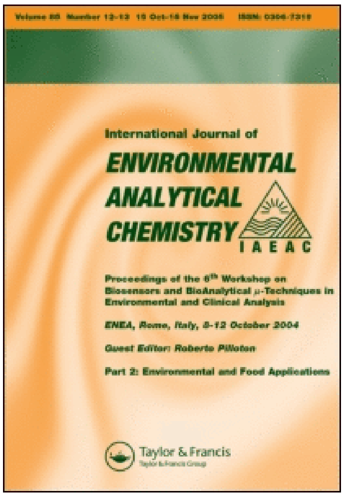

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A. Martín-Esteban[a]; P. Fernández[a]; C. Cámara[a]; G. N. Kramer[b]; E. A. Maier[c]
[a] Departmento de Química Analitica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Madrid, Spain [b] Institute for Reference Materials and Measurements, Retieseweg, Geel, Belgium [c] Measurements and Testing Programme, European Commission, Brussels, Belgium

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PREPARATION, HOMOGENEITY AND STABILITY OF POLAR PESTICIDES IN FREEZE-DRIED WATER INTERLABORATORY EXERCISE

A. MARTÍN-ESTEBAN[a], P. FERNÁNDEZ[a], C. CÁMARA[a,*],
G. N. KRAMER[b] and E. A. MAIER[c]

[a]*Departmento de Química Analitica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain;* [b]*Institute for Reference Materials and Measurements, Retieseweg, B-2440 Geel, Belgium;* [c]*Measurements and Testing Programme, European Commission, 200 Rue de la Loi, B-1049, Brussels, Belgium*

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An interlaboratory exercise was carried out to improve the state of the art of some polar pesticide determination in water (atrazine, simazine, carbaryl, propanil, linuron, fenamiphos and permethrin). The preparation, the homogeneity and the stability of freeze-dried water samples containing the above pesticides was studied. The final concentration of each pesticide in water was in the 50–80 $\mu g.l^{-1}$range with a salt content of 2.5 $g.l^{-1}$ of NaCl. After the lyophilization the residue was rehomogenised, filled into amber glass bottles and stored at -20 °C and +20 °C. Every three months, one sample was analysed to verify the stability of the residue.

All pesticides were determined by high performance liquid chromatography with diode-array detection (HPLC-DAD) except permethrin which was determined by gas chromatography with electron capture detection (GC-ECD).

The results obtained show that the atrazine, carbaryl, propanil, linuron and fenamiphos samples were homogeneously distributed, whereas simazine and permethrin were not. With respect to the stability over three months, all pesticides were stable at -20 °C. Atrazine, simazine, carbaryl, propanil and linuron are also stable for the maximum storage time at +20 °C but the concentration of fenamiphos decreased by about 70% after one month.

The results obtained in the interlaboratory study by the participants were in good agreement for many of the pesticides.

Keywords: Reference materials; atrazine; simazine; carbaryl; propanil; linuron; fenamiphos; permethrin; water; interlaboratory study

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[*] Corresponding author. Tel.: 34–1–3944318. Fax: 34–1–3944329.

INTRODUCTION

Many of our current and future environmental problems are or will be a direct result of modern society's release of a great number of pesticides into the environment. Pesticides such as organophosphorus, chlorotriazines, pyrethroids and phenylureas as well as other groups are widely employed in agriculture to eliminate weeds and parasites and often end up in surface or ground water [1,2]. The level of pesticides in these waters is rather low and their determination is still a difficult task.

Certified reference materials (CRM) are required for checking the accuracy of the determinations of these analytes but such do not yet exist due to the difficulty of their preparation. The availability of certified reference materials (CRM) would also allow the validation of analytical methods developed for this purpose. The most common analytical techniques for determining these pesticides are gas chromatography (GC) with nitrogen-phosphorus detection (NPD), electron capture detection (ECD) or mass spectrometry (MS) and high performance liquid chromatography with a diode-array detector (HPLC-DAD) [3].

A feusability project of the European Commission under the second BCR framework program was undertaken to prepare a CRM for polar pesticides (atrazine, simazine, fenitrothion, fenamiphos, parathion-ethyl, carbaryl, permethrin, propanil and linuron) at very low concentrations (0.02 to 90 $\mu g.l^{-1}$) [4]. These pesticides were dissolved in tap water containing 10 $g.l^{-1}$ of glycine as a keeper. It was found that the presence of glycine seriously affected certain analytical methods for the determination of organo-P and organo-N compounds.

In view of presence of glycine and of the low concentration of certain pesticides, a new procedure which would avoid glycine was followed for the freeze-drying of the water. The pesticides chosen for the present study were atrazine, simazine, carbaryl, propanil, linuron, fenamiphos and permethrin at concentrations within the 50–80 $\mu g.l^{-1}$ range. In order to facilitate the handling, weighing of the residue 2.5 $g.l^{-1}$ NaCl were added to the water.

