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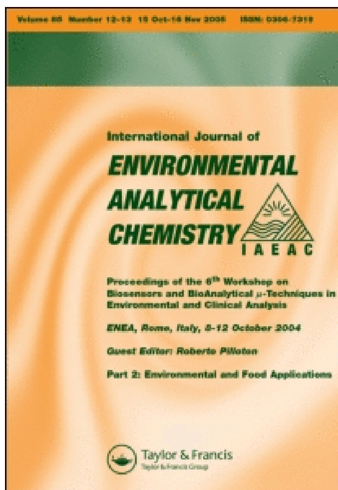


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# Global uncertainty associated with the analysis of PCBs and chlordanes in drinking water by headspace-SPME-GC/MS/MS

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Until recently, analytical results were normally stated as declared values, sporadically associated with strict uncertainty evaluation. Current trends in analytical chemistry require traceable data together with fully evaluated uncertainty. Polychlorinated biphenyls (PCBs) and chlordanes are considered in this paper from the standpoint of the uncertainty of their determination in drinking water. Using headspace solid-phase microextraction (HS-SPME) and GC-MS/MS, it was possible to reach low ng/L levels of quantification in drinking water for seven marker PCBs and two chlordane isomers, with a relative global uncertainty associated with the results of all contaminants ranging from 11 to 38%. Although the assessed relative uncertainties of the results can be considered acceptable and may even be further improved at higher concentration ranges, they may have considerable importance for levels close to the detection limits of the method, where they may represent more than 100% of variation for the stated result.

*Keywords:* Solid-phase microextraction; PCBs; Chlordane; Water; Global uncertainty

## 1. Introduction

Most of the chemical measurement results stated in the 20th century were only declarations of the result of a measurement process. Uncertainty was referred to as a calculation of repeatability or reproducibility with an estimate of systematic error. In view of the current trends in analytical chemistry, namely regarding results traceability, the 21st century will produce a measurement result as a value of a quantity with measurement uncertainty. The value of the result goes from ‘declared’ to ‘traceable’, and the uncertainty from single reproducibility with a systematic error estimate, to a full, evaluated uncertainty task. Overall, we start from changing a ‘true value’ with an error estimate and confidence limit, and end up with a possible range of values with an evaluated uncertainty, as the assessment of the doubt of the result [1].

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Error and uncertainty must be clearly distinguished. The former is a single value, defined as the difference between an individual result and the 'true value' of the measurand. As the 'true value' is never known in analytical chemistry, error is an idealized concept and thus impossible to be exactly ascertained. The latter, on the contrary, defines a range, and if assessed for an analytical procedure and sample type, it may apply to and comprise all determinations so described [2].

The presence of most environmental pollutants in the different environmental media, due to its toxicity and/or persistence, is often crucial even at very low concentrations. Analysts and environmental researchers in particular report data on the occurrence of these compounds in several matrices, but the uncertainty associated with those results is often lacking. This can hamper their interpretation and comparability, as in degradation studies, for example. Furthermore, the stated measurement results gain importance when, as an example, legal limits are to be observed, such as in the determination of pesticides (such as chlordanes) and their degradation products with a 0.1 µg/L maximum allowable level required in the European Union for potable water [3].

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants, and their presence has been confirmed in a variety of worldwide matrices such as water in the different physical states [4, 5], soil and sediments [6], and mammal tissues [7, 8].

Besides direct sources, such as storage or products incorporating PCBs, these long-lasting contaminants can arise as byproducts of fires, including bonfires, and some manufacturing processes. Although the manufacture and general use of PCBs stopped in the 1970s and their use is now banned in developed countries, PCBs are still present in products such as heat-transfer and hydraulic fluids, capacitors and transformers [9], consequently bearing a risk of direct environmental contamination. To monitor its overall occurrence and distribution, seven marker PCBs are currently used: PCBs 28, 52, 101, 118, 138, 153 and 180 [9–12].

Chlordane was used in the protection of several vegetable and fruit cultures, home, garden and ornamentals, or applied directly to control a variety of insects, and its use in the United States is still allowed for fire ant control. Furthermore, the presence of this insecticide in drinking water is regulated in several countries worldwide [13] and has been already reported in wild mammals [8].

Sample preparation can be understood as all the necessary steps to obtain a sample ready for instrumental analysis. A rapid and simple technique usually means faster analysis and greater sample throughput. The accuracy and precision are improved, as analyte loss (or contamination) and other systematic errors occurring in the process will be reduced due to fewer sample handling steps [14].

