International Council for the
Exploration of the Sea

C.M.1987/E:4

REPORT OF THE MEETING OF ANALYSTS PARTICIPATING IN THE HYDROCARBON INTERCALIBRATION PROGRAMME

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T A B L E O F C O N T E N T S

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1 BACKGROUND

At its meeting in 1986, the Marine Chemistry Working Group recognized that problems exist in the analysis of specific hydrocarbons, as shown by the first three intercomparison exercises (Law and Portmann, 1982; Farrington et al., 1986; Uthe et al., 1986). It was agreed at that meeting that the correct way forward was to adopt a stepwise approach over a period of years, with a first stage intended to test instrument calibration. The tentative plan prepared for this stage at that meeting is attached as Appendix 1. It was also agreed to propose that a meeting be held of analysts interested in participation in such an exercise, and who were currently using gas chromatography-mass spectrometry (GC-MS) or high-performance liquid chromatography with fluoresence detection (HPLC-UVF) techniques. This meeting to be held alongside the 1987 meeting of MCWG was to finalise details of the first stage of the proposed programme. This was subsequently approved by the ICES Council in C.Res.1986/2:16, which also stated that when the programme is underway, the costs of the reference materials used, their distribution as well as the extraordinary travel expenses of the coordinator will be met by ICES to ensure the success of this programme. Participation in the meeting and/or the proposed exercise was solicited by mail in January 1987, both via the meeting convener to individuals and by ICES to Delegates. A list of participants is given in Appendix 2.

2 INTERCALIBRATION PROPOSAL

2.1 General

It was recognized by the Group that improvements in analytical comparability were required in all matrices (water, sediment, biota). In order to prevent dilution of effort, it was agreed that for the purposes of the early stages of the programme attention would be concentrated on the measurement of aromatic hydrocarbons in biota, particularly recognising that many of the instrumental and analytical improvements would be common to analysis of all three sample types. The Group does recognize the importance of the abiotic compartment (see Appendix 3), although it is the biota which give rise to the greatest health concern.

2.2 What to Measure?

In the absence of any coordinated monitoring effort within ICES, there is no clear focus of compounds of interest. It was finally agreed, therefore, to define a primary list of compounds that may be used for monitoring and investigative purposes in the absence of evidence for the inclusion of other compounds, as has been the case for chlorobiphenyls. This list consists of 17 polycyclic aromatic hydrocarbons (PAH) selected from those fulfilling three basic criteria:

1) Compounds with 3 to 6 fused aromatic rings;

2) Compounds containing only carbon and hydrogen;

3) Only non-alkylated PAH.

This list is included as Appendix 4. (Triphenylene is included in parentheses as it poses a problem in the determination of chrysene by GC techniques which must be tackled in a later stage of the programme, but is not now a member of the primary list.)

From this list a sub-set of 10 compounds were selected for use in the first stage exercise. A list of these compounds is attached as Appendix 5, with the rationale for their selection. Compounds were also identified that have been synthesised specifically for use as internal standards in analyses conducted by capillary GC or GC-MS (Indeno[1,2,3-cd]fluroanthene) and HPLC-UVF (Benzo[b] chrysene) as they are believed not to be found in the environment from either natural or anthropogenic sources.

2.3 Prerequisites for the Exercise

Taking previous experience in intercomparison exercises into account, it is clear that unless a firm commitment to the programme is forthcoming from participants at the start of the exercise, adequate progress is unlikely to be achieved. This would include a commitment to analyse samples and report results by agreed deadlines, and to attend meetings at national expense to be held after each stage of the exercise to evaluate and learn from the results so far and to plan the next stage in detail. In addition, as a number of analytical techniques may be used in this exercise, it is necessary to establish a minimum core group of laboratories using the same technique so as to obtain a sufficient amount of data from which to evaluate the results by all techniques. In previous exercises the majority of the data have been generated by GC-MS, and current indications are that GC-MS will also be the most common technique used in the proposed exercise. It is expected that HPLC-UVF and capillary GC with flame-ionisation detection (GC-FID) will also be used by some participants.

