striped bass are an important game fish that is consumed by humans, so it is necessary to evaluate the extent to which locally caught striped bass are contaminated with MeHg.

To our knowledge, there is no commercial finfish fishery active in the bay, but spatially distributed samples should be taken over a three-year period because these are potential fisheries, which may re-open. There are however active shellfish fisheries in the bay, and their mercury and MeHg content will be studied closely.

These data should be tied to the assessment of human health impacts, as described below in section VII.

E. Evaluation of Mercury Concentration in Birds and Mammals (Bioaccumulation objective 1)

Question: Are MeHg concentrations in the biota that are at or near the top levels of the food chain high enough to necessitate toxicity testing of these biota?

Rationale: Aquatic feeding birds consume mercury through their diet, and excrete it primarily into their feathers. Because birds eat near the top of the aquatic food chain, most of the mercury they consume is already methylated and thus retained to a much greater extent than inorganic mercury. The extent to which they are being negatively affected is unknown. Studies of selected bird species will be done during breeding season by analyzing feathers and blood of adults, and by studies of clutch size and hatching success. One of the species will be cormorants, which nest in Penobscot Bay. Their mercury concentrations are notably higher than birds in cormorant colonies located at other New England estuarine sites. We also propose to study the kingfisher and a single shorebird species, the spotted sandpiper, which is the only known shorebird species that breeds in lower Penobscot. Contrasting the mercury concentrations in the sandpiper with those in cormorants and kingfisher may be particularly instructive, since these birds feed at different levels in the food chain. These field avian studies will precede the avian toxicity studies discussed in section VII, B.

We do not propose to study the bald eagle populations because their numbers are too low to enable collection of statistically meaningful data, but if enough osprey are present we will sample this species.

Mercury concentrations of river otter, harbor and grey seals living along the New England coast have recently been found to be elevated, particularly in the Penobscot Estuary. However, sampling has been limited. We propose to establish mercury concentrations of the otter and seal populations in the Penobscot Estuary, prior to initiating any toxicity tests.

Study plan: For all of these higher level consumers, if sufficient numbers of individuals are present, at least 10 samples of, brain, blood, feathers/hair, liver or eggs will be taken on an annual basis from each sampling location. The number of sampling locations are dependent on the targeted biological survey of distributions of the biota in the Penobscot system.

F. Mercury Concentration in Bioindicator Organisms (bioaccumulation objectives 1-3)

Question: Does the bioavailability of MeHg to bioindicator organisms differ at different locations resulting in higher (or lower) concentrations of mercury in biota for a given concentration of MeHg in sediment? Do MeHg concentrations differ seasonally in lower food web organisms?

Rationale: As with other metals, total ambient mercury levels may not reflect the amount of bioavailable mercury. For this reason, concentrations of metals in bioindicator organisms have been used to evaluate the spatial and temporal trends in metal contamination in coastal and freshwater environments. Bioindicator organisms are

defined as organisms that ideally occur throughout the ecosystem being studied, and have a high site fidelity. The bioindicator approach, as with NOAA's National Status and Trends Program, has enabled comparisons between regions and over different time periods to discern regions of concern for a given contaminant. The advantage of this approach is that it exploits the ability of naturally occurring resident organisms to integrate metal concentrations over time, and by definition the metal in these organisms was in a bioavailable form. Because the physiological state of a bioindicator organism can influence the final tissue concentration of a metal, it is necessary to sample organisms in a more-or-less similar physiological state and of comparable size, which often translates to similar age.

The mostly widely used bioindicator organisms to assess aquatic contamination are the bivalve molluscs, most particularly mussels. Details of how to sample and analyze mussels to reduce artifacts associated with these processes have been described extensively in NOAA's literature. These organisms have the advantage of being ubiquitous, sedentary, and sufficiently tolerant of most environmentally realistic contaminant concentrations that they are able to survive in polluted regions. Being sedentary is a distinct advantage in that one knows that the animal could only have accumulated its contaminant from one location, hence it is integrating its previous exposure history at that one location.

In the Penobscot estuary, we propose to examine the inorganic and MeHg concentrations in the blue mussel *Mytilus edulis*, the most commonly used bioindicator of coastal/estuarine contamination The advantages of using this organism are: (1) the protocols for sampling and analyzing these animals are well established; (2) there is already a substantial data base with which to compare Penobscot mussel data so that Penobscot data can be put into a broader context; (3) there are already some (but not sufficient) mussel data suggesting that mercury concentrations are elevated relative to other regions in Maine or nationwide; and (4) this mussel is harvested for human consumption (indeed Maine is the major source for this mussel nationwide).

Because *Mytilus edulis* are not present in the freshwater reaches of the river, we propose to assess the tissue concentrations of mercury in other prevalent bivalve organisms in the Penobscot River, if they are present. This might include the zebra mussel (*Dreissena polymorpha*), which is ubiquitous in northeastern N. American rivers and lakes and which is known to concentrate mercury from ambient waters. Where bivalves are present along the salinity gradient of the river, we plan to sample overlapping populations of bivalves, so that the spatial distribution of the study can be as complete as possible.

