1.0 PROJECT NAME

ASSESSMENT OF CONTAMINANTS IN SEDIMENTS FROM CASCO BAY

prepared by

DR. TERRY L. WADE, PROJECT MANAGER
GEOCHEMICAL AND ENVIRONMENTAL RESEARCH GROUP
TEXAS A&M UNIVERSITY
833 GRAHAM ROAD
COLLEGE STATION, TEXAS 77845

and

PRINCIPAL INVESTIGATORS
DRS. JAMES M. BROOKS AND THOMAS J. JACKSON

prepared for

CASCO BAY NATIONAL ESTUARINE PROGRAM

and

U.S. ENVIRONMENTAL PROTECTION AGENCY
REGION 1

SEPTEMBER 1994

2.0 PROJECT DESCRIPTION

A. Objective and Scope

Casco Bay has a wealth of natural resources and marine habitats that support a rich and diverse ecological web of life. Casco Bay's beauty, clean water, fish and waterfowl, and its deep and protected waters have made it a sought-after location for residences, business, industry and recreation. It is these very activities which threaten the environmental integrity of the Bay. However, although these threats exist, Casco Bay Program offers the opportunity to emphasize the protection of a resource as well as its restoration.

In April, 1990, Casco Bay was designated an estuary of national significance and included in EPA's National Estuary Program. The NEP goal is to protect and improve the water quality and enhance the living resources through the development of comprehensive conservation and management plans (CCMPs) that work to ensure the ecological integrity of designated estuaries.

Casco Bay encompasses the body of water enclosed by Cape Small to the northeast and Cape Elizabeth to the southwest. The Technical Advisory Committee has divided the Bay into 5 regions. These regions were chosen on the basis of geologic and other features. Casco Bay receives freshwater from rivers that discharge directly to the Bay (Fore, Presumpscot, and Royal Rivers). It also appears that the Androscoggin and Kennebec Rivers, which discharge just north of Cape Small, influence Casco Bay. For this reason, the eastern boundary of Region V extends beyond Cape Small, in order to capture the plume of the Kennebec/Androscoggin. The most densely populated portion of the Casco Bay watershed is in the Portland metropolitan area, and the Fore, and Presumpscot Rivers and Back Cove. These areas have historically received higher contaminant loadings than other regions of Casco Bay.

An extensive investigation of sediment contamination of Casco Bay was recently completed and reported by the Geochemical and Environmental Research Group (GERG). Sediment samples were collected by researchers from the University of Maine (UME), the Marine Geological Survey (MEGS), EPA and GERG on EPA and UME vessels in early August 1991. Selected

Table 1. Target Analyte List and Method Detection Limits.

Dioxin/Furan Congeners	*MDL (pg/g)
2,3,7,8-TCDD	1
1,2,3,7,8-PCDD	5
1,2,3,4,7,8-HXCDD	5
1,2,3,6,7,8-HXCDD	5
1,2,3,7,8,9-HXCDD	5
1,2,3,4,6,7,8-HPCDD	5
OCDD	10
2,3,7,8-TCDF	1
1,2,3,7,8-PCDF	5
2,3,4,7,8-PCDF	5
1,2,3,4,7,8-HXCDF	5
1,2,3,6,7,8-HXCDF	5
1,2,3,7,8,9-HXCDF	5
2,3,4,6,7,8-HXCDF	5
1,2,3,4,6,7,8-HPCDF	5
1,2,3,4,7,8,9-HPCDF	5
OCDF	10
BUTYLTINS	MDL ng Sn/g
Monobutyltin (MBT)	5
Dibutyltin (DBT)	5
Tributyltin (TBT)	5

*Based on 20 g wet weight of sediment

Butyltins are an ingredients of anti-fouling paints. The sample collection sites for butyltins will, therefore, be chosen in areas in close proximity to marinas, shipyards, docking facilities, anchoring areas and major shipping channels. A majority of the butyltin samples (20 or more) will be collected from the Inner Bay. Samples of fine grained sediments will be collected in the vicinity of major shipyards, anchoring locations (i.e. near Clapboard Island) and selected marinas. Additional samples will be collected near major shipping lanes (channels). Samples for butyltin analyses will also be collected from marina in East and West Bays. At least one sample will be collected from Outer Bay and Cape Small to determine the geographic extent of the butyltin contamination.

Dioxin/furan sediment samples will be collected from locations where their concentrations are suspected to be highest. Dioxin/furan are by-from the national Institute of Standards and Technology (NIST) will be analyzed as a laboratory reference material. This SRM does not currently have certified dioxin/furan concentrations, but our analyses of this material indicates they are present and this well homogenized material makes a good choice for a marine estuarine reference material (it was collected from Baltimore Harbor).

The methods used for this study will be those that the Geochemical and Environmental Research Group (GERG) uses for other programs including: NOAA's "National Status and Trend Program", EPA's Environmental Monitoring and Assessment Program - Near Coastal (EMAP-NC) and those approved by the U.S. Fish and Wildlife Service (FWS) for trace organic contaminant analyses. These methods have undergone extensive intercalibration with EPA, NOAA, NIST and FWS. The proposed methods are detailed in the attached Standard Operating Procedures (SOPs). The method used at GERG for dioxin/furan will be EPA Method 1613.

There is a limited amount of data for butyltin concentrations in sediment from Casco Bay and no known data for dioxin/furan. We will conduct a literature search to determine if any additional information on these contaminants exists for Casco Bay.

A comprehensive evaluation of the sediment quality of Casco Bay was prepared by GERG as a result of our initial study, including historical data (Kennicutt et al., 1994). The report from the proposed study would describe spatial trends in butyltins and dioxin/furan in the Bay. To the extent possible, the report will evaluate temporal trends in contaminant concentrations, using historical and current data. We also anticipate publishing the results of the proposed study in the peer reviewed literature, as we did for the initial study (Kennicutt et al., 1994).

D. Monitoring Parameters and Collection Frequency

Under the cost constraints of this proposed study we anticipate analyzing contaminants from 30 sites for butyltins and 30 sites for dioxin/furan from Casco Bay. The stations selected for butyltin and dioxin/furan analyses will be based on our knowledge of other sediments contaminant distribution for Casco Bay, proximity to suspected input sources and known depositional areas. Selection of the final sample suite to be

- 1.0 Executive Summary
- 2.0 Introduction and Background
  - 2.1 Overview
  - 2.2 Historical Data
    - 2.2.1 Butyltins
    - 2.2.2 Dioxin/furan
- 3.0 Sampling and Analytical Procedures
- 4.0 Trace Organic Contaminant Concentrations
  - 4.1 Butyltins
    - 4.1.1 Historical Data
    - 4.1.2 Comparison with Other U.S. Coastal Waters
  - 4.2 Dioxins and Furans
    - 4.2.1 Historical Data
    - 4.2.2 Comparison with Other U.S. Coastal Waters
  - 4.3 Summary
- Appendix A: Butyltin Analytical Results
- Appendix B: Dioxins and Furans Analytical Results
- Appendix C: Standard Operating Procedures

All data will be put into a SAS (Statistical Analysis System) data base from which standard statistical tests can be undertaken. We will provide the data to the NEP program electronically in EPA's ODES's format. We have developed all the download programs as part of our current NEP program to convert data from our in-house data bases to the ODES's format. We will comply with all applicable state and federal laws and regulations. Appended to the report will be tables of all analytical data and Standard Operating Conditions.

The Final Report (one camera-ready unbound copy and three bound copies) will incorporate management conference and agency review comments on the Draft Report. It will be submitted by 31 March 1995.

3. PROJECT ORGANIZATION AND RESPONSIBILITY

The project will be performed by personnel of GERG, College of Geosciences and Maritime Studies at Texas A&M University. Dr. James M. Brooks is the director of GERG which is located at 833 Graham Road in College Station, Texas (77845). The telephone number is (409)690-0095, and the FAX number is (409)690-0059. Organization of the project and interaction with CBNEP and USEPA is depicted in Figure 1.

Dr. Terry L. Wade is the project manager and will be responsible for the overall management and execution of the project, study design and report writing. Principal Investigators (PI) for the project include Drs. James M. Brooks and Thomas J. Jackson. The PIs will aid the Project Manager in technical aspects of the project including data interpretation and producing the final report and peer reviewed publication. Other project team members include: Dr. Gary Wolff (Data Manager); Dr. Guy Denoux (Field and Analytical Systems Manager); Mr. Hank Chambers (Laboratory Operation Manager); Mr. Bernardo Garcia-Romero (Butyltins); Ms. Laura Chambers (Dioxin/Furan); Ms. Grace Ekman (Quality Assurance Manager).

Dr. Gary A. Wolff will have primary responsibility for organization and establishment of the data base including information obtained from agency sources and from the field surveys. Dr. Guy Denoux will be in charge of sampling and will be the project administrator He will have responsibility for ensuring that the laboratory analyses passes QA/QC and is completed on time. Mr. Hank Chambers and Ms. Laura Chambers are experts in the analyses of dioxin/furan at ultra-trace concentrations (parts per trillion). They will be joined by Drs. Jackson and Wade to aid in data interpretation. Mr. Bernardo Garcia-Romero as well as Dr. Wade are experts in butyltin analyses. For additional details please see the attached resumes of these investigators.

4.0 DATA QUALITY REQUIREMENTS AND ASSESSMENTS

Accurate and precise techniques, state-of-the-art instrumentation, a formal quality assurance program, and skilled analysts are essential for production of high quality trace organic contaminant data. Laboratory personnel at GERG have extensive experience with the environmental analyses proposed. These methods have been routinely used and validated by GERG staff for a number of matrices, including sediments. The proposed sample preparation, compound purification, and instrumental analysis techniques for butyltins and dioxin/furan are well established in GERG's laboratories. GERG staff have over fourteen years combine experience in butyltin analyses and over fifteen years combined experience in EPA PCDD/PCDF methods (8280, 8290, 1613, 23, TO-9, CLP DFLM01.0 and SOW 68-C9-0019) and in method specified by other agencies (CARB 428 and NCASI 551). Acceptance of GERG's analytical proposal will ensure that high

In addition, some GERG analytical staff members assigned to this project have previously had similar roles at other facilities in numerous other national environmental monitoring projects of trace PCDD/PCDF contaminants. These relevant projects which were managed by these GERG staff include:

- Tetra Tech, Inc. - "Lower Columbia River Bi-State Program: Reconnaissance Survey" - Members of the GERG staff provided analysis of dioxins/furans by HRGC/HRMS on over 60 samples of fish tissue and 20 samples of river sediments. Full congener analysis and toxicity equivalencies were provided in Level IV data packages on all samples. In addition, these staff ground and composited over one hundred whole fish following the strict Puget Sound Estuary Program Protocols.

  Contact: Steve Ellis, Ph.D. (206) 883-1912
  Tetra Tech, Inc.
  15400 NE 90th, Suite 100
  Redmond, WA 98052

- APTUS/Westinghouse, Inc. - "Byers Warehouse Incineration Project" - Members of the GERG staff provided analysis of dioxins/furans by HRGC/HRMS on samples of slag, fly ash, ambient air, chemical waste, brine and soil for Aptus Environmental during the incineration of over 700,000 pounds of dioxin-contaminated pesticides at their Coffeyville, Kansas facility. Analysis of fly ash and slag were accomplished on a 48-hour basis over the entire course of the burn, approximately eight weeks, enabling Aptus engineers to adjust burn parameters in as close to real time as possible.

  Contact: John Blandamer (316) 251-6380
  APTUS Environmental Services
  P.O. Box 1328
  Coffeyville, KS 67337

- Battelle, N.W. Marine Research Laboratory - "Snake River Drawdown Sediment Survey" - Over 50 sediment samples were analyzed for PCDD/PCDF isomers using HRGC/HRMS via U.S. EPA Method 8290, for Battelle N.W. Marine Sciences. Complete, U.S. EPA Level IV QA/QC data packages were delivered within one week of sample receipt.

  Contact: Lisa Lefkovitz (206) 681-3654
  Battelle N.W. Marine Research Laboratory
  439 West Sequim Bay Road
  Sequim, WA 98382

Method

The American oyster (*Crassostrea virginica*) was collected at all Gulf of Mexico sites. Sampling started in January 1986.[6] Galveston Bay NS&T sampling sites include; Ship Channel (GBSC), located at the mouth of Goose Creek in Tabbs Bay, Yacht Club (GBYC), located near the judge's stand of the Houston Yacht Club, Todd's Dump (GBTD), located on a reef midway between Eagle Point and Red Fish Island, Hanna Reef (GBHR), a reef that separates East Bay from Galveston Bay, Offats Bayou (GBOB), near the 61st street bridge in Galveston, and Confederate Reef (GBCR), near Deer Island in West Bay. Details of the methods used to generate the present data have been reported previously.[7,8,9]

Results

Since the data for the contaminants is a log normal distribution, "high" concentrations have been defined as those that exceed the median plus one standard deviation for the log data.[10] Explaining the temporal variation in contaminant concentration is complicated because many biological and environmental factors may affect the measured concentration. Generally, it has been observed that populations with high contaminant concentrations are characterized as being less healthy.[9]

Chlordane

In the NS&T program, total chlordanes is defined as the sum of the components alpha-chlordane, trans-nonachlor, heptachlor and heptachlor epoxide. This definition is recognized as an under estimate of the complex mixture known as chlordane. The median concentration of total chlordanes for the Gulf of Mexico exhibits an overall decrease in concentration for the first seven years of NS&T, but the variation at each site within Galveston Bay is not covariant with the total Gulf-wide data, indicating strong local control for the source of chlordanes. The "high" concentration criterion relative to the Gulf-wide data was observed in 39 of the 102 samples for 1986-1992. GBOB was the site with the highest concentration of total chlordanes, which is probably due to its proximity to urban development where chlordane may have been used as a termiticide.

DDT and derivatives

DDT was once the most widely used insecticide in the world. The total DDTs is the sum of the p,p'- and o,p' isomers of DDT, DDD and DDE. The distribution of the concentration of DDTs for oysters from NS&T's Gulf of Mexico sites and all NS&T sites almost overlay. The Galveston Bay concentrations are distributed equally along the distribution curve. Bivalves appear to exhibit little biological effects attributable to DDT residues. Of the 102 Galveston Bay samples, 19 of the samples exceeded the criterion for "high" concentrations of having concentrations greater than the Gulf-wide median plus one standard deviation for each year. The general trend in the median total DDTs for all Gulf of Mexico sites is cyclic. The cyclic trend seems to fit the response a large-scale climatic control, such as the El Nino /Southern Oscillation, would impose on the contaminant concentration.[9] For the first seven years, GBSC was the site with the highest concentration of total DDTs.

Dieldrin

Dieldrin is synthesized by the oxidation of aldrin. It has generally been restricted to agricultural use.[11] The use of dieldrin and related aldrin were banned in the middle 1970's.[12] The "high" concentration criterion using Gulf-wide data was exceeded by 39 of

4) de Boer, J., Dao, Q.T., and van Dortmond, R. (1992); retention Times of Fifty one Chlorobiphenyl Congeners on Seven Narrow Bore Capillary Columns Coated with Different Stationary Phases., *Journal of High Resolution Chromatography*, **15**, 249-255.

5) de Voogt, P., Wells, D.E., Reutergardh, L., and Brinkman, U.A.Th. (1990); Biological Activity, Determination and Occurrence of Planar, Mono- and Di-ortho PCBs., *Intern.J.Environ.Anal.Chem*, **40**, 1-46.

6) Lazar, R., Edwards, R.C., Metcalfe,C.D.,Metcalfe,T. Gobas,F.A.P.C., and Haffner, G.D.(1992);A Simple Novel Method for the Quantitative Analysis of Coplanar (Non-ortho Substituted) Polychlorinated Biphenyls in Environmental Samples.,*Chemosphere*, **25**, 493-504.

7) tsuda,S., Kawano, M., Wakimoto,T., and Tatsukawa, R. (1993); Application f Charcoal/Silica Gel Column for Analysis of Polychlorinated Dibenzo-p-Dioxins (PCDDs) and polychlorinated Dibenzofurans (PCDFs), *Chemosphere*, **27**, 2117-2122.

8) de Boer, J., Stronck,C.J.N., van der Valk,F., Wester,P.G. and Daudt,M.J.M. (1992); Method for the Analysis of Non-ortho Substituted Chlorobiphenyls in Fish and Marine Mammals.,*Chemosphere*, **25**, 1277-1283.

The present paper reports an analytical protocol for the separation and determination of the non-orto, mono-ortho and di-orthoPCB's, Dioxins and Furans in environmental samples.

EXPERIMENTAL

Glassware, solvents and chemicals were pesticide grade or cleaned and conditioned as usual for trace organic analysis. All the native target analytes were obtained from Ultra Scientific (Kingstown, Richmond,U.S.A.) while all labeled compounds were obtained as concentrated solutions from Cambridge Isotope Laboratories (Andover, Massachusetts, U.S.A.)

Sample Preparation and Cleanup

To test the analytical protocol, commercially available eggs were spiked with the appropriate internal and recovery standards and, as required, with a mixture of all the targeted analytes or a 1:1:1:1 mixture of Aroclor 1248, Aroclor 1254 and Aroclor 1260.

The samples (10g) were weighed into 200 ml centrifuge bottles and mixed with sodium sulfate (40 g). After adding 100 ml of methylene chloride, the samples were macerated by using a Tekmar tissuemizer (Cincinnati, Ohio, U.S.A.) for 5 min. Two more extractions with 100 ml of methylene chloride were performed and, after the sample was centrifuged, the solvent extracts were combined in a 500 ml flat bottom flask. After the extraction, the samples are concentrated and the solvent is exchanged to 100 ml of hexane for the following step.

To eliminate the interference caused by lipid material, the hexane extract was treated with 40 g of a 44% mixture of concentrated sulfuric acid\silica gel (Silica Gel 60, EM Science, Gibbstown, New Jersey, U.S.A.) and the samples were shaken for 2h. After filtration and concentration, the extracts were ready for chromatographic cleanup.

Mixed-Bed Silica Column

The concentrated sample in hexane was further purified by using a 13 mm ID x 300 mm length chromatography column containing from top to bottom: 1 cm of quartz sand, 1 cm sodium sulfate 2g of silica gel (Silica Gel 60, EM Science, Gibbstown, New Jersey, U.S.A.), 8g 44% sulfuric acid /silica gel, 1g silica gel, 4g 33% 1N sodium hydroxide/silica gel, 1g silica gel and a glass wool plug. After the column was pre-rinsed with hexane, and the sample was loaded, the column was eluted with 120 ml of hexane. The collected eluate is then evaporated to 1 ml by using a rotary evaporator.

Basic Alumina Column Cleanup

The hexane concentrate obtained from the mixed-bed column is then applied to a 13 mm ID x 300 mm length chromatography column containing, from top to bottom: 1 cm of sodium sulfate, 6 g of alumina (Alumina, Activated, 80-200 mesh, EM Science, Gibbstown, New Jersey, U.S.A.). The column is then rinsed with 60 ml hexane and the eluate is discarded. The target analytes are then recovered by eluting the column with 40 ml of a 70:30 hexane-methylene chloride mixture. The collected eluate is then evaporated to 1 ml by using a rotary evaporator.

Charcoal Column Cleanup

All samples were fractionated by using a low pressure Michael-Miller type chromatographic column (10 mm ID x 300 mm length, ACE Glass Inc., Vineland, New Jersey, U.S.A.) The glass column was

8) Michele M. Schantz, Reenie M. Parris, and Stephen A. Wise (1993); NIST Standard Reference Materials (SRMs) for Polychlorinated Biphenyl (PCB) Determinations and Their Applicability to Toxaphene Measurements. *Chemosphere,* **27** , 1915-1922.

9) Gardinali, P.G., Wade, T.L., Chambers, L., and Brooks, J.M., (1994); A Complete Method for the Quantitative Analysis of Planar, Mono, and Diortho PCB's, Polychlorinated Dibenzodioxins, and Furans in Environmental Samples., (submitted for publication).

10) Tondeur, Yves, "Method 8290: Analytical Procedures and Quality Assurance for Multimedia Analysis of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry", USEPA EMSL, Las Vegas, Nevada, June 1987. (Revision 0, November 1990.)

for PCBs, chlorinated pesticides, PAHs, and inorganic constituents[4]. SRM 1941a is homogenized marine sediment with certified and non-certified values for PCBs, chlorinated pesticides, PAHs, and inorganics[5].

Presently, NIST does not have available a natural matrix SRM with certified concentrations of PCDDs and PCDFs. The concentrations of polychlorinated dioxins and furans in SRM 1945, SRM 1974, and SRM 1941a are of interest because of their high toxicity factors[6], and because the analysis of marine mammal, mussel tissues and sediments have become important tools in the determination of organochlorine contamination in the environment[7]. Because these SRMs have been demonstrated to be homogenous for other organic contaminants, they would be expected to be reliable standards for validation of polychlorinated dioxins and furans in marine mammals, mussels and sediments as well. The objective of this study was to determine the concentration of PCDDs and PCDFs in three readily available NIST SRMs to see if they would be useful as reference materials for environmental contaminant studies focusing on these analytes.

Experimental

Three to five grams of the NIST SRMs (tissues and sediments) were spiked with the appropriate internal standards. Tissues were mixed with sodium sulfate and extracted with three aliquots of methylene chloride with a Tekmar tissuemizer for 5 minutes each. Sediment samples were extracted for 18 hours with toluene in a soxhlet extractor fitted with a Dean Stark trap. A 44% mixture of concentrated sulfuric acid/silica gel was used to remove lipid interferences from the tissue samples. Further purification of all sample extracts was achieved by mixed-bed silica, basic alumina, and AX-21 Super Activated Carbon column chromatography. The extracts were reduced to a final volume of 20μL and the appropriate recovery standards added[9].

Two μL of the concentrated extract are injected into an HRGC/HRMS system capable of performing selected ion monitoring at resolving powers of at least 10,000. The identification of the sixteen 2,3,7,8-substituted isomers for which a ^{13}C-labeled standard is available is based on their elution from a DB-5MS analytical column at their exact retention time and the simultaneous detection of the two most abundant ions in the molecular ion region. The identification of OCDF is based on its retention time relative to ^{13}C-OCDD. Confirmation is based on a comparison of the ratios of the integrated ion abundance of the molecular ion species to their theoretical abundance ratios. Quantitation of the individual congeners is achieved in conjunction with the establishment of a multipoint calibration curve for each homologue, during which each calibration solution is analyzed once[10]. Concentrations of 2,3,7,8-TCDF were confirmed on a DB-225 analytical column.

Results and Discussion

Results from replicate analyses of NIST SRM 1945, SRM 1974, and SRM 1941a are given in Table 1. Included are measured concentrations of the seventeen 2,3,7,8-substituted PCDD/PCDF isomers, average concentration, relative standard deviation, and percent recovery of the isotopically labeled internal standards.

SRM 1941a, Organics in Marine Sediment contained measurable concentrations of sixteen out of seventeen 2,3,7,8-substituted PCDD/PCDF isomers within the calibration range of the instrument. In general, concentrations increase with increasing degree of chlorination, with the OCDF and OCDD analytes present at approximately

Wade T.L., Atlas E.L., Brooks J.M., Kennicutt II M.D., Fox R.G., Sericano J., Garcia-Romero B., and DeFreitas D. (1988a) NOAA Gulf of Mexico Status and Trends Program: Trace Organic Contaminant Distribution in Sediments and Oysters. *Estuaries* **11**, no. 3, 171-179.

Wade T.L., Garcia-Romero B., and Brooks J.M. (1988b) Butyltin *Environ. Sci. Technol.* **22**, 1488-1493.

(Received in Germany 2 March 1990; accepted 10 April 1990)

Brooks J.M., Wade T.L., Atlas E.L., Kennicutt II M.C., Presley B.J., Fay R.R., Powell E.N., and Wolff G. (1988) Analysis of bivalves and sediments for organic chemicals and trace elements. *Annual Report for NOAA's National Status and Trends Program*, Contract 50-DGNC-500262. 357 pp.

Clavell C., Seligman P.F., and Stang P.M. (1986) Automated analysis of organotin compounds: A method for monitoring butyltin in the marine environment. *Proceedings of the Oceans '86 Organotin Symposium*, vol. 4. pp. 1152-1154.

Garcia-Romero B. (1988) Temporal and Spatial Variations of Butyltin Concentrations in Bivalve and Sediment Samples from some Coastal Areas of the United States. Thesis, Texas A&M University, College Station, TX, 82 p.

Grovhoug J.G., Seligman P.F., Vafa G., and Fransham R.L. (1986) Baseline measurements of butyltin in U.S. harbors and estuaries. *Proceedings of the Oceans '86 Organotin Symposium*, vol. 4. pp. 1283-1288.

Hall L.W. Jr. and Pinkney A.E. (1985) Acute and sublethal effects of organotin compounds on aquatic biota: An interpretative literature evaluation. *CRC Critical Reviews in Toxicol.* **14**,159-209.

Harris J.R.W. and Cleary J.J (1987) Particle-water partitioning and organotin dispersal in an estuary. *Proceedings of the Oceans '87 International Organotin Symposium*, vol. 4. pp. 1370-1374.

Hinga K.R., Adelman D., and Pilson M.E.Q. (1987) Radiolabeled butyltin studies in the MERL enclosed ecosystems. *Proceedings of the Oceans '87 International Organotin Symposium*, vol. 4. pp. 1416-1420.

Huggett R.J., Hunger M.A. and Westbrook D.J. (1986) Organotin concentrations in the Southern Chesapeake Bay. *Proceedings of the Oceans '86 Organotin Symposium*, vol. 4. pp. 1262-1265.

Johnson W.E., Hall L.W. Jr., Bushong S.J., and Hall W.S. (1987) Organotin concentrations in centrifuged versus uncentrifuged water column samples and in sediment pore waters of Northern Chesapeake Bay tributary. *Proceedings of the Oceans 87 International Organotin Symposium*, vol. 4. pp. 1364-1369.

