METALS QA/QC

PROGRAM: SPAWAR, Task 19
PARAMETER: Metals
LABORATORY: Battelle/Marine Sciences Laboratory, Sequim, Washington
MATRIX: Stormwater

QA/QC DATA QUALITY OBJECTIVES

	Reference Method	Range of Recovery	SRM Accuracy	Relative Precision	Target Detection Limit (µg/L)
Aluminum	ICP/OES	50-150%	±20%	±50%	50.0
Iron	ICP/OES	50-150%	±20%	±50%	10.0
Manganese	ICP/OES	50-150%	±20%	±30%	0.5
Chromium	ICP/MS	50-150%	±20%	±30%	1.0
Nickel	ICP/MS	50-150%	±20%	±30%	0.05
Copper	ICP/MS	50-150%	±20%	±30%	0.05
Zinc	ICP/MS	50-150%	±20%	±30%	0.5
Arsenic	FIAS	50-150%	±20%	±30%	0.5
Selenium	FIAS	50-150%	±20%	±30%	0.2
Silver	GFAA	50-150%	±20%	±30%	0.5
Cadmium	ICP/MS	50-150%	±20%	±30%	0.05
Tin	ICP/MS	50-150%	±20%	±30%	0.5
Lead	ICP/MS	50-150%	±20%	±30%	0.05
Mercury	CVAF	50-150%	±25%	±30%	0.01

METHOD

Three (3) samples were analyzed for fourteen (14) metals: nickel (Ni), copper, (Cu), arsenic (As), selenium (Se), silver (Ag), cadmium (Cd), tin (Sn) and lead (Pb) by inductively coupled plasma mass spectroscopy (ICP/MS) following EPA Method 1638m, aluminum (Al), iron (Fe), chromium (Cr), manganese (Mn), and zind (Zn) by inductively coupled plasma optic emission spectroscopy following EPA Method 200.7 and mercury (Hg) by cold vapor atomic fluorescence (CVAF) following EPA Method 1631e.

Samples were preserved with nitric acid prior to arrival at MSL. Samples analyzed for Hg by CVAF were pre-treated with bromine chloride and stannous chloride to oxidize and convert all Hg compounds to volatile Hg, which is subsequently trapped onto a gold-coated sand trap.

HOLDING TIMES

Three (3) samples were received on 2/11/2005 and were logged into Battelle's sample tracking system. The samples were analyzed within the six month holding time for metals and 90 days for Hg. The following list summarizes all analysis dates:

Task	Date Performed
Hg	2/23/05
ICP-MS	2/22/05
ICP-OES	3/1 & 4/05

DETECTION LIMITS

The target detection limit was met for all metals, except Ni, Cu, Se and Cd. The MDL for seawater analysis by dilution is somewhat higher than

our typical MDL's for direct analysis. Sample concentrations were substantially greater than the MDL, except Se. All Se results were less than our MDL for this method. The method detection limit was met for all metals. An MDL is determined by multiplying the standard deviation of the results of a minimum of 7 replicate low level spikes by the Student's t value at the 99th percentile.

METHOD BLANKS

One method blank was analyzed with this batch of samples. Results were less than 3 times the MDL for all metals, except the TRM blank for Zn. The TRM field sample was greater than 10 x the blank concentration and therefore was not impacted by the blank contamination.

BLANK SPIKES

One sample of reagent water was spiked at several levels with metals. Recoveries were within the QC limits of 50-150% for all metals.

MATRIX SPIKES

One sample was spiked at several levels with metals. Recoveries were within the QC limits of 50-150% for all metals.

REPLICATES

One sample was analyzed in duplicate. All results were within the QC limits of ±30% (±50% for Al and Fe).

SRM

One matrix-appropriate standard reference material (SRM) was analyzed for each method; 1641d, river water, and 1640, natural water, obtained from the National Institute of Science and Technology.

SRM 1640 has 22 certified and reference metals. Recovery for all metals reported were within the control limit of ±20% of the certified or reference value. Tin and Hg are not certified in 1640. SRM 1641d is certified for Hg. Recovery for Hg was within the control limit of ±25% of the certified value.

REFERENCES

EPA. 1991. Methods for the Determination of Metals in Environmental Samples. EPA-600/4- 91-010. Environmental Services Division, Monitoring Management Branch.

METALS QA/QC

MSL Code	Rep	Sponsor I.D.	Al (µg/L) ICP-OES	Fe (µg/L) ICP-OES	Cr (µg/L) ICP-OES	Mn (µg/L) ICP-OES	Ni (µg/L) ICP-MS	Cu (µg/L) ICP-MS	Zn (µg/L) ICP-OES	As (µg/L) ICP-MS	Se (µg/L) ICP-MS	Ag (µg/L) ICP-MS	Cd (µg/L) ICP-MS	Sn (µg/L) ICP-MS	Pb (µg/L) ICP-MS	Hg (µg/L) CVAF
PROCEDURAL BLANK																
		Dissolved	3.36 U	2.51 U	0.155	0.025 U	0.074 U	0.883 U	0.283	0.158 U	1.47 U	0.04 U	0.054 U	0.50 U	0.009 U	0.00017 U
		Dissolved - OES reanalysis	3.36 U	2.51 U	0.119 U	0.025 U	N/A	N/A	0.113 U	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		TRM	3.36 U	2.51 U	0.119 U	0.025 U	0.074 U	0.883 U	0.705 b	0.158 U	1.47 U	0.04 U	0.054 U	0.50 U	0.009 U	N/A
METHOD DETECTION LIMIT			**3.36**	**2.51**	**0.119**	**0.025**	**0.074**	**0.883**	**0.113**	**0.158**	**1.47**	**0.040**	**0.054**	NA	**0.009**	**0.00012**
Project Target Detection Limit			50.0	10.0	1.00	0.50	0.05	0.05	0.50	0.50	0.20	0.50	0.05	0.50	0.05	0.01
STANDARD REFERENCE MATERIAL																
1640		Dissolved	52.8	36.3	37.4	125	26.9	83.9	54.7	28.9	26.2	7.57	24.1	1.63	29.0	NA
1640		Dissolved - OES reanalysis	54.6	34.4	39.0	123	N/A	N/A	54.1	N/A	N/A	N/A	N/A	N/A	N/A	NA
1640		TRM	N/A	N/A	N/A	N/A	26.7	82.3	N/A	25.7	21.1	7.42	22.2	1.71	31.4	NA
1640		certified/reference value	52.0	34.3	38.6	122	27.4	85.2	53.2	26.7	22.0	7.62	22.8	NC	27.9	NC
1640		range	±1.5	±1.6	±1.6	±1.1	±0.8	±1.2	±1.1	±0.73	±0.51	±0.25	±0.96	NC	±0.14	NC
		% difference	2%	6%	3%	2%	2%	2%	3%	8%	19%	1%	6%	N/A	4%	N/A
		% difference	5%	0%	1%	1%	N/A	N/A	2%	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		% difference	N/A	N/A	N/A	N/A	3%	3%	N/A	4%	4%	3%	3%	N/A	13%	N/A
1641d			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1497
1641d		certified value	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	1590
1641d		range	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	±18.0
		% difference	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	6%
ICV,CCV RESULTS																
ICV			99%	101%	99%	100%	100%	101%	101%	98%	100%	101%	100%	104%	101%	95%
CCV			99%	102%	98%	99%	101%	101%	100%	99%	99%	102%	99%	104%	105%	98%
CCV			101%	105%	98%	98%	98%	98%	100%	97%	97%	100%	99%	101%	107%	NA
CCV			100%	104%	98%	98%	96%	98%	100%	97%	96%	99%	97%	99%	109%	NA
CCV			NA	NA	NA	NA	96%	97%	NA	98%	96%	100%	100%	102%	108%	NA
ICV		OES reanalysis	98%	100%	102%	101%	100%	101%	103%	98%	100%	101%	100%	104%	101%	NA
CCV		OES reanalysis	100%	102%	99%	96%	101%	101%	100%	99%	99%	102%	99%	104%	105%	NA
CCV		OES reanalysis	100%	99%	100%	97%	98%	98%	100%	97%	97%	100%	99%	101%	107%	NA
CCV		OES reanalysis	99%	100%	100%	97%	96%	98%	100%	97%	96%	99%	97%	99%	109%	NA
BLANK SPIKE RESULTS																
		Amount Spiked	100	100	50.0	100	10.0	50.0	50.0	10.0	10.0	10.0	10.0	10.0	10.0	0.00472
		Blank	3.36 U	2.51 U	0.155	0.025 U	0.074 U	0.883 U	0.283	0.158 U	1.47 U	0.04 U	0.054 U	0.50 U	0.009 U	0.000407
		Blank + Spike	95.8	108	53.9	125	9.80	50.1	56.7	9.88	9.96	10.3	10.1	10.2	11.2	0.00484
		Amount Recovered	95.8	108	53.7	125	9.80	50.1	56.4	9.88	9.96	10.3	10.1	10.2	11.2	0.00443
		Percent Recovery	96%	108%	107%	125%	98%	100%	113%	99%	100%	103%	101%	102%	112%	94%
MATRIX SPIKE RESULTS																
		Amount Spiked	100	50.0	50.0	50.0	NS	NS	50.0	NS	NS	NS	NS	NS	NS	NS
		NI-OF23A-SDB6-FF (D) + Spike	17.1	20.4	1.02	0.154	N/A	N/A	134	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		NI-OF23A-SDB6-FF (D) + Spike	119	74.2	56.9	54.0	NA	NA	189	NA	NA	NA	NA	NA	NA	NA
		Amount Recovered	102	53.8	55.9	53.8	N/A	N/A	55.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Percent Recovery	102%	108%	112%	108%	N/A	N/A	110%	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Amount Spiked	NS	NS	NS	NS	10.0	50.0	NS	10.0	10.0	10.0	10.0	100.0	10.0	0.0102
		NI-OF23A-SDB6-FF (T)	N/A	N/A	N/A	N/A	3.83	49.4	N/A	1.22	1.47 U	0.0308	0.552	0.251	3.78	0.0118
		NI-OF23A-SBD6-FF (T) + Spike	NA	NA	NA	NA	13.6	102	NA	11.3	11.5	9.72	10.4	95.3	14.9	0.0196
		Amount Recovered	N/A	N/A	N/A	N/A	10	52.6	N/A	10.1	11.5	9.69	9.85	95.0	11.1	0.00780
		Percent Recovery	N/A	N/A	N/A	N/A	98%	105%	N/A	101%	115%	97%	98%	95%	111%	76%
REPLICATE RESULTS																
2360*1	1	NI-OF23A-SDB6-FF (D)	17.1	20.4	1.02	0.154	3.45	42.6	134	0.968	1.47 U	0.04 U	0.369	0.50 U	0.201	0.00593
2360*1	2	NI-OF23A-SDB6-FF (D)	17.6	19.4	1.06	0.153	NA	NA	133	NA	NA	NA	NA	NA	NA	0.00600
		RPD	3%	5%	6%	1%	N/A	N/A	1%	N/A	N/A	N/A	N/A	N/A	N/A	1%
2360*3	1	NI-OF23A-SDB6-FF (T)	290	388	1.47	15.1	3.83	49.4	185	1.22	1.47 U	0.04 U	0.552	0.50 U	3.78	0.0118
2360*3	2	NI-OF23A-SDB6-FF (T)	NA	NA	NA	NA	3.71	48.6	NA	1.15	1.47 U	0.0444	0.541	0.50 U	3.85	NA
		RPD	N/A	N/A	N/A	N/A	3%	2%	N/A	6%	N/A	N/A	2%	N/A	2%	N/A

U = not detected at or above detection limit; NC= not certified; N/A = not applicable; b= Sample results are less than 3x the blank.

METALS QA/QC (CONT.)

MSL Code	Rep	Sponsor I.D.	Al (µg/L) ICP-OES	Fe (µg/L) ICP-OES	Cr (µg/L) ICP-OES	Mn (µg/L) ICP-OES	Ni (µg/L) ICP-MS	Cu (µg/L) ICP-MS	Zn (µg/L) ICP-OES	As (µg/L) ICP-MS	Se (µg/L) ICP-MS	Ag (µg/L) ICP-MS	Cd (µg/L) ICP-MS	Sn (µg/L) ICP-MS	Pb (µg/L) ICP-MS	Hg (µg/L) CVAF
PROCEDURAL BLANK																
		Dissolved	3.36 U	2.51 U	0.155	0.025 U	0.074 U	0.883 U	0.283	0.158 U	1.47 U	0.040 U	0.054 U	0.50 U	0.009 U	0.00017 U
		Dissolved Hg reanalysis	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.00017 U
		Dissolved - OES reanalysis	3.36 U	2.51 U	0.119 U	0.025 U	N/A	N/A	0.113 U	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		TRM	3.36 U	2.51 U	0.119 U	0.025 U	0.074 U	0.883 U	0.705 b	0.158 U	1.47 U	0.040 U	0.054 U	0.50 U	0.009 U	N/A
METHOD DETECTION LIMIT			3.36	2.51	0.119	0.025	0.074	0.883	0.113	0.158	1.47	0.040	0.054	NA	0.009	0.00012
Project Target Detection Limit			50.0	10.0	1.00	0.50	0.05	0.05	0.50	0.50	0.20	0.50	0.05	0.50	0.05	0.01
STANDARD REFERENCE MATERIAL																
1640		Dissolved	52.8	36.3	37.4	125	26.9	83.9	54.7	28.9	26.2	7.57	24.1	1.63	29.0	NA
1640		Dissolved - OES reanalysis	54.6	34.4	39.0	123	N/A	N/A	54.1	N/A	N/A	N/A	N/A	N/A	N/A	NA
1640		TRM	N/A	N/A	N/A	N/A	26.7	82.3	N/A	25.7	21.1	7.42	22.2	1.71	31.4	NA
1640		certified/reference value	52.0	34.3	38.6	122	27.4	85.2	53.2	26.7	22.0	7.62	22.8	NC	27.9	NC
1640		range	±1.5	±1.6	±1.6	±1.1	±0.8	±1.2	±1.1	±0.73	±0.51	±0.25	±0.96	NC	±0.14	NC
		% difference	2%	6%	3%	3%	2%	2%	3%	8%	19%	1%	6%	N/A	4%	N/A
		% difference	5%	0%	1%	1%	N/A	N/A	2%	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		% difference	N/A	N/A	N/A	N/A	3%	3%	N/A	4%	4%	3%	3%	N/A	13%	N/A
1641d			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1497
1641d		Hg reanalysis	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1544
1641d		certified value	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	1590
1641d		range	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	±18.0
		% difference	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	6%
		% difference	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3%
ICV,CCV RESULTS																
ICV			99%	101%	99%	100%	100%	101%	101%	98%	100%	101%	100%	104%	101%	95%
CCV			99%	102%	98%	99%	101%	101%	100%	99%	99%	102%	99%	104%	105%	98%
CCV			101%	105%	98%	98%	98%	98%	100%	97%	97%	100%	99%	101%	107%	92%
CCV			100%	104%	98%	98%	96%	98%	100%	97%	96%	99%	97%	99%	109%	NA
CCV			NA	NA	NA	NA	96%	97%	NA	98%	96%	100%	100%	102%	108%	NA
ICV		Hg reanalysis	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	96%
CCV		Hg reanalysis	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	105%
ICV		OES reanalysis	98%	100%	102%	101%	100%	101%	103%	98%	100%	101%	100%	104%	101%	NA
CCV		OES reanalysis	100%	102%	99%	96%	101%	101%	100%	99%	99%	102%	99%	104%	105%	NA
CCV		OES reanalysis	100%	99%	100%	97%	98%	98%	100%	97%	97%	100%	99%	101%	107%	NA
CCV		OES reanalysis	99%	100%	100%	97%	96%	98%	100%	97%	96%	99%	97%	99%	109%	NA
BLANK SPIKE RESULTS																
		Amount Spiked	100	100	50.0	100	10.0	50.0	50.0	50.0	50.0	10.0	10.0	10.0	10.0	0.00472
		Blank	3.36 U	2.51 U	0.155	0.025 U	0.074 U	0.883 U	0.283	0.158 U	1.47 U	0.04 U	0.054 U	0.50 U	0.009 U	0.000407
		Blank + Spike	95.8	108	53.9	125	9.80	50.1	56.7	48.8	49.5	10.3	10.1	10.2	11.2	0.00484
		Amount Recovered	95.8	108	53.7	125	9.80	50.1	56.4	48.8	49.5	10.3	10.1	10.2	11.2	0.00443
		Percent Recovery	96%	108%	107%	125%	98%	100%	113%	98%	99%	103%	101%	102%	112%	94%
MATRIX SPIKE RESULTS																
		Amount Spiked	NS	NS	NS	NS	10.0	50.0	NS	50.0	50.0	10.0	10.0	100	10.0	NS
		NI-OF26-SDB6-COMP	N/A	N/A	N/A	N/A	4.62	29.1	N/A	11.0	38.3	0.04 U	0.791	0.50 U	0.512	N/A
		NI-OF26-SDB6-COMP + Spike	NS	NS	NS	NS	14.3	72.3	NS	57.6	83.8	8.67	10.1	94.4	9.86	NS
		Amount Recovered	N/A	N/A	N/A	N/A	9.68	43.2	N/A	46.6	45.5	8.67	9.31	94.4	9.35	N/A
		Percent Recovery	N/A	N/A	N/A	N/A	97%	86%	N/A	93%	91%	87%	93%	94%	93%	N/A
		Amount Spiked	100	50.0	50.0	50.0	NS	NS	50.0	NS	NS	NS	NS	NS	NS	NS
		NAB-OF9-SDB6-COMP	13.9	31.5	1.18	59.6	N/A	N/A	356	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		NAB-OF9-DB6-COMP + Spike	120	80.6	54.1	111	NS	NS	412	NS	NS	NS	NS	NS	NS	NS
		Amount Recovered	106	49.1	52.9	51.4	N/A	N/A	56.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Percent Recovery	106%	98%	106%	103%	N/A	N/A	112%	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Amount Spiked	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.0103
		NAB-OF9-SDB6-COMP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.00838
		NAB-OF9-SDB6-COMP + Spike	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.0187
		Amount Recovered	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.0103
		Percent Recovery	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	100%
REPLICATE RESULTS																
2157*9	1	NI-OF26-SDB6-COMP (D)	19.8	22.1	1.31	7.12	4.62	29.1	36.6	11.0	38.3	0.04 U	0.791	0.50 U	0.512	0.00213
2157*9	2	NI-OF26-SDB6-COMP (D)	20.9	17.1	1.17	7.04	4.76	28.7	36.9	11.0	40.8	0.04 U	0.746	0.50 U	0.463	NA
		RPD	5%	26%	11%	1%	3%	1%	1%	0%	6%	N/A	6%	N/A	10%	N/A
2157*13	1	NAB-OF9-SDB6-COMP (T)	192	847	2.11	71.3	4.37	59.5	522	4.93	14.1	0.04 U	0.551	0.50 U	3.21	0.00838
2157*13	2	NAB-OF9-SDB6-COMP (T)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00815
		RPD	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3%

U = not detected at or above detection limit; NC= not certified; N/A = not applicable; b= Sample results are less than 3x the blank.

PAHs

CLIENT ID	NI-OF23A-SDB6-FF		NI-BAY23A-SDB6-PRE		NI-BAY23A-SDB6-DUR		NI-OF26-SDB6-FF		NI-OF26-SDB6-COMP		NI-BAY26-SDB6-PRE		NI-BAY26-SDB6-DUR	
Battelle ID	S7115-P		S7116-P		S7117-P		S7111-P		S7112-P		S7113-P		S7114-P	
Sample Type	SA		SA		SA		SA		SA		SA		SA	
Collection Date	02/11/05		02/11/05		02/11/05		02/11/05		02/11/05		02/11/05		02/11/05	
Extraction Date	02/17/05		02/17/05		02/17/05		02/17/05		02/17/05		02/17/05		02/17/05	
Analysis Date	02/25/05		02/26/05		03/05/05		03/06/05		03/06/05		02/25/05		03/06/05	
Analytical Instrument	MS		MS		MS		MS		MS		MS		MS	
% Moisture	NA		NA		NA		NA		NA		NA		NA	
% Lipid	NA		NA		NA		NA		NA		NA		NA	
Matrix	WATER		WATER		WATER		WATER		WATER		WATER		WATER	
Sample Size	2.60		2.64		2.63		2.62		2.62		2.62		2.60	
Size Unit-Basis	L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID	
Units	NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID	
Naphthalene	12.63		0.76	J	1.73	J	115.33		67.79		0.72	J	2.18	J
C1-Naphthalenes	10.02		0.5	U	0.5	U	566.36		305.92		0.51	U	1.38	J
C2-Naphthalenes	11.68		0.5	U	0.5	U	1568.64		770.25		0.51	U	14.22	
C3-Naphthalenes	51.43		0.5	U	0.5	U	1695.7		836.17		0.51	U	43.47	
C4-Naphthalenes	11.93		0.5	U	0.5	U	1198.25		615.36		0.51	U	68.21	
2-Methylnaphthalene	10.41		0.36	U	0.36	U	550.31		289.36		0.36	U	1.15	J
1-Methynaphthalene	6.27		0.38	U	0.38	U	422.55		235.3		0.38	U	1.29	J
Biphenyl	1.81	J	0.47	U	0.47	U	113.71		29.82		0.47	U	0.48	U
2,6-dimethylnaphthalene	2.99	J	0.63	U	0.63	U	790.77		369.96		0.63	U	2.81	J
Acenaphthylene	0.54	U	0.53	U	11.52		0.54	U	0.54	U	0.54	U	3.71	
Acenaphthene	8.29		0.57	U	4.39		70.26		40.82		0.57	U	4.76	
2,3,5-trimethylnaphthalene	0.45	U	0.44	U	0.44	U	212.45		81.47		0.44	U	3.94	
Dibenzofuran	1.31	J	0.23	U	8.32		90.86		47.54		0.23	U	3.96	
Fluorene	3.07	J	0.52	U	2.88	J	142.16		79.66		0.52	U	3.65	
C1-Fluorenes	3.81		0.52	U	0.52	U	421.13		209.69		0.52	U	14.89	
C2-Fluorenes	21.57		0.52	U	0.52	U	634.23		333.91		0.52	U	57.66	
C3-Fluorenes	19.5		0.52	U	0.52	U	754.05		315.52		0.52	U	39.6	
Anthracene	1.93	J	0.38	U	31.98		79.35		31.18		0.39	U	12.51	
Phenanthrene	14.59		0.82	U	64.16		343.48		221.11		0.82	U	56.55	
C1-Phenanthrenes/Anthracenes	13.21		0.82	U	14.24		704.6		411.35		0.82	U	40.35	
C2-Phenanthrenes/Anthracenes	29.91		0.82	U	6.06		856.47		492.7		0.82	U	85.08	
C3-Phenanthrenes/Anthracenes	16.53		0.82	U	3.16	J	362.13		234.78		0.82	U	47.32	
C4-Phenanthrenes/Anthracenes	5.94		0.82	U	0.82	U	91.94		71.35		0.82	U	13.8	
1-Methylphenanthrene	3.55		0.46	U	3.48		205.09		109.38		0.46	U	13.4	
Dibenzothiophene	11.22		0.38	U	13.72		161.69		87.1		0.38	U	11.36	
C1-Dibenzothiophenes	16.5		0.38	U	2.29	J	309.2		163.27		0.38	U	18.55	
C2-Dibenzothiophenes	45.95		0.38	U	3.42		593.52		331.39		0.38	U	66.96	
C3-Dibenzothiophenes	41.28		0.38	U	0.38	U	402.74		255.88		0.38	U	54.3	
C4-Dibenzothiophenes	22.32		0.38	U	0.38	U	134.77		92.93		0.38	U	22.56	
Fluoranthene	11.91		3.2		295.63		765.03		291.07		3.62		235.42	
Pyrene	17.65		1.7	J	156.21		579.54		254.27		1.95	J	194.17	
C1-Fluoranthenes/Pyrenes	7.88		0.68	U	24		150.39		84.4		0.68	U	44.05	
C2-Fluoranthenes/Pyrenes	5.73		0.68	U	0.68	U	0.68	U	110.12		0.68	U	0.69	U
C3-Fluoranthenes/Pyrenes	0.69	U	0.68	U	0.68	U	0.68	U	39.92		0.68	U	0.69	U
Benzo(a)anthracene	1.58	J	1.03	U	15.4		93.72		46.25		1.39	J	33.23	
Chrysene	7.43		0.91	J	97.16		527.33		207.88		1.18	J	159.79	
C1-Chrysenes	5.36		0.45	U	6.42		96.6		45.97		0.45	U	27.54	
C2-Chrysenes	0.45	U	0.45	U	0.45	U	50.07		27.98		0.45	U	13.08	
C3-Chrysenes	0.45	U	0.45	U	0.45	U	0.45	U	0.45	U	0.45	U	0.45	U
C4-Chrysenes	0.45	U	0.45	U	0.45	U	0.45	U	0.45	U	0.45	U	0.45	U
Benzo(b)fluoranthene	2.33	J	0.88	U	65.26		581.72		230.54		0.89	U	153.21	
Benzo(j/k)fluoranthene	3.12	J	0.99	U	32.81		525.64		221.43		1	U	156.77	
Benzo(e)pyrene	4.05		0.39	U	30.72		442.13		186.04		0.39	U	126.91	
Benzo(a)pyrene	1.23	J	0.76	U	10.14		289.74		127.12		0.77	U	88.87	
Perylene	1.48	U	1.46	U	1.47	U	54.79		26.65		1.47	U	16.24	
Indeno(1,2,3-cd)pyrene	2.11	J	0.75	U	11		390.05		138.72		0.76	U	109.14	
Dibenz(a,h)anthracene	1.54	J	0.63	U	2.3	J	68.08		32.03		0.64	U	19.46	
Benzo(g,h,i)perylene	6.65		0.75	U	10.66		547.44		213.93		0.76	U	135.82	
Surrogate Recoveries (%)														
Naphthalene-d8	46		55		57		49		43		46		52	
Phenanthrene-d10	75		66		80		59		45		60		68	
Chrysene-d12	63		66		77		54		43		60		65	

PAHS QA/QC

PROJECT: Task Order TO0015/TO0019 – Contaminant Analysis of Stormwater
PARAMETER: PAH
LABORATORY: Battelle, Duxbury, MA
MATRIX: Water
SAMPLE CUSTODY: Water samples were collected 2/11/05. The samples were received at Battelle Duxbury on 2/15/05. Upon arrival, the cooler temperatures ranged from 0.8°C – 3.7°C. No custody issues were noted. Samples were logged into the Battelle LIMS and received unique IDs. Samples were stored in the access-controlled upper cold room refrigerator at 4.0°C until sample preparation could begin. Samples were extracted as one analytical batch, 05-0056, along with the appropriate quality control samples.

	Reference Method	Method Blank	Surrogate Recovery	LCS/MS Recovery	SRM % Diff.	Sample Replicate Relative Precision	Detection Limits (ng/L)
PAH	General NS&T	<5xMDL	40-120% Recovery	40-120% Recovery (target spike must be >5 x native conc.)	≤30% PD on average (for analytes >5x MDL)	≤30% RPD (calculated between the MS and MSD samples)	MDL: ~0.47 – 1.93

METHOD: Water samples were extracted for PAH following general NS&T methods. Approximately 1 liter of water was spiked with surrogates and extracted three times with dichloromethane using separatory funnel techniques. The combined extract was dried over anhydrous sodium sulfate, concentrated, processed through alumina cleanup column, concentrated, and further purified by GPC/HPLC. The post-HPLC extract was concentrated, fortified with RIS and split quantitatively for the required analyses. Extracts intended for PAH were analyzed using gas chromatography/mass spectrometry (GC/MS), following general NS&T methods. Sample data were quantified by the method of internal standards, using the Recovery Internal Standard (RIS) compounds.

HOLDING TIMES: Samples were prepared for analysis in one analytical batch and were extracted within 7 days of sample collection and analyzed within 40 days of extraction.

Batch	Extraction Date	Analysis Date
05-0056	2/17/05	2/25/05 – 3/6/05

BLANK: A procedural blank (PB) sample was prepared with the analytical batch. Procedural blank samples are analyzed to ensure the sample extraction and analysis methods are free of contamination.

05-0056 – No exceedences noted.

Comments – No target analytes were detected above the laboratory control limit (>5 x MDL), however naphthalene was detected in the procedural blank at a concentration less than the reporting limit (RL). The data was qualified with a "J" in

the procedural blank. Any authentic field sample naphthalene concentrations that are greater than the reporting limit but less than five times the concentration detected in the associated blank, were qualified with a "B". This resulted in three samples having "B" qualified naphthalene data; S7118 (OF-NAB9-SDB6-FF), S7122 (OF-NAB18-SDB6-FF), and S7125 (BAY-NAB18-SD86-D). No further corrective action was taken.

LABORATORY CONTROL SAMPLE: A laboratory control sample (LCS) was prepared with each analytical batch. The percent recoveries of target PAH were calculated to measure data quality in terms of accuracy.

05-0056 – All target analytes were recovered within the laboratory control limits (40-120%).

Comments – None.

MATRIX SPIKE/MATRIX SPIKE DUPLICATE: A matrix spike (MS) and a matrix spike duplicate (MSD) sample pair were prepared with each analytical batch. The percent recoveries of target PAH and the relative percent difference between the two samples were calculated to measure data quality in terms of accuracy and precision.

05-0056 – All target analytes were recovered within the laboratory control limits specified by the client (40-120%). All calculated RPDs were within the laboratory control limit ($\leq$ 30%).

Comments – None

SRM: A standard reference material (SRM, a certified second source standard was spiked into a natural seawater as an SRM) was prepared with each analytical batch. Surrogate corrected data has been reported for the SRM only.

05-0056 – All target analytes were recovered within the laboratory control limits specified by the client ($\leq$ 30 PD).

Comments – None.

SURROGATES: Three surrogate compounds were added prior to extraction, including naphthalene-d8, phenanthrene-d10, and chrysene-d12. The recovery of each surrogate compound was calculated to measure data quality in terms of accuracy (extraction efficiency).

05-0056 – Two exceedences noted.

Comments – Percent recoveries for all surrogate compounds were within the laboratory control limits specified by the method (40 – 120% recovery), except for naphthalene-d8 and chrysene-d12 in sample S7118 (OF-NAB9-SDB6-FF). The recoveries for these compounds were calculated to be 32% and 39%, respectively. Chromatography and calculations were reviewed. No discrepancies were found. The exceedences were qualified with an "N". No further corrective action taken.

CALIBRATIONS: The GC/MS is calibrated with a minimum of a 5 level curve. The RSD between response factors for the individual target analytes must be <25%. Each batch of samples analyzed is bracketed by a calibration check sample, run at a frequency of minimally every 10 samples. This PD between the initial calibration RF and the check should be <25% for individual analytes.

04-0103 – No calibration exceedences.

Comments – None.

PAHS QA/QC

CLIENT ID	LABORATORY CONTROL SAMPLE				MATRIX SPIKE NAB-OF18-SDB6-COMP			MATRIX SPIKE DUPLICATE-NAB-OF18-SDB6-COMP					PROCEDURAL BLANK	CLIENT ID	GG73: PCB/PESTICIDE SRM SOLUTION			
Battelle ID	BF876LCS-P				S7123MS-P			S7123MSD-P					BF875PB-P	Battelle ID	BF877SRM-P			
Sample Type	LCS				MS			MSD					PB	Sample Type	SRM			
Collection Date	02/17/05				2/11/2005			2/11/2005					02/17/05	Collection Date	02/17/05			
Extraction Date	02/17/05				2/17/2005			2/17/2005					02/17/05	Extraction Date	02/17/05			
Analysis Date	02/25/05				3/6/2005			3/6/2005					02/25/05	Analysis Date	02/25/05			
Analytical Instrument	MS				MS			MS					MS	Analytical Instrument	MS			
% Moisture	NA				NA			NA					NA	% Moisture	NA			
% Lipid	NA				NA			NA					NA	% Lipid	NA			
Matrix	LIQUID				WATER			WATER					LIQUID	Matrix	LIQUID			
Sample Size	2.00				0.825			0.825					2.00	Sample Size	2.00			
Size Unit-Basis	L_LIQUID				L_LIQUID			L_LIQUID					L_LIQUID	Size Unit-Basis	L_LIQUID			
Units	NG/L_LIQUID		Target	% Recovery	NG/L_LIQUID	Target	% Recovery	NG/L_LIQUID	Target	% Recovery	RPD (%)		NG/L_LIQUID	Units	NG/L_LIQUID	Certified Range	% Difference	
Naphthalene	579.24		1000.60	58	1379.31	2425.70	57	1415.95	2425.70	58	1.7		0.94	J Naphthalene	1064.46	1000.60 - 1000.60	6.4	
C1-Naphthalenes	0.66	U			1758.57			1835.46					0.66	U C1-Naphthalenes	0.66	U		
C2-Naphthalenes	0.66	U			1.61	U		1.61	U				0.66	U C2-Naphthalenes	0.66	U		
C3-Naphthalenes	0.66	U			1.61	U		1.61	U				0.66	U C3-Naphthalenes	0.66	U		
C4-Naphthalenes	0.66	U			1.61	U		1.61	U				0.66	U C4-Naphthalenes	0.66	U		
2-Methylnaphthalene	604.62		1002.00	60	1550.81	2429.09	64	1622.32	2429.09	67	4.6		0.47	U 2-Methylnaphthalene	891.98	1002.00 - 1002.00	11.0	
1-Methylnaphthalene	578.63		1001.20	58	1441.76	2427.15	59	1524.17	2427.15	63	6.6		0.5	U 1-Methylnaphthalene	855.14	1001.20 - 1001.20	14.6	
Biphenyl	587.69		1000.20	59	1683.06	2424.73	69	1779.39	2424.73	73	5.6		0.62	U Biphenyl	861.16	1000.20 - 1000.20	13.9	
2,6-dimethylnaphthalene	614.44		1001.00	61	1620.23	2426.67	67	1724.13	2426.67	71	5.8		0.83	U 2,6-dimethylnaphthalene	909.31	1001.00 - 1001.00	9.2	
Acenaphthylene	597.78		1000.65	60	1497.71	2425.82	62	1600.06	2425.82	66	6.3		0.7	U Acenaphthylene	877.83	1000.65 - 1000.65	12.3	
Acenaphthene	616.18		1000.75	62	1505.37	2426.06	62	1607.01	2426.06	66	6.3		0.75	U Acenaphthene	918.77	1000.75 - 1000.75	8.2	
2,3,5-trimethylnaphthalene	602.88		1000.30	60	1629.66	2424.97	67	1767.26	2424.97	73	8.6		0.58	U 2,3,5-trimethylnaphthalene	890.92	1000.30 - 1000.30	10.9	
Dibenzofuran	621.82		1002.20	62	1865.44	2429.58	77	2020.42	2429.58	83	7.5		0.3	U Dibenzofuran	933.56	1002.20 - 1002.20	5.8	
Fluorene	620.55		1000.70	62	1697.98	2425.94	70	1848.13	2425.94	76	8.2		0.68	U Fluorene	916.71	1000.70 - 1000.70	8.4	
C1-Fluorenes	0.68	U			1.65	U		1.65	U				0.68	U C1-Fluorenes	0.68	U		
C2-Fluorenes	0.68	U			1.65	U		1.65	U				0.68	U C2-Fluorenes	0.68	U		
C3-Fluorenes	0.68	U			1.65	U		1.65	U				0.68	U C3-Fluorenes	0.68	U		
Anthracene	703.01		1000.65	70	1819.86	2425.82	75	2059.39	2425.82	85	12.5		0.51	U Anthracene	1037	1000.65 - 1000.65	3.6	
Phenanthrene	677.73		1000.65	68	1837.78	2425.82	75	2059.16	2425.82	84	11.3		1.08	U Phenanthrene	1005.31	1000.65 - 1000.65	0.5	
C1-Phenanthrenes/Anthracenes	1.08	U			1292.49			1434.89					1.08	U C1-Phenanthrenes/Anthracenes	1.08	U		
C2-Phenanthrenes/Anthracenes	1.08	U			27.92			35.44					1.08	U C2-Phenanthrenes/Anthracenes	1.08	U		
C3-Phenanthrenes/Anthracenes	1.08	U			30.03			36.58					1.08	U C3-Phenanthrenes/Anthracenes	1.08	U		
C4-Phenanthrenes/Anthracenes	1.08	U			2.62	U		2.62	U				1.08	U C4-Phenanthrenes/Anthracenes	1.08	U		
1-Methylphenanthrene	693.54		1000.30	69	1890.47	2424.97	78	2124.46	2424.97	88	12.0		0.61	U 1-Methylphenanthrene	1021.46	1000.30 - 1000.30	2.1	
Dibenzothiophene	687.95		1001.00	69	1834.13	2426.67	75	2061.43	2426.67	85	12.5		0.5	U Dibenzothiophene	1019.19	1001.00 - 1001.00	1.8	
C1-Dibenzothiophenes	0.5	U			12.59			12.51					0.5	U C1-Dibenzothiophenes	0.5	U		
C2-Dibenzothiophenes	0.5	U			48.6			43.67					0.5	U C2-Dibenzothiophenes	0.5	U		
C3-Dibenzothiophenes	0.5	U			49.59			53.46					0.5	U C3-Dibenzothiophenes	0.5	U		
C4-Dibenzothiophenes	0.5	U			33.75			35.06					0.5	U C4-Dibenzothiophenes	0.5	U		
Fluoranthene	703.26		1000.50	70	1862.97	2425.45	77	2104.3	2425.45	86	11.0		0.77	U Fluoranthene	1041.81	1000.50 - 1000.50	4.1	
Pyrene	718.86		1000.50	72	1865.04	2425.45	77	2089.62	2425.45	86	11.0		0.9	U Pyrene	1067.39	1000.50 - 1000.50	6.7	
C1-Fluoranthenes/Pyrenes	0.9	U			17.67			19.81					0.9	U C1-Fluoranthenes/Pyrenes	0.9	U		
C2-Fluoranthenes/Pyrenes	0.9	U			2.17	U		2.17	U				0.9	U C2-Fluoranthenes/Pyrenes	0.9	U		
C3-Fluoranthenes/Pyrenes	0.9	U			2.17	U		2.17	U				0.9	U C3-Fluoranthenes/Pyrenes	0.9	U		
Benzo(a)anthracene	621.47		1000.60	62	1462.91	2425.70	60	1604.23	2425.70	66	9.5		1.36	U Benzo(a)anthracene	856.76	1000.60 - 1000.60	14.4	
Chrysene	730.19		1000.75	73	1556.53	2426.06	64	1657.64	2426.06	68	6.1		0.59	U Chrysene	1045.65	1000.75 - 1000.75	4.5	
C1-Chrysenes	0.59	U			17.96			26.53					0.59	U C1-Chrysenes	0.59	U		
C2-Chrysenes	0.59	U			30.65			38.76					0.59	U C2-Chrysenes	0.59	U		
C3-Chrysenes	0.59	U			1.43	U		3.54	J				0.59	U C3-Chrysenes	0.59	U		
C4-Chrysenes	0.59	U			1.43	U		1.43	U				0.59	U C4-Chrysenes	0.59	U		
Benzo(b)fluoranthene	673.96		1000.75	67	1818.68	2426.06	75	2085.1	2426.06	86	13.7		1.16	U Benzo(b)fluoranthene	935.5	1000.75 - 1000.75	6.5	
Benzo(j/k)fluoranthene	777.31		1000.65	78	1891.52	2425.82	78	2136.71	2425.82	88	12.0		1.31	U Benzo(j/k)fluoranthene	1086.15	1000.65 - 1000.65	8.5	
Benzo(e)pyrene	702.15		1001.80	70	1823.17	2428.61	75	2063.07	2428.61	85	12.5		0.51	U Benzo(e)pyrene	979.25	1001.80 - 1001.80	2.3	
Benzo(a)pyrene	629.4		1000.65	63	1716.19	2425.82	71	1960.11	2425.82	81	13.2		1	U Benzo(a)pyrene	876.77	1000.65 - 1000.65	12.4	
Perylene	656.25		1000.20	66	1707.57	2424.73	70	1955.41	2424.73	81	14.6		1.93	U Perylene	909.5	1000.20 - 1000.20	9.1	
Indeno(1,2,3-cd)pyrene	723.98		1000.60	72	1676.14	2425.70	69	1869.45	2425.70	77	11.0		0.99	U Indeno(1,2,3-cd)pyrene	1033.73	1000.60 - 1000.60	3.3	
Dibenz(a,h)anthracene	685.03		1000.55	68	1982.27	2425.58	82	2274.52	2425.58	94	13.6		0.84	U Dibenz(a,h)anthracene	916.9	1000.55 - 1000.55	8.4	
Benzo(g,h,i)perylene	705.89		1000.70	71	1939.71	2425.94	80	2258.51	2425.94	93	15.0		0.99	U Benzo(g,h,i)perylene	971.43	1000.70 - 1000.70	2.9	

