

3. EFFLUENT TOXICITY TESTING

3.1 INTRODUCTION

EA Engineering, Science, and Technology performed acute and chronic toxicity testing on residual solids discharged from the Dalecarlia and Georgetown facilities during normal cleaning operations. The toxicity testing program was conducted according to the “Study Plan for Washington Aqueduct Water Quality Studies” (dated 24 June 1999, and approved by U.S. EPA Region 3), which addresses laboratory testing to quantify of the toxicity of the effluents.

Effluent samples were collected from the Dalecarlia Basin #2 in September 1999, December 2000, April 2001, and May 2001, and from the Dalecarlia Basin #3 in May 2000. Effluent samples were collected from Georgetown Basin #2 in December 1999 and May 2000. Samples of upstream Potomac River water, and water from Dalecarlia Basin #2, were collected for use as dilution water. Sediment from the Potomac and Magothy Rivers were collected for use as control sediment.

As described in the Study Plan, toxicity tests were conducted on three fractions of the Aqueduct effluent:

- Whole effluent samples (acute toxicity tests)
- Supernatant from the settled whole effluent (chronic toxicity tests)
- Settled solids portion of the whole effluent (benthic tests)

Acute toxicity tests were performed with *Daphnia magna* (water flea), *Pimephales promelas* (fathead minnow), and *Morone saxatilis* (striped bass) as the test species. The objective of the acute toxicity testing was to determine whether the whole effluent samples were acutely toxic to the test species, based on survival, when compared to the river water control.

Chronic toxicity tests were performed on the suspended particulate phase (supernatant) using *Ceriodaphnia dubia* (water flea), *Pimephales promelas* (fathead minnow), and *Selenastrum capricornutum* (a freshwater algae) as the test species. The objective of the chronic toxicity testing was to determine whether the suspended particulate phase of the effluent was chronically toxic to the test species based on survival, reproduction (*C. dubia*), growth expressed as biomass (*P. promelas*), and cell growth expressed as cell density (*S. capricornutum*), when compared to the river water control.

Benthic toxicity tests were conducted with *Hyalella azteca* (freshwater amphipod) on the settled solids portion of the whole effluent layered over a control sediment. The objective of the benthic toxicity tests was to determine whether the settled effluent samples were toxic to *H. azteca* based on survival and growth (expressed as biomass) when compared to the control sediment.

The test organisms were exposed to a concentration series of 100, 50, 25, 12.5 and 6.25 percent of whole effluent, suspended particulate phase, or settled solids diluted with upstream Potomac River water.

Four rounds of toxicity testing were performed on the Washington Aqueduct samples. The first round of testing (Round #1), which also included preliminary acute toxicity testing, was conducted in September and December 1999. These results were presented in EA Report #3202. The results from Round #2 (May 2000), Round #3 (June 2000), and Round #4 (December 2000 - January 2001) were presented in EA Report #3626. The information included in these two reports has previously been discussed with U.S. EPA and U.S. Fish and Wildlife Service staff.. This report summarizes the data generated during the four rounds of testing and discusses the results from these toxicity tests.

3.2. METHODS AND MATERIALS

3.2.1 Sample Receipt

Discharge samples from the Dalecarlia and Georgetown basins, and samples of upstream Potomac River water, were collected by EA personnel, and hand-carried the same day to EA's Ecotoxicology Laboratory in Sparks, Maryland. At the time of the collection, bottles were also filled for chemical analyses. As specified in the Study Plan, the discharge samples were collected to be representative of the "worst-case" solids discharge concentrations that would exist during a discharge event (i.e., samples were collected at Dalecarlia when hose cleaning operations were pushing out the largest masses of solids, and at Georgetown when the front end loaders were actively pushing solids into the conduit from the deeper areas of the reservoir). Upon receipt at EA, the samples were visually inspected and compared against the chain-of-custody record. The samples were logged into the Ecotoxicology Laboratory Sample Log, and assigned a unique accession number. When not actively being processed, the samples were stored in a secured walk-in cooler in the dark at 4°C. A summary of sample collection and receipt information, along with sample descriptions, is presented in Table 3-1.

3.2.2 Test Organisms

The *Daphnia magna* (water flea) were obtained from EA's Culture Facility in Sparks, MD, and were cultured in moderately hard synthetic freshwater. The organism cultures were maintained in 8-inch culture bowls at 20°C with a 16-hour light/8-hour dark photoperiod, and fed according to EPA guidance (US EPA 1993). Gravid adults were isolated the evening before the test to ensure that neonates (young) produced were less than 24 hours old at test initiation.

