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APPENDIX A

Appendix A-1

Appendix A-1

RAPID BIOASSESSMENT SURVEY

1.00 INTRODUCTION

An environmental investigation of groundwater contamination by a heavy metals plume originating at Whyco Chromium in Thomaston, Connecticut demonstrated the likelihood that the discharge zone for the plume was a nearby portion of the Naugatuck River. In order to assess whether discharge of target metals had resulted in impairment of the ecological community of the river, a bioassessment of macroinvertebrate communities within potentially impacted reaches of the river was conducted in August, 1999. The proposed bioassessment was conducted in accordance with the recommendations of EPA's most recent revision of *Rapid Bioassessment Protocols for Use in Streams and Rivers*[1] (RBP).

Bioassessments of aquatic ecosystems are used to identify and characterize differences between ecosystems which are exposed to an environmental stressor, such as chemical discharge or nutrient enrichment, and reference ecosystems. Bioassessments use aquatic communities as sentinels of ecological impairment to natural resources. Among the most informative aquatic communities used in bioassessment studies are benthic (bottom-dwelling) macroinvertebrates. Most benthic macroinvertebrates are relatively sessile, and usually occur in great numbers and diversity. In addition, benthic macroinvertebrate communities are important food items for fish, and perform essential roles in the cycling of nutrients and organic matter. In combination, these properties make invertebrate communities ideal indicators of environmental impact.

In this bioassessment, benthic macroinvertebrate communities from potentially impacted reaches of the river and reference reaches upstream of Whyco were sampled and compared in order to evaluate potential impacts from exposure to target metals at the individual, population, and community levels. The macroinvertebrate communities of potentially target metals-impacted portions of the Naugatuck River (hereafter referred to as "study reaches") are also subject to non-target metals related causes of potential impact to the river (i.e., municipal waste water treatment plant (WWTP) discharge, a permitted Whyco NPDES outfall, and historical pollution). Additionally, Branch Brook discharges to the Study reach downstream of Whyco, and likely has some influence on the biota inhabiting the Study Reach. Accordingly, the bioassessment sampling plan was designed to identify the influences of these confounding variables by documenting the macroinvertebrate communities at several locations downstream of Whyco, allowing assessment of incremental impacts to the river due to each source. The following sections describe general concepts of target metals ecotoxicity, field sampling efforts for benthic macroinvertebrates, laboratory taxonomic procedures, and data analysis techniques employed in the bioassessment.

2.00 TARGET METALS - TOXICITY TO AQUATIC ORGANISMS

The toxicity of target metals (cadmium, Cr^{+6}, total chromium, copper, nickel, lead, and

[1] EPA 841-D-97-002, Draft Revision - July 14, 1999.

zinc) to organisms which are exposed to contaminated surface water was evaluated by reviewing toxicological literature from 1967 to 1999, including EPA's ECOTOX Database.

2.10 General Concepts of Metal Toxicity

The measured concentrations of metals in surface water do not directly reflect the toxicity or the bioavailability of the metals. Site-specific environmental factors strongly influence the toxicity and bioavailability of metals. Such factors include ionic strength, pH, reduction-oxidation potential (Eh), water hardness, sediment particle size, total organic carbon, dissolved organic carbon, and suspended particulate matter. Toxicity and bioavailability are also influenced by the species of metal present and the synergistic or antagonistic effects which may be associated with exposure to multiple contaminants. For instance, a mixture of arsenic, cadmium, chromium, copper, mercury, lead, nickel, and zinc, when combined at individual concentrations deemed protective (Dutch water quality criteria) was severely toxic to *Daphnia magna* and caused 50% mortality in Rainbow trout (*Salmo gairdneri)* (Enserink *et al.*, 1991). Even a reduction to one/fifth of the water quality criteria caused a 10% decrease in *D. magna* populations (Enserink *et al.*, 1991). The following paragraphs briefly discuss some of the influences of environmental variables on the toxicity and bioavailability of metals to aquatic.

