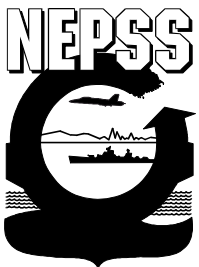




# Marine Environmental Update



## New Wetlands Protection Rule Issued

In a press release issued April 17, 2001, the Environmental Protection Agency announced an action taken jointly with the Army Corps of Engineers, which clarifies that wetlands are protected from many types of discharges that have contributed to the loss of wetlands in the United States.

Under the Clean Water Act, discharges into the waters of the United States require a permit. However, in 1997 the U.S. District Court for the District of Columbia ruled that a 1993 regulation, known as the "Tulloch Rule," should not have extended to certain discharges even when associated with activities that contribute to the loss of wetlands. Such activities can include mechanized land clearing, ditching, and channelization under certain circumstances. The U.S. Court of Appeals affirmed that court decision in June 1998. Uncertainty regarding the scope of this decision is considered to be a contributor to the destruction of many wetlands. The new rule protects wetlands by moving forward with a rule clarifying what discharges are subject to environmental review under the Clean Water Act.

This "new" rule was originally published in the Federal Register on January 17, 2001 (see *Marine Environmental Update*, Vol. FY01 No. 2). The final rule by EPA and the Corps of Engineers enhances protection of the Nation's aquatic resources, including

### ALSO IN THIS ISSUE:

<a href="#">EPA Releases Aquatic Life Criteria Update for Cadmium</a> .....	2
<a href="#">Streamlined EPA WER Procedure for Copper Discharges</a> .....	3
<a href="#">Storm Water MSGP for Industrial Activities Correction Issued</a> .....	5
<a href="#">FY2000 CNO Environmental Award Winners</a> .....	5
<a href="#">FY2000 SECNAV Environmental Awards</a> .....	8
<a href="#">FY2000 Defense Environmental Security Awards</a> .....	12
<a href="#">Rockfish and Pacific Herring Puget Sound Population Status Review</a> .....	14
<a href="#">White Abalone Listed as Endangered</a> .....	15
<a href="#">Critical Habitat for Riverside Fairy Shrimp Designated</a> .....	16
<a href="#">USCG Proposes Ballast Water Treatment Standard Approaches</a> .....	17
<a href="#">EPA State and Tribal WQS Approval Notice</a> .....	20
<a href="#">CA TMDL Program Structure, Effectiveness Report</a> .....	20
<a href="#">Analytical Chemistry Quality Requirements for EcoRisk Assessments</a> .....	23
<a href="#">Screening-Level ELISA Determination of PCBs in Seawater</a> .....	24
About the <i>Marine Environmental Update</i> .....	28

Read me on-line at: <http://meso.spawar.navy.mil/Newsltr>



SPAWAR  
Systems Center  
San Diego

wetlands, by clarifying those types of activities that are likely to result in a discharge of dredged material subject to Clean Water Act Section 404. The final rule modifies the definition of “discharge of dredged material” by clarifying what types of activities the EPA and the Corps of Engineers believe typically result in regulable discharges, based on the nature of the equipment and agency experience. The rule indicates that the Corps and EPA regard the use of mechanized earth moving equipment to conduct land clearing, ditching, channelization, in-stream mining, or other earth-moving activity in waters of the U.S. as resulting in a discharge of dredged material, unless project-specific evidence shows that the activity results in only “incidental fallback.” The rule also provides a definition of what constitutes non-regulable incidental fallback that is consistent with the recent District of Columbia Circuit court decision. The original effective date of the rule was February 16, 2001, but was changed in a Federal Register notice of February 15, 2001, to April 17, 2001.

The full text of the rule, which became effective April 17, 2001, is [available from MESO](#) (539 KB Adobe™ Acrobat™ file).

[U.S. EPA Headquarters Press Release, Tuesday, April 17, 2001.](#)

*Federal Register, Volume 66, Number 11, Wednesday, January 17, 2001, pp. 4549-4575.*

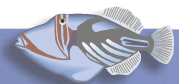
*Federal Register, Volume 66, Number 32, Thursday, February 15, 2001, p. 10367.*



## EPA Releases Aquatic Life Criteria Update for Cadmium

On April 12, 2001, the Environmental Protection Agency released its 2001 update of aquatic life criteria for cadmium (see *Marine Environmental Update*, [Vol. FY00, No. 4](#)). The freshwater CMC (Criterion Maximum Concentration) changed due to several factors including the addition of data for bull trout and rainbow trout, the elimination of some data and the recalculation of species mean acute values (SMAVs) for a few species. Two SMAVs were recalculated based on all applicable data rather than only giving preference to flow-through measured test results, as in the draft. EPA’s freshwater metals criteria are expressed as hardness dependent values because water quality characteristics such as hardness (and other parameters that vary with hardness) influence the toxicity of metals on aquatic organisms. Therefore, hardness slopes were established to normalize all freshwater acute and chronic values to the same hardness in order to derive the criteria. These hardness slopes were revised in the completed document. The revision to the acute slope was minor, but the chronic slope revision was more significant and resulted in a less stringent CCC (Criterion Continuous Concentration) compared to the draft document. The revised CCC, however, is still more stringent than EPA’s 1995 CCC.

A number of comments were received stating that the EPA should not proceed with the cadmium update until the biotic ligand model (BLM), a model that estimates the bioavailable portion of dissolved metals in the water column based on site-specific water quality parameters such as alkalinity, pH and dissolved organic carbon, is available for cadmium. To date, the EPA has not completed any BLM criteria and is still in the preliminary evaluation phase of the model for cadmium and so does not agree that the update



should wait for the development of the BLM. The cadmium criteria may be revised in the future based on the BLM, yet development is contingent upon resources and sufficient data being available to develop the model. The new criteria are shown in the following table.

	Fresh Water <sup>1</sup>		Salt Water	
	CMC (µg/L)	CCC (µg/L)	CMC (µg/L)	CCC (µg/L)
<b>Total</b>	$e^{(1.0166 [\ln (\text{hardness})] - 3.924)}$	$e^{(0.7409 [\ln (\text{hardness})] - 4.719)}$	40.28	8.846
<b>Dissolved</b>	1.0	0.15	40	8.8

Notes:  
<sup>1</sup> at 50 mg/L hardness measured as CaCO<sub>3</sub>  
 CMC conversion factor = 1.136672 – [(ln hardness)(0.041838)]  
 CCC conversion factor = 1.101672 – [(ln hardness)(0.041838)]

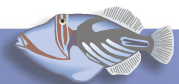
Further information can be found at <http://www.epa.gov/waterscience/criteria>. The complete text of the 2001 update of aquatic life criteria for cadmium is [available from MESO](#) (322 KB Adobe™ Acrobat™ file).

[Federal Register, Volume 66, Number 71, Thursday, April 12, 2001, pp. 18935-18936](#) (44.1 KB Adobe™ Acrobat™ file).



## EPA Releases Streamlined WER Procedure for Copper Discharges

The Environmental Protection Agency released a streamlined procedure for determining site-specific values for a Water-Effect Ratio (WER), a criteria adjustment factor accounting for the effect of site-specific water characteristics on pollutant bioavailability and toxicity to aquatic life. This guidance is intended to complement the *1994 Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (EPA-823-B-94-001). Whereas the 1994 interim procedure applies to essentially all situations for most metals, the streamlined procedure is recommended only for situations where copper concentrations are elevated primarily by continuous point source effluents. Because this is a relatively common regulatory situation, a great deal of experience is available to guide the development of a more efficient procedure. The streamlined procedure does not supersede the 1994 interim procedure, even for the limited situations to which it applies. Rather, it provides an alternative approach. In these situations the entity conducting the study may choose between using the interim procedure or using the streamlined procedure.

