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Biological Effects of Mercury-Contaminated Sediment in Northern Thunder Bay, Lake Superior

BY:

D. Milani
L.C. Grapentine

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BIOLOGICAL EFFECTS OF MERCURY-CONTAMINATED SEDIMENT IN NORTHERN THUNDER BAY, LAKE SUPERIOR

D. Milani and L.C. Grapentine

NWRI Cont. # 05-326

Environment Canada
Aquatic Ecosystem Impacts Research Branch
National Water Research Institute
867 Lakeshore Road, P.O. Box 5050
Burlington, Ontario L7R 4A6

ABSTRACT

There are elevated levels of mercury in sediments of northern Thunder Bay (inner harbour), Lake Superior. To assess impacts on invertebrate communities, sediment toxicity, and the bioavailability of this mercury and its potential for effects on fish and wildlife through biomagnification, a study was conducted involving (a) comparisons of benthic invertebrate communities and laboratory toxicological response to those established for Great Lakes reference sites, (b) comparisons of total and methyl mercury concentration in sediment and resident benthic invertebrates from Thunder Bay to those from Lake Superior reference sites, (c) analyses of the relationships of total and methyl mercury concentrations in invertebrates to those in sediment, and (d) predictions of methyl mercury concentrations in representative consumers of benthic invertebrates and their predators using screening-level trophic transfer models.

In September-October 2002, sediment, overlying water and benthic invertebrates were collected from 19 sites in northern Thunder Bay Harbour and 20 Lake Superior reference sites (located outside Thunder Bay). Samples were analyzed for total and methyl mercury concentrations and a series of physico-chemical variables were measured in the sediment and overlying water. Benthic community structure and toxicity were assessed using multivariate techniques (ordination). Mercury concentrations in sediment and two resident invertebrate taxa (chironomids and oligochaetes) collected at Thunder Bay sites were compared to concentrations at Lake Superior reference sites. Relationships between mercury in each invertebrate taxon and mercury in sediment were evaluated by regression analysis. Physico-chemical sediment and water variables were included as additional predictors. Concentrations of methyl mercury in the tissues of fish and wildlife receptors (White sucker, Yellow perch, Walleye, Great Blue heron, Mink) were predicted by multiplying measured body concentrations in the resident invertebrates by relevant biomagnification factors obtained from a review of pre-existing studies.

Sediment total and methyl mercury concentrations are elevated above reference at all Thunder Bay sites. Total mercury concentrations, which range from 0.03 to 39.7 μg/g, are above the Lowest Effect level at most sites and above the Severe Effect Level at 7 sites. Total organic carbon is also elevated in the sampling area and range from 1.1 to 25.7%.

Thunder Bay benthic communities are mostly very different than reference generally due to increased taxa diversity with the absence of a key haustoriid amphipod and enrichment of more tolerant organisms such as tubificids and chironomids or to decreased taxa diversity and increased abundance of more tolerant organisms. Enrichment is associated with increased total organic carbon in some cases. There is acute toxicity evident at five sites; toxicity can only be partially explained by mercury in some cases.

Total mercury concentrations in resident chironomids at most Thunder Bay sites are elevated above concentrations at reference sites; methyl mercury is elevated above reference at about half the Thunder Bay sites. Methyl mercury in resident oligochaetes exceeds the maximum for reference sites only at a few Thunder Bay sites. Total mercury in chironomids is significantly influenced by total mercury in sediment (adjusted $R^2 = 0.867$); for oligochaetes – with the addition of manganese. Methyl mercury in chironomids is also significantly influenced by methyl mercury in sediment (adjusted $R^2 = 0.466$); oligochaete methyl mercury is significantly influenced by sediment methyl mercury and total nitrogen (adjusted $R^2 = 0.380$). Under generally "intermediate and maximum" exposure and trophic transfer scenarios, from 7 to all Thunder Bay sites are predicted to have receptor methyl mercury concentrations elevated above the tissue residue guideline for the protection fish-consuming wildlife for two of the three fish receptors. Of these, 5 – 8 sites have predicted methyl mercury concentrations in receptors above maximum reference site concentrations. Therefore, mercury is transferred from sediment to benthic invertebrates and could bioaccumulate in receptors to levels that are not protective of adverse effects at 5 – 8 sites. The likelihood of realizing this degree of mercury biomagnification is not clear due to uncertainties associated with predicting receptor mercury concentrations. However, mercury levels in sport fish collected from the inner Thunder Bay Harbour are above guideline values, indicating that mercury is accumulating in higher trophic level organisms.

According to a decision-making framework for sediment contamination, a rule-based weight of evidence approach that combines all lines of evidence to achieve an overall assessment on a site by site basis, 9 sites require management actions.

FRENCH VERSION

Milani, D., et L.C. Grapentine. 2005. « ».

Résumé

Les concentrations de mercure dans les sédiments de la portion nord de la baie Thunder, au lac Supérieur, un secteur préoccupant, sont élevées. L'impact de ces concentrations sur les communautés d'invertébrés et la toxicité des sédiments et la biodisponibilité de ce mercure ainsi que ses effets potentiels par bioamplification pour les espèces sauvages ont été évalués en septembre et en octobre 2002. Cette évaluation reposait sur les éléments suivants:
a) comparaison à l'aide de méthodes d'analyse multivariée (ordination) des communautés d'invertébrés benthiques et de la réponse toxicologique en laboratoire à celles établies pour des stations de référence dans les Grands Lacs; b) comparaison des concentrations de mercure total et de méthylmercure dans les sédiments et chez deux invertébrés benthiques (oligochète et chironomide) de la baie Thunder à ces mêmes concentrations dans des stations de référence réparties à l'échelle du lac Supérieur; c) examen au moyen d'analyses de régression de la relation entre les concentrations de mercure total et de méthylmercure observées chez les invertébrés à celles décelées dans les sédiments; d) prévision des concentrations de méthylmercure chez des consommateurs représentatifs d'invertébrés benthiques et leurs prédateurs (meunier noir, perchaude, doré, grand héron, vison) à l'aide de modèles de transfert trophique de présélection et des facteurs de bioamplification établis dans le cadre d'études antérieures.

Des échantillons de sédiments de surface, d'eau sus-jacente aux sédiments et d'invertébrés benthiques ont été prélevés dans 19 stations réparties dans la portion nord de la baie de Thunder (arrière-port) et 20 stations de référence réparties à l'échelle du lac Supérieur. Les teneurs en mercure total et en méthylmercure de tous ces échantillons ont été déterminées. Les échantillons de sédiments et d'eau sus-jacente ont fait l'objet d'analyses prévoyant la mesure d'une série de variables physico-chimiques. Cinq stations sont considérées comme hautement toxiques, et la contamination par le mercure ne semble qu'une des causes du problème dans certains cas. De façon globale, les communautés d'invertébrés benthiques de la baie Thunder diffèrent des communautés des stations de référence. Elles s'en distinguent principalement par une augmentation de la diversité des taxons, l'absence d'une espèce clé d'amphipode et une plus grande représentation d'autres taxons, ou par une réduction de la diversité des taxons et une

augmentation de l'abondance d'organismes plus tolérants, comme les tubificides et les chironomides. Dans entre la moitié et la quasi-totalité des stations étudiées, les concentrations de mercure total et de méthylmercure décelées dans les sédiments et chez les chironomides résidants sont supérieures à celles observées dans les stations de référence. Une relation significative a été relevée entre les concentrations de méthylmercure chez les chironomides et les concentrations de ce même composé dans les sédiments (R^2 ajusté = 0,466). Selon des scénarios d'exposition généralement intermédiaire et maximale et de transfert trophique, les concentrations de méthylmercure chez deux des trois espèces réceptrices de poissons devraient excéder les valeurs indiquées dans « Recommandations canadiennes pour les résidus dans les tissus : protection des espèces fauniques » dans entre 7 et la totalité des stations de la baie Thunder. Dans 5 à 8 de ces stations, les concentrations de méthylmercure devraient dépasser les concentrations maximales décelées dans les stations de référence. Les concentrations observées chez les poissons de pêche sportive de la baie Thunder (arrière-port) dépassent les valeurs recommandées. Ce résultat atteste d'une bioaccumulation du mercure chez les organismes occupant un échelon trophique plus élevé. Selon un cadre décisionnel applicable à la contamination des sédiments et une approche à base de règles fondée sur le poids de la preuve intégrant toutes les sources de données disponibles aux fins d'une évaluation globale individuelle des stations, neuf stations devraient faire l'objet d'une évaluation des risques.

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ABBREVIATIONS, ACRONYMS AND SYMBOLS

adj	adjusted
AOC	Area of Concern
BMF	biomagnification factor
BSAF	biota-sediment accumulation factor
CI	confidence interval
CL	confidence limit
dw	dry weight
EC	Environment Canada
FCM	food chain multiplier
GLWQA	Great Lakes Water Quality Agreement
Hg	mercury; used where form (MeHg or THg) is unspecified
HMDS	hybrid multidimensional scaling
IJC	International Joint Commission
inv	invertebrate
LEL	lowest effect level
LOI	loss on ignition
max	maximum
MeHg	methyl mercury
min	minimum
MOE	Ministry of Environment
NWRI	National Water Research Institute
PCA	principal components analysis
PEL	probable effect level
QA/QC	quality assurance/quality control
RAP	Remedial Action Plan
rec	receptor
ref	reference
reg	regression
regr	regression
sed	sediment
SEL	severe effect level
THg	total mercury
TN	total nitrogen
TOC	total organic carbon
TP	total phosphorus
TRG	tissue residue guideline
wt	weight
ww	wet weight
$[x]_i$	concentration of substance x in matrix i

1 INTRODUCTION

1.1 Background and Mandate

In the 1970s, 42 locations in the Great Lakes where the aquatic environment was severely degraded were identified as "problem areas" by the International Joint Commission (IJC). Of these, 17 are along Canadian lakeshores or in boundary rivers shared by the US and Canada. In 1985, the IJC's Great Lakes Water Quality Board recommended that a Remedial Action Plan (RAP) be developed and implemented for each problem area. The RAP approach and process is described in the 1987 Protocol to the *Great Lakes Water Quality Agreement* (GLWQA). The goal is to restore the "beneficial uses" of the aquatic ecosystem in each problem area, which were now called "Areas of Concern" (AOCs). Fourteen possible "impairments of beneficial use", which could be caused by alterations of physical, chemical or biological conditions in the area, are defined in Annex 2 of the GLWQA.

The Canadian government's commitment to the GLWQA was renewed in 2000 with the Great Lakes Basin 2020 (GL2020) Action Plan, under which the efforts of eight federal departments to "restore, conserve, and protect the Great Lakes basin" over the next five years were to be co-ordinated. Environment Canada's contribution included the funding of detailed chemical and biological assessments of sediments in Canadian AOCs. The National Water Research Institute (NWRI) was given the responsibility of conducting and reporting on these assessments.

Under the terms of reference for NWRI's mandate, the Benthic Assessment of Sediment (BEAST) methodology of Reynoldson et al. (1995, 2000) was to be applied to the AOC assessments. To date, the methodology has involved evaluation of sediment contaminant concentration, laboratory toxicity, and benthic invertebrate community structure (see description below). Recent reviews of the BEAST framework have recommended the inclusion of an additional line of evidence – information on the bioaccumulation of contaminants liable to biomagnify (Grapentine et al. 2002). The study described in this document was conducted to supplement existing data to complete an overall assessment of sediments in the northern portion of Thunder Bay that are, or have been, exposed to industrial effluents.

1.2 Decision Framework for Sediment Assessment

The underlying philosophy of NWRI's approach to sediment assessment is that observations of elevated concentrations of contaminants alone are not indications of ecological degradation. Rather, it is the biological responses to these contaminants that are the concern. A recommendation on remedial activity requires evidence to be provided of an adverse biological effect either on the biota resident in the sediment, or on biota that are affected by contaminants originating from the sediment, either by physical, chemical or biological relocation.

It is recognized that to make decisions on sediment quality and the need to remediate, four components of information (in addition to knowledge on the stability of sediments) are required (Krantzberg et al. 2000):

- ***Sediment chemistry and grain size*** – Quantifies the degree to which sediments are contaminated. Indicates exposure (or at least potential exposure) of organisms to contaminants (with consideration of exposure pathways). Provides information on physicochemical attributes of the sediment to assist in the interpretation of any observed biological effects.

- ***Benthic invertebrate community structure*** – Used to determine whether natural faunal assemblages in contaminated sediments differ from those in uncontaminated reference locations. Can indicate a biological response to sediment conditions. Organisms which reside in and ingest sediments experience the most ecologically relevant exposures to contaminants present, and represent important food web components.

- ***Sediment toxicity*** - Differences in resident invertebrate communities between contaminated and uncontaminated sites alone cannot be conclusively attributed to toxic chemicals. Sediment toxicity data provides supporting evidence that responses observed in the community are associated with sediment contaminants rather than other potential stressors.

- ***Invertebrate body burdens*** - Measurements of contaminants in tissues of resident benthic fauna provide evidence of bioavailability, and that the contaminants are responsible for

observed effects on the organisms (Borgmann et al. 2001). In addition, the information can be used to assess the risk to higher trophic levels due to biomagnification. Some contaminants, although bioavailable, may not accumulate in benthic invertebrates to sufficient concentrations to induce effects. A few of these contaminants (e.g., mercury) have the property of biomagnifying up the food chain to produce adverse responses in higher trophic level organisms.

The overall assessment of a site is achieved by integrating the information obtained both within and among the above four lines of evidence. The decision framework was developed from the Sediment Triad (Long and Chapman 1985; Chapman 1996) and the BEAST (Reynoldson et al. 1995, 2000) frameworks, and is described in detail in Grapentine et al. (2002).

1.3 The BEAST

The BEAST is a predictive approach for assessing sediment quality in the Great Lakes using multivariate techniques (Reynoldson et al. 1995, 2000; Reynoldson and Day 1998). It consists of a database containing information on benthic community structure (the type and number of taxa present), selected habitat variables, and responses of four benthic invertebrates (survival, growth and reproduction) in laboratory toxicity tests. The database currently consists of nearshore reference sites that were sampled from the Laurentian Great Lakes over a three-year period. The reference sites establish normal conditions for selected endpoints, and determine the range of 'normal' biological variability. As a result, expected biological conditions are predicted by examining the relationships between variability and habitat conditions.

This assessment method has been used to assess the condition of benthic invertebrate communities and toxicity in a number of AOCs, e.g., Collingwood Harbour, St. Lawrence River (at Cornwall), Hamilton Harbour, Bay of Quinte and Peninsula Harbour (Reynoldson et al. 1995; Reynoldson 1998; Reynoldson and Day 1998; Milani and Grapentine 2003, 2004, 2005).

1.4 Potential for Biomagnification

Purpose

The purpose of the biomagnification component of this study is to determine if mercury from sediments in Thunder Bay bioaccumulate in the tissues of benthic invertebrates, and; if mercury

could potentially be transferred through benthic invertebrates to fishes, wildlife or humans. In other words: Is there evidence that mercury biomagnification is an environmental issue of concern? The results of this study should lead to one of two alternate conclusions: (a) mercury is unlikely to concentrate in the food web at levels that can cause adverse effects, or (b) mercury **could** concentrate in the food web at levels that can cause adverse effects. The determination of whether mercury biomagnification and adverse effects to higher trophic level organisms (fish, wildlife, human) are actually occurring in Thunder Bay is beyond the scope of this study, and would need to be addressed by a more comprehensive assessment such as a detailed risk assessment. The latter conclusion (b) is of **potential** biomagnification, but does not determine **actual** biomagnification.

The purpose of the biomagnification component of the study was achieved through two objectives:

A. Determining if benthic invertebrates in locations where mercury is elevated are a potential source of mercury to higher trophic levels.

B. Determining if the amount of mercury potentially available is of concern.

The first objective was addressed by comparing concentrations of mercury (Hg) in benthic invertebrates from Thunder Bay sites to those from Lake Superior reference sites, and by determining whether sediment Hg concentration is related to invertebrate (whole body) Hg concentration. For the second objective, the concentrations of Hg in selected trophically linked receptor species (i.e., consumers of benthic invertebrates and their predators) were predicted based on measured Hg in invertebrates and literature-derived biomagnification factors. (Traas et al. (2002) is an example of an application of this approach.) The predicted receptor species concentrations were then compared to an appropriate tissue mercury guideline established for the protection of higher trophic level organisms. Predictions of receptor tissue mercury concentrations focused on methyl mercury (MeHg) because it is the most toxicologically relevant and predominant form of mercury in tissues of fishes and higher trophic level receptors (USEPA 1997b; Environment Canada 2002). However, determinations of sediment Hg

distributions and bioaccumulation in benthic invertebrates were made on the basis of both total mercury (THg) and MeHg to allow comparisons with results from other studies and guidelines that involve THg.

The biomagnification modelling was broken down into four steps:

- Identification of receptors of potential concern.
- Measurement of contaminant concentrations in invertebrates and sediment.
- Selection of biomagnification factors.
- Prediction of receptor species tissue concentrations.

Knowledge of the food web structure is needed to determine relevant receptor species (fish, bird, mammal). These are identified in the following subsection. Determinations of concentrations of mercury in sediment ($[Hg]_{sed}$) and invertebrates ($[Hg]_{inv}$) are described in the experimental design and methods sections. The identified receptors determined what biomagnification factors (BMFs) to use for predicting receptor mercury concentrations and what criteria to use (e.g., guidelines for protection of wildlife consumers of aquatic biota; human health guidelines for protection from fish consumption) for comparison. The review and selection of BMFs, and the estimation of [Hg] in the tissues of receptor species are discussed in subsection 3.6.3 (Data Analyses) and Appendix A.

If the predicted contaminant concentration in the receptor exceeded the maximum (99th percentile) reference condition and the guideline, a potential risk of adverse effects due to biomagnification was concluded. Alternatively, if the predicted contaminant concentration in the receptor was less than the guideline or the maximum reference condition, no potential risk was concluded.

Identification of receptors of concern

Based on generic food webs for the Great Lakes (e.g., Diamond et al. 1994), information on fauna resident in the Thunder Bay AOC (Thunder Bay RAP Team 1991, 2000) and guidelines from Environment Canada (2000), receptors representative of four trophic levels were selected for biomagnification modelling:

- Benthic invertebrates (trophic level 1): ***Oligochaetes and Chironomids.***

- Benthivorous fish (trophic level 2): ***White sucker***. Total mercury concentrations in sucker (43 – 55 cm, n = 9) collected from the inner Thunder Bay harbour range from 200 to 800 ng/g (mean 460 ng/g) (MOE 2003). In comparison, [THg] in sucker from other areas in Lake Superior range from 80 to 490 ng/g over the period of 1985 to 2001 (MOE 2002).
- Small piscivorous fish (trophic level 3): ***Yellow perch***. The Yellow perch are part of the sport fishery in Thunder Bay, and are supported in areas such as the mouth of the Current River (Thunder Bay RAP Team 1991).
- Large piscivorous fish (trophic levels 3 and 4): ***Walleye/Lake trout***. Total mercury concentrations in lake trout (43 – 56 cm, n =13) collected in 2002 from the Thunder Bay inner harbour range from 130 ng/g to 280 ng/g (mean 200 ng/g) (MOE 2003), similar to that seen in other areas in Lake Superior (range 120 to 210 ng/g) (MOE 2002). Total Hg in Walleye (40 – 56 cm, n = 12) collected in 1998 range from 170 to 850 ng/g (mean 406 ng/g) (Stantec 2003).
- Piscivorous bird (trophic levels 3 and 4): ***Great Blue heron***. Great blue herons are widespread, and are known to breed along the shores of Lake Superior. Fishes (mostly <25 cm in length) are the preferred prey (CWS 2002).
- Piscivorous mammal (trophic levels 3 and 4): ***Mink***. Mink are associated with numerous aquatic habitats and are opportunistic feeders (CWS 2002). Mink inhabit areas throughout central and northern Ontario.

As part of the Sport Fish Contaminant Monitoring Program, regular collections of Walleye, Lake trout and White sucker (as well as other fish species) are collected from the Thunder Bay AOC (inner and outer harbours). Sport fish consumption restrictions for total mercury begin at levels above 610 ng/g and total restriction is advised for levels above 1840 ng/g for the general population (MOE 2005). For the sensitive population, restrictions begin at levels above 260 ng/g, and there is complete restriction for levels above 520 ng/g (MOE 2005). Currently, for the Thunder Bay inner harbour, there are consumption restrictions (4 meals per month) due to Hg for Walleye >60 cm long (general population) and complete restriction for Walleye >55 cm (sensitive population) (MOE 2005). For the White sucker, consumption restrictions (4 meals per month) due to Hg start at fish >40 cm long and complete restriction at fish >50 cm (sensitive population) (MOE 2005). There are also consumption restrictions due to Hg for Northern pike,

Round whitefish (sensitive population) and Ling (sensitive population) for the inner harbour (MOE 2005). A model of the feeding relationships linking these receptors with each other and benthic invertebrates and sediment is shown in Appendix A; Figure A1.

1.5 Thunder Bay Area of Concern

The Thunder Bay AOC has been the subject of two major RAP reports – Stage 1: Environmental Conditions and Problem Definition (Thunder Bay RAP Team 1991) and Stage 2: Remedial Strategies for Ecosystem Restoration (Thunder Bay RAP Team 2000). The environmental issues of concern identified in these reports are:

- Metal contamination,
- Toxic organics,
- Contaminated sediments,
- Fish consumption advisories,
- Impacted biota, and
- Beach closings (bacteria).