In natural surface waters, 30-80 percent of the copper, nickel, and zinc, and 90-95 percent of the lead may be in a particulate phase, greatly reducing toxicity and bioavailability (EPA, 1992). Both particulate and dissolved phases are detected in measurements of total metal concentrations. Because most EPA Ambient Water Quality Criteria (AWQC) and other laboratory toxicity tests are conducted using metal salts which quickly dissolve in water. comparison of total metal concentrations to benchmarks developed using dissolved metals is inherently conservative and may result in overestimating the toxicity (EPA, 1992).

The speciation and solubility of metals in natural surface waters is dependent upon pH and ionic activity. Dissolved metals complex with dissolved inorganic ligands such as SO_4^{-2} and F^- and organic ligands including humic and fulvic acids. This complexation and change in metal speciation is largely controlled by pH and the presence of organic ligands, and greatly reduces their toxicity (Freda, 1991).

The reduction of dissolved phase metals in water by complexation and immobilization directly influences toxicity to aquatic and semi-aquatic organisms by reducing the exposure point concentration.

2.20 Target Metal Toxicity

Cadmium

The chronic U.S. EPA AWQC for cadmium is 1.0 µg/l, based on a water hardness of 100 mg/l as $CaCO_3$ (EPA, 1996). Free cadmium ions (Cd^{2+}) are believed to be the bioavailable species in the dissolved phase and accumulates in microorganisms, plant and animal tissue (Wren *et al.*, 1991). Cadmium is preferentially associated with the colloidal and

particulate size fractions (Wren *et al.*, 1991).

Freshwater invertebrate (bioconcentration factor) BCFs for cadmium in water, measured over 52 weeks, ranged from 164 (*Pytiscidae* sp.) to 2200 (*Chironomidae*) (Eisler, 1985). BCFs for fish ranged from 33 (*Salmo gairdneri* over 10 weeks) to 7440 (*Gambusia affinis* over 26 weeks) (Eisler, 1985). The freshwater algae, *Chlorella vulgaris*, had a BCF of 2550 over 1.4 weeks of exposure (Eisler, 1985).

The toxicity of cadmium to aquatic invertebrates has received extensive scientific study (for example, see review by Sheedy *et al.*, 1991). Toxic responses to chronic cadmium exposure occurred at levels as low as 0.2 µg/l in *Daphnia pulex* (Wren *et al.*, 1991). Some common benthic invertebrate families exhibit marked tolerance to cadmium relative to other taxa, especially stoneflies (*Plecoptera*), caddisflies (*Trichoptera*), mayflies (*Ephemeroptera*), and crayfish (Wren *et al.*, 1991).

Chromium

The chronic EPA Ambient Water Quality Criteria (AWQC) for hexavalent chromium (Cr^{+6}) and trivalent chromium (Cr^{+3}) are 10 and 180 µg/l, respectively. The chronic EPA AWQC for Cr^{+3} is based on a water hardness of 100 mg/l as $CaCO_3$ (EPA ECO Update, 1996). Many factors influence the relative toxicity of chromium to aquatic and semi-aquatic organisms. In the case of Cr^{+6}, the AWQC document acknowledges the influence of natural environmental conditions on the toxicity of chromium. It has been demonstrated that chromium can convert from Cr^{+6} to Cr^{+3} (and vice versa) under appropriate natural conditions, which significantly lowers toxicity (US EPA, 1985). Water hardness, pH, humic acids, and temperature have also been shown to influence chromium toxicity (EPA, 1985; Stackhouse *et al.*, 1989). Table A-1 presents some toxicological response data for benthic organisms likely to inhabit the Naugatuck River.

