# APPENDIX A

***Section 5.2 from "Policy for Implementation of Toxics Standards for Inland Surface Waters, Enclosed Bays, and Estuaries of California"***

## 5.2 Site-Specific Objectives

If a priority pollutant criterion or objective is inappropriate for a particular water body (i.e., it does not protect the beneficial uses or, based on site-specific conditions, a less stringent standard may be warranted), a water quality objective that differs from the applicable criterion or objective may be developed for the site. A RWQCB may develop site-specific objectives whenever it determines, in the exercise of its professional judgement, that it is appropriate to do so. Where a priority pollutant criterion or objective is not being attained in the water body, under certain circumstances, it may be more appropriate to pursue other approaches to achieve the applicable criterion or objective rather than develop a site-specific objective. These approaches include, but are not limited to, watershed management and development of TMDLs (see Appendix 5 and Appendix 6). The RWQCB may investigate, facilitate, or implement such approaches as appropriate.

Regardless of an action taken by the RWQCB as described above, the RWQCB shall, at a public meeting, consider initiating the development of a site-specific objective under the following conditions:

(1) A written request for a site-specific study, accompanied by a preliminary commitment to fund the study, subject to development of a workplan[1], is filed with the RWQCB; and

(2) Either:

  (a) a priority pollutant criterion or objective is not achieved in the receiving water; or
  (b) a holder of an NPDES permit demonstrates that they do not, or may not in the future, meet an existing or potential effluent limitation based on the priority pollutant criterion or objective; and

(3) A demonstration that the discharger cannot be assured of achieving the criterion or objective and/or effluent limitation through reasonable treatment, source control, and *pollution prevention measures. This demonstration may include, but is not limited to, as determined by the RWQCB:

  (a) an analysis of compliance and consistency with all relevant federal and State plans, policies, laws, and regulations;
  (b) a thorough review of historical limits and compliance with those limits;

[1] The elements presented under the "Special Studies Process" in Appendix 5 should be considered in developing the site-specific objectives workplan.

(c) a thorough review of current technology and technology-based limits; and
(d) an economic analysis of compliance with the priority pollutant criterion or objective of concern.

During the period when site-specific objectives studies are being conducted, the RWQCB shall place effluent limitations based upon the applicable priority pollutant criteria or objectives into permits only in conjunction with an appropriate compliance schedule and interim requirements, as described in sections 2.1 and 2.2.

A discharger subject to a schedule for compliance with a CTR criterion or CTR criterion-based effluent limitations, as described in section 2.1, may choose to, concurrently with the actions necessary to achieve compliance, conduct the studies necessary to support the development and adoption of a site-specific objective.[2]

Following adoption of a site-specific objective by the RWQCB, existing effluent limitations shall be replaced with effluent limitations (calculated as described in section 1.4) based on the adopted site-specific objective if the analysis in section 1.3 indicates that a limitation for the pollutant is required. In the event that, for reasons beyond the control of the discharger, a decision whether or not to adopt site-specific objectives has not been made by the RWQCB before the end of the compliance schedule, the compliance schedule shall be extended for an additional period to allow time for a decision whether or not to adopt the objective. However, in no event may a compliance schedule exceed the maximum time period allowed for compliance with the CTR criteria (as described in section 2.1) or priority pollutant objectives (as described in the basin plan, if applicable), unless an exception has been granted (in accordance with section 5.3).

Development of Site-Specific Objectives

Water quality objectives shall be developed in a manner consistent with State and federal law and regulations. In accordance with the State's Porter-Cologne Water Quality Control Act (Division 7 of the Water Code), objectives must provide for the reasonable protection of beneficial uses based on consideration of the factors listed in Water Code Section 13241. In accordance with federal law (CWA) and regulations (40 CFR 131.11, revised as of July 1, 1997), the objectives must be based on sound scientific rationale and protect the designated beneficial uses of the receiving water.

The RWQCB shall use scientifically defensible methods appropriate to the situation to derive the objectives. Such methods may include U.S. EPA-approved methods (e.g., Water Effects Ratio [WER] procedure, recalculation procedure, a combination of recalculation and WER procedures, Resident Species Procedure), and/or other methods specified in the workplan.

[2] A RWQCB may include a compliance schedule in a water quality standard based on a site-specific objective. Such a compliance schedule is separate and distinct from the compliance schedules established by this Policy.

A site-specific objective adopted by the RWQCB may include a compliance schedule. However, if attainment of the potential objective(s) developed under the study is anticipated to be infeasible (as defined in 40 CFR 131.10(g), revised as of July 1, 1997), or if the RWQCB otherwise determines it is appropriate, a *use attainability analysis (UAA) may be conducted. The RWQCB shall conduct, with the participation of interested persons, as appropriate, the UAA in accordance with 40 CFR 131.10(j) (revised as of July 1, 1997). If the UAA shows that attainment of the designated beneficial use(s) is not feasible (pursuant to 40 CFR 131.10(g) (revised as of July 1, 1997), the RWQCB shall designate an alternative beneficial use or subcategory of use, and develop appropriate water quality objectives to protect the new use(s). Both the use(s) and the objective(s) established to protect it would be reevaluated during the triennial reviews of the State's water quality standards.

# APPENDIX B

## *Appendix 5 (Special Studies) from "Policy for Implementation of Toxics Standards for Inland Surface Waters, Enclosed Bays, and Estuaries of California"*

## Pre-Evaluation for Special Studies
## Decision Tree with Attached Narrative Discussion

A special study is sometimes conducted as part of a regulatory process (standard setting and permit writing) and may be conducted as part of a collaborative watershed planning effort. Special studies can provide site-specific data that can assist in decision-making regarding water quality and beneficial use issues.

Many water quality problems may be best addressed on a watershed or water body basis. The SWRCB believes that stakeholders should be able to develop flexible and innovative solutions for water quality problems in their watershed. For special studies conducted as part of a watershed management plan, the watershed management group should be involved in the design of the study, and study information should be provided back to the committee. Watershed or water body studies may gather data regarding topics such as:

- TMDLs, WLAs, and LAs (see Appendix 6);
- Regional ambient monitoring (regional ambient monitoring is the collection of scientific information regarding water quality and impacts to beneficial uses for a specified portion of, or an entire, watershed or water body); and
- Contaminant fate and transport monitoring (contaminant fate and transport monitoring is the gathering of scientific information regarding how a specific pollutant[s] moves through the environment and how the pollutant[s] degrades or is otherwise transformed in the environment).

These types of studies are useful to collect integrated, comprehensive, and systematic data regarding:

- Baseline concentrations of toxic pollutants in the water and sediment;
- Seasonal, annual, and long-term trends in water quality;
- Causes and effects of water quality problems;

- Effectiveness of a water quality control effort;

- Greater certainty regarding existing monitoring data; etc.

Any of the studies discussed below may be undertaken as part of a watershed approach to addressing regional water quality issues. Information collected as part of a watershed or water body study can be used as a way to define parameters (e.g., ambient background concentrations, mixing zones, etc.) related to the development of effluent limitations as part of the permitting process or to evaluate whether changes in water quality standards are appropriate. A watershed or water body approach is also useful to dischargers because information collected as a part of one effluent limitation or standard-setting study can be shared with other stakeholders in the same water body.

Studies for Setting Effluent Limitations

Studies regarding establishing effluent limitations can be done as part of the permitting process. Such studies may be simpler and there may be fewer interested stakeholders than studies involving more than one discharger, or an entire water body or watershed. However, when such studies are undertaken individually, the discharger, the RWQCB, and other stakeholders do not gain the benefit of data collected from others in the watershed.

Special studies may address topics such as the following:

- Determining pollutants requiring effluent limitations (see section 1.3);

- Metals translators (see section 1.4.1); or

- Mixing zones (see section 1.4.2).

Studies For Changes to Water Quality Standards

Establishing or modifying water quality standards (i.e., beneficial uses and water quality criteria/objectives) may involve complex and resource intensive studies. A detailed workplan will normally be needed because early planning and coordination with the RWQCB and U.S. EPA is critical to the development of a successful study. In addition, a workplan will normally be appropriate because there will be more stakeholder interest and involvement of other public agencies (e.g., Department of Fish and Game, U.S. Fish and Wildlife Service, etc.). Involvement in a watershed management planning effort would facilitate the sharing of information among stakeholders in the watershed, both in gathering information for the study and in sharing the results. Studies related to changes in water quality standards may address topics such as the following:

- Site-specific objective studies (see section 5.2); and
- Use attainability analysis (UAA) (see section 5.2).

Pre-Evaluation

As a first step in determining whether and how to conduct a special study, the RWQCB or other stakeholders may want to evaluate whether it would be appropriate to address a water quality issue through a watershed management approach. To do that, the factors in the following flowchart may be considered:

1. Is there a watershed management group?[a]

↓ Yes

2. Has a watershed management approach been developed?[b] →→→→→ No → GO TO start of Decision Tree[d] if water issue involves toxics.

↓ Yes

3. Are toxic pollutants part of the reason beneficial uses are impaired or water quality objectives are exceeded?[c] →→→→→ No → Issue is outside scope of this Policy.

↓ Yes

GO TO #9 in Decision Tree[d], or other point in Decision Tree as determined by stakeholders.

[a] Is there a committee of local interests in both the public and private sectors that are actively involved in the management of the watershed area?

[b] Has a watershed management approach that identifies key issues, boundaries, objectives, and early actions been developed?

[c] A study may be necessary to determine whether toxics are part of the cause of the impairment of beneficial uses. This Policy applies only to the CTR and NTR criteria; and applicable chemical-specific basin plan objectives for priority toxic pollutants.

[d] The decision tree is on page B-6.

The decision tree and associated narrative discussion in Appendix 5 are provided to assist RWQCBs and stakeholders in identifying whether there is a current or potential water quality issue requiring attention [Compliance Status], the nature of the identified water quality issue [Screening-level Evaluation], and possible action to address the issue [Potential Options].

Based on this information, the RWQCB and stakeholders can determine whether a special study is needed and the scope of the study. This approach can help avoid initiation of costly and time-consuming studies which are not appropriately designed to resolve the specific issue in question. The decision tree is not meant to preclude the exploration of any other creative solutions; it is meant to encourage constructive dialogue among stakeholders.

Two specific considerations should be kept in mind when conducting the pre-evaluation suggested by this decision tree. First, users must be familiar with the quality of the data under review and the potential need to augment data which are not of adequate quality. Second, users should know what the existing beneficial uses are (i.e., uses attained since 1975).

Special Studies Process

A. Workplan

If appropriate, the RWQCB may participate in developing a detailed workplan with interested persons (which can include, but are not limited to, U.S. EPA, the RWQCB, the SWRCB, and affected dischargers) prior to proceeding with a special study. The workplan may include the following elements:

(1) Formation of a project team for the workplan, which may include the Department of Fish and Game, the U.S. Fish and Wildlife Service, and other stakeholders;

(2) Purpose of the workplan;

(3) Responsibilities of the persons associated with the workplan;

(4) Budget and cost-sharing plan. This plan must be determined on a case-by-case basis; however, the SWRCB encourages sharing of costs (based on availability of funding), where there are multiple persons who wish to support the goals of the study;

(5) Development of the following elements:

(a) Identification of tasks(s),
(b) Purpose of tasks(s),
(c) Method by which task(s) will be implemented,

(d) Products of the tasks(s),
(e) Schedule for the task(s),
(f) Responsibility for implementing the task(s), and
(g) Budget and funding for the task(s);

(6) Administrative policies and procedures to govern oversight of the special studies process (e.g., amending the workplan, conflict resolution, etc.); and

(7) Project schedule.

B. Scientific Review Panel

If, during the data interpretation phase of a special study, the RWQCB, SWRCB, U.S. EPA, or other stakeholders have differing opinions with regard to the interpretation of data, the RWQCB and stakeholders may want to seek the advice of an independent scientific review panel. The method of selecting the panel, cost reimbursement, and other details regarding the conflict resolution process could be included in the workplan.

C. Compliance Schedule

A permit compliance schedule (as described in section 2.1) may allow sufficient time for collection of data, completion of a study, and determination of compliance measures. While special studies are being conducted, interim requirements may be established by the RWQCB (as described in section 2.2). However, in no event may a compliance schedule exceed the time period allowed in this Policy, unless an exception has been granted.

D. Environmental and Economic Impacts

To ensure that environmental and economic impacts are adequately addressed, the RWQCB staff shall, as part of the special study workplan:

(1) Comply with CEQA, if applicable; and

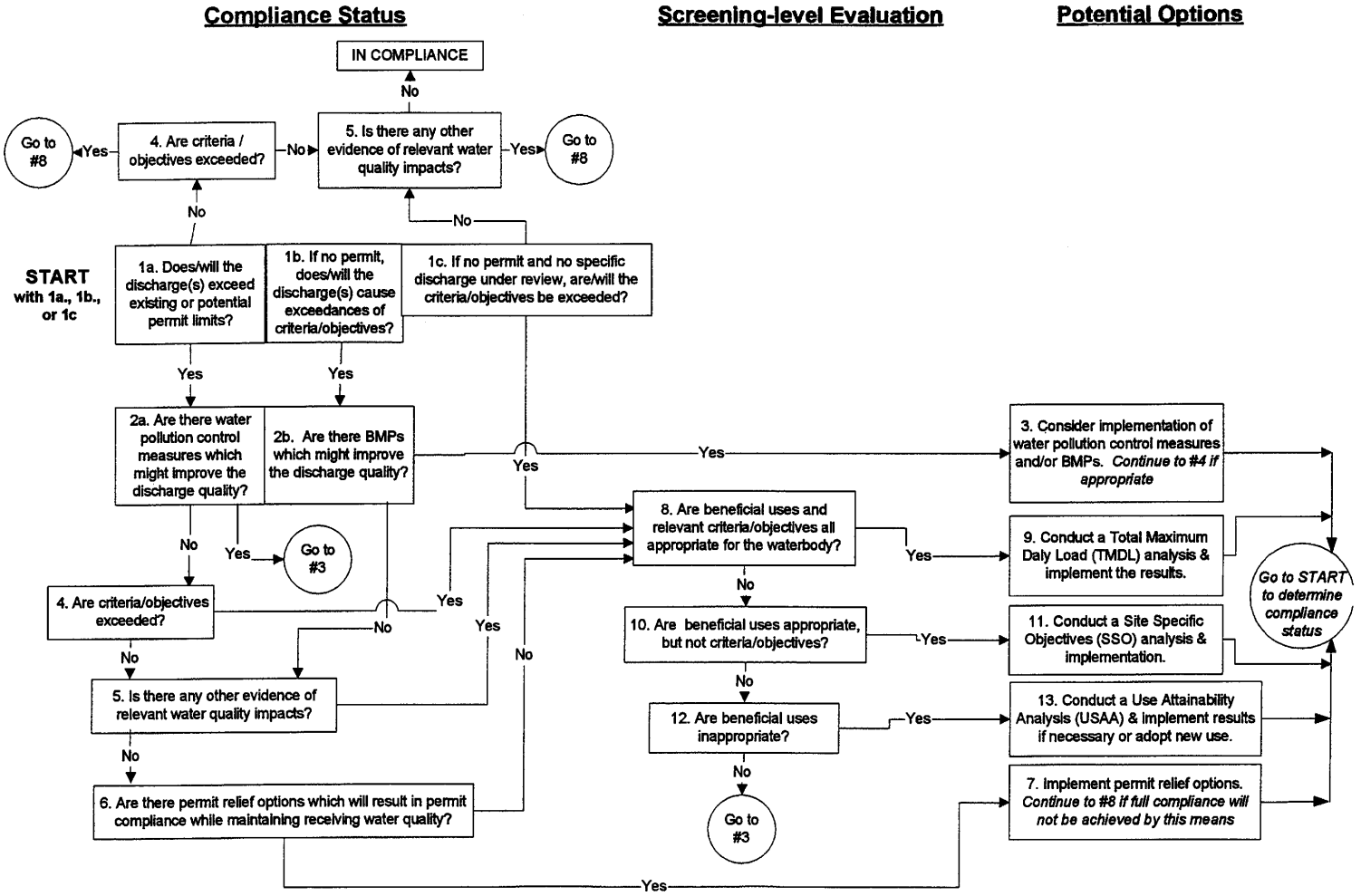
(2) Direct the preparation of an analysis documenting economic impacts if site-specific objectives or a change in designated beneficial uses is being considered under 40 CFR 131.10(g)(6), revised as of July 1, 1997.

E. Antidegradation and Other Legal Requirements

RWQCB staff shall, as part of the special study workplan, ensure compliance with SWRCB Resolution No. 68-16 (Statement of Policy with Respect to Maintaining High Quality of Waters

in California) and any other applicable legal requirements.

## Pre-Evaluation for Special Studies
## Decision Tree with Attached Narrative Discussion



**Narrative Discussion of Decision Tree:**

l a. Does/will a discharge exceed existing or potential permit limits for toxic pollutants? This question applies to discharges regulated by a National Pollutant Discharge Elimination System (NPDES) permit or Waste Discharge Requirements (WDRs). If the discharge(s) in question is not regulated by a discharge permit, proceed to #1b. It is assumed that data used to answer this question are reliable.

1b. If no permit, does the discharge(s) cause exceedances of criteria/objectives? This question primarily applies to nonpoint discharges, though it could conceivably apply to point source discharges which are not currently permitted. It is assumed that data used to answer this question are reliable.

1c. If no permit and no specific discharge(s) are under review, are criteria/objectives exceeded? It is assumed that data used to answer this question are reliable.

2a. Are there water pollution control measures which might improve the water quality? A water pollution control program may include, as appropriate: pollution control technologies; pretreatment requirements; and pollution prevention, waste minimization, and source control measures. This question is meant to elicit consideration of effluent quality control measures which could be implemented as a full or partial solution to the identified permit noncompliance issue. It is not intended as a barrier to the exploration of other potential forms of regulatory adjustment.

2b. Are there Best Management Practices (BMPs) which might improve water quality? BMPs are pollution management measures designed to reduce the water quality impacts, where they exist, associated primarily with non-point source discharges. As with #2a above, this question is meant to elicit consideration of discharge control measures which could be implemented as a full or partial solution to the identified noncompliance issue. It is not intended as a barrier to the exploration of other potential forms of regulatory adjustment.

3. Consider whether implementation of water pollution control measures and/or BMPs will lead to compliance. Simultaneously, continue to #4 if deemed appropriate, considering such questions as whether or not full compliance will be achieved by these means, or whether it would be cost effective. As stated, the simple determination that implementation of pollution control measures and/or BMPs might improve the discharge or water quality should not preclude the exploration of other potential regulatory adjustment options, as well. For clarity, the reviewer should proceed not to box four prime, but to box four.

4. Are criteria/objectives exceeded? It is assumed that data used to answer this question are reliable and appropriate hardness adjustments have been made.

5. Is there any other evidence of relevant water quality impacts? This question is meant to capture those situations, where the criteria/objective for the pollutant of concern do not exist or appear to be under protective. "Other evidence" might include: bioconcentration or biocriteria data, population studies, food web analyses, etc. Impacts to wildlife should be considered as should impacts to threatened and endangered species. The potential for impacts to be of a seasonal nature should also be considered in this pre-evaluation. "Relevant water quality impacts" are those impacts which have a demonstrable relationship to the pollutant(s) of concern.

6. Are there permit relief options which will result in permit compliance while maintaining receiving water quality? Permit relief options might include, where appropriate: development of a mixing zone, modification of the averaging periods, adoption of a variance, etc. For unpermitted discharges or pre-evaluations involving no specific discharges, the user should continue to box #8.

