COOPERATIVE RESEARCH REPORT
No. 80

REPORT ON INTERCALIBRATION ANALYSES IN ICES NORTH SEA AND NORTH ATLANTIC BASELINE STUDIES

by

G Topping and A V Holden

https://doi.org/10.17895/ices.pub.7704

ISBN 978-87-7482-565-4

ISSN 2707-7144

International Council for the Exploration of the Sea
Charlottenlund Slot, DK-2920 Charlottenlund

March, 1978

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INTRODUCTION

Following several proposals that baseline studies of pollutants in the marine environment should be established (see ICES, 1974), ICES set up a Working Group in 1971 with the responsibility of organising and implementing an International Study of the Pollution of the North Sea. Earlier ad hoc meetings in 1971 had planned a baseline survey of pollutant levels in food fish, and an associated intercalibration exercise to compare the analyses of heavy metal and organochlorine residues in two specially-prepared samples representative of the types of biological materials examined in the survey.

The results of this first international study were reported in 1973 (ICES, 1974), following which the Working Group recommended that monitoring should continue at a national level, but that further intercalibration of analytical procedures was essential. A second intercalibration of heavy metal analyses was therefore developed towards the end of 1973, although it was not possible to find a satisfactory matrix oil for a further organochlorine intercalibration sample. The countries which analysed the second heavy metal reference sample were those which had examined the first sample and the results of this second intercalibration were reported to ICES in 1975 (Topping, 1975). In January 1975 the Working Group was replaced by a new Group* which planned a further baseline survey of heavy metals and organochlorines in fish and shellfish in the North Atlantic and North Sea, and a further intercalibration exercise for both types of residues was recommended. The results of this exercise were reported in ICES(1977b).

This report presents, statistically analyses, and discusses the results obtained from all of the intercalibration programmes. As the samples used and the analytical techniques involved in the determination of the two types of residues are quite unrelated, the first section of the report deals with the trace metal analyses and the following section with those for organochlorine residues.

Note: The reference samples used in the second and third metal intercalibration exercise and the second organochlorine intercalibration exercise were also supplied to those countries taking part in the Baltic Baseline Survey. The results of these exercises, together with the results of the fish and shellfish Baseline Survey, were reported in ICES (1977a).

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* Working Group on Pollution Baseline and Monitoring Studies in the Oslo Commission and ICNAF Areas.

PART I - METALS

PREPARATION OF REFERENCE SAMPLES

Ideally the reference material circulated to participants in inter-calibration studies should be identical to the natural samples collected and analysed by them in subsequent baseline studies, i.e., it should consist of wet fish tissue. In practice this is not possible, mainly because it is difficult to produce and circulate a reliable and uniformly mixed sample of such tissue.

Previous studies with dried plant material, e.g., kale (Bowen, 1967) and orchard leaves (IDOE, 1972), however, had been quite successful. It was therefore agreed by the Working Group during the planning stages of the first exercise that the reference material for the heavy metal programme should consist of some form of dried fish material.

For the first exercise the reference fish flour was prepared from a large quantity of commercial fish meal by the MAFF Humber Laboratory, Hull, England (Windsor, 1971). The coarse material was ground in a hammer mill until a fine flour was produced. Each participant received 20 g of this material in a small plastic phial with instructions to analyse it as many times as possible using his or her own analytical method.

A number of the participants in the first exercise thought that part of the variability of the results could be caused by the heterogeneity of the fish flour. In the United Kingdom about 80% of the raw material for fish meal production is offal (heads, skeletons, and trimmings discarded from the processing industry)(Windsor, 1972). It is therefore possible that the fish flour made from this sort of material could be heterogeneous, even after repeated grinding in the hammer mill.

In an attempt to improve on the homogeneity of the reference sample a new fish flour was prepared for the second exercise using muscle (unskinned) from cod. Details of the preparation of the fish flour are given in Appendix I. Each participant received 50 g of this material in a plastic phial and 40 ml of an acidified solution containing known (but undisclosed) quantities of dissolved copper, zinc, mercury, cadmium and lead. The analyst was instructed to analyse each sample six times and report all values obtained.

The demand for fish flour by ICES and non-ICES laboratories during the second exercise had exhausted the stock of fish flour. A new fish flour was therefore prepared for the third exercise. In an attempt to produce further improvements in homogeneity, the cod muscle used in the preparation of the third fish flour was skinned prior to conversion into fish meal. Details of this preparation, which was slightly different from that used in the second exercise, are given in Appendix II.

Each participant received 100 g of this material in a plastic container together with five small phials containing 10 ml of a stock metal standard solution (1000 mg/l). Copper, zinc, cadmium and lead standard solutions were placed in plastic phials, whilst the mercury standard was contained in a glass phial.

The participant was instructed to analyse the fish flour six times using the standards provided in the phials to calibrate the method used, and report all values.

ANALYTICAL METHODS

It was agreed from the outset by the Working Groups that no attempts would be made to impose a standard method of analysis for the baseline

and intercalibration exercises. It was understood, however, that analysts producing results which were obviously unacceptable would be required to re-examine their methodology, and if necessary replace it or modify it to bring it up to the required standard.

With the exception of one analyst who used anodic stripping voltammetry, all analysts used atomic absorption methods during the three intercalibration exercises. The analysis of copper, zinc, cadmium and lead was done using both flame and flameless techniques, whereas mercury was analysed by a variety of cold vapour techniques. A broad outline of the individual techniques and the instrumentation used in these exercises is given in Appendices I and II.

PARTICIPANTS

A list of laboratories and analysts, together with details of their participation in the three exercises, is given in Table 1. Each laboratory has been assigned an identification number.

RESULTS

First Exercise

The results of the fish flour analyses submitted by the eight participating analysts (Laboratory Nos. 1, 8, 9, 11, 12a, 14, 15 and 16) are given in Table 2. With the exception of Laboratory No.8, all laboratories produced similar values for copper, in the range 17 - 20 µg/g. Zinc values fell in the range 39 - 80 µg/g, but if the results from Laboratory Nos.8 and 9 are excluded, the range contracts considerably to 66 - 80 µg/g. With the exception of Laboratory No.11, which submitted a value one order of magnitude higher than anyone else, the mercury results (0.09 - 0.23 µg/g) for most laboratories agree reasonably well. Cadmium values also fell within a small range, 1.1 - 2.5 µg/g. The agreement among analysts for lead however was poor compared to the other metals; the values ranged from 1.0 - 9.0 µg/g.

Second Exercise

Following the discussions of the results of the first exercise, it was agreed to run a second intercalibration exercise using a new fish flour, with the following modifications to provide additional information on methodology and affording the analysts an opportunity to improve their performance:

1. The fish flour was to be made from fish muscle in an attempt to improve on the homogeneity of the sample.

2. Each participant was asked to analyse the reference sample at least six times using his own method so that sufficient values would be available to allow estimation of the overall precision of the method. Each analyst was also asked to analyse the reference sample using a common procedure (see Appendix I) so that an estimate could be made of the individual analyst's performance.

3. In addition to the fish flour, each analyst was to receive an acidic solution of known metal content to be analysed at the same time. It was thought that the results from these analyses would give a measure of the accuracy of each individual method, since this was impossible to gauge from the fish flour results as the true metal content was not known.

4. Each analyst was asked to supply details of the analytical method used in his laboratory.

(a) Analysis of fish flour

The results of the fish flour analysis submitted by the eight analysts (Laboratory Nos. 1, 8, 9, 10, 11, 14, 15 and 16) using both their individual and the common procedures are given in Tables 3 and 4, respectively.

COPPER

In general, there was good agreement among analysts for copper using both analytical procedures; the range of mean values for the individual and common procedures were practically identical at 8.6 - 10.1 µg/g and 8.7 - 10.1 µg/g, respectively. With one exception, the individual laboratories produced very precise results for both procedures; coefficients of variation fell in the ranges 2.1% - 9.5% and 4.0% - 8.0% for the individual and common procedures, respectively. The coefficient of variation for the procedure adopted by Laboratory No.14 is twice as large as that of the other laboratories.

A multiple range test (see Appendix III for details) has been carried out on the results (own method) from six of the seven laboratories (Laboratory Nos. 1, 8, 9, 14, 15 and 16) submitting copper results to determine the significance of the differences between the mean values. Laboratory No.11 was excluded as the lack of relevant data precluded it from this statistical test. The test revealed the following pattern:

Laboratory No.	1	14	8	16	9	15
Mean copper value	8.62	8.63	9.5	9.6	10.0	10.1

(Key: any two or more mean values underscored by the same line are not significantly different.)

The results submitted by Laboratory Nos. 1 and 14 are significantly lower than those of Laboratory Nos. 9 and 15, but the results from Laboratory Nos. 8 and 16 are not significantly different from those of either of these pairs of laboratories. In practice, this means that Laboratory Nos. 1, 14, 8 and 16 or Laboratory Nos. 8, 16, 9 and 15 would produce data of comparable accuracy at these concentrations in a baseline study.

ZINC

The range of mean values for both procedures was very similar, namely 23 - 29.5 µg/g for the common procedure and 23 - 31.1 µg/g for the individual procedures. The coefficients of variation attained by all laboratories for both procedures are very good, ranging from 0.2% - 8.7% for the common procedure to 1.6% - 8.7% for the individual procedures.

A multiple range test was carried out on the results from six of the seven laboratories (Laboratory Nos. 1, 8, 9, 14, 15 and 16) submitting zinc values with the following results:

Laboratory No.	15	8	16	14	1	9
Mean zinc value	23	24.8	25.6	26.9	27.1	31.1

The mean value from Laboratory No. 9 is significantly higher than the others, whereas Laboratory No.15 produced a value which is significantly lower than the rest. Only 3 of the 6 laboratories produced results which are not significantly different.

MERCURY

All laboratories produced data of a very high precision using their own methods (1.4% - 6.8%) and, with the exception of Laboratory No.15, using the common procedure (1.2% - 8.3%). The ranges of mean values reported for the individual and common procedures are 0.60 - 0.83 µg/g and 0.47 - 0.83 µg/g, respectively.

A multiple range test was carried out on the results from seven of the eight laboratories (Laboratory Nos. 1, 4, 8, 9, 10, 14, 15 and 16), with the following results:

Laboratory No.	15	16	14	10	9	8	1
Mean mercury value	0.63	0.65	0.66	0.72	0.73	0.75	0.83

The mean value from Laboratory No.1 is significantly higher than the others. The remaining laboratories fall into two significantly different groups. In practice, this means that Laboratory Nos. 14, 15 and 16 or Laboratory Nos. 8, 9 and 10 would produce data of comparable accuracy, at these concentrations, in a baseline study.

CADMIUM

The range of mean values reported for the individual and common procedures are different, <0.2 - 1.1 µg/g and <0.2 - 0.55 µg/g, respectively. The precision for both methods is poor: 5.7% - 20.5% for the individual procedures and 7.7% - 27.7% for the common procedure. On these data no multiple range test was possible or indeed justifiable.

