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REPORT ON THE FOURTH ICES ORGANOCHLORINE INTERCALIBRATION EXERCISE

by

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SUMMARY

The results of a further international intercalibration of organochlorine analyses, using a natural fish oil, are described. Agreement between the 30 participants was poorer than in previous exercises, due partly to the lower concentrations present, and some analysts found the sample unusually difficult to process. The quality of many chromatograms was low, and possible reasons for the wide variation in results are discussed. The need for improvement in analytical techniques is stressed if data from different laboratories are to be assumed to have equal validity and some restriction in the choice of analytical methods may be necessary.

RÉSUMÉ

Les résultats d'un intercalibrage international en outre d'analyses d'organochlore, en utilisant une huile naturel de poisson, sont décrits. L'accord entre les 30 participants était plus médiocre que dans les exercises préalables, et quelques analystes trouvaient l'échantillon exceptionnellement difficile à analyser. Le qualité de plusieurs chromatogrammes étaient faible, et on discute les raisons possibles pour l'éscart étendu des résultats. On accentue la necessité d'améliorer les techniques analytiques si les données des different laboratoires peuvent être assumé d'avoir validité égale, et il se peut qu'on doive limiter le choix des méthodes analytiques.

INTRODUCTION

Earlier intercalibration exercises for organochlorine residue analysis were carried out, using a spiked fish oil, in 1972 and in 1974 using a spiked corn oil. The results were given in detail in ICES Co-operative Report No 80. The residue levels in the fish oil were considered to be too high by the analysts who participated, and did not constitute a sufficient test of their ability to determine residues at levels more commonly encountered in marine fish. To provide a base oil reasonably free of organochlorine residues, enabling lower concentrations of selected organochlorines to be added as spikes, the corn oil was chosen for the 1974 exercise but this was subsequently criticized as being easier to analyse, and too different from the usual types of fish extract obtained by analysts from marine fish. In October

1977 the Working Group on Marine Pollution Baseline and Monitoring proposed that, for a further intercalibration programme, an unspiked fish oil containing undisclosed amounts of organochlorines should again be circulated, and also three different mixtures of organochlorine residues for which the concentrations would be stated. The Pitlochry laboratory of the Department of Agriculture and Fisheries for Scotland, which had been responsible for the preparation and distribution of earlier samples, was asked to undertake this new exercise.

Nine different samples of fish oil were obtained from the Marfleet Refining Company Ltd, of Hull, England, and were analysed for organochlorine residues (Table 1). From this series a capelin oil of low residue content was selected for the intercalibration exercise (the capelin were caught off Iceland), and a larger consignment of the same type of oil was acquired, from which aliquots were taken for distribution to the participants.

Before the various standard solutions were prepared, enquiries were made of the airline and postal authorities regarding transport of inflammable liquids, and it was confirmed that these authorities will not permit the transport of inflammable solvents except in certain circumstances, and the proposal to distribute standard mixtures in n-hexane had to be abandoned. Suggestions were later made by the Working Group on Marine Pollution Baseline and Monitoring Studies in May 1978 that standards could be made up in mineral or silicone oil but, apart from the difficulty in preparing such solutions without the aid of normal solvents, it was considered that the oils could interfere with the absorption column chromatography commonly used for clean-up and separation purposes. The preparation of accurate standards for distribution is also time-consuming and costly, and the Pitlochry laboratory was not able to allocate funds for the work, while it was not possible for ICES to defray the expenditure incurred. Furthermore, it was considered by the Pitlochry laboratory that competent analysts should be able to prepare their own standards in pure solvents with sufficient accuracy, and the proposal to distribute standard mixtures was given up. A letter was circulated in July to nineteen laboratories giving the name and address of a source of standard pesticides of high purity. This was unfortunately not sent to latecomers in the exercise, but in any case very few recipients seem to have taken advantage of this information.

In previous exercises, carefully measured quantities of individual organochlorines in small volumes of solvent had been mixed thoroughly into a part of the accurately measured final volume of the matrix oil, and this portion of the oil subsequently added to the remainder and again mixed thoroughly for several hours. The spiked and unspiked oils were then analysed repeatedly with great care, to confirm that the measured spike concentration for each residue was in general agreement with the amount added. This procedure can be time-consuming and costly, and in view of the pressure of other commitments the Pitlochry laboratory had to abandon the preparation of a spiked version of the selected fish oil.

DISTRIBUTION OF SAMPLES

A letter from the General Secretary of ICES was circulated to all delegates in April 1978, informing them of the proposed intercalibration exercise, and by the end of August 1978 a total of 24 laboratories had been notified to the author at Pitlochry as being willing to participate. Samples of the selected fish oil were despatched to these laboratories on 21 August 1978. At that time, no laboratories in Canada, France, Spain, USSR or Poland had been named, and the General Secretary was informed. He issued a further intimation to the delegates of these countries on

1 September 1978, and laboratories in Canada, France, Spain and Poland subsequently requested samples. By 6 November 1978, 43 samples had been despatched but no results had been received from any laboratory. At this time the closing date for receipt of analyses was fixed at 31 January 1979. (See Appendix A for list of laboratories).

All recipients of the sample were asked to provide information on their analytical techniques, examples of the chromatograms of standards and sample analyses, and of a concentrate of the solvent used. The appropriate detections limits for 10 different residues were indicated, but analysts were invited to determine as many organochlorine residues as they could (Appendix B).

By 22 December 1978 only 7 sets of analyses had been reported, and by 17 January 1979 only 10 had been received. The first deadline of 31 January 1979 passed with only 12 sets of results from a total of over 40 laboratories, many having had the samples for 5 months. The names of several laboratories were provided by the Joint Monitoring Group of the Oslo and Paris Commissions in January 1979, but only two of these (Nos 10 and 11 in Appendix A) had not already been sent samples of the fish oil, and these samples were despatched on 29 January 1979. Both laboratories replied within 6 weeks reporting their results, indicating that the analysis could be completed within a relatively short time.

Up to the end of March 1979 only 19 laboratories had submitted their results. One laboratory (No 27) had used 2 separate samples of oil, having received 1 from an associated national laboratory. A second laboratory (No 16) used 2 separate groups of analysts to analyse the same sample. The total time between sample despatch and receipt of the analyses had varied at this time (among the 19 laboratories) from 5 to 29 weeks, with an average of 16 weeks.

This response was considered to have been very disappointing, in view of the fact that a high proportion of the recipients of the sample were known to have considerable experience of organochlorine analysis in fish. An interim report on the results received up to the end of March 1979 was prepared and presented to the Marine Chemistry Working Group in Lisbon in May 1979, and it was indicated that the coefficients of variation for the residues reported by most analysts were similar to those from the previous unspiked fish oil sample used for intercalibration in 1972.

Further results were received from 10 laboratories by the end of June, and this report presents the entire series of results. None of the later analysts had received a copy of the interim report before their results were despatched to the author. A further laboratory, which had encountered difficulty with the analysis due to the possible presence of toxaphene in the sample, submitted its results later but these too have been included in this final report.

It was thought probable that several laboratories had attempted to identify and quantify some substances which they would have ignored in the course of their routine work, but these results are not self-evident from the reports received. A total of 21 different residues or residue groups was reported from the ICES laboratories, 8 of them by more than half of the laboratories. Single values were given for TCNB, endosulfan, heptachlor, op-DDE, α - and β - chlordane, two for endrin and toxaphene, 3 for op-TDE, pentachlorobenzene and β - HCH, and 5 heptachlor epoxide. One laboratory also reported oxychlordane, transchlordane and transnonachlor. As no confirmatory methods were used in most cases these residues must be regarded with suspicion. Nine measured values were reported for op-DDT, while 15 were given for δ - HCH and 16 for dieldrin. HCB, DDE, TDE, DDT and PCB were measured by from 21 to 25 laboratories.

Two laboratories reported the presence of toxaphene. This pesticide is thought to be used only to a limited extent in western Europe, and it seems unlikely that fish off Iceland could be significantly contaminated by it. The distributing laboratory at Pitlochry has never detected the presence of toxaphene in any marine samples in the North Sea or eastern North Atlantic, and could not detect any peaks corresponding to toxaphene by the use of GCMS. The concentrations reported in the sample (1-5 mg/kg) were higher than the combined total of all other organochlorine residues, which also suggests an unlikely level of discharge to the marine environment in the region of Iceland. Both laboratories reporting toxaphene are very experienced, however, and the suggestion that this pesticide was present in the sample requires further consideration.

One laboratory reported only on PCB, a faulty gas chromatograph being given as the reason for a failure to measure other residues. One JMG laboratory also reported only on PCB, although this was all that had been asked of the JMG group. A few laboratories identified only 2-5 residues, but on the other hand 15 laboratories measured at least 8 different residues, excluding those reported to be below the limit of detection. The complete series of analyses reported in presented in Table 2.

STATISTICAL ANALYSIS OF RESULTS

The preliminary analysis of the data obtained by 31 March 1979 indicated that there were wide variations in the reported concentrations of some of the residues and many analysts had indicated that they had had difficulty in performing the analysis, although the capelin oil used had been extracted from fish taken in a relatively clean area of the ocean. Following the receipt of further analytical data from a number of laboratories, additional information on the analytical methods used and examples of chromatograms were requested. It was clear, as the result of an examination of this information, that a wide variety of methods had been used for preparation of the organochlorine extracts before analysis by GLC, and some of these may have influenced the analysis. The quality of the chromatograms was also very variable, ranging from the high resolution of some capillary columns to poor resolution by packed columns in a few instances. Examples are given in Appendix C.

In view of the possibility that the quality and accuracy of the final analyses might be dependent upon either the high resolution of the capillary columns (which might not require separation of residues prior to GLC analysis), or the method of treatment of the extract or of residue separation before GLC injection, the data were separated into four groups before variance analysis. These comprised six sets of data from capillary columns, six from analysts using normal GLC columns but only sulphuric acid treatment for clean-up, and two groups from analysts using absorption columns for clean-up and a preliminary separation of residues prior to analysis by GLC. The two groups comprised the results from chromatograms judged by inspection to be of higher quality (mainly due to better peak separation) and the results from a series of chromatograms which were of noticeably lower quality, sometimes showing evidence of inadequate clean-up. However, statistical analysis of the results from these groups did not reveal any clear distinction between them in respect of the mean values of the residues reported, and the results given in this report are from the combined group of analysts using pre-GLC separation and packed GLC columns.

Values for the concentrations of the eight different residues most commonly reported (including PCB as one determination) were available for statistical analysis, although sulphuric acid treatment destroys dieldrin and values for ɣ - HCH were often not given, or were below the detection limit. The results of the chemical analyses in each group are shown in Tables 3-5, and the means and coefficients of variation between analysts for each residue in Table 6. Residue concentrations differing from the mean by more than three standard deviations have been excluded from the calculations. Mean values of the concentrations found in two separate samples by laboratory No 27 have been used in the statistical analysis, but results from two separate methods given by each of laboratories 7 and 16 have been used individually.

Despite the wide variation in analytical techniques there is reasonable agreement between the mean values in the groups for most of the residues determined. No statistically significant differences between the means by the different methods were found at the 5% level for HCB, α - HCH, ɣ - HCH, dieldrin, DDE, TDE, or DDT, although this is largely due to the variance between the avlues reported within each of the three analytical groups. The mean values for PCB by the sulphuric acid clean-up method, however, were significantly higher than those obtained by the other methods. In the initial comparison, the six laboratories selected from the total using sulphuric acid were those not using capillary columns or any other pre-GLC separation process. To test whether the larger groups of laboratories using sulphuric acid had produced results differing from those of other laboratories, irrespective of the form of GLC used or other preparatory processes, data from all thirteen laboratories using sulphuric acid were pooled (Table 4).

The sulphuric acid technique, which is used to destroy interfering material in solvent extracts of organochlorines, also destroys dieldrin, but may possibly introduce substances which could be electron-capturing. Thus there may be an increase in the number of GLC peaks on the chromatograms by comparison with those from non-destructive techniques. An increase in the number of peaks could lead to a greater chance of interference with the peaks from substances originally present, or an increased risk of peaks which could be incorrectly identified. Some of the thirteen laboratories in this group from which analytical data were used in the final statistical analysis employed alkaline hydrolysis or chromic acid treatment of an aliquot of the extract in the determination of pp-DDT group residues, thus making allowance for possible interference by PCB peaks.