Krone C.A., Brown D.W., Burrows D.G., Boyar R.G., Chan S.-L., and Varanasi U. (1989) A method for analysis of butyltin species and measurement of butyltins in sediment and English sole livers from Puget Sound. Marine Environ. Res 27, 1-18.

Langston W.J., Burt G.R., and Ming Jiang Z. (1987) Tin and organotin in water, sediments, and benthic organisms of Poole Harbor. *Mar. Poll. Bull.* **18**,634-639.

Maguire R.J. (1986) Review of the occurrence, persistence and degradation of tributyltin in fresh water ecosystems in Canada. *Proceedings of the Oceans '86 Organotin Symposium*, vol. 4. pp. 1252-1255.

Maguire R.J., Tkacz R.J., Chau Y.K., Bengert G.A., and Wong P.T.S. (1986) Occurrence of organotin compounds in water and sediments in Canada. *Chemosphere* **15**,253-274.

Randall L. and Weber J.H. (1986) Adsorptive behavior of butyltin compounds under simulated estuarine conditions. *Sci. Tot. Env.* **57**,191-203.

Schatzberg P. (1987) Organotin antifouling hull paints and the U.S. Navy. A historical perspective. *Proceedings of the Oceans '87 International Organotin Symposium*, vol. 4. pp. 1324-1333.

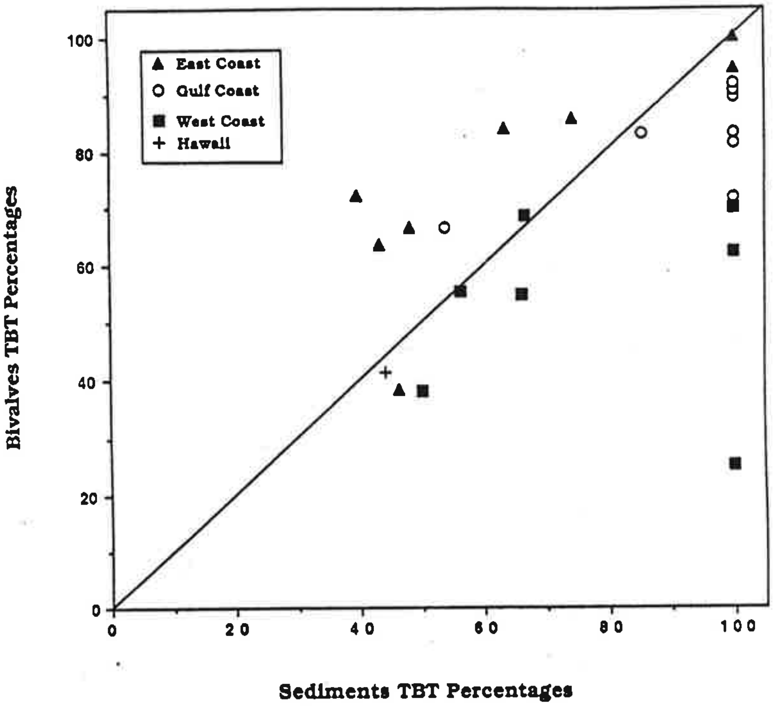


Figure 5. Comparison of TBT percentages in sediments vs. bivalves from the same site.

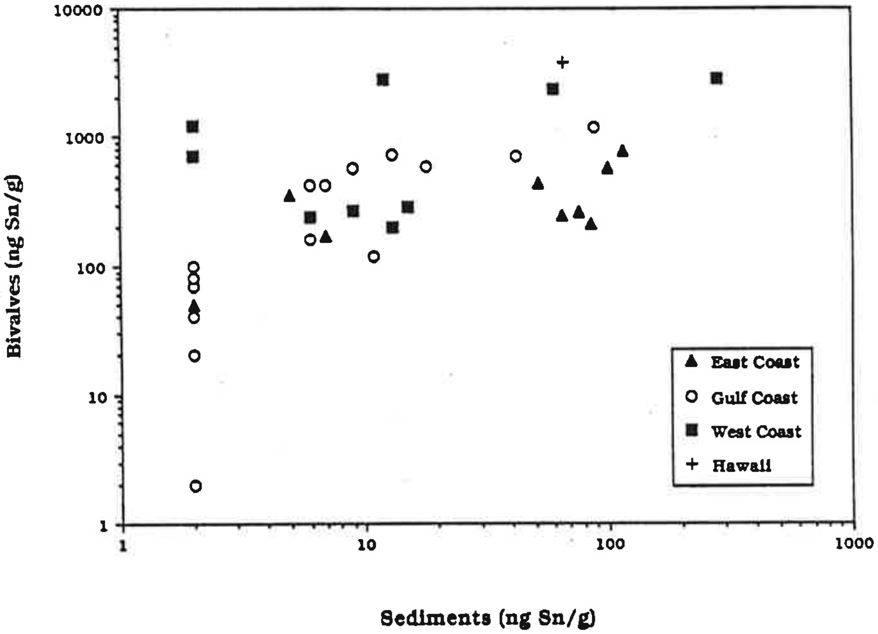


Figure 6. Concentration of total butyltins in sediments vs. bivalves from the same site.

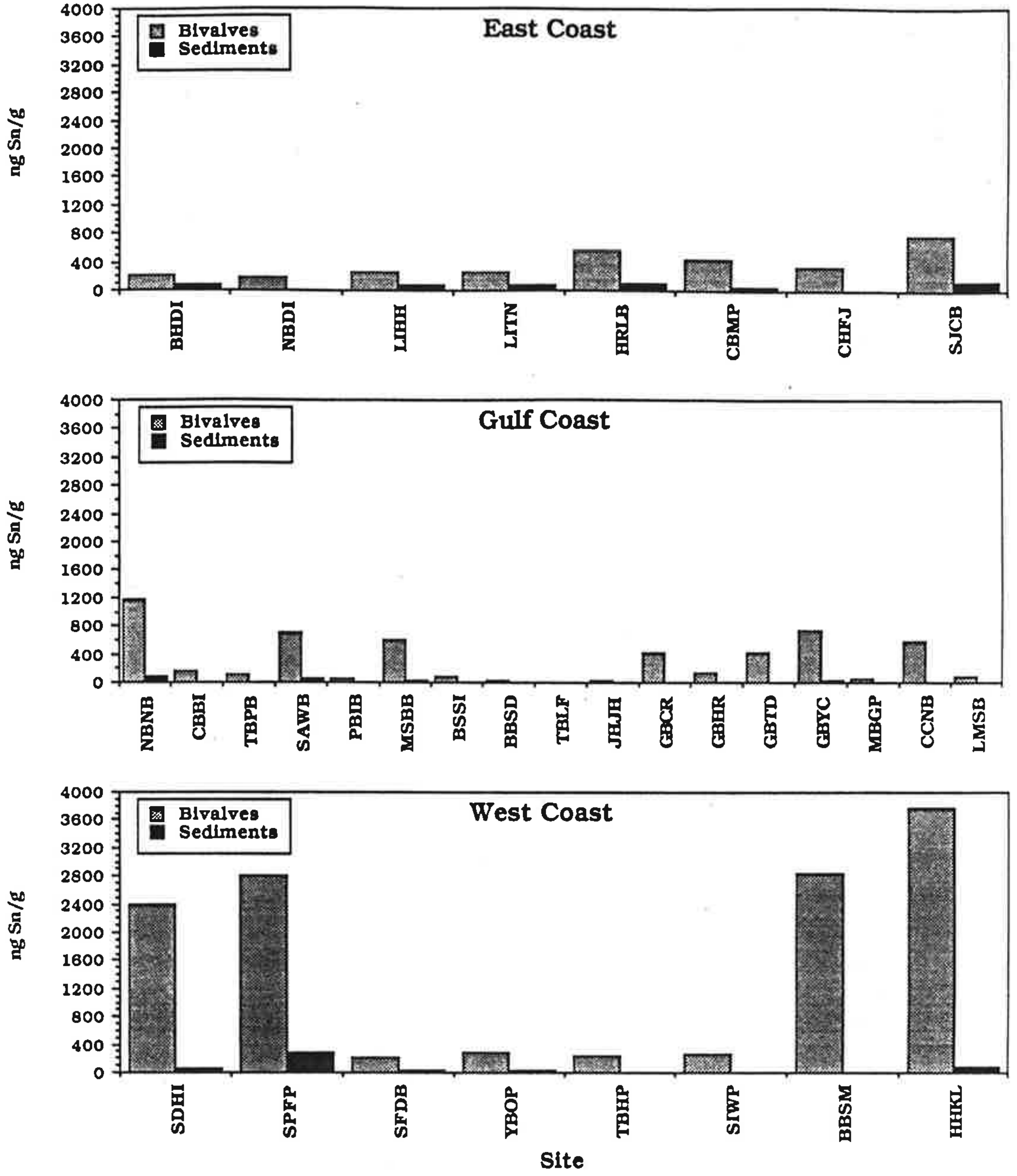


Figure 3. Comparison of total butyltin concentrations in sediments and bivalves.

with higher sediment butyltin concentrations is seen. There is not, however, a strong statistical correlation. This may in part be due to collection of sediments some distance from the bivalve collection site in some cases or difference in sediment characteristics. There was, however, not apparent correlation between sediment grain size or organic carbon content and butyltin concentration (Garcia-Romero, 1988).

degradation products tend to accumulate and, therefore, have higher concentrations than TBT (Seligman *et al.*, 1986b). DBT concentrations in Poole Harbour sediments ranged from 10 to 570 ng Sn/g (Langston *et al.*, 1987) and in Puget Sound sediment from < 3.8 to 950 ng Sn/g (Krone *et al.*,1989). Values reported here were in the lowest part of these ranges (Table 1).

The small MBT concentrations present in sediments may be a result of its higher solubility in seawater than the other butyltins. The deposition of MBT in sediments may occur through settling of particles containing MBT and may be decreased by redissolution. DBT and MBT found in sediments may be produced in the water column and then deposited to the sediments or produced from TBT degradation after deposition. MBT has been reported as the principal product of TBT degradation in sediments (Seligman *et al.*, 1988a; Stang and Seligman, 1986). The detection of TBT, DBT and MBT in 30% of the sediment samples analyzed suggests that degradation is occurring in the sediments. The partition coefficients reported for TBT and MBT indicate only a small percent of the butyltins reaching the sediments would be in the form of DBT and even less MBT would be present, unless the predominant form in seawater was DBT and MBT, which is not likely. Possibly, under certain conditions, degradation of TBT in the sediments may produce DBT and MBT, not just MBT as previously reported (Seligman *et al.*, 1988a). Another possibility is the fact that different sediment types that contain different types and amounts of organic and inorganic ligands may affect the efficiency of retention of DBT and MBT. Further studies would be required to determine if this is the case.

Butyltin concentrations and distribution in sediments may reflect partitioning processes occurring in the water column between butyltins and suspended particles. Although less than five percent of TBT present in unfiltered waters has been found to be associated with suspended particles (Valkirs *et al.*, 1987), settlings of particles containing TBT may be important process in deposition of TBT to the sediments. Mean partition coefficients determined for TBT and DBT are 4.0×10^4 and 4.5×10^2, respectively (Hinga *et al.*, 1987). This suggests that of the total water column concentration of TBT and DBT only 4.1 and 0.044%, respectively, is associated with suspended particles. This indicates that if TBT and DBT water column concentrations were equal and if equilibrium was reached, the sediment would contain 99% TBT and only 1% DBT. However, complexation of butyltins with organic or inorganic ligands complicate predictions based on these simple partition coefficient calculations.

TBT present in sediments may be a long-term source of TBT to the environment. TBT's half-life of more than 20 weeks in sediments and experimental evidences of TBT desorption processes (Clavel *et al.*, 1986; Harris and Cleary, 1987; Johnson *et al.*, 1987; Maguire, 1986; Seligman *et al.*, 1988b; Stang and Seligman, 1986; Unger *et al.*, 1987; Valkirs *et al.*, 1987) indicate TBT contaminated sediments may act as future sources of TBT to the water column. Huggett *et al.* (1986) found evidence suggesting TBT contaminated bottom sediments were a source of organotin input to estuarine waters.

Valkirs *et al.* (1986) found that 3 to 9% of the TBT associated with particulate matter was unavailable to direct hydridization. This indicates a stable association between suspended particles and TBT which may not be bioavailable. This association may be the reason that TBT has a much longer half life in sediments than in the water column (Stang

TABLE 1. Butyltin concentrations (ng Sn/g) in sediment samples from United States coastal areas.

Site Code	TBT	DBT	MBT	Σ Butyltins
EAST COAST				
BHDI	41	27	17	85
NBDI	7	5	<5	7
LIHH	35	30	11	76
LITN	28	26	11	65
HRLB	40	39	22	101
CBMP	33	6	13	52
CHFJ	5	<5	<5	5
SJCB	87	22	8	117
MEAN	35	19	10	56
GULF COAST				
NBNB	48	14	27	89
CBBI	6	<5	<5	6
TBPB	<5	<5	<5	<5
SAWB	36	6	<5	42
PBIB	<5	<5	<5	<5
MSBB	18	<5	<5	18
BSSI	<5	<5	<5	<5
BBSD	<5	<5	<5	<5
TBLF	<5	<5	<5	<5
JHJH	<5	<5	<5	<5
GBCR	6	<5	<5	6
GBHR	11	<5	<5	11
GBTD	7	<5	<5	7
GBYC	13	<5	<5	13
MBGP	<5	<5	<5	<5
CCNB	9	<5	<5	9
LMSB	<5	<5	<5	<5
MEAN	9	1	2	12
WEST COAST				
SDHI	30	21	9	60
SPFP	187	63	32	282
SFDB	13	<5	<5	13
YBOP	10	5	<5	15
TBHP	6	<5	<5	6
SIWP	9	7	<5	16
BBSM	12	<5	<5	12
MEAN	38	14	6	58
HAWAII				
HHKL	29	20	17	66
OVERALL MEAN	22	9	5	36

measured in freeze dried sediments was about twice the concentration obtained by the wet sediment extraction. Because the freeze dried sediment extraction technique provides better recoveries, all sediment samples reported here were analyzed by that technique.

Butyltins (TBT, DBT, MBT) were detected in 75% of the sediment samples analyzed (Table 1). TBT was generally the major component ranging from 40 to 100% of the total butyltins. Average butyltin concentrations for each coast were calculated by assigning concentrations below the limit of quantitation (<5 ng Sn/g) a value of zero. Sediment samples contained butyltin concentrations ranging from <5 to 282 ng Sn/g (average 36 ng Sn/g). The range of total butyltins in sediments from the East, Gulf, and West coasts was 5 to 117, < 5 to 89, and 6 to 282 ng Sn/g, respectively. The sediment sample from Keehi Lagoon, Honolulu Harbor (HHKL), Hawaii, had a total butyltin concentration of 59 ng Sn/g. Stang and Seligman (1986) report total butyltin concentrations ranging from 69 to 467 ng Sn/g in a commercial boat basin and up to 551 ng Sn/g near yacht facilities. Sediments from the Puget Sound contained total butyltin concentrations ranging from below the detection limit to 4,400 ng Sn/g with the higher levels found near boat basins and ship repair facilities. The concentrations of total butyltins reported here are below or in the lower part of these ranges. Samples for this study were not collected from areas where boats are repaired or moored.

Gulf of Mexico sediments had relatively low concentrations of butyltins with respect to the other coasts. Only NBNB (FL) and SAWB (FL) sites had TBT concentrations above 36 ng Sn/g. Sediments from these sites also contained DBT and MBT in detectable concentrations (Table 1). The sediments sampled from Louisiana (BSSI, BBSD, TBLF, and JHJH) had no detectable butyltins, and the samples from sites in Texas, when compared to East and West Coast sites, had relatively low concentrations of butyltins. No detectable butyltins were found at 47% of the Gulf of Mexico sites. West Coast butyltin concentrations were relatively high at San Pedro (SPFP), California, medium at San Diego, Harbor Island (CA), and low at the other sites.

Most environmental concerns center on TBT because it is the most toxic of the butyltins (Hall and Pinkney 1985). The average tributyltin concentration determined in sediment samples from the East and West Coast sites were 35 and 38 ng Sn/g, respectively. This is four times the average of 9 ng Sn/g for the Gulf of Mexico sites. The range of tributyltin concentration was 5 to 87 ng Sn/g, < 5 to 48, and 6 to 187 ng Sn/g for the East Coast, the Gulf of Mexico, and the West Coast sites, respectively (Table 1). These TBT concentrations are in the lower end of TBT concentrations reported for sediments in areas of high boating activity (Maguire *et al.*, 1986; Krone *et al.*,1989; Stang and Seligman,1986). This most likely results from our sampling farther from major sources of input.

TBT sediment concentrations were higher than DBT or MBT concentrations in all samples (Figure 2). Tributyltin was found in 76% of the sediment samples, while DBT and MBT were detected in 39 and 30% of the sediments, respectively. Of the 25 sites where TBT was detected, about half contained only TBT (Figure 2). This was the case for 7 of 9 of the Gulf of Mexico sites. Detectable concentrations of DBT and MBT were present only when TBT was found in the sediment samples. DBT was found in higher concentrations in all but two of the samples. The presence of degradation products of TBT may be the result major sources of TBT to the marine environment (Schatzberg, 1987; Thompson *et al.*, 1985). The negative impact of TBT in the marine environment was first observed on commercial oyster beds (Alzieu, 1986). Since then, a great number of experiments have shown that TBT is toxic even at extremely low concentrations; thus restrictions and even complete bans of the use of TBT based paints were imposed. The use of TBT-based paints are currently controlled in some European countries and in the United States.

Tributyltin associated with sediments may be remobilized into the water column. Although areas severely affected by TBT have recovered after the use of TBT was reduced, the time for a complete removal of TBT was longer than expected (Alzieu, 1986). This may be due to a combination of the long lifetime of TBT in sediments and transport of TBT from sediments to the water column (Huggett *et al.*, 1986; Seligman *et al.*, 1988a). The purpose of this study was to determine the concentration of butyltins in sediments from selected U.S. estuarine areas. In order to provide information on the role of sediments as long term sinks for butyltin compounds. The concentration of butyltins in sediments is also compared to butyltin concentrations in bivalves collected in close proximity to these sites. This provides information on weather or not bioavailable butyltins are present in these areas.

METHODS AND MATERIALS

Sample collection. Thirty-three sediment samples from coastal areas of the United States were analyzed for butyltins. Sediments and bivalves were collected as part of the NOAA's Status and Trends Mussel Watch Program (Brooks *et al.*, 1988; Boehm *et al.*, 1988). Samples were collected from three stations per site distributed in the Atlantic (8 sites), Gulf of Mexico (17 sites), and Pacific (7 sites) coasts and one site in Hawaii (Figure 1). Sample locations (latitude and longitude) have been previously reported (Wade *et al.*, 1988a). Stations at each site were from 50 to 1000 m apart. Pooled samples consisted of equal weights of sediment or homogenized bivalve tissue from each of the three stations at a site. Sediment samples were collected either by hand or from a box core using a Teflon coated scoop previously rinsed with acetone and methylene chloride. The top 1 cm of sediment was placed in combusted glass jars with Teflon-lined screw caps and frozen. Bivalves (mussels or oysters) were collected by hand, tonging, or dredging. They were shelled and placed in precombusted mason jars with Teflon-lined screw caps and then frozen until analysis. Rigorous protocols were followed in order to avoid contamination of samples.

Sample extraction. Sediment sample sets consist of 10 freeze dried sediments, a blank, and a spike blank. Twenty grams of freeze dried sediment, tripropyltin chloride as internal standard and 100 ml of 0.2% tropolone in methylene chloride were placed into a 250 ml amber bottle with a Teflon-lined screw cap. Reagent blanks and spike blanks were run in the same way as samples. Known amounts of TBT, DBT, and MBT were added to the spiked blank. Each sample was then capped with a Teflon- lined screw cap and rolled for three hours. The sample was centrifuged, and the organic phase was decanted into a 500 ml flat bottom flask. The sample was extracted two more times using 100 ml of 0.2% tropolone solution each time and rolling for 16 and 3 hours, respectively. The combined

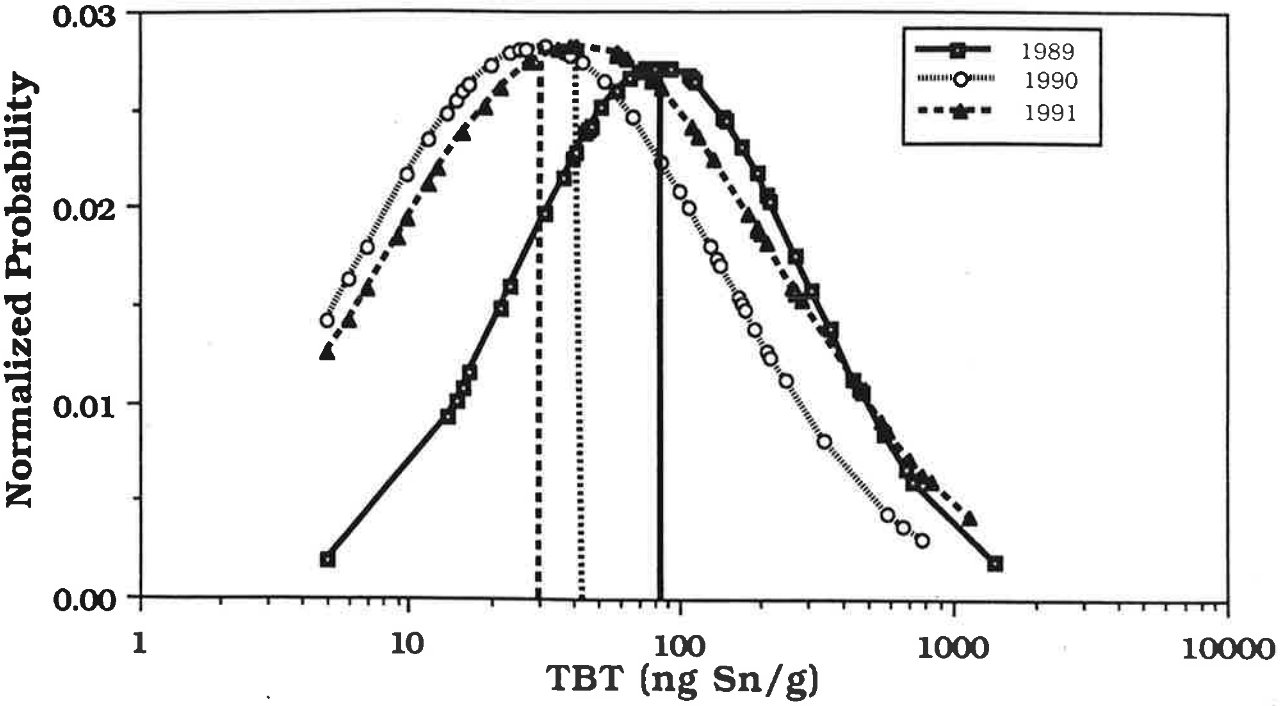


Fig. 5. Log-normal distribution of tributyltin concentrations determined in oysters in 1989, 1990, and 1991.

concentrations from 1989 to 1990 but not in 1991 may be due to the naturally higher variation of TBT concentrations near input areas (Seligman *et al.*, 1988). TBT lower-concentration ranges may therefore have decreased as a consequence of TBT regulations or changes in TBT-based paint formulations, but the effects are not as apparent at sites with high TBT concentrations. Distribution curves for DBT and MBT concentrations did not follow a log-normal distribution but also showed annual variations. This may be due to the high percentage of values below the MDL (Table 2).

Table 2a. Arithmetic and geometric means and medians (ng Sn/g)[a]

	TBT	DBT	MBT
1989			
Arithmetic mean	176	32	13
Geometric mean	85	14	8
Median	77 (2%)	12 (26%)	5 (60%)
1990			
Arithmetic mean	96	17	6
Geometric mean	30	8	6
Median	24 (17%)	5 (72%)	5 (90%)
1991			
Arithmetic mean	150	25	8
Geometric mean	43	13	6
Median	42 (17%)	8 (40%)	5 (66%)

[a] Numbers in parenthesis indicate percentage of samples below MDL.

Table 2b. Geometric mean ±1 standard deviation of the log (butyltin concentrations) (ng Sn/g)

	TBT	DBT	MBT
1989			
Plus	293	44	18
Minus	25	5	3
1990			
Plus	141	21	8
Minus	6	3	4
1991			
Plus	233	37	10
Minus	8	4	4

CONCLUSION

Oysters are valuable biomonitors for butyltins. The percentage of TBT present with respect to the total butyltins oscillated around 85% during the three years sampled. There was a decrease in the butyltin concentration from 1989 to 1990 or 1991. However, at high concentrations, there was little difference between 1989 and 1991. Environmental response to the TBT regulation in 1988 is not yet apparent. The decline between 1989 and 1990 or 1991 may have resulted from previous changes in anti-fouling paint formulation with lower TBT-release rates or the suspension of painting activities by individual boat owners after 1988. Because the newer TBT paints were formulated to last five years or more, there are many boats still in use that were painted with TBT-containing paints before the ban. Consequently, continuous monitoring is necessary to determine trends in butyltin contamination of the marine environment.

ACKNOWLEDGEMENT

This research was supported by the National Oceanic and Atmospheric Administration Grant No. 50-DGNC-5-00262 (National Status and Trends Mussel Watch Program).

of a dynamic equilibrium between uptake, metabolism, and depuration.