Of the 14 beneficial uses evaluated for the Thunder Bay AOC, 9 were determined as "impaired". The following are associated with sediment contaminants:

- Restrictions on fish consumption,
- Degradation of benthos,
- Degradation of fish populations,
- Loss of fish and wildlife habitat,
- Fish tumours, and
- Restrictions on dredging activities

The RAP Stage 2 report identified northern Thunder Bay, adjacent to Cascades Fine Paper, as a concern due to mercury contamination. Most recent assessments of sediments and biota in this part of the AOC were performed in 1993 by the MOE (Bedard and Petro 1995) and in 1997/98 (Stantec 2003). Observations were:

- Laboratory toxicity tests revealed acute toxicity to the mayfly *Hexagenia limbata* and the midge *Chironomus tentans* at 2 (of 3) sites, which were situated within the breakwall just

south or southwest of the Cascades mill effluent discharge. Effects were found to be correlated to sediment Hg, which ranged from 2.2 to 3.0 µg/g in the top 5 cm. It was concluded that toxicity appeared related to the white fibrous material present at the sites and that Hg was unlikely the sole cause of toxicity (Bedard and Petro 1995).

- Total mercury in surficial sediment (0-3 cm) in the area adjacent to Cascades is lower than that observed in the early 1970's.
- Both total and methyl mercury in resident oligochaetes were not significantly related to sediment mercury concentrations; however, there is a significant relationship for total Hg when the two most highly contaminated sites (that contain visible fibre) are removed from the analysis (Stantec 2003).
- Mercury levels in muscle of White sucker and Walleye have declined since the 1970's; however, methyl Hg concentrations for whole Walleye (collected in 1998 – overall mean 405 ng/g), are above the CCME tissue residue guideline (92 ng/g) for the protection of fish-consuming wildlife. Total Hg in mottled sculpin collected in the study area on average were up to 4-fold higher than that in reference area (located outside breakwall) (Stantec 2003).

The chief environmental issue of concern in the Thunder Bay AOC is the elevated mercury remaining in sediments in the northern part of the inner harbour and the potential risk to fish, wildlife and humans through biomagnification. The bioaccumulation component of the assessment framework is important to consider where concern exists for contaminants such as mercury and chlorinated organic compounds. These compounds can be highly concentrated in the food web without inducing effects on survival, reproduction or growth at the lower trophic levels (which are typically examined for sediment assessments). Measurement of invertebrate body burdens allows assessment of the potential for effects on higher trophic level organisms (which are more difficult to measure and typically not examined in sediment assessments) resulting from the transfer of contaminants through dietary sources. Additionally, sediment toxicity should be further evaluated as only three sites were sampled in the 1993 MOE study. While Hg was not concluded to be the sole causative agent in this MOE study, further evaluation of the relationships between toxicity and sediment Hg is warranted.

In September/October 2002, the National Water Research Institute of Environment Canada sampled northern Thunder Bay adjacent to Cascades Fine Paper to provide further information

on the degree of sediment contamination. This report presents the results of these investigations and provides a spatial description of the state of the sediments in northern Thunder Bay and the degree of contamination.

2 EXPERIMENTAL DESIGN

2.1 Sampling Design

Nineteen sampling sites were focused in the northern portion of Thunder Bay, adjacent to Cascades Fine Papers Group, Thunder Bay Inc. (Figure 1a) (16 of the 19 sites were chosen from the Stantec 1997 survey), and 20 reference sites were located east of Thunder Bay (Figure 1b). Sediment and overlying water, benthic community, sediment for toxicity tests, and resident invertebrate tissue were collected at each site. The location of sites (Table 1) were selected on the basis of (a) representing a wide range of mercury levels in sediment and areas requiring toxicity evaluation, (b) representing least contaminated/reference conditions in the area, and (c) overlapping locations of previous studies.

For the biomagnification component of the study, this mixed (gradient + control/potential impact) sampling design allowed several types of comparisons for assessing the distribution of mercury in sediment and biota. Using all sites, relationships between sediment [Hg] and invertebrate [Hg] levels were examined. In addition, Hg levels at locations in northern Thunder Bay were compared to Hg levels at reference locations. The array of the sites also allowed a spatial analysis of Hg conditions, in which locations of elevated Hg in sediment, invertebrates and receptors (predicted from models) were identified.

2.2 Measurement Endpoints for Biomagnification

Invertebrates (oligochaetes and chironomids) and sediment for mercury analyses were collected from locations of sediment deposits potentially exposed to past discharges of mercury-containing effluent, as well as from unexposed reference locations. Sediment was obtained from the top 0 - 10 cm layer of lake bed. This layer includes the vertical home range of most benthic invertebrates. Two distinct invertebrate taxa were targeted for collection from each location. Chironomids and/or oligochaetes were obtained from test and reference sites. Analyses of total and methyl mercury were performed on samples composited from organisms within each of two

taxa (i.e., taxa were analyzed separately). Invertebrates were not allowed time to clear sediment from their guts as predators would consume whole organisms. Mercury associated with sediment, as well as that incorporated into tissues, is potentially available for transfer through the food chain.

2.3 Assumptions for Potential for Biomagnification

For the prediction of Hg concentrations in the tissues of upper trophic level biota, bioaccumulation is considered to occur predominantly through dietary pathways. This is suggested by several experimental and modelling studies (Bodaly et al. 1997; Downs et al. 1998). In modelling the exposure to and uptake of Hg by receptors, several conservative assumptions (i.e., maximum potential exposure to Hg) have been made. These include:

- For fish receptor
  - Fish consume invertebrates only from the site.
  - Fish feed on same invertebrate taxa as those collected in field sampling.
- For wildlife receptor
  - 100% of the diet is fish.
  - Fish are consumed only from the site in question.
  - Fish consume invertebrates only from the site.
  - Fish feed on the same invertebrate taxa as those collected in field sampling.

In addition, the flux of mercury between sediment, water and biota compartments were considered in equilibrium.

3 METHODS

3.1 Sample Collection and Handling

Nineteen sites were sampled in northern Thunder Bay (Figure 1a) and 20 reference sites were sampled east of Thunder Bay (Figure 1b). Station co-ordinates and site depth are provided in Table 1. A list of variables measured at each site is provided in Table 2. Site co-ordinates were obtained using a differentially corrected global positioning receiver (MX300). Corrections were received from a reference station located on top of the flashing red light on the breakwall at the

south entrance of the inner harbour. An offset was added by the Technical Operations division of NWRI to the position (from the centre of the light) that was previously calculated (to within centimetres) by the Canadian Hydrographic Service using highly accurate DGPS receivers and precise survey techniques. This provided survey accuracy within 1 – 2 m.

Prior to sediment collections, overlying water samples were obtained using a van Dorn sampler, taken at 0.5 metre from the bottom. Temperature, conductivity, pH and dissolved oxygen were measured using Hydrolab apparatus. Samples for alkalinity, total phosphorus, total nitrogen, ammonia and nitrates/nitrites were dispensed to appropriate containers and stored at 4°C for later analysis.

A 40 cm × 40 cm mini-box core (inserted into the sediment) was used to obtain sediment for benthic community structure determination and sediment chemistry analysis. At each site, five replicate benthic community samples were extracted from the box core using 10 cm x 6.5 cm Plexiglas tubes. Samples were sieved through a 250-μm mesh screen and the residue preserved with 5% formalin for later identification. The remaining top 10 cm of sediment from each box core was removed, homogenised in a Pyrex dish, and allocated to containers for chemical and physical analyses of sediment. (Note: Total Hg samples collected by the methods described above were analyzed by Caduceon laboratory.) Sediment and community samples were stored at 4°C.

A mini-Ponar sampler was used to collect the sediment for toxicity test purposes. Five replicates (grabs) were collected per site, sealed in polyethylene bags, and stored in buckets at 4°C.

A mini-Ponar sampler was used to collect the resident benthic invertebrates for tissue Hg analysis. At each site, enough sediment was collected to fill 2 68-L plastic tubs (approximately 10-15 mini-Ponars per tub). A small scoop of sediment (top 10 cm) was taken from each Ponar grab and set aside in a glass tray. This was repeated until each tub was approximately 2/3 full. Ample lake water was added to each tub. Once the tubs were filled, the sediment set aside in the glass tray was homogenized, distributed to pre-cleaned polyethylene bottles for analysis of total and methyl mercury, and frozen (-20°C). (Note: These Hg samples (sediment and biota) were analyzed by Flett Research laboratory.) Invertebrates were removed from the sediment by wet

sieving with lake water using 12" stainless steel sieves (500-μm mesh). Macroinvertebrates collected on the sieve were sorted into separate taxa in glass trays using stainless steel instruments. Sorted biota were rinsed with deionized water and placed in pre-weighed and pre-cleaned (20 % HCL) 5 -mL scintillation vials, weighed, and frozen on site (-20°C). A layer of parafilm was placed between vial and cap. Invertebrate samples were later freeze-dried and reweighed. The wet:dry ratios were used in converting invertebrate tissue mercury concentrations, expressed as dry weight, to wet weight (see section 3.6.3). Stainless steel sieves and instruments were detergent washed between stations. If persistent organic matter remained on the sieve after the detergent wash (on visual inspection), a more aggressive cleaning solution was implemented (caustic ethanol). Homogenizing and sorting trays and scoops were detergent washed, rinsed with 20% HCl, and rinsed with lake water.

3.2 Taxonomic Identification

Benthic community samples were transferred to 70% ethanol after a minimum of 72 hours in formalin. Invertebrates were sorted for identification to the family level at the Invertebrate Laboratory at NWRI (Burlington, ON). Slide mounts were made for Oligochaetae and identified to family using high power microscopy.

3.3 Sediment Toxicity Tests

Toxicity tests were performed in the Ecotoxicology Laboratory at NWRI (Burlington, ON). Overlying water used in the toxicity tests was City of Burlington tap water (Lake Ontario), which was charcoal filtered and aerated for a minimum of three days prior to use. Water characteristics included: conductivity 273 – 347μS/cm; pH 7.5 - 8.5; hardness 120 - 140 mg/L; alkalinity 75 - 100 mg/L; chloride ion 22 - 27 mg/L.

Four sediment toxicity tests were performed: *Chironomus riparius* 10-d survival and growth, *Hyalella azteca* 28-d survival and growth, *Hexagenia* spp. 21-d survival and growth, and *Tubifex tubifex* 28-d survival and reproduction. Sediment handling procedures and toxicity test methods are described elsewhere (Borgmann and Munawar 1989; Borgmann et al. 1989; Krantzberg 1990; Reynoldson et al. 1991; Bedard et al. 1992; Day et al. 1994; Reynoldson et al. 1998b). Each test included control sediment for quality control purposes. This control sediment was collected from Long Point Marsh, Lake Erie, and was composed on average of 70.33% silt, 29.13% clay, 0.54% sand, and 8.1% organic carbon. All tests passed an acceptability criteria

based on percent control survival in Long Point sediment before being included in a data set, i.e., ≥ 80% for *H. azteca* and ≥70% for *C. riparius* (USEPA 1994; ASTM 1995); ≥80% for *Hexagenia* spp., and ≥75% for *T. tubifex* (Reynoldson et al. 1998b).

Water chemistry variables (pH, dissolved oxygen (mg/L), conductivity (μS/cm), temperature (° C), and total ammonia (mg/L)) were measured in each replicate test beaker on day 0 (start of test) and at the completion of the test. Tests were run under static conditions in environmental chambers at 23°C ±1 °C, under a photoperiod of 16L: 8D and an illumination of 500 - 1000 lux, with the exception of *T. tubifex* test which was run in the dark.

***Hyalella azteca* 28-day survival and growth test**

The test was conducted for 28 days using 2 -10 day old organisms. On day 28, the contents of each beaker were rinsed through a 250-μm screen and the surviving amphipods counted. Amphipods were dried at 60 °C for 24 hours and dry weights recorded. (Initial weights were considered negligible.)

***Chironomus riparius* 10-day survival and growth test**

The test was conducted for 10 days using first instar organisms. On day 10, the contents of each beaker were wet sieved through a 250-μm screen and the surviving chironomids counted. Chironomids were dried at 60 °C for 24 hours and dry weights recorded. (Initial weights were considered negligible.)

***Hexagenia* spp. 21-day survival and growth test**

The test was conducted for 21 days using pre-weighed nymphs (between 5 - 8 mg wet weight/nymph). On day 21, the contents of each jar were wet sieved through a 500-μm screen and surviving mayfly nymphs counted. Nymphs were dried at 60 °C for 24 hours and dry weights recorded. Initial mayfly wet weights were converted to dry weights (the relationship of mayfly wet weight to dry weight was previously determined by regression analysis) using the following equation: Initial dry weight = [(wet weight + 1.15)/ 7.35]. Growth was determined by final dry weight minus initial dry weight.

***Tubifex tubifex* 28-day survival and reproduction test**

The test was conducted for 28 days using sexually mature worms (gonads visible). On day 28, the contents of each beaker were rinsed through a 500-μm and 250-μm sieve sequentially. The number of surviving adults, full cocoons, empty cocoons, and large immature worms were counted from the 500-μm sieve and the number of small immature worms was counted from the 250-μm sieve. Reproduction was assessed using four endpoints: Number of surviving adults, total number of cocoons produced per adult, the percent of cocoons hatched, and total number of young produced per adult.

3.4 Sediment and Water Physico-Chemical Analyses

Overlying water

Total Kjeldahl Nitrogen (TKN), nitrates/nitrites (NO_3/NO_2), total ammonia (NH_3), total phosphorus (TP) and alkalinity were analyzed by the National Laboratory for Environmental Testing (NLET) (Burlington, ON) by procedures outlined in Cancilla (1994) and NLET (2000).

Sediment particle size

Particle size analysis (percents sand, silt, clay and gravel) was performed by the Sedimentology Laboratory at NWRI (Burlington, ON) following the procedures of Duncan and LaHaie (1979).

Sediment trace metals and nutrients

Freeze dried sediment was analysed for total mercury, trace elements, major oxides, loss on ignition (LOI), total organic carbon (TOC), total phosphorus (TP), and total nitrogen (TN) by Caduceon Laboratory (Ottawa, ON) using in house procedures or USEPA/CE (1981) standard methodologies. For sediment total mercury, 0.5g of freeze dried sediment was digested with HNO_3:HCl for two hours. $SnCl_2$ was added to reduce Hg to volatile metallic form. If there was high organic material, $KMnO_4$ was added to the digestion solution to destroy organo-mercury bonds. Hydroxyl amine hydrochloride was then added to neutralize $KMnO_4$ excess so $SnCl_2$ could react with Hg in solution. Digestion was followed by measurement using a cold vapour atomic absorption spectrometer. The detection limit was 5 ng/g dw.

Total and methyl mercury

Analysis of total and methyl mercury in sediment and resident benthic invertebrates was performed by Flett Research Ltd. (Winnipeg, MB), based on procedures of Bloom and Crecelius (1983), Horvat et al. (1993) and Liang et al. (1994). Procedures (provided by Flett Research Ltd.) are outlined below.

Total mercury in sediment

Between 100 and 1000 mg of thawed sediment sample (or spiked sediment, blanks or reference material) was digested overnight (16-18 hours) in 3 mL of 7:3 nitric/sulfuric acid at 150°C. After cooling, the sample was diluted to 25 mL with low-mercury deionized water, spiked with BrCl and allowed to react. The residual BrCl was then destroyed by addition of hydroxylamine hydrochloride. An aliquot of the sample (100 μL – 2 mL) was placed into a sparging vessel, to which was added stannous chloride. The elemental mercury produced was purged onto a gold trap with Hg-free nitrogen. The gold trap was heated with UHP argon carrier gas passing through it, and the mercury released was measured by a Brooks-Rand CVAFS model-2 detector. The detection limit was 1-5 ng/g dw.

Total mercury in biota

The same procedure as described for analysis of total mercury in sediment by Flett Research was used for biota, with the following differences in the sample digestion: up to 100 mg of thawed invertebrate sample (or spikes, blanks or reference material) was digested for 6 hours in 10 mL of 1:2.5 nitric/sulfuric acid at 250°C; after cooling, the sample was diluted to 25 mL with low mercury deionized water, spiked with BrCl and allowed to react.

Methyl mercury in sediment

Sediment was prepared for analysis by distilling 200-300 mg of homogenized sample (or spikes or blanks) in ~45 mL of low-mercury deionized water. Approximately 40 mL of distillate was collected and acidified with KCl/H_2SO_4. (Note: Since methyl mercury results were ≤0.1% of the total mercury results, a methylene chloride extraction was carried out on some of the highest total mercury samples. No significant difference in methyl mercury concentrations was observed between results obtained by either method. Therefore, it is assumed that insignificant methyl mercury production was occurring in the distillation process and thus all samples were processed

by distillation.) An aliquot of the prepared sample (1-2 mL, depending on observed interferences from the matrix) was ethylated in solution (final volume ~ 40 mL) using sodium tetraethyl borate. The solution was buffered to pH 5.5. The resulting ethylmethyl mercury was purged onto a Tenax trap with mercury-free nitrogen. The trap was heated, purged with UHP argon onto a GC column (for separation of the ethylmethyl mercury from Hg° and diethyl mercury), run through a pyrolizer (to reduce all mercury to Hg°), and then sent to a cold vapour atomic fluorescence analyser for detection. (GC oven: Perkin Elmer 8410 GC; column: chromasorb WAW-DMSC 60/80 mesh with 15% OV-3; detector: Brooks-Rand CVAFS model-2). The detection limit was 0.25 ng/g dw.

Methyl mercury in biota

Freeze dried biota (5-10 mg of homogenized sample, spike, blank or reference material) were digested overnight with ~500 µL of KOH/methanol at 75 °C. Sample aliquots (50-60 µL) were then treated and analysed as described above for the ethylation and subsequent steps in the determination of methyl mercury in sediment. The detection limit was 1.2 ng/g dw.

3.5 Biota-Sediment Accumulation Factors

Biota-sediment accumulation factors (BSAF) were calculated for each invertebrate taxa and site combination, for total and methyl mercury. The BSAF equation used was that defined by Thomann et al. (1995), and is the ratio of the metal concentration in the organism to that in the sediment:

$BSAF = [Hg]_{inv}/[Hg]_{sed}$

A BSAF assumes that the concentration of contaminant in the organism is a linear function of the contaminant concentration in the sediment.

3.6 Data Analysis

3.6.1 BEAST analysis

For the benthic community structure assessment, the BEAST model predicted the community assemblage that should occur at a test site. Using multiple discriminant analysis, environmental variables identified as predictors (latitude, longitude, depth, alkalinity, and total organic carbon;

Reynoldson et al. 1995, 2000) for the test and reference sites were merged and the model assigned a probability of the test site belonging to each of five reference faunal groups. Community data for the test site was merged with the reference site invertebrate data of the matched (group to which the test site has the highest probability of belonging) reference group only and ordinated using the ordination technique hybrid multidimensional scaling (HMDS) of Belbin (1993), with Bray-Curtis distance site × site association matrices calculated from raw data.

Toxicity data were analysed using HMDS, with Euclidean distance site × site association matrices calculated from standardized data. Toxicity endpoints for the test sites were compared to those for one group of reference sites.

Principal axis correlation (Belbin 1993) was used to identify relationships between habitat attributes and community or toxicity responses. Significant endpoints and environmental attributes were identified using Monte-Carlo permutation tests (Manly 1991). Test sites were assessed by comparison to confidence bands of appropriate reference sites. Test site toxicological responses were compared to numerical criteria previously established for each category (non-toxic, potentially toxic and toxic) and species from reference site data (Reynoldson and Day 1998).

Test data were analysed in subsets to maintain the ratio of test:reference sites ≤0.10. Multiple discriminant analysis was performed and the probability ellipses were produced using the software SYSTAT (Systat Software Inc. 2002). HMDS, principal axis correlation, and Monte-Carlo tests were performed using the software PATN (Blatant Fabrications Pty Ltd. 2001).

3.6.2 Sediment toxicity and contaminant relationships

Relationships between sediment toxicity and sediment contamination for the Thunder Bay sites were assessed graphically and by regression analysis. Initially, to examine general and dominant patterns in the data, comparisons between the toxicity responses and contaminant conditions were made based on integrative, compound variables (from multivariate ordination of measurement variables). After this, to better detect less dominant (though significant)

relationships between two or a few variables, analyses were conducted using the original measurement variables (i.e., toxicity endpoints and concentrations of individual compounds).

The sediment toxicity data for Thunder Bay sites were ordinated again by HMDS, as a single group and without the reference site data. To identify and relate the most important of the toxicity endpoints to the HMDS axes, principal axis correlation was conducted. Concentrations in sediment of 10 metals (Cd, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Zn) and 3 sediment nutrients (total N, total organic C, total P) were ordinated by principal components analysis (PCA). Data for all variables were log(x)-transformed. The eigenanalysis was performed on the correlation matrix.

Both the integrated descriptors of sediment toxicity (axes scores from the HMDS) and individual toxicity endpoints were plotted against the integrated contaminant descriptors (from PCA) as well as individual log(x)-transformed sediment contaminants (10 metals), 3 sediment nutrient variables, and grain size. To determine whether toxicity was better explained by joint consideration of the contaminant descriptors, multiple linear regression involving the contaminant descriptors as predictors was calculated with each toxicity descriptor as the response variable. The degree to which individual sediment variables account for toxicity was assessed by fitting regression models using "best subset" procedures (Draper and Smith 1998; Minitab 2000). Models were fitted for (a) all combinations of metals (b) all combinations of nutrients (c) all combinations of grain size, and then (d) all combinations of the best predictors from the three groups (This procedure was used to avoid computational difficulties arising from working with 21 predictors simultaneously.) The best models were those having maximum explanatory power (based on $R^2_{adjusted}$), minimum number of nonsignificant predictors, and minimum amount of predictor multicollinearity.