An interlaboratory exercise with 20 participants was organised in order to prepare the certification of a reference material.

EXPERIMENTAL

Preparation of Freeze-dried Water

A batch of 80 l of tap water was spiked with NaCl (2.5 $g.l^{-1}$) and with atrazine, simazine, carbaryl, propanil, linuron, fenamiphos and permethrin. The final concentration of each pesticide was 50–80 $\mu g.l^{-1}$. A blank of the same water (80 l) also containing 2.5 $g.l^{-1}$ NaCl was prepared. The freeze-dried sample program was as follows: freezing at –30 °C for 90 h, 114 h at –20 °C, 124 h at –5 °C; 138 h at 0 °C and after 207 h at + 20 °C.

The freeze-dried sample was homogenised and stored in amber glass bottles each containing about 2.5 g sample. The bottles were sealed with an aluminium crimpcap over a rubber insert lined with PTFE. Five bottles were stored at –20 °C and five at room temperature for the preliminary stability study over three months. Ten bottles were used to carry out the homogeneity study and the remaining bottles were used in the interlaboratory exercise.

Reconstitution of the Freeze-Dried Water Samples

About 0.5 g (accurately weighed) freeze-dried water residue was transferred to a clean glass bottle and dissolved in 0.5 l of bidistilled or HPLC-grade water at ambient temperature. The procedure recommended for sample reconstitution allowed two alternatives. One is based on the previous work of Barceló et al. [4] and involves the bubbling of CO_2 gas (purity > 99.998%) through the solution at a rate of 5–10 $ml.min^{-1}$ for a period of 30 min. The alternative procedure used the addition of a 10^{-3} $mol.l^{-1}$ HCl solution. Both procedures required a through magnetic stirrer.

Determination of Pesticides for Homogeneity and Stability Studies

Chemicals

High purity Milli-Q water (Millipore), RS-grade methanol and acetonitrile (Carlo Erba) were passed through a 0.45 µm nylon filter (Whatman) before use. Pesticides were obtained from Riedel-de-Häen. Stock standard solutions (1000 $mg.l^{-1}$) were prepared in acetonitrile and stored at –20 °C. Other chemicals were of analytical reagent grade.

Apparatus

The HPLC system consisted of a ConstaMetric 4100 Series high pressure pump from Thermo Separation Products coupled to a SpectroMonitor 5000 photo diode-array detector from LDC Analytical. The gas chromatograph used was a HP–5890-II with ECD from Hewlett Packard.

Sample Extraction

For atrazine, simazine, carbaryl, propanil, linuron and fenamiphos an on-line solid-phase extraction method coupled to LC-DAD was used as described previously [5]. Permethrin was determined after extraction of 250 ml of reconstituted water on a C_{18} Empore extraction disk (47 mm i.d.) using a Baker Empore Extraction System filtration apparatus from J. T. Baker. Desorption of permethrin from the disk was performed with four times 5 ml ethyl acetate. The solvent was evaporated to 5 ml under vacuum followed by flushing through an anhydrous Na_2SO_4 column. The permethrin retained in the column was eluted with four times 3 ml ethyl acetate. The eluate was evaporated under vacuum and flushed with helium to speed solvent evaporation to a final extract volume of 1 ml before GC-ECD.

Chromatographic Conditions

The determination of atrazine, simazine, carbaryl, propanil, linuron and fenamiphos was described previously[5]. After reconstitution of 0.51 1 of water, 40 ml were used for the on-line LC analysis. A Jones Chromatography precolumn (10 mm × 4.6 mm i.d.) packed with 5 μm Spherisorb ODS was coupled with the loop of a Rheodyne 7725i injection valve. The solid-phase extraction precolumn was first conditioned with 10 ml methanol and then 10 ml Milli-Q water at 1 $ml.min^{-1}$ using a Waters 590 pump. Water samples were preconcentrated on the precolumn at a flow-rate of 4 $ml.min^{-1}$. Following the preconcentration step, the injection valve was switched and the analytes were eluted in a back-flush mode and separated on a Zorbax column (25 cm × 4.6 mm i.d.) packed with 5 μm Zorbax ODS from Phenomenex. A gradient elution with a flow-rate of 1 $ml.min^{-1}$ was performed by running a mobile phase containing methanol-acetonitrile-water (25:25:50) up to methanol-acetonitrile (50:50) over 20 min followed by an isocratic mode for 10 min before returning to the initial conditions in 10 min. The pesticides were detected 220 nm for atrazine, simazine and carbaryl and 250 nm for propanil, linuron and fenamiphos. Quantitation was performed by linear calibration using peak area measurements with cyanazine as internal standard. Figure 1 shows chromatograms at 220 nm of a blank and of a water sample.

