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# Comparison of two proficiency tests on pesticide residues in olive oil conducted in 2017 and 2018

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*Dipartimento Ambiente e Salute*

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2020, v, 35 p. Rapporti ISTISAN 20/5

The Italian National Reference Laboratory (NRL) for pesticide residues annually organizes Proficiency Test (PT) for the determination of pesticide residues in olive oil. European laboratories (NRLs, official laboratories and private laboratories) especially Italian official laboratories involved in the National and European monitoring programs participate in these PTs. This participation is mandatory as set out by the ISO/IEC 17025 and verified by accreditation bodies. The main aim of these PTs is to compare the performances of European laboratories, involved in the analysis of pesticide residues in olive oil, to promote mutual acceptance of pesticide residue data regarding the analytical controls on olive oil. In this paper, two PTs of pesticide residues in olive oil named COIPT-17 and COIPT-18 are described and a comparison of their respective results is analysed.
*Key words*: Pesticide residues; Proficiency Test; Olive oil; Results Comparison

Istituto Superiore di Sanità
**Confronto di due circuiti interlaboratorio sui residui di pesticidi nell'olio d'oliva condotti nel 2017 e nel 2018.**
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2020, v, 35 p. Rapporti ISTISAN 20/5 (in inglese)

Il Laboratorio Nazionale di Riferimento italiano (LNR) per i residui di pesticidi organizza annualmente un circuito interlaboratorio per la determinazione dei residui di pesticidi nell'olio d'oliva. A questi circuito partecipano laboratori europei (LNR, laboratori ufficiali e laboratori privati) soprattutto i laboratori ufficiali italiani coinvolti nei programmi di monitoraggio nazionali ed europei. Questa partecipazione è obbligatoria secondo quanto stabilito dalla norma ISO/IEC 17025 e verificata dagli organismi di accreditamento. Lo scopo principale di questi circuiti interlaboratorio è quello di confrontare le prestazioni dei laboratori europei, coinvolti nell'analisi dei residui di pesticidi nell'olio d'oliva, per promuovere l'accettazione reciproca dei dati sui residui di pesticidi relativi ai controlli analitici sull'olio d'oliva.
In questo documento vengono descritti due circuiti interlaboratorio sui residui di pesticidi nell'olio di oliva denominati COIPT-17 e COIPT-18 e viene analizzato un confronto dei rispettivi risultati.
*Parole chiave*: Residui di antiparassitari; Circuiti interlaboratorio; Olio di oliva; Confronto dei risultati

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# ABBREVIATIONS

| | |
|---|---|
| **$AZ^2$** | Average of the Squared z-scores |
| **EUPT** | European Union Proficiency Test |
| **EURL** | European Reference Laboratory |
| **FFP RSD** | Fit-For-Purpose Relative target Standard Deviation |
| **GC** | Gas Chromatography |
| **ILAC** | International Laboratory Accreditation Cooperation |
| **ISO** | International Organization for Standardization |
| **LC** | Liquid Chromatography |
| **LOQ** | Limit of quantification |
| **MRMs** | Multi-Residue Methods |
| **MS** | Mass Spectrometry |
| **NRL** | National Reference Laboratory |
| **PT** | Proficiency Test |
| **RSD** | Relative Standard Deviation |
| **RSDr** | Repeatability Relative Standard Deviation |
| **SD** | Standard Deviation |
| **UHPLC** | Ultra High-Performance Liquid Chromatography |

## Symbols

| | |
|---|---|
| $s^*$ | *robust standard deviation* |
| $u$ | *uncertainty of the consensus value* |
| $\sigma_{EUPT}$ | *target standard deviation* |
| $Xpt$ | *consensus value* |

# PREFACE

Food safety has become a priority for all European citizens and for this reason governments and legislators have increased food controls and surveillance.

A network of National Reference Laboratories (NRLs) and Official Control Laboratories (OCLs) has been defined in order to improve and harmonize the quality, accuracy and comparability of the analytical results regarding the determination of pesticide residues in food (1).

The current European legislation on this topic requires OCLs to participate in specific Proficiency Tests (PTs), including those organized by the NRLs, in order to assess the quality of their results.

This participation is mandatory as set out by the ISO/IEC 17025 (2) and verified by accreditation bodies. In particular, all the official laboratories, involved in the EU coordinated control pesticide residue monitoring programs, follow the same European analytical quality control technical guidance document SANTE/11813/2017 (3).

Analysis of pesticide residues in food is usually carried out by using Multi-Residue Methods (MRMs) (4-6). This is a consequence of the large number of compounds enclosed in the pesticide target list of the official controls.

Generally, the performance of the participating laboratories is focused on their z-scores as reported in international protocols (7, 8). As to the use of MRMs, it is difficult to obtain an effective and suitable interpretation of the laboratories performance by using only the single z-score.

In addition to evaluate the global performance of each laboratory, normalized indexes such as Average of the Squared z-scores ($AZ^2$) are used. For both parameters, the classification was similar: 2, 3 and >3 for good, satisfactory and unsatisfactory performance, respectively.

The Italian NRL for pesticide residues annually organizes PTs for the determination of pesticide residues in olive oil. European laboratories (NRLs, official laboratories and private laboratories) especially Italian official laboratories involved in the National and European monitoring programs participate in these PTs.

In this report two PTs of pesticide residues in olive oil, named COIPT-17 and COIPT-18, are described and a comparison of their respective results is discussed.

# PROFICIENCY TESTS ON OLIVE OIL: COIPT-17 AND COIPT-18

## Rationale

In the last decade, the Italian NRL for pesticide residues has organized PTs for the determination of pesticide residues in olive oil. The European laboratories (NRLs, official laboratories and private laboratories) especially Italian official laboratories involved in the national and European monitoring programs were invited to participate.

The last two PTs organized in 2017 and 2018 were named COIPT-17 and COIPT-18. The purpose of these PTs was to determine residues of six pesticides spiked in a sample of olive oil in a defined concentration range of 0.050-0.350 mg/kg. These pesticides were selected from the same target list of twenty-six compounds, named as COIPT-17 and COIPT-18 General Protocol (Appendix A) and provided to participants in April both in 2017 and 2018. The list of compounds includes mainly those considered in the official control plans, with spiked concentration levels around their reference values set in the European Regulations.

Thirty-four laboratories agreed to participate in COIPT-17 (eighteen were Italian laboratories) and thirty-eight in COIPT-18 (twenty-three were Italian laboratories). The list of all participants is presented in Appendix B.

To assess the performance of the participating laboratories for each compound, z-score parameter was used following the norms of the International Laboratory Accreditation Cooperation (ILAC) and the International Organization for Standardization (ISO) (9-11).

Combined z-scores, such as $AZ^2$ index, have been introduced in the European Commission PT protocols, in order to evaluate each laboratory's overall performance taking into account all the pesticide analysed by MRMs (12-14). Participants were also asked for methodologies details, to compare the performance of the results obtained in relation to the methods followed.

## Test materials

The bulk material prepared for the proficiency test must be sufficiently homogeneous and stable with respect to each analyte, to ensure that all laboratories receive samples that do not differ substantially in the average concentration of the analyte.

The test material used for the two selected PTs was a kind of olive oil made by a mixture of olive pomace oils and olive oils available in a well-known Italian supermarket.

The olive oil amount for each PT was homogenized for 3 hours under magnetic stirrer. After homogenization, a sample of the test material was analysed in duplicate to verify the absence of the pesticides listed in the protocol.

A suitable portion of the blank olive oil was spiked in our laboratory with six pesticides from the possible list of compounds. Aliquots of 50 g of blank olive oil and spiked oil sample were distributed to the participants.

Some information was also provided to the laboratories with the General Protocol: the maximum number of compounds contained in the added sample (no more than 8) and their possible concentration range between 0.050-0.350 mg/kg.

Table 1 highlights the target pesticide list and the individual compounds, contained in the spiked samples, for each round.

**Table 1. Target list of compounds and individual compounds (X) actually contained in the spiked samples for both PTs**

| Target list of compounds | PT name | |
|---|---|---|
| | COIPT-17 | COIPT-18 |
| Chlorpyrifos | | |
| Chlorpyrifos-methyl | | X |
| lambda-Cyhalothrin | | X |
| Cypermethrin | | |
| Deltamethrin | | |
| Diazinon | X | X |
| Diflufenican | | |
| Dimethoate | | |
| alfa-Endosulfan | | |
| beta-Endosulfan | | |
| Endosulfan sulfate | | |
| Fenitrothion | X | |
| Fenoxycarb | | X |
| Methidathion | | |
| Oxyfluorfen | X | |
| Phosalone | | |
| Phosmet | | |
| Phosmet-oxon | | |
| Procymidone | X | |
| Quinalphos | X | |
| Kresoxim-methyl | | |
| Tebuconazole | | |
| Terbuthylazine | X | |
| Tolclofos-methyl | | X |
| Trifloxystrobin | | X |
| Trifluralin | | |

# Homogeneity and stability tests

Homogeneity and stability tests were performed for each PT. Regarding the homogeneity test, ten bottles of the spiked oil samples were randomly chosen and analyzed in duplicate. The statistical evaluation was performed according to the ISO 13528 (10).

The stability tests were performed using three bottles (chosen randomly) which were analyzed in duplicate on two occasions:

– Day 1, before the shipment of the samples;
– Day 2, shortly after the deadline for reporting results.

The acceptance criterion of the stability test for each compound is that the difference of the mean of the two days, M1 and M2, is minor of a value corresponding at $0.3 \times \sigma_{EUPT}$ where $\sigma_{EUPT}$ represents the target standard deviation (*see* Statistical evaluation of results section). All data regarding stability for the investigated pesticides are shown in Table 2.

Stability test was judged acceptable because the acceptance criterion of the stability test:

$$(M1-M2) < 0.3 \times \sigma_{EUPT}$$

is observed for all compounds in COIPT-17 and COIPT-18.

This test demonstrated that no significant decrease in the pesticide levels was showed for the duration of the PT. Regarding the homogeneity, all the six compounds in the COIPT-17 and the

other six in the COIPT-18 passed the homogeneity test and the related data are shown in Tables 3 and 4.