3.6.3 Potential for mercury biomagnification

Mercury distribution in sediment and biota

Sites in which concentrations of Hg in invertebrates ($[Hg]_{inv}$) were significantly elevated above background levels for the study area were identified by comparing $[Hg]_{inv}$ for the test sites to the upper 99th % percentile for the Lake Superior reference sites. This was done separately for total

and methyl Hg and for each invertebrate taxon. (Note: While benthic community and toxicological data for test sites were compared to reference sites sampled for the Laurentian Great Lakes region, sediment and invertebrate mercury levels at Thunder Bay sites were compared specifically to those at the Lake Superior reference sites sampled during the same period - see Figure 1b for reference locations.)

Relationships between concentrations of Hg in sediment and invertebrates were determined using regression analysis, again separately for total and methyl Hg and for each invertebrate taxon. The approach was used to estimate the degree to which Hg in invertebrates is predictable from Hg in sediment, with and without environmental covariables. Simple linear regression (ordinary least squares) was used for the single predictor ($[Hg]_{sed}$) model. "Best subset" multiple linear regression (Draper and Smith 1998; Minitab 2000) was used for the fitting of multiple predictor models. The environmental variables expected to potentially influence uptake of Hg from sediment by biota (based on reviews such as Braga et al. 2000; Lawrence and Mason 2001), including sediment concentrations of total organic C, total P, total N, Fe, and Mn, particle size fractions (sand, silt and clay), overlying water nutrients, alkalinity, dissolved O_2, pH, conductivity, temperature, and site depth were included in the models. To increase normality of data distributions and linearity of relations between variables, some data were transformed: log(x) for THg and MeHg in sediment and invertebrates; log(x) for nutrients, Fe and Mn in sediment and site depth; and arcsine-square root(x) for the particle size fractions. Normality and linearity of the water column data, which were not generally improved by transformations (pH, dissolved oxygen, conductivity), were analyzed untransformed.

All models fitted to the data included $[Hg]_{sed}$ as a free predictor (i.e., it was not forced to be in the model). The specific null hypothesis of interest was that "the effect of $[Hg]_{sed}$ on $[Hg]_{inv} = 0$, after accounting for effects of other predictors". For the best subset regressions, models were fitted for all combinations of predictors. Determination of the "best" model was based on several criteria (in roughly decreasing order of importance):

- Maximum $R^2_{adjusted}$
- Significance of partial F-tests (= t-tests) for predictors (especially $[Hg]_{sed}$)
- Significance of F-test for regression
- Variance inflation factors (VIFs) for predictors < 10

- Homoscadastic and normally distributed residuals
- Mallow's C_p statistic not >> number of predictors

Lack-of-fit tests for curvature in response-predictor relationships and interactions between predictors were performed and examined for nonsignificance. Observations having large standardized residuals or large influence on the regression were also considered in model evaluations. The best model was identified based on the overall meeting of these criteria. Both single and multiple predictor models were then examined for the degree to which $[Hg]_{sed}$ predicts $[Hg]_{inv}$, as indicated by the significance of the *t*-test of the coefficient for $[Hg]_{sed}$.

BMF literature review

A review of information on BMFs was conducted using typical methods of electronic database and chain-of-citation searches as well as consultation with leading researchers in the field of mercury ecotoxicology and risk assessment. Details on the methods and the results of the review are described in Appendix A. A summary is provided below.

The search was focused on the period 1996-2002, as a thorough review of the literature was carried out in 1997 by USEPA (1997a,b,c). Obtaining the information required to estimate mercury concentrations in receptors involved reviewing published literature, unpublished reports, databases, web pages and any other sources of data on BMFs relevant to the benthic invertebrate taxa and receptors; assessing the quality of the BMF data; and tabulating BMFs and estimates of their variability, together with information on the BMF's determination (e.g., location of study, organisms involved, proportion of receptor's diet that is invertebrates, effects of cofactors (if any), assumed ingestion rates and home ranges). The following criteria were applied to screen literature to obtain either BMFs or candidate datasets for calculating BMFs, after Suedel et al. (1994) and Gobas and Morrison (2000):

- If organisms that were presented were not from a logical food chain, or no evidence was presented that the feeding relationship between predator and prey was a functional feeding relationship, the data were not used. One exception to this rule was made in selecting a study of mink fed diets of different proportions of contaminated and uncontaminated fish

(Halbrook et al. 1997), since there was a reasonable likelihood that these fish species would have been part of their diet.

- Mean concentrations of total Hg or MeHg needed to be presented for both predator and prey, and in comparable units.
- BMFs involving Hg concentrations in feathers or fur of predators were excluded.
- Unless evidence of comparability could be found, studies from non-freshwater systems or with non-comparable species were not used. More information is presented below on the assessment of comparability of different systems and species.

There were few studies that quoted BMF estimates specifically for the receptor species and feeding relationships defined in Figure A1. Of the small number of studies that calculated BMFs that were directly comparable in part to the food chain model, most were from freshwater pelagic foodwebs. Some were also studies in different ecosystems (marine, temperate montane freshwater, tropic freshwater). Thus, it was necessary to use the most relevant studies to obtain BMFs and document the relative comparability of different species and ecosystems to those presented in the study design for this assessment. Information to support substitutions of receptor with comparable species from the literature (in applying BMF estimates) is presented in Tables A3 - A13. Species were considered the most qualitatively similar when they occupied similar habitats, had similar feeding habits and dietary composition, similar range, similar feeding substrate, and similar food ingestion:body weight ratio. Sources for this information were CCME (1999a), CWS (2002), Sample and Suter (1999), Scott and Crossman (1973), and USEPA (1997c). A breakdown of the number of BMFs obtained/calculated per feeding relationship, and the range of corresponding BMF values is presented in Table A1.

Calculation of receptor tissue mercury concentrations

It is widely recognized that mercury is transferred through trophic levels primarily in the methyl form (USEPA 1997b). It is also accepted that mercury in the tissues of fishes and higher trophic level organisms is almost entirely in the organic (methyl) form. Environment Canada (2002) states that "total mercury" concentrations in piscivorous fishes are probably ~99% methyl mercury, and note that Bloom (1992) suggests that previous studies reporting methyl mercury fractions in fishes less than 95% were likely in error. Therefore, mercury concentration in

receptors was predicted on a MeHg basis, using (a) MeHg measurements in invertebrates and (b) combined THg and MeHg BMF values (assuming that reported THg concentrations largely represent MeHg concentrations).

Concentrations of MeHg in the tissues of receptors were predicted by multiplying measured body concentrations in the resident invertebrates by the food chain multiplier relevant for the receptor:

$$C_{rec} = FCM \times C_{inv}$$

where:

C_{rec} = mean contaminant concentration in the consumer (receptor) species

C_{inv} = mean contaminant concentration in invertebrates

FCM = food chain multiplier

The FCM represents the cumulative biomagnification of a substance from one trophic level to a higher trophic level (USEPA 1997c). Whereas a BMF applies to only one trophic level-transfer, a FCM refers to one or more, and may be a multiple of more than one BMF. Thus, FCM = $BMF_1 \times BMF_2 \times BMF_3 \times \ldots \times BMF_n$, where 1,2,3,..., n are transfers of one trophic level. The BMFs used to obtain FCMs and calculate C_{rec} values are in Table A1, which shows the low, medium and high BMFs from the literature review for each transfer between trophic levels as shown in Figure A1. In Table 3, the FCM for transfer from benthic invertebrates to each receptor is estimated by multiplying the BMFs for the intermediate steps from Table A1. Low, medium and high FMC values are obtained from use of all minimum, all medium or all maximum estimates for each BMF. For the Walleye, heron and mink, it is recognized that they could be level 3 as well as trophic level 4 predators. Therefore, FCMs were estimated for both food chain pathways.

Invertebrate methyl Hg concentrations used in the predictions of Hg in receptors include observed $[Hg]_{inv}$ values for the two taxa collected from the site. These were used to obtain minimum and maximum observed $[Hg]_{inv}$ for the taxa collected from the site. "Medium" $[Hg]_{inv}$ for the site was calculated as the mean of the values. Since fish contaminant data are reported for the most part on a wet weight basis and the guidelines used in this study are also based on

wet weight, methyl Hg concentrations in invertebrates were converted to a wet weight basis. Biota comprised on average 87.7% water (range: 81.6 to 91.8%). The ratio of wet to dry weight was determined for each individual sample submitted for analysis (rather than using an overall average ratio for each taxon). Wet weights were determined using the following conversion:
$[Hg]_{inv}$ (ng/g dry weight) / (ratio of wet: dry weight) = $[Hg]_{inv}$ (ng/g wet weight)

For each site, minimum, intermediate and maximum concentrations of MeHg for each receptor were predicted by:
$[Hg]_{rec} = FCM \times [Hg]_{inv}$

using corresponding low, medium and high $[MeHg]_{inv}$ and FCMs. For the Walleye, heron and mink, FCMs for both food chain pathways were combined. From the available values, the lowest and the highest FCMs were used for the minimum and maximum prediction, and the mean of the two medium values was used for the intermediate prediction. The predicted MeHg concentrations in receptors are generic in that they are not specific to particular tissues.

3.7 Quality Assurance/Quality Control

Field replication

At two randomly selected test sites (P7 and P16) and reference sites (5108 and 5111), triplicate overlying water and sediment were collected for determination of within-site and among-sample variability. Variability in a measured analyte was expressed as the coefficient of variation (CV = standard deviation / mean × 100).

Analytical variability

Flett Research Ltd. conducted determinations of total and methyl mercury in sediment and benthic invertebrates. Quality control evaluation for these procedures included analyses of sample duplicates, matrix spikes and certified reference materials, as well as evaluation of sample recoveries. For sediment, sample duplicates were analyzed at least once every 15 samples, and matrix spikes were performed on every tenth sediment sample to determine mercury recoveries. The NRC certified sediment reference material "MESS-2" was concurrently digested and analysed for total mercury. For biota, duplicate "DORM-2" reference material,

"MQAP fish check samples", and spiked matrix duplicates were analyzed for total and methyl mercury with each lot of 10 – 20 samples. Each taxon was represented in the analyses of sample duplicates and matrix spikes.

Caduceon Environmental Laboratory analyzed sediment for trace metals (including total mercury), major oxides, total phosphorus, total nitrogen, and total organic carbon. Quality control procedures included repeat measurements, and control charting of influences, standards, and blanks. Reference material was used in each analytical run. Calibration standards were run before and after each run. Run blanks and reference standards were run 1 in 20 samples, while repeats were run 1 in 10 samples.

An inter-laboratory comparison of analyses for total Hg was conducted based on results from Flett and Caduceon Laboratories. Data were compared by regression analysis. The slope of the regression line is a measure of the overall agreement in [THg] determinations, whereas the scatter of points about the line should indicate joint laboratory measurement error.

Benthic community sorting efficiency

To evaluate control measures for benthic invertebrate enumeration (on a monthly basis), a previously sorted sample was randomly selected, re-sorted, and the number of new organisms found counted. The percent of organisms missed (%OM) was calculated using the equation:

$$\%OM = \#\ \text{organisms missed} / \text{total organisms found} \times 100$$

A desired sorting efficiency is %OM < 5%. If the %OM was > 5%, two more replicate samples were randomly selected and the %OM calculated. The average %OM was calculated based on the three samples re-sorted, and represents the standard sorting efficiency for that month. The average %OM is based on only one replicate sample if %OM is ≤ 5%).

4 RESULTS

4.1 Quality Assurance/Quality Control

Three replicate van Dorn samples were collected at all QA/QC sites, three replicate box cores were collected at site P7 and two replicate box cores at site P16 (the box core jammed preventing a third box core being collected at site P16). Variability among site replicates in a measured analyte has three sources: natural within-site heterogeneity in the distribution of the analyte in sediment or water, differences in handling among samples, and laboratory measurement error. Among-triplicate variability indicates the overall "error" associated with quantifying conditions at a site based on a single sample.

Field replication

Coefficients of variation (CV) for sediment particle size, sediment and water nutrients, and trace metals for the field-replicated sites are provided in Appendix B; Table B1. Differences in variability are seen among sites and among the parameters from the same site. Overall, variability is low, with CVs ranging from 0.4 to 113.6% (median 9.6%). The highest variability is noted for Hg, followed by Pb (66.3%) and % gravel (65.6%).

Analytical variability

Data for Flett Laboratory duplicate and repeat analyses for mercury in sediment and biota are provided in Appendix B; Table B2. There is good agreement between sample duplicates and repeats. Mean CVs for duplicate analyses are 7.3, 3.7, 15.1, and 11.6% for $[THg]_{sed}$, $[THg]_{inv}$, $[MeHg]_{sed}$ and $[MeHg]_{inv}$, respectively. These are lower than those reported for other studies using gas chromatography and cold-vapour atomic fluorescence spectroscopy (Paterson et al. 1998). Repeat analyses, performed for [MeHg], have a mean CV of 6.3%. Percent recoveries for analyses of sediment and biota samples, matrix spikes and certified reference materials are provided in Appendix B; Tables B3 and B4. Mean recoveries range from 86.8 to 98.3% for sediment and biota samples, 90.9 to 102.9% for the matrix spikes, and 87.4 to 98.1% for the reference materials. The overall range of spike recoveries (69.3 to 112.6%) is comparable to that obtained by Lawrence and Mason (2001), who used similar analytical methods.

Laboratory repeat measurements for sediment metals, major oxides and nutrients, and corresponding analyses of reference materials for Caduceon Laboratory are provided in Table B5. The mean relative percent difference (RPD) for sample repeat measurements $[(x_1 - x_2)/ ((x_1 + (x_2/2)) \times 100]$ is 4.2% (range: 0 to 48.9%). The RPD for Hg is low (0% and 3.4%), and is highest for Mo (48.9%). Mean recovery for reference materials is 99%, ranging from 89 to 127%.

The inter-laboratory comparison for analyses of total mercury in sediment is described in Appendix B. Results show a fair agreement between measurements overall; however, there are some notably large differences for some sites, mainly for the most contaminated sites located along the northern shore of the sampling area. The slope of Flett $[Hg]_{sed}$ vs. Caduceon $[Hg]_{sed}$ is 1.16 and the percent explained variability (r^2) is 78.7%.

Sorting efficiency

The mean percent community sorting efficiency for Thunder Bay samples is 1.5%. This is an acceptable low value and represents the average sorting efficiency of three sorters over a four month period.

4.2 Sediment and Water Physico-Chemical Properties

Overlying water

Conditions of overlying water 0.5 m above the sediment are similar at most sites (test and Lake Superior reference), with overlapping ranges and similar medians for each variable (Table 4; Appendix C; Table C1). Overall, Thunder Bay overlying water has slightly higher levels of nutrients (TP and TKN) and NH_3 compared to Lake Superior reference. The ranges of dissolved oxygen, pH, conductivity, alkalinity, temperature, NH_3, NO_3/NO_2, TKN, and TP across Thunder Bay sites are: 2.9 mg/L, 0.7 pH units, 94 µS/cm, 23.9 mg/L, 1.5 °C, 0.23 mg/L, 0.28 mg/L, 0.33 mg/L, and 0.06 mg/L, respectively. There are some differences in overlying water across sampling sites. Sites P1 and P2, located farthest east in the sampling area (see Figure 1a) have the highest alkalinity, conductivity, and nutrient levels (in some cases 1 – 2 orders of magnitude higher). Dissolved oxygen is ≥8.7 mg/L at all sites. Reference sites have lower temperatures overall (a depth factor – see below).

Sediment particle size

Particle size data for Thunder Bay sediment are shown in Table 5. Sediments consist mainly of silt, ranging from 12 to 78% (median 64%), and clay, ranging from 16 to 42% (median 23%), or of sand, ranging from 1 to 70% (median 5%), and silt. Sites P10, P16, P22 and P23, however, consist mainly of sand and clay, and are the coarsest sediments, containing 0.7 to 12.7% gravel. Sites P6 and P7 consist almost entirely of a white fibrous material; therefore, particle size analysis was not possible for these sites. Particle size data for reference sites are shown in Appendix C; Table C2. Percent sand ranges from 0.2 to 95% (median 12%), % silt ranges from 0 to 72% (median 23%), % clay ranges from 4 to 88% (median 32%), and % gravel ranges from 0 to 5% (median 0%). The majority of reference sites consist mainly of silt and clay or sand and silt (same as test sites); however, on average, test sites contain more silt.

Site depth

Site depths are shown in Table 1. Overall, Thunder Bay sites are more shallow than reference sites. Depths range from 1.2 to 7.4 m (median 4.2 m) for test sites and from 5.0 to 43.8 m (median 19.6 m) for reference sites.

Sediment nutrients

Total phosphorus (TP), total nitrogen (TN), and total organic carbon (TOC) in Thunder Bay sediments are shown in Table 6; reference site data are shown in Appendix C; Table C3. The Severe Effect Level (SEL; Persaud et al. 1992) is exceeded for TN at three sites (P1, P2, and P3), for TOC at four sites (P1, P3, P6, and P7) and for TP at one site (P1). Total nitrogen ranges from 687 to 5959 μg/g (median 2359 μg/g), TOC ranges from 1.1 to 25.7% (median 4.5%), and TP ranges from 470 to 2085 μg/g (median 830 μg/g). Sites P6 and P7, which consist of the white fibrous material, have the highest TOC (25.7% and 23.9%, respectively). Overall, nutrient concentrations are lower at reference sites: TN ranges from 203 to 1007μg/g (median 692 μg/g); TOC is much lower, ranging from 0 to 1.9% (median 0.5%); TP ranges from 356 to 2259 μg/g (median 545 μg/g) (Appendix C; Table C3).

Sediment trace metals

Trace metals and the corresponding provincial Lowest Effect Level (LEL) and SEL (where available) are shown in Table 6 for Thunder Bay sites and in Appendix C; Table C3 for Lake Superior reference sites. Total Hg ranges from 0.03 to 17.63 μg/g at Thunder Bay sites, with all sites exceeding the LEL with the exception of P18, P22, and P23. (Note: These total Hg results are for the sediment samples collected from the mini-box core and analyzed by Caduceon Laboratory – see Section 3.1 for explanation.) The SEL is exceeded for Hg at sites P2 (2.96 μg/g), P3 (2.52 μg/g), P5 (6.77 μg/g), and P7 (17.63 μg/g) and for Cu at P7 (120.6 μg/g) (Table 6). Total mercury concentrations are below the LEL at reference sites, ranging from 0.01 to 0.18 μg/g (median 0.02 μg/g) (Appendix C; Table C3). Sediment total Hg (and methyl Hg) was also analyzed by Flett Research Laboratory at all sites. Collection and analytical methods for the Flett samples differed from those for the Caduceon samples (see Sections 3.1 and 3.4). The Flett samples are more representative of what the resident invertebrates collected for tissue purposes were exposed to (a homogenized sediment sample was taken from each Ponar); whereas the Caduceon samples are more representative of what the whole benthic community was exposed to (the chemistry and benthic community structure samples came from the same box core). Total and methyl Hg results, analyzed by Flett Laboratory, are provided in Section 4.6 (Biomagnification Potential).

4.3 Benthic Community Structure

Five different community assemblages have been established for the Laurentian Great Lakes region (Reynoldson and Day 1998; Reynoldson et al. 2000). Thunder Bay sites have the highest probability of belonging to reference Group 1 (5 sites) and reference Group 5 (14 sites) (Table 7). The five sites that have the highest probability of belonging to Group 1 have a ≥ 63% probability of group membership (range 63 to 98%, median 96%). The 14 sites that have the highest probability of belonging to Group 5 have a ≥56% probability of group membership (range 56 to 81%, median 67%).

Abundances of predominant taxa and taxa richness for Thunder Bay sites are shown in Table 8. Complete invertebrate family counts are listed in Appendix D. Overall, Thunder Bay sites

consist mainly of Tubificidae, Sphaeriidae and Chironomidae, which are present at all sites except P6 (Sphaeriidae) (Table 8a).

Test sites predicted to Great Lakes reference group 1

Reference Group 1 is based on 108 sites found in Georgian Bay (39), the North Channel (24), Lake Ontario (21), and Lake Erie (16). This reference group is characterized by Chironomidae (midge – 12.2% occurrence), Tubificidae (oligochaete worm – 11.7% occurrence), Sphaeriidae (fingernail clam – 11.3% occurrence) and Naididae (oligochaete worm – 9.5% occurrence). To a lesser degree, Group 1 is also characterized by Valvatidae (snail – 6.0% occurrence), Sabellidae (polychaete worm – 5.9% occurrence), and Asellidae (isopod – 4.3% occurrence). These seven families make up 61% of the total families found in Group 1 (Table 8a).

Five Thunder Bay sites have the highest probability of belonging to reference Group 1: P1, P2, P3, P6, and P7 (Table 7). These sites are located along the northern shore of the sampling area – see Figure 1a). Mean abundances of predominant taxa and taxa richness for Thunder Bay sites are shown in Table 8a. Tubificidae and Chironomidae are present at all sites; there are increased abundances compared to reference at sites P1, P2 and P6 and decreased or similar abundances at P3 and P7. Site P1 also has increased abundance of Asellidae and slight increased abundances of Naididae and Sabellidae; this site is quite diverse (16 taxa). Site P6 has the greatest abundance of tubificids (305× the reference mean) and Naidiids (15× the reference mean) but Sphaeriids are absent and this site is the least diverse (4 taxa). Site P7 has decreased mean abundance of all families (except Asellidae). Taxa diversity ranges from 4 to 16 (Table 8a). The number of taxa present at P1 is above two standard deviations (SD) of the reference mean (8 taxa). The number of taxa present at the remaining sites (4 to 6 taxa) is lower than the reference mean but within two SD of the mean.

