FINAL REPORT

Derivation of Baseline Bioaccumulation Factors (BAFs) from Grand Calumet River Field Measured BAFs for Benzo[a]pyrene

Prepared for:

U.S. Steel - Gary Works
1 North Broadway
Gary, IN 46402

Submitted to:

The ADVENT Group
1925 North Lynn Street, Suite 702
Rosslyn, VA 22209

Prepared by:

GLEC
Great Lakes Environmental Center

March 24, 2000

TABLE OF CONTENTS

TABLES

FIGURES

FINAL REPORT

Derivation of Baseline Bioaccumulation Factors (BAFs) from Grand Calumet River Field Measured BAFs for Benzo[a]pyrene

1.0 INTRODUCTION

Bioaccumulation factors (BAFs) are being used increasingly by the states and the U.S. Environmental Protection Agency (U.S. EPA) to develop water quality criteria for the protection of wildlife and human health. In December 1996, the Water Pollution Control Board of the Indiana Department of Environmental Management (IDEM) adopted revisions to Indiana Rules Regarding Water Quality Standards for the Great Lakes Basin (the Indiana Rules). Those revisions included procedures for deriving BAFs to be used in the calculation of human health Tier I criteria and Tier II values, and wildlife Tier I criteria. The goal of the human health criteria is to protect humans from unacceptable exposure to toxicants via consumption of contaminated fish and drinking water, and from ingesting contaminated water as a consequence of participation in recreational activities on or around the water (IDEM, 1997).

Section 13 of the Indiana Rules (327 IAC 2-1.5-13) describes four procedures to be used to determine baseline BAFs for organic chemicals. The four procedures, in order of preference, are as follows:

1. Obtain a measured baseline BAF by conducting a field study involving the collection and analysis of samples of aquatic organisms being consumed, and the water in which they live.

2. Obtain a predicted baseline BAF using biota sediment accumulation factors (BSAFs) derived from a field study, involving the collection and analysis of samples of the aquatic organisms being consumed, and the collection and analysis of the surficial sediments.

3. Obtain a predicted baseline BAF by multiplying the bioconcentration factor (BCF) (derived from a laboratory study), by a food chain multiplier (FCM).

4. Obtain a predicted baseline BAF by multiplying the octanol-water partition coefficient (K_{ow}) for a chemical by a FCM.

The Indiana Rules state that baseline BAFs should be derived using as many of the four methods as available data allow.

The fourth procedure was used by IDEM to derive a water quality-based permit limit for benzo[a]pyrene (B[a]P) at U.S. Steel's (USS's) Gary Works Outfalls 005 and 010 (in combination, referred to as Outfall 200). The limit was derived by applying the Great Lakes Water Quality Initiative (GLWQI) (U.S. EPA, 1995a) human health criteria derivation methodology, which has also been adopted by Indiana (327 IAC 2-1.5-13) and incorporated into the U.S. EPA=s *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)* (U.S. EPA, 2000).

To supplement the available information for B[a]P, USS contracted with the ADVENT Group to perform a field study in the Grand Calumet River in the vicinity of USS's Gary Works to determine a baseline BAF using either Procedure 1 or Procedure 2. The ADVENT Group subcontracted with Great Lakes Environmental Center, Inc. (GLEC) to collect and analyze representative samples from the Grand Calumet River to derive a field-measured BAF or BSAF, from which a baseline BAF could be calculated or estimated.

1.1   BACKGROUND

A BAF is the ratio (in L/kg-tissue) of a substance's concentration in the tissue of an aquatic organism to its concentration in the ambient water, in situations where both the organism and its food are exposed to the substance, and where the ratio does not change substantially over time (U.S. EPA, 2000). A BAF that is calculated from the concentration of a chemical in the wet tissue of a specific tissue sample type (e.g., skinless fillets of a particular fish species) is specific for that sample type, and for the site from which it was collected. However, by taking into account the partitioning of the chemical within the organism and the bioavailable phase of the chemical in the water, a baseline BAF can be derived, which can be used to extrapolate from one species to another (within the same trophic level (TL)) and from one water body to another (when the conditions are similar). The lipid content of the aquatic organism is used to account for the partitioning of hydrophobic organic chemicals (HOCs, such as B[a]P) within the organism. To account for the bioavailability of the chemical, a measured baseline BAF is calculated using the freely dissolved concentration of the chemical in the water. Therefore, a baseline BAF for an organic chemical is a BAF (in L/kg-lipid) which is based on the concentration of freely dissolved chemical in the ambient water and the lipid normalized concentration in tissue (U.S. EPA, 2000).

The freely dissolved phase of a chemical in the water is the fraction, which is not complexed or associated with organic matter in the water. The phase of the chemical which is not freely dissolved is associated with dissolved organic matter (dissolved organic carbon, DOC), colloidal material, and/or suspended particles (particulate organic carbon, POC) (Hermans *et. al*., 1992). Dissolved materials are operationally defined as those that pass through a filter (e.g., 0.7 μm) (U.S.EPA, 2000), and include compounds associated with DOC, compounds associated with suspended particles with diameters less than 0.7 μm, and freely dissolved compounds. There are problems with the direct measurement of the freely dissolved concentration of chemicals, due to the difficulty in distinguishing between the components of the operationally defined dissolved fraction. However, the freely dissolved fraction can be calculated from the total concentration of the chemical in the water, and the concentrations of DOC and POC, using an empirical equation (U.S. EPA 1995b).

In order to accurately determine a field-measured BAF for B[a]P, the Indiana Rules require that the following procedural considerations be met:

a.   The field study must be conducted in the Great Lakes system with fish at or near the top of the aquatic food chain (TL-3 and TL-4).

b.   The trophic level of the fish species must be determined.

c.   The site of the field study must not be so unique that the BAF cannot be extrapolated to other locations where the criteria and values will apply.

d.   The percent lipid will either be measured or reliably estimated for the tissue used in the determination of the BAF.

e.   The concentration of the chemical in the water will be measured in a way that can be related to particulate organic carbon (POC) and dissolved organic carbon (DOC), and should be relatively constant during the steady-state time period. The freely dissolved concentration of the chemical can be determined using an empirical equation if this requirement is met.

f.   The concentration of POC and DOC in the ambient water must either be measured or reliably estimated.

When acceptable data are not available for deriving a field-measured BAF, it is recommended that a field-measured BSAF be used to predict the baseline BAF. A BSAF (kg of sediment organic carbon per kg of lipid) is the ratio of the lipid-normalized concentration of a substance in the tissue of an aquatic organism to its organic carbon-normalized concentration in surface sediment, in situations where the ratio does not change substantially over time, both the organism and its food are exposed, and the

surface sediment is representative of average surface sediment in the vicinity of the organism (U.S. EPA, 1998). BSAFs account for the partitioning of the chemical within the organism and the bioavailable phase of the chemical in the sediment because they are based on the lipid-normalized concentration of the chemical in the tissue and the organic carbon-normalized concentration in the sediment. The BSAF can be used to estimate the baseline BAF for a chemical by taking into account the disequilibrium of the sediment-water distribution of the chemical. This is accomplished through comparison to reference chemicals with similar sediment-water disequilibria (U.S. EPA, 1995a). This approach requires a field-measured BAF and BSAF for the reference chemical(s), a field-measured BSAF for the chemical of interest, and reliable K_{ow} values for both (all) chemicals. This procedure is particularly beneficial in situations where the chemical of interest is difficult to measure in the water, but is detectable in tissue and sediment samples.

1.2   PROJECT OBJECTIVE

The objective of the study was to collect sufficient information from samples collected in the Grand Calumet River in the vicinity of USS's Gary Works to determine a baseline BAF for B[a]P using Procedure 1 (field-measuring a BAF) or Procedure 2 (field-measuring a BSAF). Humans are potentially exposed to B[a]P in the Grand Calumet River by consuming fish from the river. The study objective was to measure the tendency of B[a]P to bioaccumulate in the edible tissues of the fish consumed by humans from the Grand Calumet River in the vicinity of USS=s Gary Works so that the risk to the population could be estimated.

2.0 METHODS

It was important to design and conduct the study in a manner that would ensure that the data, generated as a result of the collection and analysis of samples, were representative of the conditions to which the affected human population was being exposed. Therefore, decisions regarding the specific sample types, sampling times, sites and replication of samples were made to provide data that were representative of the exposure conditions. In addition, special attention was given to resolving the technical issues associated with proper sample collection and analysis. The study was designed with an objective of reducing sources of variability associated with sample collection and analysis, and ensuring that all the essential data were collected to successfully determine a BAF.

2.1   REVIEW OF HISTORICAL DATA AND SITE CHARACTERISTICS

In order to develop an understanding of the site conditions, the first phase of the study involved a review of historical data and a site visit. The information gathered during this phase was important to the design of the field study, to ensure that all factors which could potentially affect the validity of the BAF were considered.

Historical data were reviewed to determine flow conditions, effluent discharge rates, historical benzo[a]pyrene concentrations, sediment characteristics, fish communities, and pertinent information on the operation of USS=s Gary Works. Sources of information included:

- IDEM. June 5, 1997. *An April 1997 Examination of the Fish Community in the East Branch Grand Calumet River at the U.S. Steel Works*, USX Corporation, Gary, Indiana.

- Floyd Browne Associates, Inc. January 22, 1993. *Sediment Characterization Study, U.S. Steel, Gary, Indiana.*

- U.S. EPA. December, 1993, *Assessment and Remediation of Contaminated Sediments (ARCS) Program, Biological and Chemical Assessment of Contaminated Great Lakes Sediment.*

- U.S. Fish and Wildlife Service, U.S. Department of the Interior. November 1994. *Pre-Remedial Biological and Water Quality Assessment of the East Branch Grand Calumet River, Gary, Indiana*, June 1994.

- Grand Calumet River Sediment Dredging Plan.

- USS NPDES data.

5

!   IDEM fish contaminant data.

!   Lake Michigan Mass Balance Study data.

The east branch of the Grand Calumet River in the vicinity of USS's Gary Works, near Gary, Indiana had been previously specified as the study area. The east branch of the Grand Calumet River flows west approximately ten miles to the Indiana Harbor Canal, which discharges into the Indiana Harbor of Lake Michigan. USS's Gary Works occupies the upper five miles of the east branch (Figure 1).

The USS NPDES Permit limit for B[a]P applies to Outfall 200, which is a combination of Outfalls 005 and 010. Outfall 005 is located approximately 1,900 feet downstream of a culvert at the headwaters of the east branch of the River, and approximately 2,400 feet upstream of the Tennessee St. Bridge (Figure 1). Outfall 005 has a daily maximum discharge flow of 90.1 million gallons per day (mgd) and a maximum monthly average flow of 79.4 mgd. Outfall 010 is located approximately 1,400 feet downstream from Outfall 005. It has a daily maximum flow of 2.8 mgd, and a maximum monthly average flow of 1.96 mgd. The volume of discharge from Outfall 005 dominates the river at locations upstream of Outfall 018, which is near the Virginia St. Bridge and approximately 3,500 feet downstream of Outfall 005. Outfall flow is comprised primarily of non-contact cooling water (Lake Michigan water), the volume of which fluctuates to meet process cooling requirements.

The historical data demonstrated that the concentrations of B[a]P in Outfall 005 effluent varied. From July 1, 1997 to November 30, 1998, the average recorded B[a]P concentration was 0.0120 µg/L and the maximum recorded concentration was 2.600 µg/L at a level of detection (LOD) of 0.0230 µg/L; 23% of the monitoring data were below the LOD during this period. The reported maximum discharge concentration of B[a]P from Outfall 005 over a 3 year period was 0.0012 mg/L, representing a maximum discharge load of 0.81 lbs/day. B[a]P was also detected in wastewater collected from USS Outfalls 010 (maximum discharge load of 0.36 lbs/day), 020 (maximum discharge load of 0.013 lbs/day), and 034 (maximum discharge load of 0.024 lbs/day). However, concentrations in the ambient river water have been historically found to be below detection. This was probably due to the fact that the methods used to generate the data involved the extraction of one to two liter samples of water, which is not sufficient volume to quantify the low levels present in the diluted river water. Levels of B[a]P in Lake Michigan have been found to be in the range of 1 to 10 picograms/L (Burkhard 1999a), utilizing large volume extractions. The historical B[a]P information for outfall discharges, ambient Grand Calumet River water and Lake Michigan water was used to determine the sample collection sites and the volume of water that would be extracted for the determination of a BAF using Procedure 1 (see Section 2.2.1 Water Sample

Collection Design).

The fish community surveys demonstrated that common carp (*Cyprinus carpio*) and goldfish (*Cauratus arassius*) were the dominant fish species in the east branch of the Grand Calumet River, but that smallmouth bass (*Micropterus dolomieu*), largemouth bass (*Micropterus salmoides*) and channel catfish also inhabited the River. IDEM reported undetectable levels of benzo[a]pyrene (<0.7 mg/kg) in carp collected from the east branch of the Grand Calumet River. However, detectable levels of 1,1N-dichloro-2, 2-bis(p-chlorophenyl)ethane (*p,p*N-DDD), a breakdown product of the pesticide DDT, were reported for many of IDEM=s carp samples. The fish survey and historical tissue contaminant information was used to help to decide which fish species to target for collection, which analytical methods to employ to achieve a low level of detection, and which analyte to designate as a reference chemical in the event that a field-measured BSAF was required (see Section 2.2.3 Sediment Sampling Design).

