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AMBIENTE E SALUTE

ISTITUTO SUPERIORE DI SANITÀ

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Dipartimento Ambiente e Salute

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The Italian National Reference Laboratory for pesticide residues in products of Animal Origin and commodities with high fat content (NRL-AO) organizes yearly Proficiency Tests (PTs) on olive oil in cooperation with the International Olive Council. Laboratories invited to participate in these PTs are Mediterranean laboratories of the International Olive Council and European laboratories (NRLs, official control laboratories and private laboratories), involved in the national and European monitoring programs for pesticide residues in food. This report describes the last PT named COIPT-15. The exercise consisted in the determination of unknown seven different pesticides in a spiked olive oil sample, chosen from a target list of twenty-seven compounds. Forty-five laboratories submitted results with thirty participants, who analysed all the seven spiked compounds. The majority of participants obtained a satisfactory performance (z-score) for all tested pesticides.

Key words: National Reference Laboratory; International Olive Council; Pesticide residues; Proficiency Test; Olive oil

Istituto Superiore di Sanità
Circuito interlaboratorio su residui di antiparassitari in olio di oliva nel 2015.
Tiziana Generali, Patrizia Stefanelli, Silvana Girolimetti, Danilo Attard Barbini
2017, v, 41 p. Rapporti ISTISAN 17/38 (in inglese)

Il Laboratorio Nazionale di Riferimento italiano per residui di antiparassitari in prodotti di origine animale e alimenti ad alto contenuto di grasso annualmente organizza dei circuiti interlaboratorio sull'olio di oliva in collaborazione con il Consiglio Oleicolo Internazionale. I laboratori invitati a partecipare in questi circuiti interlaboratorio sono Paesi mediterranei del Consiglio Oleicolo Internazionale e laboratori europei (Laboratori Nazionali di Riferimento, laboratori di controllo ufficiali e laboratori privati) coinvolti nei piani di controllo nazionali ed europei per il monitoraggio dei residui di antiparassitari negli alimenti. Questo rapporto descrive l'ultimo circuito interlaboratorio denominato COIPT-15. L'esercizio interlaboratorio ha riguardato la determinazione incognita di sette residui di pesticidi addizionati in un campione di olio di oliva, scelti da una lista prestabilita di ventisette composti. Quarantacinque laboratori partecipanti hanno fornito i risultati di cui trenta hanno analizzato tutti i composti addizionati. La maggior parte dei partecipanti ha ottenuto una soddisfacente prestazione (z-score) per tutti gli antiparassitari oggetto del test.

Parole chiave: Laboratorio Nazionale di Riferimento; Consiglio Oleicolo Internazionale; Residui di antiparassitari; Circuito interlaboratorio; Olio di oliva

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ABBREVIATIONS

ADI	*Acceptable Daily Intake*
ARfD	*Acute Reference Dose*
AZ^2	*Average of the Squared z-scores*
CAS	*Chemical Abstract Service*
EC	*European Commission*
EU	*European Union*
EUPT	*European Union Proficiency Test*
FFP	*Fitness for Purpose*
GAP	*Good Agricultural Practice*
GC	*Gas Chromatography*
IEC	*International Electrotechnical Committee*
ILAC	*International Laboratory Accreditation Cooperation*
ISO	*International Organization for Standardization*
LC	*Liquid Chromatography*
LOD	*Default Lowest Limit*
MRL	*Maximum Residue Limit*
MS	*Mass Spectrometry*
NRL-AO	*National Reference Laboratory - Animal Origin*
PPP	*Plant Protection Product*
PT	*Proficiency Test*
RL	*Reporting Limit*
RSD	*Relative Standard Deviation*
SD	*Standard Deviation*
UHPLC	*Ultra High Performance Liquid Chromatography*

Symbols

s^*	*robust standard deviation*
u	*uncertainty measurement*
σ_{EUPT}	*standard deviation for proficiency assessment*
X	*consensus value*

PREFACE

Food safety is a priority in Europe: governments and regulators have been increasing the controls and surveillances on food and they have been established a network of National Reference Laboratories (NRLs) and official control laboratories. The overall objective is to improve the quality, accuracy and comparability of the analytical results regarding the determination of pesticide residues in food.

Current European legislation on pesticides in and on food requires the official laboratory participation in specific proficiency tests, particularly those organized by the NRLs. Regular participation in Proficiency Test (PT) programs is considered a suitable external quality control system for assessing reliability of their results (1).

