NC Division of Water Quality
Environmental Sciences Section
Aquatic Toxicology Unit

October 27, 2004

MEMORANDUM

To: Michelle Woolfolk
DWQ Modeling/TMDL Unit

Through: Jimmie Overton, ESS
Matt Matthews, ATU

From: Sandy Mort, Environmental Biologist
ATU

Subject: Stressor Study Toxicity Screening Report

Attached is ATU's report on Microtox solid-phase toxicity screening of sediments and *Daphnia magna* feeding inhibition toxicity testing on surface waters collected for the 2004 stressor study on 4 impacted watersheds. This report also includes sediment and surface water risk assessment evaluations of metals and semi-volatile organic compound data relative to selected ecological toxicity screening benchmarks. Watershed surveys and field measurements for these sites are provided under separate cover in the *2004 Stressor Surveys* report prepared by the ESS Intensive Survey Unit.

As always, if you have any questions regarding this data, please do not hesitate to contact me at (919) 733-2136.

cc: Dianne Reid, ESS
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Steve Tedder, WSRO
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Toxicity Screening Investigations Conducted on Sediments and Surface Waters for TMDL Stressor Study of 4 Biologically Impaired Watersheds in North Carolina

prepared:
October 27, 2004

by:
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Executive Summary

Thirteen sites in 4 freshwater watersheds were sampled by ESS staff for surface sediments and waters from May through August 2004 as part of a TMDL stressor study of biologically impaired watersheds. Watersheds investigated included the Neuse (4 streams), Roanoke (2 streams), Cape Fear (1 stream) and Yadkin (3 streams). Reference sediments were also collected from each watershed. A total of 45 surface sediment grab samples were collected for the study. Sediment sample characterization included chemical and physical analyses and toxicity screening using the Microtox® solid-phase test (SPT) method for sediments. Additionally, 9 surface water samples were collected during base flow conditions at integrator locations and sub-lethal toxicity evaluated using a *Daphnia magna* feeding inhibition test method. Sediment semi-volatile organic, PAH and metals data was compared to ecological toxicity screening values and indicated the potential for toxicity due to PAHs and metals existed at several of the sites. This potential toxicity, based on SPT toxicity responses, was likely mitigated by sediment component-toxicant complex formation, reducing toxicant bioavailability to the test organisms. Sediment toxicity classification guidelines based on dry-weight normalized SPT data indicated none of the sediments would likely be appreciably toxic to aquatic biota. Final site toxicity rankings were based on clay-normalized data to account for apparent toxicity effects related to absorption of Microtox bacteria to sediment clay particles, resulting in apparent biasing of the SPT data overestimating sediment toxicity. Based on average watershed clay-normalized SPT toxicity rankings the Roanoke basin sediments showed the highest levels of toxicity response, followed by the Neuse, Yadkin and Cape Fear basin sediments. Surface water integrator location feeding inhibition toxicity data indicated no EC50 responses <100% sample. Lowest EC20 results were identified at two Neuse basin sites. This report details the toxicity screening results and toxicity interpretation.

Introduction

Thirteen sites in 4 freshwater watersheds were sampled by ESS staff for surface sediments and waters from May through August 2004 as part of a TMDL stressor study of biologically impaired watersheds. The DWQ Modeling and TMDL unit specified watersheds for evaluation. Watersheds investigated included the Neuse (4 streams), Roanoke (2 streams), Cape Fear (1 stream) and Yadkin (3 streams). Reference sediments were also collected from each watershed. A total of 45 surface sediment grab samples were collected for the study. Sediment sample characterization included chemical and physical analyses and toxicity screening using the Microtox® solid-phase test (SPT) method for sediments. Nine surface water samples were also collected during base flow conditions at watershed integrator locations and sub-lethal toxicity evaluated using a *Daphnia magna* feeding inhibition test method. ATU staff performed all toxicity testing. The DWQ Chemistry Laboratory and NCSU Soils Laboratory also performed additional sediment and surface water chemical and physical analyses.

Methods

Toxicity sample collection

Fine-grained sediment grab samples were collected in depositional areas to optimize toxicity identification. Samples were collected to depths of typical benthic activity, 4-6 cm below ground surface (bgs). Two to five grab samples were collected at each sediment location and analyzed individually. One replicate was collected at each site. ESS's Biological Assessment Unit identified suitable reference sediment sites for each watershed and 2 to 5 grabs were also collected at each reference site, with no replicates. Sediment toxicity samples were collected directly into organic-cleaned glass jars with organic-cleaned stainless steel spoons. HDPE and glass jars were used for residue samples, and soil bags were used for particle-size samples. Base flow integrator surface water samples for toxicity testing were collected directly into 4L LDPE cubitainers. Samples for toxicity testing were collected and stored with no headspace. All samples were protected from light and stored on ice from collection through transport to the testing laboratory, where they were stored at 0.0-4.0°C until analysis. Particle size samples were air-dried before delivery to NCSU.

Sample preparation

Prior to analysis indigenous organisms, large pieces of plant material, and pebbles were removed from sediment samples, and samples were mixed to a homogenous texture and color. Sample mixing was performed in the sample container or with organic-cleaned stainless steel spoons and bowls.

Microtox toxicity analysis

Each grab sediment was analyzed at a minimum in triplicate by the Microtox SPT method [2]. A NOAA modification [9] of the standardized Microtox method was used to accommodate 3 replicate sample analyses in one analytical run. All sediment Microtox

analyses were completed within 7 days of collection. Seven-gram (7.00 ±0.05 g) sub-samples of homogenized sediment were stirred in 35.0 ml of 3.50% NaCl diluent for 10 minutes. Three sub-samples of sediment-NaCl suspension were each serially diluted to 5 sediment concentrations. After thermal-equilibration of sediment dilutions and 3.50% NaCl control solutions, the test organism, *Vibrio fischeri*, was added to each test solution. After a 20 minute temperature-controlled exposure period, sediment suspensions were filtered and light output produced by sediment-exposed bacteria compared to light output of bacteria in control solutions. Toxicity to the test bacterium is manifested by reduced light output relative to triplicate preparations of control organisms accompanying each set of sediment dilutions. *Vibrio fischeri* (strain NRRL B-11177) is a marine bioluminescent bacterium cultured under standardized conditions to optimize toxic response. The organism is supplied in a lyophilized form and reconstituted at the time of use. Toxicity system evaluation included Zn and phenol reference toxicant analyses with each lot of bacteria, and during each month of Microtox testing.

