

## **Workplan for:**

### **Assessment of Endocrine Disruption in Southern California Coastal Fish: Phases I and II**

#### **Organization proposing the project**

County Sanitation Districts of Los Angeles County (Public Agency)

Project Administrator: Shannon Grund  
562-902-4288 x2843  
[sgrund@lacsdc.org](mailto:sgrund@lacsdc.org)

Technical Supervisor: Joseph Gully  
562-908-4288 x2818  
[jgully@lacsdc.org](mailto:jgully@lacsdc.org)

#### **Third party completing the project**

Southern California Coastal Water Research Project (SCCWRP)

Project Manager: Steven Bay  
714-755-3204  
[steveb@sccwrp.org](mailto:steveb@sccwrp.org)

#### **Names and qualifications of key project team members**

Steven Bay, Principal Scientist, Toxicology Department

Doris Vidal-Dorsch, Scientist

Both key project team members have extensive prior experience with the type of work proposed. Including: 1) design, coordination, and analysis of large-scale surveys of fish endocrine disruption, 2) development of gene expression microarrays for fish and invertebrates, and 3) exposure of marine flatfish to contaminants and mixtures such as hormones, trace metals, and wastewater effluent. Brief resumes are attached in Appendix A.

#### **Name and location of project**

Name: Assessment of Endocrine Disruption in Southern California Coastal Fish: Phases I and II

Location: The project will include analysis of samples collected from the nearshore coastal environment at depths of 30-60 meters, offshore of multiple watersheds in Ventura, Los Angeles, Orange, and San Diego counties. Watersheds include Ventura Coastal, Santa Monica Bay, and Los Angeles River. Laboratory experiments will be conducted at SCCWRP's research facilities in Costa Mesa, CA.

#### **Project description**

Marine life inhabiting southern California's coastal waters are exposed to a wide variety of contaminants, both as the result of legacy discharges (e.g., PCB and DDT) and the current use and discharge of a wide variety of contaminants of emerging concern (CECs). CECs include current use pesticides (e.g., pyrethroids), pharmaceuticals and personal care products

(e.g., synthetic estrogen, psychoactive drugs, antibacterials) and industrial/commercial compounds in widespread use (e.g., flame retardants, plastics components). Recent research in southern California has demonstrated that there is widespread exposure of marine life to some CECs and indicators of endocrine disruption, including elevated vitellogenin, and atypical hormone concentrations have been detected in flatfish fish collected from nearshore waters. However, it is not known whether the observed effects are caused by legacy contamination, current discharges of CECs or other contaminants, or other factors entirely. The difficulty in determining the cause of these biological responses is that current tools for monitoring fish condition cannot distinguish between the effects of different chemicals. As a result, studies to identify the cause of biological effects observed in the environment must rely on expensive and time-consuming chemical analyses, which are often inconclusive due to limitations in the methods or the lack of thresholds for interpretation.

Rapid advances in biotechnology have resulted in new tools that measure changes in gene expression, using microarray technology to investigate the response of organisms to environmental stressors. These gene microarrays can potentially provide a rapid and comprehensive evaluation of an organism's response to contaminants, through measuring changes in the activity of a large number of genes associated with multiple important physiological processes, such as hormone regulation, reproduction, immune response, and contaminant detoxication. Previous research, supported in part by this SEP program, successfully developed and applied a preliminary gene microarray for use with marine fish which demonstrated that this technology has promise as a monitoring tool to help detect and investigate the impacts of contaminants on local fish.

The two-phase project described in this workplan will develop a gene expression monitoring tool for flatfish and begin to establish the technical foundation needed to apply this tool in monitoring studies. The first phase of the project will develop an improved flatfish gene microarray that is specific for the hornyhead turbot, a widespread species used for environmental monitoring and research. This microarray will provide more detailed and more reliable gene expression information, as compared to currently available systems. The second phase of the project will use this new microarray to establish a library of gene response patterns, with regard to endocrine disruption, for: contaminants such as estradiol (hormone); legacy pollutants (e.g., DDT and PCBs); and complex mixtures (e.g., wastewater effluent, receiving water). Results from this study are expected to provide several products of direct relevance to priority issues for water quality management, specifically: 1) a new tool for evaluation of endocrine disruption and other aspects of fish health; 2) improved knowledge of the influence of legacy contamination on potential endocrine disruption; and 3) identification of biological indicators that can be used to detect exposure of fish to wastewater effluent in receiving waters. Both phases of the project will be fully funded by this SEP, along with a final report and communication of the results to stakeholders.

#### **Relationship to SEP categories:**

- **Pollution prevention.** Tools do not currently exist to enable water quality management agencies to assess the impacts of numerous unmonitored contaminants of emerging concern (CECs) on fish health, and differentiate these impacts from those of legacy contaminants that are already subject to regulation. This project will develop and

investigate a tool (i.e., gene expression microarray) with the potential to provide information on fish responses to CECs. Successful development of this tool will provide a means to evaluate whether CECs found in municipal wastewater discharges are associated with biological impacts on resident fish, and help identify the specific types of CECs responsible. This information will help enable regulatory agencies to assess the potential for environmental harm due to specific CECs, and thus prioritize these compounds with regard to the need for future management actions to reduce pollution loads.

- **Environmental restoration.** This project does not include environmental restoration activities. However, the methods developed as a result of this project may provide a sensitive means to monitor restoration effectiveness. Measurements of gene expression provide detailed and sensitive information regarding the health status of fish. Successful development of the microarray tool described in this proposal will provide a means for assessing changes in fish condition as a result of future pollution prevention or restoration actions. For example, this tool might be used to help measure the effects of remediation of legacy contamination on the Palos Verdes Shelf on fish hormone systems.
- **Environmental auditing.** Similar to its value for assessing the impacts of environmental restoration activities, a fish gene expression microarray will provide a method for monitoring the effectiveness of environmental management activities and regulations with respect to protecting fish health. A biological method for measuring attainment of protection goals is needed, because chemical water quality criteria do not exist for many contaminants of concern, especially when present as complex mixtures in discharges or sediments.
- **Development of education materials.** The results of this project will be reported in several formats accessible to local stakeholders. The final project report will be posted for public review and download on SCCWRP's web site ([www.sccwrp.org](http://www.sccwrp.org)), selected results will be published in scientific journals and SCCWRP's Annual report, and presentation of the results will be given at local conferences/workshops. In addition, verbal summaries of the results will be provided to managers of key water quality management agencies that serve on SCCWRP's Commission (including EPA region 9, State Water Resources Control Board, Regional Water Quality Control Board, Ocean Protection Council, and County Watershed Protection Agencies).

#### **Project benefits to water quality**

No water quality changes are expected to occur as a direct result of the project activities. However, this project will develop tools needed to detect water quality impacts from contaminants of emerging concern and to identify those contaminants of greatest concern with respect to biological effects. When fully developed and applied in future programs, these tools will assist in the maintenance of water quality (by providing more informative monitoring data) and improvement of water quality (through more effective prioritization of contaminants of concern).

#### **Project benefits to public**

This project will improve the ability of water quality management agencies to investigate and determine the cause of pollution impacts on fish. Application of these tools in future programs are expected to benefit the public through helping to preserve important beneficial

uses of coastal waters related to marine ecosystem protection and fishing. These benefits will be realized through an increased ability of management agencies to identify contaminant threats to coastal water quality before severe impacts have occurred, and thus develop and implement appropriate responses in a timely fashion.

### **Support for project**

The following agencies and/or individuals have expressed support for this workplan:

1. Santa Ana Regional Water Quality Control Board (Wanda M. Cross, Chief, Coastal Waters Planning Section)
2. California Ocean Science Trust (Skyli McAfee, Executive Director)
3. California Coastal Commission (Dr. Jack Gregg, Water Quality Program Supervisor)
4. City of San Diego (Timothy D. Stebbins, Ph.D., Ocean Monitoring Program Director)
5. City of Los Angeles (Mas Dojiri, Ph.D., Environmental Monitoring Division Manager)

Copies of these support documents are provided in Appendix B.

### **Quality Assurance Project Plan (QAPP)**

A QAPP has been developed previously for this SEP project (Appendix C). The current work will adhere to relevant portions of the QAPP.

### **Description of work**

Work on this project is organized into four tasks: 1) Project Management; 2) Microarray Development; 3) Gene Response Analysis; and 4) Reporting.

**Project Management:** The objective of this task is to coordinate and monitor progress on other tasks, manage grant funds and subcontracts, and provide written updates on progress to the Project Administrator. Project updates will be in the form of quarterly progress reports to the Project Administrator that summarize activities for each task, describe proposed work for the following quarter, and provide an accounting of funds expended.

Work Products: Quarterly progress reports.

**Microarray Development:** A gene expression microarray for the hornyhead turbot (*Pleuronichthys verticalis*) will be developed. Liver RNA will be sequenced using a high-throughput direct sequencing method. After mRNA is purified from the sample, it is fragmented, reverse-transcribed into cDNA duplexes, amplified via PCR and sequenced. Each sequencing run is expected to provide information on approximately 5 million different fragments, representing about 25,000 unique transcripts. Liver samples from multiple fish exposed to a variety of different stressors will be combined for sequencing, in order to obtain a diverse set of RNA. Several sequencing runs may be needed to provide sufficient coverage for assembling the hornyhead turbot transcriptome. The fragment sequences will be matched and assembled to longer contiguous sequences (transcripts). The transcripts will be annotated by comparing them to databases of identified proteins, in order to identify the likely gene represented by each sequence. Not all transcripts will be successfully annotated (i.e., gene identified) due to limited genomic information specific to the hornyhead turbot,

however this should not preclude use of the transcript for the microarray. The Agilent eArray system will be used to select sequences with a length of 50-70 bases (oligos) for each transcript. These oligos will be used to create the probes for hornyhead turbot microarray that represents approximately 15,000 genes.

Work Products: Data file containing annotation results and a microarray profile ready for production.

**Gene Response Analysis:** This task will apply the microarray developed in Task 2 to describe gene response patterns in fish exposed to several types of chemical stressors. This task will use liver samples of hornyhead turbot obtained from both field and laboratory exposure studies conducted at SCCWRP. Gene responses to three types of stressors will be analyzed in this project: legacy contaminants; model endocrine disruptors; and complex mixtures. To investigate gene responses to legacy contaminants, hornyhead turbot will be exposed to varying concentrations of one or more legacy contaminants (e.g. DDTs, PCBs, etc.) that are representative of environmental exposure conditions in southern California. Liver samples will be collected at two time points (e.g. 4 days and 1 week) to investigate the interactions of time and exposure level on gene response patterns. The RNA of replicate samples of animals from each exposure treatment will be extracted and applied to the gene microarray. The microarrays will then be washed and scanned with a laser-based detection system (e.g. Agilent, Palo Alto, CA). Gene expression in chemical-exposed and control fish will be compared to determine the relative change in gene expression due to the stressor. The gene expression responses to model endocrine disruptors and complex mixtures will be investigated using preserved liver samples obtained from previous studies. These samples are expected to include fish exposed to estradiol and treated municipal wastewater, and also fish from reference and contaminated locations in southern California. Statistical methods will be used to identify differentially expressed genes in each treatment type. Based on previous experience, we estimate that 100-500 genes will be differentially expressed for each contaminant. We will apply a variety of computational analysis tools to identify a set of differentially expressed genes, which uniquely identify exposure to a type of stressor.

Work Products: A gene expression profile for each stressor type.

**Reporting:** A draft report that summarizes the results of the project will be produced. This report will include a description of the microarray development and gene expression analysis methods used in the study, results, and an interpretation of the results. The draft report will be submitted to the project manager and Regional Board for review, and then revised as necessary to address any comments received. In addition, a verbal summary of the study will be presented to technical staff from local water quality management agencies and an article describing the study will be published in the SCCWRP annual report.

Work Products: Draft and final reports.

**Budget**

**Line item budget:  
Assessment of Endocrine Disruption in Southern California Coastal Fish: Phases I and II**

Task #	Description	Personnel Services	Direct Expenses (Travel, Supplies)	Consultant Services	Total Cost
1	Project management	\$11,765	\$100		\$11,865
2	Microarray development	\$14,678	\$10,000	\$18,900	\$43,578
3	Gene response analysis	\$59,348	\$35,000	\$21,000	\$115,348
4	Reporting	\$15,209			\$15,209
	Total Project				\$186,000

Consultant services include RNA sequencing and assembly, microarray probe design, and statistical analysis.

### Performance monitoring

Satisfactory performance of the work will be assessed on the basis of completion of the task products described in the workplan, in accordance with the schedule of completion.

### Schedule of completion

Activities (X) and milestones (M) per calendar quarter are indicated by task. The organization proposing the project is ultimately responsible for meeting the milestones.

Task #	Description	First Year of Project				Second Year of Project			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	Project management	X	X	X	X	X	X	X	X
2	Microarray development	X	X	M1	M2				
3	Gene response analysis			X	X	M3	X	M4	
4	Reporting	X	X	X	X	X	X	X	M5

### Milestones and percent liability excused

- M1: Completion of RNA sequencing (15%)
- M2: Completion of microarray design (30%)
- M3: Completion of laboratory dose experiments (60%)
- M4: Completion of gene expression data analysis (90%)
- M5: Submission of final report (100%)

Appendix A

Resumes For Key Personnel

STEVEN M. BAY, Principal Scientist

Southern California Coast Water Research Project  
3535 Harbor Blvd, Suite 110  
Costa Mesa, CA 92626  
[steveb@sccwrp.org](mailto:steveb@sccwrp.org)  
Phone: 714-755-3204  
Fax: 714-755-3299

**EDUCATION:**

B.S., Marine Biology, California State University, Long Beach, 1976  
M.S. Biology, California State University, Long Beach, 1982

**RESEARCH EXPERTISE:**

The design of research and interpretation of data to understand the relationship between sediment contamination and biological effects is Mr. Bay's primary research focus. As the director of SCCWRP's Toxicology Laboratory, Mr. Bay directs research to develop sediment toxicity test methods having improved sensitivity and ecological relevance. His current research includes projects to develop sediment quality assessment methods, improve Toxicity Identification Evaluation (TIE) methods and evaluate the impacts of contaminants of emerging concern on fish. Mr. Bay also works closely with California environmental management agencies to improve approaches for water and sediment quality assessment. Current activities in this area include a multi-year project to develop the technical foundation for assessing sediment quality in bays and estuaries as part of the sediment quality objectives program for the California Water Resources Control Board.