7. Implement permit relief options. Continue to #8 if full compliance will not be achieved by these means. The development of permit relief options would occur through a request to the RWQCB.

8. Are beneficial uses and criteria/objectives both appropriate for the water body? To answer this question, a screening-level evaluation may be necessary, including an evaluation of the associated regulatory history; the site-specific conditions; and the status of current, applicable scientific understanding. It is assumed that data used to answer this question are reliable.

   This question is best answered when a watershed stakeholder group has formed and collectively either: 1) evaluated the condition of the watershed through a watershed management plan, 2) evaluated the condition of the watershed through less formal means, or 3) convened discussions regarding the condition of the watershed. If one does not currently exist, a watershed stakeholder group should be formed if it appears to be a useful forum for discussion and review. The following more specific questions may apply:

   - Is the water effluent dominated, agricultural drainage water dominated, etc.? These water bodies may be likely candidates for the appropriate application of regulatory adjustments (e.g., SSO or UAA).

   - Were the current beneficial uses applied on a national, state-wide, or region-wide basis or have they been specifically designated for the water body in question? While not the

only candidates, water bodies for which beneficial uses have been applied on a national, state-wide, or region-wide basis may be candidates for the appropriate application of regulatory adjustments (e.g., SSO).

- Are there rare, threatened, or endangered species, or ecological conditions which the currently applied beneficial uses do not adequately describe or the water quality objectives do not fully protect?

- Has the beneficial use and the water quality necessary to maintain the beneficial use been attained since 1975?

- How do anti-degradation requirements apply?

- Are elevated constituents the result of 1) natural phenomena or 2) anthropogenic activities that ceased prior to 1975?

- Do the currently designated beneficial uses protect all existing and appropriate potential uses?

- Are natural, ephemeral, intermittent, or low flow conditions or water levels preventing the attainment of the designated non-existing uses?

- Are there human-caused conditions or sources of pollution which prevent attainment of the uses but either cannot be remedied or would cause greater environmental damage if corrected?

- Does the presence of dams, diversions, or other types of hydrologic modifications preclude the attainment of designated non-existing beneficial uses?

- Do the physical conditions of the water body preclude attainment of aquatic life protection uses (i.e., lack of proper substrate, cover, flow, depth, pools, riffles, and the like)?

- Does attainment of designated beneficial uses require the application of controls which would result in substantial and widespread economic and social impact?

- Have the appropriate water characteristics (e.g., hardness, pH) been accounted for in the CTR criteria?

- Has an appropriate set of species been evaluated in setting the CTR criteria and toxicity objective?

9. Conduct a total maximum daily load analysis and implement the results. Conducting a TMDL could result in, among other things, waste load allocations, BMP implementation for non-point dischargers, and/or effluent trading options for point and non-point source dischargers. (See Appendix 6 regarding TMDLs.)

10. Are beneficial uses appropriate but not criteria/objectives for toxic pollutants? See #8 above.

11. Conduct a site-specific objectives analysis. An SSO study will include one or more of the following activities:

    - Recalculation of objective;
    - Water effects ratio or other similar method; or
    - Any scientifically defensible process.

    U.S. EPA's "Guidelines for Deriving Numerical Aquatic Site Specific Water Quality Criteria by Modifying National Criteria," dated 1984 (EPA-600/3-84-099) provides guidance for conducting an SSO study.

    U.S. EPA's "Water Quality Standards Handbook" dated 1994 also provides general guidance in this area.

12. Are beneficial uses inappropriate? See #8 above.

13. Conduct a use attainability analysis (UAA) and implement the results. When a use is proposed for dedesignation, i.e., removed or replaced with a subcategory requiring less stringent standards, a UAA is necessary. In a case where a use is proposed to be added, a UAA is not necessary. A new use designation can be added for a water body following the normal public review process. A UAA will determine if physical, chemical, and/or biological factors affect the attainability of a designated use via a water body survey and assessment. An analysis of economic factors can also be included to determine whether substantial and widespread economic and social impacts would be caused by stringent pollution control requirements.

    U.S. EPA's "Technical Support Manual: Water body Survey and Assessment for Conducting Use Attainability Analyses" dated 1983 provides guidance for conducting a UAA as does Region 9's Interim Final "Guidance for Modifying Water Quality Standards and Protecting Effluent-Dependent Ecosystems" dated 1992. U.S. EPA's "Water Quality Standards Handbook" dated 1994 also provides general guidance in this area.

# APPENDIX C

## EFFECT OF THE NUMBER AND RANGE OF GMAVS ON FAV

The FAV is the estimate of the GMAV of a theoretical 5$^{th}$ percentile most-sensitive genus from the known distribution of GMAVs. In general, the FAV estimate is more certain when the range (variance) among the four lowest GMAVs is low. Similarly, the FAV estimate is more certain when the range (variance) among the P values for the four lowest GMAVs is low (this range decreases as the number of GMAVs increase). The CMC, or the acute SSO, is calculated as the FAV divided by 2. An understanding of GMAVs and FAVs can be gained from numeric examples and graphs in the following section.

**TABLE C-1. FAV CALCULATION PROCEDURE** {Calculations for select chemicals can be done at USEPA Region 7 Website: http://www.epa.gov/region7/water/equ.htm}

- For each genus for which one or more SMAVs are available, the GMAV should be calculated as the geometric mean of the SMAVs available for the genus.
- Order the GMAVs from high to low.
- Assign ranks, R, to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.
- Calculate the cumulative probability, P, for each GMAV as R/(N+1).
- Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are fewer than 59 GMAVs, these will always be the four lowest GMAVs).
- Using the selected GMAVs and Ps, calculate:

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2 / 4}{\Sigma (P) - ((\Sigma(\sqrt{P}))^2 / 4}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P}))) / 4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

### *FAV Calculation Examples*

The examples provided in this section are based upon an entirely hypothetical set of GMAVs. The types of outcomes illustrated are general and should apply to any dataset, but the magnitude of the changes will differ as a function of the actual number and the exact values of GMAVs. The examples illustrated in Figures C-1 and C-2 were developed from the following hypothetical

baseline situations:

- the four lowest GMAVs are 2.5, 4.1, 9.6 and 20.3 $\mu$g/L, and
- the total number of GMAVs are either low (N = 10), intermediate (N = 20), or high (N = 40).

Baseline Results

The hypothetical baseline data will yield FAVs of 1.209, 2.223, and 5.336 $\mu$g/L for Ns of 10, 20, and 40, respectively. Clearly, the FAV is lower as the number of GMAVs decreases, even with the identical four lowest GMAVs. This is because of the increased variability (range) of the P values (a function of N) used in the calculation of the FAV (Figure C-1). Adding GMAVs to the dataset without introducing a new value into the four lowest GMAVs will always increase the FAV.

The actual FAVs calculated by the USEPA method are included in the legend and are not derived using the apparent regressions that are included simply for the sake of illustration.

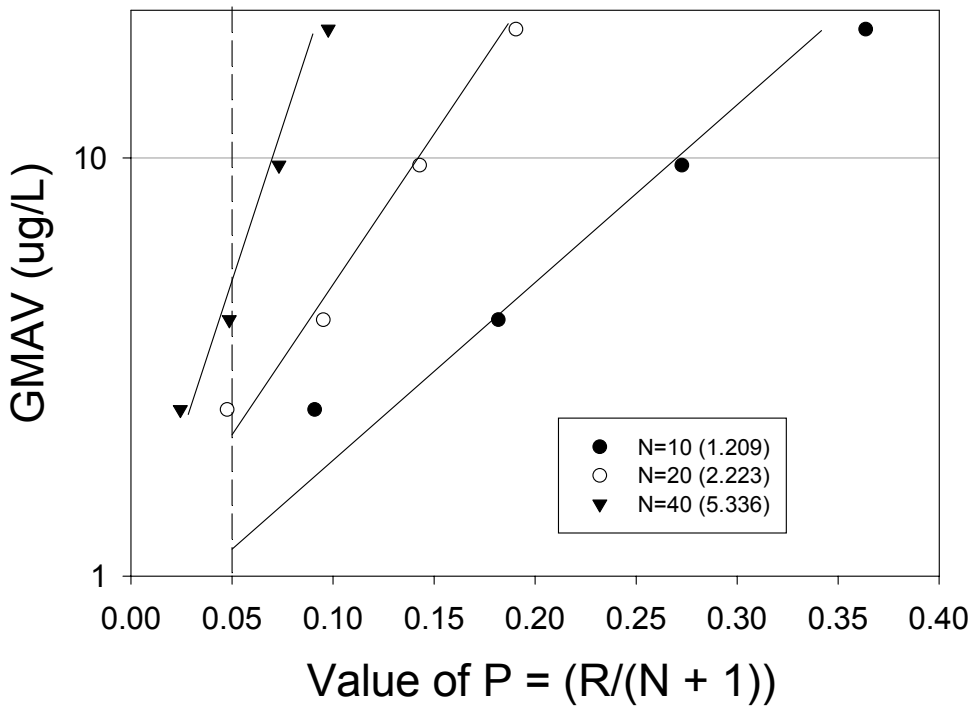



**FIGURE C-1. AN ILLUSTRATION OF THE EFFECT OF NUMBER OF GMAVS (N = 10, 20, OR 40) ON THE VALUES OF P FOR THE FOUR LOWEST GMAVS.**

Effect of GMAV Range

The hypothetical baseline data included GMAVs that ranged from 2.5 to 20.3 $\mu$g/L. The FAV calculation was shown to be influenced by the number of GMAVs through the effect of N on the range of P values (Figure C-1). The FAV is also sensitive to the range among the four lowest GMAVs. If the four lowest GMAVs were 2.5, 2.8, 3.1, and 3.9 $\mu$g/L the resultant FAVs would be 2.139, 2.422, and 2.897 $\mu$g/L for N of 10, 20, and 40, respectively (Figure C-2). Minimizing the range among the four lowest GMAVs decreases the uncertainty of the 5$^{th}$ percentile GMAV estimate.

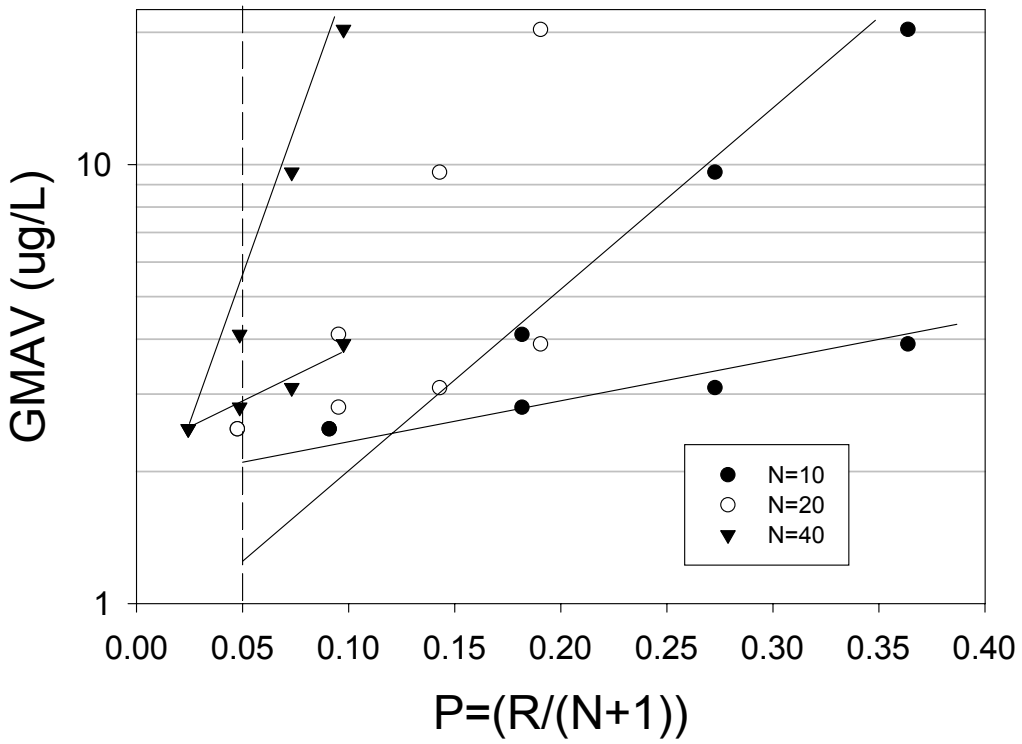



**FIGURE C-2. AN ILLUSTRATION OF THE EFFECT OF RANGE, OR VARIATION, AMONG THE FOUR LOWEST GMAVS ON THE FAV ESTIMATE.**

Lines have been excluded for the case of N=20 in order to simplify the graph. Note that each dataset has a common lowest GMAV of 2.5 $\mu$g/L. The actual FAVs as computed by the USEPA procedure were 1.209, 2.223, and 5.336 for the baseline data and N of 10, 20, and 40, respectively. The actual FAVs for the narrower range of GMAVs were 2.139, 2.422, and 2.897 for N of 10, 20, and 40, respectively.

Recalculation Procedure for FAVs

The recalculation procedure involves the addition of new data and the deletion of unsatisfactory data (both with USEPA approval), and, if desired, the deletion from the national dataset of nonresident and nonsurrogate species according to USEPA guidelines. Any net increase or decrease in the number of GMAVs as a result of the addition or deletion of data from the criteria dataset, without changing the four lowest GMAVs, will alter the FAV in a predictable direction (Figure C-3). The influence of changing N is greater when there is a large range represented by the four lowest GMAVs as compared to when there is a small range (Figure C-2).

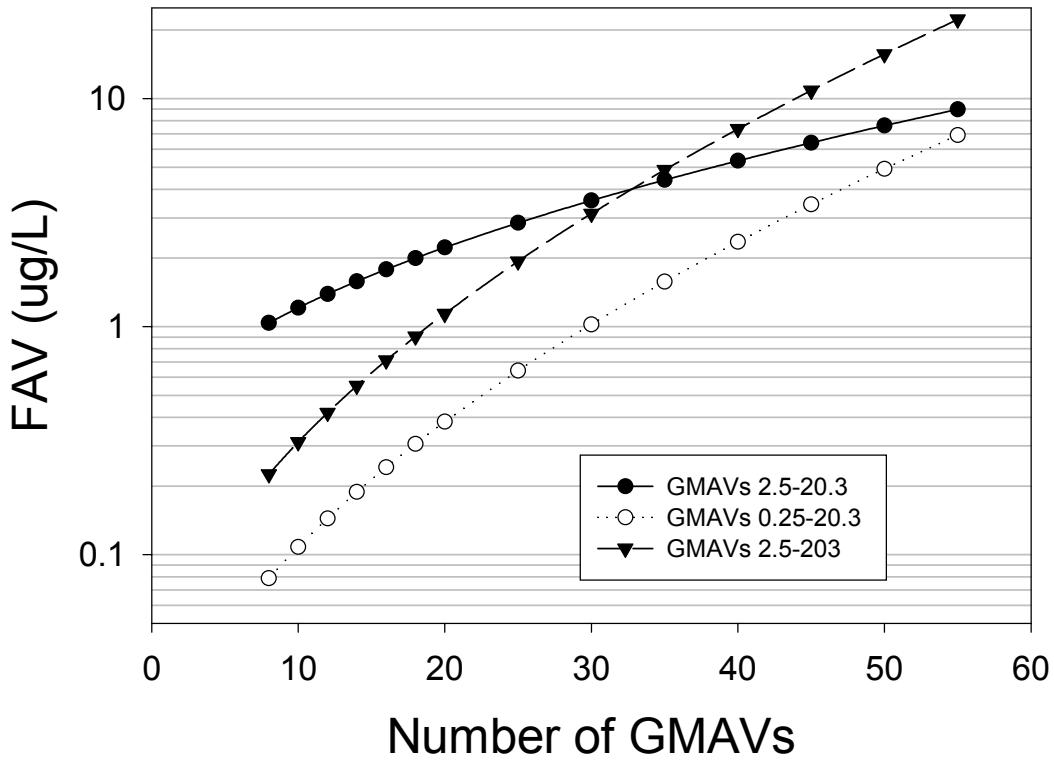



**FIGURE C-3. ILLUSTRATION OF THE TREND IN FAV AS THE NUMBER OF GMAVS INCREASES FROM 8 TO 55.**

The magnitude of the trend is dependent upon the range or variability among the four lowest GMAVs (e.g., 2.5-20.3; 0.25-20.3; 2.5-203).

Recalculation with only resident species and surrogate species can provide a taxonomically more appropriate SSO, but the procedure can have a multitude of outcomes with respect to the resultant FAV. In addition to changes in the number of GMAVs (usually resulting in a decrease due to deletion of nonresident and nonsurrogate species) there is also the likelihood of changes in the four lowest GMAVs. The following three examples indicate the complexity of these changes.

Case 1. Starting with the hypothetical baseline data (see the first paragraph in FAV Calculation Examples), reduce the total GMAVs by 20 percent and add a resident species that is more sensitive than any in the national dataset. The four lowest GMAVs are now 1.0, 2.5, 4.1, and 9.6.

Case 2. Starting with the hypothetical baseline data (see the first paragraph in FAV Calculation Examples), reduce the total GMAVs by 20 percent and delete the most sensitive species as a nonresident, nonsurrogate species. The four lowest GMAVs are now 4.1, 9.6, 20.3, and 25.0.

Case 3. Starting with the hypothetical baseline data (see the first paragraph in FAV Calculation Examples), reduce the total GMAVs by 20 percent, add a resident species that is more sensitive than any in the national dataset, and delete the two most sensitive species as nonresident, nonsurrogate species. The four lowest GMAVs are now 1.0, 9.6, 20.3, and 25.0.

**TABLE C-2. COMPARISON OF THE FAV VALUES THAT RESULT FROM THE BASELINE DATASET AND THE THREE CASES ILLUSTRATING POTENTIAL OUTCOMES OF THE RECALCULATION PROCEDURE.**

| | **FAV Values** | | |
|---|---|---|---|
| | **Number of GMAVs in Baseline Dataset** | | |
| | **N = 10** | **N = 20** | **N = 40** |
| **Baseline** | 1.209 | 2.223 | 5.336 |
| **Case 1** | 0.4704 | 0.8166 | 1.8107 |
| **Case 2** | 2.2843 | 3.6860 | 7.3548 |
| **Case 3** | 0.4312 | 1.0188 | 3.5252 |

In Case 1, the FAVs are lower than the baseline for two reasons: the 20 percent lower Ns, and the addition of the new most sensitive species. Note that for the cases where the initial Ns were 20 and 40, the FAV would have to be lowered to 0.5 if the newly added most-sensitive species was recreationally, commercially, or ecological important, i.e., the FAV = 1.0 ÷ 2. The same outcome would have resulted from simply adding the new "important" species data to the national dataset.

In Case 2, the FAVs are higher than the baseline, despite the 20 percent lower Ns, due to the deletion of the one most-sensitive species from the national dataset.

In Case 3, the FAVs are lower than the baseline because of the 20 percent lower Ns and the addition of the new most-sensitive species. Note that with a low initial N of 10, the FAV is lower in case 3 than in case 1, despite the loss of two of the most-sensitive species from the database in case 3; this is a result of the greater range of the GMAVs for the remaining four most-sensitive species.

In conclusion, it is important to understand that there are several factors that can influence the FAV resulting from the recalculation procedure.

- The number of GMAVs in the initial and final datasets;
- The range or variation among the four most sensitive GMAVs in the initial and final datasets;
- Changes in the absolute values of the GMAVs for the four most sensitive species in the initial and final datasets.

