Quality Assurance Project Plan (QAPP)

for

Environmental Toxicity in St. Lucie Estuary, Florida

[DEP DIRECT CONTRACT #SP-581]

Prepared for

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6.2.3. Proposed Research

See pages 1-4 of the research proposal for DEP ContractSP-581.

6.2.4. Project Goals

See pages 3-4 of the research proposal for DEP ContractSP-581. Presently Florida is experiencing a drought, thus the results from this study may be atypical.

6.2.5. Sample Collection/Sample Preparation Procedures

A stratified -random sampling design similar to those used in previous surveys (Long et al., 1996) will be applied for this study. Strata boundaries are normally established to coincide with the dimensions of major basins, bayous, waterways, etc. in which hydrographic, bathymetric and sedimentological conditions were expected to be relatively homogeneous. This approach combines the strengths of a stratified design with the random-probabilistic selection of sampling locations, allowing the data generated within each stratum to be attributed to the dimensions of that stratum. Therefore, these data can be used to estimate the spatial extent of toxicity with a quantifiable degree of confidence (Heimbuch, et al., 1995).

The sample collection sites and two alternate sites are provide in Tables 6.1 and 6.2 and their respective map locations in Figures 6.1 and 6.2.

Sediment

Toxicity and chemistry samples are collected with a Kynar-coated 0.1m^2 Young-modified van Veen grab sampler deployed from a boat. The grab sampler and sampling utensils are acid washed with 10% HCl and then rinsed with D.I.U.F. water at the beginning of each study, and thoroughly cleaned with acetone and site water before collection of samples at each site. Usually, 3 or 4 deployments of the sampler (minimum of 3) are required to provide a sufficient volume of surficial sediment for the toxicity tests and chemical analyses. The upper 2-3 cm of the sediment are sampled to ensure the collection of recently deposited materials. Sediments are removed with a high-impact styrene, sterile scoop and composited in an acetone rinsed, high-density, polyethylene (HDPE) bucket. Between each deployment of the sampler the bucket is covered with a HDPE lid to minimize sample oxidation and exposure to atmospheric contamination. The material is carefully homogenized in the field with an acetone rinsed, heavy-duty, polyethylene paddle before being distributed to prepared sample containers. Samples are immediately placed on ice. Chemistry, P450 RGS, and AVS samples are frozen as soon as possible.

Samples for toxicity, chemistry analyses, and the benthic community analyses are collected concurrently. One sample for benthic community analyses is collected at each site with a Young-modified, petite (0.413 cm^2) van Veen grab. The entire contents of an acceptable grab (at least 5-cm deep at the center of the grab) are retained and sieved in the field with a 0.5-mm sieve. Materials retained on the sieve are immediately preserved in 10% buffered formalin solution with a Rose Bengal stain. Samples are rejected if the jaws of the grab are open, if the sample is partly washed out, or if the sample is less than 5 cm deep.

Table 6.1. Sampling sites.

Sample	Ranlondd	Ranlatdd	Class
1	-80.32000	27.24077	zone 1
2	-80.29897	27.23751	zone 1
3	-80.30609	27.23463	zone 1
4	-80.28617	27.22734	zone 1
5	-80.28393	27.21729	zone 1
6	-80.28083	27.21358	zone 1
1	-80.26449	27.17834	zone 2
2	-80.26013	27.17771	zone 2
3	-80.25996	27.17262	zone 2
4	-80.25720	27.16572	zone 2
5	-80.25282	27.16268	zone 2
6	-80.25640	27.16338	zone 2
1	-80.25787	27.21159	zone 3
2	-80.25679	27.20889	zone 3
3	-80.26537	27.20608	zone 3
4	-80.25858	27.20197	zone 3
5	-80.27018	27.18796	zone 3
6	-80.26115	27.18665	zone 3
1	-80.23513	27.21603	zone 4
2	-80.21415	27.21153	zone 4
3	-80.23114	27.21502	zone 4
4	-80.21209	27.20741	zone 4
5	-80.23396	27.20720	zone 4
6	-80.25723	27.20317	zone 4
1	-80.20654	27.20478	zone 5
2	-80.21183	27.19990	zone 5
3	-80.20464	27.19712	zone 5
4	-80.20868	27.18760	zone 5
5	-80.20320	27.18344	zone 5
6	-80.20011	27.18400	zone 5
NMFS24	-80.26617	27.19650	NMFS
NMFS22	-80.19133	27.17350	NMFS
NMSF10	-80.16817	27.17167	NMFS
NMSF4	-80.16950	27.16333	NMFS
NMFS30	-80.15433	27.15783	NMFS
NMFS31	-80.14083	27.15633	NMFS
NMFS38	-80.25600	27.20317	NMFS

Table6.2. Alternate sampling sites.