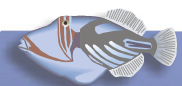




The streamlined procedure involves the sampling of two events, spaced at least one month apart. Flow during each event should be stable, and water quality unaffected by recent rainfall runoff events. Samples of effluent and upstream water are to be taken. These are mixed at the design low-flow dilution, to create a simulated downstream sample, to be used as the site-water sample in toxicity tests spiked with various concentrations of soluble copper salts. In a manner similar to the interim procedure, the side-by-side, laboratory-water and site-water toxicity tests are run to obtain the 48- hour acute EC50 with either *Ceriodaphnia dubia* or *Daphnia magna*. The result may be expressed as either dissolved or total recoverable copper. After adjusting for any hardness differences, the WER for the sample is the lesser of (a) the site-water EC50 divided by the laboratory-water EC50, or (b) the site-water EC50 divided by the documented Species Mean Acute Value. The geometric mean of the two (or more) sampling event WERs is the site WER. The design of the streamlined procedure is intended as a more efficient approach for generating the information needed to make a pollution control decision. The intent is to provide a method that is both easier for the performing organization to carry out, and easier for the regulatory agency to review. The streamlined procedure omits laboratory or field measurements that experience with the interim procedure has shown to be of little practical value. The design is also intended to be inherently less subject to random sampling variability, thereby allowing a reduction in the number of samples while maintaining reliability.

The following table shows the differences between the 1994 interim procedure and the streamlined procedure.

Characteristic	1994 Interim Procedure	Streamlined Procedure
Applicability	Universal	Copper from continuous discharges
Minimum number of sampling events	3	2, with recommended restrictions
Minimum number of WER measurements	4	2
Minimum number of WER measurements considered in obtaining final site WER	3	2
Preparation of constructed downstream water	Mix effluent and upstream samples at the dilution ratio occurring at the time of sampling	Mix effluent and upstream samples at the design low-flow dilution ratio
Calculation of sample WER	Site water LC (Lethal Concentration) ÷ Lab water LC	Site water LC ÷ The greater of: (a) Lab water LC, or (b) SMAV
Calculation of final site WER	Complicated scheme with six “if...then...else” clauses and 12 possible paths	Geometric mean of the two measurements





SPAWAR  
Systems Center  
San Diego

Further information may be found at <http://www.epa.gov/ost/standards>.

U.S. EPA Office of Water, [Streamlined Water-Effects Ratio Procedure for Discharges of Copper](#),  
EPA-822-R-01-005, March 2001 (117 KB Adobe™ Acrobat™ file).



## Correction to Final Reissuance of NPDES Storm Water MSGP for Industrial Activities Issued

On March 23, 2001, the Environmental Protection Agency published a new, corrected, version of the NPDES Storm Water Multi-Sector General Permit (MSGP), published in the Federal Register of October 30, 2000 (65 FR 64746). The October 2000 permit (see *Marine Environmental Update*, [Vol. FY01 No. 1](#)) replaced the first version issued on September 29, 1995 (60 FR 50804) and amended on February 9, 1996 (61 FR 5248), February 20, 1996 (61 FR 5248), September 24, 1996 (61 FR 50020), August 7, 1998 (63 FR 42534) and September 30, 1998 (63 FR 52430). This correction is subsequent to an initial correction notice published January 9, 2001 (66 FR 1675).

[Federal Register, Volume 66, Number 57, Friday, March 23, 2001, pp. 16233-16237](#) (235 KB Adobe™ Acrobat™ file).



## FY2000 CNO Environmental Award Winners

On May 02, 2001, the winners of the Chief of Naval Operations (CNO) Environmental Awards for FY2000 were announced. The Environmental Awards recognize ships, installations, and individuals or teams, for their exceptional environmental stewardship. Fifty-three winners and two honorable mentions were selected from a field of 99 nominees for 19 awards given in six categories. The CNO winners are:

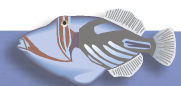
### **Natural Resources Conservation (Large Installation)**

Naval Air Station Pensacola  
Naval Security Group Activity, Northwest  
U.S. Naval Academy

### **Cultural Resources Management (Installation, Individual/Team)**

#### ***Individual/Team***

Mr. Jennings Bunn, U.S. Forces, Marianas, Guam  
Mr. Douglas P. Lister, Naval Station, Patuxent River  
Mr. James V. Sartain, Naval Surface Warfare Center  
Dahlgren Division, Coastal Systems Station





SPAWAR  
Systems Center  
San Diego

**Installation**

Commander U.S. Naval Forces Marianas

Naval Air Station Patuxent River

Naval Magazine Indian Island

**Environmental Quality (Industrial Installation, Overseas Installation)**

**Individual: Navy**

Commander Navy Region Southwest Oil Spill Working Group, Naval Aviation Depot, North Island

**Industrial Installation: Navy**

Naval Aviation Depot, Jacksonville

Pearl Harbor Naval Shipyard

Puget Sound Naval Shipyard

**Non-Industrial Installation: Navy**

Naval Security Group Activity, Northwest

Naval Undersea Warfare Division, Newport

Naval Security Group Activity, Winter Harbor

**Overseas Installation: Navy**

Commander Fleet Activities Sasebo

U.S. Naval Air Facility Atsugi

U.S. Naval Station Rota

**Large Ship: Navy**

USS ARCTIC (AOE 8)

USS BONHOMME RICHARD (LHD 6)

USS CARL VINSON (CVN 70)

**Large Ship: Honorable Mention**

USS ABRAHAM LINCOLN (CVN 72)

USS HARRY S TRUMAN (CVN 75)

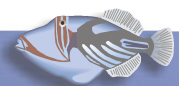
**Small Ship: Navy**

USS ANZIO (CG 68)

USS CURTS (FFG 38)

USS SAN JACINTO (CG 56)

USS WINSTON S. CHURCHILL (DDG 81)





SPAWAR  
Systems Center  
San Diego

## **Pollution Prevention (Non-Industrial Installation, Individual/Team)**

### ***Individual/Team: Navy***

Mr. Paul Cronenberger, Naval Support Activity, Souda Bay  
Mr. Awal Almasri, U.S. Naval Support Activity, Bahrain  
Field Activity Support and Tech Transfer (FASTT) Team  
Pearl Harbor Naval Shipyard  
Public Works Center Pear Harbor Solid Waste Branch Team

### ***Industrial Installation: Navy***

Naval Submarine Base Bangor  
Norfolk Naval Shipyard  
Pearl Harbor Naval Shipyard

### ***Non-Industrial Installation: Navy***

Naval Base Ventura County  
Naval Station Pascagoula  
Naval Undersea Warfare Center Division Newport

### ***Weapon System Acquisition Team: Navy***

ARLEIGH BURKE-Class Destroyer DDG 51  
H-1 Upgrades Acquisition Program  
LEWIS AND CLARK-Class (T-AKE) Project Team

## **Environmental Restoration (Installation)**

### ***Individual/Team: Navy***

Naval Weapons Industrial Reserve Plant, McGregor, TX

### ***Installation: Navy***

Naval Air Facility El Centro  
Naval Air Station Patuxent River  
Puget Sound Naval Shipyard

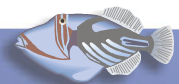
## **Recycling (Industrial, Non-Industrial)**

### ***Individual/Team: Navy***

Airborne Expendable Countermeasures Program  
Commander Navy Region, Southwest Metro Recycling Team

### ***Non-Industrial Installation: Navy***

Commander Navy Region, Southwest  
Naval Air Station, New Orleans  
Naval Air Station, Whidbey Island





**SPAWAR**  
Systems Center  
San Diego

Further information may be found at <http://web.dandp.com/n45/2000awards.html>.



