

THE MUSSEL WATCH: INTERCOMPARISON OF TRACE LEVEL CONSTITUENT DETERMINATIONS

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Abstract — The U.S. National Mussel Watch Program initially used split-sample analyses for interlaboratory quality control purposes. These indicated the possibility of interlaboratory analytical discrepancies as well as problems in the split-sample technique itself. For the third year of the program, two mussel homogenates were produced to serve as intercomparison samples — one for metals and organics, the other for radionuclides. The results obtained using these homogenates are encouraging in that generally good agreement is seen among analyses done by several labs in diverse pollutant classes. We conclude from this experience that a quality-control program relying on the analysis of large homogeneous samples of the matrix being dealt with is an essential part of any extensive, multilaboratory analytical program.

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INTRODUCTION

The U.S. National Mussel Watch [1-4] is a coastal monitoring program that uses indigenous bivalve mollusc tissues as collectors of pollutants and indicators of related chemical change. Mussel Watch began indigenous bivalve mollusc tissues as collectors of pollutants and indicators of related chemical change. Mussel Watch began in 1976 with the collection of mussels, *Mytilus edulis* and *M. californianus*, and oysters, *Crassostrea* sp., from more than 100 sites on the East, West, and Gulf Coasts. Soft tissues from most samples were analyzed for trace metals, total hydrocarbons (petroleum and combustion products, and biogenic hydrocarbons isolated by the respective procedures), chlorinated hydrocarbons and radionuclides. Each class of pollutants was measured by at least two different laboratories.

Intercomparability of results among laboratories has been a major concern within the program. This was initially addressed through split samples: organisms from the Newport-Narragansett Bay, Rhode Island, and the Bodega Head, California, sites, were collected on nearly monthly schedules and split among the participating laboratories. These samples were analyzed routinely, and data from laboratories that measured the same constituents were compared; however, any differences detected were difficult to interpret because of the small numbers of samples available from each split. The "standard materials" used in intercalibration exercises, and referred to as such in a previous article [1], consisted of these split samples and various *ad hoc* intercomparison materials. Although not of matrices identical to those being analyzed in Mussel Watch, standards or reference materials were available [from International Atomic Energy Agency (IAEA), Environmental

Protection Agency (EPA), etc.] that contained most of the trace metals or radionuclides being measured. Some Mussel Watch participants measured such materials, but there was no formal intercomparison based on them. Standard materials, along the lines of the U.S. National Bureau of Standards (NBS) Standard Reference Materials (SRMs), were not available for any constituents measured except trace metals.

It was initially assumed that large enough groups of mussels of approximately the same size from the same collection would exhibit very similar, if not identical, concentrations of pollutants. To date, however, comparatively few data exist that permit estimates of variance of pollutant concentrations within a mussel population. The data that were available in support of this assumption have never been adequately tested. Since we did not know the inherent between-sample variability of the split samples, any statistical analyses of these runs of data could offer only weak evidence of the intercomparability of the data sets from participating laboratories.

The need for intercomparison studies as a part of multilaboratory programs is obvious, but not always realized. Ideally, a standard procedure for all analyses could be devised and utilized which would presumably provide comparable results. In reality, this does not work for at least two reasons: (a) identical techniques in different laboratories do not necessarily give similar results and (b) investigators simply cannot agree on which of their collective techniques is the one to use. Some of the International Council for the Exploration of the Sea (ICES) and IAEA intercomparisons are examples of (a). The most notable (and noble) attempt at (b) was the 1975 Bureau of Land Management (BLM) Benchmark Studies meetings, which brought together most of the scientists in the

forefront of marine environmental analytical chemistry for two separate 3-d meetings, one on organics and one on metals, to hammer out standard techniques for analysis of all the water, sediment and organism samples to be collected in the Outer Continental Shelf Benchmark Studies. The closest this effort came to success was a draft document which included two to four techniques for every type of analysis, because no evidence could be brought to bear that any one method was consistently better than any other, and the participants understood the cost involved in converting to a technique that most had neither equipment for nor experience in. It was for such reasons that the laboratories in the Mussel Watch program analyzed the mollusc samples using techniques appropriate to each laboratory. Although each laboratory is most certain of data generated by what each considers its best technique, comparison of results using such methods may be a problem. In the absence of appropriate certified SRMs, and with the problems associated with split samples, recourse to a group of reference material samples of known homogeneity appeared indispensable.

There have been several intercomparison exercises which have assessed the capability of several laboratories to arrive at comparable results when asked to analyze for stated constituents in similar samples or aliquots of a large single sample. Sometimes "standard" samples, such as the NBS SRMs, have been available so that both accuracy and precision could be addressed. Historically, for environmental work, however, SRMs have been available only for trace metals, and, until very recently, only in bovine liver and orchard leaves. Organizations such as ICES and IAEA have produced homogenates in several different matrices and conducted intercomparison exercises which have consistently shown wide-ranging results [5-7]. Several exercises have been attempted in recent years in the area of organic analysis of environmental samples as interest, awareness and capabilities to measure organic

contaminants at environmental levels have increased.

A brief review of such studies indicates improvement over the years in the ability of several laboratories to analyze for the same constituents and arrive at comparable results, and we feel that the current study is another very significant indication that progress has been made. Farrington et al. [8] analyzed a fish oil and found widely varying results in total hydrocarbons and pristane. Some 10 years later, Wise et al. [9] showed much better agreement, but there were still significant differences for the same constituents in a mussel tissue homogenate sample.

An attempt by the U.S. EPA Environmental Research Laboratory at Narragansett (ERLN) to prepare a reference material from ocean clams for metals analysis resulted in samples for which the relative standard deviation for many trace elements was $< 7\%$ (P. F. Rogerson, in preparation). This success encouraged us to produce a similar mussel reference material which would be suitable for both organic and trace metal analysis. Preliminary metals analysis of this material (mussel homogenate I) showed a high degree of uniformity (data presented below), and we proceeded to establish the concentrations of metals and organics in it and to initiate intercomparison studies among the Mussel Watch laboratories using it as an intercomparison sample. Preliminary results of these analyses were encouraging, and analysis of polycyclic aromatic hydrocarbons (PAHs) was initiated. As a basis for intercomparison of analyses of transuranic radionuclides, a second mussel reference material (mussel homogenate II) was prepared in quantity sufficient to provide the number of much larger aliquots required. This article presents an assessment of the level of agreement between laboratories reached during the first 3 years of the program, and discusses discrepancies that were observed.