The high mean value for the PCB residues reported by the group of analysts using sulphuric acid does not appear to be explained by interference with other residues. The peaks of TDE and DDT can sometimes be separated from the major PCB peaks on a good GLC column, but the use of alkaline hydrolysis prior to PCB quantification will remove any interference by these substances. One laboratory (No 7) used both alkaline hydrolysis and chromic acid on an aliquot of the fish oil extract which had received sulphuric acid treatment, while a second aliquot was separated on a silica gel TLC plate as an alternative method of analysis. The latter method gave a significantly lower value for the PCB concentration ($p < 0.10$). It is also worth noting that one laboratory (No 1) determined the PCBs by perchlorination to decachlorobiphenyl after sulphuric acid treatment.

Of the thirteen laboratories in this group, seven reported values over 1000 mg/kg, but only one of four using capillary columns did so (No 31). Of the six reporting values below 1000 mg/kg (190-750 mg/kg) three used capillary columns (Nos 10, 32, 34), one (No 23) had experienced difficulty with the sensitivity of the chromatograph and No 35 used a more elaborate procedure for separating the PCB residues than was generally employed. Among the thirteen laboratories using alternative clean-up

techniques (Table 5) only two reported PCB values over 1000 mg/kg (Nos 3 and 28). Laboratory No 3 used a 1:1 mixture of Aroclor 1254 and 1260 for reference, but was not confident of the accuracy of the PCB value determined, believing it to be probably between 0.9 and 2.5 mg/kg.

Only the mean of the PCB values determined by use of the sulphuric acid technique differed significantly from that using other techniques. The means of the other six residues (excluding dieldrin, which was not determined with the use of sulphuric acid were not distinguishable statistically between the methods. The use of different reference formulations for PCBs, or different numbers of peaks, in calculating the PCB content could not be shown to influence the PCB value significantly. One other high value of 8000 mg/kg PCB was reported, by laboratory No 13, which determined only HCB, α - HCH and PCB. Clean-up was by silica column, with no further residue separation. This value would have been excluded from the statistical analysis as lying well outside the $\pm$ 3 sd range, had the laboratory been one of those using the analytical techniques considered in Tables 3-5.

A number of laboratories used packed columns but no pre-GLC residue separation, but most of them also employed sulphuric acid as a clean-up procedure. Consequently the statistical analysis of the results obtained by this group (Table 7) yielded mean values of the most commonly reported residues which were not significantly different from those in the sulphuric acid group. However, comparison with the results from the group using other forms of clean-up and pre-GLC separation (Table 5) showed that the means for DDT were different ($0.05 > p > 0.01$), as well as the means for PCB ($p < 0.01$). The latter difference has already been noted for the analysts using sulphuric acid clean-up. The numbers of laboratories in these groups are inevitably small, and not every laboratory was able to report on every residue subjected to the statistical analysis, so that deductions from this analysis must be made with caution. Of the compounds under consideration, the major interferences between residues, if no pre-GLC separation is undertaken, are those between the PCB group and the DDT group. DDE often exceeds the interfering PCB peak considerably, and the error for this substance will in such circumstances be small. However, pp'-TDE and pp'-DDT may be more significantly affected, but of the laboratories in Table 7 determining these residues all but one used an alkaline reaction to remove the TDE and DDT, thus giving a means of estimating the size of the interfering PCB peaks and correcting for this interference.

The use of high resolution capillary or WCOT columns for the gas chromatographic analysis should make the use of pre-GLC residue separation unnecessary. However, there is a possibility that the large number of peaks resolved on a capillary column, especially if temperature programmed, may lead to confusion regarding the identity of individual peaks. The timing of peak elution is critical, and is normally achieved by automatic timers, but care is clearly necessary to maintain uniform temperature and gas flow conditions, which could otherwise cause serious errors in peak identification from automatic recognition systems based on elution time. One laboratory using a temperature programmed capillary column submitted chromatograms showing 65-70 peaks following acid treatment of the extract from the ICES sample, while the same extract divided (without pre-treatment by sulphuric acid) on a silica column into two fractions produced a total of about 150 peaks, of which over 50 would be easily measurable (more than 5% fsd). Although incorrect peak identification may lead to errors in quantification in some instances it is unlikely to provide the explanation for the generally higher concentrations of PCBs reported following the use of sulphuric acid as a clean-up procedure.

The data from the groups of analyses between which no significant differences could be detected have been combined in Table 8. It will be seen that the coefficients of variation between laboratories for the various residues lie between 32% and 71%. As recorded earlier, many analysts found difficulty in analysing the fish oil, and commented that the chromatograms were not within their usual experience. In the view of the distributing laboratory, however, samples of clupeoid fish, or of the liver of gadoids, both of which contained relatively high levels of lipid, frequently yield chromatograms of a quality similar to that of the intercalibration sample.

The largest coefficient of variation found was for γ - HCH (71.1%), but the mean of the concentrations reported, 11 µg/g, was the lowest of the eight residues studied in the statistical analysis. Five of the twenty laboratories reporting on this substance could only state that the concentration was below their level of detection, and one reported an abnormally high value. For residue concentrations in the range 50-100 µg/g (or 500-1000 µg/g for a mixture of PCBs), the coefficients of variation found were 33-50%, which indicates that 19 out of 20 analysts obtained values for most of the residues which spanned at least an order of magnitude. This cannot be considered a satisfactory measure of agreement among a group of laboratories many of which have been engaged on organochlorine analysis for a number of years.

In 1972 a fish oil was also used as a basis for preparing an intercalibration sample (No 2A), spikes of several organochlorine compounds being added to it (No 2B). Residue concentrations found in the unspiked oil were higher than in the capelin oil used in the latest exercise (No 4), but the coefficients of variation for the residues found were generally within the same range (Table 9). The much higher spike concentrations added to the oil in the 1972 exercise produced better agreement among analysts, as did the lower spike concentrations added to an uncontaminated (essentially residue-free) corn oil in 1975. This supports the view that estimating spike concentrations (by difference from the matrix), or at least higher concentrations, results in a higher level of agreement (lower coefficient of variation) than the determination of low concentrations in natural fish oils. This in turn suggests that agreement among analysts on the residues found in a natural sample containing very low concentrations, as in a fish tissue of low lipid content, would be even poorer. Unfortunately such samples tend to give rise to many returns from analysts in which residues are only indicated as being present at less than the limit of detection, information not amenable to subsequent statistical treatment.

METHODS OF ANALYSIS

Appendix D gives details of the analytical procedures used by the participants. A few laboratories used more than one preparatory technique, but several used more than one GLC column. Six laboratories used well-coated or capillary GLC columns (one using two different types). In the other 24 laboratories 35 packed columns were employed with a wide variety of packing, although most columns were 1.5-2.0 m in length.

The extraction/solution stage involved the use of hexane or petroleum ether in 22 instances, pentane in three and dichloromethane or cyclohexane each in one laboratory. In four instances mixtures of solvents were employed, toluene/ethyl acetate in one, acetone/hexane in one and acetone/acetonitrite/hexane in two cases.

Clean-up of the oil extract or solution was carried out by a variety of methods. In 13 laboratories sulphuric acid was used, and alumina columns in 11. Other methods used were liquid/liquid partition (2), Florisil columns (3), silica on TLC plates or columns (2), sapanification (1) and gel permeation (1).

Pre-GLC residue separation was made on Florisil columns in 5 laboratories and by silica columns or TLC plates in 15.

The GLC stage was temperature-programme in 7 instances, from a total of 42 columns, and automatic integration of peaks used by 7 laboratories.

Confirmation by GCMS, for at least some of the residues, was used in 5 laboratories, PCB confirmation and quantification by perchlorination in 2, and chemical reaction of DDT group residues (chromic oxide, alkaline hydrolysis and a magnesium oxide micro-reactor as a GLC pre-column) in 9 laboratories.

Solvent purity, as judged by chromatograms after usually one hundredfold concentration, was in most cases very good, and only in one or two cases were peaks in the solvent likely to interfere with residues in the sample.

The quality of the chromatograms submitted was very variable. Estimates of the resolution of the GLC columns were made by calculating the number of theoretical plates for a dieldrin or DDE peak and, excluding the capillary columns, values ranged from 800 to 4000 on columns of 1.5-2 m in length. More than half of the columns used gave values of more than 2000 plates, and about one third exceeded 3000 plates. However, comparison of chromatograms of standard mixtures with those from sample extracts showed in many instances that the baseline of the latter samples was markedly non-linear, making quantification of peak height or area difficult. The sensitivity available (or used) in some instances was inadequate to detect more than a few peaks, while in other cases a large number of unidentified peaks were present in the pesticide fraction after removal of PCBs. Laboratories not using pre-GLC separation techniques were more liable to have mis-interpreted the identity of peaks, or to have incurred errors in quantification due to interference of one residue by another.

SOURCES OF VARIATION

The extent of variation between analysts is shown by the ranges of values reported for the residues most commonly determined, as summarised below.

HCB	40-116 µg/kg
α - HCH	1-131
ɣ - HCH	2-136
Dieldrin	12-129
pp'-DDE	16-340
pp'-TDE	5-180
pp'-DDT	30-305
PCB	190-8000

These ranges cover one and in some instances two orders of magnitude, but in the latter case elimination of one outlier will reduce the range to about one order of magnitude. Nevertheless even this degree of variation between laboratories suggests that there is room for considerable improvement, and some possible sources of error can be considered.

The samples distributed, which can be considered to be homogeneous aliquots from a large volume of oil, should have given no problems in obtaining an extract of all organochlorine residues, unlike fish tissue samples which require an extraction stage. The latter itself could provide a source of variance in the analysis of samples for routine monitoring, but this should have been absent in the exercise under discussion.

All solutions of the fish oil requires a clean-up stage to remove lipids and pigments which otherwise interfere in the GLC analysis. Serious interference is revealed by unstable or wandering recorder baselines, and in some cases these were present in the chromatograms submitted. If the cleaned-up extract is further processed to obtain two or more fractions containing different groups of residues, that containing PCBs (normally the first to elute from an adsorbent column) will be relatively free of interfering substances (lipids, co-extractives). Subsequent fractions may contain significant amounts, particularly if the lipid loading on an adsorbent column is excessive, and the baseline of the chromatogram may be unstable. If sulphuric acid is used for the clean-up stage, some reaction products from the destruction of lipids and other co-extractives may remain and interfere in the chromatograms.

In view of the large number of individual compounds which are likely to have been present in the sample, good separation of the individual GLC peaks is essential. This was achieved by most of the analysts who used capillary columns without the need to subdivide the extract into fractions before GLC injection, although there was some variation in the quality of chromatograms even using capillary GC. One laboratory used silica columns to separate the residues before GLC on a well-coated open tube (WCOT) and achieved good separation of residues (these results were included with capillary column results for the purpose of statistical analysis).

Where capillary or WCOT columns were not used, pre-GLC separation or residues was essential for satisfactory separation on GLC columns. Chromatograms of single cleaned-up extracts containing all residues generally suggested that there was some confusion of identity of the peaks, particularly between PCB peaks and those of pesticides. Separation was usually made on silica columns, which were more effective than Florisil for this purpose.

The sensitivity of the GLC detector was inadequate, in many instances, for the purpose of obtaining peaks large enough for accurate measurement of peak heights, and higher concentrations of the residues could have been obtained by, for example, evaporating an eluate to a smaller volume. Approximately one third of the laboratories produced chromatograms which demonstrated effective peak separation with adequate peak heights. Half were from capillary columns, and of the others none had been produced from sulphuric acid clean-up.

Chemical reaction was used in a few instances for confirmation of DDT group residues, mostly when no pre-GLC separation had been made. The DDT and TDE residues were estimated by difference following alkaline hydrolysis, and two analysts used chromic oxide to remove DDE for the same purpose. In all cases PCBs are likely to be the interfering peaks. The difference based on peak area calculation is more accurate than that from peak height unless the retention times of the interfering peaks are identical, which is rarely the case.