**PROFESSIONAL EXPERIENCE:**

Principal Scientist, Southern California Coastal Water Research Project. Westminster, CA.  
1980-Present.  
Biologist, US Army Corps of Engineers. Los Angeles, CA. 1980.  
Instructor, Fullerton College. Fullerton, CA. 1978  
Teaching Assistant, California State University, Long Beach. Long Beach, CA. 1977-1979.  
Research Assistant, Reish Marine Studies Inc. Los Alamitos, CA. 1977-1978.  
Research Assistant, University of Southern California. Los Angeles, CA. 1976-1977.

**PROFESSIONAL APPOINTMENTS:**

Chair, Joint Task Group for Echinoderm Fertilization and Development Test section of Standard Methods - American Water Works Association  
Vice Chair, Santa Monica Bay Restoration Commission Technical Advisory Committee  
Outreach Subcommittee Chair, Society of Environmental Toxicology and Chemistry, Education Committee  
Reviewer for Environmental Science and Technology, Environmental Toxicology and Chemistry, Water Environment Research, Marine Environmental Research

## PROFESSIONAL SOCIETIES:

Society for Environmental Toxicology and Chemistry

## SELECTED PUBLICATIONS:

J-W Kwon, K.L. Armbrust, D. Vidal-Dorsch, S.M. Bay. 2009. Determination of 17 $\alpha$ -Ethinylestradiol, Carbamazepine, Diazepam, Simvastatin, and Oxybenzone in Fish Livers. *Journal of AOAC International* 92:1-10

Baker, M.E., B. Ruggeri, J. Sprague, C. Eckhardt, J. Lapira, I. Wick, L. Soverchia, M. Ubaldi, A.M. Polzonetti-Magni, D. Vidal-Dorsch, S. Bay, J.R. Gully, K. Kelley, D. Schlenk, E.C. Breen, R. Šášík, G. Hadiman. 2009. Analysis of Endocrine Disruption in Southern California Coastal Fish using an Aquatic Multi-Species Microarray. *Environmental Health Perspectives*: 117:223-30.

S. Bay, Walter Berry, Peter Chapman, Russell Fairey, Tom Gries, Edward Long, Don MacDonald, S.B. Weisberg. 2007. Evaluating consistency of best professional judgment in the application of a multiple lines of evidence sediment quality triad. *Integrated Environmental Assessment and Management* 3:491-497.

Bay, S. M., T. D. Lorenson, E. Y. Zeng, and K. Tran. 2003. Temporal and spatial distributions of contaminants in sediments of Santa Monica Bay, California. *Marine Environmental Research* 56:255-276.

Greenstein, D., S.M. Bay, A. Jirik, J. Brown, and C. Alexander. 2003. Toxicity assessment of sediment cores from Santa Monica Bay, California. *Marine Environmental Research*, 56:277-297.

Zeng, E.Y., S.M. Bay, D. Greenstein, C. Vista, C. Yu, and K. Ritter. 2003. Toxic effects of polychlorinated biphenyl accumulation in sea urchins exposed to contaminated sediments. *Environmental Toxicology and Chemistry*, 22:1065-1074.

Bay, S.M., D.J. Greenstein, P. Szalay and D.A. Brown. 1990. Exposure of scorpionfish (*Scorpaena guttata*) to cadmium: biochemical effects of chronic exposure. *Aquatic Toxicology* 16:311-320.

## Doris E. Vidal-Dorsch

Southern California Coastal Water Research Project  
3535 Harbor Blvd. Suite 110  
Costa Mesa, CA 92626-1437  
Office Phone: 714-755-3216  
Fax: 714-755-3299  
Email: dorisv@sccwrp.org

### EDUCATION

1993 B.S. Civil Engineering Instituto, Tecnológico del Mar, Mexico  
2001 Ph.D. Integrative Biology, University of California at Berkeley

### PROFESSIONAL EXPERIENCE

2001 to present. Scientist. Southern California Coastal Water Research Project  
1997 to 2001. Graduate Instructor. University of California at Berkeley  
1996 to 2001. Graduate Researcher. University of California at Berkeley  
2001-2001. Consulting Student Internship, Birkenstock USA, Novato, CA.  
1998-1998. Book Translator, University of California at Berkeley, Berkeley CA.  
1995-1996. Research Intern, Municipal Government. Ursulo Galvan, Ver. Mx.  
1991-1993. Student Researcher, ITMAR Instituto Tecnológico de Mar. Veracruz, Ver. Mx.  
1992-1992. Research Assistant, Instituto de Limnología, Chapala UNAM. Chapala, Jal. Mx.  
1990-1991. Research Assistant, SEPESCA Secretaría de Pesca/ITMAR. Veracruz, Ver. Mx.

### SELECTED PUBLICATIONS

Baker M. E., Ruggeri B., Sprague J., Eckhardt C, Lapira J., Wick I., Soverchia L., Ubaldi M.,  
**Vidal-Dorsch D. E.**, Bay S. M., Gully J. R., Kelley K, Schlenk D., Šášik R. and G. Hardiman.  
2008. Analysis of Endocrine Disruption in Southern California Coastal Fish using an Aquatic  
Multi-Species Microarray. *Environmental Health Perspectives* 117:223-30s.

J. W. Kwon, K. L. Armbrust, K. Xia, **D. E. Vidal-Dorsch**, S. M, Bay. Determination of 17 $\alpha$ -  
ethynylestradiol, carbamazepine, diazepam, simvastatin, and oxybenzone in fish livers. *Journal  
of AOAC International* 92:1-10

**Vidal, D. E.**, and S. M. Bay. 2005. Comparative sediment quality guideline performance for  
predicting sediment toxicity in southern California, USA. *Environmental Toxicology and  
Chemistry*. 24: 3173-3182.

**Vidal, D. E.**, Bay S. M., and D. Schlenk. 2005. Effects of dietary selenomethionine on larval  
rainbow trout (*Oncorhynchus mykiss*). *Archives of Environmental Toxicology and Chemistry*. 49  
pp 71-75.

**Vidal D. E.** and A. J. Horne. 2003. Inheritance of Mercury Tolerance in the Aquatic  
Oligochaete *Tubifex tubifex*. *Environmental Toxicology and Chemistry*. 22: 2130-2135.

Vidal D. E. and A. J. Horne. 2003. Mercury Toxicity in the Aquatic Oligochaete *Sparganophilus pearsei* II: Autotomy as a Novel Form of Protection. *Archives Environmental Toxicology and Chemistry*. 45(4) pp 462-467.

### **SYNERGISTIC ACTIVITIES**

*Session Chair*, California and the World Ocean 2006, September 17-20, 2006, Long Beach, CA.

Annual Meeting of SETAC, 2005, November 13-17, 2004, Baltimore, MD.

Annual Meeting of SETAC, 2008, November 16-20, 2008, Tampa, FL.

*Short Courses*: "Environmental Fate and Effects of Emerging Contaminants" Annual Meeting of SETAC, 2008, November 16-20, 2008, Tampa, FL.

"Oligochaetes and toxicity bioassays". Short course *chair*. 9th symposium on aquatic oligochaeta. October 6-10 2003, Wageningen, the Netherlands.

*Society Appointments*: Latin America GU Coordinator- Society of Environmental Toxicology and Chemistry. North America Membership Committee- SETAC

*Grant Reviewing*: National Science Board 2020 Vision for the National Science Foundation (NSB-05-142). Georgian National Science Foundation (GNSF), 2006 & 2007.

*Database Development*: "California Sediment Quality Objectives Database" developed as part of the State Water Resources Control Board's effort to develop sediment quality objectives (SQOs) for enclosed bays and estuaries.

*Participation of Groups Underrepresented in Science*: Sigma Xi Ambassadorship for the Packard Initiative in Mexico.

Appendix B

Documents of Support

1. Santa Ana Regional Water Quality Control Board (e-mail from Wanda Cross, Chief, Coastal Waters Planning Section)

Hi Steve,

Unfortunately, I do not have time to write a letter of support for the project. However, I would note that Santa Ana Regional Board staff has fully supported the need to develop this kind of biological gene expression tool that will work effectively in the field, to monitor and assess water quality changes or impairments. This support was demonstrated through Santa Ana Regional Board approval to provide funding through a Supplemental Environmental Project (SEP) to SCCWRP.

The Santa Ana RB SEP helped to evaluate along the Southern CA Bight the utility of a valid gene microarray. The two-phased project described in your current workplan will help in developing such a tool using flatfish, and begin to establish the technical foundation needed to apply this tool in monitoring studies. This proposed project would be the logical next steps to developing an improved flatfish gene microarray and establishing a library of gene response patterns for specific contaminants responsive to this level of tool.

If this project is funded and completed, we see it as a valuable tool to assess our waterbodies with a potentially more accurate and representative methodology. Please feel free to forward my email to LA Regional Water Board staff. They or you can give me a call too.

Sincerely,  
Wanda

Wanda M. Cross  
Chief, Coastal Waters Planning Section  
Santa Ana Regional Water Quality Control Board  
3737 Main Street, Suite 500  
Riverside, CA 92501-3348  
(951)782-4468  
fax: (951)781-6288  
E-mail: [wcross@waterboards.ca.gov](mailto:wcross@waterboards.ca.gov)  
Website: [www.waterboards.ca.gov/santaana](http://www.waterboards.ca.gov/santaana)

2. California Ocean Science Trust (e-mail from Skyli McAfee, Executive Director)

-----Original Message-----

**From:** Skyli McAfee [mailto:skyli.mcafee@calost.org]

**Sent:** Sunday, August 29, 2010 9:38 AM

**To:** Gully, Joe

**Subject:** Re: [ctag] Support for Endocrine Disruptor Microarray (SEP)

Dear Joe,

I am writing with my strong endorsement of the proposed Supplemental Environmental Project, entitled, "Assessment of Endocrine Disruption in Southern California Coastal Fish: Phase I and II," proposed by the Sanitation Districts of Los Angeles County.

As you're aware, thousands of compounds of emerging concern are present in California waterways, and we have little understanding of their effects on aquatic organisms and ecosystems. Sub-lethal effects, including disruption of endocrine systems and concomitant behavioral changes in fishes, for example, may prove to have profound detrimental consequences that are yet poorly understood. The first step toward gaining understanding of these effects is the development of tools that can be used by resource managers to identify which of numerous compounds is associated with the impact. The proposal uses state of the art techniques and provides an opportunity for significant - much needed- advancement in our ability to distinguish the offending compounds, and thus is an important step forward in effective water quality management.

The proposal is exciting and well prepared, and I look forward to hearing the results.

Best,

Skyli

3. California Coastal Commission (e-mail from Dr. Jack Gregg, Water Quality Program Supervisor)

-----Original Message-----

**From:** Jack Gregg [mailto:jgregg@coastal.ca.gov]

**Sent:** Tuesday, August 31, 2010 3:32 PM

**To:** steveb@sccwrp.org

**Subject:** Support for Assessment of Endocrine Disruption proposal

Dr. Bay - I would like to add my voice of support for this proposal. The Coastal Commission and its staff are dedicated to protecting California's unique coastal resources from the impacts of development, including polluted runoff and sediments. As we begin to address "contaminants of emerging concern", one of the biggest obstacles is measuring

the impacts of these chemicals in the environment. The development of the flatfish gene expression tool and other aspects of this proposal will take recent advances in biotechnology and apply them towards getting data that coastal managers will be able to use in making decisions or making recommendations to decision-making bodies like the Coastal Commission. Good luck with this proposal.

Jack H. Gregg, Ph.D., R.G.  
Water Quality Program Supervisor  
California Coastal Commission  
45 Fremont Street, Suite 2000  
San Francisco, California 94105  
(415) 904-5246

4. City of San Diego (e-mail from Dr. Tim Stebbins, Ocean Monitoring Program Director)

-----Original Message-----

From: Stebbins, Tim [mailto:TStebbins@sandiego.gov]  
Sent: Monday, September 06, 2010 10:27 AM  
To: Gully, Joe  
Subject: RE: Support for Endocrine Disruptor Microarray (SEP)  
Importance: High

Hi Joe,

My apologies for not responding immediately before your email could slide below my field of vision. Anyway, the City of San Diego (Public Utilities Department) enthusiastically supports this project. We also look forward to continued collaborations with LACSD, SCCWRP and other agencies on future projects related to EDCs (or CECs or TORCs).

Please let me know if you need additional information.

Best regards,

Tim

Timothy D. Stebbins, Ph.D.  
Senior Marine Biologist  
Ocean Monitoring Program Director  
Environmental Monitoring & Technical Services Division Laboratory  
2392 Kincaid Road, San Diego, CA 92101 USA  
Voice: 619-758-2329  
Email: tstebbins@sandiego.gov

5. City of Los Angeles (e-mail from Dr. Mas Dojiri, Environmental Monitoring Division Manager)

CITY OF LOS ANGELES  
CALIFORNIA



ANTONIO R. VILLARAIGOSA  
MAYOR

BOARD OF  
PUBLIC WORKS  
COMMISSIONERS  
CYNTHIA M. RUIZ  
PRESIDENT  
ANDREA A. ALARCÓN  
VICE PRESIDENT  
PAULA A. DANIELS  
PRESIDENT PRO-TEMPORE  
STEVEN T. NUTTER  
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TRACI J. MINAMIDE  
CHIEF OPERATING OFFICER  
VAROUJ S. ABKIAN  
ADEL H. HAGEKHALIL  
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ASSISTANT DIRECTORS

ENVIRONMENTAL MONITORING DIVISION  
12000 VISTA DEL MAR, SUITE 504  
PLAYA DEL REY, CA 90295  
TEL: (310) 848-5610  
FAX: (310) 848-5731

August 30, 2010

Joseph R. Gully  
Supervising Environmental Scientist  
Ocean Monitoring and Research  
Los Angeles County Sanitation Districts  
1955 Workman Mill Rd.  
Whittier, CA 90601

**Assessment of Endocrine Disruption in Southern California Coastal Fish: Phases I and II**

Dear Mr. Gully:

The Environmental Monitoring Division (EMD) of the City of Los Angeles Bureau of Sanitation is pleased to support the proposed research project, "Assessment of Endocrine Disruption in Southern California Coastal Fish: Phases I and II". Recent research in southern California has demonstrated endocrine disruption, including elevated vitellogenin and atypical hormone concentrations, in some species of flatfish collected from nearshore waters. There is need to identify the causes, most likely environmental stressors, of these observed endocrine disruptions. This project potentially is very important because it investigates using microarray technology to investigate the response of organisms to environmental stressors. Microarray technology has the potential to provide a rapid and comprehensive evaluation of an organism's response to contaminants. If successful, it will provide a valuable new tool for investigating environmental stressors.