Copper

The chronic EPA AWQC for copper is 11 µg/l, based on a water hardness of 100 mg/l as CaCO3 (EPA, 1996). The toxicity of copper to aquatic animals is reduced in the presence of humic acids and selenium (EPA, 1984). Examples of chronic lethal thresholds for some common taxa include: caddisflies (*Clistornia magnifica*) = 10.39 µg/l (hardness = 26); snails (*Physa integra*) = 10.88 µg/l (hardness = 35 - 55). Midge (*Chironomidae*) emergence was impaired following 32 weeks of exposure to 30 µg/l (EPA, 1984). Changes in the number of species groups in aquatic insect communities were noted at copper (as copper sulfate) concentrations between 10.7 and 12 µg/l (Clements, *et al.*, 1988; Clements, *et al.*, 1990).

Lead

The toxicity of lead in water is greatly influenced by several environmental factors. The chronic AWQC for lead (2.5 µg/l based on a hardness of 100 mg/l as CaCO3) is dependent upon site-specific water hardness. Humic acids have been shown to reduce the

toxicity of lead in aquatic systems (Shanmukhappa, *et al.*, 1990) and lead toxicity is greater in low pH systems than in neutral or basic systems (Starodub, *et al.*, 1987; Buckler, *et al.*, 1987). Organic compounds of lead, such as tetraethyllead and tertramethyllead, are more toxic and have a greater tendency for bioaccumulation than inorganic forms (Eisler, 1988), for which body burdens tend to decrease with increasing trophic levels (Wren *et al.*, 1991). In combination, these factors reduce the certainty of applying a single protective benchmark to sites with elevated lead concentrations. In addition, the responses of test organisms to lead exposure vary greatly.

Water concentrations as low as 750 µg/l have been shown to cause sublethal lead toxicosis in tadpoles of Green frog (*Rana clamitans*) (Taylor, *et al.* 1990) and concentrations as low as 500 µg/l brought about behavioral changes in Bullfrog (*Rana catesbeiana*) tadpoles (Steele, *et al.*, 1989). The 30-day LC-50 value for the Leopard frog (*Rana pipiens*) has been reported at 105 mg/l (Eisler, 1988). Concentrations in water of 1 µg/l caused reproductive impairment in the freshwater invertebrate *Daphnia magna* (Eisler, 1988). For the Zebra mussel, the no observed effects level (NOEL) was measured at 116 µg/l, and the EC-50 concentration was 370 µg/l (Bleeker, *et al.*, 1992). Mortality rates of *Lymnaea palustris*, a freshwater snail, increased with exposure of 19 µg/l lead. The acute (48 hour) LC-50 for *Chironomus tentans* was 2.68 mg/l (Oladimeji, *et al.*, 1989).

Nickel

The chronic EPA AWQC for nickel is 100 µg/l, based on a water hardness of 100 mg/l as $CaCO_3$ (EPA, 1996). Of the four potential oxidation states of nickel, the divalent form is probably the most common and toxic in natural waters (EPA, 1986). Nickel toxicity and bioavailability is strongly influenced by alkalinity, hardness, salinity, pH, temperature, and organic acids (EPA, 1986).

Chronic exposure to water concentrations of nickel as low as 10 µg/l measurably reduced *Daphnia magna* growth (EPA, 1986). EPA (1986) also reported a chronic lethal concentration of 128.4 µg/l for the caddisfly larva, *Clistoronia magnifica*. Chronic exposure to 10 µg/l of nickel was lethal to the decapod, *Assellus aquaticus* (Sheedy et al., 1991).

Zinc

The chronic EPA AWQC for zinc is 160 µg/l, based on a water hardness of 100 mg/l as $CaCO_3$ (EPA, 1996). Many factors influence the relative toxicity of zinc to aquatic and semi-aquatic organisms. For instance the toxicity of zinc is higher to embryos and juveniles than to adults, to starved animals, at elevated temperatures, at low dissolved oxygen concentrations, and in the presence of cadmium and mercury (Eisler, 1993). The lethal limit for tadpoles of the toad, *Bufo boreas*, has been reported to lie between 100 and 500 µg/l (Porter *et al.*, 1976). Fifty percent of Narrow-mouthed toad (*Gastrophryne carolinensis*) embryos exposed to 10 µg/l zinc were dead or deformed within 7 days (Eisler, 1993). Acute LC50 (96 hour) values for freshwater invertebrates were between 32 and 40,930 µg/l (Eisler, 1993). The 10-day LC50 for midge larvae, (*Tanytarsus dissimilis*), a taxa

which may be present at the site, was 37 µg/l (Eisler, 1993). Concentrations as low as 76 µg/l inhibited reproduction in bluegills (*Lepomis macrochirus*) (Eisler, 1993).

