GEO-CENTERS

Development of Analytical And Environmental Diagnostic Techniques

Final Report
Contract Number N00173-99-C-2042
GC-3381

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14. ABSTRACT

GEO-CENTERS, INC. supports programs in the Chemical Dynamics and Diagnostics Branch of the Chemistry Division at the Naval Research Laboratory. The work accomplished encompassed a broad range of research in biotechnology, chemistry, materials science, and corrosion engineering. The work also involved application engineering for shipboard solid waste disposal systems and development of a biosensor chip for biological agent detection. GEO-CENTERS also provided research and documentation support for Navy environmental programs, including the shipboard environmental information clearinghouse, and a program to develop environmentallay sound ships for the 21st century.

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1.0 INTRODUCTION

This report is a summary of GEO-CENTERS' research efforts for the Chemical Dynamics and Diagnostics Branch of the Chemistry Division of the Naval Research Laboratory (NRL) under Contract No. N00173-99-C-2042, entitled "Development of Analytical and Environmental Diagnostic Techniques." The period of performance was from May 1999 through January 2002. The work was carried out at NRL using NRL Chemistry Division facilities and other NRL facilities, and at other locations and contractor facilities, in collaboration with government scientists and other contractors. There were four separate tasks under the contract Statement of Work. The main task categories were:

- Analysis/Control of Airborne Chemicals
- Chemical Analysis and Chemical Sensor Development
- Environmental Remediation and Site Characterization for Unexploded Ordnance
- Site Characterization and Remediation of Contaminated Soils, Groundwater, and River Sediments.

The various individual research projects covered under this report are separated as needed into the tasks provided in the contract Statement of Work.

Brief synopses or descriptions of the above task areas and their sub-tasks are provided in the following paragraphs. Where the work has been previously reported or published, summaries are provided or examples given followed by a listing of the publications containing a more detailed report. Unpublished or unreported work is presented in more detail.

2.0 ANALYSIS AND CONTROL OF AIRBORNE CHEMICALS

2.1 Synopsis

Work performed by GEO-CENTERS under this task included projects to assess air quality monitors for use aboard Navy submarines. The conversion from chlorofluorocarbon (CFC) based refrigerants to hydrofluorocarbon (HFC) materials in the Navy submarine fleet continues, as does the need for developing submarine air quality profiles. The HFCs, while less harmful to the earth's ozone layer than CFCs, can react and be subject to degradation by a submarine's hopcalite catalytic air purifiers. Breakdown products include aldehydes and ketones. After conversion to HFCs, a submarine's atmosphere must be monitored to assess conversion impact on shipboard air quality. GEO-CENTERS is well acquainted with the analytical sampling and analysis required for these sampling events. For 15 years, we have provided analytical support for this project under previous contracts at NRL.

2.2 Characterization of Trace Organic Components of Submarine Atmospheres and Evaluation of Atmospheric Monitors

GEO-CENTERS' long experience with the program and familiarity with the sampling devices, analytical instrumentation and methods required to support the submarine air quality program benefit the program with continued, consistent performance. During this contract, we assisted in the transfer of key analytical instrumentation for support of the program from NSMRL to NRL. We worked to bring the instrumentation on line and maintain it. The Hewlett-Packard 1090 Series II HPLC from NSMRL has an autosampler and replaced the older Spectra-Physics HPLC (which lacked one). Throughput for samples of ketone and aldehyde has increased by a factor of 2.5. We also transferred and made operational a 6 port Summa canister cleaning and evacuation manifold from NSMRL, which has made reconditioning of the canisters faster and more consistent.

In collaboration with scientists at NRL and our staff at the Naval Submarine Medical Research Laboratory in Groton, CT, we have started exposure measurements and experiments on acrolein, formaldehyde and ozone. The evaluations follow a similar format. Our evaluation method called for controlled exposure of badges at 5-20 ppb levels of the analytes of interests. Glass exposure chambers were fabricated at NRL and linked via inert Teflon tubing to a purified, humidity-controlled air supply capable of delivering air at a rate sufficient for the required face velocities of passive air sampling devices (up to 20 L/min). Airflow was regulated by Matheson mass flow controllers and could be adjusted from 0.1 to 20 L/min. The continuous emission sources for acrolein and formaldehyde were permeation tubes (from Kin-Tek, Inc.) emitting 500-1000 ng/min of analyte. Separate constant temperature housings with controllable airflow in and out of the permeation tube holders were built and connected to the system. For the ozone studies, an Environics Series 100 Multi-Gas Calibrator was regulated to emit between 0.1 and 0.3 ppm ozone and was connected to a separate glass chamber. A similar chamber apparatus is used for VOC experiments. Individual permeation tubes for *p*-xylene, toluene, benzene and *n*-octane will

be placed in a permeation tube holder at 40°C - each tube emits approximately 500ng/min. The diagram below shows the relationship of the basic components of the apparatus (Figure 1).

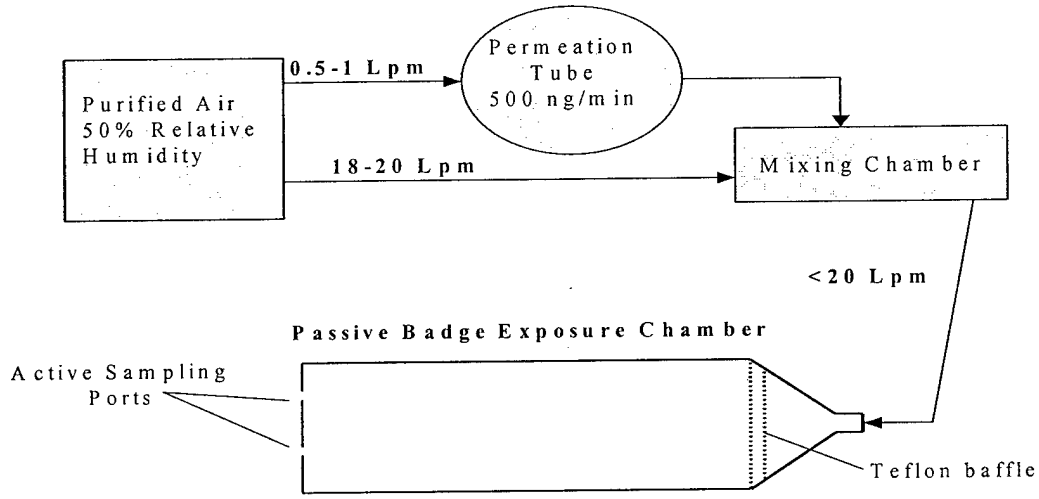

Figure 1. Passive Monitor Controlled Exposure Apparatus. Glass chamber with variable low air flow permits active sampling and controlled, consistent exposure of passive sampling badges to ppb levels of acrolein, formaldehyde, ozone and VOC's.

All the exposure levels for the 3 compounds and VOCs are in the part per billion (ppb) concentration ranges. The chambers are sampled using existing active sampling methods for ozone, VOCs, acrolein, and formaldehyde. Analysis of active samples (taken by conventional air sampling methods) is performed at NRL and by a commercial laboratory to verify the concentration levels of the analytes of interest in their respective chambers. Using the chamber apparatus, we completed 6 comparison tests of passive monitors for formaldehyde and established active sampling methods. The active samples were taken by following procedures for EPA Method TO-11 for formaldehyde. Figure 2 shows the formaldehyde concentration levels determined by both active and passive sampling in the same chamber over 4 weeks. The results show that the passive samplers can be used to quantify 10 PPB of atmospheric formaldehyde over a 28-day exposure period to within 25% of actual value.

2.3 Publications and Presentations

D.M. Bubb, B.R. Ringeisen, J.H. Callahan, M.C. Galicia, A. Vertes, J.S. Horwitz, R.A. McGill, E.J. Houser, P.W. Wu, and D.B. Chrisey, "Deposition of Intact Polyethylene Glycol Thin Films with Matrix Assisted Pulsed Laser Evaporation (MAPLE)," Applied Physics A: Mater. 73, 121-123 (2001).

Daniel M. Bubb, J.H. Callahan, J.S. Horwitz, R.A. McGill, E.J. Houser, D.B. Chrisey, R.H. Haglund, M.R. Papantonakis, M. Galicia, and A. Vertes, "Resonant IR Pulsed Laser Deposition of Polymer Films Using a Free Electron Laser," J. Vacuum Science and Technology, 19, 2698-2702 (2001).

