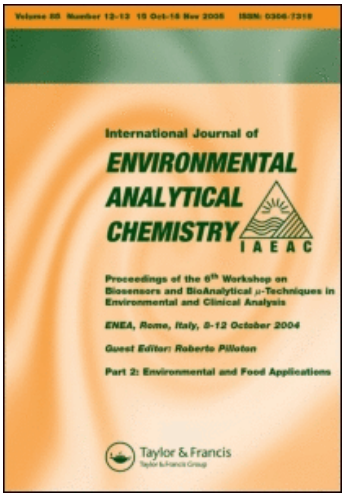

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CALIBRANTS AND CALIBRATION FOR CHLOROBIPHENYL ANALYSIS

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Guidelines are given for the preparation of calibration solutions and the calibration of the capillary gas chromatograph for the determination of chlorobiphenyls (CBs) and ways in which errors may be reduced to produce comparable data in the analysis of environmental samples. Results from a Community Bureau of Reference (BCR) intercomparison exercise indicate that calibration remains a key factor in obtaining reliable data.

KEY WORDS: Chlorobiphenyls, intercomparison, calibration, capillary gas chromatography.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are measured in most environmental analytical laboratories in a variety of matrices, and are, at present, determined more than most other organic contaminants. The financial commitment and manpower allocated to these analyses is a significant proportion of any laboratory's budget. Yet it is clear from recent intercomparison exercises (I/C)[1–3] that the quality of measurement of these compounds still requires significant improvement in most institutes. It is reasonable to conclude that any laboratory which submits results for such an exercise will report their best estimate completed under the most ideal situation. Therefore, these data generated for the I/C represent the best possible quality of measurement for each participant, which will, most likely, deteriorate further when the laboratory is under pressure to produce analytical information within a more limited time scale.

Under such circumstances the analysts and, more especially the customer, will either not know the level of accuracy and precision of the data, or be led to imagine that the data is of a higher quality than in reality. When the analyst is limited by time it is essential to know which aspects of the determination are critical for the improvement and maintenance of the quality of the data.

For the certification of reference materials, it is essential to be aware of the error sources at each stage in the determination of CBs. In 1982 the Community Bureau of Reference formed a working group on chlorobiphenyl analysis to identify the main

sources of error associated with the determination of CBs and to develop a selection of methods which could minimize these errors. Initially the working group undertook a stepwise learning programme which has now progressed to a programme of continual assessment[4,5]. Any subsequent development such as the inclusion of additional chlorobiphenyls, or changes in the methods or laboratories is evaluated in a similar way prior to adoption into the programme.

Since 1982 the working group have repeatedly demonstrated that the main sources of error in the determination of CBs in environmental matrices still occurs during the preparation and storage of primary calibration solutions and during the routine calibration of the gas chromatograph[3–5].

This paper presents the main conclusions drawn from a poster-workshop on calibration held by the BCR working group on April 9th–10th 1991 in Brussels and the results of an I/C to test the calibration procedures following the inclusion of additional CBs to the certification programme (Table 1). The information given here is not normally available in research papers and is presented in this form to assist fellow analysts in the improvement of their measurements of CBs. It may appear to be basic information that would be implicit in any trace organic analytical measurement, but it is the experience of the BCR, in general, and this working group, in particular, that it is necessary to be explicit in reporting these fundamental precautions for the preparation of calibrants for the determination of CBs. Although these guide-lines do not constitute a protocol or standard operation procedure (SOP) it may be appropriate for any laboratory to consider their incorporation into the laboratory's quality assurance/quality control manual.

CALIBRANTS

The current BCR certification programme for the determination of CBs in environmental matrices includes the original seven congeners; IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180[6] and five additional congeners 105, 128, 156, 170 which have been

Table 1 Chlorobiphenyls selected for inclusion in the BCR certification programme