# APPENDIX D

# WATER-EFFECT RATIO DETERMINATION FOR HEXAVALENT CHROMIUM

## SUMMARY

The WER procedure was selected to develop a site-specific CMC for hexavalent chromium for the effluent's receiving stream, Little Hollow Run. WER procedures followed those described in USEPA's "Interim Guidance on Determination and Use of Water-Effect Ratios for Metals" (1994). The *Daphnia magna* 48-hour static acute toxicity test was selected as the primary toxicity test for use in three seasonal WER determinations (sample dates: May 9, July 17, and September 11, 1995). The fathead minnow 48-hour static acute toxicity test was selected as the secondary toxicity test for use in one of the WER determinations (sample date: July 17, 1995). Site water used in all three WER determinations was undiluted effluent since the receiving stream originates at the discharge point of the outfall. Soft Reconstituted Water (SRW) and Moderately Hard Reconstituted Water (MHW) were used as the laboratory water for the primary and secondary toxicity tests, respectively. To assess the possible effects of hardness and pH, a MHW test was conducted with each primary toxicity test WER determination.

The primary toxicity test WERs determined from the samples collected on May 9 (spring), July 17 (summer) and September 11 (fall), 1995 were 7.92, > 24.15, and 7.29, respectively. The reason for the higher WER for the summer WER determination was due to the combined effect of a lower SRW $LC_{50}$ and a higher site water $LC_{50}$. The lower summer SRW $LC_{50}$ was apparently due to natural test variation, whereas the higher summer site water $LC_{50}$ may have been due to a change in the character of the site water such as higher pH (summer site water pH, 8.1; spring and fall site water pH, 7.5). The secondary toxicity test WER determination was considerably lower (0.88) than the primary toxicity test WERs. The lower WER for the secondary toxicity test was a validation of the primary toxicity test WERs in that less sensitive toxicity tests are expected to produce a lower WER than a more sensitive toxicity test (*D. magna* is approximately 1000X more sensitive to hexavalent chromium than fathead minnows). The final WER for hexavalent chromium determined from this study is 7.29 (lowest of the three primary toxicity test WERs) and the corresponding site-specific CMC for Little Hollow Run is 109.4 µg/L (15 µg/L hexavalent chromium x 7.29).

## METHODS

### Water-Effect Ratio

#### *Site Water*

Site water consisted solely of undiluted effluent collected from the power plant's outfall. 24-hour composite samples of the effluent discharge were collected on May 9, July 17 and September 11, 1995 for the three different WER determinations. The samples were collected in polyethylene cubitainers, which upon collection were placed in a cooler containing wet ice. The samples were transported to the testing laboratory on the day the samples were collected. Upon receipt, the samples were logged in, given an identification number, measured for temperature, pH, dissolved oxygen, specific conductivity, hardness and alkalinity, filtered through a 95 µm nylon screen (to remove unwanted organisms), and placed in a 4EC refrigerator. Copies of the chain-of-custody form for the effluent sample (including samples submitted for chemical analysis) and the effluent characterization form containing the initial water quality measurements are presented in Appendix A (not included in this case study).

***Laboratory Water***

SRW and MHW were used as dilution water for the laboratory water tests. SRW and MHW were prepared using a Standard Operating Procedure (SOP) which is based on instructions cited in Weber et al. (1991). Base water used in the preparation of the reconstituted waters was deionized water from a Millipore Milli-Q™ Plus water system. Reagent grade salts were added in the appropriate amounts to deionized water and mixed at room temperature.

A summary of the water quality characteristics of the effluent sample and the two reconstituted waters for the fall sample date are given in Table 1.

***Stock and Test Solution Preparation***

Reagent grade hexavalent chromium obtained from Aldrich Chemical Company, Inc. on December 1, 1994 was used to make the stock and test solutions in all three WER determinations. The day before the WER tests were initiated, a 250 mg/L stock solution was prepared by dissolving 70.7 mg hexavalent chromium in 100 ml Millipore (deionized) water. Test solutions (the solution to which test organisms were exposed) were prepared the day the WER tests were initiated in the following manner:

- An appropriate volume of stock solution was added to a measured volume of dilution water and mixed.
- The spiked test solution was allowed to equilibrate for three hours.
- The spiked test solution was serially diluted with unspiked dilution water using a 0.7X dilution factor.
- The diluted test solutions were allowed to equilibrate approximately one hour before initiating the tests.

A detailed description of the stock and test solution preparation is given in the laboratory information sheet in Appendix C (not included in this case study).

***WER Toxicity Test Procedures***

All procedures followed the study-specific SOP for conducting a 48-hr *D. magna* static acute toxicity test as presented in the Study Plan for this project. A summary of the test conditions were as follows:

| | |
|---|---|
| Test Chamber | 250 ml Glass Beaker |
| Depth of Solution | 40 mm |
| Volume of Solution | 125 ml |
| # of Organisms/Chamber | 5 |
| Lighting | 16:8 light dark photoperiod; 10-20 $\mu E/m^2/s$ |
| Test Initiation | Date and Time |
| Test Termination | Date and Time |

In an attempt to eliminate the occurrence of "floating" organisms, 200 µm nitex screens were inserted into each *D. magna* test beaker using a coiled polyethylene ring to maintain the screen immediately below the water's surface.

**Test Organisms**

Stock cultures of *D. magna* used in the WER were originally obtained from Aquatic BioSystems, Inc. in November 1994 (see Organism History, Appendix D – not included in this case study). *D. magna* were cultured at the testing laboratory in MHW and a natural surface water in environmental chambers under controlled conditions (temperature 20E ± 2EC; photoperiod 16 h light and 8 h dark). *D. magna* were cultured in 1 L glass beakers containing approximately 800 ml of MHW or 0.45 µm filtered Boardman river water. Daily, each beaker received 5 ml of a yeast/trout food/Cerophyl® (YTC) food suspension (see Weber et al., 1991; EPA/600/4-90/027 for procedures for preparing the food suspension) and 0.8 ml of $2.3 \times 10^8$ cells/ml of the green alga, *Selenastrum capricornutum*. Culture water in each beaker was changed a minimum of three times each week. On the days the culture water was not changed, all young were removed from each culture beaker. Survival and reproduction of culture animals and general water chemistry was measured (dissolved oxygen, pH, temperature, and specific conductivity) and recorded each time culture water was changed. Test animals were obtained from cultures where survival of culture animals was 100 percent and reproduction was ∃ 3.0 young per female per reproductive day. Twenty-four hours before the start of a test, all young were removed from the culture chambers to ensure that only daphnids less than 24-hours old would be available to start the test. Copies of the culture records for the acclimation cultures used in the fall WER are given in Appendix D (not included in this case study).

Ten organisms randomly selected from the MHW control beakers at the end of the fall sample test were measured using a Wild dissecting microscope equipped with an ocular micrometer. The average length (tip of head to base of spine) of the ten daphnids was 1.08 mm (range, 0.96 to 1.20 mm). Verification that the test organisms used in all WER tests were *D. magna* was made by processing representative individuals from the *D. magna* culture through the dichotomous key in Edmondson (1959).

**Measurements**

General water quality conditions and hexavalent chromium were measured in the effluent samples and in the test solutions of the 48-hour WER toxicity tests. The type of chemical measurements made in the effluent samples and toxicity test solutions and the method used to perform the measurement are presented in Table 2.

## RESULTS

**Water-Effect Ratio Tests**

***Primary Toxicity Test***

The average and range of pH, dissolved oxygen, temperature, and specific conductivity in the test solutions of the primary WER toxicity tests are given in Table 3. The hexavalent chromium and *D. magna* 48-hour survival measurements for the site water, SRW and MHW toxicity tests are given in Tables 4, 5, and 6, respectively. The hexavalent chromium concentrations measured at test initiation (Day 0) and at test termination (Day 2) were not appreciably different from each other and were similar to the target nominal concentrations. The Spearman-Karber method was used to calculate $LC_{50}$ values for each test using both the nominal and the measured hexavalent chromium concentrations. The average of the hexavalent chromium concentrations measured at Day 0 and Day 2 were used for the measured concentrations in the $LC_{50}$ calculation. The $LC_{50}$ values for the site water, SRW and MHW tests using the average measured hexavalent chromium concentrations were 173.37, 23.77, and 49.06 µg/L, respectively. The $LC_{50}$ value for the SRW test (23.77 µg/L) was very similar to the two hexavalent chromium $LC_{50}$ values for *D. magna* (24.2 and 22 µg/L) reported in the ambient Water Quality Criteria Document for hexavalent chromium (EPA 440/5-84-999) which were conducted at a similar hardness (45 mg/L as $CaCO_3$). Copies of the data sheets containing the individual physical, chemical and survival measurements, and printouts of the statistical analyses are given in Appendix E (not included in this case study).

***Calculation of Water-Effect Ratio***

A WER of 7.29 was calculated for the fall sample using the measured $LC_{50}$ values for the site water and SRW tests (Table 4).

## DETERMINATION OF THE FWER

**Summary of Three WER Determinations**

The $LC_{50}$ values for the primary and secondary species for each water type and the respective WERs for the spring, summer, and fall samples are summarized on Table 7. The results of the May 9, 1995 (spring) and September 11, 1995 (fall) samples were very similar. The two samples had very similar $LC_{50}$ values for all three water types and therefore had very similar WERs (spring, 7.92; fall, 7.29). The results of the July 17, 1995 (summer) sample were somewhat different than the spring and fall samples in that the summer sample WER of > 24.15 was considerably higher than the other two WERs. The relatively high summer WER was due to the SRW $LC_{50}$ for the summer WER being lower than the spring and fall WER determinations and the site water $LC_{50}$ being higher than the spring and fall WER determinations (Table 7). The reason for the lower $LC_{50}$ for the summer SRW test seems to be attributable to natural test variation. Factors such as unhealthy test organisms, differing water quality characteristics, and an error in

the dosing of hexavalent chromium do not appear to be the cause of the lower SRW $LC_{50}$ value for the following respective reasons:

1) the $LC_{50}$ value for the summer MHW test was very similar to the spring and fall tests, and the reference toxicant $LC_{50}$ value for July was within specifications,

2) the alkalinity, hardness, conductivity, and pH of the summer SRW was very similar to the spring and fall SRWs, and

3) the measured hexavalent chromium values are very similar to the target concentrations.

The reason the site water $LC_{50}$ value for the summer sample is higher than the spring and fall samples may be due to natural test variation and/or differences in the water quality characteristics of the effluent samples. The measured water quality parameters represent only a portion of all constituents in the treated fly ash effluent; other unmeasured parameters may have contributed to the differential toxicity response between seasonal tests. The water quality characteristics of the three samples (fall sample, Table 1; spring and summer samples, Appendix F – not included in this case study) are different, but the only parameter that sets the summer sample apart from both the spring and fall sample is pH which is higher in the summer sample (summer sample pH, 8.1; spring sample pH, 7.5; fall sample pH, 7.5). Call *et al*. (1981) found that hexavalent chromium was less toxic to *D. magna* in water with a higher pH which is similar to that observed in the summer test. A review of the mortality data for the three tests reveals a very similar concentration-response through the 210 µg/L nominal test concentration for all three tests, but a change in the 300 µg/L nominal test concentration was noted, in that the summer test had 55 percent survival and the other two tests had 0 percent survival. In summation, the difference in the results of the summer site water test may be due to pH, a water quality characteristic not measured or a combination of water quality characteristics, or, as in the SRW test, natural test variation.

**Quality Assurance Criteria**

***Acceptability of Laboratory Dilution Water***

As stated in the WER guidance document (EPA-823-B-94-001), two sensitive tests using the laboratory dilution water must be compared to the results obtained in another laboratory using similar water. The following table presents the three sensitive tests conducted with *D. magna* in SRW during this study and the results of two different tests obtained from the "Ambient Water Quality Criteria for Hexavalent Chromium" document (USEPA, 1985) which used a similar dilution water. Two tests, spring and fall, are within a factor 1.5X of the comparison tests which fall within the recommended criterion for lab water acceptability.

| Test Reference | $LC_{50}$, µg/L | Hardness, mg/L as $CaCO_3$ | Alkalinity, mg/L as $CaCO_3$ | pH, S.U. |
|---|---|---|---|---|
| SRW - Spring Sample | 24.84 | 44 | 34 | 7.8 |
| SRW - Summer Sample | < 13.5 | 40 | 32 | 7.8 |
| SRW - Fall Sample | 23.77 | 40 | 28 | 7.6 |
| Mount, 1982 | 24.2 | 45 | not known | not known |
| Mount and Norberg, 1984 | 22 | 45 | 43-45 | 7.2-7.4 |

***Secondary Toxicity Test***

A fathead minnow 48-hour static acute toxicity test was conducted on MHW and site water collected July 17, 1995 (summer sample). This test represented the required secondary toxicity test. The $LC_{50}$ values for hexavalent chromium in the MHW and site water were 78.71 and 69.36 mg/L, respectively, which are slightly higher than the 31 $LC_{50}$ values (mean = 41.6 mg/L; range, 17.6-66 mg/L) reported for fathead minnows in the "Ambient Water Quality Criteria for Hexavalent Chromium" document (USEPA, 1985). The WER for the secondary test (0.88) was considerably lower than the *D. magna* WERs. As stated in the WER guidance, a less sensitive test will probably give a smaller WER than a more sensitive test. The reason the more sensitive tests will result in a higher WER can be explained using a simplified example, in which the site water contains a complexing agent which renders the hexavalent chromium nontoxic. EXAMPLE: The concentration of a complexing agent in the site water is 500 µg/L and has the ability to bind 100 µg/L of the hexavalent chromium. For the *D. magna* toxicity test which has an endpoint in laboratory water of approximately 20 µg/L, it will require at least 120 µg/L of metal in the site water (100 µg/L will be bound to complexing agent) to produce the endpoint concentration of 20 µg/L, resulting in a WER of approximately 6. For the fathead minnow 48-hour acute toxicity test, which has an endpoint in laboratory water of approximately 60,000 µg/L, it will require at least 60,100 µg/L of hexavalent chromium in the site water (100 µg/L bound to the complexing agent) to produce the endpoint concentration of 60,000 µg/L, resulting in a WER of approximately 1. The difference in the WERs between the primary and secondary tests in the present study is a validation of the *D. magna* WERs because a low to negligible WER was anticipated for fathead minnows given their relative tolerance to hexavalent chromium (approximately 1000 times less sensitive than *D. magna*).

**Final Water-Effect Ratio**

The site water for all three WER determinations was undiluted effluent (no upstream receiving water), and therefore the WERs determined for each sample are the same as highest WER (hWER). The USEPA guidance states that when two or more Type 1 WERs are determined (total number in this study is three), and when less than nineteen percent of all WERs are Type 2 WERs (total number of Type 2 WERs in this study is zero), the FWER is the lowest Type 1 WER or the lowest hWER. Based on this guidance the FWER for this site determined from this study is 7.29. Thus, the corresponding site-specific water quality criterion modification for Little Hollow Run would be 15 µg/L $Cr\ 6^{+}$ x 7.29 = 109.4 µg/L.

## KEY PERSONNEL

**Principal Investigator for WER Study: Dennis McIntyre, GLEC**

**Laboratory Coordinator for WER Study: Molly Giere, GLEC**

**Project Quality Assurance Officer for WER Study: Greg Smith, GLEC**

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**TABLE 1. WATER QUALITY CHARACTERISTICS OF RECONSTITUTED AND SITE WATER USED IN THE FALL WER DETERMINATION**

| Water Quality Characteristic | Composite Effluent Sample | SRW Batch No. 9 | MHW Batch No. 82 |
|---|---|---|---|
| pH, S.U. | 7.5 | 7.6 | 7.9 |
| Dissolved Oxygen, mg/L | 9.5 | 8.6 | 8.7 |
| Specific Conductivity, µmhos/cm | 1072 | 146 | 271 |
| Alkalinity, mg/L as $CaCO_3$ | 28 | 28 | 60 |
| Hardness, mg/L as $CaCO_3$ | 416 | 40 | 84 |
| TOC, mg/L | 1 | not meas. | not meas. |
| TDS, mg/L | 884 | not meas. | not meas. |
| Hexavalent chromium, µg/L | 4.5 | < 3 | < 3 |

**TABLE 2. SAMPLING SCHEDULE AND METHOD FOR CHEMICAL MEASUREMENTS**

| Parameter | Sample Type(s) | Sampling Schedule | Method |
|---|---|---|---|
| pH | Effluent Composite | Sample Receipt | Electrometric, EPA 150.1 |
| | WER Tests | Test initiation and test termination | |
| Specific Conductance | Effluent Composite | Sample Receipt | EPA 120.1 |
| | WER Tests | Test initiation | |
| Dissolved Oxygen | Effluent Composite | Test initiation | Membrane Electrode, EPA 360.1 |
| | WER Tests | Test initiation and test termination | |
| Hardness | Effluent Composite | Sample Receipt | Titrimetric, EPA 130.2 |
| Alkalinity | Effluent Composite | Sample Receipt | Titrimetric pH 4.5, EPA 310.2 |
| Hexavalent chromium | Effluent Composite | Test initiation | EPA 218.4 |
| | WER Tests | Test initiation and test termination | |
| TOC | Effluent Composite | Test initiation | EPA 415.1 |
| TDS | Effluent Composite | Test initiation | EPA 160.1 |

**TABLE 3. AVERAGE AND RANGE OF WATER QUALITY CHEMICAL MEASUREMENTS IN *Daphnia magna* TEST SOLUTIONS OF WATER-EFFECT RATIO ACUTE TOXICITY TESTS CONDUCTED SEPTEMBER 12-14, 1995**

| Parameter | Site Water Test | | SRW Test | | MHW Test | |
|---|---|---|---|---|---|---|
| | Average | Range | Average | Range | Average | Range |
| PH, S.U. | 7.6 | 7.5-7.9 | 7.6 | 7.5-7.8 | 8.0 | 7.8-8.1 |
| Dissolved Oxygen, mg/L (% Saturation) | 8.9 (101) | 8.2-9.5 (93-108) | 8.6 (98) | 8.5-8.9 (97-101) | 8.8 (100) | 8.5-9.0 (97-102) |
| Temperature, EC | 19.8 | 19.4-20.1 | 19.9 | 19.4-20.1 | 19.9 | 19.7-20.1 |
| Specific Conductivity, µmhos/cm | 1102 | 1072-1123 | 145 | 144-146 | 276 | 255-286 |

**TABLE 4. RESULTS OF *Daphnia magna* 48-HOUR ACUTE TOXICITY TEST USING HEXAVALENT CHROMIUM ADDED TO SITE WATER (UNDILUTED EFFLUENT)**

| Results of WER Determination<br>Test Dates: September 12 - 14, 1995<br>SITE WATER | | | |
|---|---|---|---|
| Nominal Hexavalent chromium Concentration (ug/L) | Day 0 Measured Hexavalent chromium Concentration (ug/L) | Day 2 Measured Hexavalent chromium Concentration (ug/L) | Survival No. Alive (out of 20) |
| Control | 5 | 4 | 20 |
| 72 | 67 | 67 | 20 |
| 103 | 93 | 91 | 20 |
| 147 | 130 | 129 | 19 |
| 210 | 195 | 201 | 5 |
| 300 | 280 | 290 | 0 |
| 428 | 413 | 410 | 0 |
| 612 | 575 | 600 | 0 |

Calculations Based on Nominal Concentrations

$LC_{50}$ = 188.69 µg/L
95 percent confidence limits, 174.66 - 203.86 µg/L

WER = 188.69 µg/L/25.22 µg/L = 7.48

Calculations Based on Measured Concentrations (avg of day 0 and day 2 values)