Pimephales promelas (fathead minnows) were obtained from embryos spawned in EA's culture facility. Brood organisms were maintained in recirculating dechlorinated tap water at 23°C in 20-gallon aquaria. Eggs produced from the brood system were removed from the brood aquaria and placed into culture water at 25°C for the incubation period. Newly hatched larvae, less than 24 hours old, were used in the chronic toxicity testing. For use in the acute toxicity testing, the *P. promelas* larvae were gradually acclimated to 20°C and fed brine shrimp nauplii (*Artemia* sp., <24 hours old) a minimum of once daily. The larvae were 1-14 days old (hatched within a single 24-hour period) when used to initiate the acute toxicity tests.

Ceriodaphnia dubia (water fleas) were cultured at EA's culture facility in moderately hard synthetic fresh water, and maintained in an environmentally controlled room at 25°C with a 16-hour light/8-hour dark photoperiod. Organisms were fed daily a suspension of yeast/cereal leaves/trout chow supplemented with the algae *S. capricornutum* as described in US EPA (1994). Adults were maintained in individual 30-ml plastic cups (one brood female per culture cup) with a 15-ml volume. Gravid adults were re-isolated the evening before the initiation of the chronic toxicity testing to ensure that neonates produced were less than 24 hours old for test initiation. For use in the chronic toxicity testing, the less than 24-hour old neonates were released from broods of eight or more within an 8-hour period from the time of re-isolation.

Morone saxatilis (striped bass) were acquired from Horn Point Lab in Cambridge, Maryland, and from the Virginia State Fish Hatchery in Brookneal, Virginia.

Selenastrum capricornutum (freshwater algae) were cultured at EA's culture facility, following procedures detailed in US EPA (1994).

The *Hyalella azteca* (freshwater amphipod) were also obtained from EA's culture facility. The amphipods were cultured at 20°C in 10-gallon glass aquaria with a substrate of hardwood leaves and overlying water of dechlorinated municipal tap water. Prior to introduction into the aquaria, the leaves were pre-soaked or boiled to remove tannins. The cultures were fed Tetramin-B flake

food weekly in addition to the hardwood leaves. For use in testing, 7-14 day old organisms were collected from the cultures and gradually acclimated to the test temperature (23°C).

3.2.3 Laboratory Control Waters and Control Sediments

The acute and chronic toxicity tests conducted on the whole effluent and suspended particulate phase included a laboratory water control, in addition to the Potomac River dilution water control. The laboratory water used in the *D. magna* and *C. dubia* tests was moderately hard synthetic freshwater (hardness of 80-100 mg/L CaCO₃). Batches of this water were prepared per US EPA (1993) by passing deionized water through activated carbon, adding reagent grade chemicals and aerating overnight. The water was prepared at least 24 hours prior to use in testing, and kept under gentle aeration until needed. This water was also used to culture the *D. magna* and *C. dubia*.

Dechlorinated tap water was used as the laboratory control water for the *P. promelas* acute and chronic tests. Dechlorinated tap water, and moderately hard synthetic freshwater were also used as dilution water for several *M. saxatilis* acute tests. The source of the tap water was the City of Baltimore municipal water system. Upon entering the laboratory, the water passed through a high-capacity, activated-carbon filtration system to remove any possible contaminants such as chlorine and possible trace organic compounds. This water source has proven safe for aquatic organism toxicity testing at EA as evidenced by maintenance of the multigeneration *H. azteca*, and fathead minnow cultures with no evident loss of fecundity.

Natural Potomac River surficial sediment was used as the control sediment in all *H. azteca* toxicity testing, with the exception of the test performed on the effluent sample collected on 25 May 2000. These Potomac River “control sediments” were collected upstream of the Aqueduct discharges in the vicinity of Lock 5 near the C&O Canal Trail. Due to the inability to collect Potomac River sediment, caused by very high river flow, the benthic tests performed on the 25 May 2000 effluent sample utilized a control sediment collected from the Magothy River, Maryland. Sediment collected from the Magothy River has historically been non-toxic and is routinely utilized as a control sediment in EA’s toxicity tests. The overlying water in the *H. azteca* toxicity tests was dechlorinated tap water.

3.2.4 Toxicity Test Operations and Performance

The toxicity tests were performed following EA’s protocols (EA 1996) which are in accordance with US EPA guidance (1993, 1994, 2000). The test organisms were exposed for a designated

period of time to a concentration series of 100, 50, 25, 12.5 and 6.25 percent test material and a dilution water control. The *D. magna*, *C. dubia*, and *P. promelas* acute and chronic toxicity tests also included a laboratory water control. Prior to preparation of test solutions, aliquots of effluent, dilution water, and laboratory control water or control sediment were brought to the desired test temperature. Test concentrations were prepared by measuring small volumes of sample in pipets, transferring to a graduated cylinder, and bringing to volume with dilution water.