DISCUSSION

This exercise was much less satisfactory than previous intercomparison exercis. Analysts found the sample more difficult, primarily because the concentrations o. residues in the lipid were lower than in previous samples, thus requiring more efficient clean-up. The number of residues found at the level of sensitivity used by many analysts made residue separation very important, but those who employed a lower level of sensitivity could have had difficulty in identifying many of the residues. The long delay in submitting results to the author perhaps emphasises the problems experienced in the analysis of this sample.

The provision of chromatograms to accompany the analytical report, and the detailed description of techniques, proved particularly valuable and at the same time revealed a basic problem in assessing the analytical data. If the assessment is made without evidence of chromatogram quality, it is assumed that all data are equally reliable both in respect of the identity of residues and their concentrations. However, a study of the analytical methods often reveals that the identity of some residues must be questioned, while examination of the chromatograms suggests in many instances that both identity and concentration may be inaccurate.

All the exercises so far have given the participating analysts freedom to use techniques of their choice, in the hope that agreement between them in respect of the results reported may be found satisfactory. This latest exercise suggests that such will be the case only for samples which are relatively easy to analyse, with high concentrations of a few easily identifiable residues and no problems of clean-up. The spiked samples used in earlier exercises were examples. At low residue levels, for which extracts must be concentrated in order to provide measureable values of the concentrations suitable for statistical analysis, many interferences arise and good separation techniques become essential.

It is suggested that some restriction of the choice of analytical procedure may now be necessary, and that in particular either capillary GLC or alternatively packed column GLC following preliminary residue separation should be mandatory. Clean-up techniques may involve either sulphuric acid or adsorbent, but the latter seem to have certain advantages and more often produced good quality chromatograms in this exercise. With capillary columns, however, sulphuric acid seems to have been very successful.

For the purpose of statistical evaluation of the data submitted, it would also be useful to have the results of replicate results (using the complete procedure in each case, not replicate GLC injections). However, the cost of organochlorine analysis may make this prohibitive. Duplicate analyses are insufficient, unless the data for different residues are pooled and the variance for each assumed to be the same. Five or six complete analyses would be preferable, but in view of the time required for such a series it is likely that few laboratories would be prepared to undertake the work.

Calculations of residue concentrations have been based mostly on peak height measurements although a few laboratories have used integrators. With simple chromatograms these instruments should be very accurate but where peak overlaps are common, or small peaks appear on the edge of larger peaks, and where baselines are erratic, the accuracy may be less certain. Peak identification is sometimes made by computer from reference data, but variation in temperature may cause changes in elution time and consequent errors in identification.

PCB concentrations have been determined in this exercise by peak height using from 1 to 24 peaks, peak area, the use of individual isomers, one or mixed formulations as reference standards, and perchlorination to decachlorobiphenyl. It was not possible to identify any method as being of less accuracy than others, although in view of the difference between the composition of the PCB mixture in the sample and in standards it would seem desirable to use at least three of the larger peaks which did not interfere with other known residues. On capillary columns, a larger number of individual peaks can be used with greater freedom of interference. Once again, in any future exercise certain requirements could be specified in respect of PCB calculations.

The sample circulated for this exercise has been criticized as being more difficult to analyse than material normally dealt with by the participating laboratories, although earlier intercalibration samples were thought to have been too easy and atypical. In one respect the capelin oil was unusual, in that the concentrations of the residues expressed on a lipid basis were probably significantly lower than those obtained on the same basis from fish sampled in any coastal waters of Europe and North America, even in ostensibly unpolluted areas. Fish tissue samples, especially if of low lipid content, will in many cases give fewer problems in clean-up, but also lower concentrations of contaminants on a fresh weight basis, the form in which most analysts would expect to express their results. Nevertheless, if analysts are to provide information on an increasing number of pollutants, with a degree of accuracy which ensures their acceptability to authorities in other countries, intercalibration exercises in the form provided by Sample No 4 are essential. It is to be hoped that by such means laboratories will identify their weaknesses, and will improve their techniques to a standard at which agreement among analysts will be much closer. Unless this is achieved, it will be difficult to accept the validity of organochlorine analyses reported by many countries for the fish and other samples taken in their own waters, or by their commercial or research vessels. Some consideration must be given to the level of agreement to be expected among analysts before the information they report on environmental concentrations can be accepted by others.

APPENDIX A

List of participating laboratories

Number	BELGIUM
1	Dr P Hovart Rijksstation voor Zeevisserij 8400 Oostende Ankerstraat 1 BELGIUM
	CANADA
2	Mr Charles J Musial Fisheries and Environment Canada Fisheries and Marine PO Box 550 Halifax NS CANADA
3	Dr R F Addison Fisheries and Oceans Canada Ocean and Aquatic Sciences Marine Ecology Laboratory Bedford Institute of Oceanography PO Box 1006 Dartmouth NS CANADA
	DENMARK
5	Mr Allan Anderson Ministry of Environment Natural Food Institute Mørkhøf Bygade 19 DK-2860 Søborg DENMARK
	FINLAND
7	Professor R Linko Department of Chemistry and Biochemistry University of Turku SF-20500 Turku 50 FINLAND

Number	FINLAND
8	Professor Jaako Paasivirta Department of Chemistry University of Jyväskyla Kyllikinkatu 1-3 SF-40100 Jyväskylä 10 FINLAND
9	FRANCE
	Cl Alzieu Institut Scientifique et Technique des peches maritimes Rue de I'lle-d'Yeu BP 1049 44037 Nantes Cedex FRANCE
10	Centre National pour l'Exploitation des Oceans Centre Oceanologique de Bretagne BP 337 29273 Brest Cedex FRANCE
11	Laboratoire Municipal Rue du Professeur Vezes 33000 Bordeaux FRANCE
	GERMANY
13	Dr Kruger Staatliches Veterinaruntersuchu-samt fur Fische und Fischwaren Schleusenstrasse 2190 Cuxhaven FEDERAL REPUBLIC OF GERMANY
15	Institut fur Meereskunde an der Universitat Kiel Dusternbrooker Weg 20 2300 Kiel 1 FEDERAL REPUBLIC OF GERMANY

Number	GERMANY
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20	Dr E Huschenbeth Institut fur Kusten-und-Binnenfischerei Palmaille 9 2000 Hamburg 50 FEDERAL REPUBLIC OF GERMANY
21	Dipl Cheml Luckas Hygiene-Institute Rostock Lebensmittel und Ermahrungs-hygiene DDR-25 Rostock Stephanstr. 18 GERMAN DEMOCRATIC REPUBLIC
	ICELAND
22	Mrs Alda Möller Icelandic Fisheries Laboratories Skulagata 4 Reykjavik ICELAND
	IRELAND
23	Mr Dan O'Sullivan Department of Fisheries Fisheries Research Centre Abbotstown Castleknock Co Dublin IRELAND
	NETHERLANDS
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APPENDIX B

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All communications to be addressed to The Officer in Charge

Your reference

Our reference

Date

ICES ORGANOCHLORINE INTERCALIBRATION PROGRAMME

ICES Sample No 4 - Crude Fish Oil

This oil is suitable for the analysis of a number of organochlorine compounds and should be examined for the following residues, together with any others which can be identified.

Residue	Suggested Detection Limit
α - HCH, γ - HCH, HCB	0.002 mg/kg
β - HCH, pp - DDE, pp - DDD, pp - DDT, op - DDT, Dieldrin	0.01 mg/kg
PCBs	0.05 mg/kg (quote reference standard formulation)

It is recommended that an initial tenfold dilution should be used in a pure solvent. Report all results in terms of weight of oil (not volume), giving details of the analytical technique.

Specimen chromatograms of the fish oil and analytical standards are requested. A chromatogram of the solvent used (usually hexane) after 100 - fold concentration should also be provided.

Please send your results to Mr A V Holden
Freshwater Fisheries Laboratory
Faskally
Pitlochry PH16 5LB
Scotland

September 1978

A V HOLDEN

APPENDIX C

Examples of Chromatograms produced from ICES Sample No 4

A Adsorbent clean-up. No pre-GLC separation.

B Sulphuric acid clean-up, KOH treatment. No pre-GLC separation.

C Florisil clean-up. No pre-GLC separation.

D Fuming sulphuric acid clean-up. No pre-GLC separation.

E Alumina clean-up. First (PCB) fraction from silica column separation.

F GPC clean-up. First fraction from silica column separation.

G Alumina clean-up. Second (pesticide) fraction from silica column separation.

H Florisil clean-up. Second fraction from silica column separation.

J Saponification clean-up. No pre-GLC separation. Capillary column.

K Sulphuric acid clean-up. No pre-GLC separation. Capillary column.

L Silica clean-up and pre-GLC separation. First fraction. Capillary column.

M Silica clean-up and pre-GLC separation. Second fraction. Capillary column.

APPENDIX D

Laboratory No.	Extraction Solvent	Clean-up Method	Pre-GLC Separation	GLC Column (length x bore, capillary underlined)	Column Packing	Column Temperature °C (* programmed)	Confirmation Method
1	Acetone/hexane/ hexane-ether	H_2SO_4	None	2m x 3mm (o.d.)	3% SE-30 on Chromosorb Q AW-DMCS	200	PCB by perchlorination
2	Hexane	Florisil (Al_2O_3 for PCB)	Florisil TLC on Al_2O_3	(1) 1.85m x 4mm (for HCB/PCB)	3% SP-2100 on Supelcoport	200	Chemical reactions
				(2) 0.15m x 2mm (out) + 1.70m x 2mm (in) (for pesticides)	3% SP-2100/3% OV-210 on Supelcoport 1% SP-2100/2% SP-2401 on Supelcoport	200	
3	Hexane	Florisil	Florisil	(1) 1.8m x 3mm (o.d.)	4% SE-30/6% SP-2401 on Supelcoport.	215	None
				(2) 1.8m x 6mm (o.d.)	2% XE-60 on Chromosorb W	185	None
5	Pet. ether	Florisil	Silica	1.8m x 2mm	(1) 0.8% DC-200 3.2% QF-1 on Chromosorb W (2) 3% DEGS (3) OV-17	170	3 GLC columns (2) for pesticides (3) for PCB, LDE
7A	Dichloromethane	TLC on SiO_2	TLC	1.8m x 2mm	2 pts 8% QF-1 + 1 pt 4% SF-96 on Chromosorb W AW-DCMS	175-200°	
7B	Hexane	H_2SO_4	None	1.8m x 2mm	2 pts 8% QF-1 + 1 pt 4% SF-96 on Chromosorb W AW-DCMS	175-200°	CrO_3, KOH

APPENDIX (continued 1)

Laboratory No.	Extraction Solvent	Clean-up Method	Pre-GLC Separation	GLC Column (length x bore, capillary underlined)	Column Packing	Column Temperature °C (° programmed)	Confirmation Method
8	Hexane	H_2SO_4, KOH (TLC for dieldrin)	None	1.5m x 1.5m	35 pts 4% SF-96 + 65 pts 8% QF-1 on Chromosorb W	190	KOH
9	Hexane	H_2SO_4	Silica	1.9m x 4mm	3% OV-1 on Chrompart XXX	190	None
10	Hexane	H_2SO_4	None	28m x 0.25mm (WCOT)	SE-30	180-220°	None
11	Pet. ether	H_2SO_4, KOH	None	2m x 4mm	5% OV-1 on Chromosorb W-HP	210	KOH
13	Acetone/ Acetonitrile/ hexane	Silica	None	2m x 2mm	11% OV-17/QF-1	210	None
15	Hexane	Alumina	Silica	2m x 2mm	4% OV-1/6% OV-210 on Chromosorb W-AW-DMCS	220	None
16	Hexane	Alumina	Florisil	GCMS	No detail		GCMS
20	Hexane	Alumina	Silica	(1) 1.5m x 2mm	1.5% OV-1/1.95% QF-1 on Gaschrom Q	205	2 GLC columns
				(2) 1.5m x 2mm	5% QF-1/4% DC-200 on Chromosorb W-AW-DMCS		

APPENDIX (continued 2)