Currently, solid-phase microextraction (SPME) is considered a major alternative as an extraction/concentration technique to the 'classic' liquid–liquid extraction (LLE) or solid-phase extraction (SPE). SPME is solvent-free, in contrast to LLE and, to a lesser degree, to SPE, which demand large volumes of expensive and toxic solvents. In addition, SPME requires fewer steps and less sample handling than those techniques, which can greatly decrease the uncertainty introduced during sample preparation steps. Finally, SPME is by far the easiest technique to be fully automated, often allowing equal or better sensitivities than LLE or SPE for a wider range of compounds. For certain contaminants and matrices, headspace SPME (HS-SPME) may offer increased sensitivity over SPME mainly because it can produce 'cleaner' extracts, especially when the matrix contains undissolved particles or non-volatile dissolved compounds [15].

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Several works have already reported the use of SPME or HS-SPME as suitable techniques for the analysis of PCBs and different organochlorine pesticides (OPs) in water [16–20]. However, to our knowledge, there is no report on the quantification of chlordane isomers by HS-SPME, as well as on the assessment by the EURACHEM protocol of the global uncertainty associated with the quantification of both PCBs and chlordane by HS-SPME in drinking water.

Since the contamination of water supplies is a global problem, the purpose of this work was to assess the global uncertainty associated with the results of the determination of selected chlorinated pollutants in drinking-water samples, using the validation-based approach, proposed by EURACHEM [2], in order to identify the principal sources of uncertainty and, thus, aiming to improve the effective significance of such data.

## 2. Experimental

### 2.1. *Water-sample handling*

The drinking water samples used in this study were obtained from the municipal water supplier, collected before (10 samples) and after (10 samples) the water treatment plant (WTP), as well as eight commercial drinking water samples acquired on the local market.

Sampling was carried out using 250 mL dark glass bottles with Teflon stoppers, completely filled and kept refrigerated in the dark until analysis. Whenever possible, analyses were performed on sampling day. Samples were analysed in duplicate without filtration.

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### 2.2. *Chemicals and materials*

Ethanol was purchased from Riedel-de Haën (Seelze, Germany) as analytical grade. The compounds $\alpha$- and $\gamma$-chlordane were from Riedel-de Haën, PCBs congeners (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180) were purchased from Restek (Bellefonte, USA) as analytical standards. All the compounds were acquired with the highest purity available.

The organochlorine pesticides ($\alpha$- and $\gamma$-chlordane) and polychlorobiphenyls (PCBs) included in this study were used to prepare a 'working standard' (ethanol:water 60:40 (v/v)) with about 2 μg/L of each of the PCBs and chlordanes. From this solution, dilute standards were made, and water samples were spiked to the required concentration. All dilute standard solutions contained 1.8% of ethanol, and so 720 μL of ethanol was added to all samples before extraction.

Water was distilled and deionized. Helium carrier gas (99.9999% quality) was supplied by Praxair (Madrid, Spain). To minimize adsorption and loss, or desorption of the studied compounds during handling and analysis, all glass material was silanized prior to utilization. Silanization was performed by soaking glassware overnight in a 10% dichlorodimethylsilane solution in toluene, then rinsed with toluene and methanol and finally dried thoroughly for 4 h at 400°C.

The SPME device (fibre and holder) was purchased from Supelco (Bellefonte, USA). The fibre used was coated with 100 μm polydimethylsiloxane (PDMS). Magnetic bars,

PTFE coated, 20 × 7.5 mm, were used for stirring. After each extraction, stir bars were rinsed consecutively with acetone, n-hexane, ethyl acetate, diethylic ether, acetone, and finally with water, to eliminate carryover between samples.

For HS-SPME extraction, 50 mL (nominal size) crimp top HS vials (actual capacity about 55 mL), 20 mm black Viton septa and aluminium seals were used, all from Supelco (Oakville, Canada). During extraction, the SPME fibre assembly was extended so the end of the fibre was about 1 cm above the surface of the liquid. Agitation was appropriate to give a vortex depth of 0.5 cm.

Optimized HS-SPME conditions were: Headspace sampling for 80 min of 40 mL of sample (pH between 6 and 8) or standard, at 65°C, with 100 μm PDMS coated fibre. After extraction, the SPME fibre was immediately inserted in the GC injector for analysis.

### 2.3. *Instrumentation*

For the chromatographic separation and detection of the studied compounds, a Varian (Walnut Creek, USA) CP-3800 gas chromatograph, equipped with a split/splitless injector (model 1079), and a Varian Saturn 2000 ion trap detector was used. The analytical column was a Varian 60 m × 0.25 mm CP-Sil 8 CB lowbleed/MS (0.24 μm film thickness). Helium at 0.9 mL/min (constant flow) was used as carrier gas.

The analytes were desorbed from the SPME fibre in the injector at 260°C, in splitless mode. At 10 min, the split valve was opened. SPME fibres remained in the injector for at least 60 min to minimize carryover.

The chromatographic oven-temperature programme was as follows: the initial temperature of 80°C was held for 10 min after injection; at a rate of 18°C/min, it was ramped to 170°C and to 260°C at 2°C/min; then to 300°C at 3°C/min, returning afterwards to the initial value. The total run time was 80 min. The transfer line, manifold and trap temperatures were 290°C, 50°C, and 210°C, respectively.