If (and only if) it is not possible to collect sufficient numbers of bivalves from along the river, consideration should be given to using caged mussels to assess the bioavailability of mercury and its rate of bioaccumulation under the prevailing environmental conditions. Caged mussels would include *M. edulis* in the estuary and a low salinity species such as *Rangia cuneata* in the river. Protocols for handling and measuring caged mussels in such transplant studies have been developed that minimize the stress on the mussels. Under any circumstance, it is not proposed to evaluate the toxicity of mercury to the caged animals, which may already be under stress, but they can be used to assess the bioavailable mercury pools at different times and in different sections of the ecosystem.

We also propose to measure mercury concentrations in age one killifish and stickleback fish as potential bioindicator organisms, since these fish have high site fidelity and they occur over a wide salinity range within the Penobscot ecosystem. Mussels filter phytoplankton out of water for their food, whereas these fish eat smaller fish and zooplankton and benthic animals (including various larval forms). Therefore, these organisms would represent different food chains and may complement each other in evaluating the bioavailability of mercury in the Penobscot.

The samples of various feeding groups of polychaete worms, described in section V, C, can also be interpreted from a bioindicator perspective, which in this case will be representative of carnivorous, omnivorous and deposit feeding benthic invertebrates.

Study plan: Mytilus edulis will be collected throughout the estuary two to three times per year and analyzed for inorganic and MeHg. At the times of mussel collection, dissolved, particulate, and sediment mercury concentrations from these same areas will also be sampled as part of the biogeochemical studies. An attempt will be made to relate the tissue concentrations of mercury in these mussels with ambient water column and/or sediment concentrations, both seasonally and spatially. Because this mussel will not be able to survive in the low salinities in the river, attempts should be made to sample other prevalent bivalve molluscs from the shores of the river. Comparable water column and sediment mercury analyses will be performed as for *M. edulis*. Sampling, handling, and analytical procedures should follow those of NOAA's National Status and Trends Program.

Killifish (*Fundulus heteroclitus*) and sticklebacks will be sampled for mercury concentration at the same sites as water, sediment and other biota. Concentrations in the fish will be related to that in water and suspended particles and mercury bioconcentration factors will be calculated for sediments, water and food items. Spatial and temporal differences in these concentration factors should reveal trends in the changing bioavailability of mercury over these spatial scales. In addtion to stable isotope studies, analyses of stomach contents will also be carried out to help place the fish species in the food web. Where possible, one-year old fish will be sampled in the early spring and autumn (rather than young-of-the-year fish which are subject to substantial temporal changes in concentrations and burdens of MeHg). During these times growth is slow and temporal variation in tissue mercury levels is less, thus allowing for the most data comparability among sites and years.

VI. REFERENCE SYSTEM(S)

Question: Are mercury concentrations in sediments, water and biota of the lower Penobscot River and Estuary elevated as a result of industrial discharges of mercury?

Rationale: To accomplish certain objectives in the overall study, it will be useful to compare some measurements made in the Penobscot system with measurements made in nearby systems on the northeast coast of North America where point source mercury contamination is absent, and where atmospheric deposition rates and geological characteristics are similar. This approach is most valuable for comparison of sediment and water concentrations, and for comparison of selected biota. The entire food web will not be sampled in the reference system, but bioindicator organisms as well as representative species of benthic organisms, fish, birds and mammals that occur in both the Penobscot River/Estuary and in the reference systems will be studied.

If a suitable reference system can not be found, the study will have to rely only on upstream/downstream comparisons of biota and geochemical mercury concentrations to assess the impact of HoltraChem activities on the Penobscot River/Estuary. The Study Panel recognizes that both upstream/downstream comparisons and comparisons of the Penobscot River/Estuary to reference systems(s) would be a preferable and stronger scientific approach. The isotopic studies described in Section IV,E,3 may also help to distinguish the HoltraChem contribution to the river and bay from background mercury.

Study Plan: If nearby reference river/estuary systems known to be unimpacted by industrial inputs of mercury can be located, a subset of the measurements described above (Sections IV and VI) will be done for samples from several sites in these rivers and their estuaries. One possible candidate for this reference study is the Englishman's Bay and the St. Croix River system, which is north of the Penobscot River/Estuary. The Project Leader will search for additional river/estuary systems along the north-east coast that are unimpacted by industrial mercury discharges. Habitat types for the sampling of the reference systems will be as close as possible to the habitat types sampled in the Penobscot System. In addtion to the biota sampling, on three occasions during one year, samples will be taken for total and MeHg in sediment cores and water.

VII. PROPOSED STUDIES ON TOXIC EFFECTS OF MERCURY IN THE PENOBSCOT ECOSYSTEM

The charge to the Study Panel is to determine whether mercury in the river and estuary has caused or has the potential to cause harm to human health or the environment. In this regard, it is important to distinguish between contamination, which the previous sections of this Study Plan address, and harm. While harm is certainly a potential outcome of contamination, they are by no means equivalent concepts. In the previous section, the emphasis is on determining where elevated mercury concentrations in biota occur, and their pattern with respect to the plant site, methylation sites and bioaccumulation factors. In this section the question is whether the mercury concentrations are elevated enough to cause toxicity.

A. Proposed Work Involving Aquatic Organisms

The toxic effects of sediment-bound mercury and mercury in the water column will be assessed in a series of experimental laboratory studies employing standard toxicological protocols. These studies will begin in the second year, if mercury levels found by the sampling described in section V are high enough to be of concern. The threshold for concern will be established from the current toxicity literature and our consultations with toxicity experts. A detailed study design for the birds and mammals will be developed by the Project Leader in consultation with recognized mammalian and avian experts. Fledgling success will be added if recommended by the avian experts. We will concentrate on concentrations of MeHg. Work will include riverine and estuarine sites.