M.C. Galicia, A. Vertes, and J.H. Callahan, "Atmospheric Pressure Matrix-Assisted Laser Desorption Ionization in Transmission Geometry," J.H. Analytical Chemistry (in press).

D.M. Bubb, P.K. Wu, J.S. Horwitz, J.H. Callahan, M. Galicia, A. Vertes, R.A. McGill, E.J. Houser, A. Pique, and D.B. Chrisey, "The Effect of the Matrix on Film Properties in Matrix-Assisted Pulsed Laser Evaporation," Journal of Applied Physics (in press).

W.P. Gardner, R.E. Shaffer, J.E. Girard, and J.H. Callahan, "Applications of Chemometric Analysis Techniques to Direct Sampling Mass Spectrometry," Analytical Chemistry, 73, 596-605 (2001).

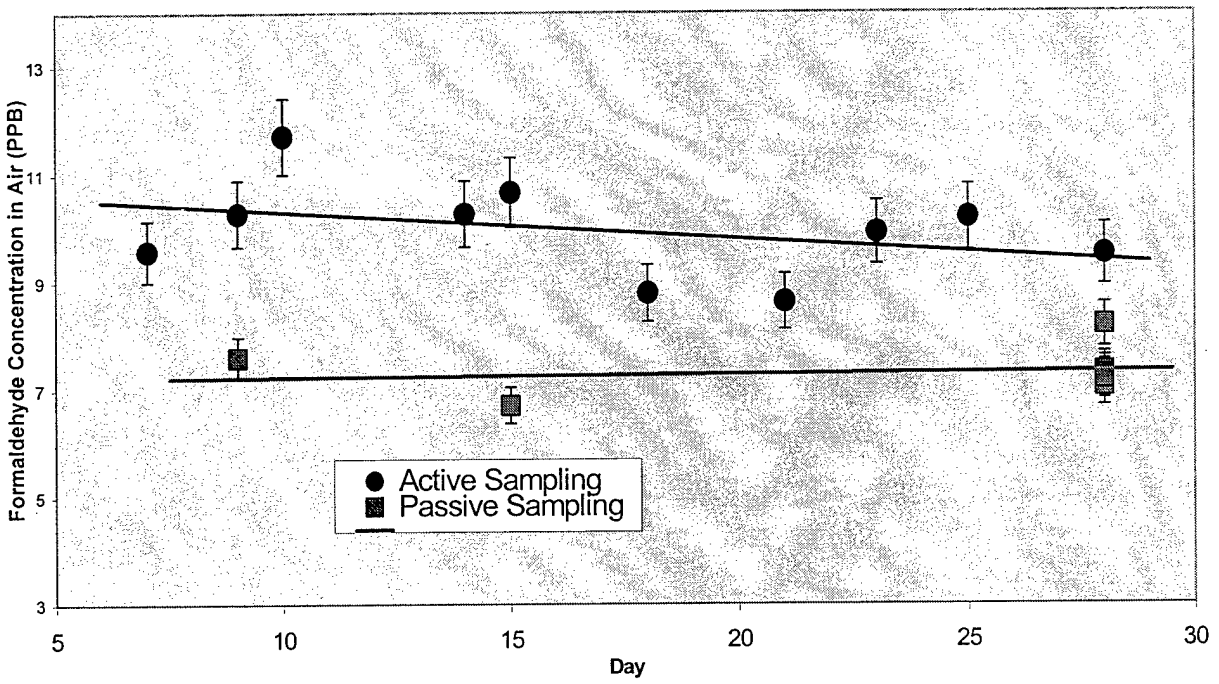

Figure 2. Comparison of Atmospheric Formaldehyde Determination by Active and Passive Sampling. Initial 28-day exposure of passive formaldehyde monitors compares favorably with results obtained by standard methods and demonstrates effectiveness of exposure apparatus for additional experiments.

3.0 CHEMICAL ANALYSIS AND CHEMICAL SENSOR DEVELOPMENT

3.1 Radionuclide and Explosives Sensor Development

3.1.1 Synopsis

The research performed by GEO-CENTERS in the Environmental and Sensor Chemistry Section (Code 6116) was in support of the Laboratory-on-a-Chip Project. The program addresses the need for developing a new class of radionuclide and heavy metal complexation agents that are tagged with near-infrared dyes and, therefore, can be extended to the implementation of a compact and portable "laboratory-on-a-chip" operable in the stringent field requirements of site characterization and remediation. The research objectives for this program are summarized as follows:

- To synthesize a new class of near-infrared tagged macrocycles that will take advantage of the inherent metal complexation properties of the macrocycle, while fluorescing in a region of the spectrum with very little background fluorescence
- To characterize the fluorescence and complexation behavior of this new class of complexation agents
- To implement these new materials into the design of a portable monitor for radionuclide and heavy metal analytes that utilizes the "laboratory-on-a-chip" technology for performing capillary electrophoresis on a microchip.

3.1.2 Overview of Research Effort

Our efforts from May 1999 to January 2002 were focused on 2 primary projects: 1) radionuclide and metal ion detection funded by DOE and 2) Explosives detection funded by ONR using microchip capillary electrophoresis. A brief review of each project is listed below.

3.1.3 Radionuclide Detection

The sensitive and selective detection of various radionuclide, lanthanide and transition metal ion on a capillary electrophoresis microchip is demonstrated using two metallochromic complexation ligands, 4-(2-pyridylazo) resorcinol (PAR) and arsenazo III (AIII), and a derivative **L** of "super-uranophile" synthesized through the coupling reaction of 4-sulfonic calix[6]arene with lissamine rhodamine B sulfonyl chloride. In order to take advantage of the compact and portable potential of a CE microchip, LED absorbance detection and fluorescence detection using a compact, 15 mW Nd/YAG laser (532 nm) were investigated on a simple cross pattern microchip with two different microchannel depths, 20 and 100 microns. Micellar electrokinetic chromatography (MEKC) was utilized on a glass microchip to resolve up to seven different PAR-metal chelates with sub ppm detection limit within a minute. Microchip determinations of uranium (VI) were performed using the colorimetric metal complexation ligand arsenazo III, utilizing a red LED

light source and a photodiode array detector. A mixture of UO_2^{2+} and thee interference metal ions were separated on a microchip, and the addition of EDTA to the background electrolyte is demonstrated to be an effective means of eliminating any interference from lanthanide and transition metal ions. Fluorescence detection of UO_2^{2+} was carried out with the calixarene derivative **L** in the presence of six competing metal ions. The resulting electropherogram indicates that **L** is able to selectively complex UO_2^{2+} despite the presence of a matrix of six impurity metal ions.

3.1.4 Explosives Detection

The sensitive detection and separation of three trinitroaromatic explosives, 1,3,5-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB) and tetryl, in the presence of ten other explosives and explosive derivatives was demonstrated using a nonaqueous solvent mixture of acetonitrile/methanol (87.5%:12.5%, v/v) containing 2.5 mM NaOH and a low concentration of surfactant. The chemical reaction of base with trinitroaromatic compounds forms colored products that can be easily detected using a green light emitting diode on the microchip. Three different surfactants, cetyltrimethylammonium bromide (CTAB), dodecyl sulfate sodium salt (SDS), and polyoxyethylene(10)iso-octylphenyl ether (Triton X-100) are compared with respect to migration times, detection limit capabilities and resolution. All separations were achieved in less than 20 s. The most sensitive analyte measured by this method was TNB, whose detection limit was as low as 240 μg/l, while TNT was detectable at 650 μg/l. By performing the separation in nonaqueous media, the glass microchip is positioned to take advantage of solid phase extraction methodologies, either on-line or ex-situ, in order to lower detection limits further, and make the microchip more amenable to traditional sampling techniques in the field.

3.1.5 Publications in Refereed Journals

Q. Lu, G.E. Collins, and J. Wang, "Microchip Detection of Nitroaromatics in Nonaqueous Media," Analytic Chemistry (submitted October 2001).

G.E. Collins, Q. Lu, S. Abubeker, and E. Vajs, "Remote Fiber Optic Flow Cell for the Detection of Uranium(VI) in Ground Water," Applied Spectroscopy (accepted December 2001).