Data acquisition and reduction was performed by the PC-based Microtox Omni® software system [3]. Data point selection was evaluated and modified as appropriate by ATU analysts. Data was calculated as mean EC50 and EC20 for each analysis, representing a 50% and 20% negative effect level (reduction of light output) relative to average control light output. Analytical variables monitored for quality control validation included control replicate CV ≤12% and sediment response linearity (R^2 ≥0.90). *Vibrio fischeri* test organisms were purchased from Azur. Sodium chloride and reference toxicant solutions were prepared by ATU from ACS-grade reagents and Type I-reagent grade de-ionized water (DIW).

Sediment characterization analyses

Sediment pH methodology referenced Standard Methods [12] soil pH method (9045C). An equivalent weight of DIW was added to wet sediment and stirred for 5 minutes, allowed to settle for 60 minutes, and pH of the supernatant measured. Percent total solids (PTS) were determined by drying duplicate portions of wet sediment to a constant weight at 103-105°C (Standard Methods, 2540G). ATU PTS data is reported. Total volatile solids (TVS, Standard Methods, 2540G) were determined from dried sample by ashing to a constant weight at 550°C. The DWQ Chemistry Laboratory-WARO branch performed the TVS analyses in triplicate. Particle-size analyses were performed by NCSU Soils Laboratory and reported as percent sand, silt, and clay.

TVS was used as a surrogate for sediment organic carbon analysis due to the inability to locate a laboratory able to perform sediment TOC analysis. The temperature of TVS ashing may have lead to a positive bias relative to actual organic carbon content due to volatilization or decomposition of non-organic sediment components, such as mineral salts [12].

Ecological screening value and toxicity criteria analyses

Analytical data for sediment and water column metals were compared to NOAA ecological toxicity screening values [4]. NOAA sediment values were used as they included data for all metals analyzed in this study, contrary to USEPA Region 4 values [14] or NC aquatic life standards. Sediment metal concentrations were compared to

conservative lower threshold effect level (TEL) screening values. Sediment concentrations below TELs would be expected to rarely result in toxicity due to metals. NOAA chronic freshwater screening values, along with the Environment Canada screening value for manganese [5], were used for surface water metal evaluations. The Environment Canada benchmark was selected since neither USEPA Region 4 nor NOAA listed a Mn value.

PAHs were quantified in two sediment samples from this study. Sediment PAH levels were compared to USEPA Region 4 and NOAA screening values. PAHs reported that did not have a PAH-specific screening value were screened against a PAH of similar structure. NOAA ARC TELs [4] were used for comparison to PAHs grouped as low and high molecular weight PAHs, and total PAHs, since these values were not included in the TEL list.

Hazard quotients (HQ) were calculated as the ratio of sample analyte concentration to the screening value, and summed to represent potential combined effects due to classes on toxicants acting on the same target organ. One-half the sample-reporting limit was used for analytes reported as not detected.

Sediment Microtox SPT data was evaluated against toxicity classification criteria developed by Environment Canada [7] and two levels of criteria developed by Ringwood *et al* [11], representing different levels of conservatism. All three classification systems evaluate sediment toxicity by comparing SPT-generated EC50 values to toxicity criteria established for samples with percent fines above or below 20%. Grain size has been identified as a potential confounding factor for Microtox testing [7,11]. When sediment fines (soil particles ≤0.063 mm) range from 5-20% test bacteria may absorb to fines, with the effect of apparent increases in toxicity (decreased EC50 values) as the percent fines increase. To account for this effect guidelines for interpretation of Microtox SPT data take into consideration sample percent fines (Table 1).

Table 1. Microtox sediment toxicity classification guidelines. Sediments are toxic if EC50s are lower than listed values. EC50 values mg dry-weight normalized sediment/L diluent.

criteria source	<20% fines	≥20% fines
Environment Canada, 2002 [7]	EC50 <1000 mg/L, or EC50 ≥1000 mg/L and sample EC50 >50% lower than reference sediment EC50 and results are significantly different	EC50 <1000 mg/L
Ringwood et al, 1997, less conservative [11]	EC50 <5,000 mg/L	EC50 <2,000 mg/L
Ringwood et al, 1997, more conservative [11]	EC50 <10,000 mg/L	EC50 <5,000 mg/L

Feeding inhibition toxicity test

The *Daphnia magna* feeding inhibition test was developed by ATU to provide a sub-lethal single sample toxicity assessment to replace USEPA-standardized acute tests employing lethality endpoints. The feeding inhibition toxicity test allows for organism exposure through direct toxicant contact, as well as through ingestion, as many toxicants will absorb to food particles, altering toxicant assimilation kinetics. In addition, the feeding inhibition test evaluates the persistence of negative effects after exposure to the toxicant has ended. The test was developed from methods described by McWilliam and Baird [9]. The feeding inhibition procedure employs the principle that feeding rate is a general response to toxicant exposure, and that feeding inhibition of some toxicants may persist after exposure to the toxicant has ended, indicating continued physiological impacts. Latent effects are identified during a post-exposure feeding period. Food (energy) intake is an important parameter at the organism as well as population level, impacting developmental rate, growth rate, fecundity, and survival, thus influencing population structure and dynamics. Studies have indicated that exposure to a variety of metals and organic chemicals results in a significant reduction in Cladoceran feeding rates, and that feeding depression is a rapid, general, indicator of toxic stress.