$LC_{50}$ = 173.37 µg/L
95 percent confidence limits, 159.24 - 188.75 µg/L

**WER = 173.37 µg/L/23.77 µg/L = 7.29**

**TABLE 5. RESULTS OF *Daphnia magna* 48-HOUR ACUTE TOXICITY TEST USING HEXAVALENT CHROMIUM ADDED TO SOFT RECONSTITUTED WATER**

| Results of WER Determination for Cardinal Plant<br>Test Dates: September 12 - 14, 1995<br>SOFT RECONSTITUTED WATER | | | |
|---|---|---|---|
| Nominal Hexavalent chromium Concentration (ug/L) | Day 0 Measured Hexavalent chromium Concentration (ug/L) | Day 2 Measured Hexavalent chromium Concentration (ug/L) | Survival No. Alive (out of 20) |
| Control | < 3 | < 3 | 20 |
| 6.6 | 6 | 5 | 20 |
| 9 | 9 | 8 | 20 |
| 13 | 12 | 11 | 20 |
| 19 | 18 | 18 | 19 |
| 27 | 25 | 25 | 7 |
| 39 | 38 | 39 | 0 |
| 56 | 52 | 54 | 0 |

Calculations Based on Nominal Concentrations

$LC_{50}$ = 25.22 µg/L
95 percent confidence limits, 23.18 - 27.45 µg/L

Calculations Based on Measured Concentrations (avg of day 0 and day 2 values)

$LC_{50}$ = 23.77 µg/L
95 percent confidence limits, 21.73 - 25.99 µg/L

**TABLE 6. RESULTS OF *Daphnia magna* 48-HOUR ACUTE TOXICITY TEST USING HEXAVALENT CHROMIUM ADDED TO MODERATELY HARD RECONSTITUTED WATER**

| Results of WER Determination for Cardinal Plant<br>Test Dates: September 12 - 14, 1995<br>MODERATELY HARD RECONSTITUTED WATER | | | |
|---|---|---|---|
| Nominal Hexavalent chromium Concentration (ug/L) | Day 0 Measured Hexavalent chromium Concentration (ug/L) | Day 2 Measured Hexavalent chromium Concentration (ug/L) | Survival No. Alive (out of 20) |
| Control | < 3 | < 3 | 20 |
| 25 | 21 | 17 | 20 |
| 36 | 32 | 34 | 19 |
| 51 | 46 | 45 | 13 |
| 74 | 68 | 70 | 1 |
| 105 | 94 | 94 | 0 |
| 150 | 136 | 145 | 0 |

Calculations Based on Nominal Concentrations

$LC_{50}$ = 54.17 µg/L
95 percent confidence limits, 49.44 - 59.35 µg/L

Calculations Based on Measured Concentrations (avg of day 0 and day 2 values)

$LC_{50}$ = 49.06 µg/L
95 percent confidence limits, 44.57 - 54.02 µg/L

**TABLE 7. WER SUMMARY**

| Species | Dilution Water | $LC_{50}$ Values | | |
|---|---|---|---|---|
| | | May 9, 1995 Sample | July 17, 1995 Sample | September 11, 1995 Sample |
| *D. magna* (primary test) | Soft Reconstituted Water | 24.84 µg/L | < 13.5 µg/L | 23.77 µg/L |
| | Moderately Hard Reconstituted Water | 53.83 µg/L | 57.10 µg/L | 49.06 µg/L |
| | Site Water | 196.66 µg/L | > 326 µg/L | 173.37 µg/L |
| | WER | 7.92 | > 24.15 | 7.29 |
| Fathead Minnow (secondary test) | Moderately Hard Reconstituted Water | not tested | 78.71 mg/L | not tested |
| | Site Water | | 69.36 mg/L | |
| | WER | | 0.88 | |

**Estimated Costs to Conduct a Water-effect Ratio Study**

As with any site-specific study, many variables and considerations factor into the level of effort and costs needed to perform a water-effect ratio (WER) study.

Many of the WERs conducted over the last ten years, however have been of a similar design for which a cost can be estimated. A WER study, based on the following set of conditions, may range from $25,000 to $30,000.

- It is a Method 1 study that is it is for a stream site in the vicinity of a plume.
- Static or static-renewal acute tests are used to derive a WER.
- Three separate WERs are conducted using the primary species and one using the secondary species.
- The chemical of interest is a metal.

Additional costs can be expected if:

- The WER study is for a large river or water body with multiple discharges and dynamic mixing situations.
- Static-renewal chronic tests or flow-through tests are used to derive a WER.
- More than three WERs are conducted.
- The costs of analysis are more expensive than routine metal analyses.

# APPENDIX E

***Section III from "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their uses" (Stephan et al. 1985)***

## MINIMUM DATA REQUIREMENTS FROM GUIDELINES FOR DERIVING NUMERICAL NATIONAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

Required Data

A. Certain data should be available to help ensure that each of the four major kinds of possible adverse effects receives adequate consideration. Results of acute and chronic toxicity tests with representative species of aquatic animals are necessary so that data available for tested species can be considered a useful indication of the sensitivities of appropriate untested species. Fewer data concerning toxicity to aquatic plants are required because procedures for conducting tests with plants and interpreting the results of such tests are not as well developed. Data concerning bioaccumulation by aquatic organism are only required if relevant data are available concerning the significance of residues in aquatic organisms.

B. To derive a criterion for freshwater aquatic organisms and their uses, the following should be available:
    1. Results of acceptable acute tests (see Section IV in Stephan et al. 1985) with at least one species of freshwater animal in at least eight different families such that all of the following are included:
        a. The family Salmonidae in the class Osteichthyes.
        b. One other family (preferable a commercially or recreationally important warmwater species) in the class Osteichthyes (e.g., bluegill, channel catfish, etc.).
        c. A third family in the phylum Chordata (e.g., fish, amphibian, etc.).
        d. A planktonic crustacean (e.g., cladoceran, copepod, etc.).
        e. A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.).
        f. An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.).
        g. A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.).
        h. A family in any order of insect or any phylum not already represented.

    2. ACRs (see Section VI) with species of aquatic animals in at least three different families provided that of the three species:
        --at least one is a fish.
        --at least one is an invertebrate.
        --at least one is an acutely sensitive freshwater species (the other two may be saltwater species).
    3. Results of at least one acceptable test with a freshwater alga or vascular plant (see Section VIII). If plants are among the aquatic organisms that

are most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.

4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available (see Section IX).

C. To derive a criterion for saltwater aquatic organisms and their uses, the following should be available:

1. Results of acceptable acute tests (see Section IV) with at least one species of saltwater animal in at least eight different families such that all of the following are included:
   a. Two families in the phylum Chordata.
   b. A family in a phylum other than Arthropoda or Chordata.
   c. Either the Mysidae or Penaeidae family.
   d. Three other families not in the phylum Chordata (may include Mysidae or Penaeidae, whichever was not used above).
   e. Any other family.
2. Acute-chronic ratios (see Section VI) with species of aquatic animals in at least three different families provided that of the three species:
   --at least one is a fish.
   --at least one is an invertebrate.
   --at least one is an acutely sensitive saltwater species (the other two may be freshwater species).
3. Results of at least one acceptable test with saltwater alga or vascular plant (see Section VIII). If plants are among the aquatic organisms most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.
4. At least one acceptable bioconcentration factor determined with an appropriate saltwater species, if a maximum permissible tissue concentration is available (see Section IX).

D. If all the required data are available, a numerical criterion can usually be derived, except in special cases. For example, derivation of a criterion might not be possible if the available ACR vary by more than a factor of ten with no apparent pattern. Also, if a criterion is to be related to a water quality characteristic (see Sections V and VII), more data will be necessary. Similarly, if all required data are not available, a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it might be possible to derive a criterion if the available data clearly indicate that the Final Residue Value should be much lower than either the FCV or the Final Plant Value.

E. Confidence in a criterion usually increases as the amount of available pertinent data increases. Thus, additional data are usually desirable.

# APPENDIX F

## RECALCULATION PROCEDURE - CASE EXAMPLE FOR ALUMINUM

### Power Station Ash Disposal Site

A state has granted an electric power company permission to construct and operate a site for the disposal of fly ash, bottom ash, construction debris, boiler refractory material, screenhouse debris, pyrites, insulation containing non-friable asbestos, wastewater lagoon sludges, coal pile sludges, spent filter materials, and spent resins, all of which are generated at a nearby power station. The disposal site, located approximately two miles from the power station, consists of an active area where waste material is currently being disposed, and a closed area containing waste material which has been covered with soil and planted for land reclamation.

The surface water runoff and leachate from the active area is collected in three sedimentation ponds before being discharged into Unnamed Tributary, approximately one-third mile upstream from its confluence with Bradley Run.

The active area of the disposal site contains three outfalls which are permitted as Outfalls 001, 002, and 003. The predominant characteristics which are common to each of these outfalls (and which are typical for fly ash effluents) are sulfates, TSS, aluminum, boron, iron, and manganese.

### Description of the Receiving Streams

Unnamed Tributary is a first order stream which originates on the southern edge of the active area of the ash disposal site. Prior to construction of the ash disposal site, Unnamed Tributary flowed through what is now part of the disposal site. During construction, the upper portion of Unnamed Tributary was diverted to an area south of the site.

Unnamed Tributary is the receiving stream for effluent discharged from Outfalls 003, 004, and 005. All three effluents enter Unnamed Tributary via a small stream (also unnamed) at a point on Unnamed Tributary approximately one-third mile upstream from its confluence with Bradley Run. Except during years of very low flow, the discharges from the outfalls are continuous throughout the year, and as a result the flow of Unnamed Tributary is continuous throughout the year downstream of the outfalls. Upstream of the outfalls, however, the flow is intermittent during low flow periods. Unnamed Tributary is a high gradient stream downstream of the outfalls, dropping approximately 350 feet in elevation over the one-third of a mile segment to the confluence with Bradley Run. The state assigned a 7Q10 design flow for Unnamed Tributary of 0 cfs.

### Existing Water Quality Standards

The effluent permit limits are based on state water quality standards for the categories: warm water fishery streams, small non-fishable streams, and wetlands. The state's chronic water quality criterion for aluminum is 0.087 mg/L.

The procedure used to recalculate the FAV for aluminum followed Appendix B of USEPA's *Interim Guidance for the Determination and Use of Water-Effect Ratios for Metals* (USEPA, 1994). The general approach to recalculating the FAV was to generate a list of species that have been found at the site, and to use this list in the process of deleting taxa from and/or adding taxa to the national data set. Appendix B of the USEPA guidance provides a straightforward step-wise process that describes the conditions necessary to add taxa to and/or delete taxa from the national data set. After the addition/deletion process is completed, the FAV is recalculated using the revised data set.

**Procedure**

The step-wise process of adding taxa to and/or deleting taxa from the national toxicity data set required the generation of a list of species that could occur at the site. This list was created using data gathered from ecological surveys conducted by a local university during 1988 through 1995 for Unnamed Tributary, Bradley Run, and a reference stream within the same watershed similar in size to Unnamed Tributary. The surveys primarily consisted of collecting and identifying organisms from the benthic community at multiple stations in each of the three streams in May and in August each year. A fish survey was conducted by the university in October 1995. The organisms recorded for Unnamed Tributary and the reference stream were included in this evaluation because they represent the organisms, which could occur at the site. Bradley Run and its fauna were included in the aluminum recalculation of the FAV because doing so would serve to protect the downstream uses of the receiving streams. The list of taxa that could occur at the site are given in Table 1.

The guidance for performing the Recalculation Procedure, as given in Appendix B of the *Interim Guidance on the Determination and Use of Water-Effect Ratios for Metals* (USEPA, 1994), requires that approved corrections and additions to the data set be included in the recalculation. Communication with USEPA headquarters at the time of this study indicated that there were no corrections or additions to the national data set for aluminum, and therefore the current national data set is consistent with Table 3 of USEPA's AWQC document for aluminum. Table 2 presents the taxa contained in the national data set for aluminum in phylogenetic order. The circled taxa in Table 2 represent the taxa that could occur at the site, and therefore are retained in the national data set. Each taxon was evaluated according to the deletion process procedures described in USEPA's guidance; and the results are as follows:

| ***Taxa*** | ***Deletion Status with Explanation*** |
|---|---|
| *Dugesia tierias* (planarian) | **Retain**. The genus *Phygocata* from the family, Planaridae, occurs in Fly Ash Run, Unnamed Tributary and Daugherty Run. |
| *Physa sp.* (snail) | **Retain**. The genus *Lymnaea* from the order, Pulmonata, occurs in Unnamed Tributary. |
| *Ceriodaphnia sp.* | **Delete**. The genus and family do not occur at the site. The order, Cladocera, occurs at the site but is represented in the data set by *Gammarus pseudolimnaeus*. |
| *Ceriodaphnia dubia* | **Delete**. The genus and family do not occur at the site. The order, Cladocera, occurs at the site but is represented in the data set by *Gammarus pseudolimnaeus*. |
| *Daphnia magna* | **Delete**. The genus and family do not occur at the site. The order, Cladocera, occurs at the site but is represented in the data set by *Gammarus pseudolimnaeus*. |
| *Gammarus pseudolimnaeus* (scud) | **Retain**. The family Gammaridae occurs in Fly Ash Run and the genus *Crangonyx* from the family Gammaridae occurs in Daugherty Run. |
| *Tanytarsus dissimilis* (midge) | **Retain**. The Tanytarsini tribe from the family Chironomidae occurs in Fly Ash Run, Unnamed Tributary, and Daugherty Run. |
| *Oncorhynchus tshawytsha* (chinook salmon) | **Delete**. The genus and family do not occur at the site. The order, Salmoniformes, occurs at the site but is represented in the data set by *Salvelinus fontinalis* (brook trout). |

| *Taxa* | *Deletion Status with Explanation* |
|---|---|
| *Oncorhynchus mykiss* (rainbow trout) | **Retain**. The occurrence of rainbow trout in Bradley Run is not known at this time. However, if rainbow trout occur in the river receiving Bradley Run either through a stocking program or through natural reproducing populations, they may periodically enter Bradley Run. |
| *Salvelinus fontinalis* (brook trout) | **Retain**. This species, *Salvelinus fontinalis*, occurs in Bradley Run. |
| *Pimephales promelas* (fathead minnow) | **Retain**. The species *Rhinichthys atratulus* (blacknose dace) and *R. cataractae* (longnose dace) from the family Cyprinidae occurs in Bradley Run. |
| *Ictalurus punctatus* (channel catfish) | **Retain**. The occurrence of channel catfish in Bradley Run has not been recorded. However, the habitat is suitable for some madtom and bullhead species, which are members of the family Ictaluridae and therefore could occur at the site. |
| *Lepomis cyanellus* (green sunfish) | **Retain**. The occurrence of the green sunfish in Bradley Run has not been recorded. However, the habitat is suitable for this common species and therefore it could occur at the site. |
| *Perca flavescens* (yellow perch) | **Retain**. The genus and family do not occur at the site. The order, Perciformes, does occur at the site as represented by *Cottus bairdi* (mottled sculpin), but this order is not represented in the data set. |

**Recalculation of Final Acute Value**

The computer program provided in USEPA's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (USEPA, 1985) was used to recalculate the FAV for aluminum using the revised data set. Four taxa, representing three GMAVs were deleted from the data set, namely, *Ceriodaphnia*, *Daphnia magna* and *Oncorhynchus tshawytsha*. The revised data set therefore had 11 GMAVs, and the four most sensitive GMAVs were used in the recalculation. A representation of the output from the computer program is as follows:

HOW MANY GMAVS ARE IN THE DATA SET?
? 11
WHAT ARE THE FOUR LOWEST GMAVS?
? 22600 (*Acroneuria sp*.)
? 22000 (*Gammarus pseudolimnaeus*)
? 10390 (rainbow trout)
? 3600 (brook trout)
FAV = 2533

The FAV for the entire national data set is 1,496 µg/L. The recalculated FAV is 2,533 µg/L, an increase in the FAV by a factor of 1.693. The chronic criterion was recalculated by multiplying the relative difference in the site-specific data set and the national data set (1.693) by the chronic criterion, 0.087 mg/L, to obtain 0.1473 mg/L.

**TABLE 1. List of Taxa Collected from Bradley Run, a Reference Stream and Unnamed Tributary 1988 through 1995**

| TAXA | Bradley Run | Reference Stream | Unnamed Tributary |
|---|---|---|---|
| Turbellaria: *Phygocata velata* | | x | |
| Turbellaria: *Phygocata c.f. morganii* | x | x | x |
| Nematoda | x | x | |
| Oligocheata | | x | |
| Oligochaeta: *Branchiobdella sp*. | x | x | |
| Oligochaeta: Aeolosomatidae | x | | |
| Oligochaeta: Haplotaxidae | x | | |
| Oligochaeta: Lumbriculidae | x | x | x |
| Oligochaeta: *Eclipidrilus sp.* | x | x | x |
| Oligochaeta: Tubificidae | x | x | x |
| Oligochaeta: Naididae | x | x | x |
| Gastropoda: *Lymnaea sp.* | | | x |
| Crustacea: *Asellus sp.* | | x | |
| Crustacea: *Asellus kenki* | | x | |
| Crustacea: *Caecidotea sp.* | x | | |
| Crustacea: Gammaridae | | x | |
| Crustacea: *Crangonyx sp.* | x | | |
| Crustacea: Copepoda | | x | |
| Crustacea: *Cambarus sp.* | x | x | x |
| Collembola | | x | x |
| Ephemeroptera: *Baetisca carolina* | x | | |
| Ephemeroptera: *Pseudocleon sp.* | x | x | x |
| Ephemeroptera: *Baetis sp.* | x | x | x |
| Ephemeroptera: *Baetis brunneicolor* | x | | |
| Ephemeroptera: *Baetis amplus* | x | | x |
| Ephemeroptera: *Seratella sp.* | x | | |
| Ephemeroptera: *Ephemerella subvaria* | x | | x |

| TAXA | Bradley Run | Reference Stream | Unnamed Tributary |
|---|---|---|---|
| Ephemeroptera: *Ephemerella dorothea* | x | | |
| Ephemeroptera: *Ephemerella invaria* | x | | |
| Ephemeroptera: *Ephemerella sp.* | x | | |
| Ephemeroptera: *Eurylophella sp.* | x | | |
| Ephemeroptera: *Cinygmula subequalis* | x | | x |
| Ephemeroptera: *Cinygmula sp.* | | | |
| Ephemeroptera: *Ephemera guttulata* | x | | |
| Ephemeroptera: *Ephemera simulans* | x | | |
| Ephemeroptera: *Ephemera sp.* | x | | |
| Ephemeroptera: *Isonychia sp.* | x | | |
| Ephemeroptera: Leptoplebiidae | x | x | |
| Ephemeroptera: *Paraleptophlebia sp.* | x | x | |
| Ephemeroptera: *Drunella sp.* | x | | |
| Ephemeroptera: *Drunella lata* | x | | |
| Ephemeroptera: *Epeorus pluralis* | x | | |
| Ephemeroptera: *Epeorus rubidius* | x | | |
| Ephemeroptera: *Epeorus sp.* | x | | x |
| Ephemeroptera: Heptageniidae | | x | x |
| Ephemeroptera: *Stenonema sp.* | x | | |
| Ephemeroptera: *Stenonema vicarium* | x | | |
| Ephemeroptera: *Stenonema femoratum* | x | | |
| Ephemeroptera: *Stenonema modestum* | | | x |
| Ephemeroptera: *Nixe sp*. | | x | |
| Plecoptera: Capniidae | x | x | x |
| Plecoptera: Chloroperlidae | x | | |
| Plecoptera: Leuctridae | x | x | |
| Plecoptera: *Leuctra sp*. | | x | |
| Plecoptera: *Paraleuctra sp*. | x | x | x |
| Plecoptera: Nemouridae | | x | x |