## FY2000 SECNAV Environmental Awards

On May 02, 2001, the winners of the FY 2000 Secretary of the Navy (SECNAV) Environmental Awards were announced. The winners are:

### **Natural Resources Conservation (Large Installation)**

#### **NAVY**

Winner: Naval Weapons Station Charleston

Runner-Up: U.S. Naval Station Guantanamo Bay

#### **MARINE CORPS**

Winner: Marine Corps Base Camp Lejeune

Runner-Up: Marine Corps Air Station Miramar

### **Natural Resources Conservation (Small Installation)**

#### **NAVY**

Winner: Naval Air Station Pensacola

Runner-Up: Naval Security Group Activity Northwest

#### **MARINE CORPS**

Winner: Marine Corps Recruit Depot Parris Island

### **Natural Resources Conservation (Individual/Team)**

#### **NAVY**

None

#### **MARINE CORPS**

Winner: Marine Corps Recruit Depot Parris Island/Mr. Ronald E. Kinlaw

### **Cultural Resources Management (Installation)**

#### **NAVY**

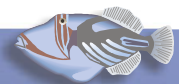
Winner: Commander U.S. Naval Forces Marianas

Runner-Up: Naval Air Station Patuxent River

#### **MARINE CORPS**

Winner: Marine Corps Base Camp Pendleton

Runner-Up: Marine Corps Base Quantico







SPAWAR  
Systems Center  
San Diego

### **Cultural Resources Management (Individual/Team)**

#### **NAVY**

Winner: Naval Air Station Patuxent River/Mr. Douglas P. Lister

Runner-Up: Commander U.S. Naval Forces Marianas/Mr. Jennings Bunn

#### **MARINE CORPS**

Winner: Marine Corps Base Hawaii/Team

Runner-Up: Marine Corps Air Ground Task Force Training Center Twenty-nine Palms/Team

### **Environmental Quality (Industrial Installation)**

#### **NAVY**

Winner: Pearl Harbor Naval Shipyard & Intermediate Maintenance Facility

Runner-Up: Naval Aviation Depot Jacksonville

#### **MARINE CORPS**

Winner: Marine Corps Air Station Cherry Point

### **Environmental Quality (Non-Industrial Installation)**

#### **NAVY**

Winner: Naval Undersea Warfare Center Division Newport

Runner-Up: Naval Security Group Activity Northwest

#### **MARINE CORPS**

Winner: Marine Corps Base Camp Lejeune

Runner-Up: Marine Corps Air Station Beaufort

### **Environmental Quality (Individual/Team)**

#### **NAVY**

Winner: WINSTON S. CHURCHILL (DDG 81) EIS Team/Team

Runner-Up: Naval Aviation Depot North Island/Mr. Theodore Beyer

#### **MARINE CORPS**

Winner: Marine Corps Recruit Depot Parris Island/Team

Runner-Up: Marine Corps Air Station Beaufort/Mr. Charles Herron

### **Environmental Quality (Overseas Installation)**

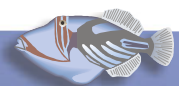
#### **NAVY**

Winner: Commander Fleet Activities Sasebo

Runner-Up: U.S. Naval Station Rota

#### **MARINE CORPS**

Winner: Marine Corps Base Camp Butler





SPAWAR  
Systems Center  
San Diego

### **Environmental Quality (Overseas Individual/Team)**

#### **NAVY**

Winner: U.S. Naval Support Activity Souda Bay/Mr. Paul Cronenberger

Runner-Up: U.S. Naval Support Activity Bahrain/Mr. Awni Almasri

#### **MARINE CORPS**

None

### **Environmental Quality (Large Ship)**

#### **NAVY**

Winner: USS CARL VINSON (CVN 70)

Runner-Up: USS BONHOMME RICHARD (LHD 6)

### **Environmental Quality (Small Ship)**

#### **NAVY**

Winner: USS SAN JACINTO (CG 56)

Runner-Up: USS ANZIO (CG 68)

### **Pollution Prevention (Industrial Installation)**

#### **NAVY**

Winner: Naval Submarine Base Bangor

Runners-Up: (Tie) Norfolk Naval Shipyard/Pearl Harbor Naval Shipyard & Intermediate Maintenance Facility

#### **MARINE CORPS**

Winner: Marine Corps Logistics Base Barstow

### **Pollution Prevention (Non-industrial Installation)**

#### **NAVY**

Winner: Naval Undersea Warfare Center Division Newport

Runner-Up: Naval Station Pascagoula

#### **MARINE CORPS**

Winner: Marine Corps Air Station Camp Pendleton

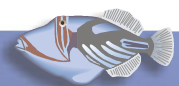
Runner-Up: Marine Corps Air Station Beaufort

### **Pollution Prevention (Individual/Team)**

#### **NAVY**

Winner: Field Activity Support And Technology Transfer Team/Team

Runner-Up: Public Works Center Pearl Harbor, Solid Waste Branch/Team





SPAWAR  
Systems Center  
San Diego

**MARINE CORPS**

Winner: Marine Corps Base Hawaii/Team

Runner-Up: Marine Corps Logistics Base Barstow/Mr. David J. Tousseau

**Pollution Prevention (Weapon System Acquisition Team)**

**NAVY**

Winner: LEWIS AND CLARK-Class Project Team

Runner-Up: H-1 Upgrades Acquisition Program Team

**MARINE CORPS**

Winner: Advanced Amphibious Assault Vehicle Program Team

**Recycling (Industrial Installation)**

**NAVY**

None

**MARINE CORPS**

Winner: Marine Corps Air Station Cherry Point

**Recycling (Non-Industrial Installation)**

**NAVY**

Winner: Commander Navy Region Southwest

Runner-Up: Naval Air Station Whidbey Island

**MARINE CORPS**

Winner: Marine Corps Base Camp Lejeune

Runner-Up: Marine Corps Recruit Depot Parris Island

**Recycling (Individual/Team)**

**NAVY**

Winner: Commander Navy Region Southwest, Metro Recycling Team/Team

Runner-Up: Airborne Expendable Countermeasures Chaff Countermeasures Recycling Program Team/Team

**MARINE CORPS**

Winner: Marine Corps Recruit Depot Parris Island/Team

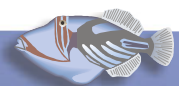
Runner-Up: Marine Corps Air Station Beaufort/Mr. James Williams

**Environmental Cleanup (Installation)**

**NAVY**

Winner: Puget Sound Naval Shipyard

Runner-Up: Naval Air Station Patuxent River





SPAWAR  
Systems Center  
San Diego

**MARINE CORPS**

Winner: Marine Corps Logistics Base Albany

Runner-Up: Marine Corps Base Camp Lejeune

**Environmental Cleanup (Individual/Team)**

**NAVY**

Winner: Naval Weapons Industrial Reserve Plant McGregor Remediation Team

**MARINE CORPS**

Winner: Marine Corps Base Camp Pendleton/Team

Runner-Up: Marine Corps Base Camp Lejeune/Team

Further information may be found at <http://www.chinfo.navy.mil/navpalib/news/envnews/Envnews.txt>.