Cis- and trans-permethrin were analysed by GC-ECD using a 30 m × 0.32 mm i.d. fused silica capillary column with a 5% phenyl-methyl silicon stationary phase (HP-5, Hewlett Packard) with helium as carrier gas (9.5 $ml.min^{-1}$) and nitrogen as make-up gas (30 $ml.min^{-1}$). A 1.5 μl aliquot of extract was injected on-column with a 3 °C oven track. The detector temperature was 350 °C. The temperature program was as follows: the initial oven temperature of 55 °C was-increased at 40 $°C.min^{-1}$ to 160 °C for 6 min, and then at 40 $°C.min^{-1}$ to 240 °C

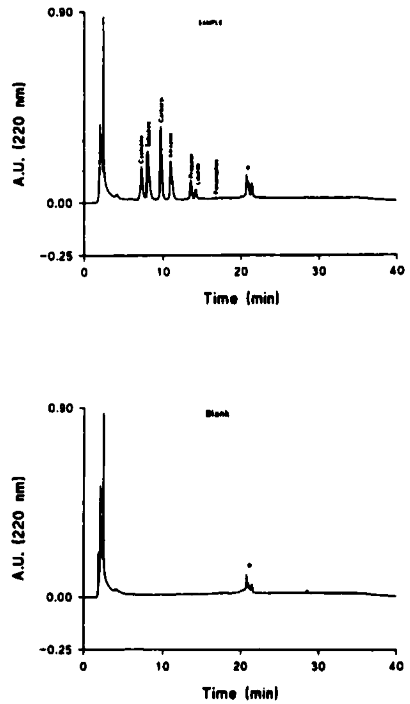


FIGURE 1 LC-DAD sample and blank chromatograms at 220 nm obtained from reconstituted freeze-dried blank (bottom) and spiked (top) water samples. LC conditions: See Experimental section. (*) Unknown peaks

for 0.5 min followed by a ramp of 3 °C.min^{-1} to 250 °C and held for 5 min to complete the program. Quantitation was performed by linear calibration over a concentration range of 100 to 400 μg.l^{-1} using 4,4'-dichlorobifenyl as internal standard.

RESULTS AND DISCUSSION

Pesticide Losses by the Lyophilization

Little is known about the behaviour of compounds during the freeze-drying process. The losses can be affected by the properties of each compound such as solubility in water and vapour pressure [4]. In order to investigate the way in which the pesticides are incorporated into the lyophilised powder, the losses of the pesticides during the freeze-drying process were calculated.

Table I shows the amount of each pesticide added before lyophilization and the percentage found after analysis of reconstituted freeze-dried water samples. The solubility in water and vapour pressure of each compound are also given [6–8]. It was expected that the higher the vapour pressure of the pesticides, the greater would be the loss. However, the results do not explain the behaviour of each compound and the high saline content in the samples may play an important role in the lyophilization process.

TABLE I Amount Added Before and Found After Lyophilization and Physical Properties of the Pesticides Studied

Pesticide	*Amount added ($\mu g.g^{-1}$)*	*Mass fraction recovered (%)*	*Vapour pressure (mPa)*	*Solubility ($mg.l^{-1}$)*
Simazine	29.94	72.5	0.0085	5
Atrazine	24.14	44.0	0.04	30
Carbaryl	22.52	37.2	0.30	40
Propanil	21.50	51.1	3.60	268
Linuron	24.21	20.6	1.40	75
Fenamiphos	27.73	14.3	0.13	700
Permethrin	23.17	4.3	0.0025	0.20

The permethrin is almost totally lost during freeze-drying, whereas simazine shows only 28% decrease.

Homogeneity Studies

The homogeneity of each pesticide in the freeze-dried water residue was measured by analysing 10 bottles (three replicates from each reconstituted water), and comparing the results obtained with the mean value ± standard deviation of ten independent determinations for one reconstituted sample. For permethrin only one determination per bottle was possible.

The results obtained are shown in Table II. No inhomogenity could be demonstrated for atrazine, carbaryl, propanil, linuron and fenamiphos. The homogeneity of simazine could not be demonstrated even using a criterion of mean value ± 2 standard deviations. The results for permethrin indicate that this compound was not suitable in such a reference material.

Stability Studies

A preliminary study was carried out by determining atrazine, simazine, carbaryl, propanil, linuron and fenamiphos in five samples stored at –20 °C and another five at room temperature for a maximum period of three months.

