1.0 EXECUTIVE SUMMARY

A pilot study was conducted during the summer of 2000 on the Kennebec River, Maine to evaluate the ability of caged freshwater mussels to monitor and assess the bioavailability of dioxins (polychlorinated dibenzo-dioxins, PCDDs), furans (polychlorinated dibenzo-furans, PCDFs), and polychlorinated biphenyls (PCBs). The caged mussel study had two purposes: (1) to determine whether this approach would be a reasonable surrogate for resident fish used in upstream versus downstream comparisons of chemical exposures associated with pulp and paper mills, and (2) to identify hotspots of polychlorinated biphenyls (PCB) contamination in the lower Kennebec River. Because of the limitations of fish sampling and dams on the river, the nearest upstream location (Norridgewock) where fish could be collected was approximately 13 miles from the mill, and the nearest downstream location (Fairfield) was approximately 11 miles from the mill. Caged mussels were deployed at these locations.

The State of Maine Department of Environmental Protection (DEP) has expressed concern regarding the ability to detect statistically significant differences in chemical exposure when comparing upstream and locations from pulp and paper mills due to declining tissue concentrations of dioxins and furans in fish. These comparisons are important because environmental regulations do not allow significant differences in upstream versus downstream exposures associated with those effluents. The Friends of Merrymeeting Bay (FOMB) have expressed concerns regarding hotspots of PCBs as well as dioxin/furan contamination on the Kennebec River associated with elevated exposures and possible adverse biological effects. FOMB and others have identified problems with monitoring indigenous fish populations for upstream/downstream comparisons at mill sites, including uncertainty associated with mobility, accumulation from other sources, previous mill discharges sequestered in sediments, and the inability to collect fish near the mill discharge. As with the PCB monitoring, FOMB supported the caged mussel pilot study anticipating that concerns regarding fish monitoring could be eliminated by using a surrogate, such as caged mussels, that could be deployed closer to the mill discharge where fish could not be collected. DEP focused on upstream/downstream locations where mussels could be compared directly with fish data.

DEP is responsible for developing a monitoring program to assess the nature and extent of dioxin and furan contamination in the waters and fisheries of the state. Maine has adopted the most stringent environmental regulations for dioxins in the US, and the primary objective of the dioxin/furan monitoring program is to assess potential ecological and human health effects by measuring chemical exposure in fish tissues. A secondary objective is to document the status and trends in of dioxin/furan exposures, evaluate progress in reducing environmental concentrations by compliance with existing regulations, and the need for even more stringent regulations. The third, and most specific objective is to determine if kraft pulp mills are discharging dioxins or furans into the rivers of Maine. A state law enacted in 1997 prohibits such discharges and requires compliance by December 31, 2002. In practice, environmental exposures of dioxins and furans estimated by measuring concentrations in fish tissues or some surrogate, cannot be higher downstream of a pulp mill discharge than upstream. This is commonly referred to as the “above/below” test.

In 2000, DEP continued to develop an appropriate "above/below" fish test, but as dioxin and furan concentrations decline, there are concerns that the existing monitoring approach may not be sufficiently sensitive to detect statistically or environmentally significant differences in exposure to properly evaluate compliance with the 1997 state law. Although concentrations of dioxins and furans measured in fish tissues were higher below than above pulp mill discharges in 1999, questions have been raised about the suitability of fish as effective monitors. These questions are related to: 1) The mobility of fish and where exposure to dioxins and furans actually occurred, 2) Whether fish accumulated dioxins and furans from sediment or food that was contaminated from previous, rather than recent mill discharges and 3) When exposure and accumulation in collected fish occurred. In response to some of these questions, DEP modified the 2000 fish monitoring program to include measuring dioxins and furans in tissues of caged mussels and in lipids of semi-permeable membrane devices (SPMDs) as potential surrogates for monitoring dioxins and furans in fish tissues.

Caged freshwater bivalves have been used to monitor dioxins and furans associated with pulp and paper mill effluents in Finland and for similar chemicals in Canada for approximately 20 years. Environment Canada has recently adopted caged bivalve monitoring as an alternative to the required adult fish survey in their Environmental Effects Monitoring (EEM) at pulp and paper mills in Canada. Standardized protocols have been adopted by the American Society for Testing and Materials (ASTM) for conducting caged bivalve studies, and a standard guide appeared for the first time in the 2001 ASTM Annual Book of Standards. Caged bivalves are a potentially powerful tool because of their ability to quantify exposure and effects over space and time. *In situ* studies with caged bivalves could complement and help establish links between various elements of the existing DEP monitoring program through the use of tissue chemistry and mussel growth measurements. This approach could also help reduce uncertainty in the current approach and answer questions within government, industry, and the public regarding chemical exposure and biological effects associated with pulp mill effluents. It is also consistent with the ecological risk assessment process of characterizing exposure through bioaccumulation and characterizing effects through mussel growth rates.