LEAD

Only five of the eight laboratories analysed the sample for lead and of these five only four reported values above the detection limit using their own analytical procedure. The ranges of values for the two procedures are significantly different, 1.3 - 2.5 µg/g for the individual procedures and 0.25 - 3.0 µg/g for the common procedure. No multiple range test was applied to these data.

(b) Analysis of the acidic solution

The results of the analyses of this solution are given in Table 5; the true values on the basis of careful laboratory preparation are quoted at the base of this table.

COPPER - Laboratory Nos. 1 and 8 were the only laboratories to report accurate values for the copper content of this solution; the remainder produced mean values which were higher than the true value. This suggests that these latter laboratories may have used working standard solutions which were weaker than the one circulated.

ZINC - Four out of five laboratories reported mean values for the zinc content which were higher than the true value; Laboratory No.1 reported a value of 0.61 µg/ml, which is 20% higher than the true value.

MERCURY - Only one laboratory reported an accurate mean value for the mercury content of the acidic solution. The remaining laboratories reported mean values which were all significantly higher than the true value; Laboratory No.1 reported a value of 0.18 µg/ml which was nearly twice the true value of 0.10 µg/ml.

CADMIUM - Surprisingly, no laboratory reported an accurate mean value for the acidic solution containing 0.10 µg Cd/ml. The actual values reported ranged from 0.06 µg/ml (Laboratory No.1) to 0.20 µg/ml (Laboratory No.14).

LEAD - The results for lead were no more accurate than those for cadmium. Lead values of 0.07 - 0.49 µg/ml were reported for an acidic solution containing 0.30 µg/ml.

Third Exercise

Although the overall results of the second exercise demonstrated that since the time of the first exercise significant improvement had been made by all the analysts in terms of comparability of data, it was generally considered by the participants that further improvements could be made. One major source of error in the second exercise was thought to arise from the working standards used by individual laboratories. If this error were eliminated, one might expect a significant improvement in the overall comparability of data. The third intercalibration exercise was planned to take this factor into account by issuing each laboratory with the same stock standards, one for each metal examined. In addition, each analyst was required to prepare his working standard from these stock standards, using exactly the same procedure, in order to minimise losses by adsorption (all metals) and by volatilization (Hg) during the bench life of these working solutions. This was felt to be necessary since all of the participants seemed to have a different method for preservation and storage of mercury standards (Table 7). Some of the participants surmised that another source of error in the intercomparison exercise might be the heterogeneity of the reference sample. The coordinator agreed to seek advice on the most practicable method of subsampling a large quantity of reference material in order to ensure that each subsample was typical of the bulk material.

Twenty-one analysts from thirteen countries took part in the third ICES intercalibration exercise. The results reported by each analyst, consisting of mean values, standard deviations and coefficients of variation, are listed in Table 8. The detection limits for each metal, expressed as µg/g, are listed in Table 9.

COPPER

Mean values of copper in fish flour, reported by twenty analysts, range from 2.69 µg/g - 5.68 µg/g (Table 8). The largest standard deviation and coefficient of variation were those of Laboratory No.14. Two of the six values quoted by this laboratory (5.6 and 8.7 µg/g) were considerably higher than the other four values which gave a mean, standard deviation and coefficient of variation of 3.38 ± 0.17 and ± 5.1, respectively. Because of the distortion that one very large variance can put on the overall statistical analysis, it was decided that there was sufficient justification for excluding the two very high values from further analyses.

Analysis of variance shows that there are significant differences among mean levels of copper as measured by the different analysts. The differences among analysts are demonstrated by means of a multiple range test (Table 10).

It can be seen that Laboratory No.5 submitted a mean copper value significantly higher than the others. Also there is a group of eleven laboratories in the middle of the range for which there is no significant difference between mean copper values. Two of these laboratories, Nos. 7 and 18, have higher variability than the others, but no special allowance has been made in computing the multiple range test which assumes the same "within group" variance.

The problems raised by the presence of outlying observations require careful consideration. In the third exercise, all participants were instructed to report every determination made. In practice, however, it is quite likely that outlying observations could have been discarded immediately on the basis of some criterion. In the analysis of the copper data, for example, if all six observations reported by Laboratory No.4 are accepted, the mean value 4.63 µg/g is very different from those reported by most other analysts. If, however, the two highest values are omitted, the results are similar to those presented by the other laboratories.

ZINC

Mean values of zinc in fish flour, reported by twenty-one laboratories, ranged from 27.8 - 52.7 µg/g (Table 8). One laboratory (Laboratory No.19) submitted two sets of results produced by different methods of analysis. It can be seen from the results that five laboratories, Nos. 5, 6, 8, 18 and 19b, produced more variable results than the others. A multiple range test was carried out using the data from the remaining sixteen laboratories, with an overall coefficient of variation of 3%, and the results are given in Table 10.

The mean values of Laboratory Nos. 14 and 13 are significantly higher than any of the other mean values and they themselves also differ significantly, while the mean zinc value of 31.0 µg/g from Laboratory No.21 is significantly lower than the other means considered. Although the remaining mean values are more closely linked together, there still exists a number of significant differences among them. The five laboratories omitted from the multiple range test tended to produce fairly extreme (both high and low) mean values for zinc in the fish flour.

TOTAL MERCURY

Mean values of total mercury in fish flour, reported by 16 laboratories, range from 0.74 - 1.26 µg/g (Table 8). The coefficients of variation for all but three of the laboratories (Nos. 2, 12 and 19) were fairly consistent (< 10%), giving an overall coefficient of variation of 7%. An analysis of variance for the 13 laboratories was computed. This showed statistically significant differences among mean levels of total mercury. The results of a multiple range test are given in Table 10.

Although there are a number of significant differences between the 13 laboratories, the differences are much reduced compared with the corresponding results of the second ICES intercalibration exercise.

LEAD

Mean values of lead in fish flour reported by twenty-one laboratories range from 0.16 - 4.0 µg/g (Table 8). An examination of the results submitted by Laboratories Nos. 2, 4, 5, 6, 9, 10, 11 and 14 was made as the analysts in these laboratories had used methods with good detection limits, i.e., < 0.02 µg/g. Within this group, mean values range from

0.16 - 2.99 µg/g and coefficients of variation range from 4% to 41%. The differences in means clearly exist within this group of analysts, the most striking features being the exceptionally large differences between laboratories and the very high variability within some. Thus, a multiple range test is once more not considered appropriate.

CADMIUM

Mean values of cadmium in fish flour reported by twenty-one laboratories range from 0.020 - 0.552 µg/g (Table 8), except for Laboratory No.7 which quoted a value < 1.8 µg/g. With such tremendous variability within laboratories, it seems inappropriate to compare differences among laboratories at this stage.

DISCUSSION

On the basis of the data presented in Table 2, it seemed reasonable to conclude that the majority of the analysts in the first exercise could produce comparable estimates for copper and zinc at the concentrations present. The results for mercury, cadmium and lead however were not so encouraging, and suggested that some improvement was needed in the techniques for these metals before more harmonious results could be obtained. A lack of homogeneity in the sample was also suggested.

The results of the second exercise indicate that there were significant differences between the working standards used by individual laboratories and that these differences might well account for a major portion of the differences produced in the fish flour analysis. It is convenient to illustrate this point by examining the mercury data in a little more detail.

The concentration of mercury in fish flour determined by the individual procedures ranges from 0.47 - 0.83 µg/g. Similarly, the levels of mercury in the reference solution range from 0.10 - 0.18 µg/ml (note that no laboratory returned levels less than the true value of 0.10 µg/ml).

The results for copper, zinc and mercury reported by each laboratory can be adjusted to take into account the differences in the strengths of the individual metal standards by multiplying each result by the following factor:

$$\frac{\text{True concentration of individual metal in acidic solution}}{\text{Reported concentration of individual metal in acidic solution}}$$

The overall comparability of the metal data is thus improved for all three metals (Table 6), e.g., the mercury value of 0.83 µg/g reported by Laboratory No.1 now becomes 0.46 µg/g and is then the lowest value rather than the highest. The ranges of values for copper and zinc also become much smaller.

Although the statistical examination of the analytical data for the fish flour used in the third exercise reveals that there are significant differences among results submitted by the individual laboratories, the values for copper, zinc and mercury confirm the tendency towards improvement in successive ICES exercises (Table 11). The overall spread of values for these metals for the majority of participating laboratories is small enough to allow meaningful comparisons of the fish and shellfish metal data collected in the baseline study, provided the methods used are not changed.

Unfortunately, the laboratories do not agree when it comes to lead and cadmium at levels of 0.X µg/g and 0.0X µg/g, respectively, which represent average values for these metals in the muscle of fish and shellfish collected from North Sea areas. There is little doubt that the inherent

differences are related to the different analytical methods used and their respective limits of detection. In selecting a method for the analysis of an element within a known concentration range, one should always be selected which has a detection limit (based on two or three times the baseline width) at least an order of magnitude lower than the lower limit of the expected concentration range. Table 9 clearly shows that about half of the laboratories which submitted data on detection levels employed analytical methods which satisfy the above criteria. Most of these laboratories produced data which not only agree reasonably well with each other but which are significantly lower than data from most of the remaining laboratories (Table 12).

All but one analyst reported that they could find no difference between their own standards and the ones issued. Laboratory No.17 reported that the copper and the mercury standards were significantly higher than the one used by their laboratory. An examination of the information supplied by Laboratory No.17 indicated that their copper and mercury standards contained no added acid. Lack of acidity could well mean that accelerated mercury losses could have taken place from their stock solution which would then make the ICES mercury stock standard appear higher.

The use of common stock standard solutions and the adoption of a common procedure for the preparation of working standards (see Appendix II) noticeably improved the overall performance in this intercalibration exercise compared to previous exercises (Table 11). Overall coefficients of variation for Hg, Cu and Zn have now been reduced to single figures compared with the double figures produced in the first and second ICES intercalibration exercises. It would be in the interest of the group as a whole that this procedure should be formally adopted by the analytical group as a permanent routine and that new participants would be obliged to adopt them.