Laboratory No.	Extraction Solvent	Clean-up Method	Pre-GLC Separation	GLC Column (length x bore, capillary underlined)	Column Packing	Column Temperature °C (° programmed)	Confirmation Method
21	Pentane	H_2SO_4	None	(1) 1.6m x 3mm	5% QF-1 on Gaschrom Q	180	MgO reaction column on GLC
				(2) 1.6m x 3mm	1.5% OV-17/2% QF-1 on Chromosorb W-AW-DMCS	195	
22	Hexane	Hexane/DMF, Alumina	None	1.5m x 3mm	1.5% SP-2250/ 1.95% SP-2401 on Supelcoport	205 and 230 (separately)	None
23	Hexane	H_2SO_4	Alumina, Silica	2m x 3mm	2.5% OV-1 on Chromosorb G-AW-DMCS	210	None (PCB only)
25	Pentane	Alumina	Silica	(1) 1.5m x 3mm	3% NPGS on Gaschrom Q	215	HCB, α- and δ-HCH, PCB, dieldrin on (1) DDE, DDD, DDTs, HE, β-HCH on (2)
				(2) 50m x 0.5mm	SE-30 (WCOT)	235	
26	Pet. ether	Alumina	Silica	1.8m x 2mm	4 pts 3% OV-210 + 1 pt 3% OV-17 on Chromosorb W-HP	190	None
27	Pentane	KOH, Alumina	None	25m	SE-52 or SE-30	100-200*	None
28	Hexane	Alumina	Silica	(1) 2m x 2mm	10% DC-200 + 7% QF-1 + 3% OV-225 on Chromosorb W-HP	205	None
				(2) 1m x 2mm	5% SE-30 on Chromosorb W-HP	200	None

APPENDIX (continued 3)

Laboratory No.	Extraction Solvent	Clean-up Method	Pre-GLC Separation	GLC Column (length x bore, capillary underlined)	Column Packing	Column Temperature °C (* programmed)	Confirmation Method
29	Hexane	Alumina	Silica	1.8m x ?	1.5% SP-2250/ 1.95% SP-2401 on Supelcoport	205	None
31	Hexane	H_2SO_4	None	(1) 20m x 0.25mm (2) 10m x 0.25mm (3) 60m x 0.25mm	OV-1 OV-101 OV-101	220 ? ?	GCMS
32	Hexane	H_2SO_4	None	45m x ?	SE-54	100-230*	KOH
34	Cyclohexane	H_2SO_4	Silica for dieldrin	25m x 0.24mm	SE-54	70-210*	KOH, Silica column
35	Hexane	H_2SO_4	None	1.8m x 2mm (i.d.)	1.5% OV-17 + 1.95% OV-210 on Gaschrom Q	210	KOH
37	Hexane	Hexane/DMF Alumina	Alc. KOH Florisil	(1) 2m x ?	6% QF-1 + 4% SE-30 on Gaschrom Q	210	KOH
				(2) 2m x ?	10% DC-200 on Gaschrom Q	205	KOH
40	Acetone/hexane	H_2SO_4	Silica	3.7m x 1.8mm	7 pts 4% QF-1 + 3 pts 2% SF-96 on Chromosorb W-AW-DMCS	170-210*	CrO_3, KOH
41	Hexane	Alumina	Silica	(1) 1m x 6mm (i.d.)	3% OV-17 on Chromosorb W-HP	185	None
				(2) 1.2m x 10mm (i.d.)	4% OV-101 on Chromosorb W-HP	230	None

APPENDIX (continued 4)

Laboratory No.	Extraction Solvent	Clean-up Method	Pre-GLC Separation	GLC Column (length x bore, capillary underlined)	Column Packing	Column Temperature °C (* programmed)	Confirmation Method
42	Hexane	Alumina	Silica	(1) 1.5m x 2mm (i.d.)	3% Dexsil 300 on Chromosorb W-AW-DMCS	200	GCMS
				(2) 1.5m x 2mm (i.d.)	3.8% SE-30 on Diatoport S	200	GCMS
44	Toluene/Ethyl acetate	Gel permeation	GPC, Florisil, Silica	1.8m x 2mm	1.5% SP-2250/ 1.95% SP-2401 on Supelcoport	190(pesticides) 200 (PCB) 165-235* (polar cpds)	GCMS

TABLE 1

Organochlorine Residues in Commercial Fish Oils (Concentrations in $\mu g\ g^{-1}$)

Information	α HCH	γ HCH	Dieldrin	pp'DDE	pp'TDE	op DDT	pp'DDT	HCB	PCB(1254)
Crude trawler[a]	0.003	0.006	0.16	0.22	0.22	0.04	0.36	0.097	1.97
Refined trawler[a]	0.003	0.007	0.17	0.28	0.25	0.06	0.34	0.080	1.92
Crude Icelandic[b]	0.002	0.003	0.14	0.57	0.17	0.08	0.24	0.116	2.62
Refined Icelandic[b]	0.002	0.004	0.18	0.58	0.15	0.03	0.12	0.101	3.15
Crude Capelin	0.001	0.011	0.049	0.090	0.005	0.021	0.110	0.058	0.38
Crude Capelin	0.012	0.007	0.03	0.06	0.04	0.03	0.06	0.034	0.56
Refined Capelin	0.010	0.009	0.06	0.07	0.05	0.02	0.08	0.037	0.60
Crude Mackerel	0.008	0.011	0.05	0.14	0.09	0.03	0.05	0.047	1.86
Refined Mackerel	0.004	0.014	0.06	0.10	0.09	0.04	0.14	0.034	1.45

[a] Mixed fish oils

[b] Cod liver oils

TABLE 2

Analyses of ICES Organochlorine Intercalibration Sample No. 4 (Results in µg/kg)

Laboratory No.	HCB	α-HCH	β-HCH	γ-HCH	Heptachlor epoxide	Dieldrin	pp'-DDE	pp'-TDE	pp'-DDT	op-DDT	PCB	Other Residues
1	-	-	-	-	-	-	-	-	-	-	1190	
2	101	91	<10	-	-	92	32	96	109	<10	191	
3	84	75	<40	<10	-	75	(164)	(231)	(203)	(216)	172	(DDT group possibly over-estimated)
5	84	57	<7	<4	51?	64	76	110	100	60?	380	?identity doubtful
7 Method A	-	-	-	-	-	-	80	80	80	-	900	
Method B	-	-	-	-	-	-	80	70	80	-	1020	
8	43	-	-	<2	-	12	33	114	220	-	1160	
9	-	12	-	<4	-	-	56	69	120	-	644	
10	-	-	-	136	-	-	16	-	30	-	750	
11	59	40	-	-	-	-	70	160	170	-	1200	
13	116	72	-	-	-	-	-	-	-	-	8000	
15	-	-	-	-	-	-	103	-	-	-	509	
16 Method 1	54	32	-	-	-	32	54	83	195	-	411	
Method 2	40	33	-	6	-	59	-	76	-	-	261	
20	42	40	<10	8	-	65	62	60	72	<10	260	
21	64	49	<10	6	-	-	100	75	100	<20	1130	
22	86	55	-	25	-	129	116	87	131	128	Present	op-TDE 74
23	-	-	-	-	-	-	-	-	-	-	270	
25	70	60	<10	20	10	100	60	50	40	40	530	
26	83	73	<10	3	<10	100	123	123	33	45	500	
27 Sample 1	63	48	<10	16	<10	80	80	54	71	<10	277	
Sample 2	69	59	<10	13	<10	64	52	36	47	<10	270	
28	41	50	80	<10	22	109	139	<20	305	<100	1630	TCNB 4, QCB12, Endosulfan 6
29	58	53	-	24	16	119	61	62	-	-	583	op-TDE 174, QCB11, Endrin 22

Table 2 (continued)

Laboratory No.	HCB	α-HCH	β-HCH	γ-HCH	Heptachlor epoxide	Dieldrin	pp'-DDE	pp'-TDE	pp'-DDT	op-DDT	PCB	Other residues
31	-	-	-	-	-	-	100	79	110	75	1300	
32	52	131	-	13		-	116	189*	171	-	467	
34	80	60	<2	6	-	90	110	<50	170	70	400	QCB6
35	-	-	-	-	-	-	74	52	158	-	190	
37	34	60	25	21	-	82	88	110	134	51	720	α-chlordane 50, β-chlordane 50
40	100	10	1	1	-	-	210	70	120	-	1500	Chlordane 240 Toxaphene 1000 - 5000
41	-	48	-	-	-	-	-	-	-	-	-	
42	58	1	<5	13	<5	49	90	<5	110	21	380	
44	70	50	-	10	20	90	340	180	90	-	700	Endrin 90, Toxaphene 2500 Heptachlor 20, Oxychlordane 20 Transchlordane 40, cis-chlordane 150 Transnonachlor 70

* With op-DDT QCB = pentachlorobenzene TCNB = tetrachloronitrobenzene

TABLE 3

Results Obtained by Analysts using Capillary Columns (µg/kg).

Laboratory No.	HCB	α-HCH	β-HCH	Dieldrin	DDE	TDE	DDT	PCB	PCB Ref.	PCB Quant..
10	-	-	136*	-[a]	16*	-	30	750	DP5	Several peaks
25	-	-	-	-	60	50	40	-		
27[d]	63 69 (66)[e]	48 59 (54)	16 13 (15)	80 64 (72)	80 52 (66)	54 36 (45)	71 47 (59)	277 270 (274)	1254	2 peaks
31	-	-	-	-[a]	100	79	110	1300*	1254	2 peaks
32	52	131*	13	-[a]	116	189*[b]	171	467	A.50	24 peaks
34	80	60	6	90[c]	110	<50*	170	400	A.60	10 peaks
Mean	66.00	57.00	11.33	81.00	90.40	58.00	96.67	472.75		
s.d.	14.00	4.24	4.73	12.72	25.74	18.36	63.49	201.41		
No. of Analyses	3	2	3	2	5	3	6	4		
* No. Omitted	0	1	1	0	1	2	0	1		

[a] H_2SO_4 treatment [b] With op-DDT [c] Separate analysis for dieldrin [d] Two samples [e] Means of two values

TABLE 4

Results Obtained by Analysts using Sulphuric Acid Pretreatment (µg/kg)

Laboratory No.	HCB	α-HCH	γ-HCH	Dieldrin	DDE	TDE	DDT	PCB	PCB Ref.	PCB Quant.
1	-	-	-	-	-	-	-	1190	1254	Perchlorination
7B	-	-	-	-	80	70	80	1020	A.50/A.60	7 peaks
8	43	-	<2*	12[a]	33	114	220	1160	1254	5 peaks
9	-	12	<4*	-	56	69	120	644	DP.5	5 peaks
10	-	-	136*	-	16*	-	30*	750	DP.5	Several peaks[c]
11	50	40	-	-	70	160	170	1200	DP.5	?
21	64	49	6	-	100	75	100	1130	1254	4 peaks
23	-	-	-	-	-	-	-	270	1254	3 peaks
31	-	-	-	-	100	79	110	1300	1254	2 peaks[c]
32	52	131*	13	-	116	189[b]*	171	467	A.50	24 peaks [c]
34	80	60	6	90[a]	110	<50*	170	400	A.60	10 peaks [c]
35	-	-	-	-	75	52	158	190	1254	Several peaks
40	100	10	1	-	210*	70	120	1500	A.50	Several peaks
Mean	64.83	34.20	6.50	-	75.60	86.13	131.72	863.15		
s.d.	21.58	22.34	4.93	-	33.01	34.61	52.66	431.84		
No. of Analyses	6	5	4	-	10	8	11	13		
* No. Omitted	0	1	3	-	1	2	0	0		

[a] Separate analysis [b] With op-DDT [c] Capillary column

TABLE 5

Results Obtained using Clean-up (without H_2SO_4), Pre-GLC Separation and Packed GLC columns (µg/kg)