CLIENT ID	LABORATORY CONTROL SAMPLE				MATRIX SPIKE OF-NAB18-SDB6-COMP			MATRIX SPIKE DUPLICATE-OF-NAB18-SDB6-COMP					PROCEDURAL BLANK	CLIENT ID	GG73: PCB/PESTICIDE SRM SOLUTION			
Surrogate Recoveries (%)														*Surrogate Recoveries (%)*				
Naphthalene-d8	61				55			58					42	Naphthalene-d8	51			
Phenanthrene-d10	71				77			84					44	Phenanthrene-d10	63			
Chrysene-d12	72				66			69					43	Chrysene-d12	66			

PCBs

CLIENT ID	NI-OF26-SDB6-FF		NI-OF26-SDB6-COMP		NI-BAY26-SDB6-PRE		NI-BAY26-SDB6-DUR		NI-OF23A-SDB6-FF		NI-BAY23A-SDB6-PRE		NI-BAY23A-SDB6-DUR	
Battelle ID	S7111-P		S7112-P		S7113-P		S7114-P		S7115-P		S7116-P		S7117-P	
Sample Type	SA		SA		SA		SA		SA		SA		SA	
Collection Date	2/11/2005		2/11/2005		2/11/2005		2/11/2005		2/11/2005		2/11/2005		2/11/2005	
Extraction Date	2/17/2005		2/17/2005		2/17/2005		2/17/2005		2/17/2005		2/17/2005		2/17/2005	
Analysis Date	3/5/2005		3/5/2005		3/5/2005		3/6/2005		3/6/2005		3/6/2005		3/6/2005	
Analytical Instrument	MS		MS		MS		MS		MS		MS		MS	
% Moisture	NA		NA		NA		NA		NA		NA		NA	
% Lipid	NA		NA		NA		NA		NA		NA		NA	
Matrix	WATER		WATER		WATER		WATER		WATER		WATER		WATER	
Sample Size	2.62		2.62		2.62		2.60		2.60		2.64		2.63	
Size Unit-Basis	L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID	
Units	NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID	
Cl2(8)	0.07	U	0.07	U	0.07	U	0.07	U	0.07	U	0.07	U	0.07	U
Cl3(18)	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U
Cl3(28)	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U
Cl4(44)	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U
Cl4(49)	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U
Cl4(52)	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U
Cl4(66)	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U
Cl4(77)	0.14	U	0.14	U	0.14	U	0.14	U	0.14	U	0.14	U	0.14	U
Cl5(87)	0.23	U	0.23	U	0.23	U	0.24	U	0.24	U	0.23	U	0.23	U
Cl5(101)	0.23	U	0.23	U	0.23	U	0.24	U	0.24	U	0.23	U	0.23	U
Cl5(105)	0.11	U	0.11	U	0.11	U	0.11	U	0.11	U	0.11	U	0.11	U
Cl5(114)	0.23	U	0.23	U	0.23	U	0.24	U	0.24	U	0.23	U	0.23	U
Cl5(118)	0.07	U	0.07	U	0.07	U	0.07	U	0.07	U	0.07	U	0.07	U
Cl5(123)	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U
Cl5(126)	0.12	U	0.12	U	0.12	U	0.12	U	0.12	U	0.12	U	0.12	U
Cl6(128)	0.27	U	0.27	U	0.27	U	0.27	U	0.27	U	0.27	U	0.27	U
Cl6(138)	0.27	U	0.27	U	0.27	U	0.27	U	0.27	U	0.27	U	0.27	U
Cl6(153)	1.98	J	1.66	J	0.27	U	1.13	J	0.27	U	0.27	U	0.27	U
Cl6(156)	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U
Cl6(157)	0.15	U	0.15	U	0.15	U	0.15	U	0.15	U	0.14	U	0.15	U
Cl6(167)	0.27	U	0.27	U	0.27	U	0.27	U	0.27	U	0.27	U	0.27	U
Cl6(169)	0.11	U	0.11	U	0.11	U	0.11	U	0.11	U	0.11	U	0.11	U
Cl7(170)	1.05	J	0.92	J	0.19	U	0.53	J	0.19	U	0.19	U	0.19	U
Cl7(180)	2.72	J	1.93	J	0.11	U	1.61	J	0.11	U	0.11	U	0.11	U
Cl7(183)	0.58	J	0.41	J	0.19	U	0.27	J	0.19	U	0.19	U	0.19	U
Cl7(184)	0.19	U	0.19	U	0.19	U	0.19	U	0.19	U	0.19	U	0.19	U
Cl7(187)	1.01	J	0.91	J	0.19	U	0.74	J	0.19	U	0.19	U	0.19	U
Cl7(189)	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U	0.08	U
Cl8(195)	0.36	U	0.36	U	0.36	U	0.37	U	0.37	U	0.36	U	0.36	U
Cl9(206)	0.34	U	0.34	U	0.34	U	0.34	U	0.34	U	0.34	U	0.34	U
Cl10(209)	0.41	U	0.41	U	0.41	U	0.41	U	0.41	U	0.4	U	0.41	U
Surrogate Recoveries (%)														
Cl2(14)	67		52		55		66		65		59		68	
Cl3(34)	71		53		59		67		65		61		70	

PCBs QA/QC

PROJECT: Task Order TO0015/TO0019 – Contaminant Analysis of Stormwater
PARAMETER: PCB
LABORATORY: Battelle, Duxbury, MA
MATRIX: Water
SAMPLE CUSTODY: Water samples were collected 2/11/05. The samples were received at Battelle Duxbury on 2/15/05. Upon arrival, the cooler temperatures ranged from 0.8°C – 3.7°C. No custody issues were noted. Samples were logged into the Battelle LIMS and received unique IDs. Samples were stored in the access-controlled upper cold room refrigerator at 4.0°C until sample preparation could begin. Samples were extracted as one analytical batch, 05-0056, along with the appropriate quality control samples.

	Reference Method	Method Blank	Surrogate Recovery	LCS/MS Recovery	SRM % Diff.	Sample Replicate Relative Precision	Detection Limits (ng/L)
PCB	General NS&T	<5xMDL	40-120% Recovery	40-120% Recovery (target spike must be >5 x native conc.)	≤30% PD on average (for analytes >5x MDL)	≤30% RPD (calculated between the MS and MSD samples)	MDL: ~0.09 – 0.53

METHOD: Water samples were extracted for PCB following general NS&T methods. Approximately 2 liters of water was spiked with surrogates and extracted three times with dichloromethane using separatory funnel techniques. The combined extract was dried over anhydrous sodium sulfate and concentrated. The extract was then fortified with RIS and split quantitatively for the required analyses. Extracts were analyzed using gas chromatography/mass spectrometry (GC/MS). The method is based on key components of the PCB congener analysis approach described in EPA Method 1668A. Sample data were quantified by the method of internal standards, using the Recovery Internal Standard (RIS) compounds

HOLDING TIMES: Samples were prepared for analysis in one analytical batch and were extracted within 7 days of sample collection and analyzed within 40 days of extraction.

Batch	Extraction Date	Analysis Date
05-0056	2/17/05	3/5/05 – 3/7/05

BLANK: A procedural blank (PB) sample was prepared with the analytical batch. Procedural blank samples are analyzed to ensure the sample extraction and analysis methods are free of contamination.

05-0056 – No exceedences noted.

Comments – No target analytes were detected in the procedural blank.

LABORATORY CONTROL SAMPLE: A laboratory control sample (LCS) was prepared with each analytical batch. The percent recoveries of target PCB were calculated to measure data quality in terms of accuracy.

05-0056 – All target analytes were recovered within the laboratory control limits (40-120%).

Comments – None.

MATRIX SPIKE/MATRIX SPIKE DUPLICATE: A matrix spike (MS) and a matrix spike duplicate (MSD) sample pair were prepared with each analytical batch. The percent recoveries of target PCB and the relative percent difference between the two samples were calculated to measure data quality in terms of accuracy and precision.

05-0056 – All target analytes were recovered within the laboratory control limits specified by the client (40-120%). All calculated RPDs were within the laboratory control limit ($\leq$ 30%).

Comments – None

SRM: A standard reference material (SRM, a certified second source standard was spiked into a natural seawater as an SRM) was prepared with each analytical batch. Surrogate corrected data has been reported for the SRM only.

05-0056 – All target analytes were recovered within the laboratory control limits specified by the client ($\leq$ 30 PD).

Comments – None.

SURROGATES: Two surrogate compounds were added prior to extraction, including PCB 14 and PCB 34. The recovery of each surrogate compound was calculated to measure data quality in terms of accuracy (extraction efficiency).

05-0056 – Percent recoveries for all surrogate compounds were within the laboratory control limits (40 – 120% recovery).

Comments – None.

CALIBRATIONS: The GC/MS is calibrated with a minimum of a 6-point curve. The co-efficient of determination must be $\geq$ 0.995 for each target analyte. Each batch of samples analyzed is bracketed by a calibration check sample, run at a frequency of every 12 hours (minimally). This PD between the initial calibration RF and the check should be <20% for individual analytes; 15% on average. Additionally an ICC check was run with the initial calibration. The PD for the ICC should be < 15%, for each analyte.

05-0056 – No calibration exceedences.

Comments – None.

PCBs QA/QC (CONT.)

CLIENT ID	LABORATORY CONTROL SAMPLE				MATRIX SPIKE-NAB-OF18-SD86-COMP				MATRIX SPIKE DUPLICATE-NAB-OF18-SD86-COMP					PRODECURAL BLANK		GG73: PCB/PESTICIDE SRM SOLUTION					
Battelle ID	BF876LCS-P				S7123MS-P				S7123MSD-P					BF875PB-P		BF877SRM-P					
Sample Type	LCS				MS				MSD					PB		SRM					
Collection Date	2/17/2005				2/11/2005				2/11/2005					2/17/2005		2/17/2005					
Extraction Date	2/17/2005				2/17/2005				2/17/2005					2/17/2005		2/17/2005					
Analysis Date	3/5/2005				3/6/2005				3/7/2005					3/5/2005		3/5/2005					
Analytical Instrument	MS				MS				MS					MS		MS					
% Moisture	NA				NA				NA					NA		NA					
% Lipid	NA				NA				NA					NA		NA					
Matrix	LIQUID				WATER				WATER					LIQUID		LIQUID					
Sample Size	2.00				0.825				0.825					2.00		2.00					
Size Unit-Basis	L LIQUID				L LIQUID				L LIQUID					L LIQUID		L LIQUID					
Units	NG/L LIQUID		Target	% Recovery	Q NG/L LIQUID		Target	% Recovery	NG/L LIQUID		Target	% Recovery	RPD (%)	NG/L LIQUID		NG/L LIQUID		Certified Value	+/-	Passing %Difference	Passing %Difference
Cl2(8)	20.94		40.12	52	57.93		97.26	60	65.69		97.26	68	12.5	0.09	U	28.71		34.24	2.88	23.41	8.5
Cl3(18)	23.48		40.12	59	63.17		97.26	65	72.28		97.26	74	12.9	0.11	U	33.26		32.93	0.30	15.92	0.1
Cl3(28)	21.18		40.04	53	58.47		97.07	60	66.61		97.07	69	14.0	0.11	U	31.4				15	
Cl4(44)	23.36		40.08	58	67.71		97.16	70	80.77		97.16	83	17.0	0.19	U	31.8		32.86	0.59	16.8	1.5
Cl4(49)	26.89		40.16	67	72.7		97.36	75	86.35		97.36	89	17.1	0.19	U	0.19	U			15	
Cl4(52)	22.65		40.00	57	64.69		96.97	67	76.93		96.97	79	16.4	0.19	U	30.17		33.07	0.38	16.16	7.7
Cl4(66)	16.82		40.04	42	56		97.07	58	65.49		97.07	67	14.4	0.19	U	23.19		32.82	0.62	16.9	28
Cl4(77)	17.85		40.00	45	60.54		96.97	62	69.94		96.97	72	14.9	0.18	U	24.75		33.55	1.10	18.29	23.7
Cl5(87)	25.33		40.00	63	82.81		96.97	85	96.23		96.97	99	15.2	0.31	U	35.34		33.1	0.27	15.82	5.9
Cl5(101)	23.84		40.08	59	72.85		97.16	75	86.65		97.16	89	17.1	0.31	U	31.49		32.56	0.47	16.43	1.9
Cl5(105)	23.38		40.04	58	78.35		97.07	81	90.65		97.07	93	13.8	0.14	U	34.77		32.67	1.01	18.09	3.2
Cl5(114)	0.31	U			0.74	U			0.74	U				0.31	U	0.31	U			15	
Cl5(118)	16.89		40.04	42	59.07		97.07	61	70.11		97.07	72	16.5	0.1	U	30.68		32.74	1.06	18.23	3.2
Cl5(123)	0.11	U			0.26	U			0.26	U				0.11	U	0.11	U			15	
Cl5(126)	21.05		40.24	52	73.18		97.55	75	83.73		97.55	86	13.7	0.16	U	32.14		33.22	1.38	19.14	1
Cl6(128)	20.67		40.24	51	70.52		97.55	72	81.83		97.55	84	15.4	0.35	U	28.54		32.94	0.27	15.83	12.6
Cl6(138)	23.4		40.08	58	83.59		97.16	86	93.09		97.16	96	11.0	0.35	U	32.27		32.43	0.38	16.18	1
Cl6(153)	23.31		40.04	58	78.01		97.07	80	91.91		97.07	95	17.1	0.35	U	31.88		32.64	0.62	16.91	0.4
Cl6(156)	0.1	U			0.24	U			0.24	U				0.1	U	0.1	U			15	
Cl6(157)	0.19	U			0.46	U			0.46	U				0.19	U	0.19	U			15	
Cl6(167)	0.35	U			0.86	U			0.86	U				0.35	U	0.35	U			15	
Cl6(169)	23.83		40.16	59	80.81		97.36	83	95.7		97.36	98	16.6	0.15	U	0.15	U			15	
Cl7(170)	20.19		40.20	50	66.97		97.45	69	81.17		97.45	83	18.4	0.25	U	28.45		32.72	0.54	16.66	11.6
Cl7(180)	23.14		40.16	58	78.45		97.36	81	93.41		97.36	96	16.9	0.14	U	32.98		32.96	0.32	15.97	1
Cl7(183)	24.37		40.16	61	83		97.36	85	96.91		97.36	100	16.2	0.25	U	0.25	U			15	
Cl7(184)	24.68		40.16	61	80.23		97.36	82	94.21		97.36	97	16.8	0.25	U	0.25	U			15	
Cl7(187)	20.63		40.12	51	73.67		97.26	76	89.41		97.26	92	19.0	0.25	U	31.89		32.75	0.30	15.93	1.7
Cl7(189)	0.11	U			0.26	U			0.26	U				0.11	U	0.11	U			15	
Cl8(195)	20.93		40.12	52	72.11		97.26	74	85.1		97.26	87	16.1	0.48	U	28.04		32.83	0.66	17	12.8
Cl9(206)	22.82		40.12	57	74.32		97.26	76	90.8		97.26	93	20.1	0.44	U	32.94		32.02	0.59	16.85	1
Cl10(209)	29.36		40.04	73	81.36		97.07	84	99.02		97.07	102	19.4	0.53	U	40.51		32.99	0.45	16.36	21.1
Surrogate Recoveries (%)																					
Cl2(14)	58				67				78					40		55					
Cl3(34)	59				69				79					40		56					

PESTICIDEs

CLIENT ID	NI-OF23A-SDB6-FF		NI-BAY23A-SDB6-PRE		NI-BAY23A-SDB6-DUR		NI-OF26-SDB6-FF		NI-OF26-SDB6-COMP		NI-BAY26-SDB6-PRE		NI-BAY26-SDB6-DUR	
Battelle ID	S7115-P		S7116-P		S7117-P		S7111-P		S7112-P		S7113-P		S7114-P	
Sample Type	SA		SA		SA		SA		SA		SA		SA	
Collection Date	02/11/05		02/11/05		02/11/05		02/11/05		02/11/05		02/11/05		02/11/05	
Extraction Date	02/17/05		02/17/05		02/17/05		02/17/05		02/17/05		02/17/05		02/17/05	
Analysis Date	02/26/05		02/26/05		02/26/05		02/26/05		02/26/05		02/26/05		02/26/05	
Analytical Instrument	ECD		ECD		ECD		ECD		ECD		ECD		ECD	
% Moisture	NA		NA		NA		NA		NA		NA		NA	
% Lipid	NA		NA		NA		NA		NA		NA		NA	
Matrix	WATER		WATER		WATER		WATER		WATER		WATER		WATER	
Sample Size	2.60		2.64		2.63		2.62		2.62		2.62		2.60	
Size Unit-Basis	L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID	
Units	NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID	
2,4'-DDD	0.63	U	0.62	U	0.62	U	0.62	U	0.62	U	0.62	U	0.63	U
2,4'-DDE	1.16		0.52	U	0.52	U	0.52	U	0.52	U	0.52	U	0.53	U
2,4'-DDT	0.37	U	0.37	U	0.37	U	0.37	U	0.37	U	0.37	U	0.37	U
4,4'-DDD	0.73	U	0.72	U	0.72	U	3		2.1		0.73	U	1.19	
4,4'-DDE	0.53	U	0.52	U	0.52	U	0.52	U	0.82		0.52	U	0.71	
4,4'-DDT	0.45	U	0.45	U	0.45	U	0.45	U	4.58		0.45	U	3.37	
aldrin	0.3	U	0.3	U	0.3	U	0.3	U	0.3	U	0.3	U	0.3	U
a-chlordane	0.29	U	0.29	U	0.29	U	0.29	U	1.7		0.29	U	0.47	J
g-chlordane	0.31	U	0.31	U	0.31	U	0.31	U	0.31	U	0.31	U	0.31	U
a-BHC	0.26	U	0.26	U	0.26	U	0.26	U	0.26	U	0.26	U	0.26	U
b-BHC	0.36	U	0.36	U	0.36	U	0.36	U	0.36	U	0.36	U	0.36	U
d-BHC	0.3	U	0.29	U	0.3	U	0.3	U	0.3	U	0.3	U	0.3	U
Lindane	0.38	U	0.37	U	0.38	U	0.38	U	0.38	U	0.38	U	0.38	U
cis-nonachlor	0.5	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.5	U
trans-nonachlor	0.31	U	0.31	U	0.31	U	0.31	U	1.62		0.31	U	0.65	
Chlorpyrifos	0.39	U	0.39	U	0.39	U	0.39	U	0.39	U	0.39	U	0.39	U
oxychlordane	0.3	U	0.3	U	0.3	U	0.3	U	0.3	U	0.3	U	0.3	U
dieldrin	0.59	U	0.58	U	0.58	U	0.58	U	0.58	U	0.58	U	0.59	U
endosulfan I	0.21	U	0.21	U	0.21	U	0.21	U	0.21	U	0.21	U	0.21	U
endosulfan II	0.53	U	0.52	U	0.53	U	0.53	U	0.53	U	0.53	U	0.53	U
endosulfan sulfate	0.5	U	0.49	U	0.49	U	0.5	U	0.5	U	0.5	U	0.5	U
endrin	0.58	U	0.57	U	0.57	U	0.57	U	0.57	U	0.57	U	0.58	U
endrin aldehyde	0.65	U	0.64	U	0.65	U	0.65	U	0.65	U	0.65	U	0.65	U
endrin ketone	0.68	U	0.67	U	0.68	U	0.68	U	0.68	U	0.68	U	0.68	U
heptachlor	8.67		0.44	U	0.45	U	0.45	U	0.45	U	0.45	U	0.45	U
heptachlor epoxide	1.21	U	1.19	U	1.2	U	1.2	U	1.2	U	1.2	U	1.21	U
Hexachlorobenzene	0.64	U	0.28	J	0.63	U	0.63	U	0.63	U	0.63	U	0.64	U
methoxychlor	0.75	U	0.74	U	0.74	U	9.57		6.99		0.75	U	0.75	U
Mirex	0.48	U	0.47	U	0.47	U	0.47	U	0.47	U	0.47	U	0.48	U
Surrogate Recoveries (%)														
Cl2(14)	91		73		85		90		77		64		79	
Cl3(34)	97		70		84		108		66		66		73	
Cl5(104)	86		70		82		95		67		64		77	
Cl5(112)	81		72		82		65		55		67		71	

PESTICIDEs QA/QC

PROJECT: Task Order TO0015/TO0019 – Conataminant Analysis of Stormwater
PARAMETER: Pesticides
LABORATORY: Battelle, Duxbury, MA
MATRIX: Water
SAMPLE CUSTODY: Water samples were collected 2/11/05. The samples were received at Battelle Duxbury on 2/15/05. Upon arrival, the cooler temperatures ranged from 0.8°C – 3.7°C. No custody issues were noted. Samples were logged into the Battelle LIMS and received unique IDs. Samples were stored in the access-controlled upper cold room refrigerator at 4.0°C until sample preparation could begin. Samples were extracted as one analytical batch, 05-0056, along with the appropriate quality control samples.

	Reference Method	Method Blank	Surrogate Recovery	LCS/MS Recovery	SRM % Diff.	Sample Replicate Relative Precision	Detection Limits (ng/L)
PESTICIDE	General NS&T	<5xMDL	40-120% Recovery	40-120% Recovery (target spike must be >5 x native conc.)	≤30% PD on average (for analytes >5x MDL)	≤30% RPD (calculated between the MS and MSD samples)	MDL: ~0.38 – 1.58

METHOD: Water samples were extracted for pesticide following general NS&T methods. Approximately 2 liters of water was spiked with surrogates and extracted three times with dichloromethane using separatory funnel techniques. The combined extract was dried over anhydrous sodium sulfate, concentrated, processed through alumina cleanup column, concentrated, and further purified by GPC/HPLC. The post-HPLC extract was concentrated, fortified with RIS and split quantitatively for the required analyses. Extracts intended for pesticide analysis were solvent exchanged into hexane and analyzed using a gas chromatography/electron capture detector (GC/ECD). Sample data were quantified by the method of internal standards, using the Recovery Internal Standard (RIS) compounds.

HOLDING TIMES: Samples were prepared for analysis in one analytical batch and were extracted within 7 days of sample collection and analyzed within 40 days of extraction.

Batch	Extraction Date	Analysis Date
05-0056	2/17/05	2/25/05 – 2/28/05

BLANK: A procedural blank (PB) was prepared with the analytical batch. Blanks are analyzed to ensure the sample extraction and analysis methods were free of contamination.

05-0056 – No exceedences noted.

Comments – No target analytes were detected in the procedural blank.

LABORATORY CONTROL SAMPLE: A laboratory control sample (LCS) was prepared with the analytical batch. The percent recoveries of target pesticides were calculated to measure data quality in terms of accuracy.

05-0056 – All target analytes were recovered within the laboratory control limits specified by the client (40-120%).

Comments – None.

MATRIX SPIKE/MATRIX SPIKE DUPLICATE:

A matrix spike (MS) and a matrix spike duplicate (MSD) sample pair were prepared with each analytical batch. The percent recoveries of target pesticides and the relative percent difference between the two samples were calculated to measure data quality in terms of accuracy and precision.

05-0056 – All target analytes were recovered within the laboratory control limits specified by the client (40-120%). All calculated RPDs were within the laboratory control limit ($\leq$ 30%).

Comments – None

SRM:

A standard reference material (SRM, a certified second source standard was spiked into a natural seawater as an SRM) was prepared with each analytical batch. Surrogate corrected data has been reported for the SRM only.

05-0056 – Two exceedences noted.

Comments – All target analytes were recovered within the laboratory control limits specified by the client ($\leq$ 30 PD), except for 2,4-DDD and 2,4-DDT. The percent differences calculated for these two compounds are 58.5% and 51.0%, respectively. Chromatography and calculations were reviewed. No discrepancies were found. The data has been qualified with an "N". Accuracy for this compound has adequately been demonstrated in the LCS, MS, and MSD QC samples.

SURROGATES

Four surrogate compounds were added prior to extraction, including PCB 14, PCB 34, PCB 104, and PCB 112. The recovery of each surrogate compound was calculated to measure data quality in terms of accuracy (extraction efficiency).

05-0056 – Percent recoveries for all surrogate compounds were within the laboratory control limits (40 – 120% recovery).

Comments – None.

CALIBRATIONS:

The instrument is calibrated with a 5-level (minimum) calibration, ranging in concentration from ~0.001 ng/uL to ~0.125 ng/uL. Calibration checks are analyzed minimally every 10 samples. The samples must be bracketed by passing calibrations.

04-0275 – No exceedences noted.

Comments – All calibration criteria were met except for two percent differences calculated for HCB in two calibration checks. However since this compound was not detected in any field samples, and accuracy for this compound was adequately demonstrated in all other QC samples, no further corrective action was taken.

PESTICIDES QA/QC (CONT.)

CLIENT ID	LABORATORY CONTROL SAMPLE			MATRIX SPIKE-NAB-OF18-SDB6-COMP			MATRIX SPIKE DUPLICATE-NAB-OF18-SDB6-COMP				PROCEDURAL BLANK		CLIENT ID	GG73: PCB/PESTICIDE SRM SOLUTION			
Battelle ID	BF876LCS-P			S7123MS-P			S7123MSD-P				BF875PB-P		Battelle ID	BF877SRM-P			
Sample Type	LCS			MS			MSD				PB		Sample Type	SRM			
Collection Date	02/17/05			2/11/2005			2/11/2005				02/17/05		Collection Date	02/17/05			
Extraction Date	02/17/05			2/17/2005			2/17/2005				02/17/05		Extraction Date	02/17/05			
Analysis Date	02/25/05			2/27/2005			2/27/2005				02/25/05		Analysis Date	02/25/05			
Analytical Instrument	ECD			ECD			ECD				ECD		Analytical Instrument	ECD			
% Moisture	NA			NA			NA				NA		% Moisture	NA			
% Lipid	NA			NA			NA				NA		% Lipid	NA			
Matrix	LIQUID			WATER			WATER				LIQUID		Matrix	LIQUID			
Sample Size	2.00			0.825			0.825				2.00		Sample Size	2.00			
Size Unit-Basis	L_LIQUID			L_LIQUID			L_LIQUID				L_LIQUID		Size Unit-Basis	L_LIQUID			
Units	NG/L_LIQUID	Target	% Recovery	NG/L_LIQUID	Target	% Recovery	NG/L_LIQUID	Target	% Recovery	RPD (%)	NG/L_LIQUID		Units	NG/L_LIQUID	Certified Range	% Difference	
2,4'-DDD	25.61	40.12	64	67.68	97.26	70	75.58	97.26	78	10.8	0.81	U	2,4'-DDD	50.48	31.30 - 31.84	58.5	N
2,4'-DDE	23.38	40.01	58	59.57	97.00	61	68.91	97.00	71	15.2	0.69	U	2,4'-DDE	21.94	31.35 - 31.89	30.0	
2,4'-DDT	21.07	40.23	52	52.73	97.53	54	59.9	97.53	61	12.2	0.48	U	2,4'-DDT	15.3	31.20 - 31.48	51.0	N
4,4'-DDD	26.02	40.01	65	68.94	96.98	71	77.31	96.98	80	11.9	0.95	U	4,4'-DDD	24.59	31.44 - 32.30	21.8	
4,4'-DDE	25.33	40.01	63	65.61	96.98	68	74.09	96.98	76	11.1	0.68	U	4,4'-DDE	27.41	31.46 - 31.78	12.9	
4,4'-DDT	28.23	40.02	71	88.61	97.02	91	98.05	97.02	101	10.4	0.59	U	4,4'-DDT	31.36	31.28 - 31.66	1.0	
aldrin	24.44	40.01	61	64.72	97.00	67	73.01	97.00	75	11.3	0.4	U	aldrin	24.22			
a-chlordane	23.46	40.03	59	63	97.04	65	71.99	97.04	74	12.9	0.38	U	a-chlordane	26.5	31.36 - 31.74	15.5	
g-chlordane	23.1	40.06	58	62.91	97.12	65	71.26	97.12	73	11.6	0.4	U	g-chlordane	0.4	U		
a-BHC	23.05	40.02	58	61.5	97.01	63	70	97.01	72	13.3	0.34	U	a-BHC	0.34	U		
b-BHC	26.04	40.01	65	71.33	96.98	74	81.52	96.98	84	12.7	0.47	U	b-BHC	0.47	U		
d-BHC	26.74	40.02	67	75.54	97.01	78	86.61	97.01	89	13.2	0.39	U	d-BHC	0.39	U		
Lindane	26.6	40.01	66	72.67	96.99	75	82.78	96.99	85	12.5	0.49	U	Lindane	30.23	31.39 - 31.71	3.7	
cis-nonachlor	25.29	40.03	63	66.56	97.04	69	74.86	97.04	77	11.0	0.65	U	cis-nonachlor	0.65	U		
trans-nonachlor	24.46	40.06	61	67.21	97.11	69	76.42	97.11	79	13.5	0.4	U	trans-nonachlor	27.77	31.56 - 32.00	12.0	
Chlorpyrifos	26	40.10	65	75.11	97.21	77	86.23	97.21	89	14.5	0.51	U	Chlorpyrifos	0.51	U		
oxychlordane	24.48	40.03	61	66.19	97.04	68	74.74	97.04	77	12.4	0.39	U	oxychlordane	0.39	U		
dieldrin	25.77	40.01	64	66.66	96.99	69	75.03	96.99	77	11.0	0.76	U	dieldrin	28.21	31.34 - 31.76	10.0	
endosulfan I	25.15	40.03	63	73.26	97.04	75	73.82	97.04	76	1.3	0.27	U	endosulfan I	0.27	U		
endosulfan II	24.17	40.02	60	65.82	97.02	68	76.63	97.02	79	15.0	0.69	U	endosulfan II	0.69	U		
endosulfan sulfate	25.59	40.02	64	74.76	97.01	77	84.21	97.01	87	12.2	0.65	U	endosulfan sulfate	0.65	U		
endrin	25.18	40.01	63	72.34	97.00	75	81.04	97.00	84	11.3	0.75	U	endrin	0.75	U		
endrin aldehyde	19.49	40.01	49	51.31	96.99	53	65.18	96.99	67	23.3	0.85	U	endrin aldehyde	13.8			
endrin ketone	26.63	40.02	67	72.67	97.01	75	82.31	97.01	85	12.5	0.89	U	endrin ketone	0.89	U		
heptachlor	25.65	40.00	64	77.01	96.98	79	87.83	96.98	91	14.1	0.59	U	heptachlor	29.59	31.39 - 31.87	5.7	
heptachlor epoxide	25.41	40.01	64	66.79	96.98	69	76.31	96.98	79	13.5	1.58	U	heptachlor epoxide	27.77	31.36 - 31.90	11.4	
Hexachlorobenzene	28.14	40.06	70	72.56	97.12	75	82.38	97.12	85	12.5	0.83	U	Hexachlorobenzene	32.05	31.35 - 31.63	1.3	
methoxychlor	29.49	40.01	74	91.3	97.00	92	101.57	97.00	103	11.3	0.98	U	methoxychlor	7.8			
Mirex	26.25	40.03	66	69.14	97.05	71	77.47	97.05	80	11.9	0.62	U	Mirex	29.19	31.41 - 32.31	7.1	
Surrogate Recoveries (%)													*Surrogate Recoveries (%)*				
CI2(14)	71			81			95				51		CI2(14)	69			
CI3(34)	72			76			87				51		CI3(34)	68			
CI5(104)	69			80			92				50		CI5(104)	60			
CI5(112)	72			77			95				53		CI5(112)	69			

TSS

SAMPLE LABEL	TSS (mg/L)
NI-OF23A-SDB6-FF	9.104
NI-BAY23A-SDB6-PRE	3.361
NI-BAY23A-SDB6-DUR	4.271
NI-OF26-SDB6-FF	14.714
NI-OF26-SDB6-COMP	21.742
NI-BAY26-SDB6-PRE	2.899
NI-BAY26-SDB6-DUR	12.674

SDB7- 4/27/2005

METALS

MSL		Sponsor	Al (µg/L)		Fe (µg/L)		Cr (µg/L)		Mn (µg/L)		Ni (µg/L)		Cu (µg/L)		Zn (µg/L)	
Code	Rep	I.D.	ICP-OES		ICP-OES		ICP-OES		ICP-OES		ICP-MS		ICP-MS		ICP-OES	
2360*10		NI-OF23A-SDB7-FF (T)	1448		2557		9.61		44.2		11.8		40.8		289	
2360*5		NI-OF23A-SDB7-FF (D)	11.1		12.4		0.295		2.57		1.41		3.69		33.4	
2360*9		NI-OF26-SDB7-COMP (T)	3753		5767		20.2		194		15.0		89.3		546	
2360*4		NI-OF26-SDB7-COMP (D)	121		103		1.90		23.6		5.95		18.9		79.5	
2360*8		Field Blank - Filtered	3.36	U	2.66		0.119	U	0.025	U	0.436		0.883	U	11.9	

MSL		Sponsor	As (µg/L)		Se (µg/L)		Ag (µg/L)		Cd (µg/L)		Sn (µg/L)		Pb (µg/L)		Hg (µg/L)	
Code	Rep	I.D.	ICP-MS		ICP-MS		ICP-MS		ICP-MS		ICP-MS		ICP-MS		CVAF	
2360*10		NI-OF23A-SDB7-FF (T)	0.648		1.47	U	0.109		1.26		2.45		21.9		0.0164	
2360*5		NI-OF23A-SDB7-FF (D)	0.208		1.47	U	0.04	U	0.0564		0.50	U	0.223		0.00404	
2360*9		NI-OF26-SDB7-COMP (T)	2.62		1.61		0.311		6.35		0.891		77.5		0.0494	
2360*4		NI-OF26-SDB7-COMP (D)	1.15		1.47	U	0.04	U	0.882		0.50	U	1.50		0.00547	
2360*8		Field Blank - Filtered	0.168	U	1.47	U	0.04	U	0.054	U	0.50	U	0.0602		0.000871	

SAMPLE ID	DISSOLVED COPPER (µg/L)	TOTAL COPPER (µg/L)
NI-BAY23A-SDB7-PRE	2.3	5.0
NI-BAY23A-SDB7-DUR	2.8	5.3
NI-BAY26-SDB7-FF	50	112
NI-BAY26-SDB7-PRE	2.3	4.2
NI-BAY26-SDB7-DUR	1.7	2.7

SAMPLE ID	DISSOLVED ZINC (µg/L)	TOTAL ZINC (µg/L)
NI-BAY23A-SDB7-PRE	16.96	16.47
NI-BAY23A-SDB7-DUR	13.19	18.47
NI-BAY26-SDB7-FF	588.41	917.30
NI-BAY26-SDB7-PRE	15.39	22.72
NI-BAY26-SDB7-DUR	6.22	6.97

METALS QA/QC

PROGRAM: SPAWAR, Task 19, batch 2
PARAMETER: Metals
LABORATORY: Battelle/Marine Sciences Laboratory, Sequim, Washington
MATRIX: Stormwater

QA/QC DATA QUALITY OBJECTIVES

	Reference Method	Range of Recovery	SRM Accuracy	Relative Precision	Target Detection Limit (µg/L)
Aluminum	ICP/OES	50-150%	±20%	±50%	50.0
Iron	ICP/OES	50-150%	±20%	±50%	10.0
Manganese	ICP/OES	50-150%	±20%	±30%	0.5
Chromium	ICP/MS	50-150%	±20%	±30%	1.0
Nickel	ICP/MS	50-150%	±20%	±30%	0.05
Copper	ICP/MS	50-150%	±20%	±30%	0.05
Zinc	ICP/MS	50-150%	±20%	±30%	0.5
Arsenic	FIAS	50-150%	±20%	±30%	0.5
Selenium	FIAS	50-150%	±20%	±30%	0.2
Silver	GFAA	50-150%	±20%	±30%	0.5
Cadmium	ICP/MS	50-150%	±20%	±30%	0.05
Tin	ICP/MS	50-150%	±20%	±30%	0.5
Lead	ICP/MS	50-150%	±20%	±30%	0.05
Mercury	CVAF	50-150%	±25%	±30%	0.01

METHOD

Nine (9) samples were analyzed for fourteen (14) metals: nickel (Ni), copper, (Cu), arsenic (As), selenium (Se), silver (Ag), cadmium (Cd), tin (Sn) and lead (Pb) by inductively coupled plasma mass spectroscopy (ICP/MS) following EPA Method 1638m, aluminum (Al), iron (Fe), chromium (Cr), manganese (Mn), and zinc (Zn) by inductively coupled plasma optic emission spectroscopy following EPA Method 200.7 and mercury (Hg) by cold vapor atomic fluorescence (CVAF) following EPA Method 1631e.