2.4 The first Stage

It is proposed to circulate two standard solutions for analysis by participants:

Solution 1 - This will contain all 10 PAH at declared concentrations. It will be used for instrument calibration and to prepare a single set of dilutions to be used to check linearity of response over at least a decade of concentrations. The laboratories own standard mixtures should also be analysed against this standard solution. The PAH concentration as circulated should be around 50 ng per µl per component.

Solution 2 - This will contain the PAH at undeclared concentrations of 0 to 100 ng per µl per component, to be analysed by participants against solution 1 as a standard. Other compounds may also be present, making identification necessary as well as quantification.

Both solutions will be prepared in acetonitrile so as to be compatible with the three analytical techniques expected to be employed. Six replicate injections of each solution and of the dilutions used for calibration will be required.

It was decided not to use internal standards of any sort at this stage as only standard solutions are to be analysed and there is no need at this stage to assess recovery efficiency. They will certainly be included in later stages, however, as the programme approaches the analysis of real samples.

A detailed protocol to be prepared will include a requirement for supplying all primary data to the coordinator, with details of instrumentation, operating conditions, identity of analysts, and further details to be specified.

2.5 Preparation of Solutions

A number of organisations around the world have proven expertise in the preparation and verification of standard solutions such as those proposed above. Instead of purchasing pure standards of individual standards for preparation of solutions by the coordinator, it would if possible be preferable to purchase verified standard solutions for use in our programme. The solutions would then have been prepared by a thoroughly competent and independent body, and the load on the coordinator would also be reduced. If solutions that have already been prepared were used, then some adjustments may be necessary to our proposed list of PAH to be determined. These possibilities will be explored by the coordinator.

2.6 Timescale

As questions of the availability of standard solutions and firm commitment from participants cannot be answered until some time after the 1987 meeting of MCWG it seems most unlikely that the first stage of the programme can be completed in time for a report to be made to the 1988 meeting of MCWG. It is proposed, therefore, that a detailed proposal be prepared and submitted to the 1987 Statutory Meeting together with a provisional list of participants. Provisionally, it is proposed that solutions be circulated in November 1987, with the submission of results to be made by the end of February 1988. A preliminary analysis of the data could then be made in time for the participants to meet in May or June 1988. This meeting would review the results of the first stage of the exercise and then plan the second stage in detail. A paper indicating the preliminary results of the first stage would be presented to the 1988 Statutory Meeting, and a report summarising the results of the first stage and details of the proposed second stage would be presented at the 1989 meeting of MCWG.

It was also agreed to collate all available information on past and present comparative exercises involving PAH analysis and coordinated by other bodies such as BCR, and to distribute this information to all potential participants as soon as possible after the meeting.

2.7 Coordinator

In the absence of other volunteers, the Chairman agreed to act as coordinator of the proposed first stage exercise, with the proviso that if the number of participants is greater than 15 and the preparation and verification of the standard and intercomparative solutions is to be carried out by the coordinator from pure PAH, then his resources may be inadequate.

3 RECOMMENDATIONS

1) It is recommended that an analytical intercomparison exercise for PAH be organized, based on a stepwise approach. The basic requirement is for at least 12 laboratories who will provide a firm statement of intent to participate in the programme for at least 3 years, and to report results by the agreed deadlines. They should use either GC-MS, GC-FID or HPLC-UVF techniques for PAH analysis.

   Unless this minimum requirement is met, then the Group recommends that the exercise should not proceed.

2) Participants shall attend, at national expense, meetings to be held at the end of each stage of the programme to:

   a) assess the results to date,

   b) recommend methodological improvements to be adopted in the light of that experience, and

   c) plan future stages of the programme.

   These meetings are considered to be essential for the success of the proposed programme.

