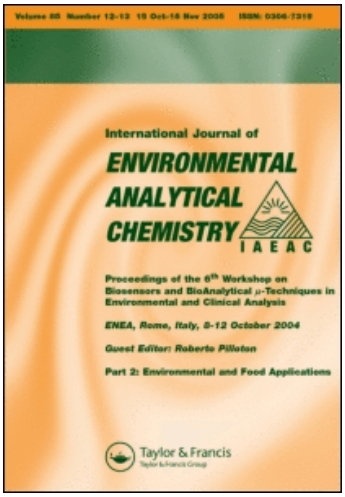

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PREPARATION, HOMOGENEITY AND STABILITY STUDIES OF FREEZE-DRIED WATER CONTAINING PESTICIDES

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The preparation, the homogeneity and the stability of freeze-dried water samples containing pesticides of various chemical groups (Atrazine, Simazine, Fenitrothion, Fenamiphos, Parathion-ethyl, Carbaryl, Permethrin, Linuron and Propanil) was studied. The concentration of pesticides in various batches of water varied from 0.02 to 90 µg/l. All freeze-dried water samples were stored at -20°C, +20°C and +40°C in amber bottles and analyzed after successive storage periods up to one year. Liquid chromatography with diode array and gas chromatography with nitrogen-phosphorus and electron-capture detection were used for the final determination of the pesticides after reconstitution of the water samples and extraction. The results indicate that the stability of the pesticides is dependent on the compound, its concentration in the freeze-dried residue and the storage temperature. All pesticides exhibited a sufficient stability during storage at -20°C. The interlaboratory exercise conducted with these samples gave data for certain compounds (e.g. carbaryl) indicating that the applied freeze-drying procedure of spiked water can be applied for the preparation of a candidate reference materials to be certified for some of the studied pesticides.

KEY WORDS: Reference materials, atrazine, simazine, fenitrothion, fenamiphos, parathion-ethyl, carbaryl, permethrin, linuron, propanil, water, stability, interlaboratory study.

INTRODUCTION

The current generation of pesticides such as organophosphorus, chlorotriazines, pyrethroids and phenylureas as well as other groups, are determined in water matrices in different monitoring programmes, throughout Europe[1–5] and the USA[6–8]. The extraction of the pesticides from various types of waters is usually performed by liquid-liquid extraction (LLE) with dichloromethane[1,5,6], by off-line solid-phase extraction with conventional C18 bonded silica cartridges[4,8], by extraction with Empore disks[9] or by on-line precolumn technology[2–4]. The pesticides in the final extracts are most often determined by gas chromatography with a nitrogen-phosphorus detector (GC-NPD) or mass spectrometric detection (GC-MS)[1,4,6,8] and by liquid chromatography with UV or diode array detection and post-column fluorescence derivatization[2,3,5–7]. One of the major problems in the different monitoring programmes of the current generation of pesticides is the unavailability of certified reference materials to evaluate the performance of the analytical systems. Although water samples containing pesticides were recently distributed in ampoules by the US EPA through different US laboratories[7] for an interlaboratory study, problems such as the instability of the pesticides remain evident.

Very little information is available in literature concerning the increase of the pesticide's stability in aqueous samples. It has been reported that the stability in water is critical for 26 organophosphorus pesticides stored at 4°C for 14 days in biologically inactivated well-water[6]. Certain organonitrogen herbicides[3,10] and carbamate insecticides[5] have also shown instability when stored in similar conditions. Within Europe, the Community Bureau of Reference (BCR) of the European Commission has developed a project for the preparation of candidate water reference materials certified for several families of pesticides; the main results being reported in the present paper. First a procedure for freeze-drying aqueous solutions containing different pesticides was developed and the homogeneity and stability of the freeze-dried samples were studied. The selection of the pesticides was based on their usage and occurrence in Europe[1-5,11] and their differing functional chemical groups. Four batches of freeze-dried samples were prepared. A first batch of river water was freeze-dried after spiking with a pesticide mixture at a level of 11 to 100 ug/l. Since the requirements for monitoring pesticides in drinking water samples are very stringent within Europe, two additional water batches were prepared with pesticide concentrations in the range of 0.02 to 7 ug/l. Glycine was added to all three batches prior to freeze-drying. This glycine acts as a "keeper" substance during lyophilisation and as an adsorbing substrate for the water phase[12] allowing an easier dissolution of the residue when reconstituting the water samples. A further river water spiked with permethrin and simazine without addition of glycine was also prepared for the preliminary studies.

The objectives of the project were: (i) to assess the stability of lyophilized water samples containing selected pesticides at concentrations of 0.02 to 90 µg/l with 6 to 10 g/l glycine at different storage temperatures over one year and the effects of the glycine addition on the preparation and the stability of the samples, (ii) to set up an interlaboratory study with the lyophilized materials among different European laboratories with the ultimate objective to prepare and certify a candidate reference material for its pesticides content.

EXPERIMENTAL SECTION

Sample collection and preparation of freeze-dried water

Batch 1: High spiking level Forty litres of water (Llobregat river, Barcelona) were freeze-dried after addition of glycine up to 4 g/l and enrichment with Atrazine, Cyanazine, Simazine, Fenitrothion, Fenamiphos, Tetrachlorvinphos, Parathion-ethyl and Carbaryl to reach a final concentration of the various compounds of 11 to 110 µg/l. Ten litres of the same water containing 10 g/l glycine were also lyophilised and were used as blank samples. The bulk water residues were homogenized and stored into amber glass bottles each containing 9.9 g freeze-dried residue under an argon atmosphere. The bottles were sealed with a PTFE insert. A total of 5 blank samples were stored at room temperature, 5 bottles containing the pesticide enriched residue were stored at -20°C and 15 at room temperature. These samples were used for preliminary stability studies.

Batch 2: Low-spiking level Two batches (2A and 2B) of 150 l drinking water were spiked with Atrazine, Simazine, Fenitrothion, Fenamiphos, Parathion-ethyl, Carbaryl, Permethrin, Linuron and Propanil in order to reach a final concentration of 0.2 to 7 µg/l for each pesticide. Glycine was added at a concentration of 6 g/l. A control blank was

prepared by freeze-drying 40 l of the same drinking water with 6 g/l glycine but without any addition of pesticides. After freeze-drying, the residue of each batch was homogenized for two hours. Thirty amber glass bottles with PTFE inserts were filled with approx. 2.5 g residue. The remaining bulk material was rehomogenised for 10 min and an additional batch of thirty bottles was filled. Two bottles out of each batch were set aside for the homogeneity studies. This procedure was repeated until 150 bottles of each batch 2A and 2B were filled. The Institute of Freshwater Ecology (Dorset) studied the homogeneity for permethrin, and the Department of Environmental Chemistry (Barcelona) studied the homogeneity of the remaining pesticides. The remaining bottles from batch 2A and 2B were subsequently used in the interlaboratory exercise involving 20 European laboratories and also served for the control of the stability of the samples over the entire duration of this exercise.