The participating labs and the pollutant class(es) analyzed by each are shown in Table 1.

Table 1.

	Trace metals	Total hydrocarbons	Chlorinated hydrocarbons	Radionuclides
U.S. EPA Environmental Research Laboratory/Narragansett (ERLN)	X	X	X	
Woods Hole Oceanographic Institution (WHOI)		X	X	X
University of California/Berkeley Bodega Marine Laboratory (BML)		X	X	
San Jose State University, Moss Landing Marine Laboratory (MLML)	X			
Scripps Institution of Oceanography (SIO)	X			X
University of New Orleans, Center for Bio-organic Studies (UNO/CBS)		X	X	

EXPERIMENTAL METHODS

Production of homogenates

A large number of mussels (*M. edulis*) was collected by commercial dredge from a single site in Narragansett Bay. Prior analyses of organisms from this site had shown moderate levels of each of the pollutants of interest. The mussels were frozen in the shell within 6 h of collection, and were kept frozen at -20°C until the homogenate was prepared. At that time, the mussels were thawed and shucked into glass carboys that had previously been acid-stripped and solvent-washed. Excess fluid was discarded to facilitate the homogenization procedure, and the carboys were refrigerated until the next day. The ~ 38 liters of mussel soft parts and entrained fluid were transferred to a stainless steel Hobart VCM-40 commercial kitchen chopper/mixer and blended for approximately 10 min. For mussel homogenate I, the resultant slurry was transferred (using Teflon funnels) to 976 30-ml Teflon bottles (also acid-stripped and solvent-washed) and immediately frozen at -20°C ; they were maintained at this temperature until analysis (P. F. Rogerson, in preparation). The process for homogenate II was identical, except that the slurry was transferred to 144 acid-stripped 250-ml linear polyethylene bottles. For the intercom-

parison exercise, samples were shipped by air, frozen on dry ice.

Trace metal procedures

ERLN. The mussel tissue was oven-dried at 95°C to constant weight, then digested in concentrated HNO_3 in a simple reflux system. The digestate was filtered on transfer to 50-ml volumetric flasks and brought up to volume in 5% HNO_3 . For the present study, solution concentrations for Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were determined using a Perkin-Elmer model 603 atomic absorption spectrophotometer (AAS), operated according to the manufacturer's recommended flame techniques. In addition to the 50 mussel samples and method blanks, quality control materials, such as spiked samples and the above-mentioned ocean clam homogenate, were analyzed. Resultant concentration, weight and statistical data were reduced by computer.

MLML. The oven-dried tissue was digested using quartz-redistilled 70% HNO_3 (QHNO_3). Samples were charred at 350°C to remove lipid, and then further oxidized with 30% reagent grade H_2O_2 [10]. The resulting solutions were diluted to a volume of 20 ml with 1% QHNO_3 . Samples were subsequently analyzed for Ag, Al, Cd, Cr, Cu,

Fe, Mn, Ni, Pb and Zn on a Perkin-Elmer model 603 AAS coupled to an HGA 500 graphite furnace.

Chlorinated hydrocarbon procedures

BML. The split samples of Bodega Head mussels were extracted by acid digestion, followed by an acid cleanup. Details of the procedure are described by Risebrough et al. [11]. The mussel homogenate samples were freeze-dried behind protective layers of polyurethane foam and then soxhlet-extracted with either methylene chloride or benzene. These solvents were removed by rotary evaporation and the sample taken up in hexane. Column chromatography was performed on deactivated Fluorisil with a hexane fraction, followed by two additional fractions of 30% CH_2Cl_2 in hexane and 50% CH_2Cl_2 in hexane. These fractions were then reduced in volume and analyzed. Packed column analyses were performed on a Tracor MT-220 gas chromatograph (GC) equipped with 2 m \times 4 mm inner diameter, glass columns containing a mixture of 1.5% SP-2250/1.95% SP-2401 on 100/120 mesh Supelcon (AW-DMCS) or 3% OV-1 on 100/120 mesh Supelcoport and ^{63}Ni electron capture detectors.

Additionally, sidearm saponification columns [12] were utilized to convert *p,p'*-DDD and *p,p'*-DDT, if present, to their ethylene derivatives, thus providing confirmation of their identity. Polychlorinated biphenyls (PCBs) were quantified by comparing the combined heights of three peaks eluting after *p,p'*-DDE and with relative retention times of 1.25, 1.48 and 1.74 with the same peaks in an Aroclor 1254 standard.

Capillary column gas chromatography was performed using a Carlo Erba 2150 GC with a ^{63}Ni Brechbuhler micro-electron capture detector equipped with an SE-54 fused-silica 30-m column. An internal standard containing a C-10 alkyl bromide and decachlorobiphenyl (DCB) was co-injected to assist in the determination of retention

indices, to determine sensitivity of the instrument (detector sensitivity and split ratio) and for quantitation.

ERLN. Wet tissues were homogenized twice with acetone using a Polytron probe and centrifugation to separate the layers. This step was repeated twice with Freon-113, after which the extracts were combined and partitioned with water and Freon. The Freon extracts were passed through a precolumn of activated silica gel, reduced in volume using a Kuderna-Danish concentrator and the solvent was changed to hexane. PCBs and other compounds of interest were eluted from a second column of 5% H_2O (w/w) deactivated silica gel with pentane and blown down to final volume. Sample extracts were analyzed by gas chromatography on a 30-m SE-54 glass capillary column in a Hewlett-Packard 5840A GC equipped with ^{63}Ni electron capture detector and a splitless injection port.

UNO/CBS. Thirty grams (wet weight) of mussel homogenate was extracted three times in a Brinkman Polytron tissue homogenizer with hexane/isopropanol (3:1, v/v). The extracts were then combined and washed three times with an aqueous saturated sodium sulfate solution and once with distilled water. The washed extract, now in hexane, was concentrated to about 2 ml in a rotary evaporator and fractionated on a Florisol chromatography column. The PCB fraction was eluted from the column with 6% diethyl ether in hexane. Prior to gas chromatographic analysis, the PCB fraction was brought to a final volume of about 2 ml in a rotary evaporator.

