Report of the first session of the

WORKING PARTY ON BIOLOGICAL ACCUMULATORS of the
ADVISORY COMMITTEE ON MARINE RESOURCES RESEARCH

Rome, 9-13 December 1974

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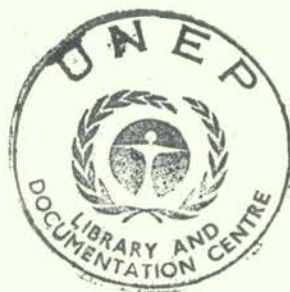United Nations Environment Programme

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

UNITED NATIONS ENVIRONMENT PROGRAMME

REPORT OF THE FIRST SESSION OF THE
FAO. Advisory Committee on Marine Resources Research.
ACMRR WORKING PARTY ON BIOLOGICAL ACCUMULATORS

FAO Headquarters, Rome, Italy, 9-13 December 1974

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, March 1975

PREPARATION OF THIS REPORT

This report was prepared as part of a co-operative project of the United Nations Environment Programme entitled

Effects of Pollutants on Living Aquatic Resources and Scientific Basis for Monitoring

with the Food and Agriculture Organization of the United Nations as co-operating agency.

CONTENTS

REPORT OF THE
FIRST SESSION OF THE ACMRR WORKING PARTY
ON BIOLOGICAL ACCUMULATORS

Rome, Italy, 9-13 December 1974

1.   OPENING OF THE SESSION

The first session of the ACMRR Working Party on Biological Accumulators was held at FAO Headquarters, Rome, from 9 to 13 December 1974, under the chairmanship of Dr. J.E. Portmann. Dr. Kasahara, Director, Fishery Resources and Environment Division, welcomed the members (listed in Annex III) to Rome and opened the session on 9 December.

2.   ADOPTION OF THE AGENDA

The Agenda (Annex I) was adopted without changes.

3.   ELECTION OF RAPPORTEUR

Dr. R.F. Addison was elected Rapporteur of the session.

4.   DISCUSSION OF TERMS OF REFERENCE

The meeting opened with a general discussion of the Terms of Reference (Annex II) and their interpretation. It was generally accepted that pollutant levels in sea water might be assessed through analyses of their concentrations in "accumulator" organisms; in this context, the GESAMP definition of marine pollution was accepted, which reads:

"Introduction by man, directly or indirectly, of substances or energy into the
   marine environment (including estuaries), resulting in such deleterious effects
   as harm to living resources, hazard to human health, hindrance to marine
   activities, including fishing, impairing the quality for use of sea water and
   reduction of amenities."

It may be agreed that analyses of organisms might offer some advantages over direct analyses of sea water: these included (a) simplification of analyses through an organism's concentration of pollutants to levels often some orders of magnitude greater than those in sea water; (b) the fact that pollutant levels in an organism may have more significance than those in sea water, since the latter may not indicate concentrations which are biologically available; (c) if the accumulator organisms selected are also used as food or in the production of food stuff, their analysis would provide information relevant to food contamination, as well as to environmental monitoring. However, it was recognized that the value of accumulator organisms might change; thus the present advantages offered by their concentration of pollutants might be offset by improvements in procedures for the direct analysis of sea water. And even at present, direct analysis of sea water (or sediments) was preferable for some pollutants.

Unless calibrated by measurements of levels in water (usually necessary on a site by site basis) analysis of pollutant levels in accumulator organisms seemed capable of providing comparative, rather than absolute, information. Since accumulation of pollutants varied with several environmental factors, it might be difficult to infer a pollutant's concentration in sea water from its concentration in an organism. However, it should be possible to detect spatial differences or temporal changes in environmental quality through such analyses. Dr. Naeve pointed out that FAO was presently involved in the planning of the GFCM Coordinated Mediterranean Pollution Project including pilot projects on baseline studies and monitoring of metals and persistent organic compounds in marine organisms and that the Working Party's recommendations would be useful in that and in similar programmes.

Finally, the members felt that some riders should be attached to the Terms of Reference.

(a)   Referring to paragraph ii, since no accumulator organism was likely to be distributed globally, it would be difficult to recommend a "standardized" organism although the possibility of using well-defined bacterial cultures would be discussed. However, different organisms could be used to monitor different areas, provided some overlap in range occurred - this would allow comparison of different organisms in the same environment.

