The Collection and Preservation of Open Ocean Marine Organisms for Pollutant Analysis

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Such pollutants as petroleum hydrocarbons, pesticides, and certain trace metals may exist in very low concentrations in open-ocean organisms. This, coupled with the fact that any ocean-going vessel is an abundant source of these same materials, makes the collection of uncontaminated samples for pollutant analysis very difficult. We have recently undertaken a program to examine present levels of concentration of a variety of pollutants in samples from the open Atlantic Ocean; specifically this now includes chlorinated hydrocarbons, petroleum residues, and some toxic elements in a wide variety of organisms, collected at many different places and depths, and in sediments. It is our purpose here to describe the sources of contamination that beset sampling for such purposes, and the procedures we have adopted to avoid contamination; also we present some data that we believe confirms that the procedures described are successful solutions to the problems posed.

Sources of Contamination. The ordinary day-to-day activities associated with the operation of a ship are potential sources of contamination of organisms collected from that ship. Specifically one may list as serious problems: 1) pumping of the bilge, 2) discharge of the septic tank, 3) dumping of garbage, debris or engine room waste, 4) cleaning of boilers, especially on steam ships by blowing the tubes, and 5) chipping of paint by deck personnel. In some cases either hydraulic lines and pumps or heat exchangers may operate with fluids rich in polychlorinated biphenyls (PCBs), presenting specific sources of these compounds; we are fortunate in that PCB's are not used in these ways on our own research fleet. Especially at times just following departure from insect-ridden ports, housekeeping activities in the galley or quarters may present the hazard of pesticide contamination. The fluorinated compounds present in most "spray cans" and many refrigeration systems, are often very difficult to resolve analytically from the chlorinated hydrocarbons, and thus represent an analytical

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complication that is best minimized. The hull and upper works of any ship, even in the absence of paint-chipping operations, are constant sources of paint flakes of various sizes, but our analyses have shown these not to be serious sources of chlorinated hydrocarbons. Paint flakes are perhaps the most serious problem to the trace-element analyses. Once the samples have been collected, it must be borne in mind that all plastics are more or less freely penetrated by the organic pollutants to be sought, and that many are sources of such compounds as PCBs. Conversely, glass and metals are sources of contamination to the trace-element analyst. If cleaning operations are required, it must be borne in mind that a great variety of "soaps" contain either chlorinated hydrocarbons (for bacteriostasis) or petroleum residues or both.

General Shipboard Precautions. It is essential to discuss the nature of the sampling program thoroughly with the ship's master before starting work. It is also desirable to post notices on the ship's bulletin boards in which the objectives of the sampling are described, explaining why the collecting procedures will differ from those to which the ship's crew is accustomed. An agreement should be made between the master and scientific party that none of the activities that are significant sources of contamination will be undertaken without prior cognizance of the scientists on watch; if possible, the hazardous activities should be scheduled to be well separated, in time, from any part of the pollutant-collecting or handling program. It is recognized that these activities are vital parts of the ship's functioning, but we have found that they can usually be performed at times and in places that have no measurable effect on the collection of materials for pollutant analysis. All that has mostly been required has been thorough understanding of the problem (on each side), and mutual good will.

Methods of Collecting. For the present program we have had to use a variety of collecting techniques:

a) Plankton have been collected in regular 3/4 or 1-m plankton nets, with either #6 or #10 nylon mesh netting.

b) Larger surface organisms (Sargassum, squid, flying fish) have been collected by dip netting or jigging.

c) Still larger organisms (dolphin, shark, swordfish) have been collected by hook and line or by long-lining.

d) Mesopelagic organisms have been collected with Isaacs-Kidd mid-water trawls.

e) Benthic organisms have been collected with anchor dredges or beam trawls.

f) Sediments have been collected with a 21-cm diameter corer of special design (1).

Each of these operations has special problems of its own, but some general precautions are common to most; unless otherwise specified these are more pertinent to organic than to inorganic pollutant analyses:

General Precautions:

1. Once the samples are retrieved they must be carefully handled and quickly transferred to the containers in which they will be preserved.

2. Specimens should never, and collecting gear should as little as possible come in contact with the surfaces of the ship.

3. Neither samples nor gear should be washed with the ship's salt water.

4. Hands, tools, sorting trays and storage containers should be liberally washed with pharmaceutical-grade 95% ethanol (prepared from ethylene hydration, if possible); the surfaces of large organisms that may have been contaminated during landing also should be washed thus. We have found that, in an emergency, good quality 190-proof white rum is an acceptable substitute, at least in respect to analysis of chlorinated hydrocarbons. This unusually serendipitous observation was very gratifying.

5. Handling and sorting gear must be of metal, glass or enamel; heavy-duty aluminum foil is very useful to cover other sorts of surfaces.

6, Preservation must be at deep-freeze temperatures (minus 10° to minus 20° C), and in glass or metal; glass screw-cap jars are useful only if under-cap-liners are made of washed aluminum foil. Large organisms or organs may be securely wrapped in heavy-duty ethanol-washed aluminum foil. As far as possible, the freezer used for "pollutant-study" samples should be reserved for this purpose.

Special Precautions:

a) Plankton. To our surprise we have no evidence that use of our usual polyvinyl chloride (PVC) cup and ring (with nylon netting sock to retain the plankton) is contraindicated for "pesticide" collecting; so far we have compared results using the PVC cups and rings, with an all-copper facsimile, and found no systematic difference. For assurance, however, we recommend the use of metal cups and rings.