Test sites predicted to Great Lakes reference group 5

Reference Group 5 is based on 75 sites from Lake Superior (30), Georgian Bay (19), the North Channel (12), Lake Michigan (7), Lake Ontario (5) and Lake Huron (2). This group is characterized mainly by Haustoriidae (44.2% occurrence - consisting almost entirely of the amphipod *Pontoporeia hoyi*). To a lesser degree, Group 5 is also characterized by Tubificidae

(16.5% occurrence), Sphaeriidae (11.7% occurrence), Chironomidae (9.9% occurrence), and Lumbriculidae, Enchytraeidae, and Naididae (oligochaete worms – 1.9 to 6.8% occurrence). These seven most prominent families make up 96% of the total families found in Group 5 reference sites (Table 8b). Fourteen Thunder Bay sites have the highest probability of belonging to reference Group 5 (Table 7). Mean abundances and taxa richness for these Thunder Bay sites are shown in Table 8b. Haustoriidae (the most prominent taxa of the Group 5 reference sites) is absent from all 14 Thunder Bay sites. Tubificidae, Chironomidae and Sphaeriidae, present at all sites, are in increased abundances at all sites compared to reference (up to 81-fold for tubificids). Lumbriculidae and Enchytraeidae are either absent or present in fairly low abundance at most sites except P16 and P22 (lumbriculids only) and P23. Naidiids are present at all sites (except P4) in increased abundance compared to reference. Generally, sites are quite diverse, with the number of taxa present ranging from 5 to 17 (Table 8b). All sites except P4 are above the reference mean (6 taxa); 9 sites (P11 to P23, IB2) are above 2 SD of the reference mean.

BEAST (benthic community) evaluation

Results of the BEAST evaluation are summarized in Table 8. Ordinations are shown in Appendix E; Figures E1 to E4 (stress ≤ 0.154). Four separate ordinations were performed each with a subset of between 2 – 7 Thunder Bay sites. The ordinations of test sites maximally predicted to reference Group 1 are shown in Figures E1 and E2. Sites P6 and P7 were analyzed separately because these two sites lack particle size data (see section 4.2 for further explanation). Ordinations of the 14 test sites maximally predicted to reference Group 5 are shown in Figures E3 and E4. Relationships between the habitat variables and community response are also shown in Figures E1 to E4. Overall, significant habitat variables that are most highly correlated to the ordination axes scores are Hg, TOC, and site depth. (Organic contaminants are not included in BEAST analyses; therefore, their contributions are not known.) Taxa and environmental variables that are maximally correlated with the site locations are shown as vectors in the ordinations.

Most Thunder Bay sites (16/19) are either different or very different from reference, falling in Bands 3 and 4 (Table 8, Appendix E; Figures E1 to E4). One site is possibly different from reference (P22) and two sites are equivalent to reference (P3 and P7). Macroinvertebrate

families that are most highly correlated to the ordination axes scores include Chironomidae (Figures E1 and E4; $r^2 = 0.55, 0.65$), Tubificidae (Figure E2; $r^2 = 0.52$), and Sphaeriidae and Haustoriidae (Figure E3; $r^2 = 0.65, 0.56$). Examination of the relationship between environmental variables and ordination axes scores reveals some significant relationships. For each ordination, the most highly correlated variables are: site depth and NO_3/NO_2 (Figure E1; r^2= 0.17-0.18); TOC (Figure E2; r^2=0.36); Hg and site depth (Figure E3; r^2= 0.26), and; Hg and TOC (r^2=0.37, 0.26). Of the five test sites maximally predicted to reference Group 1, the departure of P6 is the most severe (i.e. located the farthest away from the reference centroid), likely due to the great abundance of tubificid worms at this site (Table 8a, Figure E2). Site P6 is located along an increasing gradient of TOC (Figure E2). Sites P1 and P2 are also either very different or different than reference and these sites are associated with increased abundances of several taxa. There does not appear to be any measured environmental variable associated with P1 and P2. Test sites that are maximally predicted to reference Group 5 are either different or very different than reference with the exception of P22. The movement of these Band 3 and Band 4 sites outside of reference is associated with increased abundance of several families (Figures E1 to E4), as well as decreased abundance of Haustoriidae (Figure E3). Some sites are located along an increasing gradient of Hg and TOC (Figure E3).

4.4 Sediment Toxicity Tests

Mean species survival, growth, and reproduction in Thunder Bay sediments are shown in Table 9. The established numeric criteria for each category (non-toxic, potentially toxic and toxic) for each species are also included. Acute toxicity is evident at five sites. Site P3 is most toxic site, and is acutely toxic to all four laboratory organisms. Site P7 is acutely toxic to two of four organisms, *Hexagenia* and *Hyalella*; sites P1, P6, and P12 are acutely toxic to *Chironomus*, *Hexagenia* and *Hyalella*, respectively.

BEAST (toxicity) evaluation

Results of the BEAST multivariate toxicity evaluation are summarized in Table 9. Ordinations, summarized on 2 of 3 axes are shown in Appendix F; Figures F1 to F3 (stress = 0.08 – 0.09). The use of multivariate assessment for toxicity test endpoints is advantageous as it reduces the redundancy between endpoints, and also down weights the *Tubifex* endpoints (i.e., the *Tubifex*

test has four measurable endpoints while the other tests have two (Reynoldson 1998). Endpoints contributing most to the ordinations ($r^2 \geq 0.67$) include *Hyalella* survival and *Tubifex* young production. The relationship between the habitat variables and toxicity is also shown in Appendix F; Figures F1 to F3. Mercury is not significant in the first (Figure F1) and is very weakly correlated ($r^2 = 0.06$) in the second ordination (Figure F2). Mercury is the most significant variable in the third ordination (Figure F3); however, the correlation is not high ($r^2 = 0.12$). Total organic carbon is the most significant variable (r^2=0.18, 0.23) in the first two ordinations (Figures F1 and F2).

The majority of Thunder Bay sites (13/19) are non-toxic (Band 1). Five sites are severely toxic (P1, P3, P6, P7 and P12 – Band 4). (There are no sites in Bands 2 or 3.) The movement of the severely toxic sites outside of reference is associated with decreased amphipod survival (Figures F1 and F3), decreased mayfly survival (Figure F2), and decreased chironomid growth (Figure F2). These endpoints are shown as vectors in Figures F1 to F3; sites are located along the same or similar vector lines in the opposite direction to the endpoint vectors. The departure of site P3 and P12 is most severe; these sites are located the farthest away from the reference centroid. Site P3 is associated with decreased *Hexagenia* survival (vector is shown opposite to P3; Figure F2) while P12 is associated with decreased *Hyalella* survival (vector is shown opposite to P12; Figure F3). The position of Hg in the ordination indicates that increased Hg is associated with P3, although the correlation is weak. There does not appear to be any measured environmental variable associated with the movement of site P12 outside of reference. It is possible that some unmeasured or undetermined stressor is causing toxicity to *Hyalella* at P12. Site P1 is oriented opposite to the *Chironomus* growth vector, indicating that decreased midge survival is associated with this site (Figure F2). Sites P6 and P7 are associated with decreased *Hyalella* survival (shown as a vector in Figure F1). Elevated TOC and Cu are associated with P6.

4.5 Sediment Toxicity and Contaminant Concentrations

HMDS and principal axis correlation

The ordination of the multiple measurements of sediment toxicity by HMDS for the Thunder Bay sites produced two descriptors of sediment toxicity (Appendix G; Figure G1). The resultant axes represent the original 10-dimensional among-site resemblances well (stress = 0.149).

Principal axis correlation produces a vector for each toxicity endpoint along which the projections of sites in ordination space are maximally correlated. *Chironomus* survival is negatively correlated to Axis 1; therefore, the greater the toxicity to midge survival, the higher its score for Axis 1. *Hexagenia* survival is positively correlated to Axis 2; therefore, the greater the toxicity to mayfly survival, the lower its score for Axis 2.

Principal components analysis

The concentrations of 10 metals (Cd, Cr, Cu, Fe, MeHg, Mg, Mn, Ni, Pb, and Zn), sediment (TN, TOC, TP), and overlying water nutrients (TKN, TP, NO_3/NO_2) were ordinated by PCA. The first and second principal components (PC1 and PC2) account for 47% and 29% of the variation, respectively (total of 76%). The remaining components each account for ≤8%; therefore, most of the structure in the data is captured in two components or dimensions. All nutrient variables (except NO_3/NO_2 and sediment TP) as well as Cd, Cu, MeHg, Pb, and Zn are negatively loaded for PC1; sites elevated in these metals and nutrients score low for PC1. Overall, the magnitude of the negative loadings range from –0.079 (Pb) to –0.339 (TOC)). For PC2, all measurement variables are positively loaded except MeHg and Pb. These two principle components combined – denoted as "metPC1" and "metPC2"– are considered fair descriptors of general metal contamination and nutrient enrichment.

Toxicity-contaminant relationships

The integrated descriptors of sediment toxicity (Axis 1 and 2 scores from the HMDS) and the important individual toxicity endpoints (survival of *Hyalella* and *Hexagenia*, growth of *Chironomus*) are plotted against the contaminant descriptors metPC1 and metPC2 (Appendix G; Figure G2). Relationships among individual measurement variables were also evaluated by plotting the integrated toxicity descriptors (HMDS Axes 1 and 2) as well as the most significant toxicity endpoints against individual concentrations of metal, nutrient and grain size distribution (Figures G3 to G6).

General contaminant descriptor relationships

Using the integrated toxicity descriptors, sediment toxicity is related to sediment contaminant levels (Figure G2 [top]). For the Axis 2 toxicity descriptor, the contaminant descriptors

("metPC1" and "metPC2") account for 33% of the variability (P =0.016 for the regression). Both "metPC1" and "metPC2' are significant predictors (P = 0.043 and P = 0.026, respectively). There are no significant relationships for the HMDS Axis 1 toxicity descriptor.

ToxAxis2 = - 0.000 + 0.125 metPC1 + 0.179 metPC2

Slightly stronger relationships are found between some individual toxicity endpoints (significant at $p \leq 0.01$) and the integrated metal contaminant descriptors (Figure G2 [bottom]). There is no significant relationship for *Hyalella* survival.

For *Chironomus* growth (Crgw), the regression is significant for metPC2 at P = 0.005; the model accounts for 34% of the variability.

Crgw = 0.363 – 0.0381 metPC2

For *Hexagenia* survival (Hlsu), the regression is significant at P =0.001, and accounts for 59% of the variability. Both "metPC1" and metPC2 are significant predictors (P = <0.001, 0.046):

Hlsu = 85.1 + 6.37 metPC1 + 3.76 metPC2

<u>*Individual contaminant relationships*</u>

Regression of the toxicity descriptor HMDS Axis 1 and 2 and the measurement contaminant, nutrient, and grain size also produce significant relationships (Figures G3 and G4). For the Axis 1 toxicity descriptor, 83% of the variability is explained by the following predictors:

- log total Hg
- log Mn
- arcsine square root sand
- log NO_3/NO_2
- pH
- log total P (water)

The regression is significant at P<0.001, all predictors are significant at $P \leq 0.030$, and all variance inflation factors (VIF) are < 3. Predictors with positive regression coefficients (total Hg, Mn, Sand, and NO_3/NO_2) are potentially toxic to *Chironomus* survival, whereas those with negative coefficients (pH, TP (water)) are possibly toxic to *Tubifex* young production.

*ToxAxis 1 = 14.0 + 1.12 log**THg** + 1.50 logMn + 0.837 ARCSand + 7.76 logNO_3/NO_2*
- 2.36 pH - 2.02 logTP(W)

For the HMDS Axis 2 descriptor, the model that best explains the variation does not include Hg. For the Axis 2 toxicity descriptor, the regression is significant at $P < 0.001$, and 76% of the variability is explained by the following predictors:

- log Fe
- log Pb
- log Zn
- arcsine square root silt
- log NO_3/NO_2

$$ToxAxis\ 2 = 1.77 + 3.49\ logFe + 4.71\ logPb - 3.01\ logZn + 11.5\ logNO_3/NO_2 + 1.68\ ARCsilt$$

Predictors are all significant at $P \leq 0.026$ and all VIF are ≤ 3.3. Predictors with positive regression coefficients (Fe, Pb, silt and NO_3/NO_2) are potentially toxic to *Chironomus* growth, whereas the predictor with a negative coefficient (Zn) is possibly toxic to *Hexagenia* survival and growth.

Regression of the individual toxicity endpoints and the individual measurement contaminant, nutrient, and grain size variables also produce significant relationships (Figures G5 and G6). All individual endpoint regressions are significant at $P \leq 0.012$. After dropping terms that were not significant ($P > 0.05$) or had high (>10) variance inflation factors, the models below best explained the most variance in each toxicity endpoint among sampling sites. The *Hexagenia* model includes methyl Hg.

For *Chironomus* growth, the following model explains 77% of the variation, with predictors significant at $P \leq 0.009$:

$$Crgw = 2.06 - 1.55\ log\ Cr + 0.542\ log\ Mg - 0.572\ log\ Pb + 0.558\ log\ Zn - 1.25\ log\ NO_3/NO_2$$

For *Hyalella* survival, the following model explains 36% of the variation, with predictors significant at $P \leq 0.008$:

$$Hasu = -69.2 - 78.4\ log\ TN + 138\ log\ TP(S)$$

For *Hexagenia* survival, 86% of the variation is explained by a combination of metals, water nutrients and grain size (predictors significant at $P \leq 0.036$); however, 57% of the variation is explained by methyl Hg.

$$Hlsu = -26.3 + 150 \log Fe + 111 \log NO_3NO_2 + 56.1 \log Pb + 24.8\ silt$$

$$Hlsu = -12.2 - 43.7 \log \mathbf{MeHg}$$

4.6 Biomagnification Potential

4.6.1 Sediment mercury levels

Total mercury (Flett laboratory)

On a dry weight basis, lower [THg] are found in the Lake Superior reference sediments (range 5 to 83 ng/g, median 19 ng/g); Thunder Bay [THg] range from 106 to 39700 ng/g (median 711 ng/g), with the highest [THg] found at P6 and P7 (Figure 2, Table 10).

The LEL for THg (200 ng/g) is not exceeded at any reference site, while it is exceeded at all Thunder Bay sites except P16, P22, P23, and IB2. The SEL (2000 ng/g) is exceeded at four sites: P1, P6, P7, and just slightly at P10. The highest THg concentrations are found in sediments collected along the northern shore of the sampling area, while in general, lowest concentrations of THg are present at sites at the southern part of the sampling area.

Methyl mercury (Flett laboratory)

Methyl mercury concentrations (Figure 3, Table 10) are lowest at Lake Superior reference sites, ranging on average from 0 to 0.36 ng/g dry wt (median 0.05 ng/g). All reference sites, with the exception 5103, are below the detection limit of 0.25 ng/g. Thunder Bay [MeHg] range from 1.50 to 49.77 ng/g (median 5.50 ng/g). The highest concentrations occur at P6 and P7, the same as that observed for THg. The mean fraction of methyl mercury relative to total mercury at Thunder Bay sites is 0.93% (95% CI of 0.11 to 1.74%), but at three outlying sites (P3, P22, P23) the percent methyl Hg is 2.60, 3.25, and 1.89%, respectively. The percent methyl Hg is lower at reference sites at 0.33% (95% confidence interval of -0.47 – 1.13%), but at one outlying site - 5101 – the percent methyl mercury is 1.58%.

Methyl mercury-total mercury relationship

The relationship between methyl mercury and total mercury in the sediment (log-transformed) is shown in Figure 4. A significant strong positive correlation ($P<0.001$, $r^2 = 0.839$) exists between the methyl and total mercury concentrations in the sediment.

Comparison of sediment mercury at test sites to reference sites

For total mercury, all test sites are above the 99th percentile of the Lake Superior reference sites (Figure 2). Almost all Thunder Bay sites are 1 to 4 orders of magnitude higher in [THg] than the maximum [THg] of the reference sites. The median [THg] of the Thunder Bay sites 37× the median of the reference sites.

A similar pattern is observed for methyl mercury (Figure 3). All test sites exceed the upper 99th percentile of the reference sites by 1 to 4 orders of magnitude. The median [MeHg] of the Thunder Bay sites 110× the median of the reference sites.

4.6.2 Invertebrate mercury levels

Two separate taxa (chironomids and oligochaetes) could not be analyzed at all sites due to insufficient tissue quantity. Chironomid tissue was analyzed at all test sites but there was insufficient tissue at six reference sites. Oligochaetes could not be analyzed at five test sites and five reference sites. There is only one site where there was insufficient tissue quantity for both taxa – reference site 5100.

Total mercury

On a whole-body, uncleared-gut basis, chironomids show a greater range of total Hg accumulation across sites (42 to 2764 ng/g, reference median 75 ng/g, test median 252 ng/g) compared to the oligochaetes (27 to 654 ng/g, reference median 252 ng/g, test median 471 ng/g; Table 11). However, oligochaetes have slightly higher [THg] than chironomids at 10 of 14 test sites and at 9 of 10 reference sites. (Oligochaetes could not be analyzed at all sites due to insufficient tissue.) Concentrations of THg in chironomids and oligochaetes at test and reference sites are significantly correlated ($r=0.504$, $P=0.012$).

Methyl mercury

Chironomids show a greater range of methyl Hg accumulation across sites (7.7 to 138.0 ng/g; reference median 14.0, test median 60.0 ng/g) compared to the oligochaetes (1.1 to 46.0 ng/g; reference median 10.0, test median 4.6; Table 12). Chironomids also have higher [MeHg] than oligochaetes at all test sites (~1 to 2 orders of magnitude higher), and at 8 of 10 reference sites, different than that seen for THg. The correlation between chironomids and oligochaetes for [MeHg] (test sites only) is significant (r=0.534, P=0.049).

Comparison of mercury in benthic invertebrates at test sites to reference sites

Figures 5 to 8 show the concentrations of total and methyl mercury in chironomids and oligochaetes at test sites compared to the Lake Superior reference sites.

Chironomids – Total Hg All 19 test sites except 1B2 are above the 99th percentile of the reference site concentrations (Figure 5). Excluding reference, the lowest total Hg accumulation in chironomids is at P13 to P23 and IB2, which show very similar concentrations. The greatest accumulation occurs at P7 and P6, where there is the presence of white fibrous material.

Oligochaetes – Total Hg 3 of 14 test sites are slightly above the 99th percentile of the reference site concentrations (Figure 6). Excluding reference, the lowest total mercury accumulation in oligochaetes is at P2, and the greatest accumulation is seen at P7 (same as chironomids) and P10 and P12, which show very similar concentrations.

Chironomids – Methyl Hg 9 of the 19 test sites are above the 99th percentile of the reference site concentrations (Figure 7). Excluding reference, the lowest methyl mercury accumulation in chironomids occurs at P9, and the greatest accumulation occurs at P12, P22, P23, and P7, which show similar concentrations.

Oligochaetes – Methyl Hg 2 of the 14 test sites are above the 99th percentile for the reference site concentrations (Figure 8). Among test sites, the lowest methyl mercury accumulation is at P9 (same as observed for total Hg), and the greatest accumulation is seen in oligochaetes from

P7 and P22 (similar to what is seen for total Hg, as well as that seen for chironomids). (Site P22 is only slightly above the 99th percentile.)

4.6.3 Biota-sediment accumulation factors

Biota-sediment accumulation factors (BSAFs) for total and methyl mercury are shown by area (reference and Thunder Bay) for each taxon in Figure 9. For THg, BSAFs for Thunder Bay sites (based on whole-body, uncleared-gut concentrations) are similar for both taxa, ranging from 0.03 to 2.11 and from 0.02 to 2.74 for chironomids and oligochaetes, respectively. The BSAFs for test sites are higher for MeHg than THg, ranging from 1.2 to 51.1 and from 0.2 to 6.4 for chironomids and oligochaetes, respectively. The BSAFs for reference sites are much higher than Thunder Bay sites. With the exception of site 2514 (oligochaete BSAF of 0.5), BSAFs for THg are >1, ranging from 1.2 to 66, and for MeHg range from 5 to 1320 for reference sites.

4.6.4 Relationships between mercury concentrations in benthic invertebrates and sediment

Total mercury

Concentrations of THg in each invertebrate taxon vs. THg in sediment are plotted in Figure 10, with fitted regression lines using sediment [THg] alone as the predictor (Model A). For chironomids, the slope is significant ($P \leq 0.001$) and the $R^2_{adj} = 0.867$. The slope is also significant for the oligochaetes; however, the relationship is weaker ($P = 0.023$, $R^2_{adj} = 0.146$). Predictions of $[THg]_{inv}$ are improved slightly for the chironomids with NO_3/NO_2 and for the oligochaetes with sediment manganese as additional predictors (Model B) (Table 13); R^2_{adj} values are increased to 0.884 and 0.363 for the chironomids and oligochaetes, respectively (the slope for the oligochaetes significant at $P \leq 0.001$). For both taxa, $[THg]_{sed}$ is the strongest predictor ($P \leq 0.001$) in the Model B scenarios; coefficients for NO_3/NO_2 and manganese are positive.