Detection was made by resonant collision-induced dissociation (CID) tandem mass spectrometry (MS/MS) with the most influent parameters set to achieve maximum sensitivity. The emission current was set to 60 μA for all MS segments and the axial modulation voltage to 4.0 V. The most prominent ion was selected in each case for CID MS/MS, and the two most abundant ions in MS/MS spectra were selected for quantification.

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## 3. Results and discussion

### 3.1. *Chromatographic separation*

Capillary GC-MS is a powerful multidimensional technique. However, because of the great number of PCB congeners, any of the analytical columns available at the present can resolve all the 209 congeners in one run, and thus several coelutions are observed [21]. This allows the determination of PCBs by conventional EI-MS, even in the selected ion-monitoring mode (SIM), to exhibit higher detection limits than with electron capture detector (ECD).

Recently, tandem mass spectrometry (MS-MS) analysis by ion trap mass spectrometry systems became a competitive technique for the determination of these

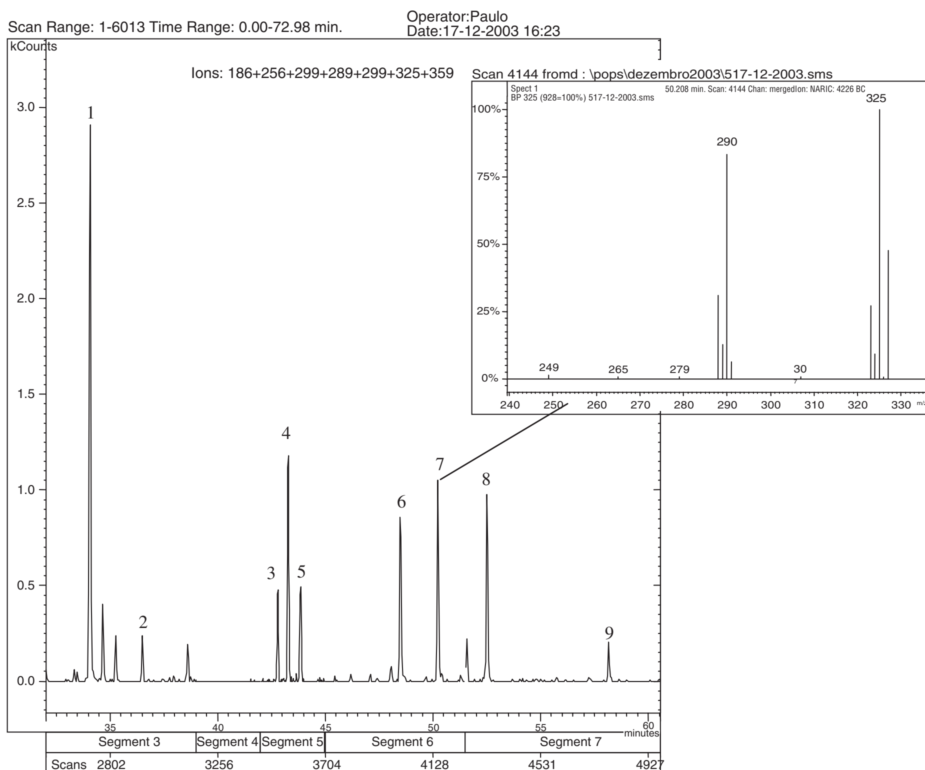


Figure 1. Selected ions chromatogram and mass spectrum of a water sample spiked with PCBs (15 ng/L) and chlordanes (24 ng/L) (1: PCB 28; 2: PCB 52; 3: $\gamma$- Chlord; 4: PCB 101; 5: $\alpha$-Chlord; 6: PCB 118; 7: PCB 153; 8: PCB 138; 9: PCB 180).

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compounds [22], due to the greater selectivity of this technique, which can even be enhanced by the use of 60 m capillary columns.

The analytes were identified and quantified by extracting the characteristic ions of each of the studied PCBs and chlordanes, monitored at the specific retention time, within a peak window of $\pm$0.2 min.

All the studied compounds were below the detection limits of the technique on the analysed samples, and so a spiked sample was necessary to show the separation obtained in real samples. Figure 1 represents the chromatogram of a water sample spiked with selected PCBs and chlordanes. It can be seen that baseline separation was possible between all the studied compounds, with a very low background level for the selected monitoring ions.

### 3.2. *Method validation*

The current trends in analytical chemistry set the method validation as an important issue of global quality associated with analytical data. Indeed, the fitness-for-purpose of analytical methods applied for routine testing is most commonly assessed through method validation studies, which provide data on overall performance parameters and on individual influence factors. The following parameters are those currently considered more important in quantitative analytical methods validation.