The historical information regarding sediments in the study area indicated that the sediments were highly impacted by organic and inorganic contaminants, including B[a]P and *p,p*N-DDD.

2.2   STUDY DESIGN

The study was designed to determine a field-measured BAF, while allowing for the determination of a field-measured BSAF in the event that the concentration of B[a]P in the water could not be accurately quantified. Therefore, the success of the study was contingent upon the collection of representative samples of water, biota and sediment. By selecting the appropriate number of samples, with an appropriate time interval between sampling events, the uncertainty associated with the derived baseline BAF could be reduced. Historical data and current stream flow data were used to gain an understanding of the variability associated with the water body, and consideration was given to the potential spatial and temporal differences between collected samples.

When ecosystems are at steady-state or conditions close to steady-state with respect to contaminants, a limited number of sampling events are necessary for a successful field study because representative samples can easily be collected in one event. B[a]P can be expected to achieve steady-state in less than a year (Hawker and Connell 1987). Anecdotal evidence suggests that B[a]P could enter the Grand Calumet River through groundwater, atmospheric deposition, stormwater and release from sediment. Although it can reasonably be concluded from examining the historical conditions that these potential sources and the east branch of the Grand Calumet River are close to steady-state conditions, it was important to test this assumption. Therefore, several sampling events were planned to collect water

samples to assess the temporal variability in contaminant concentrations; samples of both biota and sediment were collected during two of the water sampling events.

2.2.1 Water Sample Collection Design

Water sampling events were planned to capture the variation in flow that is typical for the east branch of the Grand Calumet River. Because the flow of the upper reaches of the river is USS effluent dominated, variation in river flow can be predicted from the variation in the flow of the largest (in mgd) outfalls (005, 018 and 019). The 1998 to 1999 average total monthly flow from the three largest outfalls typically ranged between 140 and 200 mgd (Figure 2). Therefore, water samples were collected in July, October, November and December to observe the effect (if any) of flow on B[a]P concentrations, and to assess temporal variability. The sampling events in October, November and December were planned to occur approximately every two weeks, based on U.S. EPA recommendations for assessing the temporal variability of a hydrophobic contaminant with a log K_{ow} of approximately 6.

For the first sampling event, sampling stations were established at locations that were representative of the range of exposure conditions for the sample species, in order to assess spatial variability in B[a]P concentrations (Figures 1 and 3). As was specified in the Work Plan/Quality Assurance Project Plan (WP/QAPP), the following locations were targeted for sampling, after a hydraulic mixing zone study was performed:

!   The zone of initial dilution for Outfall 005;

!   Immediately outside the near-field mixing zone for Outfall 005, within the near-field mixing zone for Outfall 010;

!   Outside the far-field mixing zones for Outfalls 005 and 010;

!   Near the downstream boundary of USS's property, downstream of Outfall 034.

The sampling locations for subsequent events were to be determined based on the observed spatial variability for the analytical results from the first set of samples. Based on the mixing zone study results and the analytical results, the sampling locations selected for subsequent events were:

!   Immediately upstream of the Tennessee Street Bridge, just downstream of Outfall 010;

! Immediately upstream of the Virginia Street Bridge, just downstream of Outfall 018.[1]

In order to increase the likelihood of precisely and accurately quantifying the level of B[a]P in the ambient water, the study was designed to collect −100 L water samples during the first sampling event. Based on the analytical results from the first set of samples, 10 L water samples were to be collected during subsequent events.

2.2.2 Target Species

Because the study was designed to derive a baseline BAF for B[a]P to be used to develop human health criteria, aquatic species were targeted that were representative of those which humans commonly consume from the study area. The goal was to apply the following general guidelines in the selection of the sample species:

! The species are commonly consumed in the study area and are of commercial, recreational or sustenance fishing value.

! The species represent trophic levels 3 and 4.

! The species have a wide geographic distribution. This would allow the derived baseline BAF to be extrapolated to other similar situations, especially within the Grand Calumet River system.

! The sample species are typical of the natural population. The collected organisms are healthy and are at a critical life stage to insure that the levels of bioaccumulated contaminant(s) are representative.

! Migratory species are avoided or are sampled near the end of their residence time in the river.

One adult bottom dwelling/feeding fish species, such as carp (TL 2.2 to 3.1, depending on size) or channel catfish (TL 2.8 to 4.2, depending on size), and one adult pelagic/predator fish species, such as smallmouth bass (TL 3.4 to 3.9, depending on size) or largemouth bass (TL 3.5 to 3.8, depending on size) were targeted for collection (U.S. EPA 1995c). Two species were targeted for collection to permit monitoring of a wide variety of habitats, feeding strategies, and physiological factors that result in

---

[1] A scientifically defensible BAF is one that is calculated from water samples which are representative of the fish exposure, whether the water is collected near the source of the chemical, or miles downstream of the source. As part of a follow-up study, it was determined that the B[a]P concentrations at the Tennessee and Virginia St. Bridges were representative of the concentrations throughout the Grand Calumet Watershed, and therefore of the fish exposure.

differences in the bioaccumulation of contaminants. Bottom-feeding species may accumulate high contaminant concentrations from direct physical contact with contaminated sediment, and/or by consuming benthic organisms and epibenthic organisms that live in or on contaminated sediment. Predator species are good indicators of persistent pollutants that may be biomagnified through several trophic levels. Channel catfish and smallmouth bass were the preferred species for collection. However, we recognized that if these species were not available, other species would have to be substituted.

It is likely that resident fish swim the entire length of the east branch of the Grand Calumet River, so it is also likely that a fish caught at any of the water sample locations would have been exposed to the conditions at the other water sample locations. Nevertheless, the study was designed to collect fish at the same locations as the water samples. Although the WP/QAPP specified the collection of fish during each water sampling event, we received a recommendation from the U.S. EPA (Burkhard 1999b) subsequent to the first sampling event, to collect fish only once. Therefore, during the period from October to December when water sampling events were planned for every two weeks, fish were collected once.[2]

Edible sized fish were targeted for collection. The goal was for each sample to consist of a minimum of three fish, which were to be composited in the laboratory.

2.2.3 Sediment Sampling Design

Because sediments act as "contaminant sinks," integrating contamination over long time periods, time-integrated sampling is not as important as is the case with the collection of water samples. Therefore, sediment sample collection was planned for the relatively low-flow summer time period (during the water collection event) at all four sampling locations. Sediment samples were also collected in October at the Tennessee and Virginia St. Bridge sites.

The concentrations of contaminants in sediments can vary significantly over spatial areas, so multiple samples were collected at each site for compositing in the laboratory. The following general guidelines were followed for sediment sampling:

! Surficial sediments are generally representative of the depth of exposure, so samples were

---

[2] For the determination of a BAF using Procedure 1, water samples should be collected prior to the collection of fish samples. This allows the investigators to reasonably assume that the water samples are representative of the exposure conditions for the target species. Ideally during this study, fish should have been collected in December (during or after the last water sampling event) for the determination of a BAF using data from the October through December water samples. Since fish samples were collected during the first water sampling event in October, NPDES monitoring data were analyzed to assess whether the B[a]P concentrations measured during the four sampling events from October to December were representative of the concentrations to which the fish were exposed. The results of the analysis overwhelmingly demonstrated that the data were representative.

collected from the top 1 cm.

! Samples were collected in deposition zones and scouring zones were avoided.

! Samples were not collected following flood conditions or heavy storms.

! The sampling area was representative of the area of exposure. Therefore, although hot spots may have been sampled, the sampling efforts were not concentrated solely in those areas, to avoid overestimating contaminant exposure conditions.

! Sediments and biota were collected at common locations, so that sediment samples would be representative of the area in which the fish were caught.

Sediment samples were collected with the understanding that they would only be analyzed if the concentration of B[a]P in the water was below the level of detection. If the analysis of sediment samples became necessary, then the study would determine a BSAF for B[a]P in the river, from which a baseline BAF could be predicted (Procedure 2). Procedure 2 requires the establishment of a relationship between the BSAF for the chemical of interest (i.e., B[a]P) and a reference chemical (U.S. EPA, 1998). It is best that the reference chemical and the chemical of interest have similar characteristics (e.g., log K_{ow} values, physico-chemical properties) and the reference chemical must be detectable in the water. Historical data indicated the presence of *p,pN*-DDD in some tissue and sediment samples collected from the Grand Calumet River; water sample results were not available. *p,pN*-DDD has a log K_{ow} value of 6.06, which is very similar to the log K_{ow} value for B[a]P of 5.98. Because the physico-chemical properties for the two chemicals are different, *p,pN*-DDD is not an ideal reference chemical for B[a]P. However, the historical data did not clearly indicate that a more appropriate choice (e.g., a high molecular weight polynucleated aromatic hydrocarbon (PAH) with a log K_{ow} ~6) would be feasible. Therefore, *p,pN*-DDD was selected as the reference chemical if Procedure 2 was used to estimate a baseline BAF.

2.2.4   Other Water Quality Parameters

The measurement of parameters other than those which are directly used to calculate BAFs and BSAFs is important for understanding the system from a chemical and a biological perspective, and therefore significantly increase the ability to interpret data. Therefore, the following parameters were measured during the field visits.

! Water temperature

! pH
! Dissolved oxygen (D.O.)

11

These parameters can influence the bioavailability of contaminants by aquatic organisms.

2.3   SAMPLE COLLECTION

The general sampling procedures described in Section 5 of the WP/QAPP regarding mobilization, station positioning, avoiding contamination of samples, decontamination of field equipment, sample handling, preservation and storage, and field sample documentation were followed. This section provides the sampling details which were specific to the study.

2.3.1   Mixing Zone Study

Prior to the collection of samples, a mixing zone study was conducted in July to define the zones specified in Section 2.2.1 of this report. The physical mixing zone boundary for Outfall 005 was determined using Rhodamine B dye. Concentrated liquid dye solution was pumped into Outfall 005 (maximum daily discharge = 90 mgd) via a manhole located approximately 200 feet upstream of the Outfall 005 NPDES sampling location. The dye was pumped using a Fluid Metering Incorporated (FMI) pump Model QG-50 (3/8 inch piston). The dye-pumping rate was approximately 9 mL/min for approximately 3.5 hours.

Grab samples were collected from the following four transects located downstream of Outfall 005: 1) approximately halfway between Outfall 005 and Outfall 010; 2) immediately downstream of Outfall 010; 3) at the Tennessee St. Bridge; and 4) at the Virginia St. Bridge. Four samples were collected immediately beneath the surface, across each of the four river transects, and the fluorescence of each sample was measured using a Turner Fluorometer at an excitation wavelength of 540 nm and an emission wavelength of 585 nm. The differences in fluorescence readings across the transects and down the river were used to evaluate the boundaries of the near-field and far-field mixing zones of Outfalls 005 and 010.

2.3.2 Water Sample Collection

Water samples were collected to determine B[a]P concentration, DOC and POC the weeks of July 12, October 14, November 1, November 29 and December 13, 1999. The July sampling involved the collection of both large volume (approximately 100 L) and 10 L samples. The ~100 L samples were collected in an effort to decrease the analytical quantitation limit, and therefore increase the likelihood of detecting B[a]P at reportable levels. The 10 L samples were collected and analyzed in July to assess the

volume of sample that should be collected during future sampling events. Ten-liter samples were collected during the subsequent sampling events.

2.3.2.1 July Collection of Water Samples

Due to the large volume of water (approximately 100 L) which was collected during the July sampling event, it was necessary to partially field process, the ~100 L samples which were collected for the analysis of HOCs (i.e., B[a]P, surrogate compounds and possibly *p,p N*-DDD). Samples were collected on July 12, 13 and 14 by GLEC and ADVENT Group field technicians in the following order: 1) downstream of Outfall 034; 2) outside the far-field mixing zone of Outfalls 005 and 010; just downstream of Outfall 018; 3) just downstream of Outfall 010; and 4) just downstream of Outfall 005. The ~100 L samples were filtered to separate the dissolved and particulate fractions, and pumped through columns of XAD-2 resin, a macro reticular bead (styrene-divinylbenzene copolymer) that preferentially isolates nonpolar organic compounds from the water.

Immediately prior to the sampling event, a primary and secondary XAD-2 column for each ~100 L sample was prepared in GLEC=s laboratory. Secondary columns were utilized to capture any contaminant mass which could not be retained on the primary column due to insufficient binding sites. The columns were constructed from cleaned (solvent rinsed with acetone, 1:1 hexane:dichloromethane, and methanol, and muffled at 450EC for 5 hours) sections of 2" diameter, threaded stainless steel pipe. Both the primary and secondary columns were packed with a stainless steel screen and glass wool, followed by 400 g and 200g, respectively, of purified[3] XAD resin, type Suplepak 2B purchased from Supelco. The XAD resin in the columns was rinsed, and the columns were filled with HPLC grade water and sealed. To prevent channeling during the extraction procedure, a stainless steel screen and glass wool were included at the top of the column. The columns were transported to the field in the upright position.