## FY2000 Defense Environmental Security Awards

On May 03, 2001, the Department of Defense announced the eight recipients of the Department of Defense Environmental Security Awards for their outstanding achievements in natural resources conservation, cultural resources management, environmental quality, pollution prevention, and environmental restoration. The award winners are:

### Natural Resources Conservation

***Large Installation***

*Winner*

Naval Weapons Station Charleston, South Carolina

*Honorable Mention*

U.S. Army, Alaska

Marine Corps Base Camp Lejeune, North Carolina

Eglin Air Force Base, Florida

### Cultural Resources Management

***Installation***

*Winner*

US Army Air Defense Artillery Center and Fort Bliss, Texas

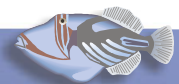
*Honorable Mention*

Marine Corps Base Camp Pendleton, California

U.S. Naval Forces Marianas, Guam

Elmendorf Air Force Base, Alaska

Defense Supply Center Richmond, Defense Logistics Agency, Virginia





**SPAWAR**  
Systems Center  
San Diego

**Individual/Team**

*Winner*

Cultural Resources Management Team, Fort McCoy, Wisconsin

*Honorable Mention*

Marine Corps Base Hawaii

Mr. Douglas P. Lister, Naval Air Station Patuxent River, Maryland

Vandenberg Air Force Base, California

**Environmental Quality**

**Industrial Installation**

*Winner*

Pearl Harbor Naval Shipyard and Intermediate Maintenance Facility, Hawaii

*Honorable Mention*

Lake City Army Ammunition Plant, Missouri

Marine Corps Air Station Cherry Point, North Carolina

Vandenberg Air Force Base, California

**Overseas Installation**

*Winner*

Marine Corps Base Camp Butler, Okinawa, Japan

*Honorable Mention*

409th Base Support Battalion, Grafenwoehr, Germany

U.S. Fleet Activities Sasebo, Japan

Aviano Air Base, Italy

**Pollution Prevention**

**Non-Industrial Installation**

*Winner*

U.S. Army Transportation Center and Fort Eustis, Virginia

*Honorable Mention*

Marine Corps Base Camp Pendleton, California

Naval Undersea Warfare Center Division, Newport, Rhode Island

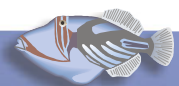
Offutt Air Force Base, Nebraska

Defense Supply Center Richmond, Defense Logistics Agency, Virginia

**Individual/Team**

*Winner*

Field Activity Support and Technology Transfer Team, Naval Sea Systems Command, Virginia



*Honorable Mention*

Pollution Prevention Action Team, Fort Bliss, Texas  
Marine Corps Base Hawaii  
Mr. R. Michael Willard, Patrick Air Force Base, Florida  
Energy Efficiency Team, Defense Commissary Agency, Virginia  
Mr. Steven J. Harris, Defense Logistics Information Service, Defense Logistics Agency, Michigan

**Environmental Restoration**

***Installation***

*Winner*

Offutt Air Force Base, Nebraska

*Honorable Mention*

Fort George G. Meade, Maryland  
Marine Corps Logistics Base, Albany, Georgia  
Puget Sound Naval Shipyard, Washington  
Defense Supply Center Philadelphia, Defense Logistics Agency, Pennsylvania  
Johnson Atoll, Defense Threat Reduction Agency, Virginia

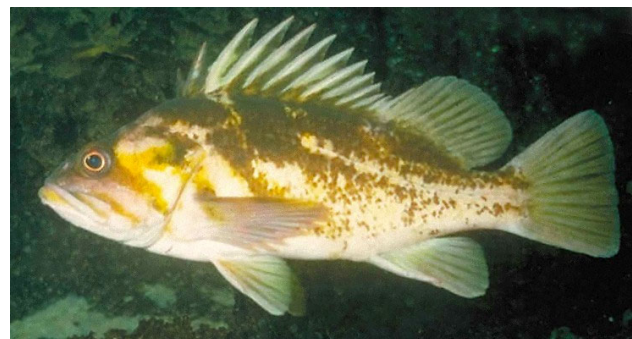
Further information may be found at

<https://www.denix.osd.mil/denix/Public/News/OSD/SecDef00/secdef00.html>.

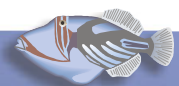


## NMFS Completes Rockfish and Pacific Herring Puget Sound Population Status Review

On April 03, 2001, the National Marine Fisheries Service (NMFS) completed a status review for copper rockfish (*Sebastes caurinus*), quillback rockfish (*S. maliger*), brown rockfish (*S. auriculatus*), and Pacific herring (*Clupea harengus pallasii*) populations in the eastern North Pacific Ocean. After reviewing the available scientific and commercial information, NMFS has determined that the petitioned populations of the three rockfish species in Puget Sound, WA, do not warrant listing as threatened or endangered at this time. NMFS also concludes that the petitioned Pacific herring populations are part of a larger



*The copper rockfish, Sebastes caurinus.*





SPAWAR  
Systems Center  
San Diego

distinct population segment (DPS) that qualifies as a species under the ESA but does not warrant listing as threatened or endangered at this time. Reference materials regarding this determination can be obtained at <http://www.nwr.noaa.gov/1salmon/salmesa/pubs.htm>.

[Federal Register, Volume 66, Number 64, Tuesday, April 03, 2001, pp. 17659-17668](#) (75.7 KB Adobe™ Acrobat™ file).



## White Abalone Listed as Endangered

On May 29, 2001, National Marine Fisheries Service published a final rule listing white abalone (*Haliotis sorenseni*) as an endangered species. The NMFS has decided it is not prudent to designate critical habitat because identification of such habitat is expected to increase the threat of poaching for white abalone. The proposed rule to list white abalone as an endangered species was issued on May 05, 2000 (65 FR 26167) and came as a result of the white abalone status review and an evaluation of the factors affecting the species.

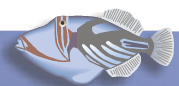
The available white abalone landings data and analysis of fishery-independent data indicate that over the last thirty years, white abalone has declined in abundance by over 99%. Some factors affecting white abalone include:

- The present or threatened destruction, modification, or curtailment of its habitat or range;
- Over-utilization for commercial, recreational, scientific, or educational purposes;
- Disease or predation;
- Inadequacy of existing regulatory mechanisms; and
- Other man-made factors affecting its continued existence.

Most of the remaining survivors are old and so scattered that they will be unable to find mates to spawn successfully and regularly produce viable cohorts of juveniles. While the NMFS recognizes that many of the existing conservation measures help protect the remaining white abalone, they do not yet provide for white abalone conservation on a scale that is adequate to protect the species and that they are in danger of extinction throughout all or a significant portion of their range and therefore warrant listing as an endangered species throughout its range in the United States and Mexico.

Activities that the NMFS believes could potentially harm white abalone, and result in violation of Endangered Species Act Section 9 take prohibitions include, but are not limited to:

- Coastal development that adversely affects white abalone (*i.e.*, dredging);
- Destruction/alteration of white abalone habitat, such as harvesting of algae;
- Discharges or dumping of toxic chemicals or other pollutants (sewage, oil, gasoline) into areas supporting white abalone;





SPAWAR  
Systems Center  
San Diego

- Interstate and foreign commerce of white abalone and import/export of white abalone without a permit; and
- Collecting or handling of white abalone in the United States.

The effective date of this rule is June 28, 2001. The complete text of *Status Review of White Abalone (Haliotis sorenseni) Throughout its Range in California and Mexico* is [available from MESO](#) (2.15 MB Adobe™ Acrobat™ file).

[Federal Register, Volume 66, Number 103, Tuesday, May 29, 2001, pp. 29046-29055](#) (73.7 KB Adobe™ Acrobat™ file).



## Critical Habitat for Riverside Fairy Shrimp Designated

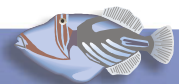
On May 30, 2001, the Fish and Wildlife Service designated critical habitat for the Riverside fairy shrimp (*Streptocephalus woottoni*), pursuant to the Endangered Species Act of 1973, as amended (see *Marine Environmental Update Bulletin*, [September 21, 2000](#)). A total of approximately 2,790 hectares (6,870 acres) in Los Angeles, Orange, Riverside, San Diego, and Ventura counties, California, is designated as critical habitat. The areas designated as critical habitat currently provide all of those habitat components necessary to meet the primary biological needs of the Riverside fairy shrimp, as described in the Recovery Plan, and defined by the primary constituent elements.