Q. Lu, J.H. Callahan, and G.E. Collins, "The Selective Detection of Uranium(VI) on a Microchip Using a Derivatized 4-Sulfonic Calyx[6]arene," J. Chem. Soc., Chem. Commun. 1913 (2001).

Q. Lu and G.E Collins, "Microchip Separations of Transition Metal Ions via LED Absorbance Detection of Their PAR Complexes," Analyst, 126, 429 (2001).

G.E. Collins and Q. Lu, "Microfabricated Capillary Electrophoresis Sensor for Uranium (VI)," Anal. Chim. Acta, 181 (2001).

G.E. Collins and Q. Lu, "Radionuclide and Metal Ion Detection on a Capillary Electrophoresis Microchip Using LED Absorbance Detection," Sensor and Actuators B76, 244 (2001).

3.1.6 Papers Presented at Professional Society Meetings

G.E. Collins, G. Deng, and Q. Lu, "Microchip Separation of Toxic Metal Ions," presented at the 9th International Meeting on Chemical Sensors, Boston, MA, July 7-10, 2001.

G.E. Collins and Q. Lu, "Rapid Microchip Separation of Trinitroaromatics Explosives in Nonaqueous Media," presented at the American Chemical Society 223rd National Meeting, Orlando, FL, April 7-11, 2001.

G.E. Collins and Q. Lu, "Selective Detection of Uranium(VI) Using a Capillary Electrophoresis Microchip," presented at the American Chemical Society 220th National Meeting, Washington, DC, August 20-24, 2000.

G.E. Collins and Q. Lu, "Sensitive and Selective Metal Ion Analysis Utilizing the Laboratory-on-a-Chip," presented at the 8th International Meeting on Chemical Sensors, Basel, Switzerland, July 3-5, 2000.

3.2 Clean Air Act Compliance Support and Air Permit Database

3.2.1 Synopsis

Under this work area, GEO-CENTERS provided support for air permitting issues, Clean Air Act (CAA) compliance, the NAVFAC ODS Conversion Guide, and various meetings and Web sites for our CNO N45 customers (especially N451E, N451K, and N456) and OASN(I&E). Specific deliverables and major accomplishments in this work area are described below.

3.2.2 Meeting Support

- Co-authored papers with our Navy customers, for presentation at numerous meetings and conferences, such as the Annual Air & Waste Management Association Conference (Orlando, FL), and attended technical sessions.

- Prepared materials for, attended, and provided minutes for various bi-monthly CAA Services Steering Committee (SSC) meetings, and monthly Navy CAA Steering Committee meetings, as well as numerous Subcommittee (such as Ranges) meetings and teleconferences, as needed, including preparing Vehicle Inspection/Maintenance reports, and updating Subcommittee membership databases.

- Supported the annual Navy/Marine Corps CAA Conference (Seattle, WA, and Jacksonville, FL), participating in technical training sessions on air emissions, coordinating a permit panel session, and preparing briefings and speeches on CAA accomplishments, new air-quality standards, and more.

- Supported CNO at numerous EPA meetings (such as the EPA CAA Advisory Committee public meeting) on an as-needed basis, preparing summaries.

- Participated in and provided summaries for the NOVA Inspection/Maintenance Workgroup and other conference calls with Navy representatives, and attended the annual Navy Environmental Planning Conference.

- Helped plan and attended the Aircraft Particulate Matter Emissions Workshop (San Diego, CA), coordinating with the Navy Aircraft Environmental Support Office and with speakers on presentations; distributing read-ahead materials; providing onsite support; summarizing proceedings; drafting the overview briefing, compiling information on particulate-matter composition, sources, and status of aircraft emissions.

- Supported the Navy OGC Conference, developing the CAA enforcement update briefing, range issues briefing, and handouts (including an ArcView map), helping present a CAA general conformity issues briefing, and summarizing the nonroad engines, ranges, and conformity sessions.

- Supported the CAA Conformity and Aircraft Emissions Working Integrated Product Team (WIPT) for the Joint Strike Fighter (JSF) meetings.

- Prepared numerous briefings for meetings, conferences, and training courses, such as the following: CAA update and environmental law, EPA-enforcement policy, diesel-fuel standards, global climate change, the history and future of NSR and Title V programs, environmental and health program status for Vieques (to Senator Clinton's staff), and DOD's responses to EPA on the burden and cost of the general conformity rule to DOD and to DOJ on boiler information.

3.2.3 Navy CAA Compliance and Title V Permit Database

- Supported the Navy effort in developing Environmental Impact Statements (EISs), and researched EISs developed for NAS Oceana, NAS Lemoore, and MCAS Miramar, and more.

- Served as expert reviewers of and commentators on various environmental documents, some to determine the effects of EPA policy on Navy facilities. Examples: Navy and Air Force conformity methodologies; aircraft-emissions and construction calculations for numerous EISs; Navy CAA General Conformity Guidance; a summary of DOD installations to be affected by EPA's proposed rescission of revoked area status for certain CAA nonattainment areas, and a GIS tool to map their status; a summary of areas granted exemptions from CAA conformity requirements; a regulatory impact analysis on EPA Diesel Fuel rule for the EPA docket; the Navy Commanding Officer's Guide to Environmental Compliance Ashore; the semi-annual Regulatory Agenda; the draft DOD Environmental Health Risk Policy; the CAA chapter of the PR-03 Environmental

Requirements Guidebook; bills introduced in Congress; the Notice of Intent to the Navy over Vieques training-range operations; the Federal Register database; the Report on Economic Analysis of a Multi-Emissions Strategy and Analysis of the U.S. Electricity Sector; EPA's guidance on source determinations and permitting of combined heat and power facilities; Navy policy on marine engine emissions; the draft Public Health Assessment report on Vieques air pollution; a proposed justification for EPA to include an "equivalency" provision in the surface coating National Emission Standards for Hazardous Air Pollutants (NESHAP)s; two revisions of the MCAS El Toro Reuse General Conformity Analysis; a draft permit application for a proposed marine turbine engine test cell; permits/applications at facilities; draft operating CAA permits at Navy facilities, and the status and types of permits in various regions, States, and their emission estimates; EPA's Interim Guidance on CERCLA; the stack height permit provision; the Airfield and Airspace Study for the Introduction of F/A-18 E/Fs to the East Coast; the CAA compliance poster, measure of merit, and associated definitions; the proposed regional haze rule change; and a proposed new utility complex selling steam to the Navy.

- Prepared point papers on CAA general conformity issues, NSR, the revised NAAQS, the regional haze rule, the Wildland Fire Policy, the status of ozone formation research and regulations, aircraft totals and corresponding emissions, the status of baghouse control technology and dioxin emission controls, and the Vehicle Inspection and Maintenance Program.

- Monitored the latest trends in permit conditions in non-Navy-facility permits through Web searches, to stay current with non-Navy Title V permit issues and inform our Navy customers. Example topics of research: ozone formation, emission offsets, EPA policies and proposals, training opportunities on risk assessment, status of CAA exemptions, EPA's particulate matter programs, the DOD database of boiler inventory data, power ratings for typical recreational vehicles, possible impacts of permits on incinerators on Navy ships, the San Diego State Implementation Plan status, regulations governing hazardous material transportation aboard aircraft, alternate operating scenarios and National Security Emergency alternatives in Title V permits, aircraft operations and emissions, emissions calculation methods for fine particulate matter, and alternative fuel vehicle refueling requirements.

- Prepared numerous environmental documents, such as the DASN(E))'s certificate of appreciation for Outstanding Executive Agent Leadership, a CAA Reference Book, a history of opacity measurement in regulations, Title V permitting and the hospital/medical/infectious waste incinerator NESHAP, summaries of the methodology and results in determining the need for EISs, possible impacts of CAA permit programs, New Source Performance Standards, DOD's industrial combustion sources, a strategy for conducting aircraft engine particulate matter emissions study, a summary of the relationship between Title V permitting, State permit programs, and the Stratospheric Ozone regulations specified in Title VI.

- Compiled, maintained, edited, and revised all responses to the semi-annual IPR data call for the Title V permit status and all reports for the Navy facility database, the nonattainment database, and an ArcView map showing current and projected nonattainment areas.

- For New Source Review (NSR) compliance, attended technical training sessions; reviewed ongoing activity in the program and the Environmental Appeals Board decision in the NSR case against the Tennessee Valley Authority; and developed fact sheets and an action plan to review the Services' position, the draft NSR guidance for the Services, a Subcommittee report on Title V permits/NSR program summaries, a 5-page NSR summary, and information on the current status of Navy Title V facilities and available training.

