

ESTIMATING THE UNCERTAINTY RELATED TO GC-MS ANALYSIS OF ORGANO-CHLORINATED PESTICIDES FROM WATER

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Abstract

The objective of this study was to assess the importance of estimating the uncertainty related to the results of analysis of water samples containing organo-chlorinated pesticides such as: aldrin ($C_{12}H_8Cl_6$), endrin ($C_{12}H_8Cl_6O$), *p,p'*-dichlorodiphenyltrichlorethane ($C_{14}H_9Cl_5$), and γ -hexachlorocyclohexane ($C_6H_6Cl_6$). The measurements were made using the gas chromatography – mass spectrometry (GC-MS) technique. In order to simplify the calculations and to stress the contribution of the uncertainty of each phase in the analytical procedure to the overall uncertainty, the present study refers exclusively to waters that do not contain dissolved fraction, while the contribution of the staff and the environmental factors to the uncertainty are not taken into consideration. Nevertheless, the steps for water sampling, for the solvent extraction and rotary evaporator concentration, for preparing the standard solutions and plotting the calibration graph, were taken into consideration.

Rezumat

Obiectivul acestui studiu a fost evaluarea importanței estimării incertitudinii asociate rezultatului analizei unor pesticide organo-clorurate din apă: aldrin ($C_{12}H_8Cl_6$), endrin ($C_{12}H_8Cl_6O$), *p,p'*-diclor-difenil-triclorețan ($C_{14}H_9Cl_5$) și γ -hexaclorociclohexan ($C_6H_6Cl_6$). Determinările au fost efectuate utilizând tehnica gaz-cromatografie cuplată cu spectrometrie de masă. Pentru simplificarea modului de calcul și pentru o mai bună evidențiere a contribuțiilor incertitudinii fiecărei etape din procedura analitică la incertitudinea totală, studiul se referă numai la cazul apelor fără fracție dizolvată, iar contribuțiile personalului și factorilor de mediu la incertitudine sunt neglijate. S-au avut în vedere etapele de prelevare a probei de apă, extracția cu solvent și concentrarea la rotaevaporator, prepararea soluțiilor de etalonare și trasarea curbei de etalonare.

Keywords: gas chromatography - mass spectrometry (GC-MS); uncertainty; organo-chlorinated pesticides; water.

Introduction

Analysts are aware that no experimental measurement can ever be perfect. Quantitatively it will mean that the result of an individual measurement of a certain quantity consists of the measured value itself plus an error. The factors “error” and “uncertainty” are interchangeable. Quite often, the quantity of the error is associated with a particular measurement method, since it is obvious that certain measurement methods give less errors than others. The capacity to determine the uncertainty of an experiment plays an important role in understanding and reporting the results of that experiment. It is necessary for an analyst to know how to determine the uncertainty, both in order to be able to interpret the measurements conducted by others and to conduct new measurements in the future.

According to the definition, the uncertainty of measurement is a parameter associated with the result of a measurement and characterizes the dispersion of values that can reasonably be attributed to the measurand [1, 8]. The information describing in detail the estimation and evaluation of an uncertainty associated with a result can be found in the SR ENV 13005:2003 Guide, and in the EURACHEM/CITAC Guide CG4 [5, 7], specifically for chemical analyses. The estimation of the uncertainty of measurement, together with developing a method and determining the other key parameters of performance, is an important step in the procedure of validation of a quantitative method (in which a numeric value for a measurand is being determined). The estimation of uncertainty is a general method of quality assurance in the laboratory. According to SR EN ISO/CEI 17025:2005, all certified laboratories must possess and apply procedures for the estimation of the uncertainty of the measurement [4].

The following practical reasons justify the determination of the uncertainty of the measurement:

- the uncertainty of a result is a quantitative indicator of its quality. The true value of a result is given by the level of trust one can place within it.
- the expression of the uncertainty of a result warrants the comparison of results obtained in different laboratories, or the results obtained in the same laboratory, or with the standard values cited in the literature; this information can often prevent the unproductive repetition of tests.
- the uncertainty of the result of a test may need to be taken into consideration by a person when interpreting the data.
- a consideration of the components of an uncertainty highlights those aspects of a test, upon which attention must be concentrated in order to constantly improve the procedure.