Batch 3: Without glycine addition Fifty litres of freshwater (From River Dorset) were spiked with permethrin and simazine at concentrations of respectively 5 and 52 µg/l. Five litres of the water remained unspiked and were used as blanks. The spiked water and the blank were lyophilized, homogenized and bottled in the dark into amber glass bottles and sealed under a nitrogen atmosphere (0.34 g of material per bottle). They were stored at room temperature. A reference extract of spiked water was prepared at the beginning of the stability study and was stored at -20°C. The pesticides were determined in a portion of this extract at each occasion of analysis during the stability study in order to assure a good long term reproducibility of the analytical method.

Reconstitution of freeze-dried water samples

The objective of the project was to prepare a water reference material. Therefore, the lyophilized samples were reconstituted in 1 l of HPLC grade water of similar high purity water. So the obtained freshwater had an ionic composition near to the original sample. The reconstitution procedure consisted in the addition of a known mass of the lyophilized residue to a known mass of HPLC grade or distilled water and in the mixing in presence of CO_2 (purity > 99.998%) at a flow-rate of less than 10 ml min^{-1}. For soft waters and in the presence of glycine, complete reconstitution was attained in less than 15 min. For the hard water samples, 2 h were needed to achieve a complete dissolution of the calcite solid formed during lyophilization. The final composition of the various reconstituted batches 2 and 3 are given in Table 1. The electrical conductivity at 25°C of

Table 1 Results of the major-ion analysis of the reconstituted samples from batch 2A, 2B and 3. All concentrations are given in nmol/l except for soluble reactive phosphate, (SRP), which is in µmol/l.

	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	NO_3^{-}	*SRP*	SiO_2
Batch 2A							
Mean	0.085	0.019	0.171	0.002	0.013	0.267	0.002
SD (n = 5)	0.008	<0.001	0.001	<0.001	<0.001	0.020	<0.001
Batch 2B							
Mean	0.113	0.025	0.099	0.003	0.014	0.264	0.001
SD (n = 5)	0.001	<0.001	0.001	<0.001	<0.001	0.055	<0.001
Batch 3							
Mean	1.99	0.085	0.440	0.050	0.380	3.46	0.027
SD (n = 4)	0.009	0.006	0.040	0.001	0.006	0.06	0.002

the final solution allows to follow up the performance of the reconstitution procedure. For batch 2A, 2.4 g of residue dissolved in 1 l exhibited a mean conductivity of (63.04 ± 3.11) μS cm^{-1} and 2.6 g residue of batch 2B a value of (65.89 ± 6.48) μS cm^{-1}. These data are comparable to the (436.3 ± 2.84) μS cm^{-1} for 0.34 g of material from batch 3 and amounted to *ca* 90% of the conductivity of the water prior to freeze-drying. The recovery of the major-ions by this procedure was typically over 80%. Other methods of reconstitution such as dissolution in presence of 0.01 $mol.l^{-1}$ HCl was also successful and more convenient although the final water composition was different from the original sample.

Determination of pesticides

Chemicals

HPLC grade water, acetonitrile gradient grade lichrosolv and methanol were passed through a 0.45 μm filter before use. Pesticide standards were purchased from Promochem (Wesel, Germany and St. Albans, UK).

Extraction

Batch 1: high-spiking level Pesticides were extracted from the reconstituted water using liquid-liquid extraction (LLE) into hexane (20 ml) and dichloromethane (2 × 40 ml). After concentration in a rotary evaporator (35°C), the extracts were carefully evaporated to dryness. Methanol was added to yield a final volume of 0.5 ml. The samples were injected onto the liquid chromatography-diode array (LC-DA) system. Three replicate determinations of all pesticides were carried out following a previous reported protocol[1]. An example of the chromatographic analysis of this mixture is shown in Figure 1, with an LC chromatogram of a sample stored at -20°C for 1 month at room temperature and a blank chromatogram.

Batch 2: low spiking level Most of the samples were analyzed by GC-NPD, three of the compounds: Linuron, Carbaryl and Fenamiphos, by LC-DAD and *cis/trans* permethrin by GC-ECD with confirmation by GC-MS. The extraction protocol and GC-NPD analysis was similar to that reported for batch 1 level but the final extract was dissolved in ethyl acetate instead of methanol. Before GC-NPD analysis, the samples were processed using Florisil columns, Supelclean 6 ml (Supelco, Bellefonte, PA, USA) and eluted with a mixture of n-hexane-diethyl ether 50:50 (v:v)[13]. An example of extract of water with and without clean-up is shown in Figure 2; it demonstrates the positive effect of using this clean-up step after LLE for these samples. With the clean-up used, all the compounds were adequately prepared for GC separation. For carbaryl, linuron and fenamiphos an on-line solid-phase disk extraction method coupled to LC-DAD was used[5]. After dissolution of the freeze-dried samples in 1 l of water, 100 ml were preconcentrated and analyzed on-line. An example of chromatogram for this sample is shown in Figure 3. Compounds 2, 5 and 7 correspond respectively to carbaryl, linuron and fenamiphos. Permethrin was determined after extraction of 1 l of sample with 500 mg of an octyl(C8) silica bonded phase extraction column and eluted with 2.5 ml methanol. The solvent finally changed to 5% acetone in hexane before GC-ECD. A confirmation by GC-MS was performed using the 183 and 163 amu ions.