3.00 FIELD INVESTIGATION

The interpretation of bioassessment results relies heavily upon the assumption that the environmental stressor of interest is the sole difference between unimpacted (reference) locations and study locations. This ideal situation rarely occurs in natural systems, which vary at macro- and micro-scales. The most important variables considered in the selection of reference sample locations for bioassessment are those that vary longitudinally along riverine ecosystems, such as bank vegetation characteristics, temperature, hydrology, and channel geomorphology (EPA-SAB, 1993). The combined influences of these variables (along with a myriad of other environmental factors such as water chemistry) exert a strong influence on the invertebrate community structure of a river. This bioassessment documented a wide range of habitat characteristics along the Naugatuck River. The most important of these characteristics are described below.

3.10 Bioassessment Reconnaissance

On August 11, 1999, a GZA ecologist conducted a qualitative biological reconnaissance of a portion of the Naugatuck River near the site. The purpose of this reconnaissance was to determine an appropriate benthic macroinvertebrate sampling method and identify suitable sampling locations. The reconnaissance focused on the reach of the river between a point upstream of the Whyco facility warehouse, presumed to be upgradient of the site groundwater discharge zone, and a point approximately 650 feet (200 meters) downstream of the facility.

The dominant river bed substrate in this portion of the river was large cobble. Neither the size or embeddedness of the cobble varied significantly within the study area. At the time of this visit, water levels in the river were abnormally low due to regional drought conditions. Water depths were generally less than 1 foot. Immediately across the river from the Whyco facility, discharge from the local WWTP substantially augmented the water level and flow rate of the river.

The numerically dominant organisms observed throughout the Study Reach were gastropods of the family *Ancylidae* (freshwater limpets). These organisms appeared to be evenly distributed throughout the Study Reach, with densities between 1 to 10 per square inch. The dominant insect taxa observed were caddisfly larvae (*Trichoptera* sp.). These organisms also appeared to be evenly distributed throughout the Study Reach. Other organisms observed included mayfly larvae *(Ephemeroptera* sp.), midge larvae (*Chironomidae* sp.), and freshwater snails. No obvious longitudinal differences in the abundance or types of macroinvertebrates were observed.

3.20 Habitat Description

The reaches of the Naugatuck River upstream of the Whyco facility are dominated by sequences of riffles (turbulent flow) and runs (approximately laminar flow), without significant occurrence of pools. Riffles and runs are known to support the most diverse and abundant benthic macroinvertebrate communities, largely due to the dominance of stable cobble substrates and the high levels of dissolved oxygen associated with these flow regimes. The dominant organisms associated with riffle/run habitats often possess morphological characteristics which prevent them from being washed downstream, such as a streamlined body profile (e.g., *Ephemeroptera* sp. (mayfly nymphs)) or cement-like secretions (e.g., some *Trichoptera* sp. (caddisfly larvae)). Riffle/run sequences are also the dominant habitat adjacent to the Whyco facility and downstream until a point approximately 160 feet (50 meters) downstream of the Whyco NPDES outfall. After this point, the river gradient decreases and large deep pools dominated by sand and silt substrates are dominant. The benthic macroinvertebrate communities associated with these substrates are generally less diverse and abundant than those which occur on cobble substrates (EPA RBP, 1999). The dominant organisms found in pools are often midge larvae (*Chironomidae* sp.) and small aquatic worms (*Oligochaeta* sp.). The riffle/run sequences resume approximately 0.7 miles (1 km) downstream of the facility.