1. Toxicity to plankton (effects objective 1)

Question: Are concentrations of mercury in selected phytoplankton and zooplankton species high enough to present imminent and substantial endangerment to these species?

Rationale: The toxicity of water column mercury will be tested using phytoplankton and zooplankton. There are standard EPA toxicological testing protocols that should be followed for both types of plankton. We will modify these for zooplankton because these protocols rely on exposing test organisms only to metal in the dissolved phase. Since phytoplankton cells accumulate metal only from the dissolved phase, this is clearly not a problem for these organisms. However, zooplankton, like all other animals, can also obtain their mercury (and other metals) from food as well as from the dissolved phase, and often the dietary source of metal accounts for more bioaccumulation than the dissolved phase. Moreover, dietary metal has proven to be orders of magnitude more toxic in zooplankton than metal obtained from the dissolved phase, primarily because the latter associates principally with the chitinous exoskeleton of microcrustaceans (the dominant zooplankton form in this system), whereas the metal assimilated from ingested food gets delivered to internal organs where it can exert toxic effects. Thus, dietary metals at concentrations approaching those found in contaminated waters have been shown to associate with the ovaries in females where they interfere with vitellogenin production. Consequently, the metals taken in from food interfere with egg production and hatching in daphnids and copepods, whereas metals taken up from the dissolved phase have proven to be innocuous at environmentally realistic concentrations. Therefore, the toxicity of water column mercury to zooplankton should be evaluated with both dissolved and particulate mercury present, which is a better approach than the EPA protocol.

Study plan: To conduct these evaluations, filtered water from sites of interest should be inoculated with EPA-recommended phytoplankton cells from axenic monocultures (obtained from national culture collections). The log-linear cell division rates in these species should be compared with growth rates in control cultures grown in mercury-free water enriched with the same nutrients and exposed to identical light and temperature regimes. Care should be taken to avoid using EDTA and other complexing agents commonly used in algal media, as these would greatly mitigate any toxic effects of the mercury on the cells. For efficiency, this work could be combined with the mercury bioaccumulation work in phytoplankton (described in section V, A). These combined bioaccumulation and toxicity studies with plankton should be conducted in a limited number of sampled waters focusing primarily on those shown to have elevated dissolved mercury concentrations.

For the zooplankton, daphnids such as *Ceriodaphnia dubea* should be used as test organisms for the Penobscot river water and the calanoid copepods *Acartia tonsa* or *Temora longicornis* for the estuarine water in which salinity exceeds 15 psu. As with the phytoplankton, initial emphasis should be place on studying the estuarine forms, and riverine zooplankton should be considered only if preliminary evidence (e.g., elevated chlorophyll levels) suggests that the riverine plankton communities are potentially important conduits of mercury. Standard toxicological protocols should be modified so that these animals are exposed to water filtered through plankton mesh (approximately 100 μm) to remove most animals but which would allow most phytoplankton cells to

pass. Thus the zooplankton will be exposed simultaneously to dissolved and particulate mercury, as they would under natural conditions. Toxic end points should include reproduction (egg clutch size) as well as growth.

2. Toxicity to fish (effects objective 1)

Question: Do fish mercury concentrations affect their reproduction?

Rationale: Recent studies have shown that the reproduction of fish can be adversely affected by exposure to environmentally realistic concentrations of MeHg. Depending on the findings for fish tissue concentrations of MeHg, attempts should be made to evaluate the toxicological effects on the local fish. Recent studies indicate that MeHg concentration of 1 µg/g dry weight in fish food is sufficient to lead to toxic effects in fish. Analysis of biochemical indicators such as hormones may reveal the toxic response of the fish species to tissue burdens of MeHg. Because reproduction is likely to be the most sensitive process in fish, emphasis should be placed on evaluating the effects of MeHg on the reproductive capabilities of local fish. There have been recent advances in understanding toxic effects on reproductive success and these should be implemented for the local fish.

Study plan: Prior to commencement of the fish toxicity studies a literature survey will be done to determine if useful toxicity information is available. If this information is not in the literature, small fish (killifish and/or sticklebacks) will be raised in the laboratory using food of varying, but environmentally realistic, mercury concentrations found. At sexual maturity, the fish will be paired and allowed to reproduce. Concentrations of mercury, testosterone and estradiol will then be measured in the fish as well as gonadal development. Spawning success and the time required to produce a clutch of eggs will as also assayed as a function of mercury concentration in fish food.

B. Toxicity to birds (effects objectives 1, 2)

Question: Are concentrations of MeHg in bird embryos high enough to be affecting reproductive success?

Rationale: Depending on the specie, birds can be high or at the top of the aquatic food chain, and so they may have particularly high mercury concentrations. If the concentrations of mercury measured in section V, E are high enough to be of concern, it will be important to determine if there are toxic effects.