- For the CNO Environmental Awards, reviewed awards packages, and judged the submittals based on criteria from OPNAV Instruction 5090.1B CH-2.

- For the DENIX CAA SSC Web Site, continually updated and made corrections to existing home pages and created new home pages. Examples: nonattainment area information, Title V/NSR and Title V permit binder pages, various EPA links, and SSC points of contact; lists of on-line information and links to EPA Region and State permit programs and spreadsheets of permit data, numerous historical documents, the Range Subcommittee Web page, a discussion area for JSF WIPT, and new Web site for N45's Navy Overseas Environmental Working Group.

3.2.4 NAVFAC ODS Conversion Guide

- Revised the hardcopy guide and made a more interactive electronic format, and designed the layout for the electronic version.

- Updated the Introduction.

3.3 Arctic Military Environmental Cooperation

AMEC is a forum for dialogue and joint activities among U.S., Russian, and Norwegian military and environmental officials that addresses Arctic environmental issues related to our militaries' unique capabilities and activities. For this task, GEO-CENTERS' primary thrust was supporting AMEC Project 2.2, Design and Construction of a Vessel for Collection and Processing of Wastes from Navy Ships, and its follow-on Project 2.2-1, Shipboard Technology Demonstration. The objective was to examine and select technologies, then design and build a vessel for the collection, processing, and environmentally sound disposal of Russian naval ship non-nuclear solid and liquid wastes in the Barents Sea region.

Project 2.2 was planned in two phases: Phase I defined the requirements, assessed Russian, Norwegian, and U.S. technologies, and proposed a notional design of the vessel; Phase II, which

was not approved, was to be final detailed design and construction of the vessel. Detailed Phase I activities included: an assessment of the magnitude of the waste disposal problem on board Russian Naval vessels; evaluation of the suitability of existing "clean ship" technologies and strategies for ship waste processing; and a cost estimate for the proposed vessel. GEO-CENTERS participated in all project meetings with other technical experts in the United States, Norway, and Russia, including a Program Officers meeting in November 2000 in Moscow, and a technology demonstration of Russian oil-water separators in St. Petersburg, Russia. GEO-CENTERS also co-authored the AMEC Project 2.2 Summary Report and abstract, which GEO-CENTERS also presented at the Waste Management 2001 conference in Tucson, AZ, in March 2001, along with photographs and a full-color briefing. We also provided input for the AMEC brochure and poster.

For Project 2.2-1, planning was completed, and the project experts had several meetings to finalize plans for a shipboard waste treatment technology demonstration. Because the three parties could not agree on the legal issues, however, this project was never initiated and is now on hold.

3.4 Environmentally Sound Ships of the 21st Century

Under this task, GEO-CENTERS supported the Shipboard Environmental Protection Process Action Team (SEPPAT) and the Ship Environmental Information Clearinghouse (SEIC) by analyzing the Fleetwide Environmental Questionnaire (FWEQ). We also researched and analyzed ODS data-call discrepancies, and researched and prepared the draft for ODS Advisory 96-01D.

3.5 Shipboard Environmental Protection Process Action Team (SEPPAT)

Under this work area, GEO-CENTERS conducted a diverse array of activities relating to the Navy's goal of achieving environmentally sound ships through technology, training, policies, and procedures. The SEPPAT charter is based on Ship Environmental Design Guidance for the Environmentally Sound Warship (prevent and control pollution) and Fleet Interface (increase knowledge and provide a forum to discuss issues). The SEPPAT identified environmental issues and, through improved communications, kept the Fleet informed about environmental programmatic developments within the Headquarters Team of CNO N45, NAVSEA, and NAVSUP. It also provided Navy Environmental Leadership with objective feedback, enhancing the marine environment and improving the quality of life for Sailors at sea.

Highlights of the achievements of the SEPPAT, which won the 2000 CNO Environmental Award, are:

- Influenced the CNO decision to require Afloat Environmental Protection Coordinator (AEPC) designation onboard each U.S. Navy ship.

- Produced the all hands environmental awareness video, "Your Ship, The Environment, and You," a high-energy video that provides an overview of the Navy's environmental program while guiding the viewer through the requirements.

- Completed the Source Segregation Container prototype test and survey.

- Compiled and distributed "The Guide to Environmental Compliance Afloat," a single-source reference that compiles regulatory requirements specific to afloat units, because AEPCs and the individual sailor did not have ready access to information. Based on the principle that Headquarters should provide information to the sailor to make environmental compliance easier.

- Visited many Naval ports to conduct Through the Fleet-Wide Environmental Questionnaire (FWEQ), and identified critical issues, explored implications, developed options, and made recommendations approved by NAVSEA and CNO. Also updated spreadsheets and database queries.

- Developed the Environmental and Natural Resources Program Manual – Environmental Compliance Afloat Excerpts, which contains sections of OPNAVINST 5090.1B, CH-1 applicable to afloat units.

- Prepared the AEPC Reference Library CD-ROM for course instructors.

- Maintained the SEPPAT membership database; hosted and provided minutes for monthly SEPPAT video teleconference (VTC) meetings; answered various environmental questions from the Fleet and other Navy activities; and prepared educational packages for schools (videos, guidebooks, CDs, SEIC newsletters, pollution whiz wheels, stickers, and posters).

3.6 Shipboard Environmental Information Clearinghouse

The primary thrust of the SEIC effort was to develop and make available to other Navy activities a comprehensive database on shipboard environmental protection systems and alternative chemicals and processes for replacing current uses of ozone-depleting substances (ODSs) within the Navy. GEO-CENTERS provided technical backup and assisted in program coordination within the Navy and between the Navy and other Federal and State agencies, and other interested parties. Specific efforts involved responding to interrogatories, coordinating workshops, meetings, and presentations; and preparing point papers, briefings, fact sheets, and newsletters. In addition to maintaining a very active and successful Web site (http://navyseic.dt.navy.mil) that includes the information, this effort addressed the following subtasks:

- **ODSs**, including alternative chlorofluorocarbon (CFC) refrigerants, alternative solvents/cleaning agents, and alternatives for Halon fire-protection systems.

- **Shipboard solid waste**, including R&D efforts, shipboard equipment, ship installations and operations, life-cycle management, Navy policy and regulations, National and international regulatory measures; and lessons learned about Navy shipboard solid waste management.

- **Shipboard liquid waste**, including oily waste, oily wastewater, compensated fuel systems, blackwater, graywater, and other liquid discharges.

- **Pollution prevention afloat (P2A) and hazardous materials**, including R&D efforts, commercial product information, shipboard equipment, ship installations and operations, life-cycle management, Navy policy and regulations, and lessons learned relating to Navy shipboard pollution prevention.

- **Navy Natural Resources Management program,** including the Marine Mammal Initiative, Operational Environmental Compliance Oversight Group (OECOG), range sustainability, and the Artificial Reef program (REEFEX).

Specific deliverables and major accomplishments in this work area are described below.

ODSs, Solid and Liquid Wastes, and P2A

- Distributed the April 2001 edition of "Shipboard Environmental Update" newsletter to over 4,500 subscribers. Continued to update distribution list as changes/additions arrived.

- Supported NAVSEA's Ship Environmental Protection Process Action Team (SEPPAT), by distributing documents; entering data and creating database tables, queries, and forms to track information from the Fleet-Wide Environmental Questionnaire (FWEQ); and preparing the FWEQ report.

- Electronically provided environmental updates and relevant news to more than 300 Navy and DOD personnel.

- Responded to Clearinghouse phone and e-mail inquiries about environmental issues from various Navy activities, DOD components, and defense contractors.

- Revised and entered Level 1 MILSPECs (i.e., those that directly reference ODSs) into the evolving DOD ODS SPECs and Standards database locally and on the Web, reviewed proposed MILSPECs/Performance Specifications/Drawings for the use of ODSs and other hazardous materials, and provided environmental and ODS certifications to NAVSEA.

- Supported CNO and NAVSEA participation in Navy Earth Day 2001, by updating graphics for the Environmental Sound Ships exhibit, setting up and manning the exhibit and solid-and-plastics-waste-processing equipment models at the Navy Memorial in

Washington, DC, answering visitor questions, and distributing handouts (posters, brochures, and newsletters).