IUPAC No.	*Name*	*Formula*
28	2,4,4′ Trichlorobiphenyl	$C_{12}H_7Cl_3$
52	2,5,2′,5′ Tetrachlorobiphenyl	$C_{12}H_6Cl_4$
101	2,4,5,2′,4′ Pentachlorobiphenyl	$C_{12}H_5Cl_5$
105	2,3,4,3′,4′ Pentachlorobiphenyl	$C_{12}H_5Cl_5$
118	2,4,5,3′,4′ Pentachlorobiphenyl	$C_{12}H_5Cl_5$
128	2,3,4,2′,3′,4′ Hexachlorobiphenyl	$C_{12}H_4Cl_6$
138	2,3,4,2′,4′,5′ Hexachlorobiphenyl	$C_{12}H_4Cl_6$
149	2,4,5,2′,3′,6′ Hexachlorobiphenyl	$C_{12}H_4Cl_6$
153	2,4,5,2′,4′,5′ Hexachlorobiphenyl	$C_{12}H_4Cl_6$
156	2,3,4,5,3′,4′ Hexachlorobiphenyl	$C_{12}H_4Cl_6$
170	2,3,4,5,2′,3′,4′ Heptachlorobiphenyl	$C_{12}H_3Cl_7$
180	2,3,4,5,2′,4′,5′ Heptachlorobiphenyl	$C_{12}H_3Cl_7$

included for toxicological reasons. CB149 was also included to evaluate the separation of CB149/118 on a 5% phenyl methyl silicone chromatographic phase (SE 54 or CPSil 8 type column) (Table 1).

Sources of calibrants

The choice of the primary calibrant material is the foundation of all subsequent measurements and ultimately the accuracy of the data. Individual, certified CBs are readily available and should be used. Where these certified reference materials (CRMs) are not available the analyst should obtain well characterized solids of known purity (not less than 99%). In the course of cross checking the calibration solution it is also essential to confirm the identity of a new batch of any uncertified material as well as the concentration of the solution. It has been known for the wrong label to have been attached to a bottle by the manufacturers.

A number of calibration solutions are offered by manufacturers which has the attraction of saving time and money. However, there has been some doubt concerning the accuracy of some of these solutions giving a measurable bias to the results[2]. In addition there is no opportunity to trace the final result to primary material. The BCR does not accept these solutions as primary calibrants in any certification exercise, unless the solutions themselves have been certified, and the working group would caution their use in any routine work, without first checking with an independent solution prepared from certified material.

Preparation of primary solutions

In view of the relatively high cost of synthesis and purification of the solid individual congeners most producers only sell the CBs in 5–10 mg quantities. The small mass and the electrostatic nature of the material can make it difficult to quantitatively transfer these compounds as solids and to prepare the primary standard solution with the required degree of accuracy. The initial manipulations in preparing these solutions, although straightforward in theory, is in practice, one of the greatest sources of bias in the whole analysis.

The responsibility for the preparation of these calibration solutions should be given to one of the most senior and experienced members of the analytical team. Formal training and practise of other staff to the required standard is an essential part of the continuity and quality assurance of any trace organic analytical laboratory.

There are two main techniques for preparation of the primary calibration solutions used by the working group.

1) Weighing by difference. When the amount of CB is restricted to 2–5 mg it is necessary to use a 5 or 6 place balance and to weigh the material into a small aluminium or tin cup and to transfer the solid to the solution flask. The tin foil is then re-weighed. The advantage of this system is that the amount of material can be weighed accurately. However, it is quite difficult to ensure that all of the material is transferred into the receiving flask and does not "fly" or stick to other surfaces. To overcome this there are two alternative approaches by direct weighing.

2) Direct weighing. If the amount of material to be weighed is <5 mg then a 5 or 6 place balance must be used. The CBs must be weighed into a tarred aluminium or tin boat, since most flasks will exceed the range of these sensitive balances. The whole boat and contents should then be transferred to the primary calibration solution flask. This reduces the risk of loss by electrostatic attraction. When the amount of material available for weighing is 5–10 mg or greater then the CBs can be weighed directly into the flask on the balance. The small metal boat would remain in the calibration solution, but this is unlikely to affect the quality of the solution in any way.

Once the CBs are in the flask and the solvent has been added then the task of preparing the solution is relatively much easier since any losses will be more obvious. The following guide-lines were given to assist in preparing the primary calibration solutions.