**Table 2. Stability tests data of COIPT-17 and COIPT-18**

| Pesticide | Concentration mg/kg | | | | |
|---|---|---|---|---|---|
| | M1 n.6 | M2 n. 6 | M1-M2 | $\sigma_{EUPT}$ | 0.3x$\sigma_{EUPT}$ |
| **COIPT-17** | | | | | |
| Diazinon | 0.125 | 0.124 | 0.001 | 0.031 | 0.009 |
| Fenitrothion | 0.343 | 0.345 | -0.002 | 0.071 | 0.022 |
| Oxyfluorfen | 0.340 | 0.326 | 0.014 | 0.074 | 0.023 |
| Procymidone | 0.280 | 0.278 | 0.002 | 0.066 | 0.020 |
| Quinalphos | 0.321 | 0.322 | -0.001 | 0.072 | 0.022 |
| Terbuthylazine | 0.173 | 0.167 | 0.006 | 0.045 | 0.014 |
| **COIPT-18** | | | | | |
| Chlorpyrifos-methyl | 0.146 | 0.147 | -0.001 | 0.034 | 0.010 |
| lambda-Cyhalothrin | 0.370 | 0.350 | 0.020 | 0.070 | 0.021 |
| Diazinon | 0.156 | 0.166 | -0.010 | 0.039 | 0.012 |
| Fenoxycarb | 0.315 | 0.305 | 0.010 | 0.073 | 0.022 |
| Tolclofos-methyl | 0.204 | 0.207 | -0.003 | 0.049 | 0.015 |
| Trifloxystrobin | 0.305 | 0.296 | 0.009 | 0.065 | 0.020 |

M1 = mean of duplicates of three bottles analysed in the first day
M2 = mean of duplicates of three bottles analysed in the second day
$\sigma_{EUPT}$ = target standard deviation
The acceptance criterion of the stability test is = (M1-M2) < 0.3x$\sigma_{EUPT}$

**Table 3. Homogeneity results (mg/kg) for COIPT-17**

| Sample number | Diazinon | Fenitrothion | Oxyfluorfen | Procymidone | Quinalphos | Terbuthylazine |
|---|---|---|---|---|---|---|
| 09 | 0.118 | 0.348 | 0.332 | 0.274 | 0.323 | 0.172 |
| 10 | 0.112 | 0.350 | 0.296 | 0.258 | 0.280 | 0.161 |
| 11 | 0.118 | 0.340 | 0.337 | 0.268 | 0.310 | 0.166 |
| 13 | 0.112 | 0.318 | 0.319 | 0.263 | 0.306 | 0.165 |
| 14 | 0.120 | 0.364 | 0.361 | 0.294 | 0.339 | 0.176 |
| 15 | 0.104 | 0.310 | 0.307 | 0.271 | 0.293 | 0.160 |
| 18 | 0.101 | 0.296 | 0.295 | 0.268 | 0.285 | 0.164 |
| 19 | 0.133 | 0.340 | 0.330 | 0.290 | 0.344 | 0.183 |
| 49 | 0.120 | 0.337 | 0.320 | 0.259 | 0.291 | 0.160 |
| 55 | 0.117 | 0.339 | 0.336 | 0.265 | 0.311 | 0.167 |
| Mean | 0.116 | 0.334 | 0.323 | 0.271 | 0.308 | 0.167 |
| SD | 0.009 | 0.020 | 0.020 | 0.012 | 0.022 | 0.008 |
| $\sigma_{EUPT}$ | 0.031 | 0.072 | 0.075 | 0.066 | 0.072 | 0.045 |
| SD/$\sigma_{EUPT}$ | 0.290 | 0.283 | 0.272 | 0.184 | 0.304 | 0.167 |
| Critical value | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| SD/$\sigma_{EUPT}$ ≤ 0.3 | yes | yes | yes | yes | yes | yes |

SD = Standard Deviation
$\sigma_{EUPT}$ = Standard Deviation target
Critical value = critical value according to ISO 13528:2015
SD/$\sigma_{EUPT}$ ≤ 0.3 = If SD/$\sigma_{EUPT}$ ≤ 0.3 the material has sufficient homogeneity

**Table 4. Homogeneity results (mg/kg) for COIPT-18**

| Sample number | Chlorpyrifos-Methyl | Lambda-Cyhalothrin | Diazinon | Fenoxycarb | Tolclofos-methyl | Trifloxystrobin |
|---|---|---|---|---|---|---|
| 43 | 0.152 | 0.345 | 0.162 | 0.296 | 0.212 | 0.298 |
| 48 | 0.140 | 0.352 | 0.153 | 0.321 | 0.201 | 0.295 |
| 55 | 0.146 | 0.370 | 0.160 | 0.313 | 0.204 | 0.306 |
| 58 | 0.150 | 0.344 | 0.168 | 0.313 | 0.204 | 0.294 |
| 71 | 0.148 | 0.349 | 0.170 | 0.299 | 0.207 | 0.312 |
| 72 | 0.153 | 0.388 | 0.155 | 0.312 | 0.208 | 0.314 |
| 73 | 0.144 | 0.339 | 0.159 | 0.300 | 0.204 | 0.306 |
| 76 | 0.145 | 0.334 | 0.164 | 0.297 | 0.205 | 0.304 |
| 82 | 0.140 | 0.336 | 0.165 | 0.287 | 0.203 | 0.305 |
| 86 | 0.154 | 0.339 | 0.158 | 0.314 | 0.208 | 0.284 |
| Mean | 0.147 | 0.350 | 0.161 | 0.305 | 0.206 | 0.302 |
| SD | 0.005 | 0.017 | 0.005 | 0.011 | 0.003 | 0.009 |
| $\sigma_{EUPT}$ | 0.034 | 0.070 | 0.039 | 0.073 | 0.049 | 0.065 |
| $SD/\sigma_{EUPT}$ | 0.149 | 0.243 | 0.140 | 0.148 | 0.065 | 0.140 |
| Critical value | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| $SD/\sigma_{EUPT} \leq 0.3$ | yes | yes | yes | yes | yes | yes |

SD Standard Deviation
$\sigma_{EUPT}$ = Standard Deviation target
Critical value = critical value according to ISO 13528:2015
$SD/\sigma_{EUPT} \leq 0.3$ = If $SD/\sigma_{EUPT} \leq 0.3$ the material has sufficient homogeneity

# Statistical evaluation of results

To evaluate the participating laboratories performance, the general protocol of European Commission Proficiency Tests (EUPT) for pesticide residues in food, adopted by the corresponding European Union Reference Laboratories (EURLs), was followed.

In this protocol, the assessment of the laboratory performance for each tested pesticide was obtained using the z-score parameter calculated by the following formula:

$$z = \frac{(xi - Xpt)}{\sigma_{EUPT}}$$

where $x_i$ is the laboratory mean, *Xpt* is the consensus value represented by the robust mean calculated according algorithm A (*see* Appendix C), $\sigma_{EUPT}$ is a Fit-For-Purpose Relative target Standard Deviation (FFP RSD) corresponding at the 25% of the robust mean value.

The usual interpretation of the z-score parameter is that values between ±2 indicate an acceptable performance, |z-score| between 2 and 3 indicate that results are questionable and some attention should be paid to the methods and/or operations in the laboratory, while |z-score| greater than 3 are considered unacceptable.

Pesticides not detected by the organizer in the spiked sample but reported by participants were considered false positive and highlighted in the final report. Compounds not detected by participants but present in the spiked sample above the specified reporting limit were considered false negative and included in calculation of z-scores.

Following the General Protocol of COIPT-17 and COIPT-18, the assigned value Xpt is the consensus value of the laboratories statistically estimated as the robust mean.

When the assigned value is calculated as a robust mean, the associate standard uncertainty ($u$, mg/kg) may be estimated using the following formula:

$$u = 1.25 \text{ x } \frac{s*}{\sqrt{n}}$$

where $s*$ is the robust standard deviation and n is the total number of results:

If the following criterion is met: $u \leq 0.3\ \sigma_{EUPT}$, then the uncertainty of the assigned value may be considered to be negligible and need not be included in the interpretation of the results of the proficiency testing.

Furthermore, a global performance (14) assessed by calculating the average of the squared z-scores ($AZ^2$) for laboratory having achieved the sufficient scope of detecting correctly at least 80 % of the analytes of interest.

The $AZ^2$ is estimated using the following formula:

$$AZ^2 = \frac{\sum_{i=1}^{n} {z_i}^2}{n}$$

The formula is the sum of the z-score value, multiplied by itself and divided by the number of z-scores (n) including those from false negatives.

The interpretation of the $AZ^2$ score is similar to that already seen for z-scores with three sub-classifications:

- Good $|AZ^2| \leq 2.0$
- Satisfactory $2.0 < |AZ^2| < 3.0$
- Unsatisfactory $|AZ^2| \geq 3.0$

In the analysis of pesticides residues in food, the evaluation of a multiresidue method includes the use of the $AZ^2$ combined parameter, even as the combined z-scores are considered to be of lesser importance than individual z-scores and should be used with caution according to ISO 13528:2015.

# COIPT-17 RESULTS

In this PT, thirty-four laboratories submitted results and twenty-three analysed all compounds: Diazinon was analysed by the majority of laboratories on the contrary of Oxyfluorfen that resulted the less analysed pesticide.

All data received are presented in the form of frequency histograms in Figure 1. The dispersion of results for each compound was evaluated performing some statistical tests (asymmetry test and normality tests using the SPSS software) (15).