3.2.4.1 Acute Toxicity Testing

Static acute toxicity tests were conducted on the whole effluent samples. The *D. magna*, *M. saxatilis*, and *P. promelas* acute toxicity tests were conducted in 250-ml glass beakers containing 200 ml of test solution. Each test concentration and control had two test replicates with ten organisms per replicate. At test initiation, ten organisms were randomly added to each replicate test chamber.

The acute toxicity tests were maintained at a target temperature of $20 \pm 1^\circ\text{C}$ with a 16-hour light/8-hour dark photoperiod. Test duration was 48 hours for the *D. magna*, and 96 hours for the *P. promelas* and *M. saxatilis* acute toxicity tests. During the exposure period, the test solutions were gently aerated at a rate of approximately 100 bubbles per minute to achieve continuous mixing of the effluent. The organisms were not fed during the test. The test organisms were observed daily and the number of live organisms per replicate was recorded on data sheets. In addition, temperature, pH, dissolved oxygen, and conductivity were measured daily in each test concentration and control. At the end of the exposure period, the 48- or 96-hour median lethal concentration (LC50) was calculated if there was at least 50 percent mortality in the 100 percent test concentration. The Acute Toxic Units (TU_a) were calculated for each acute toxicity test based on the LC50 value [$\text{TU}_a = 100/\text{LC50}$].

3.2.4.2. Chronic Toxicity Testing

Chronic toxicity tests were conducted on the suspended particulate phase, which was prepared by stirring the whole effluent sample for thirty minutes and then allowing the sample to settle for sixty minutes. The supernatant was drawn off and used to prepare the test concentrations.

3.2.4.2.1. *Ceriodaphnia dubia* Chronic Toxicity Testing

The *C. dubia* chronic toxicity tests were conducted in 30-ml plastic cups with 15 ml of test solution per cup. Each test concentration and control had ten replicate cups with one organism per cup. To initiate the chronic toxicity test, one brood of eight or more *C. dubia* neonates was used per test row according to US EPA (1994) known parentage blocking technique. The organisms were fed daily during testing with 0.2 ml suspension of yeast/cereal leaves/trout chow supplemented with algae (*S. capricornutum*). The tests were maintained at 25±1°C with a 16-hour light/8-hour dark photoperiod.

The test solution was renewed (replaced) daily by carefully transferring the test organism from each test cup into a new cup containing freshly prepared test solution. During the daily transfer, observations of mortality were recorded along with neonate counts per replicate. Temperature, pH, dissolved oxygen, and conductivity measurements were recorded on each concentration at test initiation and termination, and daily on the test solutions before and after renewal. The *C. dubia* chronic toxicity tests were terminated when at least 60 percent of the surviving dilution water control organisms had produced three broods, with a mean of at least 15 neonates per control organism.

3.2.4.2.2. *Pimephales promelas* Chronic Toxicity Testing

The *P. promelas* chronic toxicity tests were conducted in 1-L polypropylene beakers, with each beaker containing 250 ml test solution. Each test concentration had four replicates of ten organisms, for a total of 40 organisms exposed per test concentration and control. The test solution in each beaker was renewed daily. The daily solution renewals were performed by siphoning 80 percent of the old test solution from each test chamber taking care to remove debris and uneaten food from the bottom of the chamber, and then slowly siphoning new test solution into the chamber. The tests were performed at 25±1°C with a 16-hour light/8-hour dark photoperiod. Observations of mortality were recorded daily. Temperature, pH, dissolved oxygen, and conductivity measurements were recorded on one replicate of each concentration at test initiation and termination, and daily on the test solutions before and after solution renewal. The *P. promelas* larvae were fed 0.15 to 0.25 ml (ration increased with age) of a 0.05 g/ml suspension of newly hatched brine shrimp nauplii (*Artemia* sp., less than 24 hours old) three times daily.

At the end of the 7-day exposure period, the surviving larvae were rinsed, and placed in pre-weighed, oven-dried aluminum pans (one pan per replicate) and dried at 100°C for a minimum of

six hours. The total organism dry weight per replicate was divided by the number of exposed organisms to obtain a mean dry weight (biomass) for each replicate.

3.2.4.2.3. *Selenastrum capricornutum* testing

The 96-hour *S. capricornutum* chronic toxicity tests were conducted in 250-ml erlenmyer flasks with loose fitting metal lids. Each replicate chamber contained 100 ml of test solution. Each test concentration and control had three algal growth replicate test chambers, in addition to a fourth replicate designated for water quality monitoring. Prior to preparation of the test concentrations, the effluent suspended particulate phase and the Potomac River dilution water were spiked with nutrients without EDTA (US EPA 1994). Algal media without EDTA was used as the laboratory control for the *S. capricornutum* tests. The spiked effluent and river water were passed through a 0.45 µm filter prior to preparation of the test dilutions. At test initiation, each replicate was inoculated with 1 ml of a 1,000,000 cell/ml concentration of *S. capricornutum*. The flasks were placed on a shaker table and the test solutions were oscillated continuously at 100 cpm during the 96-hour exposure period. The chambers were maintained at a target temperature of 25±1°C, and were exposed to continuous illumination of 400±40 foot candles. Preparation of test dilutions and inoculum, and the inoculation of the test chambers, were performed using sterile procedures. Temperature and pH were monitored daily, and conductivity was measured at initiation and termination, in the water quality chambers. At test termination, cell growth was determined visually using a hemocytometer.