- The analytical balance must be serviced regularly by the manufacturers qualified engineer.
- The balance must be calibrated with a series of masses, to cover the range of the balance, before making a primary solution.
- Position the balance where it is free from draft, direct sunlight, changing temperature and humidity.
- Keep the electrostatic charges to a minimum. This can be done by placing the balance on a conducting mat similar to those used in the computer industry and connecting this to earth. Similarly it is possible to connect an earthing strip to the remote end of the nickel or stainless steel micro spatula. Some balances have a deionization source within the weighing cabinet which reduce the static electricity, e.g. Zerostat (Mettler balances).
- Minimize the manipulations in weighing. Where possible make one weighing of approximately the required mass into a boat or the solution flask directly. Adjust the strength of the solution by the mass of solvent rather than attempting to obtain an accurate mass of solid CB.
- Use a single solvent of known tested purity. Verify the purity of the solvent by analysing a $\times 100$ concentrate on both the electron capture and the flame ionization detector.
- Although it is quite possible to prepare calibration solutions by mass or volume, it is recommended that all measurements are made by mass, both for the solvent as well as the solute. The advantages of this system is that the mass is simple to measure, traceable to a single balance, and can be checked afterwards. Where volume is used all corrections for temperature and density also have to be made and recorded. This can also be a source of error.
- It is recommended that all primary calibration and working solutions of CBs are prepared in *iso*-octane as solvent. This is the most appropriate solvent for sample introduction into the gas chromatograph at the correct temperature and it is sufficiently non-volatile relative to other common solvents to reduce the errors associated with evaporation.

- Where all measurements are made by mass it is recommended that volumetric flasks are not used. This removes any ambiguity as to whether the solution has been prepared on a mass/mass or mass/volume basis. Also volumetric flasks are less stable to physical or mechanical abuse and it is relatively more difficult to transfer material into these flasks as opposed to a wider necked conical flask.
- When marking flasks to identify the content use a metal tag or etch the glass rather than a paper label which will take in moisture.

Storage of calibration solutions

The preservation of the quality of both composite and primary calibration solutions is vital to the whole calibration scheme. There should be a controlled, written protocol on the lifetime and storage scheme in any one laboratory. New calibration solutions should be prepared well in advance of any anticipated deterioration of the current material to maintain the "within" laboratory continuity. Each solution should be given a designated place in the refrigerator and a record kept of the mass of solution removed.

Most of the laboratories in the CB working group store the primary solutions in high quality borosilicate glass sealed either with a ground glass or teflon stopper. One laboratory uses a teflon lined ampoule with a screw cap. The solutions are then stored in the refrigerator (0–5°C) or in the freezer (−18°C). All of the solutions are weighed and recorded before and after any solution is withdrawn from the vessel.

One laboratory takes weighed aliquots of the parent calibration solution which are sealed in an amber ampoule to eliminate loss of material on storage. The main disadvantage is that great care and practise is required in sealing the ampoule to eliminate any degradation of the contents of the ampoule. The ampoule must be filled without depositing any of the solution on the inside neck of the glass. The contents must then be frozen with liquid nitrogen or solid CO_2 and the ampoule filled with argon, the neck sealed by rapidly heating the neck and allowing the molten glass to collapse inward (Figure 1). Once the ampoule is cool the seal can be checked by immersing in warm water. If there are no bubbles emerging from around the seal then it may be regarded as airtight. This technique may also be used to store sample extracts for archive purposes.

Checking the calibration solution

Once the calibration solutions have been prepared it is essential to confirm that the declared concentrations are within 5–10% of the target value.

Although the intrinsic accuracy of the balance would normally allow the calibration solution to be prepared to within ±0.2%, the effects of static electricity on the solid and the volatility of the solvent might increase this minimum value to around ±1–2%. These target accuracies can only be confirmed by analysis which would suggest that two solutions i.e. the calibrant and the test sample, should agree to within ±5% in a single laboratory.