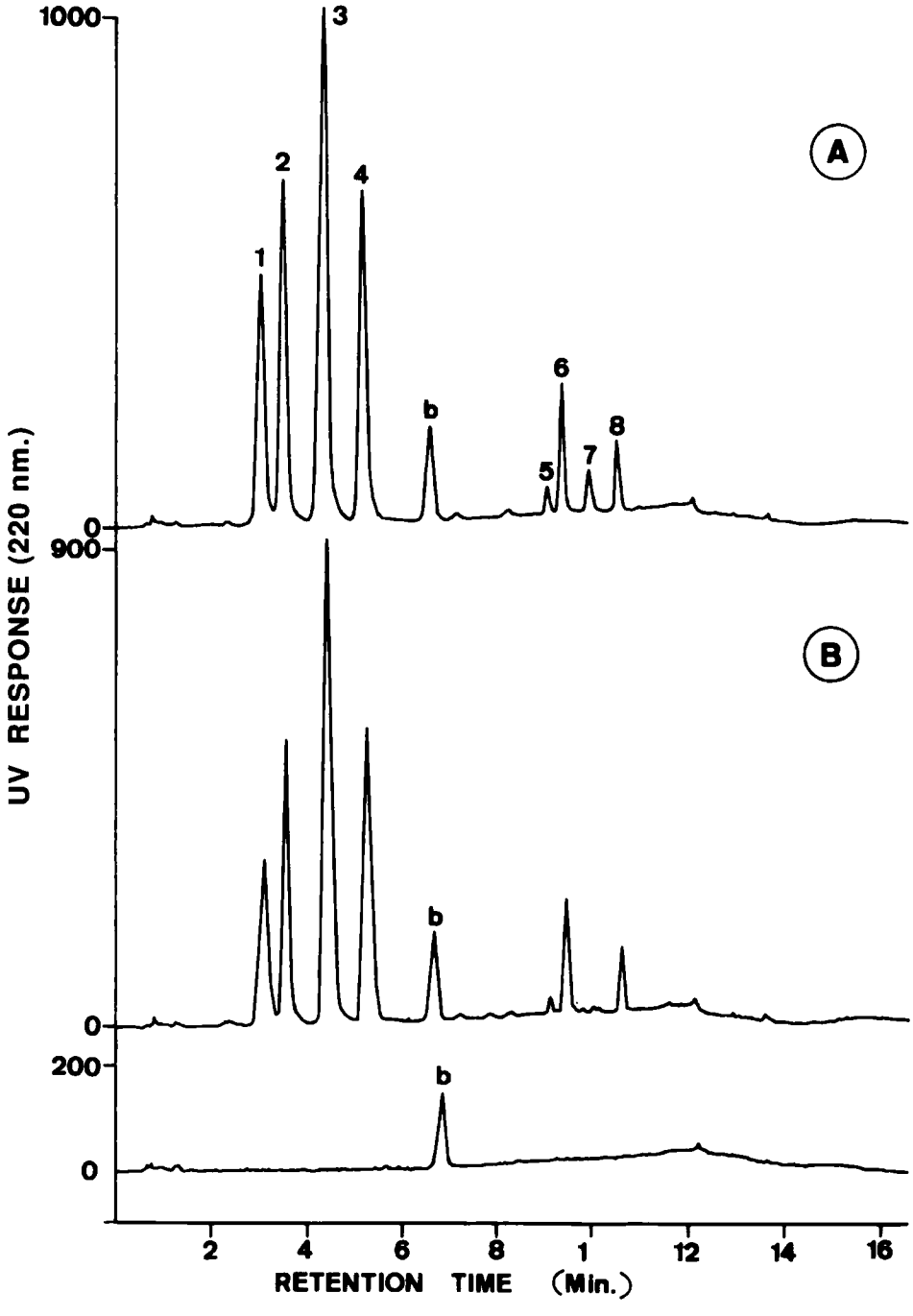

Figure 1 LC-DAD chromatogram at 220 nm obtained on the extract of 1 litre of reconstituted water containing a pesticide mixture shown in Table 2 after storage for one month at: (A) -20°C, (B) 20°C, (C) blank chromatogram; b is the glycine. For information on the peak numbers and LC conditions see Table 2 and the experimental section respectively.

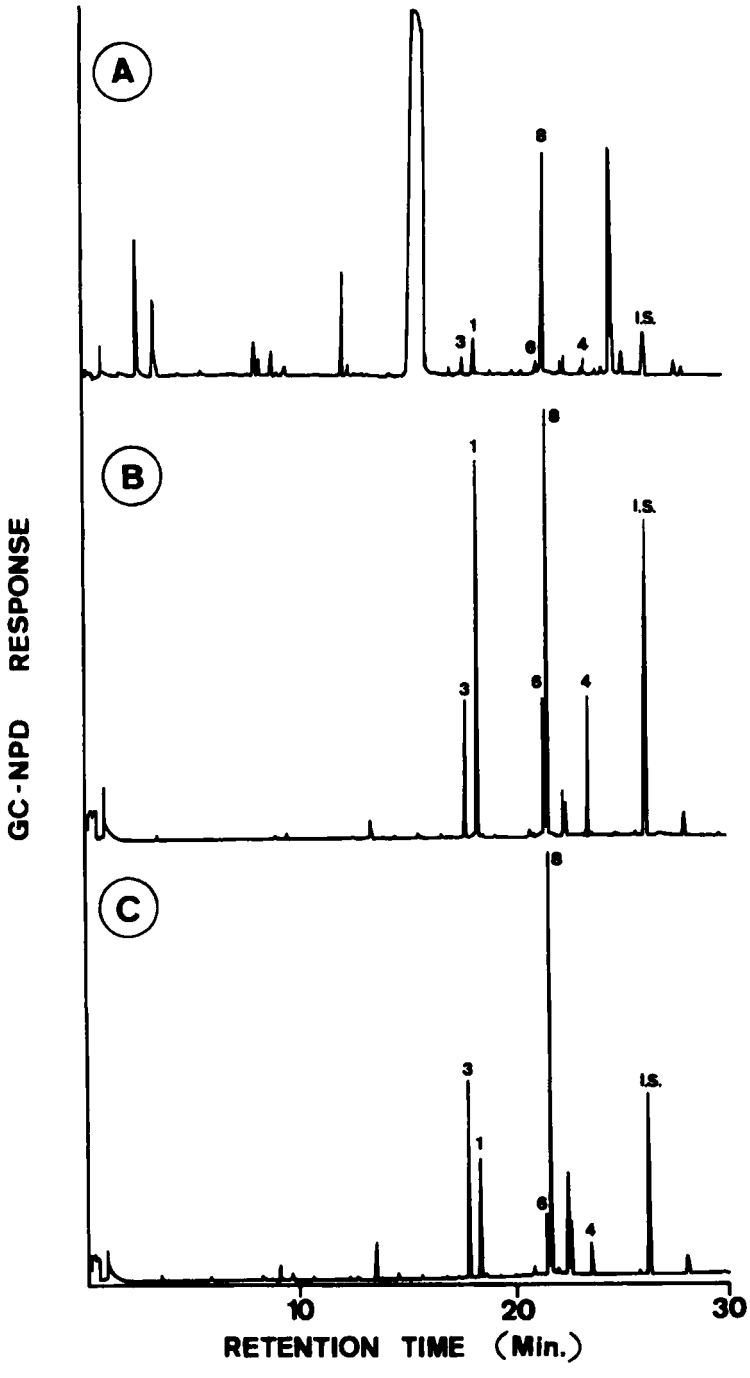

Figure 2 GC-NPD chromatogram obtained on the extracts of 1 litre of water reconstituted from the pesticide mixture indicated in Tables 3 and 4. Sample pretreatment was performed either: (A) without any cleanup (batch 2A), (B) with cleanup (batch 2A) and (C) with cleanup batch 2B. The peak numbers and LC conditions are given in Table 3 and the experimental section respectively.