The TBT concentrations determined for each site during 1989, 1990, and 1991 are shown in Fig. 1. Sites are shown in geographical order from Texas to Florida. Tributyltin concentrations ranged from <5 ng Sn/g to 1450 (TBKA), 770 (BBMB), and 1160 ng Sn/g (BBMB) in 1989, 1990, and 1991, respectively. TBT concentrations increase monotonically at some sites from 1989 to 1991, whereas, at other sites, concentrations decreased monotonically. For example, oyster TBT concentrations increased from 1989 to 1991 at CLLC, BBMB, and GBTD (Fig. 1). Decreasing TBT concentrations from 1989 to 1991 were observed for oysters from PBPH, SAWB, TBCB, MBLR, and MBEM (Fig. 1). Concentrations of TBT were the same at TBOT and GBCR during all three years. In general, higher concentrations of TBT were determined in Florida sites than in Texas, Louisiana, Mississippi, or Alabama sites. TBT was below the detection limit at one of 53 sites in 1989 and at ten and eleven sites during 1990 and 1991, respectively. Although the concentrations were low, butyltins were detected in oysters from every site sampled in at least one sampling year.

Dibutyltin concentrations determined in oysters during 1989, 1990, and 1991 are shown in Fig. 2. Dibutyltin concentrations ranged from <5 ng Sn/g to 380 (TBKA), 160 (TBKA), and 200 ng Sn/g (TBKA), in 1989, 1990, and 1991, respectively. Sites sampled in Florida had the highest DBT concentrations. With the exception of five sites (CBJB, TBKA, CBFM, BBMB, and BRFS), the annual variation of DBT concentrations did not mimic the annual variation of TBT concentrations. Ship and boating activities have been cited as potential factors that may affect DBT fluctuations (Short and Sharp, 1989; Uhler *et al.*, 1989). Furthermore, the commercial usage of DBT as a stabilizer for plastics, including PVC pipes, may be another important source of input to the marine environment and may result in DBT fluctuations that do not mimic TBT fluctuations (Fent *et al.*, 1991; Maguire, 1991). At this point, it is not possible to estimate the influence of the factors discussed above on the DBT concentrations present in the oysters. Monotonic increases or decreases of DBT were observed at specific sites during the three-year period. For example, Middle Bank (BBMB, Figs 1 and 2) not only showed increasing concentrations of TBT during the three-year sampling period but also showed a steady increase in DBT in the sample period. DBT was detected in 39, 38, and 33 out of the 53 sites sampled in each of the three years. In many instances, DBT was not detected in any of the sampling years.

Regional MBT concentrations are shown in Fig. 3. Since the MBT concentrations are low, annual variations in MBT concentrations for each site are large. The precision of MBT determination is also not as good as that of TBT and DBT (Wade *et al.*, 1988). Monobutyltin concentrations ranged from <5 ng Sn/g to 145 (NBNB), 25 (CCIC), and 42 ng Sn/g (TBKA), in 1989, 1990, and 1991, respectively. Generally, sites with high TBT concentrations had high MBT concentrations. MBT was detected in 21, four, and nineteen of the 53 sites during 1989, 1990, and 1991, respectively. During all three years, MBT was detected at only three sites in Florida (CBJB, TBKA and CBFM) and at one site in Texas (CCIC). The fact that MBT was found in lower concentrations than DBT and DBT was found in lower concentrations than TBT is consistent with the fact that TBT is the major constituent of anti-fouling paints, while DBT and MBT are environmental-breakdown products of TBT. This may indicate that only a limited degradation of TBT has occurred or that the more water-soluble DBT and MBT were assimilated by the oysters at a slower rate than TBT.

Annual variation of butyltins in the Gulf of Mexico

A graphic representation of the TBT data for the 53 sites sampled in 1989, 1990, and 1991 is shown in Fig. 4. The graph is a plot of 1989 concentrations against those of 1990 and 1991. The x and y scales are identical. If no change occurs in the TBT concentration at a site, those data will be plotted on the center line. Sites that fall below the line show a decrease, whereas points that rise above the line show an increase compared with 1989. Two other lines also appear in Fig. 4. These are the lines that form the boundary of sites with a factor of two increase (top line) or decrease (bottom line). Only six sites for 1990 and eight for 1991 of the 53 sites plotted for each year are above the center line. Hence, over 85% of the TBT concentrations in 1990 and 1991 were less than the concentration measured in oysters at that site in 1989. There were 30 sites (57%) in 1990 and 20 sites (38%) in 1991 that had decreases, of more than a factor of two. There was only one site that had an increase of TBT concentration of more than a factor of two.

In order to detect temporal trends, the butyltin oyster concentrations for the entire Gulf of Mexico from 1989 to 1991 are compared. Annual variations of butyltins for the entire Gulf of Mexico are not readily apparent in Figs. 1, 2, or 3, where only annual concentrations at individual sites are compared. Comparisons of arithmetic mean, geometric mean, and medians (Table 2) for butyltin concentrations determined during 1989, 1990, and 1991 are based only on the 53 sites that were sampled in all three years. All these parameters were calculated by assigning 5 ng Sn/g to all those samples with concentrations below the limit of detection. The percentage of samples below the detection limit is listed in Table 2. The median and geometric means are similar in all cases, whereas the arithmetic mean is always higher. The median or the geometric mean appears to be the better estimator of the central tendency of the data. On the basis of the median or the geometric mean, there was a decrease in TBT oyster concentrations when 1989 is compared with 1990 or 1991.

A complete view of butyltin concentrations for the whole Gulf of Mexico for a given year can be achieved

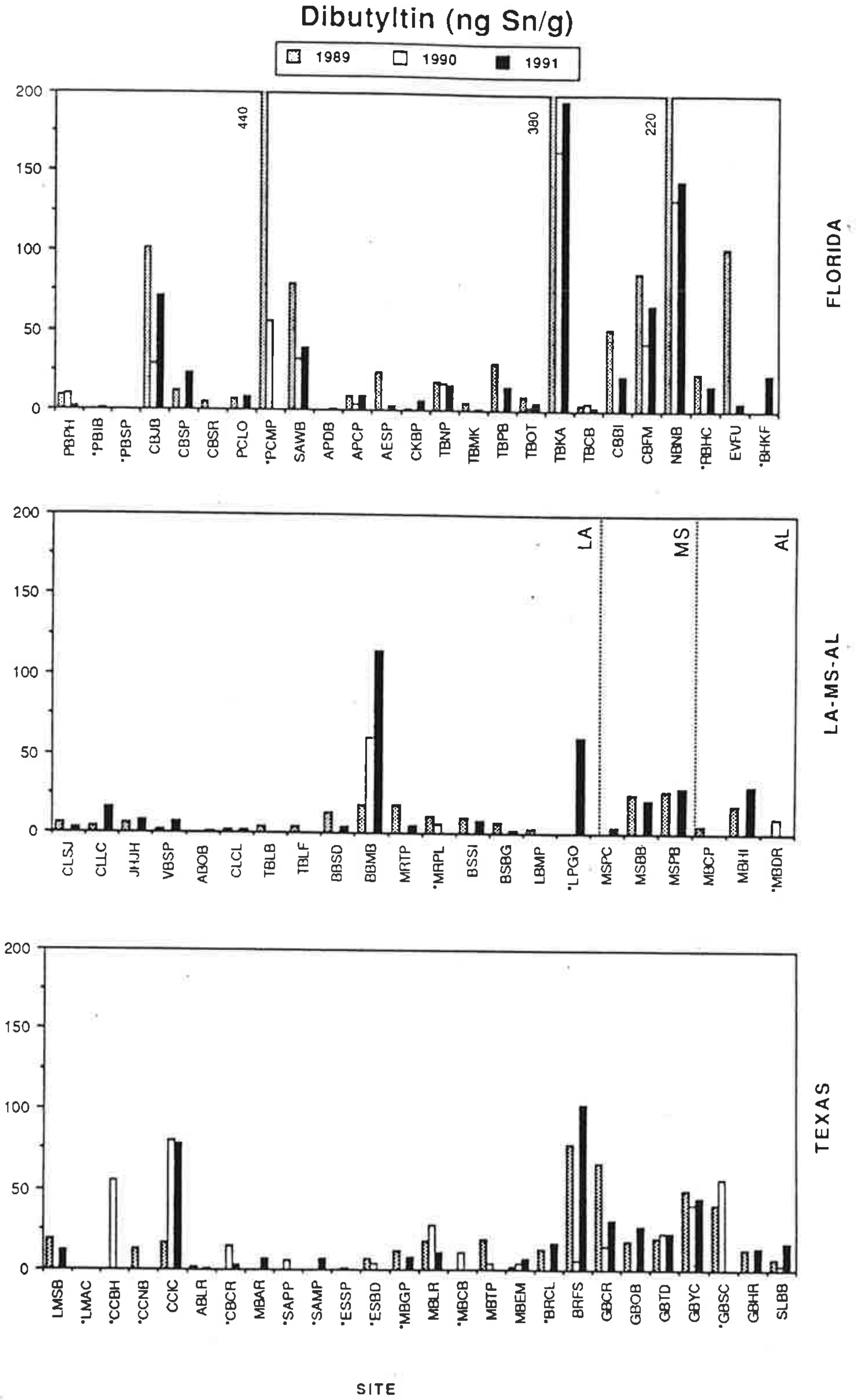


Fig. 2. Geographical distribution of dibutyltin concentrations in *C. virginica* from the Gulf of Mexico coast. Asterisks indicate those sites which were not sampled in consecutive years.

1990, and 1991 were compared. In order to simplify the presentation of data, the sites sampled have been divided into three geographical zones: Florida, Louisiana–Mississippi–Alabama (LA MS AL), and Texas. Only 73% of the sites reported were sampled during all three years. In some instances, some sites were not sampled because no oysters were available. Butyltin concentrations in oysters are reported in ng Sn/g dry weight (Maguire, 1991). Sites with an incomplete set of data are indicated with an asterisk in Table 1 and Figs 1, 2 and 3.

The concentrations of total butyltins in 1989, 1990, and 1991 ranged from below the limit of detection (<5 ng Sn/g) to 1880 (TBKA), 850 (TBKA), and 1300 ng SN/g (BBMB), for 1989, 1990, 1991, respectively. In general, the butyltin concentrations decreased from 1989 to 1990 and then increased slightly between 1990 and 1991.

Table 1. Sampling locations and site designators

Designation	Site	Location	Latitude (deg)	Latitude (min)	Longitude (deg)	Longitude (min)
		TEXAS				
LMSB	South Bay	Lower Laguna Madre	26	02·58	97	10·49
LMAC[a]	Arroyo Colorado	Laguna Madre	26	16·80	97	17·30
CCBH[a]	Boat Harbor	Corpus Christi	27	50·00	97	23·00
CCNB[a]	Nueces Bay	Corpus Christi	27	51·70	97	21·00
CCIC	Ingleside Cove	Corpus Christi	27	50·30	97	14·25
ABLR	Long Reef	Aransas Bay	28	03·30	96	57·50
CBCR[a]	Copano Reef	Copano Bay	28	08·20	97	07·58
MBAR	Ayres Reef	Mesquite Bay	28	10·30	96	49·70
SAPP[a]	Panther Pt. Reef	San Antonio Bay	28	13·20	96	43·00
SAMP[a]	Mosquito Point	San Antonio Bay	28	19·00	96	42·20
ESSP[a]	South Pass Reef	Espiritu Santo Bay	28	17·83	96	37·50
ESBD[a]	Bill Days Reef	Espiritu Santo Bay	28	25·00	96	27·00
MBGP[a]	Gallinipper Pt.	Matagorda Bay	28	35·00	96	34·00
MBLR	Lavac River Mouth	Matagorda Bay	28	39·30	96	35·00
MBCB[a]	Carancahua Bay	Matagorda Bay	28	40·00	96	23·20
MBTP	Tres Palacios Bay	Matagorda Bay	28	39·00	96	15·50
MBEM	East Matagord[a]	Matagorda Bay	28	42·30	95	53·00
BRCL[a]	Cedar Lakes	Brazos River	28	51·50	95	27·90
BRFS	Freeport River	Brazos River	28	55·00	95	20·50
GBCR	Confederate Reef	Galveston Bay	29	15·75	94	50·50
GBOB	Offatts Bayou	Galveston Bay	29	16·70	94	50·70
GBTD	Todd's Dump	Galveston Bay	29	30·10	94	54·00
GBYC	Yacht Club	Galveston Bay	29	37·00	94·	59·50
GBSC[a]	Ship Channel	Galveston Bay	29	42·50	94	59·50
GBHR	Hanna Reef	Galveston Bay	29	29·50	94	42·50
SLBB	Blue Buck Point	Sabine Lake	29	48·00	94	54·42
		LOUISIANA				
CLSJ	St. Johns Island	Calcasieu Lake	29	50·00	93	32·00
CLLC	Lake Charles	Calcasieu Lake	30	03·50	93	17·50
JHJH	Joseph Harbor Bayou	Joseph Harbor Bayou	29	37·75	92	45·75
VBSP	Southwest Pass	Vermillion Bay	29	34·70	92	04·00
ABOB	Oyster Bayou	Atchafalaya Bay	29	13·00	91	08·00
CLCL	Caillou Lake	Caillou Lek	29	15·25	90	55·50
TBLB	Lake Barre	Terrebonne Bay	29	15·00	90	36·00
TBLF	Lake Felicity	Terrebonne Bay	29	16·00	90	24·50
BBSD	Bayou St. Denis	Barataria Bay	29	24·10	89	59·80
BBMB	Middle Bank	Barataria Bay	29	17·20	89	56·60
MRTP	Tiger Pass	Mississippi River	29	08·69	89	25·67
MRPL[a]	Pass a Loutre	Mississippi River	29	04·30	89	04·60
BSSI	Sable Island	Breton Sound	29	24·70	89	28·70
BSBG	Bay Gardene	Breton Sound	29	35·87	89	38·50
LBMP	Malheureux Point	Lake Borgne	29	52·30	89	40·70
LPGO[a]	Gult Outlet	Lake Ponchartrain	30	02·20	89	03·00
		MISSISSIPPI				
MSPC	Pass Christian	Mississippi Sound	30	19·75	89	19·58
MSBB	Biloxi Bay	Mississippi Sound	30	23·38	88	15·42
MSPB	Pascagoula Bay	Mississippi Sound	30	21·05	88	37·00
		ALABAMA				
MBCP	Cedar Point Reef	Mobile Bay	30	19·40	88	07·30
MBHI	Harbor Island	Mobile Bay	30	33·59	88	02·80
MBDR[a]	Dog River	Mobile Bay	30	35·50	88	02·72
		FLORIDA				
PBPH	Public Harbor	Pensacola Bay	30	34·80	87	11·50
PBIB[a]	Indian Bayou	Pensacola Bay	30	30·83	87	04·00
PBSP[a]	Sabine Point	Pensacola Bay	30	20·80	87	08·10
CBJB	Joes Bayou	Choctawhatchee Bay	30	24·70	86	29·55
CBSP	Shirk Point	Choctawhatchee Bay	30	28·95	86	28·60
CBSR	Off Santa Rosa[a]	Choctawhatchee Bay	30	23·50	86	10·60
PCLO	Little Oyster Bay	Panama City	30	15·00	85	40·87
PCMP[a]	Municipal Pier	Panama City	30	08·20	85	37·50
SAWB	Watson Bayou	St. Andrew Bay	30	0·850	85	37·58
APDB	Dry Bar	Apalachicola Bay	29	41·50	85	05·00
APCP	Cat Point Bar	Apalachicola Bay	29	43·00	84	52·50
AESP	Spring Creek	Apalachee Bay	30	30·50	84	19·38
CKBP	Black Point	Cedar Key	29	10·25	83	03·00
TBNP	Navarez Park	Tampa Bay	27	48·30	82	45·28
TBMK	Mullet Key Bayou	Tampa Bay	27	37·17	82	43·62
TBPB	Papys Baypu	Tampa Bay	27	50·72	82	36·75
TBOT	Old Tampa Bay	Tampa Bay	28	01·48	82	37·95
TBKA[a]	K. Airport	Tampa Bay	27	54·46	82	27·29
TBCB	Cockroach Bay	Tampa Bay	28	40·55	82	30·56
CBBI	Bird Island	Charlotte Harbor	26	31·00	82	02·60
CBFM	Fort Meyers	Charlotte Harbor	26	38·64	81	52·48
NBNB	Naples Bay	Naples Bay	26	00·00	81	32·00
RBHC[a]	Henderson Creek	Rookery Bay	26	01·83	81	43·75
EVFU	Faka Union Bay	Everglades	25	54·27	81	30·60
BHKF[a]						

[a] Sites that were not sampled consecutively from 1989 to 1991.

Table 5. Comparison of ER-L, ER-M, Apparent Effects Thresholds, and Washington State Sediment Quality Criteria Concentrations for Selected Chemicals in Sediments and Values Measured in Casco Bay (after Long and Morgan, 1990; Washington State Dept. of Ecology Sediment Management Standards, Chapter 173–204 WAC)

						Casco Bay Regions[f]									
						Inner Bay		West Bay		East Bay		Cape Small		Outer Bay	
chemical analyte	ER-L[a]	ER-M[b]	AET[c]	deg of confidence[d]	WSSQC[e]	min	max	min	max	min	max	min	max	min	max
					Polychlorinated Biphenyls (ppb)										
total PCBs	50	400	370	M/M	240	7.31	484.97	1.58	16.32	8.89	37.30	0.44	40.02	5.50	30.67
					DDT and Metabolites (ppb)										
DDT	1	7	6	L/L		0.49	4.28	<0.20	0.96	0.40	2.01	<0.20	0.86	0.47	1.52
DDD	2	20	NSD[g]	M/L		0.67	15.09	0.08	1.49	0.31	1.98	<0.07	0.62	0.34	2.04
DDE	2	15	NSD	L/L		0.18	3.84	<0.06	1.14	0.07	0.48	<0.06	0.40	0.06	0.63
total DDT	3	350	NA[h]	M/M		1.63	20.42	<0.20	3.10	0.82	4.16	<0.20	1.89	1.03	4.12
					Other Pesticides (ppb)										
lindane	NA	NA	NSD	NA		<0.07	0.48	<0.07	0.22	<0.07	0.35	<0.07	0.11	<0.07	0.34
chlordane	0.5	6	2	L/L		0.15	4.91	0.07	0.98	0.16	1.91	<0.07	1.32	0.13	1.89
heptachlor	NA	NA	NSD	NA		0.08	0.13	<0.04	0.05	<0.04	0.13	<0.04	<0.04	<0.04	0.04
dieldrin	0.02	8	NA	L/L		<0.16	0.94	<0.16	<0.16	<0.16	0.43	<0.16	2.46	<0.16	1.40
aldrin	NA	NA	NSD	NA		<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28
endrin	0.02	45	NSD	L/L		<0.06	0.84	<0.06	0.21	<0.06	0.17	<0.06	<0.06	<0.06	0.55
mirex	NA	NA	NSD	NA		<0.04	0.29	<0.04	0.08	<0.04	0.49	<0.04	0.66	<0.04	0.16

[a] ER-L, effects range-low. [b] ER-M, effects range-median. [c] AET, apparent effects threshold. [d] L, low; M, medium; H, high. [e] WSSQC, Washington State Sediment Quality Criteria, calculated ppb dry wt based on 2% TOC. [f] ppm dry weight. [g] NSD, not sufficient data. [h] NA, not available.

Table 6. Comparison of ER-L, ER-M, Apparent Effects Thresholds, and Washington State Sediment Quality Criteria Concentrations for Selected Chemicals in Sediments and Values Measured in Casco Bay (after Long and Morgan, 1990; Washington State Dept. of Ecology Sediment Management Standards, Chapter 173–204 WAC)

						Casco Bay regions[f]									
						Inner Bay		West Bay		East Bay		Cape Small		Outer Bay	
chemical analyte	ER-L[a]	ER-M[b]	AET[c]	deg of confidence[d]	WSSQC[e]	min	max	min	max	min	max	min	max	min	max
		Polynuclear Aromatic Hydrocarbons (ppb dry wt surrogated corrected)													
acenaphthene	150	650	150	L/L	320	2	81	<1	3	2	19	<1	13	2	6
anthracene	85	960	300	L/M	4 400	6	255	3	15	8	107	<1	99	14	50
benz[a]anthracene	230	1600	550	L/M	2 200	30	655	12	56	34	481	1	360	48	173
benzo[a]pyrene	400	2500	700	M/M	1 980	43	741	17	100	50	498	1	433	62	209
benzo[e]pyrene	NA[h]	NA	NSD[g]	NA		37	514	14	74	37	276	1	271	48	140
biphenyl	NA	NA	NSD	NA		3	29	<2	7	4	12	<2	10	4	12
chrysene	400	2800	900	M/M	2 200	44	766	19	74	47	530	1	398	53	192
dibenz[a,h]anthracene	60	260	100	M/M	240	3	105	3	41	7	58	<0	64	11	73
2,6-dimethylnaphthylene	NA	NA	NSD	NA		4	130	1	9	3	28	<1	17	5	14
fluoranthene	600	3600	1000	H/H	3 200	90	1444	34	144	82	639	2	522	118	304
fluorene	35	640	350	L/L	460	4	201	1	7	4	96	<1	27	6	15
1-methylnaphthalene	NA	NA	NSD	NA		3	81	1	7	3	31	<1	20	5	11
2-methylnaphthalene	65	670	300	L/M	760	5	95	2	11	5	37	<1	34	8	17
1-methylphenanthrene	NA	NA	NSD	NA		10	311	5	14	0	68	<1	49	8	33
naphthalene	340	2100	500	M/H	7 400	8	135	2	14	7	46	<2	41	12	26
perylene	NA	NA	NSD	NA		17	216	9	56	31	110	<4	94	21	77
phenanthrene	225	1380	260	M/M	2 000	42	1036	17	71	41	550	1	269	57	160
pyrene	350	2200	1000	M/M	20 000	82	1552	31	137	78	560	2	562	1127	302
2,3,5-trimethylnaphthalene	NA	NA	NSD	NA		3	187	1	4	2	34	<1	9	3	6
total PAH	4000	35 000	22 000	L/L		911	20 748	421	1901	1059	7340	16	7454	1312	4004

[a] ER-L, effects range-low. [b] ER-M, effects range-median. [c] AET, apparent effects threshold. [d] L, low; M, medium; H, high. [e] WSSQC, Washington State Sediment Quality Criteria, calculated ppb dry wt based on 2% TOC. [f] ppm dry weight. [g] NSD, not sufficient data. [h] NA, not available.

the PAHs in this study are combustion related and thus may be in a sequestered form that significantly reduces their toxicity.

Long and Morgan (*16*) estimated that median concentrations of total PCB above 400 ppb dry wt elicits a toxic response in most benthic organisms. For this study, only one site (SW-2) is above this threshold. The DDT concentrations are low compared to concentrations known to cause a toxic response in most benthic organisms (*16*). Chlordane concentrations are "low" based on the definition of O'Connor (*15*) and should pose little or no threat of toxic biological effects (*16*).

A number of different approaches to determining the trace metal concentrations in sediments which lead to a biological response have been used, resulting in a large and confusing literature. Thomas (*20*) briefly describes eight different approaches to setting toxicity criteria for sediments, but no actual data are presented. Pavlov (*21*) compared results from one of these approaches, the equilibrium partitioning approach, to results from other commonly used methods. He shows that the concentration of a given metal needed to elicit a biological response, as determined by equilibrium partitioning and other methods, does not vary widely (except for Hg). The threshold concentrations for toxicity are much higher than those found in Casco Bay sediment.

None of the metal concentrations in the Casco Bay sediments are as high as Long and Morgan's (*16*) ER-M,

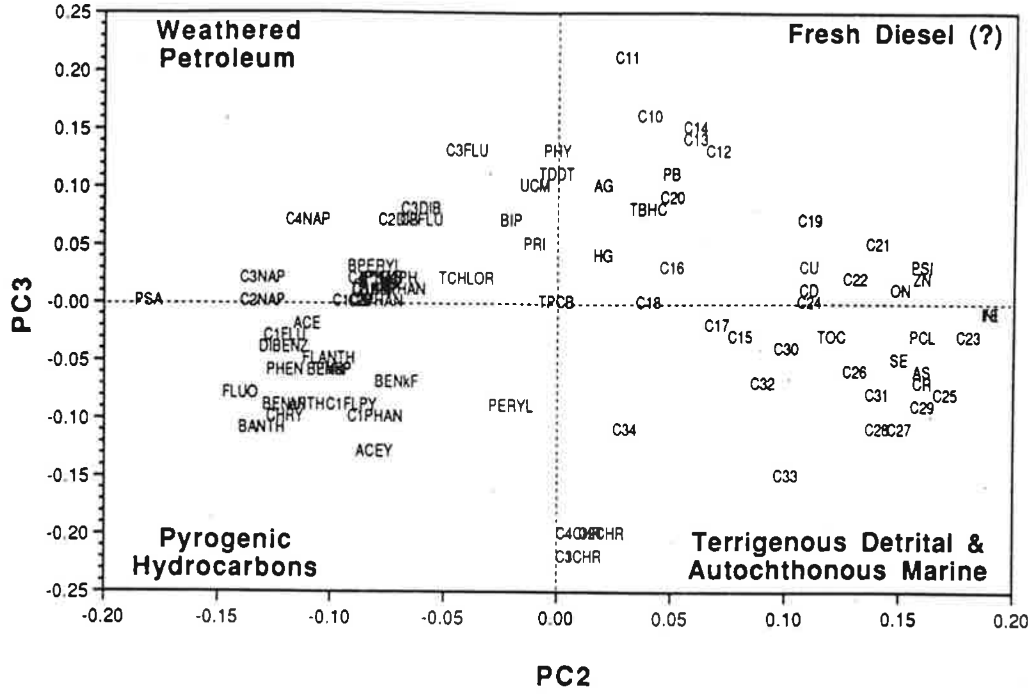


Figure 9. Relationship between PC2 and PC3 for PCA of Casco Bay contaminant data.

ilarly loaded, suggesting that biogenic materials are an important contributor to the organic richness of the sediments (*17*). Hydrocarbons loaded negatively in both PC 2 and PC 3 (lower left quadrant, Figure 9) consist primarily of four- and five-ring aromatics that are generated from both natural and anthropogenic combustion processes. A combustion origin for these hydrocarbons is also supported by the covariance of the parent two- and three-ring aromatics which are structurally stable at high temperatures (*11–14*). The departure of the alkylated chrysenes from this trend suggests either a biogenic source for these compounds or possibly some interference in their analysis from biogenic material. Hydrocarbons loaded negatively in PC 2 and positively in PC 3 (upper left quadrant, Figure 9) include two- and three-ring aromatics containing a C_2 or greater alkylation. These compounds are the most abundant aromatic hydrocarbons in petroleum and petroleum byproducts. Pristane and UCM are similarly loaded, suggesting a weathered petroleum origin (*18, 19*). The source represented by the hydrocarbons that are loaded positively in both PC 2 and PC 3 (upper right quadrant, Figure 9) is equivocal. These consist primarily of *n*-alkanes in the range C_{10}–C_{22}, which might represent a relatively unweathered petroleum product, i.e., diesel fuel. Alternatively, the covariance of these hydrocarbons with the metals Pb, Ag, and Hg and total DDTs and BHC concentrations (Figure 9) suggest possible inputs from runoff associated with either agricultural or industrial activities. Principal component 4 (5.4% of the total variance) is characterized by high positive loadings for most of the chlorinated hydrocarbons analyzed and is less straightforward to interpret. It should be noted that the organochlorine compounds are generally low and near the method detection limit, thus indicating a relatively "noisy" data set.