(b)   The terms "standardization" in paragraph iii implied specific and highly detailed analytical directions (recipes), whereas it should be recognized that analytical procedures are invariably modified to suit the particular facilities available, or the demands of a particular analytical problem: general analytical guidelines rather than specific recipes should, therefore, be provided. But, since analytical procedures would vary from laboratory to laboratory, the importance of intercalibration exercises should be stressed.

(c)   Specifically paragraph iv would be interpreted as "To advise on laboratory and field studies aimed at calibrating the accumulation capacity of selected species, to be used as a sensitive tool for indirectly measuring the low concentrations in the water".

5.   REVIEW OF WORKING PAPERS AND GENERAL DISCUSSION

The background papers prepared for the meeting (see list of documents, Annex IV) were then discussed, after each had been summarized by its author. The purpose of environmental monitoring was considered. It was agreed that one intention was to provide an indication of the risk of future trouble, since an increase in pollutant level in an organism, or its environment, would constitute a danger signal - even if the pollutant was not known to have any specifically detrimental biological effect. It was suggested also that monitoring implied control at some stage; this, in turn, implied that there should exist criteria of acceptable levels of pollutants, and these should be related to biological effects, such as those summarized in bioassay results, or in ecological indices. This connexion reflects the relationship of this Working Party to others dealing with bioassays and ecological indices. It was agreed that, since it was impossible to screen the entire marine ecosystem for all possible compounds, some selection had to be made: this selection would have to be based on either known or predicted biological effects. Thus, anticipation of biological effects through appropriate biological indices appeared particularly important, since only after such an effect is detected, or predicted, would it make sense to undertake a monitoring programme.

Discussion then turned to experience and problems in using accumulator organisms to detect and measure specific pollutants in the marine environment. Such monitoring programmes were already under way. Thus, routine detection of certain radio nuclides in the marine environment was based on their uptake by certain molluscs and macrophytes. Although such uptake varied with environmental factors, the concentration factors achieved (of the order of 10^5) were crucial in allowing the detection of such elements. However, since macrophytes are restricted to the euphotic zone, their application as accumulators is limited. As the accumulation of metals by macrophytes is based on their functioning essentially as ion-exchange resins, it may turn out that a non-biological ion-exchange accumulator would be more useful.

The possibility of using plankton for pollutant accumulation attracted some interest. Phytoplankton appear to reflect short-term fluctuations in organochlorine residue input to their environment, and so could be used to monitor this. This accumulation seems to be a passive adsorption process. Where accumulation involves active metabolism, as is the case with some metals, plankton are less suitable because accumulation factors vary widely with environmental conditions, and with species. Thus, natural plankton communities, whose composition and environment may differ widely, show highly variable concentrations of (presumably) accumulated metals. A particular problem in plankton analysis is that of contamination of samples by the surface film during towing: the materials analysed (especially organics) may reflect their distribution in the surface film rather than in the plankton.

Benthic invertebrates have been used successfully in environmental monitoring, for metals, organochlorines and petroleum hydrocarbons. <u>Mytilus</u> seemed to be most widely used, perhaps because of its ubiquity. Being sessile, its accumulation of pollutants reflected the exposure of its local environment. It was, however, noted that it may be preferable to have information about a particular body of water rather than about a point past which it flowed and, in this case, an organism associated with that body of water, e.g., a pelagic accumulator, would be required. Like other accumulators, molluscs showed varying efficiencies in accumulation in response to environmental factors, and considerable calibration work to define these factors would be desirable.

One attempt to develop a rather "standard" accumulator organism used marine bacterial cultures. However, these were not used <u>in situ</u>; instead, the water to be analysed had to be brought to them. This necessitated sampling and transporting 10 to 20-litre water samples, which would have therefore been available for conventional analyses; the main advantage of using bacteria to isolate pollutants from the water was that they would indicate pollutant levels which were "biologically available".

Marine vertebrates have been used to accumulate pollutants from various environments. Thus, metals, e.g., Hg, are accumulated by fish and some observed variations in tissue concentrations were consistent with expected differences in the areas from which fish had been sampled. Similarly, organochlorine levels in fish can show clear spatial or temporal variations, consistent with expected differences between the areas or periods at which the fish were sampled. Similar comparisons could be made with marine mammals and it was suggested that repeated sampling from a "tagged" marine mammal might allow fairly sensitive detection of temporal changes. A particularly interesting example involved comparison or organochlorine levels in Baltic herring sampled from different regions. Even though samples were taken from commercial catches and were not deliberately selected to minimize the possible effects of known biological factors which affect residue accumulation, clear regional and temporal differences could still be seen. As noted above, such analyses provided data useful to both environmental monitoring and food contamination surveys.