Working from a boat, each of the four ~100 L samples was pumped into a previously decontaminated stainless steel container, from mid-stream and mid-depth using a peristaltic pump and flexible Teflon7 tubing. Ten-liter samples were also pumped from each location into previously cleaned amber glass bottles with Teflon7-lined lids. The samples were transported to the on-site shore station. The volume of the ~100 L samples was recorded and the samples were partially processed at the shore station. The 10 L samples were packed in ice and shipped by overnight courier to the laboratory for

---

[3] The XAD purification procedure consisted of a hot water rinse; a deionized water rinse; sequential 24-hour Soxhlet extractions with the following solvents: methanol, acetone, hexane, dichloromethane; sequential 4-hour Soxhlet extractions with the following solvents: hexane, acetone, methanol. It was packed in methanol.

processing and analysis. Subsamples for POC and DOC analysis were also shipped on ice to the laboratory via overnight courier.

Each ~100 L sample was poured into an elevated stainless steel container capable of holding the entire volume. Ten micrograms (10 μg) of surrogate chemical (d_{10}-pyrene) was added as a 1 μg/mL solution in acetone. The sample was continuously gently stirred with a paddle-type mixer while being pumped using a positive displacement pump through 0.7 μm ashed glass fiber filters (to collect the particulate fraction), and through the primary and secondary XAD columns (to collect the dissolved fraction by absorption) (Figures 4). The pressure on the filter was monitored, and the flow was adjusted to maintain a pressure differential across the filter of less than 5psi to prevent any change in the pore size of the filter. The flow rate never exceeded 750 mL/min. When the flow had been reduced to <200 mL/min, the glass fiber filter was replaced. The filters were wrapped in solvent-rinsed aluminum foil and frozen for subsequent extraction. A 10 L post-XAD resin column sample was also collected to evaluate the efficiency of the XAD columns. Following sample processing, the XAD columns were re-sealed and maintained on ice with the tops of the columns elevated during transport to the laboratory. Upon arrival at the laboratory, each column was labeled with a unique identification number, and stored at 4EC until they could be processed for analysis.

2.3.2.2 October, November and December Collection of Water Samples

Duplicate 10 L samples were collected on October 14, 1999 by GLEC and ADVENT Group field technicians from locations just upstream of the Tennessee and Virginia St. Bridges (for a total of four samples). Both sets of duplicate samples were collected into previously decontaminated amber glass bottles with Teflon7-lined caps from mid-depth using a peristaltic pump and Teflon7 tubing. The sample bottles were completely surrounded with ice immediately upon returning to shore, and were transported by hand to GLEC=s Traverse City laboratory the following day.

On November 1, November 29 and December 13, 1999, duplicate 10 L samples were collected by Core Laboratories, Valparaiso, IN from the same locations (just upstream of the Tennessee and Virginia St. Bridges) using the same techniques as were used for the October 14 sampling events. Decontaminated bottles were provided by GLEC, and the samples were shipped on ice by overnight courier to GLEC=s Traverse City, MI laboratory.

Upon arrival at the laboratory, each sample was labeled with a unique identification number and

stored at 4EC until it could be processed for analysis. All samples were processed within 7 days of sample collection.

2.3.2.3 Other Water Quality Parameters

Water temperature, pH, and D.O. were measured in the field during the July 12 and October 14 events using a pre-calibrated Hydrolab7 unit.

2.3.3 Biota Sample Collection

Fish were collected using hand held electrofishing equipment. A Smith-RootJ electrofishing unit equipped with an eight horsepower generator, DC/AC Pulsator and hand held wand electrodes were operated from a shallow draft boat. The unit was set to deliver approximately 6 to 8 DC pulsed amperes. Stunned fish were collected from the water by dip netting, and were held inside pre-cleaned coolers on ice until processing. Edible sized fish (greater than 6 inches) were preferentially selected and dip netted.

In July, fish were collected starting with the most downstream location and proceeding upstream. Only common carp and goldfish were found at the most downstream location, Outfall 034, although considerable time and effort was expended searching for other species. At Outfall 018 (the Virginia St. Bridge location for subsequent sampling events), common carp were plentiful, and one goldfish and one white sucker were also collected. The goldfish from Outfall 018 was not processed due to its small size (<6 inches). At Outfall 010 (the Tennessee St. Bridge location for subsequent sampling events), common carp were collected as well as one goldfish, one bluegill and one green sunfish; all but the carp were too small for processing. At Outfall 005, common carp, one white bass, one white perch, one white sucker and one bluegill were collected. The white perch and bluegill were too small for processing.

In October, fish were collected first from the Virginia St. Bridge location, followed by the Tennessee St. Bridge. At the Virginia St. Bridge location, common carp, carp/goldfish hybrids and chinook salmon were the only fish found. The hybrids were not processed for analysis. At the Tennessee St. Bridge location, common carp, carp/goldfish hybrids, chinook salmon, largemouth bass and bluegill were found. The largemouth bass and bluegill were too small for processing. The presence of chinook salmon in the fall spawning season was typical of all Lake Michigan tributaries, and was not a function of habitat.

All fish were weighed (to the nearest g) and measured (to the nearest mm) and skin-off filets were removed from the fish and frozen. In the laboratory, fish filets were ground separately and equal portions of the same species from one location and sampling event were composited (Tables 1 and 2).

2.3.4   Sediment Sample Collection

Sediment was collected from the water sample collection sites in July and October, starting with the most downstream location and proceeding upstream. Samples were collected following the collection of water and biota using a pre-cleaned 0.5 sq. ft. Ponar sediment dredge, which was mounted to a hand held pole. At each station, a shallow draft boat was anchored and one sediment grab sample was collected from five locations: one sample at each of the four corners of the boat, and one sample from a randomly selected side of the boat. This distribution of sampling locations represented an approximate 5 m x 5 m depositional area at each station. Each of the five sediment samples was treated as a separate sample, although they would eventually be composited in the laboratory. Each sample was emptied into a shallow pan and the top one centimeter was scraped off the sample using a decontaminated stainless steel spoon. The surficial sediment sample was placed into a labeled solvent rinsed glass jar with a Teflon7 cap. The jars were placed on wet ice and transported to the laboratory, where they were stored at 4EC.

2.4   ANALYTICAL METHODS

2.4.1   Water Sample Processing for HOC Analysis

2.4.1.1 Extraction of XAD Columns

The XAD columns were processed according to the Lake Michigan Mass Balance Method (EPA 1997). Analyte was recovered from the XAD resin samples with a combination of acetone rinsing of the resin and Soxhlet extraction of the acetone-rinsed resin. More specifically, each column was rinsed with one bed volume of acetone, and the rinsate from each of the primary and secondary columns was collected in a separate container for liquid-liquid extraction. The resin was then removed from each column with acetone rinses and collected in a beaker as a slurry. The acetone/resin slurry was poured into a glass wool-plugged glass extraction thimble which was suspended above a clean beaker to collect the acetone as it drained. This acetone was combined with the previously collected acetone rinse. The resin was spiked with a surrogate compound (1 mL of a 1 ppm solution of d_{10}-anthracene), and Sohxlet extracted for a 16 hours with 1:1 hexane:acetone.

A liquid-liquid extraction was performed on the acetone rinsate. The rinsate was transferred to a separatory funnel and approximately 300 mL of HPLC-grade water was added to facilitate separation of the organic layer. The rinsate was extracted with one 200 mL portion of hexane and two 100 mL portions of hexane. The organic layers from the three extractions were combined and dried over sodium sulfate.

The solvent from both extraction techniques was combined in Kuderna-Danish (K-D) apparatus

16

and reduced in volume to approximately 10 mL over steam. The extract was further concentrated to approximately 0.5 ml under a gentle stream of nitrogen, quantitatively transferred to a 2 mL glass vial, solvent exchanged into n-octane and naturally evaporated to a volume of approximately 0.1 mL. Extracts were stored at -10EC until analysis.

The primary column from Outfall 005 was eluted prior to extraction, to compare the effectiveness of the two techniques. The column was rinsed with acetone, and the rinsate was collected for liquid-liquid extraction. The XAD was then eluted with one bed volume of 15:85 acetone:hexane, two bed volumes of hexane, and one bed volume of 1:1 ether hexane. The eluate was combined with the extract from the liquid-liquid back extraction of the acetone rinsate, and concentrated. The XAD resin was Soxhlet extracted, and the concentrated extract was analyzed separately from the eluate.

2.4.1.2 Extraction of the Particulate Fraction

The particulate fractions of the ~100 L water samples were collected on 0.7 µm glass fiber filters, as described in 2.3.2.1. The filters were stored at -10EC until extraction in pre-cleaned aluminum foil. The filters were placed in a glass Soxhlet extraction thimble with 20g of sodium sulfate, spiked with 1 mL of a 1 µg/mL solution of d_{10}-anthracene as a surrogate compound, and extracted for 16 hours with 1:1 hexane:acetone. Each extract was concentrated for GC/MS analysis in K-D apparatus, solvent exchanged to n-octane, and naturally evaporated to an approximate volume of 0.1 mL, as described in the Section 2.4.1.1.

2.4.1.3 C_{18} Solid Phase Extraction of 10 L Samples

In July, 10 L whole water samples were collected at the same sites as the ~100 L samples and were transported to GLEC=s laboratory for analysis. The samples were filtered (0.7 µm glass fiber filter) and the filtrate was collected and spiked with 1 mL of a 1 µg/mL solution in acetone of d_{10}-pyrene as a surrogate compound. The spiked filtered water was extracted using 90 mm C_{18} extraction disks (Supelco catalog #57170-U). The analytes were eluted from the extraction disks using the following sequence of solvents: acetone, 1:1 acetone:hexane, hexane. The extract was dried over sodium sulfate and concentrated using the procedure described in Section 2.4.1.1.

The 10 L samples which were collected in October, November and December were spiked with 1 mL of a 1 µg/mL solution of d_{10}-pyrene and extracted as whole water samples (i.e., unfiltered) with C_{18} disks. The analytes were extracted from the C_{18} disks by Soxhlet extraction, after spiking with a second surrogate solution (1 mL of a 1 µg/mL solution of d_{10} anthracene), for 16 hours using 1:1 acetone:hexane.

The sample extracts were cleaned by quantitatively transferring the extracts to a washed (50 mL of hexane) 19 cm x 9 mm ID glass column containing from bottom to top, glass wool, 0.5 cm of sodium sulfate, 2.0 g of deactivated silica gel[4], and 0.5 cm of sodium sulfate. The columns were eluted with 60 mL of 15:85 dichloromethane:hexane, and the eluates were concentrated. Sample extracts (Samples #2073, 2075, 2079-2082) which were insufficiently cleaned using silica gel, were further cleaned using gel permeation chromatography (GPC). The extracts were concentrated using the procedure described in Section 2.4.1.1.

2.4.2   Tissue Sample Processing

Prior to extraction and analysis, the frozen fish fillets were partially thawed. Fillets from each fish were ground separately, and equal portions of tissue from similar size fish of the same species, collected in the same location and on the same date were composited (Table 1).

Each sample was prepared for analysis by mixing approximately 20 grams of ground tissue with sufficient sodium sulfate to dry the sample. The samples were spiked with 1 mL of 1 µg/mL d_{10}-pyrene solution[5], and extracted with 1:1 dichloromethane:hexane using standard homogenization techniques. The percent lipid in each sample was determined in a separate extraction using 3:2 hexane:isopropanol (Bligh and Dyer). The tissue extract for the analysis of HOCs was concentrated to a volume containing approximately 0.3 g of lipid/mL, and a quantitative amount (typically 75%) of the extract was subjected to GPC to remove lipids, followed by normal phase chromatography with silica gel to remove cholesterol-like compounds (see Section 2.4.1.3). The column eluate was concentrated using the procedure described in Section 2.4.1.1.

2.4.3   Sediment Sample Processing

Sediment samples were to be processed only if it was necessary to determine a BSAF. Because quantifiable levels of B[a]P were found in both the ~100 L and the 10 L July water samples, we concluded that a BAF could be calculated. Therefore, it was not necessary to derive a BSAF, and the

---

[4] 60-200 mesh silica gel, Soxhlet extracted with 1:1 hexane:dichloromethane for 16 hours, and dried at room temperature. The cleaned silica gel was activated at 225EC for 18 hours, then stored in a dessicator. The activated silica gel was 1% deactivated (1 mL of water distributed in 100 g of silica gel), and allowed to equilibrate for 18 hours in a dessicator. After 5 days, a new batch of deactivated silica gel was prepared.

[5] A target spiking level of approximately 10 to 20 times the expected average B[a]P sample concentration was intended.

sediment samples were not processed.