Study plan: Prior to commencement of bird toxicity studies a literature survey will be done to determine if useful toxicity information is available. If this information is not in the literature, toxicity of mercury to bird reproduction could be assessed using cormorants, spotted sandpipers, kingfisher, ospreys, and other aquatic feeding birds that are known to feed and breed in the Penobscot River/Estuary. Attention will focus only on those species with sufficiently high presence in the region to obtain statistically significant results. Concentrations of MeHg in eggs will be assessed, and if egg concentrations are high enough to be of concern, egg-injection experiments similar to those designed by Dr. Gary Heinz of the USGS will be carried out to determine MeHg exposure thresholds for bird embryos. These threshold values will be compared to mercury concentrations of eggs taken from nests located along the Penobscot River/Estuary.

A detailed study design for the bird toxicity studies will be developed by the Project Leader in consultation with recognized avian experts. Fledgling success will be added if recommended by the avian experts.

C. Toxicity to mammals (effects objectives 1, 2)

Question: Are concentrations of MeHg in mammals (specifically river otter and seals) high enough to be affecting their health?

Rationale: The river otter and seals found in the Penobscot River/Estuary are solely fish eaters. Recent studies have shown that concentrations of neural transmitters in the brains of river otter are lowered with increasing concentrations of mercury in the brain. Some of the river otter taken for this study were from the Penobscot River/Estuary, and they were found to have mercury concentrations in their brains that were within the range of toxic effects. Similarly, concentrations of mercury in seals in the Penobscot River/Estuary have recently been found to be high in comparison to other nearby estuaries. There is concern for endocrine disruption in these animals.

If the concentrations of mercury measured in section V, E are as high as those found in the previous other and seal studies, it will be important to determine if there are toxic effects.

Study plan: The Project Leader will review this literature and consult with qualified animal toxicologists to determine the significance of the mercury concentrations found in animals sampled for the work described in section V, E. If they are high enough to be of concern, a detailed study plan for assessing the possible toxic effects of mercury on these animals (i.e. depressed or changed concentrations of neural transmitters and hormones) will be designed during the second year of the project by the Project Leader in consultation with the Study Panel and qualified toxicologists.

VIII. PROPOSED STUDIES OF HUMAN HEALTH RISK ASSESSMENT (effects objective 2)

Question: Are concentrations of mercury in finfish and shellfish in the Penobscot river and Bay high enough to present imminent and substantial endangerment to human consumers?

Rationale: The Study Panel has considered how best to assess whether and to what extent humans living near the lower Penobscot River and Penobscot Bay are exposed to mercury from consumption of locally caught fish and shellfish. Our approach is iterative, and will begin with characterization of the mercury in fish and shellfish from the Penobscot River and Bay. Based on the results from such measurements, the need for and approach to assess human exposures will be developed as described below and in Appendix C.

The conventional pattern used for site-specific exposure studies involves studies at three levels of increasing complexity: (1) determination of the MeHg concentrations in fish and shellfish species in the Penobscot River and Bay that are consumed by local residents, (2) characterization of the amount of fish and shellfish from the River and Bay that is consumed by humans, especially by those who fish or catch shellfish in the Penobscot, and (3) characterization of other sources of MeHg exposure to this population. Fish consumed by humans includes fish caught by recreational fishing, fish caught by

local commercial fisheries and fish caught elsewhere. Items (2) and (3) could be augmented with biomarker data such as mercury concentrations in hair. This would give an estimate of overall exposure to mercury but would not indicate the relative importance of various exposure sources. Such an approach would be accompanied by a questionnaire regarding the frequency, types and sources of seafood consumed by those participating in the study. In addition to providing information on the mix of local and commercial seafood consumed, the questionnaire results would help to identify the relative contribution from locally consumed species. As discussed in Appendix C, there is also a question concerning the human population to be studied if the fish and shellfish sampling indicates that additional studies are justified. One approach would be to attempt to identify recreational anglers in the River or Bay and obtain information about how often they fish, what they typically catch, and what they consume of their catch. An alternative would be to survey general population of residents who live near the River downstream from the plant and near the northern end of Penobscot Bay.

Whatever approach may be taken to assess human exposure in a population representative of either recreational anglers or local residents, both require data collection on the mercury concentration of locally caught seafood and both require information about the rate at which locally caught seafood is consumed relative to other seafood. The available approaches differ in how exposure is estimated; one approach relies on a calculation based on estimated consumption weighted by the mercury concentrations of various species, while the second approach uses hair as a biomarker and would provide a more direct measure of exposure. As described below, a phased approach will be followed.

The first step in assessing whether there is concern for humans who are consuming fish and shellfish from the Penobscot is to compare the mercury concentration data for Penobscot fisheries to established EPA and Maine DEP guidelines. These concentrations will be obtained as described in section VI-D.

The US EPA has set a criterion of 0.3 ppm as the starting point for determining whether reductions in inputs of mercury into a water body are required. Based on the EPA approach, Maine DEP has set a criterion for MeHg in fish tissue of 0.2 ppm.[1] The Maine criterion is more stringent than the default EPA value because Maine residents consume more that the U.S. average amount of seafood. Given that these concentration limits were derived to protect human health, one might reasonably conclude that fish with concentrations above the specified limits would endanger human health if consumed.