Samples were preserved with nitric acid prior to arrival at MSL. Samples analyzed for Hg by CVAF were pre-treated with bromine chloride and stannous chloride to oxidize and convert all Hg compounds to volatile Hg, which is subsequently trapped onto a gold-coated sand trap.

HOLDING TIMES

Nine (9) samples were received on 5/03/2005 and were logged into Battelle's sample tracking system. The samples were analyzed within the six month holding time for metals and 90 days for Hg. The following list summarizes all analysis dates:

Task	Date Performed
Hg	5/20/05
ICP-MS	5/11/05
ICP-OES	5/23/05

DETECTION LIMITS

The target detection limit was met for all metals, except Ni, Cu, Se and Cd. The MDL for seawater analysis by dilution is somewhat higher than

our typical MDL's for direct analysis. Sample concentrations were substantially greater than the MDL, except Se. The method detection limit was met for all metals. An MDL is determined by multiplying the standard deviation of the results of a minimum of 7 replicate low level spikes by the Student's t value at the 99th percentile.

METHOD BLANKS

One method blank was analyzed with this batch of samples. Results were less than 3 times the MDL for all metals.

BLANK SPIKES

One sample of reagent water was spiked at several levels with metals. Recoveries were within the QC limits of 50-150% for all metals.

MATRIX SPIKES

One sample was spiked at several levels with metals. Recoveries were within the QC limits of 50-150% for all metals.

REPLICATES

One sample was analyzed in duplicate. All results were within the QC limits of ±30% (±50% for Al and Fe).

SRM

One matrix-appropriate standard reference material (SRM) was analyzed for each method; 1641d, river water, and 1640, natural water, obtained from the National Institute of Science and Technology.

SRM 1640 has 22 certified and reference metals. Recovery for all metals reported were within the control limit of ±20% of the certified or reference value. Tin and Hg are not certified in 1640. SRM 1641d is certified for Hg. Recovery for Hg was within the control limit of ±25% of the certified value.

REFERENCES

EPA. 1991. Methods for the Determination of Metals in Environmental Samples. EPA-600/4- 91-010. Environmental Services Division, Monitoring Management Branch.

METALS QA/QC (CONT.)

MSL Code	Rep	Sponsor I.D.	Al (µg/L) ICP-OES		Fe (µg/L) ICP-OES		Cr (µg/L) ICP-OES		Mn (µg/L) ICP-OES		Ni (µg/L) ICP-MS		Cu (µg/L) ICP-MS		Zn (µg/L) ICP-OES		As (µg/L) ICP-MS	
PROCEDURAL BLANK																		
		Dissolved	3.36	U	2.51	U	0.119	U	0.025	U	0.074	U	0.883	U	0.248		0.158	U
		TRM	3.36	U	2.51	U	0.119	U	0.025	U	0.074	U	0.883	U	0.113	U	0.158	U
METHOD DETECTION LIMIT			**3.36**		**2.51**		**0.119**		**0.025**		**0.074**		**0.883**		**0.113**		**0.158**	
Project Target Detection Limit			50.0		10.0		1.00		0.50		0.05		0.05		0.50		0.50	
STANDARD REFERENCE MATERIAL																		
1640		Dissolved	56.3		34.0		37.4		119		26.0		78.1		53.7		26.2	
1640		TRM	N/A		N/A		N/A		N/A		25.3		81.4		N/A		25.2	
1640		certified/reference value	52.0		34.3		38.6		122		27.4		85.2		53.2		26.7	
1640		range	±1.5		±1.6		±1.6		±1.1		±0.8		±1.2		±1.1		±0.73	
		% difference	8%		1%		3%		2%		5%		8%		1%		2%	
		% difference	N/A		N/A		N/A		N/A		8%		4%		N/A		6%	
1641d			NA		NA		NA		NA		NA		NA		NA		NA	
1641d		certified value	NC		NC		NC		NC		NC		NC		NC		NC	
1641d		range	NC		NC		NC		NC		NC		NC		NC		NC	
		% difference	N/A		N/A		N/A		N/A		N/A		N/A		N/A		N/A	
ICV,CCV RESULTS																		
ICV			99%		96%		99%		99%		101%		101%		98%		99%	
CCV			99%		106%		101%		97%		98%		100%		103%		101%	
CCV			100%		107%		98%		99%		98%		96%		102%		97%	
CCV			98%		102%		99%		100%		93%		94%		101%		93%	
BLANK SPIKE RESULTS																		
		Amount Spiked	500		500		100.0		100		10.0		50.0		100.0		10.0	
		Blank	3.36	U	2.51	U	0.245	U	0.038	U	0.005	U	0.015	U	0.248		0.008	U
		Blank + Spike	587.0		499		99.6		97.3		9.60		49.3		98.0		9.66	
		Amount Recovered	587.0		499		99.6		97.3		9.60		49.3		97.8		9.66	
MATRIX SPIKE RESULTS																		
		Amount Spiked	NS		NS		NS		NS		10.0		50.0		NS		10.0	
		NI-OF26-SDB7-COMP (D)	N/A		N/A		N/A		N/A		5.95		18.9		N/A		1.15	
		NI-OF26-SDB7-COMP (D) + Spike	NA		NA		NA		NA		15.3		65.2		NA		11.5	
		Amount Recovered	N/A		N/A		N/A		N/A		9.35		46.3		N/A		10.4	
		Percent Recovery	N/A		N/A		N/A		N/A		94%		93%		N/A		104%	
		Amount Spiked	500		500		100		100		NS		NS		100		NS	
		NI-OF23A-SDB7-FF (D)	11.1		12.4		0.295		2.57		N/A		N/A		33.4		N/A	
		NI-OF23A-SDB7-FF (D) + Spike	583		515		97.8		100		NA		NA		129		NA	
		Amount Recovered	572		503		97.5		97.7		N/A		N/A		95.6		N/A	
		Percent Recovery	114%		101%		98%		98%		N/A		N/A		96%		N/A	
		Amount Spiked	NS		NS		NS		NS		NS		NS		NS		NS	
		NI-OF23A-SDB7-FF (T)	N/A		N/A		N/A		N/A		N/A		N/A		N/A		N/A	
		NI-OF23A-SDB7-FF (T) + Spike	NA		NA		NA		NA		NA		NA		NA		NA	
		Amount Recovered	N/A		N/A		N/A		N/A		N/A		N/A		N/A		N/A	
		Percent Recovery	N/A		N/A		N/A		N/A		N/A		N/A		N/A		N/A	
REPLICATE RESULTS																		
2360*4	1	NI-OF26-SDB7-COMP (D)	121		103		1.90		23.6		5.95		18.9		79.5		1.15	
2360*4	2	NI-OF26-SDB7-COMP (D)	130		107		2.00		23.9		5.94		18.6		81.6		1.14	
		RPD	7%		4%		5%		1%		0%		2%		3%		1%	
2360*6	1	NAB-OF9-SDB7-COMP (D)	13.2		14.3		1.60		95.9		8.68		37.8		709		20.2	
2360*6	2	NAB-OF9-SDB7-COMP (D)	NA		NA		NA		NA		NA		NA		NA		NA	
		RPD	N/A		N/A		N/A		N/A		N/A		N/A		N/A		N/A	

PAHs

CLIENT ID	NI-OF23A-SDB7-FF		NI-BAY23A-SDB7-PRE		NI-BAY23A-SDB7-DUR		NI-OF26-SDB7-FF		NI-OF26-SDB7-COMP		NI-BAY26-SDB7-DUR	
Battelle ID	S7470-P		S7471-P		S7472-P		S7467-P		S7468-P		S7469-P	
Sample Type	SA		SA		SA		SA		SA		SA	
Collection Date	4/28/2005		4/28/2005		4/28/2005		4/28/2005		4/28/2005		4/28/2005	
Extraction Date	5/4/2005		5/4/2005		5/4/2005		5/4/2005		5/4/2005		5/4/2005	
Analysis Date	5/18/2005		5/18/2005		5/18/2005		5/18/2005		5/19/2005		5/17/2005	
Analytical Instrument	MS		MS		MS		MS		MS		MS	
% Moisture	NA		NA		NA		NA		NA		NA	
% Lipid	NA		NA		NA		NA		NA		NA	
Matrix	WATER		WATER		WATER		WATER		WATER		WATER	
Sample Size	1.63		2.65		2.65		2.65		2.65		2.65	
Size Unit-Basis	L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID		L_LIQUID	
Units	NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID	
Naphthalene	7.27	J	1.78	J	1.81	J	31.04		23.38		1.08	J
C1-Naphthalenes	3.97	J	1.8	J	1.45	J	104.31		30.76		0.48	J
C2-Naphthalenes	0.81	U	0.5	U	0.5	U	596.52		135.99		0.5	U
C3-Naphthalenes	0.81	U	0.5	U	0.5	U	1768.67		356.54		0.5	U
C4-Naphthalenes	0.81	U	0.5	U	0.5	U	3442.96		618.61		0.5	U
2-Methylnaphthalene	3.45	J	1.56	J	1.27	J	71.55		22.19		0.45	J
1-Methynaphthatene	2.05	J	0.99	J	0.79	J	91.18		24.49		0.29	J
Biphenyl	1.74	J	0.74	J	0.97	J	20.27		11.62		0.47	U
2,6-dimethylnaphthalene	3.32	J	0.62	U	1.38	J	189.35		46.1		0.62	U
Acenaphthylene	2.8	J	5.99	J	5.09	J	9.48		23.33		0.55	J
Acenaphthene	1.38	J	2.31	J	3.91	J	45.81		18		1.08	J
2,3,5-trimethylnaphthalene	0.71	U	0.44	U	0.44	U	324.46		63.78		0.44	U
Dibenzofuran	2.66	J	5.42	J	8.38		23.65		23.44		0.96	J
Fluorene	2.32	J	2.18	J	3.48	J	73.86		26.77		0.59	J
C1-Fluorenes	0.84	U	0.51	U	0.51	U	425.27		86.92		0.51	U
C2-Fluorenes	0.84	U	0.51	U	0.51	U	2010.84		472.99		0.51	U
C3-Fluorenes	0.84	U	0.51	U	0.51	U	2810.13		579.32		0.51	U
Anthracene	3.32	J	13.01		14.29		29.06		70.69		0.49	J
Phenanthrene	40.71		83.94		104.68		175.36		536.24		1.26	J
C1-Phenanthrenes/Anthracenes	20.74		13.51		16.21		1037.32		389.54		0.81	U
C2-Phenanthrenes/Anthracenes	46.63		15.77		18.94		2983.94		772.76		0.81	U
C3-Phenanthrenes/Anthracenes	26.37		0.81	U	0.81	U	2432.11		703.14		0.81	U
C4-Phenanthrenes/Anthracenes	1.32	U	0.81	U	0.81	U	1319.2		243.89		0.81	U
1-Methylphenanthrene	5.19	J	2.75	J	3.51	J	248.61		92.73		0.46	U
Dibenzothiophene	4.08	J	4.14	J	10.46		125.25		62.99		0.38	U
C1-Dibenzothiophenes	6.35	J	0.38	U	2.6	J	725.47		184.2		0.38	U
C2-Dibenzothiophenes	24.16		0.38	U	2.88	J	2136.8		528.37		0.38	U
C3-Dibenzothiophenes	25.02		0.38	U	2.22	J	2414.49		632.8		0.38	U
C4-Dibenzothiophenes	17.42		0.38	U	0.38	U	1103.85		361.94		0.38	U
Fluoranthene	67.87		233.86		274.95		154.05		1578.13		4.3	J
Pyrene	66.39		134.26		154.19		302.64		1414.83		3.19	J
C1-Fluoranthenes/Pyrenes	21.84		20.16		19.85		446.97		481.58		1.25	J
C2-Fluoranthenes/Pyrenes	31.4		0.68	U	0.68	U	489.83		542.98		0.68	U
C3-Fluoranthenes/Pyrenes	20.02		0.68	U	0.68	U	343.23		352.32		0.68	U
Benzo(a)anthracene	9.73	J	7.01		7.99		36.69		406.38		0.88	J
Chrysene	50.53		100.65		95.68		172.27		1215.77		2.25	J
C1-Chrysenes	37.74		0.44	U	6.97		163.47		359.05		0.44	U
C2-Chrysenes	44.68		0.44	U	0.44	U	186.11		228.17		0.44	U
C3-Chrysenes	45.78		0.44	U	0.44	U	174.12		196.87		0.44	U
C4-Chrysenes	19.66		0.44	U	0.44	U	70		112.59		0.44	U
Benzo(b)fluoranthene	28.09		45.43		44.35		102.67		1159.48		1.78	J
Benzo(j/k)fluoranthene	20.9		35.69		33.48		85.78		1174.32		1.98	J
Benzo(e)pyrene	29.71		24.08		23.93		101.85		883.27		1.36	J
Benzo(a)pyrene	16.7		7.31		6.5		67.79		805.61		1.4	J
Perylene	5.31	J	1.46	U	1.46	U	21.74		204.2		1.46	U
Indeno(1,2,3-cd)pyrene	20.3		9.09		8.74		89.03		1068.22		1.45	J
Dibenz(a,h)anthracene	3.99	J	1.14	J	1.24	J	17.81		197.81		0.3	J
Benzo(g,h,i)perylene	63.1		8.16	J	9.08	J	120.13		1044.55		1.79	J

CLIENT ID	NI-OF23A-SDB7-FF		NI-BAY23A-SDB7-PRE		NI-BAY23A-SDB7-DUR		NI-OF26-SDB7-FF		NI-OF26-SDB7-COMP		NI-BAY26-SDB7-DUR	
Surrogate Recoveries (%)												
Naphthalene-d8	49		44		40		45		38	N	58	
Phenanthrene-d10	76		69		65		70		73		70	
Chrysene-d12	92		90		87		84		86		87	

PAHs QA/QC

PROJECT: Task Order TO0015/TO0019 – Contaminant Analysis of Stormwater
PARAMETER: PAH
LABORATORY: Battelle, Duxbury, MA
MATRIX: Water
SAMPLE CUSTODY: Water samples were collected 4/28/05. The samples were received at Battelle Duxbury on 5/3/05. Upon arrival, the cooler temperatures ranged from 2.2°C – 3.2°C. One sample, BAY-NI26-SDB7-Pr, was broken upon receipt. The project manager was informed of this issue, and relayed it to the client. The lab was instructed to proceed with the remaining samples. No other custody issues were noted. Samples were logged into the Battelle LIMS and received unique IDs. Samples were stored in the access-controlled upper cold room refrigerator at 4.0°C until sample preparation could begin. Samples were extracted as one analytical batch, 05-0129, along with the appropriate quality control samples.

	Reference Method	Method Blank	Surrogate Recovery	LCS/MS Recovery	SRM % Diff.	Sample Replicate Relative Precision	Detection Limits (ng/L)
PAH	General NS&T	<5xMDL	40-120% Recovery	40-120% Recovery (target spike must be >5 x native conc.)	≤30% PD plus variance (for analytes >5x MDL)	≤30% RPD (calculated between the MS and MSD samples)	MDL: ~0.50 – 1.93

METHOD: Water samples were extracted for PAH following general NS&T methods. Approximately 1 liter of water was spiked with surrogates and extracted three times with dichloromethane using separatory funnel techniques. The combined extract was dried over anhydrous sodium sulfate, concentrated, processed through alumina cleanup column, concentrated, and further purified by GPC/HPLC. The post-HPLC extract was concentrated, fortified with **R**IS and split quantitatively for the required analyses. Extracts intended for PAH were analyzed using gas chromatography/mass spectrometry (GC/MS), following general NS&T methods. Sample data were quantified by the method of internal standards, using the **R**ecovery Internal Standard (RIS) compounds.

HOLDING TIMES: Samples were prepared for analysis in one analytical batch and were extracted within 7 days of sample collection and analyzed within 40 days of extraction.

Batch	Extraction Date	Analysis Date
05-0129	5/04/05	5/17/05 – 5/19/05

BLANK: A procedural blank (PB) sample was prepared with the analytical batch. Procedural blank samples are analyzed to ensure the sample extraction and analysis methods are free of contamination.

05-0129 – No exceedences noted.

Comments – No target analytes were detected above the laboratory control limit (>5 x MDL), however naphthalene and 2-Methylnaphthalene were detected in the procedural blank at a concentration less than the reporting limit (RL). The data was qualified with a "J" in the procedural blank. All authentic field sample concentrations for these compounds were either greater than five times the

concentration in the associated blank, or less than the RL.

LABORATORY CONTROL SAMPLE: A laboratory control sample (LCS) was prepared with each analytical batch. The percent recoveries of target PAH were calculated to measure data quality in terms of accuracy.

05-0129 – All target analytes were recovered within the laboratory control limits (40-120%).

Comments – None.

MATRIX SPIKE/MATRIX SPIKE DUPLICATE: A matrix spike (MS) and a matrix spike duplicate (MSD) sample pair were prepared with each analytical batch. The percent recoveries of target PAH and the relative percent difference between the two samples were calculated to measure data quality in terms of accuracy and precision.

05-0129 – All target analytes were recovered within the laboratory control limits specified by the client (40-120%). All calculated RPDs were within the laboratory control limit (≤ 30%).

Comments – None

SRM: A standard reference material (SRM, a certified second source standard was spiked into a natural seawater as an SRM) was prepared with each analytical batch. Surrogate corrected data has been reported for the SRM only.

05-0129 – All target analytes were recovered within the laboratory control limits specified by the client (≤ 30 PD).

Comments – None.

SURROGATES: Three surrogate compounds were added prior to extraction, including naphthalene-d8, phenanthrene-d10, and chrysene-d12. The recovery of each surrogate compound was calculated to measure data quality in terms of accuracy (extraction efficiency).

05-0129 – One exceedence noted.

Comments – Percent recoveries for all surrogate compounds were within the laboratory control limits specified by the method (40 – 120% recovery), except for naphthalene-d8 in sample S7468 (OF-NI26-SDB7-FF). The recovery for this compound was calculated to be 38%. Chromatography and calculations were reviewed. No discrepancies were found. The sample prep records indicate an emulsion formed during the extraction of this sample, and that this extract had difficulty passing through the alumina cleanup column. The exceedences were qualified with an "N". No further corrective action taken.

CALIBRATIONS: The GC/MS is calibrated with a minimum of a 6 level curve. The RSD between response factors for the individual target analytes must be <25%, the mean RSD < 15%. Each batch of samples analyzed is bracketed by a calibration check sample, run at a frequency of minimally every 10 samples. This PD between the initial calibration RF and the check should be <25% for individual analytes, and again the mean PD should be <15%.

05-0129 – No calibration exceedences.

Comments – None.

PAHs QA/QC (CONT.)

CLIENT ID	LABORATORY CONTROL SAMPLE				MARTIX SPIKE-NI-OF23A-SDB7-FF				MATRIX SPIKE DUPLICATE-NI-OF23A-SDB7-FF						PROCEDURAL BLANK		060504-01: DUXBURY SEAWATER		GG73: PCB/PESTICIDE SRM SOLUTION						
Battelle ID	BG248LCS-P				S7470MS-P				S7470MSD-P						BG247PB-P		BG275PB-P		BG276SRM-P						
Sample Type	LCS				MS				MSD						PB		PB		SRM						
Collection Date	5/4/2005				4/28/2005				4/28/2005						5/4/2005		5/4/2005		5/4/2005						
Extraction Date	5/4/2005				5/4/2005				5/4/2005						5/4/2005		5/4/2005		5/4/2005						
Analysis Date	5/17/2005				5/18/2005				5/18/2005						5/17/2005		5/17/2005		5/17/2005						
Analytical Instrument	MS				MS				MS						MS		MS		MS						
% Moisture	NA				NA				NA						NA		NA		NA						
% Lipid	NA				NA				NA						NA		NA		NA						
Matrix	LIQUID				WATER				WATER						LIQUID		LIQUID		LIQUID						
Sample Size	2.00				0.5				0.5						2.00		2		2.00						
Size Unit-Basis	L LIQUID				L LIQUID				L LIQUID						L LIQUID		L LIQUID		L LIQUID			Certified		Passing	Actual
Units	NG/L LIQUID		Target	% Recovery	NG/L LIQUID		Target	% Recovery	NG/L LIQUID		Target	% Recovery	RPD (%)		NG/L LIQUID		NG/L LIQUID		NG/L LIQUID			Value	+/-	%Difference	%Difference
Naphthalene	686.93		1000.60	69	2051.39		4002.40	51	1973.1		4002.40	49	4.0		1.22	J	4.35	J	1022.26			1000.6			2.2
C1-Naphthalenes	0.66	U			2801.42	NA			2651.74	NA					0.66	U	2.28	J	1353.64						
C2-Naphthalenes	0.66	U			2.65	UNA			1236.05	NA					0.66	U	0.66	U	0.66	U					
C3-Naphthalenes	0.66	U			2.65	UNA			2.65	UNA					0.66	U	0.66	U	0.66	U					
C4-Naphthalenes	0.66	U			2.65	UNA			2.65	UNA					0.66	U	0.66	U	0.66	U					
2-Methylnaphthalene	699.17		1002.00	70	2138.17		4008.00	53	2035.88		4008.00	51	3.8		0.6	J	1.92	J	938.81			1002			6.3
1-Methynaphthalene	701.45		1001.20	70	2124.31		4004.80	53	2032.5		4004.80	51	3.8		0.5	U	1.35	J	940.5			1001.2			6.1
Biphenyl	683.83		1000.20	68	2186.57		4000.80	55	2056.94		4000.80	51	7.5		0.62	U	0.62	U	927.81			1000.2			7.2
2,6-dimethylnaphthalene	671.72		1001.00	67	2148.4		4004.00	54	2025.97		4004.00	51	5.7		0.83	U	0.83	U	898.7			1001			10.2
Acenaphthylene	696.61		1000.65	70	2330.15		4002.60	58	2230.88		4002.60	56	3.5		0.7	U	0.7	U	965.76			1000.65			3.5
Acenaphthene	705.01		1000.75	70	2351.79		4003.00	59	2236.53		4003.00	56	5.2		0.75	U	0.75	U	972.35			1000.75			2.8
2,3,5-trimethylnaphthalene	696.21		1000.30	70	2463.11		4001.20	62	2320.61		4001.20	58	6.7		0.58	U	0.58	U	953.76			1000.3			4.7
Dibenzofuran	688.85		1002.20	69	2360.31		4008.80	59	2254.7		4008.80	56	5.2		0.3	U	0.65	J	953.67			1002.2			4.8
Fluorene	716.57		1000.70	72	2582.59		4002.80	64	2455.24		4002.80	61	4.8		0.68	U	0.57	J	985.56			1000.7			1.5
C1-Fluorenes	0.68	U			2.72	UNA			2.72	UNA					0.68	U	0.68	U	0.68	U					
C2-Fluorenes	0.68	U			2.72	UNA			2.72	UNA					0.68	U	0.68	U	0.68	U					
C3-Fluorenes	0.68	U			2.72	UNA			2.72	UNA					0.68	U	0.68	U	0.68	U					
Anthracene	764.04		1000.65	76	2991.32		4002.60	75	2868.53		4002.60	72	4.1		0.51	U	0.51	U	1024.04			1000.65			2.3
Phenanthrene	739.01		1000.65	74	2909.16		4002.60	72	2772.41		4002.60	68	5.7		1.08	U	1.67	J	1001.33			1000.65			0.1
C1-Phenanthrenes/Anthracenes	1.08	U			2310.61	NA			2235.23	NA					1.08	U	1.08	U	1.08	U					
C2-Phenanthrenes/Anthracenes	1.08	U			87.84	NA			88.37	NA					1.08	U	1.08	U	1.08	U					
C3-Phenanthrenes/Anthracenes	1.08	U			58.53	NA			51.04	NA					1.08	U	1.08	U	1.08	U					
C4-Phenanthrenes/Anthracenes	1.08	U			4.32	UNA			4.32	UNA					1.08	U	1.08	U	1.08	U					
1-Methylphenanthrene	769.45		1000.30	77	3032.32		4001.20	76	2916.84		4001.20	73	4.0		0.61	U	0.61	U	1029.72			1000.3			2.9
Dibenzothiophene	727.14		1001.00	73	2828.38		4004.00	71	2702.91		4004.00	67	5.8		0.5	U	0.5	U	991.17			1001			1
C1-Dibenzothiophenes	0.5	U			138.06	NA			130.53	NA					0.5	U	0.5	U	0.5	U					
C2-Dibenzothiophenes	0.5	U			29.62	JNA			33.61	NA					0.5	U	0.5	U	0.5	U					
C3-Dibenzothiophenes	0.5	U			31.49	JNA			36.17	NA					0.5	U	0.5	U	0.5	U					
C4-Dibenzothiophenes	0.5	U			24.27	JNA			22.05	JNA					0.5	U	0.5	U	0.5	U					
Fluoranthene	821.94		1000.50	82	3251.87		4002.00	80	3170.94		4002.00	78	2.5		0.77	U	1.55	J	1104.38			1000.5			10.4
Pyrene	824.46		1000.50	82	3271.32		4002.00	80	3176.64		4002.00	78	2.5		0.9	U	1.01	J	1107.39			1000.5			10.7
C1-Fluoranthenes/Pyrenes	0.9	U			29.03	JNA			31.31	JNA					0.9	U	0.9	U	0.9	U					
C2-Fluoranthenes/Pyrenes	0.9	U			23.18	JNA			25.07	JNA					0.9	U	0.9	U	0.9	U					
C3-Fluoranthenes/Pyrenes	0.9	U			24.42	JNA			30.07	JNA					0.9	U	0.9	U	0.9	U					
Benzo(a)anthracene	829.99		1000.60	83	3342.41		4002.40	83	3185.28		4002.40	79	4.9		1.36	U	0.31	J	876.32			1000.6			12.4
Chrysene	823.75		1000.75	82	3181.59		4003.00	78	3113.69		4003.00	77	1.3		0.59	U	0.72	J	857.67			1000.75			14.3
C1-Chrysenes	0.59	U			34.57	NA			38.44	NA					0.59	U	0.59	U	0.59	U					
C2-Chrysenes	0.59	U			51.96	NA			58.75	NA					0.59	U	0.59	U	0.59	U					
C3-Chrysenes	0.59	U			36.83	NA			44.77	NA					0.59	U	0.59	U	0.59	U					
C4-Chrysenes	0.59	U			2.36	UNA			2.36	UNA					0.59	U	0.59	U	0.59	U					
Benzo(b)fluoranthene	851.64		1000.75	85	3402.73		4003.00	84	3290.1		4003.00	81	3.6		1.16	U	1.16	U	893.46			1000.75			10.7
Benzo(j/k)fluoranthene	881.56		1000.65	88	3353.44		4002.60	83	3254.85		4002.60	81	2.4		1.31	U	1.31	U	921.45			1000.65			7.9
Benzo(e)pyrene	776.8		1001.80	78	3028.88		4007.20	75	2982.69		4007.20	74	1.3		0.51	U	0.51	U	815.6			1001.8			18.6
Benzo(a)pyrene	839.45		1000.65	84	3257.28		4002.60	81	3122.06		4002.60	78	3.8		1	U	1	U	882.23			1000.65			11.8
Perylene	819.83		1000.20	82	3320.82		4000.80	83	3226.69		4000.80	81	2.4		1.93	U	1.93	U	866.73			1000.2			13.3
Indeno(1,2,3-cd)pyrene	812.86		1000.60	81	3339.86		4002.40	83	3224.82		4002.40	80	3.7		0.99	U	0.99	U	853.56			1000.6			14.7
Dibenz(a,h)anthracene	882.52		1000.55	88	3440.18		4002.20	86	3281.85		4002.20	82	4.8		0.84	U	0.84	U	924.45			1000.55			7.6
Benzo(g,h,i)perylene	814.87		1000.70	81	3214.16		4002.80	79	3128.86		4002.80	77	2.6		0.99	U	0.99	U	954.95			1000.7			14.6

CLIENT ID	LABORATORY CONTROL SAMPLE				MARTIX SPIKE-NI-OF23A-SDB7-FF				MATRIX SPIKE DUPLICATE-NI-OF23A-SDB7-FF						PROCEDURAL BLANK		060504-01: DUXBURY SEAWATER		GG73: PCB/PESTICIDE SRM SOLUTION						
Surrogate Recoveries (%)																									
Naphthalene-d8	83				51				43						83		52		68						
Phenanthrene-d10	80				72				68						78		71		75						
Chrysene-d12	102				89				86						98		87		95						

PCBs

CLIENT ID	NI-OF23A-SDB7-FF		NI-OF26-SDB7-COMP	
Battelle ID	S7470-P		S7468-P	
Sample Type	SA		SA	
Collection Date	4/28/2005		4/28/2005	
Extraction Date	5/4/2005		5/4/2005	
Analysis Date	5/29/2005		5/30/2005	
Analytical Instrument	MS		MS	
% Moisture	NA		NA	
% Lipid	NA		NA	
Matrix	WATER		WATER	
Sample Size	1.63		2.65	
Size Unit-Basis	L_LIQUID		L_LIQUID	
Units	NG/L_LIQUID		NG/L_LIQUID	
Cl2(8)	0.11	U	0.07	U
Cl3(18)	0.13	U	0.08	U
Cl3(28)	0.13	U	0.08	U
Cl4(44)	0.24	U	0.14	U
Cl4(49)	0.24	U	0.14	U
Cl4(52)	0.24	U	4.31	
Cl4(66)	0.24	U	3.9	
Cl4(77)	0.23	U	0.14	U
Cl5(87)	0.38	U	5.13	
Cl5(101)	0.38	U	29.3	
Cl5(105)	0.17	U	3.34	
Cl5(114)	0.38	U	0.23	U
Cl5(118)	0.12	U	7.05	
Cl5(123)	0.13	U	0.08	U
Cl5(126)	0.19	U	0.12	U
Cl6(128)	0.43	U	5.89	
Cl6(138)	0.43	U	74.73	
Cl6(153)	0.43	U	164.58	
Cl6(156)	0.12	U	7.02	
Cl6(157)	0.23	U	0.14	U
Cl6(167)	0.43	U	3.92	
Cl6(169)	0.18	U	0.11	U
Cl7(170)	0.3	U	55.33	
Cl7(180)	0.17	U	228.53	E
Cl7(183)	0.3	U	38.24	
Cl7(184)	0.3	U	0.18	U
Cl7(187)	0.3	U	84.98	
Cl7(189)	0.13	U	3.89	
Cl8(195)	0.59	U	11.77	
Cl9(206)	0.54	U	8.3	
Cl10(209)	0.66	U	1.5	J
Surrogate Recoveries (%)				
Cl2(14)	71		82	
Cl3(34)	76		84	

PCBs QA/QC

PROJECT: Task Order TO0015/TO0019 – Contaminant Analysis of Stormwater
PARAMETER: PCB
LABORATORY: Battelle, Duxbury, MA
MATRIX: Water
SAMPLE CUSTODY: Water samples were collected 4/28/05. The samples were received at Battelle Duxbury on 5/3/05. Upon arrival, the cooler temperatures ranged from 2.2°C – 3.2°C. One sample, BAY-NI26-SDB7-Pr, was broken upon receipt. The project manager was informed of this issue, and relayed it to the client. The lab was instructed to proceed with the remaining samples. No other custody issues were noted. Samples were logged into the Battelle LIMS and received unique IDs. Samples were stored in the access-controlled upper cold room refrigerator at 4.0°C until sample preparation could begin. Samples were extracted as one analytical batch, 05-0129, along with the appropriate quality control samples.

	Reference Method	Method Blank	Surrogate Recovery	LCS/MS Recovery	SRM % Diff.	Sample Replicate Relative Precision	Detection Limits (ng/L)
PCB	General NS&T	<5xMDL	40-120% Recovery	40-120% Recovery (target spike must be >5 x native conc.)	≤30% PD on average (for analytes >5x MDL)	≤30% RPD (calculated between the MS and MSD samples)	MDL: ~0.09 – 0.53

METHOD: Water samples were extracted for PCB following general NS&T methods. Approximately 1 liter of water was spiked with surrogates and extracted three times with dichloromethane using separatory funnel techniques. The combined extract was dried over anhydrous sodium sulfate and concentrated. The extract was then fortified with RIS and split quantitatively for the required analyses. Extracts were analyzed using gas chromatography/mass spectrometry (GC/MS). The method is based on key components of the PCB congener analysis approach described in EPA Method 1668A. Sample data were quantified by the method of internal standards, using the Recovery Internal Standard (RIS) compounds

HOLDING TIMES: Samples were prepared for analysis in one analytical batch and were extracted within 7 days of sample collection and analyzed within 40 days of extraction.

Batch	Extraction Date	Analysis Date
05-0129	5/4/05	5/28/05 – 5/30/05

BLANK: A procedural blank (PB) was prepared with the analytical batch. Blanks are analyzed to ensure the sample extraction and analysis methods were free of contamination.

05-0129 – No exceedences noted.

Comments – No target analytes were detected in the procedural blank.

LABORATORY CONTROL SAMPLE: A laboratory control sample (LCS) was prepared with each analytical batch. The percent recoveries of target PCB were calculated to measure data quality in terms of accuracy.

05-0129 –One exceedence noted.

Comments – All target analytes were recovered within the specified laboratory control limits (40-120%), except for PCB 169. This analyte was over-recovered at

141%. It was also over-recovered in both the MS and MSD samples. Chromatography and calculations were reviewed. No discrepancies were found. The exceedence has been qualified with an "N". Since PCB 169 was not detected in any field samples, the affect of this exceedence on the data is minimal. No further corrective action is necessary.

MATRIX SPIKE/MATRIX SPIKE DUPLICATE: A matrix spike (MS) and a matrix spike duplicate (MSD) sample pair was prepared with each analytical batch. The percent recoveries of target PCB and the relative percent difference between the two samples were calculated to measure data quality in terms of accuracy and precision.

05-0129 – Three percent recovery exceedences noted.
No RPD exceedences noted.