For both studies, freshwater mussels (*Elliptio complanata*) were collected from Nequasset Lake, a relatively clean lake within the Kennebec watershed in Woolwich, Maine, caging individuals of a minimum size range, and transplanting them to upstream and downstream (dioxin/furan) and gradient (PCB study) location on the Kennebec River. *Elliptio* were deployed for 53 days. After retrieval, the soft tissues of mussels were measured for PCBs or dioxins and furans, percent lipids, and percent moisture. Survival and growth of caged mussels indicated they were all in adequate health to accumulate ambient dioxins, furans, and PCBs if present. Mean concentrations of total PCBs in mussels increased from below detection at the beginning of the test to 2.7 to 188 ug/kg-dw at the lower Kennebec River stations at the end of the test. Most of the total PCB concentrations measured in mussel tissues were between 20 and 60 ug/kg-dw (~4 to 12 ug/kg-ww). The three highest values were above the fish tissue action level (FTAL) for screening evaluations of 11 ug/kg-ww for cancer endpoints. No measurements were above the FTALs of 43 ug/kg-ww for non-cancerous endpoints. The highest concentration of total PCBs (188 ug/kg-dw, ~37.6 ug/kg-ww) was measured in mussel tissues from midstream just below the Augusta Sewage Treatment plant at South Augusta and in the vicinity of a midstream outfall pipe. The second highest concentration of total PCBs (125 ug/kg-dw, ~25 ug/kg-ww) was measured in

mussels deployed on the west side of the Kennebec River, just below the former Williams gravel/asphalt facility (now Ferraiolo) in Farmingdale.

Mean concentrations of total PCDD/PCDF in mussels increased from below detection at the beginning of the test to 4.33 and 4.67 ng/kg-ww at the upstream and downstream stations, respectively, at the end of the test. These concentrations are both above the FTALs for screening evaluations of 1.5 ng/kg-ww for cancer endpoints and 1.9 ng/kg-ww for non-cancerous endpoints. The concentrations of dioxins and furans measured in mussel tissues are approximately four orders of magnitude lower than most of the PCB concentrations measured in mussel tissues. The units of the dioxin measurements (ng/kg-ww = parts per trillion) are three orders of magnitude lower than the PCB units (ug/kg-dw = parts per billion).

There was no statistically significant difference between upstream and downstream total PCDD/PCDF concentrations at the end of the test. More individual dioxin/furan congeners were measured in mussel tissues from both upstream and downstream locations than in SPMDs or fish tissues. Given that the downstream site was located 11 miles away from the mill, this result was encouraging. However, concentrations of the most predominant dioxins and furans in mussel tissues were not significantly higher downstream than upstream. In fact, the predominant dioxins (123478-HpCDD and OCDD) were higher upstream than downstream.

The concentrations of total dioxins and furans in fish tissues were significantly higher 11 miles downstream (4.19 ng/kg-ww) than 13 miles upstream (2.76 ng/kg-ww) of the mill. These data suggest that fish are more efficient accumulators of dioxins and furans than mussels or SPMDs, and the existing fish monitoring approach is appropriate. However, on a lipid-normalized basis, concentrations of total dioxins/furans in fish collected at upstream and downstream stations are not significantly different. As with the data for mussels and SPMDs, the lipid-normalized concentrations for fish are higher upstream than downstream, but not significantly different. These data reinforce the significance of the important questions mentioned earlier regarding where the fish were exposed to dioxins and furans, whether they accumulated dioxins and furans from sediment or food that was contaminated from previous, rather than recent mill discharges, or how long ago exposure and accumulation occurred.

These questions, as well as concerns regarding upstream and downstream comparisons, may be addressed, at least in part, by using a weight of evidence approach and a more careful scrutiny of the total concentrations of dioxins and furans measured in each test matrix (mussels, SPMDs, fish), the lipid normalized concentrations, and the individual congener analysis. A more direct approach would be to repeat the caged mussel pilot study with more stations closer to the mill. Downstream mussels accumulated 13 congeners, SPMDs, 12, and fish only 5. Upstream mussels accumulated 15 congeners, SPMDs 11, and fish only 4. Although the fish appeared to be the most suitable monitoring tool based on total dioxins and furans, the congener analysis and the lipid-normalized data suggest that they are not. On a congener basis the data suggest that mussels and SPMDs are more representative of all dioxin and furan exposures. The data further suggest that the upstream and downstream locations are inappropriate since the upstream station appears to be contaminated by another source upstream of Norridgewock. The downstream station was

too far away to be sure that the fish are being exposed to dioxins and furans from the SAPPI mill in Hinckley. While the experimental design in the caged mussel pilot study was appropriate for comparing dioxin and furan exposures with those in fish and SPMDs, it was not appropriate for addressing the upstream/downstream issues concerning these potential fish surrogates. That would be a gradient design as used in most effluent monitoring studies. Caged mussels and SPMDs should have been placed as close to the pulp mill discharge as possible for a more accurate evaluation of their ability to detect upstream/downstream differences.