Based on data on mercury in fish tissue introduced during the trial and on data recently reported by the US EPA, it appears highly likely that the Maine DEP fish tissue criterion will be exceeded for some fish species. For example, data on the mercury concentrations in American eels in the Penobscot River show average concentrations of about 0.5 ppm. Similarly, the average concentration of mercury in fish from Maine in the data available for the US EPA's National Fish Tissue Study is slightly over 0.45 ppm; above the Maine DEP and US EPA guideline concentrations.[2] All of the EPA samples come from Maine lakes, so no Penobscot River fish are included in this study. The fact

[1] See *Procedure for Developing Fish Tissue Action Levels*, Maine Bureau of Health, available at http://www.maine.gov/dhs/ehu/fish/actionlevels.shtml

[2] Results from the first two years (of a four-year study) from EPA's National Fish Tissue Study are now available. Information about this study is available at http://www.epa.gov/waterscience/fishstudy/.

that fish in many areas of Maine are elevated in Hg will have to be kept in mind when assessing the fish and shellfish Hg data from the Penobscot system.

A component of the fisheries sampling effort will be to develop a better understanding of the species and amounts of seafood produced for human consumption. In our work to date, we have not identified resident fish species that humans consume in the River south of the plant except for American eels. The amount of local consumption of eels was described to us as limited to nonexistent. With exceptions noted in Appendix C, we do not yet have much information about fish and shellfish species consumed from Penobscot Bay.

Study plan: Mercury concentration of finfish and shellfish will be determined as described in section V, D, and these concentrations will be assessed based on the Maine DEP concentration of limit of 0.2 ppm and the EPA concentration limit of 0.3 ppm (see Appendix C for details). Concentrations will be assessed for areas where there are both open and closed fisheries. The need for and design of follow-on human exposure studies will be evaluated based on the mercury concentrations of various fish and shellfish.

IX. DATA SYNTHESIS AND ANALYSIS

The first and second charges to the Panel were to determine: 1) the extent of the existing harm to the Penobscot River and Bay south of the plant site, and 2) the need for a remediation plan, if any. We propose to address these two charges by considering the following:

1) Do fish and/or shellfish exceed the EPA or Maine DEP guidelines for human consumption?
2) Do mercury concentrations in aquatic biota, birds or mammals result in measurable toxic effects?
3) Do toxicity tests indicate that there are toxic levels of mercury in the water and/or sediments?
4) Is there evidence that excessive levels of mercury are linked to the HoltraChem plant site?

The data that will be used for answering questions 1 through 3 are obvious, and described in Sections VI and VII above. Data from all sections of the proposal will be needed to answer question 4, including measurements on the system itself as well as upstream and downstream comparisons, and comparisons to a reference system.

Data for mercury concentrations in the various compartments within the Penobscot ecosystem will be analyzed statistically to assess trends in mercury concentrations and bioavailability, and to relate bioaccumulation and effects to specific environmental or biological factors (e.g., sediment grain size, presence of certain plankton species, dissolved organic content of the water, temperature, etc.). Appropriate statistical treatment of data through parametric and non-parametric tests, principal components analysis, etc. will be applied.

If remediation is deemed necessary, the data collected for the studies described here will be useful in making choices about specific remediation approaches. Because of the physical and biological complexity of this estuarine ecosystem, we think it is likely that a mechanistic model will be useful in this process, although it is premature at this point to identify the specific model that we would use to evaluate mercury's fate and

effects in the Penobscot. With a model, sensitivity analyses can be performed to assess possible radiation approaches, e.g., the likely impacts of dredging a particular salt marsh, which has been identified as a source of active methylation, on fish tissue mercury concentrations.

Appendix A: Historic and Cumulative Releases from the Site.

The amount of mercury released from the plant over time and the pathways through which it was released are highly uncertain. Information was presented during the trial that indicates that in the early years of plant operation (1967 to 1970), mercury losses from the plant averaged 107 pounds per day. Of this amount, it was estimated that 19 pounds were lost through brine sludge, but that some of the mercury in the brine sludge was recycled back into the system (from Joint Exhibit 64, Stipulations Regarding Testimony of Peter DeAngelis). Until 1970, the brine sludge was discharged directly into the Penobscot River. In 1970, a pond was constructed to receive the brine sludge, and it is estimated that direct discharges to the river were reduced. Exhibit 64 indicates that more mercury was discharged through air emissions than through the facility outfall.

The large uncertainties in the historical releases from the HoltraChem plant are typical of chlor-alkali facilities. In a July 2004 report to EPA, the Chlorine Institute summarized mercury purchases, use, and releases from U.S. chlor-alkali plants. This report indicates that in 2003, the most recent year for which data are available, 38 tons of mercury was used at the 9 plants currently operating. Of this 38 tons, 8 tons were reported as released to the environment, while 30 tons were describe as unaccounted for mercury. This is in spite of much increased scrutiny of mercury releases in recent years and enhanced controls to prevent environmental releases. The amount of mercury released and unaccounted for in 2003 works out to about 23 pounds per day per operating plant, of which 18 pounds are unaccounted for and 5 pounds per day are reported as released. This wide range of release rate estimates, from 5 pounds per day to over 100 pounds per day, suggests that perhaps the cumulative releases from the plant over its 33 year operating life were between 30 and 640 tons, or between roughly 1 and 20 tons per year. As a point of comparison, the average wet deposition measured at the Acadia National Park monitoring station is about 8 μg per m^2 per year. If this deposition rate is representative of that throughout Maine, the annual average wet deposition over the state works out to about 0.75 tons per year.