To summarize, it seemed clear that to monitor differences in environmental quality from place to place, or from time to time, is practicable, at least for some pollutants. The choice of accumulator organism and the frequency of sampling would be dictated by the kind of information required. Thus, short-term (of the order of days or weeks) fluctuations in organochlorine input to an area could be detected through phytoplankton analyses, whereas examination of longer-term trends would require a longer-lived organism. Likewise, regional differences of the order of 100-1,000 km could be detected through comparisons of fish, provided their migrations were small compared to the scale of regions being compared. The Baltic herring study mentioned above allowed comparisons of locations of the order of 100 km apart. For smaller-scale regional comparisons, sessile organisms such as fixed macrophytes, or sessile molluscs, would be preferred.

Petroleum hydrocarbons appeared to be somewhat less amenable to monitoring via biological accumulators than are, say, chlorinated hydrocarbons or metals. First, various hydrocarbons occur in natural components of various organisms. As they generally exist in very complex mixtures, more scope for selective accumulation, degradation and elimination of different pollutant compounds exists than is the case for other pollutants.

Furthermore, the petroleum hydrocarbons appear to be degraded or eliminated unchanged from various organisms relatively rapidly; concentration factors may, therefore, be lower and any accumulation might be more transient than that observed with other pollutants. Nevertheless, some correlations have been noted between hydrocarbon levels in bivalves and their environment. Specific aromatic compounds could be used as tracers for petroleum detection.

Finally, it was clear that if reliable estimates of seawater concentrations of pollutants were to be made from analysis of accumulator organisms, considerable development and calibration work must be carried out. This should include an examination of several candidate species from a range of trophic levels and laboratory studies on their capacity to accumulate, degrade and eliminate their pollutant burden.

The Chairman and Technical Secretary of the Working Party on Ecological Indices summarized their conclusions for this Working Party at the end of their meeting. Topics and problems of mutual interest were identified.

6. OUTLINE OF A FIELD MANUAL

The Working Party decided that its views and recommendations could be presented in the form of a guideline manual on the use of bioaccumulation in monitoring programmes. An outline of the contents of this was prepared (Annex V), listing the topics to be covered and the areas of responsibility of each author.

7. FURTHER WORK PROGRAMME

It was anticipated that the various sections would be completed by the members during the intersessional period and that a draft of the manual would be available to the secretariat end of April 1975.

8. DATE AND PLACE OF NEXT SESSION

It was proposed to hold the second session of the Working Party again at FAO Headquarters, Rome. The week of 14-18 July 1975 has been envisaged tentatively.

It was recommended that the second session should be devoted primarily to the finalization and adoption of the Guideline Manual.

9. ANY OTHER MATTERS

No other matters were discussed.

10. ADOPTION OF THE DRAFT REPORT

The draft report was adopted on 13 December 1974. Editorial changes were left to the secretariat.

11. CLOSING OF THE MEETING

Dr. Kasahara thanked the members of the Working Party for their constructive work and closed the session on 13 December 1974.

AGENDA

1. Opening of the Session

2. Adoption of the Agenda

3. Election of Rapporteur

4. Discussion of Terms of Reference

5. Review of Working Papers

6. Outline of Field Manual

   (a) Ongoing Programmes
   (b) Bioaccumulator Species
   (c) Standard Working Methods
   (d) Calibration of Accumulation Capacities
   (e) Accumulation Methods in Monitoring

7. Further Work Programme

8. Date and Place of Next Session

9. Any Other Matter

10. Adoption of Draft Report

11. Closing of the Meeting

TERMS OF REFERENCE

As a basis for using bioaccumulators in monitoring programmes to detect contaminants in the marine environment, especially at low level concentrations, taking into consideration both the methods based on the sampling of natural populations and the development of standardized, even clonal, organisms;

(i)   to review on-going research and operational programmes, and present knowledge in the field of biological accumulation of contaminants;

(ii)   to evaluate bioaccumulator species presently used for detection of pollutants and to advise on other organisms suitable for standardized use in monitoring programmes;

(iii) to recommend standardized working methods with emphasis on sampling and analytical techniques and experimental procedures;

(iv)   to advise on laboratory and field studies aimed at calibrating the accumulation capacity of selected species, to be used as a sensitive tool for measuring low concentrations of pollutants;

(v)   to develop guidelines for pilot studies aimed at introducing bioaccumulation methods into monitoring programmes.