Sincerely,

Mas Dojiri, PhD  
Division Manager

MAD:GEM

c: Gerry McGowen

INCORRESPONDENCE\Letters of Support\LACSD CEC Microarray Project, 100830.doc

DOC #

SEP 03 2010 AM 08:14

1671635

Gully J

AN EQUAL EMPLOYMENT OPPORTUNITY — AFFIRMATIVE ACTION EMPLOYER

Recycle and make toner/ink and wash

Appendix C

Quality Assurance Project Plan

**ASSESSMENT OF ENDOCRINE DISRUPTION IN SOUTHERN CALIFORNIA  
COASTAL FISH**

**Quality Assurance Project Plan**

**Southern California Coastal Water Research Project**

7171 Fenwick Lane  
Westminster, CA 92683

November 5, 2004

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Quality Assurance Project Plan  
Southern California Coastal Water Research Project

PROJECT: **ASSESSMENT OF ENDOCRINE DISRUPTION IN SOUTHERN CALIFORNIA COASTAL FISH**

PREPARED BY: Southern California Coastal Water Research Project  
7171 Fenwick Lane  
Westminster, CA 92683

1. **APPROVED BY:**

\_\_\_\_\_  
Regional Board Staff  
Los Angeles Regional Water Quality Control Board

\_\_\_\_\_  
Date

\_\_\_\_\_  
Steven Bay, Project Manager  
Southern California Coastal Water Research Project

\_\_\_\_\_  
Date

\_\_\_\_\_  
Darrin Greenstein, QA Officer  
Southern California Coastal Water Research Project

\_\_\_\_\_  
Date

\_\_\_\_\_  
Rich Gossett, QA Officer  
CRG Marine Labs

\_\_\_\_\_  
Date

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Kevin Kelley  
California State University at Long Beach

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Date

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Dan Schlenk  
University of California at Riverside

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Date

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Michael Baker  
University of California at San Diego

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Date

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### 3. DISTRIBUTION LIST

The final QAPP will be kept on file at SCCWRP. The following individuals will receive copies of the approved QAPP and any subsequent revisions:

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Control Board  
320 W. 4<sup>th</sup> Street Suite 200  
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310/533-5190

## 4. PROJECT/ TASK ORGANIZATION

### 4.1 Involved Parties and Roles.

SCCWRP is a joint powers agency that was formed by several government agencies with the mission to gather the necessary scientific information to effectively, and cost-efficiently, protect the Southern California aquatic environment. As the lead agency in this project, SCCWRP will organize the field sampling, sample processing, analysis of samples and data, and all report preparation.

The primary goal of the Quality Assurance (QA) plan is to ensure that the data generated by field and laboratory personnel meet standards for published data in the peer-reviewed literature. Field and lab personnel will follow standard operating procedures (SOP) for sampling and laboratory analysis.

Doris Vidal and Steve Bay will be the SCCWRP coordinators for this study and have established a project team for planning and conducting the study (Figure 1). Doris Vidal will manage the day-to-day activities of the project, which includes communicating with the others agencies involved in the project, coordinating activities, assessing results and preparing the data report.

Kevin Kelley at the California State University at Long Beach will develop and perform the molecular analyses of the plasma samples (except vitellogenin). Daniel Schlenk at the University of California at Riverside will develop and perform the vitellogenin analyses of the plasma samples. Michael Baker at the University of California at San Diego will develop and analyze the microarray analyses. CRG Marine Laboratories, located in Torrance, will perform the chemical analyses of the liver samples.

**Table 1.** (Element 4) Personnel responsibilities.

Name	Organizational Affiliation	Title	Contact Information (Telephone number, fax number, email address)
Steve Bay	SCCWRP	Project Manager	Tel: (714) 372-9204 Fax: (714) 894-9699 <a href="mailto:steveb@sccwrp.org">steveb@sccwrp.org</a>
Doris Vidal	SCCWRP	Assistant Project Manager	Tel: (714) 372-9216 Fax: (714) 894-9699 <a href="mailto:dorism@sccwrp.org">dorism@sccwrp.org</a>
Darrin Greenstein	SCCWRP	QA Officer	Tel: (714) 372-9224 Fax: (714) 894-9699 <a href="mailto:darring@sccwrp.org">darring@sccwrp.org</a>
Kevin Kelley	CSULB	Task Manager	Tel: (916) 341-5494 FAX: (916) 341-5296 <a href="mailto:kmkelley@csulb.edu">kmkelley@csulb.edu</a>
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Daniel Schlenk	UCR	Task Manager	Tel: (909) 787-2018 <a href="mailto:daniel.schlenk@ucr.edu">daniel.schlenk@ucr.edu</a>
Richard Gossett	CRG Marine Laboratory	Laboratory Director	Tel (310) 533-5190 <a href="mailto:crqlabs@sbcglobal.net">crqlabs@sbcglobal.net</a>

#### **4.2 Quality Assurance Officer Role**

Darrin Greenstein is SCCWRP's Quality Assurance Officer. Darrin's role is to establish the quality assurance and quality control procedures found in this QAPP as part of the different lab analysis procedures. Darrin will also coordinate the communication of all quality assurance and quality control issues contained in this QAPP to the other institutions.

Darrin will also review and assess all procedures during the life of the study against QAPP requirements. Darrin will report all findings to Doris Vidal, including all requests for corrective action. Darrin may stop all actions if there are significant deviations from required practices or if there is evidence of a systematic failure.

#### **4.3 Persons Responsible for QAPP Update and Maintenance.**

SCCWRP's Project Manager and Quality Assurance Officer may make changes and updates to this QAPP after a review of the evidence for change. Doris Vidal will be responsible for making the changes.

#### 4.4 Organizational Chart and Responsibilities

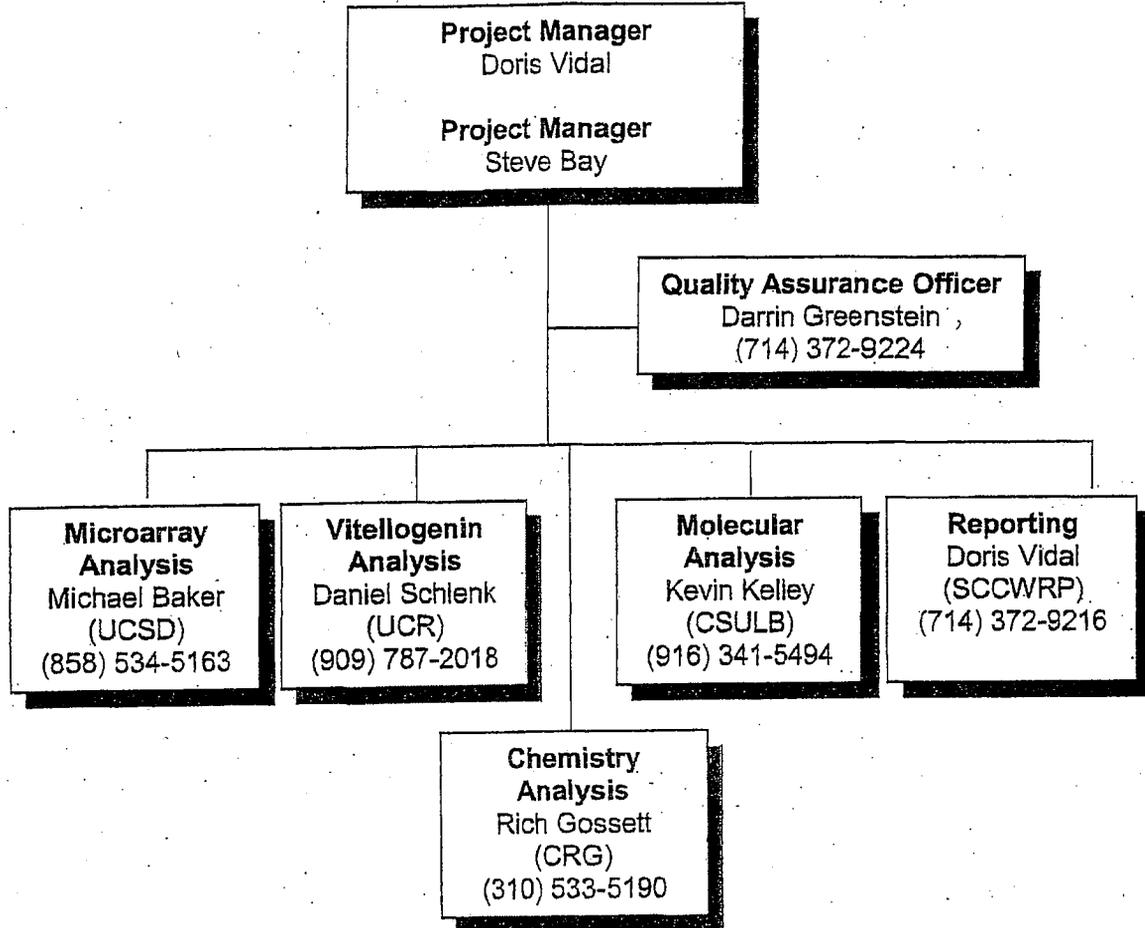


Figure 1: Organization chart

## 5. PROBLEM DEFINITION / BACKGROUND

### 5.1 Problem Statement

Endocrine disruption is currently an area of great concern. A great body of evidence indicates that several classes of environmental contaminants, such as pesticides and surfactants, interfere with normal hormonal activity (endocrine disruption) in fish and other vertebrates. In fish, endocrine disruption can affect reproduction, early development, general metabolism, growth, osmoregulation, responses to stress, and immune functions. These different body systems can be easily affected because the chemical structures of some anthropogenic contaminants are very similar to those of hormones. For example, contaminants such as DDT can mimic estrogen. When DDT enters the body of male fish it can disrupt the reproductive physiology of the fish by triggering the production vitellogenin, an egg yolk protein found in females. The potential of ecosystem impact from endocrine disruptor contaminants is still unknown.

Fish from water bodies receiving municipal wastewater or urban runoff discharges worldwide have been found to show evidence of endocrine disruption. Fish may be exposed to elevated concentrations of endocrine disruptor compounds (EDCs) near municipal wastewater discharges. Some EDCs can exert effects at low concentrations in the environment. Different substances known to be hormone-mimicking compounds are commonly present in southern California water bodies (pesticides, pharmaceuticals, surfactants, and phthalates). Preliminary studies have shown that endocrine disruption is occurring in southern California coastal fish. Despite the knowledge we have of the adverse effects caused by EDCs and the evidence from previous research there is very little known about endocrine disruption in southern California fish. This insufficient information is mainly a result of the lack of readily available tools for measuring endocrine disruption in fish. Without the proper measurement tools, no one can survey fish to assess exposure and determine effects. Without the proper measurement tools, managers cannot monitor fish populations of southern California and determine if endocrine disruption is an environmental hazard.

## **5.2 Decisions or Outcomes**

The goal of this study is to improve the measurement tools needed for examining endocrine disruption in southern California fish. The second goal is to determine, with the newly developed tools, endocrine disruption in some populations of southern California fish near impacted and non-impacted areas. This study will benefit the Regional Board by describing the effects and magnitude of endocrine disruption among local fish, which will assist in determining if endocrine impacts are substantial and action is needed. Another benefit will be the development of screening tools to provide effective endocrine disruption monitoring of marine fish.

## 6. PROJECT/ TASK DESCRIPTION

### 6.1 Work Statement and Produced Products

Activities for this project and important dates are outlined by task in Table 2. Tasks 1 and 2 are Project Administration, and development of this Quality Assurance Project Plan, respectively. Task 3 is validation of the Vitellogenin ELISA method for endocrine disruption monitoring. Task 4 is validation of Microarray method. Samples of Horneyhead turbot, English sole and California Halibut flatfish from impacted and non-impacted sites will be collected to enable a determination of the effect of endocrine disruptors.

The project will provide quarterly progress reports during the life of the project that include summaries of analytical results (Task 5). At the end of the project, SCCWRP will provide a full listing, summary and analysis of the data collected, describing the endocrine disruption in southern California fish (Task 6).

**Table 2.** (Element 6) Project schedule.

Activity	Anticipated date of completion	Deliverable	Deliverable due date
<b>Task 1.</b> Project Administration	11/01/04 and quarterly	Quarterly progress report	11/01/04 and quarterly
<b>Task 2.</b> Quality Assurance Project Plan Development	11/05/04	Quality Assurance Project Plan	11/05/04
<b>Task 3.</b> Tool Validation			
Vitellogenin ELISA	12/01/05	Summary of ELISA Assays	12/20/05
Microarray	12/01/05	Summary of Microarray Assays	12/20/05
<b>Task 4.</b> Analyses of Field Collected Samples			
Microarray Analyses	9/01/06	Summary of Test Results	9/30/06
Chemical Analysis	7/01/06	Summary of Test Results	7/30/06
<b>Task 5.</b> Effects Assessment	12/01/06	Tables and Figures	12/20/06
<b>Task 6.</b> Draft and final report	4/01/07	Draft report	4/30/07
	7/01/07	Final report	7/30/07

## 6.2 Analyses of Samples and Measurement Techniques

Chemical and molecular constituents selected for this study include endocrine disruptor compounds and proteins found in tissue and plasma. Analytical constituents associated with this project are outlined in Table 3.