Habitat classifications were performed and documented in accordance with the RBP, and are included as Appendix A-1a. Habitat classifications were based on the numerical scoring system presented in the RBP. Individual sample station characteristics such as substrate type, stream velocity, and riffle frequency were scored, and the individual scores were summed to yield a total Habitat Quality index. Higher scores suggest more suitable habitat for macroinvertebrate communities.

Habitat classification scores for selected background locations were 144 and 162, while scores for samples selected to represent potentially impacted areas ranged from 144 to 173. The habitat classifications demonstrate that the reference locations selected are similar (but not identical) to potentially impacted locations. Figure A-1 depicts the locations of the river reaches used to define background and potentially impacted biological and chemical conditions for the river.

3.30 Sampling Methods

The "Single Habitat Approach" described in the RBP was used to conduct the bioassessment. This method calls for the collection of composited samples from 325-feet (100-meter) reaches. Composites are composed of samples collected from riffles and runs representing different water velocities. Because of the relatively short linear extent of the Study Reaches and the high density of macroinvertebrates observed during a reconnaissance investigation, individual sample reaches established for this bioassessment were approximately 100 feet (30 meters) long. We established two 100 feet (30 meter) background reaches (BG-1 and BG-2) slightly upstream of Whyco in order to document the benthic macroinvertebrate community in unaffected portions of the

the river. Four additional 100 feet (30 meter) reaches were established adjacent and downstream of the Whyco facility to document the benthic macroinvertebrate communities in potentially impacted portions of the river. Three reaches were situated to allow documentation of the benthic community immediately downstream of the two permitted discharges; i.e., one downstream of the municipal WWTP discharge pipe (WINV-4), one immediately upstream of the NPDES outfall (WINV-3), and one immediately downstream of the Whyco NPDES outfall (WINV-2). The last reach (WINV-1) was established approximately 0.4 miles (0.7 km) downstream of the Whyco facility. Whereas all of the study reach samples are potentially exposed to Site groundwater contaminants, samples WINV-2 and WINV-1 were intended to represent water quality conditions as influenced by all potential contaminant sources, including the municipal WWTP, Whyco 's permitted discharge, and Site groundwater discharge.

Because water depths within the Study reach were less than 1.5 feet, samples were collected with a 0.5 mm mesh "Surber Sampler" (see photo below). The sampler net was placed perpendicular to the bottom and 1 ft^2 of the substrate upstream of the net was manually lofted and brushed to dislodge benthic macroinvertebrates, which were trapped in the net as they floated downstream. This sampling method has the advantage of sampling the entire benthic community, whereas some other methods (e.g., kick-net) skew the collection towards larger, surface-dwelling organisms. The process was repeated in three riffle/runs within each 100 feet (30-meter) reach. Organisms from each of the three sub-samples were composited, preserved in 90 percent ethyl alcohol, and returned to GZA's ecological laboratory for identification.

Use of a Surber Sampler to collect benthic macroinvertebrate samples.

Standard water quality parameters were measured at each 100 feet (30 meter) reach at the time of sampling. These parameters included dissolved oxygen, temperature, and pH. Variations of any of these three parameters within the Study Area were noted, and were considered during analysis of biological data.

3.40 Laboratory Analysis

Macroinvertebrate samples were subsampled and sorted by a GZA biologist using a 10x to 70x stereo dissecting microscope. Random sub-sampling of approximately 100 organisms from each composited sample was conducted according to the recommendations of the RBP. Sub-sampling was conducted by evenly spreading out all of the material from a composite sample in a tray divided into a 4 x 3 grid. Based on a cursory examination, an appropriate number of individual grids (either 1 or 2) were randomly selected for microscopic evaluation and removal of organisms. After subsampling, organisms were sorted by taxonomic order[2] and were identified to the lowest practical taxonomic level.

3.50 Chemical Analyses and Water Quality Parameters

In order to document water quality during the bioassessment we analyzed the study reach for target metals (cadmium, Cr^{+6}, total chromium, copper, nickel, lead, and zinc), dissolved oxygen (D.O.) pH, hardness, conductivity, and temperature. As shown on Table 8, none of the target metals were detected in surface water samples at concentrations above Method Detection Limits. Results of other analyses are presented in the habitat classification sheets (Appendix A-1a).