Appendix B: Organization and Management

The Study Panel, consisting of J. Rudd, C. Whipple, and N. Fisher, will act as an oversight and advisory group to the Consultant who will act as the Project Leader, and the Study Panel will have ultimate responsibility concerning design and changes to the study plan. Significant decisions by the Consultant will require the Study Panel's approval, and may also require approval by the Court. The Study Panel will also participate in interpretation and analysis of data. It is anticipated that the Study Panel will have conference calls with the Consultant on biweekly or as needed schedule to stay appraised of progress and to assist with problems that may arise

The Consultant serves as a Principal Investigator and Project Leader, and is responsible for bringing in outside contractors as needed to implement the study plan. The outside contractors will be chosen in consultation with the Study panel. Specific responsibilities of the consultant include ensuring that sampling plans are designed such that an appropriate number of samples are collected, permits needed for sampling are obtained, and that proper quality control of samples, sample analyses, and data are maintained. The Consultant will be responsible for preparing budgets for all work to be done and for keeping the Court informed of budgetary issues. The Consultant will require that any contractor personnel who do field work have appropriate health and safety training and equipment.

We envision that the consultant would essentially work full time on the project for the next several years, concentrating on data collection during the ice-free season and data analyses during winter. The Consultant will contract directly with the Court and will submit quarterly progress reports to the Study Panel. The Study Panel will submit quarterly reports of its activities, and the progress of the study to Judge Carter. The Study Panel reports will be forwarded to the plaintiffs and defendants.

An annual meeting will be held in the late winter of each year of the study. The purpose of the meeting will be to review progress and deficiencies of the study and to receive input from those attending the meeting on how the project design could be improved for the upcoming field season.

The criteria used to select the Consultant, who will be recommended to Judge Carter as the Project Leader, are as follows:

1. Experience with mercury in aquatic systems.
2. Field experience.
3. Scientific knowledge of mercury in the environment.
4. Standing in the scientific/technical community.
5. A biological background.
6. Experience with data management and analyses of large projects.
7. Demonstrated success in managing interdisciplinary mercury projects.
8. Institutional capabilities.
9. Availability for this project to be their sole responsibility.
10. Perceived flexibility in working with the Study Panel.
11. Clarity of written and verbal communications.
12. Willingness to relocate to the Penobscot River/Estuary during the summer field season.
13. Ability to get along with people.

14. Will get tasks done on time.

Appendix C: Comments Regarding Comparisons of Fish Hg Concentrations or Intake Rates with Reference Levels

In examining the total risk from recreational fishing, ideally, one would like to know how often individuals fish in the River or Bay, how much they catch, and how much of what they catch they eat or give away for others to eat. This information is typically obtained through a creel survey. As noted in Section VIII, it may be useful to collect hair samples from individuals who are queried regarding they seafood consumption habits.

In our review of the trial exhibits, other documents, and in conversations with individuals knowledgeable about the lower Penobscot, we have not found any indication that a creel study has been done. A statewide survey of recreational fishing was cited during the trial and included as an exhibit, but based on the specific types of fish found in the Penobscot River, we do not believe that this survey is applicable for estimating consumption on the River. Based on our discussions with staff at Maine DEP and DMR and from our trip down river by boat, we have obtained the impression that not too many people fish in the river south of Veazie dam but north of Verona Island. Obtaining a better estimate of how many people fish in the River and during what part of the year they fish is necessary to determine the feasibility and design of a creel study.

Striped bass are also found in the River and Bay. However, they are not resident in the River and their mercury concentrations may be due in part, perhaps predominantly, to consumption of ocean fish, rather than to mercury in the Penobscot system. Good data are available on lobster landings all along the Maine coast, including some information on lobster prevalence in the estuary, but measurements of mercury concentrations in lobster are scarce.

In her report on the Penobscot River prepared for the Study Panel, Karen Merritt did not identify any information relevant to human consumption of seafood from the Penobscot River south of the Veazie Dam and north of Verona Island. With respect to seafood sources south of Verona Island and into the estuary, she notes:

> …while NOAA ELMR data suggest that blue mussels, sea scallops and softshell clams are somewhat common in the estuary, the estuary has been closed for shellfish harvesting since December 1,1999. Maine DMR legally prohibits the harvesting and sale of all shellfish found north of an E-W line drawn between Fort Point and West Penobscot (Closed Area 35, effective December 1999) (www.maine.gov/dmr/rm/public_health). Furthermore, in June 2004, DMR officially closed the western shore of West Penobscot as far south as the entrance to Castine (on approximately the same parallel as Turtle Head on Islesboro) (Closed Area 36, effective June 1, 2004). While shellfish living in these closed areas may certainly be actively bio-accumulating what toxins are present, the closures effectively limit concerns regarding potential human exposure through shellfish consumption.

The extent of this posting is illustrated by the order and map at http://www.state.me.us/dmr/rm/public_health/closures/35.pdf. In addition to this

prohibition against harvesting shellfish in the estuary, there is a statewide advisory regarding freshwater fish consumption due to mercury.

The extent to which there is compliance by private citizens with these advisories and with the prohibition against harvesting shellfish is not known. Efforts to develop such information should begin with discussions with people in the Maine DEP and DMR regarding recreational fishing in these areas. The purpose of these discussions is to explore the feasibility of a survey that may be performed in this part of the river system.