3.2.4.2.4. Statistical Analyses

The results of the chronic toxicity tests were statistically analyzed according to US EPA (1994) guidance to determine if any suspended particulate phase concentration was significantly different ($p=0.05$) from the dilution water control with respect to survival, reproduction (*C. dubia*), growth expressed as biomass (*P. promelas*), and cell growth expressed as cell density (*S. capricornutum*). The short-term chronic toxicity test endpoints were expressed as the No Observed Effect Concentration (NOEC), the Lowest Observed Effect Concentration (LOEC), and the Chronic Value (ChV). The definitions of these chronic endpoints follow US EPA (1994) and are as follows:

- The NOEC is the highest concentration of toxicant to which organisms are exposed in a full or partial life-cycle test, which causes no statistically significant adverse effect on the observed parameter (usually hatchability, survival, growth, or reproduction).

- The LOEC is the lowest concentration of toxicant to which organisms are exposed in a full or partial life-cycle test, which causes a statistically significant adverse effect on the observed parameter (usually hatchability, survival, growth, or reproduction).
- The ChV is a value lying between the NOEC and the LOEC, derived by calculating the geometric mean of the NOEC and LOEC. The term is sometimes used interchangeably with Maximum Acceptable Toxicant Concentration (MATC).

The Chronic Toxic Units (TU_c) were calculated for each chronic toxicity test based on the ChV value [$TU_c=100/ChV$].

3.2.4.3. Benthic Toxicity Testing

The *H. azteca* benthic toxicity tests were conducted in 300-ml lipless glass beakers containing control sediment and test dilution. The tests were performed with eight replicates per test concentration and control. To prepare the test material, the whole effluent sample was diluted with upstream Potomac River water. The 100, 50, 25, 12.5, and 6.25 percent effluent dilutions were added to the test beakers and allowed to settle over the control sediment. The May 2000 *H. azteca* testing utilized 175 ml of dilution over 100 ml of sediment, while the December 2000 testing consisted of 225 ml dilution layered over 50 ml of sediment. The sediment and effluent dilutions were added to the chambers 24 hours prior to introduction of the test organisms. The beakers were left undisturbed overnight so as to allow any suspended sediment particles in the water column to settle. At test initiation, ten organisms were randomly introduced into each replicate beaker. The test chambers were placed in a water bath and maintained at the target temperature of $23\pm 1^\circ\text{C}$ with a 16-hour light/8-hour dark photoperiod. The *H. azteca* were fed 1 ml/replicate of YCT (a suspension of yeast, ground cereal leaves, and trout chow) daily.

The overlying water in the exposure chambers was renewed twice daily using a water delivery system (Zumwalt et al. 1994). Fresh dechlorinated tap water was slowly added to each replicate, displacing the water already in the beaker through a notch cut into the top of the beaker. The notch was sealed with fine mesh screen to prevent any organisms from being flushed out of the test chamber. Temperature, pH, dissolved oxygen, and conductivity measurements were recorded daily on the overlying water in one replicate of test concentration and control. The overlying water was gently aerated during the test, in such a way as to not disturb the settled solids.

At the end of the 10-day exposure period, the surviving organisms from each replicate were retrieved by screening through a 250 µm sieve. The number of surviving *H. azteca* from each replicate was recorded, and the organisms of each replicate were placed in a dried, pre-weighed tin and placed in a drying oven at 100°C for at least six hours. The tins were then removed from the oven, placed in a desiccator to cool, and each pan was weighed to the nearest 0.01 mg to determine a mean dry weight per replicate. The results of the 10-day *H. azteca* toxicity tests were statistically analyzed to calculate the IC25 and IC50 values. The inhibition concentration (ICp) is the point estimate of the toxicant concentration that would cause a given percent reduction in a non-quantal biological measurement such as fecundity or growth. For example, an IC25 would be the estimated concentration of toxicant that would cause a 25 percent reduction in biomass. The Acute Toxic Units (TU_a) were calculated based on the IC25 value [TU_a=100/IC25].