Gas chromatographic analyses were carried out on a Hewlett-Packard 5700 series GC equipped with a ^{63}Ni electron capture detector, a 30 m \times 0.3 mm inner diameter glass capillary column coated with SE-54, and a glass capillary inlet system. The inlet system was operated in the splitless mode. Data were acquired and processed on a Hewlett-Packard Model 3354B laboratory

data system. An internal standard method of quantification was used to quantitate the PCBs in the homogenate as Aroclor 1254. The areas of 14 specific peaks in the PCB mixture were used, along with the area of the internal standard peak in the computerized calculations. The resulting quantitative data were then adjusted for percent recovery based on the recovery of a chemically similar standard that was added to each sample prior to extraction and fractionation.

WHOI. The samples were freeze-dried and then extracted three times with hexane in the presence of glass beads in a Vertis homogenizer equipped with a nylon impeller. PCBs were isolated from the extract by column chromatography on alumina (5% H₂O) over silica gel (5% H₂O). The PCBs in the concentrated column chromatography fraction were analyzed by gas chromatography on a 1.8 m × 2 mm inner diameter glass column packed with 1.5% OV-17/1.95% OF-1 on chromosorb W-HP 100/120 mesh installed in a Perkin-Elmer model 900 GC equipped with ⁶³Ni electron capture detector. Recoveries of chlorinated hydrocarbons, based on several analyses of DDT spikes, were 80% or better.

Total hydrocarbon procedures

ERLN. Wet tissues were digested with an equal volume of 4 N NaOH overnight at 37°C and extracted three times with CH₂Cl₂, with centrifugal separation of the layers. The CH₂Cl₂ extracts were combined and passed through an activated silica gel column. Volume reduction was accomplished through Kuderna-Danish evaporation and the solvent was changed to hexane. Fractions were cut on a 5% w/w H₂O deactivated silica gel column by elution with pentane for fraction 1 (F-1, mostly alkanes) and 2% CH₂Cl₂ in pentane for fraction 2 (F-2, mostly aromatic hydrocarbons and alkenes). The column chromatographic fractions were analyzed by glass

capillary column gas chromatography on a 30-m SE-54 column in a Hewlett-Packard 5840 GC using flame ionization detection and splitless injection. For total saturates and total aromatics, areas on sample chromatograms were planimeted and quantified using areas obtained from hydrocarbon standards.

Fraction 2 was further examined using the Finnigan 1015 electron impact (70 eV) mass spectrometer (MS). This instrument was interfaced to a Shimadzu GC4CM GC equipped with a splitless injector and a 30-m SE-54 glass capillary column. The aromatic compounds to be quantitated were first identified by comparisons of retention time and mass spectra with standards (when available) and peaks in extracted ion current plots were then integrated using a program in the Riber 400 software package. Final quantification was done by comparison with standards run in an identical manner. Where standards were not available, concentrations were estimated by comparison with the response of the nearest eluting appropriate standard.

BML. The extracts produced by the extraction and separation procedures previously described for electron capture determinations were used in the determination of total hydrocarbons. Flame ionization gas chromatography was performed on Hewlett-Packard 5840A GCs equipped with automatic liquid sampling and Grob-type capillary inlet systems. AA-grade efficiency 30-m glass capillary columns (J&W Scientific), rated at a minimum of 2,500 effective plates per meter, were used for all analyses. Aliphatic (saturate) fractions were analyzed using a nonpolar SP-2100 phase column, whereas aromatic fractions were analyzed using a more polar SE-54 phase.

UNO/CBS. Thirty grams (wet weight) of mussel homogenate was digested in 15 g 4 N KOH at 90°C for 3 h. The nonsaponifiable lipids were then extracted three times

in glass-distilled diethyl ether. The diethyl ether extraction solvent was displaced by pentane prior to fractionation of the extract on a silica gel chromatographic column. The aliphatic fraction (F-1) was eluted with pentane and the aromatic fraction (F-2) with 20% dichloromethane in pentane. Prior to analysis, each fraction was concentrated to ~3 ml in a rotary evaporator and then brought to a final volume of ~200 μ l in an evaporative concentrator equipped with a modified micro-Snyder distillation column.

The total saturated and total aromatic hydrocarbon analyses were performed on a Hewlett-Packard 5700 series GC equipped with a hydrogen flame ionization detector, a 30 m \times 0.3 mm inner diameter glass capillary column coated with SE-52 and a glass capillary inlet system. The inlet system was operated in the splitless mode. Data were acquired and processed on a Hewlett-Packard 3354B data system. An internal standard method of quantification was used to quantitate the hydrocarbons in the F-1 and F-2 fractions. This was accomplished through the use of a computer program designed to integrate all of the resolved and unresolved hydrocarbons in the gas chromatogram as a single peak. The resulting total area of the chromatogram was used along with the area of the internal standard peak in the quantitative calculations. These data were then adjusted for percent recovery on the basis of the recovery of saturated and aromatic standards, which were added to the sample prior to extraction and fractionation.

Individual PAHs in the F-2 fraction were analyzed on a Hewlett-Packard Model 5985A GC-MS-DS system using the chromatographic conditions just described. An internal standard method of quantification was also used to quantify individual PAHs. Peak areas used in the quantitative calculations were generated from extracted ion current profiles of the molecular ions of the PAHs of interest and the internal standard. Accurate quantitative data were reported for those PAHs for which authentic stan-

dards were available. Polynuclear aromatic hydrocarbons for which authentic standards were not available were reported as equivalents of the internal standard. All of the quantitative data was adjusted for percent recovery based on the recovery of a PAH standard that was added to the sample prior to extraction and fractionation.

WHOI. Each 15-g wet sample was digested with 10 ml 6 N NaOH in a screw-cap centrifuge tube and extracted three times with diethyl ether. The ether was evaporated and replaced by hexane. Two hydrocarbon fractions, F-1 (essentially alkanes and cycloalkanes) and F-2 (aromatic hydrocarbons and alkenes), were isolated from the hexane extract by column chromatography on alumina over silica gel (both 5% deactivated with H₂O).

Both column chromatography fractions were analyzed by glass capillary GC on a 20 m \times 0.32 mm inner diameter SE-54 column installed in a Hewlett-Packard model 5840A GC equipped with a split/splitless injector. Compounds were quantified by comparison of peak heights of standards using the same conditions as sample analysis. Recoveries were calculated using the internal standards n-C14, n-C22, n-C28, hexamethyl- and hexaethylbenzene, and by measuring recoveries of a reference mixture of several representative hydrocarbons added to two portions of subsamples. The unresolved complex mixture signal was measured by planimetry.