Laboratory No.	HCB	∝-HCH	ɣ-HCH	Dieldrin	DDE	TDE	DDT	PCB	PCB Ref.	PCB Quant.
2	101	91	-	92	32	96	109	191	1254	3 peaks
3	84	75	<10*	75	-	-	-	1700*	1254/1260	5 peaks
5	84	57	< 4	64	76	110	100	380	1254	Several peaks
7A	-	-	-	-	80	80	80	900	A.504A.60(1:1)	7 peaks
15	-	-	-	-	103	-	-	509	A.60	3 peaks
20	42	40	8	65	62	60	72	260	1254	4 peaks
25	70	60	20	100	-	-	-	530	1254	1 peak
26	83	73	3	100	123	123	33	500	1254	DCB
28	41	50	<10*	109	139	<20*	305*	1630*	1254	3 peaks
29	58	53	24	119	61	62	-	583	A.50	Several peaks
37	34	60	21	82	88	110	134	720	1260	?
42	58	1*	13	49	90	< 5*	110	380	1254	5 peaks
44	70	50	10	90	340*	180	90	700	1254	?
Mean	65.91	60.90	14.14	85.91	85.40	102.63	91.00	513.91		
s.d.	21.32	14.93	7.73	21.20	31.16	38.79	30.38	208.81		
No. of Analyses	11	10	7	11	10	8	8	11		
* No. Omitted	0	1	3	0	1	2	1	2		

TABLE 6

Mean Values and Coefficients of Variation of Data Obtained by Different Analytical Techniques (µg/kg)

Residue	Capillary GLC	Sulphuric Acid Clean-up	Adsorbent Clean-up, pre-GLC Separation
HCB	66.00 (21.2)*	64.83 (33.3)	65.91 (32.3)
α-HCH	57.00 (7.4)	34.20 (65.3)	60.90 (24.5)
ϑ-HCH	11.33 (41.7)	6.50 (75.8)	14.14 (54.7)
Dieldrin	81.00 (15.7)	-	85.91 (24.7)
DDE	90.40 (28.5)	75.60 (43.7)	85.40 (36.5)
TDE	58.00 (31.7)	86.13 (40.2)	102.63 (37.8)
DDT	96.67 (65.7)	131.72 (40.0)	91.00 (33.4)
PCB	472.75 (42.6)	863.15 (50.0)	513.91 (40.6)

* % coefficient of variation in parentheses

TABLE 7

Results Obtained by Analysts using Packed GLC Columns without Pre-GLC Residue Separation (µg/kg)

Laboratory No.	HCB	α-HCH	γ-HCH	Dieldrin	DDE	TDE	DDT	PCB
1	-	-	-	-	-	-	-	1190
7B	-	-	-	-	80	70	80	1020
8	43	-	< 2	12	33	114	220	1160
11	50	40	-	-	70	160	170	1200
13	116	72	-	-	-	-	-	8000*
21	64	43	6	-	100	75	100	1130
22	86	55	25	129	116	87	131	-
35	-	-	-	-	74	52	158	190*
Mean	71.80	54.00			78.83	93.00	143.17	1140.00
s.d.	29.67	13.49			28.40	38.73	50.68	72.46
No. of analyses	5	4			6	6	6	5
*No. omitted	0	0			0	0	0	2

TABLE 8

Pooled Values of Analytical Results

Residue	No. of Values	No. Omitted	Mean (µg/kg)	s.d.	c.v. %
HCB	22	0	67.09	21.93	32.7
α-HCH	22	1	48.86	21.27	43.5
γ-HCH	14	6[c]	11.36	8.08	71.1
Dieldrin	17	0	78.76	29.49	37.4
pp'-DDE	24	3	79.50	30.19	38.0
pp'-TDE	21	5[d]	88.14	33.89	38.4
pp'-DDT	23	2	113.13	49.88	44.1
PCB[a]	15	1	451.40	204.36	45.3
PCB[b]	13	0	863.15	431.84	50.0

[a] excluding values from sulphuric acid pre-treatment

[b] from sulphuric acid pre-treatment only

[c] 5 values below limit of detection

[d] 3 values below limit of detection

TABLE 9

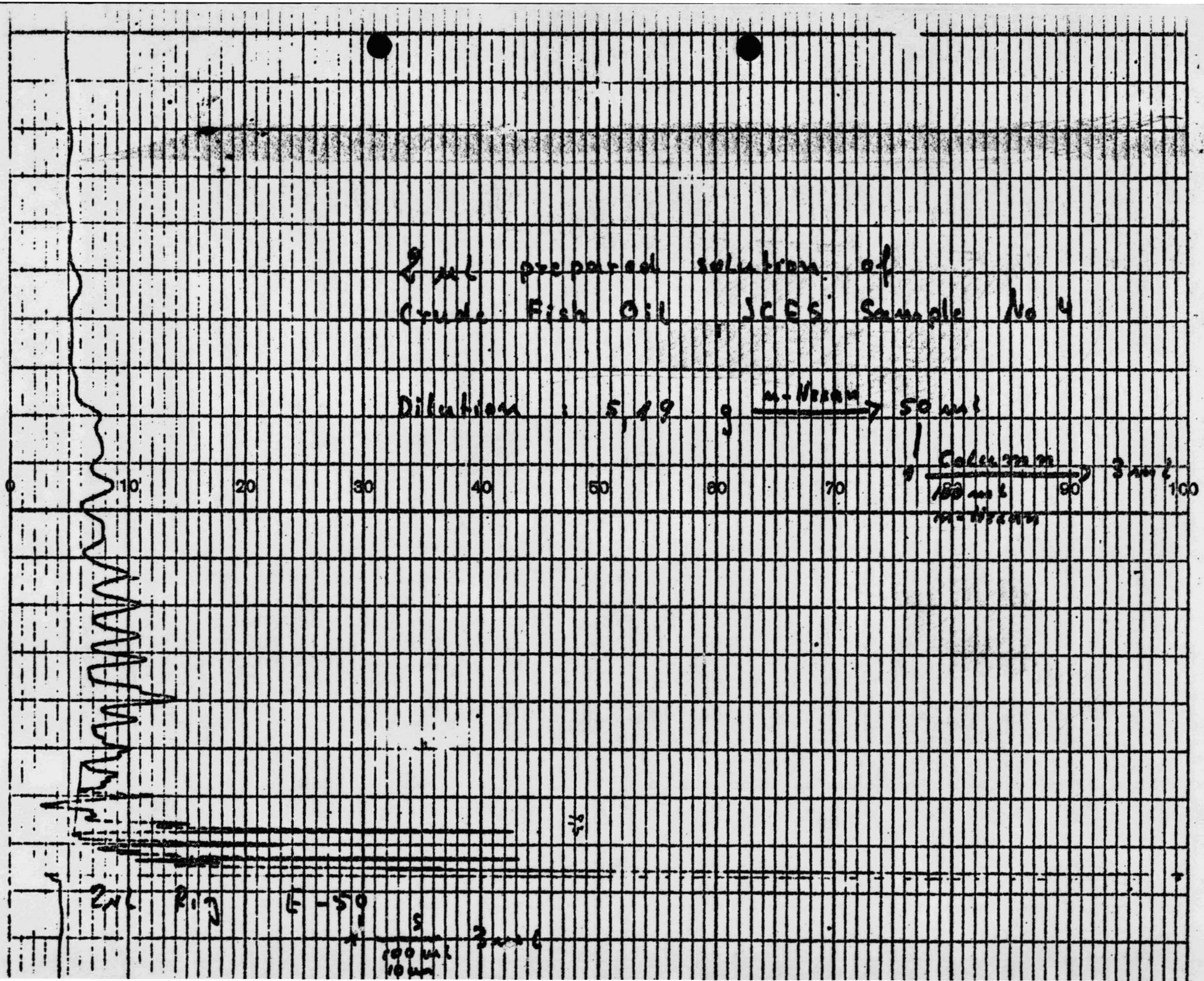
Residue Concentrations (µg/kg) and Coefficient of Variation* from Analyses of Oils in 1972, 1975 and 1979

	2A (1972) Unspiked Fish Oil	2B (1972) Spike in Fish Oil	3B (1975) Spike in Corn Oil	4 (1979) Unspiked Fish Oil
HCB	-	-	46 (40.6)	67 (32.7)
α-HCH	-	-	41 (15.5)	49 (43.5)
γ-HCH	80 (70)	750 (13.5)	52 (27.9)	11 (71.1)
Dieldrin	115 (55)	1440 (6.8)	93 (24.3)	79 (37.4)
pp-DDE	450 (30)	5260 (19.7)	101 (13.5)	80 (38.0)
pp-TDE	290 (29)	3040 (17.8)	103 (10.2)	88 (38.4)
pp-DDT	430 (21)	4990 (10.6)	93 (6.5)	113 (44.1)
PCB	1890 (48)	9960 (10.6)	96 (9.0)	451[a] (45.3)
				863[b] (50.0)

* % coefficient of variation in parentheses

[a] Without sulphuric acid treatment

[b] With sulphuric acid treatment

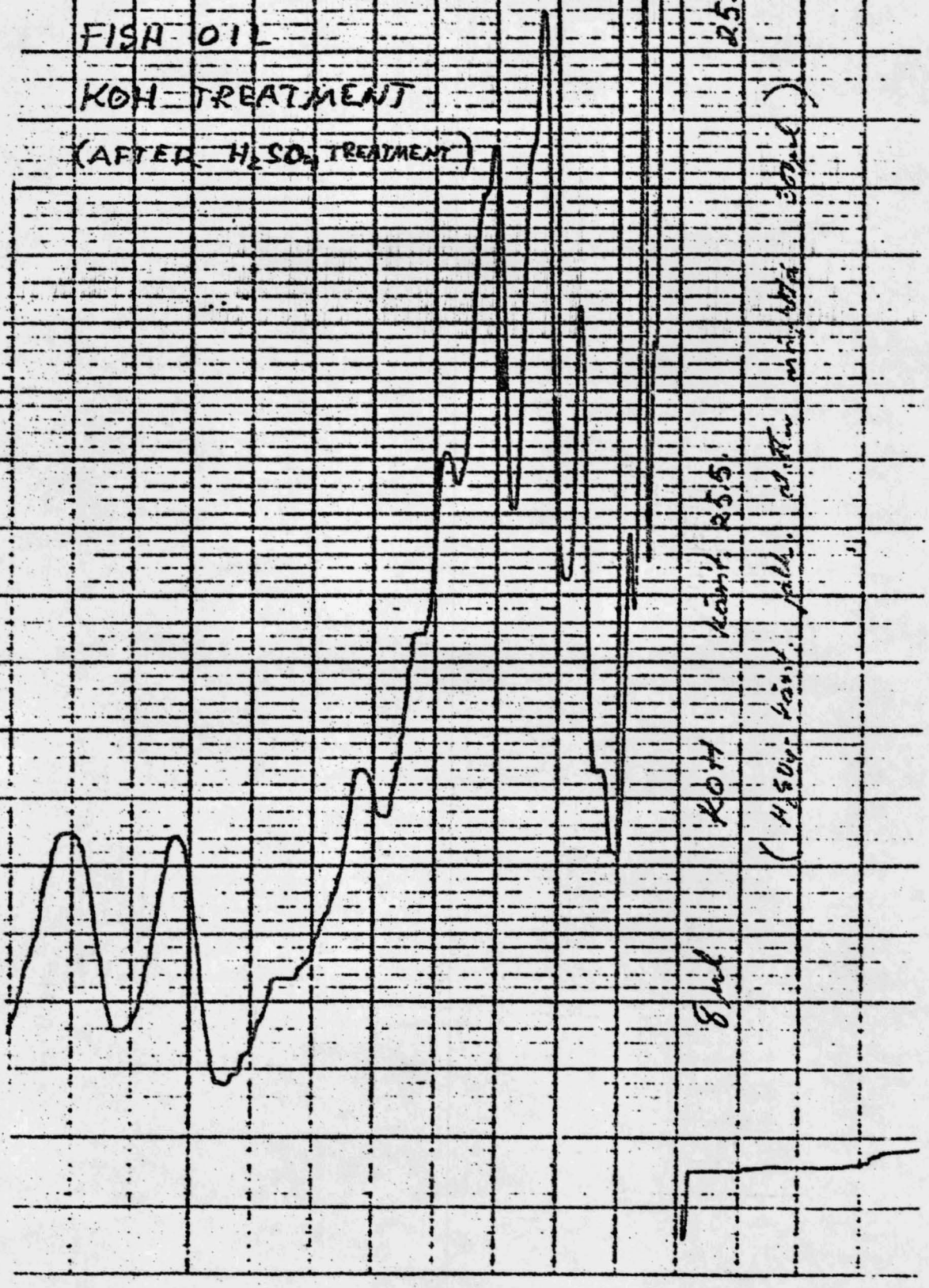

2 ml prepared solution of
Crude Fish Oil : SCES Sample No 4
Dilution : 5.19 g → 50 ml
0
10
20
30
40
50
60
70
80
90
100