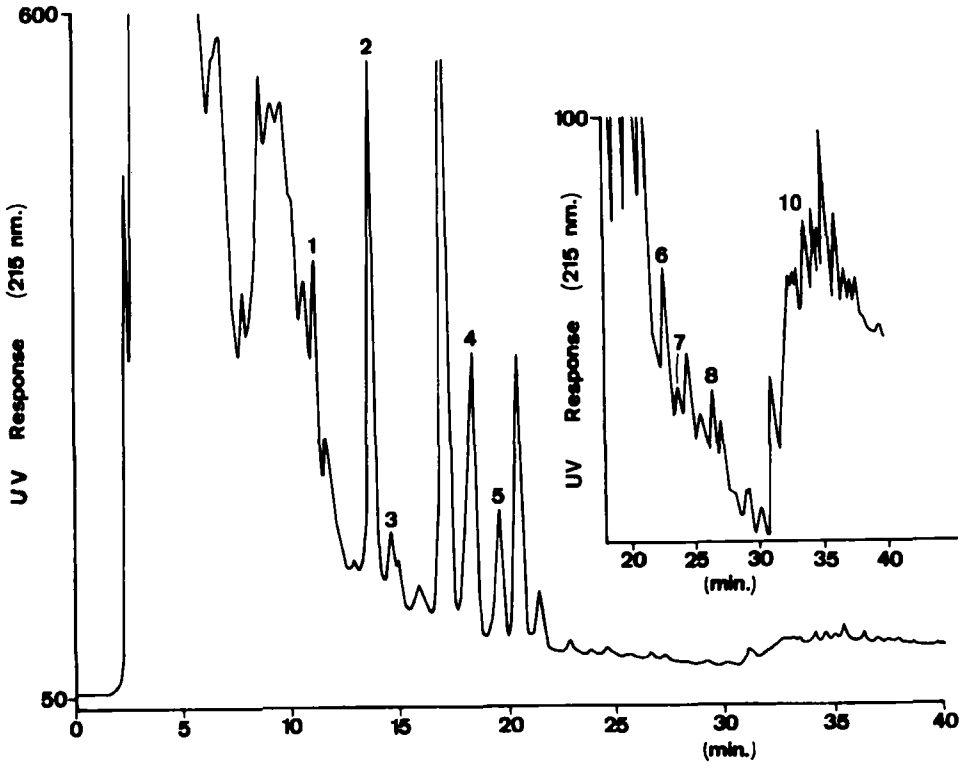

Figure 3 On-line LC-DAD analysis at 215 nm of 100 ml of reconstituted water from batch 2A after storage at -20°C. Quantitation was only performed for compounds 2, 5 and 7 (carbaryl, linuron and fenamiphos). The concentration of the different pesticides is shown in Table 3.

Batch 3: No glycine addition The samples were analyzed by GC-ECD for *cis*-permethrin, GC-NPD for simazine and confirmed by GC-MS. 250 ml of sample was extracted as described for permethrin above. An example of the chromatograms for permethrin and simazine are shown in Figure 4.

Chromatographic conditions

Batch 1: High-spiking level Two 64-high pressure pumps from Knauer (Bad-Homburg, Germany) assured the elution. Detection was performed with a Chrom-A-Scope rapid scanning UV/VIS detector from Barspec (Rehovot, Israel). Samples were injected via a 20 µl loop from Rheodyne (Cotati, California, USA). LiChrocart cartridge columns (12.5 cm × 4.0 mm i.d.) packed with 4 mm LiChrospher 100 RP-18 from Merck (Darmstad, Germany) were used. A gradient elution was programmed to pass from methanol-acetonitrile-water (25:25:50) up to methanol-acetonitrile (50:50) in 12 min at a flow rate of 1 $ml.min^{-1}$. The UV detection was performed at 220 nm for the chlorotriazines, linuron and carbaryl and at 205 nm for the organophosphorus pesticides.

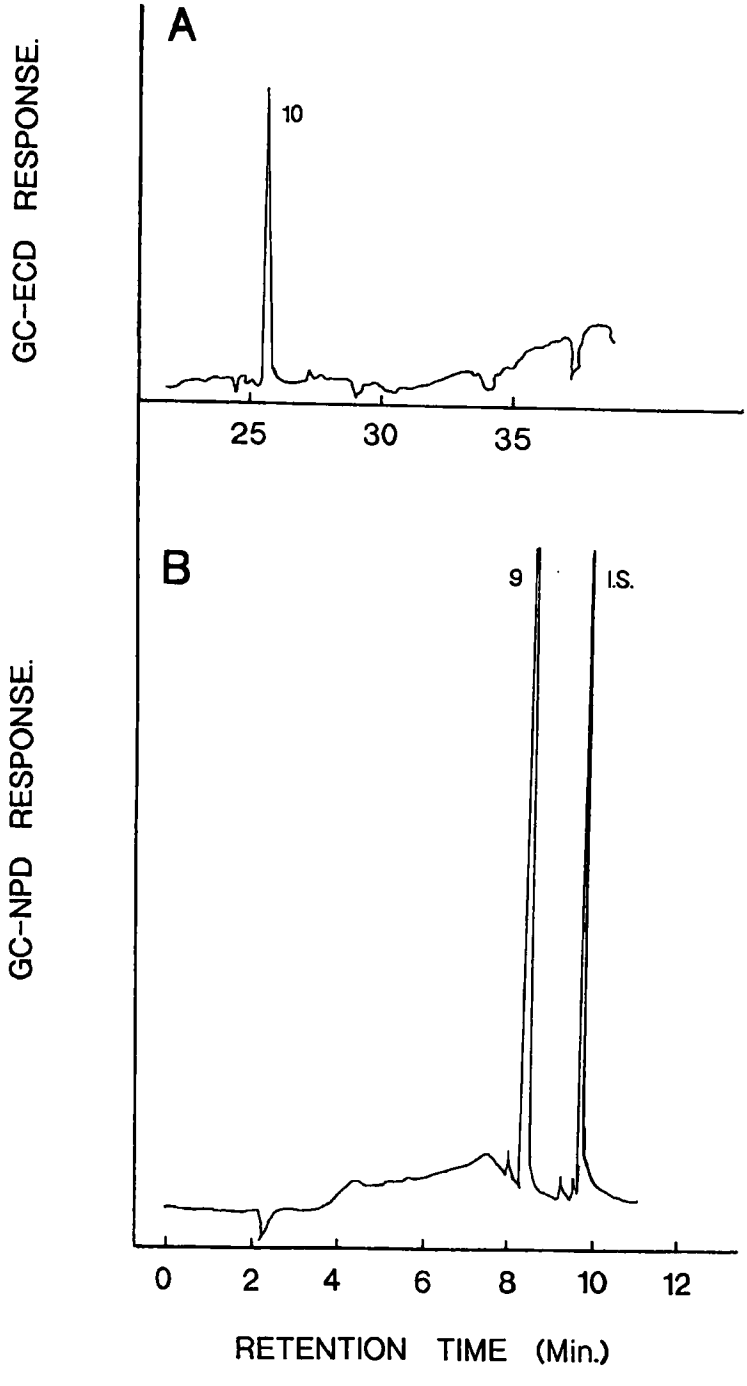

Figure 4 Chromatograms from the analysis of *cis*-permethrin (peak 10) and simazine (peak 9) by GC/ECD and GC/NPD respectively of samples reconstituted from batch 3. The freeze-dried samples were prepared without the addition of glycine.