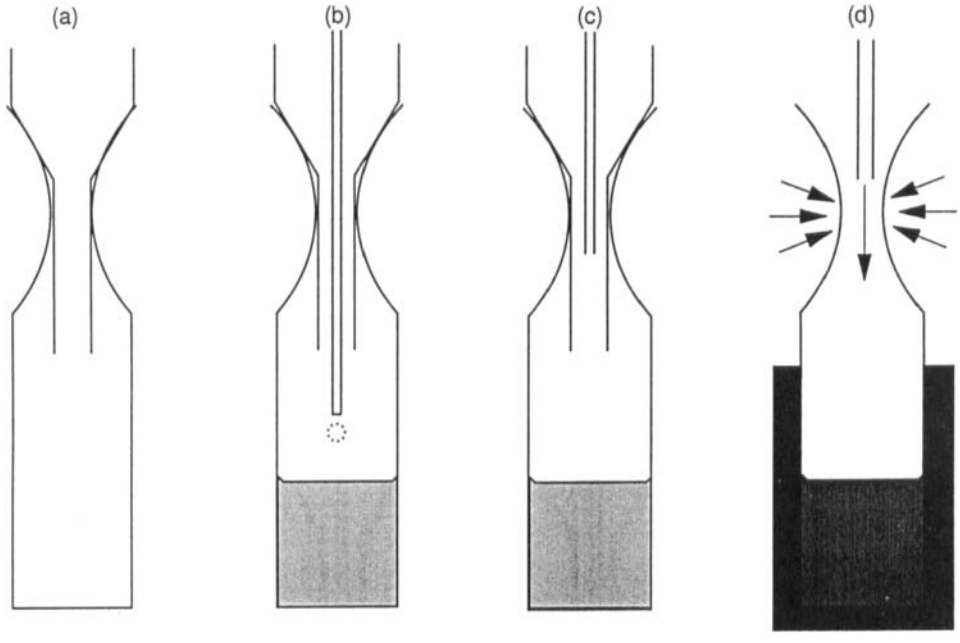

Figure 1 Technique of filling and sealing ampoules to prevent contact of the filling solution with the inside of the ampoule. (a) Insert glass protective sleeve into the neck of the ampoule. (b) Insert pipette/dispenser and fill the ampoule. (c) Withdraw the pipette into the protective sleeve and withdraw both. (d) Cool the ampoule base and content in liquid nitrogen or solid CO_2. Flush the ampoule with argon. Heat the neck with a hot multiflame gun. Allow the neck to collapse and withdraw the head to seal.

There are a number of recognized ways in which these nominal concentrations can be confirmed and so eliminate any gross errors associated with inaccurately prepared calibration solutions.

The first "in-house" method is to prepare two independent sets of standards and check one against the other. This must be done in the event of a new or novel analysis where few laboratories are undertaking this type of determination. If the solutions agree with the target values to within $\pm 5\%$ then they are acceptable. Between 5–10% variation will indicate a small difference between solutions, but is acceptable within the normal errors encountered in these analyses. If, however, the difference is greater than 10% for any individual CB then a third solution must be prepared and compared with the first two potential calibrants to confirm which solution is in error.

Fortunately, this rather protracted and costly approach is only necessary when working with CBs which are relatively scarce or when calibration solutions are prepared to this level of accuracy and traceability for the first time. Generally, it is more preferable to exchange the new solution with another laboratory experienced in these analyses and which operates a similar quality control system. Once the accuracy of the solution has been confirmed then this can be used to verify any future new solutions PROVIDED THAT THE INTEGRITY OF THE ORIGINAL HAS BEEN MAINTAINED AND IS NOT IN QUESTION, i.e. when a fresh solution is to be prepared because the existing one is suspect, because of evaporation losses etc.

Preparation of working solutions

Once the primary calibration solution of each congener has been prepared composite working solutions containing each of the CBs and an appropriate internal standard[5] can be made.

Each laboratory will have its particular own scheme and protocol depending on the applications and the sensitivity of the ECD. However, there are some aspects of preparation, storage and use which need to be common to all laboratories.

Each solution for the multi-level calibration should be prepared independently from the primary solutions. This may require a series of dilutions to reach the desired concentration, but intermediate solutions should not be used for calibration at a different level. Calibration solutions should never be prepared by serial dilution since an error in the initial solution will simply be transferred through the calibration scheme, giving a good correlation, but a high risk of an undetected bias.

All solutions except the final working one should be weighed and either ampouled or stored in the refrigerator. Losses from *iso*-octane solutions are, generally, relatively small provided that the solutions are not left for extended periods in a warm room. Normal ground glass stoppers will, for example, limit the loss of *iso*-octane to no more than 2% over a six to nine month period for a 100 ml conical flask which is 50% full. Losses in the refrigerator can be monitored and the mass adjusted to the last recorded value prior to use.

The daily working solutions can be kept in a cool place, but it is essential that they are permitted to equilibrate with the room temperature before being used to calibrate the GC. Alternatively, the working calibration solutions can be stored in a container with a small amount of *iso*-octane to saturate the atmosphere of the enclosure. This keeps the solutions at room temperature and almost eliminates the loss by evaporation due to the partial pressure of the *iso*-octane in the container.