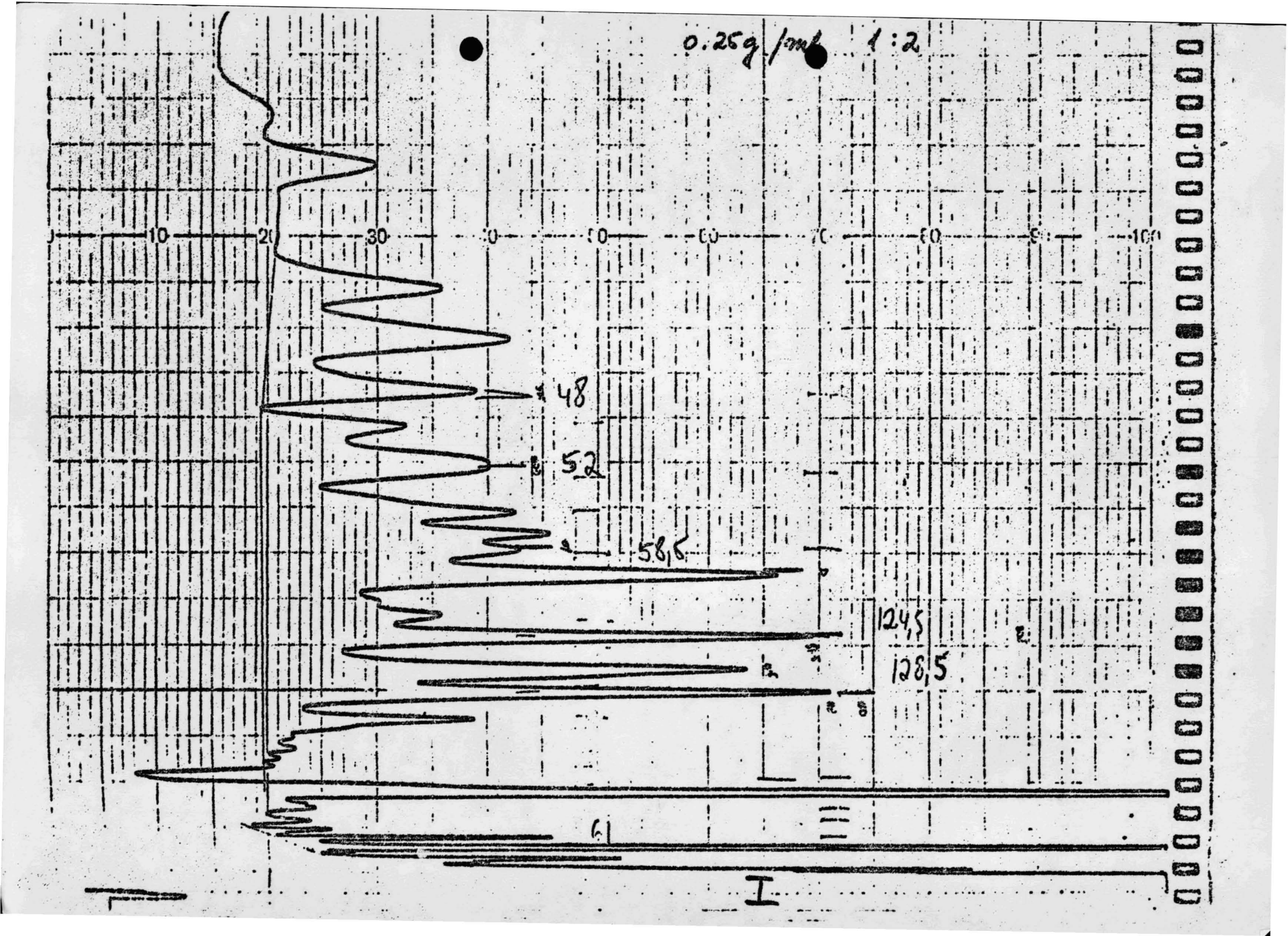
HITACHI PERKIN-ELMER 18.5×52

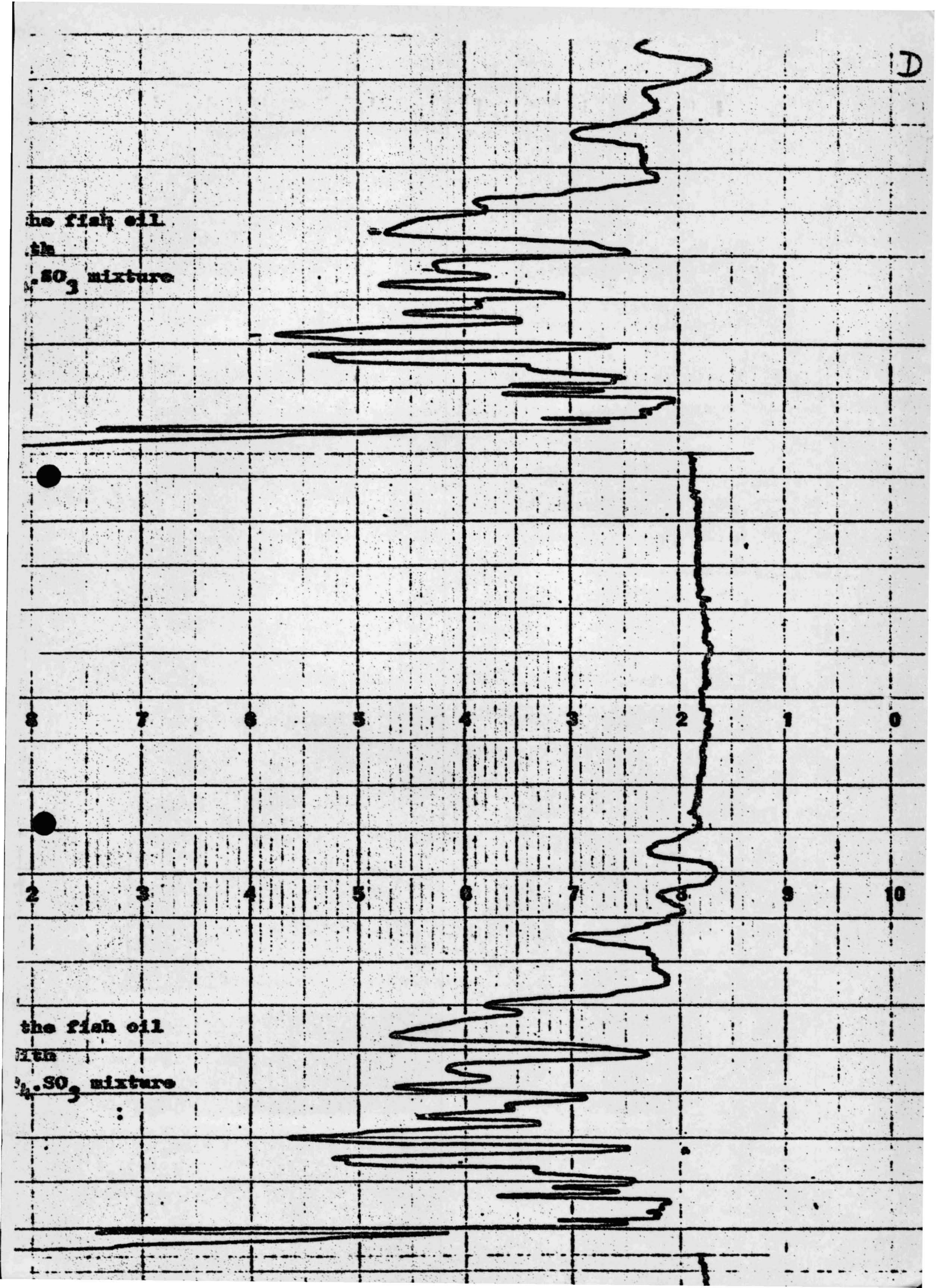
FISH OIL

KOH TREATMENT

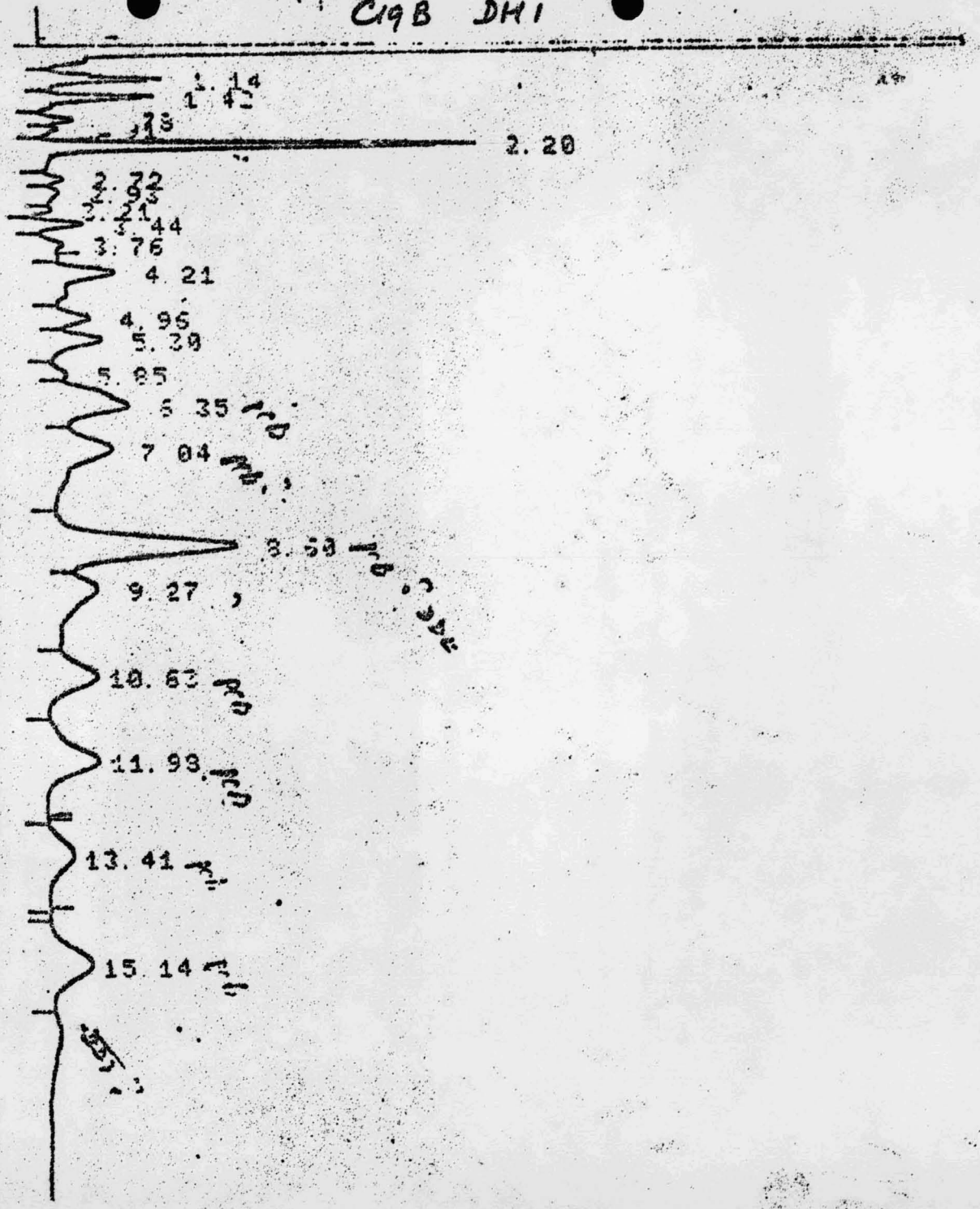
(AFTER H_2SO_4 TREATMENT)