The overall findings in these three ICES exercises compare very favourably (Table 13) with the results from recent intercomparison studies using biological materials (Bowen, 1967; IDOE, 1972; and Berlin et al., 1974). In addition, the ICES exercises reveal that results can be influenced by the different working standards used by the participants, a fact either ignored or undetected in other studies. All of these recent studies reveal that individual analysts can produce very precise data for the reference sample(s) but that there are significant differences among the mean values quoted by some participants. Berlin et al. (1974), who compared the analysis of Hg, Cd and Pb in blood, urine and water samples carried out by a number of medical laboratories in Europe, received values for individual metals which differed by 1-2 orders of magnitude, e.g., Pb, Hg and Cd values for one blood sample fell in the range 1 - 115 µg Pb/100 ml, 0.2 - 9.0 µg Hg/100 ml and 0 - 11.0 µg Cd/ml, respectively. On the basis of their overall findings, they concluded that at the present time a comparison of levels of heavy metals in biological fluids could not be directly made when the measurements are performed by different laboratories. During the baseline studies of the International Decade of Ocean Exploration (IDOE), the National Bureau of Standards circulated three samples (orchard leaves, bovine liver and tuna tissue) to a number of American and European laboratories. In the report of these baseline studies (IDOE, 1972), NBS stated that there were serious deficiencies in the analyses of Zn, Cd, Hg and Pb and that participants should review in detail their techniques for measuring these elements. They further recommended that a laboratory intercomparison should be conducted annually to determine how rapidly current analytical methodology is improving.

SUMMARY

1. The ICES Working Group on Pollution Baseline and Monitoring Studies in the Oslo Commission and ICNAF Areas, together with the former ICES Working Group on Pollution Studies in the North Sea, have successfully carried out three intercomparison exercises for heavy metals using three separate fish flour reference samples. The results are quite good compared with the results of other similar exercises.

2. Throughout these exercises there has been an overall improvement in the analytical performance of those laboratories which have participated in more than one exercise.

3. On the basis of the results of the third exercise, it is considered that the majority of analysts who participated in the 1975 ICES fish baseline study produced comparable data for copper, zinc and mercury.

4. The discovery of the differences in the quality of working standards and the adoption of common standard stock solutions and common procedures for the preparation of working solutions has been an important factor in the success of this exercise. The Working Group agreed that these procedures should be adopted in future studies.

5. Considerably more work is needed on the analytical techniques for cadmium and lead before the results from the majority of laboratories are comparable.

6. Further intercalibration exercises will be necessary to assess this improvement and to determine any additional factors which may be affecting the degree of comparability.

PART II - ORGANOCHLORINES

PREPARATION OF ORGANOCHLORINE SAMPLES

Experience in the preparation of organochlorine intercalibration samples and in the design of an appropriate programme of distribution, analytical reporting and examination, had already been obtained by several laboratories in ICES member countries which had previously participated in OECD studies, and the opportunity was therefore taken to enable further samples to be used by both organisations in 1972 and 1974. Attempts to prepare homogenates of fish tissue of acceptably uniform composition, and to provide a uniformly mixed sample of such tissue to which had been added known quantities of organochlorine compounds, had proved unsuccessful, and in consequence fish or vegetable oils were selected to form the matrix for the ICES samples.

Organochlorine residues of the type under examination in the baseline studies are highly lipophilic, and the appropriate techniques of analysis involve the extraction of lipid material and the subsequent separation of the organochlorine residues from the lipids and co-extracted substances. The selection of an oil as a matrix for the intercalibration samples thus by-passed only the initial tissue preparation and extraction stages, but the analysis of the oil would still require the separation (or destruction) of lipids, other treatment (as appropriate) prior to gas liquid chromatographic (GLC) analysis, the GLC analysis itself involving both identification and quantification of residues, and any subsequent confirmation of identity.

The first ICES intercalibration sample for organochlorine analysis was prepared from a fish oil selected for its relatively low initial concentrations of such residues. One half of the oil sample made available was subdivided into suitable aliquots for distribution as a control or blank (Sample 2A) and the other half was spiked with quantities of a selected (but undisclosed) group of organochlorine compounds. To ensure that the original residues would represent only a small proportion of the total residues in the spiked sample, the additions were approximately an order of magnitude larger than the residues originally present.

Appropriate quantities of the compounds to be added (available in a high standard of purity) were weighed out, dissolved in a small volume of purified n-hexane, and thoroughly mixed into a larger volume taken from the total available quantity of fish oil. This treated volume of fish oil was then incorporated into the remaining (much larger) volume of oil, and thorough mixing given by mechanical stirring for several hours. Suitable aliquots of this spiked oil were distributed as Sample 2B. The two samples were provided in glass bottles closed with screwcaps containing PTFE seals.

The participating laboratories were asked to analyse the two samples by the techniques which they normally employed for the examination of fish tissues (Appendix IV) and to identify and quantify as many organochlorine residues as possible, within the limits of their usual practice. The results were to be reported to the organisers of the exercise, after which they would receive in confidence a statement of the identities and concentrations of the compounds added to Sample 2A to produce Sample 2B. It was thereby hoped that any advantage gained from prior knowledge of the content of the spike could be avoided.

The success of the first intercalibration exercise provided encouragement for the preparation of a further sample for organochlorine analysis, which would present a greater but more realistic challenge to the analysts. The original oil contained easily detectable residues, as the spike concentrations added were considerably greater than are likely to be found

in most environmental samples, particularly from relatively unpolluted areas. The second intercalibration sample was therefore designed to contain, in spiked form, concentrations of residues which were approximately one order of magnitude lower than in the earlier sample. This necessitated a different, cleaner matrix oil. At the same time, it was considered appropriate to include certain residues which are commonly detectable in environmental samples but which are not often reported by analysts, or which might be incorrectly identified.

No fish oil could be found with residues sufficiently low to provide a suitable matrix, i.e., with concentrations an order of magnitude less than the proposed spike concentrations, although fish oils from various sources including Australia were tested. Consequently, a vegetable (maize) oil was finally selected, although it was recognised that some methods of analysis might be easier to perform with this type than with a fish oil.

The spiked oil was prepared in a manner identical to that used for the earlier samples, and the two aliquots 3A (unspiked) and 3B (spiked) were distributed towards the end of 1974 or early in 1975, in association with the third fish flour sample. The samples were dispatched in glass bottles with screwcaps sealed with metal foil. Analysts were again asked to identify and quantify organochlorine residues using their normal analytical methods (Appendix IV) and to report the results to the organisers, following which they would be provided in confidence with the true values for the spike addition. (The same sample numbers, and the same confidential procedure, were adopted for the concurrent OECD exercise, a few analysts being participants in both programmes.)

RESULTS

1972 Exercise

The results of the first intercalibration exercise for organochlorine analysis were reported in ICES Cooperative Research Report, No.39 (ICES, 1974), but are reproduced in revised form in Tables 15-17. Table 14 lists the participating laboratories (for both exercises) and identifies them by letters for use in subsequent tables.

Only the laboratories understood to be participating in the baseline surveys or coordinated monitoring are included. One laboratory (M) originally reported very low values, but concentrations of organochlorine residues found by the same laboratory in fish from the North Sea were slightly higher than those reported by other laboratories, and an error was subsequently found in the calculation of the intercalibration results from this laboratory. The revised values have therefore been included in the tables. Laboratory K submitted intercalibration data after the Analysts Group had met to discuss the initial results, but these two have been included.

It will be seen from Table 15 that all eight participating laboratories were able to determine the three residues of the pp'-DDT group and PCB in the unspiked sample, but fewer identified or determined α-HCH, dieldrin and op-DDT. The α-HCH emerges early in GLC analysis and is often ignored or not easily identified. Dieldrin may be confused with pp'-DDE unless the technique is capable of separating them, and Laboratories E and H used an acid clean-up procedure (ICES, 1974) which would destroy dieldrin. op-DDT (an impurity in some commercial grades of pp'-DDT) is often hidden by PCB peaks unless the technique used is able to separate PCB from other residues. PCB residues also interfere in the determination of pp'-DDE, unless the latter is in considerable excess, but only one laboratory (J) corrected for PCB interference. The

concentrations in Sample 2A were relatively high by comparison with most fish tissue samples, and the coefficients of variation among the analysts for the residues most frequently determined were 20 - 47%.

Table 16 presents the results obtained for the spike additions to Sample 2A, and most analysts were able to determine all the added compounds with the exception of op-DDT. Table 17 summarises the statistical analyses of these results. For the six residues which were determined by the majority of analysts, in only one instance was a value reported which was rejected as grossly outside the range expected. The coefficients of variation ranged from 6.9% to 18.9%, a relatively high level of agreement, probably due to the easily-determined concentrations involved. With the exception of op-DDT, for which only two values were reported, the mean percentage recoveries of the residues (based on the amounts stated to have been added) varied from 93.3% to 106.0%, indicating that the extraction and clean-up techniques employed had been very successful.

1974 Exercise

The results reported for the second ICES organochlorine intercalibration exercise, based on Samples 3A and 3B, are given in Tables 18-20. The group of laboratories involved included some which had not taken part in the previous exercise. The lower concentrations of residues in the unspiked oil 3A (Table 18) were generally below the limits of detection attained by most analysts, these limits in the majority of cases being less than 10 µg/kg. Only one laboratory (G) reported substantially higher and positive values, which were of the same order as those which that laboratory found for the spiked sample. In view of the doubts raised regarding the precision of the method used, the calculated values of the spike addition from Laboratory G have been excluded from the subsequent statistical analysis.

The added spike consisted of eight individual compounds and Aroclor 1254. Most participants were able to identify correctly and determine all but β-HCH. Table 19 presents the calculated values of the spike addition after subtracting from the analyses of Sample 3B the positive values reported for Sample 3A. "Less than" values given for the latter, irrespective of the limit stated, were ignored. Three types of PCB mixtures were reported to have been used as reference standards, but Aroclor 1260 (a 60% chlorinated mixture) appears to have caused the estimate of an excessively low result for Laboratory B, while Laboratory E obtained a very high value (standard not stated).

The statistical analysis of the results is summarised in Table 20. Apart from the data from Laboratory G, already referred to, certain values have been excluded where they were found to differ from the mean by more than three standard deviations. These include two values for β-HCH below the detection limit, one value for DDE (5.4 x s.d.), two values for TDE (3.6 x s.d.), one value for DDT (5.5 x s.d.) and the two values for PCB previously mentioned (6.1 x s.d. and 10.2 x s.d.). The coefficients of variation among the laboratories for the different residues range from 5.0% to 40.6%, the latter value (for HCB) being appreciably higher than the next highest (27.9% for γ-HCH). The level of agreement is generally good, and particularly so for the PCB and DDT group residues most commonly determined (5.0 - 13.2%).

The mean percentage recoveries of the added compounds (80.7 - 104.4%) were also very satisfactory, and again the PCB and DDT group provided particularly good values (92.0 - 102.9%). The laboratories did not, in general, indicate whether the DDE value reported had been corrected for PCB interference, although this is not likely to have caused any appreciable error.