In the ATU feeding inhibition method integrator location surface waters were diluted with a non-toxic surface water routinely used by ATU for aquatic toxicity testing. A known concentration of green algae *Selenastrum capricornutum* was added as food material to replicate test sample and control solutions. This algae is the same species used for organism rearing. Absorption of toxicant to algal cells adds an ingestion exposure in addition to direct contact with dissolved toxicant in the test medium. Three to six day old *Daphnia magna* neonates are added to test solutions for 24-hour toxicant exposure period. At the end of the 24-hour exposure *Daphnia* are transferred to treatments consisting of control water and known concentrations of *Selenastrum* for an additional 4 hours. *Selenastrum* concentrations are measured by absorbance at the beginning and end of the 24 and 4-hour periods. Effect levels were calculated with the ToxCalc™ software [13] as inhibition of *Daphnia* feeding rates relative to feeding rates of organisms in control solutions.

Water and sediment organic compound and metals analytical data

Study surface water and sediment analytical data for PAHs, SVOCs and metals used for comparison to ecological screening values and toxicity data in this study were originally reported in the ESS stressor survey report prepared by the Intensive Survey Unit [8].

Results and Discussion

Sediment data

Comparison of site sediment Microtox SPT EC50 results to toxicity classification guidelines, as well as comparison to ecological screening values for metals, indicates

that the sediments collected in this study are not likely significantly toxic to aquatic biota. Table 2 lists sample locations, sediment characterization data, and dry weight normalized and clay-normalized Microtox SPT data. Dry weight normalized Microtox EC50 data is shown in Figure 1. Comparison of Microtox EC50 dry weight normalized data to Environment Canada [7] and two Ringwood *et al* [11] toxicity classification guidelines that differ in their level of conservatism (Table 3), both utilizing sediment percent fines criteria, indicates that all sediment sites were classified as non-toxic. The two sets of Ringwood *et al* guidelines differ in the level of conservatism used for the designation of "toxic" sediments.

Based on observed relationships between Microtox EC50 values and sediment characteristics clay-normalized data likely provides the most appropriate means to compare toxicity levels observed in this study. Multiple correlation analyses were performed (data not shown) to investigate equilibrium partitioning theory relationships, including decreased toxicity due to metals as the proportion of organic carbon or fines increased in the sediment matrix [1]. A weak negative correlation ($R^2 = 0.35$) was observed for Microtox EC50 values and percent clay, indicating a possible adsorption of test bacterium by sediment fines and probable false positive toxicity indications based on dry-weight normalized data. Consequently, sediment in this study was ranked for SPT toxicity (Table 4) following percent clay-normalization (Figure 2).

Ecological screening value assessment did not indicate a substantial potential for toxicity due to metals. Although HQs >1 indicate a potential for toxicity, all sediments in this study had $\sum HQ_{metals}$ between 1.0 and 3.65, based on conservative NOAA TEL values. Table 5 lists sediment metal analytical data as well as NOAA TEL, less conservative NOAA PEL, and USEPA Region 4 screening values for freshwater sediments. The only metal-specific HQ >1.0 was Pb for Black River from the Cape Fear basin (NOAA TEL $HQ_{Pb} = 1.1$). The relatively low SPT toxicity levels observed are likely a result of the comparatively low metal concentrations and mitigation of metal toxicity due to complex formation with organic carbon or sorption to sediment fines. Metal-sulfide complex formation may also inhibit metal bioavailability, but it is likely that any M-S complexes were disrupted during the sample collection and preparation processes for Microtox testing. Acid-volatile sulfide (AVS) levels in the sediments were not evaluated in this study.

SVOCs or pesticides were reported for each of the sediments in this study but all data, other than the HQ-specified PAHs were tentatively identified or were estimated concentrations. Four high molecular weight PAHs were quantified in Heatherly Creek sediments, with a NOAA TEL $\sum HQ = 3.9$. Ten PAHs were identified in Black River sediments, with a $\sum HQ_{total\ PAHs} = 42$. Based on clay-normalized SPT EC50 data both these sites ranked low in relative toxicity. The lack of observed toxicity is likely due to reduced PAH toxicity resulting from relatively high TVS (organic carbon) and fines content of the Black River sediments, and the low PAH concentrations of the Heatherly Creek sediments combined with the fines content, resulting in reduced bioavailability of the PAHs at both locations. Reduced partitioning potential of high molecular weight PAHs in an aqueous medium, and sequestration of aged-PAHs in sediment voids may also have contributed to reduced toxicity.

Clay-normalized data (Table 4) indicates that Roanoke basin sediments represented 3 of 4 sites with lowest EC50s (Town Fork Creek, Snow Creek and Smith Creek). The Neuse basin reference site, Little River, had the 2nd lowest EC50. In each of the 4 sample basins the reference sediments did not exhibit the highest EC50s. Sediments with the highest clay-normalized EC50 values were Core Creek (Neuse River basin) and Heatherly Creek (Yadkin River basin).

Simple linear correlation analyses showed a weak/moderate negative correlation of dry-weight normalized sediment SPT EC50 data and NOAA TEL HQs ($R^2 = 0.32$). A weak/moderate negative correlation ($R^2 = 0.43$) was also observed for NOAA sediment TEL HQs and dry weight normalized SPT EC50 data, indicating that TVS data likely provided a reasonable substitute for TOC data.