The accumulation of paint flakes from the ship's hull in plankton samples is a serious problem for trace-metal measurements (possibly for some other pollutants) on samples collected in the upper 50 m. This problem may be overcome by using closing-nets for deep collections or by using an extensible boom to place nets to one side of the ship, clear of the bow wave, for shallow collections. For any analysis other than chlorinated hydrocarbons, a plankton sample should be summarily discarded if it exhibits visible fragments of paint.

Upon completion of a plankton tow the net is not washed down because the ship's sea water system may be made of PVC or copper piping. The plankton sock is thoroughly drained, carefully everted and the "cake" of plankton is transferred from the sock to a preserving bottle by means of a spatula.

b) and c) Large organisms: Dip nets, jigs, hook and line or long-line fishing gear should be kept as clean as possible. A sample should, ideally, be transferred directly to a preserving container or dissecting tray (see washing instructions above); in the cases of such lively fish as dolphin, or of large creatures like sharks, contact with the vessel's deck is usually unavoidable, and appears to be tolerable so long as the specimen surface is cleaned promptly and thoroughly.

d) Mid-water trawl specimens are shaken directly from the trawl's cod-end to a large enamel or aluminum sorting tray. It has been convenient to keep a few gallons of sea-water freshly dipped from the vessel's down-wind side, for sluicing of the end of the trawl; organisms collected from cold, deep water stay healthy longer when this sluicing water is well chilled (5° C or so).

Sorting and measuring of trawled specimens for pollutant analysis should be done as quickly as possible, especially since many oil-storing invertebrates

begin to "leak" as a result of trauma and present sources of cross-contamination.

e) Benthic organisms can rarely be retrieved even so cleanly as are the trawled mid-water creatures. It is likely that the surfaces of specimens from dredges or beam trawls will always require thorough rinsing before preservation. For cleaning of surface mud, rinsing with freshly dipped sea water (see above "e") will be desirable before alcohol washing.

f) Sediment samples collected with the 21-cm diameter corer are transferred as quickly as possible to a deep freeze in which the core-tube can be kept upright until fully frozen. After freezing, the ends of the tube are cleaned and covered with washed aluminum foil; the tubes can then be stored horizontally, still frozen, until wanted. The cores are extruded from the tube by thawing slightly with a hot-air-jet, and are then cut with a band-saw into segments 1-cm, or more, thick. Trimming the outer half-centimeter or so of the 21-cm diameter slices, using the same sort of precautions that are involved in sorting fresh specimens, yields specimens with minimum likelihood of contamination.

Analytical Data. In Table 1 are shown data from the analyses for chlorinated hydrocarbons of a variety of specimens collected and handled as described above; a fuller report and discussion of these results is in press (2). It appears to us that the fact that we have been able to collect and retrieve for analysis specimens exhibiting as little as 0.01 µg DDT per kilogram fresh weight, and as little as 5 (or less) µg PCB per kg, confirms that the precautions outlined have been successful; as we have discussed elsewhere, the internal consistency of the data also argue strongly against their having been affected by specimen contamination.

Unpublished data showing hydrocarbon concentrations (Teal and Burns, personal communication) are also available for some of these samples. It is our feeling that these values also confirm minimum specimen contamination.

Conclusions: The set of precautions outlined above, carefully adhered to, appears sufficient to ensure a high yield of open-ocean samples uncontaminated by materials deriving from the ship, the collectors, or the laboratory environment. Although the precautions may seem overly cautious, we are convinced that few,

TABLE 1

Chlorinated Hydrocarbons in North Atlantic Organisms (µg per kg fresh weight)
(Values in brackets are µg/kg lipid)

Sample #	Identification	Date Collected	Position	Total DDT	PCB (as Aroclor 1254)
A II-59-29	Sargassum	Nov. 70	26°N; 36°W	0.5	10
A II-59-39	Sargassum	Dec. 70	35°N; 48°W	0.2	20
A II-59-22	Zooplankton (#6 mesh)	Nov. 70	23°41'N; 34°29'W	<0.01	300
A II-59-36	Zooplankton (#6 mesh)	Dec. 70	30°52'N; 47°30'W	<0.01	450
K-19-4-10	Zooplankton (#6 mesh)	March 71	30°N; 60°W	.7	110 (19300)
K-19-4-28	Zooplankton (#6 mesh)	April 71	32°N; 64°W	9.5	7 (925)
A II-59-1	Flying Fish (*Cypselurus exsiliens*) (muscle)	Nov. 70	14°N; 19°W	0.6 (179)	1.4 (410)
K-19-4-17	Flying Fish (*Prognichthys rondeletii*) (muscle)	March 71	30°N; 60°W	<4 (1480)	4

TABLE 1 (Continued)

Sample	Identification	Date Collected	Position	Total DDT	PCB (as Aroclor 1254)
A II-59-14	Trigger Fish (*Canthidermis maculatus*) (muscle)	Nov. 70	19°N; 30°W	0.1 (120)	1.9 (1900)
A II-59-11	Dolphin (*Coryphaena equiselis*) (liver)	Nov. 70	17°N; 28°W	95 (1990)	1056 (21,000)
A II-59-27	Dolphin (*Coryphaena hippurus*) (muscle)	Nov. 70	25°N; 36°W	3 (3300)	10 (10,000)
A II-59-2	Shark (*Carcharhinus longimanus*) (liver)	Nov. 70	14°N; 22°W	100 (206)	300 (620)
A II-59-33	Mesopelagic Fish (*Chauliodus danae*) (5, whole)	Dec. 70	28°N; 45°W	12 (1510)	59 (7300)
A II-59-34	Mesopelagic Crustacean (*Systellaspis debilis*) 17, whole)	Dec. 70	28°N; 45°W	5.7 (169)	35 (1040)

if any, of them could safely be omitted. If one were collecting from a vessel using PCB-rich hydraulic fluid, for instance, new precautions would be indicated.

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