DISCUSSION

The results from the first intercalibration exercise were very encouraging, although the relatively high concentrations present provided little challenge to a competent analyst. The second exercise for organochlorines called for a more careful technique and a lower level of detection, but despite this the agreement among analysts was generally good for the more commonly determined residues, the coefficient of variation between laboratories being of the order of ± 10%. For the less common determinations, of HCB, HCH isomers and dieldrin, the coefficients of variation were mostly above ± 20%. Recovery values in both exercises were good, generally in the range of 90 - 110%, which is quite satisfactory for monitoring purposes. It must be emphasised that these results were obtained without any attempt to restrict the range of analytical techniques used by the various laboratories, and although an examination was made of the methods used in both exercises (Appendix IV) no evidence was found that the separation or GLC procedures had influenced the results. (However, the use of sulphuric acid in the clean-up stage will destroy dieldrin, as has already been mentioned).

The coefficients of variation between ICES analysts in the two organochlorine exercises compare favourably with those found in other similar studies, some using the same samples. Table 21 summarises the values reported in these studies for the residues most commonly measured. There was a significant improvement in the agreement among analysts between the first and second ICES intercalibration exercises for organochlorines, and the comparison with the groups of analysts examining Samples 2B and 3B was also good.

Further organochlorine intercalibration samples have been requested to provide a continuing assessment of the agreement among the analysts reporting the results of their examination of fish samples from national programmes. These intercalibration samples could include residues not normally found by all countries (e.g., certain compounds used on a large scale only in North America). If the concentrations are required to be similar to those normally encountered in fish and shellfish, it may be necessary to abandon the practice of spiking unless a new matrix, free of residues but otherwise similar to fish oil, can be obtained. As the percentage recovery of added organochlorines has been high in the exercises so far, the knowledge of the added concentrations may not be necessary in the future and a natural fish oil could then be used. A further possibility could be the use of known additions which are disclosed prior to analysis, the analysts being instructed to ensure that their technique can achieve at least a 95% level of recovery, with a within-laboratory coefficient of variation of no more than ± 10% before reporting the analysis of fish and shellfish samples. A between-laboratory coefficient of variation of ± 15% is probably the best attainable, unless the analyses of field samples are adjusted to compensate for the errors detected in the analysis of known standards.

The ICES exercises were not able to test extraction techniques or variations in the preliminary handling of tissue samples, but the extraction techniques generally used for biological samples are believed to be very efficient. Larger variations could be incurred in the methods of handling and dissecting fish and in weighing aliquots of tissue if air drying takes place. It is considered that the analytical techniques available, and the expertise of the personnel using them, are adequate for the current and contemplated

international programmes of monitoring organochlorine residues in fish and shellfish. If the investigations should at any time be extended to sediment or water samples, it may be necessary to examine the variations resulting from the use of different extraction techniques, although the subsequent analytical processes will probably differ from those already examined in the two ICES intercalibration exercises.

It seems unnecessary at the present time to examine more critically the accuracy of determinations made for organochlorine residues one order of magnitude lower than those present in Sample 3B. These levels were in the range 0.04 - 0.20 mg/kg with the exception of PCB, but the reported values for Sample 3A suggest that most analysts would not expect to estimate values below 0.010 mg/kg. However, if analyses of sediment and, particularly, of water were to be required in the future, the techniques of sampling clean-up and analysis will need considerable improvement. All solvents and adsorbents must be of the highest purity and specially prepared, and laboratory working conditions maintained at a standard higher than generally exists at the present time.

PART III

RECOMMENDATIONS FOR FUTURE WORK

In view of the benefits gained from the three intercalibration exercises it is timely to contemplate the style and frequency of future work of this kind. Recognising the NBS recommendation (IDOE, 1972) that intercalibration should be at frequent intervals, the efforts involved for what are relatively small groups of analysts preclude repetition of exercises more often than every 2 or 3 years.

Since no single reference material can adequately test the range of natural variation for all the metals encountered in baseline monitoring, two or more preparations are required for future intercalibration exercises. (For example, the 2nd and 3rd fish flours contained mercury at concentrations typical only of the upper limit encountered in the fish baseline monitoring programmes.)

Experience in these exercises has suggested that the uniform presentation of data is essential. It would certainly assist future coordinators of this type of exercise if the participants could present their data in a standard format with more details on calculations, limits of detection and perhaps any comments relevant to the conduct of the exercise and its background of field analysis.

Those responsible for monitoring programmes, which should include intercalibration exercises, must obviously give some thought to whether or not the analytical methods adopted by some laboratories are sufficiently good to deal with, for example, the low levels of lead and cadmium currently encountered in the uncontaminated environment; provided that knowledge of such values is fundamental to the interpretation of data derived from these studies. Information solely of academic value should not be the subject of a major international programme.

When intercalibration samples of known residue content (or two samples for which the difference is known) are used, an estimate of the accuracy of the analytical techniques can be made, and it may be possible to differentiate between methods in this respect. It is essential that the original bulk mixture which is used to prepare individual intercalibration reference samples is homogeneous. For samples of unknown composition, only the extent of agreement among analysts can be determined, as by a multiple range test, but it cannot be assumed that the mean or median value is necessarily a close approximation to the true value. For metal analyses, some method of spiking and suitable matrix could be sought, although such a technique is sometimes criticised on the grounds that the added residues may be more easily released (and thus determined) than those present naturally. Failure to achieve a satisfactory determination of the quantities added, in the presence of a natural matrix, would nevertheless call for further investigation.

The above remarks relate particularly to intercalibration samples for heavy metals. The samples used for intercalibration of organochlorine analyses, while believed to be truly homogeneous and containing a known but undisclosed mixture of commonly occurring organochlorine compounds, were nevertheless somewhat easier to analyse than the various fish or shellfish tissues examined in the baseline studies. As with metal residues, it has not so far been found possible to make appropriate additions of selected substances in known quantities to fish tissue in such a way that the added substances can be considered to be bound to the matrix in a manner identical to that in which they are found in the natural environment.

The magnitude of the concentrations added must be sufficiently greater than those initially present in the matrix for an accurate assessment of their recovery to be possible, and yet not so large as to make the analyses unrealistic, in relation to concentrations found in environmental samples. A matrix free of organochlorine contaminants, but otherwise representative of tissues normally analysed, has so far not been found. This should ideally be of animal origin, to resemble more closely the composition of fish tissue, as vegetable protein (which can be found free of organochlorine contaminants) is more easily processed and therefore less demanding of the analyst.

ACKNOWLEDGMENTS

Thanks are due to Miss A Shanks of the Statistics and Computing Team, DAFS, Marine Laboratory, Aberdeen, for the statistical analyses presented in this paper. Thanks are also due to Dr Burgess, MAFF, Torry Research, Aberdeen and Mr Wignall, MAFF, Humber Laboratory, Hull, for arranging the preparation of the fish flour used in this exercise, and to Dr R Scott and his colleagues, Macaulay Institute for Soil Research, Aberdeen, for their help with the final grinding of the fish flour and for the advice received during the preparation of this reference sample.

REFERENCES

Anderson, D.H., Murphy, J.J. and White, W.W., 1972. Gelatine as matrix for mercury reference material. Anal.Chem., 44:2099-2100.

Berlin, A., Lanwerys, R., Bucket, J.P., Roels, H., Del Castillo, P., and Smeets, J., 1974. Intercomparison programme on the analysis of lead, cadmium and mercury in biological fluids. Contrib. No.141, Int.Symp. - Environment and Health CEC-EPA-WHO, Paris, June 1974.

Bowen, H.J.M., 1967. Comparative elemental analyses of a standard plant material. Analyst, 92:124-131.

Holden, A.V., 1975. The OECD International Co-operative Studies of Organochlorine Residues in Wildlife. In: Environmental Quality and Safety Suppl., III (ed. F. Coulston and F. Karte) Pesticides, pp.40-46. Publ. G. Thieme (Stuttgart).

ICES, 1974. Report of the Working Group for the International Study of the Pollution of the North Sea and its Effects on Living Resources and their Exploitation. Coop.Res.Rep., No.39.

ICES, 1977a. Studies of the pollution of the Baltic Sea, by the ICES/SCOR Working Group on the Study of Pollution of the Baltic. Coop.Res. Rep., No.63.

ICES, 1977b. A baseline study of contaminating substances in living resources in the North Atlantic. Coop.Res.Rep., No.69.

IDOE, 1972. Baseline Studies of Pollutants in the Marine Environment and Research Recommendation. The IDOE Baseline Conference, May 24-26, 1972, New York.

OECD,(in prep.). Report on OECD Programmes of Wildlife Sampling and Analysis for Environmental Contaminants.

Pavlou, S.F. and Hom, W., 1976. Interlaboratory calibration results from chlorinated hydrocarbon analyses in marine sediments. Marine Chemistry, 4:155-163.

Snelson, J.T., 1976. Analysis of organochlorine residues in butter fat. Codex Cttee on Pesticide Residues. Int. Collaborative Study. Publ. PB 256, Austr. Governm. Publ.Serv. (Canberra).

Topping, G., 1975. An appraisal of the 1973 ICES Trace Metal Intercalibration Exercise. ICES, Doc. C.M.1975/E:36 (mimeo.).

Uthe, J.F., Armstrong, F.A.J. and Tam, K.C., 1971. Determination of trace amounts of mercury in fish tissues, results of a North American Check Sample Study. J.Ass. of Offic. Anal.Chem., 54: 866-869.

Windsor, M.L., 1971. Fish meal. Torry advis. note (49).

Windsor, M.L., 1972. British developments in meal technology. Fishing News Int., 11(9):37-41.