2.4.4   GC/MS Analysis

All samples were analyzed using a Hewlett-Packard 5890 gas chromatograph equipped with a 5971 mass spectral detector (GC/MS). A 30m, 0.25 mm ID DB-5 chromatography column was used for all analyses. GC parameters were as follows: injector temperature 250°C; detector temperature 280°C; initial oven temperature 50°C for 4 minutes, ramping to 265°C at a rate of 10E/min and holding at 265°C for 15 minutes. Data were acquired in selective ion monitoring (SIM) mode, to specifically look for the characteristic ions of the analytes. The mass groups were changed four times using three masses per group, with dwell times of 75 milliseconds per mass. Specifically, 1) from approximately 30.0 to 33.5 min, data for 187, 188 and 189 m/z were acquired to look for surrogate compound d_{10}-anthracene; 2) from approximately 37.0 to 41.0 min, data for 211, 212 and 213 m/z were acquired to look for the surrogate compound d_{10}-pyrene; 3) from approximately 43.0 to 47.0 min, data for 236, 239 and 240 m/z were acquired to look for the internal standard d_{10}-chrysene; and 4) from approximately 49.0 to 54.0 min, data for 250, 252 and 253 m/z were acquired to look for B[a]P. The times are approximate because as conditions changed (e.g., the column was clipped), the retention times changed.

A linear calibration curve was prepared for each analyte following the analysis of four standard solutions; calibration curves ranged from 0.5 to 10.0 µg/mL for high range samples, and from 0.025 to 0.1 µg/mL for low range samples. Instrument performance was evaluated daily by analyzing solutions containing compounds at concentrations which tested GC performance, MS sensitivity, MS calibration, response factor reproducibility and GC stability.

Sample extracts were warmed to room temperature, and the volume was adjusted to 90 µL. Half (45 µL) was archived, and 5 µL of 100 µg/mL d_{12}-chrysene was added as an internal standard to the other 45 µL. A 1 µL injection of the internal standard spiked extract was made on the GC. Because the original analysis of many of the sample extracts had significant interferences, the archived portion underwent secondary clean-up and re-analysis. The cleaned-up extracts for these samples were concentrated to 90 µL, and therefore represented a 50% (1:1) dilution of the original extract. Results were reported using a Custom Report created in Version A-03.00 ChemStation software.

2.4.5   POC/DOC Analysis

Water samples were shipped to Midwest Laboratories, Inc., Omaha, Nebraska for POC and DOC

analysis. Approximately 1.5 L samples were filtered through 0.7 μm filters. The filtrate was analyzed for DOC using catalytic combustion (EPA Method 415.1) on a Shimadzu TOC 5000. The filter was burned in a high temperature (>1000°C) combustion carbon analyzer, Leco WR12 Carbon Determinator, to determine POC.

2.5   QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

As specified in the QAPP, quality assurance/quality control (QA/QC) procedures were followed to assess the accuracy, precision, completeness, representativeness and comparability of the data. The analysis of replicate samples allowed the assessment of precision; the analysis of procedural blank samples, matrix spike samples, surrogate spike recoveries and the use of an internal standard allowed the assessment of accuracy. Representativeness was controlled by collecting water samples which were separated by time, distance and varying flow conditions, and by compositing tissue samples from multiple individuals of the same size and species, when possible. Comparability was assured through adherence to Standard Operating Procedures (SOPs) which were based on EPA methods. All instruments used in the processing and analysis of samples were calibrated, and daily checks of performance, calibration and reproducibility were conducted, as specified in the SOPs. If the daily criteria were not met, the source of the problem was determined, and the appropriate remedial actions were followed. The analysis of samples did not continue until the criteria were met.

2.6   CALCULATIONS FOR THE DETERMINATION OF A FIELD-MEASURED BAF AND A BASELINE BAF FOR B[a]P

The analytical results were entered into a spreadsheet to calculate the BAFs. Field-measured BAFs (in L/kg-tissue) for each species of fish were calculated using the equation:

$$BAF = C_t / C_w$$

where:

C_t = Concentration of B[a]P in the wet tissue (μg/kg-tissue)

C_w = Concentration of B[a]P in the whole water (μg/L-water)

Baseline BAFs (in L/kg-lipid), which take into account the partitioning of B[a]P within the organism and the bioavailable phase of B[a]P in the water, were calculated based on the lipid normalized concentration

of B[a]P in the tissue and the freely dissolved concentration of B[a]P in the water:

$$\text{Baseline BAF}_l^{fd} = C_l / C_w^{fd}$$

where:

C_l =   lipid-normalized concentration of B[a]P in tissues of biota (µg/kg-lipid)

C_w^{fd} =   freely dissolved concentration of B[a]P in water (µg/L-water)

The tissue results were lipid-normalized using the equation:

$$C_l = \frac{C_t}{\text{decimal fraction of lipid}}$$

where:

C_l =   lipid normalized concentration of B[a]P in tissue (µg/kg-lipid)

C_t =   concentration of B[a]P in tissue (µg/kg wet weight tissue)

The freely dissolved concentration of B[a]P in the water was calculated as follows:

$$C_w^{fd} \approx C_w^{t} / \left[\left(1 + \text{POC} \bullet K_{ow} \right) + \left(\text{DOC} \bullet K_{ow} / 10 \right) \right]$$

where:

C_w^{fd} =   freely dissolved concentration of B[a]P in the water (µg/L-water).

C_w^{t} =   total concentration of B[a]P in the water (µg/L-water).

POC   =   concentration of particulate organic carbon in the water (kg/L-water).

DOC   =   concentration of dissolved organic carbon in the water (kg/L-water).

K_{ow} =   octanol-water partition coefficient for B[a]P (5.98).

3.0 RESULTS AND DISCUSSION

3.1 RESULTS OF MIXING ZONE DEMONSTRATION

The results of the mixing zone demonstration indicated that the effluent flow from Outfall 005 completely mixed with the receiving water immediately downstream of the outfall, and that the water at the surface of the river was well mixed at the other downstream sampling locations (Table 3). The dye fluorescence of the water samples collected at transects 1 through 4 from the four positions across the river was very similar. Samples collected from Transect 5 immediately downstream of Outfall 018 indicated substantial dilution of the upstream water with Outfall 018 water. The nominal dye concentration at that location was reduced by a factor of approximately four relative to the upstream concentrations. However, samples collected from the sampling positions furthest from the outfall (Positions C and D) demonstrated that effluent from Outfall 018 was not completely homogeneous with the upstream water along the south bank of the river.

These results indicate that the river was dominated by flow from Outfall 005 downstream to Outfall 018. Therefore, the July sampling stations at Outfall 005 and 010 were within the near-field mixing zone of Outfall 005, and the far-field mixing zone for Outfalls 005 and 010 was between Outfalls 010 and 018. Therefore, the sampling location just upstream of the Virginia St. Bridge near Outfall 018 was outside the far-field mixing zone for Outfalls 005 and 010.

3.2 WATER SAMPLE RESULTS

3.2.1 Water Samples Collected in July

The analytical results for the ~100 L and 10 L samples collected in July are presented in Table 4.

The dissolved fraction results represent the B[a]P that was adsorbed onto XAD resin in the primary and secondary columns. Quantifiable concentrations of B[a]P were detected in all the secondary columns, although the amounts were usually less than half of the amount measured in the primary columns. The concentration of B[a]P was below the quantitation level in the 10 L post-XAD sample for the Outfall 005 location, which provided us with reasonable confidence that the XAD columns efficiently adsorbed the analytes. However, due to the low levels of B[a]P in the dissolved fraction, a much larger post-XAD sample would have been required in order to determine conclusively that there was no analyte break-through.

Because quantifiable levels of B[a]P were detected in the water samples, we concluded that a field-measured BAF could be calculated, and that it would be unnecessary to determine a BSAF. Therefore, the water sample extracts were not analyzed for the reference chemical *p,p'*-DDD.

22

The primary column XAD resin, through which water from the Outfall 005 location was pumped, was eluted prior to being extracted, as described in Section 2.4.1.1, so that the results from the two contrasting sample processing techniques could be compared. Although the U.S. EPA=s Lake Michigan Mass Balance study method for processing XAD resin calls for extraction, as summarized in Section 2.4.1.1, EPA has not evaluated the efficiency of elution as an alternative. Since elution is considerably less labor-intensive than Soxhlet extraction, it was worthwhile to determine the validity of this alternative. The elution recovered 0.85 ng/L, but the subsequent extraction of the same column recovered an additional 0.17 ng/L. Therefore, it was determined that the XAD resin samples should be Soxhlet extracted, rather than simply being eluted.

The results from the Soxhlet extraction of both the XAD-processed dissolved fraction and the particulate fraction were summed to determine the whole water concentration for each sample. The empirical equation for determining the freely dissolved concentration of B[a]P was applied to the sum for the calculation of a baseline BAF for B[a]P. The empirical equation was utilized, rather than using the dissolved fraction results, because the dissolved B[a]P included not only the freely dissolved fraction but also the B[a]P associated with DOC and suspended particles with diameters less than 0.7 μm.

The results for the 10 L samples, which were collected in July, could not be used for the calculation of a baseline BAF. Those samples were processed with the intention of determining what volume of sample should be collected during the subsequent sampling events. At that time, it was our understanding that the preferred approach was to determine the dissolved and particulate concentration of the chemical of interest separately (U.S. EPA 1998c). Due to the hydrophobic nature of B[a]P (log K_{ow} 5.98), if dissolved B[a]P was detectable in a 10 L sample, we reasoned that the particulate fraction would also be detectable. Therefore, only the dissolved fraction of the 10 L samples collected in July was analyzed to help decide if subsequent water sample collection efforts should involve the collection of 10 L samples, rather than ~100 L samples. Because the particulate fractions were not analyzed, the results for these 10 L samples could not be used for the calculation of a field measured BAF for B[a]P. The collection of 10 L samples was preferred for subsequent sampling events because the samples could be processed in the laboratory using well-established techniques, which are less cumbersome than the techniques used to process samples in the field.

The dissolved B[a]P results for the ~100 L and 10 L samples differed significantly at some sample locations (most notably at the Outfall 018 and 034 locations); the quantified amount in the ~100 L samples was less than the amount in the 10 L samples at all locations. No clear explanation could be found to account for this discrepancy. However, the combined results for the dissolved and particulate fractions for the ~100 L samples were similar to the results for the whole water 10 L samples collected

from October to December (see Section 3.2.2), with mean concentrations of B[a]P of 20.2 ng/L and 20.8 ng/L, respectively. The results for the ~100 L samples were used to calculate BAFs for the fish collected in July.

3.2.2   Water Samples Collected in October, November and December

The analytical results for the 10 L samples which were collected on October 14, November 1, November 29 and December 13, 1999 are presented in Table 5. All samples were collected and processed in duplicate.

The variability of water sample results was examined to determine how the data should be combined for the calculation of a BAF. Visual plots and linear regression were used to evaluate the temporal trends within sites, and the spatial variability between sites for levels of total B[a]P, DOC, POC and freely dissolved B[a]P (calculated using the equation in Section 2.5). There were not temporal changes in DOC or POC at the Tennessee St. and Virginia St. Bridge sites. The Virginia St. Bridge site had decreasing values for total B[a]P and freely dissolved B[a]P (negative slope in regression, p =.01) over time, while the Tennessee Bridge site showed no trend. Differences between B[a]P concentrations for duplicate samples were greater at the Tennessee St. Bridge than at the Virginia St. Bridge, possibly due to incomplete mixing at mid-depth at the Tennessee Bridge location. The results for each of the sites were averaged and a t-test was applied to evaluate the differences in the means (Table 6). The DOC was the only parameter which had statistical significance between the two sites. The freely dissolved concentration of B[a]P, which is dependent on DOC, was not significantly different. The fact that the average whole water and freely dissolved B[a]P concentrations for each of the sites were essentially the same suggests that the values for the two sites can be combined for the derivation of the BAF. Combining values is further justified by examining plots for the freely dissolved B[a]P concentration values, which show consistent overlap in values (Figure 5).

We were unable to find conclusive historical evidence of the existence of temporal variability in the east branch of the Grand Calumet River. Additionally, a correlation between flow and B[a]P concentration has not been established, especially considering that the flow is a function of manufacturing requirements, rather than natural conditions. Therefore, it is reasonable to assume that the water concentrations of B[a]P, DOC and POC determined over the period from October to December 1999 are representative of the average concentrations in the Grand Calumet River in the vicinity of USS=s Gary Works and that B[a]P concentrations are close to steady-state.

3.3 TISSUE SAMPLE RESULTS

Common carp was the predominant species of fish present in the river during both the July and October sampling events. The common carp which were captured for tissue analysis were all large enough to be considered adults with an average length and weight of 508 mm and 2409 g, respectively (Carlander, 1969). The aquatic habitat in the study area was ideal for common carp, consisting of large shallow pools and glides with a soft silt bottom and some large woody debris for cover (Pflieger, 1975 and Jenkins and Burkhead, 1993). There was a shallow pool just upstream of U.S. Steel Outfall 005 containing aquatic vegetation which was ideal for common carp spawning and rearing of young (Jenkins and Burkhead 1993). Many small common carp (< 200 mm) were observed while electrofishing in this area. The fish have no choice but to move downstream from this point, into the flow of the effluent discharges, because there is no flow upstream of the shallow pool. Although common carp have been known to migrate occasionally in search of food or reproductive habitat, they are considered a non-migratory species (Pflieger 1975, Becker 1983, and Jenkins and Burkhead 1993). Since both food and reproductive habitat were present in the study area, it is likely that the majority of the individuals found there were resident fish. And, although the carp were free to swim the entire length of the river and the Indiana Canal, there was no obvious reason for them to spend much time in Lake Michigan; carp prefer soft-bottomed, warm streams with turbid waters over clear, cold waters with sandy beach habitat like that found in Lake Michigan (Becker, 1983).