Identification of peaks was based on retention indices of compounds and co-injection, and, for aromatic hydrocarbons, additional identification by mass spectra. The aromatic/olefinic fraction was analyzed on a Finnigan 1015 quadrupole MS coupled to a Varian GC equipped with a split/splitless injector and with a 17 m \times 0.32 mm inner diameter SE-54 glass capillary column installed. Quantitative analyses for selected aromatic hydrocarbons were achieved by analyzing known standard solutions of aromatics us-

ing the same conditions and plotting a response vs. ion current curve. Ion currents were integrated from mass plot data using an integrator program in the Riber 400 software package.

Radionuclide procedures

SIO. Frozen bivalves, without broken shells, were washed free of loose particulates, opened and allowed to partially thaw before 500 to 600 ml of the soft tissues and within-shell liquids were collected into a cleaned, tared, 1-liter Pyrex beaker. Mussel byssal threads were removed and placed into a separate small beaker. Tissue wet weights were determined before the samples were dried to constant dry weight at 110°C. The dried samples were ashed at 500°C and the ash weights determined. The ashed material was wetted with distilled water before appropriate yield monitors of ^{242}Pu and ^{243}Am were added. A series of wet oxidation steps were performed on the ashed samples with 8 N HNO_3 and H_2O_2 . The final sample digest was diluted to 800 ml and subjected to a precipitation with AlPO_4 . The clear supernatant was saved for ^{137}Cs determination and the transuranics were further purified from the gel-like precipitate.

The AlPO_4 precipitate was solubilized with 6 N HCl , adjusted to 9 N HCl and oxidized with a small addition of H_2O_2 . This sample was then placed on an anion-exchange column and eluted with a series of HCl concentrations. The 9 N HCl fraction, containing Am, was collected and saved for further purification. Pu was eluted with 1.2 N HCl and H_2O_2 . The Pu fraction was subjected to a second anion column purification before the Pu was finally electroplated on a stainless steel disk for measurement by alpha-spectrometry.

The 9 N HCl Am fractions were evaporated to dryness, dissolved with a combination of distilled water and concentrated HNO_3 and then extracted with dibutyl-*N*-

N-diethyl-carbamyl-phosphonate (DDCP). After DDCP extraction, the Am was subjected to a complex series of cleanup steps before finally being electroplated and measured by alpha-spectrometry. These procedures are detailed in ref. 3.

WHOI. Bivalves were opened, the byssal threads removed and the soft tissues and within-shell liquids collected in cleaned, tared, 1-liter Pyrex beakers. Fresh weights were determined, and the samples were then dried to constant weight at about 95°C. The dried samples were ashed slowly at 500°C and the ash weights determined. The ashed samples were digested in a large volume of 8 N HNO_3 , to which the appropriate carriers and radioisotopic yield monitors were added. After thorough digestion, Pu was collected on an anion-exchange column, then reduced on the column with NH_4I , eluted, subjected to several cleanup steps and finally electroplated on stainless steel disks for measurement by alpha-spectrometry.

Cs was separated from the eluate of the first column, after dilution and pH adjustment, by adsorption on ammonium phosphomolybdate (AMP). The AMP was collected, cleaned up and destroyed by NaOH , and the Cs was separated from other alkali metals on a cation-exchange column and precipitated as chloroplatinate. The ^{137}Cs content was measured by beta-counting.

Am was collected from the AMP supernatant by co-precipitation with the hydroxides of Fe and Nd. After separation and a complex series of final cleanup steps, the Am was electroplated and measured by alpha-spectrometry.

These procedures were modified from Wong et al. [13] and Livingston et al. [14]. Several references to the experiences of WHOI with these methods in radioanalytical intercomparison exercises are collected in the study by Bowen et al. [15].

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RESULTS AND DISCUSSION

Trace metals

Results of split-sample analyses done early in the program are listed in Table 2 in the study of Goldberg et al. [1], which indicated that SIO and MLML provided similar results for Zn, Cu, Cd, and Ni, but that Pb and Ag were systematically higher in the MLML results.

Initial characterization of mussel homogenate I was carried out by ERLN on 50 of the 976 aliquots, and MLML subsequently analyzed five for intercomparison purposes. Results from both laboratories are summarized in Table 2 for Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. MLML also analyzed for Ag. For all elements except Al in the initial 50 aliquots, the relative standard deviation was 6% or less, indicating very good uniformity across the entire homogenate. The relative standard deviation for Al was 19%. The wet/dry ratio of 6.38 ± 0.09 indicates uniform moisture content and therefore good mixing in a purely physical sense as well.

Figure 1 is a plot of the relationship between means of the results from MLML and ERLN. The MLML values are shown as percent of ERLN values, with error bars to indicate one standard deviation on either side of the mean. This presentation shows graphically that for Cr, Cu, Fe, Mn, Ni and Zn, the analyses by the two labs agreed to within 10% or less, Al and Pb within 15%

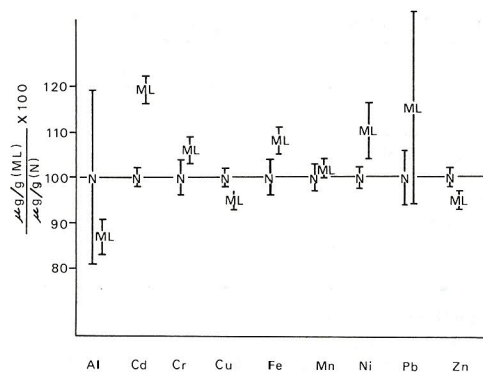


Fig. 1. Trace metals in ERLN mussel homogenate I. Plot of Moss Landing (ML) vs. ERLN (N) values as a percent of Narragansett values. Error bars indicate ± 1 SD.

and Cd 20%. In the cases of Al and Pb, the uncertainty in the analysis was such that the difference was not statistically significant. Cd was the only case in which a significant difference of more than 10% was observed. SIO did not analyze mussel homogenate I for trace metals.