Table 1

COUNTRIES/INSTITUTES PARTICIPATING IN ICES TRACE METAL INTERCALIBRATION EXERCISES

Country	Institute	Lab.No.	Exercises in which laboratories have participated		
			1	2	3
Belgium	Ministère de l'Agriculture, Institut de Recherches Chimiques, Tervuren	1	x	x	x
	Vrije Universiteit, Brussels	2			x
Canada	Environment Canada. Fisheries & Marine Service	3			x
	Research and Development Directorate, Halifax Laboratory, Nova Scotia	4			x
	Ministry of Agriculture and Food, Provincial Pesticide Residue Testing Laboratory, Guelph, Ontario	5			x
Denmark	Grønlands Geologiske Undersøgelser, Copenhagen	6			x
	Institute of Petrology, University of Copenhagen	7			x
France	Institut scientifique et technique des Pêches maritimes, Nantes	8	x	x	x
Germany (Fed.Rep. of)	Bundesforschungsanstalt f. Fischerei, Hamburg	9	x	x	x
Iceland	Marine Research Institute, Reykjavik	10		x	x
Netherlands	Netherlands Institute for Fishery Investigations, IJmuiden	11	x	x	x
Norway	The Official Norwegian Quality Control Institute for Canned Fish Products, Stavanger	12a	x	x	
	Government Vitamin Institute, Directorate of Fisheries, Bergen	12b			x
Portugal	Instituto Nacional de Investigação, Lisbon	13			x
Sweden	Statens Naturvårdsverk, Drottningholm	14	x	x	x
United Kingdom					
England	MAFF Fisheries Laboratory, Burnham-on-Crouch	15	x	x	x
Scotland	DAFS Marine Laboratory, Aberdeen	16	x	x	x
U.S.A.	Marine Research Laboratory, University of Connecticut	17			x
	Middle Atlantic Coastal Fisheries Centre, Milford Laboratory, Connecticut	18			x
	US Dept. of Commerce, NOAA National Marine Fisheries Service, Maryland	19			x
	Environmental Protection Agency, South Ferry Road, Narragansett, R.I.	20			x
Ireland	Department of Agriculture & Fisheries, Fisheries Division, Dublin	21			x

Table 2

RESULTS OF 1ST INTERCALIBRATION EXERCISE

FISH FLOUR ANALYSES (µg/g)

Lab. No.	Copper	Zinc	Mercury	Cadmium	Lead	No. of Analyses
1	17	80	0.11	-	1.9	2
8	11	55	0.17	2.5	9.0	1
9	19	39	-	-	-	1
11	-	-	1.1	-	-	1
12a	20	75	0.17	1.3	1.0	1
14	-	71	0.09	2.4	7.1	4
15	17	75	0.23	1.3	8.4	1
16	20	66	0.22	1.1	5.7	1

Table 3

ICES 2nd INTERCALIBRATION EXERCISE

RESULTS OF FISH FLOUR ANALYSES (/ug/g) USING INDIVIDUAL METHOD

Lab. No	Copper			Zinc			Mercury			Cadmium			Lead		
	Mean+ Value	sd*	CV**	Mean Value	sd	CV	Mean Value	sd	CV	Mean Value	sd	CV	Mean Value	sd	CV
1	8.62	0.54	6.3	27.1	0.4	1.5	0.83	0.04	4.8	0.73	0.11	15.1	1.3	0.2	15.3
8	9.5	0.9	9.5	24.8	1.8	7.2	0.75	0.04	5.3	0.7	0.1	14.3			
9	10.0	0.6	6.0	31.1	1.6	5.1	0.73	0.05	6.8	0.43	0.04	9.3	1.7	0.1	5.9
10							0.72	0.01	1.4						
11	8.3			32			0.6			1.1					
14	8.63	1.60	18.5	26.9	1.3	4.8	0.66	0.04	6.1	1.12	0.23	20.5	<3		
15	10.1	0.5	4.9	23	2	8.7	0.63	0.03	4.8	<0.02			2.5	0.2	8.0
16	9.6	0.2	2.0	25.6	1.0	3.9	0.65	0.04	6.2	0.35	0.02	5.7	1.9	0.3	15.9

+ based on 6 replicate analyses

* sd = standard deviation

** CV = Coefficient of Variation

Table 4

ICES 2nd INTERCALIBRATION EXERCISE

RESULTS OF FISH FLOUR ANALYSES (µg/g) USING COMMON METHOD

Lab. No.	Copper			Zinc			Mercury			Cadmium			Lead		
	Mean+ Value	sd*	CV**	Mean Value	sd	CV	Mean Value	sd	CV	Mean Value	sd	CV	Mean Value	sd	CV
1	9.86	0.79	8.0	27.4	0.06	0.2	0.83	0.01	1.2	0.47	0.13	27.7	0.25	0.01	4.0
9	8.7	0.6	6.9	29.5	2.1	7.1				0.55	0.08	14.5	3.0	0.2	6.7
10							0.48	0.04	8.3						
14	10.1	0.6	6.0	23.9	0.3	1.2	0.65	0.02	3.1	0.40	0.05	12.5	1.4		
15	10.1	0.5	5.0	23	2	8.7	0.47	0.07	14.9	<0.2			2.5	0.2	8.0
16	9.9	0.4	4.0	25.7	0.9	3.5	0.62	0.03	4.8	0.39	0.03	7.7	2.0	0.2	10.0

+ based on 6 replicate analyses

* sd = standard deviation

** CV = Coefficient of Variation

Table 5

ICES 2nd INTERCALIBRATION EXERCISE

RESULTS OF THE ANALYSIS OF THE ACIDIC REFERENCE SOLUTION

METAL CONCENTRATION (µg/ml)

Lab. No.	Copper		Zinc		Mercury		Cadmium		Lead	
	Mean	sd*	Mean	sd	Mean	sd	Mean	sd	Mean	sd
1	0.392	0.012	0.608	0.036	0.18	0.004	0.061	0.0006	0.249	0.005
8	0.40	0.03	0.53	0.04	0.14	0.005	0.12	0.007		
9	0.50	0.02	0.54	0.04	0.10	0.008	0.07	0.01	0.07	0.02
10					0.125	0.002				
14	0.460	0.018	0.548	0.001	0.110	0.004	0.201	0.004	0.340	0.009
15	0.48		0.50		0.124		0.15		0.49	
16	CONTROL LABORATORY									
True Value	0.40		0.50		0.10		0.10		0.30	

sd* sd = standard deviation

Table 6

ICES 2nd INTERCALIBRATION EXERCISE

COMPARISON OF "ADJUSTED" VALUES OF MERCURY, COPPER AND LEAD IN FISH FLOUR WITH THE ORIGINAL REPORTED VALUES

Lab. No.	REPORTED VALUES (µg/g)			ADJUSTED VALUES (µg/g)		
	Copper	Zinc	Mercury	Copper	Zinc	Mercury
1	8.62	27.1	0.83	8.8	22.3	0.46
8	9.5	24.8	0.75	9.5	23.4	0.54
9	10.0	31.1	0.73	8.0	28.8	0.73
10			0.72			0.58
14	8.63	26.9	0.66	7.5	24.5	0.60
15	10.1	23	0.63	8.4	23	0.51
16	9.6	25.6	0.65	9.6	25.6	0.65

Table 7

SUMMARY OF PREPARATION OF MERCURY STANDARDS BY ANALYSTS IN 2nd EXERCISE

Lab.No.	Stock Solution	Intermediate Solution	Daily Working Solution
14	1000 µg/ml BDH standard solution	10 µg/ml (0.1 N HCl) weekly	0.1 µg/ml (0.1 N HCl) daily
8	100 µg/ml. Prepared in laboratory using $HgCl_2$ every 4 months. 5% conc. HNO_3	1.0 µg/ml 5% HNO_3 daily	0.1 - 1.0 µg/ml 5% conc. HNO_3 daily
9	1000 µg/ml "Merck Titrisol" 1N HNO_3 every 3 months		0.004 µg/ml daily
1	500 µg/ml. Prepared in laboratory 1N H_2SO_4 (+$KMnO_4$) every month		0.02 µg/ml 1N H_2SO_4(+$KMnO_4$) daily/weekly
15	1000 µg/ml. Prepared in laboratory 5% conc. HNO_3 ~ 6 months	10 µg/ml 5% conc. HNO_3 monthly	0.1 µg/ml 5% HNO_3 daily
16	1000 µg/ml BDH 1N HCl 6 months		10 µg/ml 0.01 N HCl (+$KMnO_4$) daily
10	100 µg/ml 1N HNO_3 1 month		0.02 µg/ml 1N HNO_3 1-5 days
11	1000 µg/ml BDH 1N HCl		0.002-0.010 µg/ml 10% H_2SO_4/40% HNO_3 weekly

Table 8

ICES 3rd INTERCALIBRATION EXERCISE

RESULTS OF FISH FLOUR ANALYSIS (µg/g)

Lab. No.	Copper			Zinc			Mercury			Cadmium			Lead		
	Mean value	s.d.*	C.V.**	Mean value	s.d.	C.V.	Mean value	s.d.	C.V.	Mean value	s.d.	C.V.	Mean value	s.d.	C.V.
1	3.09	0.17	5.6	38.5	0.5	1.2	0.80	0.03	3.4	0.053	0.022	40.8	2.08	0.08	3.7
2	3.05	0.19	6.1	35.9	1.5	4.2	1.26	0.15	12.1	0.123	0.006	4.6	2.99	0.13	4.4
3	3.42	0.20	5.8	36.6	0.9	2.5	0.88	0.04	4.1	0.023	0.003	12.2	0.52	0.01	2.7
4	4.63	2.2	47.2	36.9	0.5	1.3	0.89	0.08	8.5	0.060	0.0005	5.0	1.45	0.21	14.6
5	5.68	0.77	13.5	49.5	5.5	11.2				0.177	0.059	33.3	0.25	0.07	26.5
6	2.69	0.63	23.6	27.8	6.8	24.6	0.94	0.05	5.3	0.036	0.009	24.4	0.59	0.10	16.2
7	3.53	0.66	18.6	33.7	2.1	6.2				<1.8			1.08	0.45	41.5
8				33.8	5.4	16.0	0.90	0.05	5.6	0.41	0.05	12.2	4.00	0.4	10.0
9	3.82	0.13	3.3	39.2	1.1	2.9	0.81	0.02	2.9	0.028	0.002	6.4	0.53	0.06	10.5
10	3.35	0.09	2.7	38.3	0.9	2.3	0.93	0.02	2.8	0.022	0.013	59.5	0.16	0.06	40.8
11	3.86	0.28	7.2	37.6	1.6	4.4				0.055	0.012	21.1	0.51	0.08	16.6
12+	4.2	0.02	0.05	36.0	0.14	-	0.79	0.12	15.2	0.042	0.056	133.3	0.81	0.02	2.5
13	4.37	0.62	14.2	52.7	0.8	1.6	0.74	0.03	4.5	0.552	0.041	7.5	1.04	0.26	24.9
14	4.22	0.50	11.9	42.8	1.6	3.7	0.82	0.02	2.6	0.020	0.008	42.0	0.21	0.03	14.4
15	3.72	0.17	4.6	34.8	1.7	4.9	0.83	0.01	1.2	<0.2			0.53	0.27	49.9
16	3.67	0.20	5.5	40.3	1.1	2.8	0.90	0.03	3.7	<0.030			0.34	0.07	20.5
17	3.38	0.17	5.1	31.4	1.3	4.1	0.80	0.02	2.8	0.39	0.05	12.0	2.30	0.06	2.8
18	3.58	0.62	17.3	28.3	8.6	30.4	0.90	0.04	4.0	<0.24			3.00	1.35	45.1
19a,b	3.84	0.08	2.1	35.6	0.8	2.3	0.90	0.16	18.1	0.17	0.12	69.9	1.18	0.21	18.0
				41.7	3.4	8.2									
20	4.14	0.52	12.5	41.8	1.6	3.8				0.12	0.04	33.3	0.85	0.18	21.2
21	3.01	0.84	27.9	31.0	0.94	2.9				0.125	0.02	16.0	1.62	0.5	30.9

+Mean values were based on a standard addition technique and not on the analysis of six replicates. Because of this the results from Laboratory 12 have been excluded from the multiple range test analysis.