The present study was elaborated in consideration of the last reason

mentioned above, that is to identify the components of an uncertainty and to focus attention upon those components that can lead to the improvement of the performances of a method. It is a well known fact that the global uncertainty of the method used for the determination of organo-chlorinated pesticides in water is given by the uncertainty due to the processing of the sample (sampling and processing), the uncertainty due to the method of preparing the standard solutions and the uncertainty associated with plotting the calibration graph. The data obtained during the development of the method and its *in-house* validation as well as from the quality control of the results, were used for the estimation of the uncertainty of measurement when, by the gas chromatography method coupled with mass spectrometry, organo-chlorinated pesticides (aldrin, endrin, *p,p'*-DDT – dichloro diphenyl trichlorethane, and γ -HCH – hexa chloro cyclohexane) were determined.

The study intended to quantify the pesticide content and estimate the incertitude. Due to their oleophilic properties and high persistence, these pesticides have the tendency to bio-accumulate in food. Holding a priority position on the list of toxic xenobiotics, the use of pesticides in Romania (Order No. 161/2006) as well as in most of the other countries in the world, has been banned [6]. Residues and metabolites of these compounds can still be determined in samples taken from the environment or food [3].

Materials and Methods

An Agilent Technologies 6890-type gas chromatograph, coupled with a MSD 5973 type detector were used for the analysis of pesticides. A capillary column HP-5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m) was used for the separation of components, while for the analysis the SIM-acquisition mode was applied (SIM – selective ion monitoring). The general procedure is schematically represented in Figure 1.

Standard material certified by Supelco was employed for the calibration of the instrument. The certified purities for each component were: aldrin 96.8%, endrin 99.1%, *p,p'*-DDT 98.2%, and γ -HCH 99.7%. Standard solutions of different concentrations, prepared in volumetric flasks with adequate pipettes, were used for calibration (Figure 2).

The content of organo-chlorinated pesticides in water was calculated according to the following equation:

$$\text{Organo-chlorinated pesticides [mg/L]} = C \cdot \frac{V \times 100}{V_a \times R \times 100}$$

where: C – concentration given by the instrument [ng/ μ L]; V – volume of petroleum ether used for redissolving the sample concentrate [mL]; V_a – volume of water sample analyzed [mL]; R – pesticides' recovery coefficient [%].

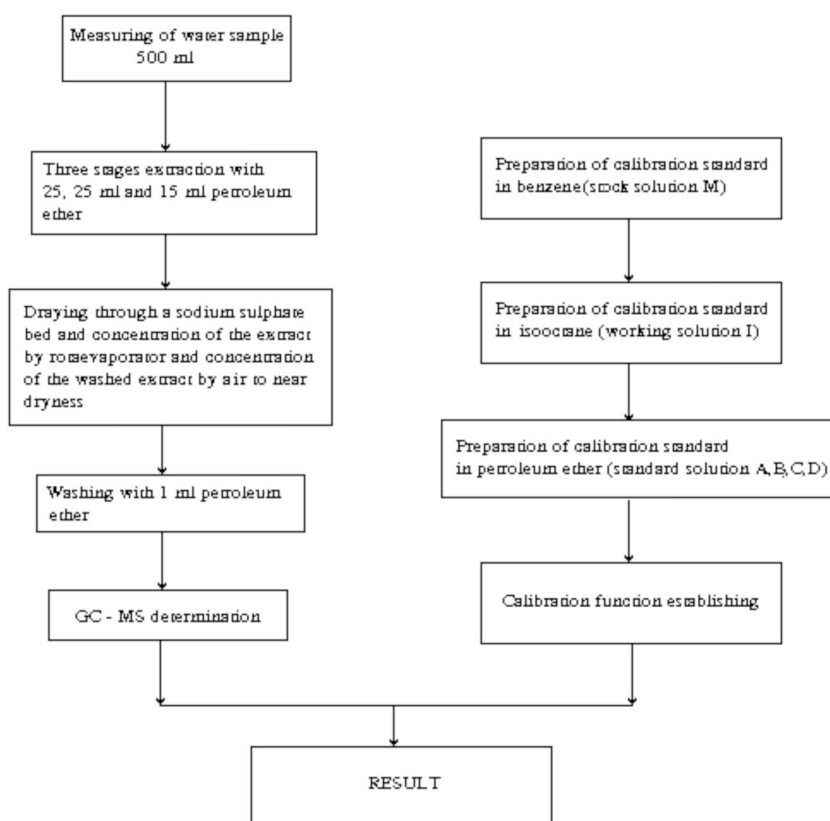


Figure 1
Flux diagram of pesticides analysis in water.

