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REPORT ON INTERCALIBRATION ORGANIZED AND CO-ORDINATED BY IAEA's INTERNATIONAL LABORATORY FOR MARINE RADIOACTIVITY

(document prepared by the International Laboratory for Marine Radioactivity
of the International Atomic Energy Agency)

PROVISIONAL REPORT ON MEDPOL II INTERCOMPARISON EXERCISES ON MUSSEL TISSUE AND MARINE SEDIMENT

1. INTRODUCTION

In late 1983 and early 1984 samples were distributed by the IAEA Monaco Laboratory to institutes participating in MEDPOL II. The samples were to be used for two analayses of pollutants -- (1) trace metals, (2) chlorinated hydrocarbons. Two types of marine materials were distributed. The first was a homogenized coastal marine sediment SD-N-1/2, and the second was a lyophilized mussel soft tissue MA-M-2. The two materials were divided for distribution in containers suitable for organic and inorganic analyses (details are provided below).

The following sections discuss the topics - sample preparation, trace metal results and chlorinated hydrocarbon results. It must be noted that results were requested to be returned to the Monaco Laboratory by September 1984, but all the results are not yet available. Follow up reminders were sent out to participants in late 1984 and early 1985. The following discussions relate the status at the end of February 1985.

2. SAMPLE PREPARATION

A large effort is required to prepare samples of marine environmental materials for intercalibration runs on pollutants. A considerable amount of this work relates to establishing the homogenity of the samples with respect to both trace metals and chlorinated hydrocarbons. There follows an explanation of the procedure used for the sample MA-M-2 (mussel tissue). The essential aspects of this part of the preparation are the same as for SD-N-1/2, and here only a description relating to the mussel tissue is presented to avoid unecessary repetition.

2.1 Preparation of material

About 600 kg of Mediterranean mussels (*Mytilus galloprovincialis*) were prepared in the following way:

Mussels were opened with stainless steel knives. Soft tissues were separated from the shells which were discarded. Then, soft tissues were spread on aluminium trays covered with plastic sheets and were lypholised according to the following steps:

1. Freezing for about 2 hours at -35°C.
2. Obtainment of a vacuum of 10^{-1} Torr in about 45 minutes.
3. Drying at a maximum temperature of 20°C for about 8 hours.
4. Drying at a temperature of 40°C for about 15 hours.

Then, the lyophilised tissues were ground in a mixer (made of stainless steel and glass only) and the resulting powder was put in paper and plastic bags.

2.2 Sieving

In principle, the particle size of the homogenised powder should be as small as possible in order to get an homogeneous material. It was obvious that this powder contained large aggregates of fibres, so that it was concluded that sieving was unavoidable. This procedure, however, may cause large loss of material if the threshold particle size chosen is too low. Therefore, a compromise between particle size and recovery was undertaken first. A preliminary sieve analysis gave the following results:

Particle size	U.S. Mesh No.	% of weight
> 300 µm	< 50	5.3
150 - 300 µm	50 - 100	16.2
75 - 150 µm	100 - 200	45.1
< 75 µm	> 200	33.4

On the basis of these results, it was decided to retain the fraction corresponding to the particle size < 150 µm.

In consequence, the total homogenate was sieved through stainless steel sieves and the resulting powder, which had a particle size < 150 µm, was put in 20 l polypropylene bottles which had been previously washed with dilute nitric acid and demineralized water.

The stainless steel sieves were carefully washed with detergent, a dilute EDTA solution and demineralized water, and then oven dried before use.

During sieving of successive lots of powder it was realized that the mean particle size increased, this being due to the fact that the residue of the sieving was a more and more portion of the total. Since this resulted in a large loss of material, it was decided to powder and to sieve again the residue of the first sieving. Powdering was done with the help of a porcelaine ball mill (200-300 g were powdered at a time for about 15 hours). The resulting powder was sieved and added to the powder which had been already prepared.

By proceeding in this way, the residue of the total operation was very low and the following results were obtained:

Total amount of powder	=	17.970 kg
Final residue (after sieving)	=	0.915 kg
Loss due to sieving	=	5.1%
Total recovery	=	94.9%

2.3 Homogenization procedure and distribution of samples

This operation was performed in a stainless steel rotating container. The container was washed with detergent, tap water, a diluted EDTA solution and finally with demineralized water before use. After complete drying of the apparatus, the total powder was put in it. The "dead" volume was more than the half of the total volume of the container. The powder was then homogenized by rotating the container for 100 hours. It was then transferred to 20 l polypropylene bottles. The bottles were well stoppered and the powder was stored until the homogenity testing was completed.