- Resolved action items generated from the Fleet Environmental Protection Conference and collected data for the Fleet-Wide oily-rag management plan.

- Prepared/presented over 30 briefings, each of which had several drafts and iterations before being finalized, predominantly for NAVSEA and NSWCCD environmental program managers, relating to training personnel on environmental laws applicable to Navy ships; CVN refrigerant conversions; P2A; NAVSEA ODS elimination; ODS refrigerant conversion for the CVN-69/70; CFC-12 conversion; Military Environmental Information Systems; AC&R conversions; SEIC resources for Navy/DOD use; ODS replacement in cleaning shipboard and diver life-support systems; new European Union ODS regulations, and the electronic version of the Navy Guide to ODS Replacement.

- Participated in/attended numerous meetings, often preparing minutes and action items. Some examples: annual Earth Technologies Forum; monthly P2A Equipment Transition/Program Implementation meeting (Philadelphia, PA); annual Navy P2 Conference (Norfolk, VA); annual Fleet Environmental Protection Conference (Norfolk, VA, and San Diego, CA); USCG Environmental Forum 2000; lessons-learned on Norfolk installation meetings for conversions; DOD/DOE's 11th Annual Solvent and Toxic Chemical Substitution Workshop (Scottsdale, AZ); DOD meeting on DENIX updates; Workshop on the Importance of the Military Organizations in Stratospheric Ozone Protection and Climate Protection (Brussels, Belgium), where we also chaired a breakout session on Shipboard CFC Refrigerant Replacement; annual Joint-Service Pollution Prevention meeting (San Antonio, TX); and the Services/DLA/OSD ODS meeting.

- Hosted numerous meetings, such as the Navy ODS Reserve meeting between DLA and Navy representatives (Richmond, VA); an ODS meeting between NAVAIR and NAVSEA representatives; a meeting on ODS Reserve Issues and a tour of the Defense Supply Center (Richmond, VA) facility; the many DOD Pollution Prevention Committee, ODS Reserve Supply Support, and Joint Service ODS Subcommittee meetings; and a Navy FWEQ meeting.

- Produced and distributed the following multi-media CD-ROMs: ODS Resources; NAVSEA Shipboard Environmental Protection RDT&E Symposium; Revisions D and E of the Afloat Environmental Protection Coordinator (AEPC) Library; Military Environmental Information Systems; and the Navy Facility Guide to ODS Replacement.

- From the 2000 ODS Data Call, developed a year-by-year chart showing when the Navy will be ODS-free, and analyzed the 2001 ODS Data Call.

- Prepared many various environmental documents for NAVSEA, DOD, and CNO. Examples: NAVSEA's annual report to ASN (RD&A) on contracts requiring ODS use;

the draft ODS Advisory 96-01D (not yet issued); the P2A Equipment Installation Guide for the CV-67; the P2A and ODS sections of the CNO N45 FY-00 Accomplishments List; Navy ODS Reserve draw-down curves for NAVSEA's PR-03 and POM-04 budget planning; information on HCFC-22 alternatives; DOD's responses to EPA's request for information on expanding the Significant New Alternatives Program (SNAP) and to Congressional inquiries about Halon alternatives; the advantages of HFC-236fa conversion for the N45 Web site; an ODS Waiver analysis; an ODS chart; DOD input for military exemptions for mission-critical equipment to new European Union ODS regulations; background information on Navy ODS elimination at facilities (for an interview of the Navy Chief of Civil Engineers); a technical paper on the Navy's Shipboard CFC-114 Elimination Program; NAVAIR Substitution Guide mailing list updates; CFC-113 data to support Foreign Military Sales (FMS); and an updated NAVSEA 05L1 organization chart.

- Served as expert reviewers/commentators on ODS conversions and environmental documents. Examples: the first HFC-236fa conversion on a DDG-51-class ship; ODS Supply Support messages; various EPA documents for DOD (such as SNAP, Halon Emission Reduction Rule, and proposed HCFC Allowance System rule); the Navy's ODS Reserve Authorized Users List (AUL) and on-line AULs for NAVSUP, NAVSEA, and DSCR, which we also continually updated; Fleet Casualty Reports; EPA's toxicity assessment of alternative solvents; draft Environmental Final Governing Standards for the UK; ODS parts of the Commanding Officers' Guide to Environmental Compliance; draft U.S. Negotiating Position for the Meeting of the Open-Ended Working Group on the Montreal Protocol; draft OSD point paper on ODS for the DOD Live-Fire Test & Evaluation and DUSD(I&E) meeting; Fleet Industrial Supply Center San Diego procedures for ODS Reserve requisitions; OPNAVINST 5090.1B Chapter 19, Change 3; technical data on a new refrigerant analyzer; DLA action plan for conducting laboratory analyses and reclaiming ODS Reserve CFC-114 turn-ins; and ODS training material (for the Navy Advanced Environmental Management Course and the Surface Warfare Officer School).

Navy Natural Resources Management (NNRM)

- Coordinated and prepared and distributed minutes, agendas, action items, and participant lists for the CNO's 10 Natural Resources Working Group teleconferences, and helped track progress of and maintained the master list of Integrated Natural Resources Management Plans (INRMPs) for Navy compliance with the Sikes Act. Also continuously updating the master Plan of Action and Milestones (POA&M) and other spreadsheets and attended the Conservation Committee Meeting and other meetings.

- Supported development of the CNO's policy on feral animals on military bases, by reviewing and commenting on the draft policy; conducting research on the trap-neuter-release option; and hosting and preparing the minutes, action items, and participant lists for the Feral Animals Teleconference.

- Supported the Navy's REEFEX program, by reviewing the draft health and ecological risk assessments, the PCB-leachate study results, and the legal constraints; developing and revising the REEFEX POA&M; hosting a 2-day Inter-Agency (Navy/EPA) REEFEX Technical Working Group meeting and attending three others. Also researched the history and practices of other artificial-reef programs in the United States and wrote point papers on both REEFEX and ship-sinking exercises (SINKEX).

- Supported programs relating to range and OPAREA sustainability, endangered species, and other issues, by helping to prepare articles for Navy and outside journals on NNRM and encroachment problems. Attended the NDIA Environmental Symposium (Austin, TX) and the annual technical symposium of the Strategic Environmental Research and Development Program (SERDP), to stay current on vital issues; and wrote the draft Strategic Plan for, and reviewed and commented on the draft NNRM program's mission, vision, and strategy statement. Also co-authored a feature article for the Fall 2001 Currents magazine about NNRM, and coordinated with CNO N45P for its publication, and attended and summarized the annual NNRM Conference (San Diego, CA). In addition, drafted ideas for the CNO's first newsletter about NNRM (Navy & Nature News, which was put on hold), researched and prepared a draft report of past successful endangered-species-relocation projects, and wrote a point paper on two versions of the Agency for Toxic Substances and Disease Registry (ATSDR)'s Vieques soil-toxicology reports.

- Supported the MMI and OECOG, hosting several IPT video teleconferences and meetings, and preparing minutes and action items, arranging/hosting a Keystone Dialogue meeting on marine mammals, and serving as expert reviewer and commenter on the Methodology for Risk Assessment: Injury to Marine Mammals from an Underwater Explosion document.

- Prepared numerous briefings, each of which had several drafts and iterations before being finalized and presented within and outside the Navy, predominantly dealing with MMI, INRMPs, Ships as Man-Made Reefs, and range sustainability issues. Examples: DASN(E) Briefing to the Marine Mammals Commission; CNO N456's Environmental Manager Course Overview and CECOS Civil Engineer Corps Indoctrination Seminar presentations to update officers on current DOD and Navy issues and initiatives; and current issues at N45.

- Gathered data on cruise-ship technologies to be entered into a database.

- Distributed copies of the NATO CCMS Black Sea Integrated Coastal and Shelf Zone Monitoring and Modeling (INCOM) Program report/science plan, Arctic Environmental Atlas, AEPC Reference Library CD, Oil-Spill Contingency Plan, and Solid Waste Management Guide.

4.0 ENVIRONMENTAL REMEDIATION AND SITE CHARACTERIZATION FOR UNEXPLODED ORDNANCE (UXO)

For this task, GEO-CENTERS' support came in the form of logisitics management. The Company had the responsibility to coordinate, transport, set-up, and help operate MTADS instrumentation designed for use in detecting unexploded ordnance at various sites around the country. Our support staff consisted of one individual who would coordinate with NRL scientists and drive the equipment and instrumentation to the site, help conduct the surveys, and then drive the equipment back to NRL. A listing of UXO surveys that GEO-CENTERS supported during the contract is listed below.