Methyl mercury

The relationships between MeHg in benthic invertebrates and MeHg in sediment are weaker than those for total Hg (Figure 11, Table 13). With $[MeHg]_{sed}$ alone as the predictor (Model A), the regression is significant for the chironomids ($P \leq 0.001$, $R^2_{adj} = 0.466$), but not for the

oligochaetes ($P = 0.333$, $R^2_{adj} = 0$). For the chironomids, the regression accounts for more variability in $[MeHg]_{chir}$ with sediment total nitrogen, site depth, and temperature in the model ($P \leq 0.001$, $R^2_{adj} = 0.634$) (Model B). Total nitrogen, depth and temperature have negative coefficients, and MeHg is the strongest predictor ($P \leq 0.001$) in the model. For the oligochaete, the regression becomes significant with sediment total nitrogen in the model ($P = 0.001$, $R^2_{adj} = 0.380$). The coefficient for total nitrogen is negative and is a more significant predictor than MeHg. This suggests that low nitrogen conditions are important in the uptake of MeHg in oligochaetes. Although the reference site $[MeHg]_{sed}$ used in determination of the relationship between $[MeHg]_{inv} - [MeHg]_{sed}$ are below the detection limit, the formal designation of the detection limit based on EPA methods is 3× SD observed at very low concentrations (Robert Flett, Flett Research Ltd., Winnipeg, MB, pers. comm.). Thus, 'real' values can be obtained below the detection limit, although the measurement error is larger closer to the detection limit (Flett, pers comm.).

Relationships between $[MeHg]_{inv}$ and $[THg]_{sed}$ were also examined and found to be weaker than $[MeHg]_{inv} - [MeHg]_{sed}$. With $[THg]_{sed}$ alone as the predictor, the regression is significant for the chironomids but accounts for less variability than with $[MeHg]_{sed}$ ($P \leq 0.001$; $R^2_{adj} = 0.335$); the regression for the oligochaetes is not significant ($P = 0.635$; $R^2_{adj} = 0$).

4.7 Predictions of Methyl Mercury Concentrations in Receptors

4.7.1 Presentation of model outcomes

Predicted concentrations of methyl mercury in each receptor species at each sampling site, calculated by multiplying observed methyl mercury concentrations in invertebrates (wet weight values – from Table 12) by the appropriate FCMs (from Table 3), are shown in Table 14 and Figure 12. Receptor MeHg concentrations are presented for "minimum", "intermediate" and "maximum" levels of mercury exposure and uptake scenarios. In each subfigure, predicted $[MeHg]_{rec}$ for the five receptors are presented in bar charts comparing reference and test sites. In the bar charts, which have the same logarithmic scales in all subfigures, two criteria concentrations are marked: (1) the 99th percentile of the predicted $[MeHg]_{rec}$ for the reference sites, and (2) tissue residue guideline (TRG) for the fishes. The tissue residue guideline (TRG) applies only to the fish receptors and it refers to the concentration of MeHg in the diets of

wildlife that consume aquatic biota. The TRG used for MeHg is the lowest of the reference concentrations derived by Environment Canada (2002) for the protection of wildlife receptors in the AOC that consume aquatic biota: 92 ng/g ww. This pertains to the American mink (Table 12 of Environment Canada 2002). The recommended TRG for the protection of *all* wildlife species – 33 ng/g ww – was not considered appropriate because it is based on the reference concentration for the Wilson's Storm Petrel, which is not native to the Thunder Bay area.

4.7.2 Exceedences of criteria

Methyl Hg – minimum

The low predictions of $[MeHg]_{rec}$ result in two Thunder Bay sites (P6, P7) exceeding the TRG for perch only (Table 14a, Figure 12a). Of these two sites, P6 is also above the 99th percentile for the reference sites. Exceedence of the TRG is also predicted for the perch at 1 reference site (2513). For the perch and the sucker, the minimum, intermediate, and maximum predicted $[MeHg]_{rec}$ for some reference and test sites are the same. This is because 1) low, intermediate and high invertebrate tissue Hg concentrations are the same as only 1 taxa could be analyzed, and 2) there is only one BMF value for the sucker and perch, thus the low, medium and high FCMs are the same (see Table 3).

Methyl Hg – intermediate

The medium predictions of $[MeHg]_{rec}$ result in 7 Thunder Bay sites exceeding the TRG for perch and 12 sites exceeding the TRG for Walleye (Table 14a, Figure 12a). Of these, 5 sites also exceed the 99th percentile for the reference sites for the perch and Walleye. Exceedence of the TRG is also predicted for the perch and Walleye at 1 reference site (2513).

Methyl Hg – maximum

The maximum predictions of $[MeHg]_{rec}$ result in 10 Thunder Bay sites exceeding the TRG for perch and all sites exceeding the TRG for Walleye (Table 14a, Figure 12a). Of these, 8 sites also exceed the 99th percentile for the reference sites for perch and Walleye. In comparison, there is no reference site exceedence of the TRG predicted for the sucker, 1 reference site exceedence of the TRG predicted for the perch, and 17 predicted for the Walleye.

4.7.3 Overall patterns

Beyond the comparisons of predicted $[MeHg]_{rec}$ for test sites to reference sites and to the TRG, patterns are evident in the differences in predicted $[MeHg]_{rec}$ among the five receptors, and among the three exposure and uptake scenarios.

Among receptors

Predicted $[MeHg]_{rec}$ generally increases with the trophic level of the receptor, with differences of 2× to 50× between sucker and mink predictions (Table 14, Figure 12). Consequently, the number of sites at which $[MeHg]_{rec}$ exceeds the TRG and the amount by which the TRG is exceeded increases overall with the trophic level of the receptor. However, the number of exposed sites at which predicted $[MeHg]_{rec}$ are above the 99th percentile of reference site concentrations is the same among receptors. This is because within a series (i.e., any of the minimum/intermediate/ maximum groups) all derive from the same $[MeHg]_{inv}$ values. Differences among predicted $[MeHg]_{rec}$ values reflect differences among uptake pathways in the FCMs from Table 3. The pattern of variability among sites is the same for all receptors within a scenario (i.e., the $[MeHg]_{rec}$ values are fully correlated among receptors).

Among exposure and uptake scenarios

Looking at differences in results across the three exposure and effect scenarios for the same receptor, predicted $[MeHg]_{rec}$ (for all sites) ranges on average 6× (sucker) to 179× (Walleye). The predicted [MeHg] range for the heron (49×) is narrower than the Walleye range because the FCM (Table 3) for the minimum exposure and effect scenario for the heron is larger than that of the Walleye. The predicted [MeHg] range for the mink (182×) is similar to that of the Walleye. The number of Thunder Bay sites for which $[MeHg]_{rec}$ values exceed the TRG increases from minimum to maximum scenario. In the minimum predictions, no Thunder Bay or reference site $[MeHg]_{rec}$ values exceed the TRG, except for the perch, for which two test sites and one reference site exceed the TRG. In the intermediate scenario, 0 sites based on sucker, 7 sites based on perch and 12 sites based on Walleye have $[MeHg]_{rec}$ greater than the TRG. The reference sites exceedences are 0 for sucker, and 1 for perch and Walleye. In the maximum scenario, 0 sites based on sucker, 10 sites based on perch and all 19 sites based on Walleye have $[MeHg]_{rec}$ greater than the TRG, while the reference sites exceedences are 0 for sucker, 1 for

perch and 17 sites for the Walleye (Table 14). Differences among scenarios increase overall with trophic level of the receptor due to the increase in variability in the FCMs as the trophic pathway lengthens.

5 DISCUSSION

5.1 Mercury Concentrations at Thunder Bay Sites Relative to Reference Sites

5.1.1 Sediment

Concentrations of total Hg in the upper 10 cm layer of sediment sampled in 2002 from all Thunder Bay sites are greater than [THg] in sediment at references sites (Figure 2). The maximum $[THg]_{sed}$ are 39700 ng/g and 38900 ng/g (at the sites with heaviest white fibrous material); however, most sites have concentrations between the LEL (200 ng/g) and the SEL (2000 ng/g). Reference sites are in the range of 5 to 83 ng/g, which compare to background concentrations of 10 to 700 ng/g for the Great Lakes, and overlap the range in concentration at reference sites sampled along the north shore of Lake Superior in May 2002 (range 8 to 169 ng/g; Grapentine et al. 2005a). The highest Hg concentration observed in Thunder Bay is greater than the maximum concentration of 5568 ng/g observed for sites in the St. Lawrence River (at Cornwall) and similar to the maximum concentration observed for contaminated sites in Peninsula Harbour (32160 ng/g) (Grapentine et al. 2005a, b). Mean total Hg concentrations in surficial sediments from the current study are generally similar to those reported by Stantec (2003) in their 1997 study (16 concomitant sites) with some exceptions (Appendix H; Table H1). Total [Hg] at sites P2 and P3 are 5.9× and 5.8× higher, respectively, in 1997 than in 2002. Total [Hg] at sites P6 and P7 (where the white fibre is present), are 8.1× and 4.5× higher, respectively, in 2002. Total [Hg] at remaining sites are generally similar (from 1.2× to 2.0× higher in 2002). Mean methyl Hg concentrations in the current study are also generally similar that that found in 1997 (9 concomitant sites); the greatest difference is for site P1 (2× higher in 1997). The observed differences in Hg concentrations between the studies may reflect small scale heterogeneity in the area. Also, differences in [MeHg] between the studies could be due to the depth at which the samples were taken (1997 samples were collected from the top 0-3 cm sediment whereas they were collected from the top 0-10 cm sediment in the current study). The CCME (1999b) freshwater sediment quality guideline (Probable Effect Level) for total Hg is 486

ng/g, which is exceeded at 12 of the 19 sites. Within the sampling area, sediment contamination is highest at sites located along the northern shore of the sampling area. For MeHg, a similar pattern is observed (Figure 3). Sediment [MeHg] is strongly related to sediment [THg] (Figure 4), with [MeHg] making up an overall average of 0.9% and 0.3 % of the [THg] for Thunder Bay and reference sites, respectively. The percentage of MeHg to THg at Thunder Bay and references sites is similar to that observed in the St. Lawrence River and Peninsula Harbour sites (0.8 and 0.4% and 0.5 and 0.2%, respectively) (Grapentine et al. 2005a,b). The spatial pattern of these results is strong evidence for a local (as opposed to regional) source of Hg to this area of Thunder Bay.

5.1.2 Benthic invertebrates

Benthic community structure and toxicity

Thunder Bay sites have the highest probability of belonging to 2 benthic community groups: reference group 1 (5 sites) and reference group 5 (14 sites). The sites that are maximally predicted to Group 1 are located in the most northern part of the study area, closest to the Cascades settling ponds. These sites tend to be the most shallow and have the highest total organic carbon, which likely explains why these sites are predicted to a different reference group than the remaining 14 sites (TOC and site depth, as well as latitude, longitude and alkalinity are the habitat variables used as predictors in the model – see Section 3.6.1). These five sites that are closest to Cascades are less diverse than reference (except P1), and 3 of the 5 sites have increased abundances of tubificids and chironomids. The remainder of sites (14 sites predicted to Group 5) are characterized by the absence of a key haustoriid amphipod and increased diversity and taxa abundance (except P4).

The northern part of the study area is also where most of the toxicity is observed (4 of the 5 sites). *Hexagenia* and *Hyalella* are the most sensitive of the four laboratory organisms, showing an acutely toxic response at three sites each (2 of the 3 sites are the same). The midge shows an acutely toxic response at two sites and the oligochaete worm at one site. Toxicity-contaminant relationships (regressions) reveal that sediment mercury only partially explains toxicity in some cases. For example, just a little over half of the variability (57%) in mayfly survival is explained by sediment $[MeHg]_{sed}$ alone. The nature of the substrate (white fibrous material) likely poses a problem for the mayflies as this is not suitable material for building tubes. (*Hexagenia* build U-

shaped tubes, preferably in silt bottoms, and filter water through these tubes with their gills.) Pulp fibres were noted at sites P1, P2, P3, with greatest quantity at sites P6 and P7. Reduced *Hyalella* survival does not appear to be explained by sediment mercury (no significant relationships were found).

Tissue concentrations

In general, $[THg]_{inv}$ for the Thunder Bay sites are several fold the $[THg]_{inv}$ for the reference sites. Median [THg] for Thunder Bay sites are 1.8× and 5.8× higher than the reference site medians for oligochaetes and chironomids, respectively. For MeHg, the test: reference site ratio is 3.8 for chironomids, while for the oligochaetes, [MeHg] is similar for reference and test sites. (The median value is actually 2.3× higher for the reference sites.) Total [Hg] in chironomids is greater than that found in reference sediment at 18/19 Thunder Bay sites; for the oligochaetes, total [Hg] is greater than that found in reference sediment at 3/14 Thunder Bay sites. Fewer exceedences by individual Thunder Bay sites of the 99th percentile of the reference sites are observed for methyl Hg than for total Hg. Methyl Hg accumulates in the chironomids to higher concentrations than that found in reference sediment at 9/19 Thunder Bay sites; for oligochaetes, methyl Hg accumulates to higher concentrations than that found in reference sediment at 2 of 14 sites. Mean total [Hg] in oligochaetes are from ~1.6 to 43× lower than those reported by Stantec (2003) in their 1997 study at 7 concomitant sites (Appendix H, Table H1). The greatest difference is for site P1. Mean methyl [Hg] in oligochaetes (2 concomitant sites) is 1.8× higher at site P7 and 12× lower at site P13 in 2002. Oligochaete [Hg] reported in Appendix H; Table H1 for the current study were converted from dry weight [Hg] (moisture content corrected), whereas the oligochaetes were analyzed as wet tissue in the Stantec study. This does not likely account for differences in [Hg] between the studies as tissue samples in the current study were freeze dried and therefore the conversions should be fairly reliable. Also, the mercury analyses were performed by the same laboratory (Flett Research) using the same analytical methods. Baker et al. (2004) found [Hg] in freeze dried fish fillet samples that were back calculated significantly lower than [Hg] that were based on wet weight tissues. However, in subsequent analyses, they found no significant differences. Small loss of Hg due to freeze drying was therefore not ruled out in this study (Baker et al. 2004).

In general, the sites that have the highest BSAFs are those with the lowest total sediment Hg concentrations (reference sites). For oligochaetes, BSAFs are greater for methyl Hg than total Hg for the reference sites, but the test site BSAFs are similar for total and methyl Hg. For chironomids, BSAFs are greater for methyl Hg than total Hg at all sites. On average, oligochaetes have slightly higher BSAFs for total Hg, whereas chironomids have higher BSAFs for methyl Hg. Tremblay et al. (1996b), in a study of two reservoirs and a natural lake in Quebec, reported BSAFs for detritivorous insects to be 1.9 – 2.8 for total Hg and 5.2 – 22.6 for methyl Hg, similar to that observed in Thunder Bay. The BSAFs for chironomids in the current study are similar to that observed for Jellicoe Cove (Peninsula Harbour) sediments for total Hg, but are lower for methyl Hg (Jellicoe Cove BSAFs: THg up to ~ 3 and MeHg up to ~ 300) (Grapentine et al. 2005a). For reference sites, BSAFs are much higher than the test sites. With the exception of site 2514 (oligochaete BSAF =0.5), BSAFs for total Hg are >1, ranging from 1.2 to 66; BSAFs for methyl Hg range from 5 to 1320 (Figure 9). Tissue concentrations do not increase as much as sediment concentrations at highly contaminated sites; therefore, the higher BSAFs observed for the reference sites are not unusual. Gut contents are included in the mercury analyses of the biota, which could obscure true BSAFs. As the amount of sediment in the gut increases, the measured BSAF will converge to 1. A true BSAF < 1 will be overestimated because the concentration in the sediment is greater than the tissue concentration, whereas a true BSAF >1 will be underestimated because sediment concentrations are lower than that found in the tissue (Bechtel Jacobs 1998).

5.2 Effects of Mercury in Sediment on Mercury in Invertebrates

The log-log relationships between $[THg]_{sed}$ and $[THg]_{inv}$ across sites is strong for 1 of the 2 taxa (chironomids – Figure 10). The log-log relationships between $[MeHg]_{sed}$ and $[MeHg]_{inv}$ are weaker than those for total Hg for both taxa; however, the relationship is significant for the chironomids (Figure 11). For the chironomids, $[MeHg]_{sed}$ alone significantly predicts $[MeHg]_{inv}$; however, the $[MeHg]_{inv}$ - $[MeHg]_{sed}$ relationship improves when considering reduced sediment total nitrogen as well as temperature and depth (Table 13). For oligochaetes, sediment total nitrogen is required to significantly predict $[MeHg]_{inv}$ (Table 13). As can be seen from the R^2_{adj} - values of the oligochaete model, only 38.0% of the variation in $[MeHg]_{inv}$ is explained by the $[MeHg]_{sed}$ and total nitrogen, with total nitrogen being the more significant predictor. (The

amount explainable by $[MeHg]_{sed}$, the partial r^2, which is proportional to the P (predictor) for $[MeHg]_{sed}$ would be lower.) Therefore, while $[MeHg]_{sed}$ is a statistically significant predictor, other factors (reduced sediment total nitrogen) are important in determining the uptake of methyl Hg in the oligochaetes.

Concentrations of Hg in the benthic invertebrates were measured without clearing their guts. Thus, a fraction of the observed $[Hg]_{inv}$ could include sediment-bound Hg in the gut. This is relevant for assessing uptake of Hg by predators of invertebrates, which consume whole organisms, and can also factor in the strength of the $[THg]_{sed}$ - $[THg]_{inv}$ relationships. Concentrations of total Hg in sediment are generally 1 – 2 orders of magnitude greater than those for methyl Hg, and total Hg vary more among sites.

Other studies have reported significant relationships between [Hg] in sediment and [Hg] in benthic invertebrates. Bechtel Jacobs (1998) reviewed data from 15 studies of [Hg] in freshwater benthic invertebrates and sediment. In 13 of these studies, invertebrate guts were not cleared. Slopes of $\log[THg]_{inv}$ vs. $\log[THg]_{sed}$ regressions were 0.327 ± 0.246 (mean ± S.E), and the mean r^2 was 0.12. This is similar to the slope observed for the chironomids for Thunder Bay and reference sites (0.421), whereas the slope for the oligochaetes is 0.148 for total Hg. Tremblay et al. (1996b) found a correlation between [MeHg] in chironomids and $[MeHg]_{sed}$ of $r=0.78$ ($P<0.005$, $n=18$) for a series of Quebec lakes, slightly weaker than the correlation between $[MeHg]_{chir}$ and $[MeHg]_{sed}$ in the present study ($r=0.93$, $p<0.005$, $n=39$). Sediments of Tremblay et al. (1996a) and Bechtel Jacobs (1998) were much less contaminated with Hg (≤ 350 ng/g dw) than Thunder Bay sediments. An assessment of bioaccumulation by chironomids from Hg-contaminated and reference sediments in the St. Lawrence River (at Cornwall) and Peninsula Harbour AOCs, using the same methods as the current study, shows good agreement between the studies for $\log[Hg]_{inv}$ vs. $\log[Hg]_{sed}$. The corresponding slope coefficients (Cornwall / Peninsula Harbour /Thunder Bay) are:

- THg in chironomids = 0.570 / 0.431 / 0.421
- MeHg in chironomids = 0.160 / 0.163 / 0.233

Results from this assessment indicate that $[MeHg]_{inv}$ is largely determined by $[MeHg]_{sed}$ for the chironomids. The positive relationships between sediment and chironomid methyl Hg concentrations is evidence that mercury transfers from sediment into the food web.

5.3 Predicted Methyl Mercury Concentrations in Receptor Species

5.3.1 Integration of prediction outcomes

Models involving a range of biomagnification conditions were used to predict potential [Hg] in receptors. Five receptor species were considered to encompass the trophic levels linking sediments to the top predators, where biomagnification is expected to be greatest. Three levels of dietary exposure and trophic transfer of Hg were assumed: minimum and maximum scenarios to bracket the range of potential outcomes and an intermediate scenario to characterize "average" conditions. Conclusions determined from overall evaluations of the model outcomes should consider:

- $[MeHg]_{rec}$ relative to the TRG;
- $[MeHg]_{rec}$ for exposed sites compared to $[MeHg]_{rec}$ for references sites;
- How many receptors are predicted to exceed the criteria at each site;
- How many of the exposure and uptake scenarios result in exceedences; and
- How many sites exceed the criteria.

On the whole, a minority of the 19 Thunder Bay sites are predicted to have $[MeHg]_{rec}$ higher than the TRG and the 99th percentile for the reference sites $[MeHg]_{rec}$. Figure 12a shows the sites meeting this condition for all exposure and uptake scenarios for the fish receptors. For the sucker, no test sites are predicted to result in such "hits" for any scenario. For the perch, $[MeHg]_{rec}$ predictions resulted in 1 hit for the minimum scenario, 5 hits for the intermediate scenario, and 8 hits for the maximum scenario. Walleye $[MeHg]_{rec}$ predictions resulted in 0 sites for the minimum scenario, 5 hits for the intermediate scenario, and 8 hits for the maximum scenario.

The TRG applies to concentrations of methyl Hg in fishes, and are for the protection of wildlife or human consumers of fishes. Some data are available for direct evaluation of the predicted tissue mercury levels for heron and mink. Wolfe et al. (1998) reviewed total Hg and methyl Hg toxicity and tissue residue data associated with adverse effects for birds and mammals. (As

noted above, nearly all mercury in fishes and higher trophic level animals should be in the methyl form.) For Great Blue heron, liver concentrations > ~6000 ng THg/g ww correlated with chronic adverse effects. A conservative residue threshold for major toxic effects in water birds was concluded to be 5000 ng THg/g ww in liver. For mink, a similar criterion of 5000 ng MeHg/g ww in muscle or brain was suggested. This value of 5000 ng/g corresponds to 3.7 on the log-scales in Figure 12b (shown as a red dashed). For the heron receptor, the highest predicted $[MeHg]_{rec}$ in any of the scenarios is 1856 ng/g ww, and for the mink, the highest $[MeHg]_{rec}$ prediction is 2730 ng/g ww (site P23 - Table 14b). Thus, $[MeHg]_{rec}$ is not predicted to exceed the tissue residue benchmarks suggested by Wolfe et al. (1998) for heron or for mink.