3.2.5. Reference Toxicant Tests

In conformance with EA's quality assurance/quality control program, reference toxicant tests were performed on the in-house cultured organisms and on the acquired organisms stocks. The results of each reference toxicant test were compared to EA's established control chart limits. The reference toxicants used for this study were sodium chloride (NaCl) for *C. dubia*, cadmium chloride (CdCl₂) for *P. promelas* used in the chronic toxicity tests, potassium chloride (KCl) for *D. magna*, *P. promelas* and *M. saxatilis* used in the acute toxicity tests, potassium dichromate (K₂Cr₂O₇) for *S. capricornutum*, and copper sulfate (CuSO₄) for *H. azteca*.

3.2.6. Archives

Original data sheets, records, memoranda, notes and computer printouts are archived at EA's Baltimore Office in Sparks, Maryland. These data will be retained for a period of 5 years unless a longer period of time is requested by the US Army Corps of Engineers - Baltimore District.

3.3. RESULTS

3.3.1 Acute Toxicity Testing

The results of the acute toxicity testing conducted on effluent samples discharged from the Washington Aqueduct facilities are summarized in Table 3-2. The test results indicate that (with one exception) the whole effluent samples collected for the preliminary testing and for Rounds #1 through #4, were not acutely toxic to the test organisms. The 48- and 96-hour LC50 values

were >100 percent effluent (TUa <1.0) for *D. magna*, *P. promelas* and *M. saxatilis*. Although the *P. promelas* acute toxicity test conducted during Round #1 on the Georgetown #2 effluent had unacceptable control mortality, it appears that the sample has some level of dose-related acute toxicity which resulted in a 96-hour LC50 value of 29.3 percent effluent (Table 3-2).

The Study Plan included whole effluent acute toxicity testing using striped bass (*M. saxatilis*) prolarvae; and included a cautionary statement that:

“As ASTM Standard Guide E 1241-92 observes, “striped bass embryos and larvae are difficult to work with,” the proposed aeration/mixing of the test solutions may not be an acceptable practice for the proposed striped bass prolarvae testing and the results will need to be interpreted with caution.”

As toxicity tests could only be conducted when discharges occur, which are in turn limited by Potomac River minimum flow requirements, substantial efforts were made by EA and Aqueduct staff to conduct this component of the acute toxicity testing program.

A minimum of ten acute toxicity tests were initiated with *M. saxatilis* on Aqueduct effluents, in addition to EA's QA program requirement that reference toxicant tests performed on each lot of acquired organisms. Further, two different sources of eggs and larvae were used (Horn Point Lab in Cambridge, Maryland, and the Virginia State Fish Hatchery in Brookneal, Virginia).

Only one *M. saxatilis* test had acceptable control survival (minimum of 90 percent control survival), per US EPA (1993) guidelines. The acceptable test was an unaerated reference toxicant test conducted in May 2000 (Round #2), using prolarvae. During the corresponding May 2000 test using the Georgetown #2 effluent, the test solutions were aerated throughout the 96-hour exposure period to maintain complete mixing of the effluent sample, per instructions from U.S. F&WS staff. It is EA's opinion that the gentle aeration caused excessive stress to the test organisms, resulting in >10 percent mortality in all effluent concentrations and in the control. Although the effluent test had unacceptable control mortality (40 percent), the results indicate that the effluent sample was not acutely toxic to the test organisms. More specifically, even though the May 2000 Georgetown #2 test had 40 percent mortality in the controls, survival in the 12.5, 25, 50 and 100 percent effluent exposure concentrations were 55%, 75%, 55% and 80 percent survival, respectively. Thus, the LC50 value is >100 percent effluent [<1.0 TUa], and that result is presented in Table 3-2. Due to limited seasonal availability, EA was unable to acquire additional lots of prolarvae in 2000.

The striped bass larvae acquired in Spring 2001 for subsequent toxicity testing had just begun to feed, and thus were more sensitive to stress from feeding problems and other conditions unrelated to effluent toxicity. The toxicity tests that were conducted with the organisms in the larval stage were performed both with and without aeration of test solutions, and with a variety of dilution/control waters, in efforts to achieve a successful test with reliable results. However, these tests all had high mortality across test concentrations, including the controls.

In our opinion, the unacceptable tests with *M. saxatilis* were a result of the age and sensitivity of available test organisms, and the aeration of the exposure solutions during testing. They were not caused by, nor indicate the presence of, acute toxicity associated with the Aqueduct effluent samples.

3.3.2 Chronic Toxicity Testing

Table 3-3 summarizes the results of the chronic toxicity tests performed on the suspended particulate phase of the discharge samples collected during Rounds #1 through #4. The Georgetown #2 sample collected on 1 December 1999 (Round #1) and the Dalecarlia #2 sample collected on 18 December 2000 (Round #4) were not chronically toxic to the three test species. The NOEC values for *C. dubia*, *P. promelas* and *S. capricornutum* were 100 percent effluent, and the chronic values (ChV) were >100 percent effluent.