The carp tissue sample results are presented in Table 7. The composite sample from four common carp collected at the Tennessee St. Bridge location in October had a higher concentration of B[a]P than all of the other samples, including the composite sample from five common carp collected at the Virginia St. Bridge in the same time period. It is unreasonable to assume that the substantial difference in these results was due to differences in exposure. The sites were in close proximity (the distance between Outfall 005 and Outfall 018 is approximately 5000 ft.) and there were no barriers preventing fish from moving from one location to the other. Differences in the size/age of the fish which comprised the composite samples was also an improbable explanation. Although the uptake and metabolism of contaminants can vary for different life stages, the two samples represented composites from similar size (and age) fish, with the exception of one considerably larger fish which was included in the October Virginia St. Bridge sample (Table 2). Because equal portions of each fish were composited, it is unlikely that one-fifth of the sample would have been sufficiently different to account for the difference between the results from the two locations. The difference could be attributable to unequal distribution of the sexes of the fish that comprised each composite, or to natural variability. Because the

sex of each fish was not recorded, this theory could not be validated.

The few fish of species other than carp, which were available for collection, were small in size, with the exception of the salmon collected in October. Due to the migratory nature of salmon, this species was not an ideal choice of fish to be used to determine a BAF. In addition, in the U.S. EPA=s *Assessment and Remediation of Contaminated Sediments (ARCS) Program*, fish consumption risk estimates were based on pumpkinseed, golden shiner and carp sampled throughout the entire Grand Calumet River/Indian Harbor Canal area; salmon were not used at all (U.S. EPA 1994). Nevertheless, in an effort to collect data for higher trophic levels than carp, all fish greater than 6 inches in length were processed and analyzed. Those samples consisted of single fish samples of white sucker and white bass and composite samples of salmon. The results for these samples are presented in Table 8, and were used to calculate BAFs.

3.4 QA/QC RESULTS

The data reports, including QA/QC sample results, are compiled in the Appendix. QA/QC results are summarized as follows:

! All the procedural blank samples had undetectable concentrations of B[a]P.

! The criterion for surrogate recovery in the 10 L water samples and in the tissue samples was 25 to 120 % (U.S. EPA 1991). All but one (2068 rep 1) of the tissue samples met the criterion. Three of the sixteen 10 L water samples had surrogate recoveries below 25% for the d_{10}-pyrene surrogate. The low recoveries for the three 10 L samples were expected; the extracts of samples #2063 and 2065 may have experienced significant loss as a result of total evaporation of the Soxhlet extraction solvent, and approximately 30% of sample #2072 was lost during filtration (Table 5). The variation in surrogate recoveries did not effect the reproducibility of the data, and B[a]P concentrations were not adjusted for surrogate recovery.

! The tissue matrix spike sample had 40% recovery.

! Results for laboratory replicate samples were all within the criterion of 30% relative standard deviation.

! The calibration checks for GC performance, MS calibration and sensitivity, internal standard area stability, and analyte response stability were met each day that analyses were performed.

3.5 OTHER WATER QUALITY PARAMETER RESULTS

Field measured parameters are summarized in Table 9. The warm water temperature contributed to the lack of suitable habitat for fish species other than carp. The D.O. and specific conductance readings were within a range adequate for the support of aquatic life. The pH was alkaline; however, the bioaccumulation of nonpolar compounds such as B[a]P is generally not affected by high pH (API 1997).

3.6   CALCULATED BAFs

BAFs for TL 2.4 (common carp) were calculated using several combinations of data. A BAF was calculated for the carp samples collected in July using the average tissue B[a]P concentrations for composite carp samples collected from the Outfall 005, 018 and 034 locations and the average water B[a]P concentration for the four locations (Outfalls 005, 010, 018 and 034); the Outfall 010 composite carp result was not included because of the size (and trophic level) difference. All other BAFs for carp were calculated using the average results for water samples collected at the Tennessee and Virginia St. Bridge locations during four sampling events from October to December. Because samples were collected during times which spanned the range of flow conditions, these results were representative of the average exposure conditions for fish residing in the upper reaches of the east branch of the Grand Calumet River.[6] The carp which were collected at these same sites were estimated to be between two and seven years old (Carlander 1969). Because B[a]P can reach steady-state in less than one year under relatively constant exposure conditions, and because carp are not migratory, we assumed that the carp had been exposed to the average B[a]P concentrations over a time period sufficient to reach steady-state. Therefore, the combined water result was used to calculate the BAF for all of the carp, regardless of when they were collected. Due to the site-to-site differences in the carp tissue sample results from the October sampling event, BAFs were calculated separately for the two sample collection locations; a BAF for the arithmetic mean of the two samples was also calculated (Table 10).

The salmon were present in the river in October due to their migratory behavior and therefore had not been exposed to the Grand Calumet River over an extended period of time. Consequently, the BAF for salmon was calculated using the arithmetic mean of the results for water samples collected at the Tennessee and Virginia St. Bridge locations on October 14, 1999 (Table 10).

All other processed and analyzed fish tissue samples were collected in July in the upper reaches of the east branch of the river. Due to the small size of the fish, it could not be assumed that they had been exposed to the Grand Calumet River water sufficiently long to reach steady-state. Therefore, BAFs

---

[6] As stated earlier, the representativeness of the water results was confirmed in an analysis of the NPDES monitoring data.

were calculated for these samples using the combined results from the dissolved and particulate fractions for water collected at Outfalls 005, 010 and 018 (0 value = 25.9 ng/L) (Table 10). Due to the uncertainty of these values (see Section 3.2.1), we felt that the water concentrations for samples collected at the Tennessee and Virginia St. Bridge locations during four sampling events from October to December may have been more representative of the conditions to which these fish were exposed. Therefore, for comparative purposes, a second set of BAFs was calculated using these water results (Table 10).

3.7 UNCERTAINTY OF THE CALCULATED BAF VALUES

Uncertainty estimates reflect the variability associated with the calculated BAF values; the more representative the data are of the true conditions, the lower the uncertainty values. Conventional parametric uncertainty estimates are based on the assumption of a normal distribution of results (or log-transformed results) for all factors used in the calculation. While this assumption is supported for the water data (Table 5), it is not supported for the carp tissue data (Table 7). While most of the carp samples had B[a]P concentrations below 1.0 µg/kg-tissue, the sample collected from the Tennessee St. Bridge location in October had a B[a]P concentration of approximately 10 µg/kg-tissue. Similarly, the percent lipid results for the carp samples range from 1% to 10%. The observed natural variability of results is not normally distributed for the carp tissue samples evaluated in this study. Therefore, conventional parametric statistical techniques cannot be applied for the estimation of uncertainty.

When conventional parametric statistical techniques do not apply, it is appropriate to use nonparametric techniques to estimate uncertainty. These techniques assume that the true value lies within the range of the observed values. Therefore, the most appropriate method to account for the uncertainty in the BAF is to conclude that the observed BAFs bracket the true BAF. Using this approach, we concluded that the B[a]P log-baseline BAF for trophic level 2.4 carp in the upper east branch of the Grand Calumet River was between 2.43 and 4.55.

4.0 CONCLUSIONS

The study successfully calculated baseline BAFs for benzo[a]pyrene from the Grand Calumet River field-measured BAFs for a range of trophic levels (TL 2.4 to TL 4.0) (Table 10). The calculated log-baseline BAFs ranged between 2.23 and 4.55. The BAFs which were calculated for the common carp (TL 2.4) (log baseline BAF ranging between 2.43 and 4.55) had significantly greater statistical power than those which were calculated for the other trophic levels. The greater statistical power of the TL 2.4 BAFs was due to the fact that the BAFs were based on results from multiple water samples, which were evaluated for representativeness, and multiple composite tissue samples. None of the other trophic level BAFs calculated in this study were based on the same magnitude (and representativeness) of samples because the fish were not available. Common carp were the predominant fish, and in many locations the only fish, of edible size by humans in the Grand Calumet River.

The study objective was to measure the tendency of B[a]P to bioaccumulate in the edible tissues of the fish consumed by humans from the Grand Calumet River in the vicinity of USS=s Gary Works, so that the risk to the human population could be estimated. Humans who are fishing in the study area are probably most successful at catching common carp. Therefore, the field measured BAFs which are the most representative of the exposure to humans are those for the common carp (TL 2.4).

The degree of bioaccumulation and biomagnification that occurs in different species within the same water body can be affected by a number of site-specific factors. Factors which may have influenced the calculated BAFs in the east branch of the Grand Calumet River include the following:

! Because common carp feed primarily on detritus and benthic invertebrates, they can receive significant exposure to contaminants from the sediment. Historical data demonstrate that the sediments in the study area are highly contaminated with B[a]P. Therefore, the highest BAFs which were calculated for TL 2.4 may be artificially high. This factor may limit the applicability of the calculated baseline BAF for TL 2.4 to other water bodies.

! The higher trophic level fish collected and analyzed during this study may not have reached steady state due to age, size and/or duration of exposure. Until conditions are close to steady state, the rates of uptake and depuration by an organism are not at equilibrium. BAFs which are calculated from the results of samples collected when conditions are not close to steady state could be artificially high or low, depending on whether the rate of uptake or depuration is higher. Due to lack of adequate habitat and

the overall degraded conditions, it may be impossible for predator fish to reach maturity while residing in the east branch of the Grand Calumet River.

! The food web in the east branch of the Grand Calumet River is disfunctional. The EPA recommends using the food chain model by Gobas (1993) for predicting chemical residues in organisms, which are then used to estimate BAFs for each species in the food chain. However, before this model can be applied in the east branch of the Grand Calumet River to estimate BAFs for TL 3 or TL 4 fish, site-specific data on the structure of the food chain and the water/sediment quality characteristics must be gathered (U.S. EPA, 1998). It is clear from our observations that, because TL 3 and TL 4 fish are mostly absent, the analysis would be extremely problematic.

Additionally, the literature undisputedly demonstrates that B[a]P does not conform to the paradigms of bioaccumulation and biomagnification of other hydrophobic organic chemicals (Broman *et al.*, 1990; Corner *et al.*, 1976; Kolok *et al.*, 1996; Neff, 2001; Niimi, 1987; Niimi and Dookhran, 1989; Spacie *et al.*, 1983; Stein *et al.*, 1984; Thomann and Komlos, 1999; Varanasi *et al.*, 1985; White *et al.*, 1998; Whittle *et al.*, 1977). B[a]P is rapidly metabolized and is biodiminished as trophic level increases. It is not a scientifically defensible approach to apply the Gobas model to calculate a B[a]P BAF for TL 4 fish from the measured BAF for TL 2.4 fish, because FCMs in the Gobas model were developed using polychlorinated biphenyls and other organochlorines, which are not readily metabolized and do biomagnify (Gobas, 1993; U.S. EPA, 1995b).

The information gathered in this study indicate that the calculated TL 2.4 BAFs may be the most scientifically valid BAFs for estimating the risk to humans in the east branch of the Grand Calumet River. Although the Indiana Rules specify that TL 3 and TL 4 fish must be used in the calculation of a BAF, the scarcity of higher trophic level species make it impractical to consider applying a TL 3 or TL 4 BAF for the derivation of human health criteria for this location. Because the bioaccumulation factors for B[a]P are likely to decrease with increasing trophic levels[7] (U.S. EPA 1995c), the use of the highest TL 2.4 log-

---

[7] In a follow-up study, the FCM ratio between trophic levels 4 and 3 for B[a]P was predicted to be between 0.009 and 0.06. Conversely, the Gobas model estimates the ratio to be approximately 1.5.

baseline BAF of 4.55 for the derivation of criteria may be the approach that is the most protective of human health.

5.0 REFERENCES

API. 1997. Bioaccumulation: How chemicals move from the water into fish and other aquatic organisms. API 4656. American Petroleum Institute, Washington, DC.

Becker, G. C. 1983. Fishes of Wisconsin. University of Wisconsin Press. Madison, Wisconsin. 1052.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37(8):911-917.

Broman, D., C. Naf, I. Lundbergh and Y. Zebuhr. 1990. An *in situ* study on the distribution, biotransformation and flux of polycyclic aromatic hydrocarbons (PAHs) in an aquatic food chain (seston - *Mytilus edulis* L. - *Somateria mollissima* L.) from the Baltic: An ecotoxicological perspective. *Environ. Toxicol. and Chem.*, Vol. 9, 429-442.

Burkhard, L. 1999a. Personal communication (telephone conversation with Robin A. Silva-Wilkinson, GLEC, Traverse City, MI on April 9, 1999). United States Environmental Protection Agency, Mid-Continent Ecology Division, Duluth, MN.