During the transfer operation, 10 samples were collected in small plastic flasks for checking the homogeneity with respect to five trace elements. Ten samples were also collected in glass flasks for the homogeneity testing of PCB's. The samples were taken randomly from the bulk of powder.

After the homogeneity testing had been completed, the powder was roughly divided into two equal parts. One part was distributed into 200 glass flasks equipped with aluminium stoppers for the analysis of PCB's (each flask contained at least 35 g). The other part was distributed into 362 glass flasks equipped with plastic stoppers for the analysis of trace elements (each flask contained at least 25 g). All glass flasks were carefully washed with detergent, tap water, dilute nitric acid demineralized water, and were completely dried before use.

2.4 Determination of the water content of the material

As the samples were intended for distribution to many laboratories located in different countries, it was recognised that their water content may change with the ambient humidity and temperature. Therefore, it was appropriate to recommend a standard drying procedure to the participating laboratories, and to request them to report their results on a dry-weight basis (the water content being determined on a separate subsample, and not the one used for the actual analysis).

The water content of the mussel tissue homogenate as determined by drying to a constant weight at 85°C was found to be approximately 6.5%. At this temperature, constant weight was reached after a drying time of 24 hours. A higher temperature was judged to be undesirable as it would cause some degradation of the organic matter. Therefore, it appeared appropriate to recommend to the participants a drying temperature of 85°C for 24 hours for the determination of the water content.

2.5 Homogeneity testing of trace elements

About 500 mg of each sample taken for the homogeneity control of trace elements were weighed accurately into teflon beakers, and were mineralized in the following way:

A small amount of concentrated nitric acid was added to each beaker contents by successive additions of 1 ml aliquots, and drying between each addition. The samples were heated to about 60°C. Nitric acid was continually added until the black colour of the residue disappeared. The residue was finally taken up in concentrated hydrochloric acid and the final solution (25 ml) was made up in 0.1N HCl for each sample.

Reagent blanks were prepared in the same manner as the sample solution by using the same amounts of acids. Only "Suprapur" acids and high purity demineralized water were employed.

The concentrations of Cd, Cu, Mn and Pb in the sample solutions were determined by flameless atomic absorption spectroscopy using a Perkin Elmer Model 403 spectrophotometer. The concentrations of zinc were determined by flame atomic absorption spectroscopy.

For trace elements the sequence of measurements was the following:

Standard, Solution No. 1, Solution No. 2 ...Solution No. 10, Standard, Solution No. 1, Solution No. 2, ...Solution No. 10, Standard, ... and so on until each solution was analysed 10 times. The absorbance measurements in each series were then related to the absorbance of the standard solution measured just before that series. By proceeding in this way, the variations of sensitivity of the apparatus were corrected and a systematic measurement error for a given solution was avoided.

Blank absorption values were negligible for Cd, Cu, Mn and Pb. For zinc, it was necessary to subtract a small blank value and the solutions had to be diluted by a factor of ten before analysis. The elements were chosen for this homogeneity control exercise as they can be easily determined by A.A.S. with good accuracy and precision.

For each trace element a one-way variance analysis was performed in order to compare the variability "between samples" and the variability "within samples". The variance "between samples" is estimated by $\hat{\sigma}_1^2$ while the variance "within samples" is estimated by $\hat{\sigma}_2^2$. Thus:

$\hat{\sigma}_1^2 = B/(m-1)$

$\hat{\sigma}_2^2 = C/(n-m)$

with:

B = $\sum_{i=1}^{m} l_i (\bar{y}_i - \bar{\bar{y}})^2$

C = $\sum_{i=1}^{m} \sum_{j=1}^{l_i} (y_{ij} - \bar{y}_i)^2$

n = total number of determinations = 100

m = number of samples = 10

and:

l_i = number of determinations for the samples i = 10

$\bar{y}_i$ = mean value for 10 determinations of the sample i

y_{ij} = value of the jth determination of the sample i

$\bar{\bar{y}}$ = overall mean =

The total variance $\hat{\sigma}^2$ is estimated by:

$\hat{\sigma}^2 = A/(n-1)$

with:

A = $\sum_{i=1}^{m} \sum_{j=1}^{l_i} (y_{ij} - \bar{\bar{y}})^2$

The variances $\hat{\sigma}_1^2$ and $\hat{\sigma}_2^2$ significantly differ if the value of F = $\hat{\sigma}_1^2 / \hat{\sigma}_2^2$ is higher than the critical value given in Snedecor's table with:

(m-1) degrees of freedom for $\hat{\sigma}_1^2$

(n-m) degrees of freedom for $\hat{\sigma}_2^2$

The significance level which was chosen for the critical value was % = 0.05.