Of the five habitat components in the final rule, the North San Diego County Critical Habitat Unit, San Diego County, California (Map Unit 4, 372 Ha (920 Ac)), includes essential vernal pool habitat and associated watersheds at Marine Corps Base Camp Pendleton and one pool complex within the City of Carlsbad. This unit encompasses approximately 312 ha (770 ac) in non-training areas within Camp Pendleton. These include pool complexes and lands within the associated watersheds in the Wire Mountain Housing Area, within the Cocklebur Sensitive Area, and lands leased to the State of California and included within San Onofre State Park.

The FWS removed the original proposed Marine Corps Air Station, Miramar critical habitat unit from the final rule due to the completion and approval of MCAS Miramar's Integrated Natural Resources Management Plan.

Activities that may appreciably diminish the value of critical habitat to the degree that they affect the survival and recovery of the Riverside fairy shrimp and may be considered an adverse modification of critical habitat include, but are not limited to:

1. Any activity, including the regulation of activities by the Corps of Engineers under section 404 of the CWA or activities carried out by or licensed by the U.S. Environmental Protection Agency, that could alter the watershed, water quality or quantity to an extent that water quality becomes unsuitable to support Riverside fairy shrimp, or any activity that significantly affects the natural hydrologic function of the vernal pool system and/or ephemeral pond or depression;







SPAWAR  
Systems Center  
San Diego

2. Road construction and maintenance, right-of-way designation, and regulation of agricultural activities, or any activity funded or carried out by the Department of Transportation or Department of Agriculture that results in discharge of dredged or fill material, excavation, or mechanized land clearing of ephemeral and/or vernal pool basins;
3. Regulation of airport improvement or maintenance activities by the Federal Aviation Administration;
4. Military training and maneuvers on Camp Pendleton and Miramar, and other applicable Department of Defense lands; and
5. Construction of roads and fences along the international border with Mexico, and associated immigration enforcement activities by the Immigration and Naturalization Service; and
6. Licensing of construction of communication sites by the Federal Communications Commission.

The effective date of this rule is June 29, 2001.

[Federal Register, Volume 66, Number 104, Wednesday, May 30, 2001, pp. 29383-29414](#) (563 KB Adobe™ Acrobat™ file).



## NMFS Issues Regulations Governing Humpback Whale Approaches in AK Waters

On May 31, 2001, the National Marine Fisheries Service issued a final rule to establish measures to protect humpback whales, *Megaptera novaeangliae*, in waters within 200 nautical miles (370.7 km) of Alaska. Under these regulations it is unlawful for a person subject to the jurisdiction of the United States to approach, by any means, with some exceptions, within 100 yards (91.4 km) of a humpback whale.

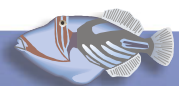
The complete text of this rule is [available from MESO](#) (62.4 KB Adobe™ Acrobat™ file).

*Federal Register, Volume 66, Number 105, Thursday, May 31, 2001, pp. 29502-29508.*



## USCG Releases Potential Ballast Water Treatment Standard Approaches

On May 01, 2001, the U.S. Coast Guard requested comments on four potential approaches to setting standards for Ballast Water Treatment and on several specific questions related to setting, implementing, and enforcing such standards. Following discussions within the Workgroup and within the Ballast Water and Shipping Committee, the following options (in no order of preference) have been identified:



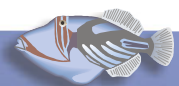


SPAWAR  
Systems Center  
San Diego

1. The standard should be based on the theoretical effectiveness of ballast water exchange (BWE) in replacing water (100% for empty-refill exchange (ERE) and 95% for flow-through exchange (FTE)).
2. The standard should be set as equivalent to the measured effectiveness of BWE. This effectiveness could be expressed as an average across all vessel types and all taxa, as a specific profile across taxonomic groups within vessel types, or as some intermediate combination of these.
3. The standard should be based on the measured capabilities of the best available technology. As in #2 (above), this level of treatment could be determined as an overall average, or within discrete groupings of vessels and taxa.
4. The standards should be based on the biological requirements, as empirically estimated or modeled, of receiving systems. The standards would be expressed as absolute concentrations of organisms from foreign waters (or dissimilar bioregions?) permissible in ballast water discharged in U.S. waters.

In addition to the specific options identified above, it is the committee's opinion that the following elements would be important components of a solicitation for comment:

1. Background description of the need for and legislative context around the standard (National Invasive Species Act (NISA) narrative standard, International Maritime Organization efforts).
2. Summary of the state-of-knowledge regarding BWE and technology effectiveness.
3. Delineation of the concept of "the standard" vis-à-vis the terms within which the standard may be applied (*i.e.*, required technology performance *vs.* grandfathering provisions, timing of the review and revision of the standard, *etc.*).
4. Outline fundamental approaches to setting the standard:
  - a. Approaches based on BWE, as currently specified by congress, under NISA:
    - Standard based on the theoretical effectiveness of BWE in replacing water (100% for ERE and 95% for FTE).
    - Standard set as equivalent to the measured effectiveness of BWE. This effectiveness could be expressed as an average across all vessel types and all taxa, as a specific profile across taxonomic groups within vessel types, or as some intermediate combination of these.
  - b. Approaches not related to BWE, but which are used in other standard-setting efforts:
    - Standard based on the measured capabilities of the best available technology. As in #2, this level of treatment could be determined as an overall average, or within discrete groupings of vessels and taxa.
    - Standards based on the biological requirements, as empirically estimated or modeled, of receiving systems.

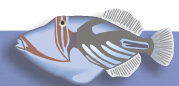




SPAWAR  
Systems Center  
San Diego

5. Solicit detailed input on the above options for standards in general, and for issues related to setting (quantifying) and implementing standards for ballast water discharges, including:
  - a. Issues related to setting the standard:
    - BWE as the basis for the standard, as opposed to other bases, such as best available technology or the biological capacity of the receiving systems.
    - BWE is the basis, the metric used to quantify effectiveness (*i.e.*, the theoretical effectiveness of exchange, the water volume exchanged as estimated with physical/chemical markers, or the biological effectiveness as measured with biological markers.
    - The specificity in determining effectiveness of either BWE or best available technology (*i.e.*, for each vessel, vessel class, or across all vessels).
    - Consideration of the probability of safe and effective BWE (including vessels declaring No Ballast On Board) in estimating the effectiveness of BWE.
    - Use of the absolute concentrations of organisms *vs.* the percent inactivation or removal of organisms.
  - b. Issues related to implementing the standard:
    - Different initial standards for existing and yet-to-be-built vessels.
    - Incremental refinements (quantitative level or taxonomic breadth) in the standard over time.
    - The period of approvals and the timing of revisions.
    - If Best Available Technology is the basis for standards, the definitions of “best” and “available.”
    - The use of indicators to characterize or monitor effectiveness, such as:
      - A single organism type (like dinoflagellate cysts) that serves as a lone indicator of effectiveness.
      - A limited set of indicators representative of near-coastal zooplankton.
      - Phytoplankton and bacteria that provide a profile of effectiveness across broad taxonomic groupings.
      - Physical surrogates for organisms, such as microspheres, that mimic the passive entrainment of organisms in water.
      - The percent reduction in all organisms regardless of type (as measured through ATP reduction, for example), providing a blanket estimate of system effectiveness.
      - Other methods of characterizing the effectiveness of BWM measures that could be alternatives to the above list.
6. Invite open-ended discussion of these or other issues relative to setting and implementing a standard for ballast water treatment.

The *Summary and Recommendations On Ballast Water Discharge Standards From the Ballast Water and Shipping Committee To the Aquatic Nuisance Species Taskforce* is [available from MESO](#) (751 KB Adobe™ Acrobat™ file). Comments are due on or before July 02, 2001. For further information contact





**SPAWAR**  
Systems Center  
San Diego

Dr. Richard Everett, Project Manager, Office of Operating and Environmental Standards (GMSO), Coast Guard, (202) 267-0214. For questions on viewing or submitting material to the docket, contact Dorothy Beard, Chief, Dockets, Department of Transportation, (202) 366-9329.