Sample	Ranlondd	Ranlatdd	Class
1_1	-80.32097	27.24835	zone 1
1_2	-80.31671	27.24417	zone 1
2_1	-80.29121	27.23621	zone 1
2_2	-80.29969	27.23686	zone 1
3_1	-80.30405	27.23466	zone 1
3_2	-80.30567	27.23883	zone 1
4_1	-80.28772	27.22741	zone 1
4_2	-80.28456	27.22827	zone 1
5_1	-80.28139	27.21973	zone 1
5_2	-80.28299	27.22228	zone 1
6_1	-80.28022	27.20821	zone 1
6_2	-80.27172	27.20910	zone 1
1_1	-80.26094	27.18009	zone 2
1_2	-80.26500	27.17792	zone 2
2_1	-80.26008	27.17386	zone 2
2_2	-80.26008	27.17551	zone 2
3_1	-80.25783	27.17080	zone 2
3_2	-80.26035	27.17144	zone 2
4_1	-80.25437	27.16879	zone 2
4_2	-80.25745	27.16818	zone 2
5_1	-80.25286	27.16154	zone 2
5_2	-80.25255	27.16198	zone 2
6_1	-80.25739	27.15843	zone 2
6_2	-80.25371	27.15969	zone 2
1_1	-80.25558	27.21264	zone 3
1_2	-80.25667	27.21455	zone 3
2_1	-80.25840	27.20375	zone 3
2_2	-80.26144	27.20297	zone 3
3_1	-80.26659	27.20555	zone 3
3_2	-80.26843	27.20397	zone 3
4_1	-80.26168	27.19732	zone 3
4_2	-80.26074	27.20186	zone 3
5_1	-80.26656	27.18997	zone 3
5_2	-80.27074	27.18967	zone 3
6_1	-80.26256	27.18617	zone 3
6_2	-80.26399	27.18707	zone 3
1_1	-80.23478	27.21591	zone 4
1_2	-80.23350	27.21632	zone 4
2_1	-80.21193	27.21310	zone 4
2_2	-80.21647	27.21030	zone 4
3_1	-80.23199	27.20501	zone 4
3_2	-80.23009	27.21107	zone 4
4_1	-80.20973	27.20995	zone 4
4_2	-80.21160	27.20557	zone 4
5_1	-80.24208	27.21045	zone 4
5_2	-80.24485	27.20447	zone 4
6_1	-80.25030	27.20093	zone 4
6_2	-80.25630	27.20250	zone 4
1_1	-80.20950	27.20432	zone 5
1_2	-80.20974	27.20472	zone 5
2_1	-80.20635	27.19886	zone 5
2_2	-80.21198	27.20086	zone 5
3_1	-80.20497	27.19541	zone 5
3_2	-80.20196	27.19497	zone 5
4_1	-80.20852	27.18835	zone 5
4_2	-80.20924	27.18982	zone 5
5_1	-80.20451	27.19039	zone 5
5_2	-80.20294	27.18465	zone 5
6_1	-80.19963	27.18377	zone 5
6_2	-80.19740	27.18485	zone 5

Figure 6.1 here

Figure 6.2 here

On occasion a site that has been randomly generated cannot be sampled. Reasons may include, the site is too shallow or there is no dredging or anchoring allowed in the area. If this occurs the first alternative site should attempted to be sampled. If this site is deemed inaccessible then the second alternative should be attempted, etc. It is important to maintain this procedure for selecting the alternative site and to record the reasons for inaccessibility of the primary and any subsequent alternate sites on the log sheets.

Water

Water samples are collected for the analysis of semi-volatile pesticide and nonylphenol, octylphenol and their respective alkylphenol ethoxylates from a depth of 1-meter using a submersible marine pump. Only the dissolved phase of the sample (defined by the filter pore size) was collected. The particulates trapped by the filter will not be analyzed for reasons of cost. The pump is joined to a length of Teflon tubing which connects directly with two in-line, stainless steel filter holders housing a 1 µm pore size multi-grade GMF glass fiber filter (Whatman #1841070) and a 0.7 µm pore size GF/F filter (Whatman #1825150), respectively. The filtered water passes directly into a labeled and pre-cleaned, 20L, stainless steel canister, which is sealed with an airtight lid. An additional 4L volume of water is filtered into pre-cleaned, amber, glass bottles for nonylphenol, octylphenol and their respective alkylphenol ethoxylates analysis. The sampling equipment is cleaned after each sample by pumping a solution of 7:3 organic free water:methanol through the entire pump, tubing, and filtration system. The used solution is collected in a waste canister for disposal at an approved facility. A field blank is collected each sampling day by pumping 10L of organic free water through the sampling and filtration system into a clean stainless steel canister and into additional 4L amber glass bottles. Several extra canisters of water will be collected from the control site for use as matrix spikes in the laboratory. All samples are accompanied by chain of custody forms which included the date and time of sample collection and the site number (Figure 6.3).

6.2.6. Sample Handling, Identification, Preservation and Transportation

Immediately prior to sampling a station, a sample container for each constituent is redundantly labeled on the lid and the side of the container with a permanent marker. Labeling includes a unique station ID number/code, sample date and project code. At each station a log sheet is filled in at the time of sampling that records local conditions, precise location, actual sample time and a list of all samples collected. All sample container lids are sealed with electrical tape to minimize possible cross contamination while in storage and shipment. Sediment samples for chemical analyses are kept in coolers with ice or in an on-board freezer until they are delivered to an on-shore laboratory where they are stored frozen until shipment. Samples for toxicity bioassays and grain size analyses are stored in coolers with ice until they are delivered to an on-shore laboratory where they are stored in coolers with ice or refrigerated. Benthic community samples are preserved with buffered formalin in the field. Since these samples are preserved, there is no need to refrigerate or pack in ice. However, they are kept in a cool environment and out of direct sunlight.

Samples for toxicity testing and chemistry analyses are shipped in ice chests packed with water ice, dry ice or blue ice to the testing laboratories by overnight courier. Samples for toxicity tests are chilled on ice until extractions or tests are initiated. Sediment samples for chemical analyses are frozen until thawed for analysis. Sediment and water sample handling procedures are summarized in Table 6.3.