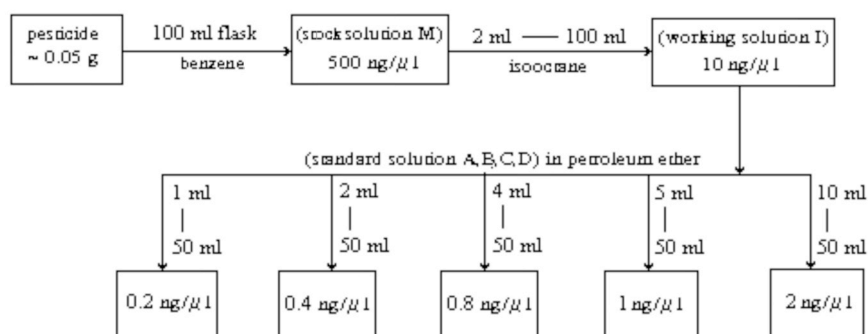


Figure 2
Preparation of standard solutions.

Results and Discussion

In order to estimate the uncertainty, we registered data regarding the quantification of the uncertainty associated to each individual source as well as data resulting from the study of validation. It was supposed that the uncertainty due to any difference between the measurement response on comparative marker and the analyte in the sample was small as compared to the overall uncertainty of the result [4].

The following steps were carried out in estimating the uncertainty: specification of the measurand, identification and analysis of the sources of uncertainty, quantification of the same, calculating the combined uncertainty.

Taking into account the extensive analysis method, the measurand was best specified by describing the different steps of the analytical method and by determining the equation of the measurand. For this specific case human error, the environmental conditions during transport and storage and the non-homogeneity of the sample were not taken into consideration as possible sources of uncertainty. The uncertainty was considered the result of several factors: sample processing, the recovery process, the preparation of the standard solutions and the uncertainty associated with the plotting of the calibration curve. The identification of all possible sources of uncertainty for this complex analytical procedure was performed by plotting Ishikawa's cause-effect diagram (Figure 3). The parameters of the equation were represented by the main lines of the diagram. Then, other factors were added to the diagram, considering each phase of the analytical procedure, until the contributing factors became sufficiently vague.

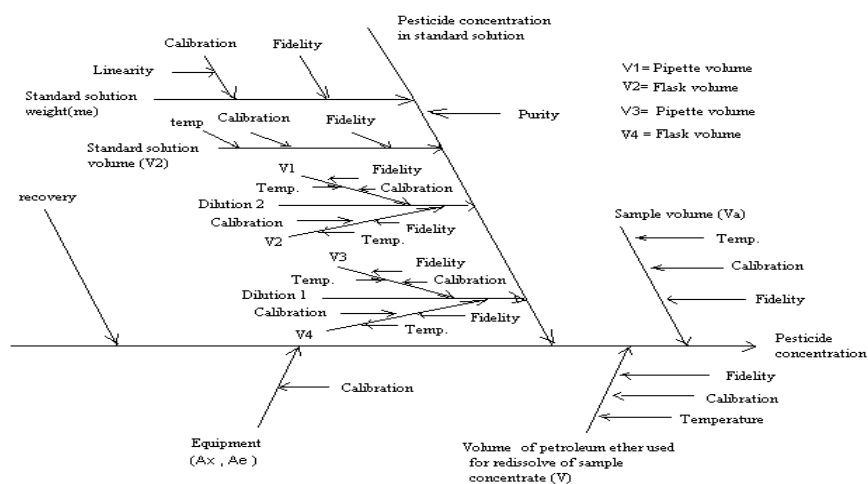


Figure 3
Ishikawa's cause-effect diagram.