CALIBRATION

The correct characterization of the response curve of the ECD of each GC instrument is the second critical parameter in obtaining reliable CB analyses. Previously, it had been recommended[5] that only the most linear part of the response curve of the detector should be calibrated for use. However, this approach had a number of drawbacks since the detector response is never truly linear and the concentration of different CBs in the environmental samples cover a wider range than any "linear" portion of the ECD curve. An alternative approach, which has a considerable practical advantage and greater accuracy, is to use a multi-level calibration.

Intially, the response curve of the detector is determined with a sufficient number of calibration solutions (ca 10–12). The concentration of the different CBs in these solutions can be approximately the same, giving an increased detector response with increasing chlorination, as occurs in many of the industrial formulations or the concentration of each CB can be adjusted in relation to the detector response factor to obtain a constant peak height. It is not essential to determine the response curve

for every CB since congeners of similar structure and chlorination respond in the same way. The test mixture should, at least, contain examples of a tetra to hepta or octa chlorobiphenyl (e.g. CB28, 118, and 180 would be the minimum selection). Once the response curve has been established it needs only be checked and updated every 6 months, after the foil has been cleaned or replaced, or prior to a certification programme. The multi-level calibration is then based on this curve (Figure 2). The number of calibration levels is dependent on the change of the response/mass v mass profile such that an additional calibration point is added each time the response/mass ratio changes by more than 5%. In most cases five calibration levels are sufficient to accurately describe the ECD response curve to within ±5% over a concentration range of two decades. This, of course, takes some considerable analysis time, but the more recent series of gas chromatographs have relatively stable detectors and their response only change dramatically after being contaminated with dirty extracts, or after major maintenance.

Previous practice has been to fully calibrate the GC every day at one or two calibration levels. However, it is now possible to use five point calibration which remains within ±5% for over a week of continual use. A single calibration level can be injected each day to monitor the stability of the detector. Using this approach it is possible to document the long term stability of the ECD as part of the essential quality of the measurements being made.

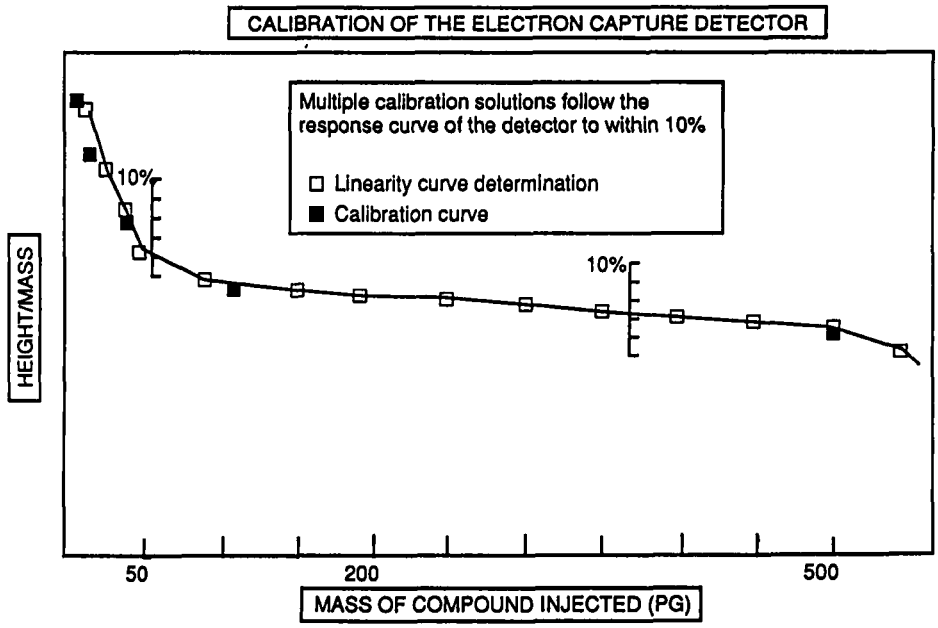

Figure 2 Multi-level calibration of the electron capture detector. The response curve of the detector is determined over the required working range and a plot of peak height/mass versus mass injected is made. The number of calibration points required are determined by the change in height/mass. An additional level is added when the change exceeds 5%. In this example a 5 calibration points will describe the detector response over the range 5–500 pg on column.

