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National Status and Trends Program for Marine Environmental Quality: Benthic Suveillance and Mussel Watch Projects Sampling Protocols

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Rockville, Maryland
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NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
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SAMPLE PROTOCOLS - BENTHIC SURVEILLANCE

Site and station designations. A National Benthic Surveillance Project site bears the name of the general coastal or estuarine region sampled, and is defined by the individual stations in that area where bottom sediments are obtained. In most cases, three stations comprise a site. Trawls for bottomfish are conducted as close to the sediment stations as possible.

Sediment samples. Sediment samples are obtained by a specially constructed box core device or a standard Smith-MacIntyre bottom grab. National Benthic Surveillance Project sediment samples are analyzed for selected organic compounds, trace elements, total organic carbon, moisture content, and particle size. NOAA compiled a field sampling manual (Lauenstein and Young, 1986) to ensure uniformity for sediment and fish sample collection.

Organic analyses - Surface skims taken from the top 3 cm of three separate box core or grab samples are composited in the laboratory to yield one sediment sample per station. Composites are represented by approximately equal weights of material from each of the three skims. Skim samples are extracted and processed wet; a separate aliquot is used to determine dry weights.

Major and trace element analyses - Three cores from each box core or grab are composited to produce a single sample per station. Prior to compositing in the laboratory, overlying water is drained from the plastic cylinders containing the frozen core samples by piercing the lower end caps and allowing the cores to thaw in a vertical position. Suspended material in the water settles onto the surface of the sediment as the water drains. The top 3 cm of each drained core is extruded, and the outermost cylindrical layer is removed and discarded. The remainder is dried separately and homogenized before compositing with equal weights of material from the other two core samples.

Fish tissue samples. Contaminant concentrations are measured in three biological matrices for the National Benthic Surveillance Project: fish liver tissue, fish stomach contents, and fish bile. Dissection and preservation protocols designed to minimize risk of contamination are strictly observed in the field.

Fish liver tissue - Chlorinated organic compounds and trace elements are analyzed in target fish species liver samples. For the organic analyses of liver samples collected along the Pacific and northeast Atlantic coasts, three composites of ten livers each are prepared. Because target fish are smaller on the southeast Atlantic and Gulf coasts, individual composites are derived from between ten to twenty livers. For trace elements and iron in fish liver, measurements are performed on ten individual livers at each site.

Fish stomach contents - Chlorinated organic compounds, aromatic hydrocarbons, and trace element analyses are performed on target fish stomach contents at each site. One measurement per site results from the analysis of a single composite of the contents of ten individual stomaches.

Fish bile - Metabolic products of aromatic hydrocarbons are measured in target fish bile samples. Bile samples at a site can consist of discrete contributions from ten individual fish, or one to three composites of ten individuals each, depending upon species and gall bladder size (Krahn, 1984).

Storage temperatures. All sediment and tissue samples collected for chemical analysis are shipped frozen from the field to the laboratory, and are stored at -80^{o} C.

SAMPLE PROTOCOLS - MUSSEL WATCH

Site and station designations. A National Mussel Watch Project site bears two names, a location and a site name. The location name refers to a general geographic area (e.g. Boston Harbor). The site name defines a more specific location within the location designator (e.g. Boston Harbor, Deer Island). In most cases, bivalve populations, that are sampled, are stable and so the same population may be sampled year after year. Each site is comprised of three bivalve mollusc and three sediment stations. Sediment collections are made as close to the bivalve collection site as possible.

Sediment samples. Sediment samples are obtained by a specially constructed Kynar-coated Young modified Van Veen bottom grab or a stainless box corer. National Mussel Watch Project sediment samples are analyzed for selected organic compounds, trace elements, total organic carbon, dry weight, coprostanol, and particle size.

Organic analyses - Surface skims taken from the top 1 cm of three separate box core or grab samples are composited in the field to yield one sediment sample per station. Composites are represented by approximately equal weights of material from each of the three skims. Samples are stored in either Teflon jar or glass jars with an aluminum foil lid liner.

Major and trace element analyses - Surface skims are once again taken with samples stored in either Teflon jars or in ziplock bags. Station samples are composited in the field. Samples for major and trace elements, and organic analyses are frozen in the field for shipment back to the laboratory.