Quantification of the uncertainty sources

Uncertainty due to the preparation of standard solutions

The uncertainty associated with the volumetric glassware has three components: one resulting from the calibration, one from the repeatability of filling and one due to the difference in temperature between the calibration temperature and the room temperature in the laboratory. The deviation value from the nominal volume of the glassware is given by the manufacturer as $\pm A(\text{mL})$ without any further information. Consequently, a triangular distribution is supposed, that allows calculation of the standard uncertainty value dividing the tolerance by $\sqrt{6}$. The manufacturer has calibrated the glassware at a temperature of 20°C , while the temperature in the laboratory may vary with $\pm 4^{\circ}\text{C}$. The uncertainty due to this effect was calculated by estimating the temperature range and taking into account the coefficient of volume expansion:

$$u_{\text{vol}} = \Delta t \times \delta \times V$$

where:

u_{vol} – volume's incertitude due to temperature difference [mL];

Δt – difference between working temperature and calibration temperature of glassware [$^{\circ}\text{C}$];

δ – volume's expansion coefficient [$^{\circ}\text{C}^{-1}$];

V – measured volume [mL].

Since the volume of a liquid expands much more than the volume of a glassware, only the former was taken into consideration. The volume expansion coefficient for water is $2.1 \times 10^{-4} \text{ }^{\circ}\text{C}^{-1}$, for petroleum ether is $0.9 \times 10^{-3} \text{ }^{\circ}\text{C}^{-1}$, for benzene is $1.18 \times 10^{-3} \text{ }^{\circ}\text{C}^{-1}$, and for *iso*-octane is $1.06 \times 10^{-3} \text{ }^{\circ}\text{C}^{-1}$.

The volume variation given by the filling repeatability was determined by carrying out a filling test for each type of glassware used. The standard deviation of repeatability after ten fillings with water to the mark and weighing of these volumes was taken as such, without further calculations, since it was already calculated as standard deviation.

Table I represents a synthesis of the data obtained and shows the combined uncertainty of the glassware used for sampling the water to be analyzed and the glassware used to prepare the standard solutions.

The uncertainty associated with the analyte used for the preparation of the standard solutions has three sources of uncertainty: the balance sensitivity, the linearity and the repeatability of the measurement. The balance sensitivity was not taken into account since measurements were made on the same balance by difference and on a short interval, whereas for linearity the manufacturer's data indicate a value of $\pm 0.2 \text{ mg}$. The uncertainty is evaluated by supposing a

rectangular distribution, in order to convert the linearity contribution into standard uncertainty, which means that the indicated value is divided by $\sqrt{3}$.

Table I

The combined uncertainty of the volumetric glassware used for the preparation of standards

Nominal volume [mL]		Temperature influence	Calibration	Repeatability	Combined uncertainty [mL]
		<i>Standard uncertainty</i> [mL]	<i>Standard uncertainty</i> [mL]	<i>Standard uncertainty</i> [mL]	
V_1	2	0.0055	0.0041	0.008	0.011
V_2	100	0.245	0.041	0.08	0.261
V_3	10	0.0245	0.02	0.046	0.056
V_4	50	0.104	0.025	0.06	0.122

V_1, V_2, V_3, V_4 – volumes used for the preparation of standard solution [mL].

The contribution of linearity was taken into consideration twice in calculating standard uncertainty, once for the tare and once for the mass of the standard. Table II represents a synthesis of the data obtained and shows the combined uncertainty that resulted from weighing the mass of standard.