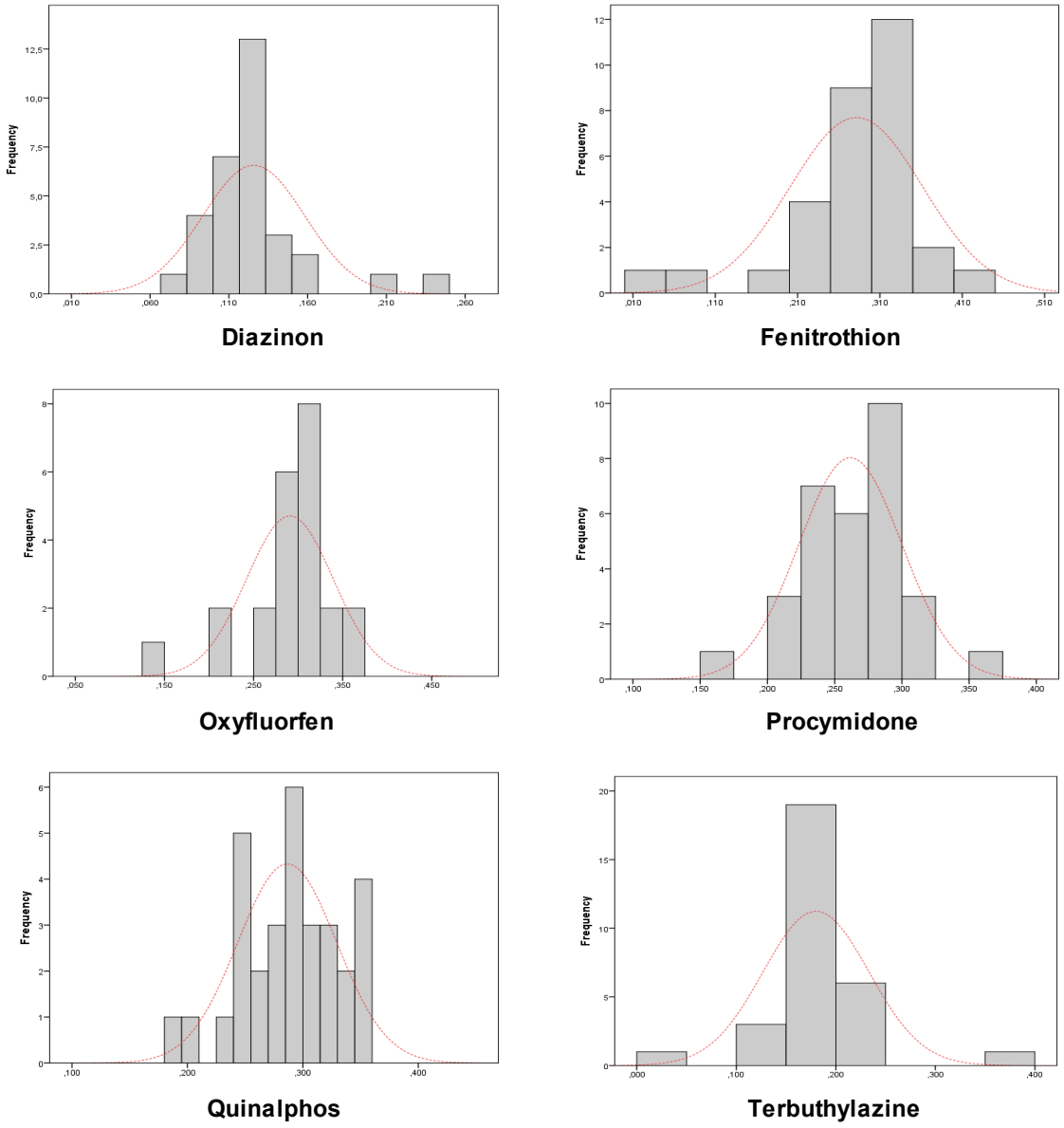


**Figure 1. COIPT-17: frequency histograms of Diazinon, Fenitrothion, Oxyfluorfen, Procymidone, Quinalphos and Terbuthylazine results**

Four compounds (Diazinon, Fenitrothion, Oxyfluorfen and Terbuthylazine) presented distributions asymmetric while the other two (Quinalphos and Procymidone) were normally distributed. In Figure 2 the results (mg/kg) have been presented in graphical form in combination with recovery data.

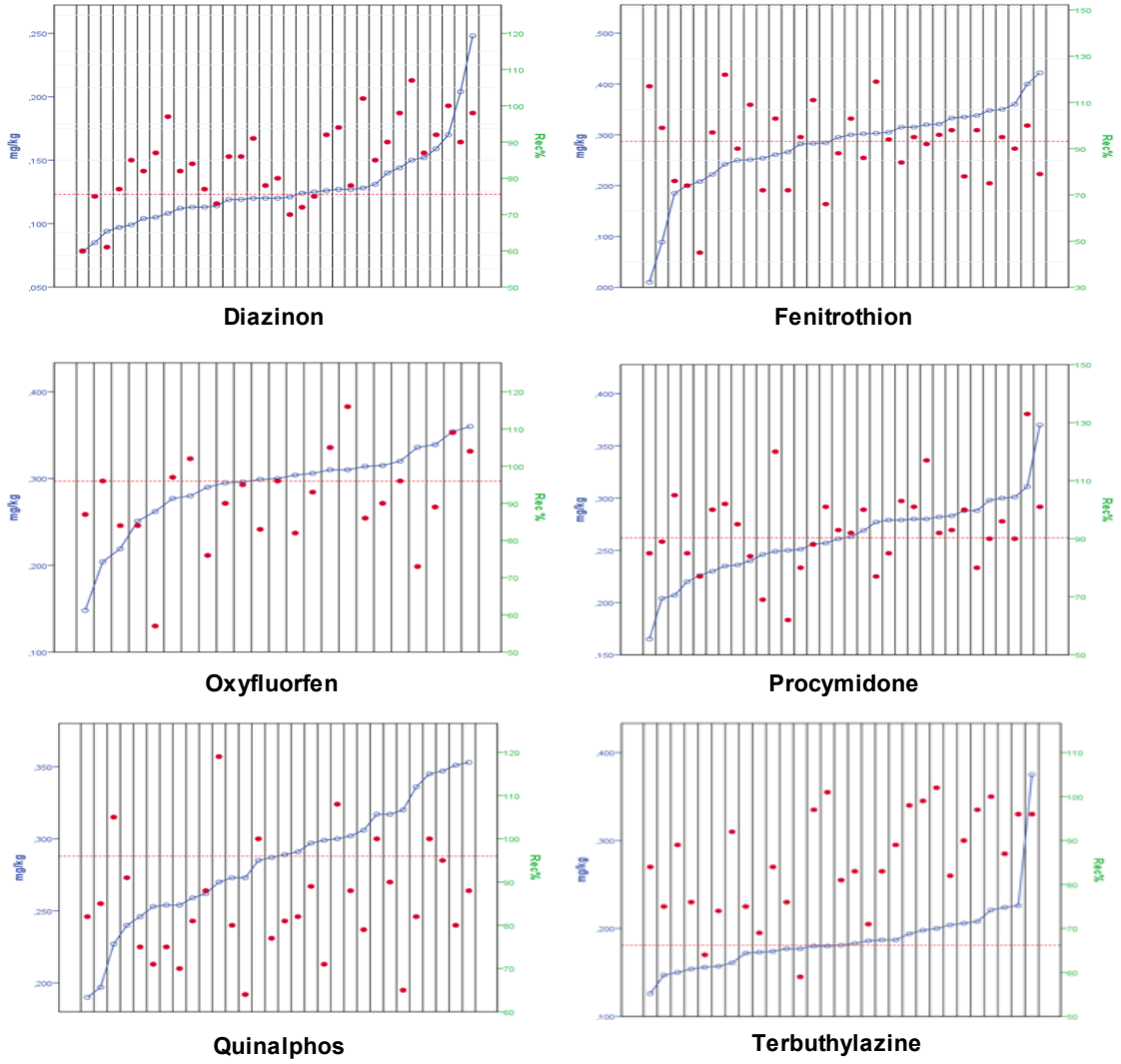


**Figure 2. COIPT-17: results (mg/kg) in combination with recovery data (%)**
**The dots tied by a line show the results while the free dots the reported recovery.**
**The dotted line represents the Robust mean value (see Table 5)**

Recovery is one of the validation parameters necessary to verify the ability of a method to identify residues of pesticides in food and feed according to the European analytical quality control technical guidance document SANTE/11813/2017.

Mean recoveries from initial validation should be within the range 70-120% with an associated repeatability RSDr ≤ 20%

A practical default range of 60-140% may be used for individual recoveries in routine analysis.
All the statistical parameters calculated for COIPT-17 are presented in the Table 5.

In some cases, the recoveries reported in the Figure 2 were not consistent with the results found in the spiked samples when compared to the *robust mean* values.

Observing the Quinalphos graph, it is possible to note that the majority recovery values supplied by participants are well below the respective provided results. The situation is exactly the opposite as regards the Terbuthylazine graph where the majority of recoveries were above the respective results.

The Robust RSD% values calculated and presented in the Table 5, were found be good in the range 13-18 % for five compounds with a maximum value of 22% for Fenitrothion.

**Table 5. Statistical parameter of COIPT-17 compounds**

| Parameter | Diazinon | Fenitrothion | Oxyfluorfen | Procymidone | Quinalphos | Terbuthylazine |
|---|---|---|---|---|---|---|
| Mean *(mg/kg)* | 0.127 | 0.280 | 0.294 | 0.262 | 0.286 | 0.181 |
| Median *(mg/kg)* | 0.120 | 0.298 | 0.302 | 0.262 | 0.289 | 0.180 |
| Robust mean *(mg/kg)* | 0.123 | 0.287 | 0.300 | 0.262 | 0.288 | 0.181 |
| Spiked value *(mg/kg)* | 0.135 | 0.346 | 0.324 | 0.281 | 0.317 | 0.202 |
| $s^*$ *(mg/kg)* | 0.023 | 0.062 | 0.042 | 0.034 | 0.044 | 0.029 |
| $\sigma_{EUPT}$ *(mg/kg)* | 0.031 | 0.072 | 0.075 | 0.066 | 0.072 | 0.045 |
| Robust RSD *(%)* | 18 | 22 | 14 | 13 | 15 | 16 |
| $u$ *(mg/kg)* | 0.005 | 0.014 | 0.011 | 0.007 | 0.010 | 0.006 |

$s^*$ = robust standard deviation
$u$ = uncertainty of the consensus value

# COIPT-18 RESULTS

Thirty-eight laboratories submitted results and thirty-two (equal to 84%) analysed all compounds. The normal distribution of all results presented in the COIPT-18 was evaluated by the Shapiro Wilks test. Five compounds (Chlorpyrifos-methyl, lambda-Cyhalothrin, Diazinon, Fenoxycarb and Trifloxystrobin) resulted normally distributed, while only one compound Tolclofos-methyl appeared to have a not normal distribution. For this PT, all results are showed as frequency histograms in Figure 3.