Surface water data

None of the surface water samples collected for this study resulted in feeding inhibition EC50 values <100% sample, while 5 of 8 had EC20 values <100% sample (Table 7). EC20 data is provided as all EC50 results were >100%sample. Twenty percent inhibition levels represent an effect-level generally considered ecologically significant. The EC20 values for NEU-KRC (EC20 = 7.36%) and NEU-SFR (EC20 = 38.4%) were substantially lower than the other sites, and may indicate the potential for significant inhibition to water column organisms at these sites. *Daphnia magna* feeding inhibition toxicity tests were run on waters from 8 watershed integrator locations. Waters were not collected at the reference sediment locations and no feeding inhibition test was performed on the Cape Fear basin sample collected at Black River at SR 1780 due to a lack of suitable test organisms. All feeding inhibition tests were initiated the day after sample collection, except for the Yadkin basin sample collected at Town Creek at I-85. The test for this sample was started 5 days after collection and the data should be considered un-reliable due to the potential for chemical or biological alteration of the toxicants in the sample matrix.

All water sample metal concentration comparisons to ecological screening values resulted in $\sum$HQs >1.0 for the analyzed metals (Table 6), ranging from 7.0 to 74 using NOAA and Environment Canada values. Metal concentrations at 5 sites exceeded the NC aquatic life standard for Fe (Table 6). No other NC aquatic life standards for metals were exceeded, although NC does not have aquatic life standards for Al or Mn, and all sites had HQs >1 for Al, Mn or both. The screening values used for calculation of HQs exceeded NC standards for As, Cd and Zn, but all sites were reported at less than sample reporting limits for these metals. The metal $\sum$HQs >1 were due to elevated Al, Mn and Fe concentrations in the samples, as well as sample reporting limits less than screening values for Cd, Cr, and Pb. All sample data for Cr and Pb were also reported as less than sample reporting limits. No correlation was observed between feeding inhibition EC20 results and freshwater $\sum$HQs for metals, nor between feeding inhibition EC20 results and sediment clay-normalized SPT EC50 data. The lack of correlation of feeding inhibition EC20 data to metal concentrations may be due to the relatively small number of data points, reduced bioavailability of the metals in the sample matrices due to ligand-metal complex formation, or a lack of sensitivity of the test organisms.

Recommendations for future studies

In light of the experience gained in this study, the first undertaken by DWQ utilizing in-house sediment toxicity analyses, several suggestions are provided for future studies:

- Matching of sediment test site and reference site particle-size characteristics (%fines differ by <30%) provides a more reliable comparison of toxicity levels.
- Evaluate site-specific sampling protocols relative to the number of grabs and the potential for compositing for analyses, without substantially reducing the ability to identify "hot-spots".
- Collect grab sediments by combining adequate sediment volume for all parameters into a single stainless steel bowl and mix until homogenized, then aliquot to individual containers. This will reduce variability between individual containers from a collection site.
- Run the same analytical scans on all sediments and waters, including reference sites.
- Run TOC on all sediments and waters as organic carbon plays a dominant role in metal and non-polar organic toxicant bioavailability. Run adequate sediment replicates to appropriately characterize the sample.
- Consider pore water toxicity or analytical testing to enhance toxicant assessment when significant levels of sediment toxicity are observed.
- Use AVS analysis of sediments to better characterize metal bioavailability where metals may be contributing to observed toxicity.
- Evaluate the use of alternative osmotic adjustments or freshwater bacteria for Microtox studies of freshwater systems to eliminate potential toxicant alterations due to the saline media required to accommodate the marine bacterium.
- Employ additional organisms and endpoints for sediment toxicity assessments to improve the reliability of the assessment. Cladoceran whole-sediment methods are recommended for these types of investigations and utilize currently available ATU organisms and equipment. Microtox analysis of surface waters may also be used to provide an additional low-cost, rapidly obtained endpoint suitable for a single sample collection.

Table 2. TMDL stressor study sediment characterization and toxicity data.

													as mg sediment/L	
Site Code	ref.	Site ID	depth, cm	date collected	Soil pH	PTS	%TVS	%sand	%silt	%clay	total %fines	Microtox SPT EC50, mg dry sedmt/L diluent	Microtox SPT EC20, mg dry sedmt/L diluent	EC50 - %clay normalized	EC20 - %clay normalized
NEUr-LR	X	Little River @ SR 1416	4	5/20/2004	7.04	55.16	7.01	44.99	41.99	13.02	55.02	10,660	<2,142	1,358	<272.9
NEU-PC		Perry Crk at SR 2006	4	5/5/2004	7.11	66.60	1.59	85.12	12.52	2.52	15.05	>111,300	28,290	>2,746	698.0
NEU-CC		Core Crk @ NC55	6	5/11/2004	6.82	39.73	6.19	77.30	14.54	8.17	22.70	>79,760	12,360	> 6,374	987.7
NEU-SFR		S. Flat R. @ SR 1109	6	5/13/2004	7.02	67.12	3.73	61.15	28.23	10.62	38.85	20,730	<4,850	2,154	<503.9
NEU-KRC		Knap of Reeds Crk ds Butner	6	5/19/2004	7.02	74.50	1.80	86.86	9.36	3.78	13.14	>110,100	>101,600	>4,072	>3,758
CPFr-AC	X	Averts Crk @ SR 1418	5	6/16/2004	6.41	64.70	2.17	81.86	14.14	4.00	18.14	>93,250	19,660	>3,653	770.1
CPF-BR		Black River @ SR 1780	5	6/15/2004	5.32	46.04	7.15	38.32	47.96	13.73	61.69	>42,210	<30,070	>5,670	<4,039
ROAr-SnC	X	Snow Crk @ SR 1673	5	6/29/2004	6.37	69.20	1.63	92.83	3.55	3.61	7.16	55,020	11,000	1,943	388.6
ROA-SmC		Smith Crk @ US 1	6	6/21/2004	6.51	64.44	3.67	88.29	6.31	5.41	11.72	37,220	<11,400	1,968	<602.9
ROA-TFC		Town of Fork Crk @ SR 1973	5	6/23/2004	6.27	60.42	2.75	88.07	7.44	4.50	11.94	14,590	3,734	642.1	164.3
YADr-MR	x	Mitchell River @ SR1001	6	7/14/2004	6.30	64.80	4.88	86.19	7.69	6.12	13.81	>53,610	>9,017	>3,210	>540.0
YAD-HC		Heatherly Crk @ Hwy 268	5	7/13/2004	6.50	70.38	1.36	93.23	1.49	5.28	6.77	>140,500	>49,240	>7,259	>2,544
YAD-TC		Town Crk @ I-85	6	8/25/2004	7.21	59.37	3.70	73.48	17.30	9.23	26.53	22,910	9,674	2,069	873.5

Table 3. Site sediment toxicity classification. Site EC50 mg/L dry weight Microtox SPT data compared to toxicity classification guidelines.