Table II

The combined uncertainty resulting from weighing the mass of standard

	Nominal mass [g]	Linearity		Repeatability		Combined uncertainty [mg]
		<i>Tolerance</i>	<i>Standard uncertainty</i> [g]	<i>Mass</i> [g]	<i>Standard uncertainty</i> [g]	
<i>Standard mass</i>	0.05	0.2	0.12	0.00527	0.00527	0.17

Since no information about uncertainty was given by the supplier in the analysis certificate for the standard matter used for the preparation of the standard solutions, purity was not taken into consideration as a potential source of uncertainty. Still, the purity of standard was taken into consideration when the concentration was calculated. The uncertainty given by the volumes and the mass of pesticide was combined in order to give the standard uncertainty of the standard solutions used for the calibration of the instrument. The uncertainty resulting from the preparation of the standard solution was determined from the relation used for the calculation of the concentration and the Kragten's method (Table III):

$$C = 10^6 \times \frac{mV_1V_3}{V_2V_4}$$

where: C – concentration of the standard solution [ng/μL]; m – mass of standard compound [g]; V_1, V_2, V_3, V_4 – volumes used for the preparation of the standard solution [mL].

Table III

Kragten's diagram used for the calculation of uncertainty associated with the preparation of the endrin standard solution, at a concentration of 0.2 ng/ μ L

No.	A	B	C	D	E	F	G
1.			m [g]	V_1 [mL]	V_2 [mL]	V_3 [mL]	V_4 [mL]
2.	Source	Value	0.05	2	100	1	50
3.		Uncertainty	0.00017	0.011	0.261	0.056	0.122
4.	m [g]	0.05	0.05017	0.05	0.05	0.05	0.05
5.	V_1 [mL]	2	2	2.0011	2	2	2
6.	V_2 [mL]	100	100	100	100.261	100	100
7.	V_3 [mL]	1	1	1	1	1.056	1
8.	V_4 [mL]	50	50	50	50	50	50.122
9.							
9.	C [ng/ μ L]	0.2	0.20068	0.20011	0.19896	0.2112	0.199513
10.	$u(y, x_i)$	–	0.00068	0.00011	-0.00104	0.0112	-0.00049
11.	$u(y)^2, u(y, x_i)^2$	0.00012723	4.624E-07	1.21E-08	1.08E-06	0.000125	2.37E-07
12.	$u_{c\text{ prep et}}(c)$	0.01127976	–	–	–	–	–

m – mass of standard compound [g]; V_1, V_2, V_3, V_4 – volumes used for the preparation of standard solution [mL]; C – concentration of the standard solution [ng/ μ L]; $u(y, x_i)$ – differences of cells C9–G9 minus the value given in B9-cell; $u(y)^2, u(y, x_i)^2$ – values of cells C10–G10 are squared and summed to give the value shown in B11-cell; $u_{c\text{ prep et}}(c)$ – combined standard uncertainty, which is the square root of B11-cell.

The values of volume V_3 were replaced gradually in the formula for the calculation of concentration and in the Kragten's diagram. Thus, the standard compound uncertainties of all the calibration solutions were obtained and they were further combined to get to the determination of the uncertainty resulting from the phase of calibration solutions' preparation (Table IV).

Table IV

Combined standard uncertainty of the calibration solutions

	Concentration [ng/ μ L]					$u_{\text{prep et}}(c)$
	0.2	0.4	0.8	1	2	
$u_{c\text{ prep et}}(c)$	0.0113	0.0115	0.0124	0.0130	0.0174	0.0298
RSD%	5.64	2.88	1.55	1.30	0.87	–

$u_{\text{prep et}}(c)$ – uncertainty associated with preparation of the calibration solutions [ng/ μ L]; $u_{c\text{ prep et}}(c)$ – combined standard uncertainties for the preparation of calibration solutions, at different concentrations [ng/ μ L]; RSD% – relative standard deviation [%].

Uncertainty resulting from sampling and processing $u_c(V)$

In calculating this type of uncertainty, only the uncertainty associated with the volumetric glassware was taken into consideration. The uncertainty associated with the operator or the concentration of the sample could not be

evaluated. Table V shows the combined uncertainty that resulted from sampling and processing the water samples to be analyzed.

Table V

Combined standard uncertainty of the volumetric glassware used for the sample processing

Nominal volume [mL]	Temperature influence	Calibration	Repeatability	Combined uncertainty [mL]
V	2	0.004	0.004	0.008
V_a	500	0.242	2.041	2.38
$u_c(V)$	–	–	–	2.38

V – volume of pipette [mL]; V_a – volume of cylinder [mL]; $u_c(V)$ – combined standard uncertainty of the volumetric glassware used for the sample processing [mL].