Table 3. (Element 6) Analytical constituents for tissue and plasma analyses.

Analyte
<b>Tissue Constituents</b>
PCBs <sup>1</sup>
Pesticides <sup>2</sup>
Pharmaceuticals <sup>3</sup>
<b>Plasma Constituents</b>
Proteins <sup>5</sup>

<sup>1</sup> Total PCBs

<sup>2</sup> Pesticides include: DDT, DDE, lindane, chlordane, and Atrazine.

<sup>3</sup> Pharmaceuticals include: ethynylestradiol, diethylstilbestrol, and 4-hydroxytamoxifen.

<sup>5</sup> Proteins include: cortisol, estradiol, IGF, and vitellogenin

## 7. QUALITY OBJECTIVES AND CRITERIA

Data Quality Objectives (DQOs) are quantitative and qualitative statements that clarify study objectives, and specify the tolerable levels of potential errors in the data (U. S. EPA, 2000). As defined in this plan, DQOs specify the quantity and quality of data required to support the study objectives. DQOs are generally used to determine the level of error considered to be acceptable in the data produced by the sampling or monitoring program. They are used to specify acceptable ranges of laboratory performance. Each data quality category is described below. Numerical DQOs for the constituents being sampled are listed in Table .

### 7.1 Precision

The precision objectives apply to duplicate and split samples taken during laboratory analysis as part of periodic QC checks. Precision describes how well repeated measurements agree. The evaluation of precision described here relates to repeated measurements/samples taken in the field (i.e. field replicates) or the laboratory. Precision is measured as relative percent difference (Table 4).

### 7.2 Accuracy

Accuracy describes how close the measurement is to its true value. Accuracy is the measurement of a sample of known concentration and comparing the known value against the measured value. The accuracy of chemical measurements will be checked by performing tests on laboratory control standards (LCS) prior to and/or during sample analysis at the Laboratory. A standard is a known concentration of a certain solution. Standards can be purchased from chemical or scientific supply companies. Standards might also be prepared by professional partners (e.g. a commercial or research laboratory). The concentration of the standards will be unknown to the analyst until after measurements are determined. The concentration of the standards should be within the mid-range of the equipment. Accuracy is measured as percent recovery (Table ).

### 7.3 Completeness

Completeness is the fraction of planned data that must be collected in order to fulfill the statistical criteria of the project. There are no statistical criteria that require a certain percentage of data. However, it is expected that 90% of all measurements could be taken when anticipated. This accounts for adverse weather conditions, safety concerns, and equipment problems. We will determine completeness by comparing the number of measurements we planned to collect compared to the number of measurements we actually collected that were also deemed valid. An invalid measurement would be one that does not meet the sampling methods requirements and the data quality objectives. Completeness results will be checked quarterly. This will allow us to identify and correct problems.

#### 7.4 Representativeness

Representativeness will be addressed by examining within-station variation (i.e., how representative is a particular sample to the specific station being assessed?). This study addresses this by measuring multiple samples, in order to characterize variability. If funding is available additional constituents may be analyzed.

**Table 4.** (Element 7) Measurement quality objectives:

Analyte	% Recovery <sup>1</sup>	RPD <sup>2</sup>	Method Detection Limit	Completeness
<b>Tissue Constituents</b>				
PCBs <sup>3</sup>	70-130%	0-30%	10 ug/kg*	90%
Pesticides <sup>4</sup>	70-130%	0-30%	2 ug/kg*	90%
Pharmaceuticals <sup>5</sup>	70-130%	0-30%	10 ug/kg*	90%
<b>Plasma Constituents</b>				
Proteins <sup>6</sup>	70-130%	0-30%	0.1 ng/ml*	90%

<sup>1</sup> For constituents <10x the method detection limit in the non-spiked samples.

<sup>2</sup> For constituents >10x the method detection limit.

<sup>3</sup> Total PCBs

<sup>4</sup> Pesticides include: DDT, DDE, lindane, chlordane, and Atrazine.

<sup>5</sup> Pharmaceuticals include: ethynylestradiol, diethylstilbestrol, and 4-hydroxytamoxifen.

<sup>6</sup> Proteins include: cortisol, estradiol, IGF, and vitellogenin

\*This value is an estimation.

## **8. SPECIAL TRAINING NEEDS/CERTIFICATION**

### **8.1 Specialized Training or Certifications**

CRG Laboratory holds State ELAP certifications for analysis of the constituents. Each project manager provides specialized training to his or her personnel. Details of the training are available from each lab.

Standard Operating Procedures (SOPs) for field, laboratory, and data management tasks will be developed and updated on a regular basis in order to maintain procedural consistency. The sampling SOP has been attached in Appendix C. The maintenance of an SOP Manual will provide project personnel with a reference guide for training new personnel as well as a standardized information source that personnel can access.

### **8.2 Training and Certification Documentation**

Laboratories will maintain records of their training. Those records can be obtained if needed from the lab through their Quality Assurance Officer.

### **8.3 Training Personnel**

Each lab task manager Officer provides training to personnel.

## 9. DOCUMENTS AND RECORDS

All data and reports generated by this project will be stored at SCCWRP. Records that pertinent to this study will be maintained at their respective offices. Copies of all records held will be provided to SCCWRP and stored in the project file.

Persons responsible for maintaining records for this project are as follows: Doris Vidal will maintain all sample collection, sample transport, analyses forms, all records associated with the receipt and analysis of samples analyzed for all parameters, and all records submitted. Each lab's Quality Assurance Officer will maintain the lab's own records. Steve Bay will oversee the actions of these persons and will arbitrate any issues relative to records retention and any decisions to discard records.

All data will be entered into an electronic database using a set of standardized data protocols for data entry and sharing. Database tables will include information on the results of the different analyses.

Copies of this QAPP will be distributed to all parties involved with the project, including analysis task managers. Any future amended QAPPs will be distributed in the same fashion. The task managers are responsible for distributing the QAPP to their laboratory staff. All originals of this and subsequent amended QAPPs will be held at SCCWRP. Copies of versions, other than the most current, will be discarded so as not to create confusion.

### 13. ANALYTICAL METHODS

#### 13.1 Analysis Methods

The samples will be analyzed for chemistry as indicated below.

##### 13.1.2 Organics

Gas chromatography/mass spectrometry (GC/MS, EPA 8270) will be used to analyze concentrations of organophosphate pesticides. A modification of this method is used in order to reach the low method detection limits. One gram of liver tissue is used and concentrated to 100-300  $\mu$ l. The samples are analyzed on a 60 m column temperature programmed at 2.5°C per minute, 90 min runs.

**Table 5.** (Element 13) Analytical methods.

Analyte	Method	Modifications to Method	Method Detection Limit
<b>Organics</b>			
Organophosphate Pesticides	EPA 8270	2 L sample volume 60m column, 90 min run time	.2 ug/kg
<b>Vitellogenin ELISA</b>	Method in development for this project	None	NA
<b>Microarray</b>	Method in development for this project	None	NA
<b>Molecular Analyses of Plasma</b>	Method in development for this project	None	NA

NA= Not available

#### 13.2 Sample Disposal

The amounts of plasma and tissue sampled are minimal. Tissue will be disposed of according to the health and safety regulations of each analytical lab.

#### 13.3 Corrective Action

Corrective action is taken when an analysis is deemed suspect for some reason. These reasons include exceeding RPD ranges and/or problems with spike recoveries or blanks. The corrective action varies somewhat from analysis to analysis, but typically involves the following:

- A check of procedures.
- A review of documents and calculations to identify possible errors.
- Correction of errors.
- A re-analysis of the sample extract, if sufficient volume is available, to determine if results can be improved.

- A complete reprocessing and re-analysis of additional sample material, if sufficient volume is available and if the holding time has not been exceeded.

The QA Officers will each have systems in place to document problems and make corrective actions.

The deliverable package will include hard copy and Electronic Data Deliverable (EDD). The hard copy will include standard narratives identifying any analytical or QA/QC problems and corrective actions, if any. The following QA/QC elements will be included in the data package: sample collection, extraction, and analysis dates and times, results of method blanks, summary of analytical accuracy, summary of analytical precision, and reporting limits. The electronic data files will contain all information found in the hard copy reports submitted by the laboratories. Individual data sets will be submitted as either Microsoft Excel® workbook files or as Microsoft Access® database files.

## 14. QUALITY CONTROL

Lab QA/QC samples are used to evaluate the analytical process for contamination, accuracy, and reproducibility. Internal laboratory quality control checks will include method blanks, matrix spike/matrix spike duplicate (MS/MSDs), and duplicates. These QA/QC activities are discussed below.

### Blanks

Blanks help verify that the equipment, sample containers, and reagents are not a source of contamination, and that the sampling techniques used are non-contaminating.

Method blanks will be run by the analytical laboratory to determine the level of contamination associated with laboratory reagents and equipment. A method blank is a sample of a known matrix that has been subjected to the same complete analytical procedure as the submitted samples to determine if contamination has been introduced into the samples during processing. Results of method blank analysis should be less than the reporting limits for each analyte.

### Duplicates

Duplicates are part of the QA/QC program to assess variability. Field duplicates are used to assess variability attributable to collection, handling, shipment, and storage.

### Spikes

Spikes are used to assess precision and accuracy of the laboratory analytical method, and to evaluate matrix interference. The matrix spike/matrix spike duplicates (MS/MSD) approach will be used with the field samples. A MS sample is an aliquot of a field sample into which the laboratory adds a known quantity of a compound. Reported percent recovery of the known compound in the sample indicates matrix effect on the analysis. A MSD sample is a duplicate aliquot of the matrix spike sample that is analyzed separately. The MSD results are compared to the matrix spike results to assess the precision of the laboratory analytical method. Duplicate results are evaluated by calculating the relative percent difference (RPD) between the two sets of results. This serves as a measure of the reproducibility (precision) of the sample results. The acceptable RPD limits are shown in Table . The RPD is calculated as:

Relative percent difference =  $100 \times (\text{sample 1} - \text{sample 2}) / \text{average}$

## **15. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE**

### **15.1 Analytical Instruments**

The all laboratories will maintain its equipment in accordance with its SOPs, which include those specified by the manufacturer and those specified by the method.

## **16. INSTRUMENT/ EQUIPMENT CALIBRATION AND FREQUENCY**

All laboratory equipment is calibrated based on manufacturer recommendations and accepted laboratory protocol. All laboratories maintain calibration records as part of the method SOPs and details are described in the attached QA plan documents.

**17. INSPECTION/ ACCEPTANCE FOR SUPPLIES AND CONSUMABLES**

Laboratories will maintain logbooks for all consumables that are checked against all materials received.

## 18. NON-DIRECT MEASUREMENTS

This study will not incorporate existing data or other non-direct measurements, as this is the first study of its kind.

## 19. DATA MANAGEMENT

Analysis results will be electronically sent to Doris Vidal following the completion of quality control checks by each of the laboratories. Data will be screened for the following major items:

- A 100 percent check between electronic data provided by the laboratory and the hard copy reports
- A check for laboratory data report completeness
- A check for typographical errors on the laboratory reports
- A check for suspect values

Laboratories will provide data in both hard copy and electronic format. The required form of electronic submittals will be provided to the laboratories to make sure the files can be imported into the project database with a minimum of editing. The data will be managed at SCCWRP.

Following the initial screening, a more complete QA/QC review process will be performed, which will include an evaluation of method and equipment blank contamination, and analytical accuracy and precision. Accuracy will be evaluated by reviewing MS/MSD and LCS recoveries; precision will be evaluated by reviewing MSD and laboratory sample duplicate RPDs.

## **GROUP B: ASSESSMENT AND OVERSIGHT**

### **20. ASSESSMENTS AND RESPONSE ACTIONS**

The Assistant Project Manager (Doris Vidal) will be responsible for the day-to-day oversight of the project. The SCCWRP QA Officer will conduct periodic reviews of the data and relay any problems to the Assistant Project Manager. The SCCWRP QA Officer has the power to halt all sampling and analytical work by SCCWRP, CRG Laboratory, California State University at Long Beach, University of California at Riverside, or University of California at San Diego if the deviation(s) noted are considered detrimental to data quality.

## 21. REPORTS TO MANAGEMENT

The status of data collection during this project will be reported by the Project Manager, Doris Vidal to the LARWQCB on a quarterly basis beginning November 5, 2004 and continuing until the completion of the project in May 30 of 2007. A draft final project report will be filed no later than July 30, 2007.

Table 6. (Element 21) QA management report.

Report	Due by
Quarterly progress reports	November 05, 2004 and quarterly thereafter
Draft final report for review	May 30, 2007
Final Report	July 30, 2007

## **GROUP C: DATA VALIDATION AND USABILITY**

### **22. DATA REVIEW, VERIFICATION, AND VALIDATION**

The specifics of data review, validation, and verification are detailed in the Quality Objectives section (Element 7) of this document. Project personnel will perform laboratory and equipment blanks as described in that section to maintain quality assurance and control of the data collected.

### 23. VERIFICATION AND VALIDATION METHODS

Laboratory validation and verification of the data generated is the responsibility of each laboratory. Each laboratory supervisor maintains analytical reports in a database format as well as all QA/QC documentation for the laboratory.

Doris Vidal and Steve Bay are responsible for oversight of data collection and the initial analysis of the raw data obtained from the field and the contracted laboratory. Their responsibilities also include the generation of rough drafts of quarterly and final reports. Doris Vidal has final oversight on the submission of quarterly and final reports.

## 24. RECONCILIATION WITH USER REQUIREMENTS

The data will be evaluated for values that appear to be anomalous. Measurements that are deemed suspect will be checked for errors in calculations or procedures. Samples with errors that cannot be reconciled through a review process will be removed prior to statistical analysis. Acceptable data will be assessed for the presence of endocrine disruption of different endpoints and compared to determine if the analyzed fish are impacted.