Comments – All target analytes were recovered within the specified laboratory control limits (40-120%), except for PCB 169 in samples S7470MS and S7470MSD (background OF-NI23A-SDB7-FF) and PCB 209 in sample S7470MS. All exceedences were due to over-recoveries. Chromatography and calculations were reviewed, no discrepancies were found. The exceedences were qualified with an "N". Since PCB 169 was not detected in any field samples, and PCB 209 was not detected above the RL, the affect of these exceedences on the data is minimal. No further corrective action is necessary.

SRM: A standard reference material was prepared with each analytical batch. The percent difference (PD) between the measured value and the certified range was calculated to measure data quality in terms of accuracy. The MQO criteria of 30% PD was added to the variance of each analyte. The variance of each analyte is determined by dividing the range value by the target.

05-0129 – All PDs were within the specified laboratory control limits.

Comments – None.

SURROGATES: Two surrogate compounds were added prior to extraction, including PCB 14 and PCB 34. The recovery of each surrogate compound was calculated to measure data quality in terms of accuracy (extraction efficiency).

05-0129 – Percent recoveries for all surrogate compounds were within the laboratory control limits (40 – 120% recovery).

Comments – None.

CALIBRATION: The GC/MS is calibrated with a minimum of a 6-point curve. The co-efficient of determination must be ≥ 0.995 for each target analyte. Each batch of samples analyzed is bracketed by a calibration check sample, run at a frequency of every 12 hours (minimally). This PD between the initial calibration RF and the check should be <20% for individual analytes; 15% on average. Additionally an ICC check was run with the initial calibration. The PD for the ICC should be < 15%, for each analyte.

05-0129 – One exceedence noted.

Comments – In mid C1466.d PCB 105 was over-recovered and had a PD of 31%. Two samples S7468 and S7478 (Samples OF-NI26-SDB7-Comp and OF-NAB18-SDB7-Comp, respectively) had PCB 105 detected in them. Chromatography and calculations were reviewed. No discrepancies were found. The deviation has been documented and the data reviewed. No further corrective action was taken.

PCBs QA/QC (CONT.)

CLIENT ID	LABORATORY CONTROL SAMPLE			MATRIX SPIKE-NI-OF23A-SDB7-FF			MATRIX SPIKE DUPLICATE-NI-OF23A-SDB7-FF				PROCEDURAL BLANK		050504-01: DUXBURY SEAWATER		GG73: PCB/PESTICIDE SRM SOLUTION					
Battelle ID	BG248LCS-P			S7470MS-P			S7470MSD-P				BG247PB-P		BG275PB-P		BG276SRM-P					
Sample Type	LCS			MS			MSD				PB		PB		SRM					
Collection Date	5/4/2005			4/28/2005			4/28/2005				5/4/2005		5/4/2005		5/4/2005					
Extraction Date	5/4/2005			5/4/2005			5/4/2005				5/4/2005		5/4/2005		5/4/2005					
Analysis Date	5/28/2005			5/29/2005			5/29/2005				5/28/2005		5/28/2005		5/28/2005					
Analytical Instrument	MS			MS			MS				MS		MS		MS					
% Moisture	NA			NA			NA				NA		NA		NA					
% Lipid	NA			NA			NA				NA		NA		NA					
Matrix	LIQUID			WATER			WATER				LIQUID		LIQUID		LIQUID					
Sample Size	2.00			0.5			0.5				2.00		2		2.00					
Size Unit-Basis	L_LIQUID			L_LIQUID			L_LIQUID				L_LIQUID		L_LIQUID		L_LIQUID		Certified		Passing	Actual
Units	NG/L_LIQUID		Target	% Recovery	NG/L_LIQUID		Target	% Recovery	NG/L_LIQUID		Target	% Recovery	RPD (%)	NG/L_LIQUID		NG/L_LIQUID		NG/L_LIQUID		Value	+/-	%Difference	%Difference
Cl2(8)	27.49		40.12	69	98.76		160.48	62	111.42		160.48	69	10.7	0.09	U	0.09	U	27.52		34.24	2.88	38.41	19.6
Cl3(18)	32.94		40.12	82	117.38		160.48	73	123.26		160.48	77	5.3	0.11	U	0.11	U	31.47		32.93	0.30	30.92	4.4
Cl3(28)	29.26		40.04	73	118.72		160.16	74	114.24		160.16	71	4.1	0.11	U	0.11	U	30.54					
Cl4(44)	34.28		40.08	86	134.47		160.32	84	124.95		160.32	78	7.4	0.19	U	0.19	U	30.39		32.86	0.59	31.8	7.5
Cl4(49)	40.18		40.16	100	150.87		160.64	94	145.68		160.64	91	3.2	0.19	U	0.19	U	0.19	U				
Cl4(52)	31.65		40.00	79	122.5		160.00	77	119.2		160.00	75	2.6	0.19	U	0.19	U	30.03		33.07	0.38	31.16	9.2
Cl4(66)	31.54		40.04	79	141.85		160.16	89	118.9		160.16	74	18.4	0.19	U	0.19	U	30.09		32.82	0.62	31.9	8.3
Cl4(77)	31.71		40.00	79	160.6		160.00	100	131.34		160.00	82	19.8	0.18	U	0.18	U	31.48		33.55	1.10	33.29	6.2
Cl5(87)	35.98		40.00	90	165.64		160.00	104	136.48		160.00	85	20.1	0.31	U	0.31	U	34.88		33.1	0.27	30.82	5.4
Cl5(101)	34.94		40.08	87	155.41		160.32	97	124.41		160.32	78	21.7	0.31	U	0.31	U	31.45		32.56	0.47	31.43	3.4
Cl5(105)	32.22		40.04	80	187.32		160.16	117	144.07		160.16	90	26.1	0.14	U	0.14	U	33.85		32.67	1.01	33.09	3.6
Cl5(114)	0.31	U			1.23	U			1.23	U				0.31	U	0.31	U	0.31	U				
Cl5(118)	32.35		40.04	81	163.33		160.16	102	126.35		160.16	79	25.4	0.1	U	0.1	U	29.41		32.74	1.06	33.23	10.2
Cl5(123)	0.11	U			0.43	U			0.43	U				0.11	U	0.11	U	0.11	U				
Cl5(126)	29.27		40.24	73	166.74		160.96	104	130.52		160.96	81	24.9	0.16	U	0.16	U	32.45		33.22	1.38	34.14	2.3
Cl6(128)	29.39		40.24	73	149.58		160.96	93	117.39		160.96	73	24.1	0.35	U	0.35	U	27.53		32.94	0.27	30.83	16.4
Cl6(138)	33.24		40.08	83	176.99		160.32	110	139.78		160.32	87	23.4	0.35	U	0.35	U	31.99		32.43	0.38	31.18	1.4
Cl6(153)	34.07		40.04	85	168.47		160.16	105	131.73		160.16	82	24.6	0.35	U	0.35	U	30.86		32.64	0.62	31.91	5.5
Cl6(156)	0.1	U			0.4	U			0.4	U				0.1	U	0.1	U	0.1	U				
Cl6(157)	0.19	U			0.76	U			0.76	U				0.19	U	0.19	U	0.19	U				
Cl6(167)	0.35	U			1.42	U			1.42	U				0.35	U	0.35	U	0.35	U				
Cl6(169)	56.68		40.16	141	N 309.8		160.64	193	N 248.63		160.64	155	N 21.8	0.15	U	0.15	U	0.15	U				
Cl7(170)	29.13		40.20	72	163.63		160.80	102	131.34		160.80	82	21.7	0.25	U	0.25	U	27.28		32.72	0.54	31.66	16.6
Cl7(180)	29.47		40.16	73	175.36		160.64	109	146.13		160.64	91	18.0	0.14	U	0.14	U	29.53		32.96	0.32	30.97	10.4
Cl7(183)	32.99		40.16	82	169.17		160.64	105	137.46		160.64	86	19.9	0.25	U	0.25	U	0.25	U				
Cl7(184)	34.92		40.16	87	163.2		160.64	102	132		160.64	82	21.7	0.25	U	0.25	U	0.25	U				
Cl7(187)	30.23		40.12	75	152.19		160.48	95	127.03		160.48	79	18.4	0.25	U	0.25	U	30.46		32.75	0.30	30.93	7
Cl7(189)	0.11	U			0.42	U			0.42	U				0.11	U	0.11	U	0.11	U				
Cl8(195)	29.27		40.12	73	148.26		160.48	92	120.19		160.48	75	20.4	0.48	U	0.48	U	27.7		32.83	0.66	32	15.6
Cl9(206)	33.76		40.12	84	172.85		160.48	108	143.4		160.48	89	19.3	0.44	U	0.44	U	32.46		32.02	0.59	31.85	1.4
Cl10(209)	46.77		40.04	117	223.66		160.16	140	N 182.47		160.16	114	20.5	0.53	U	0.53	U	42.96		32.99	0.45	31.36	30.2
Surrogate Recoveries (%)																				
Cl2(14)	87				74				77					77		68		80		
Cl3(34)	94				79				82					80		70		82		

PESTICIDEs

CLIENT ID	NI-OF23A-SDB7-FF		NI-OF26-SDB7-COMP	
Battelle ID	S7470-P		S7468-P	
Sample Type	SA		SA	
Collection Date	4/28/2005		4/28/2005	
Extraction Date	5/4/2005		5/4/2005	
Analysis Date	5/14/2005		5/14/2005	
Analytical Instrument	ECD		ECD	
% Moisture	NA		NA	
% Lipid	NA		NA	
Matrix	WATER		WATER	
Sample Size	1.63		2.65	
Size Unit-Basis	L_LIQUID		L_LIQUID	
Units	NG/L_LIQUID		NG/L_LIQUID	
2,4'-DDD	1	U	7.52	
2,4'-DDE	0.11	J	0.52	U
2,4'-DDT	0.59	U	5.98	
4,4'-DDD	1.17	U	6.55	
4,4'-DDE	0.3	J	9.29	
4,4'-DDT	0.74	J	16.1	
aldrin	0.49	U	0.3	U
a-chlordane	0.51	J	8.56	
g-chlordane	1.35		14.36	
a-BHC	0.42	U	0.26	U
b-BHC	0.58	U	0.36	U
d-BHC	0.48	U	1.62	
Lindane	0.61	U	0.37	U
cis-nonachlor	0.79	U	3.16	
trans-nonachlor	0.3	J	6.48	
Chlorpyrifos	0.63	U	0.39	U
oxychlordane	0.48	U	0.3	U
dieldrin	0.94	U	2.53	
endosulfan I	0.09	J	0.21	U
endosulfan II	0.85	U	5.98	
endosulfan sulfate	0.8	U	33.23	
endrin	0.92	U	0.57	U
endrin aldehyde	1.04	U	6.25	
endrin ketone	1.09	U	0.67	U
heptachlor	0.72	U	0.44	U
heptachlor epoxide	1.93	U	1.19	U
Hexachlorobenzene	1.01	U	0.62	U
methoxychlor	2.41		15.05	
Mirex	0.76	U	0.47	U
Surrogate Recoveries (%)				
CI2(14)	91		89	
CI3(34)	88		95	
CI5(104)	85		90	
CI5(112)	90		92	

PESTICIDEs QA/QC

PROJECT: Task Order TO0015/TO0019 – Contaminant Analysis of Stormwater
PARAMETER: Pesticides
LABORATORY: Battelle, Duxbury, MA
MATRIX: Water
SAMPLE CUSTODY: Water samples were collected 4/28/05. The samples were received at Battelle Duxbury on 5/3/05. Upon arrival, the cooler temperatures ranged from 2.2°C – 3.2°C. One sample, BAY-NI26-SDB7-Pr, was broken upon receipt. The project manager was informed of this issue, and relayed it to the client. The lab was instructed to proceed with the remaining samples. No other custody issues were noted. Samples were logged into the Battelle LIMS and received unique IDs. Samples were stored in the access-controlled upper cold room refrigerator at 4.0°C until sample preparation could begin. Samples were extracted as one analytical batch, 05-0129, along with the appropriate quality control samples.

	Reference Method	Method Blank	Surrogate Recovery	LCS/MS Recovery	SRM % Diff.	Sample Replicate Relative Precision	Detection Limits (ng/L)
PESTICIDE	General NS&T	<5xMDL	40-120% Recovery	40-120% Recovery (target spike must be >5 x native conc.)	≤30% PD plus variance (for analytes >5x MDL)	≤30% RPD (calculated between the MS and MSD samples)	MDL: ~0.27 – 1.58

METHOD: Water samples were extracted for pesticide following general NS&T methods. Approximately 2 liters of water was spiked with surrogates and extracted three times with dichloromethane using separatory funnel techniques. The combined extract was dried over anhydrous sodium sulfate, concentrated, processed through alumina cleanup column, concentrated, copper cleaned, and further purified by GPC/HPLC. The post-HPLC extract was concentrated, fortified with RIS and split quantitatively for the required analyses. Extracts intended for pesticide analysis were solvent exchanged into hexane and analyzed using a gas chromatography/electron capture detector (GC/ECD). Sample data were quantified by the method of internal standards, using the Recovery Internal Standard (RIS) compounds.

HOLDING TIMES: Samples were prepared for analysis in one analytical batch and were extracted within 7 days of sample collection and analyzed within 40 days of extraction.

Batch	Extraction Date	Analysis Date
05-0129	5/04/05	5/14/05 – 5/16/05

BLANK: A procedural blank (PB) was prepared with the analytical batch. Blanks are analyzed to ensure the sample extraction and analysis methods were free of contamination.

05-0129 – No exceedences noted.

Comments – No target analytes were detected in the procedural blank.

LABORATORY CONTROL A laboratory control sample (LCS) was prepared with the analytical batch. The percent recoveries of target pesticides were calculated to measure data quality in

SAMPLE: terms of accuracy.

05-0129 – All target analytes were recovered within the laboratory control limits specified by the client (40-120%).

Comments – None.

MATRIX SPIKE/MATRIX SPIKE DUPLICATE: A matrix spike (MS) and a matrix spike duplicate (MSD) sample pair were prepared with each analytical batch. The percent recoveries of target pesticides and the relative percent difference between the two samples were calculated to measure data quality in terms of accuracy and precision.

05-0129 – All target analytes were recovered within the laboratory control limits specified by the client (40-120%). All calculated RPDs were within the laboratory control limit ($\leq$ 30%).

Comments – None

SRM: A standard reference material (SRM, a certified second source standard was spiked into a natural seawater as an SRM) was prepared with each analytical batch. Surrogate corrected data has been reported for the SRM only.

05-0129 – All percent differences for reported target analytes were within the laboratory control limits (<30% difference plus variance).

Comments – None.

SURROGATES Four surrogate compounds were added prior to extraction, including PCB 14, PCB 34, PCB 104, and PCB 112. The recovery of each surrogate compound was calculated to measure data quality in terms of accuracy (extraction efficiency).

05-0129 – Percent recoveries for all surrogate compounds were within the laboratory control limits (40 – 120% recovery).

Comments – None.

CALIBRATIONS: The instrument is calibrated with a 6-level (minimum) calibration, ranging in concentration from ~0.001 ng/uL to ~0.125 ng/uL. The initial correlation coefficient must be > 0.995. Calibration checks are analyzed minimally every 12 hours. The samples must be bracketed by passing calibrations. Calibration checks must have a percent difference $\leq$ 25%.

05-0129 – No exceedences noted.

Comments – None.

PESTICIDEs QA/QC (CONT.)

CLIENT ID	LABORATORY CONTROL SAMPLE			MATRIX SPIKE: NI-OF23A-SDB7-FF			MATRIX SPIKE DUPLICATE-NI-OF23A-SDB7-FF				PROCEDURAL BLANK		050504-01: DUXBURY SEAWATER		GG73: PCB/PESTICIDE SRM SOLUTION					
Battelle ID	BG248LCS-P			S7470MS-P			S7470MSD-P				BG247PB-P		BG276PB-P		BG276SRM-P					
Sample Type	LCS			MS			MSD				PB		PB		SRM					
Collection Date	5/4/2005			4/28/2005			4/28/2005				5/4/2005		5/4/2005		5/4/2005					
Extraction Date	5/4/2005			5/4/2005			5/4/2005				5/4/2005		5/4/2005		5/4/2005					
Analysis Date	5/16/2005			5/14/2005			5/14/2005				5/16/2005		5/16/2005		5/14/2005					
Analytical Instrument	ECD			ECD			ECD				ECD		ECD		ECD					
% Moisture	NA			NA			NA				NA		NA		NA					
% Lipid	NA			NA			NA				NA		NA		NA					
Matrix	LIQUID			WATER			WATER				LIQUID		LIQUID		LIQUID					
Sample Size	2.00			0.5			0.5				2.00		2		2.00					
Size Unit-Basis	L LIQUID			L LIQUID			L LIQUID				L LIQUID		L LIQUID		L LIQUID		Certified		Passing	Actual
Units	NG/L LIQUID	Target	% Recovery	NG/L LIQUID	Target	% Recovery	NG/L LIQUID	Target	% Recovery	RPD (%)	NG/L LIQUID		NG/L LIQUID		NG/L LIQUID		Value	+/-	%Difference	%Difference
2,4'-DDD	33.25	40.12	83	126.17	160.48	79	121.46	160.48	76	3.9	0.81	U	0.81	U	65.74					
2,4'-DDE	29.51	40.01	74	116.42	160.05	73	110.03	160.05	69	5.6	0.69	U	0.69	U	22.4		31.62	0.27	30.86	29.2
2,4'-DDT	26.38	40.23	66	110.77	160.93	69	104.02	160.93	65	6.0	0.48	U	0.48	U	27.15		31.34	0.14	30.46	13.4
4,4'-DDD	33.01	40.01	83	128.85	160.02	81	123.46	160.02	77	5.1	0.95	U	0.95	U	24.85		31.87	0.43	31.36	22
4,4'-DDE	32.85	40.01	82	125.33	160.02	78	118.75	160.02	74	5.3	0.68	U	0.68	U	29.28		31.62	0.16	30.51	10.6
4,4'-DDT	32.99	40.02	82	130.94	160.09	81	124.55	160.09	77	5.1	0.59	U	0.59	U	27.74		31.47	0.19	30.61	11.9
aldrin	27.61	40.01	69	105.56	160.06	66	97.41	160.06	61	7.9	0.4	U	0.4	U	21.28					
a-chlordane	29.84	40.03	75	113.71	160.11	71	108.08	160.11	67	5.8	0.38	U	0.38	U	26.85		31.55	0.19	30.61	14.9
g-chlordane	28.59	40.06	71	107.53	160.26	66	103.45	160.26	64	3.1	0.4	U	0.4	U	0.4	U				
a-BHC	23.22	40.02	58	84.2	160.06	53	72.71	160.06	45	16.3	0.34	U	0.28	J	0.32	J				
b-BHC	26.75	40.01	67	100.77	160.02	63	93.5	160.02	58	8.3	0.47	U	0.47	U	0.47	U				
d-BHC	31.05	40.02	78	123.12	160.07	77	113.38	160.07	71	8.1	0.39	U	0.39	U	0.39	U				
Lindane	26.45	40.01	66	103.5	160.04	65	92.09	160.04	58	11.4	0.49	U	0.49	U	22.74		31.55	0.16	30.51	27.9
cis-nonachlor	33.3	40.03	83	124.04	160.11	77	119.47	160.11	75	2.6	0.65	U	0.65	U	0.65	U				
trans-nonachlor	30.91	40.06	77	117.4	160.22	73	112.61	160.22	70	4.2	0.4	U	0.4	U	27.72		31.78	0.22	30.7	12.8
Chlorpyrifos	32.53	40.10	81	127.35	160.40	79	121.06	160.40	75	5.2	0.51	U	0.51	U	0.51	U				
oxychlordane	28.88	40.03	72	108.92	160.11	68	102.68	160.11	64	6.1	0.39	U	0.39	U	0.39	U				
dieldrin	32.5	40.01	81	119.69	160.03	75	115.7	160.03	72	4.1	0.76	U	0.76	U	27.43		31.55	0.21	30.66	13.1
endosulfan I	31.23	40.03	78	114.7	160.11	72	110.45	160.11	69	4.3	0.27	U	0.27	U	0.27	U				
endosulfan II	31.53	40.02	79	121.66	160.08	76	116.01	160.08	72	5.4	0.69	U	0.69	U	0.69	U				
endosulfan sulfate	35.11	40.02	88	133.4	160.07	83	127.81	160.07	80	3.7	0.65	U	0.65	U	0.65	U				
endrin	34.35	40.01	86	136.19	160.05	85	128.49	160.05	80	6.1	0.75	U	0.75	U	0.75	U				
endrin aldehyde	27.27	40.01	68	105.84	160.03	66	99.74	160.03	62	6.3	0.85	U	0.85	U	0.85	U				
endrin ketone	35.14	40.02	88	132.66	160.06	83	129.21	160.06	81	2.4	0.89	U	0.89	U	0.89	U				
heptachlor	29.47	40.00	74	114.63	160.02	72	104.8	160.02	65	10.2	0.59	U	0.59	U	25.28		31.63	0.24	30.76	20.1
heptachlor epoxide	28.54	40.01	71	102.62	160.02	64	98.63	160.02	62	3.2	1.58	U	1.58	U	23.46		31.63	0.27	30.86	25.8
Hexachlorobenzene	30.22	40.06	75	114.74	160.24	72	107.22	160.24	67	7.2	0.83	U	0.83	U	27.24		31.49	0.14	30.46	13.5
methoxychlor	33.31	40.01	83	133.39	160.05	82	127.62	160.05	78	5.0	0.98	U	0.98	U	0.98	U				
Mirex	34	40.03	85	124.94	160.13	78	121.75	160.13	76	2.6	0.62	U	0.62	U	29.34		31.86	0.45	31.41	7.9
Surrogate Recoveries (%)																				
CI2(14)	93			83			76				85		79		87					
CI3(34)	99			87			76				86		80		84					
CI5(104)	91			80			77				86		77		75					
CI5(112)	96			82			79				96		81		86					

TSS

SAMPLE LABEL	TSS (mg/L)
NI-OF23A-SDB7-FF	63.571
NI-BAY23A-SDB7-PRE	5.536
NI-BAY23A-SDB7-DUR	6.232
NI-OF26-SDB7-FF	145.558
NI-OF26-SDB7-COMP	162.415
NI-BAY26-SDB7-PRE	4.519
NI-BAY26-SDB7-DUR	4.165

DOC

SAMPLE LABEL	DOC (mg/L)
NI-OF-23A-SDB7-FF	3.796
NI-OF-23A-SDB7-FF	3.748
NI-OF-23A-SDB7-FF	3.810
NI-BAY23A-SDB7-PRE	2.144
NI-BAY23A-SDB7-PRE	2.074
NI-BAY23A-SDB7-PRE	2.059
NI-BAY23A-SDB7-DUR	3.111
NI-BAY23A-SDB7-DUR	3.243
NI-BAY23A-SDB7-DUR	3.284
NI-OF26-SDB7-FF	47.653
NI-OF26-SDB7-FF	49.174
NI-OF26-SDB7-FF	49.197
NI-OF26-SDB7-COMP	1.089
NI-OF26-SDB7-COMP	0.798
NI-OF26-SDB7-COMP	0.841
NI-BAY26-SDB7-PRE	1.789
NI-BAY26-SDB7-PRE	1.695
NI-BAY26-SDB7-PRE	1.643
NI-BAY26-SDB7-DUR	2.874
NI-BAY26-SDB7-DUR	3.120
NI-BAY26-SDB7-DUR	3.047

APPENDIX E

TIE1 Report

Please note that the report in this appendix was generated with slightly different acronyms from those used throughout the body of the report and other appendices. The differences are as follows:

MAIN REPORT	THIS APPENDIX
NAV	NAVSTA
SUB	SUBASE

Additionally, one outfall identified as OF23CE in the report and other appendices is identified as OF23C+e in this appendix.

Nautilus Environmental

Toxicity Identification Evaluation (TIE) Study of San Diego Bay Stormwater

February 18, 2004 Sampling Event
FINAL REPORT
Response to External Comments Included

Prepared for: Computer Sciences Corporation
4045 Hancock Street
San Diego, CA 92110

Space and Naval Warfare Systems Center
San Diego (SPAWAR)
53560 Hull Street
San Diego, CA 92152-5001

Prepared by Nautilus Environmental
5550 Morehouse Drive, Suite 150
San Diego, CA 92121

Submitted: April 26, 2006

Data Quality Assurance:

- Nautilus Environmental is a certified laboratory under the State of California Department of Health Services Environmental Laboratory Accreditation Program (ELAP), Certificate No. 1802.
- All test results included in this report have met internal Quality Assurance/Quality Control (QA/QC) requirements, as well as minimum acceptability criteria as outlined in their respective protocols.
- All data have been reviewed and verified.
- Any test data discrepancies or protocol deviations have been noted in the summary report pages.

Results verified by: **Chris Stransky, Laboratory Manager** Date: **April 26, 2006**

1.0 INTRODUCTION

From February through July 2004, preliminary screening and Toxicity Identification Evaluation (TIE) studies were performed on stormwater samples collected from six storm drain outfalls (NAVSTA: OF 9; OF 11; and OF 14; SUBASE: OF 11B; OF 23c+e; and OF 26) discharging into San Diego Bay, San Diego, California. Stormwater toxicity to several marine species, including *Mytilus galloprovincialis* (blue mussel), *Atherinops affinis* (topsmelt), and *Americamysis bahia* (opossum shrimp) has been documented in previous monitoring surveys. Confirmation studies using the blue mussel, opossum shrimp, and *Menidia beryllina* (inland silverside) were performed at the AMEC Earth & Environmental Aquatic & Terrestrial Toxicology Laboratory (AMEC) located in San Diego, California. Inland silversides were used in place of topsmelt due to lack of availability. Toxicity to mussel larvae was confirmed for all six samples. One sample (SUBASE OF 23 c+e) also exhibited marked toxicity to the opossum shrimp. No toxicity to the silversides was observed in any of the samples tested. Subsequently, Phase I TIEs using the blue mussel were initiated for all six sites, and a single Phase I TIE was initiated with opossum shrimp on SUBASE OF 23 c+e. Metals, particularly zinc and copper, were largely responsible for toxicity in all six samples tested. Results from the SUBASE OF 11B Phase I TIE also identified the presence of an organic toxicant. TIE sample manipulations were performed using methods outlined by the U.S. Environmental Protection Agency (EPA). All biological testing was conducted at AMEC. Supporting analytical testing was conducted in partnership with Calscience Environmental Laboratories (CEL), located in Garden Grove, California. Results of the screening studies, Phase I TIEs, and Phase II/III TIEs are presented in this report. Screening studies were initiated on 19 February 2004. Phase I testing was initiated on 27 February 2004. Phase II/III TIEs were initiated between 3 April and 15 July 2004, and identification of the organic constituent found in SUBASE OF 11B is ongoing.

2.0 MATERIALS AND METHODS

2.1 Test Material

Stormwater samples were collected on 18 February 2004 between 4:25 and 6:30 PM under the supervision of SPAWAR personnel. The samples were collected using peristaltic pumps and contained in plastic bags lining 19-L plastic buckets. As soon as sampling was completed, the buckets were placed in a 4°C cold room and stored overnight. AMEC personnel picked up the samples the following morning and transported them to AMEC for testing. Upon arrival at the laboratory, each sample was assigned a tracking number, and water quality measurements of

temperature, pH, dissolved oxygen (DO), conductivity, alkalinity, and hardness were recorded (Table 1).

Table 1. Water Quality Parameter Measurements upon Sample Receipt.

Site ID	Date Collected	Date Received	Temp. (°C)	pH (units)	DO (mg/L)	Cond. (µmhos/cm)	Alkalinity (mg/L $CaCO_3$)	Hardness (mg/L $CaCO_3$)
NAVSTA OF 9	2/18/04	2/19/04	15.0	7.38	10.7	1316	20	132
NAVSTA OF 11	2/18/04	2/19/04	14.7	7.34	9.8	142	18	24
NAVSTA OF 14	2/18/04	2/19/04	14.4	7.48	10.0	1956	20	192
SUBASE OF 11B	2/18/04	2/19/04	14.4	7.45	10.1	299	27	125
SUBASE OF 23c+e	2/18/04	2/19/04	14.9	7.12	9.8	156	16	26
SUBASE OF 26	2/18/04	2/19/04	15.6	7.58	10.2	317	27	61

Temperature and conductivity were measured with an Orion 130 meter. DO was measured using a YSI 55 meter, and an Orion 250A+ meter was used to measure pH. Alkalinity (Hach Method 8203) and hardness (Hach Method 8213) were checked using Hach digital titrators (Model 16900). The samples were held at 4°C in the dark at AMEC. Appropriate chain-of-

custody (COC) procedures were followed during all phases of this study. Copies of the COC forms for this study are attached in Appendix F.

2.2 Test Design and Bioassay Procedures

The overall experimental design incorporated a number of features to facilitate comparisons of sensitivity between species, and identifying the presence and degree of both acute and chronic toxicity. The Navy's stormwater permit requires evaluation of acute toxicity with both opossum shrimp (*Americamysis bahia*) and topsmelt (*Atherinops affinis*) (inland silversides, *Menidia beryllina*, were substituted for topsmelt during this study). However, in case the samples were not sufficiently toxic to elicit acute responses, the test design incorporated the 7-day chronic test procedures. Thus, if the samples exhibited acute toxicity within the first 96 hours of exposure, the tests could be terminated and TIEs initiated. However, if no acute toxicity was observed, it would still be possible to default to the sublethal growth endpoint to evaluate differences between samples and species. Similarly, the 48-hour mussel embryo development using *Mytilus galloprovincialis* test was incorporated into the study design because of its known sensitivity to copper, and its comparatively short exposure duration. Thus, if results for the mussels appeared correlated with those obtained with opossum shrimp and/or inland silversides, subsequent TIE characterization could be conducted in a more cost-effective manner and with less sample volume than could be achieved using 96-hour or 7-day exposure durations.

The results of the screening tests were used to select samples that would be amenable to follow-up investigation of the cause of toxicity. In general, TIEs have the highest probability of success if conducted on samples that produce well-defined toxic responses that do not dissipate quickly over time. Consequently, a degree of response that can be clearly separated from the control is highly desirable. While this ultimately depends on the number of replicates used and the reproducibility of the test methods, our experience suggests that a 30-percent difference from the control usually provides sufficient resolution against which to judge the effectiveness of the various treatments used to determine the general characteristics of the toxicant and, ultimately, to identify and confirm the cause of toxicity.

The blue mussel embryo development assay was performed in accordance with "Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs (E724-94)" (ASTM 1994). Procedures for testing stormwater using the opossum shrimp and inland silverside survival and growth tests followed "Short-Term Methods for Estimating the

Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition (EPA/821/R-02/014)," (EPA 2002).

Procedures for performing Phase I TIEs are outlined in "Methods for Aquatic Toxicity Identification Evaluations – Phase I Toxicity Characterization Procedures, Second Edition (EPA/600/6-91/003)" (EPA 1991), "Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I (EPA/600/6-91/005F)" (EPA 1992), and "Marine Toxicity Identification Evaluation (TIE) – Phase I Guidance Document" (EPA 1996). Procedures for performing Phase II and III TIEs are outlined in "Methods for Aquatic Toxicity Identification Evaluations – Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity (EPA/600/R-92/080)" (EPA 1993a), and "Methods for Aquatic Toxicity Identification Evaluations – Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity (EPA/600/R-92/081)" (EPA 1993b), respectively.

2.2.1 Screening Bioassays

Blue Mussel Embryo Development Test

Carlsbad Aquafarms in Carlsbad, CA supplied the blue mussel *Mytilus galloprovincialis*. The mussels were transported to AMEC in ice chests via same-day courier service. In the laboratory, the organism receipt date and arrival condition were recorded in a logbook. The mussels were then acclimated to test temperature and salinity, and observed each day prior to test initiation for any indications of significant mortality (>10%).

Mussel embryos were exposed to stormwater for a period of 48 hours to evaluate effects on percent-normal embryo development. Sample concentrations of 12.5, 25, 50, and 68 (the highest testable concentration) percent were tested concurrently with a negative control. Due to the low salinities of the samples, hypersaline brine was added to each sample to raise the salinity to 32 ppt. The volume of hypersaline brine required to adjust the salinity determined the highest testable concentration for each sample. An additional control composed of hypersaline brine and deionized water was also tested to ensure any observed toxic effects were not due to the brine.

Test solutions were prepared using graduated cylinders and pipettes. Measurements of pH, DO, temperature, and salinity were recorded for each test concentration and control. Five replicate test chambers were prepared for each test concentration and control. Replicates

consisted of 30-ml shell vials containing 10 ml of test solution. Test solutions were acclimated to 15°C in temperature-controlled environmental chambers prior to initiation.

In order to spawn the mussels, brood stock were exposed to heated ultraviolet (UV) treated seawater (27-29°C) in shallow plastic trays. Within 30-60 minutes, the mussels began to spawn. Spawning individuals were removed and isolated in individual 250-ml beakers containing 20°C seawater. After allowing individuals to continue to spawn for 30 minutes, eggs were examined under a compound microscope in order to determine egg quality. The three "best" egg stocks (as defined by microscopic observations of egg shape, color, and opacity) were poured into 1-L Erlenmeyer flasks and each was fertilized with sperm from at least three different males. Fertilization was allowed to continue for twenty minutes. Each sperm-egg stock mixture was then poured through a 20-µm screen allowing sperm to pass through while retaining fertilized eggs. The three embryo stocks were allowed to develop for approximately two hours in a 15°C environmental chamber. A 1-ml aliquot was then removed from each embryo stock and examined under a compound microscope. The embryo stock that exhibited the furthest development (i.e., most number of cleavages per cell) was diluted to a concentration of 200 embryos/ml, and 1 ml of this stock was added to each vial to initiate testing. A 16:8 hour light:dark illumination cycle was provided for the duration of the test. Test chambers were covered with a clear plexiglass sheet to reduce evaporation and prevent test solution contamination.

Temperature, pH, DO, and salinity were measured daily in surrogate test chambers for each concentration and control. At test termination, larvae in each test chamber were preserved with 1 ml of seawater-buffered Formalin prior to evaluation. A subsample of 100 bivalve embryos from each test chamber was counted under a compound microscope at 400x magnification. The embryos were classified as normal or abnormal. Normally developed embryos have a distinct D-shape with complete formation of the shell.

A concurrent reference toxicant test (positive control) using copper (II) chloride ($CuCl_2$) was conducted in conjunction with the stormwater tests.

Opossum Shrimp and Inland Silverside 7-Day Survival and Growth Tests

Juvenile opossum shrimp were purchased from Aquatic Biosystems of Fort Collins, CO. The organisms were placed in plastic bags containing oxygenated culture water, packed in insulated containers, and transported to AMEC via overnight delivery service. Upon arrival at AMEC, water quality parameters of temperature, pH, DO, and salinity were measured and recorded in a

logbook. The condition of the organisms was also noted. The mysids were then acclimated to test salinity and temperature, and observed prior to test initiation for any indications of stress (e.g. abnormal swimming behavior) or significant mortality (>10%). The mysids were fed *Artemia* nauplii to satiation during holding. Mysids were 6 days old upon arrival at AMEC and 7 days old upon test initiation.

Juvenile silversides were purchased from Aquatic Biosystems of Fort Collins, CO. The organisms were placed in plastic bags containing oxygenated culture water, packed in insulted containers, and transported to AMEC via overnight delivery service. Upon arrival at AMEC, their condition was noted, and water quality measurements of temperature, pH, DO, and salinity were recorded in a logbook. The fish were then acclimated to test salinity and temperature, and observed prior to test initiation for any indications of stress (e.g. abnormal swimming behavior) or significant mortality (>10%). The silversides were 9 days old upon arrival at AMEC and 10 days old upon test initiation; they were fed *Artemia* nauplii to satiation during holding.

These tests estimate chronic toxicity by evaluating survival and growth of opossum shrimp or inland silversides over a 7-day exposure period. Sample concentrations of 25, 50, and 100 percent were tested along with a negative control. Due to the low salinities of the samples, Forty Fathoms™ sea salt was added to each sample to raise the salinity to 32 ppt. An additional control composed of Forty Fathoms™ sea salt and deionized water was also tested to ensure observed mortality was not due to the addition of artificial salt rather than other toxic constituents.

Test solutions were prepared using graduated cylinders and pipettes. Measurements of pH, DO, temperature, and salinity were recorded for each test concentration and control. Eight (mysids) or five (silversides) replicate test chambers were prepared for each test concentration and control. Replicates for the mysid test consisted of 400-ml plastic cups containing 250 ml of test solution. Replicates for the silverside test consisted of 1-L glass jars containing 500 ml of test solution. Test solutions were acclimated to 25°C in temperature-controlled environmental chambers prior to initiation, for both the shrimp and silverside tests.

Five organisms were counted and transferred from holding bowls into individual plastic soufflé cups. A second technician verified counts and condition of all test organisms prior to addition of the organisms to the test chambers, and again when test initiation was complete. A 16:8 hour light:dark illumination cycle was provided for the duration of the test. Test chambers were covered with a clear plexiglass sheet to prevent evaporation and cross-contamination of the test

solutions.