5.3.2 Uncertainty in the prediction of mercury concentrations in receptors

The prediction of the potential transfer of methyl Hg from benthic invertebrates to the trophically linked receptor species involves several simplifying assumptions, each of which is associated with some degree of uncertainty in its relevance to conditions in the Thunder Bay sampling area. While it is beyond the scope of this study to quantify these uncertainties, those considered most important are identified here.

Assumptions regarding the modelling of Hg biomagnification include those dealing with the exposure of the receptors to Hg, and those dealing with the effects of Hg on the receptors. Regarding the latter category, some of the sources of uncertainty discussed by USEPA (1997c) could apply to the present study:

- Validity of the biomagnification model;
- Variability of the calculated BMFs and FCMs;
- Selection of the receptors of concern;
- Trophic levels at which receptors feed;
- Limitations of the toxicity database (with respect to the determination of TRGs); and
- Effects of environmental cofactors and multiple stressors.

Among these sources, the greatest contributor to uncertainty in predicting the trophic transfer of mercury could be the large ranges in the selected BMF and FCM values. These range over 1 to

1.5 orders of magnitude between lowest and highest, and include all BMFs judged to be potentially applicable to Thunder Bay. Further validation of their relevance would require field studies beyond the scope of this assessment. Owing to limitations of the available data and the desire to minimize assumptions about the distributions of the data, a probabilistic approach was not applied to predict receptor mercury concentrations. Rather, low, medium and high FCMs were used to define the range of possible outcomes and intermediate values that "balance" the minimum and maximum rates of biomagnification. Another problem inherent in the literature-derived BMF data is the difficulty in assigning prey and predator species to discrete trophic levels due to omnivory. When omnivory is integrated with a continuous measurement of trophic position (e.g., using stable isotope methods), estimates of BMFs will generally be higher for each discrete trophic level (Vander Zanden and Rasmussen 1996). Correct determination of trophic levels is also limited by how well the composition of a predator's diet is quantified. Often the information necessary to clearly establish this is not available in the published studies.

Another potentially large source of uncertainty in predictions of $[MeHg]_{rec}$ relates the exposure of receptors to Hg. These assumptions (listed in Sec. 2.3) are recognized as being conservative and limited in their representation of natural conditions. Spatial (and perhaps temporal) heterogeneity in the distribution of total and methyl Hg throughout the study area, and aspects of receptor ecology challenge the maximum exposure scenario. A particularly important source of uncertainty could be the assumption of 100% residency of all consumers in the food chain on each site. The degree to which this assumption is unrealistic is proportional to the size of the foraging areas of the receptor species relative to the area of contaminated sediment. Given that the sampling sites could be on the order of 10 × 10 m to 100 × 100 m (= 0.01 to 1.0 ha), the 100% residency assumption is likely unrealistic. According to data compiled in the Wildlife Exposure Factors Handbook (USEPA 1993), feeding territory sizes for Great Blue heron range from 0.6 ha to 0.98 km^2, and distances they travel from heronry to foraging grounds range from 1.8 to 8 km. Home range sizes of mink are reported as 7.8 to 1626 ha, and 1.85 to 5.9 km of stream/river. These foraging/home range areas substantially exceed the site boundaries of the study. If areas outside of the Hg-contaminated zones of the Thunder Bay River are not equally Hg-contaminated, the actual $[MeHg]_{rec}$ would be lower than those predicted by the models.

5.3.3 Observed mercury levels in receptors from Thunder Bay

Comparison of actual [Hg] in fishes, heron and mink collected from the Thunder Bay AOC to the predicted $[MeHg]_{rec}$ are a means of qualitatively ground truthing the prediction model. Although fish and wildlife receptors may not feed as assumed by the prediction model (i.e., focus on single sites), and exposure histories can be difficult to determine, sources of mercury from beyond Thunder Bay should be low and contribute little to receptor mercury burdens, because expected foraging areas (at least for the fishes) are likely smaller than the Thunder Bay area. (Grapentine et al. 2005a estimated the maximum individual foraging areas of the Longnose sucker and lake trout to be 428 m^2 and 3459 m^2, respectively, based on models of Minns et al. (1996).) Measured [Hg] in recently sampled receptors indicate actual, as opposed to potential, biomagnification.

The most recent survey of sport fish contaminant levels include collections of White sucker and Lake trout (which occupy the same trophic position as Walleye) from the inner Thunder Bay Harbour in 2002 (MOE 2003a). These collections occurred around the mouth of the Kam and Mission Rivers, which is approximately 6-8 km south of the sampling area. Concentrations of Hg in suckers (43 – 55 cm length) are reported as ranging from 200 to 800 ng/g ww; concentrations in trout (43 – 56 cm length) are reported as ranging from 130 to 280 ng/g ww. The observed values for the sucker are high relative to the predicted [Hg], whereas the observed values for the trout are similar to the predicted [Hg]. Even the lowest measured sucker [Hg] substantially exceeds the highest maximum-scenario prediction of 55 ng/g ww (site P23 – Table 14a), whereas the observed trout values fall within the intermediate scenario. The higher Hg observed in sucker could result from the fact that suckers are more associated with sediments in diet and habit than the trout (Scott and Crossman 1973), and likely have more restricted habitat use areas (Minns et al. 1996).

Young-of-the-year white sucker and adult Walleye were collected in the actual Thunder Bay study area in 1998 by Stantec Consulting Ltd. (Stantec 2003). Total mercury was analyzed in all fish samples and methyl mercury was analyzed on a subset of the Walleye samples. Total Hg levels are reported as ranging from 11 to 86 ng/g ww for the young-of-year sucker and from 170 to 850 ng/g ww for the adult Walleye (length 40 – 56 cm). The observed values for the young

suckers fall within the intermediate and maximum scenarios; observed values for the Walleye (which are higher than those reported for the Lake trout by the MOE - see above), fall within the intermediate and maximum scenarios.

Observations of [Hg] in receptor species residing in the Thunder Bay AOC suggest that mercury does accumulate in tissues of higher trophic level members of aquatic food webs. It is also evident that the receptor methyl Hg concentrations predicted from the screening level approach of this assessment are not overestimating actual levels for the highest fish predator (Walleye or trout). Methyl Hg predictions are underestimating actual levels for the adult benthivorous fish (sucker), collected from 6-8 km south of the sampling area, but are similar to actual concentrations reported for young-of-year suckers collected within the sampling area.

5.4 Potential Risk of Adverse Effects of Mercury due to Biomagnification

Concluding that mercury originating from contaminated sediment could concentrate in the food web at levels that can cause adverse effects depends on establishing that:

(1) Mercury in invertebrates from sites exposed to industrial effluents is elevated relative to concentrations in invertebrates from reference sites;
(2) Mercury in invertebrates is related to mercury in sediment; and
(3) Predicted levels of mercury in receptors at exposed sites exceed the TRG and exceed levels in receptors at reference sites.

Results show that at nearly all Thunder Bay sites for THg and at ~half of the Thunder Bay sites for MeHg, chironomid [Hg] are significantly higher than concentrations for the reference sites However, this is not the case for the oligochaetes where few sites are significantly higher than concentrations for the reference sites for either total or methyl Hg. Measured mercury concentration in invertebrates is related to mercury concentration in sediment for total Hg. For the biologically relevant methyl Hg, measured [MeHg] in the chironomids is related to [MeHg] in the sediment; for the oligochaetes, $[MeHg]_{olig}$ is related to $[MeHg]_{sed}$ and total nitrogen. Regarding the trophic transfer modelling, based on outcomes for Walleye under the intermediate and maximum mercury exposure and uptake scenarios, 5 to 8 test sites could be considered "of

concern" because of predicted $[MeHg]_{rec}$ exceeding the TRG and the maximum reference site concentration (Figure 12a).

Regarding the overall assessment of sediment conditions based on the integrated framework outlined in Section 1.2, the biomagnification line of evidence can differ from the other three lines of evidence. If fish and wildlife receptors are the concern, the appropriate spatial and temporal boundaries for assessing potential biomagnification are not the same as those for assessing sediment contaminant concentrations, sediment toxicity and benthic invertebrate communities. Activities of fishes, birds and mammals are not limited to individual sites to the same degree as contaminants and invertebrates. Whereas incorporating invertebrate contaminant bioaccumulation information into the framework works well on a site-by-site basis, fish and wildlife data require some form of spatial averaging or weighting to reflect realistic contaminant exposure conditions. On a per site basis, fish and wildlife biomagnification predictions remain "theoretical" or overly conservative.

One way of addressing the problem is to assess exposure to contaminants across areas of sediment comparable to the foraging areas of the receptors, as suggested by Freshman and Menzie (1996). Their "average concentration with area curve" exposure model involves determining the average concentration of a contaminant for increasing areas of soil, starting with the most contaminated site up to and beyond the foraging area of the receptor of interest. The average contaminant concentration for a section of soil corresponding to the foraging area is then compared to appropriate benchmark adverse effect levels. Exceedence of the benchmark by the average contaminant concentration is considered a potential impact to the receptor individual. An example of where this technique was applied is Jellicoe Cove, Peninsula Harbour (Grapentine et al. 2005a). The application of this method requires a grid-type or other statistically suitable array of sampling sites designed to representatively quantify contaminant conditions across the study area. A rough characterization of conditions across the study area, obtained by averaging mercury concentrations for the sites within the study area, could be possible for the Thunder Bay study area.

The application of tissue Hg residue data that are associated with adverse effects in other studies to evaluate potential risks to the receptors in the present study carries some uncertainty. The data come from different tissues, species, environmental conditions and study types (e.g., field vs. lab). In addition, Hg detoxification and a possible ameliorative effect of dietary selenium may contribute further uncertainty in the extrapolation of results from one set of conditions to another (USEPA 1997c). The TRGs also typically include uncertainty factors. For example, the methyl Hg reference concentration (92 ng/g wet wt) incorporates an uncertainty factor of five (Environment Canada 2002). Considering these uncertainties and the generally conservative ("worst case") assumption of the trophic transfer model, quantifying the probability that mercury from sediments in Thunder Bay could cause adverse effects to receptors is difficult.

The likelihood of realizing the degree of mercury biomagnification predicted for the receptor species is not clear, due to uncertainties associated with predicting receptor [MeHg] values and conservative assumptions of the assessment. Comparing results from the screening level model used in this study to actual values in fish collected from the inner Harbour show that Hg is accumulating to higher levels than predicted from the model in some cases. Reducing uncertainty in the predictions of mercury biomagnification in Thunder Bay would be best achieved by identifying a more narrow range of appropriate BMFs, and by quantifying the actual exposures of receptors to dietary mercury. However, Hg data for the inner Thunder Bay Harbour clearly show that Hg is accumulating in higher trophic organisms to levels above guidelines, evidence of an actual problem in the Harbour.

5.5 Decision-Making Framework for Sediment Contamination

The main findings of the study are summarized in a sediment decision-making framework table (Table 15). The framework, described in Grapentine et al. (2002), is a rule-based, weight of evidence approach that that combines all lines of evidence to achieve an overall assessment on a site by site basis. Table 15 depicts the results for the contaminant analysis, community structure, toxicity and biomagnification components of the study for each site, and provides the interpretation and management recommendations for the site. A "+" denotes that there is indication of contamination or an adverse biological condition and a "-" denotes that there is no indication of contamination or an adverse biological condition.

Contaminant column

A "+" in the contaminant column indicates an elevation of contaminants above a threshold. In this case it is specific for Hg and indicates sites where sediment Hg concentrations exceed the provincial SEL.

- 7 sites have sediment total Hg concentrations above the SEL (both Caduceon and Flett Hg data considered - i.e., data from Tables 6 and 10).

Toxicity column

A "+" in this column indicates strong evidence of toxicity (i.e., a site falls in either of Band 3 or 4 from the BEAST analysis).

- 5 sites are in Band 4 (severely toxic).

Community structure

A "+" in the community structure indicates strong evidence of community alteration (i.e., a site falls in either of Band 3 or 4 from the BEAST analysis).

- 5 sites are in Band 3 and 11 sites are in Band 4.

Biomagnification column

A "+" in the column is determined by (a) a significant positive relationship between [MeHg] in the sediment and [MeHg] in the resident benthic invertebrates (determined with either Models A or B) for the study area, (b) using the *intermediate* and *maximum* uptake and exposure scenario, values are > TRG and > the predicted maximum reference concentration.

- 5 – 8 sites fall into this category.

6 CONCLUSIONS

The purpose of the study was to determine if contaminants are causing deleterious effects on benthos and whether contaminants (mercury) could potentially be transferred from sediments through benthic invertebrates to fish, wildlife or humans in the northern Thunder Bay inner Harbour. This was addressed by:

1. Determining if contaminants are having an effect on the community composition of the benthic invertebrates in the system;
2. Determining whether the sediments are causing any toxic effect on laboratory benthic invertebrates;
3. Determining if total and methyl Hg are bioaccumulating in resident benthic macroinvertebrates to higher concentrations than in unexposed reference sites;
4. Testing if concentrations of total and methyl Hg in invertebrates are related to concentrations in sediment; and
5. Predicting if concentrations of methyl Hg in consumers of benthic invertebrates and their predators (i.e., trophically linked receptor species) reach levels associated with adverse effects.

- Sediment total and methyl Hg levels are elevated above reference at all Thunder Bay sites. The highest Hg concentrations are found along the northern shore of the study area, and at the sites that contain the white fibrous material. **The spatial pattern of these results is strong evidence for a local (as opposed to regional) source of Hg to the area.**

- Total and methyl mercury concentrations in 1 of the 2 resident invertebrate taxa assessed (chironomids) at the majority of Thunder Bay sites are elevated above those at reference sites. **This indicates that benthic invertebrates accumulate Hg.**

- Concentrations of total mercury in sediment are strongly predictive of concentrations in resident chironomids. **This indicates that sediment [THg] affects invertebrate [THg].** Methyl mercury in sediment is significantly predictive of methyl mercury in chironomids. **This indicates that sediment [MeHg] affects invertebrate [MeHg].**

- Most Thunder Bay sites have different communities generally due to:
    - Increased diversity with the absence of the pollution-sensitive haustoriids and enrichment of more tolerant organisms such as tubificids and chironomids based on the other changes in the community (e.g., P5 to P23, IB2); or
    - Decreased taxa diversity and increased abundance of more tolerant organisms such as tubificids and chironomids (e.g. P2, P6); or
    - High species diversity, increased abundances of several taxa (e.g., P1).
- Enrichment is associated with increased total organic carbon in some cases.

- There is strong evidence of sediment toxicity at five sites (see Figure 13 for the location of these sites). Four of the five sites are located along the northern shore of the sampling area. Increased methyl mercury may partially explain toxicity to the mayfly; however, there may be unmeasured stressors involved as well as substrate related issues with respect to the white fibrous material.

- Under the intermediate and maximum mercury-exposure and uptake assumptions, the number of sites to also have predicted [MeHg] in receptors higher than the TRG and the 99th percentile of the reference site $[MeHg]_{rec}$ is 5 – 8 sites (see Figure 13 for the location of these sites). **This indicates that mercury could bioaccumulate in Yellow perch and Walleye to levels that are not protective of adverse effects at 5 – 8 sites.**

- Risk management evaluation is recommended for 9 sites. For 8 of the 9 sites this is due to biomagnification. For one site (P1), it is due to elevated sediment [Hg] and concurrence of sediment toxicity and benthic alteration.

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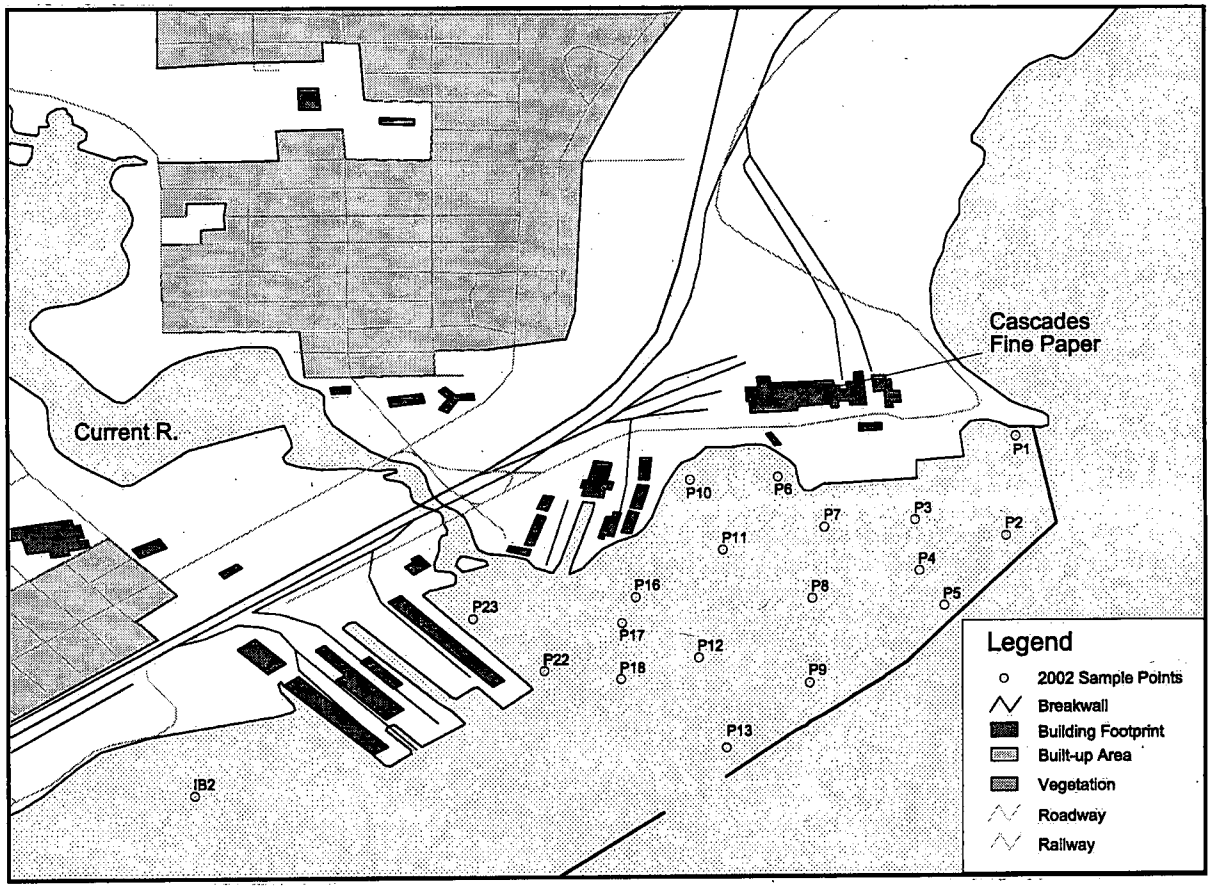


Figure 1a. Location of sites in northern Thunder Bay.

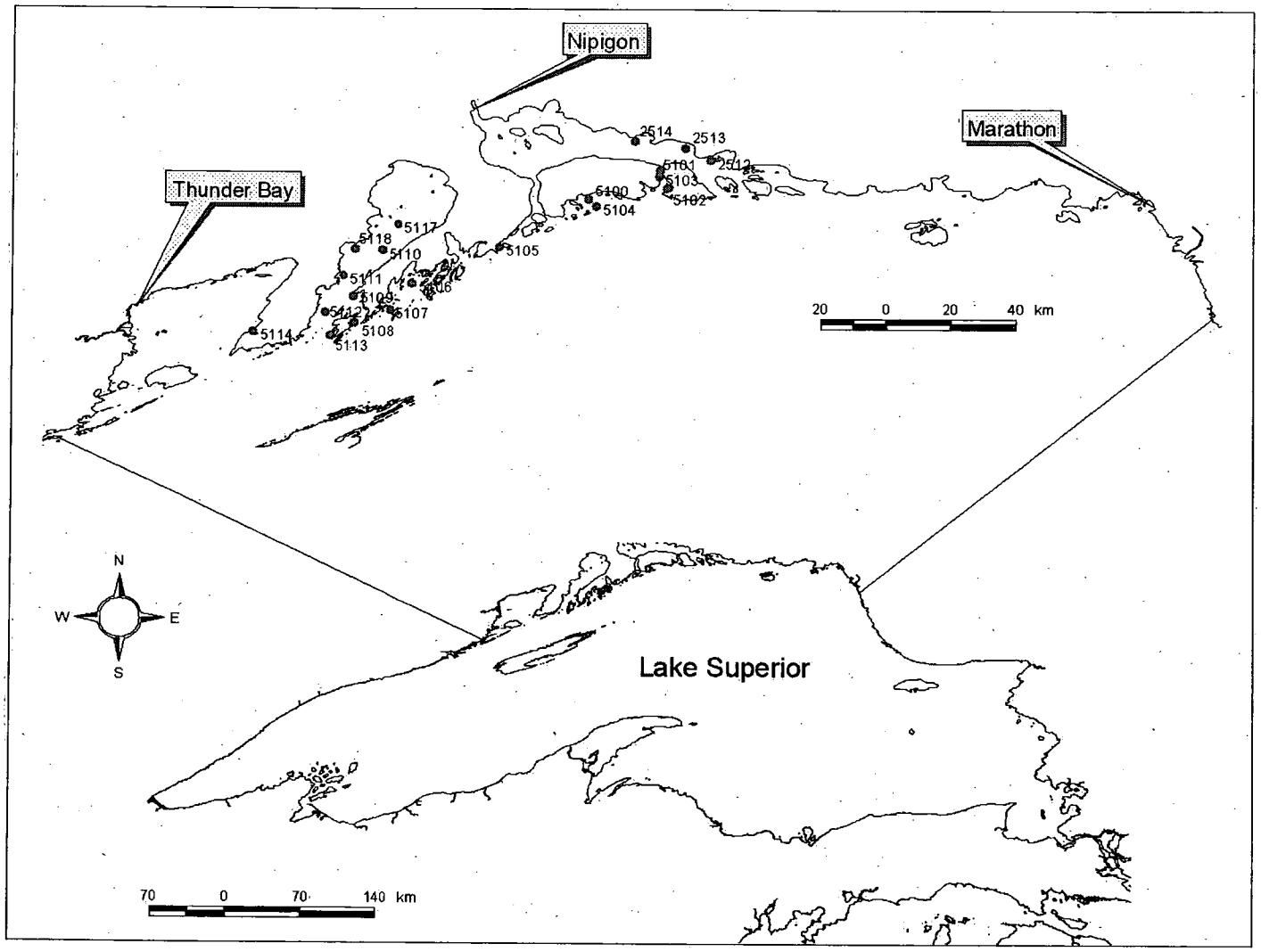


Figure 1b. Lake Superior reference site locations.