Based on the information we have been able to develop to date, it is not clear that there are sufficient numbers of recreational anglers on the River to allow for a creel survey. If it is determined that recreational fishing on the Penobscot occurs south of Veazie dam and that a creel survey is feasible, and if fish and shellfish tissue sampling exceeds the Maine DEP criterion, we will recommend that one be performed. Depending on what is learned in a creel survey concerning consumption of self-caught fish, additional fish sampling may be required to understand the MeHg concentrations of the consumed fish and shellfish. If a creel survey appears to be difficult due to a limited number of recreational anglers, an alternative would be to conduct a survey of seafood consumption rates and sources, perhaps in conjunction with collection of hair samples, amount residents along the lower part of the River and northern part of the Bay. Such an approach would provide a more representative picture of exposures and sources than a creel survey of recreational anglers, but might miss the most highly exposed populations. Before any such study was designed, appropriate expertise would be brought in to write the questionnaire and to determine the size of a study population needed for statistically valid results. Other considerations include the likelihood that consumption of fish and shellfish from the River or Bay varies with the season.

For commercial fisheries, we have identified no information indicating that any commercial finfisheries are operating in the Penobscot River or Bay, although eels are fished commercially and exported. There are active lobster, crab and clam fisheries, and we will analyze catch from these fisheries for THg and MeHg concentrations.

With respect to an overall human exposure assessment, in addition to information about locally caught and consumed seafood, data on the total consumption of seafood by those individuals who fish in Penobscot River or Bay is necessary to characterize total seafood consumption. This would allow the contribution to MeHg exposure from locally caught seafood to be evaluated along with information about the total mercury exposures of these individuals.

Data from the Center for Disease Control's National Health and Nutrition Evaluation Survey (NHANES) indicate that about 6% of US women of childbearing age are exposed to mercury at levels above that associated with the EPA reference dose. The percentage of women in Maine above the RfD is likely to be higher than 6%, for several reasons. First, according to EPA's Exposure Factors Handbook, people in New England eat more seafood, on average, than in other regions of the US. Second, census data indicate that, on average, Maine residents spend more time participating in recreational fishing than the US average. Third, the results for the first two years of EPA's National Fish Tissue Study indicate that wild freshwater fish in Maine have higher average mercury levels than fish in most other states. In contrast to average concentration in Maine of 0.45 ppm, the average concentration in all the samples in EPA's study is 0.22 ppm. Of the 44 states for which data are reported (no fish tissue data are available for

AK, DE, HI, MD, MO, or RI), Maine has the third highest average concentration. So some fraction of Maine women of childbearing age, perhaps 10% to 15%, are already exposed to MeHg at levels above that recommended as the maximum safe level by EPA, and any incremental exposure to MeHg should be avoided. Whether the presence of mercury in fish in the Penobscot River would present "an imminent and substantial endangerment to public health" to such women is a relevant question. While an objective of this study is to assess the degree to which past and ongoing releases from the Holtrachem site contribute to human exposure, some fraction of Maine residents are exposed to mercury at levels above EPA recommendations absent any contributions from the plant.

These data, viewed along with the fact that fish consumption advisories to avoid excessive mercury exposures have been set on a statewide basis in Maine, suggest that mercury in freshwater fish is too high in most lakes and rivers. The Study Plan includes measurements designed to determine the extent to which mercury levels in biota in the Penobscot River and Estuary are elevated due to releases from the Holtrachem Site or are reflective of high regional mercury levels. The point of these comparisons is not to ignore a potential source of human health risk, but rather to understand whether the problem is local or regional.

The available data do not clearly resolve this question. For example, the mercury concentrations in eels (based on very limited sampling) appear similar to those of eels sampled at other Maine locations. Similarly, mercury concentrations in lobsters sampled along the Maine coast do not indicate that concentrations in lobster meat are higher near the Penobscot Estuary than in other locations (however, lobster tomalley may be slightly higher). Conversely, mercury levels in mussels sampled in the Penobscot River appear significantly higher than is typical for mussels in Maine and elsewhere. All of these datasets include relatively limited numbers of samples.

A similar difficulty will accompany the determination of whether a nonhuman species is endangered by high levels of mercury in the Penobscot system. Data reviewed by the Study Group suggests that mercury levels in loons increase with proximity to the Site. Mercury in top piscivorous birds along the river, e.g., eagles and osprey, appear to be high, but whether they are high in comparison to other Maine locations has not been established.

The data mentioned here suggest that further study is needed to determine whether mercury concentrations in biota may endanger human health and the environment. Whether these conditions are the result of Holtrachem mercury releases or reflect regional mercury contamination is a key objective of the Study Plan.

Table I. Chronology of Proposed Studies

The following table describes the chronology of the field and laboratory studies that will be done over a three year time period. In addition to this work, we propose a fourth year during which time the lead consultant and Study Panel would synthesize the data and write a comprehensive report for submission to Judge Carter.

	Year 1												Year 2												Year 3											
	j	f	m	a	m	j	j	a	s	o	n	d	j	f	m	a	m	j	j	a	s	o	n	d	j	f	m	a	m	j	j	a	s	o	n	d
IV, Biogeo. studies																																				
A, site description[1]																																				
B, aerial deposition																	*	*	*	*	*	*	*													
C, 1, Hg in water																																				
2, past & present discharges																																				
a, particulate Hg																																				
b, Hg isotopes																*	*	*	*	*	*	*	*													
3, ongoing inputs													?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
4, mass flux																																				
D, sediment accum.																*	*	*	*	*	*															
E, 1, % MeHg																																				
2, MeHg flux from wetlands																																				
3, MeHg isotopes																												*	*	*	*	*	*	*		
V, Bioaccumulation																																				
A, Food web isotopes																	*	*	*	*	*	*	*	*	*	*										
B, plankton																																				
C, benthos																																				
D, higher tropic levels																																				

Chronology cont.