| TAXA | Bradley Run | Reference Stream | Unnamed Tributary |
|---|---|---|---|
| Plecoptera: *Amphinemoura nigritta* | | x | |
| Plecoptera: *Amphinemoura delosa* | x | x | x |
| Plecoptera: *Amphinemoura wui* | | x | x |
| Plecoptera: *Yugus arinus* | | | x |
| Plecoptera: *Pteronarcys proteus* | x | | x |
| Plecoptera: *Haloperla sp.* | x | | x |
| Plecoptera: *Talloperla sp*. | x | x | x |
| Plecoptera: *Acroneuria sp.* | x | | x |
| Plecoptera: *Acroneuria abnormis* | x | | x |
| Plecoptera: *Acroneuria caroliniensis* | x | | x |
| Plecoptera: *Beloneuria sp.* | | x | |
| Plecoptera: *Paragentina sp.* | x | | |
| Plecoptera: *Cultus sp.* | x | x | |
| Plecoptera: *Isoperla sp.* | x | x | |
| Hemiptera: *Microvelia sp.* | x | x | x |
| Hemiptera: *Rhagovelia sp.* | x | | |
| Hemiptera: *Trepobates sp.* | | x | |
| Megaloptera: *Nigronia fasciatus* | x | x | x |
| Megaloptera: *Nigronia serricornis* | x | | x |
| Megaloptera: *Cordulegaster sp.* | | x | |
| Megaloptera: *Sialis latreille* | x | x | x |
| Trichoptera: *Glossoma sp.* | x | | |
| Trichoptera: Hydropsychidae | x | x | x |
| Trichoptera: *Diplectrona modesta* | x | x | x |
| Trichoptera: *Neophylax mitchelli* | x | x | |
| Trichoptera: *Cheumatopsyche sp.* | x | | |
| Trichoptera: *Hydropsyche sp.* | x | | x |
| Trichoptera: *Hydropsyche betteni* | x | x | x |
| Trichoptera: *Symphitopsyche alhedra* | x | | |

| TAXA | Bradley Run | Reference Stream | Unnamed Tributary |
|---|---|---|---|
| Trichoptera: *Symphitopsyche bifida* | x | | |
| Trichoptera: *Symphitopsyche slossonae* | x | x | x |
| Trichoptera: *Symphitopsyche riola* | x | | x |
| Trichoptera: *Symphitopsyche etnieri* | x | | |
| Trichoptera: *Symphitopsyche morosa* | x | | |
| Trichoptera: *Symphitopsyche sparna* | x | | |
| Trichoptera: *Symphitopsyche sp.* | x | | x |
| Trichoptera: *Hydatophylax sp.* | | x | |
| Trichoptera: *Cernotina sp.* | | x | x |
| Trichoptera: *Micrasma sp.* | x | | |
| Trichoptera: *Cyrnellus sp.* | | | x |
| Trichoptera: *Chimera sp.* | x | | |
| Trichoptera: *Dolophiloides sp.* | x | x | x |
| Trichoptera: *Oligostomis sp.* | | x | |
| Trichoptera: *Goera sp.* | x | | x |
| Trichoptera: Rhyacophilidae | | x | |
| Trichoptera: *Rhyacophila nigrita* | | x | |
| Trichoptera: *Rhyacophila sp.* | x | x | x |
| Trichoptera: *Rhyacophila fuscula* | x | | x |
| Trichoptera: *Lype diversa* | x | | x |
| Trichoptera: *Psychomyia sp.* | x | | |
| Trichoptera: *Scatobiellia sp.* | x | | |
| Trichoptera: *Hydroptila sp.* | x | x | |
| Trichoptera: *Lepidostoma sp.* | x | x | x |
| Trichoptera: *Pycnopsyche divergens* | | x | |
| Trichoptera: *Pycnopsyche guttifer* | | x | |
| Trichoptera: *Pycnopsyche scabripennis* | | | x |
| Trichoptera: Philipotamidae | | x | |
| Trichoptera: *Wormaldia sp.* | | x | x |

| TAXA | Bradley Run | Reference Stream | Unnamed Tributary |
|---|---|---|---|
| Trichoptera: *Polycentropus sensu lato* | x | x | x |
| Trichoptera: *Polycentropus sp.* | x | x | |
| Coleoptera: *Ilybius sp.* | | x | |
| Coleoptera: *Liodessus/Uvarus sp.* | | x | |
| Coleoptera: *Agabus sp.* | | | x |
| Coleoptera: *Pyrrhalta sp.* | | x | |
| Coleoptera: Curculionidae | | x | |
| Coleoptera: *Hydrobiomorpha sp.* | | x | |
| Coleoptera: *Bagous sp.* | | | x |
| Coleoptera: *Helophorus sp.* | | x | |
| Coleoptera: *Helichus sp.* | x | | |
| Coleoptera: *Ouliminus sp.* | x | | |
| Coleoptera: *Promoresia sp.* | x | | |
| Coleoptera: *Optioservus sp.* | x | x | |
| Coleoptera: *Dubiraphia sp.* | x | | |
| Coleoptera: *Stenelmis sp.* | | x | |
| Coleoptera: Hydrophilidae | | x | x |
| Coleoptera: Melyridae | | x | |
| Coleoptera: *Ectopria sp.* | x | x | x |
| Coleoptera: *Psephenus herricki* | x | x | |
| Coleoptera: *Stenus sp.* | x | x | |
| Lepidoptera: *Vogita sp.* | | x | |
| Diptera: *Blephericera sp.* | x | | x |
| Diptera: *Probezzia sp.* | | | x |
| Diptera: Dolichopodidae | x | | |
| Diptera: Cecidomyiidae | | x | |
| Diptera: *Athrix sp.* | x | | |
| Diptera: *Bezzia sp.* group | x | x | x |
| Diptera: *Atrichopogon sp.* | x | x | |

| TAXA | Bradley Run | Reference Stream | Unnamed Tributary |
|---|---|---|---|
| Diptera: Chironomidae | x | x | x |
| Diptera: Chironominae | x | x | x |
| Diptera: Tanytarsini | x | x | x |
| Diptera: Orthocladinae | x | x | x |
| Diptera: Tanypodinae | x | x | x |
| Diptera: Empididae | | | x |
| Diptera: *Wiedemannia sp.* | | | x |
| Diptera: Simuliidae | x | x | |
| Diptera: *Prosimulium sp.* | x | | |
| Diptera: *Simulium sp.* | x | | |
| Diptera: *Simulium notiale* | | x | |
| Diptera: *Simulium tuberosum* group | x | x | x |
| Diptera: *Stratiomys sp.* | | | x |
| Diptera: *Chelifera sp.* | x | x | |
| Diptera: *Chrysops sp.* | x | x | x |
| Diptera: *Ormosia sp.* | x | x | x |
| Diptera: *Dicranota sp.* | x | x | x |
| Diptera: *Hemerodromia sp.* | x | x | x |
| Diptera: *Oreogeton sp.* | | x | |
| Diptera: *Antocha sp.* | | | x |
| Diptera: *Tipula sp.* | x | x | x |
| Diptera: *Tipula abdominalis* | | | x |
| Diptera: *Pedicia sp.* | | x | x |
| Diptera: *Hexatoma sp.* | x | x | x |
| Diptera: *Molophilus sp.* | x | x | x |
| Diptera: *Pseudolimnophila sp.* | x | x | x |
| Diptera: Sciomyzidae | | x | |
| Salmoniformes: *Salvelinus fontinalis* (Brook trout) | x | | |
| Cypriniformes: *Rhinichthys atratulus* (Blacknose dace) | x | | |

| TAXA | Bradley Run | Reference Stream | Unnamed Tributary |
|---|---|---|---|
| Cypriniformes: *Rhinichthys cataractae* (Longnose dace) | x | | |
| Perciformes: *Cottus bairdi* (Mottled sculpin) | x | | |

**TABLE 2. LIST OF TAXA IN THE NATIONAL DATA SET FOR ALUMINUM AND THEIR STATUS FOR THE RECALCULATION OF THE FINAL ACUTE VALUE**

| Phylum | Class | Order | Family | Genus | Species | Recalculation status | GMAV[a], µg/L |
|---|---|---|---|---|---|---|---|
| Platyhelminthes | Turbellaria | Tricladida | Planariidae | *Dugesia* | *D. tierias* | Retain | >23,000 |
| Mollusca | Gastropoda | Pulmonata | Physidae | *Physa* | | Retain | 30,600 |
| Arthropoda | Crustacea | Cladocera | Daphnidae | *Ceriodaphia* | | Delete | 2,640 |
| Arthropoda | Crustacea | Cladocera | Daphnidae | *Ceriodaphia* | *C. dubia* | Delete | |
| Arthropoda | Crustacea | Cladocera | Daphnidae | *Daphnia* | *D. magna* | Delete | 38,200 |
| Arthropoda | Crustacea | Amphipoda | Gammaridae | *Gammarus* | *G. pseudolimnaeus* | Retain | 22,000 |
| Arthropoda | Insecta | Plecoptera | Perlidae | *Acroneuria* | | Retain | >22,600 |
| Arthropoda | Insecta | Diptera | Chironomidae | *Tanytarsus* | *T. dissimilis* | Retain | >79,000 |
| Chordata | Osteichthyes | Salmoniformes | Salmonidae | *Oncorhynchus* | *O. tshawytscha* | Delete | >40,000 |
| Chordata | Osteichthyes | Salmoniformes | Salmonidae | *Oncorhynchus* | *O. mykiss* | Retain | 10,390 |
| Chordata | Osteichthyes | Salmoniformes | Salmonidae | *Salvelinus* | *S. fontinalis* | Retain | 3,600 |
| Chordata | Osteichthyes | Cypriniformes | Cyprinidae | *Pimephales* | *P. promelas* | Retain | 35,000 |
| Chordata | Osteichthyes | Siluriformes | Ictaluridae[b] | *Ictalurus* | *I. punctatus* | Retain | >47,900 |
| Chordata | Osteichthyes | Perciformes | Centrarchidae | *Lepomis* | *L. cyanellus*[c] | Retain | >50,000 |
| Chordata | Osteichthyes | Perciformes | Percidae | *Perca* | *P. flavescens* | Retain | >49,000 |

[a] Genus mean acute value.
[b] Family not recorded, but habitat is suitable for members of this family to occur at site.
[c] Species not recorded, but habitat is suitable for species to occur at site.

**Estimated Costs to Conduct a Recalculation Procedure Study**

The activities needed to perform the recalculation procedure consist of 1) a visit to the site to become familiar with the relevant characteristics; 2) preparation of a study plan; 3) data acquisition; 4) recalculation; 5) report preparation; and 6) follow-up interaction with stake-holders and regulatory agencies. The costs to perform all of the above steps with the exception of step 3, data acquisition, can range from $7,500 to over $10,000 depending on the complexity of the site and the amount of follow-up activities. The costs for data acquisition are dependent upon whether existing data are available for the site and reference condition for the site. If the appropriate data are available, the acquisition of data from the sources may only add a nominal amount to the total costs for the study. If data are not available, field studies will need to be conducted that survey multiple assemblages over different seasons at both the site of interest and at least one reference condition to adequately list the organisms that "occur at the site." The latter effort can add $10,000 to over $50,000 to the study, with the lower estimate for a smaller site consisting of wadeable streams and the higher end for larger sites containing water bodies requiring boats for collecting the organisms.

# APPENDIX G

# DEVELOPMENT OF SITE-SPECIFIC WATER QUALITY CRITERIA: A CASE STUDY USING THE RESIDENT SPECIES PROCEDURE

Here we describe the development of site-specific water quality criteria using USEPA's Resident Species procedure for copper in Blaine Creek, Kentucky (KCPo and AEPSC 1992, Dobbs *et al*. 1994) to identify critical steps in the creation of site-specific water quality criteria and discuss relevant issues that arise during the process.

## JUSTIFICATION

The operation permit for Big Sandy Power Plant limited maximum concentrations of total recoverable copper in the plant's effluent. Big Sandy is a coal-fired power plant managed by Kentucky Power Company (KPCo) and American Electric Power Service Corporation (AEPSC). Fly ash produced during coal combustion is sluiced to a 44 ha settling pond, where physical and chemical treatment of the ash slurry occurs. A tower discharge structure equipped with a skimmer allows overflow from the pond into a 190 m effluent ditch that discharges to Blaine Creek. Based on analysis of outfall data, KPCo determined that compliance with these limits was not possible. KPCo and AEPSC then requested permission from the Kentucky Department for Environmental Protection to develop a copper site-specific water quality criterion for a segment of Blaine Creek.

Site-specific criteria recognize that the physical, chemical, and biological conditions at a site can influence the toxicity of a substance to aquatic organisms. These conditions vary among sites, and may be significantly different from those in which national and statewide criteria were developed. A copper site-specific criterion was appropriate for Blaine Creek because the recommended national and statewide criterion protects sensitive species that are not present in this stream, and laboratory tests from which the recommended national and statewide criterion were computed exposed organisms to dissolved forms of these chemicals. Often, only small fractions of the total concentrations of copper in effluents from a power plant are soluble. Furthermore, analysis of biological data collected by KPCo for several years suggested that the fly ash pond discharge had no deleterious effects on aquatic life use in Blaine Creek (KPCo and AEPSC, 1992; Van Hassel et al., 1988). In fact, the presence of fly ash pond discharge actually maintained the aquatic life use during critical (low flow) conditions prior to flow regulation at Yatesville Dam. Approval and adoption of the proposed site-specific criterion would allow KPCo to continue to discharge treated fly ash without costly alternative technologies. Such technologies would require initial capital investments in the range of 2.6 to 31 million dollars, and would not provide measurable benefits to the Blaine Creek biota since water use classifications were being maintained without them.

## STUDY AREA

Blaine Creek is a fifth-order tributary to the Big Sandy River, located in eastern Kentucky. The Blaine Creek watershed covers an area of 686 $km^2$ and lies in the Western Allegheny Plateau ecoregion, which is characterized as having low to high hills, mixed mesophytic forest, and alfisol-type soils (Omernik, 1987). Blaine Creek receives treated fly ash water from KPCo Big Sandy power plant about 3.2 km upstream of the Big Sandy River confluence. The Big Sandy

power plant is coal-fired, and its two units have a combined generating capacity of 1,060 megawatts (MW). Fly ash produced during the coal combustion process is sluiced to a 44 hectares settling pond that discharges into Blaine Creek. At the time this study was undertaken, the discharge contributed <10 percent of the creek's flow. Historically, the discharge has comprised as much as 75 percent of creek flow during low flow conditions. In 1991, Blaine Creek was impounded by a U.S. Army Corps of Engineers dam located near Yatesville, Kentucky. Because of this flow regulation, the fly ash discharge would make up no more than 33 percent of total stream flow under worst-case conditions (stream low flow and effluent maximum design flow).

***Copper Speciation***

The fraction of dissolved copper in Blaine Creek water downstream of the fly ash pond discharge was used as an estimate of bioavailable copper. Analyses of total recoverable and dissolved copper were performed on samples from the discharge and Blaine Creek during 1990 and 1991. Total recoverable copper was determined using USEPA methods 220.2 or 200.7 (Kopp and Mckee, 1983). Dissolved copper was determined by filtration through a 0.45 $\mu$m filter at the time of collection, followed by acidification with $HNO_3$ (0.5 percent). Analysis of the preserved samples was similar to USEPA method 220.2, but without a digestion step. The ratio of dissolved to total recoverable copper was used to adjust the final criterion value in terms of bioavailable copper.

The geometric mean ratio of dissolved to total recoverable copper in the discharge from 1990 to 1991 was 0.77 and indicated that approximately three-quarters of the copper was in bioavailable form (Table 1). There was a distinct difference between the two years. During 1990, the geometric mean of the ratio was 0.67 for the discharge; in 1991, copper was almost completely in the dissolved form with the geometric mean ratio of 0.90. This difference in the relative amount of dissolved copper may be attributed to climate factors and decreasing suspended solid levels in the discharge. Very wet conditions prevailed in 1990 (causing substantial stormwater runoff into the fly ash pond), whereas drought conditions were evident in 1991.

**Table 1. COPPER SPECIATION STUDY OF BIG SANDY PLANT ASH POND DISCHARGE AND BLAINE CREEK SAMPLES DOWNSTREAM OF DISCHARGE (μG/L), 1990 AND 1991**

| | Outfall | | | Blaine Creek Below Outfall | | |
|---|---|---|---|---|---|---|
| ***Date*** | *Total* | *Dissolved* | *Ratio*[a] | *Total* | *Dissolved* | *Ratio*[a] |
| 7 Jul 90 | 23 | 14 | 0.61 | 4 | 3 | 0.75 |
| 17 Jul 90 | 23 | 18 | 0.78 | 6 | -[b] | - |
| 19 Jul 90 | 23 | 18 | 0.78 | 6 | -[b] | - |
| 24 Jul 90 | 12 | 11 | 0.92 | 5 | 3 | 0.60 |
| 31 Jul 90 | 19 | 12 | 0.63 | 5 | 4 | 0.80 |
| 7 Aug 90 | 13 | 14 | 1.0 | 4 | 3 | 0.75 |
| 14 Aug 90 | 23 | 3 | 0.13 | 6 | 5 | 0.83 |
| 21 Aug 90 | 8 | -[b] | - | 10 | 6 | 0.60 |
| 28 Aug 90 | 31 | 12 | 0.39 | 13 | 4 | 0.31 |
| 4 Sep 90 | 15 | 14 | 0.93 | 15 | 6 | 0.40 |
| 9 Sep 90 | -[b] | -[b] | - | 4 | 2 | 0.50 |
| 11 Sep 90 | 11 | 8 | 0.73 | 5 | 9 | 1.0 |
| 18 Sep 90 | 6 | 6 | 1.0 | 4 | -[b] | - |
| 24 Oct 91 | 4 | 3 | 0.75 | 2 | 1 | 0.5 |
| 29 Oct 91 | 6 | 6 | 1.0 | 3 | 3 | 1.0 |
| 5 Nov 91 | 20 | 20 | 1.0 | 7 | 5 | 0.71 |
| 12 Nov 91 | 24 | 25 | 1.0 | 8 | 7 | 0.88 |
| 18 Nov 91 | 24 | 27 | 1.0 | 5 | 5 | 1.0 |
| 25 Nov 91 | 19 | 21 | 1.0 | 7 | 5 | 0.71 |
| 5 Dec 91 | 24 | 23 | 0.93 | 5 | 1 | 0.20 |
| 9 Dec 91 | 28 | 30 | 1.0 | 10 | 2 | 0.20 |
| 16 Dec 91 | 42 | 25 | 060 | 4 | 2 | 0.50 |
| 23 Dec 91 | 43 | 35 | 0.81 | 4 | 2 | 0.50 |
| Geometric Mean | 17 | 14 | 0.77 | 6 | 3 | 0.58 |

The ratio is equal to the dissolved copper levels divided by the total recoverable value. All analyses based on split samples.

[a] For samples in which the dissolved copper was greater than total copper the ratio was set equal to one.

[b] Sample lost due to contamination or not taken.

For regulatory purposes, the samples of concern are from the downstream site after complete mixing of Blaine Creek and the discharge. The geometric mean dissolved-to-total-copper ratio for the 1990 samples ($n$ = 10) was 0.62: for the 1991 samples ($n$ = 10), it was 0.54. When the two studies were pooled, the geometric mean of the ratio for all samples ($n$ = 20) was 0.58. These

results suggest that a substantial fraction of the copper in Blaine Creek below the fly ash pond discharge was not in a highly bioavailable form. Considering the importance of copper speciation in regulating toxicity, application of criteria based on total recoverable copper levels may be over protective. Adequate protection to aquatic life in Blaine Creek would require adjustment of the national FAV with the geometric mean ratio of dissolved to total recoverable copper (from the fully mixed downstream location).