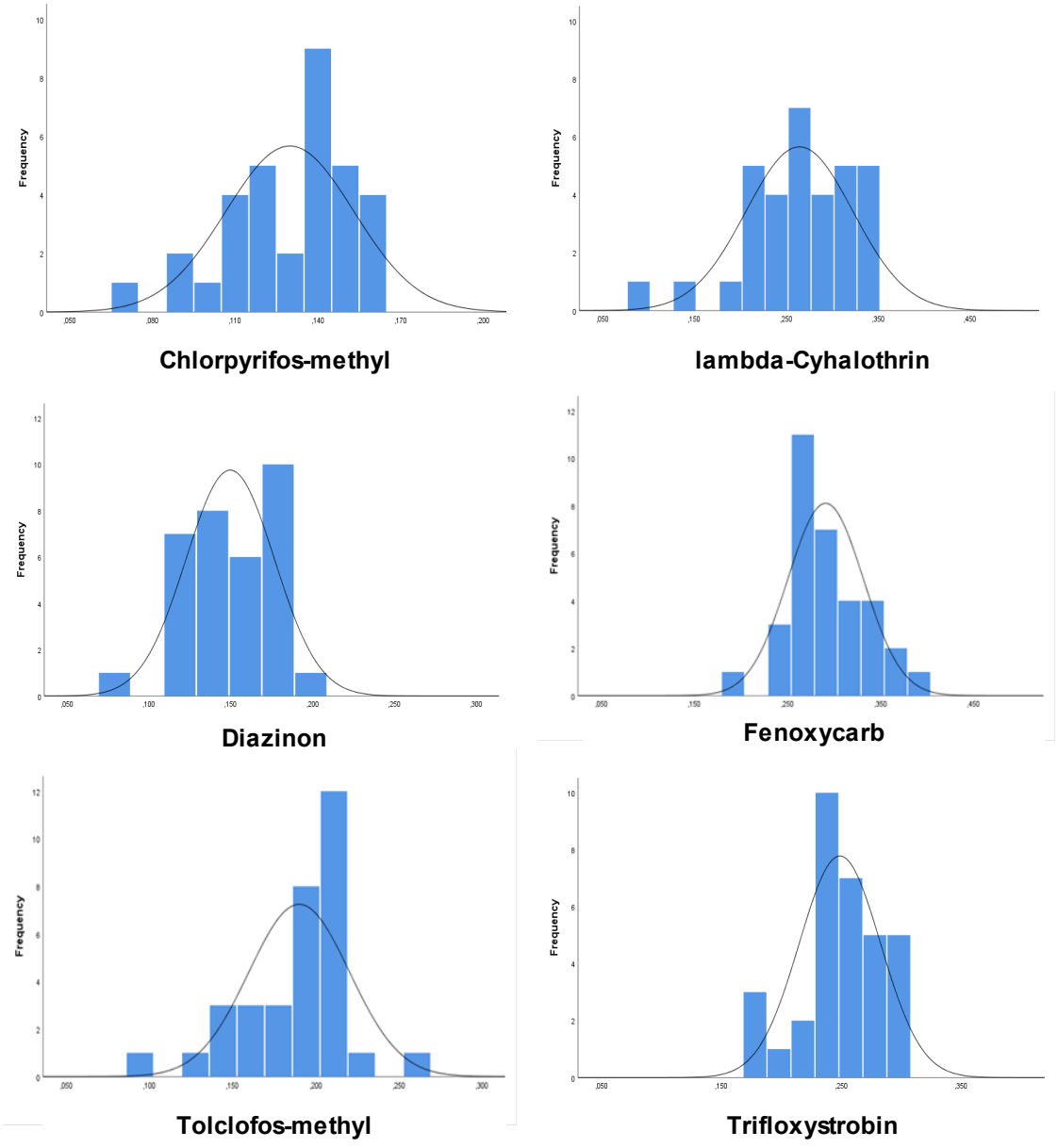


**Figure 3. COIPT-18: frequency histograms of Chlorpyrifos-methyl, lambda-Cyhalothrin, Diazinon, Fenoxycarb, Tolclofos-methyl and Trifloxystrobin**

The results of COIPT-18 in combination with recoveries are presented in Figure 4.

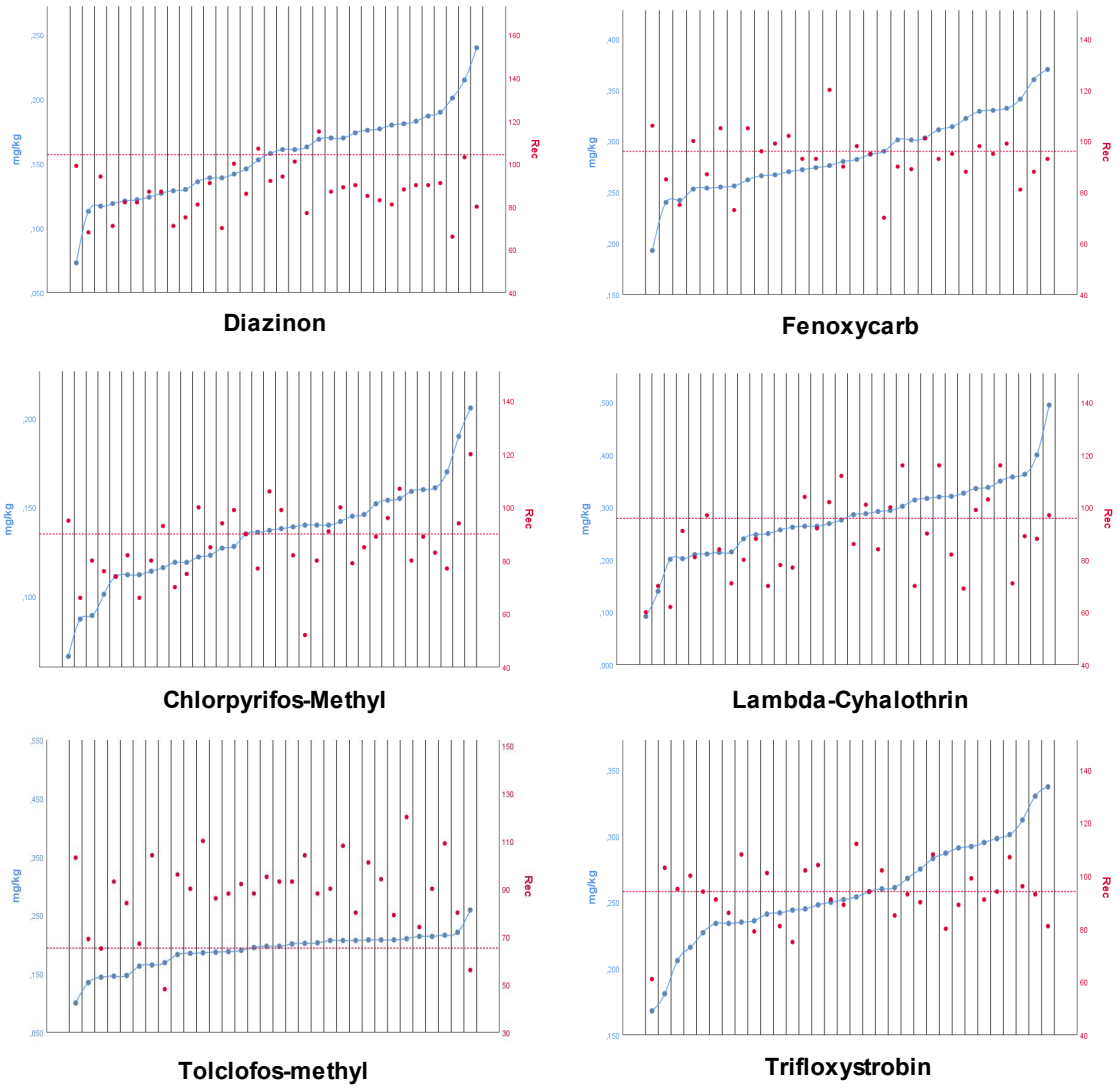


**Figure 4. COIP-18: results (mg/kg) in combination with recovery data (%)**
**The dots tied together by a line show the results and the free dots the reported recovery.**
**The dotted line represents the Robust mean value (see Table 6)**

All the statistical parameters calculated for COIPT-18 are presented in the Table 6.

In the case of COIPT-18 too, the recoveries reported in the Figure 4 not always were consistent with the results found in the spiked samples when compared to the robust mean values.

However, only in the case of Tolclofos-methyl the recovery trend leads to higher values than the corresponding results provided by the laboratories.

In the COIPT-18 the range of Robust RSD %was good, from 13 to 21% for five compounds with a maximum value of 22% in the case of lambda-Cyhalothrin (Table 6).

**Table 6. Statistical parameters of COIPT-18 compounds**

| Parameter | Chlorpyrifos-methyl | lambda-Cyhalothrin | Diazinon | Fenoxycarb | Tolclofos-methyl | Trifloxystrobin |
|---|---|---|---|---|---|---|
| Mean *(mg/kg)* | 0.136 | 0.279 | 0.157 | 0.291 | 0.192 | 0.258 |
| Median *(mg/kg)* | 0.138 | 0.276 | 0.161 | 0.282 | 0.199 | 0.252 |
| Robust mean *(mg/kg)* | 0.135 | 0.279 | 0.157 | 0.290 | 0.194 | 0.258 |
| Spiked value *(mg/kg)* | 0.158 | 0.333 | 0.171 | 0.304 | 0.216 | 0.277 |
| *s** *(mg/kg)* | 0.025 | 0.063 | 0.032 | 0.039 | 0.026 | 0.039 |
| $\sigma_{EUPT}$ *(mg/kg)* | 0.034 | 0.070 | 0.039 | 0.073 | 0.049 | 0.065 |
| Robust RSD *(%)* | 19 | 22 | 21 | 13 | 14 | 15 |
| *u* *(mg/kg)* | 0.005 | 0.013 | 0.007 | 0.008 | 0.006 | 0.008 |

*s** = robust standard deviation
*u* = uncertainty of the consensus value

# ASSURANCE OF THE RESULTS VALIDITY FROM COIPT-17 AND COIPT-18

In accordance with the standard ISO/IEC 17025: 2018, the assurance of the validity of the results becomes a strategic element in the quality system of a laboratory. An important tool for monitoring the performance is the use of internal quality control materials (recovery) in addition to the participation in interlaboratory comparisons such as the proficiency tests. In particular, the recovery results provide an important information on the trueness parameter. With Multiresidues Methods (MRMs) recoveries should be determined over as wide a range of analyte concentration because the recovery of the compounds may be concentration dependent. At low concentrations, a larger recovery range is observed (from 70% to 120%); this could be attributed to potential chemisorption on the matrix or irreversible adsorbed onto surfaces of the analytical vessels. It is common practice that pesticide residues analysis results are not corrected for recovery, when the recovery rates range between 80 and 120%, according to the control technical guidance document SANTE/11813/2017 (3).

An example of the dispersion of recoveries relating to the same Multi-Residue Method used by an individual laboratory participating in COIPT-17 and COIPT-18 is shown in Figure 5. Two graphic forms are presented: a control chart and the corresponding frequency distribution containing 392 recovery values.