The coefficient of variation was calculated for each element as the ratio $S_{\bar{y}i} / \bar{\bar{y}}$ where:

$S_{\bar{y}i}$ = estimator of the standard deviation of the $\bar{y}_i$'s

$\bar{\bar{y}}$ = overall mean

We have: $S^2_{\bar{y}i} = \frac{\sum (\bar{y}_i - \bar{\bar{y}})^2}{m-1}$ and $S_{\bar{y}i} = \sqrt{S^2_{\bar{y}i}}$

2.6 Homogeneity testing of chlorinated hydrocarbons

Ten samples, each weighing approximately 20 g, were randomly taken in the bulk of the powder and analysed for their PCB contents by gas chromatography. Chlorinated hydrocarbons were analysed both with packed columns and capilliary columns.

For all hydrocarbons except toxaphene the pp'DDD coefficients of variation below 20% were obtained by packed columns. Results obtained by capilliary columns are in good agreement with those obtained by packed

columns, but coefficients of variation are generally larger.

2.7 Discussion of homogeneity results

The results obtained for trace element concentrations were compared with those results reported in the literature for soft tissues of mussels from the Mediterranean.

Results obtained for Cd and Zn are of the same order of magnitude as those reported some years ago for mussels from the same area (Fowler and Oregioni, 1976). They are, however, higher for manganese and lower for lead and copper.

Very low values for the coefficients of variation were obtained for the concentrations of some typical trace elements determined in 10 samples randomly taken in the bulk of the powder. Therefore, the probability that this material is homogeneous for all trace elements is very high. On the other hand, the reasonably small values obtained for the coefficients of variation of the measurements of PCB's is also a strong indication of homogeneity of this material with respect to chlorinated hydrocarbons.

3. TRACE METALS

Twenty-one laboratories were designated to participate in these intercomparison exercises. Until March 1985, only 7 of them have sent their results.

Owing to the limited amount of data, therefore, a detailed statistical evaluation of the results is not possible. At this moment, one can only attempt a rough evaluation of the analytical quality of the results obtained in these MEDPOL intercomparison exercises.

Each participant received a flask containing about 25 g of lyophilised material MA-M-2 (mussel tissue) taken from a batch of 17 Kg of homogenized powder (grain-size < 150 um). He received also a flask containing about 25 g of SD-N-1/2 material taken from a batch of 100 Kg of a fine-grained estuarine sediment from the North Sea. Each participant was requested to determine separately the moisture content by drying to a constant at 85° (for the mussel tissue) and 105° (for the sediment), and to report his results on a dry-weight basis.

For each laboratory the mean value of the individual determinations was computed, as well as the corresponding coefficient of variation. The methods applied by each laboratory to the determination of trace elements were:

ASV: anodic stripping voltammetry (or other technique)
Flame-AAS: atomic absorption spectroscopy (flame technique)
GF-AAS: " " " (graphite furnace technique)
Hydride-AAS: " " " (hydride generation technique)
CV-AAS: " " " (cold-vapour technique)

No actual results from Monaco or other laboratories are reported here since until the MEDPOL and worldwide exercises are complete the data must remain confidential.