					Env. Canada, 2002 [7]		Ringwood, *et al* [11]		Ringwood, *et al* [11] (more conservative)	
Site Code	ref.	Site ID	date collected	total %fines	<20% fines, toxic	≥20% fines, toxic	<20% fines	≥20% fines, toxic	<20% fines	≥20% fines, toxic
NEUr-LR	x	Little River @ SR 1416	5/20/2004	55.02		NO		NO		NO
NEU-PC		Perry Crk at SR 2006	5/5/2004	15.05	NO		NO		NO	
NEU-CC		Core Crk @ NC55	5/11/2004	22.70		NO		NO		NO
NEU-SFR		S. Flat R. @ SR 1109	5/13/2004	38.85		NO		NO		NO
NEU-KRC		Knap of Reeds Crk ds Butner	5/19/2004	13.14	NO		NO		NO	
CPFr-AC	x	Averts Crk @ SR 1418	6/16/2004	18.14	NO		NO		NO	
CPF-BR		Black River @ SR 1780	6/15/2004	61.69		NO		NO		NO
ROAr-SnC	x	Snow Crk @ SR 1673	6/29/2004	7.16	NO		NO		NO	
ROA-SmC		Smith Crk @ US 1	6/21/2004	11.72	NO		NO		NO	
ROA-TFC		Town of Fork Crk @ SR 1973	6/23/2004	11.94	NO		NO		NO	
YADr-MR	x	Mitchell River @ SR1001	7/14/2004	13.81	NO		NO		NO	
YAD-HC		Heatherly Crk @ Hwy 268	7/13/2004	6.77	NO		NO		NO	
YAD-TC		Town Crk @ I-85	8/25/2004	26.53		NO		NO		NO

ref = reference sediment site

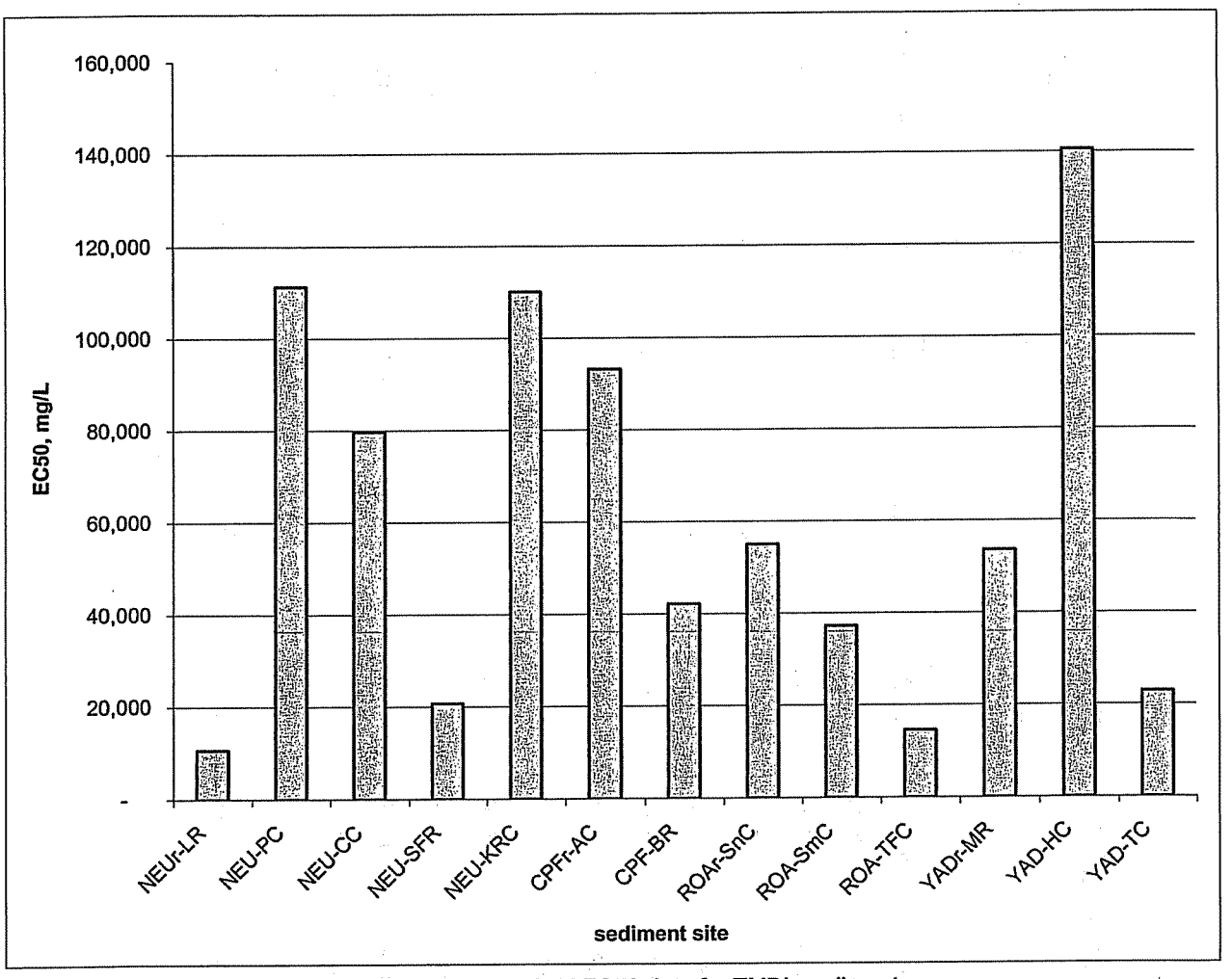

Figure 1. Dry weight normalized Microtox SPT EC50 data for TMDL sediments. Data as EC50 in mg/L dry weight sediment.