Data from plasma samples will be evaluated for significant protein production or inhibition using analysis of variance (ANOVA) with Dunnet's test, or with Steel's Many-One rank tests when assumptions of normality or homoscedasticity are not met. Comparisons will be made against reference areas. Results obtain from traditional molecular techniques adapted for the target species will be compared to the results obtained with the microarray.

The relationships among molecular analysis and chemistry parameters present in liver tissue will be assessed using Spearman nonparametric correlation. These procedures will assist us to understand the extent and magnitude of endocrine disruption in southern California flat fish.

**APPENDIX A: SAMPLING PROCEDURES**

## ENDOCRINE DISRUPTION IN COASTAL FISH Sampling Protocol

### Overview of Sampling Design

Blood, liver, gonad, and muscle tissue samples for endocrine disruption measurements will be obtained from flatfish collected from mainland shelf. Because proteins and enzymes that affect the endocrine disruptor measurements begin to degrade rapidly after a fish has died, trained personnel will collect blood and dissect needed tissues on board immediately after catching. Dead fish should not be sampled. All tissues will be appropriately prepared (as described below) and then frozen on dry ice for transport back to SCCWRP or other designated locations for long-term storage at -80°C.

### METHODS

**Equipment and Procedures:** Fish from different locations will be caught by trawling and dissected on board during the Southern California Regional Bight 03 survey. Staff from SCCWRP, California State University at Long Beach (CSULB), University of California at Riverside (UCR), Los Angeles County Sanitation district (LACSD) and/or additional field crew will carry out the field sample collection. Other samples from selected bays and harbors areas may be collected if samples can be obtained without added sampling effort (i.e., extra fish are available during a cruise when dissection personnel are present).

**Species:** Ideally a minimum of five fish (maximum of 15) of the following fish species will be processed at each site for the ED study: Hornyhead turbot, California halibut and English sole. The target species will be dissected as soon as possible after each trawl. Fish for the ED study will come from the same trawls as the fish collected for the flatfish bioaccumulation study, or from additional trawls when necessary. The same specimens will not, however, be shared between the two studies.

**Bleeding:** Blood sampling is top priority in the sampling protocol for each individual fish, and one person will be dedicated solely to this task. Bleeding is carried out while the heart is still beating, and thus this must be done as an initial step in the protocol (as soon as possible after catching). After sorting from the trawling net, fish will be placed into holding buckets containing clean seawater. Fish will then be placed ventral side down on a clean board, and a 26-gauge needle and heparinized syringe will be used to draw the blood from the aorta at the caudal peduncle. This blood vessel runs along the ventral side of the spine (along the same region as the lateral line in most flatfish). Blood will be held on

wet ice until centrifugation (depending of the size of the fish, 0.5 to 1 ml of whole blood should be extracted from each fish).

**Plasma Isolation:** Blood, collected in the presence of heparin (coated syringe barrels), must be centrifuged at 500x g to separate plasma and cells using a portable centrifuge. Following centrifugation, the plasma is removed and placed into microfuge tubes (see Aliquotting) and frozen on dry ice. Samples on dry ice should be subsequently stored at  $-80^{\circ}\text{C}$  until further distribution and use in assays.

**Tissue Collection:** Dissections will be conducted on clean boards using scissors, scalpels, and forceps. Liver, gonads, and muscle tissue will be removed from each fish, sectioned into aliquots as necessary (see next section), packaged, and immediately frozen on dry ice or preserved. Aluminum foil squares (6 x 6 cm) labeled with a permanent marker (making sure that the label shows) will be used for tissue storage. After it has been wrapped with aluminum foil, the tissue should be placed inside a plastic bag, which has been previously placed directly on the top surface of a dry ice block so the tissue freezes quickly. All frozen samples belonging to a single fish should be kept in a single plastic bag during storage. Dissection tools will be cleaned between samples in deionized water. Prior to each day of sampling, instruments will be cleaned by a detergent scrub and deionized water rinses.

Tissue samples for histopatology will be placed in tissue cassettes and preserved with Dietrich (Kahle's) solution until further distribution. All other tissues will be kept frozen on dry ice and transported to CSULB or SCCWRP for storage at  $-80^{\circ}\text{C}$  until further distribution for analyses.

**Aliquotting:** Prior to freezing the blood plasma samples, two aliquots of 200  $\mu\text{l}$  (for CSULB) and 100  $\mu\text{l}$  (for UCR) should be placed in microfuge tubes and frozen until later transport to the analyzing laboratories, the rest of the sample should be given to SCCWRP.

It is important to practice clean dissecting procedures with the tissues to prevent cross contamination. When liver and muscle tissue are removed it is important to work on a clean Teflon square or other clean area before splitting them into aliquots. Take particular cautions to avoid contamination of muscle samples with skin or other external debris.

Muscle will be obtained by stripping ~5 g of lateral epaxial muscle tissue without skin (two aliquots are necessary). The first aliquot should be the size of a pea and placed in a vial containing RNase stopper, and frozen for analysis by CSULB. The other aliquot of approximately 4 g is going to be used for chemical analysis and should be sent wrapped in foil and frozen.

The liver tissue will be aliquoted in three different parts. The first one, about the size of a pea, should be placed in a vial containing RNAse stopper. After the tissue has been placed in RNAse stopper the vial should be placed frozen on dry ice. A second aliquot of approximately 0.2 g should be frozen for analysis by UCR. The rest of the liver should be frozen for chemical analysis by LACSD.

One gonad should be placed on aluminum foil and stored on dry ice (for UCR). The second gonad after dissected should be placed in a tissue cassette and put in Dietrich solution. If only one gonad is available, it should be preserved in fixative for histological analysis.

**Record keeping and labeling:** Standard length, species, sex, estimated maturity; etc will be recorded for each specimen. Besides record keeping, it is important that each vial or foil is properly labeled with water resistant markers.

A weekly report and a copy of the field data should be faxed to SCCWRP to the attention of Doris Vidal (714) 894-9699. This report should describe the number of stations sampled, the number of fish and species collected at each station, and information pertaining to additional samples collected from stations not listed on the given map. In addition to the weekly information an electronic datasheet version of the data should be provided to Doris Vidal at SCCWRP at the end of the sampling effort with the following information:

Station ID		
Latitude	Weight (g)	Tissue Type (See code)
Longitude	Standard Length (cm)	Aliquot ID (See code)
Sample ID	Fish Anomalies	Sex/ Maturity (See code)
ID	Dissection Time (Beginning of	Date
Species	blood extraction)	Comments

The labels should contain the following information (Codes are explained in Table 1):

Station ID (Last 2 #)	Species (HT, CH, ES)	Aliquote ID (SC, UC, LB, LA)
Sample ID	Tissue Type (L, G, M, P)	Date (Day/Month/Year)

Example:            00                            P                            S  
                           Station ID   Tissue Type   Aliquot ID  
                           01                            HT                            07/16/03  
                           Sample ID   Species   Date

Assessment of Endocrine Disruption in Southern California Coastal Fish  
Quality Assurance Project Plan  
November 5, 2004

Note: for tissue cassettes the labels should contain: station id, sample id, and species only

**Storage:** samples will be stored by the lead sample collection agency until the end of sampling effort.

## MATERIALS

### Materials required:

- 2 permanent markers
- 2 Pencils
- Water resistant paper or notebook
- 2 clear metric ruler or fish measuring board
- Data sheets
- Whirl-packs of plastic vials (6 vials per fish processed)
- 6 foil squares per fish (6x6cm<sup>2</sup> pre-labeled with permanent marker) for tissue storage
- 10 lbs of dry ice per day
- Bag of wet ice per day
- 1.5-ml microfuge tubes (4 per fish processed) for primary plasma collection and aliquotting
- 1.5-ml microfuge tubes (2 per fish processed) for aliquotting
- Bags for organizing groups of samples (by fish)
- 1 auto-pipettes (2-200  $\mu$ l)
- Pipette tips (200  $\mu$ l)
- Heparin (ammonium salt; Sigma #H6279) solution (0.18%) in ddH<sub>2</sub>O
- Syringes (1 ml) (1 per fish processed; pre-treated by washing syringe barrel in Na-heparin solution)
- 26 gauge syringe needles (1 per fish processed)
- 500 ml of Dietrich's solution
- 1 Tissue cassettes (per fish) for the gonad collection.
- 2 sets of forceps
- 2 sets of scissors
- 2 scalpels with disposable blades
- 2 clean boards
- Portable centrifuge
- Ice cooler
- Plastic bottle with distilled water
- Roll of paper towels
- Gloves
- Small ice chest
- 10 Clean Teflon squares
- Tape
- 5 big clear plastic bags
- Liquid waste plastic container
- Sharps' collector container

**Table 2.** Code classification

Category	Name	Code
Species	Hornyhead	HT
	turbot	
	English sole	ES
	California halibut	CH
Tissue Types	Plasma	P
	Liver	L
	Gonads	G
	Muscle	M
Sampling Agencies	CSULB	CL
	LACSD	LA
	SCCWRP	SC
	UCR	UR
Sex and Maturity	Unidentifiable	U
	Male Mature	MM
	Female Mature	FM
	Male Immature	MI
	Female Immature	FI

**Table 3.** Tissue aliquotting, storage and distribution.

<b>Tissue Type</b>	<b>Aliquot Total</b>	<b>Amount</b>	<b>Storage</b>
Plasma	3	200 $\mu$ l 100 $\mu$ l Reminder	Vial with RNase stop Vial + Frozen Vial + Frozen
Gonad	2	1 (whole) 1 (whole)	Aluminum foil + Frozen Tissue cassette (fixed)
Muscle	2	Pea size ~ 5 g	Vial with RNase stop Aluminum foil + Frozen
Liver	3	Pea size ~ 0.2 g Reminder	Vial with RNase stop Aluminum foil + Frozen Aluminum foil + Frozen

Write an estimation of the weight of the liver on the comments section of the field sheet.

**APPENDIX B, CRG MARINE LABORATORIES' QUALITY ASSURANCE  
PROGRAM DOCUMENT**

**CRG MARINE LABORATORIES**

2020 Del Amo Blvd, Torrance, California 90501, (310) 533-5190

**QUALITY ASSURANCE PROGRAM DOCUMENT**

## **1.0 TABLE OF CONTENTS**

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## 2.0 INTRODUCTION

- 2.1 CRG Marine Laboratories, Inc., Torrance, CA (CRG) is committed to providing quality environmental analytical services to all of its clients. To maintain this high level of quality, an extensive Quality Assurance Program (QA) has been implemented within CRG. The purpose of this manual is to document the QA practices utilized by CRG. It describes the applications and concepts employed to assure that results generated by CRG are in control, scientifically valid, of known highest possible quality, and can be used with a high degree of confidence by the client or user.
- 2.2 The format of this manual is patterned after that outlined in the California Department of Health Services Application for Environmental Laboratory Accreditation.

This document is intended for use as a reference document to CRG's Quality Assurance Program. It is designed to assist all staff members to perform the operations necessary to comply with all client and contractual requirements and to ensure that data produced by CRG conforms to the highest standards set by state and/or federal regulations.

## ORGANIZATIONS AND RESPONSIBILITY

CRG operates two environmental laboratories at the following locations:

2020 Del Amo Blvd., Suite 200  
Torrance, CA 90501.

355 Van Ness, Suite 115  
Torrance, California 90501

### Quality Assurance Staff Responsibilities

The Laboratory Director is ultimately responsible and accountable for all activities related to the generation of technical data by or for CRG. In order to carry out these QA responsibilities and facilitate the integration of QA into all data generation activities, certain responsibilities have been delegated to other CRG employees.

The **Laboratory Director** is responsible for the following activities:

Provides leadership and technical direction for the organization

Removes barriers that limit the ability of individuals to obtain their goals and introduces change as a positive opportunity for the growth of the individual and CRG

Ensures that adequate QA/QC provisions are developed and incorporated into all laboratory data generation activities

Ensure that adequate resources are provided to meet these objectives

Ensure that specific QC procedures conform to the requirements specified by the client or project manager

Participates in appropriate certification programs and audit programs to establish credibility and demonstrate proficiency

Ensure that deficiencies or problems identified through audits are corrected as expeditiously as possible

Ensure that all routinely used analytical and administrative procedures are covered by well-written Laboratory Operating Procedures (LOP)

Ensure that all staff members are adequately qualified and trained to perform assigned tasks

Ensure that equipment is adequately maintained for the intended use

Ensure that the laboratory is a safe, efficient, and productive work environment.

The **Quality Assurance Specialist** is responsible for the following activities:

Maintain and update the Quality Assurance Program and this QA Manual

Serve as a QA liaison with clients and project managers

Coordinate accreditation/certification and auditing activities

Assess the adequacy of QC activities within the laboratory and keep the Laboratory Director informed of their effectiveness

Ensure that data is validated with respect to QC criteria

Ensure that all chain-of-custody requirements are met

Issue and evaluate the analyses of performance evaluation samples

- H. Ensure that audit results are communicated with the appropriate staff and corrective actions are taken when needed
- I. Identify and recommend staff training needs
- J. Work with the various laboratory staff to assure that LOPs are documented and meet the established quality standards

3.2.3 The **Organics Supervisor** is responsible for the following activities:

- A. Develop, update, and implement modern state-of-the-art instrumental analysis techniques to cost-effectively meet CRG's requirements
- B. Provide organic analytical testing services including priority pollutants and other regulated organic chemicals to CRG's clients
- C. Validate data generated by the Organic Chemistry Section to assure that all quality objectives are met
- D. Responsible for financial performance of the Organic Chemistry Section
- E. Provide necessary training for all subordinates
- F. Provide a safe working environment.