At the time of shipment, all samples are organized chronologically by station and sample type (e.g. bioassay, chemical analysis etc.). Any discrepancies between sample containers and log sheets are resolved. Sediment samples are packed into coolers with appropriate packing material and coolant. Benthos samples are packed into plastic-lined boxes with absorbent material. Duplicate chain of custody sheets are compiled for each container enumerating each sample by unique station ID, number of containers per station, and collection date and time. One sheet is retained and one is sent by overnight delivery to the receiving laboratory with the samples. Receiving laboratories are notified of sample shipments at the time they are sent.

The filtered surface water samples in 20L, stainless steel canisters and 4L amber, glass bottles are placed in coolers with dry ice as soon as possible or immediately upon returning to shore. They are then shipped to the testing laboratory by overnight courier usually within 24 hours of their collection. Chain of custody forms are included with each sample as described above (Figure 6.3). Water samples are refrigerated upon arrival at the laboratory and are extracted within 7-days of collection. Sampling handling procedures are summarized in table 6.3.

Table 6.3. Sediment sample handling procedures.

Sample Type	Field Holding Conditions :	Lab Holding Conditions :	Shipping
Benthos	10% buffered formalin/R. Bengal	10% buffered formalin/R.Bengal	end of cruise
Sediment Grain Size (Benthos sample)	cooler filled with ice chips	ice/refrigerate	end of cruise with blue ice
Total Organic Carbon (Benthos sample)	cooler filled with ice chips	freeze	end of cruise with dry ice
AVS	cooler filled with ice chips	freeze	end of cruise with dry ice
Metals/Organics	cooler filled with ice chips	freeze	end of cruise with dry ice
Sediment Grain Size (Chem. Sample)	cooler filled with ice chips	ice/refrigerate	end of cruise with blue ice
Total Organic Carbon (Chem. Sample)	cooler filled with ice chips	freeze	end of cruise with dry ice
P450/Microtox	cooler filled with ice chips	ice/refrigerate	every 4-5days with blue ice
Amphipod	cooler filled with ice chips	ice/refrigerate	every 4-5days with blue ice
Porewater	cooler filled with ice chips	ice/refrigerate	every 4-5days with blue ice
Surface water	air-tight stainless steel canisters	ice/refrigerate	within 24hrs of collection

Figure 6.3 here

6.2.7. Measurement methods

Chemical Analytical Methods

Sediment

Organic Chemicals

Organic chemical analyses are preformed by methods outlined in NOAA Technical Memorandum NOS ORCA 130 (1998). Method detection limits for the two analytic al methods used for organic chemicals (gas chromatography/mass spectrometry and gas chromatography electron capture) are given in table 6.6.

Trace Elements

Trace element analyses are preformed by methods outlined in NOAA Technical Memorandum NOS ORCA 130 (1998). Method detection limits for the three analytical techniques (Cold vapor atomic absorption, flame atomic absorption, graphite furnace atomic absorption) are given in table 6.5.

Water

All glassware in contact with the sample is cleaned with phosphate-free detergent, rinsed with tap water to remove all traces of detergent, rinsed again with distilled water and then finally with high purity acetone prior to baking at 400 ^{O}C for a minimum of 4 hours. All equipment that can not be baked is rinsed with a solution of 70:30 water:methanol prior to use and in between each sample collection. Filters used in the field collection are baked for 4 hours at 450ºC.

Gas chromatograph performance normally is indicated by peak shape and by the variation of the target-compound response factors relative to response factors obtained using a new capillary column and freshly prepared calibration solutions. If peak shape deteriorates or if response factor fail to meet the calibration criteria, either change the injection liner or perform maintenance on the capillary column to bring the gas chromatograph into compliance.

Mass spectrometer performance is checked prior to the analysis of any samples or every 24 hours thereafter during a series of analyses to ensure performance according to the perfluorotributylamine (PFTBA) criteria outlined by the instrument manufacturer. Parameters in the tuning software initially optimize the resolution as masses 69, 131, 264, and 502 in the spectrum of PFTBA.

Prior to the analysis of each sample set and every 24 hours thereafter during a series of analyses, calibration solutions containing all target analytes are measured to ensure that the GC/MS or GC/ECD is in compliance with the established criteria. Initial calibration data acquired are acceptable if the relative standard deviation is less than or equal to 35 percent for response factors calculated across the working concentration range. Calibration-curve fitting routines are used, provided back calculation of the calibration-standard concentration agrees within $\pm$ 20 percent of the expected value.

Qualitative indentation (GC/MS) is based on the expected retention time (RT) of the GC peak of the quantitation ion for the target analyte of interest and needs to be within $\pm$10 second of the expected retention time based on the relative retention time of the target

compound or surrogate. Confirmation of target compounds are based on comparisons of relative integrated abundance values of three significant ions monitored with the relative integrated abundance values obtained from calibration solutions analyzed by the GC/MS.

Analyte concentrations are reported as follows: if the concentration is less than the detection limit (see tables) than the concentration is reported as less than the detection limit; if the concentration is greater than the detection limit, then the concentration is reported as the concentration to three significant figures.

Semi-volatile pesticides

Upon arrival at the lab or within 7-days of collection, two 10L portions of each sample are measured into clean stainless steel canisters for duplicate processing. Duplicate matrix spike samples will be formulated by the addition of all compounds of concern to a 10L portion of water collected from the control site. Field blanks will be processed as site samples. Each sample canister is pressurized with high purity nitrogen, forcing the water sample through a certified solid phase extraction (SPE) cartridge containing 500 mg of hyper cross-linked styrene-divinylbenzene copolymer, ENV+ (Jones Chromatography) extraction resin. After extraction, the ENV+ cartridge is dried with nitrogen and eluted with certified high purity solvents (6 mL of dichloromethane (DCM) followed by 9 mL of 3:1 acetone:acetonitrile). This 15 mL extract is concentrated to a final volume of 0.5 mL under nitrogen and analyzed by two gas chromatograph (GC)-mass spectrometer (MS) instruments to provide the most comprehensive screening of the pesticides (Table 6.6).