Polychlorinated biphenyls

In the Mussel Watch Program, PCBs are quantified as the Aroclor mixture which most closely resembles that found in each particular sample. Some samples have been reported as two different mixtures. For the reference material, PCBs are reported as Aroclor 1254, although chlorinated biphenyls may be present beyond the usual 1254 range. The results in Table 3 show that the analyses by three of the laboratories agree to within one standard deviation, while results from the fourth lab are high by a factor just less than two. The agreement of the three is remarkable, with the mean values falling within 11% of each other, and it should be noted that the fourth lab used a quantification procedure different from that used by the other three. Given the absence of accepted environmental standards for trace organic analysis, the "correctness" of these values cannot be established. The sources of disagreement may be identified by future

Table 2. ERLN mussel homogenate I trace metal concentration (in $\mu\text{g/g}$ dry weight)

Element	ERLN (n = 50)		MLML (n = 5)	
	Mean	SD	Mean	SD
Al	270	52	236	12
Cd	2.08	0.04	2.48	0.07
Cr	2.15	0.08	2.28	0.06
Cu	12.8	0.3	12.2	0.2
Fe	450	18	486	12
Mn	26.8	0.7	27.3	0.4
Ni	6.84	0.17	7.53	0.38
Pb	9.11	0.55	10.5	1.94
Zn	135	3	128	3
Ag	—	—	0.13	0.01

Table 3. ERLN mussel homogenate I: Mussel Watch intercomparison results (total hydrocarbons and PCBs)

Material	ERLN	UNO/CBS	WHOI	BML
Total saturates [(F-1)($\mu\text{g/g}$)]	61 \pm 17 (6)	87 \pm 5 (10)	111 \pm 13 (4)	87 \pm 27 (3)
Total aromatics [(F-2)($\mu\text{g/g}$)]	21 \pm 4 (6)	67 \pm 13 (10)	38 \pm 9 (4)	41 \pm 6 (3)
PCBs [as Aroclor 1254] ($\mu\text{g/kg}$)	470 \pm 45 (12)	895 \pm 273 (10)	412 \pm 23 (4)	510 \pm 140 ^a (3) 580 \pm 23 ^b (3)

^aCapillary column GC.^bPacked column GC.Concentrations given on dry weight basis as mean \pm SD, with number of samples analyzed in parentheses. Analyses performed by GC. UNO/CBS values adjusted for percent recovery.

studies involving extract exchanges and joint chromatogram interpretations.

Analyses of split samples from Bodega Head collected and analyzed in 1976 and 1977 are given in Table 4. Even though the concentrations of DDE and PCBs were lower by an order of magnitude than the PCB concentrations in mussel homogenate I, the agreement between the two laboratories involved is reasonable. There are still some problem areas which need improvement. For example, two of the duplicate measurements were high. These were in the first set of samples analyzed in the program and may be explained as random error or by some contamination event in the laboratory. We now know that such disagreements of duplicates are uncommon and indicate some problem requiring reanalysis of a set of duplicates.

Total hydrocarbons

The total hydrocarbon values result from gas chromatographic analyses of two column chromatographic fractions, a nonpolar saturated fraction (F-1) and an aromatic fraction (F-2). These two fractions are defined operationally by each laboratory's separation procedure. Quantitation was accomplished by integration of the unresolved complex mixture of hydrocarbon material on gas chromatograms of samples and comparison with areas obtained from appropriate standards. The values for F-1 and F-2 are shown in Table 3. For the F-1 values, the mean value for the four labs is 86 ± 20 (23%) $\mu\text{g/g}$ dry weight, with two labs very close to this value, and one about 30% high and one about 30% low. The statistical uncertainty around this mean lies

Table 4. DDE and PCB concentrations in *M. californianus* from Bodega Head: Comparison of split-sample analyses

Sample code	DDE (10^{-9} g/g dry wt)				PCB (10^{-9} g/g dry wt as 1254 mixture)			
	BML	WHOI duplicates			BML	WHOI duplicates		
		a ^a	b	Average		a	b	Average
760324	17	34	35	35	10	36	67 ^b	52
760603	10	20	21	21	19	17	35	26
760729	23	15	13	14	16	27	97 ^b	62
761006	7	13	15	14	18	20	16	18
770104	4.6	19	17	18	27	17	16	17
770202	24	19	21	20	34	25	34	30
770303	15	14	15	15	21	14	15	15
Average	14			20	20			31

^aDuplicate analyses of subsamples of homogenate of 20–30 mussels.^bPoor reproducibility (see text).

Sample code is date of collection given as year, month and day.

well within the uncertainties of the individual laboratories. This is consistent with a reproducibility of better than 70% and no discernible lab-to-lab bias.

The F-2 results are not as consistent as the F-1 results. The mean value for the four labs is 42 ± 19 (45%), which is very close to two of the values but 50% higher or lower than the other two. The standard deviation of the mean is about twice that of the most variable individual lab value. This indicates that there may be some systematic bias between the laboratories, perhaps due to the different procedures used to separate the aromatic fractions. The absence of established environmental standards makes this interpretation difficult to substantiate, although future studies involving extract exchanges may eliminate instrumental variations. Other aspects of analyses for intercomparison of hydrocarbon measurements by laboratories involved in the Mussel Watch program are given by Farrington et al. [2].

Polycyclic aromatic hydrocarbons

Three of the laboratories — UNO/CBS, WHOI and ERLN — analyzed the F-2 fractions by GC-MS for a number of aromatic compounds. For the PAH intercomparison, a list of 26 molecular weights, with corresponding compound names, was distributed and the labs were asked to report results at those values (Table 5). For example, the results obtained at mol wt 128 are reported as naphthalene because the data gathered at 128 are a measure of the amount of the single compound naphthalene present in the sample. However, PAH analyses are often not single-compound analyses because at a particular molecular weight there are several different isomers of similar hydrocarbons present, and these may not be resolved with the techniques employed. In addition, few standards are available for the alkylated aromatics, and so response factors must be extrapolated. Results of this type should not be assumed to be single-compound analyses, but rather to be an indication of the level of

PAHs present at that molecular weight, with an identification, where possible, of the major compound(s) or class of compounds present.

Table 5 shows the results of the aromatic hydrocarbon intercomparison. In general, the measurements made at these individual molecular weights are quite variable. Some of the compounds measured show much greater between-laboratory variation than do others. For example, the means of the naphthalene values are widely divergent between labs, while the fluoranthene/pyrene values are much more consistent. Similar variations in the standard deviations of individual laboratory measurements are also apparent. At mol wt 192 (C-1 phenanthrene/anthracene), the different values show standard deviations that vary from <10% to >130%. Although the large differences in the standard deviations of some of these measurements are not easily explained, there is a possible explanation for the divergence of the naphthalene values. The naphthalenes may be too volatile to be consistently retained, and the small differences in methods between laboratories may yield divergent recovery percentages.

