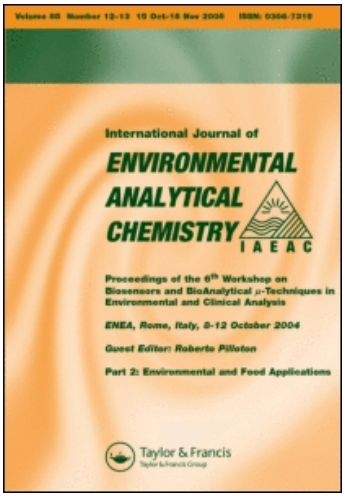


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# Assessment of the performance of nine Greek laboratories through a proficiency test on the analysis of grapes for the determination of residues of pesticides

George E. Miliadis[a]; Konstantinos S. Liapis[a]; Panayota Th. Malatou[a]
[a] Benaki Phytopathological Institute, Kifissia, Greece

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# ASSESSMENT OF THE PERFORMANCE OF NINE GREEK LABORATORIES THROUGH A PROFICIENCY TEST ON THE ANALYSIS OF GRAPES FOR THE DETERMINATION OF RESIDUES OF PESTICIDES

GEORGE E. MILIADIS*, KONSTANTINOS S. LIAPIS
and PANAYOTA TH. MALATOU

*Benaki Phytopathological Institute, 7 Ekalis Str., Kifissia, 145 61, Greece*

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A test material of grape homogenate spiked with 10 pesticides at concentrations 0.005–1.25 mg/kg was distributed to 11 Greek laboratories for analysis. The test material was checked by the organizer for homogeneity and stability and found homogeneous and stable. Ten laboratories reported results, one of them considered to be an independent laboratory. The assigned value for each analyte was the spiking concentration and the target standard deviation was evaluated from the Horwitz equation. Out of 36 *z*-score values 31 were acceptable, 4 questionable and 1 unacceptable. The overall performance of the laboratories was also estimated, one laboratory failing to the sum of squared *z*-test.

*Keywords:* Pesticides; Grapes; Proficiency test

## INTRODUCTION

Proficiency testing of laboratories is a requirement for accreditation together with the use of validated methods. The Greek National Accreditation Council (E.SY.D.) assessments use information from the proficiency tests to assess the reliability of the data produced from the laboratories. It is also known that food analysis for the detection and determination of pesticide residues at the parts-per-billion levels required by the EU and other legislative bodies presents particular analytical difficulty. As a result the quality of some data has in the past been proved to be inadequate. Customers of the laboratory who, in the case of state laboratories, may be the consumer, demand independent proof of reliability and competence. In addition, laboratory accreditation and proficiency testing are important requirements of the

*Corresponding author. Fax: +30-210-8077506. E-mail: bpipest@otenet.gr

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EU Directive for laboratories in the Member States that generate monitoring data for submission to the Commission [1].

To the best of our knowledge, there are 16 laboratories in Greece performing food analysis for pesticide residues on a regular basis. Seven of them are state laboratories, which perform the official control, six are owned by private companies, performing analysis when a certificate is required for a product and, three are University laboratories, mainly conducting research studies. All the private and two of the state laboratories are either already accredited or are undergoing the procedure for accreditation assessment.

Two 3-day, seminars were organized in the year 2001 by the Benaki Phytopathological Institute on analytical aspects concerning residues of pesticides in food and water. Laboratories known to perform such analyses in Greece were invited and participated in those seminars. As a result of discussions during the meeting, it was decided that the host laboratory would organize a proficiency test for the participating laboratories, to evaluate their performance. Eleven laboratories accepted the invitation. Five of these were state laboratories, four were owned by private companies and two were University laboratories.



## EXPERIMENTAL

The general rules of AOAC International's harmonized protocol for proficiency testing of analytical laboratories [2] and the FAPAS protocol for food analysis performance assessment scheme [3] were roughly followed. Each laboratory was given a code number, known only to the particular laboratory and the organizer. A list with 61 pesticides, their maximum residue limits (MRL) and their limits of quantitation (LOQ) in grapes was distributed to the 11 participating laboratories and they were asked to indicate which pesticides they could analyze. Ten pesticides were then selected by the organizer to be included in the test based on the following criteria: the pesticides should be (a) used in viticulture in Greece, (b) belong to different chemical classes, and c) be analyzed by most of the participating laboratories, according to their statement, as shown in the second column of Table I. A spiking solution was then prepared in acetone containing the 10 selected pesticides.