The stability at –20 °C and at 20 °C was evaluated by comparing results obtained at +20 °C or –20 °C versus those of samples recently prepared (t = 0):

$$R = X/X_{(t=0)}$$

The combined uncertainty (Ut) of the measurements

$$Ut = (CV^2 + CV^2_{(t=0)})^{1/2} \cdot R/100$$

where X is the mean value of the measurements made in different periods and CV the coefficient of variation of X.

Figures 2 and 3 plot R versus storage time for each pesticide at –20 °C and 20 °C, respectively. Each pesticide is considered stable when R value is within the interval 1 - Ut and 1 + Ut. The results clearly indicate that all the pesticides are stable in the freeze-dried powder for the storage time of three months at both temperatures, except for fenamiphos at 20 °C, whose concentration decreased about 71% and 77% after one and three months of storage, respectively.

These results are in agreement with those reported in the literature[4]. Thus, atrazine, simazine, carbaryl, propanil and linuron showed good stability for three months at 20 °C in freeze-dried material, whereas fenamiphos suffered a loss of 25%. This different behaviour might be related to the different type of water used and the absence of glycine in the present study. The different behaviour may indicate that glycine gave a kind of protection to the samples.

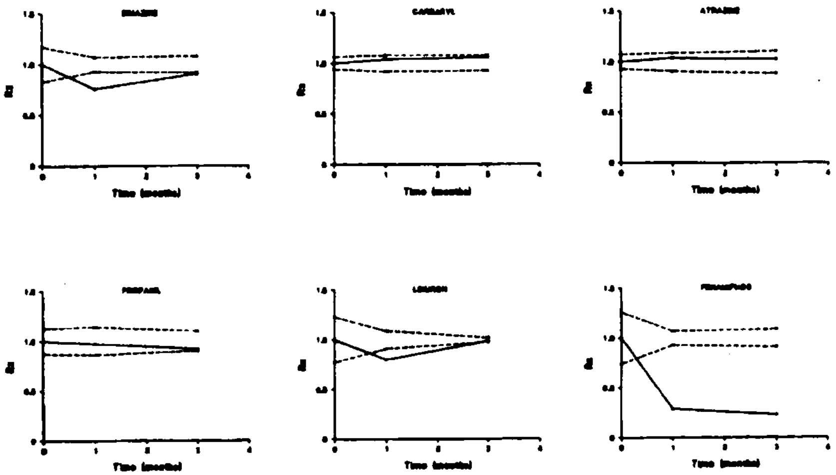


FIGURE 2 Stability of pesticides in freeze-dried material at +20°C for three months. (–): R; (- - -): 1 + Ut and 1 - Ut

TABLE II Results of the Sample Homogeneity Study for Selected Pesticides. Concentrations are Given in μg.g^{-1} of Reconstituted Water with Standard Deviations (x ± SD)

	M-33	*M-5*	*M-47*	*M-52*	*M-2*	*M-63*	*M-29*	*M-41*	*M-17*	*M-61*
Simazine	20.96 ± 0.98	24.00 ± 0.19	16.88 ± 0.20	19.65 ± 0.80	20.16 ± 0.53	26.86 ± 0.27	19.77 ± 0.82	23.24 ± 0.59	23.63 ± 0.40	15.80 ± 0.75
Carbaryl	9.11 ± 0.30	8.75 ± 0.20	8.89 ± 0.80	8.67 ± 0.19	9.06 ± 0.28	8.76 ± 0.53	8.74 ± 0.54	8.24 ± 0.40	9.69 ± 0.82	9.34 ± 0.75
Atrazine	9.81 ± 0.81	9.74 ± 0.63	9.53 ± 0.54	9.64 ± 0.79	9.74 ± 0.62	9.32 ± 0.42	10.35 ± 0.83	9.58 ± 0.54	10.45 ± 0.65	10.36 ± 0.75
Propanil	10.07 ± 1.00	10.49 ± 1.21	10.55 ± 0.87	9.86 ± 0.80	10.55 ± 1.01	10.31 ± 1.10	12.86 ± 0.70	10.85 ± 1.20	12.09 ± 0.95	11.72 ± 0.86
Linuron	4.96 ± 0.82	5.27 ± 0.58	5.22 ± 0.41	5.03 ± 0.62	5.26 ± 0.68	5.60 ± 0.47	5.48 ± 0.33	5.99 ± 0.52	5.22 ± 0.79	5.84 ± 0.60
Fenamiphos	4.74 ± 0.63	4.92 ± 0.39	4.88 ± 0.60	4.35 ± 0.73	3.79 ± 0.51	4.25 ± 0.29	4.71 ± 0.51	4.45 ± 0.82	4.21 ± 0.61	4.59 ± 0.82
c-Permethrin	0.18 ± 0.06	0.15 ± 0.05	0.29 ± 0.04	0.16 ± 0.02	0.23 ± 0.03	0.20 ± 0.03	0.11 ± 0.02	0.10 ± 0.02	0.14 ± 0.02	0.21 ± 0.03
t-Permethrin	0.82 ± 0.14	0.23 ± 0.04	1.09 ± 0.19	0.91 ± 0.15	0.84 ± 0.14	0.68 ± 0.12	0.47 ± 0.08	0.74 ± 0.13	0.79 ± 0.13	0.71 ± 0.12