Survey	Location	Date
L-Range	Army Research Laboratory, Blossom Point Facility, MD	August 1999
Impact Area	Badlands Bombing Range, SD	September 1999
Anacostia Annex	Naval District of Washington, DC	November 1999
JPG IV	Jefferson Proving Ground, IN	August 2000
Fort Ord	Monterey, CA	March 2001
Federal Property	Spring Valley, Washington, DC	June 2001

5.0 SITE CHARACTERIZATION AND REMEDIATION OF CONTAMINATED SOILS, GROUNDWATER, AND RIVER SEDIMENTS

5.1 Synopsis

Under the contract, GEO-CENTERS supported the Navy shipyard site assessment and characterization program at NRL. GEO-CENTERS developed extensive experience sampling and characterizing estuarine and riverine sediments, nepheloid layer and waters for hydrocarbon contamination at various sites (Charleston, Norfolk, Philadelphia, San Diego, Pearl Harbor, Washington, Latvia) resulting in presentations, NRL Technical Memorandum, a book chapter, and 2 NRL Technical Reports. Also under this contract, GEO-CENTERS collaborated with NRL to develop strategies for evaluating the performance of *in situ* bioremediation treatments using stable isotope monitoring of the metabolism of the contaminant carbon into bacteria and carbon dioxide. GEO-CENTERS gained extensive experience collaborating with NRL on protocol development for contaminant analyses in a variety of media. We have supported a number of both basic and applied research projects during the course of the contract.

5.2 Hexavalent Chromium Reduction

5.2.1 Project Summary

Hexavalent chromium is a common industrial pollutant of soil, water and sediment. Chromium in this form is toxic to most organisms and may be carcinogenic. Another form of chromium,

Cr(III), is both less toxic and less soluble at typical environmental pH. Therefore, the reduction of Cr(VI) to Cr(III) is an effective means of remediation. *Shewanella oneidensis*, a Gram negative bacterium, is able to rapidly reduce Cr(VI) to Cr(III) under both aerobic and anaerobic conditions. However, before an effective bioremediation protocol utilizing this organism can be designed, information about the effects of various physical, chemical, and biological conditions on the rate at which it can reduce chromium must be elucidated. The focus of this year's work was to establish; a) physical and chemical conditions at which *S. oneidensis* reduces Cr(VI) at the highest rate, and b) the effect of the presence of other bacteria on this rate, and c) whether clay or other soil components will sorb the chromium ions and decrease reduction rates.

5.2.2 General Procedures

S. oneidensis cultures are grown either in Luria Bertani broth or minimal salts plus lactate at room temperature either in an anaerobic hood or on bench top. Growth is monitored by plating appropriate dilutions on LB agar. Rates of Cr(VI) reduction are determined by acidifying aliquots of the culture, reacting with diphenyl carbazide and spectrophotometric analysis.

5.2.3 General Results

Under appropriate conditions, *S. oneidensis* is capable of very fast reduction of Cr(VI) to Cr(II); 100 μm to less than 5 μm in 10 minutes, and 50 ppm (ca. 1mM) to undetectable levels in 24 h.

Reduction rate is directly proportional to cell density over a wide range of densities. However, at low cell density there is a distinct lag period before the onset of reduction is evident.

Rate does not seem to be effected by prior exposure to Cr(VI) i.e. the Cr(VI) reductase system does not appear to be inducible.

Growth under anaerobic conditions is slower than aerobic, and anaerobic cultures never become as dense as aerobic. However, anaerobic reduction is faster on a per cell basis than is aerobic.

The presence of 10% (w/v) garden soil or illite clay does not affect reduction of Cr(VI) in aqueous phase (supernatants). However, there is evidence that small amounts of Cr(VI) do sorb to illite clay. We are investigating the use of AQDS and other electron shuttles to enhance reduction of this sorbed chromium.

Shewanella oneidensis competes well against many of the bacteria tested. In rich media (LB) *S. oneidensis* reaches stationary phase in about 48 h (aerobically) as fast or faster than many environmental isolates. Many laboratory strains of bacteria are able to grow much faster than this in rich media, and so are likely to overgrow *S. oneidensis* under these conditions.

5.2.4 Publications

P.H. Pritchard, J. Jones-Meehan, W. Straube, C. Nestler, and L.D. Hansen, "Polycyclic Aromatic Hydrocarbons (PAHs): Improved Land Treatment with Bioaugmentation," Chapter in a CRC book on the SERDP project, chapter complete, book being prepared.

5.2.5 Posters (accepted)

W. Straube, Tyrone L. Daulton, Brenda J. Little, and Joanne Jones-Meehan, "Cr(VI) Reduction by *Shewanella oneidensis* in Mixed Culture and Soil Slurry," 102nd General Meeting of the American Society of Microbiology, Salt Lake City, UT, 2002.

W. Straube, Tyrone L. Daulton, Brenda J. Little, and Joanne Jones-Meehan, "*Shewanella oneidensis* Reduces Cr(VI) in Aerobic Mixed Culture and Soil Slurries," Sixth International Symposium on Environmental Biotechnology, Veracruz, Mexico, 2002.

W. Straube, C.C. Nestler, L.D. Hansen, D. Ringelberg, P. Pritchard, and J.M. Jones-Meehan, "Remediation of Polyaromatic Hydrocarbons (PAHs) Through Landfarming with Biostimulation and Bioaugmentation," Sixth International Symposium on Environmental Biotechnology, Veracruz, Mexico, 2002.

5.3 Methane Hydrate Program

5.3.1 Synopsis

A facility at the Naval Research Laboratory (NRL) has been developed to extract, trap, cryogenically distill and graphitize carbon from a suite of organic and inorganic carbon pools for analysis by Accelerator Mass Spectrometry (AMS). The system was developed to investigate carbon pools associated with the formation and stability of methane hydrates. However, because the carbon compounds found in hydrate fields are ubiquitous in aquatic ecosystems, this apparatus is applicable to a number of oceanographic and environmental sample types. Targeted pools are dissolved methane, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), solid organic matrices (e.g., seston, tissue and sediments), biomarkers and short chained (C_1-C_5) hydrocarbons. In most instances, the extraction, distillation and graphitization are continuous within the system, thus, minimizing the possibility of fractionation or contamination during sample processing. A variety of methods are employed to extract carbon compounds and convert them to CO_2 for graphitization. Dissolved methane and DIC from the same sample are sparged and cryogenically separated before the methane is oxidized in a high temperature oxygen enriched helium stream. Solid material is oxidized in sealed, evacuated tubes. Technology is being developed to oxidize DOC to CO_2 with a 1200W ultraviolet photo-oxidation lamp. Microbial biomarkers will be separated and concentrated by preparative capillary gas-chromatography (PCGC) for compound specific radiocarbon analysis. With this system, up to 10 samples, standards or blanks can be analyzed per day.

5.3.2 Publications

J.W. Pohlman, K.S. Grabowski, D.L. Knies, T.M. Deturk, D.J. Treacy, Jr., and R.B. Coffin, "Sample Distillation/Graphitization Apparatus from Carbon Pool Analysis by Accelerator Mass Spectrometry," Nucl. Instr. and Meth. B, 172, 428-433 (2000).

K.S. Grabowski, D.L. Knies, T.M. Deturk, D.J. Treacy, Jr., J.W. Pohlman, R.B. Coffin, and G.K. Hubler, "A Report on the Naval Research Laboratory AMS Facility," Nucl. Instr. and Meth. B, 172, 34-39 (2000).

5.3.3 Book Chapters

J. Pohlman, L.A. Cifuentes, and T.M. Iliffe, "Food Web Dynamics and Biogeochemistry of Anchialine Caves: A Stable Isotope Approach," In: H. Wilkens, D.C. Culver, W.F. Humphreys, (editors), Ecosystems of the World 30: Subterranean Ecosystems, Elsevier, New York, pp. 345-357 (2000).

5.3.4 Presentations

R.B. Coffin, K. Grabowski, I. MacDonald, L. Cifuentes, J. Pohlman, "Analysis of Ocean Methane Hydrate and Fate," American Society of Limnology and Oceanography (ASLO) Conference, Santa Fe, NM, February 14-17, 1999.