First, the sample extracts are analyzed by a Varian 3800 GC coupled to a Saturn 2000 ion trap MS operating in split-less mode with a J&W Scientific DB-17MS (50% methyl-50% phenyl polysiloxane) 30 meter, 0.25 mm i.d., 0.25 µm in film thickness capillary column. The carrier gas is ultra high purity helium at a constant flow rate of 1.0 mL/min controlled by a constant flow pressure program. The GC is operated at an injector port temperature of 260 ^{O}C and an initial oven temperature of 130 ^{O}C. The temperature program for the GC oven is as follows: 130 ^{O}C for 1 min., 5 ^{O}C/min to 280 ^{O}C, then hold for 6 min. at 280 ^{O}C. The GC-MS interface temperature is maintained at 280 ^{O}C and the ion trap temperature is 220 ^{O}C. The ion trap MS operates in selective ion storage (SIS) mode, scanning for ions with masses of 70-450.

The second analysis is conducted on a Hewlett Packard (HP) 5890 GC coupled to a HP 5989A quadrapole MS. The GC is operated in split-less mode with a J&W Scientific DB-5 (95% dimethyl-5% diphenyl polysiloxane) 25 meter, 0.20 mm i.d., 0.33 µm in film thickness capillary column. The carrier gas is ultra high purity helium at a constant flow rate of 1.12 mL/min controlled by a constant flow pressure program. The GC is operated at an injector port temperature of 250 ^{O}C and an initial oven temperature of 130 ^{O}C. The temperature program for the GC oven is as follows: 130 ^{O}C for 1 min., 6 ^{O}C/min to 280 ^{O}C, then hold for 5 min. at 280 ^{O}C. The GC-MS interface temperature is maintained at 280 ^{O}C. The source and quadrapole temperatures for NCI operation are 200 and 100 ^{O}C, respectively. The MS is operated in negative chemical ionization (NCI) mode with selective ion monitoring of the characteristic negative ion fragments of the desired compounds.

A field blank, matrix spike and matrix spike duplicate are analyzed with each batch of water samples. Method detection limits are given in table 6.6.

Nonylphenol, octylphenol and their respective alkylphenol ethoxylates

Extraction of nonylphenol (NP), octylphenol (OP) and their respective alkylphenol ethoxylates (APnEOs) (n = 1 – 3) from water is performed by SPE as follows: SPE cartridges (Isolute ENV+, 500 mg / 6 mL cartridges from IST) are conditioned with 12 mL dichloromethane (DCM), 6 mL acetone and 12 mL organic-free deionized water (solvents are pesticide grade from Burdick & Jackson) in a vacuum manifold; approximately 4L of sample is passed through each cartridge. The cartridges are then dried with nitrogen and eluted with 6 mL of DCM. The extract is concentrated under a gentle nitrogen flow and exchanged to hexane to a final volume of 1 mL.

Pentafluorobenzoylchloride (PFBC) is used to derivatize alykyphenols (APs) and APnEOs before GC-MS analysis. The method used was modified from Wahlberg et al. (1990). 5 µL of pyridine and 10 µL of PFBC is added to the final hexane extracts (1 mL) from the SPE. The extracts are placed in a water bath at 60°C for 15 min. Next, 10 mL of a sodium borate buffer (pH 8.5) are added to the extracts. After mixing and allowing for phase separation, the organic layer is removed. Two more extractions with 2 mL of hexane are performed, and the hexane fractions are pooled and passed through a sodium sulfate column. Finally, extracts are concentrated to 1 mL with a gentle nitrogen flow.

GC-MS: PFBC derivatives of APs and APnEOs are analyzed by a Hewlett Packard 5890 Series II gas chromatograph with a HP 7673 GC/SFC autosampler. The column is a J&W Scientific DB-17MS 30 m x 0.25 mm, 0.25 µm film thickness. Carrier gas is helium at a 1.12 mL/min flow rate. The oven program is 130°C for 4 min, raise to 170°C at 20°C/min, to 250°C at 7°C/min, then to 300°C at 10°C/min and hold for 20 min. The sample injection volume is 2 µL in splitless mode. The injector and interface temperatures are 250 and 300°C respectively. The detector for analysis is a Hewlett Packard 5989A MS mass spectrometer operating in negative chemical ionization (NCI) mode with methane as the ionizing gas (CH_4-NCI). Data Acquisition is achieved in selective ion monitoring (SIM) mode with no less than three ions monitored.

A field blank, matrix spike and matrix spike duplicate are analyzed with each batch of water samples. Method detection limits are given in table 6.7.

Table 6.4. Method detection limits for organic chemicals in sediment.