TABLE I Data on the pesticides used and the number of participating laboratories

| *Analyte* | *Number of laboratories* | *MRL* (mg/kg) | *LOQ*[a] (mg/kg) | *C* (mg/kg) | $\sigma_{Horwitz}$ |
|---|---|---|---|---|---|
| Azinphos-methyl | 8 | 1 | | 0.625 | 0.107 |
| Chlorpyriphos-ethyl | 9 | 0.5 | 0.05 | 0.3125 | 0.0595 |
| λ-Cyhalothrin | 4 | 0.2 | | 0.2 | 0.0408 |
| Diazinon[b] | 10 | 0.02 | 0.02 | 0.0075 | 0.00251 |
| α-Endosulfan[b] | 7 | 0.5 | 0.05 | 0.01 | 0.0032 |
| Malathion[b] | 8 | 0.5 | | 0.005 | 0.00177 |
| Methamidophos | 7 | 0.01 | 0.01 | 0.375 | 0.0695 |
| Parathion | 8 | 0.5 | | 0.4375 | 0.0793 |
| Procymidone | 6 | 5 | 0.02 | 1.25 | 0.193 |
| Vinclozolin | 7 | 5 | 0.05 | 0.5 | 0.0888 |

[a]EU, indicative; [b]pesticides for which spiking concentration was lower than the LOQ.

The test material was grapes grown in Corinth, Peloponnese. The field treatment included only the use of sulfur three months prior to harvest. The grapes were shipped to the organizer's laboratory, where, after removal of the stems, approximately 10 kg of grapes were chopped and homogenized with a high-speed mixer. The homogenate was divided into two equal portions. The first portion was used as control and the second portion was fortified with the spiking solution, under continuous blending, so that the concentrations of the 10 pesticides in the grape homogenate were between 0.005 and 1.25 mg/kg, as shown in Table I. In the same table the MRLs and the LOQs are also shown for each pesticide in grapes. The control samples and the spiked grape test material were weighed (~220 g) in polyethylene bags and stored for one night at $-18^{\circ}$C prior to shipment to the participating laboratories, on 16 January 2002. The number and the names of the pesticides used were not announced to the participating laboratories.

Ten bags of the test material, randomly selected, were analyzed in duplicate by the organizer for controlling the homogeneity of the material. The method used was the Dutch multiresidue [4] and the determination was performed by GC with NP and EC detectors, as well as by negative chemical ionization mass spectrometry. For quantitation matrix-matched standard solutions were used. Two extra bags were stored at $-18^{\circ}$C for 21 and 42 days respectively and each analyzed in duplicate for confirming the stability of the pesticides for the time period of the test.



## RESULTS AND DISCUSSION

The assigned value for the concentration of the analytes was decided to be the spiking concentration for the following reasons: (a) the number of participating laboratories was insufficient to produce a robust mean value, (b) the grape test material was known and proved to be free of the analytes and, (c) the homogeneity of the test material for the analytes was found satisfactory.

As target value for the standard deviation the value evaluated from the Horwitz equation $\sigma_H = 0.02c^{0.8495}$ was chosen, since for most analytes it produces values of target RSD very close to 20%, which is the required RSD for obtaining recoveries 60–140%, as set by the Commission guidelines document for the acceptability of analytical performance [5].

The homogeneity of the test material was assessed by estimating the sampling variance $s_s^2$ and the analytical variance $s_a^2$ by one-way analysis of variance (ANOVA). Both F-test and $s_s/\sigma_H$ were carried out. The results of the homogeneity test were satisfactory for all analytes except for methamidophos.Two of the analytes (azinphos methyl and $\alpha$-endosulfan) were found with a statistically significant difference between samples (failed the F-test) but the test material was considered sufficiently homogeneous for them, as $s_s/\sigma$ was less than the maximum recommended value of 0.3 for endosulfan or 0.4 for azinphos methyl, as recommended by Thompson and Lowthian [6]. The reason for the homogeneity test being found unacceptable for methamidophos is possibly that the extraction method used was unsatisfactory for this analyte, as it presented a very low mean recovery equal to 43% ($N = 20$) and a high RSD = 20%, while the RSD for all other analytes was <10%. A summary of the results of the homogeneity test, as well as the results of the stability test are presented in Table II. In the same table the results of the analysis of the test material

TABLE II Results of homogeneity and stability tests and results of the analysis by the independent laboratory for the ten analytes applied

| *Analyte* | *Spiking C* (mg/kg) | *Homogeneity* (mg/kg) *2nd day* ($N=20$) | *Stability* (mg/kg) $N=2$ | | *Independent lab* (mg/kg)[a] |
|---|---|---|---|---|---|
| | | | *21st day* | *42nd day* | |
| Azinphos methyl | 0.625 | $0.635 \pm 0.046$ | 0.636 | 0.557 | – |
| Chlorpyriphos ethyl | 0.3125 | $0.353 \pm 0.021$ | 0.304 | 0.313 | 0.214 |
| λ-Cyhalothrin | 0.2 | $0.204 \pm 0.014$ | 0.194 | 0.190 | 0.208 |
| Diazinon[b] | 0.0075 | $0.0083 \pm 0.0009$ | 0.0071 | 0.0056 | 0.0065 |
| Endosulfan-α[b] | 0.01 | $0.0091 \pm 0.0009$ | 0.0091 | 0.0107 | 0.005 |
| Malathion[b] | 0.005 | $0.0053 \pm 0.0002$ | 0.0047 | 0.0052 | 0.005 |
| Methamidophos | 0.375 | $0.161^{c} \pm 0.031$ | 0.143[c] | 0.132[c] | 0.329 |
| Parathion | 0.4375 | $0.497 \pm 0.028$ | 0.439 | 0.449 | 0.380 |
| Procymidone | 1.25 | $1.300 \pm 0.0665$ | 1.235 | 1.248 | 0.900 |
| Vinclozolin | 0.5 | $0.526 \pm 0.019$ | 0.470 | 0.478 | 0.408 |