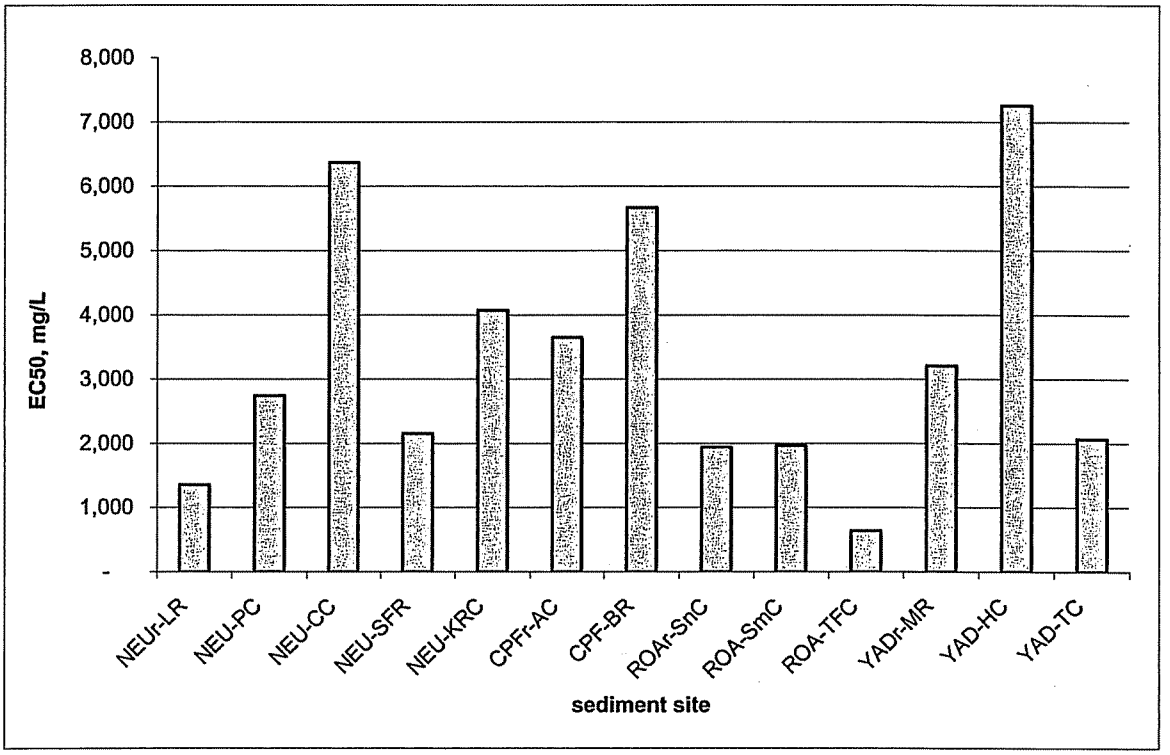

Figure 2. Percent clay-normalized Microtox SPT EC50 data for TMDL sediments. Data as EC50 in mg/L percent clay normalized sediment.

Table 4. Numerical rank of clay-normalized sediment EC50 Microtox SPT toxicity data. 1 = lowest EC50, 13 = highest EC50

Site Code	Basin	Site ID	rank
ROA-TFC	ROA	Town of Fork Crk @ SR 1973	1
NEUr-LR	NEU-reference	Little River @ SR 1416	2
ROAr-SnC	ROA- reference	Snow Crk @ SR 1673	3
ROA-SmC	ROA	Smith Crk @ US 1	4
YAD-TC	YAD	Town Crk @ I-85	5
NEU-SFR	NEU	S. Flat R. @ SR 1109	6
NEU-PC	NEU	Perry Crk at SR 2006	7
YADr-MR	YAD- reference	Mitchell River @ SR1001	8
CPFr-AC	CPF- reference	Averts Crk @ SR 1418	9
NEU-KRC	NEU	Knap of Reeds Crk ds Butner	10
CPF-BR	CPF	Black River @ SR 1780	11
NEU-CC	NEU	Core Crk @ NC55	12
YAD-HC	YAD	Heatherly Crk @ Hwy 268	13

Table 5. Sediment ecological screening values for metals.

metal	EPA R4, effects value	NOAA TEL	NOAA PEL	NOAA, lowest ARCs	NEU-PC	NEU-PC HQs	NEU-CC	NEU-CC HQs	NEU-SFR	NEU-SFR HQs	NEU-KRC	NEU-KRC HQs	CPF-BR	CPF-BR HQs
	mg/kg, dry wt.	mg/kg, dry wt.	mg/kg, dry wt.	mg/kg, dry wt.	mg/kg, dry wt.		mg/kg, dry wt.		mg/kg, dry wt.		mg/kg, dry wt.		mg/kg, dry wt.	
Al				25,500	1200	0.047	4800	0.19	3100	0.12	1500	0.059	13,000	0.51
As	7.24	5.9	17		0.33	0.056	0.68	0.12	0.56	0.095	0.86	0.051	0.80	0.14
Cd	0.676	0.596	3.53		<0.20	0.17	<0.2	0.17	<0.2	0.17	<0.20	0.17	0.25	0.42
Cr	52.3	37.3	90		2.2	0.059	4.2	0.11	8.2	0.22	46	0.51	8.8	0.24
Cu	18.7	35.7	197		2.4	0.067	1.2	0.034	3.6	0.10	2.90	0.015	14	0.39
Fe				188,400	4100	0.022	3300	0.018	5000	0.027	9500	0.050	9,600	0.051
Pb	30.2	35	91.3		2.3	0.066	9.4	0.27	7.3	0.21	3.5	0.038	40	**1.1**
Mn				630	280	0.44	45	0.071	180	0.29	160	0.25	100	0.16
Ni	15.9	18	35.9		0.71	0.039	1.3	0.072	1.9	0.11	2.8	0.078	3.1	0.17
Zn	124	123.1	315		12	0.097	9.0	0.073	10	0.081	14	0.044	53	0.43
ΣHQs						**1.07**		**1.12**		**1.41**		**1.27**		**3.65**