3) The first stage of the exercise should concentrate on the analysis of standard solutions of 10 PAH as described in detail above, with a view to checking instrument calibration as a source of error.

4) It is recommended that Robin Law prepare a paper for presentation at the 1987 Statutory Meeting containing full details of the proposed intercomparison exercise.

5) It is recommended that MCWG contact bodies with acknowledged expertise in the field of preparation and certification of standard reference materials, such as NRC Canada or NBS in the USA, to see whether they can assist in the preparation of suitable standard materials for use in the proposed intercomparative programme.

4 REFERENCES

Law, R.J. and Portmann, J.E. 1982. Report on the first ICES intercomparison exercise on petroleum hydrocarbon analyses in marine samples. ICES Coop. Res. Rep. No. 117, 55 pp.

Farrington, J.W., David, A.C., Livramento, J.B., Clifford, C.H., Frew, N.M. and Knap, A. 1986. ICES/IOC intercomparison exercises on the determination of petroleum hydrocarbons in biological tissues (mussel homogenate) - ICES (2/HC/BT) ICES, Coop. Res. Rep. No. 141, 75 pp.

Uthe, J.F., Musial, C.J. and Sirota, G.R. 1986. Report on the intercomparative study 03/HC/BT on the determination of polycyclic aromatic hydrocarbons in biological tissue. ICES Coop. Res. Rep. No. 141, 10 pp.

APPENDIX 1

The tentative plan agreed at MCWG 1986 for the first stage of a hydrocarbon intercalibration programme was as follows:

To circulate for analysis by GC-MS or HPLC-UVF the following solutions:

1) A standard solution containing 6 aromatic hydrocarbons at declared concentrations.

2) Solution 1, containing the same 6 compounds at concentrations which are approximately indicated, to be determined.

3) Solutions 2 and 3, containing the same 6 compounds at unknown concentrations, higher in one solution than the other, to be determined.

4) Blank solution for analysis.

5) Internal standard solution containing deuterated aromatic hydrocarbons for use in GC-MS analysis and, if feasible, a separate solution for use with HPLC-UVF.

APPENDIX 2

LIST OF ATTENDEES

Name	Address
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John Calder	National Oceanic and Atmospheric Administration Ocean Assessments Division NOMA 32 Rockville, MD 20852 UNITED STATES OF AMERICA
Manfred Ehrhardt	Institut für Meereskunde an der Universität Kiel Düsternbrooker Weg 20 D-2300 Kiel FEDERAL REPUBLIC OF GERMANY
Jarle Klungsøyr	Institute of Marine Research P.O. Box 1870 5011 Bergen-Nordnes NORWAY
Robin Law (Chairman)	Ministry of Agriculture, Fisheries and Food Fisheries Laboratory Burnham-on-Crouch, Essex CM0 8HA UNITED KINGDOM
Michael Marchand	IFREMER Centre de Brest P.O. Box 337, 29273, Brest FRANCE
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APPENDIX 3

Seawater is the medium which marine organisms primarily are exposed to and from which they receive, either directly by uptake through gills and the body surface or indirectly by ingestion of prey, their body burden of contaminants. It would seem logical therefore to link investigations or survey programmes of contaminants in marine organisms to analagous investigations of these same materials in seawater. This approach offers a number of advantages. If, e.g., lipophilic contaminants such as hydrocarbons are to be analyzed, the lipid matrix of seawater is far less complex in composition than the lipid matrix of organisms. Thus, with relatively moderate effort, seawater can be screened for the occurrence of lipophilic contaminants other than hydrocarbons which might affect organisms but which would be far more difficult to detect in them.

The development of analytical techniques for analyzing lipophilic organic trace constituents of seawater has reached a stage where this approach becomes feasible. Its tentative application has led to the detection of a large number of organic compounds, generally more polar than hydrocarbons, which may have detrimental effects on marine organisms. In addition their composition and structure often provide information on degradation pathways of lipophilic contaminants in the environment. Among these compounds are quinones and many other aromatic as well as aliphatic carbonyl compounds.