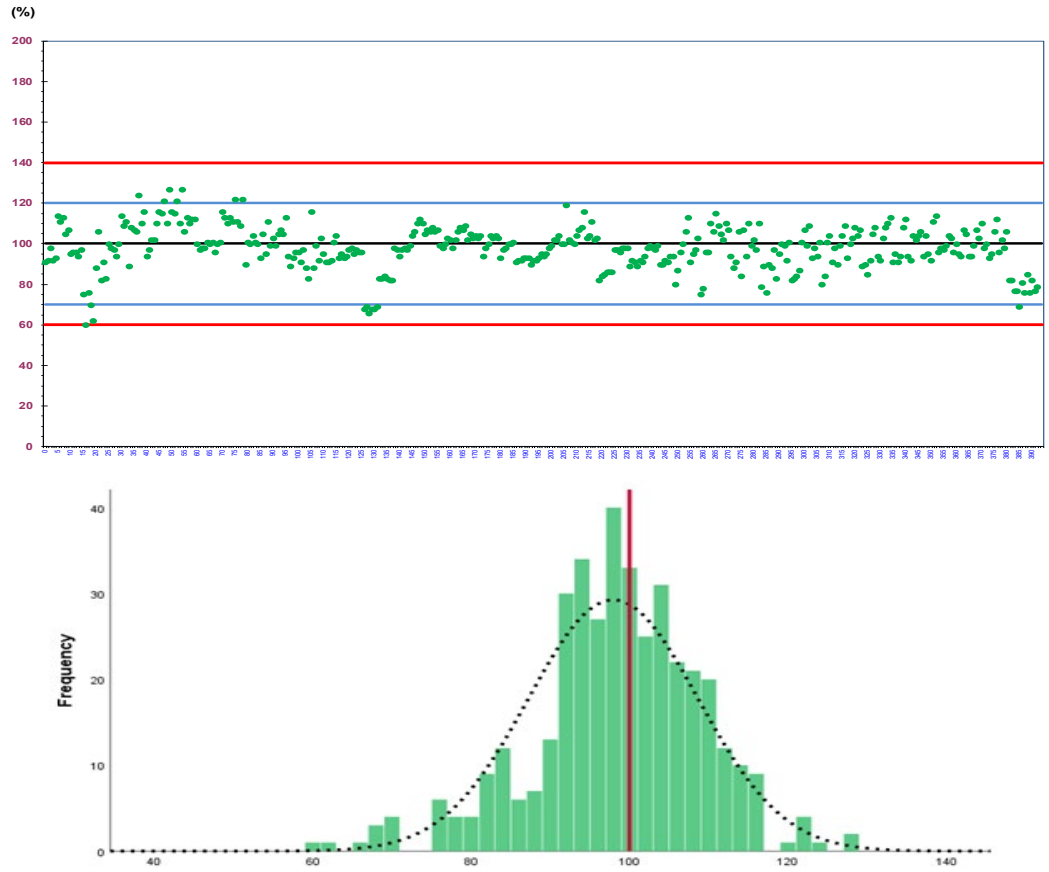


**Figure 5. Example of recoveries dispersion related to the same method used by a single laboratory participating in COIPT-17 and COIPT-18**

The control chart, shows a very high percentage (96%) of data considered acceptable as in the range 70-120% with an overall mean value of 98%. The recovery frequency in Figure 5 gives a much better understanding of the spread. The shape of the normal distribution curve depends on the spread in the results and the central value of the distribution curve (mean value of 98%) is very near to the reference value of 100% (vertical line) corresponding to the central line of the recovery control chart. Always according to the control technical guidance document SANTE/11813/2017 (3), also other validation parameters of MRMs have been performed to provide evidence that a method is fit for the intended purpose. A minimum of 5 replicates is required at the Limit of Quantification Level (LOQ) and at least one other higher level with mean recoveries within the range 70-120% and an associated repeatability RSDr ≤ 20%. Figure 6 presents validation data of the method used by the same individual laboratory cited in Figure 5 and more precisely the combination of the recoveries means with the corresponding repeatability RSDr of two concentration levels. These data come from the validation results obtained during the characterization of the testing method, employed by the single laboratory to analyse the proficiency test samples (16).

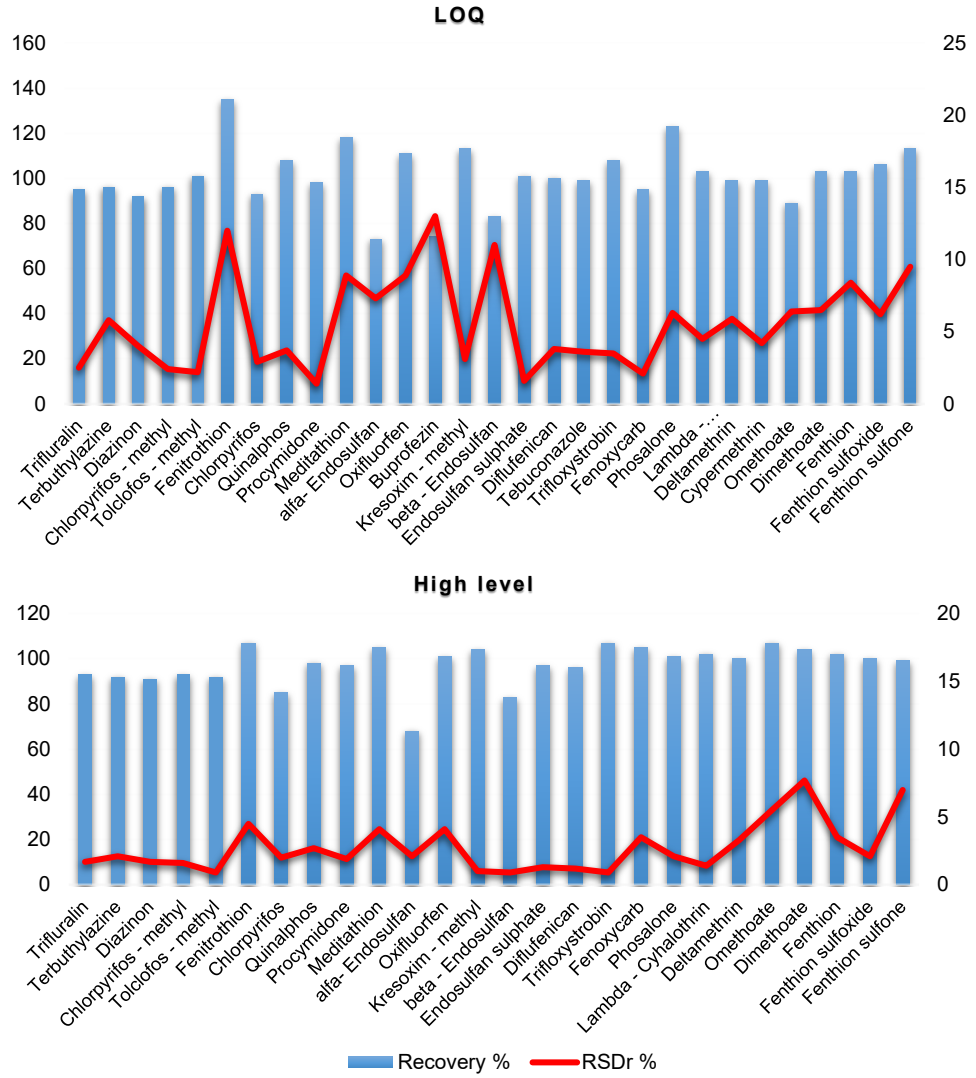


**Figure 6. Mean recovery histograms (LOQ equal to 0.05 mg/kg and High level equal to 0.5 mg/kg) in combination with the repeatability relative Standard Deviation**

As shown in the graphs of Figure 6 repeatability results are excellent with all values lower than the 20% acceptance criterion for both the high concentration level and the LOQ level.

Regarding the average percentage recovery values, only for 2 compounds, Fenitrothion and Phosalone, at the LOQ level the values obtained were slightly higher than the 120%.

These two recoveries may be due to the influence of the oil matrix on the final analytical determination. It is known that when the concentration level decreases, the effect of the number of interfering compounds that affects the recovery results also increases.

The overall assessment of the performance of participants in the two PTs is discussed in Figures 7-9. Figure 7, containing 376 values, compares the recovery data reported by all participants in the COIPTs during the rounds 2017 and 2018, using again the graphical tools of the control chart and the frequency distribution of recoveries.

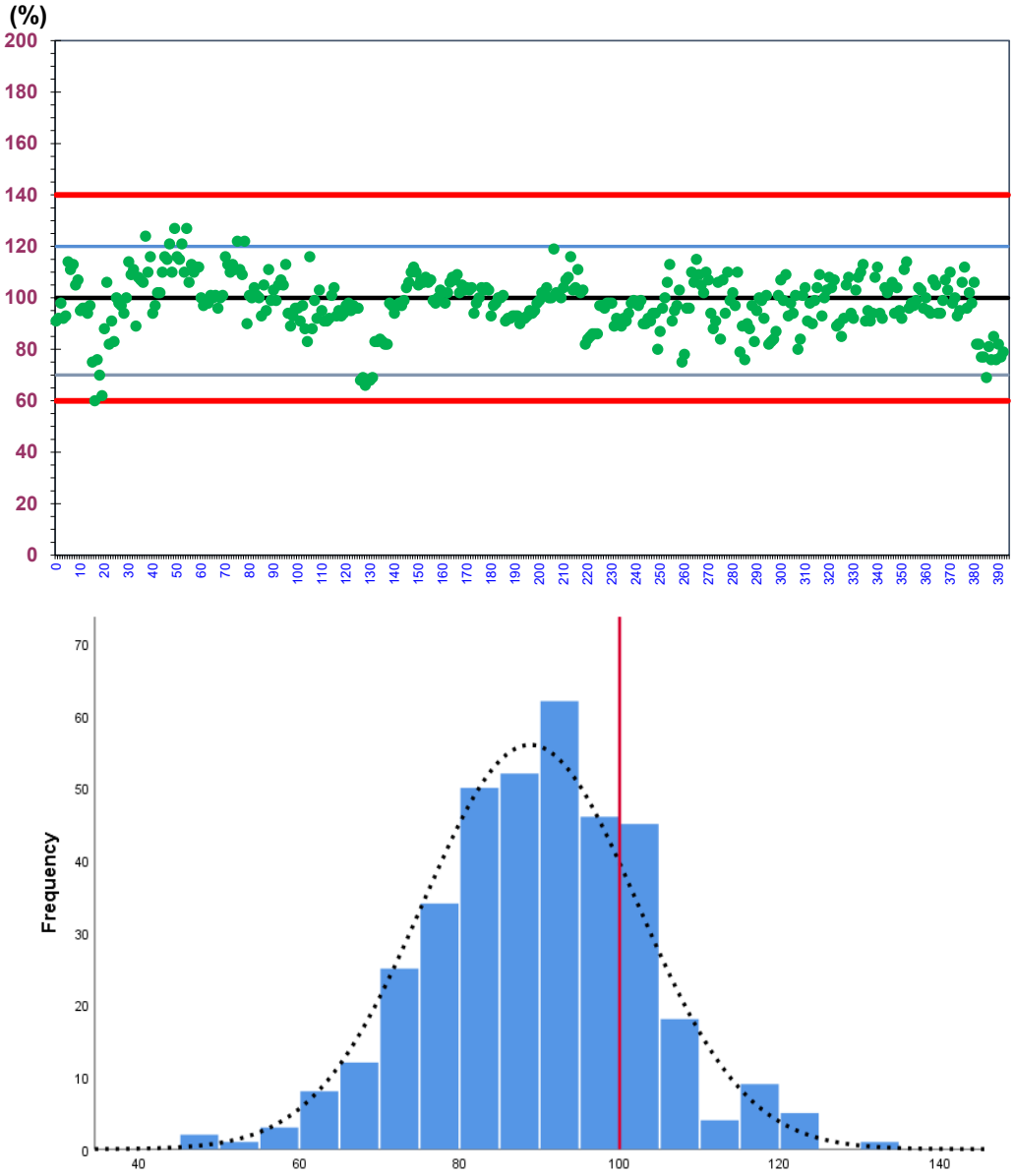


**Figure 7. Control chart of mean recoveries supplied by participants in the COIPT-17 and COIPT-18**

The Control Chart evidences a slight negative bias, as well as displayed and confirmed in the frequency distribution. The dispersion of the data appears uniform and in the most cases below the reference value of 100%. Moreover, the overall mean value was 89%. A limited number of cases is observed with recovery data below 60%; three of which have been correct for recovery as stated by the participant laboratory. In general, for pesticide residues in food, single recovery data are acceptable in the range 60-140%, as defined in the SANTE document guide (3).