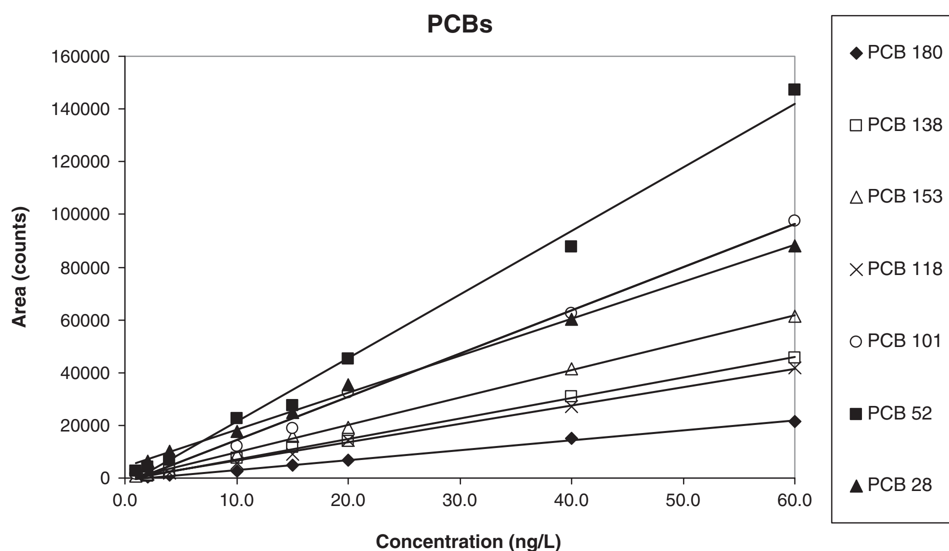


Figure 2. Calibration curves obtained for each studied PCBs.

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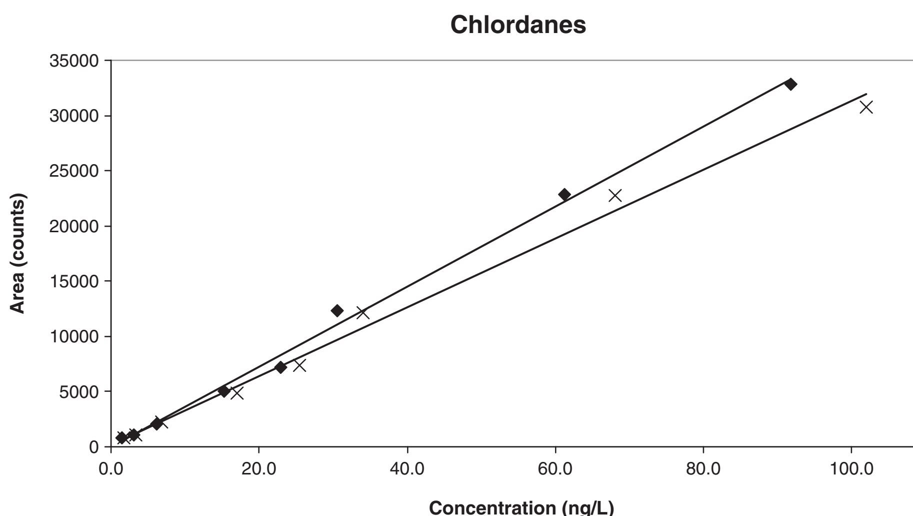


Figure 3. Calibration curves obtained for each studied chlordanes (◆: $\gamma$-chlordane; ×: $\alpha$-chlordane).

**3.2.1. Linearity.** Linearity is generally not quantified, as significant non-linearity is usually corrected by the use of non-linear calibration functions or eliminated by the choice of more restricted operating ranges. Any remaining deviations from linearity are normally sufficiently accounted for by overall precision estimates covering several concentrations, or within any uncertainties associated with calibration [2].

In the present work, eight calibration standards were analysed in duplicate for the calibration of each substance, except for PCBs 28, 138 and 180—seven standards (figures 2 and 3). For the compounds studied here, the higher squared correlation

coefficient ($R^2$) was 0.9990 (PCB 153) and the lower 0.9928 ($\alpha$-Chlord). The calibration functions were linear within the concentration range considered (on average 1–60 ng/L for PCBs and 2–95 ng/L for chlordanes).

**3.2.2. Detection limit.** Uncertainties near the detection limit may require careful consideration, as the basic form of the 'law of propagation of uncertainties' used in EURACHEM guide may cease to apply accurately in this region [2]. Nevertheless, the determination of this parameter is not of direct relevance to uncertainty estimation.

Although there are other methods to calculate the detection limit, based for example on the signal-to-noise ratio, this parameter was calculated by the sum of the intercept and three times $s_{y/x}$ ($s_{y/x} = [\sum(y_i - y_{i\text{calc}})^2/(n-2)]^{1/2}$, with $y_i$ denoting the experimental values and $y_{i\text{calc}}$ being calculated from the calibration curve; see table 1). This ranged from 2 (PCBs 153 and 138) to 10 ng/L ($\alpha$-Chlord).