Test solutions were renewed once per day, and organisms were fed two times per day. Temperature, pH, DO, and salinity were measured daily in both freshly prepared test solutions, and test solutions collected from the test chambers for each concentration and control. Survival status was recorded for each test chamber once per day. At test termination, final observations were made and test animals were prepared for weight determination.

Dry weights were determined by placing organisms from each test chamber into individual tared aluminum pans and drying them in an oven at 60°C for 24 hours. After drying, pans were weighed on a Mettler 240AE balance to the nearest 0.01 mg.

Acute $CuCl_2$ reference toxicant tests (positive control) were conducted within the same week of these chronic tests.

2.2.2 Phase I TIE Treatments

Phase I treatments are designed to remove, inhibit, or potentiate a particular classes of compounds that may be present in the sample, thereby isolating the toxic signal. Selected treatments were applied in this study; detailed descriptions of each treatment are provided below, and a general schematic of Phase I TIE characterization procedures is shown in Figure 1.

Filtered, natural seawater (mussel larvae) or artificial seawater (opossum shrimp) was used as dilution and control water for these studies. Untreated control water was tested concurrently with the "Baseline" (untreated) stormwater tests for each site and species. Aliquots of the appropriate control water underwent each of the Phase I manipulations (method controls) and were tested alongside the treated stormwater samples. The method controls are used to assess whether the sample manipulations resulted in adverse effects due to the procedures themselves.

Baseline Tests

Baseline tests were performed concurrently with the Phase I TIE treatments to compare the response in untreated stormwater to responses obtained after the manipulations. Treatments that altered the toxicity compared with the toxicity of the baseline test were used to identify classes of toxic compounds present in the sample.

EDTA Metal Chelation

The addition of ethylenediaminetetraacetic acid (EDTA) was used to determine the extent of toxicity attributable to divalent cationic trace metals (EPA 1991). EDTA chelates divalent cationic trace metals, thereby reducing their bioavailability. EDTA was added to the method controls and all stormwater dilutions at exposure concentrations of 30 and 60 mg/L.

Solid-Phase Extraction

Solid-phase extraction (SPE) with a C_{18} column was used to determine the extent of toxicity associated with nonpolar organic compounds. It has been found that C_{18} columns also have the ability to remove some metals as well (EPA 1991). A 5-ml capacity Baker brand column was used for this procedure. Post-filtered SPE columns were labeled, wrapped in airtight resealable bags, and held at 4°C for potential subsequent Phase II testing.

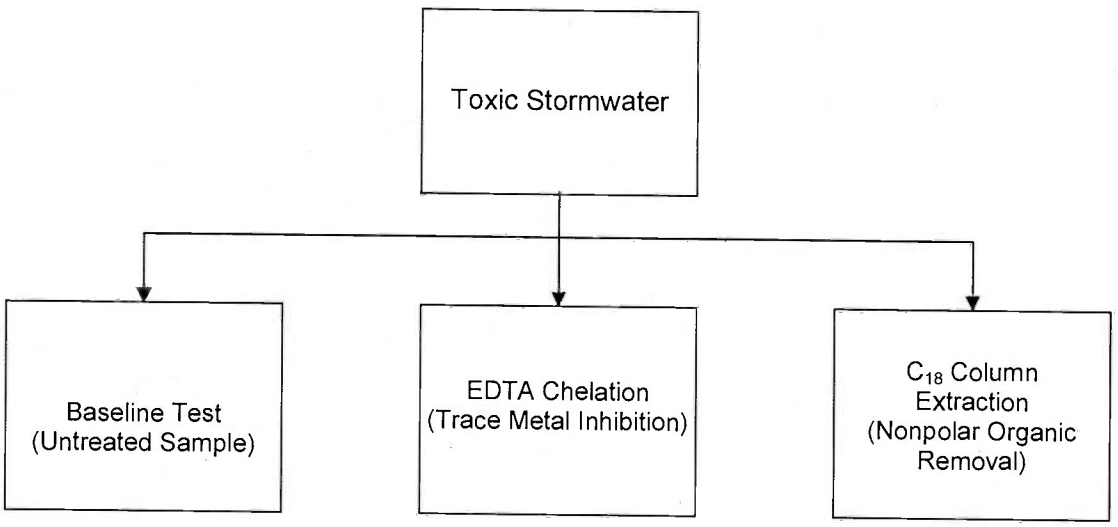


Figure 1. Schematic diagram of Phase I TIE sample treatments used for San Diego Bay stormwater samples.

2.2.3 Phase I TIE Bioassays

Blue Mussel Embryo Development Test

A dilution series was prepared for each treatment to evaluate its effectiveness at different concentrations. Bioassays were conducted following the same methods for organism procurement, test initiation, monitoring and termination previously described for screening tests. The experimental design, including number of replicates, concurrent controls and test concentrations, is summarized in Table 2.

Table 2. Phase I TIE Toxicity Test Experimental Design – Blue Mussel

Test Procedure	Replicates	Test Solutions
Baseline Tests (NAVSTA OF 9, OF 11, OF 14, SUBASE OF 11B, OF 23 c+e, and OF 26)	2	Lab Control, Brine Control, 25, and 50%[a]
Phase I Manipulations (EDTA addition [b] and C_{18} column extraction)	2	Method Control, 25, and 50%[a]
Reference Toxicant Test	5	0, 2.5, 5, 10, 20, and 40 µg/L Cu

[a] Toxicity to blue mussels observed in all six screening bioassays was sufficient to test a 50% dilution as the highest concentration for all sites.
[b] EDTA was added to test solutions for final concentrations of 30 and 60 mg/L across concentrations.

Opossum Shrimp 7-Day Survival and Growth Test

Because the opossum shrimp test requires daily renewal of test solutions, the remaining sample volume was insufficient to test multiple concentrations. Consequently, the TIE treatments were performed only on 100% sample. Fresh aliquots of SUBASE OF 23 c+e stormwater were treated with EDTA each day three hours prior to test solution renewal. However, due to the time associated with C_{18} column extraction, a sample volume adequate for the test initiation and all of the daily renewals was prepared the day prior to test initiation. All remaining aspects of the tests pertaining to organism procurement, test initiation, monitoring and termination were conducted following the same methods as previously described for the screening tests. Experimental design, including number of replicates, concurrent controls, and test concentrations is summarized in Table 3.

Table 3. Phase I TIE Toxicity Test Experimental Design – Opossum Shrimp

Test Procedure	Replicates	Test Solutions
Baseline Test (SUBASE 23 c+e)	5	Lab Control, Salt Control, and 100%
Phase I Manipulations		

(Round One) (EDTA addition [a] and C_{18} column extraction)	5	Method Control and 100%
Reference Toxicant Test	8	0, 25, 50, 100, 200, and 400 µg/L Cu

[a] EDTA was added to test solutions for final concentrations of 30 and 60 mg/L.

2.2.4 Phase II/III TIEs

During Phase II TIE procedures, additional manipulations and measurements are performed in an effort to identify and confirm the contaminants that are responsible for toxicity. Specific Phase II methods depend on the results obtained during Phase I testing. Confirmation of suspected toxicants is performed during Phase III of the TIE, which uses a combination of statistical and experimental procedures to provide additional evidence that supports the identification process. The Phase II and III TIE procedures were conducted using the mussel embryo development test because the treatments could be completed more rapidly (48-hour end-point) and cost-effectively than with opossum shrimp, which require a 7-day exposure period to achieve the sub-lethal endpoint. Conclusions regarding the cause(s) of toxicity to opossum shrimp were based on inferential comparisons to the mussel data, and known sensitivities to the contaminants identified.

C_{18} Column Methanol Elutions- SUBASE OF 11B

Non-polar organic compounds bound to C_{18} columns can be removed from the columns using methanol. Two types of methanol elutions were performed for this study: one used only 100 percent methanol, and the other used a concentration gradient of methanol. The first elution method was used with C_{18} columns from Phase I in order to confirm that non-polar organic toxicants had been retained on the columns. After recovery of toxicity was successful, six L of the remaining SUBASE OF 11B stormwater were filtered through six additional C_{18} columns. Following a confirmatory elution of one column with 100 percent methanol to ensure that toxicity had not dissipated in the sample over time, the remaining columns were subsequently eluted sequentially with the following series of methanol/water fractions to elute compounds based on their polarity: 0 (Control), 50, 75, 80, 85, 90, 95, and 100 percent methanol. This step not only isolates the toxic constituent in one fraction, it also eliminates all of the organic constituents found in the other fractions. This makes it easier to detect the toxicant using analytical techniques such as GC/MS, since there are fewer peaks in the sample to cause interferences.

For each set of elutions, 2 ml of the appropriate methanol concentration was pumped through the columns using a peristaltic pump set at an approximate rate of 1 ml per minute. For elutions conducted using methanol/water fractions, care was taken to ensure that the columns did not dry out between fractions. Extracts were collected into 2-ml amber glass Voa® vials.

The extracts were added to clean dilution water at concentrations that were 2X (3 April and 8 May) and/or 4X (3 April, 8 May, and 15 July) the concentration of that in the original stormwater sample. Concurrent method controls consisted of: 1) clean dilution water passed through the C_{18} column; 2) a methanol control equivalent to the highest concentration achieved in the tested fractions. Bioassays were conducted following the same methods for organism procurement, test initiation, monitoring and termination as previously described for the screening and Phase I tests. The experimental design, including number of replicates, concurrent controls and test concentrations, for these tests is summarized in Table 4.

Table 4. Phase II TIE Toxicity Test Experimental Design – Blue Mussel

Test Procedure	Replicates	Dilution Series
Baseline Test (SUBASE OF 11B)	2	Lab Control, Brine Control, 25, and 50%[a]
C_{18} Column Elutions		
3 April	5	Method Controls, 25, 50, and 100%[b]
8 May	5	Method Controls, and 100%
15 July	5	Method Controls, 50, 75, 80, 85, 90, 95, and 100%[c]
Reference Toxicant Tests	5	0, 2.5, 5, 10, 20, and 40 µg/L Cu

[a] The highest testable concentration due to the addition of hypersaline brine was 59%.
[b] Dilution series was created after the 100% methanol eluted fraction was added back to dilution water at 2X the original concentration.
[c] Dilution series refers to the concentration of methanol filtered through the column. All extracts were added back to dilution water at 4X the original concentration.

Copper and Zinc Mixture Studies

Based on Phase I TIE and analytical chemistry results, studies were conducted to evaluate the toxicity of copper and zinc to mussel larvae. Four bioassays were conducted using clean laboratory seawater and analytically verified trace metal stock solutions: 1) a mixture of copper and zinc at concentrations based on the ratio of the two metals in the stormwater samples (excluding SUBASE 23 c+e); 2) a mixture of copper and zinc at concentrations based on the ratio of their individual Median Effect (EC_{50}) Concentrations; 3) a copper reference toxicant test;

and 4) a zinc reference toxicant test. Results from these studies were used to evaluate the extent to which each of the two metals contributed to toxicity in the stormwater samples, and if the two metals exhibited additive or synergistic toxicity. All aspects of these bioassays were conducted similarly to screening tests.

2.3 Statistical Analyses

Proportional data (e.g., percent normal embryos, percent survival) were arcsine square-root transformed prior to analysis. Growth data were analyzed without transformation. To determine if parametric or non-parametric statistical methods could be applied to the data, the data were evaluated for normality (Shapiro-Wilks Test) and homogeneity of variance (Bartlett's Test). Depending on the results of these tests, Steel's Many One Rank Test (non-parametric) or Dunnett's Test (parametric) was used to identify significant differences between each concentration and the appropriate control (brine or salt). Minimum Significant Differences (MSDs) were calculated as a percentage of the control response for each test, based on Dunnett's t-statistic. Note that this procedure likely overestimates test sensitivity in cases where the test endpoints were determined with non-parametric methods.

Median Lethal (LC_{50}), and/or EC_{50} values were also calculated for all tests that exhibited a dose-response curve. These endpoints were calculated with Maximum Likelihood Probit, or Trimmed Spearman-Karber methods. ToxCalc Comprehensive Toxicity Data Analysis and Database Software, Version 5.0, or the Comprehensive Environmental Toxicity Information System (CETIS), version 1.0, was used for these analyses.

2.4 Analytical Chemistry

Based on historical chemical and toxicological data available for the six stormwater outfalls, subsamples from each site were analyzed for a suite of total trace metals, including antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, selenium, silver, thallium, vanadium, and zinc. Because C_{18} columns can bind some trace metals in addition to non-polar organic substances, subsamples were also collected following C_{18} column extraction and analyzed for the same suite of trace metals to determine if a reduction in toxicity following C_{18} extraction was due to removal of trace metals. Finally, due to the possibility of anionic surfactants in the samples, each sample was analyzed for methylene-blue active substances (MBAS), a colorimetric method that detects anionic surfactants. Analytical measurements were performed by CEL.

2.5 Quality Assurance

AMEC implements quality assurance (QA) procedures in accordance with our internal QA Plan, which is based on applicable protocols and guidance documents. These procedures encompass all aspects of testing, including the source, handling, condition, receipt, and storage of samples and test organisms, and the calibration and maintenance of instruments and equipment. All data generated by the laboratory are monitored for completeness and accuracy at the end of each day, and at the end of each individual test period. Laboratory controls are conducted concurrently with every assay. In addition, reference toxicant tests are performed concurrently with every assay, or on a monthly basis, to confirm that test organism quality, and laboratory conditions and procedures, remain consistent over time.

3.0 Results and Discussion

Detailed descriptions of the results from screening tests, as well as the Phase I and Phase II/III TIEs are presented in the following sections. Tables and figures summarizing the toxicity data are presented in Appendix A. Statistical summaries and raw bench datasheets are presented in Appendix B. Appendix C contains reference toxicant test results, as well as a laboratory quality control chart for each species. The analytical chemistry report from CEL is in Appendix D, and the sample receipt information and COC forms, are contained in Appendices E and F, respectively.

3.1 Screening Bioassays

3.1.1 Blue Mussel Embryo Development Tests

All six stormwater samples exhibited appreciable toxicity to blue mussel embryos; no normal development was observed in the highest testable concentration (68 percent) of each sample, and the EC_{50}s ranged from 16 to 38 percent stormwater (Table 5). SUBASE OF 26 was the most toxic sample tested and NAVSTA OF 9 was the least toxic. Based on these data, all of these samples exhibited sufficient toxicity to trigger a Phase I TIE.

3.1.2 Opossum Shrimp Survival and Growth Tests

At 96 hours, survival in all six undiluted stormwater samples ranged between 55 and 90 percent, compared with 95 to 100 percent in the controls. However, only one of the samples (SUBASE OF 23 c+e) exhibited at least a 30 percent reduction in survival relative to the controls; this effect was also statistically significant. These data are included in Table 6.

At the end of the 7-day exposure period, mean survival in the undiluted stormwater samples ranged from 50 to 88 percent. NAVSTA OF 11, OF 14, and SUBASE OF 23 c+e were the sites exhibiting statistically significant decreases in survival. Of these, only SUBASE OF 23 c+e exhibited a response in excess of 30 percent (Table 6). By way of comparison, laboratory seawater controls exhibited a mean survival of 93 percent, and survival among the artificial salt controls ranged from 93 to 95 percent. With respect to test organism growth, all six sites exhibited significantly reduced biomass compared to the artificial salt controls (Table 6). Mean values for biomass in undiluted stormwater ranged from 0.06 mg per shrimp (SUBASE OF 23 c+e) to 0.20 mg per shrimp (NAVSTA OF 9). In contrast, control biomass ranged from 0.25 to 0.30 mg per shrimp in laboratory seawater, and 0.22 to 0.28 mg per shrimp in solutions of artificial sea salts. Although sublethal responses were apparent to varying degrees in all six of the samples tested, budget constraints did not allow for conducting chronic Phase I TIEs on all samples. Consequently, a single Phase I chronic TIE was conducted on SUBASE OF 23 c+e, the sample that exhibited the greatest toxicity to opossum shrimp.

3.1.3 Inland Silverside Survival and Growth Tests

Silversides exhibited markedly less sensitivity to the stormwater samples than mussels or mysids. None of the samples tested resulted in any statistically significant reductions in survival or growth. The lowest survival was associated with SUBASE OF 23 c+e; in undiluted sample, mean survival was 88 percent at 96 hours, and mean survival and biomass were 84 percent and 0.49 mg per fish, respectively, after 7 days of exposure. All of these values were within 10 percent of the same endpoints exhibited by the artificial salt control and were not statistically significant. These data are shown in Table 7.

Table 5. Pre-TIE screening test results using the blue mussel for 48-hour embryo development.

Site ID	Mean Normal Development (%)					NOEC [a]	EC_{25}	EC_{50}
	0%	12.5%	25%	50%	68%		(% Sample)	
Lab Control 1	81	NA	NA	NA	NA	NA	NA	NA
Brine Control 1	80	NA	NA	NA	NA	NA	NA	NA
NAVSTA OF 9	NA	82	81	5.4	0.00	25	32	38
NAVSTA OF 11	NA	77	79	0.27	0.32	25	31	34
NAVSTA OF 14	NA	77	62	0.00	0.00	25	25	27
Lab Control 2	81	NA	NA	NA	NA	NA	NA	NA
Brine Control 2	75	NA	NA	NA	NA	NA	NA	NA
SUBASE OF 11B	NA	81	69	1.0	0.00	25	28	32
SUBASE OF 23c+e	NA	73	0.00	0.00	0.00	12.5	15	19
SUBASE OF 26	NA	70	0.20	0.00	0.00	12.5	14	17

Table 6. Pre-TIE screen test results using the opossum shrimp for a) 96-hour survival, b) 7-day survival, and c) 7-day growth.

a)

Site ID	Mean Survival (%)				NOEC [a]	LC_{25}	LC_{50}
	0%	25%	50%	100%		(% Sample)	
Lab Control 1	95	NA	NA	NA	NA	NA	NA
Salt Control 1	100	NA	NA	NA	NA	NA	NA
NAVSTA OF 9	NA	100	93	90	100	>100	>100
NAVSTA OF 11	NA	100	98	85	100	>100	>100
NAVSTA OF 14	NA	93	98	85	100	>100	>100
Lab Control 2	95	NA	NA	NA	NA	NA	NA
Salt Control 2	98	NA	NA	NA	NA	NA	NA
SUBASE OF 11B	NA	98	100	85	100	>100	>100
SUBASE OF 23c+e	NA	93	93	55	50	83	>100
SUBASE OF 26	NA	95	95	88	100	>100	>100

b)

Site ID	Mean Survival (%)				NOEC [a]	LC_{25}	LC_{50}
	0%	25%	50%	100%		(% Sample)	
Lab Control 1	93	NA	NA	NA	NA	NA	NA
Salt Control 1	95	NA	NA	NA	NA	NA	NA
NAVSTA OF 9	NA	98	93	88	100	>100	>100
NAVSTA OF 11	NA	100	95	78	50	>100	>100
NAVSTA OF 14	NA	93	95	75	50	>100	>100
Lab Control 2	93	NA	NA	NA	NA	NA	NA
Salt Control 2	93	NA	NA	NA	NA	NA	NA
SUBASE OF 11B	NA	95	100	83	100	>100	>100
SUBASE OF 23c+e	NA	83	80	50	50	63	>100
SUBASE OF 26	NA	95	95	85	100	>100	>100

c)

Site ID	Mean Biomass (mg)				NOEC [a]	EC_{25}	EC_{50}
	0%	25%	50%	100%		(% Sample)	
Lab Control 1	0.30	NA	NA	NA	NA	NA	NA
Salt Control 1	0.28	NA	NA	NA	NA	NA	NA
NAVSTA OF 9	NA	0.28	0.25	0.20	50	88	>100
NAVSTA OF 11	NA	0.25	0.21	0.10	25	50	81
NAVSTA OF 14	NA	0.21	0.19	0.18	25	24	>100
Lab Control 2	0.25	NA	NA	NA	NA	NA	NA
Salt Control 2	0.22	NA	NA	NA	NA	NA	NA
SUBASE OF 11B	NA	0.24	0.22	0.16	50	90	>100
SUBASE OF 23c+e	NA	0.13	0.12	0.06	<25	16	59
SUBASE OF 26	NA	0.31	0.22	0.17	50	74	>100

[a] NOEC statistical comparisons based on the salt control

NA - Not applicable

Table 7. Pre-TIE screen test results using the inland silverside for a) 96-hour survival, b) 7-day survival, and c) 7-day growth.

a)

Site ID	Mean Survival (%)				NOEC [a]	LC_{25}	LC_{50}
	0%	25%	50%	100%		(% Sample)	
Lab Control 1	100	NA	NA	NA	NA	NA	NA
Salt Control 1	96	NA	NA	NA	NA	NA	NA
NAVSTA OF 9	NA	100	100	96	100	>100	>100
NAVSTA OF 11	NA	100	96	100	100	>100	>100
NAVSTA OF 14	NA	100	100	100	100	>100	>100
Lab Control 2	100	NA	NA	NA	NA	NA	NA
Salt Control 2	100	NA	NA	NA	NA	NA	NA
SUBASE OF 11B	NA	100	96	96	100	>100	>100
SUBASE OF 23c+e	NA	100	96	88	100	>100	>100
SUBASE OF 26	NA	100	96	96	100	>100	>100

b)

Site ID	Mean Survival (%)				NOEC [a]	LC_{25}	LC_{50}
	0%	25%	50%	100%		(% Sample)	
Lab Control 1	92	NA	NA	NA	NA	NA	NA
Salt Control 1	92	NA	NA	NA	NA	NA	NA
NAVSTA OF 9	NA	100	100	88	100	>100	>100
NAVSTA OF 11	NA	100	96	100	100	>100	>100
NAVSTA OF 14	NA	100	100	100	100	>100	>100
Lab Control 2	100	NA	NA	NA	NA	NA	NA
Salt Control 2	100	NA	NA	NA	NA	NA	NA
SUBASE OF 11B	NA	100	96	96	100	>100	>100
SUBASE OF 23c+e	NA	96	92	84	100	>100	>100
SUBASE OF 26	NA	96	96	96	100	>100	>100

c)

Site ID	Mean Biomass (mg)				NOEC [a]	EC_{25}	EC_{50}
	0%	25%	50%	100%		(% Sample)	
Lab Control 1	0.46	NA	NA	NA	NA	NA	NA
Salt Control 1	0.50	NA	NA	NA	NA	NA	NA
NAVSTA OF 9	NA	0.47	0.57	0.46	100	>100	>100
NAVSTA OF 11	NA	0.48	0.48	0.48	100	>100	>100
NAVSTA OF 14	NA	0.49	0.49	0.53	100	>100	>100
Lab Control 2	0.50	NA	NA	NA	NA	NA	NA
Salt Control 2	0.55	NA	NA	NA	NA	NA	NA
SUBASE OF 11B	NA	0.50	0.49	0.54	100	>100	>100
SUBASE OF 23c+e	NA	0.52	0.50	0.49	100	>100	>100
SUBASE OF 26	NA	0.55	0.51	0.51	100	>100	>100

[a] NOEC statistical comparisons based on the salt control

NA - Not applicable

3.2 Phase I TIE

Phase I TIEs were initiated on samples that exhibited clear evidence of toxicity during the screening tests. On this basis, all of the samples tested with mussels qualified for a TIE. Conversely, no TIEs were pursued with silversides because none of the samples resulted in any adverse effects. While adverse effects on growth were observed in all of the samples tested with opossum shrimp, generally only limited effects were observed with the survival endpoint. Since it was not feasible to perform TIEs on all six samples with 7-day opossum shrimp chronic toxicity tests, the TIE investigation with this species was limited to the sample that produced the greatest level of toxicity; i.e., SUBASE OF 23 c+e.

3.2.1 Blue Mussel

Baseline Tests

Although all of the test samples exhibited toxicity during the initial toxicity tests conducted 19 February 2004, toxicity had diminished in most of the samples when re-tested on 27 February concurrently with the Phase I TIE manipulations. Toxicity dissipated completely in NAVSTA OF 9, and decreased to less than a 30-percent effect in the 50-percent solutions of NAVSTA OF 11, and OF 14, and in SUBASE OF 11B. All three of these samples had previously exhibited 99 to 100 percent abnormal larvae at this concentration when first tested. SUBASE OF 26 and SUBASE OF 23 c+e still retained most of their original toxicity. These data are shown in Figure 2.

Toxicant Characterization

The results of the Phase I TIE treatments are summarized in Table 8. EDTA treatments essentially eliminated the remaining toxicity in samples NAVSTA OF 11 and OF 14, as well as SUBASE OF 23 c+e and OF 26. While EDTA increased the proportion of normal larvae in SUBASE OF 11B, it did not completely eliminate toxicity.

Extraction through SPE columns eliminated toxicity in NAVSTA OF 11 and OF 14, and in SUBASE OF 11B (Table 8). C_{18} extraction did not eliminate toxicity in SUBASE OF 23 c+e or OF 26.

Based on the effectiveness of the EDTA treatments, these data suggest that toxicity in samples NAVSTA OF 11 and OF 14, and SUBASE OF 23 c+e and OF 26 was due to divalent cationic metals. Divalent metals contributed to the toxicity observed in SUBASE OF 11B, but a non-

polar organic constituent also contributed to toxicity in this sample, as indicated by the additional reduction in toxicity in a sub-sample treated with a C_{18} column, compared with the EDTA treatment. The presence of a toxic organic constituent in SUBASE OF 11B was verified by testing a methanol elution of the C_{18} column; toxicity was recovered at both 2X and 4X add-backs, suggesting relatively good recovery from the column. These data are also shown in Table 8.

Note that the conclusion of divalent cationic metals being the primary cause of toxicity is based on the effectiveness of EDTA in removing toxicity. While reduction of toxicity following extraction with C_{18} SPE columns is generally attributed to the presence of non-polar organic toxicants, metals concentrations can also be reduced by C_{18} extraction (USEPA 1991). For this study, metals concentrations were measured before and after C_{18} treatment to determine the extent to which they were reduced following C_{18} extraction. These data are presented in Figure 3 for copper and zinc, and clearly demonstrate that concentrations of these two metals were appreciably reduced by extraction with C_{18} columns. Thus, the presence of an organic constituent must be confirmed by: 1) a comparative lack of effect of EDTA; and 2) toxicity in a solvent elution of the SPE column. Conversely, while C_{18} columns did reduce copper and zinc concentrations in SUBASE OF 23 c+e and OF 26, there was sufficient metal remaining in these filtered samples to result in toxicity (Figure 3).

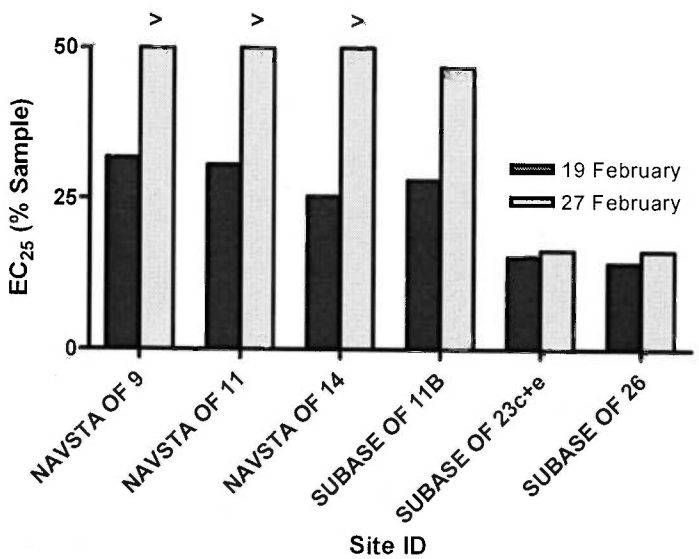


Figure 2. Changes in toxicity of San Diego Bay stormwater samples to blue mussel embryos over time. EC25 values increased for each sample between the intital screens (19 February) and the Phase I TIE baseline tests (27 February).

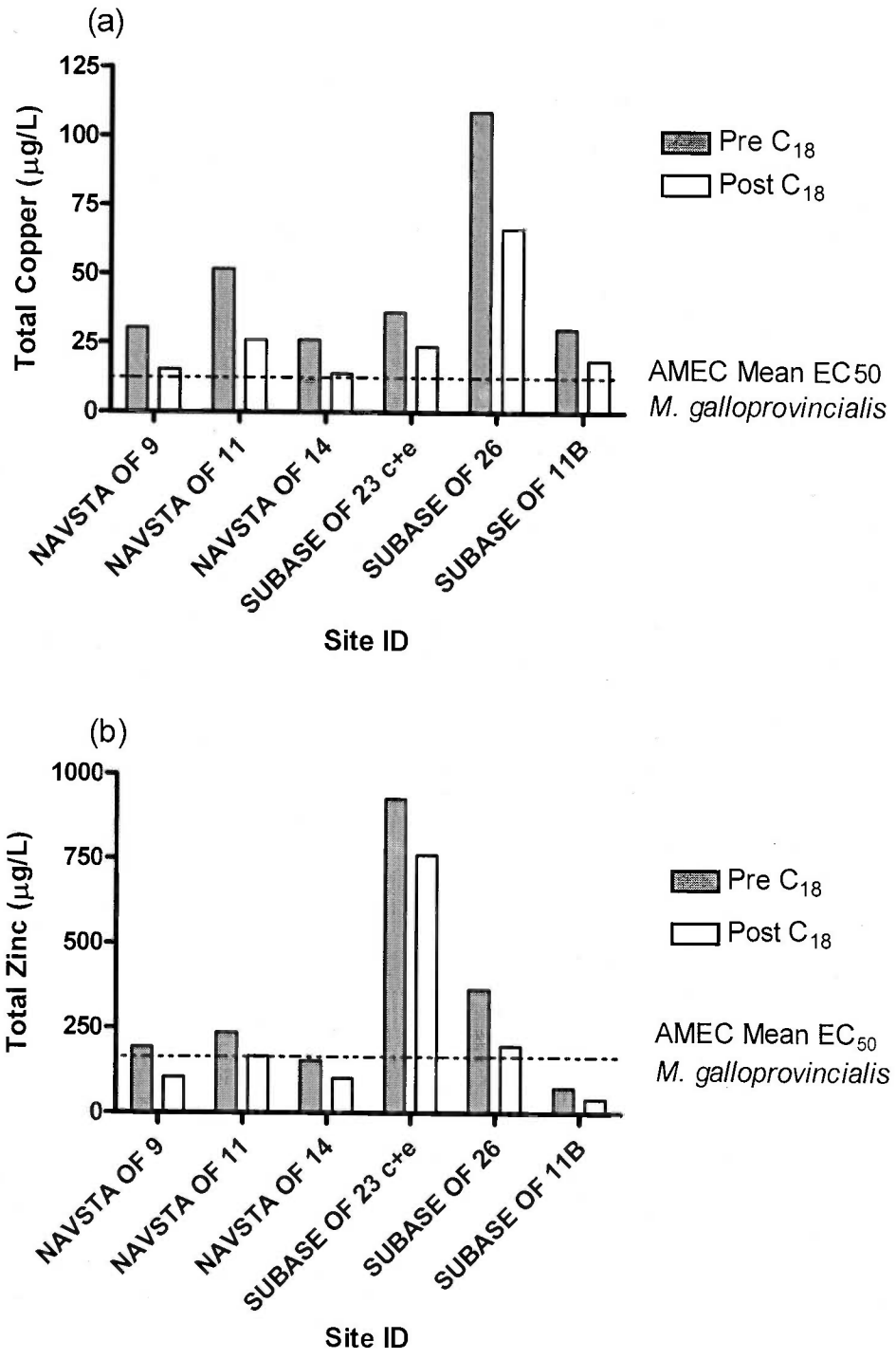


Figure 3. Total Copper (a), and Total Zinc (b) measurements for San Diego Bay stormwater samples before and after C_{18} column extraction. Mean EC_{50} values for blue mussel embryos are displayed on each figure.

Table 8. Blue mussel Phase I TIE results.

Site ID	Conc. (%)	Mean Normal Development (%)						
		Screen	Phase I Baseline	30 mg/L EDTA	60 mg/L EDTA	C_{18} Extraction	2x Methanol C_{18} Elution	4x Methanol C_{18} Elution
NAVSTA OF 9	Method Control [a]	80	96	91	97	92	NT	NT
	50	5.4	92	96	91	97	NT	NT
NAVSTA OF 11	Method Control [a]	80	96	91	97	96	NT	NT
	50	0.0	76	93	96	92	NT	NT
NAVSTA OF 14	Method Control [a]	80	96	91	97	96	NT	NT
	50	0.0	73	96	91	93	NT	NT
SUBASE OF 11B	Method Control [a]	75	96	94	93	93	98	98
	50	1.0	68	73	81	98	0.0	0.0
SUBASE OF 23 c+e	Method Control [a]	75	96	94	93	96	NT	NT
	50	0.0	0.0	88	94	0.0	NT	NT
SUBASE OF 26	Method Control [a]	75	96	94	93	89	NT	NT
	50	0.0	0.0	92	93	1.0	NT	NT

[a] Method controls and C_{18} column elutions were prepared using hypersaline brine and deionized water.

NT - Not Tested

3.2.2 Opossum Shrimp

Baseline Test

The results of the baseline test on SUBASE OF 23 c+e initiated 27 February concurrently with the Phase I TIE manipulations were similar to those obtained in the original screening test initiated 19 February, suggesting that toxicity did not dissipate appreciably over this time period. This result is similar to that observed with the mussel larvae test for this sample. At the end of the 7-day exposure period, the baseline test resulted in 44 percent survival, and a mean biomass of 0.10 mg per shrimp. These data are shown in Table 9, which also includes the results of the TIE treatments.

Toxicant Characterization

Addition of EDTA eliminated adverse effects on both survival and growth of opossum shrimp. In

contrast, extracting the sample with a C_{18} column did not improve either of these parameters, compared with the baseline results (Table 9). Overall, these data provide strong evidence that divalent cationic metals were the cause of toxicity to mysids in this sample. These results are consistent with those obtained with the mussel larvae tested with the same sample.

Table 9. Opossum shrimp Phase I TIE results.

Treatment	Mean Survival (%)		Mean Biomass (mg)	
	0%	100%	0%	100%
Lab Control	96	NT	0.28	NT
Salt Control	100	NT	0.27	NT
Baseline	NT	44	NT	0.10
30 mg/L EDTA	96	96	0.24	0.29
60 mg/L EDTA	100	96	0.28	0.28
C_{18} Column Extraction	96	20	0.42	0.07

[a] NOEC calculations based on comparisons against the brine control.
NT - Not Tested

3.3 Phase II/III TIE Bioassays

3.3.1 Copper and Zinc Mixture Studies

The results of the Phase I TIE manipulations strongly suggested that divalent cationic metals were the primary cause of toxicity in the samples tested. Metals concentrations in the samples were then compared with available toxicity data to evaluate which of the metals might be contributing to toxicity. Based on a review of metals concentrations in the samples (Table 10), it appeared that copper and zinc were the two most likely causes of toxicity that could be attributed to divalent metals. For example, total copper concentrations in the samples ranged between 26.0 and 109 µg/L; these values exceed our long-term laboratory mean EC_{50} value of 13.8 µg/L for mussel larvae exposed to copper by factors of 2 to nearly 8-fold. Similarly, values of zinc in the samples ranged from 75.8 to 927 µg/L; according to the ECOTOX database, concentrations of zinc exceeding 145 µg/L would be expected to result in adverse effects to mussel larvae. Not only were concentrations of these metals sufficiently elevated to be suspected as causes of toxicity, the range and pattern of concentrations also suggested that they could be related to toxicity. Moreover, they were both reduced substantially by extraction with C_{18} columns. In contrast, the other metals measured were either: 1) below detection limits;

2) exhibited fairly consistent concentrations across samples; or 3) were not appreciably affected by extraction with C_{18} columns.

To help evaluate the extent to which each metal contributed to toxicity and to understand how they interacted when present in solution together, a series of tests were performed to identify the level of toxicity associated with each metal and their degree of interaction. Zinc and copper were tested alone, and as mixtures at two different ratios (4.5:1 and 13.6:1) to evaluate whether the ratios affected the interactive characteristics of the metals.

The EC_{50} estimates determined for copper and zinc alone were 9.6 and 160 µg/L, respectively. These values are likely conservative as they were obtained in laboratory seawater. Regardless of the ratios tested, toxicity appeared to be additive, in mixtures of the two metals in laboratory seawater, the EC_{50}s for the two mixtures were 1.2 and 1.3 total TUs, respectively. Figure 4 shows the response curves for zinc and copper individually, as well as for the two mixtures. Clearly, similar dose-responses were exhibited in all four of the tests, suggesting similar modes of action and additive toxicity. Details of metal concentrations, TUs and observed responses are shown in Appendix Tables A-13 through A-15.

Applying these laboratory-derived EC_{50} estimates to metals concentrations measured in the actual samples suggested that, in most cases, the predicted toxicity over-estimated the actual toxicity observed in the original screening tests (Table 11). In other words, there was frequently less toxicity present in the original samples than would have been predicted on the basis of additivity and the concentrations of total metals present. These data suggest that at least some portion of the metals present in the samples was not bioavailable. On average, the actual TUs in the stormwater samples were 64 percent of those that would have been predicted on the basis of the toxicity of copper and zinc in laboratory seawater.