Batch 2: Low-spiking level Carbaryl, linuron and fenamiphos were analyzed by the procedure described in detail previously[5]. After the reconstitution of 1 l of water, 100 ml were used for the on-line LC analysis using a solid-phase disk extraction. Prefiltration of the water samples was done using a 0.45 µm PTFE filter. The LC procedure was the same as the one described above for batch. 1. The detection was performed at 215 nm with an external calibration procedure from 0.5 to 10 $\mu g.ml^{-1}$. After the membrane disks were placed in the disk-holder, the holder was fit in a MUST column switching device from Spark Holland (AS Emmen, The Netherlands) and connected to an SSI Model 300 LC pump from Scientific Systems Inc., (State College, PA, USA) which delivered the water samples containing the pesticides. The disks were first conditioned by flushing 10 ml of methanol and then 10 ml of HPLC water at 1 $ml.min^{-1}$. Following the preconcentration step, the MUST valve was switched and the components were desorbed and separated in a LiChrocart cartridge column (25 cm × 4.6 mm i.d.) packed with 5 µm Zorbax SB-C8 (purchased to Rockland technologies through Chrompack, Middleburg, NL) by using the following gradient elution program: from 35% of A [acetonitrile-methanol (50:50)] and 65% of B [acetonitrile-water (10:90)] to 70% A/30% B in 20 min; [from 70% A/30% B] to [100% A] in 6 min; [from 100% A] to initial conditions in 15 min at a flow rate of 0.8 $ml.min^{-1}$. The disks were cleaned by flushing 5 ml of acetonitrile followed by 5 ml of water through the disks after each analysis. The memory effect of the disks was estimated by the determination of the pesticide in the flushing solvents and was found to be less than 1% of the concentration of the sample. Desorption of the pesticides from the membrane-disk holder was done in the back-flush mode thus preventing chromatographic tailing[15].

For the low-spiking level, the lower amount of the pesticides: simazine, atrazine, propanil, fenitrothion and parathion-ethyl, were analyzed by gas chromatography-nitrogen phosphorus detection (GC-NPD). In this instance a clean-up step was required to eliminate the interferences due to the presence of glycine in the matrix. Although glycine was added at a lower concentration compared to the high -spiking level, (0.6% versus 1%), the effect of the interferences in the analytical determination was proportionally more important (see Figure 2) because the concentration of pesticides in the river water was a factor of 50–100 times lower. Following the Florisil clean-up, the extracts were injected onto the column of a gas chromatograph (HRGC 5300 mega Series, carlo Erba, Milano) equipped with a nitrogen-phosphorus (NPD-40) detector. A 15 m × 0.15 mm i.d. fused silica capillary column coated with chemically bonded cyanopropylphenyl DB 225 (J and W Scientific, Folsom, CA, USA). Hydrogen was the carrier gas with a flow speed of 50 $cm.min^{-1}$ and helium the make-up gas with a flow rate 30 $ml\text{-}min^{-1}$. The temperatures of the injector and detector were held at 300°C and 320°C, respectively. The columns temperature programme ranged from 70°C to 220°C at $6°C.min^{-1}$. Quantitation was performed by a linear calibration over a concentration range of 1 $ng.l^{-1}$ to 10 $\mu g.l^{-1}$ with cyanazine as internal standard.

Permethrin was analyzed by GC-ECD (8700 series Perkin-Elmer) using a 30 m × 0.25 mm i.d. fused silica capillary column with a 5% phenyl-methyl silicon stationary phase (DB5, Jones Chromatography) with helium as carrier gas (50 $ml.min^{-1}$) and nitrogen as make-up gas. The injector and the detector were at 310°C and 350°C respectively and the injector was operated in split-less mode for 30 s after a 2 µl injection. The temperature program was as follows: oven at 50°C for 2 min, increased at $30°C.min^{-1}$ to 170°C and then at $10°C.min^{-1}$ to 240°C for 7 min. Then followed a ramp of $2°C.min^{-1}$ to 280°C and held for 2 min to complete the programme. Quantitation was performed by a linear calibration over a concentration range of 0.1 to 2 $\mu g.l^{-1}$ using phosalone as internal standard.

RESULTS AND DISCUSSION

Homogeneity studies

The homogeneity of the pesticides in samples set aside during the bottling procedure from batch 1 and 3 was determined in three replicates of the reconstituted water. The pesticide concentrations in the water, together with their standard deviations, are shown in Table 2 and indicate a high degree of homogeneity in these preparations. For the low-spiking level, the homogeneity was measured by analyzing six replicate samples (five for permethrin) for batches 2A and 2B for the nine pesticides (Table 3). The result for permethrin indicate that the lyophilized samples from batch 2A and 2B are too heterogeneous with respect to their permethrin content and therefore permethrin was not included in the stability study of these batches. Permethrin is very lipophilic compared with the other compounds studied and is suspected to behave differently during freeze-drying in the presence of glycine. In general, all the other compounds exhibited a good homogeneity in the samples analyzed.

Stability studies

Batch 1: high-spiking level The stability of the pesticides was tested in the freeze-dried material in order to determine its suitability for the interlaboratory exercise. For batch 1 and 3 (high-spiking level), the sets of samples were kept at -20°C and at +20°C for a period of one year. Assuming that the samples stored at -20°C in the dark are stable, the results obtained at this temperature may be compared with those obtained after certain storage periods at higher temperatures. Table 4 gives the ratios, $R_{+20°C}$, of the mean values of the three measurements made at the different periods of time (3, 6 and 12 months) against the mean value obtained for three determinations made on samples stored at -20°C. The same table also lists the uncertainty $U_{+20°C}$ calculated from the coefficient of variation (CV) of each set of three measurements at the different periods (3, 6 and 12 months):

$$U_{+20°C} = (CV^2_{+20°C} + CV^2_{-20°C})^{1/2} R_{+20°C} \quad (1)$$

Table 2 Results of the study of the homogeneity of samples from batch 1 and 3 for selected pesticides. The concentrations are given in μg kg^{-1} of reconstituted water with the standard deviations (SD) as shown. The peak numbers refer to those in Figures 1, 2 and 4.

Compound	*Peak No*	*1*	*2*	*3*	*Mean*	*SD*
Batch 1						
Cyanazine	1	53	54	59	55	3
Simazine	2	52	56	51	53	3
Carbaryl	3	51	54	51	52	1
Atrazine	4	54	55	52	54	1
Fenitrothion	5	16	15	14	15	0.7
Fenamiphos	6	97	99	89	95	5
Tetrachlorvinphos	7	11	11	10	11	0.7
Parathion-ethyl	8	47	52	49	49	3
Batch 3						
Simazine	9	41	42	37	40	3
Permethrin	10	3.7	4.2	3.9	3.9	0.3

Table 3 Results of the study of the homogeneity of samples from batch 2A and 2B for selected pesticides. The concentrations are given in $\mu g\ kg^{-1}$ of reconstituted water with the standard deviations (SD) as shown. The peak numbers refer to those in Figures 1 to 4.