**3.2.3. Precision.** The main precision measurements include repeatability standard deviation ($S_r$), reproducibility standard deviation ($S_R$), and intermediate precision, which can be defined as $S_{Zi}$, with '$i$' denoting the number of varying factors (time, equipment and operator) [23]. The observed precision of an analytical procedure is an essential component of overall uncertainty.

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The experiments needed to obtain an estimate of the method precision should be designed so as to take into consideration the scope of the method. Several situations can arise from this; in extreme cases, the method scope covers only a single matrix type and a single analyte concentration, or it may cover a range of matrices and a range of concentrations. Bearing in mind that each analysis represents a complete application of the method, including sample preparation steps, precision study experiments should be spread in order to vary as many method parameters as possible. Typically, these are: calibration, reagents (this can include SPME fibres) and analyst (if the method is to be routinely used by a number of different analysts). In those cases where the method is to be used for the determination of an analyte in a range of sample matrices and at a range of concentrations, the precision study must consider also a range of representative samples, when available.

Table 1. General characteristics of the analytical method by HS-SPME-GC/MS/MS.

| Compound | Linearity range (ng/L) | $R^2$ | Detection limit[a] (ng/L) | Accuracy[b] (% recovery ± SD) | Precision[c] (% CV ± SD) |
|---|---|---|---|---|---|
| $\gamma$-Chlordane | 2–92 | 0.9959 | 7 | 92 ± 21 | 32 ± 8 |
| $\alpha$-Chlordane | 2–102 | 0.9928 | 10 | 105 ± 27 | 38 ± 6 |
| PCB 28 | 2–60 | 0.9978 | 3 | 94 ± 6 | 19 ± 1 |
| PCB 52 | 1–60 | 0.9937 | 5 | 100 ± 20 | 21 ± 3 |
| PCB 101 | 1–60 | 0.9963 | 4 | 97 ± 14 | 22 ± 5 |
| PCB 118 | 1–60 | 0.9976 | 3 | 99 ± 27 | 32 ± 8 |
| PCB 153 | 1–60 | 0.9990 | 2 | 98 ± 35 | 30 ± 1 |
| PCB 138 | 2–60 | 0.9987 | 2 | 105 ± 26 | 27 ± 3 |
| PCB 180 | 2–60 | 0.9974 | 4 | 114 ± 30 | 37 ± 4 |

[a] Calculated by 3 × $s_{y/x}$/calibration curve slope, with ($s_{y/x} = [\sum(y_i - y_{i\text{calc}})^2/(n-2)]^{1/2}$, where $y_i$ denotes the experimental values and $y_{i\text{calc}}$ is calculated from the calibration curve).
[b] Averaging the three standard levels spiking on the four water samples.
[c] Averaging the two levels studied.

In the present case, precision was evaluated by intermediate precision, varying three factors: standard solutions, SPME fibre and time (coefficient of variation of at least six assays performed in three different days with a different fibre in 50% of the analysis). In addition, two concentration levels were studied: 40 and 4 ng/L for PCBs and 64 and 6 ng/L for chlordanes. The results obtained are presented in table 1 and are reasonable, taking into consideration the low levels in study and that intermediate precision varying three factors can be considered a good estimate of the overall precision in laboratory routine conditions.

**3.2.4. Accuracy or bias.** The accuracy of an analytical method is usually determined by the study of relevant certified reference materials (CRM), by participation in proficiency testing schemes, or by spiking. Using spiking experiments, accuracy can be expressed as analytical recovery and should be shown to be negligible or corrected for, but in either case, the uncertainty associated with the determination of the bias remains an essential component of overall uncertainty.

The recovery for a particular sample, $R$, can be considered as comprising three components [24]:

1. $\overline{R}_m$: an estimate of the mean method recovery obtained, for example, by the analysis of a CRM or a spiked sample. The uncertainty, $U_{\overline{R}_m}$, is composed of the uncertainty in the reference value and the uncertainty in the observed value.
2. $\overline{R}_s$: a correction factor that accounts for differences in the recovery for a particular sample compared with the recovery observed for the material used to estimate $\overline{R}_m$. This factor can be evaluated by analysing a representative of spiked samples and concentrations.
3. $\overline{R}_{rep}$: a correction factor to take account of the fact that a spiked sample may behave differently to a real sample with an incurred analyte. This factor is usually assumed to be equal to 1.

The estimate of the recovery for a particular sample is obtained by combining the three factors multiplicatively ($R = \overline{R}_m \times \overline{R}_s \times \overline{R}_{rep}$). In this particular case, $R$ was calculated, assuming that $\overline{R}_s$ and $\overline{R}_{rep}$ equal 1.

Accuracy was expressed as the percentage of recovery, obtained by the addition of three standard levels, in triplicate, to four different drinking water samples (two collected before the WTP and two after). The results are comparable with those obtained by other authors [16, 18, 19] using similar techniques, and are presented in table 1.