Uncertainty resulting from recovery

The standard relative uncertainty associated with recovery was calculated from the values obtained when the average recovery for each analyte was determined on enriched distilled water samples, while the validation study of the method was carried out (Table VI).

Table VI

Standard relative uncertainty associated with recovery

Analyte	Aldrin	Endrin	<i>p,p'</i> -DDT	γ -HCH
$\bar{R}$ [%]	92	83	97	85
$u(R)$ [%]	1.61	4.28	11.59	6.66
$u(R)/R$	0.0170	0.0515	0.1195	0.0783

$\bar{R}$ – average recovery of every analyte [%]; $u(R)$ – standard uncertainty [%]; $u(R)/R$ – relative standard uncertainty.

Uncertainty associated with the calibration of measuring equipment

From the study of method validation, it resulted that the equipment calibration is best performed by plotting some linear calibration curves. In order to determine the uncertainty associated with the plotting of the linear calibration curve, the uncertainty of the values on the X-axis was considered by far lower than that of the values on the Y-axis. Therefore, the uncertainty associated with the plotting of the calibration curve does not reflect either the uncertainty of standards or the correlations introduced by the successive dilutions of the same stock solution. The calibration curve used for the calibration of the equipment is described by the following equation:

$$Y_j = A + BC_i$$

where: Y_j – the “j” measurement of the calibration signal “i” [Abs]; A – intercept [ng/μL]; B – slope of curve; C_i – concentration of the calibration solution “i” [ng/μL].

Starting from the results obtained after plotting the calibration curves in the phase of method validation, a series of parameters (Table VII), which are important for the calculation of the uncertainty associated with the calibration curve $u(C_0)$, were calculated: the slope (B), the intercept with the X-axis (A), the standard deviation of the slope (S_A), the standard deviation of the intercept (S_B), and the standard residual deviation of the regression curve (S_0).

Table VII

Values of A , B , S_A , S_B , and S_0 , resulted from plotting the calibration curves

Analyte	Calculated parameter				
	A	B	S_A	S_B	S_0
Aldrin	-151.2	93695	847	858.5	1387.9
Endrin	-761.9	21076.8	616.7	625.1	1010.5
p,p' -DDT	248.3	35597.4	528.4	871.1	722.5
γ -HCH	-1735.7	47344.1	1810.3	1835	2966.4

The uncertainty associated with the linear calibration curve $u(C_0)$, was calculated according to the equation [2]:

$$u_{\text{reg}}(C_0) = \frac{S_0}{B} \sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(Y_j - \bar{Y})^2}{B^2 \sum_{i=1}^n (C_i - \bar{C})^2}}$$

where:

$u_{\text{reg}}(C_0)$ – uncertainty associated with the linear calibration curve;

S_0 – residual standard deviation of calibration curve;

B – slope of curve;

p – number of repeated samples (unknown sample or standard);

n – number of calibration solutions, for plotting the calibration curve;

Y_j – the measured value of the unknown sample signal;

$\bar{Y}$ – average value of the signals of calibration solutions;

C_i – measured value of the unknown sample concentration [ng/ μ L];

$\bar{C}$ – average value of the calibration solutions concentration [ng/ μ L].

Taking the above relation as starting point, for each analyte and for each concentration of the calibration solutions the uncertainty associated with the calibration curve $u_{\text{reg}}(C_0)$ was calculated (Table VIII).

Table VIII
Uncertainty associated with the calibration curve $u_{\text{reg}}(C_0)$

C_0 [ng/μL]	γ -HCH		Aldrin		Endrin		p,p' -DDT	
	$u_{\text{reg}}(C_0)$	RSD%	$u_{\text{reg}}(C_0)$	RSD%	$u_{\text{reg}}(C_0)$	RSD%	$u_{\text{reg}}(C_0)$	RSD%
0.2	0.09109	45.5	0.021333	10.7	0.068829	34.4	0.022778	11.4
0.4	0.077736	19.4	0.018147	4.5	0.058277	14.6	0.02279	5.7
0.8	0.092934	11.6	0.023303	2.9	0.071639	8.9	0.024645	3.1
1	0.103594	10.4	0.025967	2.6	0.08013	8.0	0.025631	2.6
2	0.189849	9.5	0.04476	2.2	0.144918	7.3	—	—

RSD% – relative standard deviation [%].