Burkhard, L. 1999b. Personal communication (telephone conversation with Robin A. Silva-Wilkinson, GLEC, Traverse City, MI on July 27, 1999). United States Environmental Protection Agency, Mid-Continent Ecology Division, Duluth, MN.

Carlander, K.D. 1969. Handbook of Freshwater Biology, Volume I. The Iowa State University Press, Ames, Iowa.

Corner, E.D.S., R.P. Harris, K.J. Whittle, P.R. Mackie. 1976. Hydrocarbons in marine zooplankton and fish. In *Effects of Pollutants on Aquatic Invertebrates* (Edited by Lockwood, A. P. M.), Cambridge University Press, Cambridge. 71-105.

Gobas, F.A.P.C. 1993. A model for predicting the bioaccumulation of hydrophobic organic chemicals in aquatic food webs: application to Lake Ontario. Ecological Modeling 69:1-17

Hawker, W. and D. W. Connell. 1987. Influence of partition coefficient of lipophilic compounds on bioconcentration kinetics with fish. Pergamin Press. 22(6):701-707.

Hermans, J.H., F. Smedes, J.W. Hofstaat and W.P. Cofino. 1992. A method for estimation of chlorinated biphenyls in surface waters: influence of sampling method on analytical results. Environ. Sci. Technol. 26:2028-2035.

IDEM. 1997. IDEM Indiana rules regarding water quality standards for the Great Lakes basin. Indiana Department of Environmental Management, Indiana.

Jenkins, R. E. and N. M. Burkhead. 1993. Freshwater fishes of Virginia. American Fisheries Society. Bethesda, Maryland. 1080.

Kolok, A. S., J.N. Huckins, J.D. Petty, J.T. Oris. 1996. The role of water ventilation and sediment

injestion in the uptake of benzo[a]pyrene in gizzard shad (*Dorosoma cepedianum*). *Environ. Toxicol. Chem.*, Vol. 15, 1752-1759.

Neff, J. 2001. *Bioaccumulation in Marine Organisms. Effects of Contaminants from Oil Well Produced Water*. Elsevier Science Publishers, Amsterdam. (in press).

Niimi, A. J., 1987. Biological half-lives of chemicals in fishes. *Reviews of Environ. Contam. and Toxicol.*, Vol. 99.

Niimi, A. J. and G.P. Dookhran. 1989. Dietary absorption efficiencies and elimination rates of polycyclic aromatic hydrocarbons (PAHs) in rainbow trout (*Salmo gairdneri*). *Environ. Toxicol. and Chem.*, Vol. 8, 719-722.

Pflieger, W. L. 1975. The fishes of Missouri. Missouri Department of Conservation. Columbia, Missouri. 372 .

Spacie, A., P.F. Landrum and G. Leversee. 1983. Uptake, depuration, and biotransformation of anthracene and benzo[a]pyrene in bluegill sunfish. Ecotox. and Environ. Safety. Vol. 7, 330-341.

Stein, J. E., T. Hom and U. Varanasi. 1984. Simultaneous exposure of English sole (*Parophrys vetulus*) to sediment associated xenobiotics: Part 1 - Uptake and disposition of ^{14}C-polychlorinated biphenyls and ^{3}H-benzo[a]pyrene. *Mar. Environ. Res*., Vol 13, 97-119.

Thomann, R. V. and J. Komlos. 1999. Model of biota-sediment accumulation factor for polycyclic aromatic hydrocarbons. *Environ. Toxicol. and Chem.*, Vol. 18, No. 5, 1060-1068.

U.S. EPA 1991. Guidance on assessment and control of bioconcentratable contaminants in surface waters. Draft, 40 CFR Lut 136, Fed Reg 56:13150-13151, Washington, DC.

U.S. EPA 1994. Assessment and Remediation of Contaminated Sediments (ARCS) Program - Baseline human health risk assessment: Grand Calumet River/Indian Harbor Canal, Indiana Area of Concern. October 1994. EPA - 905-R94-025. Great Lakes National Program Office, Chicago, IL.

U.S. EPA. 1995a. Water quality guidance for the Great Lakes system: supplementary information document (SID). EPA-820-B-95-001. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

U.S. EPA. 1995b. Great Lakes water quality initiative technical support document for the procedure to determine bioaccumulation factors - March 1994. EPA-820-8-95-005. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC. 92 pp. + appendices.

U.S. EPA 1995c. Trophic level and exposure analyses for selected piscivorous birds and mammals. U.S. Environmental Protection Agency, Office of Science and Technology, Office of Water, Washington, D.C.

U.S. EPA 1997. Lake Michigan Mass Balance (LMMB) study methods compendium. Volume 2: organic and inorganic sample analysis techniques. EPA 905-R-97-012b. Great Lakes National

Program Office, Chicago, IL.

U.S. EPA. 1998. Ambient water quality criteria derivation methodology human health: technical support document, final draft. EPA-822-B-98-005. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

U.S. EPA. 1998c. Draft guidance for designing and conducting a field study to determine bioaccumulation factors and biota-sediment accumulation factors for nonpolar organic chemicals. Unpublished. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

U.S. EPA. 2000. Methodology for deriving ambient water quality criteria for the protection of human health (2000). EPA-822-B-00-004. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Varanasi, U., W.L. Reichert, J.E. Stein, D.W. Brown and H.R. Sanborn. 1985. Bioavailability and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from an urban estuary. *Envir. Sci. Technol.*, Vol 19. No. 9, 836-841.

White, P. A., J.B. Rasmussen and C. Blaise. 1998. Genotoxic substances in the St. Lawrence system II: Extracts of fish and macroinvertebrates from the St. Lawrence and Saguenay Rivers, Canada. *Environ. Toxicol. and Chem.*, Vol. 17, No. 2, 304-316.

Whittle, K. J., J. Murray, P.R. Mackie, R. Hardy and J. Farmer. 1977. Fate of hydrocarbons in fish. *Rapp. P.-V. Reun. Cons. Int. Explor. Mer*, Vol. 171, 139-142.

TABLE 1. FISH SAMPLES COLLECTED IN JULY

SPECIES	SAMPLE NUMBER	INDIVIDUAL FISH	
		Length (mm)	Weight (g)
Outfall 005			
Common carp *(Cyprinus carpio)*	2086	770	7750
		665	3200
		650	3200
White bass *(Morone chrysops)*	2090	255	285
White sucker *(Catostomus commersoni)*	2091	260	168
Outfall 010 (Tennessee St. Bridge)			
Common Carp *(Cyprinus carpio)*	2085	660	3500
		700	4200
Outfall 018 (Virginia St. Bridge)			
Common Carp *(Cyprinus carpio)*	2084	305	401
		220	225
		235	178
White sucker *(Catostomus commersoni)*	2089	215	98
Outfall 034			
Common Carp *(Cyprinus carpio)*	2083	703	4500

TABLE 2. FISH SAMPLES COLLECTED IN OCTOBER

SPECIES	SAMPLE NUMBER	INDIVIDUAL FISH	
		Length (mm)	Weight (g)
Tennessee St. Bridge			
Common carp *(Cyprinus carpio)*	2067	491	1500
		428	1000
		408	750
		340	500
Chinook salmon *(Onchorhyncus tshawytscha)*	2102	960	7600
		767	5000
		770	5250
Virginia St. Bridge			
Common carp *(Cyprinus carpio)*	2068	765	7250
		494	2000
		488	1250
		369	500
		340	750
Chinook salmon *(Onchorhyncus tshawytscha)*	2098	582	1250
		630	2100
		617	1500

TABLE 3.   **RHODAMINE DYE FLUORESCENCE OF WATER SAMPLES COLLECTED DURING THE MIXING ZONE DEMONSTRATION**

Transect #	Description	Position A	Position B	Position C	Position D
1	Immediately downstream of outfall 005	013	012	013	014
2	Midway between outfall 005 and outfall 010	014	013	013	013
3	Immediately downstream of outfall 010	011	013	013	013
4	Tennessee Street Bridge	012	012	012	013
5	Virginia Street Bridge	003	004	009	011

TABLE 4. ANALYTICAL RESULTS FOR WATER SAMPLES COLLECTED IN JULY

LOCATION	SAMPLE DESCRIPTION	BENZO[a]PYRENE (ng/L)	POC[a] (mg/L)	DOC[b] (mg/L)
Outfall 005	87.9L, dissolved fraction, adsorbed onto XAD resin	1.36	1.50	5.7
	87.9L particulate fraction	12.79		
	10L, dissolved fraction	1.73		
Outfall 010 (Tennessee St. Bridge)	89.7, dissolved fraction, adsorbed onto XAD resin	0.97	0.44	4.6
	89.7L particulate fraction	18.08		
	10L, dissolved fraction	1.54		
Outfall 018 (Virginia St. Bridge)	89.7L, dissolved fraction, adsorbed onto XAD resin	1.44	6.50	5.0
	89.7L particulate fraction	43.15		
	10L, dissolved fraction	13.37		
Outfall 034	86.2L, dissolved fraction, adsorbed onto XAD resin	0.5	4.00	4.0
	86.2L particulate fraction	2.67		
	10L, dissolved fraction	3.01		

[a] Particulate organic carbon
[b] Dissolved organic carbon

TABLE 5. ANALYTICAL RESULTS FOR FIELD DUPLICATED 1O LITER WHOLE WATER SAMPLES

SAMPLING EVENT	TENNESSEE ST. BRIDGE			VIRGINIA ST. BRIDGE		
	Whole Water Benzo[a] pyrene (ng/L)	POC[a] (mg/L)	DOC[b] (mg/L)	Whole Water Benzo[a] pyrene (ng/L)	POC[a] (mg/L)	DOC[b] (mg/L)
October 14, 1999	30.59[c]	1.00	4.0	27.33[c]	1.20	2.0
	14.53[c]	1.00	3.9	58.37	1.20	1.9
November 1, 1999	37.81	0.36	2.9	23.09	0.76	2.0
	13.57[d]	0.52	2.7	22.93	0.38	3.1
November 29, 1999	17.62	3.60[e]	3.2	10.36	0.50	2.3
	5.85	0.80	2.9	11.06	0.50	2.4
December 13, 1999	16.17	0.80	2.8	5.57	0.80	2.3
	31.25	0.70	2.5	7.41	0.70	2.5

[a]   Particulate organic carbon

[b]   Dissolved organic carbon

[c]   Significant loss of analytes may have occurred as a result of total evaporation of the Soxhlet extraction solvent.

[d]   Approximately 30% of the sample was lost during filtration. Results were not corrected to account for this loss.

[e]   Sample was visibly higher in particulates than its duplicate sample, possibly due to sampling error.

TABLE 6.   ARITHMATIC MEAN (± 1 STANDARD DEVIATION) FOR WATER SAMPLES COLLECTED FROM OCTOBER TO DECEMBER

LOCATION	WHOLE WATER BENZO[a]PYRENE (ng/L)	FREELY DISSOLVED BENZO[a]PYRENE (ng/L)	POC (mg/L)	DOC (mg/L)
Tennessee St. Bridge	20.92	10.24	0.75[a]	3.11
	(±10.96)	(±7.22)	(±0.22)	(±0.55)
Virginia St. Bridge	20.77	10.76	0.76	2.31
	(±17.23)	(±6.54)	(±0.31)	(±0.38)
Statistical Significance[b]	0.98	0.88	0.96	0.005
Combined results for Tennessee and Virginia St. Bridge Samples	20.84	10.50	0.75	2.71
	(±13.95)	(±6.66)	(±0.26)	(±0.62)

[a]   The outlier value (3.60 mg/L) for November 29 (see Table 4) was probably caused by contamination with sediment, due to sampling error. This value was replaced with its replicate value of 0.8 mg/L.

[b]   Values #0.005 indicate statistical significance.

TABLE 7.   ANALYTICAL RESULTS FOR COMMON CARP (TROPHIC LEVEL 2.4[a])
             TISSUE SAMPLES

SAMPLE LOCATION	SAMPLE DATE	BENZO[a]PYRENE (ug/kg -tissue)	LIPID[b] (%wet weight)	BENZO[a]PYRENE (ug/kg -lipid)
Outfall 005	July 1999	0.20U	10.95	1.83
	July 1999	0.04U	10.04	0.40
Tennessee St. Bridge or Outfall 010		11.03	3.00	37.2
	October 1999	10.91	2.72	
		9.96		
	July 1999	0.15[c]	1.14	13.2[c]
Virginia St. Bridge or Outfall 018		0.04U	6.36	3.02
	October 1999	0.08U	5.57	
		0.42		
Outfall 034	July 1999	0.06U 0.19U	7.87	1.59

[a]   Source, U.S. EPA 1995c
[b]   All results represent the mean of two determinations on the same extract. Multiple results for a location and date
      represent replicate sample processing and analysis of the same composite/sample.
[c]   The fish which were composited for this sample were smaller than those for other samples (see Table 1).
      Therefore, this sample may represent a higher trophic level (i.e., TL 2.6-2.8) (U.S. EPA 1995c), and was not
      averaged with the other July carp data for the calculation of a BAF.
U   Value is below the quantitation limit (ranging from 0.15 to 0.36 ug/kg, depending on the weight of tissue
      extracted), but above the method detection limit. The concentration value is estimated.