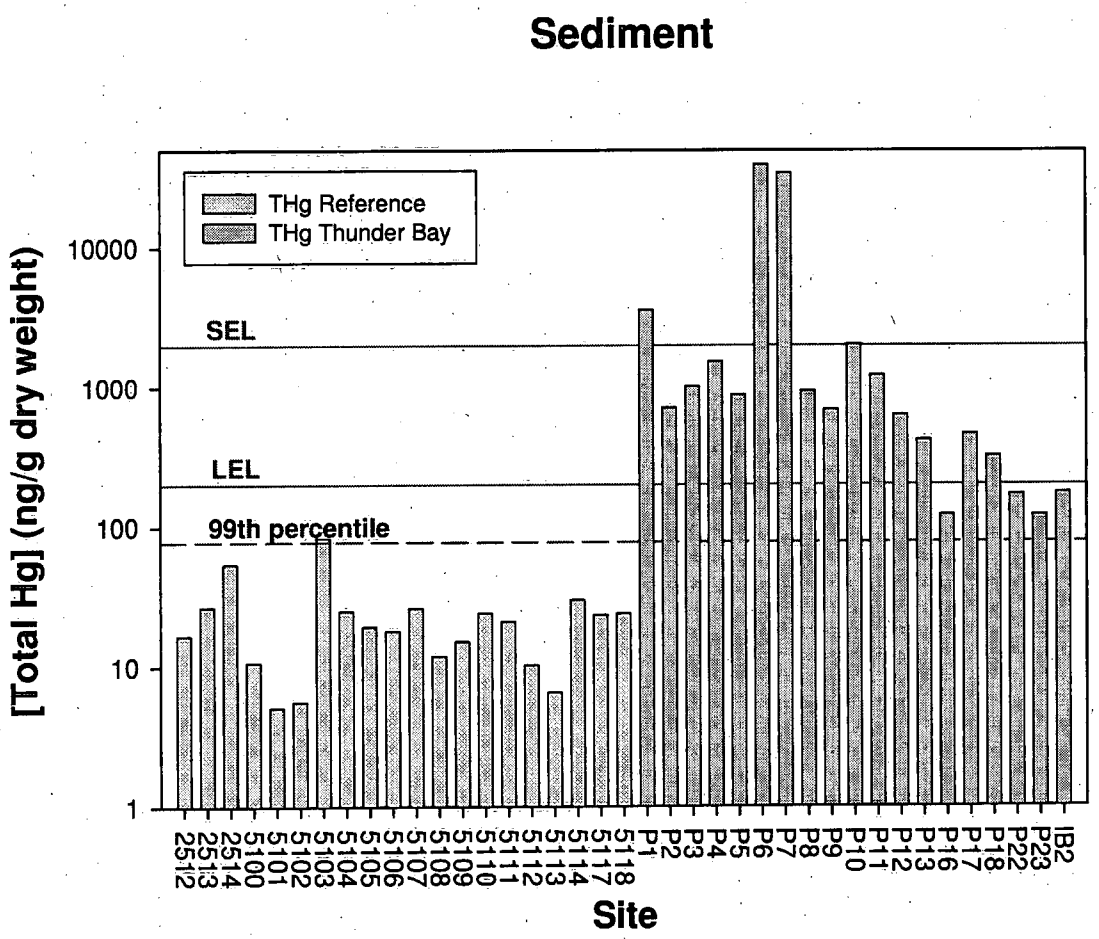


Figure 2. Total mercury (ng/g) in sediment collected from Lake Superior reference (green) and Thunder Bay (grey) sites. The dotted line indicates the 99th percentile for reference sites. LEL = lowest effect level; SEL = severe effect level.

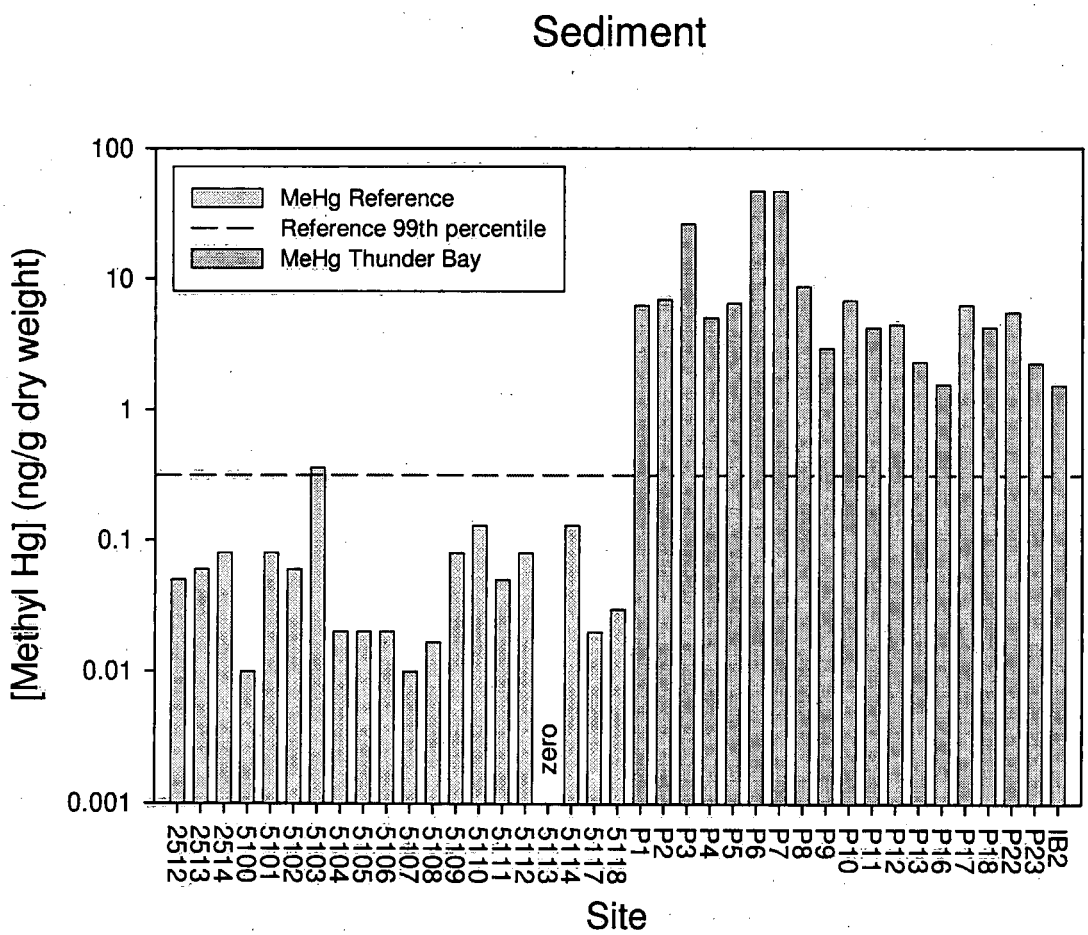


Figure 3. Methyl mercury (ng/g) in sediment collected from Thunder Bay (grey) and Lake Superior reference sites (green). The dotted line indicates the 99th percentile for reference sites.

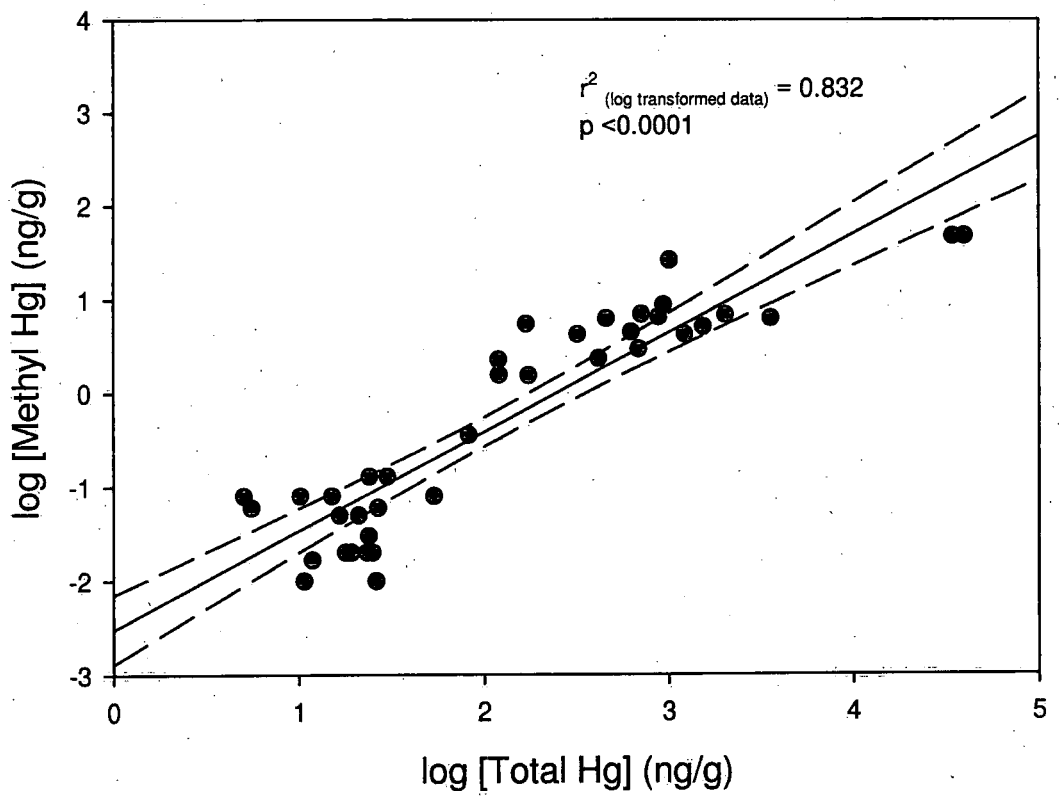


Figure 4. Log scatter plot of methyl mercury versus total mercury in sediment. The 95% confidence interval for the regression equation is shown by the dashed lines.

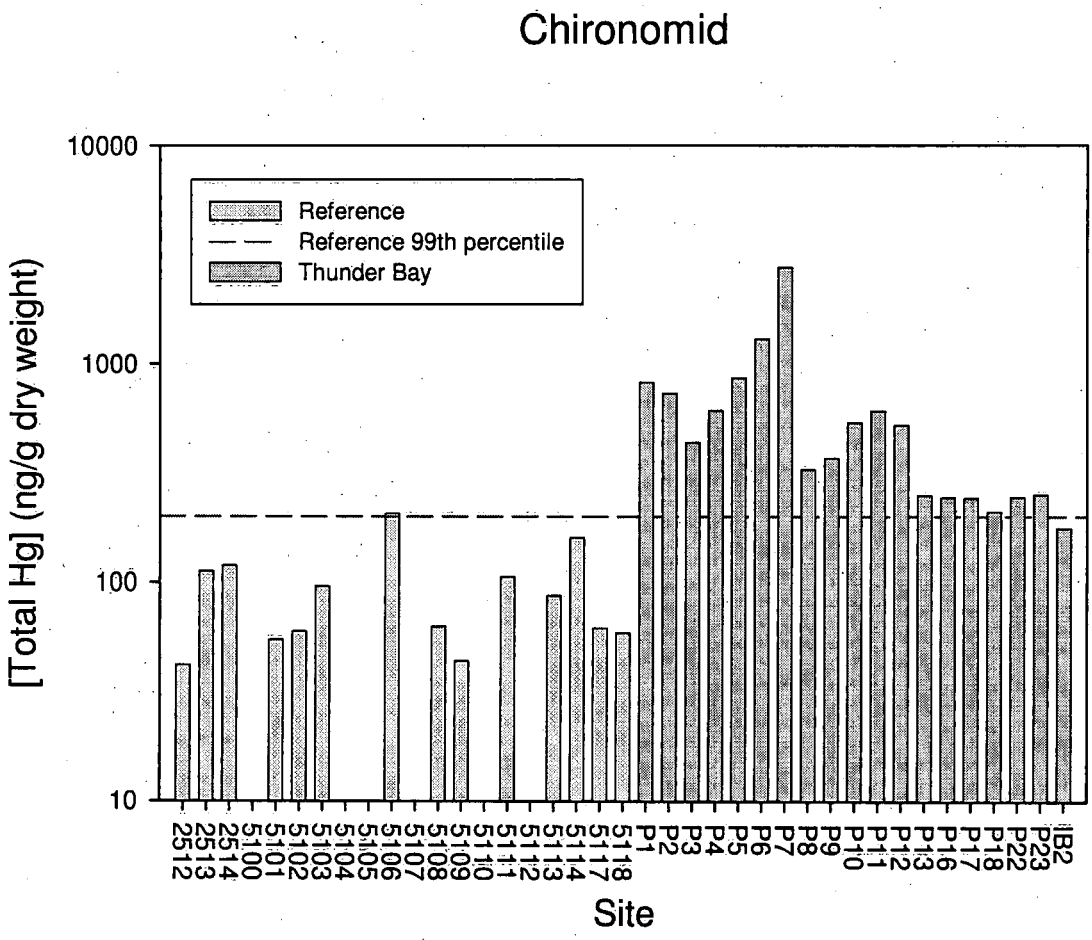


Figure 5. Total mercury in chironomids (ng/g dw) from Thunder Bay (grey) and Lake Superior reference sites (green). The dotted line indicates the 99th percentile for reference sites.

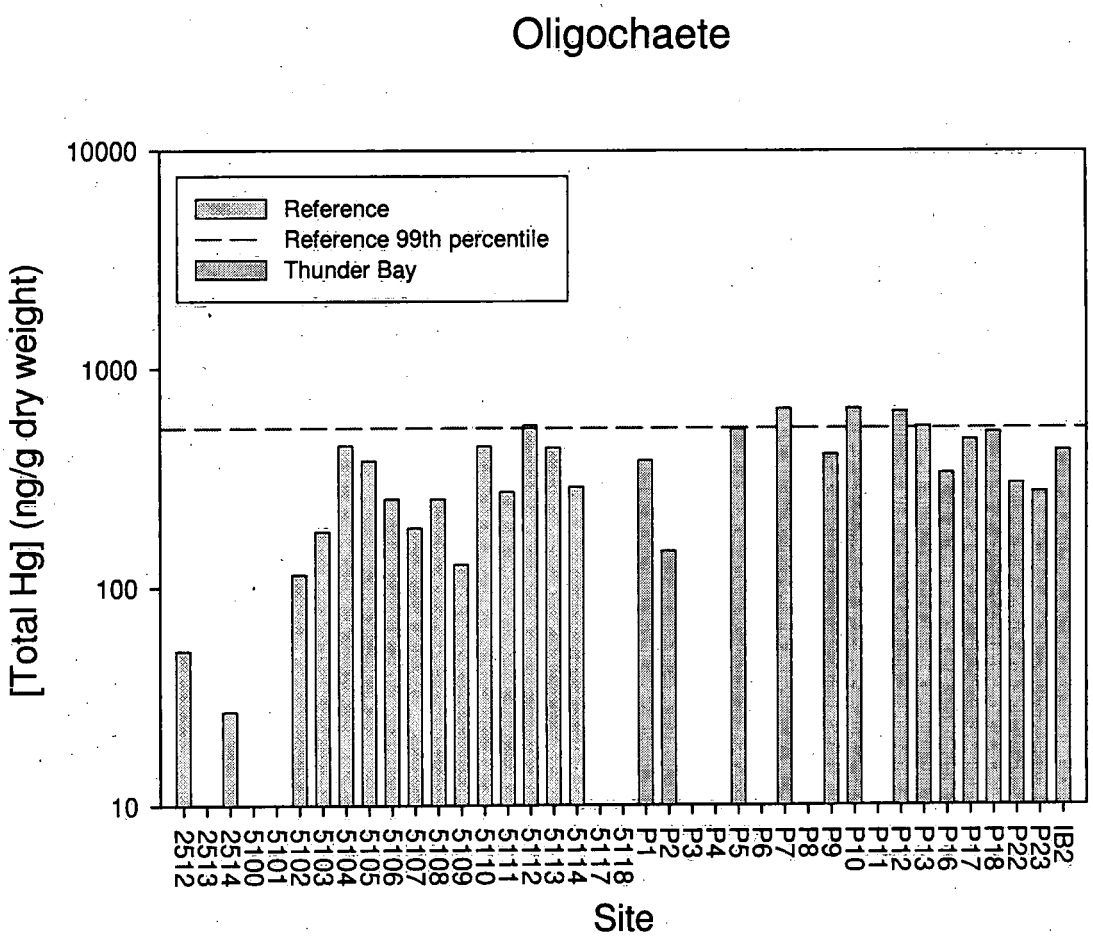


Figure 6. Total mercury in oligochaetes (ng/g dw) from Thunder Bay (grey) and Lake Superior reference sites (green). The dotted line indicates the 99th percentile for reference sites.

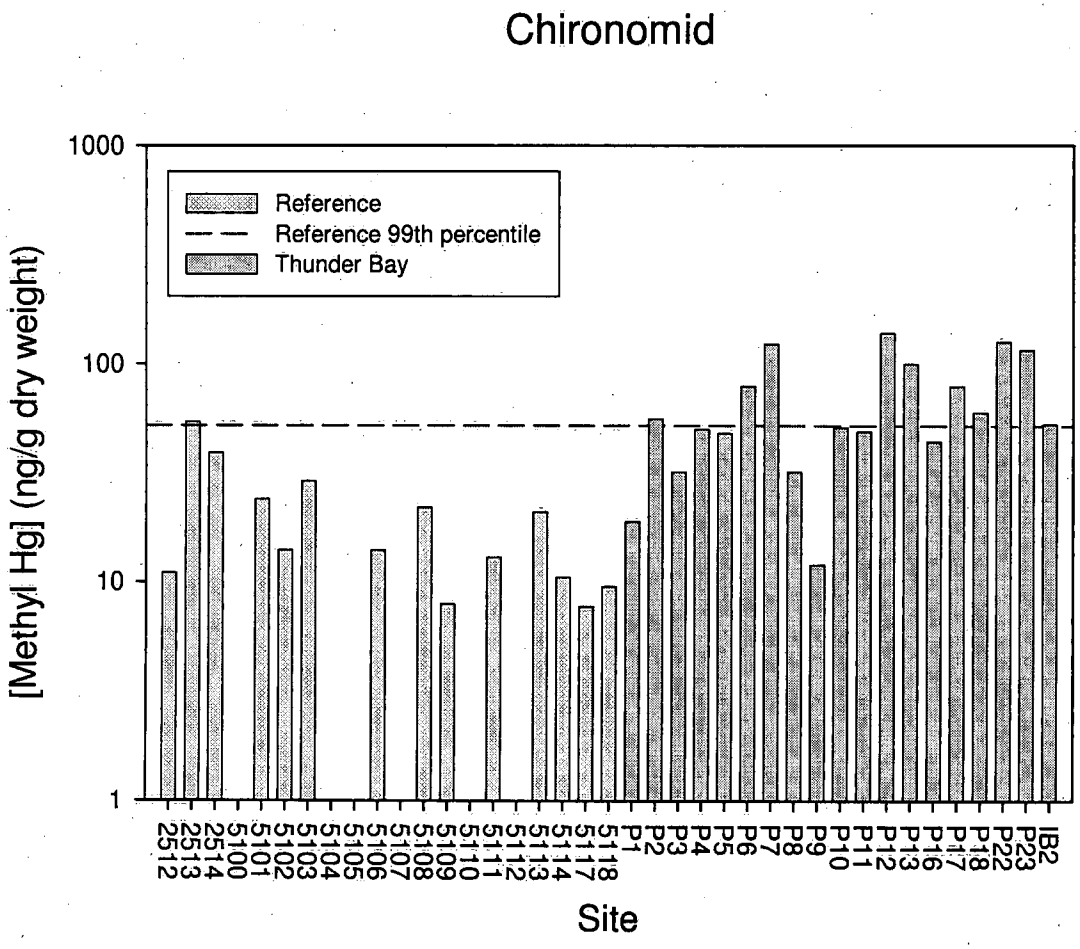


Figure 7. Methyl mercury (ng/g dw) in chironomids from Thunder Bay (grey) and Lake Superior reference sites (green). The dotted line indicates the 99th percentile for reference sites.