### 3.3. *Assessment of global uncertainty*

To date, there are several proposals and different approaches for estimating measurement uncertainty. The main approaches are the 'bottom-up' and 'top-down' methods. The former was proposed by ISO, adopted subsequently in 1995 by EURACHEM, and assesses each individual uncertainty for every single step of the measurement process. The 'top-down' approach uses validation and proficiency testing schemes data [25] to estimate the uncertainty of the method. This method has the disadvantage of not providing information about the variation of uncertainty and, thus, not allowing corrective actions on critical steps. It also depends on the availability of interlaboratory information which is often not available, mainly in research laboratories.

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In present study, the general statistical procedure described in the second edition of the EURACHEM/CITAC guide [2] was followed and adjusted to evaluate the global uncertainty associated with the analytical results. In that edition, a validation-based method for estimating measurement uncertainty is proposed, based on inter- or intra-laboratory validation studies as an extension of the method validation work.

The basic steps undertaken for calculating the measurement uncertainty were: specification (modeling the measurement process), identification (identifying sources of uncertainty), quantification (calculating the standard uncertainty), and combination (calculating the combined standard uncertainty).

The function used to calculate the final concentration in our analytical method is:

$$\text{Concentration } (X_0) = [(\bar{y}_0 - a)/b]/R, \tag{1}$$

where $\bar{y}_0$ is the average chromatographic peak area, $a$ is the calibration regression intercept, $b$ is the slope, and $R$ is the average recovery obtained during method validation. Although other contributing factors exist, the four main individual sources of uncertainty were taken into account:

(i) Uncertainty associated with standard preparation ($U_1 = u_{\text{st}}$):

$$u_{\text{st}} = \left[\sum (\Delta m_i/m_i)^2\right]^{1/2} \tag{2}$$

where $\Delta m_i$ denotes the uncertainty associated with the measure of a certain parameter, such as reagent weighing; and $m_i$ is the measured value in each of those actions.

(ii) Uncertainty associated with the calibration curve ($U_2 = s_{x_0}/x_0$):

$$s_{x_0} = (s_{y/x}/b)\left\{(1/m) + (1/n) + \left[(y_0 - y_{\text{av}})^2/b^2.\sum (x_i - x_{\text{av}})^2\right]\right\}^{1/2} \tag{3}$$

where: $s_{x_0}$ is the standard deviation of the concentration, calculated from the calibration curve; $x_0$ is the concentration calculated from the calibration curve; $s_{y/x} = [\sum(y_i - y_{i\text{calc}})^2/(n-2)]^{1/2}$, $y_i$ is the experimental value, $y_{i\text{calc}}$ is calculated from the calibration curve; $b$ is the slope of the calibration curve; $m$ represents the experimental values obtained for each $x$ value; $n$ is the number of experimental points to build the trend line; $y_0$ is the experimental value of $y$ for which the concentration $x_0$ will be calculated by the calibration curve; $y_{\text{av}}$ is the average of the $y_i$ values; $x_i$ is the concentration of the standards ($x$) used in the calibration; and $x_{\text{av}}$ is the average of the $x_i$ values.

(iii) Uncertainty associated with precision ($U_3 = u_p/x_0$):

$$u_{\text{p}} = s/n^{1/2} \tag{4}$$

where: $s$ is the standard deviation of the experimental data for precision; and $n$ is the number of assays.

Under certain conditions, it may be possible to use a single uncertainty estimate that covers all the samples types and concentrations specified in the method scope [24]. If standard deviations obtained for each representative

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sample are not significantly different, they can be pooled to give a single estimate of precision, using the following equation:

$$S_{\text{pool}} = (((n_1 - 1)S_1^2 + (n_2 - 1)S_2^2 + \cdots)/((n_1 - 1) + (n_2 - 1) + \cdots))^{1/2} \quad (5)$$

where $S_1$ is the standard deviation for matrix 1, and $n_1$ is the number of replicates for matrix 1.

Also, if there are no significant differences between the relative standard deviations obtained for different concentration levels studied, this indicates that the precision is proportional to the analyte concentration, and the relative standard deviation can be pooled to give a single estimate using the equation:

$$\text{RSD}_{\text{pool}} = (((n_1 - 1)\text{RSD}_1^2 + (n_2 - 1)\text{RSD}_2^2 + \cdots)/((n_1 - 1) + (n_2 - 1) + \cdots))^{1/2} \quad (6)$$

However, in most cases, the precision is not proportional to concentration over the entire range specified in the method scope, and it may be necessary to calculate separate uncertainty estimates.

Presently, one of the two standard deviation values obtained during precision studies was used, depending on the calibration standard analysed. For example, for standards ranging from 1 to 10 ng/L (PCBs), the precision estimate calculated with lower concentration standard was used (see 3.2.3).

(iv) Uncertainty associated with accuracy ($U_4 = u_e$):

$$u_e = s(\eta)/n^{1/2} \quad (7)$$

where $s(\eta)$ is the relative standard deviation of the recovery; and $n$ is the number of assays.