Method: GC/MS		Method: GC/ECD	
Polycyclic Aromatic Hydrocarbons	ppb (dry wt.)	PCBs & Chlorinated Hydrocarbons	ppb (dry wt.)
Naphthalene	2.2	PCB8/5	0.095
C1-Naphthalenes		PCB18/17	0.071
C2-Naphthalenes		PCB28	0.028
C3-Naphthalenes		PCB44	0.052
C4-Naphthalenes		PCB52	0.113
Biphenyl	0.3	PCB66	0.05
Acenaphthylene	0.3	PCB101/90	0.064
Acenaphthalene	0.5	PCB105	0.06
Fluorene	0.5	PCB118	0.096
C1-Fluorenes		PCB128	0.1
C2-Fluorenes		PCB138 /160	0.042
C3-Fluorenes		PCB153/132	0.073
Phenanthrene	0.8	PCB170/190	0.443
Anthracene	0.5	PCB180	0.03
C1-Phenanthrenes/Anthracenes		PCB187	0.046
C2-Phenanthrenes/Anthracenes		PCB195/208	0.028
C3-Phenanthrenes/Anthracenes		PCB206	0.031
C4-Phenanthrenes/Anthracenes		PCB209	0.037
Dibenzothiophene	0.3		
C1-Dibenzothiophenes		Endosulfan II	
C2-Dibenzothiophenes		Endosulfan I	0.1
C3-Dibenzothiophenes		Endosulfan Sulfate	0.1
Fluoranthene	1	chlorpyrifos	0.1
Pyrene	1.1	Hexachlorobenzene	0.1
C1-Fluoranthenes/Pyrenes		Tetrachlorobenzene 1,2,4,5	0.051
Benzo(a)anthracene	0.2	Tetrachlorobenzene 1,2,3,4	0.331
Chrysene	0.7	Pentachlorobenzene	0.134
C1-Chrysenes		Pentachloroanisole	0.234
C2-Chrysenes		Alpha HCH	0.024
C3-Chrysenes		Beta HCH	0.073
C4-Chrysenes		Gamma HCH (Lindane)	0.088
Benzo(b)fluoranthene	1.3	Delta HCH	0.021
Benzo(k)fluoranthene	0.5	Heptachlor	0.036
Benzo(e)pyrene	0.6	Heptachlor Epoxide	0.077
Benzo(a)pyrene	0.6	Oxychlordane	0.02
Perylene	0.6	Gamma Chlordane	0.019
Indeno(1,2,3-c,d)pyrene	0.3	Alpha Chlordane	0.062
Dibenzo(a,h)anthracene	0.5	Trans-Nonachlor	0.015
Benzo(g,h,i)perylene	1.3	Cis-Nonachlor	0.047
		Aldrin	0.05
1-Methylnaphthalene	1	Dieldrin	0.036
2-Methylnaphthalene	1.7	Endrin	0.04
2,6-Dimethylnaphthalene	2.4	Mirex	0.197
1,6,7-Trimethylnaphthalene	0.4	2,4' DDE	0.032
1-Methylphenanthrene	0.2	4,4' DDE	0.09
		2,4' DDD	0.018
		4,4' DDD	0.032
		2,4' DDT	0.046
		4,4' DDT	0.06

Table 6.5. Method detection limits for trace elements in sediment.

Parameter	ppb (dry wt.)	Analytical Method
Aluminum	440	FAA
Iron	40	FAA
Manganese	5	FAA
Arsenic	0.3	GFAAS
Cadmium	0.008	GFAAS
Chromium	0.1	GFAAS
Copper	0.44	GFAAS
Lead	0.35	GFAAS
Mercury	0.007	CVAA
Nickel	0.7	GFAAS
Selenium	0.2	GFAAS
Silver	0.03	GFAAS
Tin	0.1	GFAAS
Zinc	2.2	FAA

Table 6.6. Method detection limits for semi-volatile pesticides in water.

Compound	ng/L	Compound	ng/L
acephate	25	HCH - beta	25
acetochlor	5	HCH - delta	25
alachlor	25	HCH - gamma	25
aldrin	25	heptachlor	5
ametryn	5	heptachlor epox	5
atrazine	25	malathion	25
azinphos-methyl	20	metalaxyl	25
CEAT	125	methamidaphos	20
chlordane - alpha	5	methoxychlor	125
chlordane - gamma	5	metolachlor	25
chlorothalonil	25	metribuzin	25
chlorpyrifos	5	mirex	5
chlorpyrifos methyl	25	naled	20
CIAT	125	norflurazon	15
cyanazine	5	oxamyl	75
diazinon	25	pendimethalin	25
p,p'-dicofol	79	permethrin-cis	10
dieldrin	25	permethrin-trans	25
endosulfan sulfate	15	phorate	10
endosulfan I	75	pp-DDD	25
endosulfan II	15	pp-DDE	25
ethion	5	pp-DDT	5
ethoprop	10	simazine	75
fenamiphos	76	trans-nonachlor	25
HCH - alpha	125	trifluralin	5

Table 6.7. Method detection limits for nonylphenol, octylphenol and their alkylphenol ethoxylates in water.

Analyte	*ng/L at 1-L Volume*
octylphenol	159
o1eo	3031
o2eo	1144
o3eo	302
o4eo	143
o5eo	17
nonylphenol	65
np1eo	49
np2eo	53
np3eo	393
np4eo	256
np5eo	520

Sediment Toxicity Testing

NOAA requires its contractors and research collaborators to engage in substantial, explicit and documented quality control and quality assurance protocols. This is to ensure that data produced by different laboratories for studies in different estuaries and coastal bays are consistent and comparable. In most instances, the sediment toxicity testing procedures are standardized with specific experimental controls and data reporting procedures. Details of the proposed toxicity testing procedures can be found in he following documents.

- Amphipod bioassay ASTM E-1367, 1992 (Thursby et al., 1997)
- Sea urchin Fertilzation Test: USGS Standard Operating Procedure F10.6
- Microbial Bioluminescence (Microtox) Test: EPA/Puget Sound Estuary Program Testing Protocol
- Cytochrome P450 Test: ASTM E 1853-96
- Acetylchlolinesterase (AChE) Test: Van Dolah, et al., 1997; Key et al., 1998.
- Strand Breakage “Comet assay” : Steinert, et al., 1998 Mutation Research 399: 65-85
- Juvenile clam bioassay: Chung, K.W. 1999; Fulton, et al., 1999.