***Toxicity Tests – Materials and Methods***

Acute toxicity tests were performed on nine species that reside in Blaine Creek to determine 48-hours $LC_{50}$ values for copper. These organisms fulfilled all requirements of the national guideline procedures (Stephan et al., 1985). Integration of results was based on the USEPA's resident-species procedure (USEPA, 1983; Carlson et al., 1984).

Blaine Creek water was used as dilution water for all tests and was collected 10 km upstream of the discharge, near Fallsburg, Kentucky, in 19 L polycarbonate carboys and stored at room temperature for a period not exceeding 14 days before use. The water was filtered (1.6 $\mu$m) due to high levels of suspended solids during initial water collection trips. During the study, Blaine Creek was typified by highly variable flow due to stream terrain of the watershed, which causes frequent flooding with associated elevated levels of suspended solids. The decision to filter the water for all tests was made to provide dilution water with more consistent characteristics because of the effect that suspended solids have on copper bioavailability.

Species used for toxicity testing were selected on the basis of potential residency in the Blaine Creek watershed, fulfillment of the national guidelines selection criteria (Stephan et al., 1985), state requirements, and availability of the tests organisms of a suitable age. Residency of a species was based on long-term fish and macroinvertebrate biosurvey data from various stream sites (KPCo and AEPSC 1992, Van Hassel et al., 1988). The only organism that was originally sought but could not be found in sufficient quantity was the bluntnose minnow (*Pimephales notatus*). The fathead minnow (*P. promelas*) was used as a surrogate species. Nine different species were tested to fulfill the national guidelines requirement (Stephan et al., 1985) for eight species in selected families and the state of Kentucky's requirements for a species from a copper-sensitive family (Daphnidae).

The following organisms were obtained from either house cultures or commercial sources: *Daphnia pulex, P. promelas, Lepomis macrochirus, Physella* sp. and *Chronomus riparius*. The salamander *Eurycea bislineata*, crayfish *Orconcectes* sp., and mayflies *Stenonema* sp. and *Isonychia bicolor* were collected from locales where their relative population abundance and condition were known from prior collection experience. After collection and transportation of organisms to the lab in coolers that had been chilled to the collection temperature, the organisms were allowed to acclimate to Blaine Creek water for a minimum of 48 hours. Temperature acclimation of test organisms was not a major concern in this study because collection sites remained within 2° of 20°C over the course of the study. The period of acclimation was an acceptable balance between allowing the organisms to recover from the stress of the handling and the potential problem of reduced health after long-term holding.

Acute toxicity tests lasted 48 hours and used 1.6 $u$m filtered Blaine Creek water as the dilution water. All tests were conducted at 20°C with 16:8 light:dark photoperiod. Standard conditions were three replicate test containers containing 10 organisms each, with five concentrations and a control for each test. Method development before performing definitive tests consisted of a series

of screening tests with each species to optimize holding conditions and test parameters for nonstandard species. This design was essential for performing valid toxicity tests. Testing methods generally followed those outlined by Weber (1991) and Standard Methods for the Examination of Water and Wastewater (APHA et. al., 1985). The screening tests resulted in selection of optimal test concentrations, which led to narrower confidence intervals. Samples for total recoverable copper determination were taken at the beginning and end of the test and analyzed in accordance with USEPA method 2007 (Kopp and Mckee, 1983). Species-specific conditions are described below.

Testing of *D. pulex* and *P. promelas* followed closely the protocols described by Weber (1991), except three replicates (instead of two) of 10 organisms each were used and the test duration for both organisms was 48 hours. *Daphnia pulex* were cultured in filtered Baine Creek water for at least one month before testing. Neonates were < 24 hours hold at test initiation and were tested in 100 ml Pyrex® beakers containing 50 ml test solution.

Three-week-old *P. promelas* from in-house cultures were tested in 1 L glass beakers with 750 ml test solution. The minnows were reared in dechlorinated tap water (treated New River, VA water) and transferred to Blaine Creek water one week before testing. Juvenile *L. macrochirus* (35 mm length) were obtained from Kurtz Fish Hatchery (Elverson, PA) and were acclimated for one week in Blaine Creek water. The average wet weight at the time of testing was 0.51 gram per fish. Fish were tested in 17 L polycarbonate vessels containing 7 L test solution for 48 hour.

The two-lined salamander, *E. bislineara*, was collected from an unnamed tributary to Little Scary Creek (near Winfield, WV) and transported to the lab in coolers chilled at 20°C. Organisms were acclimated before use to Blaine Creek water for 2 days without feeding. Salamanders (average length 40 mm) were tested in covered 17 L polycarbonate vessels containing 6 L tests solution. An equal number of washed stones of similar size (10-20 cm maximum diameter) were placed in each container to provide refugia.

Crayfish (*Orconectes* sp.) were collected from Sinking Creek (Newport, VA) and transported in chilled coolers. Crayfish were acclimated for 48 hours in Blaine Creek water before testing. Low dissolved oxygen (DO) in screening tests necessitated gently aerating 5 L tests solution with Pasteur pipettes in 17 L polycarbonate vessels. Crayfish (30-40 mm in length) were used in the tests and were checked for exoskeleton condition before use.

Mayflies (sixth-to-eighth-instar *Stenonema* sp. and *I. bicolor*) were collected from Sinking Creek and acclimated for 48 hours to Blaine Creek water before use. Mayflies were tested in 2 L Nalgene® (Rochester, NY) containers washed cobble added to provide substrate. Current was provided by a stir bar positioned over a magnetic stir plate in the center of the container. Stir-bar speed was determined from trial and error before conducting the definitive tests.

*Physella* sp. (<10 mm diameter) were obtained from in-house cultures at Virginia Tech (Blacksburg, VA) and acclimated in Blaine Creek water for 48 hours before use. They were tested in loosely covered 350 ml glass culture dishes filled to within 1.5 cm of the top with test solution to prevent the snails from avoiding the toxicant. Snails were considered dead after 48 hours if no movement of the foot or antenna was evident in the test solution after being placed in control water for 5 minutes.

*Chironomus riparius* was obtained from in-house cultures at Virginia Tech and acclimated to Blaine Creek water for 48 hours. Second instar midges were tested in 350 ml glass culture dishes using 250 ml test solution and 10 ml of glass beads (150-300 $\mu$m) as inert substrate.

DO, pH, and temperature were monitored daily at all concentrations when a sufficient volume was available. Alkalinity and hardness were measured at the beginning and the end of the tests in the control and the highest level. Water samples (50 ml) were collected at the surface of the containers from all treatment levels at the beginning and end of the tests. The samples were preserved with 150 $\mu$l of 50 percent $HNO_3$ and shipped to a commercial lab for total recoverable copper analysis by USEPA method 200.7 (Kopp and Mckee, 1983).

The trimmed Spearman-Karber method (Hamilton et al.,1977) was used to calculate 48 hours median lethal concentrations and respective 95 percent confidence intervals. Total recoverable copper measurements taken at the beginnings of the tests were used for calculation of $LC_{50}$ values.

Integration of test results was conducted using USEPA's FAV equation (Stephan et al., 1985, Erickson and Stephan, 1988). The FAV equation is an extrapolation procedure that plots the log of the acute values against the cumulative probability of the relative sensitivity of the species in the database to the chemical. The cumulative probability of a species is determined by dividing the rank of each acute value by the total sample size in the database plus one. The FAV is the concentration that corresponds to a 0.5 cumulative probability level estimated by using the best-fit line through the four acute values closest to the 0.05 level. At the 0.05 level, 95 percent of the species in the statistical population that the database represents will be less sensitive, whereas 5 percent will be more sensitive. This procedure is designed to protect 95 percent of the species within the entire database. To obtain the CMC, the FAV is divided by two. For the purposes of this study, the criterion continuous concentration (CCC) was derived by dividing the site-specific FAV by the ACR of 2.823 from national criteria document (USEPA 1984).

***Toxicity Tests – Results and Discussions***

Based on the results of the nine definitive acute toxicity tests, *D. pulex* was the most sensitive taxon tested, with a $LC_{50}$ of 17 $\mu$g/L Cu (Table 2). This finding is consistent with the USEPA database (USEPA, 1984), which lists species in the family Daphnidae among the least tolerant taxa. The four most sensitive species in the Blaine Creek database were from a broad range of taxonomic groups and included *Physella* sp. ($LC_{50}$ 109 µg/L).

**TABLE 2. RESULTS OF ACUTE TOXICITY TESTS WITH COPPER USING DILUTION WATER FROM BLAINE CREEK.**

| Rank | Species | $LC_{50}$ (µg/L) | 95 percent Confidence limits |
|---|---|---|---|
| 1 | *Daphnia pulex* (water flea) | 37 | 35-38 |
| 2 | *Physella* sp. (snail) | 109 | 100-118 |
| 3 | *Isonychia bicolor* (mayfly) | 223 | 162-109 |
| 4 | *Pimephales promelas* (fathead minnow) | 283 | 242-334 |
| 5 | *Stenonema* sp. (mayfly) | 453 | 372-551 |
| 6 | *Eurycea bislineata* (salamander) | 1,120 | 872-1,450 |
| 7 | *Chironomus riparius* (midge) | 1,170 | 946-1,450 |
| 8 | *Orconectes* sp. (crayfish) | 2,370 | 1,830-3,070 |
| 9 | *Lepomis macrochirus* (bluegill sunfish) | 4,300 | 3,350-5,520 |

All tests were conducted at hardness values between 100 and 120 mg/L as $CaCO_3$.

To compare the $LC_{50}$ values from Blaine Creek database directly with those of similar species as reported in the national criteria document (USEPA,1984), values were normalized to a hardness of 50 mg/L as $CaCO_3$, based on the following equation (USEPA, 1984):

$$LC_{50} \text{ at hardness of } 50 = e^{Y}$$

$$Y = [\ln (LC_{50}) - 0.9422 (\ln (\text{test hardness}) - \ln (50)]$$

where: 0.9422 is the pooled slope between hardness and $LC_{50}$ values for all species in the USEPA database.

The two databases have a similar ranking of species sensitivity (Table 3). The hardness-adjusted $LC_{50}$ value for D. pulex (17.3 µg/L) in Blaine Creek water is lower than the species mean acute value (25.42 µg/L) reported by the USEPA. However, the GMAV for *Daphnia* (17.08 µg/L), used by USEPA to calculate the FAV, was almost identical to the previously recorded value. The response of 51.0 µg/L for the snail (*Physella* sp.) is quite similar to that of 39.33 µg/L for *Physa*, which is the most sensitive snail in the USEPA database. The acute values for both the fathead minnow (*P. promelas*) and the crayfish (*Orconectes* sp.) are in close agreement with the two databases. No comparisons were possible for the two mayflies and the salamander tested because the USEPA database does not contain any similar species.

**TABLE 3. COMPARISON OF THE BLAINE CREEK AND USEPA (USEPA, 1984) ACUTE DATABASE FOR COPPER**

| | $LC_{50}$ (µg/L) | |
|---|---|---|
| Test species | Blaine Creek | USEPA |
| *Daphnia pulex* | 17.3 | 25.42 |
| *Daphnia* | - | 17.08 |
| *Physella* sp. | 51.0 | - |
| *Physa* | - | 39.33 |
| *Isonychia bicolor* | 109 | - |
| *Pimephales promelas* | 133 | 115.5 |
| *Pimephales* | - | 91.29 |
| *Stenonema* sp. | 212 | - |
| *Eurycea bislineata* | 524 | - |
| *Chironomus* | 547 | 76.92 |
| *Orconectes* | 1,110 | 1,397 |
| *Lepomis macrochirus* | 2,010 | 1,017 |

All acute values were standardized to a hardness of 50 mg/L as CaCO.

There were discrepancies between the two databases for species that were less sensitive to copper. The $LC_{50}$ value of 547 $\mu$g/L for *Chironomus* is appreciably higher than the GMAV of 76.92 $\mu$g/L reported by USEPA. This difference may be due to the use of different species of *Chironomus* and/or different test procedures. The $LC_{50}$ value for bluegill sunfish was twice as high than reported by USEPA and may be due to the effect of site water on the bioavailability of copper at higher test concentrations. A blueish-white precipitate was observed at higher test concentrations (> 1,000 $\mu$g/L nominal), and the quantity appeared to increase with dose, indicating loss of copper from the test solution. The precipitate formed relatively rapidly and was evident at the higher test levels within hours. The observation that the bioavailability of a metal changes during the exposure period was also reported by Parkerton and colleagues (Parkerton et al., 1988) who found that the toxicity of zinc is reduced by predosing site water 24 hours before exposure of test organisms.

Water quality parameters were similar for all toxicity tests; typical values for the dilution water were hardness, 100 - 120 mg/L as $CaCO_3$,; alkalinity, 50 - 60 mg/L as $CaCO_3$,: pH, 7.5 - 7.8 DO levels were within 80 percent of saturation for all tests. Because the fly ash pond discharge caused a substantial increase in the hardness of the water downstream (i.e., 100-173 mg/L as $CaCO_3$ after addition of an effluent at 326 mg/L), the dilution water hardness was lower downstream of the discharge after complete mixing.

In conducting the necessary acute toxicity tests, a number of practical decisions affect the relevance of the resident species procedure in accounting for the site-specific factors that may modify chemical toxicity. The first decision that affects the results is species selection. Although species selection guidelines are relatively broad (Stephan et al., 1985), the actual species selected are based on practical considerations. The availability of test organisms usually is a key factor in the selection process. Ideally, tests organisms should be from the system being studied. Because this process can be difficult and have a negative impact on the ecosystem, representative

organisms from a variety of sources are used. This typically leads to reliance on standard test species as much as possible. Although the selected species should represent ecologically important species covering a full range of sensitivity to the chemical of interest, the reliance on standard test organisms can lead to a prevalence toward the use of more sensitive species. The more representative the tests species are of the ecosystem being protected, the more accurate the resulting criteria will be for protecting aquatic life in a specific ecosystem. A site-specific-criteria demonstration should not, by its very design, be subject to overprotective assumptions that often characterize national criteria.

In addition to differences in species composition between a site and the national database, the resident species procedure is designed to account for the effect of site water on chemical toxicity. The USEPA site-specific guidance suggests collection of dilution water from a pristine site for use as dilutent (USEPA, 1984). In this study, water was collected 10 km upstream of the discharge because of limited river access and concern for nonpoint sources of pollution directly above the discharge. Because the effluent substantially changed the chemical characteristics of the creek under low flow conditions (at least historically), the water used for toxicity testing was probably not representative of downstream conditions. Collection of water below the discharge, after complete mixing, would more accurately reflect the effect of site water in the area of concern. The authors support the recent change in the USEPA site-specific guidelines that recommends using water from downstream of any potential pollutant sources, after all are well mixed with the receiving system (USEPA, 1992). Additional factors that can affect the relevance of the procedure are site water availability, whether the water is filtered, and water storage conditions.

***Criteria Derivation and Evaluation***

As discussed earlier, a number of alternative procedures are available to derive WQC. Based on the information available from Blaine Creek, three alternatives to derive site-specific WQC for copper are possible: (a) recalculation procedure, (b) resident-species procedure, and (c) recalculation modified by copper speciation data. These three alternatives result in distinctly different criteria that are summarized in Table 4. Choice of the most appropriate procedures should be driven by an evaluation of site-specific factors that are most important in a specific situation and should be considered the total sum of data available for a site.

**Table 4. CALCULATION OF THE CONTINUOUS MAXIMUM CRITERION (CMC) FOR COPPER, APPLICABLE TO BLAINE CREEK USING DIFFERENT PROCEDURES AT A HARDNESS OF 50 MG/L (UNLESS OTHERWISE NOTED, EXPRESSED IN TERMS OF μG/L CU)**

| Criterion derivation procedure | Database size | FAV[a] | CMC | CMC at hardness 173 mg/L |
|---|---|---|---|---|
| National | 41 | 18.5 | 9.23 | 29.7 |
| Site-specific | | | | |
| Recalculation | 33 | 19.4 | 9.68 | 31.2 |
| Recalculation with metal speciation | 33 | 33.4 | 16.7 | 53.8 |
| Resident species | 9 | 10.1 | 5.1 | 16.2 |
| Resident species[b] | 33 | 36.3 | 18.1 | 58.4 |

[a] Final acute value.
[b] Example of the role database size has in calculating FAV.

***Recalculation Procedure***

The first step to determine if a site-specific WQC study is necessary should be recalculation of the FAV. The recalculation procedure entails no lab or fieldwork, as nonresident species are removed from the national criteria database and the FAV is recalculated (USEPA 1983, Carlson et al. 1984). Because this procedure is designed to correct for differences in species composition between the national database and a specific site, it is an inexpensive method to determine if differences in species composition are a prominent factor for a specific situation. For Blaine Creek, the recalculation procedure involves mainly removing non-indigenous cold-water species such as the northern squawfish from the national database. The recalculation procedure ($n$ = 33) results in a FAV of 19.4 $\mu$g/L at a hardness of 50 $\mu$g/L (Table 4). This is slightly higher than the national value (18.5 $\mu$g/L), indicating only a slight difference in species sensitivity after nonresident species are excluded. The CMC, using the mixed downstream site hardness, would be 31.2 $\mu$g Cu/L (Table 4). Although this procedure can address whether differences in species composition is a factor, it does not address whether there is a site-water effect. Based on the results of the recalculation procedure, additional work using a procedure that considers site-water effects would be recommended.

***Resident Species***

After generating $LC_{50}$s for the nine species in the database, the next step was to integrate this information using the FAV equation. The site-specific FAV based on the Blaine Creek database ($n$ = 9) was estimated as 22.0 $\mu$g/L Cu. At the mean test hardness of 112 mg $CaCO_3$/L, the FAV was assessed as 10.1 $\mu$g/L Cu. This is less than two-thirds of the national FAV of 18.5 $\mu$g/L at a hardness of 50 mg/L. At the downstream hardness of 173 mg/L as $CaCO_3$, the resulting CMC is 16.4 and the CCC is 11.5 $\mu$g/L compared to the statewide values of 29.7 and 21.1 $\mu$g/L, respectively. This result was unexpected because $LC_{50}$ values for similar species in both databases were generally within a factor of two (Table 3) and because the lowest acute value used to calculate the FAV was almost identical (Table 5). The results of the calculation process erroneously imply that the Blaine Creek database represents a more sensitive ecosystem than the

national database, yet the species-by-species comparison of the two databases does not support this conclusion. Furthermore, low values of the calculated criteria are not consistent with long-term biosurvey data from Blaine Creek, which show the designated aquatic life use being supported (KPCo and AEPSC, 1992). Van Hassel and collaborators (1988) found no statistical correlations between macroinvertebrate taxa richness and measured in-stream concentrations of several toxic metals, including copper.

**Table 5. COMPARISON OF LOWEST FOUR ACUTE VALUES USING THE RESIDENT SPECIES AND RECALCULATION PROCEDURES FOR BLAINE CREEK (ALL VALUES ADJUSTED TO A HARDNESS OF 50 MG/L AS $CACO_3$).**

| Resident species (n = 9) | | | Recalculation (n = 33) | | |
|---|---|---|---|---|---|
| Cumulative Probability | Genus | GMAVs[a] | Cumulative Probability | Genus | GMAVs |
| 0.10 | *Daphnia* | 17.3 | 0.03 | *Daphnia* | 17.08 |
| 0.20 | *Physella* | 51.0 | 0.06 | *Ceriodaphnia* | 18.77 |
| 0.30 | *Isonychia* | 109 | 0.09 | *Gammarus* | 25.22 |
| 0.40 | *Pimephales* | 133 | 0.12 | *Plumatella* | 17.05 |

[a] Genus mean acute values.