Based on these interpretations, the distribution of samples in a scores cross-plot of PC 2 versus PC 3 (Figure 10) can be used to assess the regional influence of a variety of sources. Sediments exhibiting a predominantly biogenic influence from detrital and autochthonous sources (positive scores for PC 2, negative scores for PC 3) are found in the upper East Bay (EB-3, -5, -6, -7, and -8), and also at Outer Bay sites OB-1 and OB-12 and Inner Bay site IB-9. In contrast, the lower East Bay (EB-1, -2, -4, -9, and -10), as well as Outer Bay site OB-15 and shallow water site SW-15, contains a greater component originating from pyrogenic sources (negative scores for PC 2 and PC 3). Site CS-4 in Cape Small exhibits a composition similar to the lower East Bay sites. These distributions are significant in that the sites that are similar in composition are geographically clustered. This suggests subtle differences in the principal sources of hydrocarbons in the upper and lower East Bay.

Sites characterized by inputs of weathered petroleum (negative scores for PC 2, positive scores for PC 3) include the Inner Bay and shallow water sites nearest the city of Portland (IB-1 and -2 and SW-3, -4, and -5). This is probably the result of chronic inputs from runoff and point sources associated with urban activities. Surprisingly, however, the sandy sediments from Cape Small (CS-1, -2, -3, -5, -7, and, to a lesser extent CS-6) have contaminant compositions that are nearly identical to site IB-1. This is illustrated in the scores cross-plot in Figure 10, where the majority of Cape Small sites plot intermediate between the lower East Bay and shallow water sites SW-3 and SW-4 from the Inner Bay. This likely reflects aromatic hydrocarbon inputs from both pyrogenic and petroleum sources at these locations and suggests that, despite significantly lower concentrations, the assemblage of contaminants in Cape Small sediments is similar to those at some contaminated Inner Bay sites. Sites showing a relative enrichment in C_{10}–C_{22} *n*-alkanes (positive scores for PC 2 and PC 3) include nearly all the West Bay sites and shallow water sites SW-9, -10, -11, and -13 within the West Bay. Several nearby sites also exhibit a similar composition. These include Outer Bay site OB-13 and Inner Bay sites IB-6 and IB-10. Thus, although the origin of this compositional feature is uncertain, it appears to manifest itself over a limited portion of Casco Bay, suggesting a localized source. Several Outer Bay sites (OB-

Table 3. Casco Bay Estuary Program Site Rankings Based on Selected Metal Data, 1991 (ppm dry wt)

station no.	Ag (μg/g)	Ag ranking	Cd (μg/g)	Cd ranking	Hg (μg/g)	Hg ranking	Pb (μg/g)	Pb ranking	Zn (μg/g)	Zn ranking	total ranking
CS-7	0.05	1	0.069	5	<0.006	1	17.1	3	31	2	12
CS-3	0.06	1	0.053	3	0.008	1	17.6	4	35	4	13
CS-2	0.07	1	0.060	4	0.019	2	17.8	5	34	3	15
CS-1	0.05	1	0.071	6	<0.006	1	14.1	2	39	6	16
CS-5	0.09	3	0.036	1	0.031	3	20.0	6	38	5	18
CS-6	0.07	1	0.051	2	0.046	6	20.8	9	46	9	27
SW-8	0.09	3	0.150	14	0.019	2	20.5	7	34	3	29
SW-15	0.08	2	0.192	21	0.048	7	13.6	1	28	1	32
SW-7	0.07	1	0.155	15	0.032	4	24.7	13	46	9	42
EB-4	0.10	4	0.076	7	0.058	10	23.3	11	59	11	43
EB-10	0.08	2	0.121	10	0.069	15	20.6	8	56	10	45
OB-11	0.10	4	0.168	17	0.049	8	25.5	14	43	8	51
EB-1	0.11	5	0.127	12	0.059	11	26.2	16	62	12	56
WB-3	0.11	5	0.258	28	0.031	3	20.5	7	69	14	57
EB-2	0.11	5	0.175	19	0.077	20	25.8	15	68	13	72
SW-5	0.12	6	0.245	27	0.062	13	27.5	20	40	7	73
OB-1	0.14	8	0.118	9	0.065	14	27.7	21	88	27	79
WB-6	0.11	5	0.088	8	0.057	9	31.7	30	92	29	81
WB-8	0.13	7	0.293	30	0.077	20	26.8	17	68	13	87
SW-10	0.16	10	0.486	48	0.037	5	22.2	10	73	16	89
WB-7	0.11	5	0.312	32	0.071	17	27.1	18	80	20	92
SW-9	0.17	11	0.400	38	0.037	5	25.5	14	87	25	93
OB-15	0.16	10	0.155	15	0.102	28	29.3	24	75	17	94
SW-12	0.25	16	0.355	35	0.048	7	29.4	25	71	15	98
SW-4	0.19	12	0.213	24	0.097	27	32.0	32	35	4	99
SW-14	0.16	10	0.414	40	0.082	22	24.3	12	75	17	101
SW-13	0.15	9	0.125	11	0.073	18	31.5	28	101	36	102
OB-10	0.14	8	0.156	16	0.081	21	33.8	38	82	22	105
OB-2	0.12	6	0.133	13	0.058	10	37.7	49	92	29	107
OB-13	0.15	9	0.268	29	0.082	22	30.6	27	82	22	109
OB-8	0.14	8	0.176	20	0.087	24	35.7	43	76	18	113
SW-6	0.13	7	0.435	45	0.061	12	31.7	30	78	19	113
OB-5	0.15	9	0.200	22	0.085	23	34.7	40	81	21	115
OB-4	0.17	11	0.226	25	0.104	29	33.1	36	75	17	118
WB-2	0.17	11	0.358	36	0.076	19	29.7	26	92	29	121
WB-1	0.15	9	0.430	42	0.087	24	28.4	22	93	30	127
OB-7	0.16	10	0.245	27	0.113	32	35.8	44	75	17	130
WB-4	0.17	11	0.444	46	0.082	22	28.6	23	94	31	133
WB-9	0.36	21	0.302	31	0.087	24	31.9	31	93	30	137
OB-9	0.17	11	0.174	18	0.113	32	38.3	51	91	28	140
CS-4	0.20	13	0.208	23	0.190	43	32.4	34	88	27	140
WB-5	0.15	9	0.529	52	0.069	16	27.4	19	140	45	141
SW-11	0.16	10	0.239	26	0.096	26	37.6	48	95	32	142
IB-5	0.20	13	0.325	33	0.094	25	38.1	50	84	23	144
EB-3	0.19	12	0.431	43	0.112	31	33.2	37	87	26	149
EB-9	0.19	12	0.401	39	0.148	36	32.1	33	92	29	149
OB-12	0.19	12	0.434	44	0.118	33	35.1	41	92	29	159
OB-6	0.26	17	0.592	58	0.106	30	32.8	35	86	24	164
EB-7	0.20	13	0.608	59	0.153	37	31.6	29	100	35	173
OB-3	0.20	13	0.327	34	0.141	35	40.7	52	109	41	175
IB-10	0.23	14	0.501	50	0.170	39	36.0	45	98	34	182
EB-8	0.23	14	0.720	60	0.181	42	34.1	39	97	33	188
IB-6	0.25	16	0.392	37	0.195	44	41.2	53	104	38	188
IB-8	0.24	15	0.573	56	0.168	38	35.3	42	104	38	189
EB-6	0.29	19	1.320	63	0.137	34	33.2	37	105	39	192
IB-9	0.23	14	0.557	53	0.173	40	36.2	46	106	40	193
EB-5	0.23	14	0.794	61	0.176	41	37.0	47	101	36	199
IB-7	0.32	20	0.424	41	0.234	45	42.1	55	106	40	201
SW-1	0.46	23	0.488	49	0.264	46	55.5	58	95	32	208
IB-4	0.27	18	0.571	55	0.274	49	41.5	54	102	37	213
IB-2	0.46	23	0.524	51	0.271	48	49.9	57	109	41	220
IB-3	0.39	22	0.574	57	0.264	46	48.5	56	109	41	222
SW-2	0.57	24	0.478	47	0.392	50	70.3	60	117	43	224
IB-1	0.57	24	0.564	54	0.269	47	55.6	59	125	44	228
SW-3	0.78	25	0.908	62	0.424	51	75.6	61	112	42	241

n-alkanes in the range C_{10}–C_{22} are positively loaded in PC 3, as are the more highly alkylated (C_2 and higher) two- and three-ring aromatics: naphthalenes, fluorenes, phenanthrenes, and dibenzothiophenes. Pristane, phytane, and UCM hydrocarbons are also loaded positively in PC 3. In contrast, *n*-alkanes in the range C_{23}–C_{34} along with C_{15} and C_{17} are loaded negatively in PC 3. Aromatic hydrocarbons loaded negatively in PC 3 include most parent two- and three-ring compounds, their methyl-substituted homologs, and most four- and five-ring aromatic compounds.

Together, the loadings for PC 2 and PC 3 discriminate sources of organic and inorganic materials in the Casco Bay sediments. Hydrocarbons loaded positively in PC 2

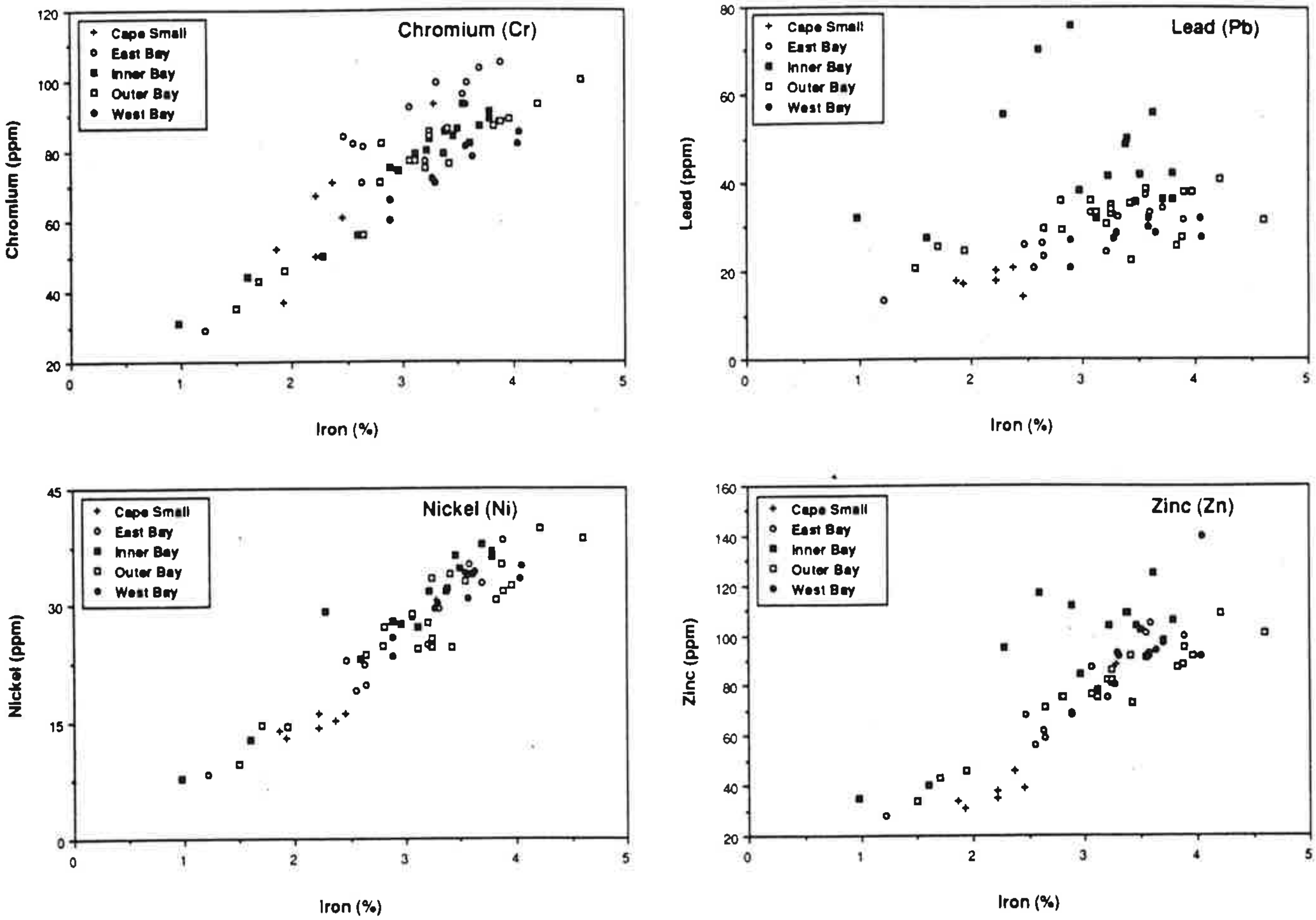


Figure 5. Relationship between chromium, lead, nickel, and zinc concentrations (ppm dry weight) and iron content (% dry weight) in sediments from Casco Bay.

Outer Bay, three East Bay, and one Cape Small sites. Eight of the 10 most highly contaminated stations are located in the Inner Bay region, including the six highest stations. The lowest levels of organic contaminants are in the Cape Small and West Bay regions. High levels of a variety of organic contaminants tend to occur at the same location.

For inorganic contamination, only those metals believed to be influenced by anthropogenic inputs were used to rank the sample locations, i.e., Ag, Cd, Pb, Zn, and Hg. Based on the summation of inorganic contaminant rankings, 25% of the locations with the highest levels were as follows: 12 Inner Bay, three East Bay, and one Outer Bay locations. Nine of the 10 highest locations are in the Inner Bay region, including the eight highest stations. Lowest metal concentrations occur in the Cape Small region. Eleven stations are ranked in the highest 25% on both the inorganic and organic contaminant rankings (Figure 6). They are almost exclusively Inner Bay locations, i.e., 9 of 11.

Principal Components Analysis. A total of four significant principal components (PC) were extracted from the Casco Bay data. PC 1 accounts for 48.9% of the total variance. The loadings for this PC show the sand content of the sediments inversely correlated with all other measured variables. PC 1 is inversely correlated with sand content and positively correlated with the TOC content of the sediments (Figure 7). This principal component reflects differences in the concentration of the targeted analytes due to variations in sediment texture. This finding is more significant than might appear at first consideration, as it implies that regional differences in concentrations result in part from sediment accumulation patterns. Thus, areas of fine-grained sediment accumulation such as the Inner Bay have high scores for PC 1 and exhibit high concentrations, while sediments in areas that are characterized by a dynamic physical environment and little sediment accumulation such as the Outer Bay have low scores for PC 1 and exhibit lower concentrations. It is also notable that both the organic and inorganic contaminants exhibit the same general trend. Shallow water samples SW-1 and SW-2 were identified as outliers because their compositions were anomalous relative to the other sediments (extreme enrichment in PAH and PCB, respectively). These samples were excluded from the PCA analysis.

PC 2 (12.3% of the total variance) and PC 3 (6.1% of the total variance) are related to the *composition* of organic and inorganic contaminants in the sediments. Since principal components are orthogonal, the processes governing PC 2 and PC 3 are independent of PC 1. Hence, the information contained in these principal components is more representative of contaminant sources in the sediments and is not related to absolute concentrations.

PC 2 is correlated positively with the Fe and saturated hydrocarbon content of the sediments (Figure 8). This most likely reflects a detrital component enriched in plant wax *n*-alkanes and inorganic clastics derived from continental erosion (*8-10*). A loadings cross-plot for PC 2 versus PC 3 (Figure 9) shows that, although all *n*-alkanes are positively loaded in PC 2, C_{23}, C_{25}, C_{27} and C_{29} *n*-alkanes have the highest loadings, consistent with this interpretation. Figure 9 also shows that nearly all the aromatic

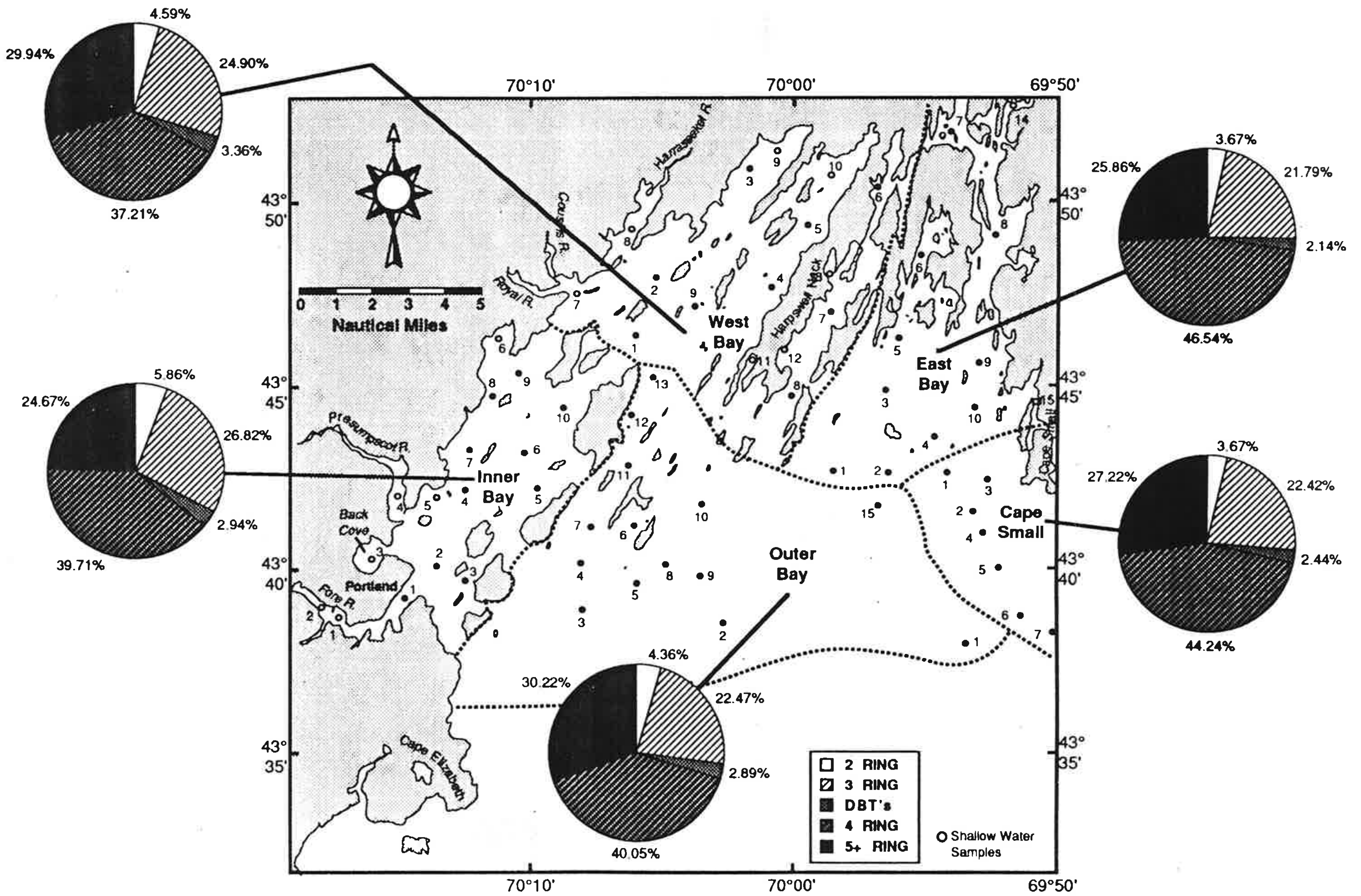


Figure 3. Average PAH compositions in sediments by region within Casco Bay.

complete the dissolution. Various dilutions were made on the clear digest solutions to bring them within the calibration of the AAS. Standard reference materials and blanks were digested and analyzed with every batch of samples.

Concentrations of Fe, Mn, and Zn were determined by flame AAS using a Perkin-Elmer Model 306 instrument, following the manufacturer's recommendations with only slight modifications. Calibration curves were constructed from commercial standards. Concentrations of Ag, As, Cd, Cr, Cu, Ni, Pb, and Se were determined with a Perkin-Elmer Zeeman 3030 instrument equipped with an HGA-600 furnace and AS-60 autosampler. Matrix modifiers and analytical conditions for the furnace and spectrophotometer were based on the manufacturer's recommendations, with modifications as appropriate to maximize sensitivity and minimize interferences. Mercury was determined by cold vapor AAS following a slightly modified EPA Method 245.5 aqua-regia/permanganate digestion. A headspace sampling procedure was used to remove Hg from the digest in contrast to the more common stripping procedure. A UV monitor (Laboratory Data Control Co.) with a 30-cm path length cell was used for Hg detection and quantification.

Organic Carbon and Grain Size. Organic carbon (OC) was determined by detection of CO_2 by an infrared spectrometer after combustion in an O_2 stream (LECO WR-12 total carbon system). Samples were acidified using dilute HCl in methanol and then dried. Method blanks and duplicate samples were analyzed every 20 samples. Data are reported as micrograms of carbon per gram of dry weight. All glassware and utensils are preheated prior to use.

Sediment grain size was determined by the procedure of Folk (*4*), utilizing sieving to separate gravel and sand fractions from the clay and silt fractions. The latter fractions were subsequently separated by the pipet (settling rate) method. Detailed descriptions of the methods utilized in measuring OC and grain size are reported in Brooks *et al.* (*5*).

Principal Components Analysis (PCA). The organic and inorganic data were analyzed using PCA (*6*). The results of PCA are highly dependent on the pretreatment or scaling of the data matrix. The data for this study consist of a wide variety of analytes that range several orders of magnitude in their absolute values. Because PCA is a least-squares method, variables with large variance will have large loadings. To avoid this bias, the entire data matrix was first scaled by dividing each variable by the standard deviation. This scaling assigns every variable a variance of 1.0 so that each variable has the same influence in the PCA model. The technique of cross-validation was used to establish the significance of each principal component (*7*). PCA was performed on a personal computer using the program SIRIUS (Pattern Recognition Systems A/S, Bergen, Norway).

Results

Hydrocarbons. Aliphatic hydrocarbons were detected at all stations sampled. The majority of resolved alkanes had odd-carbon chain lengths with 23–33 carbons indicative of plant biowaxes (Figure 2; refs 8–10). N-C_{15}, *n*-C_{17}, *n*-C_{19}, *n*-C_{21}, and pristane were often more abundant than the co-occurring even carbon-numbered normal alkanes and phytane, suggesting a phytoplankton input (*8-10*). Total alkanes and unresolved complex mixture (UCM) concentrations varied from 151 to 10 078 ppb dry wt and from 2 to 335 ppm dry wt, respectively. PAHs were also detected at all locations sampled. The predominant PAHs are highly condensed ring structures with few alkylations indicating a pyrogenic or combustion source (Figure 3; refs 11–14). Four-ring and larger PAHs account for more than 60% of sedimentary PAHs in Casco Bay. Total PAH concentrations varied from 16 to 20 798 ppb dry wt.

The western part of Casco Bay (Inner Bay) is most highly contaminated with PAH. Sediments from the Fore River area and locations close to Portland contain the highest concentrations of PAH. In general, contaminants decrease in concentration with distance from populated areas. However, regionally elevated PAH concentrations are also present at a few sites in East Bay and Cape Small. One station in the Cape Small (CS-4) region was unusual compared to other sites in the region. Most Cape Small stations contained <1.0% organic carbon and more than 65% sand, whereas sediment from station CS-4 contained 2.7% organic carbon and only 29.9% sand. Total alkanes, UCM, and total PAH concentrations were elevated at this location as well. Sediments at station EB-9 also had high concentrations of total PAH. An organic carbon content of 4.6% at EB-9 is the highest for all of the sediments sampled.

PCBs and Pesticides. Total PCB concentrations for the study area range from 0.4 to 485 ppb dry wt with a median concentration of 15 ppb. Total PCBs are highest in the Inner Bay in close proximity to Portland. Concentrations are lowest in Cape Small and West Bay with a few anomalous values in East Bay. The site from Cape Small with a total PCB concentration of 40 ppb dry wt has a higher TOC content (2.8%) than other samples from Cape Small.

Total DDT concentrations for the study area range from below the method detection limit (0.25 ppb) to 21 ppb dry weight. The DDTs were dominated by the *p,p'*-isomers. This is expected since technical-grade DDT is primarily the *p,p'*- isomer (75–85%). In the environment, DDT is metabolized to DDD and DDE. In some samples, DDD is the major metabolite while in other samples DDE predominates. Samples from the Inner Bay and associated shallow water sites exhibit DDD > DDE while at most other locations DDE > DDD. There is a relatively high percentage of undegraded DDT in Casco Bay sediments. The geographic distribution of total DDT concentrations is similar to that found for PCBs. The Inner Bay has the highest concentration in Casco Bay. East Bay and Outer Bay have intermediate concentrations, West Bay has lower concentrations, and the Cape Small region has the lowest concentrations.