If one eliminates the naphthalene series from consideration, there are nine compounds or classes which all three laboratories determined. Table 6 lists these values as percentages of the mean for each determination. Of the 27 values reported, only four differ from the mean by more than 50% of the mean, 85% of the data falling with $\pm 50\%$ of the mean. This is a remarkably good agreement for state of the art analysis for trace organics, especially considering that standards for many of these are not available. Averaging these relative values yields averages that are also very consistent. If one of the laboratories were biased either high or low, it should show up in these relative averages. However, the means of the three labs' values are within about $\pm 10\%$ of each other, which indicates that results from no one lab are consistently higher or lower than results from the other two.

Table 5. ERLN mussel homogenate I: Mussel Watch intercomparison results (polycyclic aromatic hydrocarbons)

Mol wt	Compound	ERLN	UNO/CBS	WHOI
128	Naphthalene	4.8 ± 1.9	96 ± 118 (61 ± 43)	2.8 ± 0.8
142	Methylnaphthalene	3.0 ± 1.0	20 ± 15	4.0 ± 2.6
156	C-2 Naphthalene	10 ± 5	6.8 ± 8.2 (10 ± 8)	6.5 ± 2.0
170	C-3 Naphthalene	14 ± 3	0.7 ± 0.7 (1.4 ± 0.7)	3.0 ± 2.5
178	Phenanthrene/anthracene	13 ± 5	32 ± 40	7.9 ± 1.6
192	C-1 Phenanthrene/anthracene	15 ± 4	3.4 ± 7.5 (8.2 ± 11)	15 ± 1.4
206	C-2 Phenanthrene/anthracene	38 ± 15	68 ± 60 (84 ± 55)	58 ± 14
220	C-3 Phenanthrene/anthracene	78 ± 34	91 ± 88 (113 ± 85)	38 ± 14
184	Dibenzothiophene	1.3 ± 0.4 ^a	ND	ND
198	C-1 Dibenzothiophene	6.4 ± 2.4 ^a	0.7 ± 0.7 (1.4 ± 0.7)	ND
212	C-2 Dibenzothiophene	21 ± 9 ^a	4.1 ± 4.1 (6.2 ± 4.1)	ND
226	C-3 Dibenzothiophene	32 ± 14 ^a	6.2 ± 6.1 (8.9 ± 5.5)	NR
202	Fluoranthene	56 ± 18	42 ± 37	80 ± 12
202	Pyrene	46 ± 13	34 ± 31	92 ± 14
216	C-1 Fluoranthene/pyrene	24 ± 7	24 ± 23 (30 ± 23)	40 ± 7
230	C-2 Fluoranthene/pyrene	28 ± 9	21 ± 28 (30 ± 29)	NR
244	C-3 Fluoranthene/pyrene	22 ± 8	ND	ND
228	Benz[a]anthracene/chrysene	29 ± 6	28 ± 32 (31 ± 33)	47 ± 6
242	C-1 Benz[a]anthracene/chrysene	18 ± 6	12 ± 17 (25 ± 17)	NR
256	C-2 Benz[a]anthracene/chrysene	18 ± 6	ND	ND
270	C-3 Benz[a]anthracene/chrysene	5.9 ± 5.9	ND	ND
252	C _n H _{2n+z} Z = -28	68 ± 18	15 ± 3 (16 ± 14)	65 ± 32
266	C-1 C _n H _{2n+z} Z = -28	14 ± 5	ND	NR
280	C-2 C _n H _{2n+z} Z = -28	8.4 ± 5.8	ND	NR
294	C-3 C _n H _{2n+z} Z = -28	ND	ND	NR
276	Benzoperylene	22 ± 5	ND	ND
No. of observations		6	10	4

^aIdentifications based on mass spectra only.

Analyses performed by GC-MS. Concentrations in µg/kg dry weight reported as mean ± SD. Parentheses indicate mean ± SD calculated without one reading which was greater than 3 σ from the mean of the other readings.

ND, not detected; NR, not reported.

The results reported here are a significant improvement over those of a similar exercise reported by Wise et al. [9]. We think that substantial further improvements in reproducibility and in lowering the detection limit of quantitative GC-MS can be achieved by quantitation using single-ion monitoring. In the present program, this was not done because of the need to have full mass spectra available for structure identification prior to determining concentrations from

reconstructed molecular ion profiles. Interlaboratory comparison of PAH measurements in tissue homogenates using GC-Flame Ionization Detection (GC-FID), GC-MS and High-Pressure Liquid Chromatography Ultra-Violet Fluorescence (HPLC-UVF) should be undertaken for some selected PAHs to cross-check among analytical measurement techniques as well as among laboratories. This would be another significant step toward demonstrating

Table 6. Comparison of PAH results from analysis of ERLN mussel homogenate I

Compound	Mean \pm SD $\mu\text{g/kg}$ dry weight)	% of Mean		
		ERLN	UNO/CBS	WHOI
Phenanthrene/anthracene	18 \pm 13	74	181	45
C-1 Phenanthrene/anthracene	13 \pm 4	118	64	118
C-2 Phenanthrene/anthracene	60 \pm 23	63	140	97
C-3 Phenanthrene/anthracene	76 \pm 38	102	148	50
Fluoranthene	59 \pm 19	94	71	135
Pyrene	57 \pm 31	80	59	160
C-1 Fluoranthene/pyrene	31 \pm 8	77	96	128
Benz[a]anthracene/chrysene	36 \pm 10	81	87	132
C _n H _{2n+z} = -28	50 \pm 29	137	32	131
Mean \pm SD		92 \pm 24	98 \pm 49	111 \pm 39

85% of values lie within $\pm 50\%$ of mean values, based on data presented in Table 5.

the feasibility of having an NBS reference material for PAH in tissue.

Radionuclides

Split-sample analytical results from the first year were presented in Table 3 of the study by Goldberg et al. [1], and were generally in agreement within a factor of two.

Results of analysis of radionuclides in mussel homogenate II by WHOI and SIO are presented in Table 7. The analysis of seven aliquots by WHOI indicates that the reference material is uniform in terms of moisture content as well as ^{238}Pu , $^{239,240}\text{Pu}$, ^{137}Cs , and ^{241}Am . There is excellent interlaboratory agreement in the $^{239,240}\text{Pu}$ analysis, and the ^{241}Am analyses fall within one standard deviation of each other. The ^{238}Pu level is very close to detection limits, but there is good agreement there also. SIO did not measure ^{137}Cs . The uncertainties quoted are, as is customary, ± 1 sigma, and are based only on counting statistics; these most probably are substantial underestimates of the overall uncertainty of such analytical data.