	Year 1											Year 2											Year 3											
	j	f	m	a	m	j	j	a	s	o	n	d	j	f	m	a	m	j	j	a	s	o	n	d	j	f	m	a	m	j	j	a	s	o	n	d
E, birds & mammals																																				
F, bioindicator organisms																																				
VI, Reference System(s)																																				
VII, Toxic Effects																																				
A, 1, plankton																																				
2, fish													*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
B, Birds													*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
C, Mammals													*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
VII, Human health																																				

* Studies will commence only if concentrations of mercury in wildlife species and/or in biota that could be potentially be used for human consumption are high enough to be of concern.

[1] Site characterization will be completed prior to the start of the main project.

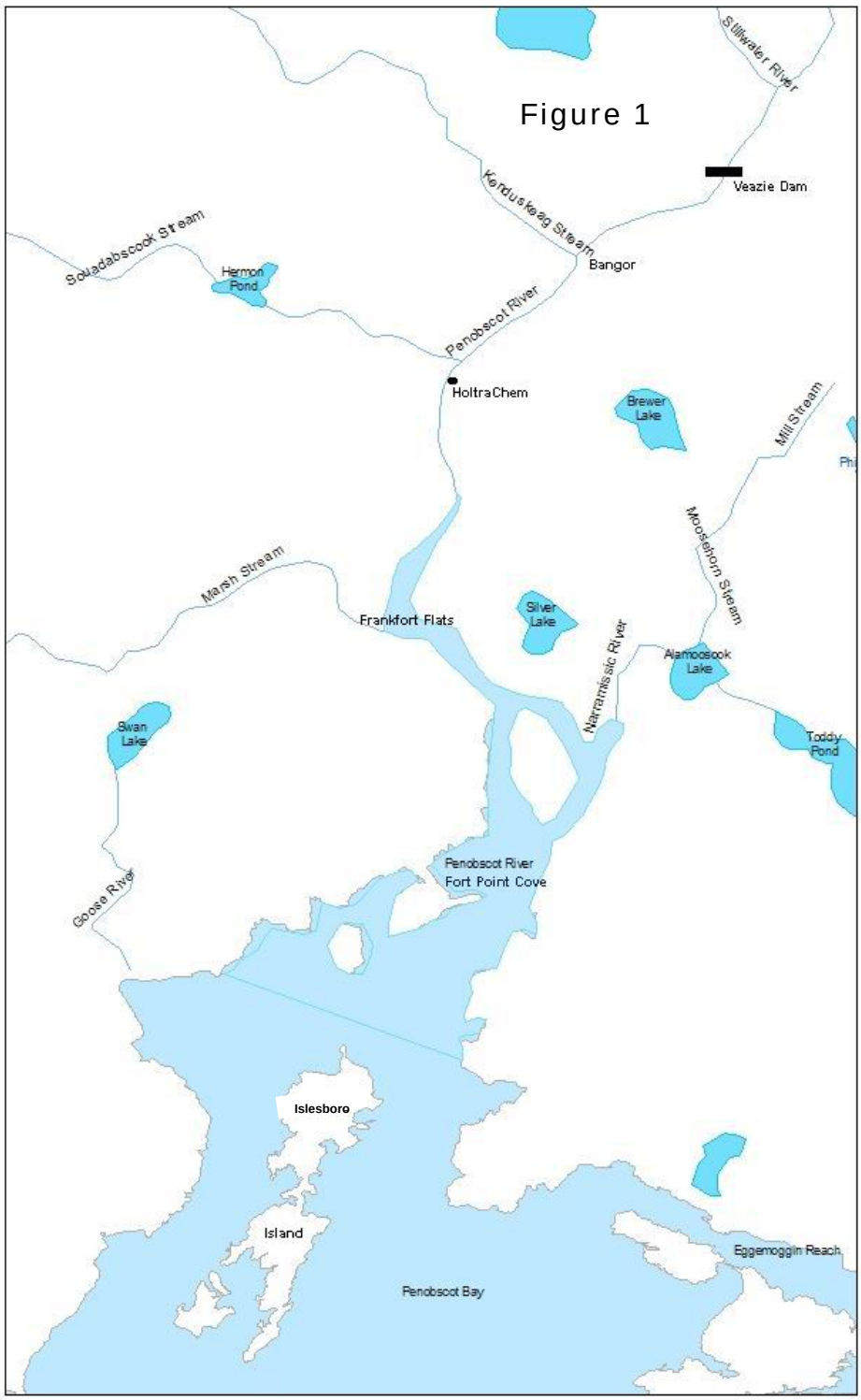
Figure 1
Stillwater River
Veazie Dam
Kenduskeag Stream
Bangor
Soladabscook Stream
Hermon Pond
Penobscot River
HoltraChem
Brewer Lake
Mill Stream
Marsh Stream
Frankfort Flats
Silver Lake
Mooseshorn Stream
Alamoosook Lake
Narramissic River
Swan Lake
Toddy Pond
Penobscot River
Fort Point Cove
Goose River
Islesboro
Island
Eggemoggin Reach
Penobscot Bay