Combined and extended uncertainty

The combined uncertainty was calculated from the first rule of combination (square root of the sums of squares) of the uncertainty associated with recovery u_R , with sample processing u_V , with the preparation of the calibration solutions $u_{\text{pre et}}$ and with the calibration of the equipment u_{etal} :

$$u_c(C_0) = C_0 \sqrt{\left(\frac{u_R}{R}\right)^2 + \left(\frac{u_V}{V}\right)^2 + \left(\frac{u_{\text{pre et}}(C_0)}{C_0}\right)^2 + \left(\frac{u_{\text{reg}}(C_0)}{C_0}\right)^2}$$

where:

$u_c(C_0)$ – combined uncertainty [ng/μL];

C_0 – concentration [ng/μL];

u_R – uncertainty associated with recovery [%];

R – recovery [%];

u_V – uncertainty associated with sample processing (volume) [mL];

V – volume [mL];

$u_{\text{pre et}}(C_0)$ – uncertainty associated with preparation of the calibration solutions [ng/μL];

$u_{\text{reg}}(C_0)$ – uncertainty associated with the calibration of the equipment [ng/μL].

In order to calculate the extended uncertainty a coverage factor of 2, corresponding to a level of trust of 95% was used.

The values of extended uncertainty for each analyte and at different concentrations are presented in Table IX.

Table IX
Extended uncertainty of analytes at different concentrations

C_0 [ng/μL]	u_{ext} [ng/μL]			
	Aldrin	Endrin	p,p' -DDT	γ -HCH
0.2	0.048	0.141	0.069	0.187
0.4	0.045	0.125	0.108	0.172
0.8	0.059	0.167	0.199	0.234
1	0.068	0.193	0.246	0.272
2	0.119	0.358	—	0.517

u_{ext} – extended uncertainty [ng/μL]; C_0 – concentrations of analyte [ng/μL].

Conclusions

The four sources of uncertainty taken into consideration in this study were the uncertainty associated with recovery, with sample processing, with the preparation of the calibration solutions and with the plotting of the calibration curve of the equipment. The fact that the uncertainty associated with the calibration of an instrument plays the most important part in the overall uncertainty urges us to pay great attention when carrying out an instrumental measurement. Also, it is important to keep under perfect conditions, to check and to calibrate the equipment, as often as necessary.

Of the four measured analytes, the highest extended uncertainty was associated with γ -HCH and endrin. The uncertainty values were in perfect correlation with the higher standard residual deviations obtained for these compounds when plotting the calibration curves.

It has been noticed that the importance of uncertainty for the value of the measured concentration becomes acceptable towards the middle of the calibration interval. Therefore, it is necessary to process the water samples in such a way that, after extraction and concentration, the concentration of the unknown sample will be in this range.

References

1. Brownlee K. A., Statistical theory and methodology in science and engineering, 2nd edition, Krieger Publishing Co., New York, 1984.
2. Pană M., Analytical control of pesticides, PhD Thesis, University of Bucharest, 2006 (in Romanian).
3. Wittkowski R., Matissek R., Capillary gas chromatography in food control and research, Technomic Publishing Company, Pennsylvania, 1993.
4. ***, Cerințe generale pentru competența laboratoarelor de încercări și etalonări, SR EN ISO/CEI 17025:2005.
5. ***, Ghid pentru exprimarea incertitudinii de măsurare, SR ENV 13005:2003.
6. ***, Ordin nr. 161/16 februarie 2006 pentru aprobarea Normativului privind clasificarea calității apelor de suprafață, în vederea stabilirii stării ecologice a corpurilor de apă, Monitorul Oficial, Partea I, nr. 511, 13 iunie 2006.
7. ***, Quantifying uncertainty in analytical measurement, EURACHEM/CITAC Guide CG4, 2nd edition, 2000.
8. ***, Vocabulaire international des termes fondamentaux et généraux de métrologie, ISO, 2e édition, Genève, 1993.

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