The following narrative summarizes the QA/QC requirements of NOAA using an example of the amphipod mortality test.

Each sediment sample is logged on a standardized form (not prescribed by NOAA but approved by NOAA) and assigned a sample tracing number at the time of arrival. The sample number is used to track the sample from arrival, through testing, and for disposal. Proper state and federal regulations are followed to insure the safe disposal of all samples. The original form is maintained in a permanent. The information on the login sheet serves as documentation of proper handling within the laboratory, as well as how the sample was held. Arrival and collection dates are recorded. Samples must be

grouped according to their time of collection since testing of each sample must begin within 10 days of collection.

The comparability of data among various test series will be assured with written procedures, e.g., a standard operating procedure, of the laboratory. All data entries are checked for errors in transcription, calculation or computer output by no less than two individuals. All sample logs and data forms will be reviewed to ensure that requirements for sediment holding times, data quality assessments, and equipment maintenance have been met. Data that do not meet those requirements will be reported with an accompanying explanation of the problems that were encountered.

The quality of test animals will be assessed before the beginning of a test and throughout the test. All organisms not collected at the test temperature and salinity will be acclimated to those conditions and then held for an additional 48 hours before testing can begin. During acclimation and holding, the general health of the organisms will be monitored daily. If total mortality exceeds acceptable limits (i.e., 10%), then that batch of animals will not be used for toxicity testing. All toxicity test chambers must be randomized such that testing will be conducted without laboratory personnel being aware of sample identities, i.e., testing will be "blind." Replicates of each treatment will be assigned a code number during testing and will be randomized in each testing sequence.

Proper overlying water quality and other conditions necessary to the survival of the organisms will be maintained and documented. There should be no violation of the controlling environmental parameter listed in the ASTM method.

During each toxicity test, the quality of organisms used will be monitored with positive and negative control treatments. The negative control sediment could be the "home" sediment for the test animals or from another justifiable site. Mean survival of amphipods should be greater than 90% among replicates, and greater than 80% within each replicate test chamber. These tests should be run with each batch of testing with field sediments.

The positive control, or reference toxicant, will be used to document the sensitivity of each batch of test organisms. Quite often this toxicant is sodium dodecyl sulfate (SDS) but other toxicants can be used. The positive control tests will consist of 96-hour water-only exposures. LC50 values are calculated for each test run, and these values should be incorporated into a control chart. The control chart provides an insight into the level of quality control within the entire facility. The results of each positive control test should be within the upper and lower boundaries of the control chart. The boundaries are 2 standard deviations above or below the mean of the last 20 reference toxicant tests at the laboratory.

Criteria for Acceptance: The fundamental criterion for judging the acceptability of sediment toxicity tests is the achievement of 90% survival in the negative control sediment. If mean mortality is greater than 10%, re-testing of samples should be considered. However, the magnitude of deviation below 90% and the degree of variability among the replicates of both the negative control and sample sediments should be considered, and discussion held with the NOAA COTR before deciding whether to re-test.

Significant toxicity is inferred when sample survival is statistically less than that of the negative control (alpha = 0.05) and mean sample survival in test sediment is equal to or less than 80% of the mean negative control. Analysis of test results with *Ampelisca abdita* shows that the beta-error associated with 20% difference from the control is less than 10% (i.e., the power of the test is greater than 90%).

6.2.8. Quality Control Procedures

Sediment Chemistry

The quality of the chemistry data generated by the National Status and Trends Program is overseen by a performance based quality assurance program (Cantillo and Lauenstein, 1993; Cantillo and Lauenstein, 1995). All NS&T cooperating laboratories are required to participate. Brief and general descriptions of the procedures are out lined below.

Metals

Quality control samples were processed in a manner identical to actual samples. A method blank was run with every 20 samples, or with every sample set, whichever was more frequent. If corrected blank concentrations for any component were above three times MDL, the whole sample set was re-extracted and reanalyzed. If insufficient sample was available for re-extraction, the data was reported and appropriately qualified. Matrix spike/matrix spike duplicate (MS/MSD) samples were run with every 20 samples, or with every sample set, whichever was more frequent. The appropriate spiking level was ten times the MDL. Reference materials were extracted with each set of sediment samples and were analyzed when available. The method detection limit was determined following the procedures outlined in CFR 40, part 136 (1999).

Organics

A method blank was run with every 20 samples, or with every sample set, whichever was more frequent. If blank levels for any component were above three times MDL, samples analyzed in that sample set were re-extracted and reanalyzed. If insufficient sample was available for extraction, the data was reported and appropriately qualified. Matrix spike/matrix spike duplicate samples were run with every 20 samples, or with every sample set, whichever was more frequent. Surrogate standards were spiked into every sample and quality control sample.

PCBs & chlorinated pesticides

All samples and quality control samples were spiked with DBOFB, PCB 103 and PCB 198. The surrogate standard solution was spiked into the samples prior to extraction in an attempt to minimize individual sample matrix effects associated with sample preparation and analysis. A matrix spike and a duplicate were analyzed with each sample set or every 20 field samples, whichever was more frequent. The acceptable matrix spike recovery criteria were 50 - 125% recovery for at least 80% of the analytes. Criterion for duplicates was ≤30% relative percent difference (RPD). The method detection limit was determined following the procedures outlined in CFR 40, part 136 (1999). Most target compounds, surrogates and internal standard were resolved from one another and from interfering compounds. When they were not, coelutions were

documented. A standard reference material sample was analyzed per batch of sediment samples or every 20 samples whichever was more frequent.