4.00 DATA ANALYSIS METHODS

Several analytical methods were used to characterize the macroinvertebrate communities sampled. Methods included several ecological indices related to community structure and health. Note that the presence of differences between macroinvertebrate sampling stations is not necessarily an indication of community structure impairment, and must be interpreted with consideration given to habitat characteristics and other potentially confounding variables (see Section 2.00). Analytical methods used for this bioassessment are described below.

4.10 Community Diversity and Evenness

The health of a biological community is often related to the number of different species represented (diversity) and the level of dominance exhibited by any one species (evenness). Several metrics which relate to both diversity and evenness were calculated.

Taxa Richness

[2] Taxonomy is the science of categorizing organisms according to body form and structure (which are generally presumed to relate to evolutionary ancestry), with the most basic taxonomic level being the species, and all higher taxonomic levels consisting of groups of species with similar characteristics. The higher taxonomic levels, in ascending order from species are genus, family, order, class, phylum, and kingdom. Criteria for placing organisms within a particular taxonomic group are increasingly broader as you move up the taxonomic levels, so that more species are included under each category. For this evaluation, most organism were identified to the family, or lower, and each uniquely named group of individuals will be referred to as a "taxon".

Taxa Richness

Taxa Richness is the total number of taxa identified within a sample. The higher the Taxa Richness, the more diverse the community. Communities with greater diversity are generally considered to be healthier as compared to communities with lower diversity.

Percent Contribution of Dominant Taxon

The Percent Contribution of Dominant Taxon metric compares the abundance of the numerically dominant taxon to the total number of organisms in the sample and is calculated as

$$\frac{\textit{Total Abundance of all Taxa}}{\textit{Abundance of Dominant Taxon}}$$

In general, invertebrate communities which are dominated by one or a few taxa are indicative of environmental stress; therefore, the health of the community is considered to decrease with increasing Percent Contribution of Dominant Taxon values. Note that the results of this metric are dependent upon the overall level of taxonomic resolution on a sample-specific basis. Therefore, this metric has been calculated at the family level of taxonomic resolution.

Simpson's Index of Diversity

Simpson's Index of Diversity (Simpson, 1949) takes into account both taxa richness and taxa evenness (i.e., the equitability of taxa abundances; see Shannon's Index of Evenness below). The values generated by the index increase with increasing diversity. Although Simpson's Index is heavily influenced by the most abundant taxa in the sample, it has been shown that for samples containing more than 10 taxa, evenness plays an important role in determining the index value (May, 1975). The Simpson Diversity Index is calculated as:

$$D = \sum \frac{n_i(n_i - 1)}{(N(N - 1))}$$

where D = Diversity, n_i = the number of individuals in the $_i$th taxa, and N = abundance.

Shannon's Index of Diversity

Shannon's Index of Diversity can be referred to as an index of

heterogeneity because it takes into account both taxa richness and taxa evenness. The values generated by the index increase with increasing diversity. The Shannon Index is most significantly influenced by taxa richness, and is dependent upon the proportional abundances of individual taxa within a sample. Shannon's Index of Diversity is calculated as:

$$H' = -\sum \frac{n_i}{N} \left(\ln \frac{n_i}{N}\right)$$

where H' = Diversity, and n_i and N are as noted above.

Shannon's Index of Evenness

"Evenness" in community ecology is a term that refers to the equitability between the abundance of each taxon within the community. Ultimate hypothetical evenness would be a community in which all species had the same abundance. Community health is considered to increase with increasing evenness because there is more balance among species with different ecological roles. Shannon's Index of Evenness is calculated by dividing Shannon's Index of Diversity by the natural log of taxa richness. The evenness index is expressed as a decimal between 0 and 1, with increasing values reflecting more even taxa distribution. Shannon's Index of Evenness is calculated by dividing Shannon's Index of Diversity by the natural log of taxa richness:

$$E = \frac{H'}{\ln S}$$

where E = Evenness and S = taxa richness

3.20 Indices of Environmental Stress Within Benthic Communities

Two metrics were calculated which indicate whether the biological community is stressed by an environmental factor.