As emphasized above, in all interlaboratory exercises one of the fundamental elements is the use of a performance indicator to quantify the analytical performance of each participant (12). The z-score is frequently advised as such performance indicator. In the COIPT-17 and COIPT-18 the z-score is calculated for each laboratory/pesticide combination according the following equation presented in the Statistical evaluation of results section:

$$z = \frac{(xi - Xpt)}{\sigma_{EUPT}}$$

In Figure 8 are presented as histograms the z-score values calculated for COIPT-17.

The horizontal bars represent the set of z-score values between ±2 supplied by participants for each compound, and each individual value is indicated by a thin line.

With the label written in normal font the questionable z-scores between 2 and 3 are indicated, while the label in bold font represents the z-scores value >3 that are considered unacceptable.

In this PT the majority of z-scores calculated were in the range of ±2 especially for three compounds: Oxyfluorfen, Procymidone and Quinalphos.

Two compounds, Diazinon and Fenitrothion, obtained one questionable z-score each, while four unacceptable z-scores were submitted: one for Diazinon and Fenitrothion and 2 for Terbuthylazine.

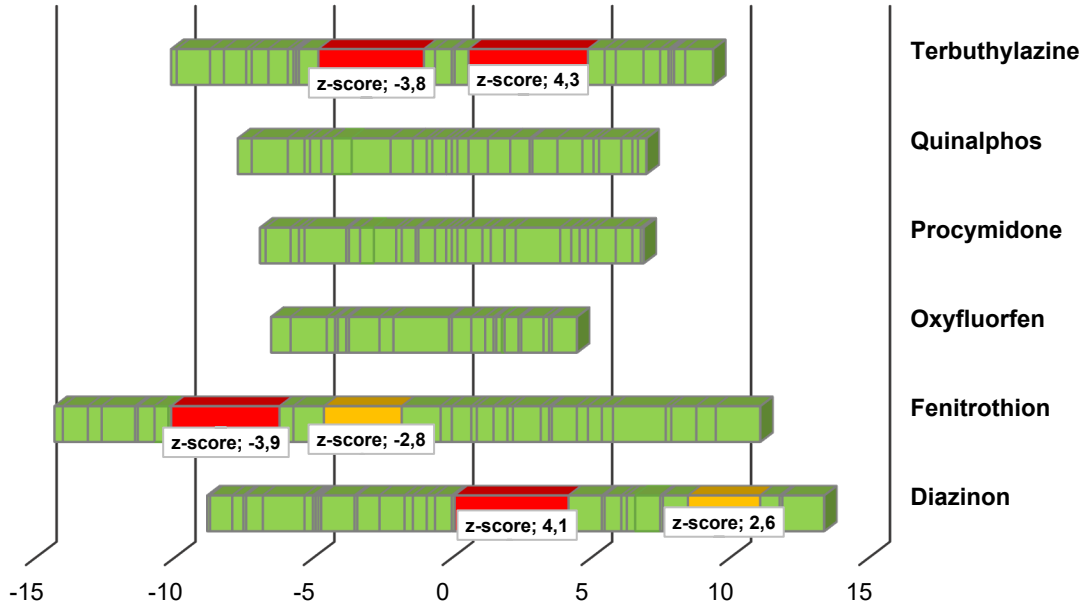


**Figure 8. z-scores values clustered for COIPT-17**

The z-scores of the COIPT-18 are also represented in the same way in Figure 9.

Besides in this case three compounds have z-score values between ±2: Fenoxycarb, Tolclofos-methyl and Trifloxystrobin.

Chlorpyrifos-methyl and Diazinon obtained one and two questionable z-score values respectively, and finally lambda-Cyhalothrin presented a questionable z-score together with a value even if slightly higher than 3 considered as an unacceptable z-score.

Based on the z-score values, laboratory performances are considered more than satisfactory for both PTs but it is clear that in the COIPT-18 the trend of the z-scores with only one unacceptable z-score was considered better if compared to the four of the COIPT-17.

Moreover, it is possible notes that the z-scores data set for both PTs shows a deviation towards a negative bias as confirmed by the comparison of these values with the data of the average recoveries presented in Figure 7.

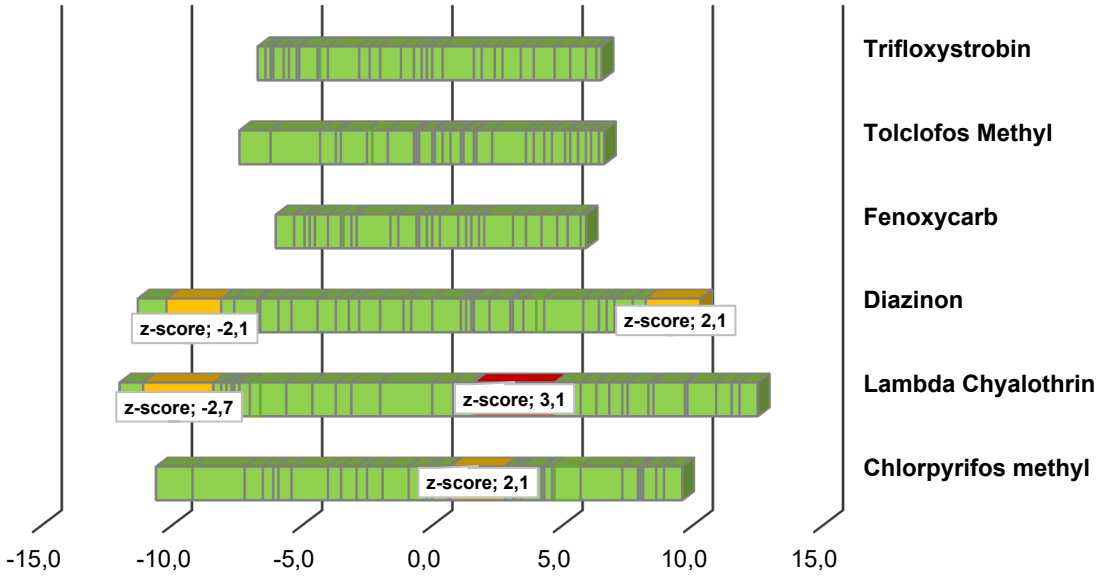


**Figure 9. z-scores values clustered for COIPT-18**

# COMPARISON RESULTS OF THE TWO PTS

## Discussion

Participation on a routine basis in PTs is one disposable tool for laboratories to monitor their competence in the pesticide residues analysis and as consequence to improve their performances.

This last assumption is confirmed by the comparison of the z-score values trend presented in COIPT-17 and COIPT-18.

Further confirmation of this aspect is highlighted by the example in Figure 10 where the z-scores obtained for the Diazinon analysed in both the PTs mentioned have been compared. For some laboratories, the negative performance of Diazinon in COIPT-17 has definitely improved in the following COIPT-18.

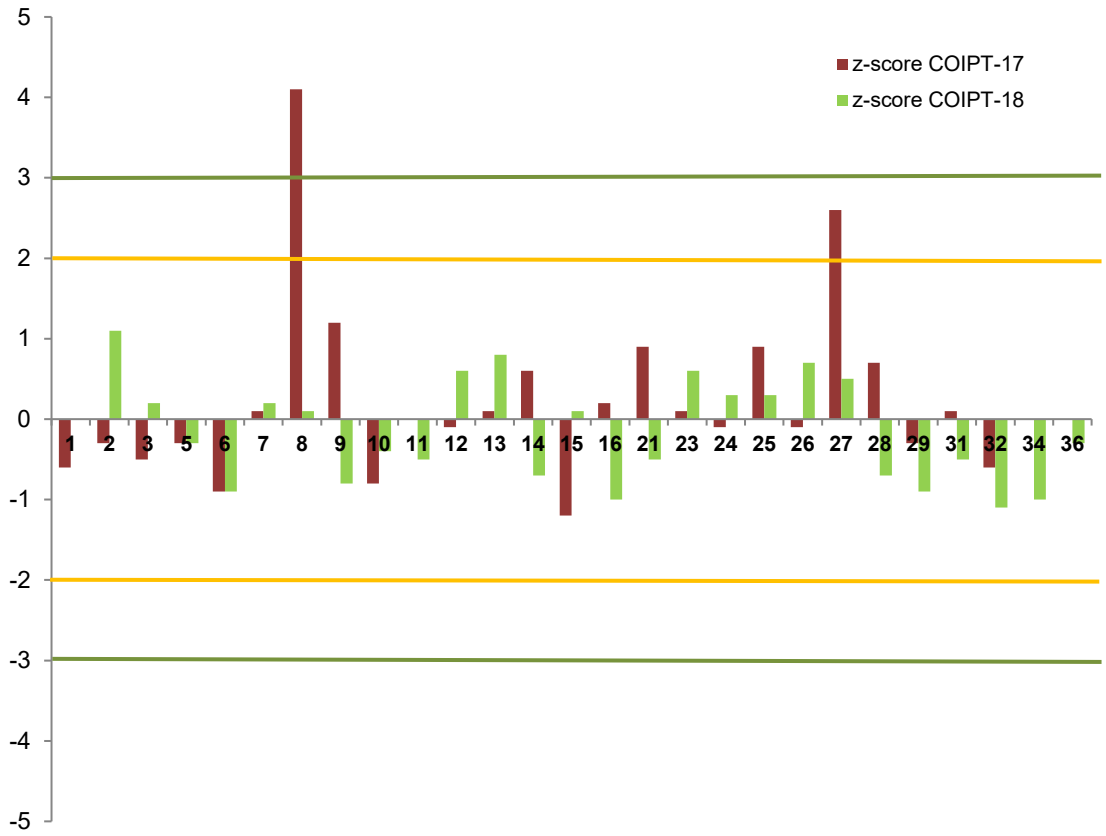


**Figure 10. Comparison of Diazinon z-scores data**

The performance evaluation of MRMs by PTs requires the use of overall indexes, or combined z-score values, as well as the single z-score (*see* Statistical evaluation of results).