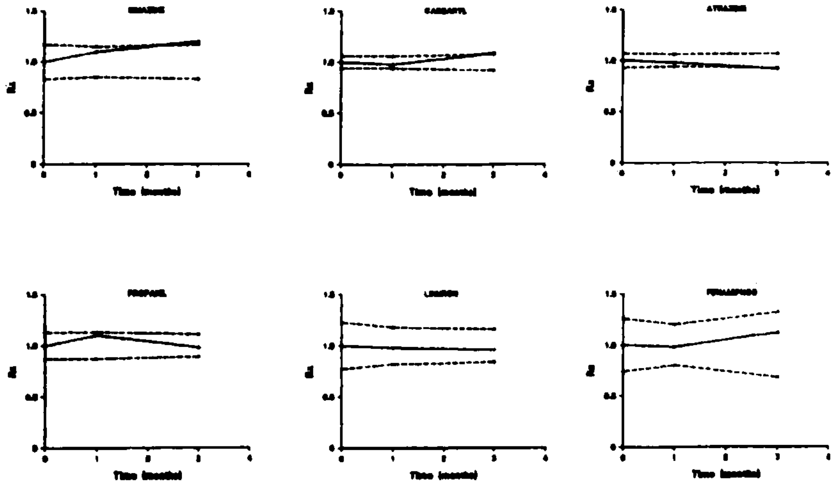


FIGURE 3 Stability of pesticides in freeze-dried material at –20°C for three months. (–): R; (- - -): 1 + Ut and 1 - Ut

It seems that most of the fenamiphos loss occurred during the first month of storage. Similar behaviour has been reported for trifluralin and pendimethalin by Senseman et al. [9] who observed significant reductions in pesticide recoveries after 10 days compared to 3 days of storage on solid-phase extraction disks.

Therefore further experiments must be carried out to permit an analysis of the kinetics and to accurately determine the maximum storage time without fenamiphos losses for the preparation of a possible CRM.

Interlaboratory Study

Analytical methods

Table III summarises the different pretreatment, clean-up, chromatographic separation and final detection techniques used by the 20 laboratories which participated in the programme.

The pesticides were preconcentrated by solid-phase extraction onto different sorbent materials (e.g. C_8, C_{18}, PLRP-S). When GC was used the pesticides extract was dried on anhydrous Na_2SO_4 and preconcentrated by rotary evaporation or under dry N_2 gas.

TABLE III Summary of Pretreatment. Chromatographic Analysis and Detection Techniques used by the Different Participant Laboratories

Preconcentration/extraction	*Chromatographic analysis*	*Detection*
SPE(C_{18})/Drying Na_2SO_4/ Rotary evaporation	GC on capillary columns	NPD
SPE (C_8,C_{18},PLRP-S)	HPLC on C_{18} columns	UV
SPE (C_{18})	GC on capillary columns	MS
SPE (C_{18})/Drying Na_2SO_4/ Turbovap concentration	GC on capillary columns	ECD FTD

Separation and detection was generally performed by capillary column GC (e.g. CpSil5, DB 5) with ECD, NPD and flame thermoionic detector (FTD), or reverse-phase HPLC (e.g. Zorbax ODS, Kromasil ODS) with DAD and UV detection.

The results of the intercomparison exercise were discussed by the participants in a technical meeting.

Each participant received one freeze-dried water sample of pesticides and one blank to be analysed. Each bottle contained about 2.5 g residue. Those applying two different analytical methods received two bottles of each freeze-dried sample.

Each laboratory was requested to make a minimum of five independent analyses of both the water blank and spiked water samples on two different days. To estimate the pesticide recovery from one litre of water the standard addition method was applied for each pesticide or was estimated by reextraction. The methods included extraction, clean-up and/or fractionation steps chosen by each participant. For GC analysis, at least two internal standards were used for the final determination. For LC, the use of one internal standard was required. The participants were allowed to choose between two GC columns of different polarity to confirm the identity and purity of each pesticide peak.

Each participant had to verify the linear response of the detector in the calibration range for each pesticide. A chromatogram of the blank, the sample and of one of the standard solutions used were also required. Each participant received from the coordinator pure pesticides of certified purity with which to prepare the standard solutions or to verify the laboratory working solutions. Between 3 to 5 calibration points were requested.