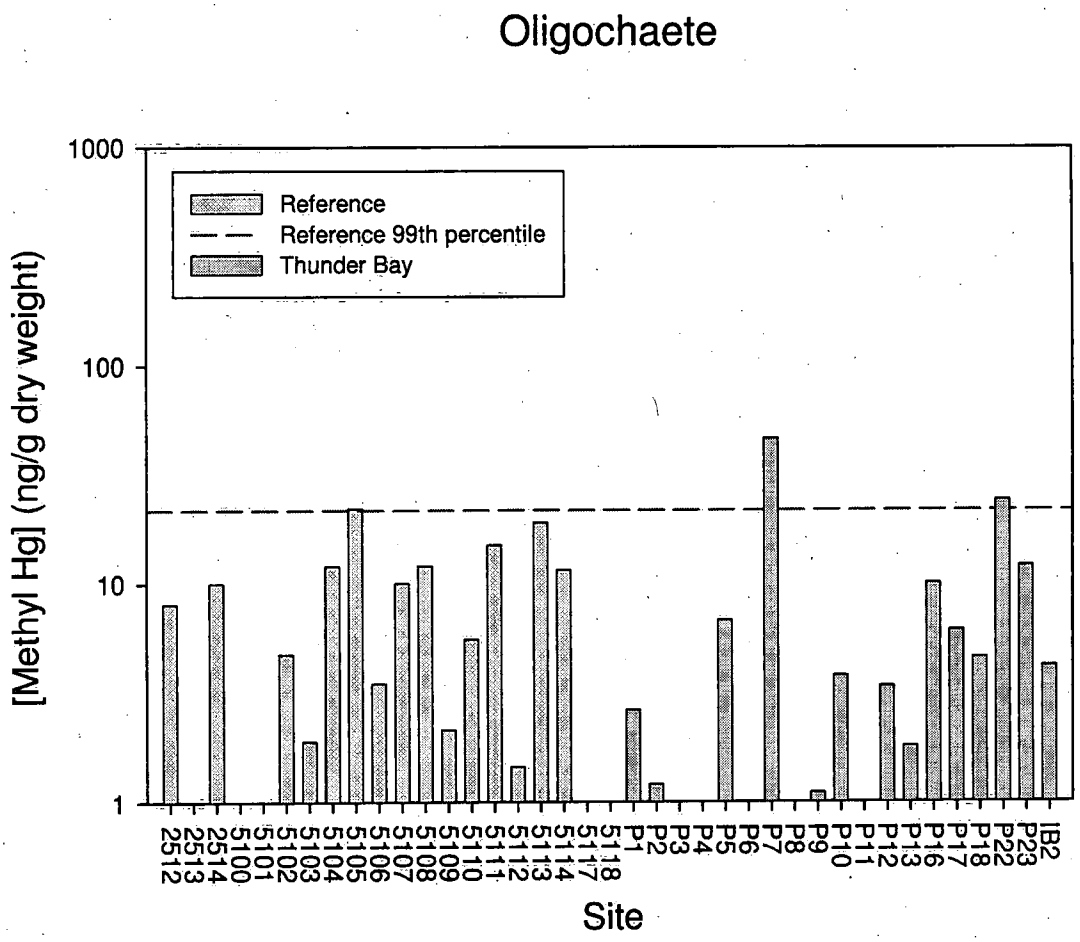


Figure 8. Methyl mercury (ng/g dw) in oligochaetes from Thunder Bay (grey) and Lake Superior reference sites (green). The dotted line indicates the 99th percentile for reference sites.

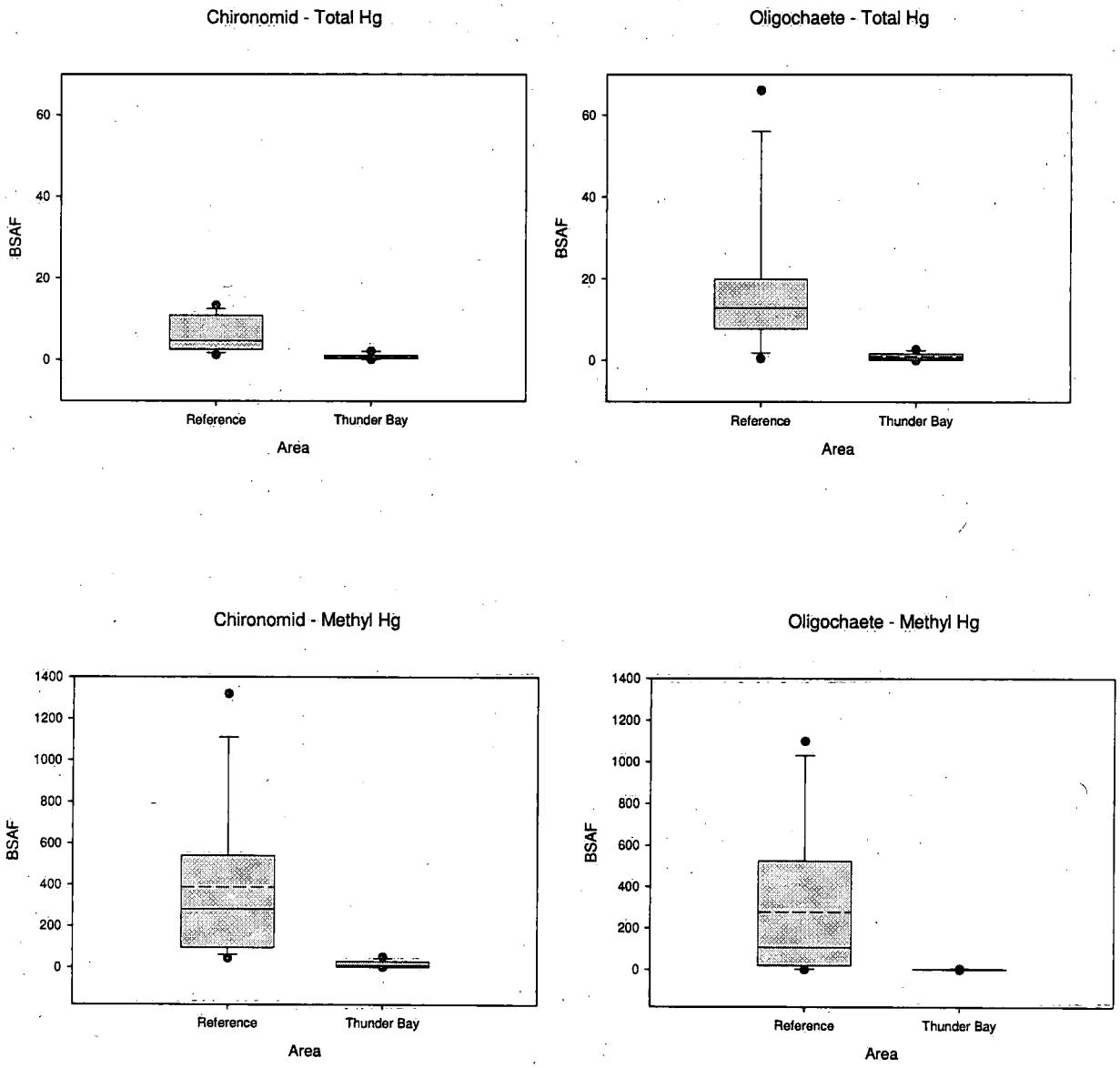


Figure 9. Biota-sediment accumulation factors for Thunder Bay and Lake Superior reference sites. Boxplots of BSAFs (= $[Hg]_{inv}$ / $[Hg]_{sed}$) for each taxon within areas show 90th and 10th percentile (whiskers above and below boxes), inter-quartile ranges (box boundaries closest and farthest from zero), median (horizontal line within boxes) and mean (dotted line).

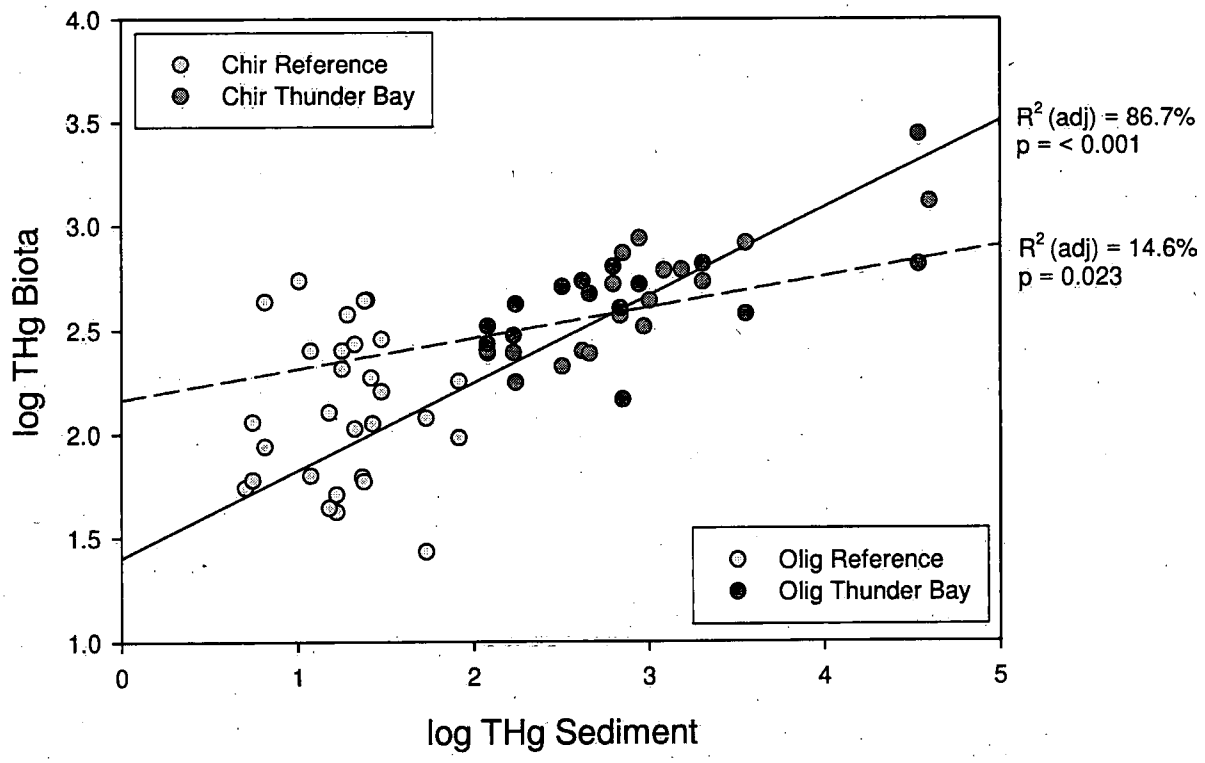


Figure 10. Relationships between total mercury in benthic invertebrates and total mercury in sediment. Separate regression lines are shown for each taxon.

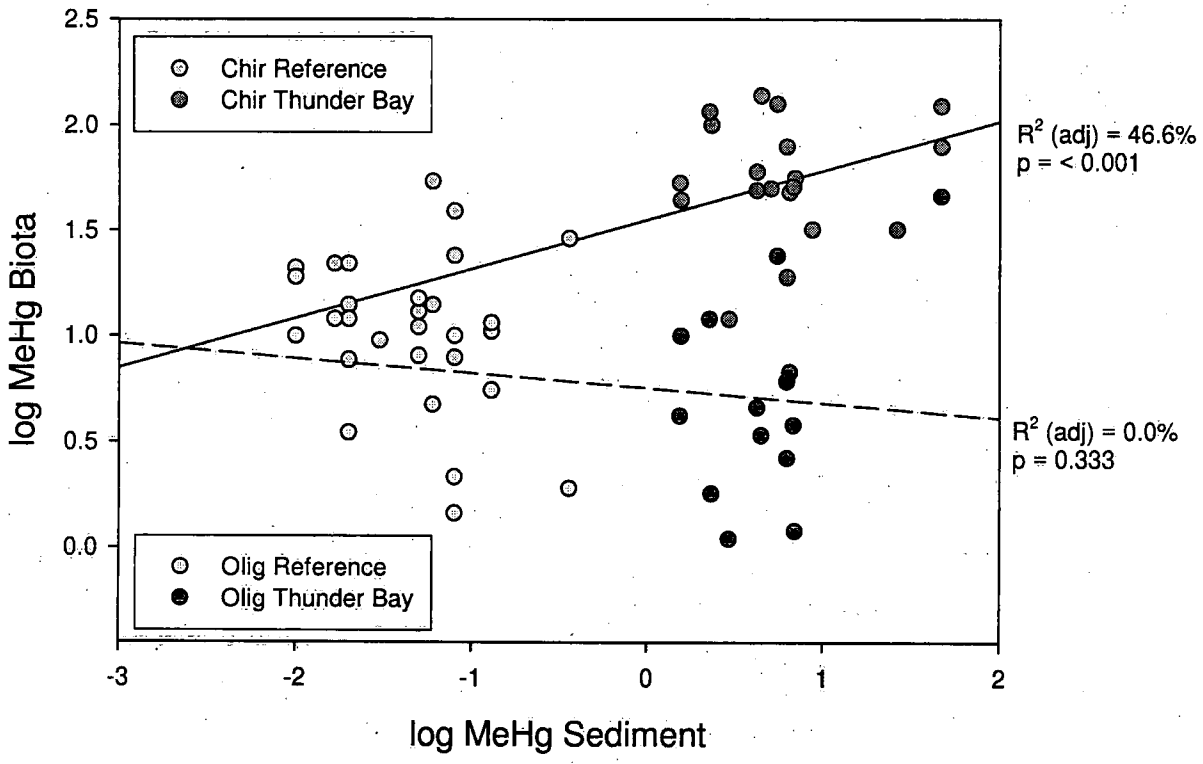
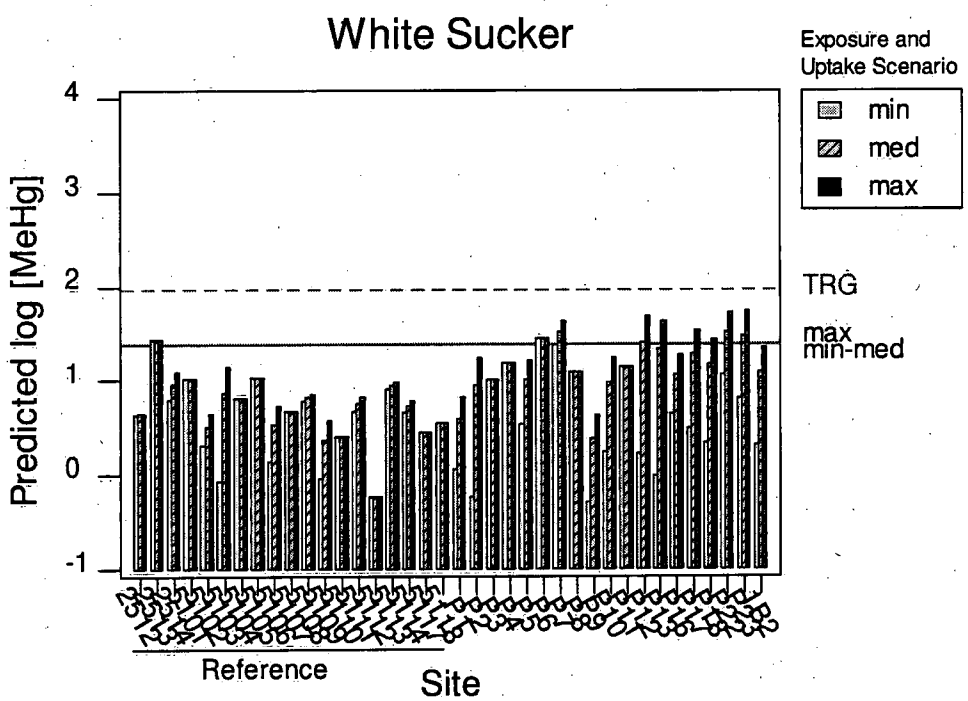


Figure 11. Relationships between methyl mercury in benthic invertebrates and methyl mercury in sediment. Separate regression lines are shown for each taxon.



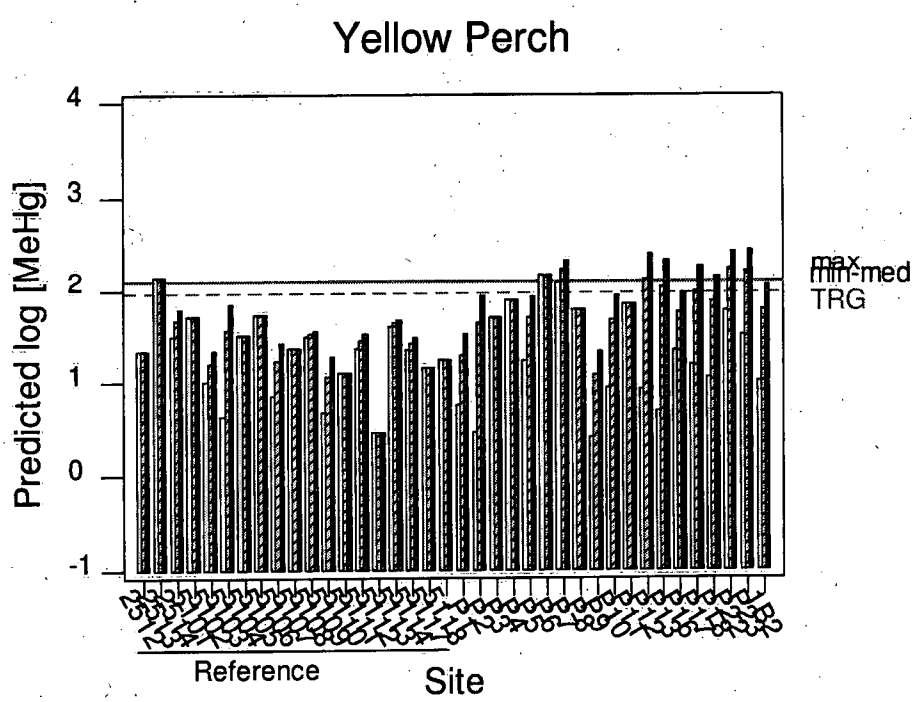


Figure 12a. Predictions (minimum, intermediate, and maximum) of methyl mercury concentrations (ng/g ww) in fish receptor species. Charts compare predicted [MeHg] among receptors and between reference and test sites. The 99th percentile of the predicted [MeHg] for reference sites for each scenario is indicated on the chart (min, med, max). The tissue residue guideline (92 ng/g ww, Environment Canada), is indicated is shown by the red dotted line.

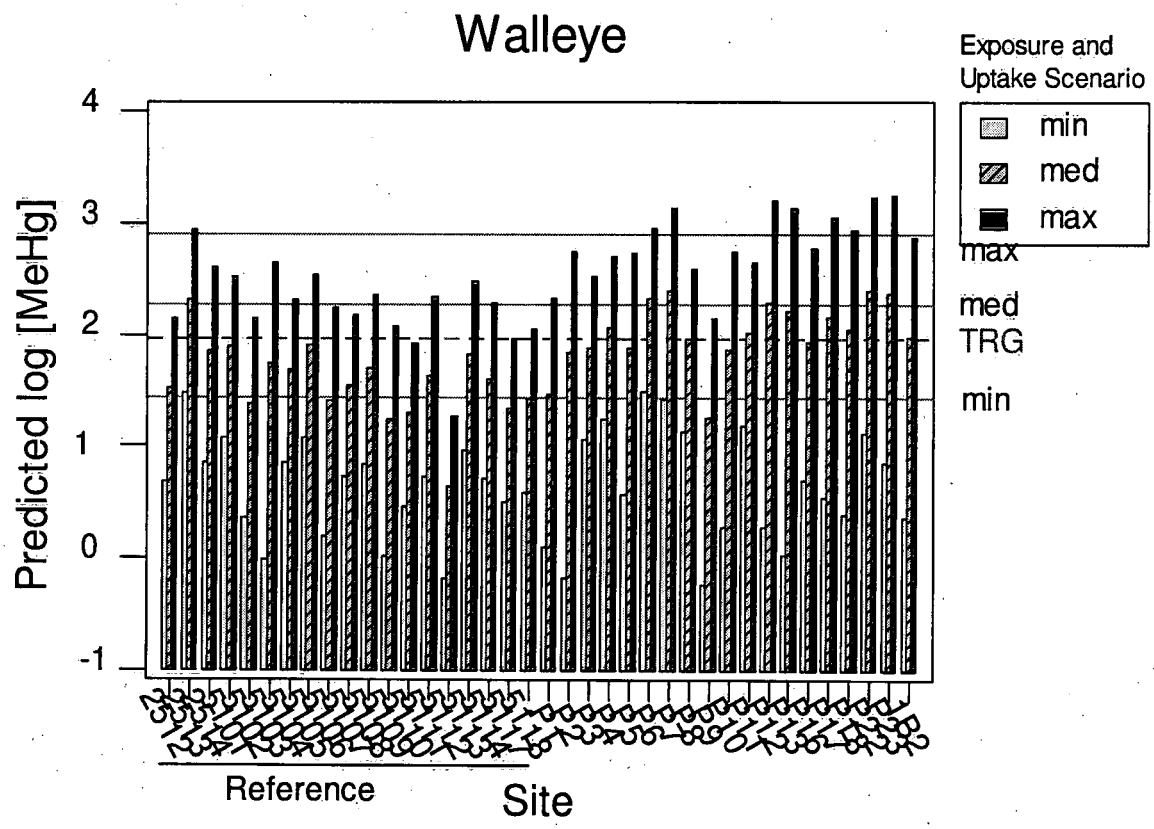


Figure 12a. Continued.

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National Water Research Institute
Environment Canada
Canada Centre for Inland Waters
P.O. Box 5050
867 Lakeshore Road
Burlington, Ontario
L7R 4A6 Canada

National Hydrology Research Centre
11 Innovation Boulevard
Saskatoon, Saskatchewan
S7N 3H5 Canada

NATIONAL WATER RESEARCH INSTITUTE
INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX

Institut national de recherche sur les eaux
Environnement Canada
Centre canadien des eaux intérieures
Case postale 5050
867, chemin Lakeshore
Burlington, Ontario
L7R 4A6 Canada

Centre national de recherche en hydrologie
11, boul. Innovation
Saskatoon, Saskatchewan
S7N 3H5 Canada

Environment Canada Environnement Canada

Canada