The overall assessment of laboratory performance, as already mentioned, was evaluated using the ($AZ^2$) parameter for both PTs. $AZ^2$ values are showed as graphical representations in Figure 11 in the case of COIPT-17 and in Figure 12 for COIPT-18.

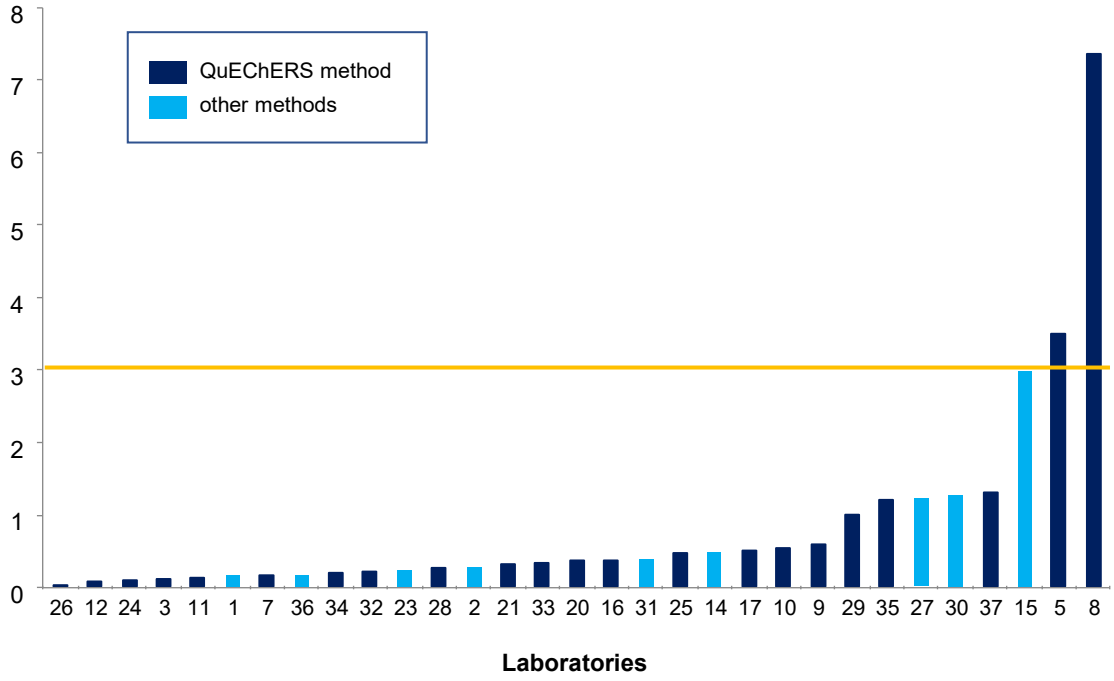


**Figure 11 COIPT-17: graphical of $AZ^2$ values**

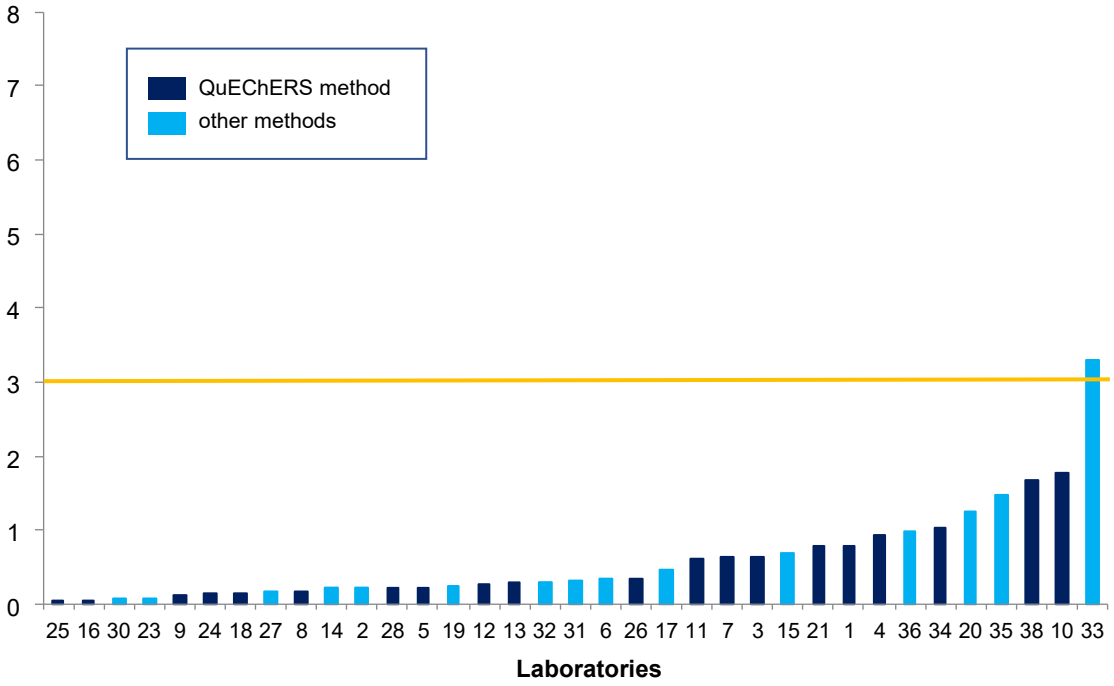


**Figure 12. COIPT-18: graphical representation of $AZ^2$ values**

Comparing the two Figures 11 and 12, it is evident that in the case of COIPT-17 two laboratories have obtained unsatisfactory values of $AZ^2$, identified by a numerical value > of 3 (with a numeric value close to 8 for the Laboratory 8), while only one laboratory in the case of COIPT-18 obtained a disappointing performance with a value slightly higher than 3.

In the two figures the laboratories are also distinguished according to the different methodologies used for the PT.

Participants were requested to provide a detailed description of the analytical methodology employed. In COIPT-17, the majority of the laboratories (77%) used the QuEChERS (Quick Easy Cheap Effective Rugged and Safe) methodology (17) as well as in COIPT-18 (67%). This is clearly evident in Figures 11 and 12 where laboratories that used QuEChERS method have been highlighted in darker colour.

In 2009, this method became the official method for the analysis of pesticide residues in food of plant origin, such as fruits, vegetables, and cereals (18); it is currently applied for food with high content of fats (as olive oil) and food of animal origin. Figure 11 shows that most of the laboratories having used the QuEChERS methodology obtained ''very'' satisfactory $AZ^2$, except for two laboratories. In Figure 12 where the data of the COIPT-18 are summarized it can be seen instead that all participants that have used the QuEChERS obtained more than satisfactory results.

Other methods presented by laboratories in addition to QuEChERS are listed below:

- Method EURL-FV (2012-M6). "Validation Data of 127 Pesticides Using a Multiresidue Method by LC-MS/MS and GC-MS/MS in Olive Oil" (19);
- UNI EN 1528 parts 1-4 in 1997 (20-23);
- Method described in the publication of Lentza-Rizos *et al.* (24).

Finally, some participants described experimental methods developed internally.

The instrumental detection techniques used by the majority of the laboratories were:

GC (Gas Chromatography) coupled with Mass Spectrometry Detector (MSD), Mass Spectrometry Ion Trap Detector (MSITD), Time of Flight (TOF) MS detector, HRMS (High Resolution Mass Spectrometry) orbitrap detector, MS/MS detector;

LC (Liquid chromatography) coupled with MS/MS detector or UHPLC (Ultra High-Pressure Liquid Chromatography) MS/MS.

In some cases, selective detectors have been used coupled with GC as Electronic Capture Detector (ECD), Flame Photometric Detector (FPD), Thermoionic Nitrogen Phosphorous Detector (NPD), followed by a confirmation in GC-MS.

A small number of laboratories routinely use liquid chromatography with mass spectrometry absolutely necessary for determining certain polar pesticides in complex matrices.

In the large part of the cases the quantification has been carried out with matrix calibration at single or multiple levels. Some participants used the solvent calibration and few laboratories performed the standard addition procedure.

# CONCLUSIONS

Participation in PTs has become a very common practice for pesticide-residue laboratories in order to assess the quality of their results. This participation is mandatory for European laboratories involved in the official controls of pesticide residues in food, and is requested by the ISO/IEC 17025.

The Italian NRL for pesticide residues annually organizes PTs for the determination of pesticide residues in olive oil. European laboratories (NRLs, official laboratories and private laboratories), especially Italian official laboratories involved in the National and European monitoring programs, participate in these PTs.

In this paper, two PTs of pesticide residues in olive oil named COIPT-17 and COIPT-18 are described and a comparison of their respective results is discussed.

The outcome of both PTs can be considered satisfactory from several points of view. First of all, the good participation of laboratories: thirty-four for the COIPT-17 and thirty-eight for COIPT-18. Another positive aspect is the good value obtained for the robust RSD parameter, calculated for all compounds for both PTs, with values between 13-21% for COIPT-17 and 13-22% for COIPT-18. Laboratory performance for each compound, defined by the z-score values, is also considered more than satisfactory for both PTs with a deviation towards a negative trend confirmed by the corresponding average recoveries presented.

The same applies to the overall performance of the laboratories in both PTs, indicated by the $AZ^2$ parameter.

Based on the z-score values, laboratory performances for each compound are considered more than satisfactory for both PTs with a deviation towards a negative bias confirmed by the trend of the corresponding average recoveries presented

Even regarding the global performance both PTs have been obtained good results.

Only two laboratories for COIPT-17 and one in the case of COIPT-18 have obtained unsatisfactory values of $AZ^2$.

In conclusion, from the comparison between these two PT it is possible to underline how the results of COIPT-18 are better than the good ones of COIPT-17.

This consideration is a further confirmation of the importance for laboratories to participate in these PTs on a regular basis, to improve their performances in the analysis of pesticide residues in olive oil.