The highest values of total chlordane are at Inner Bay sites. East Bay and Outer Bay sites are intermediate, while West Bay and Cape Small sites exhibit the lowest concentrations. Total chlordane concentrations range from below the method detection limit (0.25 ppb) to 4.9 ppb dry wt. Other organochlorine pesticides including aldrin, BHC, dieldrin, endosulfan (I, II, and sulfate), endrin, endrin aldehyde, heptachlor, heptachlor epoxide, toxaphene, and hexachlorobenzene were near or below the method detection limit (<0.25 ppb).

Trace Metals. Sediment trace metal data show considerable geographic variation with generally higher values

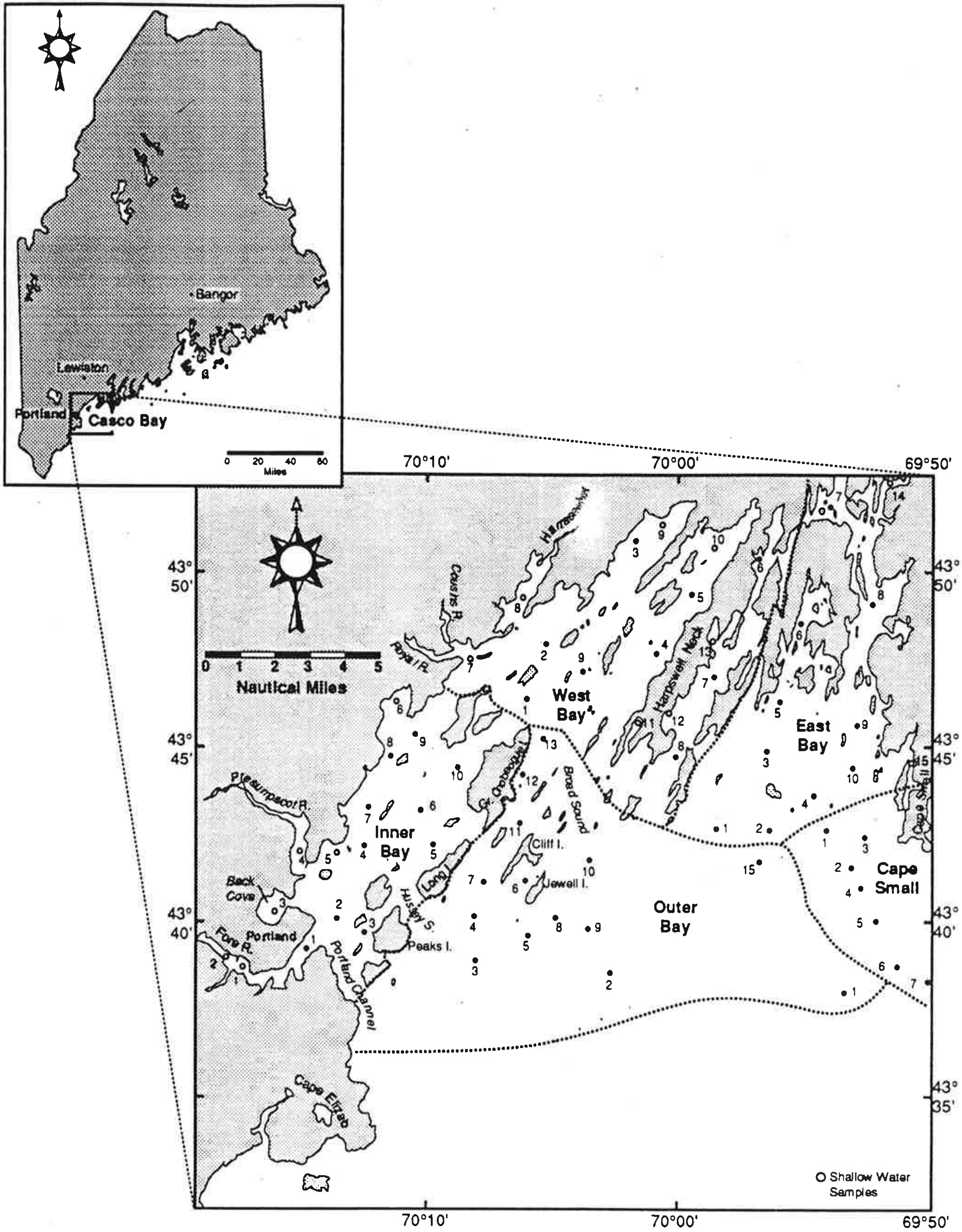


Figure 1. Location map for the Casco Bay study.

Hydrocarbons, Pesticides, and PCBs. The extraction method is that of Wade *et al.* (2). A total of 10 g of freeze-dried sediment was Soxhlet-extracted with methylene chloride and concentrated in Kuderna-Danish tubes. The extracts were fractionated by alumina:silica gel (80–100 mesh) chromatography. The extracts were sequentially eluted from the column with 50 mL of pentane (aliphatic fraction) and 200 mL of 1:1 pentane–dichloromethane (aromatic/PCB/pesticide fraction) and concentrated for instrumental analysis.

8.2a The IPR requirements apply to TCDD and TCDF only and must be performed using the same procedures used to generate succeeding sample data, i.e., any modifications made to the procedures must be verified by a new IPR test.

8.5.2a The acceptance criteria for blanks apply to TCDD and TCDF only and to any interferences with the analysis of those compounds.

8.7a If spiked sample analysis is requested, spike only TCDD and TCDF.

12.3a Silica column may be optimized for TCDD and TCDF.

12.4a Alumina column cleanup may be optimized for TCDD and TCDF.

12.5a AX-21 column cleanup may be optimized for TCDD and TCDF.

13.3a The GC temperature program and data collection parameters may be optimized for TCDD and TCDF.

14.3a Calibration verification applies to unlabeled and labeled TCDD and TCDF only.

14.4.1.1a Criteria for $^{13}C_{12}$-1,2,3,7,8,9-HxCDD do not apply.

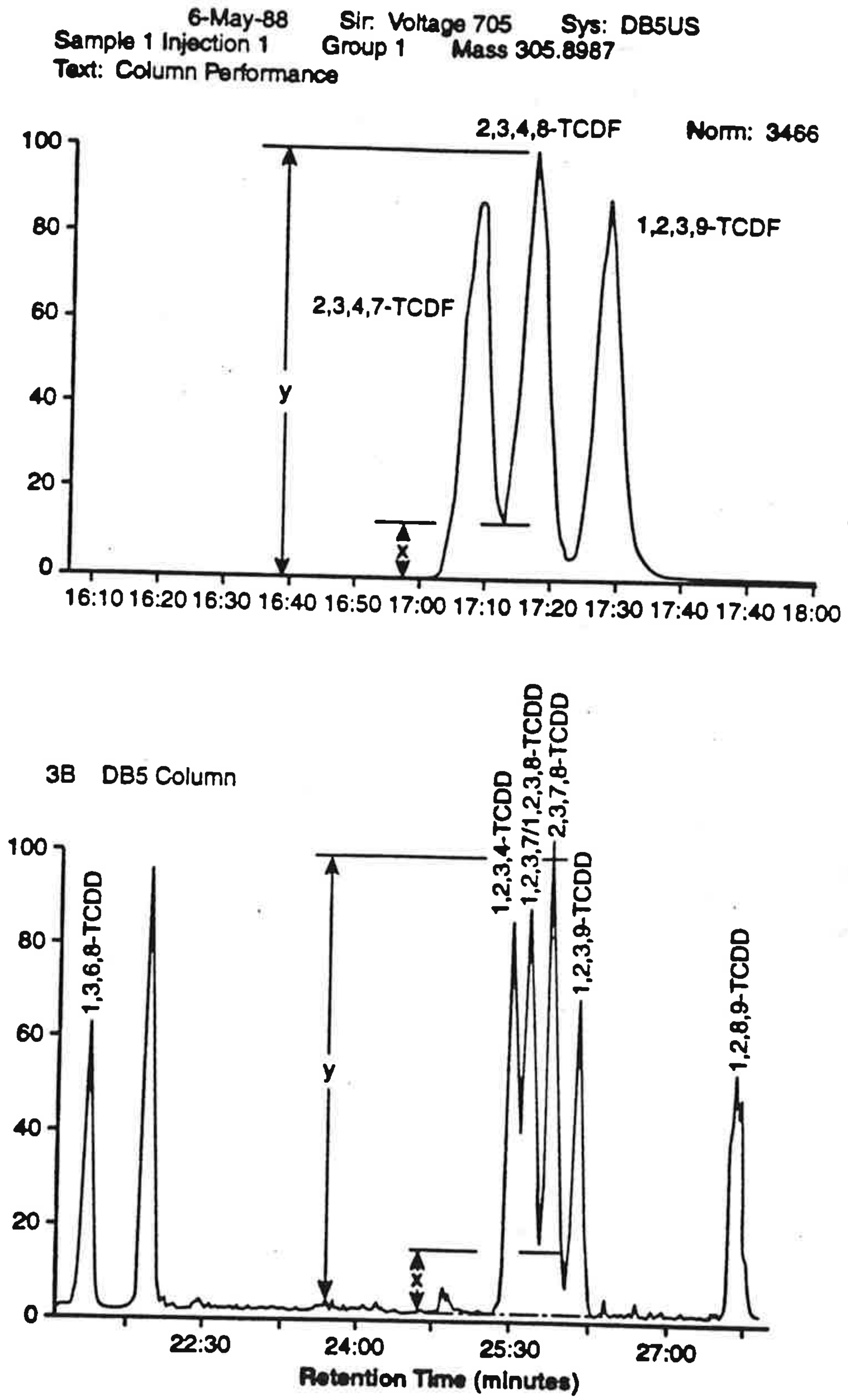
14.5a The OPR solution contains TCDD and TCDF only, and only those compounds are evaluated.

15A Confirmatory-column analysis is required when 2,3,7,8-TCDF is detected.

16.1a 2,3,7,8-TCDD and 2,3,7,8-TCDF are determined by isotope dilution.

16.1.1a Does not apply.

16.1.2a Does not apply.



82-020-07A

Figure 3. Valley Between 2,3,7,8- Tetra-Dioxin and -Furan Isomers and Other Closely Eluted Isomers

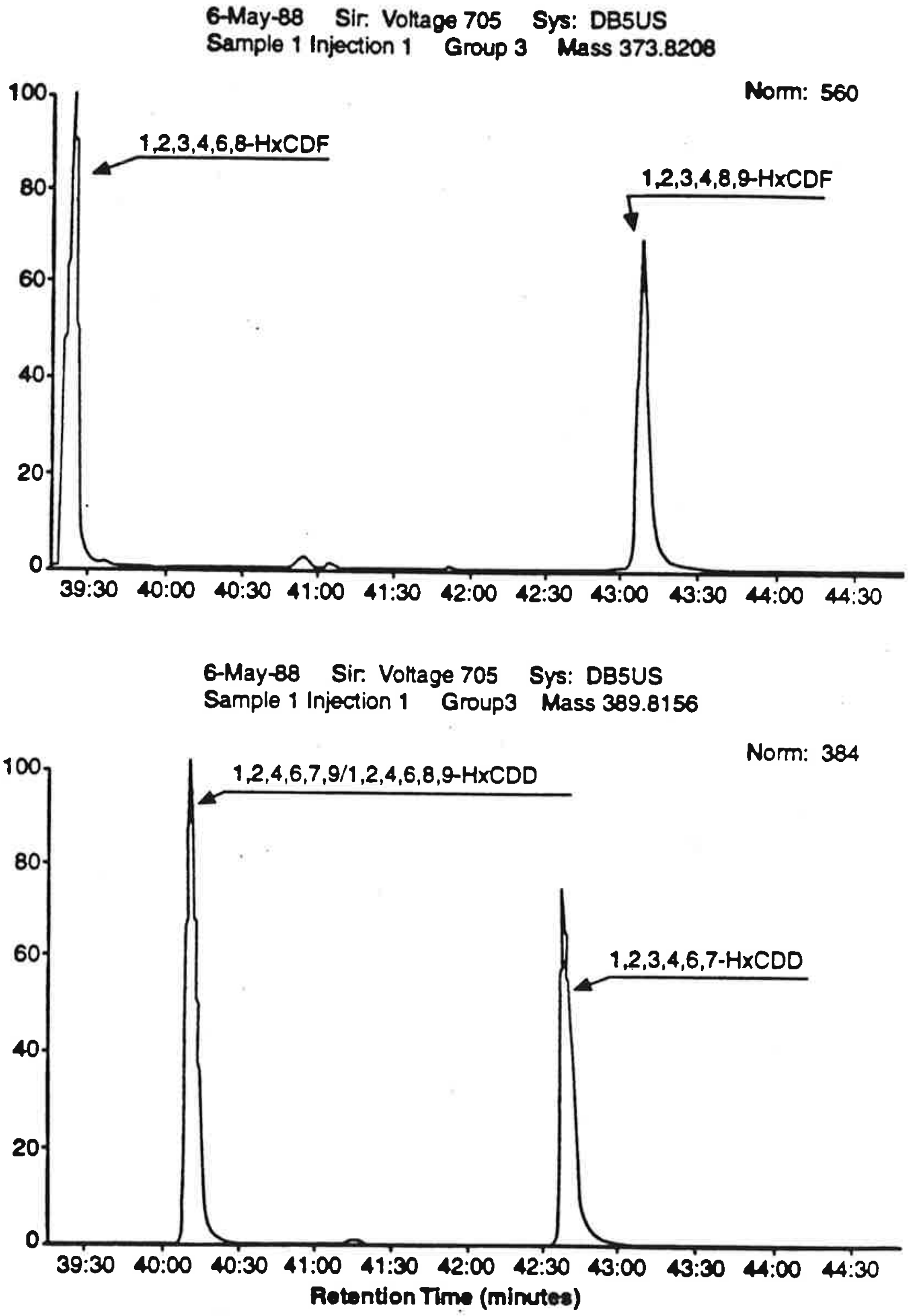
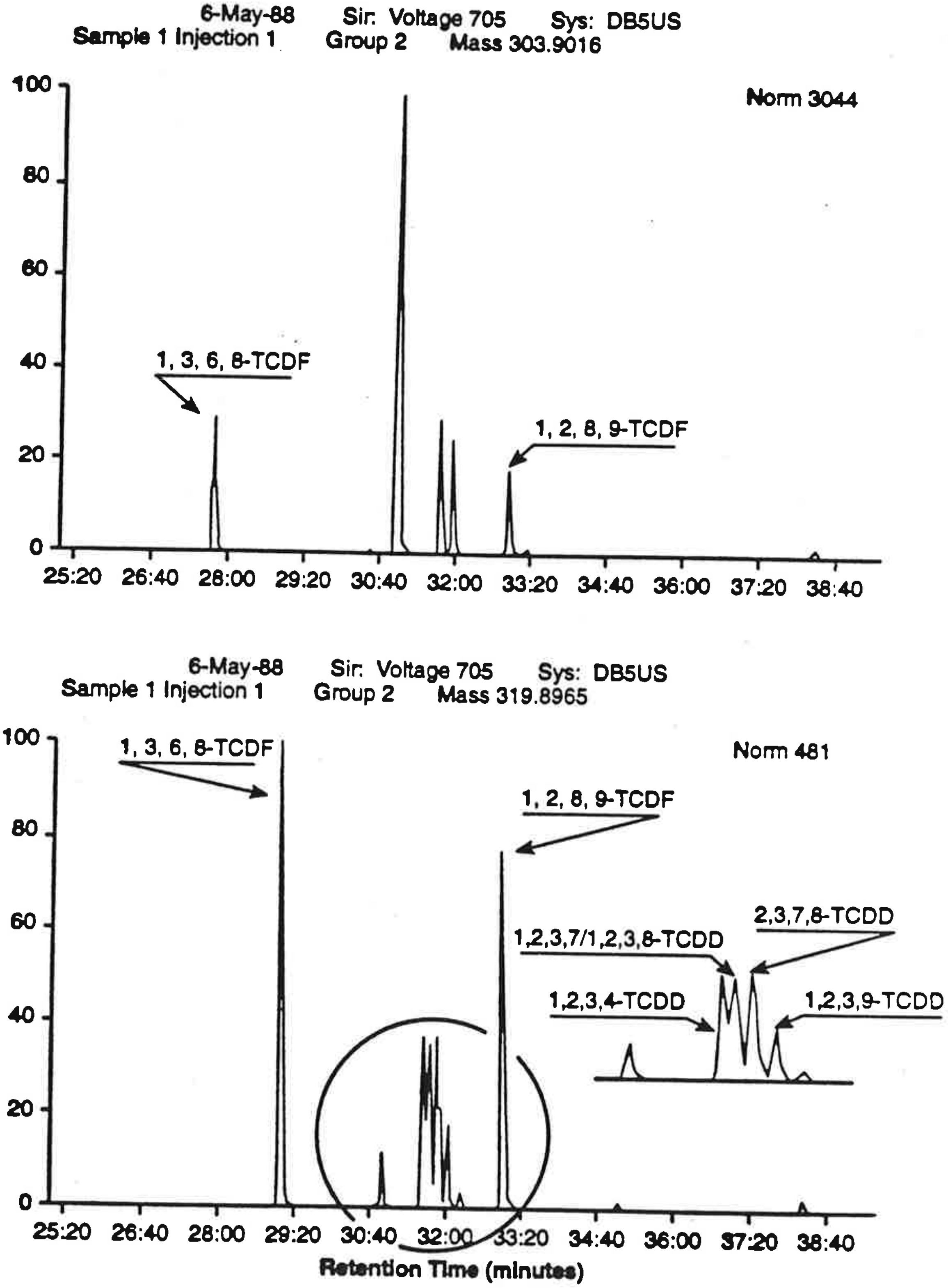


Figure 2C. First- and Last-Eluted Hexa-Dioxin and -Furan Isomers

82-020-05A



82-020-03A

Figure 2A. First- and Last-Eluted Tetra-Dioxin and -Furan Isomers

Table 8. Sample Phase and Quantity Extracted for Various Matrices

Sample Matrix[1]	***Example***	***Percent Solids***	***Phase***	***Quantity Extracted***
Single-phase				
Aqueous	Drinking Water Groundwater Treated Wastewater	<1	–[2]	1000 mL
Solid	Dry Soil Compost Ash	>20	Solid	10 g
Organic	Waste Solvent Waste Oil Organic Polymer	<1	Organic	10 g
Multi-phase				
Liquid/Solid				
Aqueous/Solid	Wet Soil Untreated effluent Digested municipal sludge Filter cake Paper pulp Tissue	1–30	Solid	10 g
Organic/solid	Industrial sludge Oily waste	1–100	Both	10 g
Liquid/Liquid				
Aqueous/organic	In-process effluent Untreated effluent Drum waste	<1	Organic	10 g
Aqueous/organic/solid	Untreated effluent Drum waste	>1	Organic & solid	10 g

1. The extract matrix may be vague for some samples. In general, when the CDDs and CDFs are in contact with a multiphase system in which one of the phases is water, they will be preferentially dispersed in or adsorbed on the alternate phase, because of their low solubility in water.
2. Aqueous samples are filtered after spiking with labeled analogs. The filtrate and the materials trapped on the filter are extracted separately, and then the extracts are combined for cleanup and analysis.

Table 6. Reference Compounds for Quantitation of Unlabeled and Labeled PCDDs and PCDFs

Compound	Reference for Quantitation
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxCDD	—[1]
1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF
OCDD	$^{13}C_{12}$-OCDD
OCDF	$^{13}C_{12}$-OCDD
$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
$^{37}Cl_4$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD

1. 1,2,3,7,8,9-HxCDD is quantified using the average responses for the $^{13}C_{12}$-1,2,3,4,7,8-HxCDD and the $^{13}C_{12}$-1,2,3,6,7,8-HxCDD.

Table 4 (continued). Concentration of Solutions Containing Lableled and Unlabeled PCDDs and PCDFs—Calibration and Verification Solutions

Compound	*CS1 (ng/mL)*	*CS2 (ng/mL)*	*VER[6] CS3 (ng/mL)*	*CS4 (ng/mL)*	*CD5 (ng/mL)*
2,3,7,8-TCDD	0.5	2	10	40	200
2,3,7,8-TCDF	0.5	2	10	40	200
1,2,3,7,8-PeCDD	2.5	10	50	200	1000
1,2,3,7,8-PeCDF	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1000
OCDD	5.0	20	100	400	2000
OCDF	5.0	20	100	400	2000
$^{13}C_{12}$-2,3,7,8-TCDD	100	100	100	100	100
$^{13}C_{12}$-2,3,7,8-TCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	100	100	100	100
$^{13}C_{12}$-PeCDF	100	100	100	100	100
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
$^{13}C_{12}$-OCDD	200	200	200	200	200
Cleanup Standard					
$^{37}Cl_4$-2,3,7,8-TCDD	0.5	2	10	40	200
Internal Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100

[6] Section 14.3—calibration verification solution.

Table 3A. Theoretical Ion Abundance Ratios and QC Limits

Number of Chlorine Atoms	m/z's Forming Ratio	Theoretical Ratio	QC Limits[1]	
			Lower	Upper
4[2]	M/M+2	0.77	0.65	0.89
5	M+2/M+4	1.55	1.32	1.78
6	M+2/M+4	1.24	1.05	1.43
6[3]	M/M+2	0.51	0.43	0.59
7	M+2/M+4	1.05	0.88	1.20
7[4]	M/M+2	0.44	0.37	0.51
8	M+2/M+4	0.89	0.76	1.02

1. QC limits represent ±15% windows around the theoretical ion abundance ratios.
2. Does not apply to $^{37}Cl_4$-2,3,7,8-TCDD (cleanup standard).
3. Used for $^{13}C_{12}$-HxCDF only.
4. Used for $^{13}C_{12}$-HpCDF only.

Table 3. Descriptors, Exact Masses, m/z Types, and Elemental Compositions of the PCDDs and PCDFs

Descriptor	*Accurate m/z*[1]	*m/z Type*	*Elemental Composition*	*Compound*[2]
1	292.9825	Lock	$C_7 F_{11}$	PFK
	303.9016	M	$C_{12} H_4 {}^{35}Cl_4 O$	TCDF
	305.8987	M+2	$C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O$	TCDF
	315.9419	M	${}^{13}C_{12} H_4 {}^{35}Cl_4 O$	TCDF[3]
	317.9389	M+2	${}^{13}C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O$	TCDF[3]
	319.8965	M	$C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD
	321.8936	M+2	$C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O_2$	TCDD
	327.8847	M	$C_{12} H_4 {}^{37}Cl_4 O_2$	TCDD[4]
	330.9792	QC	$C_7 F_{13}$	PFK
	331.9368	M	${}^{13}C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD[3]
	333.9339	M+2	${}^{13}C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O_2$	TCDD[3]
	375.8364	M+2	$C_{12} H_4 {}^{35}Cl_5 {}^{37}Cl O$	HxCDPE
2	339.8597	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	341.8567	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF
	351.9000	M+2	${}^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	353.8970	M+4	${}^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF[3]
	354.9792	Lock	$C_9 F_{13}$	PFK
	355.8546	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD
	357.8516	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PeCDD
	367.8949	M+2	${}^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD[3]
	369.8919	M+4	${}^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PeCDD[3]
	409.7974	M+2	$C_{12} H_3 {}^{35}Cl_6 {}^{37}Cl O$	HpCDPE
3	373.8208	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF
	375.8178	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O$	HxCDF
	383.8639	M	${}^{13}C_{12} H_2 {}^{35}Cl_6 O$	HxCDF[3]
	385.8610	M+2	${}^{13}C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF[3]
	389.8157	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O_2$	HxCDD
	391.8127	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O_2$	HxCDD
	392.9760	Lock	$C_9 F_{15}$	PFK
	401.8559	M+2	${}^{13}C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O_2$	HxCDD[3]
	403.8529	M+4	${}^{13}C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O_2$	HxCDD[3]
	430.9729	QC	$C_9 F_{17}$	PFK
	445.7555	M+4	$C_{12} H_2 {}^{35}Cl_6 {}^{37}Cl_2 O$	OCDPE

Table 2. Retention Times and Minimum Levels for PCDDs and PCDFs

Compound	Retention Time Reference	Relative Retention Time	Minimum Level[1]		
			Water (pg/L; ppq)	Solid (ng/kg; ppt)	Extract (pg/µL; ppb)
Compounds using $^{13}C_{12}$-1,2,3,4-TCDD as the injection internal standard					
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	0.999-1.001	10	1	0.5
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	0.999-1.001	10	1	0.5
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	0.999-1.001	50	5	2.5
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF	0.999-1.001	50	5	2.5
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	0.999-1.001	50	5	2.5
$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD	0.931-0.994			
$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD	0.993-1.036			
$^{37}Cl_4$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD	1.002-1.013			
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1.091-1.371			
$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1.123-1.408			
$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD	1.134-1.428			
Compounds using $^{13}C_{12}$-1,2,3,7,8,9-HxCDD as the injection internal standard					
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	0.999-1.001	50	5	2.5
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	0.999-1.001	50	5	2.5
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	0.999-1.001	50	5	2.5
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	0.999-1.001	50	5	2.5
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	0.999-1.001	50	5	2.5
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	0.999-1.001	50	5	2.5
1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0.986-1.016	50	5	2.5
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	0.999-1.001	50	5	2.5
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	0.999-1.001	50	5	2.5
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	0.999-1.001	50	5	2.5
OCDF	$^{13}C_{12}$-OCDD	0.995-1.013	100	10	5.0
OCDD	$^{13}C_{12}$-OCDD	0.999-1.001	100	10	5.0
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.947-0.992			
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.940-1.006			
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.993-1.017			
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.971-1.000			
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.974-1.002			
$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.975-1.006			

Reference (cont.)