0.25g/ml
1:2
10
20
30
0
0
60
70
80
90
100
48
52
58,5
124,5
128,5

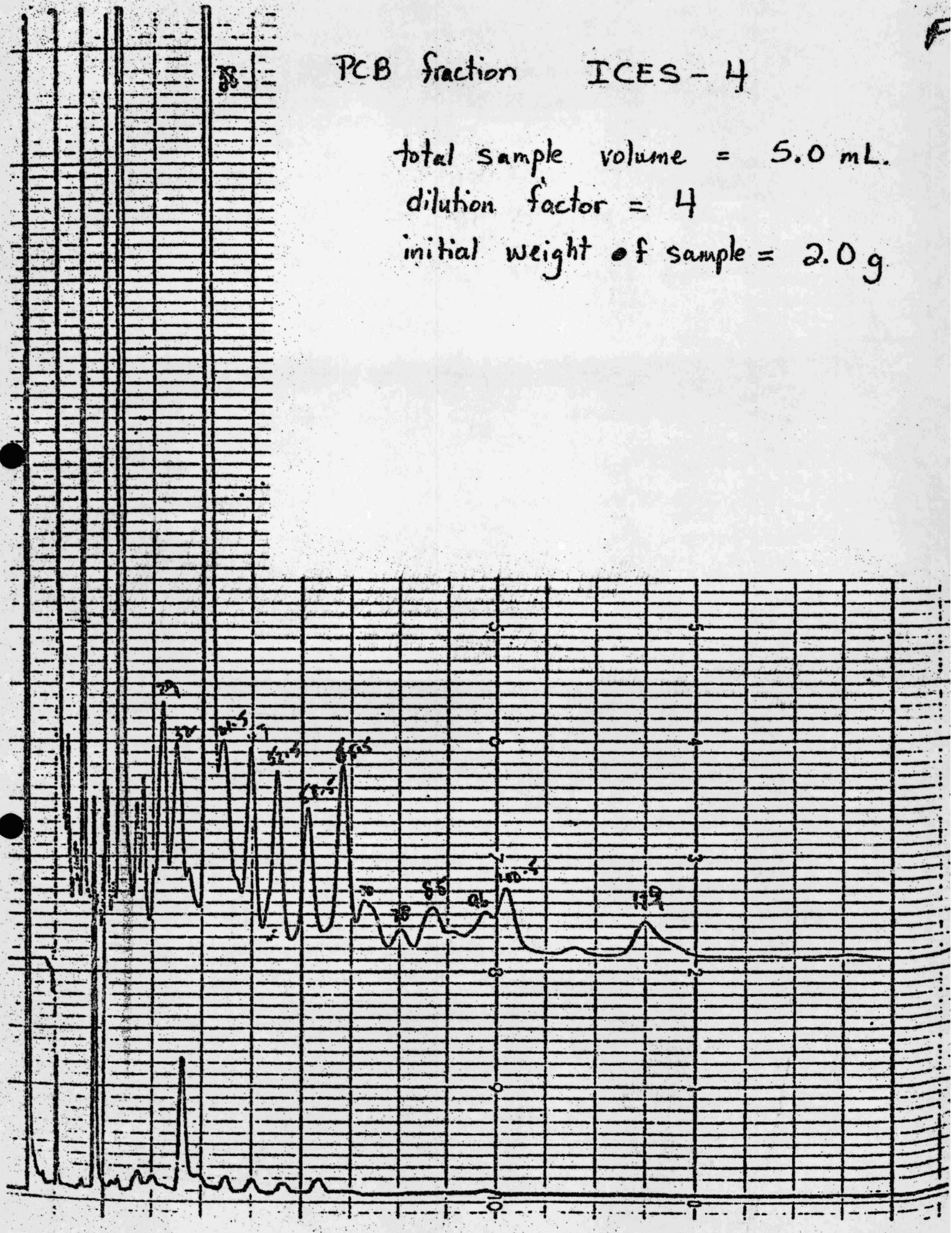

D
he fish oil
th
SO_3 mixture
8 7 6 5 4 3 2 1 0
2 3 4 5 6 7 8 9 10
the fish oil
ith
SO_3 mixture

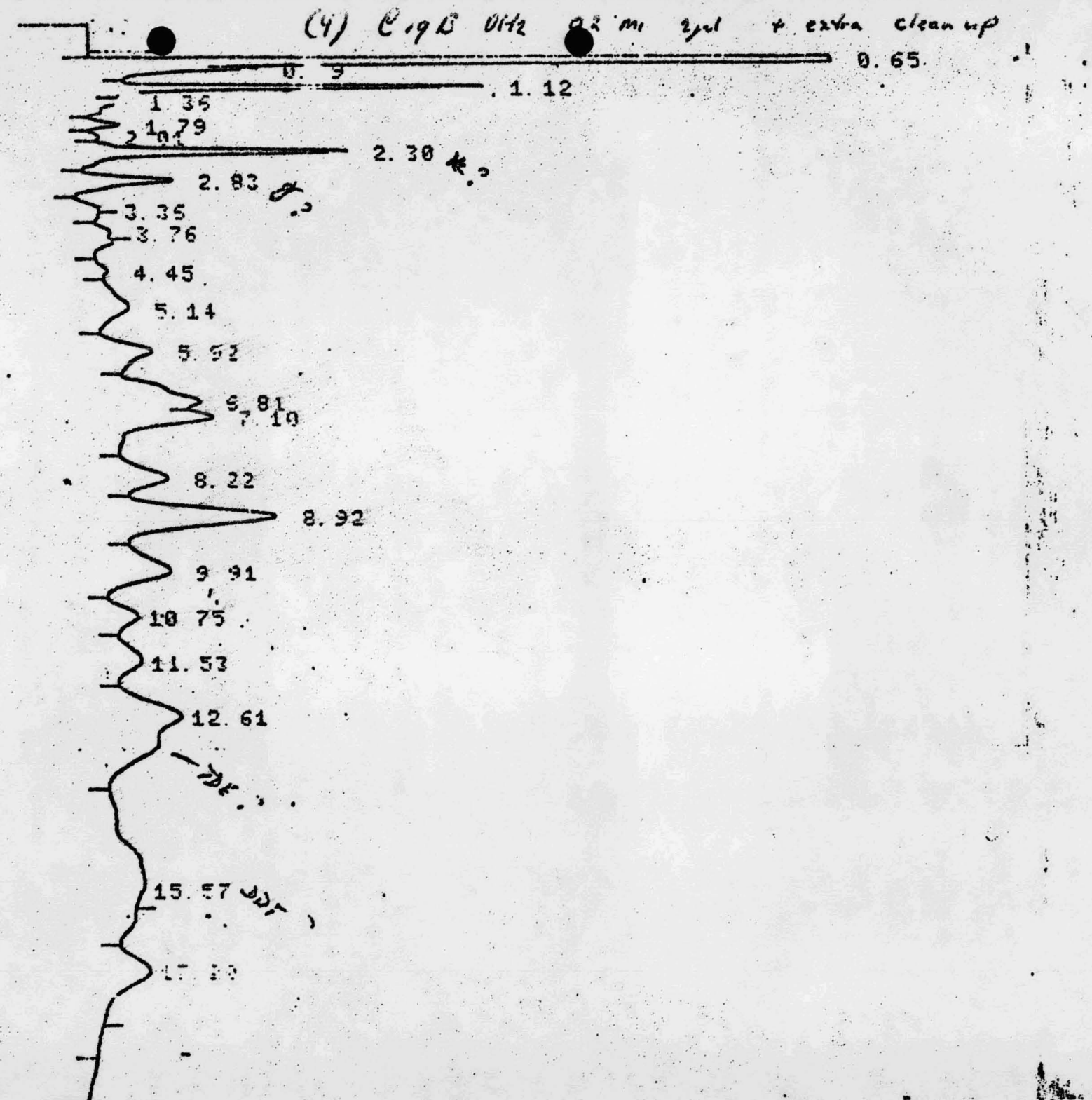
C19B DH1

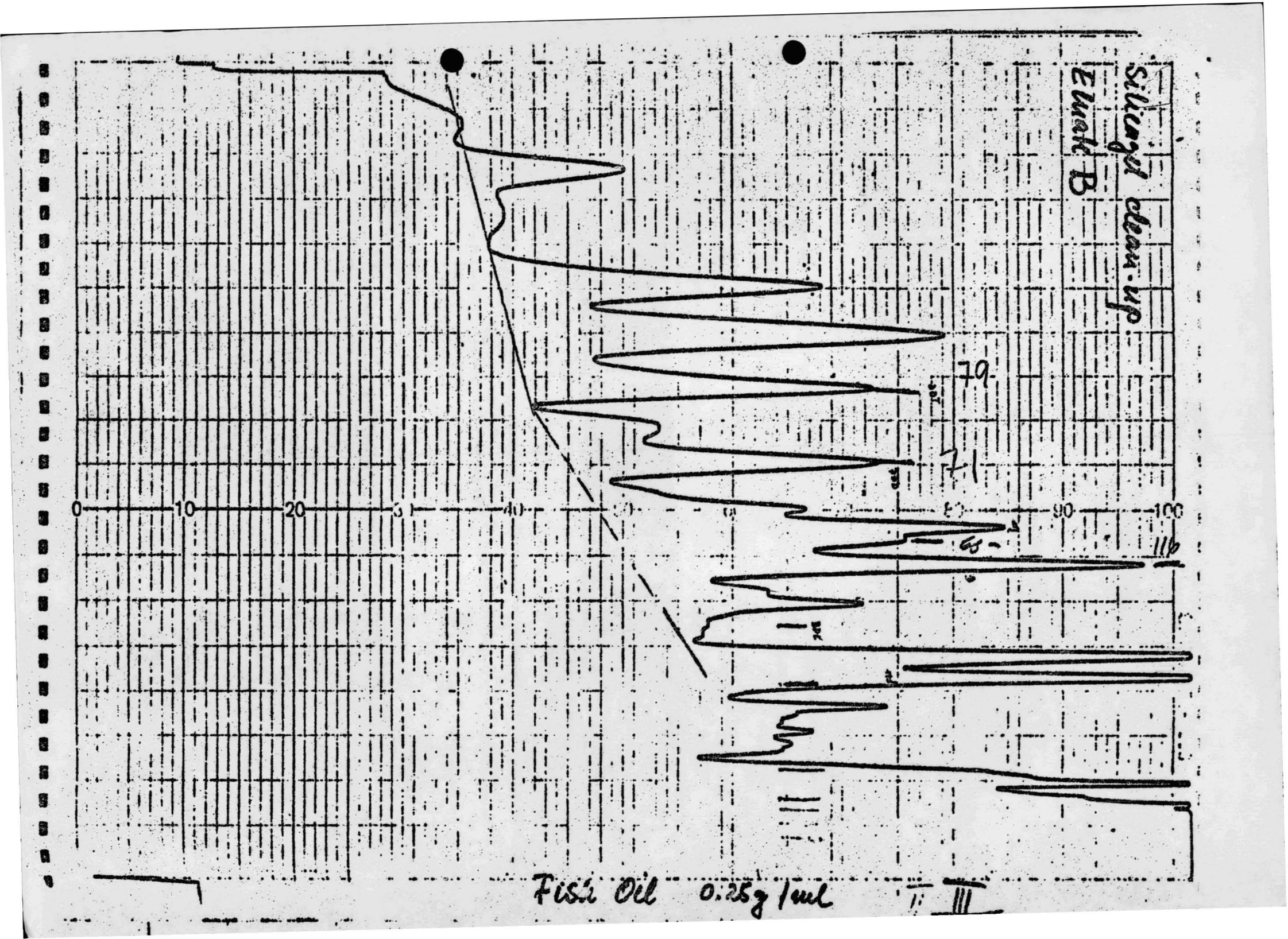

1.14
1.45
1.78
2.20
2.72
2.93
3.21
3.44
3.76
4.21
4.96
5.30
5.85
6.35
7.04
8.58
9.27
10.63
11.93
13.41
15.14