R.B. Coffin, T.J. Boyd, M.T. Montgomery, J.W. Pohlman, C.S. Mitchell, J.K. Steele, and B.J. Spargo, "Transport and Degradation of PAHs in the Tidal Region of the Anacostia River," SETAC 20th Annual Meeting, Philadelphia, PA, November 14-18, 1999.

T.J. Boyd, R.B. Coffin, M.T. Montgomery, B.J. Spargo, J.K. Steele, and J.W. Pohlman, "Coupling Contaminate Fate and Transport with Biodegradation: Is a Small Tidal Basin a Source or a Sink for Hydrocarbons?," SETAC 20th Annual Meeting, Philadelphia, PA, November 14-18, 1999.

M.T. Montgomery, T.J. Boyd, J.K. Steele, J.W. Pohlman, R.B. Coffin, D.M. Ward, B.J. Spargo, and D.C. Smith, "Intrinsic Hydrocarbon Bioremediation of Sediments in the Charleston Harbor System," 21st Annual Meeting of the Society of Environmental Toxicology and Chemistry, Nashville, TN, November 12-16, 2000.

M.T. Montgomery, T.J. Boyd, R.B. Coffin, B.J. Spargo, J.K. Steele, J.P. Pohlman, D.M. Ward, and D.C. Smith, "Bacterial Adaptation for Intrinsic Bioremediation of PAHs in Sediments," presented at the 11th Annual West Coast Conference on Contaminated Soils, Sediments and Water, San Diego, CA, March 19-22, 2001.

J.W. Pohlman, R. Campos, A.M. Pohlman, K.M. Johnson, and R.B. Coffin, "Nitrification Induced Inhibition of Heterotrophic Bacterial Production in the Hypoxic Region of the Mississippi River Plume (MRP)," American Society of Limnology and Oceanography (ASLO) Conference, Albuquerque, NM, February 12-16, 2001.

5.4 Harbor Study Program

5.4.1 Synopsis

As a part of a continuing ecological and remediation study, our scientists regularly collected field samples of sediment, soil, and water at harbors utilized by the U.S. Navy. We also helped initiate similar studies in Latvia, making several sampling trips and training Latvian scientists to conduct field sampling and analyses on their own. The basic research focused on three separate program areas.

Intrinsic PAH Biodegradation: Novel molecular techniques were being developed for detecting and predicting intrinsic bioremediation by natural bacterial assemblages. Using Denaturing Gradient Gel Electrophoresis of 16S rRNA (DGGE) and Fluorescent In Situ Hybridization (FISH), we helped investigate the impact of hydrocarbon amendments on assemblage composition and rates of bacterial metabolism. We found that heterotrophic production is less affected in chronically contaminated than pristine environments. This assay is being used to locate submerged source areas of fresh petroleum hydrocarbons. Also, we found major changes in bacterial assemblage composition in less than 72 h from the addition of naphthalene to samples from chronically contaminated environments. Progress in this area will add to the lines of evidence of intrinsic bioremediation in estuarine sediment. This work is funded by ONR, SERDP, NRL, and Delaware Sea Grant (to Dave Kirchman).

Bacterial Attachment: We also investigated the role of initial bacterial attachment mechanisms on the rate of degradation of high molecular weight aromatic hydrocarbons like PAHs and lignins. Bacteria that have developed specific attachment mechanism, such as binding proteins, may have a selective advantage in degrading aromatic associated with aggregates and inorganic surfaces. It may be that attachment is a precursor to effective degradation as opposed to disassociation of aromatics from the surface or aggregate. This work was funded by ONR and NRL core.

Organic Matter Cycling in Coastal Waters: This was a new start NRL core project examining biodegradation of allocthonous and anthropogenic organic carbon in estuarine systems. The mechanisms, rates and efficiencies of organic carbon mineralization by aquatic bacterial assemblages were examined as part of an approach employing analytical chemistry, optical sciences, molecular biology and remote hyperspectral imaging.

5.4.2 Sampling Events

Intrinsic PAH Bioremediation in Submerged Estuarine Sediments

Description: During eight research cruises in the Charleston Harbor Estuary from January 1999 to October 2001, the team of scientists from the Naval Research Laboratory and academia, measured several parameters in surface and bottom water, sediments and the bottom boundary layer. At various times these measurements have included PAH and metal concentration, bacterial production, nutrient concentration, CTD profiles (salinity, temperature, transmissivity), bacterial assemblage characterization (plasmid profiling, DGGE of 16S rRNA), particulate organic matter concentration, chlorophyll concentration, electron acceptor utilization, 14C-PAH (naphthalene, phenanthrene, fluoranthene) mineralization, sediment size distribution, benthic meiofaunal characterization, allozyme analyses of mysid shrimp communities, and naphthalene inhibition of bacterial production. During some of the samplings, sediment traps have been deployed in the Cooper River, upriver, downriver and at the former Navy Yard. Separate collections were recovered during incoming and outgoing tides to estimate the flux of PAHs and metals through the sample site and exporting downriver. In addition to this work at Charleston, we have performed less comprehensive studies of other aquatic systems, such as the waterways around Philadelphia Navy Yard (5 samplings), Norfolk Naval Station (5 samplings), Washington Navy Yard (3 samplings), Pearl Harbor (2 samplings), and San Diego Bay (2 samplings).

Effect of Tidal Intrusion on Intrinsic Bioremediation in Groundwater

Description: The effect of tidal intrusion on the biodegradation of petroleum in groundwater was examined during 2 samplings in Philadelphia and 4 in Norfolk from June 1999 to October 2000. We found up to 20-fold variation in groundwater bacterial production over the tidal cycle. In addition, mineralization rate of petroleum hydrocarbons varied over 10-fold over the same period. Tidal intrusion may force circulation of groundwater through the impacted area, removing metabolically inhibiting waste products and providing rate limiting nutrients that may enhance biodegradation. Tidal flushing may enhance rates of petroleum biodegradation in impacted nearshore sites and may make intrinsic bioremediation a viable remedial options for attenuation of impacted groundwater once the source material has been removed.

Charleston, SC

10/13/99	Sample sediment at Beazer
	Sample sediment and water from local rivers and in estuary
3/28/01	Sample sediment at Beazer
	Sample sediment and water from local rivers and in estuary
1/23/01	Sample sediment at Beazer
9/09/01	Sample sediment at Beazer
	Sample sediment and water from local rivers and estuary

Norfolk, VA

7/08/99	Sampled groundwater at the Naval Station
7/22/01	Sampled goundwater at the Naval station
8/26/01	Sampled sediment and water from local rivers
1/28/02	Sampled sediment and water from local rivers

Pearl Harbor, HI

7/00	Sampled harbor sediment and water column
8/01	Sampled harbor sediment and water column

Riga, Latvia

5/99	Sampled soil, harbor sediment, and groundwater at Liepaja Harbor
10/01	Sampled soil, harbor sediment, and groundwater at Liepaja Harbor

5.4.3 Presentations and Publications

T.J. Boyd, M.T. Montgomery, R.B. Coffin, **S.R. Reatherford,** and **C.V. Badger,** "Characterization of Intrinsic PAH Bioremediation in Groundwater During Tidal Cycles at the Naval Station Norfolk," NRL Technical Report (submitted).

J.W. Pohlman, R.B. Coffin, C.S. Mitchell, M.T. Montgomery, B.J. Spargo, J.K. Steele, and T.J. Boyd, "Transport, Deposition, and Biodegradation of Particle Bound Polycyclic Aromatic Hydrocarbons in a Tidal Basin of an Industrial Watershed," Environ. Monitor. Assess. (accepted).