Compound	*Peak No.*	*1*	*2*	*3*	*4*	*5*	*6*	*Mean*	*SD*
Batch 2A									
Simazine	1	1.70	2.05	1.96	1.89	2.01	1.75	1.89	0.14
Carbaryl	2	2.60	3.00	2.74	3.01	2.68	3.01	2.84	0.18
Atrazine	3	0.60	0.66	0.62	0.59	0.66	0.57	0.62	0.04
Propanil	4	5.70	5.72	6.02	6.23	5.80	6.20	5.94	0.24
Linuron	5	3.30	3.73	3.38	3.54	3.55	3.43	3.49	0.15
Fenitrothion	6	0.03	0.03	0.04	0.03	0.04	0.03	0.03	0.005
Fenamiphos	7	1.07	0.89	0.80	0.84	1.01	0.87	0.91	0.10
Parathion-ethyl	8	0.11	0.11	0.13	0.12	0.12	0.11	0.12	0.008
Permethrin	10	0.87	0.44	0.03	7.32	1.09	–	1.95	3.03
Batch 2B									
Simazine	1	0.80	0.94	1.01	0.77	0.94	0.87	0.88	0.09
Carbaryl	2	5.01	5.55	4.94	5.10	5.01	5.10	5.11	0.22
Atrazine	3	1.30	1.42	1.45	1.37	1.50	1.33	1.39	0.07
Propanil	4	1.34	1.45	1.55	1.75	1.62	1.54	1.54	0.14
Linuron	5	6.90	6.45	6.55	6.60	6.84	6.70	6.67	0.17
Fenitrothion	6	0.02	0.03	0.02	0.04	0.03	0.03	0.03	0.007
Fenamiphos	7	3.97	3.70	4.00	3.94	4.20	4.10	3.97	0.16
Parathion-ethyl	8	0.21	0.24	0.27	0.30	0.33	0.37	0.29	0.06
Permethrin	10	0.32	0.19	0.60	0.24	0.25	–	0.32	0.16

Table 4 The ratios (R_T) from the analysis of samples from batch 1 and 3 together with the uncertainties, U_T, calculated from the mean concentrations of samples stored at 20°C compared with the mean concentration of 3 samples stored at -20°C. The uncertainty was determined from the coefficient of variation (CV) of each set of 3 measurements at the different storage intervals according to eqn. (1). The superscript (a) denotes the samples from batch 3.

Compound	R_T			U_T		
	(months)			*(months)*		
	3	*6*	*12*	*3*	*6*	*12*
Cyanazine	0.67	0.32	0.12	0.06	0.03	0.02
Simazine	1.02	0.98	0.98	0.08	0.07	0.06
Carbaryl	1.11	1.10	0.85	0.06	0.12	0.07
Atrazine	1.05	1.05	0.96	0.05	0.08	0.05
Fenitrothion	0.46	–	–	0.05	–	–
Fenamiphos	0.75	0.66	0.54	0.06	0.03	0.05
Tetrachlorvinphos	–	–	–	–	–	–
Parathion-ethyl	1.10	1.12	0.87	0.12	0.10	0.05
Permethrin[a]	1.08	1.32	1.10	0.37	0.14	0.17
Simazine[a]	1.09	1.33	0.77	0.18	0.28	0.11

$CV_{-20°C}$ is the coefficient of variation at -20°C and $R_{+20°C}$ is the ratio of the mean values at the storage temperature compared with the mean value of the concentrations of samples stored at -20°C.

From the results in Table 4 it can be concluded that simazine, carbaryl, atrazine, parathion-ethyl and permethrin exhibited a good long-term stability in the lyophilized

samples. Cyanazine and fenamiphos suffered a loss of 34% and 25% respectively after three months storage at room temperature. Fenitrothion and tetrachlorvinphos were particularly unstable in these conditions (see also Figure 1). The trend in stability may be explained by considering the properties of the individual pesticides, eg. their vapour pressure, aqueous solubility and degradation rate. In Table 5 the two physicochemical parameters are shown for the different compounds[14–16]. It is interesting to note that the more stable compounds have low vapour pressures and medium water solubilities (up to 40 $mg.l^{-1}$) suggesting that these parameters affect the way in which the pesticides are incorporated in the lyophilized powder. Unfortunately, little is known about the processes occurring during freeze-drying although the role of glycine is likely to be important in soft waters. In hard waters, the precipitation of calcite and coprecipitation of trace solutes is important. The pesticides may be incorporated or occluded in the crystal structure of the calcium carbonate solid thus inferring a degree of protection from the external environment. Lipophilic compounds such as permethrin are mainly associated with colloid and particulate components in the water and thus may behave differently from the more water soluble compounds. The higher the vapour pressure of the pesticide, the greater is the loss expected during freeze-drying. This may be compensated by increasing the amount of compound added to the water prior of freeze-drying. The results are also in agreement with reported literature on the degradation of pesticides under environmental conditions. For example, short half-lives of 1–2 days have been reported for fenitrothion and tetrachlorvinphos whereas cyanazine has a reported half-life of less than 50 days[13]. This is much shorter than for the other triazines, e.g. atrazine with 140 days[17–18]. The order of the reported half-lives also agree with the results obtained here for the more stable compounds, eg. only *ca.* 2% losses have been estimated for atrazine and simazine under environmental conditions during a period of 20 days. The instability of cyanazine is attributed to the oxidation of the cyanazine group[18]. The relatively high vapour pressure of fenitrothion and fast degradation rate in environmental samples explain the observed instability. Similar conclusions have been reported in a recent study by the National Pesticide Survey of the US EPA, leading to the exclusion of this compound in a survey of 121 pesticides[6].

Batch 2: low spiking level The results for batch 2 at the low-spiking level were analyzed as above by assuming that the samples stored at -20°C in the dark are stable and

Table 5 Vapour pressure and aqueous solubility of the selected pesticides from references 14–16.

Compound	*Vapour pressure (mPa)*	*Solubility (mg/l)*
Cyanazine	0.0002	170
Simazine	0.0085	5
Carbaryl	0.30	40
Atrazine	0.04	30
Fenitrothion	7.20	30
Fenamiphos	0.13	700
Tetrachlorvinphos	4.00	11
Parathion-ethyl	0.60	15
Propanil	3.60	268
Linuron	1.40	75
Permethrin	0.0025	0.20

therefore serve as comparators for the samples stored at +20°C. Tables 6 and 7 show the ratios, $R_{+20°C}$, of the mean values of the three measurements on samples stored at +20°C for different periods against the mean value for three determinations on samples stored at -20°C for batches 2A and 2B respectively. In the same tables, the uncertainties, $U_{+20°C}$, calculated from the coefficients of variation, eqn.(1), are reported for the different storage times. As shown, propanil and linuron exhibit good stability at +20°C over the trials. Other compounds were stable for a period of at least a month, i.e. carbaryl, atrazine, simazine, fenamiphos and parathion-ethyl. Fenitrothion suffered the highest degradation in agreement with the results from the high-spiked samples. These results are in broad agreement with those from batch 1 at the high-spiking level discussed above. However a comparison of results of Table 4, 6 and 7, indicate a greater percentage degradation in the second set of samples than in those with higher pesticide concentrations, eg. atrazine and simazine. This means that small losses of the pesticides

Table 6 The ratios (R_T) of the mean values of the three measurements on samples from batch 2A (low-spiking level) at different storage times (3, 6 and 12 months) against the mean value obtained for three determinations made on samples stored at -20°C. The uncertainty (U_T) was obtained from the coefficient of variation (CV) of each set of three measurements at the different periods (1, 3 and 6 months).