*Standard deviation

**Coefficient of variation

Table 9

DETECTION LIMITS (EXPRESSED AS µg/g FISH MEAL) OF ANALTYICAL TECHNIQUES EMPLOYED BY EACH LABORATORY

Lab No.	Cu	Zn	Hg	Cd	Pb
1	0.5	5	0.2	0.02	1
2	0.1	2.5	0.2	0.005	0.01
3	0.02	0.05	0.02	0.005	0.02
4	0.01	0.002	0.0004	0.0005	0.005
5	0.08	0.6	-	0.006	0.007
6	0.02	1	-	0.002	0.002
7	0.01	1.8	-	1.8	0.1
8	-	2	0.02	0.05	1.5
9	0.008	0.004	0.02	0.001	0.004
10	0.03	0.3	0.005	0.001	0.01
11	0.048	0.078	0.001	0.0014	0.02
12	1.6	0.8	0.001	0.005	0.05
13	0.02	0.1	0.05	-	-
14	0.3	1	0.02	0.001	0.01
15	0.2	1	0.005	0.2	0.4
16	0.1	0.1	0.03	0.03	0.2
17	0.05	0.25	0.02	0.06	0.35
18	1.0	0.25	0.14	0.20	1.5
19	0.04	0.15	-	0.015	0.2

Table 10

ICES 3rd INTERCALIBRATION EXERCISE

RESULTS OF MULTIPLE RANGE TESTS - COPPER, ZINC AND MERCURY DATA

Copper

Lab. No.	6	21	2	1	10	4	17	3	7	18	16	15	9	19	11	20	14	13	5
Mean value	2.69	3.01	3.05	3.09	3.35	3.38	3.38	3.42	3.53	3.58	3.67	3.72	3.82	3.84	3.86	4.14	4.22	4.37	5.68

Zinc

Lab. No.	21	17	7	15	19a	2	3	4	11	10	1	9	16	20	14	13
Mean Value	31.0	31.4	33.7	34.8	35.6	35.9	36.6	36.9	37.6	38.4	38.5	39.2	40.3	41.8	42.8	52.7

Mercury

Lab. No.	13	17	1	9	14	15	3	4	8	16	18	10	6
Mean value	0.74	0.80	0.80	0.81	0.82	0.83	0.88	0.89	0.90	0.90	0.90	0.93	0.94

Table 11

SUMMARY TABLE FOR THOSE LABORATORIES WHICH PARTICIPATED IN ALL THREE EXERCISES

	Laboratory Nos							
	1	8	9	11	14	15	16	C.V.*
COPPER								
1st	17	11	19	-	-	17	20	21%
2nd	8.62	9.5	10.0	8.3	8.63	10.1	9.6	8%
3rd	3.09	-	3.82	3.86	4.22	3.72	3.67	10%
ZINC								
1st	80	55	39	-	71	75	66	23%
2nd	27.1	24.8	31.1	32	26.9	23	25.6	12%
3rd	38.5	33.8	39.2	37.6	42.8	34.8	40.3	8%
MERCURY								
1st	0.11	0.17	-	1.1**	0.09	0.23	0.22	38%
2nd	0.83	0.75	0.73	0.6	0.66	0.63	0.65	12%
3rd	0.80	0.90	0.81	-	0.82	0.83	0.90	5%

* overall coefficient of variation

** value excluded from the calculation of the coefficient of variation

Table 12

ICES 3rd INTERCALIBRATION EXERCISE

COMPARISON OF FISH FLOUR DATA (µg/g) FOR CADMIUM AND LEAD ON THE BASIS OF DETECTION LIMITS

Laboratory Number	CADMIUM Detection Limits		LEAD Detection Limits	
	≤0.002 µg/g	>0.002 µg/g	≤0.01 µg/g	> 0.01 µg/g
1	-	0.53	-	2.08
2	-	0.123	2.99	-
3	-	0.023	-	0.52
4	0.060	-	1.45	-
5	-	0.177	0.25	-
6	0.036	-	0.59	-
7	-	< 1.8	-	1.08
8	-	0.41	-	4.00
9	0.28	-	0.53	-
10	0.022	-	0.16	-
11	0.055	-	-	0.51
12	-	0.042	-	0.81
13	-	0.552	-	1.04
14	0.020	-	0.21	-
15	-	< 0.2	-	0.53
16	-	< 0.030	-	0.34
17	-	0.39	-	2.30
18	-	< 0.24	-	3.00
19	-	0.17	-	1.18
Mean value	0.040	0.201*	0.88	1.44

* < signs have been ignored in computing mean value and the data for Laboratory number 7 has been excluded from the calculation of the mean

Table 13

COMPARISON OF ICES 3rd EXERCISE WITH OTHER INTERCALIBRATION STUDIES

METAL	INTERCALIBRATION SAMPLE	NO OF ANALYSTS	MEAN* VALUE	RANGE OF VALUES	C.V**	REFERENCE
Mercury	Fish Flour	6	0.84	0.80-0.90	5.0	This Study***
	Standard Plant Material	9	0.150	-	5.3	Bowen 1967
	Wet Fish	29	1.36	0.93-1.80	16.9	Uthe et al. 1971
	Wet Fish	29	0.10	0.03-0.21	60.0	Uthe et al. 1971
	Wet Fish	29	4.26	2.8 -5.4	18.1	Uthe et al. 1971
	Orchard Leaves	11	0.15	0.10-0.20	24.6	IDOE 1972
	Gelatine Standard	19	2.0	1.88-2.18		Anderson et al. 1972
	Blood	18	2.6	2 -9	80.5	Berlin et al. 1974
	Urine	29	7.5	1 -87	20.6	Berlin et al. 1974
Copper	Fish Flour	6	3.73	3.07-4.22	10.0	This Study***
	Standard Plant Material	88	4.81	-	15.3	Bowen 1967
	Orchard Leaves	10	11.6	9.9 -13.0	7.7	IDOE 1972
Zinc	Fish Flour	7	38.1	33.8-42.8	8.0	This Study***
	Standard Plant Material	77	31.9	-	15.1	Bowen 1967
	Orchard Leaves	11	24.2	18 -29	13.6	IDOE 1972

* Mean value expressed in /ug/g for solid material; /ug/100 ml for blood and /ug/l for urine.

** C.V = coefficient of variation.

*** Represents those analysts who participated in all 3 intercalibration exercises.

Table 14

LABORATORIES PARTICIPATING IN ORGANOCHLORINE INTERCALIBRATIONS

Identification Letter	Address	Country
A	Bedford Institute, Halifax	Canada
B	National Food Institute, Søborg	Denmark
C	Bundesforschungsanstalt, Hamburg	Germany (Fed. Rep. of)
D	Netherlands Institute for Fishery Investigations, IJmuiden	Netherlands
E	Institute of Marine Research, Bergen	Norway
F	Laboratory of Phytopharmacy, Oeiras	Portugal
G	Oceanographic Laboratory, Santander	Spain
H	Special Analytical Laboratory, Stockholm	Sweden
I	MAFF Fisheries Laboratory, Burnham-on-Crouch	UK (England)
J	DAFS Freshwater Fisheries Laboratory, Pitlochry	UK (Scotland)
K	Institute for Sea Research, Texel	Netherlands
L	Rijksuivelstation, Leiden	Netherlands
M	Station de Phytopharmacie, Gembloux	Belgium
N	Fisheries and Marine Services, Halifax	Canada

Table 15

RESULTS OF ORGANOCHLORINE ANALYSES FOR SAMPLE 2A

(Concentrations in µg/kg)

Laboratory	γ-HCH	Dieldrin	pp'-DDE	pp'-TDE	pp'-DDT	op-DDT	PCB	PCB Ref.
C	120	200	340	280	440	-	3900	1254[b]
E	-	-	370	310	370	160	2200[a]	A.50[c]
F	-	60	340	190	330	-	1230	1254
H	<10	<100	510	250	520	Present	1900	A.50
I	-	90	420	320	410	-	1400	1254
J	130	70	470	270	610	<20	1400	1254
K	-	210[a]	780[a]	480[a]	440[a]	-	2600[a]	1254
L	-	-	380	230	340	-	1400	1254
M	60[a]	70[a]	450[a]	250[a]	400[a]	-	1000[a]	1254
Mean	80	115	450	290	430	-	1890	
s.d.	56	63	137	83	89	-	907	
c.v.(%)	70	55	30	29	21	-	48	

a Values submitted after analysts' meeting

b Aroclor 1254

c Clophen A.50

Table 16

VALUES OF ESTIMATED SPIKE CONCENTRATIONS FROM SAMPLE 2B

(corrected for positive values reported for 2A)

(Concentrations in µg/kg)

Laboratory	γ-HCH	Dieldrin	pp'-DDE	pp'-TDE	pp'-DDT	op-DDT	PCB	PCB Ref.
C	30[a]	1500	7300	2600	4500	Present	8300	1254[b]
E	-	-	6300	3500	5800	700	10400[a]	A.50[c]
F	630	850	4780	2420	4500	-	9650	1254
H	730	1500	5200	2900	4700	Present	9100	A.50
I	700	1400	4100	4000	5100	-	9000	1254
J	670	1300	5100	3000	4800	300	10600	1254
K	890[a]	1400[a]	5700[a]	2600[a]	5000[a]	-	11800[a]	1254
L	720	1600	4100	2700	4600	-	10300	1254
M	880[a]	1400[a]	4800[a]	3600[a]	5900[a]	-	10500[a]	1254
Amount added	800	1500	5000	3000	5000	300	10000	1254

a Values submitted after analysts' meeting

b Aroclor 1254

c Clophen A.50

Table 17

STATISTICAL ANALYSIS OF RESULTS REPORTED FOR SPIKE ADDITION 2B - 2A

Compound	No. of Values	Values omitted	Mean (µg/kg)	True Value (µg/kg)	Mean % Recovery	S.D. (µg/kg)	Coeff. of Variation (%)
γ-HCH	6	30	750	800	93.8	± 101	13.5
Dieldrin	7	850	1440	1500	96.0	± 98	6.8
pp'-DDE	9	Nil	5260	5000	105.2	±1037	19.7
pp'-TDE	9	Nil	3040	3000	101.3	± 540	17.8
pp'-DDT	9	Nil	4990	5000	99.8	± 530	10.6
op-DDT	2	Nil	500	300	166.7	-	-
PCB	9	Nil	9960	10000	99.6	±1060	10.6

Table 18

RESULTS OF ORGANOCHLORINE ANALYSES FOR SAMPLE 3A
(concentrations in µg/kg)

Laboratory	HCB.	α-HCH	β-HCH	γ-HCH	Dieldrin	DDE	TDE	DDT	PCB	PCB Ref.
A				1	5	4	9	11	27	1254[a]
B	2	n.d.		2	n.d.	n.d.	n.d.	n.d.	n.d.	1260[b]
C	<1	6		7	3	3	<1	<1	2	
D	<10	<10	<10	<10	<10	<30	<10	<20	<300	
E							22	59	470	
F					7	15	6	6	14	1254
G		87		59	80	40	Nil	170		
H	<5	<5	<5	<5	<5	<5	<10	<5	<50	A.50[c]
I	<2	<2	<5	<2	<5	<5	<10	<20	<50	1254
J	<2	<5	<10	<5	5	5	<5	<5	<20	1254
N					<1	<1		<2	<10	1254