[a]Analyzed following the 42nd day; [b]spiking concentration lower than the limit of quantitation; [c]low recovery (43%) attained with the method used.



by the pesticide residues laboratory of the General State Chemical Laboratory, which was considered to be an independent laboratory, are also given. The results of the stability test were found to be within the $\bar{x} \pm 3s$ range, except for diazinon on the 42nd day, showing good agreement with the results of the homogeneity test ($\bar{x}$ and $s$). It was therefore suggested that the material was stable over the period of the test.

Nine of the 11 laboratories that received the test material submitted results, one laboratory submitted results as an independent laboratory as already mentioned, and one laboratory did not submit any result. Unfortunately by mistake the spiking concentrations of 3 analytes (diazinon, α-endosulfan and malathion) were lower than the LOQs. As soon as this was discovered the participating laboratories were informed of the names of these analytes. It is interesting to note that quantitation of the three analytes was not difficult for the organizer during the homogeneity and stability tests. However only three laboratories submitted results for diazinon and none did so for α-endosulfan or malathion. The participating laboratories did not know the names and numbers of the seven other laboratories.

The laboratories used their routine analytical methods and did not correct the results for the recovery. The performance of the laboratories was assessed with the $z$-score, calculated as

$$z = \frac{x - \hat{x}}{\sigma},$$

where $x$ is the reported result, $\hat{x}$ the assigned value and $\sigma$ the target value for standard deviation. Values $|z| \leq 2.0$ are considered as acceptable, $2.0 < |z| < 3.0$ questionable and $|z| \geq 3.0$ as unacceptable. False positives (pesticides found in the sample while not being present) were ignored but no $z$-score was given. False negatives (pesticides from the analytes sought by a laboratory but not found) were considered as having concentration equal to the LOQ of the reporting laboratory and were given a $z$-score. The $z$-score values obtained are presented in Table III, in which 31 of the 36 values are

TABLE III Performance of the participating laboratories expressed by the $z$-score values

| *Analyte* | *Laboratory code number* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *02* | *03* | *04* | *05* | *06* | *07* | *08* | *10* | *11* |
| Azinphos-methyl | | 2.20 | −1.16 | 0.63 | −1.05 | | −2.35 | | 1.37 |
| Chlorpyriphos-ethyl | | −0.55 | −0.21 | | −1.32 | −1.86 | −1.82 | −0.60 | 0.46 |
| λ-Cyhalothrin | | | | | 0.15 | | | | |
| Diazinon[a] | | | | | −0.33 | | −0.20 | | −0.80 |
| Endosulfan-α[a] | | | | | | | | | |
| Malathion[a] | | | | | | | | | |
| Methamidophos | | | −0.36 | −1.94 | −1.40 | −1.50 | | | |
| Parathion | −1.00 | | 0.03 | | −1.13 | −2.59 | −1.25 | | |
| Procymidone | | 0.08 | | | | | −0.93 | | −0.26 |
| Vinclozolin | −1.53 | 0.28 | | −5.00 | −1.64 | | 0.13 | −2.13 | 0.20 |

[a]Analytes with $c < \text{LOQ}$.

acceptable, 4 questionable and 1 unacceptable (this was the case of a false negative result). False positives reported were methidathion at 0.04 mg/kg and parathion methyl at 0.009 mg/kg.

For obtaining an indication of the overall performance of the participating laboratories the combination score SSZ (sum of squared $z$-scores) was used. This method ignores the signs of the $z$ values, as it uses the squared ($z^2$) values. One of the laboratories (Lab 05) had a significant SSZ value at the 0.27% probability level, that corresponds to $z$-score 3, and one laboratory (Lab 07) at the 4.55% probability level, corresponding to $z$-score 2. All other laboratories had no significant (acceptable) SSZ values, showing that the results of these laboratories as a group do not indicate any unusual source of error.

Assessment for normality of the distribution of the results was performed only for analytes for which at least five laboratories had reported (azinphos methyl, chlorpyriphos ethyl, parathion and vinclozolin). Skewness and kurtosis tests by the SPSS statistical program were used and the analysis revealed a normal distribution for all four analytes checked.

In reporting the conclusions of this proficiency testing the remarkable interest in participating of the Greek pesticide residues laboratories should be mentioned. Considering the fact that nine of the participating laboratories are not accredited, and that for most of the laboratories this was their first participation in such tests, it is highly encouraging that their performance was acceptable in most cases.

It is, however, desirable that in any such future tests the laboratories should increase the number of analytes for which they can report results.

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