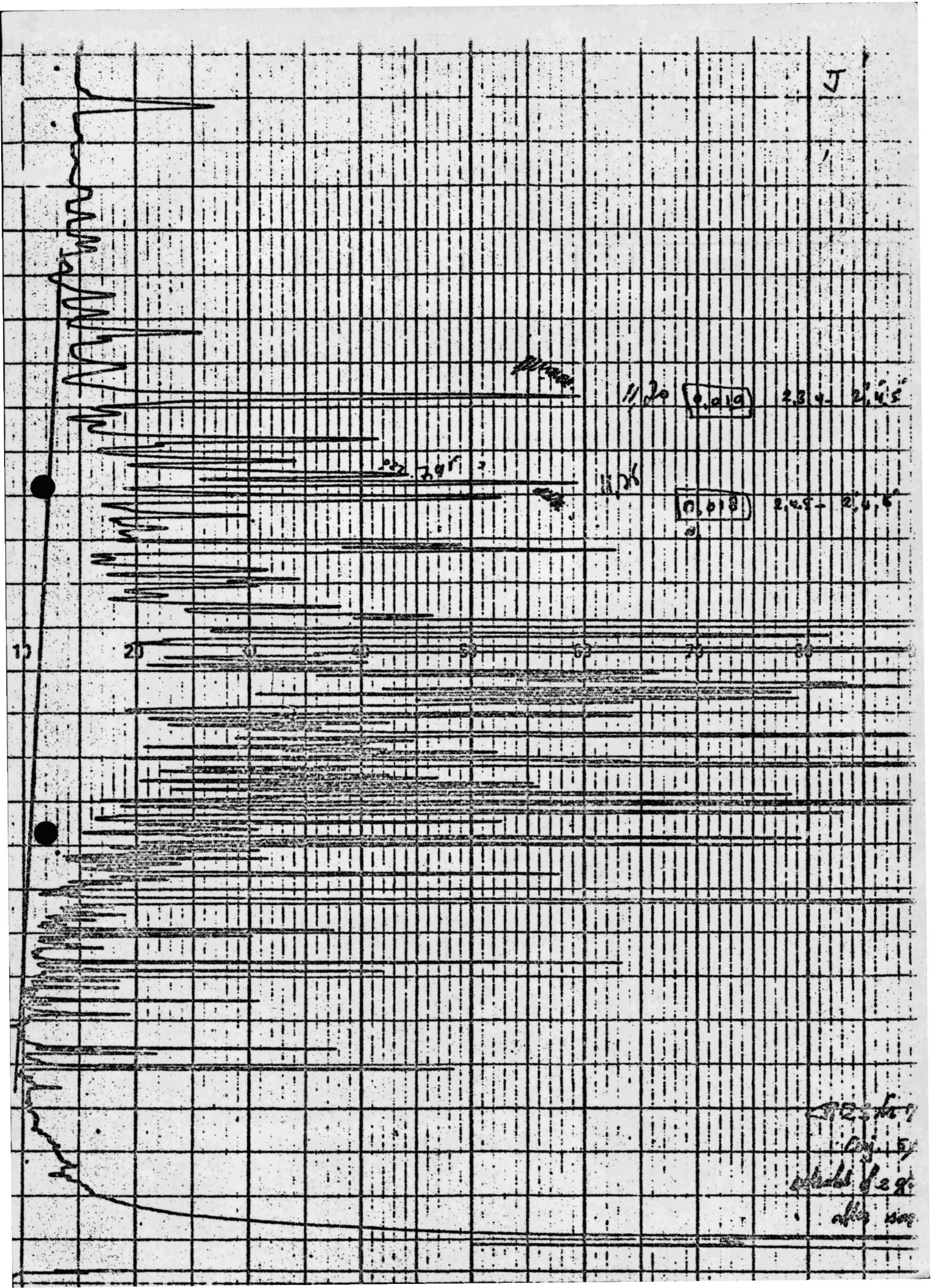
PCB fraction ICES-4

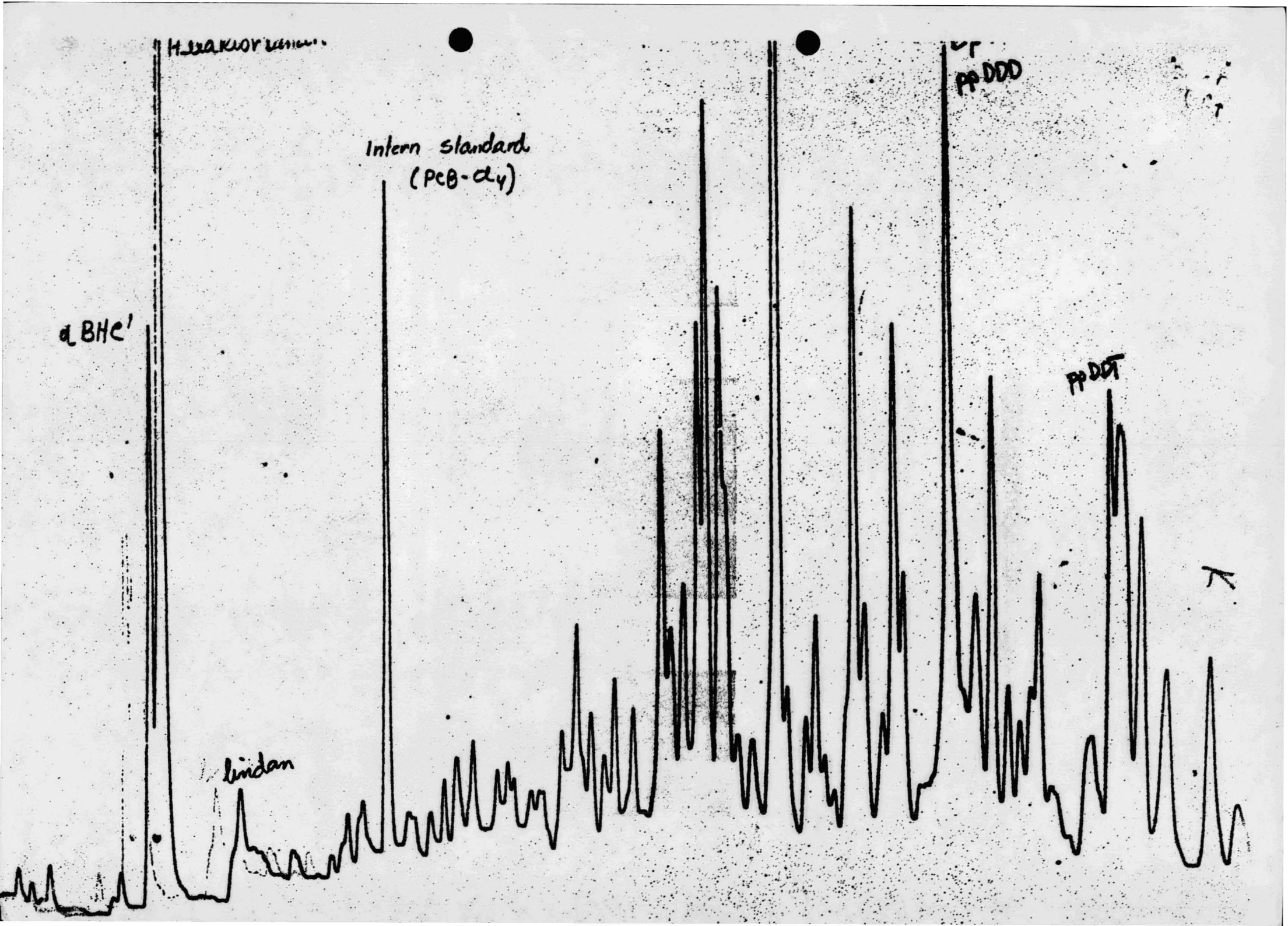
total sample volume = 5.0 mL.
dilution factor = 4
initial weight of sample = 2.0 g




(4) C19 Ultra 82 mi 2µl + extra clean up
0.65
0.9
1.12
1.35
1.79
2.30
2.83
3.35
3.76
4.45
5.14
5.92
6.81
7.10
8.22
8.92
9.91
10.75
11.53
12.61
15.57


Silicagel clean-up
Eluate B
79
71
0
10
20
30
40
50
90
100
Fish Oil 0.25g/ml

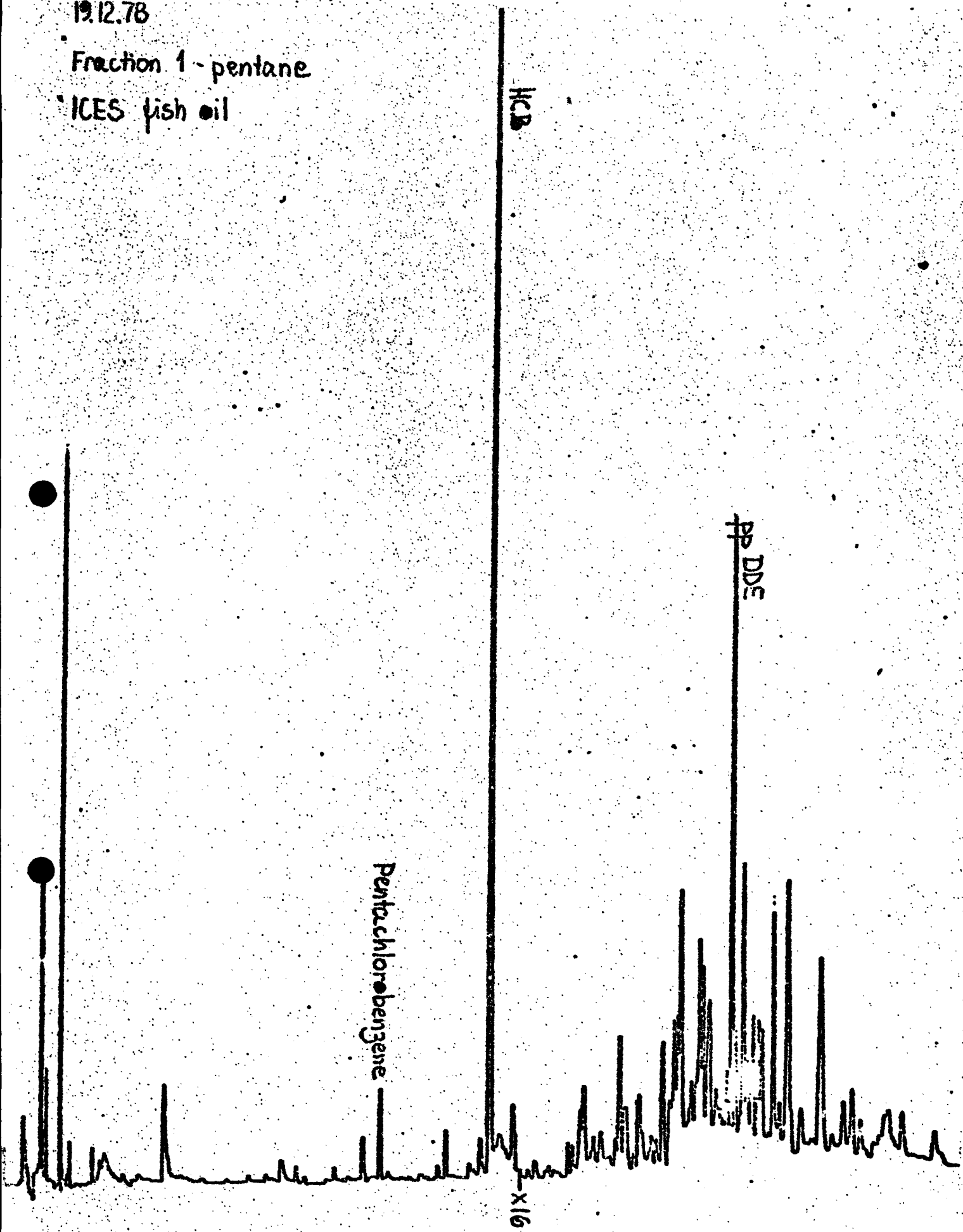

J
0,019
2,3 -
2,4,5
2,4,5 -
2,4,6
20
30
40
50
60
70
80


Hexachlorobenzene
Intern standard
(PcB-α_4)
α BHC
Lindan
PpDDD
PpDDT

12.12.78

Fraction 1 - pentane

ICES fish oil



19.12.78

Fraction 2 - pentane/diethylether

ICES fish oil