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The experiments required to evaluate the method recovery and its uncertainty will depend also on the scope of the method and the availability of suitable CRMs or interlaboratory testing data. In the present work, none of these were available, and so the recovery was studied using spiked samples.

Under optimal circumstances, when estimating $\overline{R}_m$ and $U_{\overline{R}_m}$ by spiking a 'blank sample', at least 10 portions of the bulk spiked sample should be analysed. However, not always this situation is possible due to the analysis time.

Assuming $\overline{R}_m$ and $U_{\overline{R}_m}$ have been obtained, three possible cases arise:

1. $\overline{R}_m$ is not significantly different from 1, and results are not corrected for $\overline{R}_m$;
2. $\overline{R}_m$ is significantly different from 1, and results are corrected for $\overline{R}_m$;
3. $\overline{R}_m$ is significantly different from 1, but results are not corrected for $\overline{R}_m$.

The significance test can be made by calculating $t$ as $t = (|1 - \overline{R}_m|/u(\overline{R}_m))$ and the $t$ value obtained must be compared with $t_{\text{crit}}$ (two-tailed, 95%, $n - 1$).

In the present situation, more of the obtained recoveries ($\overline{R}_m$) was found to be different from 1, and no correction to the results was made.

Global uncertainty ($U$) is then calculated by the following expression:

$$U = (U_1^2 + U_2^2 + U_3^2 + U_4^2)^{1/2} \quad (8)$$

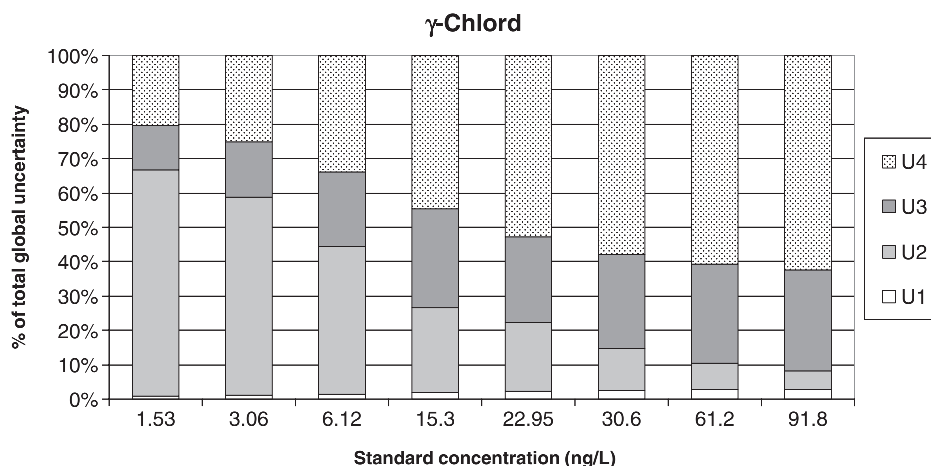


Figure 4. Contribution of each of the four uncertainties components to the global uncertainty of each calibration standard: $\gamma$-chlordane.

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Although this is the combined uncertainty, final results should be stated, unless otherwise required, with an expanded uncertainty, calculated using a coverage factor ($k$) of 2. This gives a level of confidence of approximately 95% [2].

As shown in figure 4, the contribution of each of the individual sources to the global uncertainty associated with the final result is highly dependent on the concentration level.

The main contribution for global uncertainty at the lower concentrations came from the uncertainty associated with the calibration curve, whereas at the higher concentrations, it was the uncertainty associated with accuracy assessment. This pattern observed for chlordane was identical for all the compounds studied, with similar relative contributions of each of the four uncertainty sources in all cases. In this case, the uncertainty associated with the analytical results can be globally lowered by using an internal standard. The use of an internal standard can, in most situations, lower the variability of the chromatographic data and, consequently, the uncertainty coming from the calibration regression; in addition, it can lower the uncertainty in the method recovery assessment.

As expected, the minor contribution to global uncertainty was always standard preparation, with values not higher than 5% of the global uncertainty. The results obtained in relation to the relative contribution of the uncertainty associated with accuracy assessment stress the need for a budget of recovery and precision calculation and associated uncertainties, as complete as possible, when analysing water contaminants at the ultra-trace levels. Moreover, because the method used for the uncertainty assessment may overlook some of the uncertainty sources, and absolute uncertainty can only be assessed if certified reference materials are used [26], these results point to the need to use adequate certified reference materials to improve the results' traceability.