[Federal Register, Volume 66 Number 84, Tuesday, May 01, 2001, pp. 21807-21809](#) (43.6 KB Adobe™ Acrobat™ file).



## EPA Issues State and Tribal Water Quality Standards Approval Notice; Internet Repository Available

On June 04, 2001, the Environmental Protection Agency issued a listing of State and Tribal submissions of new or revised water quality standards that the EPA approved during the period April 01, 1998, through May 30, 2000. It also contains a list of EPA actions to promulgate or remove Federal water quality standards during the same period. For each EPA approval action, this document provides a reference to the State's or Tribe's regulations that contain the State and Tribal water quality standards, followed by the date of State and Tribal adoption and/or effectiveness, the date of EPA approval, and a brief description of the EPA's approval. Additionally, this notice contains a listing of Tribes that have obtained EPA approval to administer a water quality standards program. It also contains a listing of federal water quality standards rulemakings.

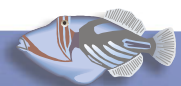
The EPA also announced the availability of an Internet repository for all water quality standards effective under the Clean Water Act. The public may view the effective Federal, State, Territory, and Tribal water quality standards at <http://www.epa.gov/ost/wqs>. This Internet repository will be updated periodically to include new and revised water quality standards approved by the EPA.

[Federal Register, Volume 66, Number 107, Monday, June 04, 2001, pp. 29951-29962](#) (76.4 KB Adobe™ Acrobat™ file).



## AB 982 PAG Releases CA TMDL Program Structure, Effectiveness Report

On April 02, 2001, the Co-Chairs of the Assembly Bill (AB) 982 Public Advisory Group (PAG) released a report that aims at providing the perspectives of a wide-range of stakeholders on efforts by the State of California to develop and implement Total Maximum Daily Loads (TMDLs) under Section 303(d) of the Clean Water Act, as amended, in California. The report contained seven summary recommendations for improving the State's implementation of the TMDL program and furthering the goal of attaining water quality standards throughout the State. The report is organized by issues as they arise in the TMDL process: (1) listing of impaired waterbodies; (2) TMDL development by Regional Water Quality Control





SPAWAR  
Systems Center  
San Diego

Boards; (3) implementation of TMDLs; and (4) assessing future effectiveness of the TMDL Program. The PAG report contained the following recommendations:

- The Legislature and the Governor should dramatically increase resources available to the State Water Resources Control Board (SWRCB) and the Regional Water Quality Control Boards (RWQCBs) in order to implement the TMDL Program in California.
- The Governor, working cooperatively with the California Congressional Delegation, should aggressively pursue additional federal funds to assist in the implementation of the TMDL Program in California.
- The SWRCB should commit to the effective and timely implementation of the TMDL Program and, to further that goal, should improve both the pace at which TMDLs are developed as well as the quality of information on which they are based.
- Through implementation of a variety of means recommended by the PAG, the SWRCB should assume greater responsibility for assuring that SWRCB and RWQCB staff have sufficient technical expertise at their disposal to efficiently develop high quality TMDLs.
- The PAG's recommendations related to the Surface Water Ambient Monitoring Program (see following article) for the State of California should be implemented immediately.
- Taking advantage of the Internet and other information technology, the SWRCB should assure that information generated from monitoring and TMDL related programs is readily accessible to the extent permissible by law.
- The SWRCB should better coordinate with other agencies where needed to assure full implementation of TMDLs.

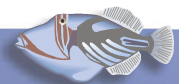
The complete text of the PAG report is [available from MESO](#) (152 KB Adobe™ Acrobat™ file).

*AB 982 Public Advisory Group, Report on the Structure and Effectiveness of California's Efforts to Develop Total Daily Maximum Loads (TMDLs) to Restore Impaired Waters and Recommendations for Future Policy Development, Presented to the State Water Resources Control Board, February, 2001.*



## CA SWRCB Proposes Comprehensive Surface Water Quality Monitoring Program

California Assembly Bill (AB) 982 (Water Code Section 13192; Statutes of 1999) requires the State Water Resources Control Board (SWRCB) to assess and report on the State monitoring programs and to prepare a proposal for a comprehensive surface water quality monitoring program. Ambient monitoring is independent of the water quality programs and serves as a measure of (1) the overall quality of water resources and (2) the overall effectiveness of Regional Water Quality Control Board (RWQCB) prevention, regulatory, and remedial actions. Current monitoring and assessment capability at the SWRCB is limited and tends to be focused on specific program needs. This has led to a fragmentation of





**SPAWAR**  
**Systems Center**  
**San Diego**

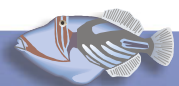
monitoring efforts resulting in gaps in needed information and a lack of integrated analyses. The SWRCB issued a monitoring program proposal designed to address a number of programmatic objectives focused on assessing the quality of the beneficial uses of the State's water resources. Some of these objectives may be satisfied with the information produced by existing monitoring efforts. However, the SWRCB proposes to restructure the existing water quality monitoring programs into a new program, the Surface Water Ambient Monitoring Program (SWAMP).

The major proposed activities of SWAMP are described below.

- The SWRCB will implement comprehensive environmental monitoring focused on providing the information the SWRCB and RWQCBs need to manage effectively the State's water resources. This will be an umbrella program that monitors and interprets data for each hydrologic unit at least one time every five years. This program shall focus on all waters of the State without bias to known impairment.
- The program will have consistent monitoring methods with respect to sampling and analysis, data quality objectives, and centralized reporting requirements. Furthermore, the monitoring efforts implemented through SWAMP will be: adaptable to changing circumstances, built on cooperative efforts, established to meet clear monitoring objectives, inclusive of already available information, implemented using scientifically sound monitoring design with meaningful indicators of water quality, comparable methods, regular reporting, and data management.
- The program will focus on spatial status and temporal trends in water quality statewide. To do this the program will determine the site-specific locations, the areal extent, and temporal trends in a number of measures of the quality of water, sediments, and biota that are widely applicable throughout the State depending on the type of water body being monitored. In watersheds, the program will implement a rotating basin framework. In coastal waters, a smaller amount of probabilistic monitoring will be completed.
- The SWRCB will also develop a Water Quality Control Policy, and a means to implement the Policy, to provide listing/de-listing criteria, an approach for setting priorities, minimum data needed to list water bodies, categories of acceptable data quality, and other factors that will allow consistent implementation of the CWA Section 303(d) requirements.

The complete text of the SWAMP proposal is [available from MESO](#) (296 KB Adobe™ Acrobat™ file).

*California State Water Resources Control Board, Proposal for a Comprehensive Ambient Surface Water Quality Monitoring Program (SWAMP) Report to the Legislature, California State Waters Resources Control Board, November, 2000.*





SPAWAR  
Systems Center  
San Diego

## Specifying and Evaluating Analytical Chemistry Quality Requirements for Ecological Risk Assessments

**R.K. Johnston, Marine Environmental Support Office.**

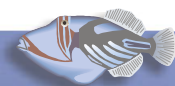
### **EXECUTIVE SUMMARY**

To achieve the goal of assessing risk to ecological systems, scientifically sound analytical chemistry data are needed. This document defines the quality assurance and quality control (QA/QC) procedures that will assure analytical chemistry data are capable of meeting the data quality objectives required for ecological risk assessments. For analysis of parts-per-billion levels of organic and inorganic contaminants in samples of water, sediment, and wildlife tissues (fish, birds, mammals, invertebrates, and plants) collected from estuarine and marine ecosystems, specialized methodologies are required that are more “research” oriented than routine methods that are generally available. A performance-based quality assurance program is described that requires the performing laboratory to demonstrate proficiency through routine analysis of certified or well-documented reference materials. The laboratory is required to initiate corrective actions if their performance falls below minimal standards.