There was some evidence of chronic toxicity associated with the effluent samples collected during Rounds #2 and #3. The Georgetown #2 sample collected on 3 May 2000, and the Dalecarlia #3 sample collected on 25 May 2000 were both chronically toxic to *C. dubia*. Although neither sample statistically ($p=0.05$) affected *C. dubia* survival, there was a significant adverse effect on reproduction in the higher concentrations of effluent. The NOEC for the Round #2 *C. dubia* test was 25 percent effluent, and the ChV was 35.4 percent effluent. For Round #3, the NOEC for *C. dubia* was 50 percent effluent and the ChV was 70.7 percent effluent.

During Rounds #2 and #3, the upstream Potomac River water, which was used as the control and dilution water for the *P. promelas* chronic toxicity tests, was toxic to the fathead minnow larvae, severely impacting both survival and growth of these test organisms. Exposure to the effluent samples produced an inverted dose response for both survival and growth, indicating that the effluent samples were not chronically toxic to the fathead minnow larvae (since there was no toxicity at the highest exposure concentrations (including 100 percent effluent)). The NOEC values for the Round #2 and #3 *P. promelas* chronic toxicity tests were 100 percent effluent (ChV >100 percent effluent).

In Round #2, the Georgetown #2 sample was chronically toxic to the *S. capricornutum*. Cell density was significantly affected in the higher concentrations of effluent, with a resulting NOEC of 12.5 percent effluent, and a ChV of 17.7 percent effluent. During Round #3, the Potomac River water (control/dilution water) was toxic to the algae, interfering with the evaluation of cell growth. Statistical analyses were inappropriate, and the toxicity of the Dalecarlia #3 sample could not be determined.

3.3.3. Benthic Toxicity Testing

A Summary of the benthic toxicity test results is presented in Table 3-4. The 10-day LC50 (survival) values from the four rounds of testing were >100 percent sample. The IC25 (growth) values ranged from 6.9 percent sample during Round #4, to 32.8 percent sample during Round #3. The Round #1 test had an IC25 of 13.9 percent sample, and the Round #2 test had an IC25 of 23.5 percent sample.

3.3.4. Reference Toxicant Testing

The results of the *C. dubia*, *D. magna*, *P. promelas*, *S. capricornutum*, and *H. azteca* reference toxicant tests were all valid and fell within the control chart limits, indicating that these organism cultures were of acceptable quality.

3.4 SUMMARY AND CONCLUSIONS

A series of acute and chronic toxicity testing was performed on effluents discharged from the Dalecarlia and Georgetown facilities during normal cleaning operations. As described in the Study Plan, toxicity tests were conducted on three fractions of the Aqueduct effluent: whole effluent samples (using acute toxicity tests); supernatant from the settled whole effluent (using chronic toxicity tests); and the settled solids portion of the whole effluent (using benthic tests). Note that the concentrations of *total* aluminum used in the toxicity tests are substantially greater than the concentrations to which organisms in the Potomac River would be exposed. This can be seen by comparing total and dissolved aluminum concentrations used in the toxicity tests (Table 4-3) with aluminum concentrations collected from the Potomac River during discharges from Outfall 002 (Table 2.1-4) and from Outfall 003 (Table 2.1-3). Recognize that the Table 4-3 concentration values are in mg/L (ppm), whereas the concentrations in Tables 2.1-3 and 2.1-4 are in µg/L (ppb). Similar comparisons of *dissolved* aluminum indicate that the concentrations used

in the toxicity tests are (in almost all cases) higher than what were measured in the Potomac during discharge events.

The acute test results indicate that (with one exception) the whole effluent samples collected for the preliminary testing and for Rounds #1 through #4, were not acutely toxic to the test organisms. The 48- and 96-hour LC50 values were >100 percent effluent (TUa <1.0) for *D. magna*, *P. promelas* and *M. saxatilis*. One fathead minnow test showed some level of dose-related acute toxicity which resulted in a 96-hour LC50 value of 29.3 percent effluent.

The chronic toxicity test results showed that in two of the four rounds, the effluent was not chronically toxic. In the other two rounds, the lowest 7-day ChV for a fish or invertebrate was 35.4 percent effluent. It is noteworthy that 7-day chronic effluent toxicity tests were conducted and reported in the Dynamac (1992, p. 72) study which showed “*that the effluent released from the sampled sedimentation basins had no effect on either mortality or growth of fathead minnows. This result is consistent with observations at the basins, where the fish communities were clearly visible.*”

For the benthic testing, the 10-day LC50 (survival) values from the four rounds of testing were >100 percent sample, but the effluent concentration causing effects on growth (the IC25 value) ranged from 6.9 to 32.8 percent effluent.