Water Chemistry

The methods for water chemistry have been used previously to analyze south Florida water samples with good success. Performance of these methods will be measured as follows.

Blanks, Duplicates, and Matrix Spikes.

Preparation and analysis of a method reagent blank for each sample set is prepared by closely matching the field collection and filtration procedure. At the end of each sampling day, 20L of organic free water is pumped out of a stainless steel canister with the submersible field pump. The pump is joined to a length of Teflon tubing which connects directly with two in-line, stainless steel filter holders housing a 1 µm pore size multi-grade GMF glass fiber filter (Whatman #1841070) and a 0.7 µm pore size GF/F filter (Whatman #1825150), respectively. The filtered field blank water passes directly into another labeled and pre-cleaned, 20L, stainless steel canister, which is sealed with an airtight lid and treated as all other field samples. Field blanks for the nonylphenol (NP), octylphenol (OP) and their respective alkylphenol ethoxylates (APnEOs) are collected in pre-cleaned, 4L, amber bottles and treated as all other field samples.

For semi-volatile pesticide analysis, each 20L sample that is collected from each site is split in the laboratory and extracted as two duplicate 10L samples. This procedure allows for sample replicates at a rate equivalent to 100 percent of the samples. Duplicate 4L volumes of water for NP, OP, and ApnEO analysis are collected from randomly selected sites for sample replicates at a rate equivalent to 5 percent of the total samples.

Matrix spikes are prepared by collecting several extra canisters and bottles of water from the control site and fortifying exactly measured portions of this water in the laboratory with all compounds of interest at a level of 3-5 times the expected concentration and a rate equivalent to 5 percent of the total samples collected. Matrix spike samples are processed and analyzed in the exact manner as all other field samples. Water chemistry matrix spike samples are analyzed in duplicate including a field blank with each batch.

Chemical Standards.

Stock standard solutions are prepared from either high purity neat materials, from the U.S. Environmental Protection Agency's Pesticide and Industrial Chemicals Repository, or as certified neats and solutions from commercial vendors (Accu Standard, New Haven, CT and Chem Service, West Chester, PA). Stock and working standards are prepared in class "A" volumetric flasks with measurements of neat materials made with certified analytical balances. Fortification and surrogate standard solutions are prepared similarly. Each stock solution is given a tracking code and this code is recorded in permanent records of the preparation procedures of each standard (calibration, internal, fortification and surrogate), and equipment maintenance, repair and calibration are maintained in laboratory notebooks. Dilutions of stock standard solutions are prepared in class "A" volumetric flasks with aliquots taken by gas tight analytical syringes. These solutions are then used to prepare the matrix spike samples

and instrument calibration standards. A tracking code is assigned to connect each dilution with its stock solution and all pertinent information is recorded in laboratory notebooks.

Method Accuaracy and Precision.
The recovery of surrogate standards will be used to monitor method performance. Commonly used compounds are d_{10}-phenanthrene (50 µL of 74.5 ng/µL solution in acetone), d_{10}-diazinon (50 µL of 20 ng/µL solution in methanol), and/or d_5-atrazine (25 µL of 48 ng/µL solution in acetone). A recovery value of > 85% for all three compounds must be maintained in order to classify an extraction as fully successful.

Analytical instruments are calibrated daily (prior to each analysis sequence, in the middle of the sequence, and at the end of the sequence) with a minimum of 5 calibration standards prepared in extract matrix solution. Normal sequence size is 40 samples. The "A" check standard is reanalyzed every 10^{th} sample within a sample batch. Calibration curves are created electronically and checked for consistency throughout the sequence. An acceptable calibration curve will have a linear slope with a linear correlation factor (r^2) of ≥ 0.985. Further more, the slope value should not vary more than +/- 5% over the course of the sequence. A print out of all calibration curves for all compounds of interest is kept on file with the chromatograms produced from each sequence.

Analytical instruments are maintained at the highest possible performance condition through routine maintenance and necessary repair. In the event of critical repair needs, authorized manufacturer service technicians are employed. Detailed logs of the daily use, number and type of sample extracts, routine maintenance, repairs, tunes, and calibrations are kept and reviewed daily.

Benthic Taxonomy and Sorting

NOAA/CCMA requires its contractors to establish and follow specific procedures and controls to assure data quality and accurate reporting of results. Although, no formalized criteria have been established for taxonomic analysis, NOAA's QA/QC requirements have been derived from currently accepted procedures in benthic ecology and are consistent with procedures used in the US Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP).

Sorting

1. A minimum of 10% of all samples sorted by each technician shall be re-sorted by a different technician to monitor performance and provide feedback necessary to maintain acceptable standards. Re-sorts shall be conducted on a regular basis on batches of 10 samples, and all results shall be documented and recorded in the QA/QC logbook for the laboratory.
2. The QC re-sort procedure is designed to provide effective and continuous monitoring of sorting efficiency. The minimum acceptable sorting efficiency is 90%. Based upon the experience of other programs using similar methods (Holland et al., 1988), however, sorting efficiencies are expected to be greater than 95%.
3. Samples sorted by a particular technician shall be randomly selected for re-sorting from a sample batch.