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# APPENDIX A
# General Protocol
# for COIPT-17 and COIPT-18

Italian National Reference Laboratory

for Pesticide Residues

*in cooperation with*

International Olive Council
Working Group on Methods for Pesticide Residues

**General Protocol**

## INTERLABORATORY TEST
## MULTIRESIDUES DETERMINATION OF PESTICIDES RESIDUES IN OLIVE OIL

### Introduction

Proceeding the series of Proficiency Tests for pesticide residues in olive oil organized in cooperation with the IOC (International Olive Council), the Italian National Reference Laboratory for pesticide residues in products of animal origin and commodities with high fat content (NRL-AO), decided to organize a new proficiency test in olive oil named ……
The target of this Proficiency Test is to compare the performances of laboratories in Mediterranean and European countries and to promote mutual acceptance of pesticide residue data regarding the analysis of olive oil.
We cordially invite to participate in this Proficiency Test all laboratories active in the analysis of pesticide residues in olive oil: Mediterranean laboratories of International Olive Council (IOC), European laboratories (NRLs, official laboratories and private laboratories) and Italian official laboratories involved in the National and European monitoring programs.

### Participation

For participating in the PT please register by filling in the enclosed registration form and returning it by e-mail to tiziana.generali@iss.it
The participation is free of charge for all laboratories.

### Sample material

The sample material used in this proficiency test is olive oil.
The test sample may contain up to 8 pesticides from the target list given in the Annex 1 with a concentration level in the range of 0.050-0.350 mg/kg.
Each participant will receive approximately 50 g of the spiked oil test sample and 50 g of the same unspiked (blank) oil.

### Analysis and reporting of results

Laboratories should:

- use their own standard operating procedures for clean-up and analytical measurement
- use their own reference standards for identification and quantification
- report one single result for each quantified analyte (the result could be the mean of two or more determinations)
- report the reporting levels for all quantified or not detected analytes and
- provide detailed method description and any additional information

Results of each analyte should be reported in mg/kg, rounded to three significant figures (0.0612, 0.164).

**Reporting forms** will be sent by e-mail (word or excel files) when the test material is dispatched. To avoid transcription errors, participants are requested to submit their reports electronically only. There will be **no extension of the deadline.**

Results should be submitted by e-mail to *organizer* before deadline.

## Statistical evaluation of results

The performance of each laboratory will be evaluated and presented in anonymous form in a report issued after the final evaluation. According with the protocol of European Proficiency Tests on pesticide residues in fruit and vegetable matrices and in food of animal origin and commodities with high fat content, a fit-for-purpose relative target standard deviation (FFP RSD) of 25% will be chosen to calculate the target standard deviation (σ) as well as the z-scores for the individual pesticides.
Results reported as < RL (RL= Reporting Limit) will be considered as not detected and will be judged as false negatives and considered in the z-score calculation.
False positive will be reported in the PT Report but no *z-score will be* calculated for this result.
The EUPT-Panel retains the right not to calculate $AZ^2$ if it is considered as not being useful or if the number of results reported by any participant is considered to be too low.
For evaluation of the overall performance of laboratories the *Average of the Squared z-scores* ($AZ^2$) will be used. Combined z-scores are considered to be of lesser importance than the individual z-scores but however it is considered useful calculate this parameter.
Laboratories should detect at **least 80%** of the analytes present in the spiked sample to achieve the *"sufficient scope"*. Only laboratories with *"sufficient scope"* will receive the $AZ^2$ ranking.

## Time schedule

| | | |
|---|---|---|
| Organiser | Announcement and invitation | |
| Participant | Registration by e-mail to organizer<br>using the attached **registration form** | |
| Organiser | Shipment of test material and sending by e-mail three forms:<br>• confirmation of sample receipt (**Form 1**)<br>• analytical data (**Form 2**)<br>• method information (**Form 3**) | |
| Participant | Confirmation of test material receipt<br>(**Form 1**) by e-mail | |
| Participant | Reporting of test results and method information<br>(**Forms 2 and 3**) by e-mail | |
| Organiser | Dispatch of the report to all participants as pdf-file | |

***** Please make sure to report your results on time as there will be **no extension of the deadline**

## Contact information

*Organizer:*

Italian National Reference Laboratory for pesticides residues in products of animal origin and commodities with high fat content
Istituto Superiore di Sanità (National Institute of Health)
Pesticide Section
Viale Regina Elena, 299 – I-00161 Roma

in cooperation with:
International Olive Council (IOC)
Madrid

**Contact person**

For any clarification, please, contact the organizer!

## Annex 1: List of possible pesticides in the spiked oil sample

Reporting Limit (RL) for all listed pesticides is 0.05 mg/kg

Chlorpyrifos
Chlorpyrifos-methyl
lambda-Cyhalothrin
Cypermethrin
Deltamethrin
Diazinon
Diflufenican
Dimethoate
alfa-Endosulfan
beta-Endosulfan
Endosulfan sulfate
Fenitrothion
Fenoxycarb
Methidathion
Oxyfluorfen
Phosalone
Phosmet
Phosmet-oxon
Procymidone
Quinalphos
Kresoxim-methyl
Tebuconazole
Terbuthylazine
Tolclofos-methyl
Trifloxystrobin
Trifluralin

## PROFICIENCY TEST FOR PESTICIDE IN OLIVE OIL

### Registration form

**Please complete the form and submit your registration by e-mail to *organizer* until ……**

| | |
|---|---|
| **Institute/Laboratory:** | |
| **Address:** | |
| **Postal code:** | |
| **City:** | |
| **Country:** | |
| **Contact person:** | |
| **Phone:** | |
| **Fax:** | |
| **e-mail:** | |

**Position of the laboratory regarding analysis of pesticides**

- ❑ National Reference Laboratory
- ❑ Official Laboratory
- ❑ Private Laboratory

**Comments**:

# APPENDIX B
# List of participants of COIPT-17 and COIPT-18

**List of participants in COIPTs in 2017 and 2018**

**BELGIUM**

Primoris Belgium (Zwijnaarde)

**FRANCE**

ITERG (Canejan)

Laboratori Du Scl De Montpellier (Montpellier)

**GERMANY**

Bavarian Health and Food Safety Authority (Erlagen)

Institut Kirchoff Berlin GMBH (Berlin)

Niedersaechsisches Landesamt Fuer Verbraucherschutz Und Lebensmittelsicherheit Lebensmittel Und Veterinaerinstitut Oldenburg (Oldenburg)

**GREECE**

Benaki Phytopathological Institute, Pesticide Residue Laboratory (Kiphissia)

CADMION (Kiato Korinthia)

Chemicotechniki Laboratories "Lagouvardou-Spantidaki O.E" (Rethymno)

General Chemical State Laboratory, Pesticide Residues Laboratory, D Chemical Division (Athens)

Food Allergens Laboratory (IONIA)

UNIHER S.A. (ENOSI IRAKLIOU) (Iraklion)

SKYLAB – Med S.A. (Athens)

**IRELAND**

Pesticide Control Laboratory, Department of Agriculture Food and Marine (Kildare)

**ITALY**

Agenzia delle Dogane Direzione Regionale per la Sicilia - Laboratorio Chimico (Palermo)

Agro.biolab Laboratory srl (Rutigliano, BA)

APPA Trento, Settore Laboratorio (Trento)

ARPA Emilia Romagna Area Fitofarmaci (Ferrara)

ARPA Friuli Venezia Giulia, Laboratorio di Udine (Udine)

ARPA Lazio, Sezione di Latina (Latina)

ARPA Puglia, Polo di Specializzazione "Alimenti" (Bari)

ARPA Liguria, Dipartimento di La Spezia, UO Laboratorio (La Spezia)

USL Toscana Centro Laboratorio Sanità Pubblica (Firenze)

INNOVHUB – SSI (Milano)

ATS Laboratorio di Prevenzione (Bergamo)

CHEMISERVICE srl (Monopoli, BA)

ATS Milano Laboratorio Prevenzione (Milano)

Istituto Superiore di Sanità, Dipartimento Ambiente e Connessa Prevenzione Primaria (Roma)

AGROBIOLAB LABORATORY Rutigliano (Bari)

IZSLER Laboratorio Pesticidi (Brescia)

IZS Abruzzo e Molise (Teramo)

IZSLT (Roma)

LABCAM srl (Albenga, SV)

MIPAAF-ICQRF, Laboratorio di Catania (Catania)

PH srl (Firenze)

Water & Life Lab srl (Bergamo)

CADIR LAB srl (Alessandria)

ANALYTICAL srl. (Firenze)

**POLAND**

Voivodship Sanitary Epidemiological Station in Warsaw Pesticide Residue Laboratory (Warsaw)

Voivodship Sanitary Epidemiological Station in Rzeszow (Rzeszow)

**SPAIN**

Borges Agricultural & Industrial Edible Oils SAU (Tàrrega Lléida)

CNTA (San Adrian Navarra)

Laboratorio Agroalimentario (Granada)

Laboratorio Arbitral Agroalimentario (Madrid)

# APPENDIX C
# Robust analysis: algorithm A

The algorithm A yields robust estimates of the mean and standard deviation of the data to which it is applied. We have followed the indication and equations descripted in Appendix C of the ISO 13528: 2015. This appendix reports in detail the calculation performed in order to obtain the robust mean ($x^*$) and the robust standard deviation ($s^*$). The algorithm A given in this appendix is reproduced from ISO 5725-5, with a slight addition to specify a stopping criterion: no change in the 3rd significant figures of the robust mean and standard deviation.

Calculate initial values for $x^*$ and $s^*$ as:

$$x^* = \text{median of } x_i \ (i = 1, 2, \ldots, p) \tag{1}$$

$$s^* = 1.483 \text{ median of } |x_i - x^*| \text{ with } (i = 1, 2, \ldots, p) \tag{2}$$

Denote the p items of data, sorted into increasing order, by:

$$x\,(1), x\,(2), x\,(3), x\,(4), \ldots.. x\,(p)$$

Update the values of $x^*$and $s^*$ as follows. Calculate:

$$\delta = 1.5\, s^* \tag{3}$$

For each $x_i$ ($i$ = 1,2, ….$p$), calculate:

$$x_i^* = \begin{cases} x^* - \delta, \text{when } x_i < x^* - \delta \\ x^* + \delta, \text{when } x_i < x^* + \delta \\ x_i \ otherwise \end{cases} \tag{4}$$

Calculate the new values of $x^*$ and $s^*$ from:

$$x^* = \sum_{i=1}^{p} \frac{x_i^*}{p} \tag{5}$$

$$s^* = 1.134 \sqrt{\sum_{i=1}^{p} \frac{(x_i^* - x^*)^2}{p-1}} \tag{6}$$

where the summation is over $i$.

The robust estimates $x^*$ and $s^*$ may be derived by an iterative calculation, i.e. by updating the values of $x^*$ and $s^*$ several times using the modified data in equations 3 to 6, until the process converges.

Convergence may be assumed when there is no change from one iteration to the next in the third significant figures of the robust mean

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