Results

The interlaboratory exercise enabled the identification of some problems and sources of error occurring in the determination of pesticides. Extensive work was carried out to evaluate the analytical data from each participant. The results were presented in the form of bar-graphs indicating the laboratory codes along with the methods used, the mean of all individual values and the mean of the laboratory mean values with the corresponding standard deviations (Figures 4–6). These bar-graphs were used in the technical discussion. Some remarks were drawn from the discussion of the results between participants.

CARBARYL

The results for carbaryl confirmed its high sensitivity to light and temperature by degradation into l-naphthol in acetonitrile. The chromatogram of some participants contained two analytical peaks for this pesticide. Therefore it was recommended to store the sample and calibrants in the dark at low temperature (in the fridge). It was also stressed to avoid excessive sample heating in particular during the concentration steps.

SIMAZINE

The agreement between laboratories was less good than for atrazine. This may be due to the lack of homogeneity of the samples for this pesticide as already suspected by the homogeneity study.

FENAMIPHOS

Fenamiphos was one of the more difficult pesticides to analyse because of the instability at room temperature. Thus it was concluded that the next evaluation of stability should be done with higher frequency than in the present study.

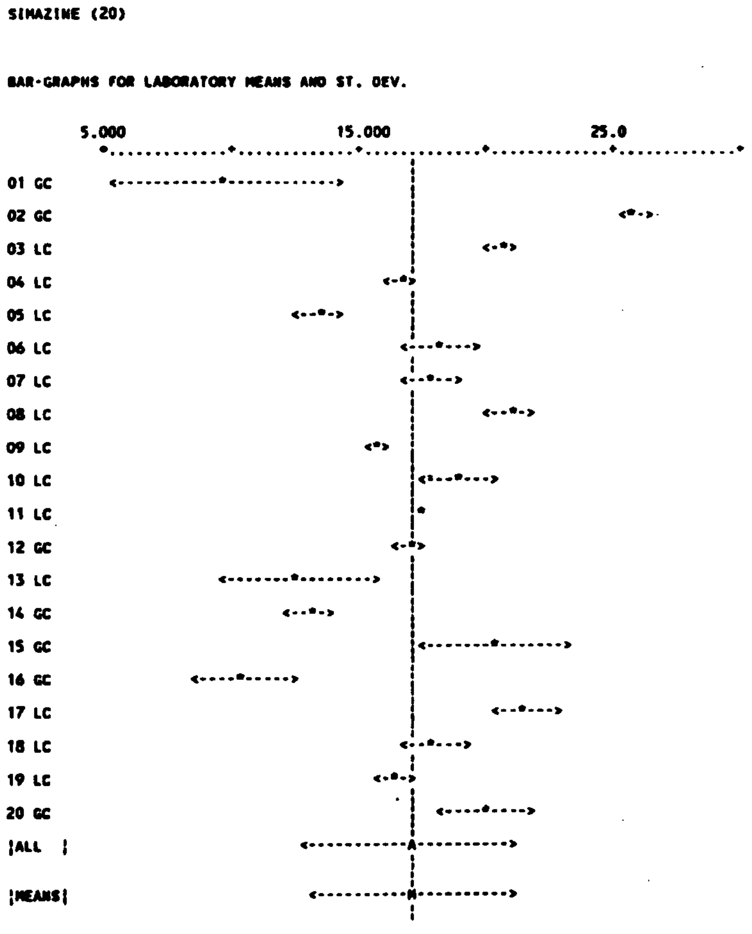
PERMETHRIN

Not many laboratories submitted results for permethrin and in general the results showed poor agreement. This was attributed to the low analyte concentration, which was close to the limit of detection of many methods. Other possible sources of error came from the insufficient separation of cis- and trans-permethrin isomers.

Table IV shows a list of values which correspond to the mean and the standard deviation of replicate determinations obtained by the different participants for each pesticide in the sample.

CONCLUSIONS

The addition of sodium chloride for the lyophilization was a clear advantage with respect to the addition of glycine used in a previous study.



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MEAN OF ALL INDIVIDUAL VALUES :   17.01815   ST.DEV. :   4.12421