Any analytical chemistry data produced for an ecological risk assessment must be of sufficient quality to satisfy the intended use of the data. The philosophy of the performance-based approach presented in this guide is that as long as proper QA/QC requirements are implemented and comparable analytical performance on standard materials is demonstrated, multiple procedures for the analysis of different compound classes used by different laboratories should yield comparable results. Performance-based QA/QC requirements are defined which require the use of accuracy materials (*e.g.*, certified or standard reference materials and laboratory control materials), calibration standards, method blanks, matrix spike samples, laboratory duplicates, internal standards, injection standards, and interlaboratory calibrations.

This guide is applicable to low parts-per-billion analyses of water, sediment, and tissue samples, unless otherwise noted. If implemented in a consistent manner, this protocol will provide the information necessary to verify the quality of the data, validate the raw data, and assess the comparability of data generated by different laboratories with different analytical procedures. The QA/QC requirements specified in this guide are the minimum requirements for any given analytical method. Additional method-specific requirements should always be followed, as long as the minimum requirements have been met.

The complete text of this guide is [available from MESO](#) (1.28 MB Adobe™ Acrobat™ file).





SPAWAR  
Systems Center  
San Diego

## Screening-Level Determination of Chlorinated Biphenyls in Seawater Matrices using Enzyme-linked Immunosorbent Assay (ELISA) Techniques<sup>1</sup>

Christine R. In, Joel M. Guerrero, Kristy M. Lane, and Robert D. George.

### BACKGROUND

Enzyme-linked Immunosorbent Assays (ELISAs) are effective analytical tools for quantitation of contaminants in environmental samples. The benefit of ELISA is the ability to quickly test or screen a large number of samples for an analyte of interest, which would otherwise require a series of conventional analyses with a much longer turn-around time. Because consistent technique and method protocol is the most critical part of performing an effective immunoassay, use in a laboratory setting is much more prevalent than field assays. However, field assays are becoming more possible with an appropriate level of laboratory training. ELISA kits are commercially available for a very large number of analytes, including polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs), but are designed for freshwater matrices. More recently, the need for monitoring activities along coastal areas, in estuaries, and in harbors has been increasing. These areas are generally high in salinity or are brackish, depending upon the degree of freshwater input, and the development or application of immunoassays for use in such matrices is of great interest for environmental site evaluation and management.

At a fundamental level, ELISAs work by a concentration-driven competition between sample analyte and enzyme-labeled analyte that bind to antibodies attached to a substrate, in this example, covalently bound to paramagnetic particles. Antibodies are derived from a biological immune response to an antigen, which, in the case of an environmental immunoassay, is the analyte of interest. The antibody is then used analytically to detect the competition between sample analyte and enzyme-labeled analyte. The magnetic particles are isolated, magnetically separated, and processed with a dye for detection by colorimetric means. A schematic of the immunoassay process is illustrated in Figure 1.

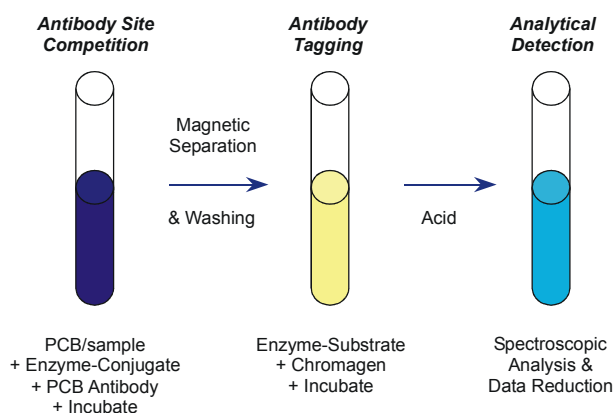


Figure 1. Illustration of Immunoassay Processing.

<sup>1</sup> Originally published in *ACS Environmental Abstracts*, 2001. For further information contact Dr. Robert George at the [Environmental Materials and Coatings Laboratory](#), Space and Naval Warfare Systems Center, Environmental Chemistry & Biotechnology – D361, San Diego, CA 92152; (619) 553-2776, DSN 553-2776.







SPAWAR  
Systems Center  
San Diego

The most convenient approach for analyte quantitation is to utilize commercial freshwater immunoassay kits with seawater samples directly. The drawback is the presence of methanol as the primary reagent in which the antibody/magnetic particles, enzyme-conjugate, and sample must coexist. Unfortunately, this leads to the precipitation of seawater salts that can act to remove analyte from solution. Thus, it is necessary to avoid conditions that lead to precipitation and this critical factor must be incorporated into all seawater immunoassay development efforts.

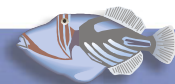
Our initial approach in this work has been to evaluate commercial freshwater ELISA kits and protocols for seawater samples in an effort to develop similar methodologies for a host of various analytes. In order to demonstrate the utility of this approach, we present preliminary results for ELISA determination of PCBs in seawater. PCBs are of particular interest for seawater leaching studies of PCB-laden solid matrices (REF A). The controlling chemistry that allows development of optimal protocols for effective ELISA response in saline matrices will be the focus of this article.

## **MATERIALS AND METHODS**

Commercial immunoassay kits were acquired from Strategic Diagnostics, Inc., and included a series of standards, a control (3 ppb Aroclor 1254 in sample diluent, a methanol-based assay reagent), assay test tubes, and associated reagents for performing immunoassays. Additional Aroclor controls at concentrations above and below 3 ppb were prepared using NIST (National Institute of Standards and Technology)-traceable Aroclor standards in methanol, which were subsequently diluted into kit-supplied diluent (methanol-based assay reagent) as appropriate. Replicate assay tubes were prepared for each standard and control in the assay. An inorganic membrane filter (Whatman™ Anotop 10 IC, 0.2 μm) was used to filter seawater samples. Spectral absorption data were collected at 450 nm using a Hach™ DR/2010 spectrophotometer and then downloaded to a computer as a text file for subsequent standard calibration curve generation, control-recovery analysis, and sample data analysis. Artificial seawater for immunoassay experiments was prepared from ACS reagent-grade salts to provide a salinity of 34 and pH of 8.0 (REF B). All laboratory glassware and sample containers were precleaned using EPA guidelines and rinsed with high purity methanol (GC-grade, 99.9+%) as a final cleaning step prior to drying *in vacuo* (60°C).

## **RESULTS AND DISCUSSION**

Preliminary immunoassay experiments performed using a freshwater ELISA kit indicated that sensitivity for Aroclor 1254 and Aroclor 1248 in seawater correlate well with results in freshwater. During this evaluation it was observed that when using a freshwater protocol, particulate formation interfered with and ultimately terminated the experiment. This is attributable to mixing of the seawater phase (sample) with the methanol phase (diluent) causing precipitation of sea salts, which likely occlude analyte and reduce the solvated analyte concentration. It is also important to note that use of a methanol-based reagent is necessary to prevent analyte adsorption onto walls of glass sample containers. Ultimately, it is necessary to 1) reduce the overall salt content in the immunoassay solution and 2) add the seawater sample to the methanol phase to avoid shocking the system by not reaching the critical salt concentration at which point precipitation occurs. Rather than decreasing the amount of sample to achieve lower salt concentration, the total amount of diluent is increased. This ensures that a sufficient amount of sample is





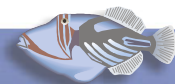
SPAWAR  
Systems Center  
San Diego

present for detection. The maximum proportion of seawater:methanol required to prevent observable precipitation was determined to be approximately 1:4, which is much less than the corresponding ratio in the freshwater protocol (1:1). Because of increased reagent volumes in the assay tubes, incubation times were increased to allow chemical reactions and magnetic separations to go to completion. The extra amount of diluent used also raises the detection limit by a dilution factor, which must ultimately be taken into consideration when calculating the sample concentration.