The resident species values are the four lowest in the Blaine Creek site-specific database. The recalculation procedure values are the four lowest in the USEPA national database.

An evaluation of the criteria derivation process indicates that the low FAV for copper in Blaine Creek using the resident species procedure is a function of the calculation process and does not represent a site-water effect or true differences in species sensitivity. The controlling factor appears to be the effect of database size in derivation of the FAV. Although previous investigators (Spehar and Carlson 1984, USEPA 1983, Carlson et al., 1984) have mentioned the role of database size on the FAV, they failed to recognize the extreme impact it may have on results of the resident species procedure. The FAV equation is designed so that the fewer available acute values, the more conservative the resulting FAV. When the resident species procedure is used for a chemical for which national WQC are based on a large number of species, the FAV equation will most likely produce a FAV that is overprotective compared to the national value. An overprotective criterion will also occur if the database has a preponderance of sensitive species rather than covering a broad range of species sensitivities.

The role of database size in the criteria development can be illustrated by considering the hypothetical situation in which the national database consists of only the nine most sensitive species instead of a total of 41. The resulting FAV would be 13.4 $\mu$g/L, which is comparable to the value based on the Blaine Creek database (10.1 $\mu$g/L) and approximately one-fourth lower than the value of 18.5 $\mu$g/L based on a database size of 41. A similar result is evident using the Blaine Creek database. Using a database size of 33 (based on the recalculation procedure), the resulting FAV of 36.3 $\mu$g/L is more than three times the FAV of 10.1 $\mu$g/L based on a database of nine. The importance of database size is dependent on how close together the lowest four acute values in the database are, as they determine the slope of the line being used to extrapolate to the

FAV. The greater the distance between the values, the steeper the slope, and the more important database size is in determining the FAV.

Two distinctly different values were obtained using the procedures just discussed (i.e., resident species and recalculation procedures; Table 4) and can be explained by noting the large difference in cumulative probability values assigned to the most sensitive genus (*Daphnia*) using the different procedures (Table 5). Table 5 compares the lowest four acute values and their respective cumulative probabilities used to extrapolate to the FAV (cumulative probability 0.05) for the Blaine Creek database and the USEPA database modified by the recalculation procedure. It is important to remember that these values were used for a log GMAV-cumulative probability plot to obtain the FAV. Because of the effect of database size, the cumulative probability assigned to *Daphnia* is three times higher using the resident species compared to the recalculation procedure. When the database is small (<20 organisms), the FAV equation must extrapolate below the lowest acute value instead of estimating the 0.05 level based on surrounding records.

Results of copper speciation analyses below the discharge clearly indicate a site-water effect reducing the amount of bioavailable copper. The FAV and CMC computed by the recalculation procedure were multiplied by the geometric mean ratio of dissolved to total recoverable copper to adjust for this site water effect. Multiplying the CMC of 31.2 $\mu$g/L (at a site hardness of 173 mg/L) by the speciation ratio of 0.58 results in a criterion of 53.8 $\mu$g/L, which accounts for both site-water effects and species composition. It should be noted that it would be inappropriate to modify a criterion derived by either the resident or indicator species procedures by this method because they already account for site-water effects.

***Conclusions***

Results of the acute toxicity tests for copper using Blaine Creek water are in general agreement with values reported by the USEPA (USEPA, 1984). However, use of the resident species procedure for Blaine Creek resulted in a proposed criterion that was overprotective when compared to national WQC for copper and other site-specific derivation procedures. Overprotection is mostly due to the effect of database size in the calculation process, rather than differences in the relative sensitivity of the test species. The FAV equation is justifiably designed to yield a more conservative criterion when a smaller database is used. For chemicals such as copper, for which a large database already exists, the resident species procedure has an inherent bias resulting in an unduly conservative value. A conservative bias is also introduced into the resident species procedure if the range of the test species contains a preponderance of sensitive organisms.

The recalculation procedure, as modified on the basis of metal bioavailability, appeared to be the most appropriate site-specific modification of the statewide or national criterion. It generated a less stringent FAV, and it was based on factors known to mitigate the toxicity of copper in aquatic environments. A higher criterion is supported by analysis of extensive data on biological monitoring at the site. In this particular case, the regulatory agency does not have to assume a conservative approach because the weight of evidence (biosurvey and chemical speciation data) supports a less stringent site-specific criterion.

The key to derive appropriate site-specific WQC is to focus on the chemical and biological factors that are most important at a specific site. Based on this research, use of the resident species procedure is not recommended unless the national WQC are derived from a small database. Use of the recalculation procedure, metal bioavailability information, the indicator

organism approach, or combination of these appears to be more appropriate and cost-effective techniques to derive site-specific criteria. Regulatory agencies should recognize that method flexibility and novel approaches are acceptable and can result in protective site-specific criteria.

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**Estimated Costs to Conduct a Resident Species Study**

The determination of a site-specific criterion using the resident species approach is an expensive undertaking. A minimum of eight species representing the required families are needed to generate the site-specific criterion. Considerable time is needed to collect an appropriate number of resident organisms in the field and to develop holding and testing procedures that will produce acceptable results. Although in-house cultures or commercial suppliers can be used for obtaining resident species, it is anticipated at least half of the species used will be obtained from the site. The case study presented above used nine species, five of which were obtained from in-house or a commercial supplier and four were collected from the site. Since tests should be replicated to capture variability of the results, a minimum of 16 tests will be conducted during the study.

The estimated costs for a resident species study in which static or static-renewal acute tests are used to derive a site-specific criterion for a metal or metalloid can range from $100,000 to $200,000. Studies in which flow-through tests are used or studies in which the measurement of the chemical of interest is more costly than that for a metal or metalloid will be more expensive.

# APPENDIX H

***Section 1.4.1 from "Policy for Implementation of Toxics Standards for Inland Surface waters, Enclosed Bays, and Estuaries of California"***

## 1.4.1 Translators for Metals and Selenium

To derive total recoverable effluent limitations for aquatic life metals and selenium criteria/objectives that are expressed in the dissolved form, a translator first must be applied to the criterion/objective to express it as total recoverable. The translator shall be the U.S. EPA conversion factor (see Appendix 3) that applies to the dissolved aquatic life metals criterion as specified in the CTR (i.e., the dissolved criterion/objective would be divided by the applicable U.S. EPA conversion factor to calculate a total recoverable criterion) unless:

A. the discharger, in the permit application, (1) commits to (a) completing a defensible site-specific translator study and (b) proposing a dissolved to total recoverable translator to the RWQCB, and (2) describes the method(s) to be used in developing the translator; and

B. the discharger, within a time period specified by the RWQCB not exceeding two years from the date of issuance/reissuance of the permit, submits to the RWQCB (1) the proposed translator, and (2) all data and calculations related to its derivation.

Site-specific translators can be developed from field data by either direct determination of the fraction dissolved, or by development of a site-specific partition coefficient that relates the fraction dissolved to ambient background conditions such as pH, suspended load, or organic carbon. The fraction of metal that is dissolved in a water body can vary depending on when and where measurements are taken. A site-specific translator must (1) account for spatial and/or seasonal variability in areas of the water body that are affected by the discharger's effluent and (2) protect against toxic effects during critical conditions. The translator shall be derived using the *median of data for translation of chronic criteria and the *$90^{th}$ percentile of observed data for translation of acute criteria. If systematic seasonal variation in the translator is demonstrated, seasonal effluent limitations may be justified. If a spatial gradient in the translator is demonstrated, the highest translator value should be used unless the permit allows for a mixing zone (in accordance with section 1.4.2), in which case measurements should be taken outside the mixing zone. The site-specific study plan (including sampling design) must be approved by the RWQCB, after consultation with the California Department of Fish and Game, prior to conducting the study. Translator studies may be conducted by one or more dischargers discharging to the same receiving water body, as described in the permit application, subject to approval by the RWQCB. The planning and undertaking of the study may follow the guidelines presented in Appendix 5, as applicable.

Alternatively, the RWQCB may consider applying a previously approved site-specific translator or translator based on a study completed prior to the adoption of this Policy if the RWQCB believes the translator adequately reflects existing conditions (including spatial and/or seasonal variability) in the areas of the water body affected by the discharger's effluent.

While a translator study is being conducted, a final effluent limitation based on the applicable U.S. EPA conversion factor shall be included in the provisions of the permit and interim requirements shall be established (in accordance with section 2.2.2). An interim deadline to submit the results of the study shall be specified by the RWQCB, and shall not exceed two years from the date of issuance/reissuance of the permit. Once the translator is developed by the discharger(s) and approved by the RWQCB, the RWQCB shall reopen the permit and a new effluent limitation shall be calculated using a method described in section 1.4 after adjusting the dissolved metal or selenium criterion/objective by dividing it by the translator. In the event a translator study is not completed within the specified time, the U.S. EPA conversion factor-based effluent limitation in the provisions of the permit shall become effective as a default limitation.

# APPENDIX I

## STATISTICAL PROCEDURE TO DETERMINE IF A SITE-SPECIFIC OBJECTIVE FOR A CHEMICAL WITH A HIGH NATURAL BACKGROUND CONCENTRATION IS EXCEEDED

The following procedure describes a t-test to determine significance if an SSO for a chemical with a high natural background concentration is exceeded. If an SSO is simply defined as the mean concentration of a chemical at the upstream (or reference) site (no input due to human activities), significant differences in average concentrations between the upstream and downstream sites ($X_d - X_u$) can be analyzed with a *t* test, provided that samples are normally distributed and have equal variances (Zar, 1999). The *t* statistic is

$$t = \frac{\overline{X}_S - \overline{X}_R}{s_{\overline{X}d - \overline{X}u}}$$

where:

$$s_{\overline{X}d - \overline{X}u} = \sqrt{\frac{s_p^{\,2}}{n_d} + \frac{s_p^{\,2}}{n_u}}$$

The pooled variance ($s^2_p$) equals the sum of squares (SS) from the study and reference sites, divided by the sum of their degrees of freedom ($v_d + v_u = n - 2$).

$$s_p^2 = \frac{\mathbf{SS}_d + \mathbf{SS}_u}{v_d + v_u} \qquad \text{and} \qquad \mathbf{SS} = \sum_{i=1}^{n} (X_i - \overline{X})^2$$

We reject the null hypothesis $H_0$: $\mu_d \# \mu_u$ if $t \exists t_{\forall(1),v.}$ when samples from the upstream and downstream sites have unequal variances. Welch's approximate *t* offers a reliable alternative test. The critical value for *t*' is Student's $t_{\forall(1)}$ with *v*' degrees of freedom, where

$$t' = \frac{\overline{X}_d - \overline{X}_u}{\sqrt{\frac{s_d^2}{n_d} + \frac{s_u^2}{n_u}}} \qquad \text{and} \qquad v' = \frac{\left(\frac{s_d^2}{n_d} + \frac{s_u^2}{n_u}\right)}{\frac{\left(\frac{s_d^2}{n_d}\right)^2}{n_d - 1} + \frac{\left(\frac{s_u^2}{n_u}\right)^2}{n_u - 1}}$$

The calculated $v$' degrees of freedom will generally include decimals lower than one, but only the next smaller integer should be used.

When concentrations of a chemical in the reference site are compared against multiple locations, the overall probability of rejecting a true null hypothesis (Type I error) may be far greater than the reported error rate for each individual test. For instance, if we perform twenty tests at the 0.05 level of significance, the probability of at least one Type I error is

$$1 - (1-0.05)^{20} = 0.64$$

Dunnett's test (1955) is specifically designed to adjust the error rate for multiple comparisons between the mean of a reference site and the means of ($k$-1) study areas. The test statistic is

$$q = \frac{X_u - X_d}{\mathbf{SE}}$$

where:

$$\mathbf{SE} = \sqrt{s^2\left(\frac{1}{n_u} + \frac{1}{n_d}\right)}$$

$s^2$ is an estimate of the error mean square (from an analysis of variance to test the null hypothesis $H_0$:

$$\mu_u = \mu_{d_1} = \mu_{d_2} = \ldots = \mu_{d_{k-1}}$$

Critical values for the Dunnett's statistic ($q'_{\forall(1),v,k}$) are available from published tables (e.g., Zar 1999). The number of samples from the reference site should be greater than the number of samples from any of the study areas. The recommended, optimal sample size for the reference site should be slightly less than

$$\sqrt{k-1}$$

times the sample size for each of the study areas.

We use simulated data on concentrations of aluminum at one reference and three study sites to illustrate an application of Dunnett's test. Aluminum concentrations are expressed in (mg/L)

Reference Site

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.6793 | 0.5138 | 0.5168 | 0.1167 | 0.5241 | 0.5955 | 0.6112 | 0.5346 | 0.4837 | 0.7029 |
| 0.6900 | 0.7090 | 0.7246 | 0.6152 | 0.6192 | 0.4560 | 0.5283 | 0.3745 | 0.1521 | 0.2344 |
| 0.7703 | 0.6988 | 0.6569 | 0.4905 | 0.5114 | 0.5195 | 0.5560 | 0.6615 | 0.2793 | 0.5210 |
| 0.6760 | 0.8210 | 0.7340 | 0.7960 | 0.8315 | 0.6755 | 0.4892 | 0.5408 | 0.3602 | 0.5258 |
| 0.5192 | 0.5281 | 0.8750 | 0.9434 | 0.8414 | | | | | |

Site 1

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.6960 | 0.6437 | 0.6424 | 0.4495 | 0.5783 | 0.4619 | 0.7810 | 0.5243 | 0.5479 | 0.4735 |
| 0.6910 | 0.4022 | 0.4355 | 0.3525 | 0.4347 | 0.2777 | 0.4713 | 1.0165 | 0.3342 | 0.8989 |
| 0.4965 | 0.3570 | 0.6228 | 0.7117 | 0.7885 | 0.7472 | 0.6538 | 0.2597 | 0.5368 | 0.4852 |

Site 2

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.6202 | 0.7330 | 0.4285 | 0.7584 | 0.8533 | 0.6029 | 0.7708 | 1.0277 | 0.4256 | 0.6523 |
| 0.7853 | 0.3032 | 0.7133 | 0.6175 | 0.8988 | 0.4975 | 0.4835 | 0.7949 | 0.6894 | 0.6831 |
| 0.4077 | 0.5171 | 0.6178 | 0.6930 | 0.6399 | 0.4603 | 0.4202 | 0.6798 | 0.5955 | 0.5000 |

Site 3

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.5092 | 0.7135 | 0.5944 | 1.0061 | 0.6414 | 0.3645 | 0.5905 | 0.5620 | 0.5237 | 0.6052 |
| 0.3857 | 0.8539 | 0.8968 | 0.5050 | 0.6330 | 0.8206 | 0.5120 | 0.7247 | 0.8545 | 0.3682 |
| 0.4925 | 0.5490 | 0.7268 | 0.9285 | 0.4859 | 0.3341 | 0.5429 | 0.8066 | 0.5864 | 0.7111 |

The first step of the analysis is to test for normality of the data, a critical assumption of Dunnett's test. Departures from the normal distribution were assessed with the Shapiro and Wilk test (1965). The assumption of normality is rejected if the computed value of the test statistic (W) is less than the critical value ($W_{\forall}$). The error rate ($\forall$) for these multiple comparisons can be adjusted with the sequential method proposed by Rice (1990), but such procedure was not necessary because all computed Ws (Table 1) were greater than 0.1. The Shapiro and Wilk tests were performed in S-Plus 6 (Insightful Corporation 2001). Next, we used the Dunnett-type test of Levy (1975) to compare the variance ($s^2$) of each study site with variance of the reference area. The test statistic is

$$q = \frac{\ln s_u^2 - \ln s_d^2}{\mathbf{SE}}$$

where:

$$\mathbf{SE} = \sqrt{\left(\frac{2}{v_u}\right) + \left(\frac{2}{v_d}\right)}$$

Variance in aluminum concentrations at the three study sites were not significantly different from variance in aluminum concentration at the reference area ($q'_{0.05(2),131,4}$ □ 2.35, Table 1). Therefore, the assumption of homogeneity of variances was satisfied for all comparisons. Differences in mean concentration of aluminum between the reference area and sites 1 and 2 were not significant (0.346, 1.064 < $q'_{0.05(1),131,4}$ □ 2.08, Table 1). The average concentration of aluminum at site 3 was significantly higher than the average concentration at the reference site (3.351 > $q'_{0.01(1),131,4}$ □ 2.97).

**TABLE 1. SUMMARY OF DATA AND RESULTS OF DUNNETT'S TEST (MEAN | Q|) COMPARING THE AVERAGE ALUMINUM CONCENTRATION IN A REFERENCE AREA WITH EACH OF THE THREE TARGET SITES**

| | *n* | Mean | SD | W | Variance \|*q*\| | Mean \|*q*\| |
|---|---|---|---|---|---|---|
| Reference | 45 | 0.5823 | 0.1795 | 0.96 | - | - |
| Site 1 | 30 | 0.5975 | 0.1651 | 0.96 | 0.493 | 0.346 |
| Site 2 | 30 | 0.6290 | 0.1641 | 0.98 | 0.528 | 1.064 |
| Site 3 | 30 | 0.7294 | 0.2315 | 0.96 | 1.506 | 3.351 |

When the data significantly departs from a normal distribution, the nonparametric, one-tailed Mann-Whitney test is recommended. It tests for differences in ranks between the reference and study sites, instead of actual measurements of aluminum concentration. There is also a nonparametric procedure to adjust the error rate for multiple comparisons (see Zar 1999: 225).

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**APPENDIX J:**
**SITE-SPECIFIC BAF CASE EXAMPLE**

# FINAL REPORT

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

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March 24, 2000

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# 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.

- 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,pN*-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,pN*-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,p'*-DDD in some tissue and sediment samples collected from the Grand Calumet River; water sample results were not available. *p,p'*-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,p'*-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,p'*-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.)

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'*-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

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:

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

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.

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.