Note: n.d. = not detected, but no limits given

a Aroclor 1254

b Aroclor 1260

c Clophen A.50

Table 19

VALUES OF ESTIMATED SPIKE CONCENTRATIONS FROM SAMPLE 3B
(corrected for positive values reported for 3A)

(concentrations in µg/kg)

Laboratory	HCB	α-HCH	β-HCH	γ-HCH	Dieldrin	DDE	TDE	DDT	PCB	PCB Ref.
A				47	72	98	236	193	1020	1254[b]
B	14	42		42	92	109	193	190	480	1260[c]
C	30	29		41	80	100	200	175	948	
D	70	50	<10	60	120	30	130	140	1100	
E							257	192	1970	
F	53	40	49	45	90	103	205	209	944	1254
G[a]		(80)		(75)	(150)	(42)	(180)	(170)		
H	56	40	45	46	53	99	200	200	1100	A.50[d]
I	55	42	<5	52	100	83	130	190	1000	1254
J	44	45	44	85	113	128	212	197	1190	1254
N					120	88	230	220	1170	1254
Amount added	53	47	57	50	100	100	210	210	1100	1254

a Corrections for 3A were large

b Aroclor 1254

c Aroclor 1260

d Clophen A.50

Table 20

STATISTICAL ANALYSIS REPORTED FOR SPIKE CONCENTRATIONS 3B - 3A

Compound	No. of Values	Values omitted*	Mean (µg/kg)	True Value (µg/kg)	Mean % Recovery	S.D. (µg/kg)	Coeff. of Variation (%)
HCB	7	Nil	46.0	53	86.8	± 18.7	40.6
∝-HCH	7	Nil	41.2	47	87.7	± 6.4	15.5
β-HCH	3	<10, <5	46.0	57	80.7	-	-
ɣ-HCH	8	Nil	52.2	50	104.4	± 14.6	27.9
Dieldrin	9	Nil	93.3	100	93.3	± 22.7	24.3
pp'-DDE	8	30	101.0	100	101.0	± 13.6	13.5
pp'-TDE	8	130, 130	216.6	210	103.1	± 22.2	10.2
pp'-DDT	9	140	196.2	210	93.4	± 12.7	6.5
PCB	8	480,1970	1059	1100	96.3	± 95.2	9.0

* All values as stated, which differ from the mean by more than three standard deviations, and all values from Laboratory G.

Table 21

COMPARISON OF PERCENTAGE COEFFICIENTS OF VARIATION BETWEEN ANALYSTS IN ORGANOCHLORINE INTERCALIBRATION STUDIES

Study	Type of Sample	No of Analysts	Type of Residue			References
			DDE	DDT	PCB	
OECD (1969)	Spiked corn oil	19	14.4	16.1	-	Holden (1975)
OECD (1972)	Spiked fish oil (2B)	15	12.8	16.2	12.0	Holden (1975)
ICES (1972)	Spiked fish oil (2B)	9	19.7	10.6	10.6	This report
OECD (1975)	Spiked corn oil (3B)	23	24.2	17.8	15.4	OECD (in prep.)
ICES (1975)	Spiked corn oil (3B)	8	13.5	6.5	9.0	This report
Univ. Washington	Marine Sediment	10	-	-	22	Pavlou and Horn (1976)
Codex	Spiked butter fat	190	27.8	21.0	-	Snelson (1976)

APPENDIX I

2nd INTERCALIBRATION EXERCISE - INFORMATION ON:

(a) Preparation of Reference Fish Flour

(b) Acidic Reference Solution

(c) Procedure for the Analysis of the Fish Flour

(d) Common Procedure

(e) Summary of Methods Employed by Participants

(a) Preparation of Reference Fish Flour

The fish flour used in this exercise was prepared from freshly caught inshore cod by the MAFF Humber Laboratory in Hull. The details of preparation of this meal are as follows:

i. Freshly caught cod from an inshore area was stored in ice after capture.

ii. The fish was filletted but the fillets were not skinned.

iii. The fillets were then steamed for ca 30 mins and then broken up into small pieces.

iv. The cooked fish was air dried in a tunnel for ca 24 hours.

v. The dried fish was minced and then repeatedly ground in a hammer mill.

(b) Acidic Reference Solution

The solution of metal ions was prepared from BDH stock standard solutions by dilution, using 1 N HCl as the diluent. The exact composition of this standard was as follows:

Copper - 0.40 µg/ml; Zinc - 0.5 µg/ml; Mercury - 0.10 µg/ml; Cadmium - 0.10 µg/ml and Lead - 0.30 µg/ml.

Each analyst or laboratory participating in this exercise received 2 plastic phials, each containing ca 25 gm fish flour, and

an additional 2 phials containing ca 20 ml of the reference solution.

(c) Procedure for the Analysis of the Fish Flour

1. The fish flour should be analysed by the analytical procedure adopted by you for the baseline survey of trace metals in 1972 and also by the "common procedure" discussed at the last meeting - details of this "common procedure" are given below.
2. All analyses should be carried out 6 times and the full results together with mean values, standard deviations and details of the analytical method should be sent to the coordinator.

(d) Common Procedure

The fish flour (ca 3 gm) should be weighed without further drying into a 100-150 ml flat-bottomed silica flask and treated with 20 ml concentrated nitric acid (Aristar or similar grade). The flask should be covered with a silica bubble stopper and allowed to stand for 1 hour at room temperature. Transfer the flask to a hot plate having a surface temperature of ca 140°C and allow the acid to reflux for ca 12 hours. The bubble stopper should then be removed and the solution slowly evaporated to a volume of 2-3 ml. After cooling, the solution and washings should be transferred to a 25 ml graduated flask and diluted to the mark using distilled water.

The solution should be centrifuged to remove any suspended matter and then examined for trace metals using standard atomic absorption techniques, correcting for non-atomic absorption using background correction.

Please analyse for as many trace metals as possible, but include Cu, Zn, Pb, Cd and Hg.

(e) Summary of the Individual Analytical Techniques Employed by Laboratories during the Second Exercise

Lab No	Mercury	Other Metals
1	Wet digestion H_2SO_4/H_2O_2 + $KMnO_4$ Cold vapour analysis.	Dry combustion at 450°C for Cu, Pb and Zn. Flameless AA for Cu and Pb. Flame AA for Zn. Wet digestion for Cd. Flameless AA for Cd. (Perkin Elmer 303)
8	Wet digestion with HNO_3/H_2SO_4 50-60°C for 2 hrs followed by $KMnO_4$ solution. Cold vapour analysis.	Wet digestion with $HNO_3/HClO_4$ AA using air acetylene flame.
9	Wet digestion using "Bethge" apparatus - HNO_3/H_2SO_4 followed by $HNO_3/HClO_4$. Cold vapour analysis; Jarrell Ash mercury analysis kit.	Wet digestion using $HNO_3/HClO_4$ Cd and Pb determined by HGA-72 (Perkin Elmer).
10	Atomic Absorption Newsletter (1971), 10:101-103. (H_2O_2 replaced by ascorbic acid). Cold vapour analysis following amalgamation procedure using gold.	

Lab No	Mercury	Other Metals
11	Wet digestion with HNO_3/H_2SO_4 air condenser. Cold vapour analysis.	Wet digestion with $HNO_3/HClO_4$ followed by flame AA Perkin Elmer.
14	Wet digestion with $HNO_3/HClO_4$ at 70°C overnight. Cold vapour analysis using IRD Mercurimeter.	Wet digestion with $HNO_3/HClO_4$ at 70°C. AA using Perkin Elmer 303 with background correction.
15	Wet digestion H_2SO_4/HNO_3 at 140°C + potassium persulphate. Cold vapour analysis.	Wet digestion, Cu, Zn, Cd and Cr by flame AA using background correction. Pb extracted with APDC and MIBK - followed by flame AA.
16	Dry combustion at 900°-1000°C followed by absorption in $KMnO_4/H_2SO_4$. Cold vapour analysis.	Wet digestion using $HNO_3/HClO_4$ Flame AA for Cu, Cd, Pb and Zn using I.L.251. (background correction incorporated into double beam system).

APPENDIX II

3rd INTERCALIBRATION EXERCISE - INFORMATION ON:

(a) Fish Flour Preparation
(b) Stock Standard Solution
(c) Procedure for the Analysis of Fish Flour
(d) Preparation and Storage of Working Standards
(e) Summary of Methods employed by Participants.

(a) Preparation of reference fish flour

400 kg of distant water cod were bought from Hull fish market and processed by MAFF Humber Laboratory as follows.

1. The fish were filletted and then skinned.
2. The fillets were then cooked continuously by indirect steam at 80 psig at a temperature 80–100°C and broken up into small pieces.
3. The cooked fish plus liquor was then dried by indirect heating (ca 80° C) to a moisture content of 20%. The drying stage was completed by air drying at ambient temperature.
4. The meal was then sieved to remove large pieces, caused by overheating and ground repeatedly in a hammer mill to a fine flour.
5. The final product (ca 20 kg) was subdivided using the classical coning and quartering technique* into 100 gm portions, which were transferred to individual 300 ml clear polystyrene containers.

   * Standard Method of Chemical Analysis by Scott and Furman 5th Edition. Vol. II. 1937 p. 1620–1624.

(b) Preparation of reference metal standard solutions

The stock solutions (2 litre) of metal standards (Cu, Zn, Cd, Pb and Hg) were prepared from BDH AA stock standards (1000 ppm) by bulking 4 x 500 ml of each metal standard. 10 ml aliquots of each standard was pipetted into individual phials with leak-proof stoppers (plastic phials were used for Cu, Zn, Pb and Cd - glass phials were used for Hg standards).

Each analyst received 100 gm of fish flour and 10 ml of each of the stock metal standard solutions.

(c) Procedure for the analysis of the fish flour

1. Before subsampling the fish flour, the container holding the flour should be inverted 3 times to thoroughly mix the sample. Once any fine dust has settled, the sample for analysis should be taken using a plastic spoon or spatula. This complete procedure should be repeated for each replicate sample.
2. The samples of fish flour should be analysed by the analytical procedure currently in use in your laboratory which should be the one you will adopt for the forthcoming fish and shellfish baseline study.
3. All analyses should be carried out 6 times. It is essential that you include the measurements of copper, zinc, lead, cadmium and total mercury in your determinations. Wherever possible analyses should be made for arsenic, chromium and organic mercury.
4. Calibration of your analytical procedure should be made using
   (a) the standards provided with the fish flour, working standards being prepared and used according to the procedure outlined below.
   (b) the standards normally adopted by your laboratory for this work.
5. On completion of this intercalibration exercise the following information should be returned to the coordinator.
   I. Full results of all metal analyses made on the fish meal.
   II. Details of the analytical procedure used for these analyses, including the detection limits, sensitivity of the procedure and blanks.
   III. Make and model of the instrumentation used in these procedures.
   IV. Xerox copies of all calibration curves and where possible xerox copies of recorded data.