Furthermore, in accordance with article 12 of Regulation (EC) 882/2004, the laboratories designated for official control have to adopt the general quality criteria for testing laboratories laid down in ISO/IEC 17025:2005 (2). 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/11975/2015 (3).

The Italian NRL for pesticide residues in products of Animal Origin and commodities with high fat content (NRL-AO) yearly organizes PTs on olive oil in cooperation with the International Olive Council, which is the only intergovernmental organization involved in the field of olive oil and table olives and has its headquarters in Madrid.

GENERAL CONSIDERATION ON MAXIMUM RESIDUE LEVEL IN OLIVE OIL

The olive tree is one of the most important and ancient crops in the Mediterranean area where 95% of the olive oil in the world is produced. Olive oil is one of the major component in the Mediterranean diet and as consequence of the high content of monounsaturated fats, the consumption of virgin olive oil helps in the reduction of coronary diseases (4).

According to the data published in November 2016 by International Olive Council, Italy is the second producer of olive oil in Europe with 475 thousand tons during 2015-2016 production. On the contrary related to consumption in Europe Italy is the main consumer with 583 thousand tons in 2015-2016 (5).

The olive tree can be attacked by a large variety of pests, resulting in a reduction in the quality and quantity of the olive fruit and oil produced. Most Plant Protection Products (PPP) used on the olive trees are insecticides, acaricides and fungicides. Herbicides are used to remove weeds from olive tree fields and for this reason a contamination of olive fruit and olive oil is likely. The traces pesticides leave in treated products are called “residues”.

A Maximum Residue Level (MRL) is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly (Good Agricultural Practice, GAP). Other considerations on the definition of MRL are linked with possible amounts of residues in food that must be evaluate as safe for consumers and must be as low as possible.

The European Commission fixes MRLs for all food and animal feed and these MRLs for all crops and all pesticides can be found in the MRL database on the Commission website.

To set any MRL for pesticides applicants e.g. producers of plant protection products, farmers, importers, EU (European Union) or non-EU countries must submit the following key points:

- use of a pesticide on the crop, e.g. quantity, frequency, growth stage of the plant (GAP);
- experimental data on the expected residues when the pesticide is applied according to GAP;
- toxicological reference values for the pesticide – chronic toxicity is measured with the Acceptable Daily Intake (ADI) and acute toxicity with the Acute Reference Dose (ARfD).

Based on the available information, the intake of residues through all food that may be treated with that pesticide is compared with the:

- ADI;
- ARfD for long and short-term intake and for all European consumer groups.

If the requested MRL is not safe, it is set at the lowest limit of analytical determination (*Limit of Determination*, LOD). That is the MRL also for crops on which the pesticide has not been used or when its use has not left detectable residues.

The default lowest limit (LOD) in EU law is 0.01 mg/kg.

The MRLs of pesticide residues are established in olives (as all crops) by the Regulation (EC) 396/2005 (6) and amendments.

To calculate MRLs in olive oil are used processing factors. Currently the followed processing factors are indicated in the Commission Implementing the Regulation (EU) 400/2014 concerning the coordinated multiannual control programme of the EU (7). These factors correspond at 5 for fat soluble pesticides and 1 for non-fat soluble pesticides.

PROFICIENCY TEST ON OLIVE OIL: THE COIPT-15

Rationale

In the last decade, many laboratories have been invited by the Italian NRL-AO to participate in PTs on olive oil: Mediterranean laboratories of the International Olive Council, European laboratories (NRLs, official control laboratories and private laboratories), involved in the national and European monitoring programs. The main aim of these PTs was to compare the performances of the laboratories in Mediterranean and European countries in order to promote mutual acceptance of pesticide residue data regarding the analytical controls of olive oil.

The last PT organized in 2015 on olive oil was named COIPT-15.

The exercise consisted in the determination of seven different pesticides in an olive oil sample spiked with a definite range of concentration (0.050-0.350 mg/kg). These pesticides were chosen from a list of twenty-seven compounds presented in COIPT-15 Announcement that was sent to participant on 23 April 2015. The possible 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.

Forty-seven laboratories agreed to participate in this PT: six NRLs, twenty-three official control laboratories (fourteen were Italian laboratories) and seventeen private laboratories. To assess the performance of the participating laboratories, z-scores are used following the norms of the International Laboratory Accreditation Cooperation (ILAC) and the International Organization for Standardization (ISO) (8, 9).

Information of analytical methodologies used was also requested of the participants. So the effects on the results using different analytical procedures were investigated. The results and information received from the participants have provided indications with respect to satisfactory and unsatisfactory performance and potential analytical problems.