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**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 (±10.96) | 10.24 (±7.22) | 0.75[a] (±0.22) | 3.11 (±0.55) |
| Virginia St. Bridge | 20.77 (±17.23) | 10.76 (±6.54) | 0.76 (±0.31) | 2.31 (±0.38) |
| Statistical Significance[b] | 0.98 | 0.88 | 0.96 | 0.005 |
| Combined results for Tennessee and Virginia St. Bridge Samples | 20.84 (±13.95) | 10.50 (±6.66) | 0.75 (±0.26) | 2.71 (±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 |
| Tennessee St. Bridge or Outfall 010 | July 1999 | 0.04U | 10.04 | 0.40 |
| | October 1999 | 11.03 | 3.00 | 37.2 |
| | | 10.91 | 2.72 | |
| | | 9.96 | | |
| Virginia St. Bridge or Outfall 018 | July 1999 | 0.15[c] | 1.14 | 13.2[c] |
| | October 1999 | 0.04U | 6.36 | 3.02 |
| | | 0.08U | 5.57 | |
| | | 0.42 | | |
| Outfall 034 | July 1999 | 0.06U | 7.87 | 1.59 |
| | | 0.19U | | |

[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]<br>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. $C_t$ = 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**

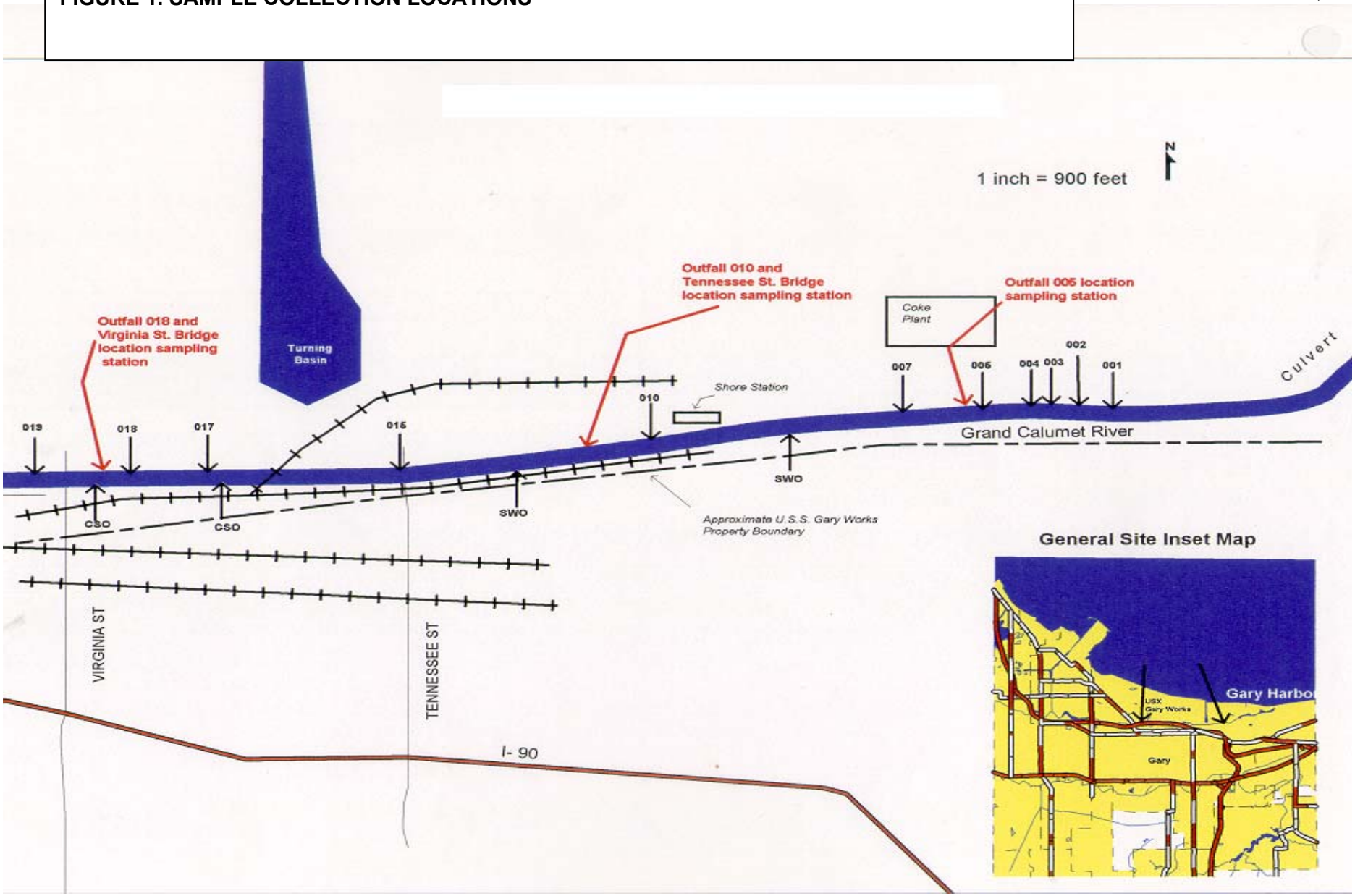


**FIGURE 2. TOTAL MONTHLY AVERAGE FLOWS - 1998 VS 1999**
**OUTFALLS 005, 018 AND 019**

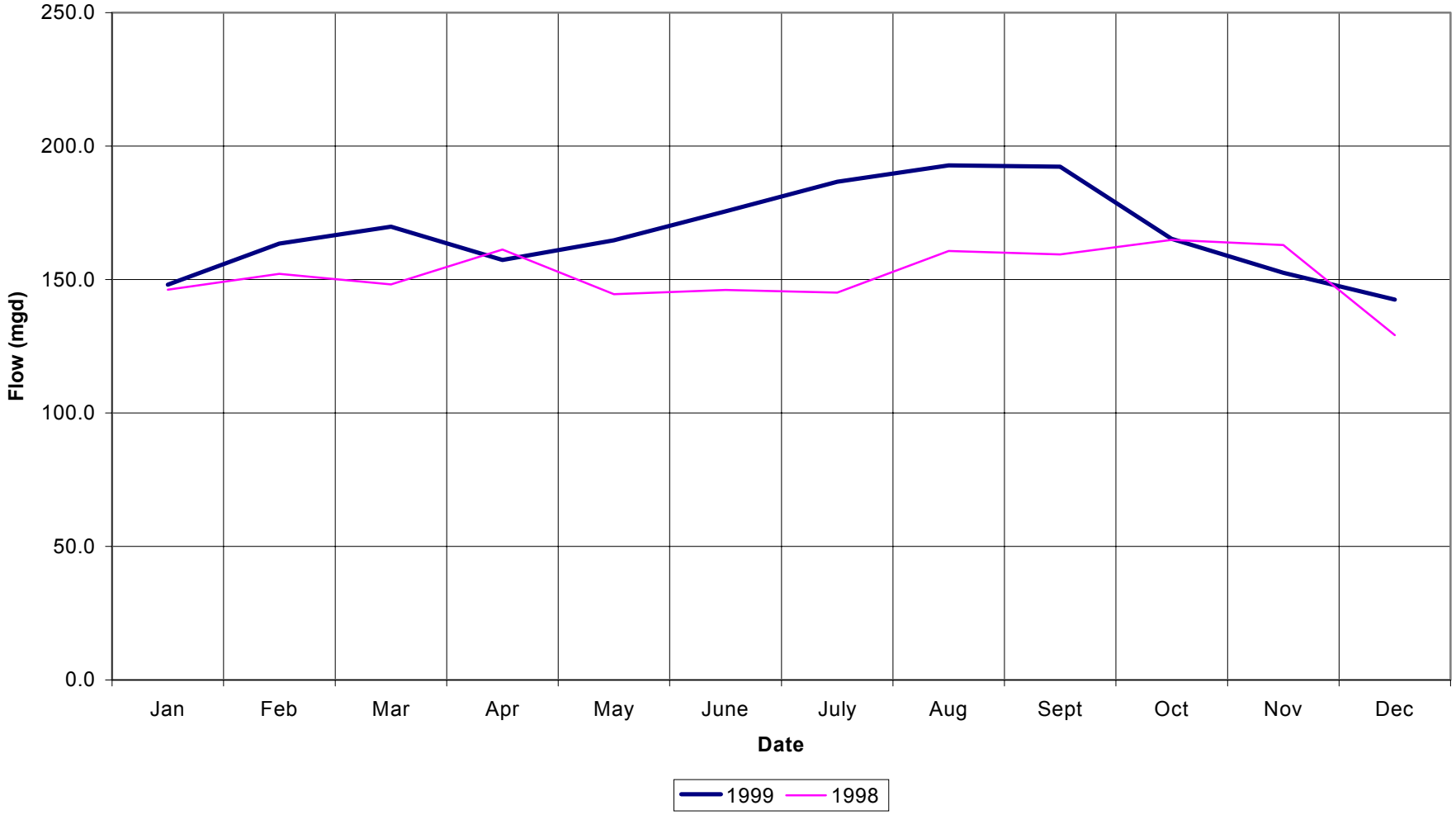


**Figure 3 Additional Sample Collection Location**

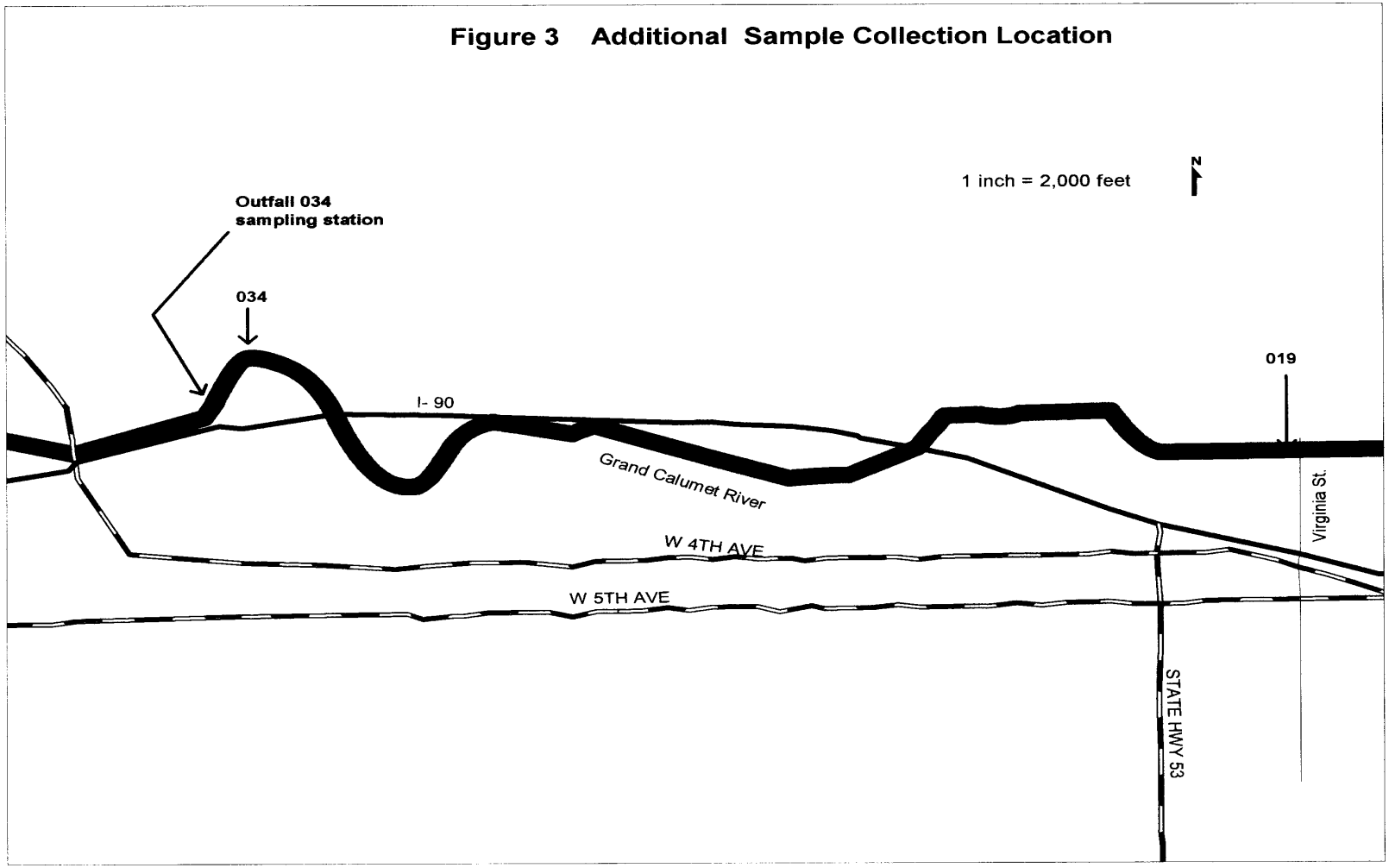


**FIGURE 4. SCHEMATIC OF SYSTEM USED TO FIELD PROCESS LARGE VOLUME WATER SAMPLES**

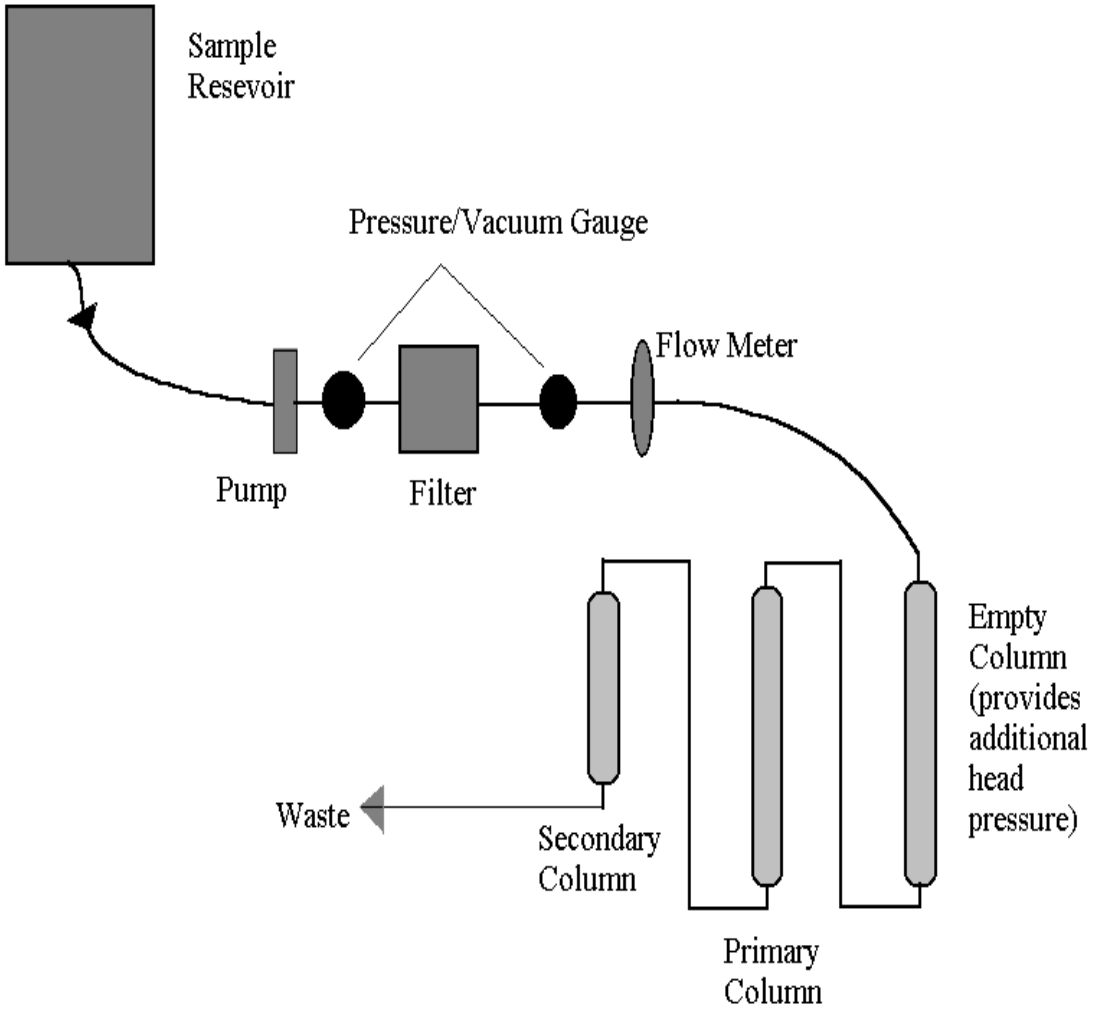
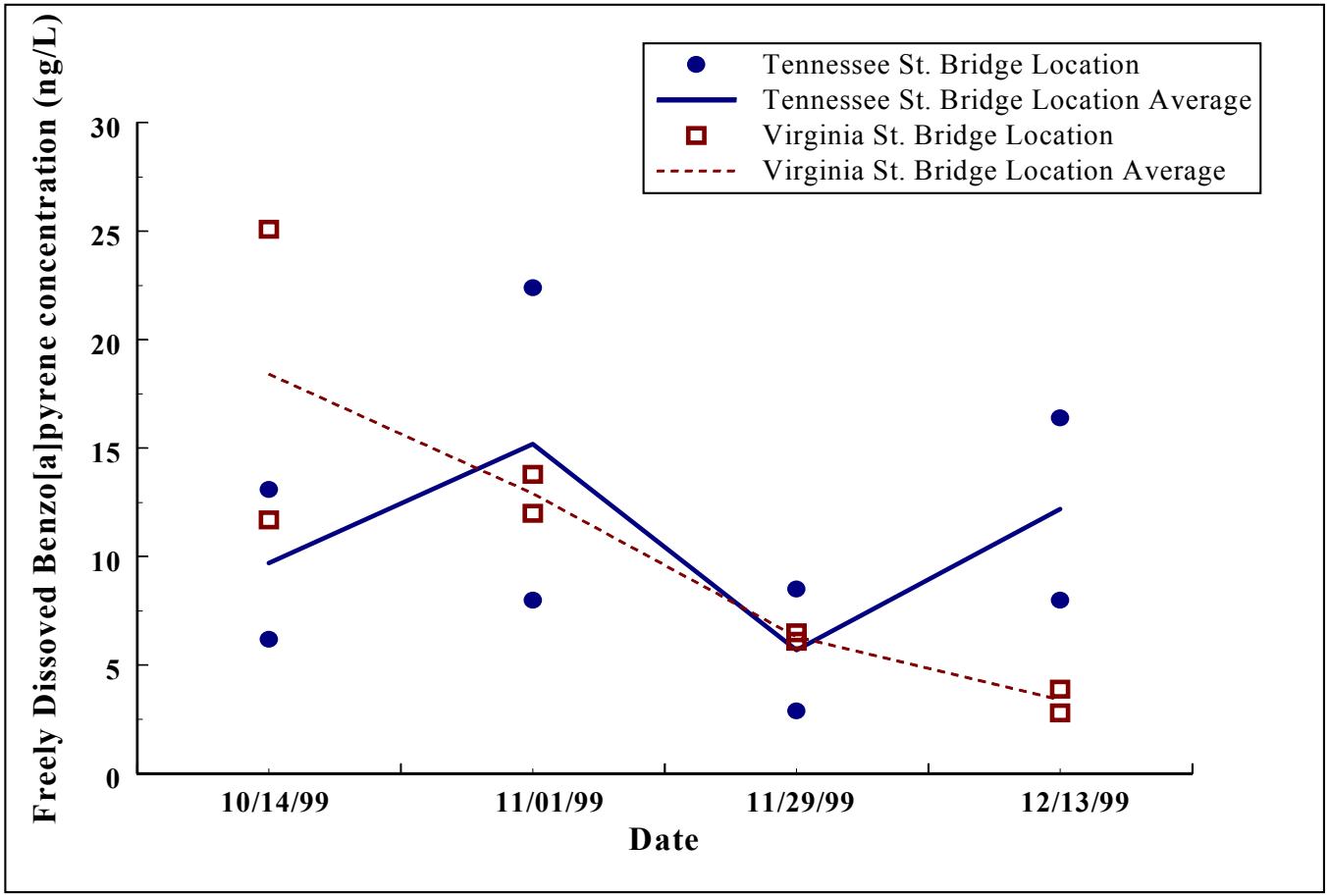


**FIGURE 5. FREELY DISSOLVED CONCENTRATIONS OF BENZO[A]PYRENE**



**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)**<br>Including:<br>a) Review of historical data and literature<br>b) Site visit<br>c) WP and QAPP development | 6 months | $15,000 |
| 2 | **Field Sampling and Laboratory Analysis**<br>Including:<br>a) Two field sampling events<br>b) Preparation and analysis of 11 composite fish tissue samples<br>c) Analysis of four 100 liter river water samples (dissolved and particulate fractions analyzed separately)<br>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**<br>Including:<br>a) Travel to meet with the client and the regulatory agency personnel<br>b) Preparation of a written response<br>c) An additional literature review<br>d) Preparation of a White Paper | 12 months | $35,000 |
| | **TOTALS** | **28 months** | **$250,000** |