T.J. Boyd, M.T. Montgomery, J.K. Steele, J.W. Pohlman, R.B. Coffin, D.M. Ward, B.J. Spargo, and D.C. Smith, "Dissolved Oxygen Saturation Controls PAH Biodegradation in Freshwater Estuary Sediments," Environ. Sci. Technol. (submitted)

M.T. Montgomery, C.L. Osburn, J.K. Steele, C.V. Badger, and T.J. Boyd, "Bacterial Adaptation to PAH Degradation in the Sediments of the Elizabeth River and Lower Chesapeake Bay," presented at the 17th AEHS Annual International Conference of Contaminated Soils, Sediments, and Water, Amherst, MA, October 22-25, 2001. (INVITED)

T.J. Boyd, M.T. Montgomery, J.K. Steele, D.M. Ward, D.C. Smith, B.J. Spargo, R.B. Coffin, J.W. Pohlman, J.G. Mueller, and D.C. Smith, "PAH Biodegradation in an Intertidal Salt Marsh," presented at the Spring Meeting of the EPA Technical Support Project Engineering Forum, San Diego, CA, May 10, 2001. (INVITED)

M.T. Montgomery, T.J. Boyd, J.K. Steele, J.W. Pohlman, R.B. Coffin, D.M. Ward, B.J. Spargo, and D.C. Smith, "Intrinsic Hydrocarbon Bioremediation of Sediments in the Charleston Harbor System," presented at the 21st Annual Meeting of the Society of Environmental Toxicology and Chemistry," Nashville, TN, November 12-16, 2000. (INVITED)

T.J. Boyd, M.T. Montgomery, J.G. Mueller, J.K. Steele, B.J. Spargo, R.B. Coffin, J.W. Pohlman, T. Demetriades-Shah, and M. Slenska, "Source Reduction Effect on Creosote PAH Bioremediation in Marsh Sediments," presented at the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 22-25, 2000. (INVITED)

M.T. Montgomery, T.J. Boyd, J.K. Steele, D.M. Ward, D.C. Smith, B.J. Spargo, R.B. Coffin, J.W. Pohlman, M. Slenska, and J.G. Mueller, "Measuring Intrinsic Bacterial Degradation of PAHs in a Salt Marsh," presented at the International Conference on Wetlands & Remediation, Salt Lake City, UT, November 16-17, 1999. (INVITED)

6.0 DARPA BIOTECH SUPPORT

6.1 Synopsis

Technology Scanning

- Scouted out new, nascent technologies relevant to program development.
- Utilized automated web-based information gathering tools.
- Attended conferences and meetings, initiated interactions with performers and developers of technologies to include:
  - Tissue engineering
  - Advanced biomaterials
  - Blood cell preservation
  - Cell-based devices
  - Fundamental homeostatic mechanisms
- Collected, interpreted, and collated information from current biotechnology press (both technical and business components) such as *BioCentury, Nature Biotechnology, etc.*
- Made original information source available to sponsor.
- Prepared notable findings in timely fashion via reports and weekly verbal/electronic mail communications.
- Provided management and oversight for consultants and subcontractors for the task.

- Assisted with technology commercialization facilitation effort.

- Travel
  - Attended conferences/meetings as directed by sponsor.
  - Performed site visits to current/prospective research sites

- Meeting support
  - Organized meetings to include technical, logistic, administrative, and travel reimbursement/honorarium for guest speakers/attendees.
  - Arranged consultant support as needed per sponsor direction.

6.2 Travel, Meetings and Conferences

6.2.1 DARPA Meetings

- February 2001, San Diego CA. Tissue-based Biosensors and Advanced Diagnostics, attended 3-day meeting and heard multiple presentations from sponsored research programs.

- March 2001, Washington, DC. Metabolic Engineering Kickoff, Helped with the planning and invited industry presenters. Listened to current performers.

- September 2001, Washington DC. Advanced Diagnostics New Start Kickoff, listened to newly funded sponsored research early milestones and goals.

6.2.2 DARPA Site Meetings

- January 29, 2001; Life Technologies, Gaithersburg MD. Discussed update and technical progress regarding gene transfer for trehalose/sucrose synthesis.

- February 26-March 2, 2001; University of Texas, San Antonio. Discussed production and testing methods for blood substitute research.

- July 6, 2001; Washington DC. Waseda University, Tokyo, met with Japanese representatives for discussion on collaborative research program in blood substitutes.
- July 16, 2001; Washington DC. Meeting at DARPA with Virginia Center for Innovative Technology and Biotech Accelerator to discuss local effort with technology commercialization.

- August 27, 2001; American Red Cross, Rockville MD. Discussed lyophilized platelet technology.

- August 30, 2001; Washington DC. Meeting at DARPA with new PM for Metabolic Engineering, Dr. Joe Bielitzki.

- September 6, 2001; TherImmune, Gaithersburg, MD. Discussed regulatory planning for lyophilized platelets.
- November 7, 2001; Bethesda, MD. Attended "Raising Money for Life Science" seminar.
- November 12, 2001; UC-Davis, site meeting regarding lyophilized platelets.
- January 15, 2002; Washington DC. Meeting at DARPA with Seraphim and Adjuvant regarding technology commercialization.

6.2.3 Meetings with Corvista Business Development for Commercialization Facilitation

Corvista was brought on as a subcontractor within another NRL contract to perform the commercialization potential and facilitation for DARPA's biotechnology programs. Our task included assistance in developing a process and tools for individual project assessment. We had meetings at DARPA as follows:

- June 15, 2001; Washington DC. Kickoff and planning meeting.
- September 4-5, 2001; Washington DC. Technology Readiness and Case Study for Commercialization.
- November 16-19, 2001; Washington DC. Commercialization Matrix and Multiple Platform Bundling Strategy Session.

Part of this effort included technical evaluation of the 70-80 funded projects to assess technical readiness. Screening was performed by reading progress reports, interviewing investigators, and scoring the technical status using the criteria outlined below.

The following documents were created by this effort and are deliverables as described in the project statement of work:

- DARPA Biotechnology: Project Update Report 07/30/01
- Strategy/Case Study Session 09/04/01
- DARPA Progress Review 11/16/01
- Investor Summary Book: Technology Transfer Opportunities December 2001

The following conferences were also attended during the completion of this effort:

- February 20-25, 2001; Tissue Engineering, Hilton Head SC

- June 24-27, 2001; BIO 2001 International Convention & Exhibition, San Diego CA
- October 13-17, 2001; American Association of Blood Banks 54th Annual Meeting, San Antonio, TX
- December 7-11, 2001; American Society of Hematology 43rd Annual Meeting, Orlando, FL

DARPA Technology Readiness Levels and Their Definitions

Technology Readiness Level	Description
Basic principles observed and reported	Lowest level of technology readiness. Scientific research begins to be translated into applied research & development. Examples include paper studies on basic properties.
Technology concept and/or application formulated	Invention begins. Once basic principles are observed, practical applications can be invented. The application is speculative and there is no proof or detailed analysis to support the assumption. Examples are still limited to paper studies.
Analytical and experimental critical function and/or characteristic proof of concept	Active R&D is initiated, to include analytical studies and laboratory studies to physically validate analytical predictions of separate elements of the technology. Examples include components that are not yet integrated or representative.
Component/breadboard/prototype validated in either laboratory or relevant environment.	Basic technological components are integrated to establish that the pieces work together. A representative model or prototype has been developed and tested in a simulated environment.
System prototype demonstration in an operational environment.	Prototype near or at planned operational system. Represents a major step up from TRL 4, requiring demonstration of an actual system prototype in an operational environment.

7.0 SUBCONTRACTORS

Over the course of this contract, GEO-CENTERS, INC. initiated a number of subcontracts for additional support of the research tasks listed at the beginning of this document. The following firms or agencies provided research support for the site characterization and remediation of contaminated soils, groundwater, and river sediments task.

- PrSM, Inc. - provided personnel for sampling events and NPDES permitting processes
- U.S. Dept of Fish & Wildlife – provided technical support for Anacostia River Assessment project
- Syracuse Research Corporation – provided technical support for Anacostia River Assessment project.
- Geosea Consulting, Inc. – also provided technical support for Anacostia River Assessment project. A 200 page report entitled, "Interpretive Summary of Existing Data Relevant to Potential Contaminants of Concern within the Anacostia River Watershed," was generated and supplied under separate cover.
- GEO-CENTERS, Inc. also initiated a subcontract with Texas A& M University for a calibration study of a new stable carbon isotope laboratory within the Chemistry building at the Naval Research Laboratory. By the end of the contract, approximately 50% of the project was completed, and the data were provided in a report supplied to GEO-CENTERS, INC. under separate cover. Copies of all reports were shared with appropriate NRL scientists.
- As noted in section 6.0 (DARPA Biotech Support), GEO-CENTERS, Inc. initiated a subcontract with Corvista, Inc. for additional aid in meeting project objectives. The contributions of the subcontractor are outlined in that section.