Compound	R_T *(months)*			U_T *(months)*		
	1	*3*	*6*	*1*	*3*	*6*
Simazine	0.60	0.35	0.20	0.04	0.04	0.01
Carbaryl	1.02	0.62	0.38	0.05	0.03	0.04
Atrazine	0.80	0.60	0.27	0.06	0.02	0.02
Propanil	1.01	0.95	0.94	0.05	0.04	0.04
Linuron	0.97	0.85	0.85	0.04	0.04	0.04
Fenitrothion	0.66	0.33	–	0.20	0.17	–
Fenamiphos	0.90	0.76	0.48	0.06	0.05	0.11
Parathion-ethyl	0.91	0.50	0.33	0.16	0.07	0.04

Table 7 The ratios (R_T) of the mean values of the three measurements on samples from batch 2B (low-spiking level) made at different storage times (3, 6 and 12 months) against the mean value for three determinations made on samples stored at -20°C. The uncertainty (U_T) was obtained from the coefficient of variation (CV) of each set of 3 measurements at the different periods.

Compound	R_T *(months)*			U_T *(months)*		
	1	*3*	*6*	*1*	*3*	*6*
Simazine	0.69	0.34	–	0.08	0.05	–
Carbaryl	0.95	0.67	0.27	0.05	0.05	0.01
Atrazine	0.86	0.77	0.67	0.04	0.05	0.06
Propanil	0.90	0.88	0.90	0.05	0.05	0.06
Linuron	1.03	0.82	0.69	0.04	0.03	0.03
Fenitrothion	0.66	–	–	–	–	–
Fenamiphos	0.85	0.76	0.60	0.04	0.02	0.03
Parathion-ethyl	0.87	0.54	0.21	0.11	0.08	0.03

during storage significantly affect the R_T values for the low-spiked samples but the same losses are insignificant, compared with analytical errors, for the determination of R_T's for the high-spiked samples. In instances where pesticide losses occur during storage, the data in Tables 4, 6 and 7 are insufficient to permit an analysis of the kinetics.

Interlaboratory exercise

After having gained the guarantee that some pesticides are stable in the freeze-dried water samples, an interlaboratory exercise was organised with 14 European laboratories.

The participants discussed the work to be done and the pesticides to be studied in a meeting before the start of the interlaboratory exercise. The preparation procedure, the homogeneity and the data on the stability obtained in the preliminary studies (batch 1). Each laboratory was asked to use its own method. The participants were warned on the presence of the lyophilisation "keeper" and on the necessity to add an additional clean-up step to the normal routine procedures for drinking water to remove it. A strict protocol was distributed describing in details the reconstitution procedure of the water samples and the minimum requirements. These included in particular: 5 independent determinations from reconstitution to final determination, an estimate of the extraction efficiency and recovery of the method (to be performed in triplicate by standard additions to the blank samples distributed); the determination for each compound of the range of the linear response of the detector; procedure blank. The results and the description of the analytical procedures had to be reported on specially prepared reporting forms. These forms already included quality control items which had to be checked. In particular, for GC methods, it was requested to verify the identity of chromatographic peaks by using a second column of different polarity. The data from the column which was estimated, by the participant, to give the most accurate results had to be delivered. At least one internal standard had to be used for the quantification. The results were corrected for the procedure blank and the recovery and were expressed on the dry residue basis. Each participant had to submit a chromatogram of the blank, the calibration solution and the sample. A special consideration was devoted to the calibration step. The quality of the calibrants used had to be demonstrated (purity and stoichiometry of crystalline compounds, certificate or evidence of traceability to certified compounds for ready to use solutions). When two different chromatographic systems were used (GC and HPLC) all above mentioned requirements had to be provided for each method.

Analytical procedures

Reconstitution of the samples The participants were requested to use the entire content of a bottle and to weigh accurately the mass of residue, approx. 2.5 g per bottle, before the reconstitution. They were asked to express their results on a mass of residue basis.

Extraction, clean-up and chromatographic conditions Several different analytical methods have been applied. Some laboratories have used different extraction methods prior to the GC or LC separation. Several participants reported that they encountered difficulties in using solid phase extraction (cartridge clogging) because of the presence of glycine. Finally, only few participants applied a clean-up procedure.

Each laboratory had to verify the dynamic range of the detector (linear range of response) for each compound and for the internal standard(s). The system had to be calibrated within this tested range. The amount of sample in ng injected into the system had to fall within this range. At least one internal standard was used for GC determinations.

COMMENTS ON THE RESULTS

Blank and recovery experiments

Non enriched (blank) samples were distributed to the participants in order to establish the extraction efficiency of their method by spiking the residue with known amounts of the pesticides. "Blank" samples were produced from the same river water as the enriched samples. The results of the recovery experiments ranged between 44% and 148%. The "blank" samples contained several pesticides e.g. parathion-ethyl, fenamiphos, propanil, linuron and carbaryl. For parathion-ethyl the concentration in the non-enriched samples appeared to be higher than in the enriched sample A. This might have resulted from a high concentration in parathion-ethyl in the collected water and a different behaviour during lyophilisation between the original and the spiked compounds. Therefore, the results for this compound were not discussed further. The results found in various laboratories for the "blank" samples are widely differing and did not allow to conclude on the real concentration of pesticides present in the collected water. Except for parathion-ethyl and at a lesser extent for fenamiphos the values for the "blank" samples were negligible towards the spiked amounts. The values for the blank, with exception of parathion and fenamiphos, found in several laboratories rather indicate contaminations within the laboratory rather than the real concentration of the pesticide in the non enriched sample.

Carbaryl: the presence of glycine did not affect too much the determination of this compound. At least eight laboratories showed comparable results using either GC or HPLC.

Atrazine: the presence of glycine affected some methods. The use of an organo-phosphorus compound as internal standard for the quantitation of atrazine and simazine was shown to be inadequate for a NPD detection. This may be due to the matrix effect which affects differently the signal of the organo-P compound than the triazines.

Simazine: some participants noticed that a low pH of the water lowers the extraction efficiency of simazine by liquid/liquid systems.

Fenitrothion: the compound was not stable in the distributed residues. No conclusions could be drawn from the interlaboratory study.

Parathion-ethyl: as already explained above, the content of parathion-ethyl in the initial water was already high and the spiked portion might have disappeared during lyophilisation.