MEAN OF MEAN VALUES           :   17.26586   ST.DEV. :   3.96871
```

FIGURE 4 Bar-graph presentation of the results obtained in the interlaboratory exercise for simazine ($\mu g.g^{-1}$) in the reconstituted sample. The results plotted correspond to three or five replicate determinations. M is the mean of the laboratory means. A is the mean of all individual results

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CARBARYL (16)

        SELECTED DATA

BAR-GRAPHS FOR LABORATORY MEANS AND ST. DEV.
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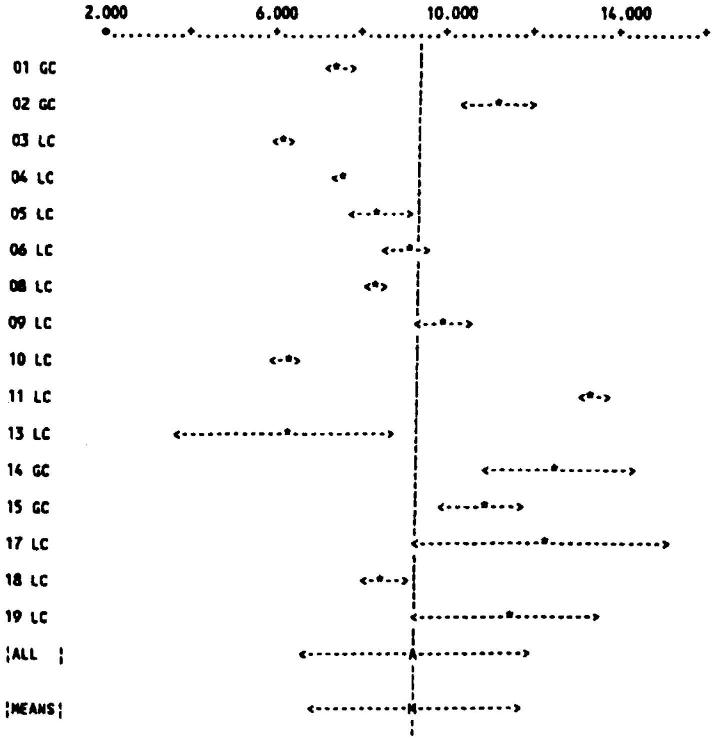

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MEAN OF ALL INDIVIDUAL VALUES :     9.48752      ST.DEV. :      2.59371

MEAN OF MEAN VALUES           :     9.42929      ST.DEV. :      2.38286
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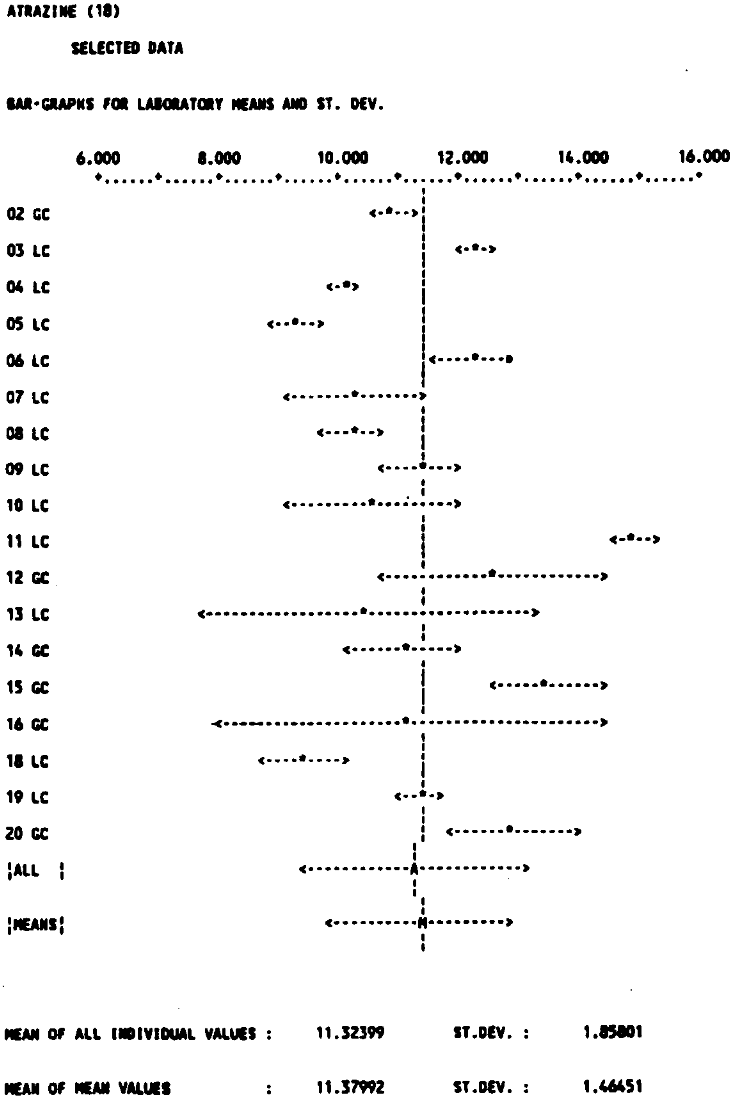
FIGURE 5 Bar-graph presentation of the results obtained in the interlaboratory exercise for carbaryl ($\mu g.g^{-1}$) in the reconstituted sample



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MEAN OF ALL INDIVIDUAL VALUES :   11.32399      ST.DEV. :     1.85801

MEAN OF MEAN VALUES           :   11.37992      ST.DEV. :     1.46451
```

FIGURE 6 Bar-graph presentation of the results obtained in the interlaboratory exercise for atrazine ($\mu g.g^{-1}$) in reconstituted sample

TABLE IV Mean Values ($\mu g.g^{-1}$) Obtained by the Different Laboratories for Each Pesticide in the Freeze-Dried Water Samples.