In order to address the relative importance of each of the metals to overall toxicity, predicted TUs for copper and zinc alone and in combination were plotted against the actual TUs determined in screening tests on the original samples (Figure 5). The relationships for copper and zinc alone were not statistically significant ($p>0.05$); however, the relationship between actual toxicity and the toxicity predicted by the combination of metals was significant ($p<0.05$). This finding clearly indicated that both metals contributed to the toxicity observed across all samples, which is consistent with the fact that concentrations of each metal varied independently across sites and both exhibited a relatively wide range of concentrations. A linear regression including both zinc and copper as separate variables was then used to predict

actual toxicity in the samples. A regression between values predicted by this equation and actual TUs observed exhibited an R^2 of 0.80 ($p<0.05$), suggesting that 80 percent of the variability in toxicity across samples could be explained by the concentrations of these two metals (Figure 6).

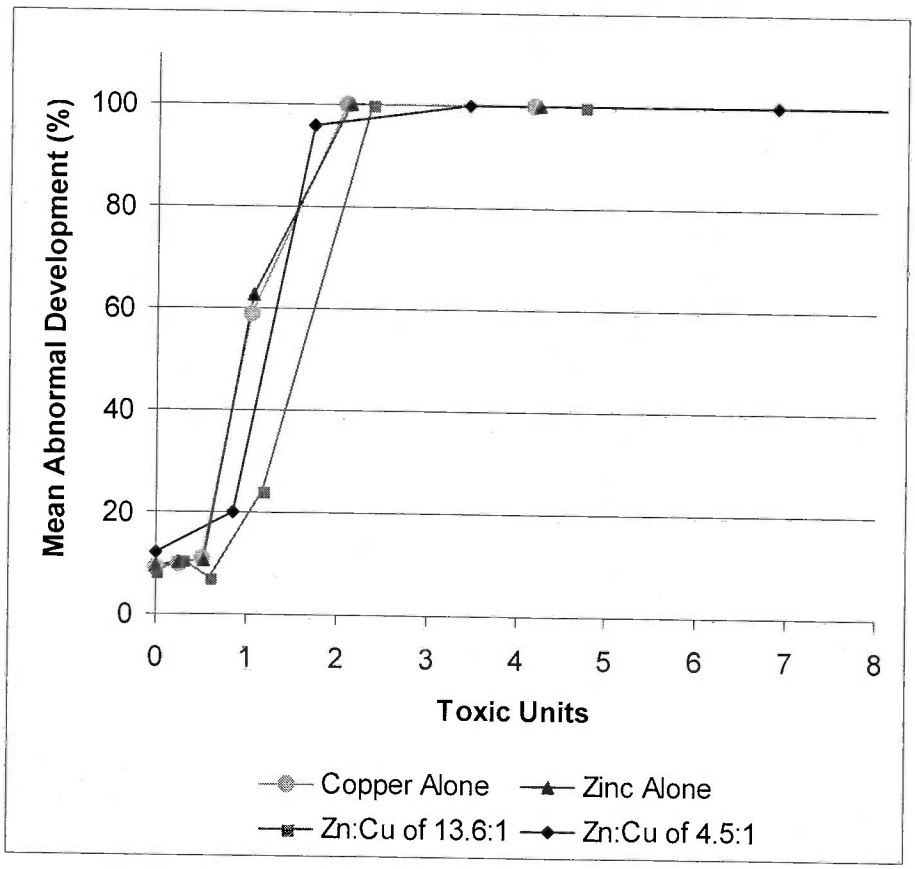


Figure 4. Response of mussel embryos to copper and zinc alone and in combination. Metals are expressed as TUs.

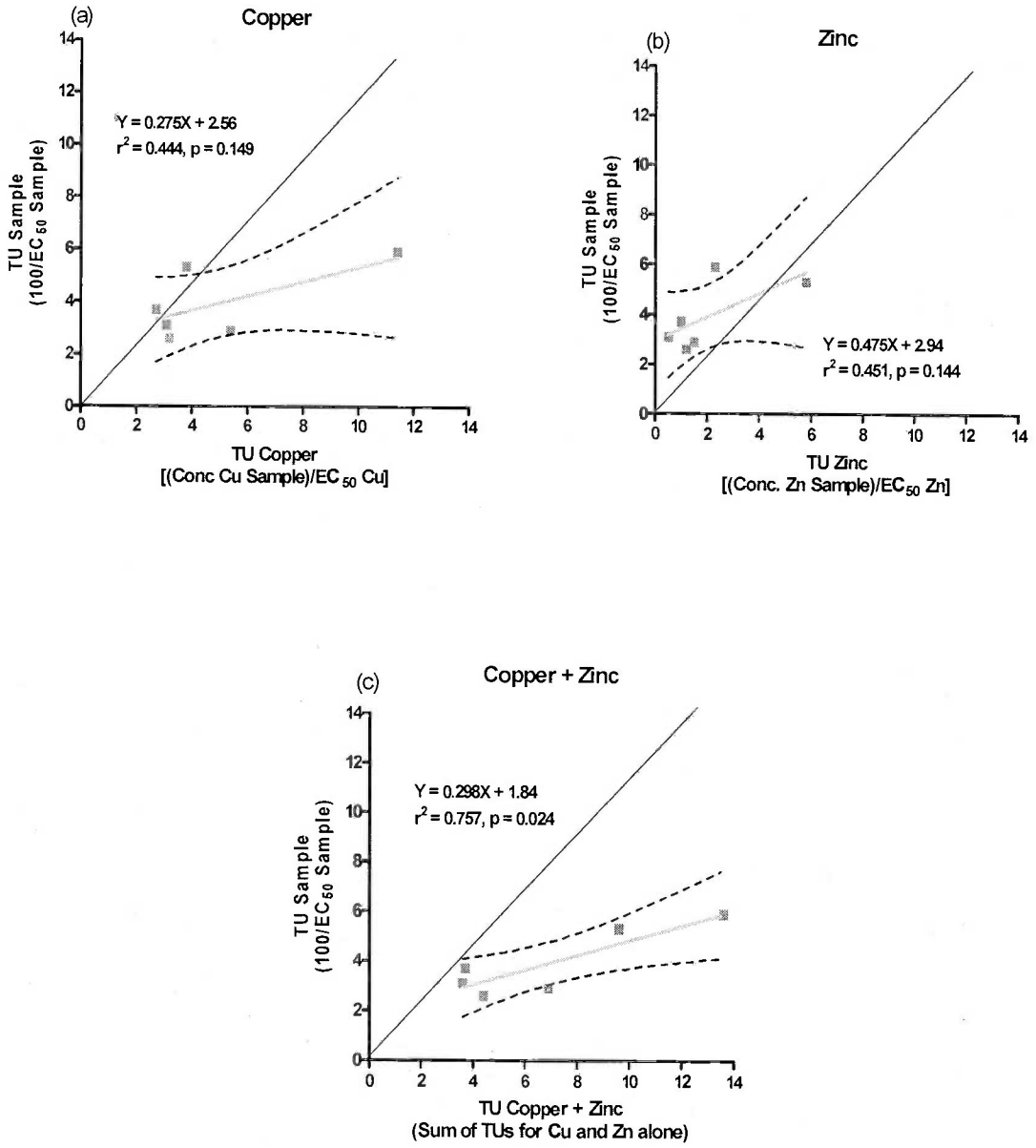


Figure 5. Comparisons of predicted TUs, based on copper and zinc, to TUs found in samples when originally tested.

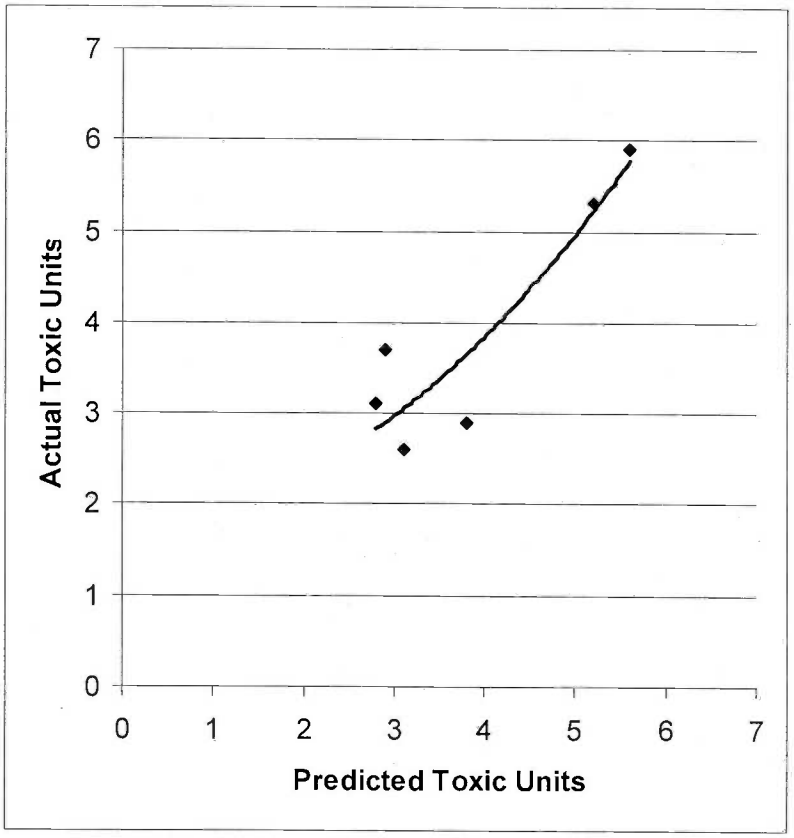


Figure 6. Comparison of actual TUs and TUs predicted from a regression incorporating copper and zinc as separate variables: $TU_{pred} = 1.88 + 0.25TU_{Cu} + 0.41TU_{Zn}$.

Table 10. Total trace metals analysis results for San Diego Bay stormwater samples.

Trace Metal	Reporting Limit (µg/L)	Measurement	Concentration (µg/L) NAVSTA OF 9	NAVSTA OF 11	NAVSTA OF 14	SUBASE OF 11B	SUBASE OF 23 c+e	SUBASE OF 26
Antimony	15.0	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Arsenic	15.0	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Barium	10.0	Pre-C_{18}	12.1	15.3	19.1	12.8	16.4	26.7
		Post-C_{18}	13.4	14.7	17.8	11.9	16.5	23.1
Beryllium	1.00	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Cadmium	5.00	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Chromium	5.00	Pre-C_{18}	ND	ND	6.32	ND	ND	ND
		Post-C_{18}	ND	ND	5.88	ND	ND	ND
Cobalt	5.00	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Copper	5.0	Pre-C_{18}	30.4	51.8	26.0	30.1	36.1	109
		Post-C_{18}	15.4	26.1	13.9	18.7	23.7	66.4
Lead	10.0	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Mercury	0.50	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Molybdenum	5.00	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Nickel	5.00	Pre-C_{18}	5.20	5.26	5.23	7.26	9.15	7.02
		Post-C_{18}	5.98	ND	ND	5.68	13.3	6.36
Selenium	15.0	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Silver	5.00	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Thallium	15.0	Pre-C_{18}	ND	ND	ND	ND	ND	ND
		Post-C_{18}	ND	ND	ND	ND	ND	ND
Vanadium	5.00	Pre-C_{18}	ND	ND	ND	6.21	ND	6.23
		Post-C_{18}	ND	ND	ND	5.15	ND	5.68
Zinc	10	Pre-C_{18}	194	236	153	75.8	927	363
		Post-C_{18}	106	166	103	42.2	761	196

Table 11. Comparisons of predicted copper and zinc TUs.

Site ID	Total Copper (ug/L)	Total Zinc (ug/L)	Screening Test EC_{50} (% Sample)	Screening Test TU[a]	Predicted Copper TU[b]	Predicted Zinc TU[b]	Predicted Copper + Zinc TU
NAVSTA OF 9	30.4	194	38	2.6	3.2	1.2	4.4
NAVSTA OF 11	51.8	236	34	2.9	5.4	1.5	6.9
NAVSTA OF 14	26.0	153	27	3.7	2.7	1.0	3.7
SUBASE OF 11B	30.1	75.8	32	3.1	3.1	0.5	3.6
SUBASE OF 23 c+e	36.1	927	19	5.3	3.8	5.8	9.6
SUBASE OF 26	109	363	17	5.9	11	2.3	14

[a] TU is equal to 100 divided by the screening test EC_{50}.

[b] TU is equal to the concentration of the trace metal in the stormwater sample divided by the reference toxicant test EC_{50}.

3.3.1 C_{18} Column Methanol Elutions

Eluting a C_{18} column used to extract a subsample of SUBASE OF 11B with a methanol gradient resulted in toxicity being recovered in the 95-percent methanol fraction, with no toxicity observed in the adjacent fractions. This suggests that the organic toxicant is relatively non-polar, as it eluted late in the methanol gradient (Table 12). At this point, we believe that the organic toxicant is not likely to be an anionic surfactant because our previous experience suggests that such surfactants typically elute in lower methanol concentrations due to their comparatively high polarity. Moreover, the MBAS measurements ranged from 0.32 to 0.66 mg/L across samples (Table 13), and these concentrations were not related to the level of toxicity observed ($p>0.05$). However, the level of MBAS measured in the SUBASE OF 11B sample did exceed the 48-hour EC_{50} to blue mussels of 0.2 mg/L (unpublished data). Thus, depending on the polarity of the actual surfactant present, it is possible that MBAS contributed to toxicity in this sample. Regardless, the identity of this organic contaminant is being further investigated using GC/MS.

Table 12. Mean normal development in different methanol fractions used to extract C_{18} columns.

Treatment (% Methanol)	Mean Normal Development (%)
Method Control	86
Methanol Control	90
50	77
75	83
80	84
85	72
90	86
95	34
100	81

Table 13. Anionic surfactant (as MBAS) analytical results for San Diego Bay stormwater samples.

Site ID	MBAS (mg/L)[a]
NAVSTA OF 9	0.32
NAVSTA OF 11	0.57
NAVSTA OF 14	0.64
SUBASE OF 11B	0.62
SUBASE OF 23 c+e	0.66
SUBASE OF 26	0.52

[a] Reporting limit is 0.10 mg/L.

4.0 CONCLUSIONS

These data provide an indication of the relative sensitivity of three species to the stormwater samples tested, as well as the cause of toxicity in these samples. Mussel larvae were clearly the most sensitive species tested, with adverse effects observed at concentrations as low as 25 percent sample. Based on the survival endpoint, opossum shrimp were less sensitive than mussel larvae; however, the chronic growth endpoint approached the sensitivity exhibited by the mussel larvae for several of the samples tested. Silversides exhibited relatively low sensitivity to the test samples; no statistically significant effects were observed in any of the samples tested.

With respect to mussel larvae, the results of the TIE clearly implicated copper and zinc as the primary causes of toxicity. In addition, an organic toxicant contributed to the toxicity of SUBASE OF 11B.

Metals were also the most likely cause of toxicity to opossum shrimp; although a TIE was only performed on the sample that exhibited the most toxicity (SUBASE OF 23 c+e), the results clearly indicated that metals were the cause of reduced survival and growth in this sample.

Given that the TIE identified copper and zinc as primary causes of toxicity, the differences in sensitivity observed between species can be explained on the basis of these two metals. Mussel larvae are clearly the most sensitive of the three species to copper; our long-term laboratory mean EC_{50} for this metal (n=20) is 13.8 µg/L, which can be compared with long-term average LC_{50}s of 125 µg/L, and 243 µg/L for silversides and opossum shrimp exposed for 7 days, respectively. Thus, given the range of copper concentrations in the samples (26.0 to 109 µg/L), mussels would have been the only species expected to exhibit a significant response. Similarly, mussels were the most sensitive species to zinc, with an EC_{50} of 160 µg/L. Opossum shrimp were less sensitive; during this TIE study, we determined that the 7-day LC_{50} for this species was 448 µg/L. The ECOTOX database contains 96-hour LC_{50} estimates for zinc that range from approximately 300 to 550 µg/L, with most of the values approaching 500 µg/L. At 96 hours, only SUBASE OF 23 c+e exhibited any significant indication of acute toxicity, and then only to opossum shrimp. Zinc was the most likely constituent responsible for this observed response; the concentration of zinc present in the sample (927 µg/L) exceeded literature values for acute toxicity by 2- to 3-fold. Moreover, comparison of the metals concentrations and degree of toxic responses exhibited by the opossum shrimp in the different samples suggests that zinc was the primary cause of toxicity to this species in SUBASE OF 23 c+e (Table 14). This sample exhibited the highest degree of toxicity to opossum shrimp and also contained the highest concentration of zinc (927 µg/L), and the only concentration of zinc that clearly exceeded the threshold for acute toxicity. Thus, the range of concentrations in the remaining samples (i.e., 75.8 to 363 µg/L) were likely at, or below, the threshold for acute toxicity, particularly if bioavailability was reduced due to binding by various ligands (e.g., dissolved organic carbon) possibly present in the samples. Silversides were the least sensitive species tested, which suggests that they are even more tolerant to zinc than opossum shrimp.

Table 14. Opossum shrimp screening test results with copper and zinc sample concentrations.

Site ID	Mean Survival (%)		Mean Biomass (mg)		Total Copper	Total Zinc
	100% Sample	Salt Control	100% Sample	Salt Control	(ug/L)	(ug/L)
NAVSTAOF 9	88	95	0.20	0.28	30.4	194
NAVSTA OF 11	78	95	0.10	0.28	51.8	236
NAVSTA OF 14	75	95	0.18	0.28	26.0	153
SUBASE OF 11B	83	93	0.16	0.22	30.1	75.8
SUBASE OF 23 c+e	50	93	0.06	0.22	36.1	927
SUBASE OF 26	85	93	0.17	0.22	109	363

[a] NOEC statistical comparisons based on the salt control
NA - Not applicable

The results for each of the samples are reviewed below in the context of the findings of the TIE investigation. These summaries emphasize the tests conducted with mussel larvae, but mysid results are included where appropriate.

NAVSTA OF 9 – This sample exhibited 2.6 TU when testèd originally and contained an estimated 3.2 TU Cu and 1.2 TU Zn. Toxicity dissipated completely when the Phase I TIE was performed, so the contribution of metals to toxicity could not be verified empirically. However, there was sufficient metal present in the sample to account for the original toxicity. Both copper and zinc were present at concentrations in excess of 1 TU, so it is possible that both metals contributed to toxicity, although their relative contributions are not known. The results of the TIE process for this sample are summarized as a flowchart in Figure 7.

NAVSTA OF 11 – This sample exhibited 2.9 TU when tested originally, and contained an estimated 5.4 TU Cu and 1.5 TU Zn. Toxicity dissipated appreciably by the time the Phase I TIE was performed, but there was enough of a response remaining to determine that EDTA removed all of the toxicity, implicating divalent metals as the cause of toxicity. Both copper and zinc were present at concentrations sufficient to result in toxicity, but their relative contributions could not be determined. As with NAVSTA OF 9, copper could have accounted for all of the toxicity, but zinc could only have accounted for partial toxicity. However, without data to document their relative bioavailability, it is not possible to know whether toxicity was due to copper alone or to a combination of copper and zinc. The results of the TIE process for this sample are summarized as a flowchart in Figure 8.

NAVSTA OF 14 – This sample exhibited 3.7 TU when tested originally, and contained an estimated 2.7 TU Cu and 1.0 TU Zn. Toxicity had decreased when the Phase I TIE was performed, but EDTA effectively removed the residual toxicity, implicating metals as the cause of toxicity. Toxicity was due to a combination of copper and zinc, as neither metal alone was present at a concentration sufficiently high enough to account for the original toxicity. These findings are summarized in the flowchart in Figure 9.

SUBASE OF 11B – This sample exhibited 3.1 TU when tested originally, and contained 3.1 TU Cu and 0.5 TU Zn. While toxicity decreased by the time the Phase I TIE was initiated, there was still sufficient residual toxicity to determine that: 1) EDTA was able to remove some of the remaining toxicity; and 2) C_{18} was able to remove all of the residual toxicity. The effectiveness of the C_{18} column could be explained on the basis of partial removal of zinc and copper from solution, but a non-polar organic constituent was also implicated as toxicity was recovered in a methanol elution of the C_{18} column. Collectively, these data suggest that toxicity was primarily due to copper, but a non-polar organic constituent also contributed to toxicity; the actual contribution of each of these constituents is problematic to determine since the relative dissipation rates are not known. Note that the identity of the non-polar organic is being investigated further. The results of the TIE process for this sample are summarized as a flowchart in Figure 10.

SUBASE OF 23 c+e – This sample exhibited 5.3 TU when tested originally, and contained 3.8 TU Cu and 5.8 TU Zn. Significant toxicity was still present when tested in conjunction with the Phase I TIE. EDTA clearly removed toxicity, implicating divalent cations as the cause of toxicity. Sufficient copper was not present to account for all of the toxicity present. Conversely, there was barely enough Zn to account for all of the toxicity. Under the assumption that not all of the metal present would be bioavailable, it would be reasonable to conclude that both metals contributed to toxicity in this sample, although the exact contribution of each cannot be established. These findings are presented in a flowchart in Figure 11.

Figure 11 also includes the TIE results for mysids. EDTA removed toxicity, indicating that divalent cations were the toxicant involved. Comparison of metals concentrations in the sample with known toxicity benchmarks suggested that zinc was responsible for toxicity.

SUBASE OF 26 – This sample exhibited 5.9 TU when tested originally, and contained 11.4 TU Cu and 2.3 TU Zn. Significant toxicity was still present when tested in conjunction with the Phase I TIE. As with SUBASE OF 23 c+e, toxicity was removed by EDTA, indicating that

divalent cationic metals were the cause of toxicity. There was clearly enough copper present to account for all of the toxicity, and sufficient zinc present to account for partial toxicity. Thus, toxicity was due to copper alone, or to a combination of copper and zinc; the exact contribution of each metal would depend on their relative bioavailability. The results of the TIE process for this sample are summarized as a flowchart in Figure 12.

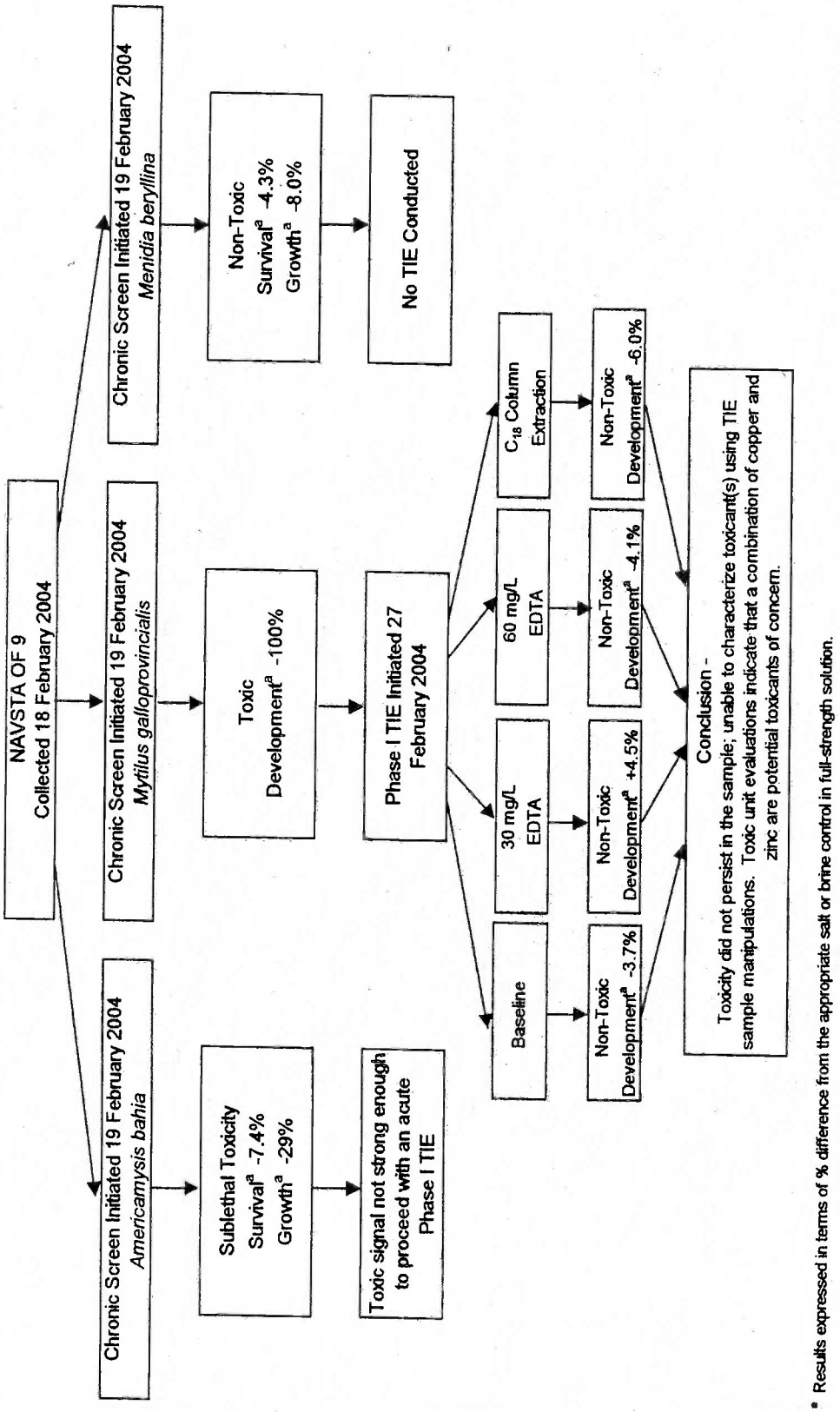


Figure 7. Summary of Results for NAVSTA OF 9 Stormwater.

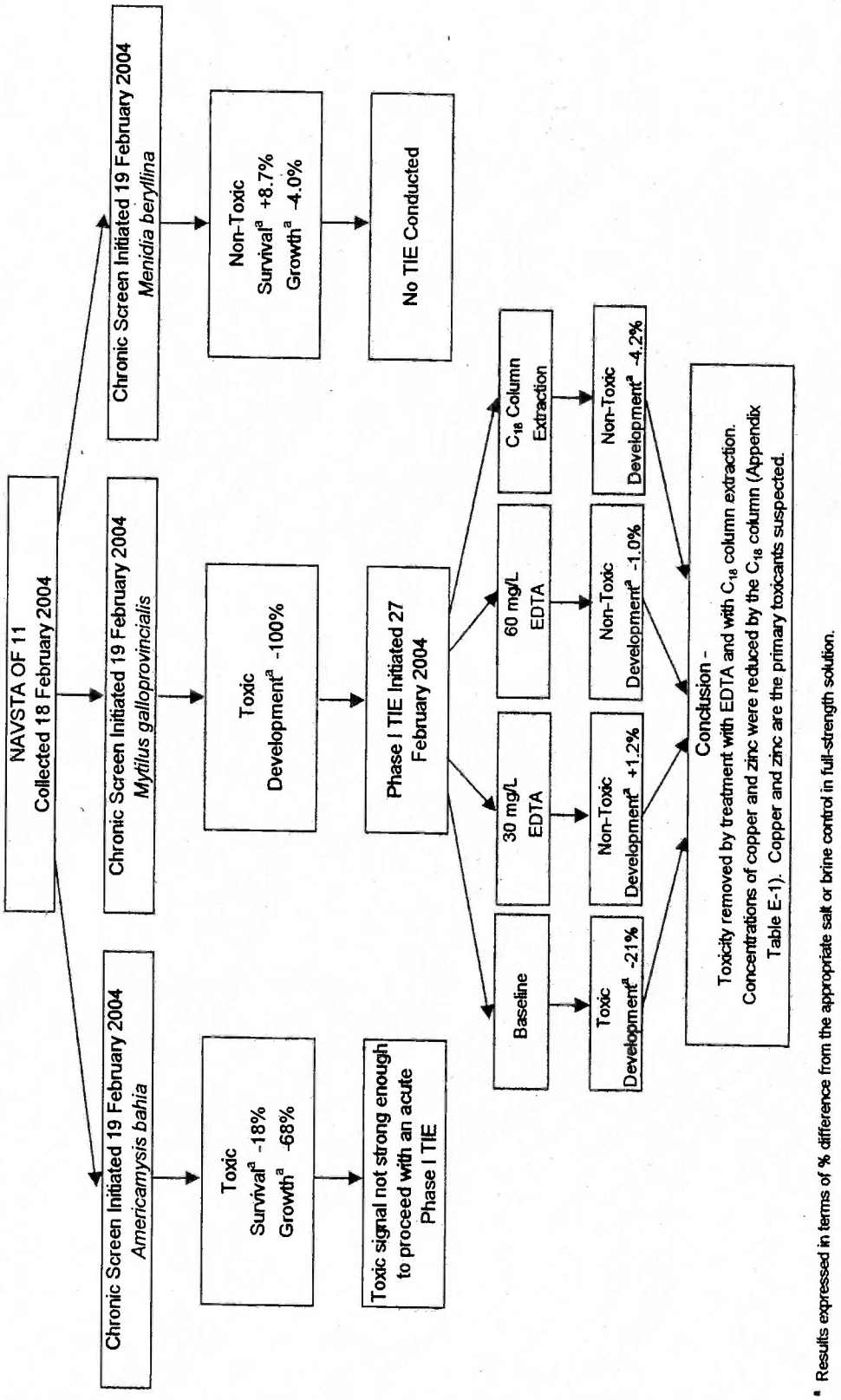


Figure 8. Summary of Results for NAVSTA OF 11 Stormwater.

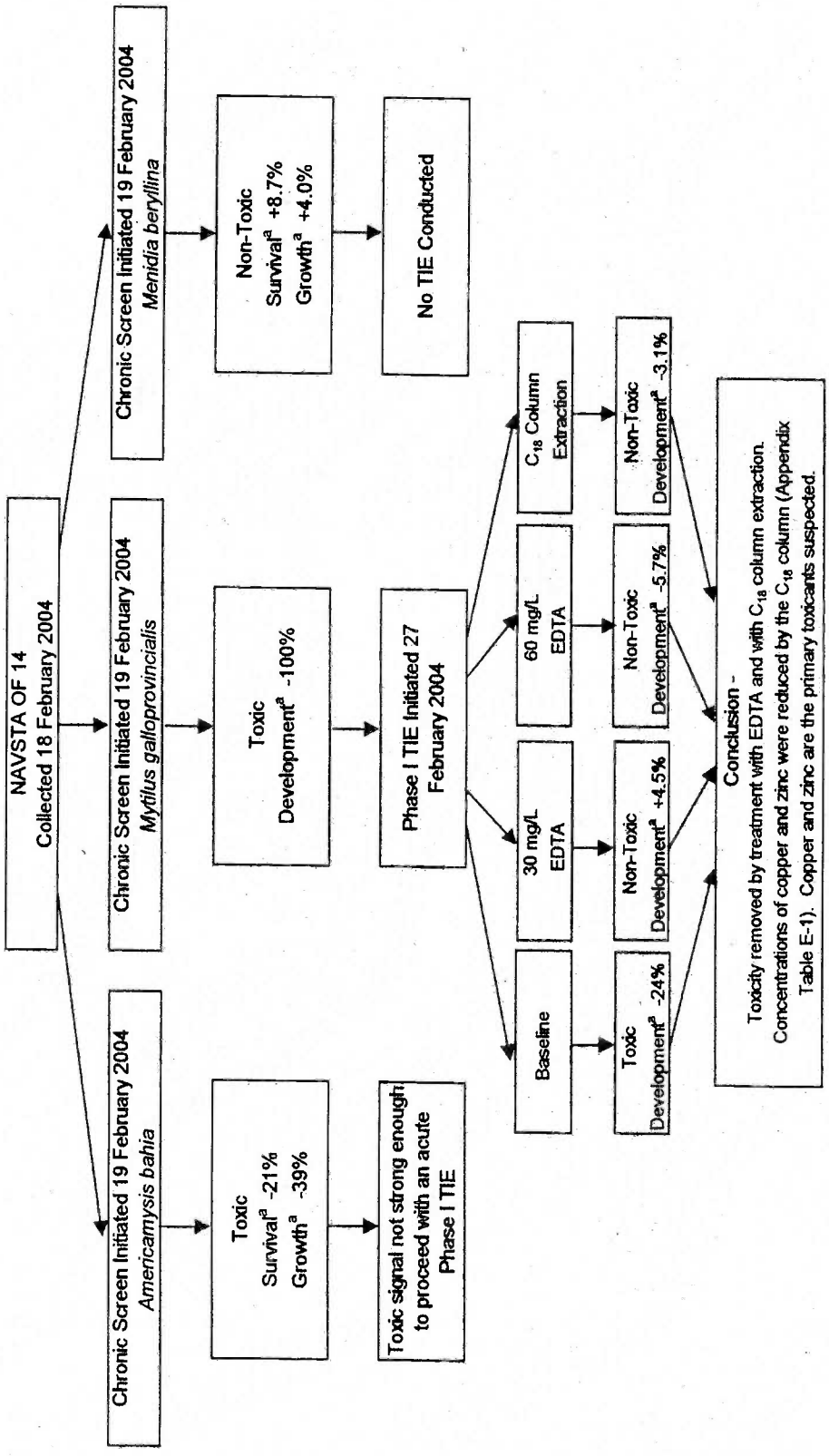
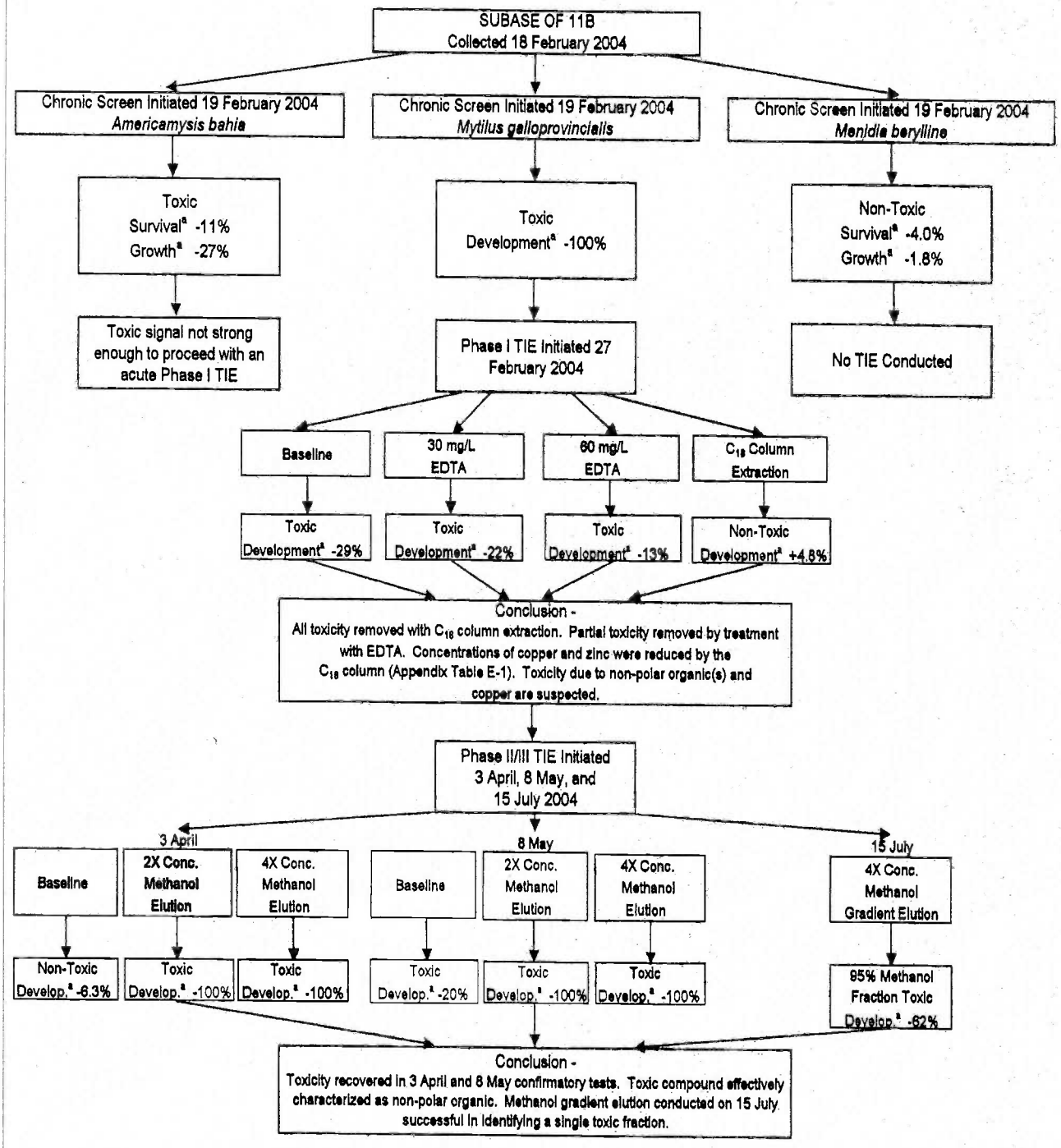


Figure 9. Summary of Results for NAVSTA OF 14 Stormwater.



[a] Results expressed in terms of % difference from the appropriate salt or brine control in full-strength solution.

Figure 10. Summary of Results for SUBASE OF 11B Stormwater.