Interpretation of these results is complicated by the fact that these tests continuously expose the test organisms to a series of effluent concentrations for 2 to 10 days (depending upon the test), whereas exposure to the Aqueduct plume is a transient phenomenon that lasts for perhaps 4-8 hours. Using the guidance presented in U.S. EPA’s (1991) Technical Support Document for Water Quality-Based Toxics Control, the lowest acute value would require a dilution factor of approximately 11:1 to be non-toxic (i.e., to yield 0.3 TUa); and the lowest chronic value would require a dilution factor of approximately 9.4:1. The benthic results would suggest that a dilution factor of 14.5 would result in no effect on organism growth. As discussed in Chapter 2, these dilution factors are easily obtained for Outfall 002, but outfall relocation would be required to achieve these values for the Georgetown Reservoir discharges from Outfall 003.

3.5 REFERENCES

Dynamac Corporation. 1992. *Impacts of Sedimentation Basin Discharges from the Dalecarlia and Georgetown Reservoirs on the Potomac River; Final Report*. Prepared for Planning Division, U.S. Army COE Baltimore District. Report dated 1 September 1992.

- EA. 1996. *Quality Control and Standard Operating Procedures Manual for the EA Ecotoxicology Laboratory. Revision No.5.* EA Manual ATS-102. Internal document prepared by EA's Ecotoxicology Laboratory, EA Engineering, Science, and Technology, Inc., Sparks, Maryland.
- EA. 1999. *Study Plan for Washington Aqueduct Water Quality Studies.* Prepared for Metropolitan Washington Council of Governments by EA Engineering, Science and Technology, Inc., Sparks, Maryland. Approved by U.S. EPA 24 June 1999.
- US EPA. 1991. *Technical Support Document for Water Quality-Based Toxics Control.* U.S. EPA Office of Water, Washington, D.C. EPA 505/2-90-001. NTIS # PB-91-127415.
- US EPA. 1993. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.* Fourth Edition. EPA/600/4-90/027F. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- US EPA. 1994. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.* Third Edition. EPA/600/4-91/002. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- US EPA. 2000. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates.* Second Edition. EPA/600/R-99/064. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, Minnesota.
- Zumwalt, D.C., F.J. Dwyer, I.E. Greer, and C.G. Ingersoll. 1994. *A water-renewal system that accurately delivers small volumes of water to exposure chamber.* Environmental Toxicology and Chemistry. 13:1311-1314.

Table 3-1 Summary of Sample Collection and Receipt Data for Samples from Washington Aqueduct

<u>Sample Identification</u>	<u>EA Accession Number</u>	<u>Collection Time and Date</u>	<u>Receipt Time and Date</u>	<u>Sample Hardness (mg/L)</u>
EFFLUENT:				
Dalecarlia Basin #2	AT9-1254	1352, 9 SEP 99	1805, 9 SEP 99	
Georgetown Basin #2	AT9-1637	1155, 1 DEC 99	1630, 1 DEC 00	
Georgetown Basin #2	AT0-386	1030, 3 MAY 00	1615 3 MAY 00	136
Dalecarlia Basin #3	AT0-436	0940, 25 MAY 00	1648, 25 MAY 00	144
Dalecarlia Basin #2	AT0-988	1220, 18 DEC 00	1605, 18 DEC 00	84
Dalecarlia Basin #2	AT1-162	1405, 12 APR 01	1645, 12 APR 01	
Dalecarlia Basin #2	AT1-224	1500, 14 MAY 01	1730, 14 MAY 01	
DILUTION WATER:				
Potomac River Water	AT9-1255	1510, 9 SEP 99	1805, 9 SEP 99	
Potomac River Water	AT9-1639	1155, 1 DEC 99	1630, 1DEC 99	
Potomac River Water	AT0-385	1410, 3 MAY 00	1615, 3 MAY 00	120
Potomac River Water	AT0-437	(a) 25 MAY 00	1648, 25 MAY 00	132
Potomac River Water	AT0-989	0830, 19 MAY 00	1045, 19 MAY 00	72
Potomac River Water	AT1-161	1305, 12 APR 01	1645, 12 APR 01	
Dalecarlia Basin #2 Water	AT1-225	1500, 14 MAY 01	1730, 14 MAY 01	
CONTROL SEDIMENT:				
Potomac River Sediment	AT9-1638	1155, 1 DEC 99	1630, 1 DEC 99	
Potomac River Sediment	AT0-389	1542, 8 MAY 00	0945, 9 MAY 00	
Magothy River Sediment	AT0-280	(a) 22 MAR 00	1225, 23 MAR 00	
Potomac River Sediment	AT0-990	0850, 19 DEC 00	1045, 19 DEC 00	

(a) Time of collection is not available.