Laboratory	*Atrazine*	*Simazine*	*Carbaryl*	*Propanil*	*Linuron*	*Fenamiphos*	*Permethrin*
01	**	9.81 ± 4.55	7.50 ± 0.27	10.47 ± 0.39	5.05 ± 0.08	1.94 ± 0.72	*
02	10.93 ± 0.32	25.83 ± 0.43	11.23 ± 0.78	10.38 ± 0.51	6.63 ± 0.33	1.43 ± 0.03	*
03	12.26 ± 0.28	20.57 ± 0.52	6.17 ± 0.14	12.15 ± 0.17	5.13 ± 0.22	1.16 ± 0.09	*
04	10.10 ± 0.26	16.76 ± 0.53	7.52 ± 0.13	**	5.66 ± 0.09	0.58 ± 0.04	**
05	9.28 ± 0.41	13.40 ± 1.04	8.50 ± 0.78	9.36 ± 0.65	4.78 ± 0.51	1.26 ± 0.23	0.62 ± 0.07
06	12.24 ± 0.66	18.28 ± 1.43	9.10 ± 0.59	12.22 ± 0.87	5.40 ± 0.44	1.28 ± 0.13	0.74 ± 0.55
07	10.29 ± 1.08	17.90 ± 0.97	*	11.82 ± 0.31	5.11 ± 0.19	*	**
08	10.26 ± 0.49	20.93 ± 0.87	8.48 ± 0.21	10.92 ± 1.41	5.00 ± 0.56	1.13 ± 0.14	0.99 ± 0.29
09	11.41 ± 0.63	15.65 ± 0.37	10.03 ± 0.59	10.95 ± 0.53	5.61 ± 0.46	*	**
10	10.54 ± 1.46	18.98 ± 1.48	6.31 ± 0.30	12.84 ± 0.14	4.44 ± 0.01	1.26 ± 0.06	0.069 ± 0.003
11	14.93 ± 0.36	17.46 ± 0.11	13.48 ± 0.30	13.94 ± 0.45	5.50 ± 0.08	1.43 ± 0.14	**
12	12.60 ± 1.89	17.07 ± 0.53	**	12.56 ± 0.50	7.35 ± 0.28	*	**
13	10.50 ± 2.73	12.64 ± 2.98	6.39 ± 2.50	11.68 ± 2.63	4.06 ± 1.00	0.78 ± 0.11	**
14	11.07 ± 0.91	13.09 ± 0.78	12.69 ± 1.77	12.00 ± 1.71	7.31 ± 1.05	0.88 ± 0.30	0.22 ± 0.13
15	13.50 ± 0.98	20.47 ± 2.91	10.92 ± 0.88	11.60 ± 0.66	5.50 ± 0.94	2.07 ± 0.13	0.98 ± 0.17
16	11.19 ± 3.25	10.41 ± 2.00	*	**	**	0.52 ± 0.36	**
17	**	21.55 ± 1.16	12.33 ± 2.87	**	**	*	**
18	9.46 ± 0.68	17.98 ± 1.27	8.66 ± 0.46	14.14 ± 3.09	5.54 ± 0.26	0.92 ± 0.56	**
19	11.37 ± 0.35	16.43 ± 0.82	9.92 ± 2.06	10.19 ± 1.87	4.84 ± 0.72	*	0.16 ± 0.03
20	12.92 ± 1.08	20.12 ± 1.73	*	**	*	**	*
Mean	11.38	17.27	9.43	11.70	5.47	1.19	0.54
SD	1.46	3.97	2.38	1.31	0.90	0.45	0.39

*Not analysed
**Outlier

The results indicate that losses of the pesticides during the lyophilization process were not in direct relation with the solubility in water or vapour pressure of the compound.

All permethrin is almost lost (95.7%) during the freeze-drying process, obliging to increase the spiking level for this compound for a possible reference material.

The results obtained in the interlaboratory study are in sufficient agreement for most of the pesticides tested. Still there are some discrepancies for permethrin which may be related to the low concentration in the material but it could be concluded that this freeze-drying procedure of water can be applied for the preparation of a reference material and the certification of some compounds filling the actual lack of CRM in this field.

Therefore, six thousand litres of water spiked with the above studied pesticides have been lyophilised and the homogeneity and the stability studies have been started. Their certification will follow the interlaboratory approach.

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