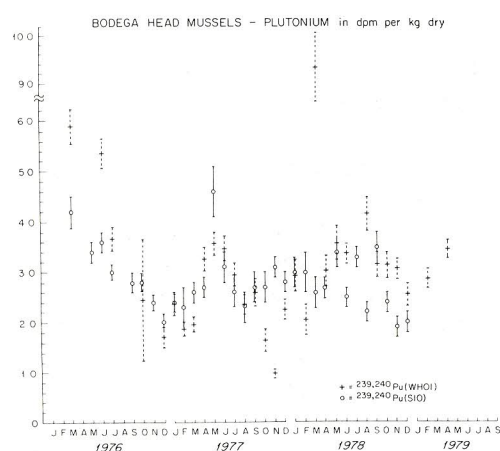
As we noted in the introduction, during the initial three years of the Mussel Watch program, only the comparisons of analyses of split samples were available to support estimates of the intercomparability of radionuclide data provided by WHOI and SIO. These data will be fully reported in a forthcoming series of articles, under various authorships, considering all the analyses that resulted from the first three years of Mussel Watch (1976-1978). It should be noted that the split-sample data, from analyses performed during these years, do not support the feeling of complacency that might be derived from the homogenate analyses summarized in Table 7, or from the split-sample data reported earlier [1]. The intercomparability of the full three years of split-sample analyses is indicated in Table 8: of the four series summarized, two (Narragansett ^{241}Am and Bodega Head $^{239,240}\text{Pu}$) show agreement, within 1 sigma confidence limits, among almost half the samples; the other two (Narragansett $^{239,240}\text{Pu}$ and Bodega Head ^{241}Am) show agreement of less than one-third. In each case, the number of ± 2 sigma agreements is close or equal to the

Table 7. ERLN mussel homogenate II: Mussel Watch intercomparison results (radionuclides)

Lab	n	Wet/Dry Weight Ratio (110°C)	Radionuclides (dpm/kg wet wt)			
			^{238}Pu	$^{239,240}\text{Pu}$	^{241}Am	^{137}Cs
WHOI	7	6.76 \pm 0.05	0.009 \pm 0.005	0.238 \pm 0.032	0.062 \pm 0.017	2.4 \pm 0.73
SIO	3	6.6	0.012 \pm 0.005	0.25 \pm 0.02	0.09 \pm 0.01	

Table 8. WHOI vs. SIO split-sample intercomparisons of transuranic data

	No. of replicate samples	No. of agreements within:	
		1 sigma	2 sigma
Narragansett Bay mussels			
^{239,240} Pu analyses	23	7	8
²⁴¹ Am analyses	17	8	8
Bodega Head mussels			
^{239,240} Pu analyses	28	12	10
²⁴¹ Am analyses	27	8	12

Fig. 2. ^{239,240}Pu in Bodega Head mussels, showing monthly values and a comparison of results of WHOI and SIO.

number of ± 1 sigma agreements. A representative plot of split-sample results (Pu in Bodega Head mussels) is shown in Figure 2. Clearly, we are not dealing with variation among the members of a single, normally distributed population. A few analyses made by each laboratory of samples split after being received (summarized in Table 9) show that each laboratory was capable of satisfactory data replication, and, consequently, that the variability among mussels from each site was not large enough to vitiate the split-sample technique. The systematic interlaboratory disagreement of perhaps twice the estimated uncertainty of the data reported indicates a clear need for more thorough intercomparisons using ho-

Table 9. Intralaboratory radionuclide duplicate analyses

Sample code	Radionuclides in dpm/kg dry wt			
	^{239,240} Pu	²³⁸ Pu	²⁴¹ Am	¹³⁷ Cs
WHOI				
FAR-780820	4.58 \pm 0.21	0.064 \pm 0.029	14.25 \pm 0.72	36.44 \pm 14.22 ^b
	4.80 \pm 0.36	0.15 \pm 0.06	22.84 \pm 1.15	39.02 \pm 4.22
CM-780812	5.44 \pm 0.17	0.21 \pm 0.04	13.46 \pm 0.95	42.46 \pm 2.76
	4.06 \pm 0.26	0.12 \pm 0.04	12.60 \pm 0.69	28.05 \pm 0.53
PLY-780926	3.74 \pm 0.56	— ^a	0.69 \pm 0.24	— ^a
	3.74 \pm 0.27	— ^a	0.96 \pm 0.19	— ^a
BH-761202	1.66 \pm 0.23	0.03 \pm 0.03	— ^a	27.94 \pm 3.02
	1.74 \pm 0.15	0.06 \pm 0.03	— ^a	33.22 \pm 4.53
AC-761014	— ^a	— ^a	1.14 \pm 0.11	— ^a
	— ^a	— ^a	1.25 \pm 0.23	— ^a
SIO				
SCI760604	1.7 \pm 0.1	— ^c	— ^c	— ^c
	1.6 \pm 0.3	— ^c	— ^c	— ^c
BB-760818	2.2 \pm 0.2	— ^c	— ^c	— ^c
	2.5 \pm 0.3	— ^c	— ^c	— ^c
SOB-770612	11.2 \pm 1.3	— ^c	— ^c	— ^c
	13.9 \pm 1	— ^c	— ^c	— ^c

^aData omitted because one of the duplicates was either lost or was an obviously bad number.

^bData by gamma-spectrometry; other ¹³⁷Cs data by beta-counting.

^cOnly ^{239,240}Pu was measured by SIO on own-split duplicates.

Sample code is station initials and date of collection as years, month and day.

mogenates over a range of concentrations. This conclusion is also supported by the results of another quality-control activity that was introduced in 1978: rather than SIO measuring all west coast and WHOI all east coast samples, alternate west coast samples were sent to WHOI, and alternate east coast samples to SIO. In virtually every east coast case, the 1978 SIO-analyzed ^{241}Am to $^{239,240}\text{Pu}$ ratio is the highest observed, whereas among the WHOI-analyzed 1978 samples, this ratio shows no systematic shift from the previous two years. The between-laboratory discrepancy was thus, in these cases, enough to erase some useful information about concentration trends over time. However, the data tend to indicate overall, as do the organic data, that between-station differences of enough magnitude to be easily interpretable have not been lost. From somewhat different points of view, these indications are discussed in forthcoming reports (3; V. T. Bowen, in preparation).

Other pollutant classes and compounds

In addition to the pollutant compounds and classes discussed in this report, measurements are being made within the Mussel Watch Program of a number of other pollutant classes and compounds. Among the petroleum compounds, these include three pentacyclic triterpanes. The procedures also detect the considerable variety of synthetic organic compounds that is present in such environmental samples. Intercalibration of the measurement of these compounds is the subject of ongoing and future intercomparison exercises.