4. The archived sample residues shall be retrieved and the sample number shall be recorded in the QC logbook.
5. Sorting efficiency (%) shall be calculated using the following formula: [# organisms originally sorted ÷ (# organisms originally sorted + additional # found in resort)] x 100.
6. The results of sample resorts may require that certain actions be taken for specific technicians. If sorting efficiency is greater than 95%, no action shall be required. If sorting efficiency is 90 to 95%, , the technician shall be retained and problem areas identified.
7. Laboratory personnel and supervisors must be particularly sensitive to systematic errors (i.e., consistent failure to represent specific taxonomic groups) that may suggest the need for further training. Sorting efficiencies below 90% require re-sorting all samples in that batch.
8. If sorting efficiency is less than 90%, organisms found in the re-sort shall be added to the original data sheet. If sorting efficiency is 90% or greater, the results shall be recorded in the QC log book; however, the animals shall be kept separate from the original sample.
9. If a sample batch fails to meet the 90% efficiency sorting criteria, all samples within the batch shall be re-sorted. An additional sample from the batch shall be randomly selected and used to check the sorting efficiency of the re-sorted batch.
10. Re-sort results shall be summarized for each technician on a QC re-sort summary sheet.

Species Identification and Enumeration

1. Approximately 10% of the samples from any given project (Delivery Order) shall be randomly selected and re-checked by an independent qualified taxonomist. Re-checks shall be performed in a timely manner so that subsequent processing steps and data entry may proceed. Each taxonomist's findings shall be separately documented in a written report, high-lighting any discrepancies in their findings. The CCMA COTR shall have final say over any discrepancy that cannot be resolved.
2. The vials containing specimens from the randomly selected sample shall be retrieved along with the original species identification sheet and information shall be recorded in the QC logbook.
3. The specimens in each vial shall be re-identified and enumerated using the procedures given in Section 1.3.5.
4. As each taxon is identified and counted, results shall be compared to the original data sheet. Discrepancies shall be double-checked to verify that the final results are correct.
5. Following re-identification, specimens shall be returned to the original vials.
6. When the entire sample has been re-identified, the total number of errors shall be computed. The total number of errors shall be based upon the number of misidentifications and miscounts. Accuracy shall be computed in the following manner: [(Total # of organisms in QC recount – total number of errors) ÷ Total # of organisms in QC recount] x 100
7. Three types of errors are to be included in the total error computation:
   - Counting error (for example, counting 11 *Gemma gemma* as 10);
   - Identification errors (for example, identifying a *Nucula annulata* specimen as *Nucula proxima*, where both are present); and

- Unrecorded taxa error (for example, not identifying *Phoronis spp*. when it is present).

8. The minimum acceptable taxonomic efficiency shall be 90%. If taxonomic efficiency is greater than 95%, no action shall be required.
9. If taxonomic efficiency is 90 to 95%, the taxonomist shall be consulted and problem areas shall be identified. Taxonomists and laboratory supervisors must be particularly sensitive to systematic errors (i.e., repeated errors for specific taxonomic groups) that may suggest the need for further training. Taxonomic efficiency below 90% shall require re-identifying and enumerating all samples in that sample batch.
10. Any species identification changes resulting from QA/QC procedures shall be recorded on the original data sheet; however, the numerical count for each taxonomic group shall not be corrected unless the overall accuracy for the sample is below 90%.
11. Treatment of the results of QA/QC audits are illustrated in the following examples:

    Example 1. Ten *Mulinia lateralis* individuals were recounted as eleven. The sample had a greater than 90% overall efficiency, therefore, the original count of ten *Mulinia* would be recorded.

    Example 2. One individual of the species *Prionospio steenstrupi* was misidentified as *Streblospio benedicti*. On the final data sheet, one *Prionospio steenstrupi* and no *Streblospio benedicti* would be recorded.

    Example 3. Ten *Nucula annulata* and no *Nucula proxima* were originally recorded. During the QA/QC check, one *N. annulata* was found to be *N. proxima.* Providing the overall efficiency was greater than 90%, nine *N. annulata* and one *N. proxima* would be recorded on the final data sheet.

    Example 4. Five *Nucula annulata* and ten *Mulinia lateralis* were originally recorded. During the QA/QC check, one *M. lateralis* was found to be a *N. annulata*. Providing the overall efficiency was greater than 90%, six *N. annulata* and nine *M. lateralis* would be recorded on the final data sheet.

    Example 5. One *Onuphidae spp*. (juvenile) was recorded on original data sheet. During the QA/QC check, this individual was not found. On the final data sheet, one *Onuphidae spp*. (juvenile) would be recorded.

    Example 6. *Terebellidae spp*. (juvenile) was found in the annelid fragment category during the QA/QC check. No *Terebellidae* were previously recorded on the data sheet. On the final data sheet, one *Terebellidae spp*. would be recorded.

12. The results from all QA/QC re-checks of species identification and enumeration shall be recorded in the QA/QC logbook that will become a part of the documentation for CCMA.
13. All corrections to data sheets shall be initialed and dated by the person making the changes.

6.2.9. Data Reduction and Reporting

See pages 8 –1 0 of the proposal to DEP Contract No. SP581.

6.2.10. Resumes

The Project team is listed on pages 10 and 11 of the proposal to DEP Contract No. SP581. Two-page resumes for each team member may be found in the proposal's Appendix.

6.2.11. References

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6.0 Research Quality Assurance Plan

6.1. Qualifying Criteria

The proposed study is a research project, involving a number of tests and new approaches for determining the biological effects associated with contaminants and other sources of environmental degradation in the St. Lucie Estuary. Certain state-of-the-art or innovative approaches to be used in the study are still in the development and verification stage. Further, it should be noted that contractors that are yet to be selected would do some of the proposed testing. All contractors or collaborators in this study will be expected to adhere to the quality assurance and quality control requirements of NOAA's National Status and Trends Program.