TABLE 8.   ANALYTICAL RESULTS FOR TISSUE SAMPLES OTHER THAN COMMON CARP

SPECIES	TROPHIC LEVEL[a]	SAMPLE LOCATION	SAMPLE DATE	BENZO[a] PYRENE (ug/kg - tissue)	LIPID[b] (%wet weight)	BENZO[a] PYRENE (ug/kg - lipid)
White sucker	2.7	Outfall 018 (Virginia St. Bridge)	July 1999	0.19	0.95	20.0
		Outfall 005	July 1999	0.35	2.2	15.9
White bass	3.9	Outfall 005	July 1999	0.06U[c] 0.05U[c]	2.5	2.2
Chinook salmon	4.0	Tennessee St. Bridge	October 1999	0.60	0.93	64.5
		Virginia St. Bridge	October 1999	0.30	1.16	25.9

[a]   Source U.S. EPA 1995c

[b]   All results represent the mean of two determinations on the same extract.

[c]   One result from the processing and analysis of duplicate portions of the same ground tissue.

U   Value is below the quantitation limit (approximately 0.14 ug/kg) but above the method detection limit. The concentration value is estimated.

TABLE 9. SUMMARY OF FIELD COLLECTED DATA

PARAMETER	Outfall 005	Outfall 010	Outfall 018	Outfall 034
Average Stream Width (feet)		60	60	90
Average Depth (feet)		2	2.5	4
Water Temperature (°C)				
July 1999	26.31	26.21	27.02	28.73
October 1999		21.85	20.53	
Dissolved Oxygen (mg/L)				
July 1999	8.13	8.33	7.38	8.31
October 1999		8.21	8.75	
Specific Conductance (ms/cm)				
July 1999	0.456	0.482	0.493	0.370
October 1999		0.645	0.504	
pH (SU)				
July 1999	9.32	9.3	9.27	9.65
October 1999		8.37	8.43	

TABLE 10.   FIELD-MEASURED AND BASELINE BAFs FOR BENZO[a]PYRENE

WATER SAMPLE DESCRIPTION	TISSUE SAMPLE DESCRIPTION	TROPHIC LEVEL	FIELD-MEASURED BAF (L/kg-tissue)	BASELINE BAF (L/kg-lipid)	log-BASELINE BAF
July all sites[a]	Common carp[b]	2.4	6.05	268	2.43
Combined Oct. to Dec[c]	Common carp[d]	2.4	510	35,190	4.55
Combined Oct. to Dec[c]	Common carp[e]	2.4	8.6	269	2.43
Combined Oct. to Dec[c]	Common carp[f]	2.4	260	11,597	4.06
Oct. 14[g]	Chinook salmon[h]	4.0	14	2,976	3.47
July[i]	White Bass[j]	3.9	2.1	314	2.50
Combined Oct. to Dec[c]	White Bass[j]	3.9	2.6	168	2.23
July[i]	White sucker[k]	2.7	10.4	2,694	3.43
Combined Oct. to Dec[c]	White sucker[k]	2.7	13	1,561	3.19

[a]   Average of results for ~100L samples collected in July from Outfalls 005, 010, 018 and 034 (dissolved + particulate fractions). $C_W = 0.020$ ug/L. $C_w^{fd} = 0.0046$ ug/L.

[b]   Average of composite samples collected at Outfalls 005, 010 and 034 in July. $C_t = 0.123$ ug/kg. $C_l = 1.3$ ug/kg.

[c]   Average of results for water samples collected at the Tennessee and Virginia St. Bridges on Oct. 14, Nov. 1, Nov. 29, and Dec. 13, 1999 (n = 16). $C_w = 0.0208$ ug/L. $C_w^{fd} = 0.0106$ ug/L.

[d]   Composite sample of common carp collected at the Tennessee St. Bridge location in Oct., 1999. $C_t = 10.63$ ug/kg. $C_l = 372$ ug/kg.

[e]   Composite sample of common carp collected at Virginia St. Bridge location in Oct., 1999. $C_l = 0.18$ ug/kg. $C_l = 3.0$ ug/kg.

[f]   Average for composite samples d and e. $C_t = 5.41$ ug/kg. $C_l = 123$ ug/kg.

[g]   Average of results for water samples collected at the Tennessee St. and Virginia St. Bridge locations on Oct. 14, 1999 (n = 4). $C_w = 0.0327$ ug/L. $C_w^{fd} = 0.0140$ ug/L.

[h]   Average for composite samples of chinook salmon collected at the Tennessee and Virginia St. Bridge locations in Oct., 1999. $C_t = 0.45$ ug/kg. $C_l = 43$ ug/kg.

[i]   Average for ~100L samples collected in July from the Outfall 005, 010 and 018 locations (dissolved + particulate fractions). $C_w = 0.0259$ ug/L. $C_w^{fd} = 0.0062$ ug/L.

[j]   White bass tissue sample collected from Outfall 005 location in July, 1999. $C_t = 0.06$ ug/kg. $C_l = 2.2$ ug/kg.

[k]   Average for white sucker tissue samples collected from the Outfall 005 and 018 locations in July, 1999. $C_t = 0.27$ ug/kg. $C_l = 17$ ug/kg.
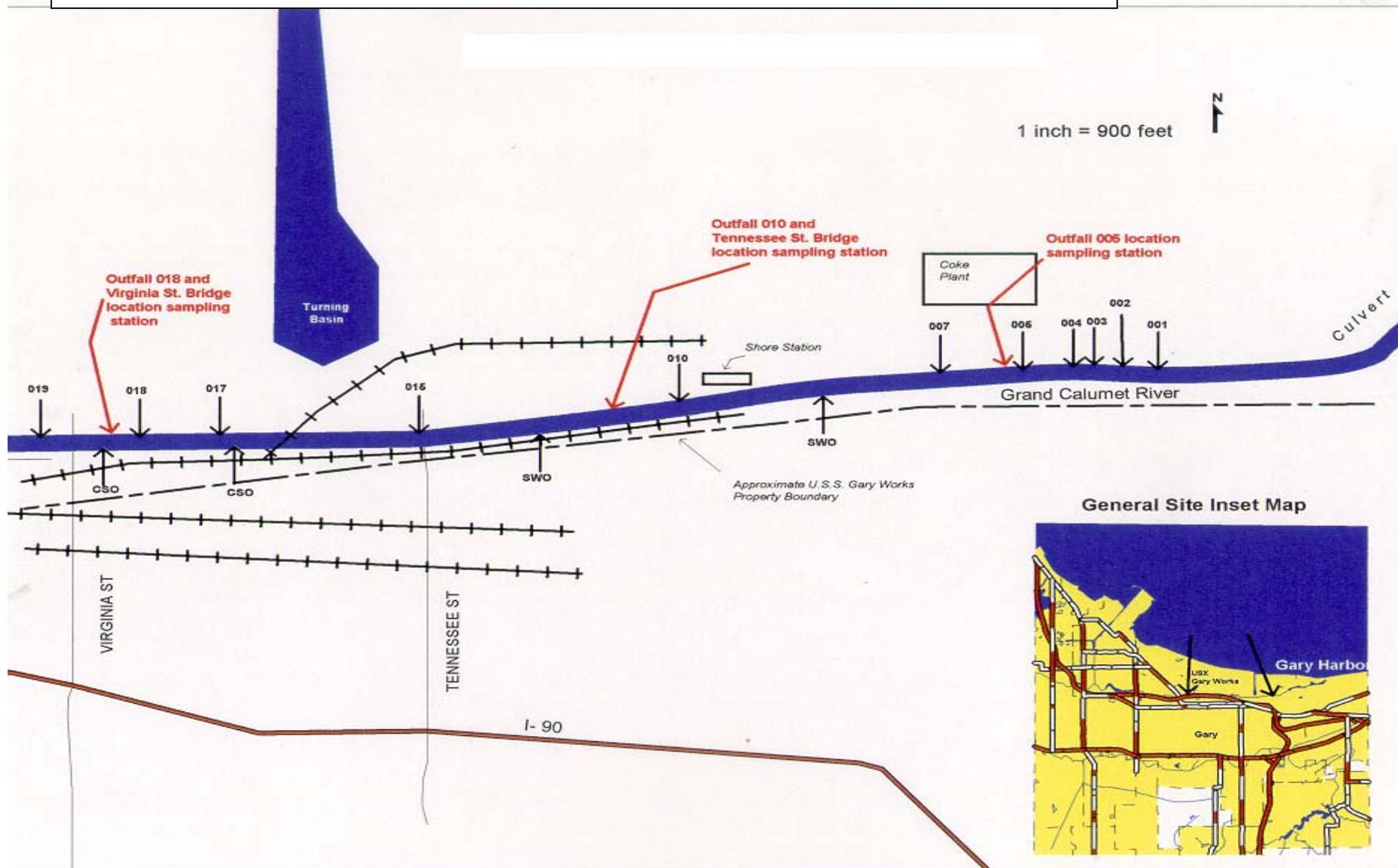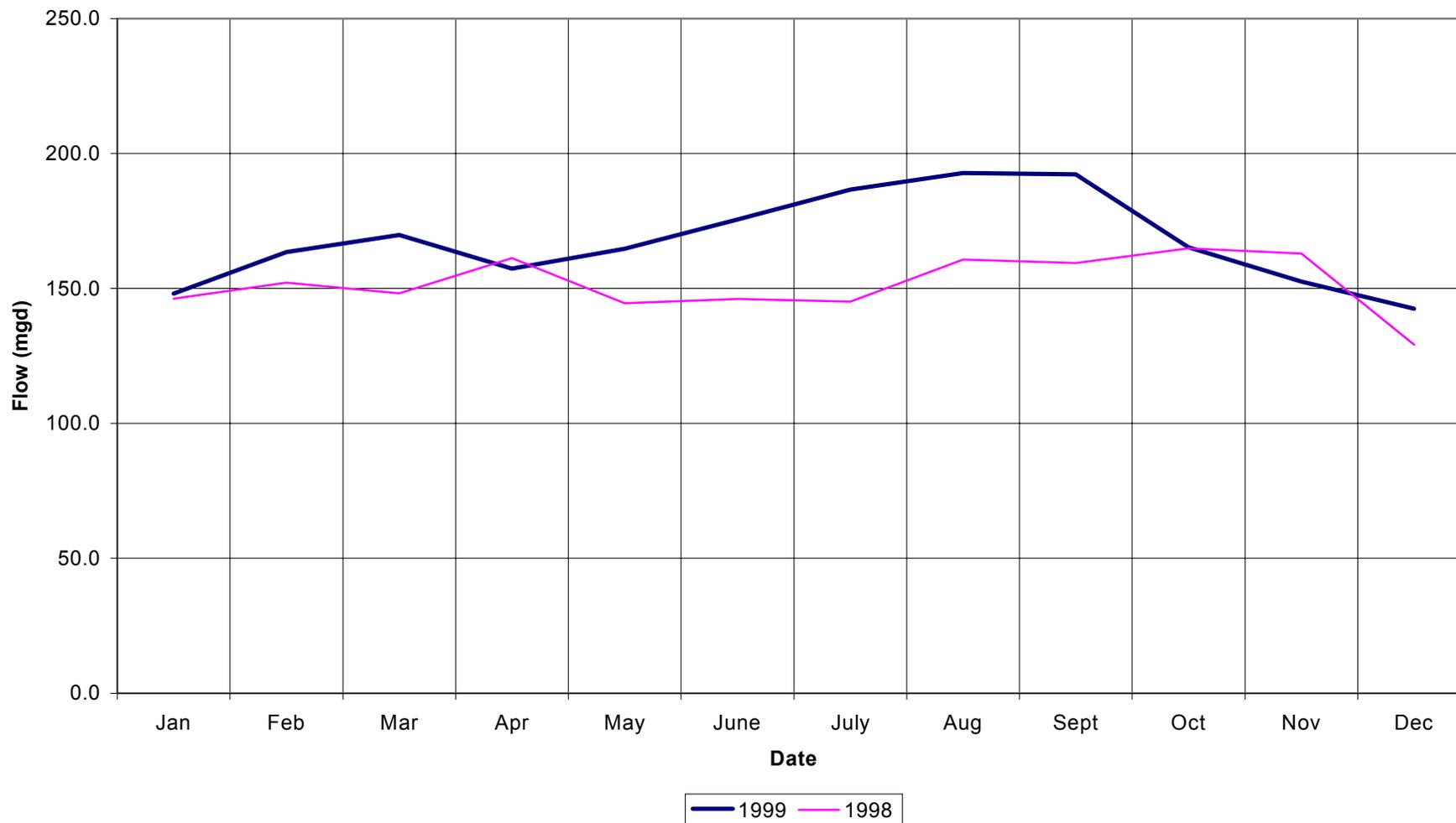
FIGURE 1. SAMPLE COLLECTION LOCATIONS