Using the above general observations and determinations as a guide, we began exploring different methodologies with the specific aim of developing a standard protocol for seawater samples which could be used as a direct replacement of the kit-supplied freshwater protocol. Determining the effectiveness of a particular protocol change requires experimental consistency and comparability for drawing conclusions from one data set to the next. To achieve this from kit to kit, a sample of 3 ppb control supplied with the previous assay kit is routinely processed with the new kit. Identical processing of each sample tube is likewise important for valid comparison among the tubes within an assay. This is evident in data clustering and skewing which results from cumulative differences in reagent exposure from one assay tube to the next. Consequently the preparation of replicate standards and controls involves separate, timed combinations, which must be performed according to the particular assay sequence assigned for sample processing and analysis of each assay tube.

For further consistency between assays, it is critical to evaluate the accuracy and sensitivity of each calibration curve. Additional assay controls (1.0 and 10 ppb) are prepared to evaluate detection accuracy in both the upper and lower regions of the calibration curve. Immunoassay sensitivity to analyte originating in the seawater matrix is evaluated by including a seawater matrix spike with known concentration. However, it is important to realize that a matrix spike prepared by introducing a methanol-based Aroclor standard into seawater will exhibit differential solubilities for each congener in the Aroclor mixture. Unlike a spike sample, a native seawater sample is only subjected to selective PCB dissolution from a solid matrix into a seawater matrix, similar to the process of dissolving pure (neat) Aroclor (REF A).

Accuracy of the immunoassay can be further validated by conventional quantitative analysis. When making comparisons between immunoassay and conventional analytical data, it is important to consider that the immunoassay detects total PCBs as Aroclor 1254, whereas conventional analyses by GC-mass spectrometry provide a measurement of PCB homologues (REF C). The summation of these homologues yields an empirical total of PCBs. In general, conventional PCB analysis (*e.g.*, using EPA method 8081, REF D) specifies extraction of the sample liquid volume and sample bottle. This results in extraction of PCBs from insoluble PCBs adhered to sample container surfaces or particulates in the liquid sample, in addition to the seawater solvated PCBs. The immunoassay only responds to PCBs that are solvated in the sample/diluent mixture, not what might be adsorbed or absorbed to particulate matter. To reduce false positives in the immunoassay, these particulates must be removed from the sample prior to analysis. This is most easily accomplished by sample filtration, also used to remove particulates that are of concern in colorimetric analyses because of scattering losses. Filtration results in the possibility of analyte being trapped in the filter, leading to lower than expected analyte concentration in the assay tube. The filters are therefore rinsed with diluent phase in order to reclaim any residual PCBs. It is advisable to determine





SPAWAR  
Systems Center  
San Diego

if there are diluent-soluble particulates present in the sample, since it is possible to extract additional PCBs not actually solvated in the seawater matrix. It is also generally advisable to avoid any aging effects related to time and temperature conditions for both the samples and assay reagents, as recommended by the assay manufacturer.

The sensitivity of the immunoassay to PCBs in seawater is illustrated for PCB controls in Figure 2 using the protocol that has been developed in this work. The data are plotted in their entirety to show the variance in the data. The slope of the best linear fit (0.99) indicates a good correlation with expected concentration. More significant variance is noted at higher concentrations, which can be most effectively addressed by dilution into the more accurate (linear) concentration range. The assay tube detection limit is ~0.5 ppb, resulting in a practical detection limit (seawater samples) of 1-2 ppb. The practical detection limit takes into consideration the dilution factor described previously for avoiding seawater salt precipitation (1:4 seawater:diluent). This detection limit is thought to be sufficient for useful screening-level determinations of PCBs in seawater.

An ELISA seawater protocol, with sufficient consistency and sensitivity to serve as a semi-quantitative analytical tool, has been developed. This adapted protocol for seawater analysis has the added potential of being applicable to ELISAs for detection of PAHs and other analytes in seawater. Current and future efforts are focused on finalizing optimal protocols for PCBs in seawater and also on evaluating these seawater protocols with freshwater ELISAs developed for other analytes. For example, preliminary results with a commercial immunoassay kit for PAHs (as phenanthrene) show good sensitivity with an approximate detection limit of 0.7 ppb in the assay tube.

## ACKNOWLEDGEMENTS

The authors thank Scott Steinert, Computer Sciences Corporation, Marine Sciences Department, for a preliminary comparison of freshwater ELISA methods for PCBs in seawater vs. freshwater samples and for validating an initial set of immunoassay parameters for seawater sample analysis.

## REFERENCES

(A) In, C. R., Lane, K. M., Guerrero, J. M., and George, R. D. "Controlled Leaching Studies of Chlorinated Biphenyls from Solid Matrices into Seawater", *ACS Environmental Abstracts*, 2001.

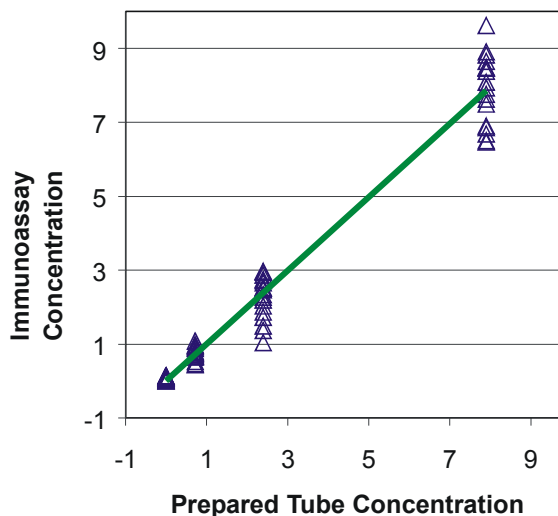
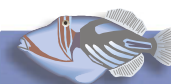


Figure 2. Detection of Immunoassay Controls Prepared in Seawater;  $y = 0.9944x + 0.0028$ ,  $r^2 = 0.9702$ .





**SPAWAR**  
**Systems Center**  
**San Diego**

(B) *Table 1: Reconstituted Saltwater for Marine and Estuarine Crustaceans*, in “Standard Guide for Conducting 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods”, ASTM E1367-92, p. 737, and references therein.

(C) “Test Methods for Determination of Pesticides and PCBs in Water and Soils/Sediment by Gas Chromatography/Mass Spectroscopy”, Method 680, Physical and Chemical Methods Branch, Environmental Monitoring and Support Laboratory, Office of Research and Development, United States Environmental Protection Agency, Cincinnati, OH, 1985.

(D) “Test Methods for Evaluating Solid Waste, Physical/Chemical Methods”, SW-846, Environmental Protection Agency, Final Update III, 1997.



The *Marine Environmental Update* is produced quarterly as an information service by the Marine Environmental Support Office (MESO) to inform the Navy environmental community about issues that may influence how the Navy conducts its operations. The contents of this document are the responsibility of the Marine Environmental Support Office and do not represent the views of the United States Navy. References to brand names and trademarks in this document are for information purposes only and do not constitute an endorsement by the United States Navy. All trademarks are the property of their respective holders. Approved for public release; distribution is unlimited.

The Marine Environmental Support Office may be reached at:

MARINE ENVIRON SUPPORT OFC  
SPAWARSYSCEN D3621  
53475 STROTHER ROAD  
SAN DIEGO CA 92152-6326

Voice: 619.553.5330/5331; DSN 553.5330/5331  
Facsimile: 619.553.5404; DSN 553.5404

E-mail: [meso@spawar.navy.mil](mailto:meso@spawar.navy.mil)  
PLAD: SPAWARSYSCEN SAN DIEGO CA

WWW: [meso.spawar.navy.mil](http://meso.spawar.navy.mil)