Bivalve mollusc samples. Whole tissue samples are analyzed for contaminant concentrations. Bivalves are not shucked in the field rather they are separated when found to be adhering to each other (as may be the case with oysters) and scrubbed with a nylon or natural fiber brush to remove adhering detritus. Cleaned samples are then prepared for shipment back to the laboratory, which usually entails bagging and freezing in dry ice. The exception to this are the gonadal index samples which are shucked and preserved immediately after collection. Both mussels and oysters are collected for the Mussel Watch Project. Mussels are taken along the West Coast and the East Coast (north of Delaware Bay). The Gulf Coast and remaining East Coast (Delaware Bay and south) provide oyster samples.

A composite sample of 30 mussels are analyzed for organic contaminants and major and trace elements at each station. Methods used for oyster samples are the same with the exception that only 20 specimens are used to quantify each analyte group. For both species 10 individuals per station are analyzed to determine the populations gonadal index.

ANALYTICAL PROTOCOLS

Compounds analyzed in the National Status and Trends Program are summarized in Tables 1-4. Tables 5 & 6 show matrices, units of measurement, general analytical methodologies employed, and references for methods.

The methods used for the analysis of organic chemicals in sediment, and tissue samples are described in a technical report prepared by NOAA's National Analytical Facility (MacLeod *et al.*, 1985) and subsequent revisions.

Analytical methods used for the analysis of trace metals and iron in fish liver tissue are described in an unpublished manual developed by the National Marine Fisheries Service (NMFS), Beaufort Laboratory in conjunction with the NMFS, Sandy Hook and Seattle Laboratories. Tissues are prepared for analysis through digestion in concentrated nitric acid within Teflon-lined Parr bombs (NMFSa).

Analytical methods for the analysis of major and trace elements in sediment, collected as part of the Benthic Surveillance Project, are described in a manual in preparation by the NMFS Sandy Hook Laboratory. Sediments are digested in concentrated hydrofluoric acid within Teflon-lined Parr bombs (NMFSb).

Table 1. Major and trace elements analyzed in the National Status and Trends Program.

Symbol	Element
Al	Aluminum
Si	Silicon
Cr	Chromium
Mn	Manganese
Fe	Iron
Ni	Nickel
Cu	Copper
Zn	Zinc
As	Arsenic
Se	Selenium
Sn	Tin
Sb	Antimony
Ag	Silver
Cd	Cadmium
Hg	Mercury
Pb	Lead

Table 2. Organic compounds (aromatic hydrocarbons) analyzed in the National Status and Trends Program.

Aromatic Hydrocarbon	CAS Number	Alternate Name
Acenaphthene	83-32-9	1,2-dihydroacenaphthalene
Anthracene	120-12-7	Paranaphthalene
Benz[*a*]anthracene	56-55-3	1,2-benzanthracene
Benzo[*a*]pyrene	50-32-8	3,4-benzpyrene
Benzo[*e*]pyrene	192-97-2	1,2-benzpyrene
Biphenyl	92-52-4	Diphenyl; phenylbenzene
Chrysene	218-01-9	1,2-benzphenanthrene
Dibenzanthracene	414-29-91	Dibenz[*a,h*]anthracene
2,6-Dimethylnaphthalene	581-42-0	---
Fluoranthene	206-44-0	1,2-(1,8-naphthalene)benzene
Fluorene	86-73-7	*o* -biphenylenemethane
1-Methylnaphthalene	90-12-0	---
2-Methylnaphthalene	91-57-6	---
1-Methylphenanthrene	832-69-9	---
Naphthalene	91-20-3	---
Perylene	198-55-0	Dibenz[*de,kl*]anthracene
Phenanthrene	85-01-8	---
Pyrene	129-00-0	Benzo[*def*]phenanthrene

Table 3. Organic compounds (pesticides) analyzed in the National Status and Trends Program.