Sediment sample HQ calculations are calculated relative NOAA TEL screening values.

Table 5., continued

metal	EPA R4, effects value	NOAA TEL	NOAA PEL	NOAA, lowest ARCs	CPF-BR	CPF-BR HQs	ROA-SmC	ROA-SmC HQs	ROA-TFC	ROA-TFC HQs	YAD-HC	YAD-HC HQs	YAD-TC	YAD-TC HQs
	mg/kg, dry wt.	mg/kg, dry wt.	mg/kg, dry wt.	mg/kg, dry wt.	mg/kg, dry wt.		mg/kg, dry wt.		mg/kg, dry wt.		mg/kg, dry wt.		mg/kg, dry wt.	
Al				25,500	13,000	0.51	13000	0.51	7,400	0.29	8,600	0.34	4,400	0.17
As	7.24	5.9	17		0.80	0.14	1.4	0.24	0.42	0.071	0.50	0.085	1.1	0.19
Cd	0.676	0.596	3.53		0.25	0.42	<0.20	0.17	0.21	0.35	<0.20	0.17	<0.20	0.17
Cr	52.3	37.3	90		8.8	0.24	11	0.29	10	0.27	9.2	0.25	24	0.64
Cu	18.7	35.7	197		14	0.39	4.5	0.13	3.5	0.10	4.9	0.14	7.4	0.21
Fe				188,400	9,600	0.051	29000	0.15	24,000	0.13	18,000	0.10	13,000	0.069
Pb	30.2	35	91.3		40	**1.1**	16	0.46	5.9	0.17	8.8	0.25	7.4	0.21
Mn				630	100	0.16	370	0.59	460	0.73	290	0.46	230	0.37
Ni	15.9	18	35.9		3.1	0.17	4.1	0.23	2.1	0.12	3.3	0.18	3.3	0.18
Zn	124	123.1	315		53	0.43	16	0.13	29	0.24	23	0.19	45	0.37
ΣHQs						**3.65**		**2.89**		**2.46**		**2.15**		**2.57**

Table 6. Sediment ecological screening value data for PAHs.

PAH	USEPA Reg. 4 ESVs	NOAA SQuiRT TEL	NOAA SQuiRT ARCs TEL	CPF-BR, µg/kg	CPF-BR USEPA HQs	CPF-BR NOAA HQs	YAD-HC, µg/kg	YAD-HC USEPA HQs	YAD-HC NOAA HQs
phenanthrene	330	41.9		920	**2.79**	**22.0**			
LMW PAHs	330		76.42	920	**2.79**	**12.0**			
fluoranthene	330	111		2,100	**6.36**	**18.9**	280	0.85	**2.52**
pyrene	330	53		1,800	**5.45**	**34.0**	180	0.55	**3.40**
benzo(a)anthracene	330	31.7		680	**2.06**	**21.5**			
chrysene	330	57.1		1,100	**3.33**	**19.3**	130	0.39	**2.28**
benzo(b)fluoranthene	330	111.0		1,700	**5.15**	**15.3**	160	0.48	**1.44**
benzo(k)fluoranthene	330	111.0		490	**1.48**	**4.41**			
benzo(a)pyrene	330	31.9		850	**2.58**	**26.6**			
indeno(1,2,3-cd)pyrene	330	53		870	**2.64**	**16.4**			
benzo(g,h,i)perylene	330	53		710	**2.15**	**13.4**			
					ΣHQs =				
HMW PAHs	655		192.95	10,300	**15.7**	**53.4**	750	**1.15**	**3.89**
Total PAHs	1,684		264.05	11,220	**6.7**	**42.5**	750	0.45	**2.84**

Table 7. *Daphnia magna* feeding inhibition toxicity test data for surface waters collected at watershed integrator locations. Data as % surface water.

Site Code	Site ID	EC50	EC20
NEU-PC	Perry Crk at SR 2006	>100	89.0
NEU-CC	Core Crk @ NC55	>100	81.3
NEU-SFR	S. Flat R. @ SR 1109	>100	38.4
NEU-KRC	Knap of Reeds Crk ds Butner	>100	7.36
CPF-BR	Black River @ SR 1780	na	na
ROA-SmC	Smith Crk @ US 1	>100	82.2
ROA-TFC	Town of Fork Crk @ SR 1973	>100	>100
YAD-HC	Heatherly Crk @ Hwy 268	>100	>100
YAD-TC	Town Crk @ I-85	>100 (a)	>100 (a)

Notes:
na = not analyzed
(a) Sample analyzed at 5-days post-collection.