Since suspended particles in seawater often are partly lipophilic in nature and thus tend to adsorb lipophilic dissolved material, their investigation would also be warranted in this context as would be the sediment surface on which they accumulate under suitable conditions. Analyses of deeper sediment layers finally would provide information on which compounds are stable over long periods of time.

APPENDIX 4

PRIMARY LIST

Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benz[a]anthracene
Chrysene
Benzo[b]fluoranthene
Benzo[j]fluoranthene
Benzo[k]fluoranthene
Benzo[a]pyrene
Benzo[e]pyrene
Perylene
Benzo[ghi]perylene
Indeno[1,2,3-cd]pyrene
Dibenz[a,c]anthracene
Dibenz[a,h]anthracene
(Triphenylene)

APPENDIX 5

Selection Criteria for the PAHs included in the intercalibration programme

1) While consideration was given to the environmental significance of the PAHs selected, the primary criteria for the inclusion of each compound was that it highlighted a particular problem associated with PAH analysis at the trace level. It is essential to identify and quantify the major potential sources of error in the determination of PAHs.

2) The initial exercise will cover the preparation, manipulation and storage of standard and sample solutions; the optimization and calibration of the instrumentation and the use of the instrument in determining PAHs in a clean matrix (i.e., pure solvent).

3) The range selected was 3-6 fused ring systems. Phenanthrene - Indeno[1,2,3-cd]pyrene. This also covers a wide boiling range and hence much of the GC retention range. Phenanthrene was selected to identify losses due to evaporation. A number of PAHs are photo-labile and precautions are necessary in handling and storing such compounds. Chrysene and benzo[a]pyrene can be ratioed to the photostable pyrene to detect losses due to photoactivity.

   Chromatographic performance, in particular the resolution of the capillary column, can be measured by observing the separations of benz[a]anthracene and chrysene on an apolar column, e.g., CPSil5 or OV1 and benzo[e]pyrene and benzo[a]pyrene on a moderately polar (OV17, CPSil9) column. More polar columns make this separation more difficult.

   Discrete identification by retention index and/or mass spectrometry is essential and although benzo[k]fluoranthene is difficult to separate from a mixture of benzo[b]fluoranthene and benzo[j]fluoranthene, quantitatively, in a mixture, it is possible to identify this component from the others with the appropriate care.

   Benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene are included to complete the volatility range.

   Comparisons of the group as a whole should identify problems associated with the cleanliness of the injector (high mass discrimination and peak tailing equally across the boiling point range), incorrect injector temperature (mass discrimination after a particular point in the chromatogram).

   Resolution (as well as discrete separation of closely adjacent pairs) can be measured between pairs, e.g., fluoranthene and pyrene, and benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene.

   All compounds, including the internal standards, are available as certified pure compounds.

PAH Compounds Selected for
Study in ICES Stepwise Approach to Analytical Control

Compound	Reten. ind. CPSil5 or OV1	Reten. ind. CPSil9 or OV17	BCF[+]
Phenanthrene	1 836	2 171	1.2×10^3
Fluoranthene	2 091	2 462	3×10^3
Pyrene	2 139	2 529	3×10^3
Benz[a]anthracene	2 516	2 935	12×10^3
Chrysene*	2 526	2 953	12×10^3
Benzo[e]pyrene	2 858	3 339	-
Benzo[a]pyrene*	2 870	3 353	28×10^3
Benzo[k]fluoranthene	2 802	3 256	28×10^3
Benzo[g,h,i]perylene	3 185	3 656	68×10^3
Indeno[1,2,3-cd]pyrene	3 131	3 610	68×10^3

*more photolabile.

[+]bioconcentration factor.

Synthetic PAH for internal standards (not found in the environment) are:

1. Indeno[1,2,3-cd]fluoranthene for HRGC.
2. Benzo[b]chrysene for HPLC.

All compounds may be analyzed by HPLC.