Chlorinated Pesticide	CAS Number	Alternate Name
Aldrin	309-00-2	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4;5,8-dimethanonaphthalene
alpha-Chlordane (cis-chlordane)	5103-71-9	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*h*- indene
2,4'- DDD	53-19-0	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]-benzene
4,4'- DDD	72-54-8	1,1-dichloro-2,2-bis (*p*- chlorophenyl)ethane
2,4''- DDE	3424-82-6	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl] benzene
4,4'- DDE	72-55-9	1,1'-(dichloroethenylidene)-bis (4-chloro-benzene)
2,4'- DDT	58633-27-5	1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl] benzene
4,4'- DDT	50-29-3	1,1'-(2,2,2-trichloro-ethylidene)bis[4-chloro-benzene]
Dieldrin	60-57-1	3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth-[2,3-*b*]-oxirene
Endrin	72-20-8	1,2,3,4,10,10-hexachloro-6,7,-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo,endo-1,4:5,8-dimethanonaphthalene
Heptachlor	76-44-8	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Heptachlor epoxide	1024-57-4	1,4,5,6,7,8,8-heptachloro 2,3-epoxy-3a,4,7,7a-tetra-hydro-4,7-methanoindane
Hexachlorobenzene	118-74-1	---
Lindane (gamma-BHC)	58-89-9	gamma-hexachlorocyclohexane
Mirex	2385-85-5	1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1*h*-cyclobuta-[*c,d*]-pentalene
trans- Nonachlor	39765-80-5	(1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*h*- indene

Table 4. Organic compounds (polychlorinated biphenyls) analyzed in the National Status and Trends Program.

Polychlorinated Biphenyl	CAS Number	Alternate Name
Dichlorobiphenyls	---	---
Trichlorobiphenyls	---	---
Tetrachlorobiphenyls	---	---
Pentachlorobiphenyls	---	---
Hexachlorobiphenyls	---	---
Heptachlorobiphenyls	---	---
Octachlorobiphenyls	---	---
Nonachlorobiphenyls	---	---

Table 5. Analytical parameters for the Benthic Surveillance Project.

Parameter	Matrix	Units[a]	Method	Reference
Organic Compounds[b]				
Pesticides, PCBs	Tissue	ng/g	GC/ECD	c
	Sediment	ng/g	GC/ECD	c
PAHs	Stomach contents	ng/g	GC/FID	c
	Sediment	ng/g	GC/FID	c
Coprostanol	Sediment	ng/g	GC/FID	c
Major and Trace Elements				
Ag, As, Cd, Cr, Ni, Pb, Sb, Se, Sn	Tissue	ug/g	Graphite AA	d
Fe, Mn, Cu, Zn,	Tissue	ug/g	Flame AA	d
Hg	Tissue	ug/g	Cold vapor AA	d
Si, Al, Fe	Sediment	%	Flame AA	e
Cr, Zn, Mn	Sediment	ug/g	Flame AA	e
Se	Sediment	ug/g	Hydride AA	e
Ag, As, Cd, Cu, Ni, Pb, Sb, Sn,	Sediment	ug/g	Graphite AA	e
Hg	Sediment	ug/g	Cold vapor AA	e
C. perfringens	Sediment	spores/g	Plate count	f

a All contaminant units are dry weight.

b Confirmation of values near detection limit is made with GC/MS.

c MacLeod, W.D., *et al.* 1985. Standard Analytical Procedures of the NOAA National Analytical Facility, 1985-6: extractable toxic organic compounds, 2nd Edition. NOAA Tech. Memo NMFS F/NWC-92, 121 pp.

d NMFSa. Analytical Methods for Trace Elements in Fish Liver Tissue. Unpublished manuscript. NOAA, National Fisheries Service, Southeast Center, Beaufort, NC.

e NMFSb. Analytical Methods for Trace Elements in Marine Sediments. Unpublished manuscript. NOAA, National Marine Fisheries Service, Northeast Center, Highlands, New Jersey.

f Bisson, J.W. and V.J. Cabelli. 1979. Membrane filter enumeration method for *Clostridium perfringens*. Appl. and Environ. Microbiol. 37: 55-6.

Table 6. Analytical parameters for the Mussel Watch Project.