INTERCOMPARISON

Prior to the certification of the spray-dried milk powder the working group undertook an intercomparison exercise to determine the accuracy of the preparation of the calibration solutions for the additional CB to be included into the BCR programme. The participants were asked to prepare these calibration solutions from solid CBs of known purity, and to determine the concentration of these congeners in a simple *iso*-octane solution. Each participant was requested to optimize the gas chromatograph according to the working group guide-lines[4,5] and to determine the response curve of the detector over the working concentration range used in the laboratory, and to make five separate, replicate determinations of each CB in the sample solution.

Twenty-four laboratories participated in this exercise, and one laboratory returned four sets of data obtained by different columns, electron capture detection and mass spectrometry. There were 323 data sets of five replicates returned from the 24 laboratories covering the 12 congeners.

Overall, 31 sets of data appeared not to agree with the values obtained by the other laboratories in the initial survey of the data. Following the technical discussion of the data, four laboratories indicated that they had either some difficulty in the preparation of the calibration solutions or that they were aware of some level of bias in the solutions they had used. The data submitted by these laboratories accounted for most of the 31 outlying values and were withdrawn from the final evaluation of the intercomparison. The selected mean values obtained by the group and the coefficient of variation for each of the twelve CBs are given in Table 2 along with the target values. With the exception of CB128 all mean values were in agreement with the target values. The difference between target and mean values for CB128 was further investigated, but the reason of the discrepancy was not determined. However, the coefficient of variation for CB128 was, at 4.7%, a little lower than some of the

Table 2 Comparison of the mean and target values for the BCR intercomparison of calibration solutions

CB IUPAC No.	*Target value ($\mu g\ g^{-1}$ mass fraction)*	*Mean value*	*No. of obs.*	*Coefficient of variation*
28	55.1	58.8	26	9.1
52	55.1	59.0	23	9.1
101	95.4	93.4	26	8.0
105	60.5	63.0	24	5.8
118	94.7	92.4	25	7.7
128	85.9	95.5	23	4.7
138	136.0	136.9	24	4.4
149	89.3	91.3	23	8.7
153	140.0	134.8	25	9.2
156	50.7	56.0	24	6.4
170	78.9	87.5	26	9.1
180	64.7	65.8	23	6.8

other CB around 9%. This improvement in the precision of the group also highlights the increase in difficulty in maintaining the required accuracy. This is also evident when comparing the mean "between" laboratory coefficient of variation of, for example, CB153 at 9.2% and the mean "within" laboratory coefficient of variation at 2.6%. The apparent level of precision demonstrated by some laboratories only serves to highlight the level of bias which can exist between the institutes.

Although there is still room to improve the performance of the group by applying all of the lessons of this exercise, these data still reflect some of the best information available on "between" laboratory agreement for these determinands. However, by comparing these data with the overall coefficient of variation for the CRMs produced[7] so far for CBs in environmental matrices (CV (overall) = 5–18%) it is clear that the preparation of calibrants and the method of calibration remain the dominant source of error in the determination of CBs.

References

1. A. V. Holden, G. Topping and J. F. Uthe. *Can. J. Fish & Aquat. Sci.*, **40**, Suppl 2, 100 (1983).
2. Report on the ICES/IOC/OSPARCOM intercomparison exercise on the analysis of chlorobiphenyls in marine media. MCWG 1991/7.2. (Brussels, March 1991). ICES, Palagade, Copenhagen, DK.
3. L. G. M. Th. Tuinstra, A. H. Roos, D. E. Wells and B. Griepink. *Mikrochim. Acta*, **1**, 1–7 (1989).
4. D. E. Wells, J. de Boer, L. G. M. Th. Tuinstra, L. Reutergardh, and B. Griepink. *Fresenius Z. Anal. Chem.*, **322**, 591–597 (1988).
5. D. E. Wells, J. de Boer, L. G. M. Th. Tuinstra, L. Reutergardh, and B. Griepink. Report on the improvement in the determination of chlorobiphenyls leading to the certification of seven CBs in two fish oils and in sewage sludge. EUR 12596 EN (1989).
6. K. Ballschmiter and M. Zell. *Fresenius Z. Anal. Chem.*, **320**, 20–31 (1980).
7. D. E. Wells, A. E. Maier and B. Griepink. *Int. J. Environ. Anal. Chem.*, **46**, 259–269 (1992).