For each element, the results reported by the participants have been compared with "provisional consensus values" established on the basis of the data evaluation of worldwide intercomparison exercises on trace element determination in the same materials (Lyophilised mussel tissue MA-M-2 and sediment SD-N-1/2). In the case of the determination of a trace element in mussel (MA-M-2/TM exercise), the estimated "modal value" (value around which a maximum of frequency of results is observed) of the distribution of the results sent by the first 30 worldwide reporting laboratories is taken as the "provisional consensus value". In the case of the sediment (SD-N-1/2 exercise) the "provisional consensus values" were those values given by IAEA in "provisional Report No. 22 - Intercomparison of trace metal measurements in marine sediment sample SD-N-1/2, October 1984). In this case, for each element, the "provisional consensus value" corresponds to the "median value" of the distribution of the "accepted results" sent by the participants. Only those results which are kept after applying a non-parametric test for detection of outliers were used for the computation of the median. One must, therefore, understand that the "provisional consensus values" are not definitive and will probably be revised in the future. We do believe, however, that the final consensus values which will be recommended later will not be very different from the present provisional consensus values. These provisional values, therefore, have been taken as a comparison basis for the provisional estimation of the analytical quality of the MEDPOL results.

When examining the results to date, we see that the results of the MEDPOL exercises, for MA-M-2 and for SD-N-1/2, are generally in good agreement with the provisional consensus values, except for chromium in sediment, for which the MEDPOL values are all significantly lower than the consensus value of the worldwide intercomparison. One determination of zinc in sediment is obviously too low and probably wrong, and one result of cadmium in the mussel seems too high when compared with the other MEDPOL results and the consensus value of the worldwide exercise. On the other hand, one determination of mercury in the mussel seems too low, and one result of nickel, also in the mussel, is too high.

When comparing the MEDPOL results all together, we find that they are relatively well grouped with, however, some spread which can reach a factor 2 or 3 between the extreme values (without considering the suspected "outliers"). Since these materials (mussel and sediment) were submitted to a careful homogenity testing procedure and, on the other hand, as the results of the worldwide intercomparison show for almost all elements dominant clusters of data around a central value, one can conclude that the observed spread in the results originates from the analytical techniques themselves rather than from other reasons.

The present status of these expercises does not allow the comparison of the results given by different techniques. However, we can see that, except for some results obtained by volammetric techniques (ASV), determinations were made by the same basic technique, namely atomic absorption spectroscopy (AAS) in its various forms (Flame, graphite furnace, etc.). We also note, however, that the results obtained by voltammetric techniques by one participant are in good

agreement with the AAS results obtained by the other participants.

For some elements (Ag, As, Co, Se in mussel, As, Se, V in sediment) one must consider that only one result was reported. The evaluation of the quality of such results is difficult, particularly when the consensus value was established on the basis of a limited number of data from the worldwide intercomparison (case of Ag in MA-M-2, for example; for Se in sediment, there is even no recommend values, and only one result in the MEDPOL exercise!). It is, therefore, absolutely necessary to get more data, particularly from the laboratories which are designated to participate in these MEDPOL exercises and have not yet sent their results, before trying to do a meaningful evaluation of the quality of trace element determinations by Mediterranean scientific institutes.

4. CHLORINATED HYDROCARBONS

For this MEDPOL intercalibration exercise 25 laboratories were designated to participate in the analysis of either or both the sediment or mussel homogenates. By the end of February 1985 only 10 laboratories had reported their results for the sediment sample, and 12 laboratories had reported their results for the mussel sample.

In essence, the only values eliminated by the statistical treatment described above in section 3 for the mussel sample came from one laboratory. For example, analytical values for HCB, HCM, pp'DDE and pp'DDD for mussel are rejected because they are definitely too high. It may be that there are fundamental errors in the calculations performed by this one laboratory, and this question is to be investigated further. In this set of results the reported concentrations of HCH, pp'DDE, pp'DDD are one order of magnitude greater than expected, while the HCB results are two orders of magnitude too high.

5. CONCLUSIONS

The two marine environmental samples prepared for the MEDPOL II intercalibration exercise on pollutant analyses have been subjected to careful evalutaion at the IAEA Monaco Laboratory. Following stringent preparation, the homogenity tests performed for trace element and chlorinated hydrocarbon contents showed that both the mussel tissue and sediment samples are very satisfactory. This conclusion is based on one-way variance analysis using Snedecor's statistical techniques. The analytical results include not only those obtained at Monaco on random samples, but also on a "worldwide" data basis.

By the end of February 1985, the data reported in the framework of MEDPOL II is still incomplete. However, a provisional analysis of both the trace metal and chlorinated hydrocarbon results indicate that an adequate degree of analytical quality control appears to be emerging. It must be stressed that a more statistically valid conclusion will only be

possible when a complete return of all results is available, and that this can be supplemented by an evaluation with respect to the "worldwide" intercalibration study.