EPT/Chironomidae and EPT Index

The ratio of *Ephemeroptera*, *Plecoptera* and *Trichoptera* abundance (EPT) to the sum of EPT and *Chironomidae* abundance was calculated for each sample. Many taxa within the EPT orders are sensitive to pollution, whereas *Chironomidae* taxa are generally considered to be pollution tolerant, therefore, the higher the ratio, the healthier a community is considered to be. This index describes communities with an unduly high proportion of *Chironomidae* as environmentally stressed. Another metric which focuses on pollution sensitive invertebrates is the EPT Index, which is the total number of EPT taxa identified in a sample. As with the EPT/*Chironomidae* index, the

It should be noted that neither of these metrics considers actual target metal sensitivity of EPT genera or *Chironomidae* sp., and the metrics are probably better suited for analysis of impacts from organic pollutants. To illustrate the uncertainty associated with use of such organism-specific metrics, GZA searched EPA's ECOTOX Database for information describing target metals toxicity to benthic organisms like those observed in the Naugatuck River. To normalize comparisons between the EPT groups and Chironomids, the search was restricted to lethal effects, and did not include such important effects as reproduction or reduced growth. We selected the lowest reported lethal concentrations for EPT and Chironomidae taxa expected to inhabit the river. Table A-1a presents the results of this search. As shown, *Chironomidae* sensitivity to the target metals is similar to that of mayflies, caddisflies, and stoneflies. Nonetheless, these two indices were calculated because of their potential to identify impacts which may be associated with organic loading from the WWTP (should it occur).

The result for each metric calculated for a study reach sample was compared to the range of results calculated for reference samples.

5.00 RESULTS

Table A-1b presents biological data for each of the six sample locations. Table A-1c presents the results of metrics, and Table A-1d presents the metric results ordered from "healthy" to "impaired" in descending order. It should be noted that some portions of the benthic macroinvertebrate community were excluded from this analysis, either because of their large size or because of the sampling and sub-sampling methods. For instance, large crayfish and small minnows were observed at all six sampling stations. As noted during the previous discussion regarding the biological reconnaissance visit, high densities of freshwater limpets were present throughout the river. However, these organisms are firmly attached to cobbles, and the sampling method was not capable of dislodging substantial numbers of limpets. Many other taxa were undoubtedly excluded from analysis by the subsampling method, which focused on as little as 8 percent of the actual sample material and associated biota.

Metrics were evaluated by comparing results between individual samples, as in Table A-1d, and by comparing the results for potentially impacted areas to the range of metric results for the reference samples.

Taxa Richness

As shown on Table A-1d, between 8 and 12 individual benthic macroinvertebrate taxa were identified in background samples. Samples from potentially impacted areas contained between 9 and 12 taxa. Therefore, this important measure of taxonomic diversity suggests that Site groundwater contaminants have not adversely affected the benthic biota of the river.

Percent Contribution of Dominant Taxon

The percent contribution of dominant taxon metric was calculated at the Family level of taxonomic resolution to allow meaningful examination of all organisms. For background samples, the dominant taxon were caddisflies of the family *Hydropsychiidae*. These organisms constituted between 50 and 64 percent of the sample. This family was also the dominant taxon in samples WINV-2 and WINV-3. Samples WINV-1 and WINV-4 were dominated by midges (family *Chironomidae*). With the exception of sample WINV-1, collected approximately 0.4 miles (700 meters) downstream of the Site, the remaining samples all had lower ("healthier") results for this metric than did background samples. The percent contribution of dominant taxon result for WINV-1 was 68 percent, slightly higher than that observed in background samples.

It is likely that the high frequency of low gradient pools between the Site and WINV-1 increased the overall abundance of *Chironomidae* at this location. These species usually dominate low gradient pooled areas, and are exported downstream with stream flow.