Table 3-2 Summary of Acute Toxicity Test Results
Washington Aqueduct

<u>Sample Location</u>	<u>Sample Date</u>	<u>Test Species</u>	<u>Testing Date</u>	<u>LC50</u>	<u>TUa</u>
<i>Preliminary testing:</i>					
Dalecarlia #2	9 SEP 99	<i>D. magna</i>	10-12 SEP 99	>100	<1.0
Dalecarlia #2	9 SEP 99	<i>P. promelas</i>	10-12 SEP 99	>100	<1.0
<i>Round #1:</i>					
Georgetown #2	1 DEC 99	<i>D. magna</i>	7-9 DEC 99	>100	<1.0
Georgetown #2	1 DEC 99	<i>P. promelas</i>	7-11 DEC 99	29.3 ^(a)	3.4
<i>Round #2:</i>					
Georgetown #2	3 MAY 00	<i>D. magna</i>	4-6 MAY 00	>100	<1.0
Georgetown #2	3 MAY 00	<i>P. promelas</i>	4-8 MAY 00	>100	<1.0
Georgetown #2	3 MAY 00	<i>M. saxatilis</i>	11-15 MAY 00	>100 ^(a)	<1.0 ^(a)
<i>Round #3:</i>					
Dalecarlia #3	25 MAY 00	<i>D. magna</i>	30 MAY-1 JUN 00	>100	<1.0
Dalecarlia #3	25 MAY 00	<i>P. promelas</i>	30 MAY-1 JUN 00	>100	<1.0
<i>Round #4:</i>					
Dalecarlia #2	18 DEC 00	<i>D. magna</i>	20-22 DEC 00	>100	<1.0
Dalecarlia #2	18 DEC 00	<i>P. promelas</i>	20-24 DEC 00	>100	<1.0

(a) Unacceptable control mortality.

Table 3-3 Summary of Chronic Toxicity Test Results – Washington Aqueduct

<u>Sample Location</u>	<u>Sample Date</u>	<u>Test Species</u>	<u>Testing Date</u>	<u>NOEC (Survival)</u>	<u>NOEC (Sub-lethal)</u>	<u>ChV</u>
Round #1:						
Georgetown #2	1 DEC 99	<i>C. dubia</i>	7-13 DEC 99	100	100 (Reproduction)	>100
Georgetown #2	1 DEC 99	<i>P. promelas</i>	7-14 DEC 99	100	100 (Growth)	>100
Georgetown #2	1 DEC 99	<i>S. capricornutum</i>	23-27 DEC 99	N/A	100 (Cell Density)	>100
Round #2:						
Georgetown #2	3 MAY 00	<i>C. dubia</i>	4-10 MAY 00	100	25 (Reproduction)	35.4
Georgetown #2	3 MAY 00	<i>P. promelas</i>	4-11 MAY 00	100 ^(a)	100 (Growth) ^(a)	>100
Georgetown #2	3 MAY 00	<i>S. capricornutum</i>	4-8 MAY 00	N/A	12.5 (Cell Density)	17.7
Round #3:						
Dalecarlia #3	25 MAY 00	<i>C. dubia</i>	30 MAY-5 JUN 00	100	50 (Reproduction)	70.7
Dalecarlia #3	25 MAY 00	<i>P. promelas</i>	30 MAY-6 JUN 00	100 ^(a)	100 (Growth) ^(a)	>100
Dalecarlia #3	25 MAY 00	<i>S. capricornutum</i>	1-5 JUN 00	N/A	^(a)	^(a)
Round #4:						
Dalecarlia #2	18 DEC 00	<i>C. dubia</i>	27 DEC 00-3 JAN 01	100	100 (Reproduction)	>100
Dalecarlia #2	18 DEC 00	<i>P. promelas</i>	27 DEC 00-3 JAN 01	100	100 (Growth)	>100
Dalecarlia #2	18 DEC 00	<i>S. capricornutum</i>	4-8 JAN 01	N/A	100 (Cell Density)	>100

(a) River water (control/dilution water) was toxic to test organisms.

Table 3-4 Summary of Benthic Toxicity Test Results – Washington Aqueduct

<u>Sample Location</u>	<u>Sample Date</u>	<u>Test Species</u>	<u>Testing Date</u>	<u>10-Day LC50</u>	<u>IC25 (Growth)</u>
Round #1:					
Georgetown #2	1 DEC 99	<i>H. azteca</i>	29 DEC 99-8 JAN 00	>100	13.9
Round #2:					
Georgetown #2	3 MAY 00	<i>H. azteca</i>	12-22 MAY 00	>100	23.5
Round #3:					
Dalecarlia #3	25 MAY 00	<i>H. azteca</i>	13-23 JUN 00	>100	32.8
Round #4:					
Dalecarlia #2	18 DEC 00	<i>H. azteca</i>	29 DEC 00-8 JAN 01	>100	6.9