12. "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety, 1979.

13. "Standard Methods for the Examination of Water and Wastewater," 16th edition and later revisions, American Public Health Association, 1015 15th St, N.W., Washington, DC 20005, 46: Section 108 (Safety), 1985.

14. "Method 613—2,3,7,8-Tetrachlorodibenzo-*p*-dioxin," 40 *CFR* 136 (49 *FR* 43234), October 26, 1984, Section 4.1.

15. Provost, L.P., and Elder, R.S., "Interpretation of Percent Recovery Data," *American Laboratory*, 15: 56-83, 1983.

16. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA EMSL, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.

17. "Standard Practice for Sampling Water," ASTM Annual Book of Standards, ASTM, 1916 Race Street, Philadelphia, PA 19103-1187, 1980.

18. "Methods 330.4 and 330.5 for Total Residual Chlorine," USEPA, EMSL, Cincinnati, OH 45268, EPA 600/4-70-020, March 1979.

19. Barnes, Donald G., Kutz, Frederick W., and Bottimore, David P., "Update of Toxicity Equivalency Factors (TEFs) for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-Dioxins and Dibenzofurans (CDDs/CDFs)," Risk Assessment Forum, USEPA, Washington, DC 20460, February 1989.

(Section 16.5) and the labeled compound recovery is within the normal range for the method (Section 17.3).

16.6.3 Additionally, if requested, the total concentration of all isomers in an individual level of chlorination (i.e., total TCDD, total TCDF, total PeCDD, etc.) may be reported by summing the concentrations of all isomers identified in that level of chlorination, including both 2,3,7,8-substituted and non-2,3,7,8-substituted isomers.

17. ANALYSIS OF COMPLEX SAMPLES

17.1 Some samples may contain high levels (>10 ng/L; >1000 ng/Kg) of the compounds of interest, interfering compounds, and/or polymeric materials. Some extracts will not concentrate to 10 µL (Section 11); others may overload the GC column and/or mass spectrometer.

17.2 Analyze a smaller aliquot of the sample (Section 16.5) when the extract will not concentrate to 20 µL after all cleanup procedures have been exhausted.

17.3 Recovery of labeled compounds: In most samples, recoveries of the labeled compounds will be similar to those from reagent water or from the alternate matrix (Section 6.6). If recovery of any of the labeled compounds is outside of the 25 to 150% range, a diluted sample (Section 16.5) shall be analyzed. If the recoveries of any of the labeled compounds in the diluted sample are outside of the limits (per the criteria above), then the calibration verification standard (Section 14.3) shall be analyzed and calibration verified (Section 14.3.5). If the calibration cannot be verified, a new calibration must be performed and the original sample extract reanalyzed. If the calibration is verified and the diluted sample does not meet the limits for labeled compound recovery, then the method does not apply to the sample being analyzed and the result may not be reported for regulatory compliance purposes.

18. METHOD PERFORMANCE

The performance specifications in this method are based on the analyses of more than 400 samples, representing matrices from at least five industrial categories. These specifications will be updated periodically as more data are received, and each time the procedures in the method are revised.

16. QUANTITATIVE DETERMINATION

16.1 Isotope dilution: By adding a known amount of a labeled compound to every sample prior to extraction, correction for recovery of the unlabeled compound can be made because the unlabeled compound and its labeled analog exhibit similar effects upon extraction, concentration, and gas chromatography. Relative response (RR) values are used in conjunction with the initial calibration data described in Section 7.5 to determine concentrations directly, so long as labeled compound spiking levels are constant, using the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A_n^1 + A_n^2)\ C_{is}}{(A_l^1 + A_l^2)\ RR}$$

where:

C_{ex} = *The concentration of the unlabeled compound in the extract and the other terms are as defined in Sections 7.5.2 and 7.6.1.*

16.1.1 Because of a potential interference, the labeled analog of OCDF is not added to the sample. Therefore, this unlabeled analyte is quantitated against the labeled OCDD. As a result, the concentration of unlabeled OCDF is corrected for the recovery of the labeled OCDD. In instances where OCDD and OCDF behave differently during sample extraction, concentration, and cleanup procedures, this may decrease the accuracy of the OCDF results. However, given the low toxicity of this compound relative to the other dioxins and furans, the potential decrease in accuracy is not considered significant.

16.1.2 Because the labeled analog of 1,2,3,7,8,9-HxCDD is used as an internal standard (i.e., not added before extraction of the sample), it cannot be used to quantitate the unlabeled compound by strict isotope dilution procedures. Therefore, the unlabeled 1,2,3,7,8,9-HxCDD is quantitated using the average of the responses of the labeled analogs of the other two 2,3,7,8-substituted HxCDD's, 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD. As a result, the concentration of the unlabeled 1,2,3,7,8,9-HxCDD is corrected for the average recovery of the other two HxCDD's.

16.1.3 Any peaks representing non-2,3,7,8-substituted dioxins or furans are quantitated using an average of the response factors from all of the labeled 2,3,7,8- isomers in the same level of chlorination.

16.2 Internal standard: Compute the concentrations of the ^{13}C-labeled analogs and the ^{37}C-labeled cleanup standard in the extract using the response factors determined from the initial calibration data (Section 7.6) and the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A_s^1 + A_s^2)\ C_{is}}{(A_{is}^1 + A_{is}^2)\ RF}$$

where:

C_{ex} = *The concentration of the compound in the extract. The other terms are defined in Section 7.6.1*

14.3.3 The peaks representing each unlabeled and labeled compound in the VER standard must be present with S/N of at least 10; otherwise, the mass spectrometer shall be adjusted and the verification test (Section 14.4.1) repeated.

14.3.4 Compute the concentration of each unlabeled compound by isotope dilution (Section 7.5) for those compounds that have labeled analogs (Table 1). Compute the concentration of the labeled compounds by the internal standard method. These concentrations are computed based on the calibration data in Section 7.

14.3.5 For each compound, compare the concentration with the calibration verification limit in Table 7. If all compounds meet the acceptance criteria, calibration has been verified. If, however, any compound fails, the measurement system is not performing properly for that compound. In this event, prepare a fresh calibration standard or correct the problem causing the failure and repeat the resolution (Section 14.2) and verification (Section 14.3.1) tests, or recalibrate (Section 7).

14.4 Retention times and GC resolution.

14.4.1 Retention times.

14.4.1.1 Absolute: The absolute retention times of the $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD GCMS internal standards shall be within ±15 seconds of the retention times obtained during calibration (Section 7.2.4).

14.4.1.2 Relative: The relative retention times of unlabeled and labeled PCDDs and PCDFs shall be within the limits given in Table 2.

14.4.2 GC resolution.

14.4.2.1 Inject the isomer specificity standards (Section 6.16) on their respective columns.

14.4.2.2 The valley height between 2,3,7,8-TCDD and the other tetra-dioxin isomers at m/z 319.8965, and between 2,3,7,8-TCDF and the other tetra-furan isomers at m/z 303.9016 shall not exceed 25% on their respective columns (Figure 3).

14.4.3 If the absolute retention time of any compound is not within the limits specified or the 2,3,7,8-isomers are not resolved, the GC is not performing properly. In this event, adjust the GC and repeat the verification test (Section 14.3.1) or recalibrate (Section 7).

14.5 Ongoing precision and recovery.

14.5.1 Analyze the extract of the ongoing precision and recovery (OPR) aliquot (Section 10.3.4 or 10.4.4) prior to analysis of samples from the same set.

14.5.2 Compute the concentration of each PCDD and PCDF by isotope dilution for those compounds that have labeled analogs (Section 7.5). Compute the concentration of each labeled compound by the internal standard method.

14.5.3 For each unlabeled and labeled compound, compare the concentration with the limits for ongoing accuracy in Table 7. If all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the range given, the adsorbent bed 2 cm long. Insert a glass-wool plug on top of the bed to hold the adsorbent in place.

12.5.2 Prerinse the column with 5 mL of toluene followed by 2 mL methylene chloride:methanol:toluene (15:4:1 v/v), 1 mL methylene chloride:cyclohexane (1:1 v/v), and 5 mL hexane. If the flow rate of eluate exceeds 0.5 mL/min, discard the column.

12.5.3 When the solvent is within 1 mm of the column packing, apply the sample extract to the column. Rinse the sample container twice with 1-mL portions of hexane and apply separately to the column. Apply 2 mL of hexane to complete the transfer.

12.5.4 Elute the interfering compounds with 2 mL of hexane, 2 mL of methylene chloride:cyclohexane (1:1 v/v), and 2 mL of methylene chloride:methanol:toluene (15:4:1 v/v). Discard the eluate.

12.5.5 Invert the column and elute the PCDDs and PCDFs with 20 mL of toluene. If carbon particles are present in the eluate, filter through glass fiber filter paper.

12.5.6 Concentrate the eluate per Section 11.4 or 11.5 for further cleanup or for injection into the HPLC or GC/MS.

12.6 HPLC (adapted from Reference 6).

12.6.1 Column calibration.

12.6.1.1 Prepare a calibration standard containing the 2,3,7,8-substituted isomers and/or other isomers of interest at a concentration of approximately 500 pg/µL in methylene chloride.

12.6.1.2 Inject 30 µL of the calibration solution into the HPLC and record the signal from the detector. Collect the eluant for reuse. The elution order will be the tetra- through octa-isomers.

12.6.1.3 Establish the collect time for the tetra-isomers and for the other isomers of interest. Following calibration, flush the injection system with copious quantities of methylene chloride, including a minimum of five 50-µL injections while the detector is monitored, to ensure that residual PCDDs and PCDFs are removed from the system.

12.6.1.4 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the PCDDs and PCDFs from the calibration standard (Section 12.6.1.1) is 75 to 125% compared to the calibration (Section 12.6.1.2). If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated system.

12.6.2 Extract cleanup: HPLC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 30 µL of extract. If the extract cannot be concentrated to less than 30 µL, it is split into fractions and the fractions are combined after elution from the column.

12.6.2.1 Rinse the sides of the vial twice with 30 µL of methylene chloride and reduce to 30 µL with the evaporation apparatus.

12.6.2.2 Inject the 30 µL extract into the HPLC.

12.2.2.2 Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis(2-ethyl hexyl) phthalate, pentachlorophenol, perylene, and sulfur.

12.2.2.3 Set the "dump time" to allow >85% removal of the corn oil and >85% collection of the phthalate.

12.2.2.4 Set the "collect time" to the peak minimum between perylene and sulfur.

12.2.2.5 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85%. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

12.2.3 Extract cleanup: GPC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 0.5 g of high molecular weight material in a 5 mL extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into aliquots for GPC and the aliquots are combined after elution from the column. The residue content of the extract may be obtained gravimetrically by evaporating the solvent from a 50-μL aliquot.

12.2.3.1 Filter the extract or load through the filter holder to remove particulates. Load the 5.0-mL extract onto the column.

12.2.3.2 Elute the extract using the calibration data determined in Section 12.2.2. Collect the eluate in a clean 400- to 500-mL beaker.

12.2.3.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

12.2.3.4 If a particularly dirty extract is encountered, a 5.0-mL methylene chloride blank shall be run through the system to check for carry-over.

12.2.3.5 Concentrate the eluate per Sections 11.2.1, 11.2.2, and 11.3.1 or 11.3.2 for further cleanup or for injection into the GC/MS.

12.3 Silica gel cleanup.

12.3.1 Place a glass-wool plug in a 15-mm-ID chromatography column. Pack the column in the following order (bottom to top): 1 g silica gel (Section 6.5.1.1), 4 g basic silica gel (Section 6.5.1.3), 1 g silica gel, 8 g acid silica gel (Section 6.5.1.2), 2 g silica gel. Tap the column to settle the adsorbents.

12.3.2 Prerinse the column with 50 to 100 mL of hexane. Close the stopcock when the hexane is within 1 mm of the sodium sulfate. Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.

12.3.3 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.

12.3.4 Rinse the receiver twice with 1-mL portions of hexane and apply separately to the column. Elute the PCDDs/PCDFs with 100 mL hexane and collect the eluate.

12.3.5 Concentrate the eluate per Section 11.4 or 11.5 for further cleanup or for injection into the HPLC or GC/MS.

11.3.6 Pour each extract through a drying column containing 7 to 10 cm of anhydrous sodium sulfate. Rinse the separatory funnel with 30 to 50 mL of toluene and pour through the drying column. Collect each extract in a 500-mL round-bottom flask. Concentrate and clean up the samples and QC aliquots per Sections 11.4 and 12.

11.4 Macro-concentration: Concentrate the extracts in separate 100-mL round bottom flasks on a rotary evaporator.

11.4.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45°C. On a daily basis, preclean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for contamination check if necessary. Between samples, three 2- to 3-mL aliquots of toluene should be rinsed down the feed tube into a waste beaker.

11.4.2 Attach the round-bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system, and begin rotating the sample flask.

11.4.3 Lower the flask into the water bath and adjust the speed of rotation and the temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of concentration, the flow of solvent into the receiving flask will be steady, but no bumping or visible boiling of the extract will occur.

NOTE: If the rate of concentration is too fast, analyte loss may occur.

11.4.4 When the liquid in the concentration flask has reached an apparent volume of 2 mL, remove the flask from the water bath and stop the rotation. Slowly and carefully, admit air into the system. Be sure not to open the valve so quickly that the sample is blown out of the flask. Rinse the feed tube with approximately 2 mL of hexane.

11.4.5 Transfer the extract to a vial using three 2- to 3-mL rinses of hexane. Proceed with micro-concentration and solvent exchange.

11.5 Micro-concentration and solvent exchange.

11.5.1 Toluene extracts to be subjected to GPC or HPLC cleanup are exchanged into methylene chloride. Extracts that are to be cleaned up using silica gel, alumina, and/or AX-21/Celite are exchanged into hexane.

11.5.2 Transfer the vial containing the sample extract to a nitrogen evaporation device. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed.

NOTE: A large vortex in the solvent may cause analyte loss.

11.5.3 Lower the vial into a 45°C water bath and continue concentrating.

11.5.4 When the volume of the liquid is approximately 100 µL, add 2 to 3 mL of the desired solvent (methylene chloride or hexane) and continue concentration to approximately 100 µL. Repeat the addition of solvent and concentrate once more.

11.5.5 If the extract is to be cleaned up by GPC or HPLC, adjust the volume of the extract to 5.0 mL with methylene chloride. Proceed with GPC cleanup (Section 12.2).

the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation.

11.1.7 The concentrated extracts of the filtrate and the particulates are combined using the procedures in Section 11.2.13.

11.2 Soxhlet/Dean-Stark extraction of solids: Extract the solid samples, particulates, blanks, and IPR/OPR aliquots using the following procedure.

11.2.1 Charge a clean extraction thimble with 5.0 g of 100/200 mesh silica (Section 6.5.1.1) and 100 g of quartz sand (Section 6.3.2).

NOTE: Do not disturb the silica layer throughout the extraction process.

11.2.2 Place the thimble in a clean extractor. Place 30 to 40 mL of toluene in the receiver and 200 to 250 mL of toluene in the flask.

11.2.3 Pre-extract the glassware by heating the flask until the toluene is boiling. When properly adjusted, 1 to 2 drops of toluene per second will fall from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 hours.

11.2.4 After pre-extraction, cool and disassemble the apparatus. Rinse the thimble with toluene and allow to air dry.

11.2.5 Load the wet sample from Sections 10.4.5, 10.5.2, 10.6.3, or 10.6.4, and any non-aqueous liquid from Section 10.5.2 into the thimble and manually mix into the sand layer with a clean metal spatula carefully breaking up any large lumps of sample. If the material to be extracted is the particulate matter from the filtration of an aqueous sample, add the filter paper to the thimble also.

11.2.6 Reassemble the pre-extracted SDS apparatus and add a fresh charge of toluene to the receiver and reflux flask.

11.2.7 Apply power to the heating mantle to begin refluxing. Adjust the reflux rate to match the rate of percolation through the sand and silica beds until water removal lessens the restriction to toluene flow. Check the apparatus for foaming frequently during the first 2 hours of extraction. If foaming occurs, reduce the reflux rate until foaming subsides.

11.2.8 Drain the water from the receiver at 1 to 2 hours and 8 to 9 hours, or sooner if the receiver fills with water. Reflux the sample for a total of 16 to 24 hours. Cool and disassemble the apparatus. Record the total volume of water collected.

11.2.9 Remove the distilling flask. Drain the water from the Dean-Stark receiver and add any toluene in the receiver to the extract in the flask.

11.2.10 For solid samples, the extract must be concentrated to approximately 10 mL prior to back extraction. For the particulates filtered from an aqueous sample, the extract must be concentrated prior to combining with the extract of the filtrate. Therefore, add one or two clean boiling chips to the round-bottom flask and attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of toluene through the top. Place the round-bottom flask in a heating mantle and apply heat as required to complete the concentration in 15 to 20 minutes. At the proper rate of

10.4.4 Spike 1.0 mL of the diluted labeled compound spiking solution into one reference matrix aliquot. This aliquot will serve as the blank. Spike 1.0 mL of the diluted precision and recovery standard into the remaining reference matrix aliquot. This aliquot will serve as the PAR (Section 14.5). Spike 1.0 mL of the diluted labeled compound spiking solution into the PAR aliquot as well.

10.4.5 Stir or tumble and equilibrate the aliquots for 1 to 2 hours.

10.4.6 Extract the aliquots using the procedures in Section 11.

10.5 Multiphase samples

10.5.1 Pressure filter the sample, blank, and PAR aliquots through Whatman GF/D glass fiber filter paper. If necessary, centrifuge these aliquots for 30 minutes at greater than 5000 rpm prior to filtration.

10.5.2 Discard any aqueous phase (if present). Remove any non-aqueous liquid (if present) and reserve for recombination with the extract of the solid phase (Section 11.2.5). Prepare the filter papers of the sample and QC aliquots for particle size reduction and blending (Section 10.6).

10.6 Sample grinding, homogenization, or blending: Samples with particle sizes greater than 1 mm (as determined by Section 10.2.2) are subjected to grinding, homogenization, or blending. The method of reducing particle size to less than 1 mm is matrix-dependent. In general, hard particles can be reduced by grinding with a mortar and pestle. Softer particles can be reduced by grinding in a Wiley mill or meat grinder, by homogenization, or by blending.

10.6.1 Each size-reducing preparation procedure on each matrix shall be verified by running the tests in Section 8.2 before the procedure is employed routinely.

10.6.2 The grinding, homogenization, or blending procedures shall be carried out in a glove box or fume hood to prevent particles from contaminating the work environment.

10.6.3 Grinding: Tissue samples, certain papers and pulps, slurries, and amorphous solids can be ground in a Wiley mill or heavy duty meat grinder. In some cases, reducing the temperature of the sample to freezing or to dry ice or liquid nitrogen temperatures can aid in the grinding process. Grind the sample aliquots from Section 10.4.5 or 10.5.2 in a clean grinder. Do not allow the sample temperature to exceed 50 °C. Grind the blank and reference matrix aliquots using a clean grinder.

10.6.4 Homogenization or blending: Particles that are not ground effectively, or particles greater than 1 mm in size after grinding, can often be reduced in size by high speed homogenization or blending. Homogenize and/or blend the sample, blank, and PAR aliquots from Section 10.4.5, 10.5.2, or 10.6.3.

10.6.5 Extract the aliquots using the procedures in Section 11.

11. *EXTRACTION AND CONCENTRATION*

11.1 Extraction of filtrates: Extract the aqueous samples, blanks, and IPR/OPR aliquots according to the following procedures.

11.1.1 Pour the filtered aqueous sample from the filtration flask into a 2-L separatory funnel. Rinse the flask twice with 5 mL of reagent water and add these rinses to the automatic sampling equipment. Solid samples are collected as grab samples using wide-mouth jars.

9.2 Maintain samples at 0 to 4°C in the dark from the time of collection until extraction. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 18).

9.3 Perform sample analysis within 40 days of extraction.

10. SAMPLE PREPARATION

The sample preparation process involves modifying the physical form of the sample so that the PCDDs and PCDFs can be extracted efficiently. In general, the samples must be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place. Table 8 lists the phase(s) and quantity extracted for various sample matrices. Samples containing a solid phase and samples containing particle sizes larger than 1 mm require preparation prior to extraction. Because PCDDs/PCDFs are strongly associated with particulates, the preparation of aqueous samples is dependent on the solids content of the sample. Aqueous samples containing 1% solids or less are extracted in a separatory funnel. A smaller sample aliquot is used for aqueous samples containing more than 1% solids. For samples expected or known to contain high levels of the PCDDs and/or PCDFs, the smallest sample size representative of the entire sample should be used, and the sample extract should be diluted, if necessary, per Section 16.5.

10.1 Determine percent solids.

10.1.1 Weigh 5 to 10 g of sample (to three significant figures) into a tared beaker.

NOTE: *This aliquot is used only for determining the solids content of the sample, not for analysis of PCDDs/PCDFs.*

10.1.2 Dry overnight a minimum of 12 hours at 110°C (±5°C), and cool in a dessicator.

10.1.3 Calculate percent solids as follows:

$$\%\ solids = \frac{weight\ of\ sample\ aliquot\ after\ drying}{weight\ of\ sample\ aliquot\ before\ drying} \times 100$$

10.2 Determine particle size.

10.2.1 Spread the dried sample from Section 10.1.2 on a piece of filter paper or aluminum foil in a fume hood or glove box.

10.2.2 Estimate the size of the particles in the sample. If the size of the largest particles is greater than 1 mm, the particle size must be reduced to 1 mm or less prior to extraction.

10.3 Preparation of aqueous samples containing 1% solids or less: The extraction procedure for aqueous samples containing less than or equal to 1% solids involves filtering the sample, extracting the particulate phase and the filtrate separately, and combining the extracts for indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits. Procedures for dilutions are given in Section 16.5.

8.1.5 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the ongoing precision and recovery aliquot that the analytical system is in control. These procedures are described in Sections 14.1 through 14.5.

8.1.6 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.4.

8.2 Initial precision and recovery (IPR): To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations.

8.2.1 For low solids (aqueous) samples, extract, concentrate, and analyze four 1-L aliquots of reagent water spiked with the diluted precision and recovery standard (PAR) (Sections 6.14 and 10.3.4) according to the procedures in Sections 10 through 13. For an alternative sample matrix, four aliquots of the alternative matrix are used. All sample processing steps that are to be used for processing samples, including preparation (Section 10), extraction (Section 11), and cleanup (Section 12), shall be included in this test.

8.2.2 Using results of the set of four analyses, compute the average concentration (X) of the extracts in ng/mL and the standard deviation of the concentration (s) in ng/mL for each compound, by isotope dilution for PCDDs and PCDFs with a labeled analog, and by internal standard for labeled compounds.

8.2.3 For each unlabeled and labeled compound, compare s and X with the corresponding limits for initial precision and accuracy in Table 7. If s and X for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any individual X falls outside the range for accuracy, system performance is unacceptable for that compound. Correct the problem and repeat the test (Section 8.2). The concentration limits in Table 7 for labeled compounds are based on the requirement that the recovery of each labeled compound be in the range of 25 to 150%.

8.3 The laboratory shall spike all samples and QC aliquots with the diluted labeled compound spiking solution (Sections 6.10 and 10.3.2) to assess method performance on the sample matrix.

8.3.1 Analyze each sample according to the procedures in Sections 10 through 13.

8.3.2 Compute the percent recovery of the labeled compounds and the cleanup standard using the internal standard method (Section 16.2).

8.3.3 The recovery of each labeled compound must be within 25 to 150%. If the recovery of any compound falls outside of these limits, method performance is unacceptable for that compound in that sample. To overcome such difficulties, water samples are diluted and smaller amounts of soils, sludges, sediments, and other matrices are reanalyzed per Section 17.

8.4 Method accuracy for samples shall be assessed and records shall be maintained.

$$RR = \frac{(A_n^1 + A_n^2)\,C_l}{(A_l^1 + A_l^2)\,C_n}$$

Where:

A_n^1 *and* A_n^2 = *The areas of the primary and secondary m/z's for the unlabeled compound.*

A_l^1 *and* A_l^2 = *The areas of the primary and secondary m/z's for the labeled compound.*

C_l = *The concentration of the labeled compound in the calibration standard.*

C_n = *The concentration of the unlabeled compound in the calibration standard.*

7.5.3 To calibrate the analytical system by isotope dilution, inject a 1.0-µL aliquot of calibration standards CS1 through CS5 (Section 6.13 and Table 4) using the procedure in Section 13 and the conditions in Table 2. Compute the relative response (RR) at each concentration.

7.5.4 Linearity: If the relative response for any compound is constant (less than 20% coefficient of variation) over the five-point calibration range, an averaged relative response may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point calibration range.

7.6 Calibration by internal standard: The internal standard method is applied to determination of non-2,3,7,8-substituted compounds having no labeled analog in this method, and to measurement of labeled compounds for intralaboratory statistics (Sections 8.4 and 14.5.4).

7.6.1 Response factors: Calibration requires the determination of response factors (RF) defined by the following equation:

$$RF = \frac{(A_s^1 + A_s^2)\,C_{is}}{(A_{is}^1 + A_{is}^2)\,C_s}$$

Where:

A_s^1 *and* A_s^2 = *The areas of the primary and secondary m/z's for the compound to be calibrated.*

A_{is}^1 *and* A_{is}^2 = *The areas of the primary and secondary m/z's for the internal standard.*

C_{is} = *The concentration of the internal standard (Section 6.12 and Table 4).*

C_s = *The concentration of the compound in the calibration standard.*

NOTE: There is only one m/z for $^{37}Cl_4$*-2,3,7,8-TCDD. See Table 3.*

7.6.2 To calibrate the analytical system by internal standard, inject a 1.0 µL aliquot of calibration standards CS1 through CS5 (Section 6.13 and Table 4) using the procedure in Section 13 and the conditions in Table 2. Compute the response factor (RF) at each concentration.

can have serious adverse effects on instrument performance. Therefore, a mass-drift correction is mandatory. A lock-mass ion from the reference compound (PFK) is used for tuning the mass spectrometer. The lock-mass ion is dependent on the masses of the ions monitored within each descriptor, as shown in Table 3. The level of the reference compound (PFK) metered into the ion chamber during HRGC/HRMS analyses should be adjusted so that the amplitude of the most intense selected lock-mass ion signal (regardless of the descriptor number) does not exceed 10% of the full-scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur during the analysis can be more effectively monitored.