Based on the high degree of similarity for this metric between the background and potentially impacted locations, and on the likelihood that *Chironomidae* are at least as sensitive to target metals as other macroinvertebrates, it can be concluded that the results for this metric do not indicate Site-related impairment of the river.

Simpson's Index of Diversity

Background sample results for this metric were 4.64 at BG-1 and 5.83 at BG-2. With the exception of WINV-1, samples from potentially impacted areas were within the range of background samples. The result for WINV-1 (2.15) was heavily skewed by the high *Chironomidae* density. As noted above, it is likely that the abundance of *Chironomidae* in this sample is related to the extensive pooled areas upstream, and associated downstream drift of these organisms.

It is our opinion that the values for this metric do not suggest site-related impact to the benthic community.

Shannon's Index of Diversity

Background sample results for this metric were 1.40 at BG-2 and 1.74 at BG-1. With the exception of WINV-1, samples from potentially impacted areas were within the range of background samples. As with other diversity measures, the result for WINV-1 (0.60) was heavily skewed by the high *Chironomidae* density. As noted above, it is likely that the abundance of *Chironomidae* in this sample is related to the extensive pooled areas upstream.

It is our opinion that the values for this metric do not suggest site-related impact

to the benthic community.

Shannon's Index of Evenness

Background sample results for this metric ranged from 0.70 at BG-1 to 0.67 at BG-2. With the exception of WINV-1, samples evenness from potentially impacted areas was essentially equal to background evenness (0.64 at WINV-3, 0.66 at WINV-4 and 0.67 at WINV-2). As with other diversity measures, the result for WINV-1 (0.27) was heavily skewed by the high *Chironomidae* density. As noted above, it is likely that the abundance of *Chironomidae* in this sample is related to the extensive low-gradient pooled areas upstream.

It is our opinion that the results for this metric do not suggest site-related impact to the benthic community.

EPT/Chironomidae and EPT Index

As previously noted, these two indices have been calculated to evaluate potential impacts associated with hypothetical nutrient and/or organic loading associated with the WWTP outfall, and are not capable of identifying target metals-related effects. Background sample results for the EPT/*Chironomidae* metric were 0.49 at BG-2 and 0.82 at BG-1. With the exception of WINV-1, the remaining three samples from potentially impacted reaches fell within the range of background samples. The result for WINV-1, 0.28, was heavily influenced by the large numbers of Chironomids and relatively small number of Trichopterans in this sample. Given the large distance between the WWTP and WINV-1, it is unlikely that this source affected the community at WINV-1.

The reference sample results for the EPT metric ranged from 6 taxa at BG-1 to 3 taxa at BG-2. All of the samples from potentially impacted areas fell within this range.

Based on these metrics, no adverse impacts to the benthic community of the river from the previously noted sources have been observed.

6.00 CONCLUSIONS

The bioassessment performed on the Naugatuck River during August, 1999 did not identify impacts to the river's benthic community resulting from the Whyco heavy metals plume. The structure and composition of the benthic macroinvertebrate community adjacent to and downstream of the facility did not vary substantially from that of upstream reference locations. The structure and composition of a sample collected approximately 0.4 miles (700 meters) downstream of the facility was substantially different than that of the remaining samples, but based on the nature of the differences, this sample location is not interpreted as being impacted by the Site's heavy metal plume. Rather, the differences can likely be attributed to habitat differences, organism drift and

associated community shifts resulting from an extensive reach of low gradient flow between the Site and this sample location.

The bioassessment was conducted during a record-setting drought event, with concomitantly low river stage. The low river stage would result in a far lower ground water to surface water dilution ratio. Therefore, the bioassessment should be regarded as being representative of "worst-case" conditions regarding organism exposure to heavy metals.

Based on the bioassessment conducted by GZA, it is our opinion that the migration of site groundwater contaminants to nearby reaches of the Naugatuck River has not measurably impaired the function or composition of the river's benthic community.

7.00 LITERATURE CITED

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