Table 8. Water column ecological screening values for metals.

metal	NC aq life stds	EPA R4, chronic SV	NOAA, CCC (chronic)	Env. CAN, 1999	NEU-PC	NEU-PC NOAA CCC HQ	NEU-CC	NEU-CC NOAA CCC HQ	NEU-SFR	NEU-SFR NOAA CCC HQ	NEU-KRC	NEU-KRC NOAA CCC HQ
	µg/L	µg/L	µg/L	µg/L		µg/L		µg/L		µg/L		
Al			87		930	**11**	640	**7.4**	380	**4.4**	55	0.63
As	50	190	150		<10	0.033	<10	0.033	<10	0.033	<10	0.033
Cd	2.0	0.66	2.2		<2.0	0.45	<2.0	0.45	<2.0	0.45	<2.0	0.45
Cr	50	11	11		<25	**1.1**	<25	**1.1**	<25	**1.1**	<25	**1.1**
Cu	7	6.54	9		<2.0	0.11	<2.0	0.11	<2.0	0.11	5.40	0.60
Fe	1000		1000		1200	**1.2**	1100	**1.1**	3300	**3.3**	630	0.63
Pb	25	1.32	2.5		<10	2.0	<10	**2.0**	<10	**2.0**	<10	**2.0**
Mn				50	180	NA	40	NA	960	NA	300	NA
Ni	88	87.71	52		<10	0.10	<10	0.10	<10	0.10	<10	0.10
Zn	50	58.91	120		<10	0.042	13	0.11	<10	0.067	25	0.21
				∑HQs =		**16**		**12**		**12**		**5.8**
				Env CAN Mn HQ =		**3.6**		0.80		**19**		**6.0**

Table 8., continued

metal	NC aq life stds	EPA R4, chronic SV	NOAA, CCC (chronic)	Env. CAN, 1999	CPF-BR	CPF-BR NOAA CCC HQ	ROA-SmC	ROA-SmC NOAA CCC HQ	ROA-TFC	ROA-TFC NOAA CCC HQ	YAD-HC	YAD-HC NOAA CCC HQ	YAD-TC	YAD-TC NOAA CCC HQ
		µg/L	µg/L	µg/L	µg/L		µg/L		µg/L		µg/L		µg/L	
Al			87		190	**2.2**	55	0.63	260	**3.0**	170	**1.95**	84	**1.0**
As	50	190	150		<10	0.033	<10	0.033	<10	0.033	<10	0.033	<10	0.033
Cd	2.0	0.66	2.2		<2.0	0.45	<2.0	0.45	<2.0	0.45	<2.0	0.45	<2.0	0.45
Cr	50	11	11		<25	**1.1**	<25	**1.1**	<25	**1.1**	<25	**1.1**	<25	**1.1**
Cu	7	6.54	9		2.1	0.23	<2.0	0.11	<2.0	0.11	<2.0	0.11	<2.0	0.11
Fe	1000		1000		1,900	**1.9**	5,500	**5.5**	550	0.55	630	0.63	340	0.34
Pb	25	1.32	2.5		<10	**2.0**	<10	**2.0**	<10	**2.0**	<10	**2.0**	<10	**2.0**
Mn				50	31	NA	3,200	NA	61	NA	38	NA	88	NA
Ni	88	87.71	52		<10	0.10	<10	0.10	<10	0.10	<10	0.10	<10	0.10
Zn	50	58.91	120		<10	0.042	<10	0.042	<10	0.042	<10	0.042	<10	0.042
				∑HQs =		**8.1**		**10**		**7.4**		**6.5**		**5.2**
				Env CAN Mn HQ =		0.62		**64**		**1.2**		0.76		**1.8**

References

1. Ankley, G.T., Di Toro, D.M., Hansen, D.J., Berry, W.J. 1996: Technical basis and proposal for deriving sediment quality criteria for metals. *Environmental Toxicology and Chemistry*: Vol. 15, No. 12, pp. 2056–2066.
2. Azur Environmental®. 1998. *Microtox Manual: Solid-Phase Test* (SPT). Carlsbad, CA.
3. Azur Environmental®. 2003. Microtox Omni™ Software for Windows® 95/98/NT, version 1.18.
4. Buchman, M.F., 1999. NOAA Screening Quick Reference Tables, NOAA HAZMAT Report 99-1, Seattle WA, Coastal Protection and Restoration Division, National Oceanic and Atmospheric Administration, 12 pages.
5. D.D. Macdonald, T. Berger, K. Wood, J. Brown, T. Johnsen, M.L. Haines, K. Brydges, M.J. Macdonald, S.L. Smith, and D.P. Shaw. 1999. A Compendium of Environmental Quality Benchmarks, Environment Canada, Georgia Basin Ecosystem Initiative, GBEI 99-01.
6. Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning: *Environ Toxicol Chem* 10:1541–1583.
7. Environment Canada. April 2002. Biological test method: reference method for determining the toxicity of sediment using luminescent bacteria in a solid-phase test. EPS 1/RM/42.
8. Environmental Sciences Branch. 2004. 2004 Stressor surveys – Yadkin River Basin, Heatherly Creek – subbasin 030703, Town Creek – subbasin 030704. Intensive Survey Unit.
9. McWilliam, RA, Baird DJ. 2002. Postexposure feeding depression: A new toxicity endpoint for use in laboratory studies with *Daphnia magna*. *Environ Toxicol Chem* 21:1198–1205.
10. NOAA. Microtox Solid-Phase Test (SPT) Ecotox SOP 00-017. December 2000.
11. Ringwood AH, DeLorenzo ME, Ross PE, Holland AF. 1997. Interpretation of Microtox® solid-phase toxicity tests: The effects of sediment composition: *Environ Toxicol Chem* 16:1135–1140.
12. Standard Methods for the Examination of Water and Wastewater, 20th edit. 1998. APHA, AWWA, WEF.
13. ToxCalc™ version 5.0. 1996. Tidepool Scientific Software, McKinleyville CA.
14. USEPA. 2001. Supplemental Guidance to RAGS: Region 4 Bulletins, Ecological Risk Assessment. Originally published November 1995. Website version last updated November 30, 2001: **http://www.epa.gov/region4/waste/ots/ecolbul.htm**.