Parameter	Matrix	Units[a]	Method	Reference
Organic Compounds				
Pesticides, PCBs	Sediment	ng/g	GC/ECD	b
	Tissue	ng/g	GC/ECD	b
PAHs	Sediment	ng/g	GC/FID-MS	
	Tissue	ng/g	GC/FID-MS	
Coprostanol	Sediment	ng/g	GC/FID	b
Major and Trace Elements				
Ag,Cd,Cr,Ni,Pb,Tl	Sediment	µg/g	Graphite AA	c
Ag,Cd,Tl	Tissue	µg/g	Graphite AA	c
Cr,Ni,Pb	Tissue	µg/g	X-Ray Fluoresence	c
Mn,Cu,Zn	Sediment	µg/g	X-Ray Fluoresence	c
Mn,Cu,Zn	Tissue	µg/g	X-Ray Fluoresence	c
Hg	Sediment	µg/g	Cold vapor AA	c
Hg	Tissue	µg/g	Cold vapor AA	c
Si,Al,Fe	Sediment	%	Graphite AA/Flame AA X-R Fluoresence	c
Si,Al,Fe	Tissue	µg/g	X-Ray Fluoresence	c
As,Sb,Se,Sn	Sediment	µg/g	X-R Fluoresence Hydride generation	c c
As,Sb,Se,Sn	Tissue	µg/g	Neutron Activation	c

[a] All contaminant units are dry weight.

[b] MacLeod, W.D., *et al.* 1985. Standard Analytical Procedures of the NOAA National Analytical Facility, 1985-6: extractable toxic organic compounds, 2nd Edition. NOAA Tech. Memo NMFS F/NWC-92, 121 pp.

c Battelle New England, 1986. Work/Quality Assurance Project Plan for Collection of Bivalve Molluscs and Surficial sediments and performance of analyses for Organic Chemicals and Toxic Trace Elements.

HISTOPATHOLOGY - BENTHIC SURVEILLANCE

Liver, kidney and gill sections from each of 30 individuals per site are excised and preserved for subsequent examination in the laboratory by histopathologists.

For histopathological analysis of liver, kidney and gill, tissues preserved in the field are routinely embedded in paraffin and sectioned at five microns (Preece, 1972). When necessary, tissues such as gills and bones are decalcified using a commercial decalcification solution prior to processing. Paraffin sections are routinely stained with Mayer's hematoxylin and eosin. For further characterization of specific lesions, additional sections are stained observing standard staining methodologies (Thompson, 1966; Armed Forces Institute of Pathology, 1968; Preece, 1972).

PROTOCOLS FOR DETERMINATION OF OTHER PARAMETERS

Taxonomic characterization of stomach content samples is conducted using quality assurance protocols developed for the EPA (Tetra Tech, Inc., 1987).

Total organic carbon is determined instrumentally, for both Benthic Surveillance and Mussel Watch Projects, with a CHN analyzer (Tetra Tech, Inc., 1986).

Sediment grain size analysis is accomplished using standard wet sieving techniques. Sediment samples are separated into two fractions: silt-clay (<64 um), and larger (Tetra Tech, Inc., 1986).

Age is determined for target fish specimens sampled for the Benthic Surveillance Project. Otoliths or other structures (e.g., scales) by which age can be estimated are collected from fish and are analyzed to determine age of specimens used for chemistry and histopathology.

HISTOPATHOLOGY - MUSSEL WATCH

Perkinsus marinus is the most important disease causing mortality in the oyster populations in the Gulf of Mexico. Incidence of infection is derived using a culture method developed by Ray (1966).

The gonadal index is derived for 30 bivalves from each Mussel Watch site. These data are primarily used to ensure that non-spawning bivalve molluscs are being collected for analysis since spawning organisms jettison gonadal associated lipids into the water, and so may be depurating themselves of lipophilic organic contaminants.

QUALITY ASSURANCE ACTIVITIES

Quality assurance (QA) protocols are an integral part of the National Status and Trends Program. QA efforts are designed to produce nationally uniform analytical results of known and accepted quality, thereby facilitating comparability among data sets. A specific objective of the QA Program is to reduce errors in measurement precision to 10 percent intralaboratory and 20 percent interlaboratory, in both sediment and tissue matrices and for both organic and trace element analyses. Attainment of this goal involves five major activities:

1. Development and use of standardized field sampling procedures and analytical protocols;
2. Conduct of interlaboratory comparisons of analytical methods;
3. Conduct of periodic quality assurance workshops;
4. Development of Standard Reference Materials (SRMs) and Reference Materials (RMs) for marine sediments and tissues;
5. Development and use of a standardized data base for QA data and information.

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