Fenamiphos: the group demonstrated a rather good comparability of results for this compound.

Propanil: severe interferences were suspected by several participants but the overall results demonstrated a good comparability as shown in Figure 5.

PROPANIL IN WATER – ng/g –

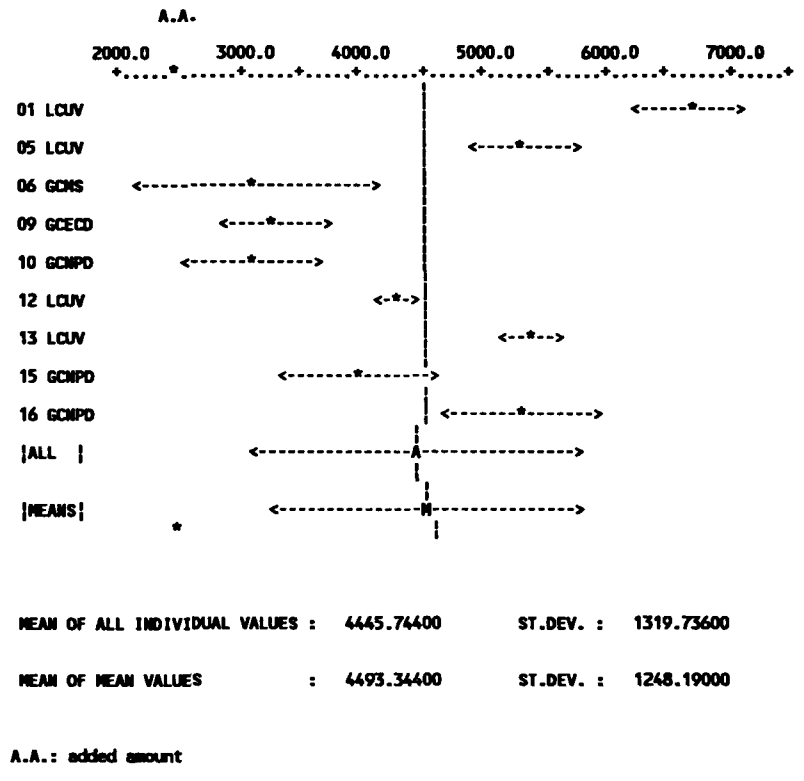

Figure 5 Bar graph presentation of the results obtained in the interlaboratory study for propanil in batch A. The laboratories 01, 05, 15 and 16 reported the presence of interfering peaks with propanil. The maximum possible value calculated from the amount spiked before lyophilisation was 2500 ng/g which is lower than all values determined by the group.

Linuron: for this compound a poor performance was achieved. Several interferences were suspected e.g. with fenitrothion, propanil after degradation in the GC-MS, similar UV spectra for propanil and linuron. The participants concluded that the methods for linuron needed to be fully revalidated in all laboratories.

The mean of mean values reported by the participants as well as the range of mean values for the blank and both batches of spiked water are listed in Table 8.

CONCLUSIONS

There is no doubt that the unavailability of certified matrix reference materials is a severe limitation on the quality assessment of analytical data of pesticides in potable

Table 8 Results obtained in the interlaboratory study for the blank sample, batch A and batch B in ng/g residue.

Pesticide	*Natural sample*			*Spiked batch A*			*Spiked batch B*		
	mean ± SD	*lower*	*upper*	*mean ± SD*	*lower*	*upper*	*mean ± SD*	*lower*	*upper*
Carbaryl	321 ± 218	113	618	1424 ± 638	567	3045	2440 ± 1672	445	6592
Atrazine	58 ± 36	33	121	147 ± 80	46	282	230 ± 201	19	578
Simazine	48 ± 47	8.5	126	360 ± 243	95	796	142 ± 113	15	359
Fenitrothion	28 ± 25	6.5	49	43 ± 23	15	75	60 ± 80	10	266
Parathion-ethyl	346 ± 171	160	675	144 ± 86	20	272	360 ± 301	81	1065
Fenamiphos	632 ± 280	363	1083	460 ± 312	120	1225	2723 ± 1879	1258	4498
Propanil	480 ± 436	180	1251	4493 ± 1248	3109	6682	1774 ± 432	683	1826
Linuron	1067 ± 364	615	1799	2229 ± 1149	926	4694	3643 ± 1453	2130	7369
tr-Permethrin	[1]	–	10.2	27.8[2]	18.2	37.3	14.6[2]	9.9	19
cis-Permethrin	[1]	–	8.1	1.1[2]	4.9	15.4	1.1[2]	6.3	14

SD: standard deviation of the mean of means
mean: mean of laboratory means
lower-upper: lowest and highest result submitted
[1]: only one set of data
[2]: only two sets of data

water and natural waters in general. This investigation highlights some of the difficulties in formulating a realistic reference matrix and offers several important conclusions and guidelines for the future development in this area. Briefly they may be summarized as follows.

(1) The main advantages of the reference powder containing a mixture of pesticides are the easy transport and storage of large quantities of material and the simplicity of the reconstitution procedure to generate a water sample very close in ionic composition to the natural samples. The lyophilized powder stored at -20°C showed no degradation or losses of any of the pesticides studied.

(2) Certain compounds can be sufficiently stabilized for storage at room temperature for at least one year depending on the concentration of the pesticide in the freeze-dried water. These include simazine, atrazine, parathion-ethyl and carbaryl at *ca* 50 µg/l with glycine addition and cis- permethrin at *ca* 4 µg/l in a hard water without glycine addition.

(3) At lower concentrations, only propanil at 1.5 and 6 µg/l and linuron at 3.5 and 6.7 µg/l with glycine addition, were stable over a six month period. Other compounds such as carbaryl, atrazine, parathion-ethyl and fenamiphos were stable over one month when stored at room temperature. Hence further development is possible with these compounds as long as the powders are stored at -20°C and exposed to ambient conditions for a maximum period of about one month. The fact that many pesticides are stable at 20°C during a period of one month makes the shipping and transportation of a candidate reference material possible.

(4) The results suggest that glycine is not always necessary for the success of the stabilization and its omission simplifies the analysis of the reconstituted water. However in this instance it is essential to use a hard water for the preparation of the lyophilized samples. Further research is needed to determine whether a homogeneous sample containing synthetic pyrethroids at low concentrations, eg < 1 µg/l, can be prepared by this technique.

(5) The results indicate that some compounds are very unstable in the freeze-dried form, e.g. cyanazine, fenitrothion and tetrachlorvinphos. For other compounds, eg. fenamiphos, the loss is directly related to the initial concentration in the freeze-dried powder.

(6) The first conclusion from the interlaboratory study was that the addition of glycine disturbed the methods foreseen for drinking water analysis - i.e. methods without clean-up or precolumns. The second conclusion was that for carbaryl, atrazine, simazine and fenamiphos, different reliable methods of determination exist which allow to envisage the certification of a material with no glycine addition. For linuron and propanil further analytical investigations are necessary.

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