NOTE: *Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source resulting in an increase in time lost in cleaning the source.*

7.1.2.2 Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10% valley) at m/z 304.9824 (PFK) or any other reference signal close to m/z 303.9016 (from TCDF). For each descriptor (Table 3), monitor and record the resolution and exact mass of three to five reference peaks covering the mass range of the descriptor. The resolution must be greater than or equal to 10,000. The deviation between the exact mass and the theoretical mass (Table 3) for each ion monitored must be less than 5 ppm.

7.2 Ion abundance ratios, minimum levels, signal-to-noise ratios, and absolute retention times: Inject an aliquot of the CS1 calibration solution (Table 4) using the GC conditions from Section 7.1.

7.2.1 Measure the SICP areas for each analyte and compute the ion abundance ratios specified in Table 3A. Compare the computed ratio to the theoretical ratio given in Table 3A.

7.2.1.1 The groups of m/z's to be monitored are shown in Table 3. Each group or descriptor shall be monitored in succession as a function of GC retention time to ensure that all PCDDs and PCDFs are detected. Additional m/z's may be monitored in each descriptor, and the m/z's may be divided among more than the five descriptors listed in Table 3, provided that the laboratory is able to monitor the m/z's of all the PCDDs/PCDFs that may elute from the GC in a given retention-time window.

7.2.1.2 The mass spectrometer shall be operated in a mass-drift correction mode, using perfluorokerosene (PFK) to provide lock masses. The lock-mass for each group of m/z's is shown in Table 3. Each lock-mass shall be monitored and shall not vary by more than ±20% throughout its respective retention time window. Variations of the lock-mass by more than 20% indicate the presence of coeluting interferences that may significantly reduce the sensitivity of the mass spectrometer. Reinjection of another

If the chemical purity is 98% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at room temperature in screw-capped vials with PTFE-lined caps. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. If solvent loss has occurred, the solution should be replaced.

6.8 Stock solutions.

6.8.1 Preparation: Prepare in nonane per the steps below or purchase as dilute solutions (Cambridge Isotope Laboratories, Woburn, MA, or equivalent). Observe the safety precautions in Section 4, and the recommendation in Section 4.1.2.

6.8.2 Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 1 to 2 mg of 2,3,7,8-TCDD to three significant figures in a 10-mL ground-glass-stoppered volumetric flask and fill to the mark with nonane. After the TCDD is completely dissolved, transfer the solution to a clean 15-mL vial with PTFE-lined cap.

6.8.3 Stock standard solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards. Reference standards that can be used to determine the accuracy of calibration standards are available from Cambridge Isotope Laboratories and may be available from other vendors.

6.9 Secondary standard: Using stock solutions (Section 6.8), prepare secondary standard solutions containing the compounds and concentrations shown in Table 4 in nonane.

6.10 Labeled-compound stock standard: From stock standard solutions prepared as above, or from purchased mixtures, prepare this standard to contain the labeled compounds at the concentrations shown in Table 4 in nonane. This solution is diluted with acetone prior to use (Section 10.3.2).

6.11 Cleanup standard: Prepare $^{37}Cl_4$-2,3,7,8-TCDD at the concentration shown in Table 4 in nonane.

6.12 Internal standard: Prepare at the concentration shown in Table 4 in nonane.

6.13 Calibration standards (CS1 through CS5): Combine the solutions in Sections 6.9, 6.10, 6.11, and 6.12 to produce the five calibration solutions shown in Table 4 in nonane. These solutions permit the relative response (labeled to unlabeled) and response factor to be measured as a function of concentration. The CS3 standard is used for calibration verification (VER).

6.14 Precision and recovery standard (PAR): Used for determination of initial (Section 8.2) and ongoing (Section 14.5) precision and accuracy. This solution contains the analytes and labeled compounds at the concentrations listed in Table 4 in nonane. This solution is diluted with acetone prior to use (Sections 10.3.4 and 10.4.4).

6.15 GC retention time window defining solutions: Used to define the beginning and ending retention times for the dioxin and furan isomers.

6.15.1 DB-5 column window defining standards, Cambridge Isotope Laboratories ED-1732-A (dioxins) and ED-1731-A (furans), or equivalent, containing the compounds listed in Table 5.

6.16 Isomer specificity test standards: Used to demonstrate isomer specificity of the GC columns employed for the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzodibenzofuran.

5.11 GC/MS interface: The mass spectrometer (MS) shall be interfaced to the GC such that the end of the capillary column terminates within 1 cm of the ion source but does not intercept the electron or ion beams.

5.12 Data system: Capable of collecting, recording, and storing MS data.

6. REAGENTS AND STANDARDS

6.1 pH adjustment and back-extraction.

6.1.1 Potassium hydroxide: Dissolve 20 g reagent grade KOH in 100 mL reagent water.

6.1.2 Sulfuric acid: Reagent grade (specific gravity 1.84).

6.1.3 Sodium chloride: Reagent grade, prepare a 5% (w/v) solution in reagent water.

6.2 Solution drying and evaporation.

6.2.1 Solution drying: Sodium sulfate, reagent grade, granular anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 400°C for 1 hour minimum, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix), that batch of reagent is not suitable for use and should be discarded. Extraction with methylene chloride (as opposed to simple rinsing) and baking at a lower temperature may produce sodium sulfate that is suitable for use.

6.2.2 Prepurified nitrogen.

6.3 Extraction.

6.3.1 Solvents: Acetone, toluene, cyclohexane, hexane, methanol, methylene chloride, and nonane; distilled in glass, pesticide quality, lot-certified to be free of interferences.

6.3.2 White quartz sand, 60/70 mesh: For Soxhlet/Dean-Stark extraction (Aldrich Chemical, Cat. No. 27-437-9, or equivalent). Bake at 450°C for a minimum of 4 hours.

6.4 GPC calibration solution: Prepare a solution containing 300 mg/mL corn oil, 15 mg/mL bis(2-ethylhexyl) phthalate, 1.4 mg/mL pentachlorophenol, 0.1 mg/mL perylene, and 0.5 mg/mL sulfur.

6.5 Adsorbents for sample cleanup.

6.5.1 Silica gel.

6.5.1.1 Activated silica gel: Bio-Sil A, 100-200 mesh (Bio-Rad 131-1340, or equivalent), rinsed with methylene chloride, baked at 180°C for a minimum of 1 hour, cooled in a dessicator, and stored in a precleaned glass bottle with screw-cap that prevents moisture from entering.

6.5.1.2 Acid silica gel (30% w/w): Thoroughly mix 44.0 g of concentrated sulfuric acid with 100.0 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a screw-capped bottle with PTFE-lined cap.

6.5.1.3 Basic silica gel: Thoroughly mix 30 g of 1N sodium hydroxide with 100 g of activated silica gel in a clean container. Break up aggregates with a

5.6 Centrifuge apparatus.

5.6.1 Centrifuge: Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum.

5.6.2 Centrifuge bottles: 500-mL, with screw-caps, to fit centrifuge.

5.6.3 Centrifuge tubes: 12- to 15-mL, with screw-caps, to fit centrifuge.

5.7 Cleanup apparatus.

5.7.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc, Columbia, MO, Model GPC Autoprep 1002, or equivalent).

5.7.1.1 Column: 600 to 700 mm long × 25 mm ID, packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).

5.7.1.2 Syringe: 10-mL, with Luer fitting.

5.7.1.3 Syringe filter holder, stainless steel, and glass fiber or PTFE filters (Gelman 4310, or equivalent).

5.7.1.4 UV detectors: 254-nm, preparative or semi-prep flow cell (Isco, Inc., Type 6; Schmadzu, 5 mm path length; Beckman-Altex 152W, 8 µL micro-prep flow cell, 2 mm path; Pharmacia UV-1, 3 mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).

5.7.2 Reverse-phase high-performance liquid chromatograph.

5.7.2.1 Column oven and detector: Perkin-Elmer Model LC-65T (or equivalent) operated at 0.02 AUFS at 235 nm.

5.7.2.2 Injector: Rheodyne 7120 (or equivalent) with 50-µL sample loop.

5.7.2.3 Column: Two 6.2 mm × 250 mm Zorbax-ODS columns in series (DuPont Instruments Division, Wilmington, DE, or equivalent), operated at 50°C with 2.0 mL/min methanol isocratic effluent.

7.2.4 Pump: Altex 110A (or equivalent).

5.7.3 Pipets.

5.7.3.1 Disposable, Pasteur, 150 mm long × 5 mm ID (Fisher Scientific 13-678-6A, or equivalent).

5.7.3.2 Disposable, serological, 10-mL (6 mm ID).

5.7.4 Chromatographic columns.

5.7.4.1 150 mm long × 8 mm ID, (Kontes K-420155, or equivalent) with coarse-glass frit or glass-wool plug and 250-mL reservoir.

5.7.4.2 200 mm long × 15 mm ID, with coarse-glass frit or glass-wool plug and 250-mL reservoir.

5.7.5 Oven: For storage of adsorbents, capable of maintaining a temperature of 130°C (±5°C).

5.8 Concentration apparatus.

5.8.1 Rotary evaporator: Buchi/Brinkman-American Scientific No. E5045-10 or equivalent, equipped with a variable temperature water bath.

the equipment or work space, and indicates that unacceptable work practices have been employed.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment for discrete or composite sampling.

5.1.1 Sample bottles and caps.

5.1.1.1 Liquid samples (waters, sludges and similar materials containing 5% solids or less): Sample bottle, amber glass, 1.1 L minimum, with screw cap.

5.1.1.2 Solid samples (soils, sediments, sludges, paper pulps, filter cake, compost, and similar materials that contain more than 5% solids): Sample bottle, wide mouth, amber glass, 500-mL minimum.

5.1.1.3 If amber bottles are not available, samples shall be protected from light.

5.1.1.4 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.

5.1.1.5 Cleaning.

5.1.1.5.1 Bottles are detergent-water washed, then solvent-rinsed before use.

5.1.1.5.2 Liners are detergent-water washed, then rinsed with reagent water (Section 6.6.1) and then solvent, and baked at approximately 200°C for a minimum of 1 hour prior to use.

5.1.2 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Glass or PTFE tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for glassware cleaning: Laboratory sink with overhead fume hood.

5.3 Equipment for sample preparation.

5.3.1 Laboratory fume hood of sufficient size to contain the sample preparation equipment listed below.

5.3.2 Glove box (optional).

5.3.3 Tissue homogenizer: VirTis Model 45 Macro homogenizer (American Scientific Products H-3515, or equivalent) with stainless steel Macro-shaft and Turbo-shear blade.

5.3.4 Meat grinder: Hobart, or equivalent, with 3- to 5-mm holes in inner plate.

5.3.5 Equipment for determining percent moisture.

5.3.5.1 Oven: Capable of maintaining a temperature of 110 ±5°C.

5.3.5.2 Dessicator.

4.1.1 The 2,3,7,8-TCDD isomer has been found to be acnegenic, carcinogenic, and teratogenic in laboratory animal studies. It is soluble in water to approximately 200 ppt and in organic solvents to 0.14%. On the basis of the available toxicological and physical properties of 2,3,7,8-TCDD, all of the PCDDs and PCDFs should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

4.1.2 It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator shall be worn when high concentrations are handled.

4.2 The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 10 through 13. The references and bibliography at the end of Reference 13 are particularly comprehensive in dealing with the general subject of laboratory safety.

4.3 The PCDDs and PCDFs and samples suspected to contain these compounds are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. The PCDDs and PCDFs are extremely toxic to laboratory animals. Each laboratory must develop a strict safety program for handling the PCDDs and PCDFs. The following practices are recommended (References 2 and 14).

4.3.1 Facility: When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

4.3.2 Protective equipment: Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be utilized. During analytical operations which may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection equipment (preferably full face shields) must be worn while working with exposed samples or pure analytical standards. Latex gloves are commonly used to reduce exposure of the hands. When handling samples suspected or known to contain high concentrations of the PCDDs or PCDFs, an additional set of gloves can also be worn beneath the latex gloves.

4.3.3 Training: Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

the gas chromatograph. The analytes are separated by the GC and detected by a high-resolution (≥ 10,000) mass spectrometer. Two exact masses (m/z's) are monitored for each analyte.

2.4 Dioxins and furans are identified by comparing GC retention times and the ion abundance ratios of the m/z's with the corresponding retention-time ranges of authentic standards and the theoretical ion-abundance ratios of the exact m/z's. Isomers and congeners are identified when the retention times and ion abundance ratios agree within predefined limits. By using a GC column or columns capable of resolving the 2,3,7,8-substituted isomers from all other tetra-isomers, the 2,3,7,8-substituted isomers are identified when the retention-time and m/z abundance ratios agree within predefined limits of the retention times and exact m/z ratios of authentic standards.

2.5 Quantitative analysis is performed by GC/MS using selected ion current profile (SICP) areas, in one of two ways.

2.5.1 For the 15 2,3,7,8-substituted isomers for which labeled analogs are available (see Table 1), the GC/MS system is calibrated and the compound concentration is determined using an isotope dilution technique. Although a labeled analog of the octachlorinated dibenzofuran (OCDF) is available, using high-resolution mass spectrometry it produces an m/z that may interfere with the identification and quantitation of the unlabeled octachlorinated dibenzo-*p*-dioxin (OCDD). Therefore, this labeled analog has not been included in the calibration standards, and the unlabeled OCDF is quantitated against the labeled OCDD. Because the labeled analog of 1,2,3,7,8,9-HxCDD is used as an internal standard (i.e., not added before extraction of the sample), it cannot be used to quantitate the unlabeled compound by strict isotope dilution procedures. Therefore, the unlabeled 1,2,3,7,8,9-HxCDD is quantitated using the average of the responses of the labeled analogs of the other two 2,3,7,8-substituted HxCDDs (i.e., 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD). As a result, the concentration of the unlabeled 1,2,3,7,8,9-HxCDD is corrected for the average recovery of the other two HxCDDs.

2.5.2 For non-2,3,7,8-substituted isomers and the total concentrations of all isomers within a level of chlorination (i.e., total TCDD), concentrations are determined using response factors from the calibration of labeled analogs at the same level of chlorination.

2.6 The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems.

3. CONTAMINATION AND INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms (References 8 and 9). Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Where possible, reagents are cleaned by extraction or solvent rinse.

3.2 Proper cleaning of glassware is extremely important, because glassware may not only contaminate the samples but may also remove the analytes of interest by adsorption on the glass surface.

7.4 Surrogate Recovery

Report surrogate recoveries for every sample analyzed.

7.5 Matrix Spike

Report matrix spike recoveries for each batch of samples analyzed.

7.6 Reference Materials

When available, report the results of the analysis standard reference materials for each batch of samples analyzed.

Note: The effective minimum performance standard can be adjusted by decreasing final sample volume, increasing sample amount and/or increasing volume injected on the GC-FPD.

6.0 CALCULATIONS

6.1 Butyltin Calculations

All calculations are based on the methods of internal standards from Section 7.8.2 of EPA SW-846 Method 8000 with the modification that all values are corrected for surrogate recoveries:

- RF = average of $(A_s \times C_{is})/(A_{is} \times C_s)$

where:

A_s = Area of analyte to be measured
A_{is} = Area of internal standard tetrapropyltin
C_{is} = Concentration of the internal standard tetrapropyltin (ng Sn/µl)
C_s = Concentration of the analyte to be measured (ng Sn/µl).

$$Ce = \frac{(A_s)(I_s)}{(A_{is})(RF)}$$

where:

Ce = Sample extract concentration (ng Sn/µl).
A_s = Area of the analyte to be measured.
A_{is} = Area of the the internal standard (tetrapropyltin).
I_s = Amount of internal standard added to each extract divided by the final extract volume (Ve).

The actual sample concentration (C) for each compound is calculated by the following formula:

$$C = (Ce) \times \frac{V_e}{V_s}$$

where:

C = Concentration in sample (ng Sn/l or g).
V_E = The final extract volume (ml).

If the average daily response factors for all analytes is within ±15 percent of the corresponding calibration curve value, the analysis may proceed. If, for any individual analyte, the daily response factor exceeds ±30 percent of the corresponding calibration curve value, a three-point calibration curve must be repeated for that compound prior to the analysis of the samples. All samples are calculated from the initial calibration.

5.2 Method Blank Analysis

An acceptable method blank analysis does not contain any target compound at concentration 3 times greater than the MDL. If the method blank does not meet these criteria, the analytical system is out of control and the source of the contamination must be investigated, corrective measures taken, and documented before further sample analysis proceeds.

5.3 Surrogate Compound Analysis

Spike all samples and quality control samples with TPT. Spike the surrogate solution into the sample prior to extraction to measure individual sample matrix effects associated with sample preparation and analysis.

The laboratory will take corrective action whenever the recovery of the surrogate is below 30 percent for water, sediment and tissue matrices.

Take the following corrective action when an out of control event occurs:

a Check calculations to assure that no errors have been made.

b. Check the internal standard and surrogate solutions for degradation, contamination, etc., and check instrument performance is checked.

c. If the surrogate could not be measured because the sample required a dilution, no corrective action is required. The surrogate recovery is properly annotated.

d. If the steps above fail to reveal a problem, reanalyze the sample or extract. If reanalysis of the extract yields surrogate recoveries within the stated limits, then report the reanalysis data . If upon reinjection QA criteria are still violated, the sample will be submitted for re-extraction if sufficient sample is available. If the sample was

Sample injections of 1 to 4 µl are made with an autosampling device.

If the response for any peak exceeds the highest calibration solution, dilute and reanalyze the extract.

Table 1. Minimum Sample Distribution to Meet QA Requirements During a Typical TBT Analysis.

Sample No.	Description	Function
1	Solvent Blank	Establish instrument background
2	Cal Ck*	Confirm "in calibration" condition
3	Sample #1	Unknown Analysis
4	Sample #2	Unknown Analysis
5	Sample #3	Unknown Analysis
6	Sample #4	Unknown Analysis
7	Sample #5	Unknown Analysis
8	Sample #6	Unknown Analysis
9	Sample #7	Unknown Analysis
10	Sample #8	Unknown Analysis
11	Sample #9	Unknown Analysis
12	Sample #10 (duplicate)**	Unknown Analysis
13	System Blank	Confirm method blank
14	Spiked Blank/[illegible]fied Sample/SRM	Complete Analytical QA***
15	Cal Ck	Confirm "in calibration" condition

* Criteria ±30% an individual analyte
** 10% of samples analyzed in duplicate
*** Criteria ±30% for all analytes

4.2.5 Calculations

Calculations are based on the methods of internal standards. The general formula for calculating butyltins is found in Section 7.8.2 of EPA SW-846 Method 8000. See Section 6.1 of this method for details of the calculations. This method is modified in that all analyte concentrations are corrected for the surrogate recoveries and all concentrations are reported as µg Sn/l or g.

4.2 High Resolution GC-FPD Analysis

4.2.1 GC Conditions

For the analysis of butyltins, the analytical system, or its equivalent, should include at a minimum:

Instrument:	Hewlett-Packard 5880A or HP 5890 Gas Chromatograph
Features:	Split/splitless capillary inlet system, HP-1000 LAS 3357 data acquisition system
Inlet:	Splitless
Detector:	Flame photometric, 610 nm filter
Column:	0.32-mm I.D. x 30-m DB-5 fused silica capillary column (J&W Scientific)
Gases:	
Carrier:	Helium 2 ml/min.
Make-Up:	Helium 33 ml/min.
Detector:	Air 100 ml/min. Hydrogen 80 ml/min.
Temperatures:	
Injection port:	300°C
Detector:	225°C
Oven Program:	60°C to 300°C, hold 10 min.

The GC oven temperature program may be modified to improve resolution.

Daily Calibration:	Mid-level calibration solution; Retention index solution

QUANTITATIVE DETERMINATION OF BUTYLTINS

1.0 INTRODUCTION

The quantitative method described in this document determines butyltins in extracts of water, sediments and tissues. The method is based on high resolution, capillary gas chromatography using flame photometric detection (GC/FPD). This method quantitatively determines tetrabutyltin (4BT), tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT).

Extracts should be prepared as described in GERG SOP's-9010, 9011 and 9012 for water, sediment and biological tissues, respectively.

Sample collection, preservation and storage times are discussed under the analytical procedures for sample extraction and purification.

2.0 APPARATUS AND MATERIALS

A gas chromatograph with a split/splitless injection system, capillary column capability and a flame photometric detector (FPD) equipped with a tin selective 610 nm filter. The output from the detector is collected and processed by an automated HP-LAS 3357 data acquisition software package.

2.1 GC Column

Use a 30-m long x 0.32-mm I.D. fused silica capillary column with DB-5 bonded phase (J&W Scientific or equivalent).

2.2 Autosampler

The autosampler is capable of making 1-4 µl injections.

3.0 REAGENTS

3.1 Calibration Solution

The calibration solution is comprised of 4BT,TBT,DBT and MBT.

5.2.4 Neutralize excess Grignard reagent by adding 5 ml of 6N HCl.

5.2.5 Shake sample vigorously and allow phases to separate.

5.2.6 Decant organic phase (top) with a pasture pipet into a 125 ml flat bottom flask.

5.2.7 Add 15 ml of a mixture of 3:1 of pentane:CH_2Cl_2 to the aqueous phase in the 50 ml centrifuge tube, shake vigorously, allow phase to separate and decant the pentane:CH_2Cl_2 into the 125 ml flat bottom flask.

5.2.8 Repeat step 5.2.7.

5.2.9 Dry sample with Na_2SO_4

5.2.10 Attach a Snyder column to the 125 ml flat bottom flask containing the combined organic phases and concentrate to 1-10 ml in a water bath (60-70°C). Further concentrate the sample to 2 ml in a concentrator tube.

5.3 SILICA/ALUMINA COLUMN CLEANUP

5.3.1 Fill the glass chromatographic column to about 20 cm with hexane. Weigh 10.0 g of silica and add the silica to the column. Gently tap the column to evenly distribute the alumina. Alteratively, a slurry of alumina in pentane may be used to pack the column.

5.3.2 Allow the silica to settle and then add 10 g of alumina to the top of the silica.

5.3.4 Drain the pentane through the column until the head of the liquid in the column is just above the top of the column. Close the stopcock to stop solvent flow.

5.3.5 Transfer the hexylated sample extract in 2 ml of hexane onto the column. Rinse the extract vial with 1 ml pentane and add it to the column.

Water Bath: heated to 60-70°C.

Graduated Cylinder: 1 or 2 l.

Disposable Glass Pasteur Pipets: 1 and 3 ml.

Syringes: 10 or 25 µl.

Teflon Boiling Chips: Solvent extracted.

Vials: 1 ml to 7 ml glass vials with Teflon-lined caps.

Gas Evaporation Unit: Nitrogen

Note: Volumetric glassware for sample measurement or introduction of internal standards must be calibrated.

4.2 Reagents

Reagent Water: Water containing no analytes above the method detection limit (i.e., HPLC water).

Sodium Sulfate: (ACS) Granular, anhydrous (purified by heating at 400°C for 4 hours in a shallow tray, or other suitable method).

Solvents: Methanol (for rinsing), methylene chloride, hexane, pentane (pesticide quality or equivalent).

Tropolone: Aldrich t8, 970-2, purity 98%

6N HCl

Hexylmagnesium Bromide: 0.5M in diethylether.

Alumina Oxide: Basic Brockmann 1, standard grade 150 mesh Aldrich 19, 744-3 or equivalent. Combust 4 hours at 400°C. Store at 120°C prior to use.

Silica Gel: Grade 923, 100-200 mesh Aldrich 21,447-7 or equivalent. Store at 170°C before use.

EXTRACTION OF SEDIMENTS FOR BUTYLTIN ANALYSIS .

1.0 INTRODUCTION

Assessment of the environmental impact of butyltins requires their measurement in sediments at trace levels (parts per billion to parts per trillion).

This standard operating procedure provides a precise and accurate method to quantitatively determine butyltin compounds in sediments. Freeze-dried samples are serially extracted with 0.2% tropolone in methylene chloride. The extract is then hexylated with a Grignard reagent. The hexylated extract is dried and concentrated. A silica/alumina column cleanup step is used before the instrumental analysis to remove matrix interferences. The protocol is designed for 20 gram samples, but sediment samples of other sizes may be collected and extracted by appropriately adjusting the volume of tropolone/methylene chloride used for the extraction. The extract is then submitted for analysis of butyltins by GERG SOP-9013.

2.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

2.1 Sample Collection

Collect and place sediment samples in precleaned mason jars.

2.2 Sample Storage

Store sediment samples in the dark at or below -20°C. Store sample extracts in the dark at 4°C.

3.0 INTERFERENCES

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to false positives in GC/FPD detection. All materials used in this method are routinely demonstrated to be free from interferences by processing procedural blanks identical to samples (one blank per 12 samples or each batch whichever is more frequent).

Jennifer M. Wong

Assistant Research Scientist

Specialities: Laboratory Management (HPLC-Pigments)
Soil Chemistry

Education: B.S. (Food Biochemistry), 1985, M.S. (Food Science and Technology), 1986, Ph.D. (Agricultural and Environmental Chemistry), 1991, University of California-Davis.

Professional Experience:

9/91-present Geochemical and Environmental Research Group, Texas A&M University, College Station, Texas. Laboratory Manager, HPLC-Pigments Laboratory.

1/87-7/91 University of California-Davis. Graduate Research Assistant - Performed research on the development of analytical methods using supercritical fluid extraction for characterizing vapor-phase toxicants for integration with the *Salmonella* mutagenicity bioassay, extracting pesticides from soil with analysis by immunoassay.