3.2.4 The **Inorganics Supervisor** is responsible for the following activities:

- A. Develop, update, and implement modern state-of-the-art instrumental analysis techniques to cost-effectively meet CRG's requirements
- B. Provide inorganic analytical testing services including metals and wet chemistry to CRG's clients

- C. Validate data generated by the Inorganic Chemistry Section to assure that all quality objectives are met
- D. Responsible for financial performance of the Inorganic Chemistry Section
- E. Provide necessary training for all subordinates
- F. Provide a safe working environment.

3.2.5 The **Microbiology Supervisor** is responsible for the following activities:

- A. Develop, update, and implement modern state-of-the-art analytical techniques to cost-effectively meet CRG's requirements
- B. Provide Microbiology analytical testing services including indicator bacteria, bacterial viruses and other microorganisms CRG's clients
- C. Validate data generated by the Microbiology Section to assure that all quality objectives are met
- D. Responsible for financial performance of the Microbiology Section.
- E. Provide necessary training for all subordinates
- F. Provide a safe working environment.

3.2.5 The **Sample Custodian** is responsible for the following activities:

- A. Receipt, login, and storage of all analytical chemistry samples
- B. Review all chain-of-custody forms, record sample condition, and resolve inconsistencies and problems
- C. Serve as liaison between Project Managers and Analysts with respect to handling rush orders

- D. Purchase, label, preserve, pack, and ship all appropriate sample containers provided to clients
- E. Ensure that all laboratory samples are ultimately disposed of according to the laboratory guidelines.

#### **4.0 QA OBJECTIVES FOR MEASUREMENT DATA**

4.1 Data Quality Objectives (DQOs) for the data collection activity describe the overall level of uncertainty that a decision-maker is willing to accept in results derived from environmental analyses. The objective of CRG's Quality Assurance Program is to ensure that the validity and reliability of the data meets client's requirements in terms of DQOs. The program follows the guidelines established by the California Department of Health Services and the U.S. EPA.

Since DQOs often vary with individual projects, CRG sets internal specifications that are strict enough to meet a majority of client's requirements. Project-specific DQO's can be found in the Quality Assurance Project Plans (QAPPs) for that project.

4.2 DQOs for analytical determinations are expressed in terms of accuracy, precision, detection limits, completeness, and comparability. Section 11 of this manual describes the types of quality control checks used to measure these objectives and the procedures used to derive them. Table 1 outlines typical accuracy, precision, and method detection limit objectives for each field of testing. Specific DQOs for each parameter are contained within the LOP used for analysis.

#### **5.0 SAMPLING PROCEDURES**

CRG provides trained staff for sample collection purposes. Proper sampling includes using appropriate equipment, containers, and preservation as well as following strict procedures for collection, storage, and transport to prevent cross contamination and loss of sample integrity.

CRG provides appropriate containers and sampling procedures to those clients who choose to perform their own sampling. CRG staff refers to EPA guidelines published in the Federal Register, 40 CFR Part 136.3 and Standard Methods for the Examination of Water and Wastewater, 20<sup>th</sup> Ed, for container selection and preservation.

## 6.0 SAMPLE CUSTODY

To produce legally defensible data, CRG maintains and demonstrates custody control of all samples. Two components of custody are addressed: physical possession and documentation.

6.1 Documentation begins with field records, including a chain-of-custody (COC) form, which follows the physical sample from the field to the laboratory. The Sample Custodian checks to insure that:

- A. The sample container is clearly marked and agrees with the information provided on the chain-of-custody sheet
- B. The evidence tape is unaltered and the container is intact
- C. The sample was supplied in the proper type of container
- D. The sample has not exceeded its maximum holding time
- E. Sufficient sample volume exists to perform the requested analyses
- F. Samples requiring analysis by a contract laboratory are packaged with an ice substitute and dunnage, and are shipped in an ice chest to the contract laboratory. A chain-of-custody sheet accompanies all samples shipped from CRG.

6.2 If samples are delivered without a COC, one is completed at the laboratory prior to acceptance of the samples. The Sample Custodian shall note on the COC any discrepancies between the physical sample and the custody record.

6.3 Once received, each sample is assigned a unique laboratory ID number and logged into a bound Sample Receiving Logbook. Key characteristics are recorded into the logbook, the COC is filed with the project file, and the sample is placed in the appropriate storage location until analysis.

## 7.0 CALIBRATION PROCEDURES AND FREQUENCY

- 7.1 All instrumentation is calibrated at a frequency that ensures the validity of the results. These procedures are carried out following USEPA guidelines and the recommendations of the instrument manufacturer.
- 7.2 Calibration standards are prepared either from purchased stock standards or from stock standards prepared in-house utilizing reagents suitable for the preparation of standards. When available, calibration standards are prepared from starting materials that are certified traceable to the National Institute of Standards Technology (NIST).
- 7.3 The following is a brief summary of the instrumentation calibration procedures employed at CRG. Detailed descriptions of these procedures are contained with the appropriate method.
- 7.3.1 The gas chromatograph or gas chromatograph mass spectrometer is calibrated using either an external calibration procedure or internal standard. For each parameter of interest, at least three to five different concentrations of standards are employed. One of the concentrations is near the Method Detection Limit (MDL) for each parameter. Concentrations of the remaining standards correspond to the expected range of concentrations found in the samples analyzed. Calibration standards are prepared by utilizing secondary dilution standards and/or stock solutions. Calibration standards may include a set of internal standards at a known constant amount. The base peak  $m/z$  shall be used as the primary  $m/z$  for quantification of the standards. Sensitivity of the instrument is checked every 10 samples by analyzing the external reference samples. If the result is not within a predetermined range, the problem is corrected, and the samples immediately following the last acceptable check are reanalyzed.
- 7.3.2 The Inductively Coupled Mass Spectrometer (ICPMS) is calibrated before each use. For each parameter of interest, at least three to five different concentrations of standards are employed. One of the concentrations is near the Method Detection Limit (MDL) for each parameter. Concentrations of the remaining standards correspond to the expected range of concentrations found in the samples analyzed. Calibration standards are prepared by utilizing secondary dilution standards and/or stock solutions. Calibration standards may include a set of internal standards at a known

constant amount. Sensitivity of the instrument is checked every 10 samples by analyzing the external reference samples. If the result is not within a predetermined range, the problem is corrected, and the samples immediately following the last acceptable check are reanalyzed

7.3.3 The performance of the balances is monitored against a set of calibration weights that are traceable to NIST (a log is maintained of these inspections)

7.3.4 Temperature records are maintained for all refrigerators, incubators, water baths, and ovens. The temperatures are monitored at a frequency determined by how often the equipment is placed in service.

## **8.0 ANALYTICAL PROCEDURES**

Analytical procedures are determined by current environmental regulations set forth by both state and federal guidelines. Analytical methods are published in CRG's Laboratory Operating Procedures Manual (LOPM). Revisions and updates of the LOPMs are developed as required. The LOPMs are numbered to correspond with their standard reference method.

8.1 The manual includes the methods employed by CRG for the analyses required to support CRG's clients

8.2 The format of the LOPM is patterned after those listed in the Code of Federal Regulations.

8.3 The LOPMs are prepared by senior members of the technical staff and approved by the Laboratory Director.

8.4 The LOPM is a controlled document. Each manual is assigned to an individual who has custodial responsibilities. Revised LOPMs are issued with a new revision letter. The custodian updates the manual and is responsible for replacing the previous section(s) with the revised section(s). This insures that the analyst is always working to the latest revision of test procedures and protocols. A history file is maintained of all revisions to the LOPM. A memorandum is attached to each revision in the history file summarizing the reason for the change.

8.5 Research and development projects and methods development projects are documented in bound laboratory notebooks.

## 9.0 DATA REDUCTION, VALIDATION, AND REPORTING

Laboratory results are communicated to CRG's clients through the analytical report delivered either electronically or by mail. This document is based on the client's laboratory order or by group of related samples.

9.1 Data reduction- Data reduction is the process by which the analyst translates raw data into a reported result that is reviewed by a second party then approved by the section supervisor before being released in the final report. All charts, printouts, and data books are archived after the results are reported. Specific calculations and verification processes are summarized in the respective LOPMs.

All determinations are performed by dedicated instrumentation equipped with a microcomputer. Results are stored in a computer file, reported in a printed report and then electronically transferred to the database. A sequence logs containing the sample position, and order of analysis is kept both electronically and hardcopy. Sample results are tracked by the computer filename cross-referenced to the unique sample ID number.

9.2 Data validation - Data validation involves ensuring the correct assignment of sample labels before instrument operation, checking the performance of the instrument, verification of successful completion of all quality-control checks, and fitness of the calculations performed by the computer.

9.3 **Data Management** - Sample analytical data including ID, date and time of collection and analyses, type of requested field and laboratory analyses, and results are entered into a Laboratory Information Management System (LIMS), which is a Microsoft Access-based database system. After data entry, all results from sample analyses and QA/QC are reviewed for accuracy and completeness and any reporting of laboratory results are based on queries from the LIMS.

9.4 **Reports** - Electronic and/or hard copy reports are provided based on client's need. The basic report includes a header containing the CRG sample ID number, date collected, date received, date processed, prepared, date analyzed, client sample information, batch ID number, replicate number, and instrument identification. Electronic data deliverables can be designed to meet any client requests and based

upon queries of the LIMS database. The section supervisor prior to release to the client reviews the final report.

## 10.0 INTERNAL QUALITY CONTROL CHECKS

Quality control measurements verify the integrity of the analytical results. While the goal of all quality control procedures remains constant, specific quality control procedures vary from method to method. Every analyst is responsible for a thorough understanding of the goals of each quality control measurements and the control analyses as required per method.

10.1 A batch is defined as a group of 20 or fewer samples of similar matrix, processed together under the same conditions and with the same reagents. Quality control samples are associated with each batch and are used to assess the validity of the sample analyses. Control limits can be found in Table 4.1 of this document. Each batch must include the following QC checks:

10.1.1 Method Blank- A method blank is a sample that contains no analytes of interest. For solid matrices, no matrix is used. The method blank serves to measure contamination associated with processing the sample within the laboratory.

10.1.2 Laboratory Control Material (LCM) or Certified Reference Material (CRM)- A LCM or CRM is a sample with a matrix similar to the client samples that contains analytes of interest at known or certified concentrations. It is used to determine the accuracy of the results based on the comparison of the measured concentration with the true value. For analytes that are greater than 10 times the MDL, the acceptable percent recovery is presented in Table 4.1.

10.1.3 10.1.3 Duplicate Analyses- Duplicate analyses are samples that have been split and processed within a single batch. They are used to determine the precision of the results based on the percent relative difference (% RSD) between the two sets of results. Control limits for %RSD are presented in Table 4.1.

10.1.4 Matrix Spike/Matrix Spike Duplicates (MS/MSD)- MS/MSD are samples of similar matrix to the client's samples that are spiked with a known amount of analyte. Spike recovery measures the effect of interferences caused by the sample matrix and reflects the accuracy of the determination. The

spike level should be at least ten times the MDL. The duplicate spike may be used to determine the precision of the analytical results similar to Section 10.1.3:

10.1.5 Tuning Check- The tuning of the mass spectrometer is checked at the beginning of each run to insure that it is providing adequate spectra.

10.1.6 Initial Calibration- Initial calibration is performed by analyzing standards of known levels of concentration. The lowest level should be less than or equal to ten times the MDL and the remaining levels should represent the entire range of expected concentrations in the samples.

10.1.7 Calibration Verification- When a calibration curve is not performed for each run, a calibration verification is performed with a standard from, preferably a second source, is used to verify that the instrument is still operating within the original calibration curve.

10.1.8 Internal Standard- An internal standard is a non-target analyte, which is added to samples and QC checks after the preparation of the sample, just prior to analysis. It is used to compensate for variations in the instrument response from one sample to the next.

10.1.9 Recovery Surrogate- A recovery surrogate is a non-target analyte or analytes that are added to the sample prior to processing. It is used to indicate the extraction efficiency and instrument variation from sample to sample.

## **11.0 PERFORMANCE AND SYSTEM EVALUATIONS**

CRG is dedicated to the continuous improvement of all of its operational systems. This is an essential part of everyone's job within CRG. Internal evaluations are conducted by staff from the Laboratory and are performed on a periodic basis.

11.1 CRG employs the philosophy of Continuous Measurable Improvement systems to evaluate its process performance and to identify opportunities for improvement on a continual basis. Five key elements are essential for the Continuous Measurable Improvement system to work efficiently. The first is to establish open and honest communication among all personnel. The second

is to encourage decision making by delegating responsibility to the lowest appropriate levels of the work force. The third is to provide positive recognition for achievements and to strive continuously to identify and strengthen areas needing improvements. The fourth is to provide employees with the knowledge, skills, motivation, and working environment to meet their full potential and find personal satisfaction in their work. The fifth is to accept the concept of change as a positive opportunity for growth for both the individual and the organization.

- 11.2 With the five key elements of this philosophy in place, all levels of personnel can develop a true quantitative measurement system for assessing the status of meeting target goals in a wide variety of processes (i.e. improved accuracy, precision, training, safety, working environment, etc.). The system begins with a quantitative evaluation of the process based on a review of both historical and current capability and performance. Individual processes are selected as proposed projects based on whether they are in statistical control, predictable, and have attained target goals. CRG then prioritizes the selected projects based on frequency and magnitude of problem recurrence. Root-cause analysis is employed to establish control and eliminate the true sources of problems. Corrective actions are taken and the process is rerun to verify stability, capability and quality. If necessary, new target goals are set for the process and the system is repeated until the acceptable goal is achieved.