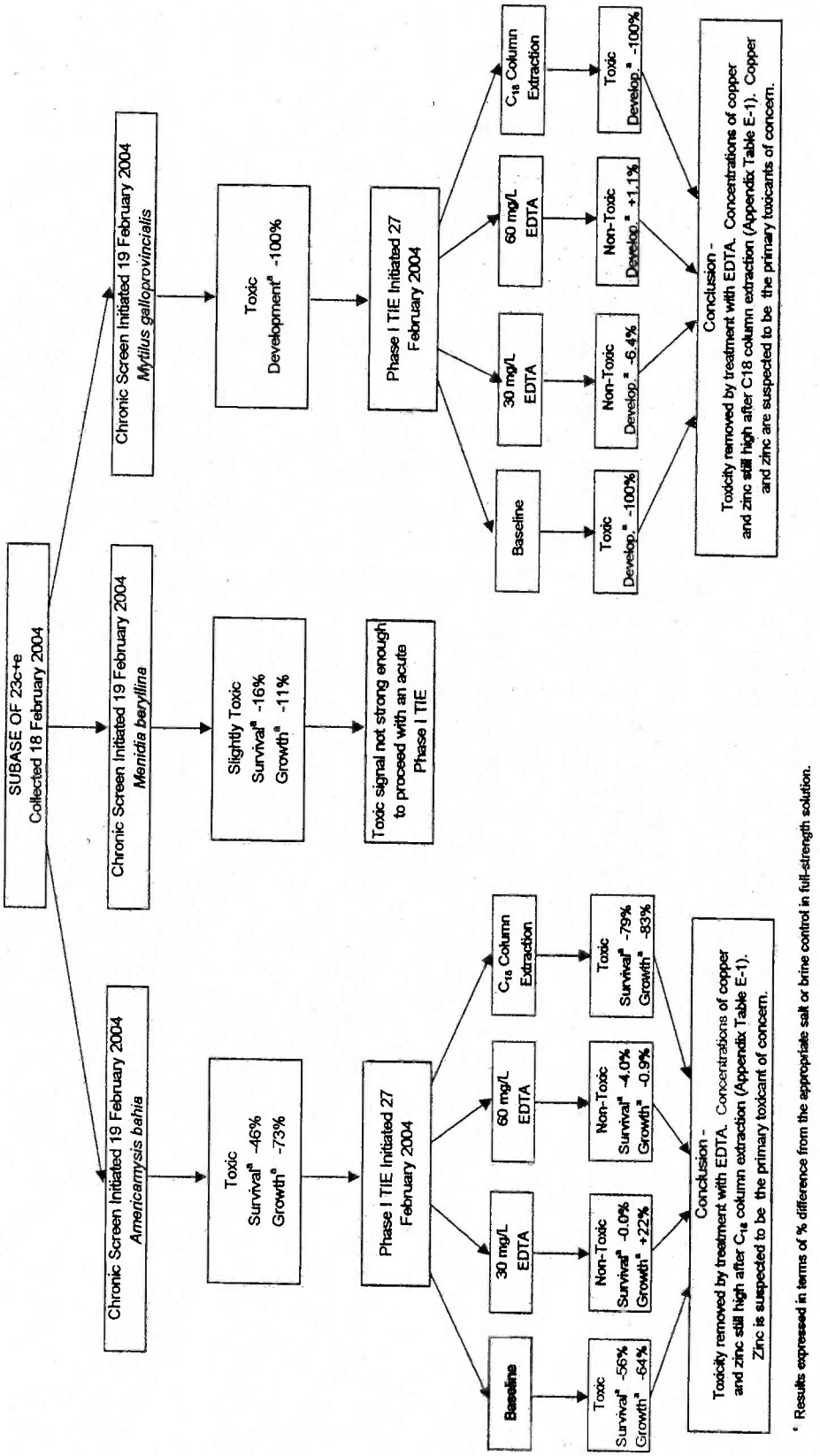


Figure 11. Summary of Results for SUBASE OF 23 c+e Stormwater.

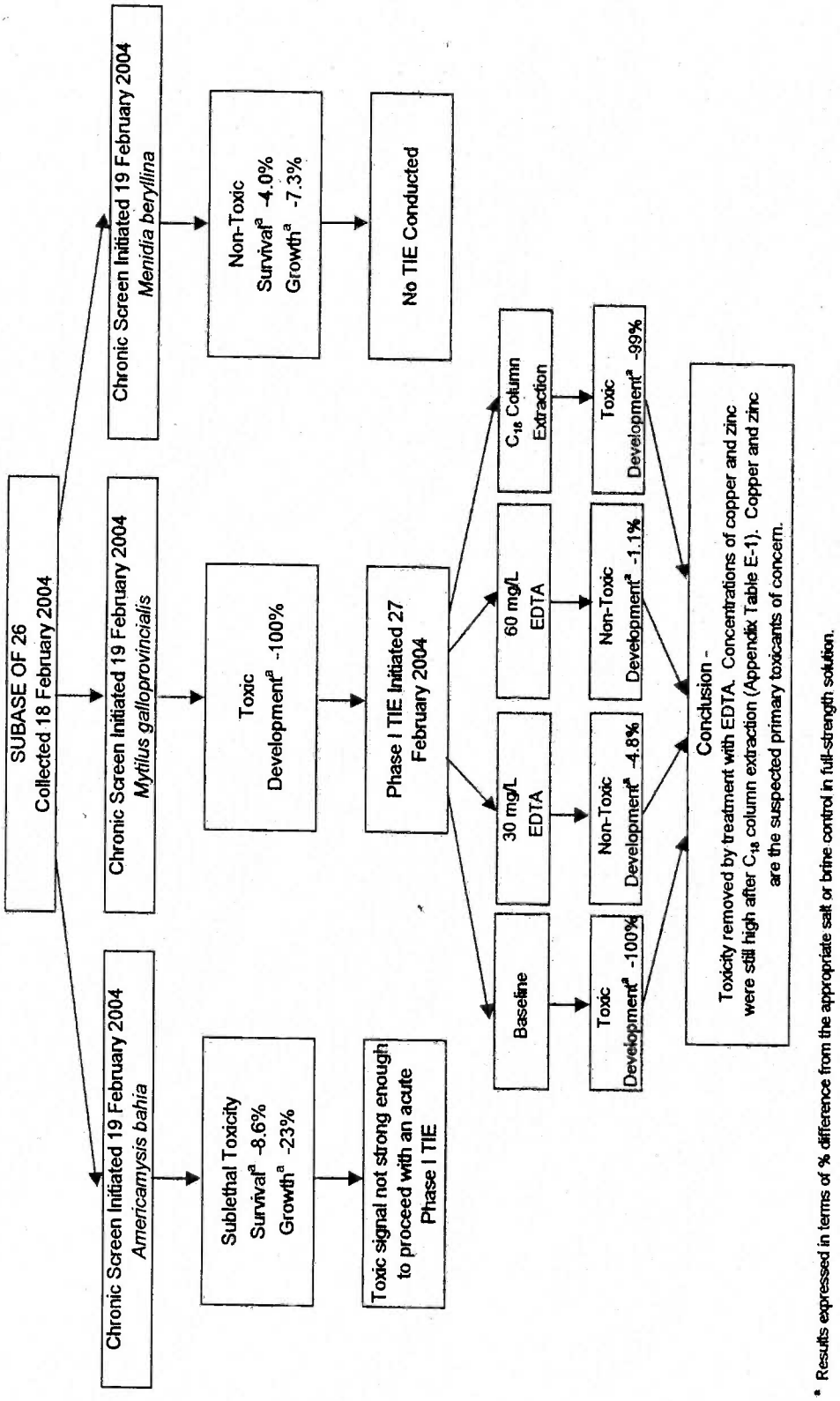


Figure 12. Summary of Results for SUBASE OF 26 Stormwater.

5.0 QA/QC

5.1 Screening Bioassays

5.1.1 Blue Mussel

Mean normal development of mussel larvae in all laboratory seawater and hypersaline brine controls tested during the screening phase of the study ranged between 75 and 81 percent. MSDs ranged between 10 and 25 percent, indicating test sensitivity was within a suitable range.

5.1.2 Opossum Shrimp

At 96 hours, control performance met the 90 percent acute criterion in all cases, with mean survival ranging from 95 to 100 percent across laboratory seawater and artificial salt controls. MSDs calculated in comparison with the artificial salt controls ranged from 5 to 11 percent across samples. At 7 days, laboratory seawater controls exhibited mean survival of 93 percent, and survival among artificial salt controls ranged from 93 to 95 percent. MSDs ranged from 6 to 15 percent. Mean control biomass ranged from 0.25 to 0.30 mg per shrimp, and 0.22 to 0.28 mg per shrimp for laboratory seawater and artificial salt controls, respectively. The control criterion for this endpoint is 0.20 mg per shrimp. MSDs calculated for the growth endpoint ranged from 16 to 31 percent, with only one site (SUBASE OF 26) exceeding 25 percent (Appendix A).

5.1.3 Inland Silversides

Both laboratory seawater and artificial salt controls met survival acceptability criteria. At 96 hours, mean control survival ranged from 96 to 100 percent across controls (> 90 percent acute criterion). MSDs ranged from 5 to 7 percent across samples. At 7-days, mean control survival and biomass ranged from 92 to 100 percent (> 80 percent chronic criterion), and from 0.46 to 0.55 mg per larva, respectively (Appendix A). The criterion for biomass is 0.50 mg per larva. Only one laboratory seawater control fell below this criterion. However, because all statistical comparisons were made using the artificial salt control, results were deemed acceptable for reporting purposes. MSDs for 7-day survival ranged from 6 to 15 percent, and those for growth ranged from 13 to 27 percent. Again, only one sample (NAVSTA OF 9) exceeded 25 percent MSD.

5.2 TIEs

5.2.1 Blue Mussel

EDTA controls exhibited a mean of 91 to 97 percent normal larvae and C_{18} controls exhibited 90 to 96 percent normal larvae, indicating that both the addition of EDTA and the C_{18} extraction process did not adversely affect the test organisms. Methanol controls in the add-back tests exhibited 94 to 99 percent normal larvae, indicating that the presence of methanol also did not adversely affect the test organisms.

5.2.2 Opossum Shrimp

For the opossum shrimp, survival and growth in the EDTA and C_{18} treatment controls were comparable to that observed in the laboratory seawater and artificial salt controls, suggesting that these treatments did not adversely affect the exposed shrimp. Mean survival ranged from 96 to 100 percent, and mean biomass ranged from 0.24 to 0.42 mg per shrimp across controls.

5.3 Reference Toxicant Tests

All reference toxicant test results were within +/- 2 standard deviations of the long-term laboratory control chart averages, suggesting that the sensitivity of the test organisms and the laboratory techniques were consistent throughout the study.

6.0 LITERATURE CITED

American Society for Testing and Materials (ASTM). 1994. Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve molluscs (E 724-89).

Tidepool Scientific Software 1992-1994. TOXCALC Comprehensive Toxicity Data Analysis and Database Software, Version 5.0.

Tidepool Scientific Software. 2001-2002. CETIS – Comprehensive Environmental Toxicity Information System, Version 1.0.

U.S. EPA. 1991. Methods for Aquatic Toxicity Identification Evaluation – Phase I Toxicity Characterization Procedures, Second Edition. U.S. EPA Office of Research and Development, Washington DC. EPA/600/6-91/003.

U.S. EPA. 1992. Toxicity Identification Evaluation: Characterization of Chronically Toxic effluents, Phase I. U.S. EPA Office of Research and Development, Washington DC. EPA/600/6-91/005F.

U.S. EPA. 1993a. Methods for Aquatic Toxicity Identification Evaluations – Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. U.S. EPA Office of Research and Development, Washington DC. EPA/600/R-92/080.

U.S. EPA. 1993b. Methods for Aquatic Toxicity Identification Evaluations – Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity. U.S. EPA Office of Research and Development, Washington DC. EPA/600/R-92/081.

U.S. EPA. 1996. Marine Toxicity Identification Evaluation (TIE) – Phase I Guidance Document. U.S. EPA Office of Research and Development, Washington DC. EPA/600/R-96/054.

U.S. EPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition. U.S. EPA Office of Research and Development, Washington DC. EPA/821/R-02/014.

Addendum Report

Evaluation of Toxicity due to Non-polar Organics in Sample NAVSTA OF11B

A follow-up investigation of toxicity attributable to non-polar organic compounds in NAVSTA Sample OF11B is included in this addendum to a final stormwater toxicity report submitted to SPAWAR August 2, 2004.

This sample exhibited 3.1 TU when originally tested. While overall toxicity decreased by the time the Phase I TIE was initiated, there was still sufficient residual toxicity to determine that: 1) EDTA was able to remove most of the remaining toxicity; and 2) extraction through a C_{18} column was able to remove all of the residual toxicity. The effectiveness of the C_{18} column in removing all of the toxicity could be partially explained on the basis of removal of zinc and copper from solution but, since EDTA failed to remove all of the toxicity, a non-polar organic constituent was also implicated as contributing some portion of the overall toxicity observed. This hypothesis was confirmed by recovery of toxicity in a methanol elution of the C_{18} column. Collectively, these data suggested that toxicity was primarily due to divalent cationic metals (e.g., copper and zinc), but a non-polar organic constituent also contributed to some of the observed toxicity. However, determining the actual contribution of each of these constituents to the toxicity originally observed in the sample is problematic because the relative dissipation rates of each of the contaminants are not known.

In an attempt to identify potential toxicants of concern recovered in the C_{18} methanol extract, subsamples of three extracts (90, 95, and 100 percent methanol) were submitted to CRG Marine Laboratories (CRG) for analysis using GCMS, as described in the attached report from CRG. These extracts were selected because toxicity was recovered in the 95-% methanol fraction, but not in either of the adjacent fractions. Thus, comparing relative concentrations in these fractions would help differentiate among constituents present in more than one fraction, in that the fraction exhibiting the highest concentration should also exhibit the greatest toxicity.
The constituents exclusively detected with certainty in only the 95 percent methanol extract were: 1) nonylphenol (NP), and 2) tetramethylbutyl phenol. Phthalate and phthalate esters were detected in all extracts, but were believed to be a result of laboratory contamination. Two additional compounds, 1-nitroso-3-piperidinol and benzoic acid, were also detected in all three extracts, and eluted early in the chromatograms. Consequently, CRG felt they were most likely caused by trace contamination of the methanol solvent. Since toxicity was not present in the 90 and 100 present methanol extracts, these compounds were not considered to be of toxicological

concern.

Because of the known properties and toxicity of NP, the concentration of this compound was subsequently quantified in the methanol extracts using GCMS. The molecular composition of NP is very similar to tetramethylbutyl phenol and the two compounds may be from a common source.

Analytical results identified five isomer peaks for NP. Total concentrations in the raw methanol extract and within the toxicity test chambers for the methanol add-back study are provided below. Summing the concentrations in the three extracts results in a final estimated concentration of 0.18 µg/L NP in the original sample.

Nonylphenol Concentrations (µg/L)
NAVSTA OF 11B

Sample OF11B	90% Extract	95% Extract	100% Extract
Methanol extract (concentrated 500x)	13.3	57.9	19.6
Toxicity test chambers with methanol extract (concentrated 4X)	0.11	0.46	0.16

A review of toxicity data in EPA's ECOTOX database found a wide range of toxicity values for nonylphenol. On the low end of the curve are NOEC and LOEC values in the range of 5 to 15 µg/L for *Daphnia magna* reproduction, fathead minnow survival, rainbow trout growth, and copepod population effects. Published acute LC50 values for *Americamysis bahia* are in the range of 50 to 100 µg/L for 4-nonylphenol (Lussier et al, 2000). Published nonylphenol LC50 values for *Mytilus edulis* range from 140 µg/L following an 850 hr exposure to 3000 µg/L following a 96-hr exposure (Granmo et al., 1989).

These published values are greater than the concentration of NPE present in the toxic methanol extract at 4X, and calculated for the OF 11B sample based on the totals found in the methanol extracts. Thus, this comparison does not provide a clear indication that there was sufficient NP (and TMBP) present to account for toxicity, although their presence in the toxic fraction is highly suggestive. Alternatively, NP could be a marker for a constituent present in the 95-percent

methanol fraction that was not amenable to analysis with GCMS.

Nonylphenol is a degradation product from a broader class of compounds known as nonylphenol ethoxylates (NPEs). The following information on NPs and NPEs was obtained from a report entitled "Assessment Report - Nonylphenol and its Ethoxylates" by Environment Canada, January 12, 2005. NPEs are common components in detergents, emulsifiers, wetting agents and dispersing agents. Nonylphenol polyethoxylate-containing products are used in many sectors, including textile processing, pulp and paper processing, paints, resins and protective coatings, oil and gas recovery, steel manufacturing, pest control products and power generation. A variety of cleaning products, degreasers and detergents are also available for institutional and domestic use. NPEs are also used in a wide range of consumer products, including cosmetics, and cleaners and paints.

NPEs and their degradation products (including NP) are not produced naturally. The mechanism of degradation is complex but, in general, there is an initial loss of ethoxylate (EO) groups from the original moiety. The intermediate and final products of metabolism are more persistent than the parent NPEs but, ultimately, are expected to undergo biodegradation. Under aerobic and anaerobic treatment conditions, biodegradation to more toxic (and estrogenic) metabolites occurs. These products are NP, nonylphenol ethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), nonylphenoxyacetic acid (NP1EC), and nonylphenoxyethoxyacetic acid (NP2EC). In aquatic environments, primary biodegradation of NPEs is fast, but the resultant products, such as NP1EO, NP2EO, NP1EC, NP2EC and NP, are moderately persistent, especially under anaerobic conditions. Unfortunately, there is currently very limited published toxicity data available for NPEs. No data were available in ECOTOX.

Although we were able to quantify the concentration of NP in the extracts, CRG was not able to quantify the concentration of any of the NP ethoxylates using GCMS. The concentration of NP corresponds well to toxicity in the methanol extract, thus NP and the similar compound recovered, tetramethylbutyl phenol, may serve as good surrogate markers for NPE and its various degradation products. Based on the current weight of evidence, the summed concentrations of the various degradation products may explain the small proportion (approximately 16 percent) of toxicity in the OF11B sample that was unaccounted for after addition of EDTA.

References

Environment Canada, 2005. Assessment Report – Nonylphenol and its Ethoxylates. http://www.ec.gc.ca/substances/ese/eng/psap/final/npe.cfm

Grammo, A., R. Ekelund, K. Magnusson, and M. Berggren. 1989. Lethal and sublethal toxicity of 4-Nonylphenol to the common mussel (*Mytilus edulis L.*). Environ. Pollut. 59(2):115-127.

Lussier, S.M., D. Champlin, J. LiVolsi, S. Poucher, and R.J. Pruell. 2000. Acute toxicity of para-Nonylphenol to saltwater animals. Environ. Toxicol. Chem 19(3):617-621.

APPENDIX F

TIE2 Report

Please note that the report in this appendix was generated with slightly different acronyms from those used throughout the body of the report and other appendices. The differences are as follows:

MAIN REPORT	THIS APPENDIX
NI	NASNI

Nautilus Environmental

Toxicity Identification Evaluation (TIE) Study of San Diego Bay Stormwater

March 19, 2005 Sampling Event
FINAL REPORT
Response to External Comments Included

Prepared for: Computer Sciences Corporation
4045 Hancock Street
San Diego, CA 92110

Space and Naval Warfare Systems Center
San Diego (SPAWAR)
53560 Hull Street
San Diego, CA 92152-5001

Prepared by Nautilus Environmental
5550 Morehouse Drive, Suite 150
San Diego, CA 92121

Submitted: April 26, 2006

Data Quality Assurance:

- Nautilus Environmental is a certified laboratory under the State of California Department of Health Services Environmental Laboratory Accreditation Program (ELAP), Certificate No. 1802.
- All test results included in this report have met internal Quality Assurance/Quality Control (QA/QC) requirements, as well as minimum acceptability criteria as outlined in their respective protocols.
- All data have been reviewed and verified.
- Any test data discrepancies or protocol deviations have been noted in the summary report pages.

Results verified by: **Chris Stransky, Laboratory Manager** Date: **April 26, 2006**

1.0 INTRODUCTION/ EXECUTIVE SUMMARY

The toxicity of stormwater samples from four outfall locations (identified as NAB OF 9, NAB OF 18, NASNI OF 23a, and NASNI OF 26) and four receiving water samples from San Diego Bay collected near each of the outfall locations was evaluated using a suite of marine test species including *Mytilus galloprovincialis* (Mediterranean mussel), *Atherinops affinis* (Pacific topsmelt), and *Americamysis bahia* (mysid shrimp). All samples were collected during a light rain event (approximately 0.1 inch), which occurred on March 19, 2005. Mussel embryo development was evaluated following a 48-hour exposure to the samples and survival of mysids and topsmelt was evaluated following an acute 96-hour exposure. Toxicity Identification Evaluation (TIE) studies were performed on samples that exhibited toxicity to any of the test species. Of the eight samples tested, three of the stormwater samples (NAB OF 9, NAB OF 18, and NASNI OF 23a) exhibited toxicity to one or more of the species tested. Two of these samples (NAB OF 18 and NASNI OF 23a) were toxic to all three species tested. Sample NAB OF 9 was toxic to mussels and mysids, but not to topsmelt. The trace metals copper and zinc were wholly responsible for toxicity to mussels in this sample. Zinc, and a possible contribution from copper were responsible for toxicity to mysids in Sample NAB OF 9. A combination of toxicants including copper, zinc, and surfactants were responsible for toxicity to mussels in both NAB OF 18 and NAB OF 23a. Evidence suggests that surfactants were responsible for all toxicity observed in NAB OF 18 and NASNI OF 23a for both mysids and topsmelt. None of the bay receiving water samples were toxic to any of the species tested. All toxicity tests and TIE procedures were performed at Nautilus Environmental's San Diego location (Nautilus). Supporting analytical testing was conducted in partnership with Calscience Environmental Laboratories (CEL), located in Garden Grove, California. Results of the screening studies, Phase I TIEs, and Phase II/III TIEs are presented in this report. Toxicity screening studies were initiated on March 19, 2005 and TIE evaluations were performed between March 24 and May 23, 2005.

2.0 MATERIALS AND METHODS

2.1 Test Material

Stormwater samples were collected on March 19, 2005 between 2:25 and 4:25 AM under the supervision of Chuck Katz at SPAWAR. The samples were collected in plastic-lined, 19-L plastic buckets using peristaltic pumps to fill each container. As soon as sampling was completed, the buckets were transported to Nautilus by SPAWAR personnel. Upon arrival at the laboratory, each sample was assigned a tracking number, and water quality measurements

of temperature, pH, dissolved oxygen (DO), conductivity or salinity, alkalinity, and hardness were recorded (Table 1).

Temperature and conductivity or salinity were measured with an Orion 130 meter. DO was measured using a YSI 55 meter, and an Orion 250A+ meter was used to measure pH. Alkalinity (Hach Method 8203) and hardness (Hach Method 8213) were checked using Hach digital titrators (Model 16900). The samples were held at 4°C in the dark at Nautilus prior to testing. Appropriate chain-of-custody (COC) procedures were followed during all phases of this study. Copies of the COC forms for this study are attached in Appendix F.

2.2 Test Design and Bioassay Procedures

The overall experimental design was built to facilitate comparisons of sensitivity between species and identify the presence and degree of acute toxicity. The Navy's stormwater permit requires evaluation of acute toxicity with both mysid shrimp (*Americamysis bahia*) and topsmelt (*Atherinops affinis*). However, the 48-hour mussel embryo development test (using *Mytilus galloprovincialis*) was also incorporated into this study design because of its known sensitivity to copper, a contaminant known to be historically relevant at these sites. TIEs were then performed using any species exhibiting toxicity to any sample material.

The results of the screening tests were used to select samples that would be amenable to follow-up investigation of the cause of toxicity. In general, TIEs have the highest probability of success if conducted on samples that produce well-defined toxic responses that do not dissipate quickly over time. Consequently, a degree of response that can be clearly separated from the control is highly desirable. While this ultimately depends on the number of replicates used and the variability of the results, our experience suggests that a minimum of a 20-percent difference from the control usually provides sufficient resolution against which to judge the effectiveness of the various treatments. These treatments can then be used to determine the general characteristics of the toxicant, and ultimately to identify and confirm the cause of toxicity.

Table 1. Water Quality Parameter Measurements upon Sample Receipt.

Site ID	Date Collected	Date Received	Temp. (°C)	pH (units)	DO (mg/L)	Conductivity (µmhos/cm) or Salinity (ppt)	Alkalinity (mg/L $CaCO_3$)	Hardness (mg/L $CaCO_3$)
NAB OF 9	3/19/05	3/19/05	15.9	7.54	8.7	8140[a]	60	794
NAB OF 18	3/19/05	3/19/05	15.9	7.53	8.5	2260[a]	55	379
NASNI OF 23a	3/19/05	3/19/05	16.6	7.71	10	443[a]	35	95
NASNI OF 26	3/19/05	3/19/05	18.1	8.07	6.8	21000[a]	162	>1000
NAB OF 9 Bay	3/19/05	3/19/05	16.0	8.20	8.3	32.1[b]	94	NA
NAB OF 18 Bay	3/19/05	3/19/05	15.3	8.14	8.0	32.1[b]	115	NA
NASNI OF 23a Bay	3/19/05	3/19/05	16.3	8.19	8.4	32.7[b]	113	NA
NASNI OF 26 Bay	3/19/05	3/19/05	16.3	8.19	8.4	32.7[b]	113	NA

Note: [a] conductivity or [b] salinity
NA - not applicable, as hardness is not measured in saline samples

The Mediterranean mussel embryo development assay was performed in accordance with "Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs (E 724-98)" (ASTM 1999). Procedures for testing stormwater using mysid shrimp and Pacific topsmelt acute survival tests followed "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. (EPA-821-R-02-012)" (EPA 2002a).

Procedures for performing Phase I TIEs are outlined in "Methods for Aquatic Toxicity Identification Evaluations – Phase I Toxicity Characterization Procedures, Second Edition (EPA/600/6-91/003)" (EPA 1991), "Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I (EPA/600/6-91/005F)" (EPA 1992), and "Marine Toxicity Identification Evaluation (TIE) – Phase I Guidance Document" (EPA 1996). Procedures for performing Phase II and III TIEs are outlined in "Methods for Aquatic Toxicity Identification Evaluations – Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity (EPA/600/R-92/080)" (EPA 1993a), and "Methods for Aquatic Toxicity Identification Evaluations – Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity (EPA/600/R-92/081)" (EPA 1993b), respectively.

2.2.1 Screening Bioassays

Mediterranean Mussel Embryo Development Test

The Mediterranean mussel, *Mytilus galloprovincialis*, was field collected by Nautilus personnel in Mission Bay, San Diego, California and transported to Nautilus in ice chests containing blue ice. In the laboratory, the organism receipt date and arrival condition were recorded in a logbook. The mussels were then acclimated to test temperature and salinity, and observed each day prior to test initiation for any indications of significant mortality (>10%).

Mussel embryos were exposed to stormwater for a period of 48 hours to evaluate effects on embryo development. Original screening tests were conducted using a sample concentration series of 12.5, 25, 50 percent, and the highest testable concentration (dependent upon the initial salinity of the sample) along with a concurrent negative control. Test solutions were prepared using graduated cylinders and pipettes. TIE testing was conducted on a reduced dilution series to focus resources on the concentrations most likely to express toxicity. Due to the low salinities of the samples, hypersaline brine was added to each sample to raise the salinity to 32 ppt. The volume of hypersaline brine required to adjust the salinity determined the highest testable concentration for each sample. An additional negative control composed of

hypersaline brine and deionized water was also tested to ensure any observed toxic effects were not due to the brine.

Measurements of pH, DO, temperature, and salinity were recorded for each test concentration and control. Five replicate test chambers were prepared for each test concentration and control. Replicates consisted of 30-ml shell vials containing 10 ml of test solution. Test solutions were acclimated to 15°C in temperature-controlled environmental chambers prior to initiation.

In order to spawn the mussels, brood stock were exposed to heated ultraviolet (UV) treated seawater (27-29°C) in shallow plastic trays. Within 60-90 minutes, the mussels began to spawn. Spawning individuals were removed and isolated in individual 250-ml beakers containing 20°C seawater. After allowing individuals to continue to spawn for 30 minutes, the quality of the eggs was examined under a compound microscope. The three "best" egg stocks (as defined by microscopic observations of egg shape, color, and opacity) were poured into 1-L Erlenmeyer flasks and each was fertilized with sperm from at least three different males. Fertilization was allowed to continue for twenty minutes. Each sperm-egg stock mixture was then poured through a 20-µm screen allowing sperm to pass through while retaining fertilized eggs. The three embryo stocks were allowed to develop for approximately two hours in a 15°C environmental chamber. A 1-ml aliquot was then removed from each embryo stock and examined under a compound microscope. The embryo stock that exhibited the furthest development (i.e., most number of cleavages per cell) was diluted to a concentration of 400 embryos/ml, and 0.5 ml of this stock was added to each vial to initiate testing. Mussel embryos were exposed to a 16:8 hour light:dark illumination cycle for the duration of the test. Test chambers were covered with a clear Plexiglas sheet to reduce evaporation and prevent test solution contamination.

Temperature, pH, DO, and salinity were measured daily in surrogate test chambers for each concentration and control. At test termination, larvae in each test chamber were preserved with 1 ml of seawater-buffered Formalin prior to evaluation. A subsample of 100 bivalve embryos from each test chamber was counted under a compound microscope at 400x magnification. The embryos were classified as normal or abnormal. Normally developed embryos have a distinct D-shape with complete formation of the shell.

A concurrent reference toxicant test (positive control) using copper (II) chloride ($CuCl_2$) was conducted in conjunction with the stormwater tests.

Mysid and Topsmelt 96-Hour Acute Tests

Juvenile mysids and topsmelt were purchased from Aquatic Biosystems of Fort Collins, Colorado. Prior to shipment, the organisms were placed in plastic bags containing oxygenated culture water, packed in insulated containers, and transported to Nautilus via overnight delivery service. Upon arrival at Nautilus, water quality parameters of temperature, pH, DO, and salinity were measured and recorded in a logbook for each species. The condition of the organisms was also noted. The organisms were then acclimated to test salinity and temperature, and observed prior to test initiation for any indications of stress (e.g. abnormal swimming behavior) or significant mortality (>10%) and were fed *Artemia* nauplii to satiation during holding. Mysids were 3-4 days old upon arrival at Nautilus and 3-4 days old upon test initiation. Topsmelt were 11-12 days old upon arrival at Nautilus and 11-13 days old upon test initiation

These tests estimate acute toxicity by evaluating survival of mysid shrimp or topsmelt over a 96-hour exposure period. Original screening tests were conducted using a sample concentration series of 25, 50, and 100 percent sample along with a concurrent negative control consisting of 32 ppt natural seawater. TIE manipulations and tests were conducted on the undiluted sample only. Test solutions were prepared using graduated cylinders and pipettes.

Due to the low salinities of the samples, Forty Fathoms™ sea salt was added to each sample to raise the salinity to 32 ppt. An additional control composed of Forty Fathoms™ sea salt and deionized water was also tested to ensure observed mortality was not due to the addition of artificial salt rather than other toxic constituents.

Measurements of pH, DO, temperature, and salinity were recorded for each test concentration and control. Four replicate test chambers were prepared for each test concentration and control. Replicates consisted of 400-ml plastic cups containing 250 ml of test solution. Test solutions were acclimated to 25°C for mysid and 20°C for topsmelt tests in temperature-controlled environmental chambers prior to initiation.

Five mysids were counted and transferred from holding bowls into individual plastic soufflé cups. A second technician verified counts and condition of all test organisms prior to addition of the organisms to the test chambers, and again when test initiation was complete. Due to their size, five topsmelt were counted and transferred from holding bowls directly into their corresponding test chambers. A second technician verified counts and condition of all test organisms when test initiation was complete. A 16:8 hour light:dark illumination cycle was provided for the duration of the test. Test chambers were covered with a clear Plexiglas sheet

to prevent evaporation and cross-contamination of the test solutions.

Test solutions were renewed at 48 hours. Mysids were fed twice per day and topsmelt once per day. Temperature, pH, DO, and salinity were measured daily in the test chambers for each concentration and control and in freshly prepared test solutions at the 48-hour renewal. Survival of organisms was recorded for each test chamber once per day. At test termination, final observations and counts were performed.

All copper chloride reference toxicant tests (positive control) were conducted within a 3-week period of these tests.

2.2.2 Phase I TIE Treatments

Phase I TIE treatments are designed to remove, inhibit, or potentiate a particular class of compounds that may be present in the sample, thereby isolating the toxic signal. Selected treatments were applied in this study; detailed descriptions of each treatment are provided below, and a general summary of Phase I TIE characterization procedures is shown in Tables 2 and 3.

Filtered, natural seawater (mussel larvae) and artificial seawater (mysid and topsmelt) were used as dilution and control water for these studies. Untreated control water was tested concurrently with the "Baseline" (untreated) stormwater tests for each site and species. Aliquots of the appropriate control water underwent each of the Phase I manipulations (method controls) and were tested alongside the treated stormwater samples. The method controls are used to assess whether the sample manipulations resulted in adverse effects due to the procedures themselves.

Baseline Tests

Baseline tests were performed concurrently with the Phase I TIE treatments to compare the organism response in untreated stormwater to responses obtained after manipulations of the sample. Treatments that altered the toxicity compared to the toxicity of the baseline test were used to identify classes of toxic compounds present in the sample.

EDTA Metal Chelation

The addition of ethylenediaminetetraacetic acid (EDTA) was used to determine the extent of toxicity attributable to divalent cationic trace metals (EPA 1991). EDTA chelates divalent cationic trace metals, thereby reducing their bioavailability. EDTA was added to the method controls and all stormwater dilutions at an exposure concentration of 60 mg/L.

Solid-Phase Extraction

Solid-phase extraction (SPE) with a C_{18} column was used to determine the extent of toxicity associated with non-polar organic compounds. It has been found that C_{18} columns also have the ability to remove some metals as well (EPA 1991). A 5-ml capacity Baker brand column was used for this procedure. Post-filtered SPE columns were labeled, wrapped in airtight re-sealable bags, and held in the dark at 4°C for potential subsequent Phase II testing.

Aeration

Aeration of the sample was used to determine the extent of toxicity associated with volatile or sublatable compounds. Sublatable compounds include surface-active compounds such as resin acids, soaps, detergents, charged stabilization polymers, and coagulation polymers used in chemical manufacturing processes. Samples were heavily aerated in 1-L glass graduated cylinders for 1-hour and any foam created was collected and stored at 4°C for subsequent testing. Samples were then siphoned out of the cylinders and held in the dark at 4°C for testing.

Combination Treatments

A combination of treatments can be used when more than one toxicant is suspected. This can occur when previous testing indicates that a particular treatment or set of treatments remove partial toxicity. By combining treatments, multiple contaminants can be inhibited, and when viewed in the context of results of prior testing, specific contaminants of concern can be isolated. A second round of Phase I TIE testing included two sets of combination treatments: 1) Solid-phase extraction + EDTA metal chelation, and 2) Aeration + EDTA metal chelation. The SPE + EDTA treatment was performed to determine the extent of toxicity related to both non-polar organic compounds and divalent cationic trace metals. EDTA, at a test concentration of 60 mg/L, was added to post-C_{18} extracted sample prior to testing. The aeration + EDTA treatment was performed to determine the extent of toxicity related to both volatile or sublatable

compounds and divalent cationic trace metals. EDTA, at a test concentration of 60 mg/L, was added to post-aerated sample prior to testing.

Aeration Foam Add-back

During the first round of the TIE, any foam produced during the aeration treatment was collected and stored in a glass beaker at 4°C. Any sublatable contaminants removed during the aeration treatment (now contained in the foam extract) were added back to laboratory dilution water at 25 percent of the original sample volume (a 4X concentration).

SPE Methanol Elution Add-back

Non-polar organic compounds bound to SPE columns can be removed from the columns using methanol. Methanol extractions were performed by pumping 2 ml of 100 percent methanol through the column using a peristaltic pump set at an approximate rate of 1 ml per minute. Extracts were collected into 2-ml amber glass Voa® vials. The extracts were then added to clean dilution water at concentrations that were two times that in the original stormwater sample. Because the extraction method is not 100 percent efficient at removing contaminants from the column, concentrating the extract in this way increases the likelihood of recovering the toxicity of a sample. Concurrent method controls consisted of: 1) clean dilution water to which methanol passed through the SPE column was added; and 2) a methanol control equivalent to the highest methanol concentration achieved in the tested fractions.

Anion Extraction of SPE Elution

Anion columns were used to determine the extent of toxicity associated with anionic compounds, in particular anionic surfactants that may have been removed from solution by the C_{18} column. Toxic C_{18} methanol extracts were added to laboratory dilution water and then pulled through an anion column. Anionic metals (e.g. aluminum, fluoride, and bromide) will not be recovered in methanol extracts, thus this class of compounds is ruled out at this point. A 3-ml capacity Burdick & Jackson brand column was used for this procedure. Post-filtered columns were labeled, wrapped in airtight re-sealable bags, and held at 4°C for potential subsequent Phase II testing.