9/87-12/87 University of California-Davis. Teaching Assistant - Taught lab techniques for microanalysis of toxicants including separation, detection and quantitative determination of toxicants using chemical and instrumental methods.

9/85-12/86 University of California-Davis. Graduate Research Assistant - Performed research investigating the role of nitrogen source on formation of pyrazine flavor components in model food systems using capillary gas chromatography.

9/84-9/85 University of California-Davis. Undergraduate Research Assistant - Performed research on the analysis of carbohydrates and fatty acids in dairy products using HPLC.

Selected Presentations:

Wong, J.M., J.E. Woodrow, and J.N. Seiber, *Recovery of Volatile Mutagens from Adsorbents using Supercritical Carbon Dioxide*, 199th National Meeting of ACS, Boston, Massachusetts. April 1990.

Seiber, J.N., D.P.H. Hsieh, N.Y. Kado, P.A. Kuzmicky, H. Ning, J.M. Wong, J.E. Woodrow, *Methods for Sampling and Analyzing Vapor-Phase Mutagens and Other Volatile Toxicants*, 200th National Meeting of the American Chemical Society (ACS), Washington, DC. August 1990.

Selected Publications:

Wong, J.M. and R.A. Bernhard, *Effect of Nitrogen Source on Pyrazine Formation*, J. Agric. Food Chem., 36(1):123-129, 1988.

Woodrow, J.E., J.M. Wong, J.N. Seiber, *Pesticide Residues in Spray Aircraft Tank Rinses and Aircraft Exterior Washes*, Bull. Environ. Contam. Toxicol., 42:22-29, 1989.

Seiber, J.N., D.P.H. Hsieh, N.Y. Kado, P.A. Kuzmicky, H. Ning, J.M. Wong, J.E. Woodrow, *Methods for Sampling and Analyzing Vapor-Phase Mutagens and Other Volatile Toxicants from Waste Sites*, In: Effective and Safe Waste Management: Modern Trends Interfacing Engineering, Chemistry, Biology and Toxicology with Site-Specific Monitoring, (R.L. Jolley, R.G.M. Wang, editors). Lewis Publishers, Inc. 1991, In press.

Wong, J.M., N.Y. Kado, P.A. Kuzmicky, H. Ning, J.E. Woodrow, D.P.H. Hsieh, J.N. Seiber, *Analysis of Volatile and Semi-volatile Mutagens in Air Using Solid Adsorbents and Supercritical Fluid Extraction*, Anal. Chem. (submitted), 1991.

Wong, J.M., Q.X. Li, B.D. Hammock, J.N. Seiber, *Method for the Analysis of 4-Nitrophenol and Parathion in Soil using Supercritical Fluid Extraction and Immunoassay*, J. Agric. Food Chem. (submitted), 1991.

Jose L. Sericano

Research Associate

Specialities: Environmental Chemistry
Marine pollution
Chlorinated Pesticides, PCBs and Hydrocarbons
Chemical Oceanography

Education: Quimico (Chemist), Universidad Nacional del Sur, Bahia Blanca, Buenos Aires, Argentina, 1975; Licenciado en Bioquimica (B.S. in Biochemistry), Universidad Nacional del Sur, Bahia Blanca, Buenos Aires, Argentina, 1976; Licenciado en Quimica (B.S. in Chemistry), Universidad Nacional del Sur, Bahia Blanca, Buenos Aires, Argentina, 1977; M.S. (Oceanography), 1986, Ph.D. (Oceanography), 1992, Texas A&M University.

Professional Experience: *Research Assistant*, Geochemical and Environmental Research Group, Texas A&M University, 1989-present; *Graduate Assistant Research*, Texas A&M University, Department of Oceanography, 1985-1989; *Research Assistant*, Instituto Argentino de Oceanografia, Bahia Blanca, Buenos Aires, Argentina, 1978-1984; Teaching Assistant (Physicochemistry), Universidad Nacional del Sur, Buenos Aires, Argentina, 1980-1982.

Selected Publications:

Pucci, A.E., R.H. Freije, R.O. Asteasuain, J.R. Zavatti, and J.L. Sericano, *Evaluacion de la contaminacion de las aguas y sedimentos de la Bahia Blanca - Informe Anual 1979. (An evaluation of the seawater and sediment pollution in Blanca Bay, Argentina - Annual Report 1979).* C.C.#52, Instituo Argentino de Oceanografia, 1979.

Pucci, A.E., R.H. Freije, R.O. Asteasuain, J.R. Zavatti, and J.L. Sericano, *Evaluacion de la contaminacion de las aguas y sedimentos de la Bahia Blanca - Informe Anual 1980. (An evaluation of the seawater and sediment pollution in Blanca Bay, Argentina - Annual Report 1980).* C.C.#56, Instituo Argentino de Oceanografia, 1980.

Sericano, J.L., and A.E. Pucci, *Cu, Cd, and Zn in Blanca Bay surface sediments*, Argentina, Marine Pollution Bulletin, 13(12): 429-31, 1982.

Sericano, J.L., and A.E. Pucci, *Chlorinated hydrocarbons in seawater and surface sediments of Blanca Bay, Argentina*, Estuarine, Coastal, and Shelf Science, 19: 27-51, 1984.

Sericano, J.L, and A.E. Pucci, *A simplified confirmatory technique for organochlorine residues*, Bulletin of Environmental Contamination and Toxicology, 33: 138-43, 1984.

Sericano, J.L., H.V. Zubillaga, and A.E. Pucci, *Behavior of hexachlorocyclohexane isomers and Zn, Cu, and Cd in freshwater-seawater mixing area*, Marine Pollution Bulletin, 15(8): 288-94, 1984.

Sericano, J.L., *Distribution of high molecular weight hydrocarbons in northern Gulf of Mexico continental slope sediments.* M.S. Thesis, Department of Oceanography, Texas A&M University, xi+123 pp, 1986.

Zubillaga, V.H., J.L. Sericano, and A.E. Pucci, *Organochlorine pesticide contents of tributaries into Blanca Bay, Argentina*, Water, Air, and Soil Pollution, 32(1-2): 43-53, 1987.

Kennicutt II, M.C., J.L. Sericano, T.L. Wade, F. Alcazar, and J.M. Brooks, *High molecular weight hydrocarbons in Gulf of Mexico continental slope sediments*, Deep-Sea Research, 34(3A): 403-24, 1987.

Wade, T.L., E.L. Atlas, J.M. Brooks, M.C. Kennicutt II, R.G. Fox, J. Sericano, B. Garcia, and D. DeFreitas, *NOAA Gulf of Mexico Status and Trends Program: Trace organic contaminant distribution in sediment and oysters*, Estuaries, 11(3): 171-79, 1988.

Wade, T.L. and J.L. Sericano, *Trends in organic contaminant distributions in oysters from the Gulf of Mexico*, Proceedings OCEANS '89 Conference, Seattle, WA, pp. 585-589, 1989.

Sericano, J.L., E.L. Atlas, T.L. Wade, and J.M. Brooks, *NOAA's Status and Trends Mussel Watch Program: Chlorinated pesticides and PCBs in oysters (Crassostrea virginica) and sediments from the Gulf of Mexico, 1986-1987*, Marine Environmental Research , 29: 161-203, 1990.

Sericano, J.L., T.L. Wade, E.A. Atlas, and J.M. Brooks, *Historical perspective on the environmental bioavailability of DDT and its derivatives to the Gulf of Mexico oysters*, Environmental Science & Technology, pp. 1541-1548, 1990.

Wade, T.L., J.L. Sericano, B. Garcia-Romero, J.M. Brooks, and B.J. Presley, *Gulf Coast NOAA National Status & Trends Mussel Watch: The first four years*, MTS'90 Conference Proceedings, 26-29 September 1990, Washington D.C. , pp. 274-280, 1990.

Sericano, J.L., A.M. El-Husseini, and T.L. Wade, *Isolation of planar polychlorinated biphenyls by carbon column chromatography*, Chemosphere, 23(7), 915-924, 1991.

Sericano, J.L., T.L. Wade, J.M. Brooks, E.L. Atlas, R.R. Fay, and D.L. Wilkinson, *National Status and Trends Mussel Watch Program: Chlordane-related compounds in Gulf of Mexico oysters, 1986-1990*, Environmental Pollution (submitted), 1992.

Sericano, J.L., T.L. Wade, A.M. El-Husseini. and J.M. Brooks. *Environmental significance of the uptake and depuration of planar PCB congeners by the American oyster (Crassostrea virginica).* Marine Pollution Bulletin, 24: 537-543, 1992.

Grace Ekman

Quality Assurance/Quality Control Manager

Education

B.S.	Chemistry, Johnson State College
M.S.	Organic Chemistry, University of Vermont, Medical and scientific courses at Alverno College, MATC, WCTC

Selected Experience

Quality Assurance/Quality Control Manager, Geochemical and Environmental Research Group, Texas A&M University. Responsible for promulgating, enacting, and enforcing all QA procedures and policies at GERG consistent with state-of-the-art quality assurance principles. The QA Manager ensures that all GERG activities are operated in a manner that provides confidence that project and QA objectives are met. The QA manager is independent of project management, reports to the Chief Chemist, and is responsible for ensuring all applicable QA policies and directives are enforced, revised and improved to provide products of the highest quality to (both internal and external) clients.

Client Services Manager, CH2M Hill, Montgomery, Alabama. Responsible for overall implementation of client services and project management functions. Responsible for preparing proposals, reports, and technical papers as well as management of large-scale laboratory projects. Monitored workload capability of the laboratory, supervised client services, data package and sample custody activities, as well as serving as the interface between client and laboratory management. Represented laboratory services to prospective and current clients, provided technical support to the staff, and provided management support to the laboratory director. Involved in preparation of marketing plans for the laboratory.

Regional Quality Assurance Manager, National Environmental Testing, Thorofare, New Jersey. As QA/QC manager for a National Environmental Testing's Atlantic Region, assisted in the definition and implementation of national, regional, and divisional QA goals. Managed the preparation of uniform SOPs and QAPPs in accordance with various regulatory protocols. Assisted in the implementation of the firm's internal QA testing program, and was responsible for the implementation of regional safety programs and safety training activities. In addition, was very active in modifications of job descriptions and in documentation of personnel training activities.

Organic Laboratory Manager/QC Supervisor, CHEM-BIOCorporation, Oak Creek, Wisconsin. Responsible for the management of all chemists in the GC/MS, GC, and extraction sections for a full service environmental laboratory. Duties included budgetary responsibility, personnel, and new equipment justification and procurement. Directed the analysis of PCB's, pesticides, herbicides, VOA, Semi-VOA, TPH, and industrial hygiene samples in multi-media matrices

As the functional laboratory manager, was responsible for heavy client contact. Answered technical questions; advised on various GC detectors; involved in scheduling samples; and developing new procedures and methodologies. Very knowledgeable of EPA regulations and protocols, drinking water regulations and AIHA procedures.

Responsible for QA/QC for organic, inorganic, and industrial hygiene samples analyzed by entire laboratory. Duties included QC data review and technical review and approval of the data packages. Additionally, responsible for QC documentation, methods and method development, SOP generation, lab audits, and proficiency samples for maintaining various state certifications.

Operations Manager, Camp, Dresser, and Mckee, Milwaukee, WI. Supervised 35 technical, sales and clerical personnel in engineering and the environmental laboratory. Developed monthly and annual budget projections. Initiated contracts, prepared proposals and made sales presentations. controlled the quality of data, research methods and report preparations. Reported to the President.

Private Consultant, Massachusetts Energy Facility Siting Council and The State of New York Power Authority, Billerica, Massachusetts. Met power company and government objectives by assessing specific environmental impacts of electrical utility construction while supervising all phases of multi-million dollar research projects. Duties included preparation of legal briefs and providing expert testimony.

Principle Environmental Engineer/Chemist, Stone and Webster Engineering Corporation, Boston, Massachusetts. Enabled major industries to fulfill complex governmental permit/licensing requirements by designing water quality studies; preparing environmental impact evaluations and related permitting applications; developing the conceptual engineering design for liquid waste water treatment and solid waste handling and disposal facilities; and by providing technical support. Provided expert testimony for related power plan siting hearings and prepared legal briefs.

Laura Chambers

Research Associate

Education

B.A., Chemistry (Phi Beta Kappa), Reed College, Portland, OR, 1988

Profile

Analytical chemist experienced in HRGC/HRMS operation and maintenance, data validation and interpretation, and quality assurance protocols. Experience with EPA methods for the analysis of environmental contaminants in complete matrices.

Career Chronology

1993-present, Research Associate, Geochemical and Environmental Research Group, Texas A&M University, College Station, TX; 1991-1993, Group Leader for Special Analytical Services Section, CHESTER LabNet, Tigard, OR; 1988-1991, High Resolution GC/MS Operator, Boise Cascade R&D, Portland, OR; 1988, Analytical/Quality Control Chemist, Optical Data, Inc., Portland, OR; 1987, Research Fellow, Neurological Sciences Institute Good Samaritan Hospital, Portland, OR

Recent Professional Experience

Trained and supervised other chemists in instrument operation, laboratory sample preparation, method QA requirements, and data validation in a commercial environmental laboratory. Response for 85% of departmental production revenues. Responsible for meeting contractual TATs and adhering to EPA guidelines.

Full responsibility for the operation and maintenance of high resolution magnetic sector mass spectrometers in a commercial production laboratory. Wrote operation and QA protocols for the instrumental analysis of contaminants in complex environmental matrices.

Responsible for analysis of various paper industry by-products using FTIR, thermal characterization, IC, bomb calorimetry, and other techniques in an industrial analytical laboratory. Responsible for operation, calibration, and maintenance of analytical instrumentation (UV-vis and FTIR spectrometers, HPLC, TGA, TMA, etc.) in an applied research laboratory.

Key Projects

Established high resolution mass spectrometry laboratories for the analysis of chlorinated dibenzo-*p*-dioxins and dibenzofurans for a major manufacturing company and for a commercial production laboratory.

Validated methods for analysis of PCDD/PCDFs, PAHs, and co-planar PCBs by high resolution mass spectrometry for use in a commercial production laboratory.

Managed laboratory procedures, instrumental measurement, quality assurance, and reporting requirements for the analysis of by-products (ash, slag, stack emissions, etc.) from a chemical waste incineration project requiring fast (i.e., 48-hour) turn-around-times, and for the analysis of PCDD/PCDFs in fish tissue and sediment for the Lower Columbia River and Snake River projects.

Participated in round-robin study establishing consensus values for PCDD/PCDFs and co-planar PCBs in fish tissues targeted for use as laboratory control samples.

Monitoring and characterization of municipal and pulp mill sludge for landfill disposal.

Implemented electronic data transfer and reporting protocols between DEC (VMS) and Apple Macintosh systems using Pathworks, formatted to accommodate EPA reporting requirements.

Conducted research on the use of uv-cured polymers for data storage/retrieval systems.

Developed novel, highly sensitive assay for protein detection using proprietary filtration techniques.

James M. Brooks
Director

Specialities:
Project Management
Environmental Chemistry
Petroleum Geochemistry

Other Expertise:
Marine Geochemistry
Environmental Assessment
Gases and Plant Pigments
Stable Isotope Geochemistry

Education: B.S. (Chemistry), Abilene Christian University, 1969; M.S., 1970, Ph.D., 1975, Texas A&M University (Oceanography).

Professional Experience: *Director,* Geochemical and Environmental Research Group, Texas A&M University, 1987-Present; *Senior Research Scientist and Senior Lecturer,* Oceanography, Texas A&M University, 1985-Present; *Associate Research Scientist*, Oceanography, Texas A&M University, 1981-1985; *Graduate Faculty Member*, Texas A&M University, 1978-Present; *Research Associate*, Ocean Affairs Board of the National Academy of Sciences, 1973.

Selected Publications (>130 publications):

Childress, J.J., C.R. Fisher, J.M. Brooks, M.C. Kennicutt II, R. R. Bidigare, and A. Anderson, *A methanotrophic marine molluscan symbiosis (Bivalvia Mytilidae): Mussels fueled by gas*, Science, 233: 1306-1308, 1986.

Kennicutt, M. C. II, J. L. Sericano, T. L. Wade, F. Alcazar and J. M. Brooks, *High molecular weight hydrocarbons in Gulf of Mexico continental slope sediments.* Deep-Sea Research, 34(3A): 403-424, 1987.

Kennicutt, M.C. II, G.J. Denoux, J.M. Brooks and W.A. Sandberg, *Hydrocarbons in Mississippi fan and intraslope basin sediments,* Geochimica et Cosmochimica Acta, 51: 1457-1466, 1987.

Brooks, J.M., M.C. Kennicutt, C.R. Fisher, S.A. Macko, K. Cole, J.J. Childress, R.R. Bidigare and R.D. Vetter, *Deep-sea hydrocarbon seep communities: Evidence for energy and nutritional carbon sources,* Science, 238:1138-1142, 1987.

Kennicutt, M.C. II, J.M. Brooks and G.J. Denoux, *Leakage of deep, reservoired petroleum to the near-surface on the Gulf of Mexico continental slope,* Marine Chemistry, 24:39-59, 1988.

Wade, T.L., E.L. Atlas, J.M. Brooks, M.C. Kennicutt II, R.G. Fox, J. Sericano, B. Garcia-Romero and D. DeFreitas, *NOAA Gulf of Mexico Status and Trends Program: trace organic contaminant distribution in sediments and oysters*, Estuaries, 11: 171-179, 1988.

Wade, T.L., B. Garcia-Romero and J.M. Brooks, *Tributyltin contamination in bivalves from U.S. coastal estuaries*, Environmental Science and Technology, 22: 1488-1493, 1988.

Kennicutt, M.C. II, J.M. Brooks, S. Macko and R.R. Bidigare, *An upper slope "cold" seep community: northern California*, Limnology and Oceanography, 34(3): 635-640, 1989.

Wade, T.L., M.C. Kennicutt and J.M. Brooks, *Gulf of Mexico hydrocarbon seep communities: III. Aromatic hydrocarbon concentrations in organisms, sediments and water*, Marine Environmental Research, 27: 19-30, 1989.

Thompson, K.F., M.C. Kennicutt II and J.M. Brooks, *Classification of offshore Gulf of Mexico oils and gas condensates*, American Association of Petroleum Geologists Bulletin, 74(2): 187-198, 1990.

Fisher, C.R., M.C. Kennicutt and J.M. Brooks, *Carbon isotopic evidence for carbon limitation in hydrothermal vent Vestimentiferans*, Science, 247: 193-197, 1990.

MacDonald, I.R., J.F. Reilly II, N.L. Guinasso, Jr., J.M. Brooks, R.S. Carney, W.A. Bryant and T.J. Bright, *Chemosynthetic mussels at a brine-filled pockmark in the northern Gulf of Mexico*, Science, 248: 1096-1099, 1990.

Brooks, J.M., M.C. Kennicutt II, T.L. Wade, A.D. Hart, G.J. Denoux and T.J. McDonald, *Hydrocarbon distributions around a shallow water multiwell platform*, Environmental Science and Technology, 24(7): 1079-1085, 1990.

Kennicutt, M.C. II and J.M. Brooks, *Unusual normal alkane distributions in offshore New Zealand sediments*, Organic Geochemistry, 15(2): 193-197, 1990.

Brooks, J.M., M.E. Field, and M.C. Kennicutt, II, *Observations of gas hydrates in marine sediments, offshore northern California*, Marine Geology, 96: 103-109, 1991.

Ondrusek, M., R.R. Bidigare, S.T. Sweet, D.A. Defreitas and J.M. Brooks, *Distribution of Phytoplankton Pigments in the North Pacific Ocean in Relation to Physical and Optical Variability*, Deep-Sea Research, 38(2): 243-266, 1991.

Brooks, J.M., M.A. Champ, T.L. Wade, and S.J. McDonald, *GEARS: Response Strategy for Oil and Hazardous Spills*, Sea Technology, pp. 25-32, April, 1991.

Selected Funding Sources (last 3 years): NSF, ONR, EPA, MMS, NOAA, U.S. FWS, Sea Grant, NURP, State of Alaska and numerous private industry projects.

Program records, precision, accuracy, and background will be assessed by the Program Director and Program Manager in consultation with the Laboratory Supervisors as they relate to project goals.

M. Performance and System Audits

The project will be open to EPA or CBNEP system audits at their discretion.

N. Corrective Action

The successful completion of the field sampling requires successful collection of the ~60 stations within the 2 to 4 day sampling period in early October as well as the receipt of these sampling within the GERG analytical facility. Although we expect to collect all the required ~60 sampling sites within the 2-4 day sampling period, we are prepared to remain longer or return for additional sampling if required.

Corrective action constitutes a variety of responses to noncompliance with calibration, SRM and MS QA limits. Responses include replacement of GC columns, cleaning of detectors, re calibration, re-extraction of samples, and repair or replacement of parts and/or instruments as necessary. If unacceptable "system blanks" are present all analyses will cease until an acceptable system blank can be produced. These criteria are monitored daily by the Laboratory QA/QC Manager.

If the response of the calibration check standard exceeds the QA criteria, a second calibration check will be analyzed. If the results are still in non-compliance a re-calibration will be performed. These criteria are monitored daily by the Laboratory QA/QC Manager. If the daily spiked blank exceeds the QA criteria, the instrumental analysis will be repeated. If the results are still in non-compliance, the Laboratory QA/QC Manager and Laboratory Supervisor will be notified and a series of spiked blanks will be processed until compliance is attained. Retention times for each analyte in a sample must be within ±30 seconds of that observed during the most recent acceptable calibration or remedial action is initiated including leak testing and column replacement, if necessary. These criteria are monitored daily by the Laboratory QA/QC Manager and Laboratory Supervisor.

- Method blank results
- Reference material or matrix spike sample amounts and percent recoveries
- Laboratory duplicate sample relative % difference
- Concentrations and % recoveries for reference sample
- Preparation and analysis dates for the actual samples and quality control samples.
- Copies of applicable analytical methodologies
- Narrative describing any method modifications, out-of-control events, and remedial action taken, etc.

Results of GERG chemical analyses of samples will be supplied according to prescribed specifications. The Project Manager will be responsible for resolving any questions concerning the specifics of the chemical analyses with the appropriate task leader prior to submitting data.

K. Data Reduction and Reporting

Validated on-line data will be formatted in Ocean Evaluation System File Type 144 and forwarded to the EPA. The following procedure will be followed:

- Obtain EPA approval to submit data.
- Perform quality control checks during data entry.
- Enter data into ODES format.
- Prepare a description of the data set, including a discussion of all quality control procedures followed during data collection, analysis, and entry.
- Certify that data are accurate.
- Send data, data set description, and data certification statement to the ODES Staff.
- Review hard copy of data and listing of errors detected during machine checks of information.
- Provide verification statement regarding any necessary data revisions or corrections to the ODES Technical Reviewers.

Samples will be collected with a Smith-McIntyre grab, ponar grab sampler or by hand. The sediment sampler will be washed with methylene chloride or other appropriate solvent prior to initial utilization and as appropriate thereafter to insure the samples are not contaminated. Samples will be taken along the sides of the corer to minimize the potential of cross-contamination. A stainless steel scoop will be used to obtain a 1- to 2-cm depth sample of the sediment from the grab. The scoop will be washed with methylene chloride between all station samplings. Care will be taken to keep all stack fumes upwind of the sampling effort. Samples will be placed in precombusted mason jars and kept on ice in an ice chest during field sampling. Upon return to port samples will be frozen and shipped to the GERG facility. All samples will be shipped by overnight courier with dry ice.

F. Analytical Procedures

The analytical procedures that will be used for CBNEP are detailed in the attached GERG Standard Operating Procedures (SOPs). The SOPs listed and attached are the methods utilized for the NOAA National Status and Trends Program, EPA's Environmental Monitoring and Assessment Program - Near Coastal (EMAP-NC) and the U.S. Fish and Wildlife Service trace organic analytical program. The method used for the dioxin/furan analyses is EPA method 1613 that employer high resolution GC/MS in order to obtain the low parts per trillion detection limits required. All methods have undergone extensive verification and intercalibration. They are high-quality analytical procedures. GERG SOPs to be utilized on this project include the following:

TRACE ORGANICS

SOP-9011	Extraction of Sediments for Butyltin Analysis
SOP-9013	Quantitative Determination of Butyltins
EPA Method 1613	Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

G. Sample Custody Procedures

The Field Sampling Chief Dr. Guy Denoux will follow routine Chain-of-Custody procedures for all samples that are collected. Once received at

- Duplicates, spike blanks, matrix spikes and/or standard reference materials must agree within ±20% on average for all analytes and no more than ±35% for any individual analyte that are present at 10 times the MDL.

If any of the above criteria are not met, the Laboratory Supervisors will notify the Project Manager and the Laboratory QA/QC Manager to discuss remedies and the status of the data.

Routine Procedures to Assess Data Precision, Accuracy and Completeness

The precision of each compound measured in each matrix will be estimated by calculating the relative percent difference (RPD) for duplicate analyses as follows:

a) calculate the relative percent difference (RPD) for each duplicate measurement,

$$RPD = \frac{2\,(X_1 - X_2)}{(X_1 + X_2)} \times 100$$

where X_1 = the value obtained for a sample, and

X_2 = the value obtained for the duplicate of that sample

The results of the MS and/or spiked blanks will be used to estimate the precision of the method. The SRMs will be used to determine the accuracy of the methods. The precision and accuracy will be determined as follows:

a) Let X = the analytical result for a given compound in a spiked blank or a MS, and thus, X - U = F, where F = the concentration of analyte from the addition of the spike, and U = the concentration for the duplicate of that sample. For a spike blank this formula simplifies to X = F.

b) Compute the percent recovery for the spike blank or MS sample:

$$\%\ Recovery = 100\ F\ /T$$

where T = the calculated concentration based on the amount of compound added to the spike blank or MS.

c) Calculate the percent recovery for the SRM