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LIST OF DOCUMENTS

ACMRR/WP-BA/1/1   Draft Agenda

2   Terms of Reference

3   List of Participants

4   List of Documents

5   Factors Affecting Organochlorine Residues Accumulation
by Marine Vertebrates
by R.F. ADDISON

6   Bioaccumulation and Metabolism of Organic Pollutants
in the Sea
by R.F. LEE

7   Primary and Secondary Producers and Transport of
Contaminants in the Marine Environment
by E.F. MANDELLI

8   Bioaccumulators of Radionuclides and Heavy Metals
by J. PENTREATH

9   The Use of Biological Accumulators in Monitoring
by D.J. REISH

10   Monitoring and Bioaccumulation
by J.E. PORTMANN

11   Feasibility of Using Benthic Organisms as Indicators
of Bioaccumulation
by T. YOSHIDA

12   Draft Report

DRAFT OUTLINE OF THE GUIDELINE MANUAL ON USE
OF BIOACCUMULATORS

A. **Introduction** (J.E. PORTMANN)

1. Purpose of monitoring — i.e. in order to establish and follow the spatial and temporal variations in contaminant levels regardless of whether or not there is an initial intention or need to control.

2. Definitions — e.g. pollution, contamination, monitoring/surveillance, bioaccumulation.

3. Why use a bioaccumulator since often analytical techniques are capable of detecting extremely low levels in the aquatic medium:

   (i) Because the level in the water may be undetectable and there is a variation in what is detectable according to laboratory

   (ii) In order to be able to monitor otherwise undetectable discharge levels

   (iii) To detect unexpected contaminated areas or discharges, especially when the levels of the contaminants are too low to measure otherwise

   (iv) In order to be able to assess relative levels of contamination area by area

   (v) Because a bioaccumulator integrates water levels which may change with time

   (vi) Because, say, a bioaccumulator is more likely to reflect the biologically significant forms of a contaminant.

4. Relationship of level found to effect. There is for all contaminants a level of presence which will constitute a lethal concentration and a lower concentration which will cause a sub-lethal effect in an organism. There is equally a no-effect level, i.e. the one at which an effect is insignificant. Presence does not equal harm, although it is a danger signal.

5. Data must be intercomparable and, whenever possible, should be obtained only for a specific purpose. Mere measurement, especially on a large scale, is wasteful of time and resources.

6. Types of bioaccumulation mentioned in Lee paper (ACMRR/WP-BA/1/6). Note that in some cases bioaccumulation is a direct result of detoxification, e.g. Cd and Pb in crustaceans.

7. In the regional or international context, monitoring/surveillance is largely a matter of establishing temporal and spatial trends.

8. It does not follow that spatial trend shown by the bioaccumulator is the same as that shown by the aquatic medium. Relative distribution may differ from site to site and, for some purposes, may have no alternative but to measure water concentration as well, at intervals.

9. For a full understanding of any one environmental situation there is a need also for data on inputs; contaminant concentrations both in sediment and in water and relative concentration in other organisms, especially ultimate target organism.

10. Basic requirements of a good bioaccumulator indicator:

   (i) Concentration factor relative to water generally $> 10^2$

   (ii) Concentration affected by minimum number of biological variables

   (iii) Accumulation linear with time at least for a number of days

   (iv) Numbers available readily at appropriate times and collection unaffected by public immotive interest

   (v) Warning given by bioaccumulator must be adequate to allow prevention of catastrophe

   (vi) Desirable to use range of organisms.

11. Bear in mind much of our knowledge has been achieved accidentally. Much information once thought extraneous now considered valuable. Full details of sample, species, age, sex, condition, size of animal and analysed organ, etc., should be recorded plus details of any sample storage, analytical methods and detection of unexpected or unidentified substances.

12. Conclusion that any monitoring programme requires _thought_ and careful planning. Not merely matter of dash-out, collect and analyse.