2.2.3 Phase I TIE Bioassays

Mediterranean Mussel Embryo Development Test

A dilution series was prepared for each treatment to evaluate its effectiveness at different concentrations. Bioassays were conducted following the same methods for organism procurement, test initiation, monitoring and termination previously described for screening tests. The experimental design, including number of replicates, concurrent controls and test concentrations, is summarized in Table 2.

Table 2. Phase I TIE Toxicity Test Experimental Design – Blue Mussel

Test Procedure	Replicates	Test Solutions
Baseline Tests (NAB OF 9, NAB OF 18 NASNI OF 23a)	5	Lab Control, Brine Control, 12.5, 25, 55 or 59%[a]
Phase I Manipulations (Round One - 3/24/05) (EDTA, SPE column, and Aeration)	5	Method Control, 12.5, 25, and 55 or 59%[a]
Phase I Manipulations[b] (Round Two – 4/8/05) (EDTA + SPE column, EDTA + Aeration, Aeration foam add-back 4X, SPE column elution 2X, and Anion extraction of SPE elution)	5	Method Control, 61%
Reference Toxicant Test	5	0, 2.5, 5, 10, 20, and 40 µg/L Cu

[a] The highest testable concentration for each of the samples: NAB OF 9 – 59%; NAB OF 18 and NASNI OF 23a - 55%.
[b] Tested only with samples NAB OF 18 and NASNI OF 23a.

Mysid and Topsmelt 96-hour Acute Test

During the initial screening tests all samples, with the exception of NAB OF18, exhibited a substantial decrease in toxicity when diluted to 50 percent. Consequently, the TIE treatments were performed only on undiluted sample to maximize the likelihood of detecting a toxic signal. Fresh aliquots of samples were treated with EDTA three hours prior to the 48-hour solution renewal. However, due to the time associated with C_{18} column extraction, a sample volume adequate for the test initiation and renewal was prepared the day prior to test initiation. All remaining aspects of the tests pertaining to organism procurement, test initiation, monitoring

and termination were conducted following the same methods previously described for the screening tests. Experimental design, including number of replicates, concurrent controls, and test concentrations is summarized in Table 3.

Table 3. Phase I TIE Toxicity Test Experimental Design – Mysids and Topsmelt

Test Procedure	Replicates	Test Solutions
Baseline Test (NAB OF 9[a], NAB OF 18 NASNI OF 23a)	4	Lab Control, Salt Control, and 100%
Phase I Manipulations (Round One – 3/30/05) (EDTA Chelation, SPE column, and Aeration)	4	Method Control and 100%
Phase I Manipulations[b] (Round Two – 4/21/05) (EDTA + Aeration, Aeration foam add-back 4X, SPE column elution 2X, and Anion extraction of SPE elution)	4	Method Control and 100%
Reference Toxicant Tests		
Mysid	4	0, 37.5, 75, 150, 300, and 600 µg/L Cu
Topsmelt	4	0, 25, 50, 100, 200, and 400 µg/L Cu

[a] Mysid only
[b] Tested only with mysids and sample NAB OF 18

2.2.4 Phase II/III TIEs

During Phase II/III TIE procedures, additional testing was performed in an effort to identify and confirm specific contaminants responsible for toxicity. Specific Phase II/III methods depended upon the results obtained during Phase I testing in which metals, specifically copper and zinc, were suspected to be a major source of toxicity. Confirmation of these suspected toxicants was performed using a combination of statistical and experimental procedures to provide additional lines of evidence that supported the identification process. The Phase II/III TIE procedures were conducted using the mysid acute survival test due to its permit compliance relevance. For comparison and clarification, results of similar Phase II/III TIE procedures performed and reported during the 2004 storm season using the Mediterranean mussel are also reported.

Copper and Zinc Mixture Studies

Based on Phase I TIE and analytical chemistry results, studies were conducted to evaluate the combined toxicity of copper and zinc to mysids. This same set of experiments and associated results for the Mediterranean mussel were previously provided to SPAWAR in a report submitted in August 2004. Four bioassays were conducted using clean laboratory seawater and analytically verified trace metal stock solutions: 1) a mixture of copper and zinc at concentrations based on the ratio of the two metals in the stormwater samples; 2) a mixture of copper and zinc at concentrations based on the ratio of their individual acute Median Lethal Effect (LC_{50}) Concentrations; 3) a copper reference toxicant test; and 4) a zinc reference toxicant test. Results from these studies were used to evaluate the extent to which each of the two metals contributed to overall toxicity in the stormwater samples, and if the two metals exhibited additive or synergistic toxicity. All aspects of these bioassays were conducted similarly to screening tests.

2.3 Statistical Analyses

Proportional data (e.g. percent normal embryos, percent survival) were arcsine square-root transformed prior to analysis. To determine if parametric or non-parametric statistical methods could be applied to the data, the data were evaluated for normality (Shapiro-Wilks Test) and homogeneity of variance (Bartlett's Test). Depending on the results of these tests, Steel's Many One Rank Test (non-parametric) or Dunnett's Test (parametric) was used to identify significant differences between each concentration and the appropriate control (brine or salt). Minimum Significant Differences (MSDs) were calculated as a percentage of the control response for each test, based on Dunnett's t-statistic. For a more detailed analysis of MSD relationships see Appendix G. Note that this procedure likely overestimates test sensitivity in cases where the test endpoints were determined with non-parametric methods.

LC_{50} and/or Median-Effect (EC_{50}) concentration values were also calculated for all tests that exhibited a dose-response curve. These endpoints were calculated with Maximum Likelihood Probit, or Trimmed Spearman-Karber methods depending on specific assumptions met by the data. Comprehensive Environmental Toxicity Information System (CETIS), version 1.025b, was used for these analyses.

2.4 Analytical Chemistry

Based on historical chemical and toxicological data available for the four stormwater outfalls, subsamples from each site were analyzed for a suite of total and dissolved trace metals. Samples were filtered through a Gelman 0.45-μm glass fiber filter at Nautilus on the day of sample receipt within 24 hours of collection for analysis of the dissolved fraction. Because C_{18} SPE columns can bind some trace metals in addition to non-polar organic substances, subsamples were also collected from NAB OF 18 and NASNI OF 23a following C_{18} SPE column extraction and analyzed for the same suite of trace metals to determine if a reduction in toxicity following C_{18} SPE extraction may be due to removal of trace metals.

Due to their prevalence in stormwater runoff, and observation of some foaming in samples when poured, surfactants were measured by analyzing methylene blue activated substances (MBAS) both prior to and after aeration of the samples. MBAS includes a common group of anionic surfactants known as linear alkyl sulfonates (LAS). Surfactants were analyzed by CEL following EPA Method 425.1.

2.5 Quality Assurance

Nautilus implements quality assurance (QA) procedures in accordance with our internal QA Plan, which is based on applicable protocols and guidance documents. These procedures encompass all aspects of testing, including the source, handling, condition, receipt, and storage of samples and test organisms, and the calibration and maintenance of instruments and equipment. All data generated by the laboratory are monitored for completeness and accuracy at the end of each day, and at the end of each individual test period. Laboratory controls are conducted concurrently with every assay. In addition, reference toxicant tests are performed concurrently with every assay, or on a monthly basis, to confirm that test organism quality, and laboratory conditions and procedures, remain consistent over time.

3.0 Results and Discussion

Detailed descriptions of the results of screening tests and all TIE procedures are presented in the following sections. Tables summarizing the toxicity data are presented in Appendix A. Statistical summaries and raw bench datasheets are presented in Appendix B. Appendix C contains reference toxicant test results, as well as a laboratory quality control chart for each species. Analytical chemistry reports from CEL are in Appendix D, and sample receipt information and COC forms, are contained in Appendices E and F, respectively.

3.1 Screening Bioassays

The results of the initial toxicity screening tests performed on March 19, 2005 are summarized in Figures 1 through 6 and Appendix Tables A-1 through A-5.

3.1.1 Stormwater Outfall Samples

Mussel Embryo Development

Three stormwater samples (NAB OF 9, NAB OF 18, and NASNI OF 23a) exhibited appreciable toxicity to mussel embryos; no normal development was observed in the highest testable concentration (57 to 69 percent) of each sample, and EC_{50} values ranged from 12 to 22 percent stormwater (Figure 1). Based on these data, all of these samples exhibited sufficient toxicity to trigger a Phase I TIE. One sample, NASNI OF 26, was not toxic to mussels with a mean of 89 percent of the embryos exhibiting normal development in the highest concentration tested (69 percent).

Mysid Shrimp Acute Survival

At 96 hours, mean survival of mysids among all four undiluted stormwater samples ranged between 5 and 95 percent, compared with 95 to 100 percent in the controls (Figure 2). Three of these samples (NAB OF 9, NAB OF 18, and NASNI OF 23a) exhibited at least a 20 percent reduction in survival relative to the controls; however, only NAB OF 9 and NAB OF 18 were statistically significant. The site with the lowest survival (NAB OF 18) exhibited an LC_{50} value of 42 percent. The LC_{50} value for NAB OF 9 exceeded 100 percent.

Pacific Topsmelt Acute Survival

Mean acute survival in the four undiluted stormwater samples ranged between 0 and 100 percent, compared with 100 percent in both controls (Figure 3). Two of these samples (NAB OF

18 and NASNI OF 23a) exhibited at least a 20 percent reduction in survival relative to the controls, and both were statistically significant. Similar to mysids, the site with the lowest survival (NAB OF 18) had an LC_{50} value of 38 percent, while LC_{50} values for all other samples exceeded 100 percent.

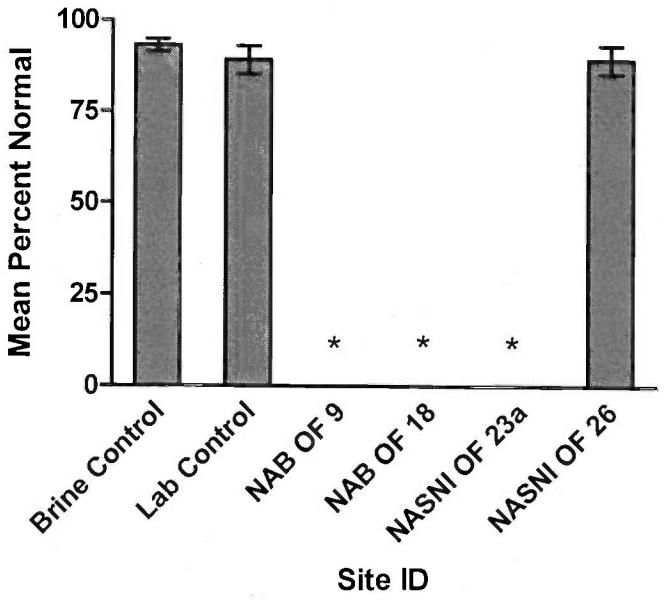


Figure 1. Stormwater Toxicity Screening Test Results for Mussel Embryo Development (100 percent sample). Mean values are presented ± 1 standard deviation. Asterisks indicate significant differences relative to the brine control.

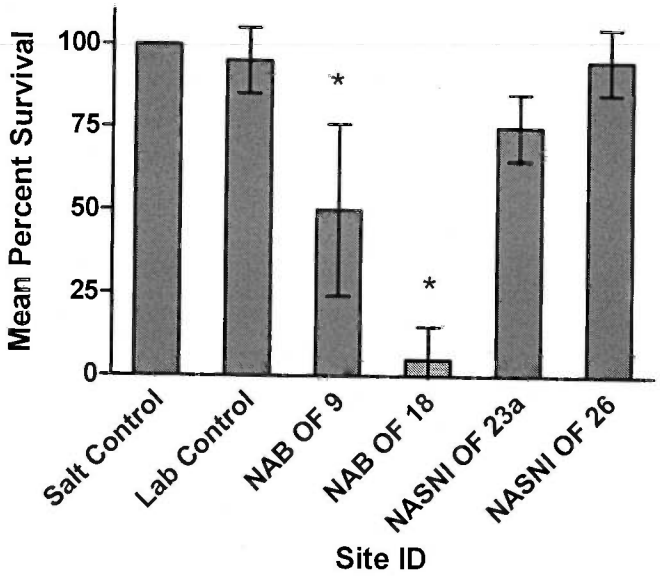


Figure 2. Stormwater Toxicity Screening Test Results for Mysid Shrimp Survival (100 percent sample). Mean values are presented ± 1 standard deviation. Asterisks indicate significant differences relative to the salt control.

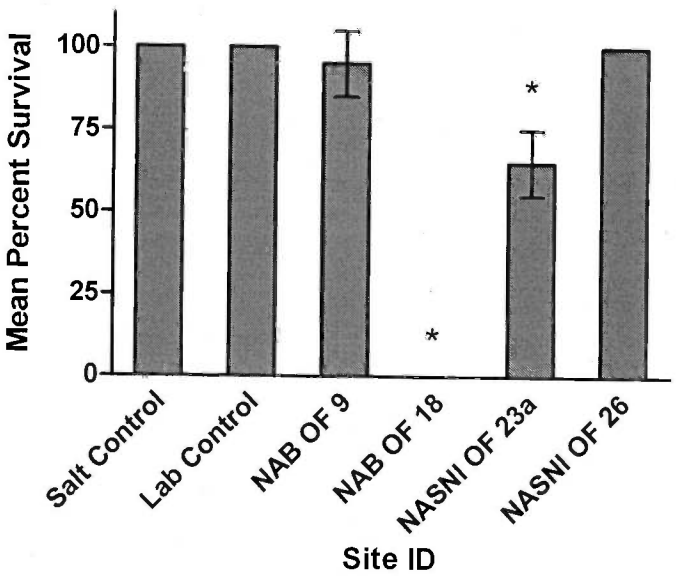


Figure 3. Stormwater Toxicity Screening Test Results for Pacific Topsmelt Survival (100 percent sample). Mean values are presented ± 1 standard deviation. Asterisks indicate significant differences relative to the salt control.

3.1.2 Bay Water Samples

All samples collected from the receiving water of San Diego Bay near each outfall were non-toxic to all three test species. Mean mussel embryo development ranged from 95 to 96 percent and mysid and topsmelt acute survival ranged from 95 to 100 percent among all four samples tested (Figures 4 through 6). Based on salinity, these samples were greater than 50 percent bay water.

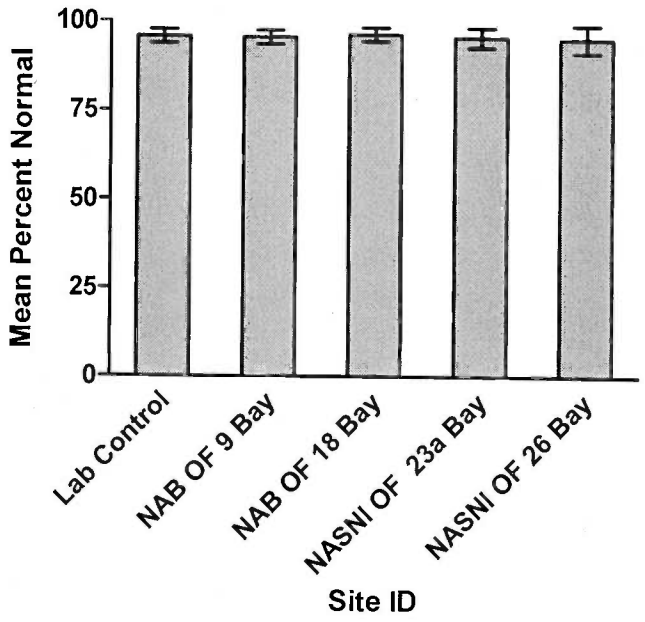


Figure 4. Bay Water Toxicity Screening Test Results for Mussel Embryo Development (100 percent sample). Mean values are presented ± 1 standard deviation.

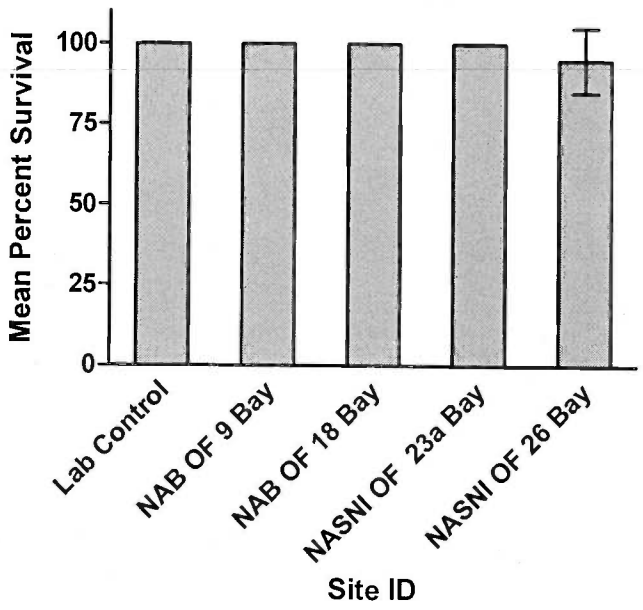


Figure 5. Bay Water Toxicity Screening Test Results for Mysid Shrimp Survival (100 percent sample). Mean values are presented ± 1 standard deviation.

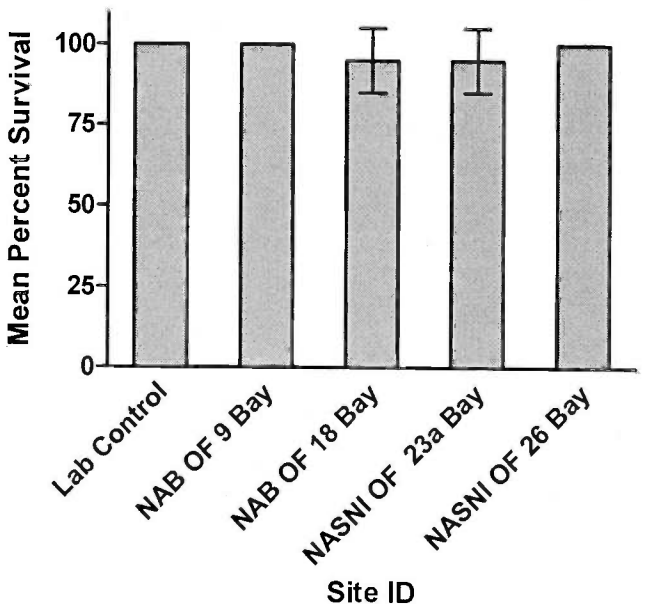


Figure 6. Bay Water Toxicity Screening Test Results for Pacific Topsmelt Survival (100 percent sample). Mean values are presented ± 1 standard deviation.

3.2 Phase I TIEs

Phase I TIEs were initiated on samples that exhibited clear evidence of toxicity during the screening tests (statistically significant and/or at least a 20 percent difference from the control). On this basis, three of the samples tested with both mussels and mysids qualified for a TIE (NAB OF 9, NAB OF 18, and NASNI OF 23a) and two of these samples (NAB OF 18 and NASNI OF 23a) qualified for a TIE using Pacific topsmelt.

3.2.1 Mediterranean Mussel

Baseline Tests

The magnitude of toxicity was similar between the screening tests conducted on March 19, 2005 and Baseline tests conducted five days later with the TIE on March 24, 2005 (Figure 7). There was, however, a slight decrease in toxicity for NASNI OF 23a, with normal development between the two test dates increasing from 24 to 88 percent in the 25 percent dilution. A second round of Baseline tests conducted on April 8, 2005 for NAB OF 18 and NASNI OF 23a remained toxic, with mean normal development of zero and one percent, respectively in a 61 percent dilution (Figure 8). Normal development in Baseline controls ranged from 90 to 98 percent.

Toxicant Characterization

Round One Test Series

Results of the initial Phase I TIE treatments performed on March 24, 2005 are shown in Figure 7 and summarized in Appendix Table A-6. The EDTA treatment essentially eliminated toxicity in NAB OF 9. While EDTA increased the proportion of normal larvae in NAB OF 18 and NASNI OF 23a, it did not completely eliminate toxicity in these samples.

Extraction through a SPE C_{18} column eliminated toxicity in NASNI OF 23a. Aeration also eliminated most of the toxicity observed in this sample. Both aeration and C_{18} treatments removed a portion but not all of the toxicity in NAB OF 18, and no toxicity was removed following these treatments in NAB OF 9.

Based on the effectiveness and specificity of the EDTA treatment, these data suggest that toxicity in sample NAB OF 9 was due largely to divalent cationic metals. Subsequent Phase I testing was, therefore, not performed for this sample.

Mean normal development in the treatment controls ranged from 92 to 98 percent, with the exception of the aeration treatment, which had slightly lower normal development between 84 and 91 percent.

Round Two Test Series

TIE results for Samples NAB OF 18 and NASNI OF 23a were investigated further on April 8, 2005 by performing a combination of characterization treatments shown in Figure 8 and summarized in Appendix Table A-7.

NAB OF 18

Addition of EDTA following both extraction through a C_{18} column and aeration treatments successfully eliminated toxicity in NAB OF 18. These treatments suggest that all observed toxicity is due to a combination of cationic trace metals and an organic that is removed or detoxified by both the C_{18} and aeration treatments. The presence of a toxic organic constituent in NAB OF 18 was verified by testing a methanol elution of the C_{18} column; toxicity was recovered in this elution at a 2X add-back, suggesting relatively good recovery from the column. Foam collected during the aeration process was also toxic when added back to dilution water at a 4X concentration. Based on prior experience, these results, in combination with the degree of foaming observed during the aeration test, are consistent with characteristics exhibited by surfactants. To further investigate this hypothesis, the toxic 2X methanol elution was pulled through an anion exchange column and retested. Toxicity of the methanol extract was eliminated following this procedure indicating that the organic toxicant in the extract is anionic, thus providing further supporting evidence that the organic toxicant of concern is an anionic surfactant.

NASNI OF 23a

Results for NASNI OF 23a were also investigated further by performing a similar combination of characterization treatments as shown in Figure 8. Addition of EDTA following the aeration treatment removed all observed toxicity in this sample. Similar to NAB OF 18, the foam add-back procedure also elicited a strong toxic response. Unlike NAB OF 18, however, the C_{18} methanol elution add-back was not toxic at 2X add-back. Although evaluation of anion toxicity in the C_{18} elution was not possible due to the lack of toxicity in the methanol extract, the results for this sample also suggest that toxicity is due to a surfactant in addition to cationic trace metals. All treatment method controls for this series of tests exceeded 90 percent normal development.

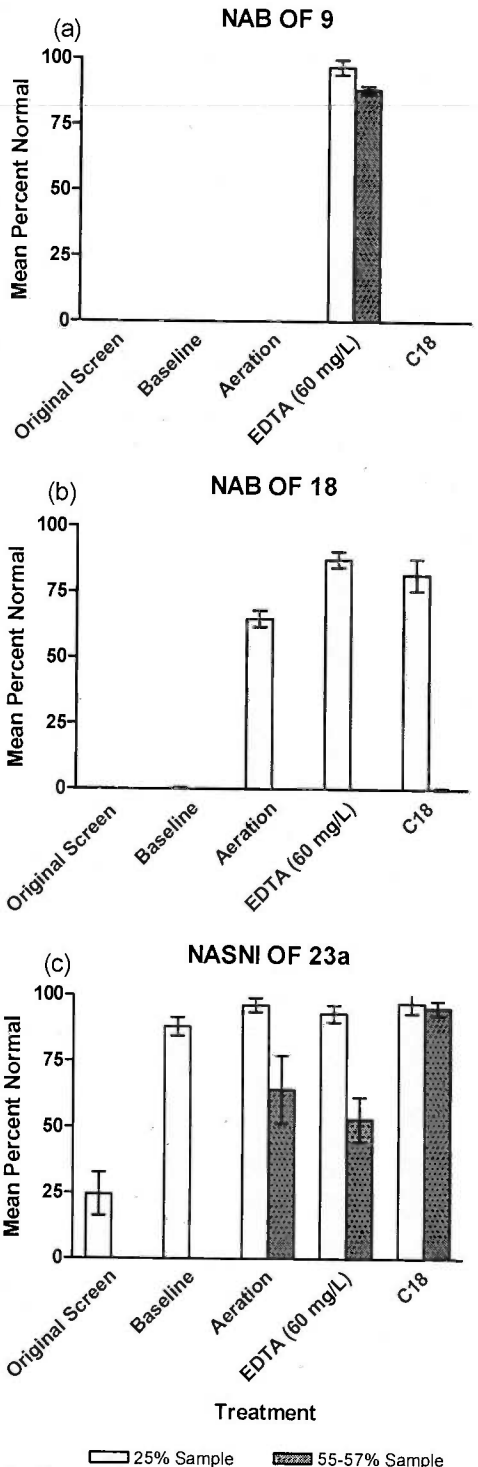


Figure 7. Mussel Phase I, Round 1 TIE results (March 24, 2005). Mean results are presented ± 1 standard deviation for: (a) NAB OF 9; (b) NAB OF 18; and (c) NASNI OF 23a. Mean normal development in the treatment controls ranged from 92 to 98 percent, with the exception of the aeration treatment at 84 to 91 percent.

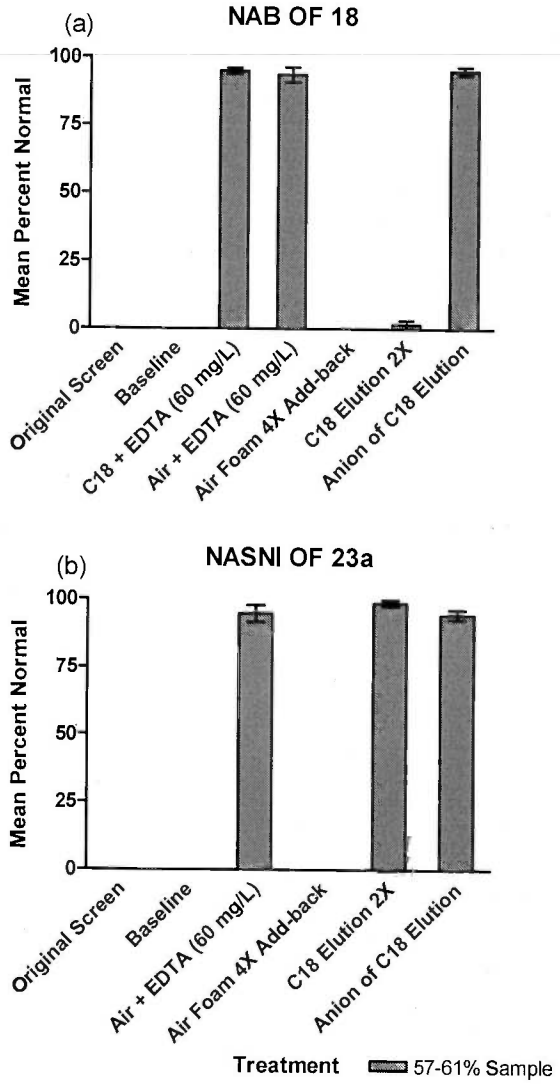


Figure 8. Mussel Phase I, Round 2 TIE results (April 8, 2005). Mean results are presented ± 1 standard deviation for: (a) NAB OF 18; and (b) NASNI OF 23a. Mean normal development in the treatment method controls ranged from 93 to 100 percent.

3.2.2 Mysid Shrimp

Baseline Test

The results of the Baseline tests for NAB OF 9 and NAB OF 18 conducted on March 30, 2005 concurrently with the Phase I TIE manipulations were similar to those obtained in the original screening test initiated eleven days prior on March 19, 2005, suggesting that toxicity did not

dissipate appreciably over this time period (Figure 9). Toxicity of NAB OF 9 actually appeared to increase slightly. Toxicity was no longer present in sample NASNI OF 23a when the first round of TIE treatments were initiated; however, the initial toxic response in the screening test was much less than that observed for NAB OF 9 and NAB OF 18. Toxicity dissipated completely in Sample NAB OF 18 by the time a second round of TIE treatments was initiated on April 21, 2005.

Mean survival of mysids was 100 percent in the Baseline control.

Toxicant Characterization

Round One Test Series

The results of initial Phase I TIE treatments performed on March 30, 2005 are shown in Figure 9 and summarized in Appendix Table A-8.

The EDTA treatment eliminated toxicity in sample NAB OF 9, but had no observable effect on toxicity of NAB OF 18.

Extraction through a SPE C_{18} column eliminated toxicity of NAB OF 18. Aeration also eliminated most of the toxicity observed in this sample. Aeration and C_{18} treatments had no effect on the toxicity of NAB OF 9.

Toxicity completely dissipated in NASNI OF 23a, eliminating any meaningful comparisons between TIE manipulations and the Baseline test for this sample.

Based on the effectiveness and specificity of the EDTA treatment, these data suggest that, like mussels, toxicity to mysids in sample NAB OF 9 was due primarily to divalent cationic metals. Subsequent Phase I testing was, therefore, not performed for this sample.

Mean survival in all method controls ranged from 90 to 100 percent.

Round Two Test Series

Results for NAB OF 18 were investigated further by performing a combination of characterization treatments on April 21, 2005. These data are shown in Figure 10 and summarized in Appendix Table A-9.

Baseline toxicity of this sample completely dissipated by the time this round of tests was

initiated almost 4 weeks post-collection. This loss of toxicity eliminates any meaningful comparisons between TIE manipulations (e.g. aeration + EDTA) and the baseline sample Extraction of methanol through the C_{18} column tested at a 2X add-back concentration, although successful for the mussel, failed to exhibit toxicity to mysids. This observation suggests that the organic toxicant of concern is more toxic to mussels than mysids if reduced toxicity following C_{18} extraction was due to the same compound for both species. Foam collected during the aeration process, however, was toxic when added back to dilution water at a 4X concentration. This treatment provides strong evidence that the primary toxic constituent of concern for mysids in NAB OF18 may also be a surfactant. Mean survival of mysids in all controls ranged from 90 to 100 percent during this series of tests.

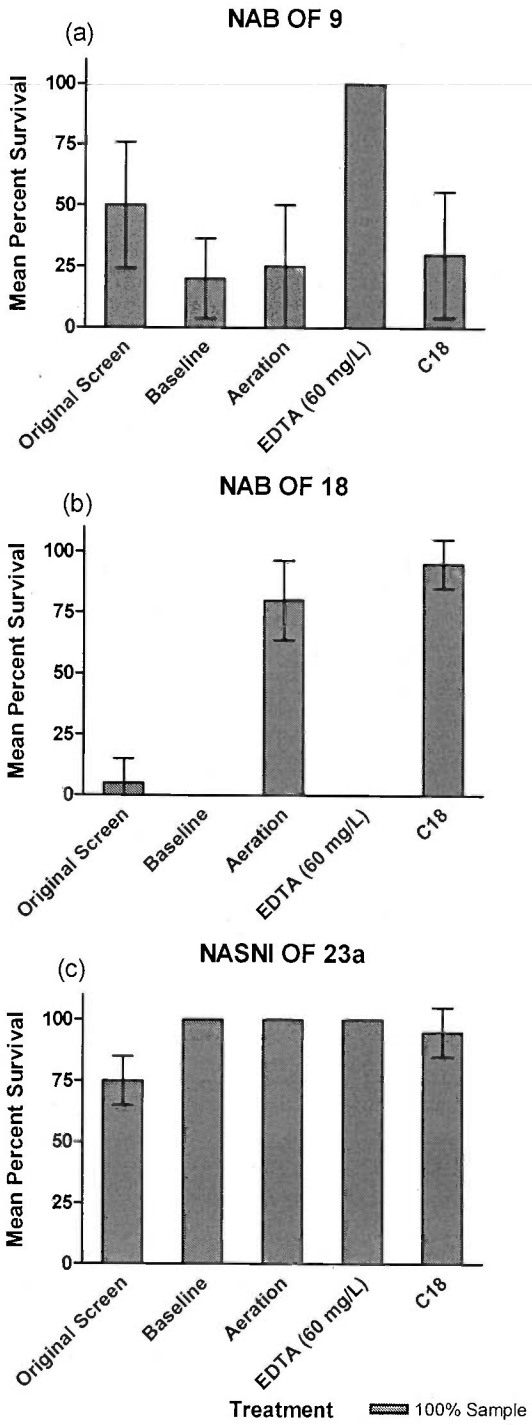


Figure 9. Mysid shrimp Phase I, Round 1 TIE results (March 30, 2005). Mean results are presented ± 1 standard deviation for: (a) NAB OF 9; (b) NAB OF 18; and (c) NASNI OF 23a. Mean survival in all controls ranged from 90 to 100 percent.

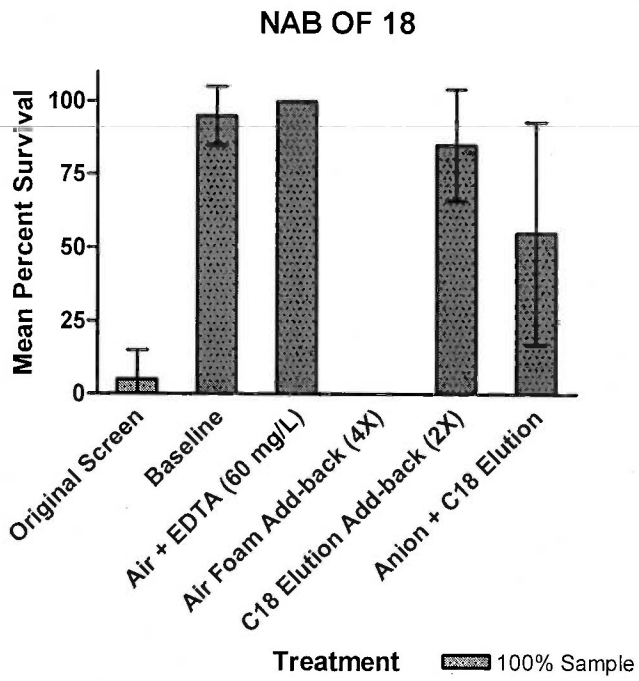


Figure 10. Mysid shrimp Phase I, Round 2 TIE results (April 21, 2005). Mean results are presented ± 1 standard deviation for NAB OF 18. Mean survival in all controls ranged from 90 to 100 percent.

3.2.2 Pacific Topsmelt

Baseline Test

Results of the Baseline test for NAB OF 18 conducted on March 30, 2005 concurrent to Phase I TIE manipulations were similar to those obtained in the original screening test initiated eleven days prior on March 19, 2005, demonstrating that toxicity did not dissipate appreciably over this time period. As with mysids, toxicity was no longer present in sample NASNI OF 23a when the first round of TIE treatments was initiated, however, the initial toxic response in the screening test was much lower than that observed for NAB OF 9 and NAB OF 18.

Mean survival of topsmelt in Baseline control was 100 percent.

Toxicant Characterization

The results of Phase I TIE treatments performed on March 30, 2005 are summarized in Figure 11 and Appendix Table A-10. Because toxicity dissipated completely in NASNI OF 23a, only results for NAB OF 18 are described.

The EDTA treatment had no observable effect on toxicity of NAB OF 18, however, both extraction through a SPE C_{18} column and aeration eliminated toxicity in this sample. These results suggest that surfactants were the primary toxicant of concern to Pacific topsmelt in this sample. Additional TIE testing was not performed using this species due to the similarity of results observed in tests with the mysids and mussels.

Mean survival of topsmelt in all method controls ranged from 90 to 100 percent.

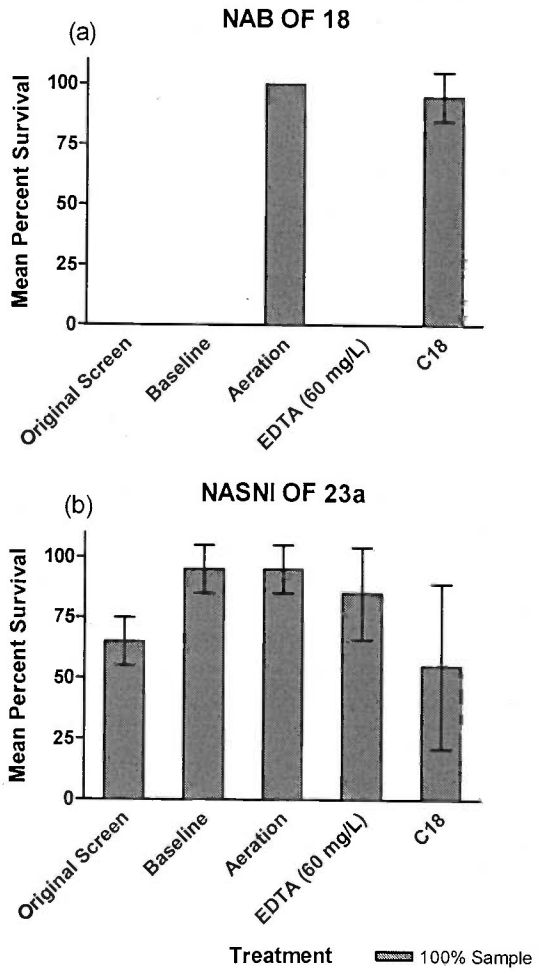


Figure 11. Pacific topsmelt Phase I TIE results (March 30, 2005). Mean results are presented ± 1 standard deviation for: (a) NAB OF 18; and (b) NASNI OF 23a. Mean survival in all controls ranged from 90 to 100 percent.