## 12.0 PREVENTIVE MAINTENANCE

- 12.1 Service contracts may be maintained for the major instrumentation and equipment that are no longer under warranty. The gas chromatographs, ICPMS instrumentation, and balances are typical examples of equipment that might be covered by a maintenance contract. Records of maintenance are kept by the person responsible for the equipment. Specific examples of routine preventive maintenance are further discussed in the following sections:

A. Hewlett Packard 5972 Gas Chromatograph/Mass Spectrometer System

1. Every six months, replace the MSD foreline pump oil and foreline trap pellets. During the fluid exchange, replace the outlet mist filter

2. Every year, check and if necessary replace the diffusion pump fluid
3. As needed, clean the ion source of the MSD (typically every six months)
4. As needed, the glass injector sleeve and injector septum for the split-splitless injector is replaced (typically once per month)
5. As needed, the gas purifiers and filters for the carrier gas are replaced

**B. Hewlett Packard 4500 ICPMS System**

1. Every six months, replace the oil and foreline trap pellets for the rough pumps. During the fluid exchange, replace the outlet mist filter
2. Every year check and replace the turbo molecular pump fluid
3. Once per month, clean the sample and skimmer cones
4. Once per week, replace the peripump tubing
5. As needed, clean the ion source of the mass spectrometer
6. Every three months, clean the nebulizer

**13.0 ASSESSMENT OF PRECISION AND ACCURACY**

13.1 CRG utilizes several methods to monitor precision and accuracy. These are designed to determine the reproducibility of the analysis (precision) or agreement of the result to the actual value of the analyte (accuracy). CRG routinely performs analysis of blind samples. This procedure is explained in section 14. The following definitions describe the types of analyses performed to assess precision and accuracy:

- A. Duplicate analyses involve performing two separate analyses of a particular parameter on the same sample. Precision is measured by the degree of agreement between the two sample results. Duplicate analyses are designed to measure the precision of a determination when the sample contains detectable amounts of the constituent
- B. Laboratory control material or certified reference materials are samples that have known concentrations of the target analytes. These concentrations are either based on a series of analyses or are certified by an external laboratory such as NIST. Accuracy is determined by comparing the measured amount of analyte recovered during analysis to the known value
- C. Sample spikes are samples that a known amount of the analyte has been added. Accuracy is determined by the amount of the added material recovered during analysis
- D. Blank spikes or water spikes are used if poor recovery from a spiked sample occurs, analysis of blank spikes is useful to determine if the poor performance is a function of the sample matrix or the analytical process. These consist of the usual sample portion of deionized water spiked with the constituent at a concentration equivalent to that of the sample spike
- E. Replicate spike analyses are employed to determine the precision and accuracy of an analysis when some or all of the parameters being determined are below the detection limit. The replicate spike procedure involves analyzing the sample and two portions of the sample spiked with a measured portion of the same analyte. Relative precision of the spikes can be determined as well as the accuracy of the analysis. Spike concentrations are sufficient to eliminate the bias that would be created by the undetectable quantity of the parameter being determined

13.2 One set of duplicate samples or spike duplicates, a LCM or CRM sample, and a method blank are analyzed with each batch of samples.

13.3 The ongoing evaluation of relative precision and accuracy performance is accomplished by the generation of control charts. Employing a minimum of 20 results, control limits are generated utilizing the mean and standard deviation of the data set. Upper

and lower "warning" limits are twice the standard deviation from the mean of the set of results for accuracy charts and twice the standard deviation from the origin for precision charts. Upper and lower "out of control" limits are three times the standard deviation from the mean for accuracy charts and three times the standard deviation from the origin for precision charts. When relative precision or accuracy results suggest atypical performance, an investigation into the problem is initiated. If a sample result is outside the out-of-control limits, the sample is reanalyzed. If samples cannot be reanalyzed, the result is flagged.

## 14.0 CORRECTIVE ACTIONS AND TRAINING

### 14.1 Corrective Actions

14.1.1 Corrective action is the process of defining- root-cause, identifying and implementing corrective action plans, educating - and training to provide system-wide solutions, and verifying that the improved system is being followed. Corrective action responses are divided into three separate categories based on the time required to complete the corrective action. An immediate corrective action occurs when a response that fully meets closure criteria can be carried out in the same time frame that the observation of the discrepancy occurs. An intermediate corrective action is one that will require a maximum of 30 days to complete the response satisfactorily. A long-term corrective action requires a time period greater than 30 days to provide a complete response. Long-term corrective actions typically involve cooperation of additional organizational elements.

14.1.2 Both intermediate and long-term corrective actions require a detailed corrective action plan showing clearly defined milestones, task descriptions, and responsibilities. CRG's Quality Assurance Specialist must approve all intermediate and long-term corrective action plans. Closure of corrective actions require verifiable, objective evidence that the corrective action be thorough, comprehensive, and will permanently prevent the problem from reoccurring. Corrective actions result from a wide variety of situations including:

A. Inspection of the sample indicates the: samples are 1) not representative of their source, 2) deteriorated, 3) improperly labeled, 4) damaged in transport, or 5) collected in an inappropriate container. In this case, the CRG Sample Custodian or Quality Assurance Specialist will notify the sample collector of the- problem(s) and request a new sample(s) to be collected following proper sample collection and handling methods

B. Samples that are not properly preserved, stored at incorrect temperatures, or exhibit deficiencies in the chain-of-custody records are not analyzed. The

CRG Sample Custodian or Quality Assurance Specialist reviews the discrepancy with appropriate personnel and new samples are collected employing correct methods

C. The required LOPM has not been followed correctly. The supervisor reviews the Method with the analyst and requests the analyst to rerun the analysis, per the method, under the supervisor's direct observation. The analyst repeats the procedure until it is correctly performed. The analyst's performance of the method's protocol and results are evaluated randomly over a minimum of a two week period to ensure adherence to all requirements of the method

D. Instrumentation malfunctions are immediately noted in the instrument logbook and the supervisor is notified. Senior technical staff with specific in-depth knowledge of the particular instrument reviews the problem and attempt to fix the instrument. Major problems may require trained field service personnel from the manufacturer to be brought in to fix the problem. If the projected downtime will extend beyond the samples required holding time, the sample will be either analyzed on another instrument or sent to an approved contract laboratory for analysis

E. When duplicate results, spike recovery results, or Quality Assurance reference samples are outside their acceptance limits, the supervisor is notified and the complete analytical procedure is reviewed with the analyst. The data entry and calculations are reviewed for transcription errors. Reagents and standards are checked to see if they were properly prepared and whether they are within their shelf life. The equipment is examined for proper performance. The calibration and maintenance record is reviewed to ensure the instrumentation is performing optimally. The methodology is reviewed to make sure that it is properly applied. Sampling and sample handling protocols are verified to ensure that the sample was collected properly and the recommended preservation and holding times were observed. If the cause of the problem is found, the Quality Assurance Specialist sends a Quality Assurance reference sample to the analyst for analysis. If the Quality Assurance check sample is acceptable, the duplicate or spike analysis is reanalyzed. However, if the same result is obtained in the repeat analysis, the problem is probably due to matrix interference effect. The results of the sample batch are reported with an accompanying explanation of possible matrix interference. If the precision of duplicate spike analyses improves and are in control, the sample batch run with the initial duplicate spike analysis sample is reanalyzed. A different scenario must be followed in circumstances such as insufficient sample or analysis of the sample after the prescribed holding time exists. In these situations, the original result is reported and accompanied by a failure report stating the circumstances that occurred in the initial and repeat analysis. If the results for

the Quality Assurance reference sample are not satisfactory, a team will be formed to identify and correct the problem. The analysis will not be resumed until the system is in control

F. CRG's internal evaluation and corrective action program and external agency audits can result in corrective actions. The response to these evaluation studies requires a written corrective action plan that has been accepted by the Quality Assurance Specialist. Closure requires objective evidence that the corrective action be thorough, complete, and will permanently solve the problem

G. CRG's Continuous Measurable Improvement program is designed to identify opportunities for improvements systematically. This program leads to specific corrective actions initiated by either a combination of senior technical staff and analysts or a team established to address the specific problem. A quantitative measurement is applied to ensure that the corrective action has had a positive impact on eliminating the problem.

## 14.2 Training

14.2.1 Educational background- the minimum qualification for conducting analyses in the laboratory is two years of college-level course work in science and two years of related analytical work experience or an equivalent combination of education and experience. These education and experience requirements provide the analysts with a proper background in the fundamentals of chemistry to assist in understanding the principles behind work that they perform.

14.2.2 Orientation- CRG provides a general orientation to working in an environmental chemistry laboratory. CRG also provides a basic safety orientation, which includes lab coats, specific safety instructions, approved footwear, location of first aid supplies, location of eyewash stations, location of emergency showers, and location of fire extinguishers.

14.2.3 Ongoing Training- CRG maintains a technical library of key journals and books for staff's use. Staffs are encouraged to join professional societies, attend conferences, and receive additional training in their technical fields.

14.2.4 Discrete Job Training- CRG Provides:

A. On-the-job training to new analysts or analysts assuming additional responsibilities.

B. Maintains a file for each employee which contains all information relating to the analysts education and training including:

Resume

Certificates from training classes and courses

Completed Training Documentation Forms

Related data

C. The following approach is used for providing staff on-the-job training:

1. Read the appropriate Laboratory Operating Procedures Method which details the analytical procedure

2. Review the associated material safety data sheets if you are not knowledgeable of the

safety hazards of the reagents used in the analysis

3. Observe the procedure in use by an analyst who is approved for performing this analysis
4. Perform the analysis under the direct supervision of a qualified analyst who will certify the successful completion of training
5. Demonstrate proficiency using the method by analyzing blind check samples
6. Document the successful completion of your training using the following Training Documentation Form:

CRG Marine Laboratories, Inc.  
2020 Del Amo Boulevard, Suite 2020  
Torrance, California 90501-1206

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**TRAINING DOCUMENTATION FORM**

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EMPLOYEE NAME \_\_\_\_\_

METHOD NUMBER	DATE COMPLETED	CERTIFIED BY

COMMENTS:

[Large empty rectangular box for entering training comments]

## 15.0 QA REPORTS

Numerical results of quality control analyses are delivered as part of the analytical report package. Reports that discuss corrective actions, Quality accomplishments, control charts, and ad-hoc inquiries are generated internally on a regular basis and made available to clients upon request.

**Table 1. Metals, Organic Chemistry and Inorganic Chemistry**

EPA METHOD	ANALYSIS	PRECISION (% RSD)	ACCURACY (% Recovery)	MDL
<b>METALS BY ICPMS- LIQUID MATRIX</b>				
200.8,	Aluminum (Al)	0-30	70-130	10 µg/L
6010	Antimony (Sb)	0-30	70-130	1.0 µg/L
	Arsenic (As)	0-30	70-130	1.0 µg/L
	Barium (Ba)	0-30	70-130	1.0 µg/L
	Beryllium (Be)	0-30	70-130	1.0 µg/L
	Boron (B)	0-30	70-130	1.0 µg/L
	Cadmium (Cd)	0-30	70-130	1.0 µg/L
	Calcium (Ca)	0-30	70-130	1.0 µg/L
	Chromium (Cr)	0-30	70-130	1.0 µg/L
	Cobalt (Co)	0-30	70-130	1.0 µg/L
	Copper (Cu)	0-30	70-130	1.0 µg/L
	Iron (Fe)	0-30	70-130	10 µg/L
	Lead (Pb)	0-30	70-130	1.0 µg/L
	Magnesium (Mg)	0-30	70-130	1.0 µg/L
	Manganese (Mn)	0-30	70-130	1.0 µg/L
	Mercury (Hg)	0-30	70-130	1.0 µg/L
	Molybdenum (Mo)	0-30	70-130	1.0 µg/L
	Nickel (Ni)	0-30	70-130	1.0 µg/L
	Selenium (Se)	0-30	70-130	1.0 µg/L
	Silver (Ag)	0-30	70-130	1.0 µg/L
	Thallium (Tl)	0-30	70-130	1.0 µg/L
	Vanadium (V)	0-30	70-130	1.0 µg/L
	Zinc (Zn)	0-30	70-130	1.0 µg/L
<b>METALS BY ICPMS- SOLID MATRIX</b>				
200.8,	Aluminum (Al)	0-30	70-130	10 mg/kg
	Antimony (Sb)	0-30	70-130	0.05 mg/kg
	Arsenic (As)	0-30	70-130	0.05 mg/kg
	Barium (Ba)	0-30	70-130	0.05 mg/kg
	Beryllium (Be)	0-30	70-130	0.05 mg/kg
	Cadmium (Cd)	0-30	70-130	0.05 mg/kg
	Chromium (Cr)	0-30	70-130	0.05 mg/kg
	Cobalt (Co)	0-30	70-130	0.05 mg/kg
	Copper (Cu)	0-30	70-130	0.05 mg/kg
	Iron (Fe)	0-30	70-130	10 mg/kg
	Lead (Pb)	0-30	70-130	0.05 mg/kg
	Magnesium (Mg)	0-30	70-130	10 mg/kg
	Manganese (Mn)	0-30	70-130	0.05 mg/kg

**Table 1. (Continued)**

METHOD	ANALYSIS	PRECISION (% RSD)	ACCURACY (% Recovery)	MDL
	Mercury (Hg)	0-30	70-130	0.01 mg/kg
	Molybdenum (Mo)	0-30	70-130	0.05 mg/kg
	Nickel (Ni)	0-30	70-130	0.05 mg/kg
	Selenium (Se)	0-30	70-130	0.05 mg/kg
	Silver (Ag)	0-30	70-130	0.01 mg/kg
	Thallium (Tl)	0-30	70-130	0.05 mg/kg
	Tin (Sn)	0-30	70-130	0.05 mg/kg
	Vanadium (V)	0-30	70-130	0.05 mg/kg
	Zinc (Zn)	0-30	70-130	0.05 mg/kg