As shown in figures 5 and 6, relative global uncertainty is highly dependent on the concentration below a certain value: *ca* 20 ng/L for PCBs and 30 ng/L for chlordanes. Above these values, the relative global uncertainty was constant and ranged from 11% (PCB 28) to 38% (PCB 153); chlordanes were present between those limits, and, providing the samples matrices are not significantly different from those used for the

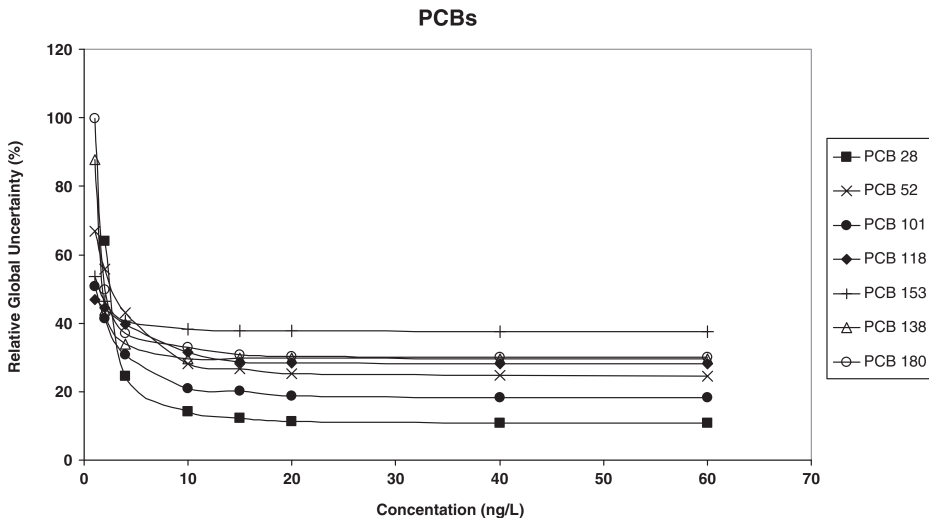


Figure 5. Relative global uncertainty associated with the quantification of each PCB.

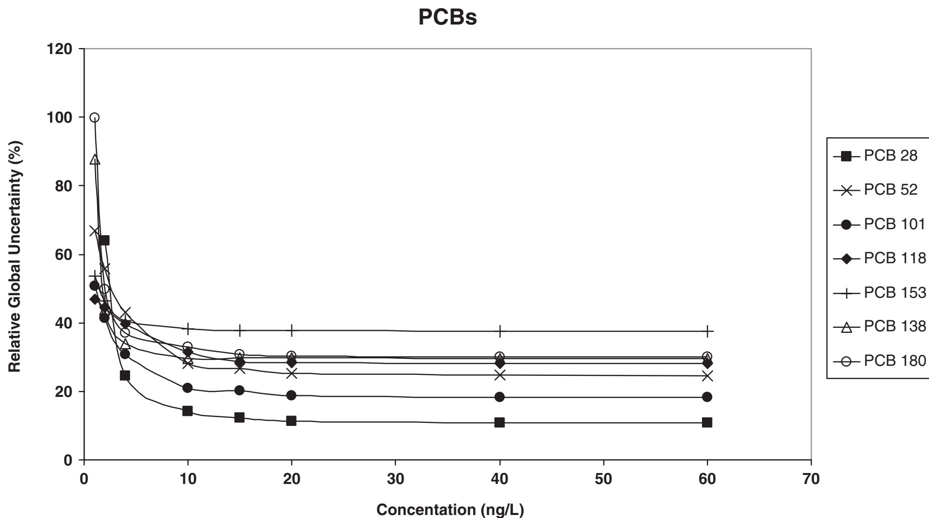


Figure 6. Relative global uncertainty (%) associated with the quantification of each chlordane (■: $\gamma$-chlordane; ×: $\alpha$-chlordane).

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method validation, these values can be used for calculating the uncertainty associated with the measurement results of new samples. The results obtained relating the main contributors to global uncertainty (recovery and calibration) and their values are accordingly to those already reported by other authors using similar analytical methods or target analytes, although some of them use different uncertainty assessment budgets [27–30].

As concentration values approach the analytical detection limit, relative global uncertainties rise to more than 100% in certain cases. This stresses the need for researchers to report their data on the incidence of some important contaminants

such as PCBs and organochlorine pesticides, together with the uncertainty associated with the results, in order to enhance the comparability of such data, especially when close to the methods detection limits. If legal limits are set, as for the presence of chlordanes in drinking water by European Union (100 ng/L), uncertainty evaluation gains extra importance. Independently of the analytical method used, it is only possible to report a positive sample when the obtained concentration minus the expanded uncertainty is higher than the legal limit.

## 4. Conclusions

Solid-phase microextraction prior to GC-MS/MS of the selected PCBs and chlordanes proved to be a suitable technique for fast extraction and analysis of drinking water samples, with acceptable global uncertainties at ng/L levels. However, when concentrations approach the detection limits of the analytical method, assessed global uncertainties increase and may represent more than 100% of the stated value. Therefore, in order to report important environmental data the assessment of the associated global uncertainty is required. Such uncertainty does not imply the absence of validity of the measurement but, on the contrary, implies increased confidence in its validity.

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