B. Monitoring Different Pollutant Classes Using Bioaccumulators

   I. ELEMENTS OTHER THAN RADIONUCLIDES, e.g. MERCURY, CADMIUM, COPPER, ZINC, LEAD AND ARSENIC (E. MANDELLI)

      1. Inputs: Sources and Routes of Entry

         (i) Man-made) via air, rivers, discharges,
         (ii) Natural) dumping and dredgings
         (iii) Physical distribution in the environment
         (iv) Chemical speciation in the environment

      2. Analytical Procedures

         (i) Sampling methods
         (ii) Sample size and storage
         (iii) Analytical techniques including preparation

      3. Biological Transfer and Transformation Process Distribution

         (i) Summary of data by metal and organism
         (ii) Laboratory uptake and loss experiments
         (iii) Field surveys
         (iv) Biological transfer
         (v) Biological transformations

4. Criteria for Selection of Species and Communities

    (i)   Availability, i.e. suitable numbers available at
          appropriate times and sites
    (ii) Ecological importance
    (iii) Commercial value
    (iv) Pure bioaccumulation capacity but of no known
          ecological or commercial importance

5. Reporting of Results

    (i)   Data coding to be adopted (i.e. standardized format)
    (ii) Evaluation of results.

II. RADIONUCLIDES (J. PENTREATH)

1. Input Sources

    Air, rivers, discharges

    Note that there are natural sources of radioactivity and radioactive
    substances present naturally in the marine environment.

2. Analytical Methods

    Brief mention of counting techniques, and spectrometry, and preparation
    of samples.

3. Uptake and Loss Studies

    (i)   Laboratory. Relative importance food versus water

    (ii) Field studies including need to balance desirability
          versus practicality

    (iii) Variation in uptake characteristics according to
          speciation of radionuclide, seasonal and other factors

4. Selection of Species

    (i)   Critical Path analysis

    (ii) Algae

    (iii) Molluscs

    (iv) Fish

5. Reporting of Results

    Assessment, need to repeat observations, scale and detail.

IV. PCBs, DDT AND OTHER CHLORINATED PESTICIDES (R.F. ADDISON, S. JENSEN)

1. <u>Introduction</u>: brief

2. <u>Inputs: Sources and Routes of Entry</u>

   (i)   Use as industrial compounds and pesticides from
         1930 on

   (ii) Physical distribution mostly absorbed.

   (Note that in (i) and in all subsequent sections there are
   variations in behaviour from group to group, but a common
   distribution and behaviour based on lipophilicity.)

3. <u>Analytical Procedures</u>

4. <u>Distribution</u>

   (i)   Uptake (by group and organism)

   (ii) Elimination (by group and organism)

   (iii) Storage concentrations affected by: (a) age
                                             (b) sex
                                             (c) nutritional
                                                  status

   (iv) Metabolism (by group and organism)

   (v)   Interactions among group members.

5. <u>Species Selection</u>

   (i)   Relation to aims of programme

   (ii) Accessibility.

V. MISCELLANEOUS SUBSTANCES (S. JENSEN, R.F. ADDISON)

1. <u>Literature Study</u>

   This should review:

   (a)   Substances already known to be accumulated in organisms
         and detected in environmental samples (examples to be given)

   (b)   Substances known to be released to the environment and
         belonging to the same chemical classes as in 1(a) but not
         yet found in nature

         A note to be included that certain substances are known
         to affect the accumulation rate of other substances (e.g. ABS).

2. <u>Laboratory Experiments</u>

Under conditions close to the natural ones (salinity,
temperature, etc.) estimate:

(a) Accumulation factors or rate; a note to be included
that it will also be necessary to establish biological
effects (lethal or sub-lethal).

(b) Elimination factors and mechanisms

(If possible, elimination should be separable into
metabolised and non-metabolised fractions.)

3. <u>Selection of Substances to be Investigated</u>

(a) In the area to be monitored, a pilot study should tell
if a primarily selected substance is present at all,
using the highest degree of analytical sensitivity

(b) When the presence of a substance has been confirmed
then try to select one or more accumulator organisms
having the highest accumulation efficiency.

4. Recommend one or more analytical methods for each substance.
These methods should be both selective and sensitive.

5. Find ways to report the presence of substance not taken into
consideration in the selection of substances to be dealt with
in the monitoring programme.

6. Unprejudiced research using general methods for separating
the substances into chemical classes.

7. A general note on the fact that groups of chemicals might,
on the basis of past experience, be considered likely to be
accumulated.