(d) Preparation and storage of working standards

Mercury

Stock solutions (1000 ppm) should be prepared 1N H_2SO_4 in 1N HCl and stored

in glass bottles. Fresh stock solutions should be prepared every 6 months or when the level of solution in the container falls below the halfway mark.

Working solutions should be prepared daily by dilution of the above stock solution using 1N H_2SO_4 together with sufficient 6% $KMnO_4$ solution to produce a distinct pink colour in the final solution. (Please check the mercury content of your potassium permanganate solution as this can contain very high levels of mercury.) In practice the working solution should be prepared immediately before use and should only have a bench life of ca 2 hrs.

Other metals

Stock solutions (1000 ppm) should be made up in 1N acid and can be stored in either glass or plastic bottles. Fresh solutions should be prepared every 6 months or when the level of the solution in the container falls below the halfway mark.

Working solutions should be prepared daily by dilution of the above stock solution using 1N acid.

(e) Summary of the individual analytical techniques employed by each laboratory during the third intercalibration exercise

No.	Mercury	Other Metals
1	Wet digestion with H_2SO_4 and H_2O_2. Cold vapour analysis using MAS 50.	Dry ashing at 450°C followed by HNO_3 and H_2O_2. Cu, Pb, and Cd - FAA - Perkin Elmer - 303, Zn - AA-Perkin Elmer 107.
2	Wet digestion with HNO_3 and H_2O_2. Cold vapour analysis using MAS 50.	Low temp ashing (Tracer Lab LTA 505) followed by dil. HCl. Cu, Pb and Cd - FAA - Perkin Elmer 300, HGA - 70. Zn - AA-Perkin Elmer 300.
3	Wet digestion with HNO_3, H_2SO_4 and $KMnO_4$. Cold vapour analysis using Perkin Elmer 403.	Wet digestion using HNO_3, H_2SO_4 and H_2O_2 for Cu, Cd, Pb and Zn. Cu, Pb and Cd - extracted with Na DDC followed by Techtron AA5. Zn - AA - Techtron AA5 As - wet digestion - followed by FAA - Perkin Elmer 403.
4	No details.	No details.
5	Not applicable.	Wet digestion using HNO_3, followed by FAA using Techtron AA5 with carbon rod Model 63.
6	Wet digestion with H_2SO_4 and $KMnO_4$. Cold vapour analysis using MAS 50.	Wet digestion followed by Anodic Stripping Voltammetry - CMGE.
7	Not applicable.	Dry ashing at 430°C followed by spectrographic analysis using Hilger Quartz spectrometer (photographic plate and Jarrel Ash micro photometer).

No.	Mercury	Other Metals
8	Wet digestion with HNO_3/H_2SO_4 and $KMnO_4$. Cold vapour analysis - Perkin Elmer 305.	Wet digestion using HNO_3 and H_2O_2. AA using Perkin Elmer 305.
9	Wet digestion using HNO_3 and $HClO_4$. Cold vapour analysis using Jarrel Ash Hg kit and Perkin Elmer 300S.	Wet digestion using HNO_3 and $HClO_4$. Cu and Zn - AA Perkin Elmer 305, Cd and Pb - FAA - Perkin Elmer HGA72.
10	Wet digestion with HNO_3/H_2SO_4 and $KMnO_4$. Hg amalgamated onto gold prior to cold vapour analysis.	Wet digestion using HNO_3 and H_2O_2. Zn - AA - Techtron AA5. Cu, Pb and Cd - FAA - Techtron AA5 with carbon rod Model 63.
11	Wet digestion. Cold vapour analysis.	Wet digestion with HNO_3 followed by FAA using Techtron CRA with carbon rod Model 63.
12b	Wet digestion with HNO_3/H_2SO_4 + V_2O_5 - Cold vapour analysis using Perkin Elmer 403.	Dry ashing followed by extraction AA Cd and Pb by extraction with Na DDC using Perkin Elmer 403.
13	Wet ashing with $HNO_3/HClO_4$ followed by cold vapour analysis using Perkin Elmer.	Wet ashing with $HNO_3/HClO_4$. Cu and Zn - AA using Perkin Elmer 403. Cd and Pb - FAA using Perkin Elmer 300SG.
14	Wet digestion with HNO_3. Cold vapour analysis using IRD double beam mercury meter.	Zn and Cu wet digestion with HNO_3. Cd and Pb - wet digestion with $HNO_3/HClO_4$. Zn and Cu - AA - using Perkin Elmer 303. Cd and Pb - FAA - using Perkin Elmer 305 and HGA 70.

No.	Mercury	Other Metals
15	Wet digestion with HNO_3/H_2SO_4. Cold vapour analysis using - A3000 single beam.	Wet digestion with HNO_3 followed by AA - Perkin Elmer 306.
16	Dry ash at 900 - 1000°C followed by absorption in $KMnO_4/H_2SO_4$. Cold vapour analysis using Techtron 120.	Wet digestion with $HNO_3/HClO_4$ followed by AA - IL 251.
17	Wet digestion using H_2SO_4. Cold vapour analysis using MAS 50.	Wet digestion using HNO_3 followed by AA - IL 151.
18	Wet digestion with HNO_3/H_2SO_4 and $KMnO_4$ at 50-60°C. Cold vapour analysis using Perkin Elmer 305.	Wet digestion with HNO_3 followed by AA - Perkin Elmer 403.
19	Wet digestion with H_2SO_4, $KMnO_4$ and H_2O_2. Cold vapour analysis using Techtron AA5.	Dry ashing at 480°C dissolution in dil. HCl/HNO_3 followed by AA using Jarrel Ash Model.

Key to Table 2

AA = Atomic Absorption Spectrophotometry

FAA = Flameless Atomic Absorption Spectrophotometry

APPENDIX III

MULTIPLE RANGE TEST

The common practice for testing the homogeneity of a set of n treatment means is to use an analysis of variance. The procedure alone, however, falls short of satisfying all the practical requirements. When the analysis of variance rejects the homogeneity hypothesis, it gives no decisions as to which of the differences among the means may be considered significant and which may not. The multiple range test pinpoints these significant differences. The data necessary to perform the test are the treatment means and the standard error of each mean. It is convenient to display the means in ranked order and to test differences in a set pattern, the largest mean minus the smallest, the largest minus the second smallest, up to the largest minus the second largest; then the second largest minus the smallest, the second largest minus the second smallest, and so on. A set of "shortest significance ranges" are calculated from tables of special "significant studentized ranges" and each difference between two means is significant if it exceeds the corresponding shortest significant range; otherwise, it is not significant.

This procedure and several alternatives are described by R O'Neill and G B Wetherill in a paper entitled "The Present State of Multiple Comparison Methods", Journal of the Royal Statistical Society, Series B, Volume 33, No.2, 1971, pp. 218-250.

APPENDIX IV

SUMMARY OF METHODS OF ORGANOCHLORINE ANALYSIS USED IN ICES INTERCALIBRATIONS 1972, 1975.

Laboratory	Extraction	Clean-up	Pre-GLC Separation	GLC columns	Temp.	Confirmation	PCB Calculation
A	Chloroform/methanol	Florisil	Florisil, 6% ether in hexane 15% ether in hexane	(1) SE-30/SP-2401 (2) XE-60		2 GLC columns Saponification	4 peaks
B	Hexane/Soxhlet	DMF/hexane, alumina	Silica, hexane	(1) DC-200/QF-1 (2) DC-200/QF-1/-OV-225 (3) OV-17		3 GLC columns	13 peaks, time x height
C	Hexane/Soxhlet	Hexane, alumina	Silica, hexane/ diethyl ether	(1) FS-1265/DC-200[a] (2) DC-200[a] (3) SF-96/QF-1[b]		GLC	4 peaks
D	Pentane	Alumina	Silica, hexane/ diethyl ether	(1) 3% DEGS (2) 3% NPGS (3) QF-1/OV-17 (4) Dexsil 300		4 GLC columns	1 or 2 peaks
E	Pet. ether, 3 times	Celite - H_2SO_4	TLC	(1) QF-1/OV-17 (2) SP2250/SP2401	190°C 200°C	2 GLC columns	5 peaks
F	Hexane/Soxhlet	DMF/Hexane, alumina	Nil	(1) DC-200 (2) QF-1/SE-30 (3) QF-1		3 GLC columns	3 peaks after alkaline hydrolysis
G							
H	Acetone/Hexane hexane/diethyl ether	H_2SO_4	Nil	(1) QF-1/SF-96	180°C		14 peaks

[a] 1972 [b] 1975

ctd.

Appendix IV (ctd)

Laboratory	Extractions	Clean-up	Pre-GLC Separation	GLC columns	Temp.	Confirmation	PCB Calculation
I	Hexane/Soxhlet	Hexane, alumina	Silica, hexane/ diethyl ether	(1) DC-200 (2) DC-200/QF-1		2 GLC columns	3 peaks
J	Hexane/Soxhlet	Hexane, alumina	Silica, hexane/ diethyl ether	(1) DC-200 (2) DC-200/QF-1	200°C 200°C	2 GLC columns	1 peak
K	Pentane/Soxhlet	Hexane, alumina	Silica, hexane/ diethyl ether	(1) OV-1/OV-210 (2) OV-17/OV-210	200°C 200°C	2 GLC columns	1 peak
L	Pentane/Soxhlet	Pentane, alumina	Silica, hexane/ diethyl ether	(1) OV-210/OV-17 (2) DEGS/H_3PO_4	195°C 195°C	2 GLC columns	2 peaks
M	Petroleum ether	(1) Pet.ether/ alumina (2) DMF/Pet.ether/ diethyl ether (3) Florisil	Silica, H_2SO_4/ Celite Pet.ether acetonitrile Alumina	OV-1/QF-1	170°C	alk.hydrolysis	5 peaks
N	Hexane/acetone	Florisil	Florisil, hexane, 30% methylene chloride in hexane	(1) OV-101 (2) Dexsil 300 (3) OV-17/QF-1 (4) SE-30/SP-2401 (5) OV-210	200°C	DDD and DDE by oxidation to DCBP DDT by alk.hydrolysis Dieldrin by chlorination and acetylation	

Indication of spine colours

Liaison Committee Reports	Red
Reports of Advisory Committee on Marine Pollution	Yellow
Fish Assessment Reports	Grey
Pollution Studies	Green
Others	Black

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