45

FIGURE 2. TOTAL MONTHLY AVERAGE FLOWS - 1998 VS 1999
OUTFALLS 005, 018 AND 019

Figure 3   Additional Sample Collection Location

**Outfall 034
sampling station**

1 inch = 2,000 feet

N

034

019

I- 90

Grand Calumet River

W 4TH AVE
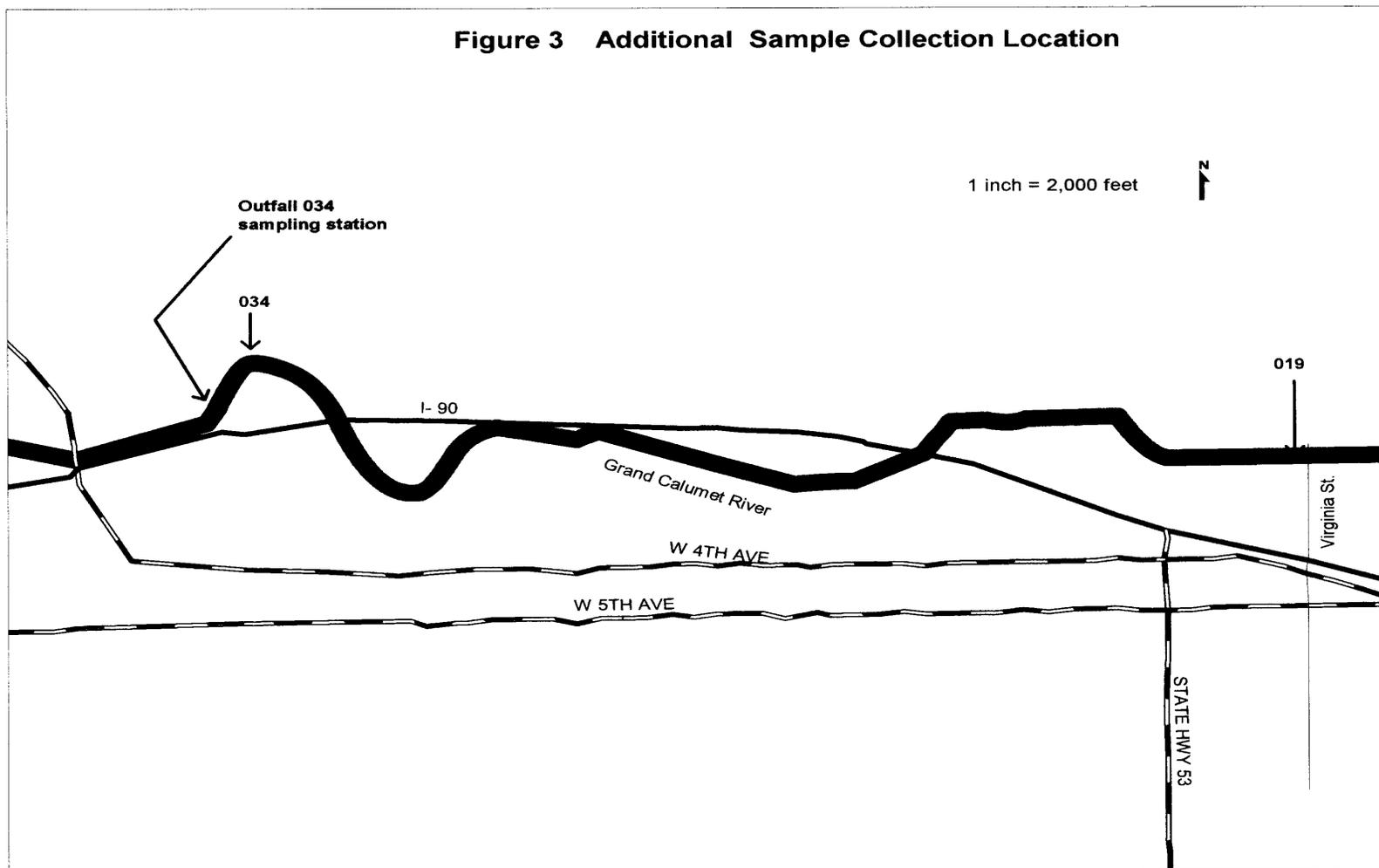
W 5TH AVE

Virginia St.

STATE HWY 53

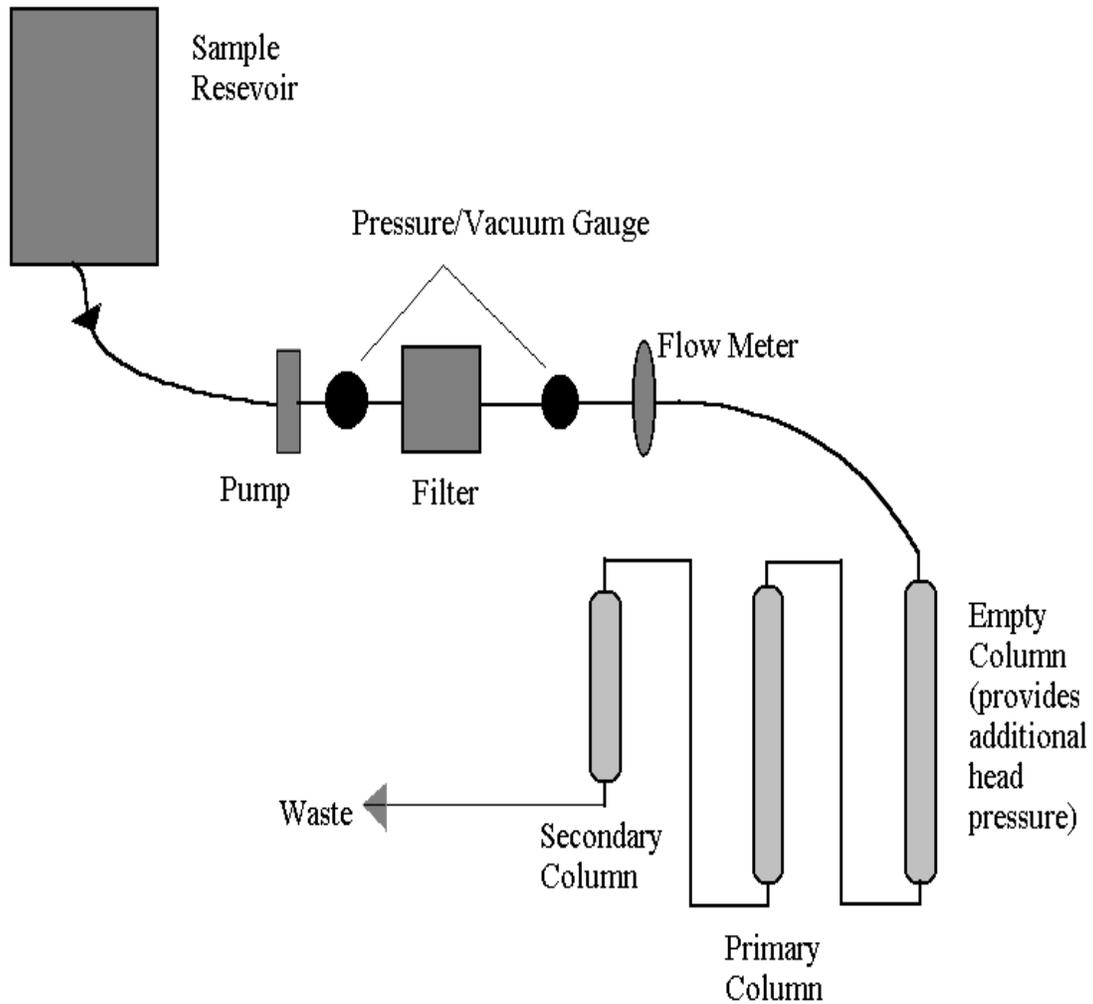**FIGURE 4.   SCHEMATIC OF SYSTEM USED TO FIELD PROCESS LARGE VOLUME WATER SAMPLES**
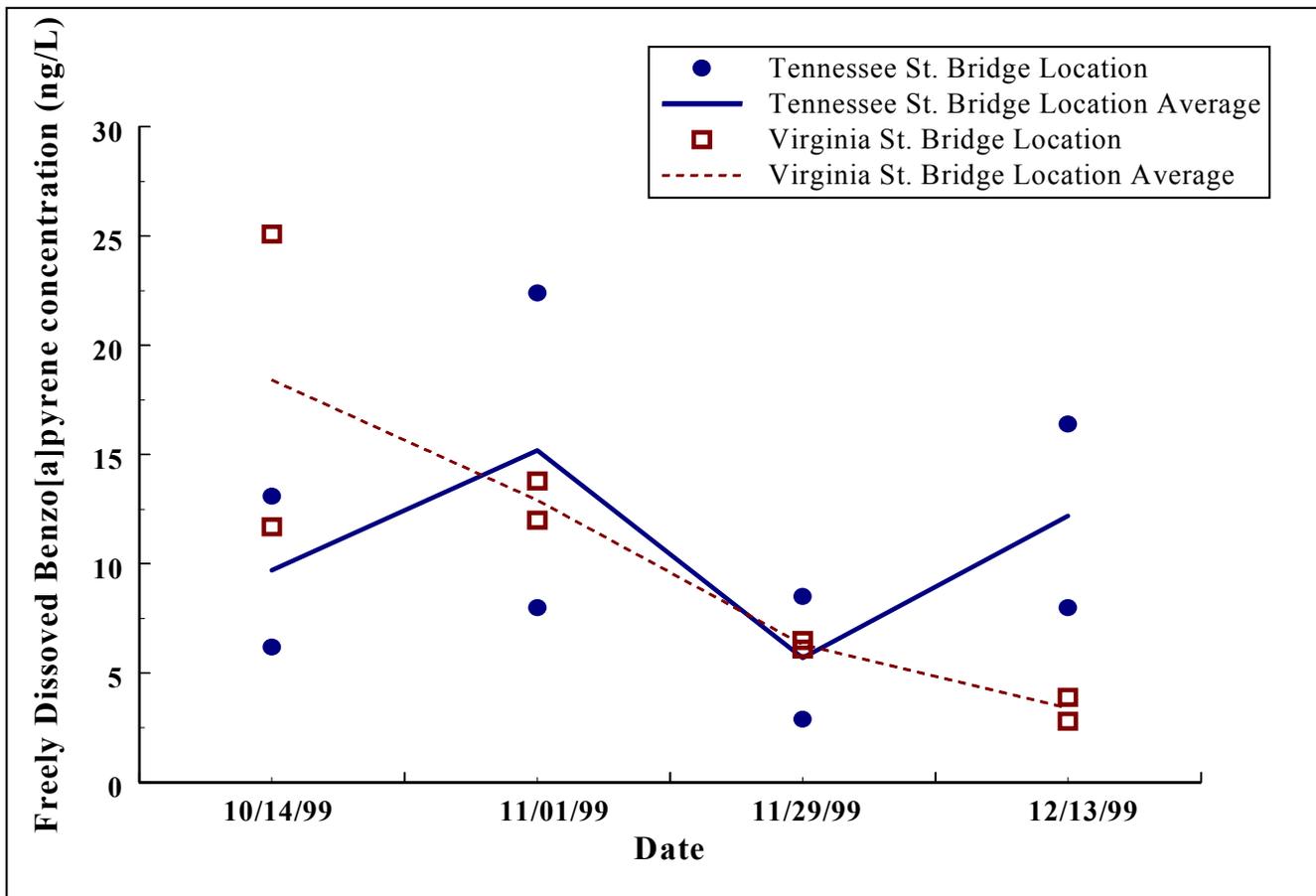
FIGURE 5. FREELY DISSOLVED CONCENTRATIONS OF BENZO[A]PYRENE

Estimated Costs to Conduct Site-specific BAF Studies

The cost for conducting a study to derive a site-specific BAF can be estimated using the previously described case study (Derivation of Baseline Bioaccumulation Factors (BAFs) from Grand Calumet River Field Measured BAFs for Benzo[a]pyrene). A few things should be noted regarding the expected costs. The costs and duration of the various phases of a BAF study are highly dependent on: 1) the nature of the chemical for which the BAF was developed, and 2) the site characteristics, including the concentration levels and the efficacy of determining a field-measured BAF versus a field-measured BSAF. Since the Grand Calumet study was the first of its kind (based on knowledge of the authors), it should be stated that as more studies are conducted costs may decrease as knowledge and efficiency are acquired. The following table provides the costs incurred in the Grand Calumet study for each phase.

PHASE NUMBER	PHASE DESCRIPTION	DURATION	COST
1	**Development of Work Plan (WP) and Quality Assurance Project Plan (QAPP)** Including: a) Review of historical data and literature b) Site visit c) WP and QAPP development	6 months	$15,000
2	**Field Sampling and Laboratory Analysis** Including: a) Two field sampling events b) Preparation and analysis of 11 composite fish tissue samples c) Analysis of four 100 liter river water samples (dissolved and particulate fractions analyzed separately) d) Analysis of twelve 10 liter river water samples	8 months	$175,000
3	**Report Preparation**	2 months	$25,000
4	**Response to Regulatory Agency Comments** Including: a) Travel to meet with the client and the regulatory agency personnel b) Preparation of a written response c) An additional literature review d) Preparation of a White Paper	12 months	$35,000
	TOTALS	**28 months**	**$250,000**