C. <u>A Matrix Table</u> (to be developed at second session)

This would be included as a quick summary of the data given in detail under section B
of the manual. By this means it would be possible to indicate wherever a particular
type of bioaccumulator is suitable for monitoring a contaminant. An entry to this
effect would be made in the appropriate box in the table. It would generally, although
not always, be possible to show whether the relationship between water concentration
and that in the organism was constant, regardless of site of collection or biological
factors. It would also be possible to indicate whether the organism concerned was most
appropriate for detection of a contaminant at a single site, at several sites (i.e.,
spatial variations) or to reveal temporal changes at one or a number of sites. Whenever
it is known that there is <u>no</u> need to select an organism according to age, size, sex,
condition, etc., an appropriate entry would also be given.

An outline of the Matrix Table is attached and a draft key (not in final form) is given
below:

	Organochlorine Pesticides and PCBs	Petroleum Hydrocarbons	Metals/Metalloids Hg, Cd, Pb, Zn, Cu, As	Radionuclides Co, Fe, Mn, Cs, Ce, Ru, Zr/Nb
Plankton				
Phytoplankton				
Zooplankton				
Macrophytes				
Red				
Green				
Brown				
Molluscs, benthic				
Bivalves				
Gastropods				
Crustaceans, benthic				
Whole				
Exo Skeleton				
Digestive Tract				
Polychaetes				
Fish				
Pelagic Muscle				
Pelagic Liver				
Demersal Muscle				
Demersal Liver				
Marine Mammals				
Blubber				
Liver				
Muscle				
Brain				

Key

Suitable	Yes	X
	No	O

Constant relationship to water level,
regardless of site   CS

Constant relationship to water level,
regardless of biological factor   CB

Suitable for single site   I

Suitable for several sites   N

Reveals temporal variations   T

No need to select according to Age/Size   A

No need to select according to Sex   M/F

No need to select according to Condition   Cond.

Thus an example of an ideal entry would be:

X CS CB N T A M/F Cond.

i.e., organism is suitable, constant relation water to organism concentration
regardless of site or biological factors, can be used to reveal spatial trends
with time and there is no need to select the organism according to age/size,
sex or condition.

D. Proposed Pilot Study in Monitoring (D.J. REISH)

    I. PREPARATIONAL PHASE

        (a) Literature review

            1. Pilot study area
            2. Important worldwide studies
            3. Summarize pertinent data by pollutant,
                organism and geographical area

        (b) Assemble research capabilities of pilot study area

            1. Personnel
            2. Laboratories
            3. Ships
            4. Training and education

        (c) Summarize input sources, types and amounts of contaminants
            of pollutants

        (d) Selection of pollutants to be monitored

        (e) Selection of species to be monitored

            1. Criteria for selection

                (i)   Food chain
                (ii)   Ecological niche
                (iii) Wide distribution
                (iv)   Availability
                (v)   Commercial importance

        2. Assemble known distributional data of species
        3. Assemble existing knowledge on the biology of
           selected species

(f) Intercalibration

(g) Sampling procedures

        1. Location - to be established in light of site visits
        2. Frequency

(h) Coordination of participating laboratories

II. SCREENING PHASE

(a) Laboratory studies - uptake of pollutants

        1. Species
        2. Pollutants

(b) Field studies - testing of procedures

(c) Evaluation

        1. Species
        2. Pollutants
        3. Number of organisms in a sample

III. OPERATIONAL PHASE - PILOT PROGRAMME

(a) Data

        1. Collection
        2. Evaluation

(b) Coordination

(c) Emergency procedures

IV. SYNTHESIS AND EVALUATION OF PILOT PROGRAMME INCLUDING
    PUBLICATION OF DATA

E. Analytical Methods (T. YOSHIDA)

  1. Brief outline of essential instrumentation per pollutant
     class and what it is capable of analysing in terms of samples/week.

  2. Whether preparation is necessary and perhaps a brief outline of
     sort of procedure which would be followed, e.g.,dissolve in acid
     extract or not direct application to instrument.

  3. Size of sample likely to be needed and brief outline of basic
     preparation for the analyst, e.g., should it be wrapped in foil
     or placed in a glass jar or polythene bag, can it be preserved
     in e.g.,Formalin or deep freeze or both. If to be dissected
     prior to sending to laboratory, basic precautions.

4. Source of variability in analytical results due to <u>analyses</u>
    procedures/errors. In one laboratory and between a number of
    laboratories.

5. On an international/regional scale the need for training of
    personnel, intercalibration and source of suitable reference
    standard materials.

6. Need or otherwise to adopt the same method, i.e. precise details,
    or merely guidelines as to methods which have been found suitable
    for particular pollutants and need to report unexpected or
    unidentifiable contaminants.