The total hydrocarbons determined in the present study consist of mixtures of petroleum compounds, combustion products and biogenic hydrocarbons. Intercomparison of the methodologies to determine their relative concentrations is an important part of ongoing intercalibration programs.

SUMMARY

Since its inception, the U.S. National Mussel Watch Program has been concerned

with interlaboratory quality control. Initial efforts to address this concern through split-sample analyses indicated several problems both in interlaboratory analytical discrepancies and with the split-sample technique itself. As discussed above, differences did appear in analyses of split samples by different laboratories looking at the same constituent. To approach the question of whether the differences were actually due to differences in laboratory techniques and not in the samples themselves, it was decided in the last year of the initial 3-year program to produce mussel homogenates for intercomparison purposes.

The results obtained through the Mussel Watch intercomparison exercise, using these homogenates, are encouraging in that generally good agreement is seen among analyses done by several labs in diverse pollutant classes. The homogenates allowed direct comparison of the analysis of aliquots of a well-characterized sample made from naturally exposed organisms. Laboratories using their own techniques were able to arrive at comparable results at environmental levels for a number of constituents. Although such intercomparisons have been run in the past, the agreement here is better, especially in some of the areas of organic chemical analysis which have caused problems in the past; the material used was readily prepared by the investigators to assure a matrix identical to that of the unknown samples to be analyzed.

Our experience confirms the need, in any extensive, multilaboratory analytical program, for a complex and extensive quality-control program. Discussions of such programs, and reports of their application, are given by Bowen et al. [15] and Bowen and Volchok [16]. Large homogeneous samples of the matrix being dealt with are an important — we think an essential — part of such programs. They must, however, be measured regularly, generally as unknowns, during the program, and attention must be paid constantly to their reported values. They must also be supplemented by as many

other sources of quality-control information as can be devised by the participants. It appears to be essential, to assure intercomparability, that quality-control samples measured by each participant include "knowns" that span all or most of the range of each constituent being sought. Quality control at these levels (10 to 15% of the sample through-put) is assuredly expensive, but the pay-off in terms of usefulness of the resulting data sets can be enormous.

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REFERENCES

1. Goldberg, E.D., V.T. Bowen, J.W. Farrington, G. Harvey, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson, E.D. Schneider and E. Gamble. 1978. The mussel watch. *Environ. Conserv.* 5:101-125.
2. Farrington, J.W., R.W. Risebrough, P.L. Parker, A.C. Davis, B. de Lappe, J.K. Winters, D. Boatwright and N. Frew. 1982. Hydrocarbons, polychlorinated biphenyls, and DDE in mussels and oysters from the U.S. coast, 1976-1978 — The mussel watch. WHOI Technical Report 82-42. Woods Hole, MA.
3. Goldberg, E.D., M. Koide, V. Hodge, J.H. Martin and A.R. Flegal. 1983. U.S. Mussel Watch: 1977-1978 results on trace metals and radionuclides. *Est. Coastal Shelf Sci.* 16:69-93.
4. Farrington, J.W., E.D. Goldberg, R.W. Risebrough, J.H. Martin and V.T. Bowen. 1983. U.S. mussel watch, 1976-1978: An overview of the trace metal, DDT, PCB, hydrocarbon, and artificial radionuclide data. *Environ. Sci. Technol.* 17:490-496.
5. Law, R.J. and J.E. Portmann. 1980. Report on the first ICES intercomparison exercise on petroleum hydrocarbons. ICES ACMP.
6. Topping, G. 1981. Report on the 6th ICES trace metal intercomparison exercise for cadmium and lead in biological tissue. ICES ACMP 1981/7.4a.
7. Uthe, J.F. and C.J. Musial. 1981. Report on the fifth ICES intercalibration study of PCBs in biological material. ICES ACMP 1981/7.4b.
8. Farrington, J.W., J.M. Teal, G.C. Medeiros, K.A. Burns, E.A. Robinson, Jr., J.G. Quinn and T.L. Wade. 1976. Intercalibration of gas chromatographic analyses for hydrocarbons in tissues and extracts of marine organisms. *Anal. Chem.* 48:1711-1716.
9. Wise, S.A., S.N. Chester, F.R. Guenther, H.S. Hertz, L.R. Hilpert, S.E. May and R.M. Parriss. 1980. Interlaboratory comparison of determinations of trace level hydrocarbons in mussels. *Anal. Chem.* 52:1828-1833.
10. Martin, J.H. and G.A. Knauer. 1973. The elemental composition of plankton. *Geochim. Cosmochim. Acta* 37:1639-1653.
11. Risebrough, R.W., B.W. de Lappe, E.F. Letterman, J.L. Lane, M. Firestone-Gillis, A.M. Springer and W. Walker II. 1980. California State Mussel Watch, Vol. III: Organic pollutants in mussels, *Mytilus californianus* and *M. edulis*. California State Water Resources Control Board, Water Quality Monitoring Report 79-22. Sacramento, CA.
12. Miller, G.A. and C.E. Wells. 1969. Alkaline pre-column for use in a gas chromatographic pesticide residue analysis. *J. Assoc. Off. Anal. Chem.* 53:548-553.
13. Wong, K.M., V.E. Noshkin and V.T. Bowen. 1970. Radiochemical procedures for the analysis of strontium, antimony, rare-earths, caesium, and plutonium in seawater samples. In Y. Nishiwaki and R. Fukai, eds., *Reference Methods for Marine Radioactivity Studies* (IAEA, Vienna) Technical Report Series No. 118, pp. 119-127.
14. Livingston, H.D., D.R. Mann and V.T. Bowen. 1975. Analytical procedures for transuranic elements in seawater and marine sediments. In T.R.P. Gibbs, Jr., ed., *Analytical Methods in Oceanography*. Advances in Chemistry Series No. 147. ACS, New York, pp. 124-138.
15. Bowen, V.T., V.E. Noshkin, H.D. Livingston and H.L. Volchok. 1980. Fallout radionuclides in the Pacific Ocean: Vertical and horizontal distributions largely from GEOSECS stations. *Earth Planet. Sci. Lett.* 49:441-434.
16. Bowen, V.T. and H.L. Volchok. 1980. Spiked sample standards: Their uses and disadvantages in analytical quality control. *Environ. Int.* 3:365-376.