This integrated pilot study compared three approaches as alternative monitoring tools for assessing the fate and effects of dioxins and furans associated with a pulp mill effluent. While water samples have been used to characterize aqueous chemical exposures for over 50 years, new elements used here include the use of caged mussels to integrate chemical exposure and associated biological effects. Caged mussels have been used for approximately 30 years, but recent refinements have increased the sensitivity of this approach to a new level, and these methods have only recently been adopted by the ASTM. SPMDs represent the newest of these methodologies and applications of this approach are still being refined. This study is unique not only in terms of comparing these three monitoring methods, but applying them in areas where they have not been commonly measured in Maine, using state-of-the-art chemical analyses with low detection limits, and using extensive experience and expertise to interpret the results of congener analysis (i.e., dioxins, furans and PCB congeners) and mussel growth rates.

There are too many uncertainties in the results from accumulation of dioxins and furans in caged mussels, SPMDs, and fish tissues to unconditionally accept the results and make important programmatic decisions regarding the utility of these three methods. Another pilot study is suggested that directly tests the utility of the caged mussel methodology (and SPMDs) using a gradient design downstream from the mill and placing cages as close as possible to the effluent discharge. The weight of evidence from bivalve biomonitoring studies conducted on dioxins, furans, and PCBs throughout the world suggest that caged bivalves can be an effective monitoring tool for pulp and paper mill effluents in the State of Maine. This is not to say that bivalves should be the only monitoring tool. Most experts have agreed that there is no perfect monitoring tool and that a weight of evidence approach should be used to make the most meaningful assessments. It seems reasonable to assume that a triad approach using caged mussels, SPMDs, and fish would provide DEP with the best possible data to make informed decisions with respect to potential exposure from dioxins and furans from pulp and paper mills and from hotspots of PCB contamination on the Kennebec River.

2.0 INTRODUCTION

A caged mussel study was conducted in the Kennebec River, Maine during the summer of 2000 to determine the applicability of this approach for monitoring PCBs, dioxins, and furans. This study was conducted under the auspices of and funded by the Maine Department of Environmental Protection (DEP), and was consistent with their environmental monitoring strategy for dioxins and furans. However, the study never would have been conducted without the development, encouragement, and assistance from the Friends of Merrymeeting Bay (FOMB), a regional environmental organization. DEP began a standardized biological monitoring program in 1983 (Davies et al. 1999) acknowledging that the best way to assess water and sediment quality is through integrated biomonitoring, as opposed to only chemical monitoring of water, sediment, and tissue. By placing emphasis on tissue chemistry and associated biological effects, it is possible to more directly determine the degree of ecological impact caused by chemical exposure. Traditional measures of water and sediment quality provide only an indirect way to assess effects because such approaches do not measure biological responses or account for the interaction of physical, chemical, or biological factors. FOMB have expressed concerns regarding hotspots of PCBs as well as dioxin/furan contamination on the Kennebec River associated with elevated exposures and possible adverse biological effects. FOMB have identified problems with monitoring indigenous fish populations. Problems with using natural fish populations for upstream/downstream comparisons for mill sites include uncertain exposures associated with the following: mobility, accumulation from other sources, previous mill discharges sequestered in sediments, and the inability to collect fish near the mill discharge. FOMB supported the caged mussel pilot study anticipating that concerns regarding fish monitoring could be eliminated by using a surrogate, such as caged mussels, that could be deployed closer to the mill discharge where fish could not be collected. DEP focused on upstream/downstream locations where mussels and SPMDs could be compared directly with fish data.

This report summarizes the tissue chemistry and effects data collected in 2000 to assess the bioavailability of dioxins and furans associated with the South African Paper and Pulp Industries, Ltd. (SAPPI) pulp and paper mill near Hinckley and characterization of PCBs along a suspect reach of the Kennebec River.

2.1 Study Objectives

The objective of the dioxin study was to determine if caged bivalves are a viable alternative to resident fish in assessing bioavailable dioxins and furans. This would be accomplished by determining whether these bivalves accumulated significantly higher concentrations of dioxins at the downstream station when compared to the station upstream of the SAPPI pulp and paper mill. The downstream station was the closest site where fish could be collected because it was only just above here that a dam prevented the fish from access to upstream habitat. The objective of the PCB study was to help identify contaminated areas and their potential sources along one suspect reach of the lower Kennebec River.