**BASE/NEUTRAL ORGANICS BY GC/MS- LIQUID MATRIX**

<b>Polynuclear Aromatic Hydrocarbons</b>				
625, 8270	1-Methylnaphthalene	0-30	70-130	5 ng/L
	1-Methylphenanthrene	0-30	70-130	5 ng/L
	2,3,5-Trimethylnaphthalene	0-30	70-130	5 ng/L
	2,6-Dimethylnaphthalene	0-30	70-130	5 ng/L
	2-Methylnaphthalene	0-30	70-130	5 ng/L
	Acenaphthene	0-30	70-130	5 ng/L
	Acenaphthylene	0-30	70-130	5 ng/L
	Anthracene	0-30	70-130	5 ng/L
	Benz[a]anthracene	0-30	70-130	5 ng/L
	Benzo[a]pyrene	0-30	70-130	5 ng/L
	Benzo[e]pyrene	0-30	70-130	5 ng/L
	Naphthalene	0-30	70-130	5 ng/L
	Fluorene	0-30	70-130	5 ng/L
	Phenanthrene	0-30	70-130	5 ng/L
	N-Nitrosodimethylamine	0-30	70-130	500 ng/L
	bis(2-Chloroethyl) ether	0-30	70-130	500 ng/L
	1,3-Dichlorobenzene	0-30	70-130	50 ng/L
	1,4-Dichlorobenzene	0-30	70-130	50 ng/L
	1,2-Dichlorobenzene	0-30	70-130	50 ng/L
	Benzyl alcohol	0-30	70-130	500 ng/L
	bis(2-Chloroisopropyl) ether	0-30	70-130	500 ng/L
	N-Nitrosodi-n-propylamine	0-30	70-130	50 ng/L
	Hexachloroethane	0-30	70-130	50 ng/L
	Nitrobenzene	0-30	70-130	100 ng/L
	Isophorone	0-30	70-130	100 ng/L
	Benzoic acid	0-30	70-130	500 ng/L
	bis(2-Chloroethoxy) methane	0-30	70-130	200 ng/L
	1,2,4-Trichlorobenzene	0-30	70-130	50 ng/L

Table 1. (continued)

METHOD	ANALYSIS	PRECISION (% RSD)	ACCURACY (% Recovery)	MDL
	4-Chloroaniline	0-30	70-130	500 ng/L
	Hexachlorobutadiene	0-30	70-130	50 ng/L
	Hexachlorocyclopentadiene	0-30	70-130	50 ng/L
	2-Chloronaphthalene	0-30	70-130	100 ng/L
	2-Nitroaniline	0-30	70-130	500 ng/L
	Dimethyl phthalate	0-30	70-130	5 ng/L
	2,6-Dinitrotoluene	0-30	70-130	50 ng/L
	3-Nitroaniline	0-30	70-130	500 ng/L
	Dibenzofuran	0-30	70-130	500 ng/L
	2,4-Dinitrotoluene	0-30	70-130	50 ng/L
	Diethyl phthalate	0-30	70-130	5 ng/L
	4-Chlorophenyl phenyl ether	0-30	70-130	50 ng/L
	4-Nitroaniline	0-30	70-130	500 ng/L
	N-Nitrosodiphenylamine	0-30	70-130	200 ng/L
	4-Bromophenyl phenyl ether	0-30	70-130	50 ng/L
	Hexachlorobenzene	0-30	70-130	50 ng/L
	Di-n-butyl phthalate	0-30	70-130	5 ng/L
	Fluoranthene	0-30	70-130	5 ng/L
	Pyrene	0-30	70-130	5 ng/L
	Butylbenzyl phthalate	0-30	70-130	5 ng/L
	3,3'-Dichlorobenzidine	0-30	70-130	50 ng/L
	bis(2-Ethylhexyl) phthalate	0-30	70-130	5 ng/L
	Chrysene	0-30	70-130	5 ng/L
	Di-n-octyl phthalate	0-30	70-130	5 ng/L
	Benzo[b]fluoranthene	0-30	70-130	5 ng/L
	Benzo[k]fluoranthene	0-30	70-130	5 ng/L
	Indeno[1,2,3-c,d]pyrene	0-30	70-130	5 ng/L
	Dibenz[a,h]anthracene	0-30	70-130	5 ng/L
	Benzo[g,h,i]perylene	0-30	70-130	5 ng/L

**ACID EXTRACTABLE ORGANICS BY GC/MS- LIQUID MATRIX**

625,	Phenol	0-30	70-130	100 ng/L
8270	2-Chlorophenol	0-30	70-130	50 ng/L
	2-Methylphenol	0-30	70-130	100 ng/L
	4-Methylphenol	0-30	70-130	100 ng/L
	2-Nitrophenol	0-30	70-130	100 ng/L
	2,4-Dimethylphenol	0-30	70-130	200 ng/L
	2,4-Dichlorophenol	0-30	70-130	50 ng/L
	4-Chloro-3-methylphenol	0-30	70-130	100 ng/L
	2,4,6-Trichlorophenol	0-30	70-130	50 ng/L

**Table 1. (continued)**

METHOD	ANALYSIS	PRECISION (% RSD)	ACCURACY (% Recovery)	MDL
	2,4,5-Trichlorophenol	0-30	70-130	50 ng/L
	2,4-Dinitrophenol	0-30	70-130	200 ng/L
	4-Nitrophenol	0-30	70-130	100 ng/L
	2-Methyl-4,6-dinitrophenol	0-30	70-130	500 ng/L
	Pentachlorophenol	0-30	70-130	50 ng/L

**BASE/NEUTRAL ORGANICS BY GC/MS- SOLID MATRIX**

8270	N-Nitrosodimethylamine	0-30	70-130	200 ng/g
	bis(2-Chloroethyl) ether	0-30	70-130	200 ng/g
	1,3-Dichlorobenzene	0-30	70-130	50 ng/g
	1,4-Dichlorobenzene	0-30	70-130	50 ng/g
	1,2-Dichlorobenzene	0-30	70-130	50 ng/g
	Benzyl alcohol	0-30	70-130	200 ng/g
	bis(2-Chloroisopropyl) ether	0-30	70-130	200 ng/g
	N-Nitrosodi-n-propylamine	0-30	70-130	200 ng/g
	Hexachloroethane	0-30	70-130	50 ng/g
	Nitrobenzene	0-30	70-130	50 ng/g
	Isophorone	0-30	70-130	100 ng/g
	Benzoic acid	0-30	70-130	200 ng/g
	bis(2-Chloroethoxy) methane	0-30	70-130	200 ng/g
	1,2,4-Trichlorobenzene	0-30	70-130	50 ng/g
	Naphthalene	0-30	70-130	10 ng/g
	4-Chloroaniline	0-30	70-130	200 ng/g
	Hexachlorobutadiene	0-30	70-130	200 ng/g
	Hexachlorocyclopentadiene	0-30	70-130	200 ng/g
	2-Chloronaphthalene	0-30	70-130	200 ng/g
	2-Nitroaniline	0-30	70-130	100 ng/g
	Dimethyl phthalate	0-30	70-130	10 ng/g
	2,6-Dinitrotoluene	0-30	70-130	50 ng/g
	Acenaphthylene	0-30	70-130	10 ng/g
	3-Nitroaniline	0-30	70-130	200 ng/g
	Acenaphthene	0-30	70-130	10 ng/g
	Dibenzofuran	0-30	70-130	200 ng/g
	2,4-Dinitrotoluene	0-30	70-130	50 ng/g
	Diethyl phthalate	0-30	70-130	10 ng/g
	4-Chlorophenyl phenyl ether	0-30	70-130	50 ng/g
	Fluorene	0-30	70-130	10 ng/g
	4-Nitroaniline	0-30	70-130	50 ng/g
	N-Nitrosodiphenylamine	0-30	70-130	200 ng/g

Table 1. (continued)

METHOD	ANALYSIS	PRECISION (% RSD)	ACCURACY (% Recovery)	MDL
	4-Bromophenyl phenyl ether	0-30	70-130	100 ng/g
	Hexachlorobenzene	0-30	70-130	50 ng/g
	Phenanthrene	0-30	70-130	10 ng/g
	Anthracene	0-30	70-130	10 ng/g
	Di-n-butyl phthalate	0-30	70-130	10 ng/g
	Fluoranthene	0-30	70-130	10 ng/g
	Pyrene	0-30	70-130	10 ng/g
	Butylbenzyl phthalate	0-30	70-130	10 ng/g
	3,3'-Dichlorobenzidine	0-30	70-130	200 ng/g
	Benz[a]anthracene	0-30	70-130	10 ng/g
	bis(2-Ethylhexyl) phthalate	0-30	70-130	10 ng/g
	Chrysene	0-30	70-130	10 ng/g
	Di-n-octyl phthalate	0-30	70-130	10 ng/g
	Benzo[b]fluoranthene	0-30	70-130	10 ng/g
	Benzo[k]fluoranthene	0-30	70-130	10 ng/g
	Benzo[a]pyrene	0-30	70-130	10 ng/g
	Indeno[1,2,3-c,d]pyrene	0-30	70-130	10 ng/g
	Dibenz[a,h]anthracene	0-30	70-130	10 ng/g
	Benzo[g,h,i]perylene	0-30	70-130	10 ng/g

**ACID EXTRACTABLE ORGANICS BY GC/MS- SOLID MATRIX**

8270	Phenol	0-30	70-130	200 ng/g
	2-Chlorophenol	0-30	70-130	200 ng/g
	2-Methylphenol	0-30	70-130	200 ng/g
	4-Methylphenol	0-30	70-130	200 ng/g
	2-Nitrophenol	0-30	70-130	200 ng/g
	2,4-Dimethylphenol	0-30	70-130	200 ng/g
	2,4-Dichlorophenol	0-30	70-130	200 ng/g
	4-Chloro-3-methylphenol	0-30	70-130	200 ng/g
	2,4,6-Trichlorophenol	0-30	70-130	200 ng/g
	2,4,5-Trichlorophenol	0-30	70-130	200 ng/g
	2,4-Dinitrophenol	0-30	70-130	200 ng/g
	4-Nitrophenol	0-30	70-130	200 ng/g
	2-Methyl-4,6-dinitrophenol	0-30	70-130	200 ng/g
	Pentachlorophenol	0-30	70-130	200 ng/g

**Table 1. (continued)**

METHOD	ANALYSIS	PRECISION (% RSD)	ACCURACY (% Recovery)	MDL
<b>CHLORINATED HYDROCARBONS BY GC/ECD- LIQUID MATRIX</b>				
608, 8080	α-BHC	0-30	70-130	2 ng/L
	β-BHC	0-30	70-130	2 ng/L
	γ-BHC (Lindane)	0-30	70-130	2 ng/L
	δ-BHC	0-30	70-130	2 ng/L
	Heptachlor	0-30	70-130	2 ng/L
	Aldrin	0-30	70-130	2 ng/L
	Heptachlor epoxide	0-30	70-130	2 ng/L
	Endosulfan I	0-30	70-130	2 ng/L
	4,4'-DDE	0-30	70-130	2 ng/L
	Dieldrin	0-30	70-130	2 ng/L
	Endrin	0-30	70-130	2 ng/L
	Endosulfan II	0-30	70-130	2 ng/L
	4,4'-DDD	0-30	70-130	2 ng/L
	Endrin aldehyde	0-30	70-130	2 ng/L
	Endosulfan sulfate	0-30	70-130	2 ng/L
	4,4'-DDT	0-30	70-130	2 ng/L
	Methoxychlor	0-30	70-130	8 ng/L
	Chlordane	0-30	70-130	10 ng/L
	Toxaphene	0-30	70-130	10 ng/L
	PCB-1016	0-30	70-130	10 ng/L
	PCB-1221	0-30	70-130	10 ng/L
PCB-1232	0-30	70-130	10 ng/L	
PCB-1242	0-30	70-130	10 ng/L	
PCB-1248	0-30	70-130	10 ng/L	
PCB-1254	0-30	70-130	10 ng/L	
PCB-1260	0-30	70-130	10 ng/L	

<b>CHLORINATED HYDROCARBONS BY GC/ECD- SOLID MATRIX</b>				
8080	α-BHC	0-30	70-130	2 µg/kg
	β-BHC	0-30	70-130	2 µg/kg
	γ-BHC (Lindane)	0-30	70-130	2 µg/kg
	δ-BHC	0-30	70-130	2 µg/kg
	Heptachlor	0-30	70-130	2 µg/kg
	Aldrin	0-30	70-130	2 µg/kg
	Heptachlor epoxide	0-30	70-130	2 µg/kg
	Endosulfan I	0-30	70-130	2 µg/kg
	4,4'-DDE	0-30	70-130	2 µg/kg
	Dieldrin	0-30	70-130	2 µg/kg

**Table 1. (continued)**

METHOD	ANALYSIS	PRECISION (% RSD)	ACCURACY (% Recovery)	MDL
<b>CHLORINATED HYDROCARBONS BY GC/ECD- SOLID (CONT.)</b>				
	Endrin	0-30	70-130	2 µg/kg
	Endosulfan II	0-30	70-130	2 µg/kg
	4,4'-DDD	0-30	70-130	2 µg/kg
	Endrin aldehyde	0-30	70-130	2 µg/kg
	Endosulfan sulfate	0-30	70-130	2 µg/kg
	4,4'-DDT	0-30	70-130	2 µg/kg
	Methoxychlor	0-30	70-130	8 µg/kg
	Chlordane	0-30	70-130	10 µg/kg
	Toxaphene	0-30	70-130	10 µg/kg
	PCB-1016	0-30	70-130	10 µg/kg
	PCB-1221	0-30	70-130	10 µg/kg
	PCB-1232	0-30	70-130	10 µg/kg
	PCB-1242	0-30	70-130	10 µg/kg
	PCB-1248	0-30	70-130	10 µg/kg
	PCB-1254	0-30	70-130	10 µg/kg
	PCB-1260	0-30	70-130	10 µg/kg

MDL: Method Detection Limits



## APPENDIX C: ACRONYMS

BMP	Best Management Practice
DQO	Data Quality Objectives
ELAP	Environmental Laboratory Accreditation Program
ED	Endocrine Disruptors
EDC	Endocrine Disrupting Compounds
ELISA	Enzyme linked immunosorbent assay method
LARWQCB	Los Angeles Regional Water Quality Control Board
LCS	Laboratory Control Standard
LOPM	Laboratory Operating Procedures Manual
MS	Matrix Spike
MS/MSD	Matrix Spike/Matrix Spike Duplicate
RPD	Relative Percent Difference
SCCWRP	Southern California Coastal Water Research Project
SWRCB	California State Water Resources Control Board
VTG	Vitellogenin