The analytical information highlighted that in some cases unsatisfactory performance could be connected with the use of selective detectors without MS confirmation or by methods excluding matrix-matched calibration and clean up step, very crucial for a matrix such as olive oil.

The instrumental measurement was not the only factor affecting the final results. Due to the complexity of analysis, problems can occur at every step in the analytical procedure, for example to not use certified reference materials.

Test materials

The test materials consisted of 6 litres of olive oil available in Italian supermarket. All the olive oil was homogenized for 3 hours under magnetic stirrer. A portion of the test material was analysed in twice to verify the absence of all listed pesticides. No levels of these compounds were found.

A portion of about 3.0 kg of the blank oil, was spiked with the following pesticides: lambda-Chyalothrin, Diazinon, alpha-Endosulfan, beta-Endosulfan, Phosalone, Kresoxim-Methyl, Trifloxystrobin. Aliquots of 50 g of this spiked oil named COIPT-15 SPIKED OIL were transferred into dark glass bottles as well as aliquots of 50 g of the blank oil named COIPT-15 BLANK OIL. Samples were sealed and stored at ambient temperature before the shipment to

participants. Each participant received one COIPT-15 SPIKED OIL sample and one COIPT-15 BLANK OIL sample. The current MRLs for these six pesticides are showed in Table 1 (10-14).

Table 1. Current MRLs for the seven pesticide spiked in the blank oil

Compounds	Current EU Regulation	MRL on olive (mg/kg)
lambda-Chyalothrin	Regulation (EU) 834/2013 Applicable from: 01/09/2013	**1**
Diazinon	Regulation (EU) 834/2013 Applicable from: 26/04/2013	**0.02***
alpha-Endosulfan	Regulation (EU) 310/2011 Applicable from: 21/10/2011	**0.05* mg/kg** on olive as sum of alpha- and beta-isomers and endosulfan-sulphate expresses as endosulfan
beta-Endosulfan	Regulation (EU) 310/2011 Applicable from: 21/10/2011	**0.05* mg/kg** on olive as sum of alpha- and beta-isomers and endosulfan-sulphate expresses as endosulfan
Phosalone	Regulation (EU) 899/2012 Applicable from: 26/04/2013	**0.02***
Kresoxim-methyl	Regulation (EU) 486/2016 Applicable from: 26/04/2016	**0.2**
Trifloxistrobin	Regulation (EU) 1902/2016 Applicable from: 24/11/2016	**0.3**

***** Limit of analytical determination

Homogeneity and stability test

Homogeneity and stability were tested according to ISO 13528:2015 and the International Harmonized Protocol.

Regarding the homogeneity test ten bottles of the spiked oil samples were randomly chosen and analysed in duplicate.

The stability test was performed using two bottles (chosen randomly) which were analysed in duplicate in two occasions:

- Day 1: during the shipment of the samples on 5th June 2015;
- Day 2: at the deadline for reporting results on 7th July 2015.

Stability test was judged acceptable as the percentage difference of the concentration, for each pesticide, was found than 10%. This test demonstrated that any significant decrease in the pesticide levels was showed for the duration of the PT. The individual results are indicated in Table 2.

Table 2. Data (mg/kg) of the stability test

Samples	lambda-Cyhalothrin	Diazinon	alpha-Endosulfan	beta-Endosulfan	Phosalone	Kresoxim-methyl	Trifloxystrobin
Day 1							
54 a	0.094	0.177	0.324	0.175	0.168	0.210	0.171
54 b	0.097	0.176	0.323	0.177	0.170	0.208	0.177
111 a	0.099	0.181	0.335	0.182	0.176	0.218	0.183
111 b	0.098	0.174	0.317	0.175	0.170	0.209	0.180
Mean 1	**0.097**	**0.177**	**0.325**	**0.177**	**0.171**	**0.211**	**0.178**
SD	0.002	0.003	0.008	0.003	0.003	0.005	0.005
Spiking level	**0.098**	**0.183**	**0.320**	**0.181**	**0.168**	**0.201**	**0.182**
Day 2							
54 a	0.079	0.195	0.280	0.164	0.164	0.201	0.173
54 b	0.097	0.170	0.350	0.187	0.177	0.223	0.190
111 a	0.083	0.156	0.240	0.148	0.160	0.193	0.165
111 b	0.086	0.186	0.335	0.182	0.175	0.212	0.186
Mean 2	**0.086**	**0.177**	**0.301**	**0.170**	**0.169**	**0.207**	**0.179**
SD	0.008	0.017	0.051	0.018	0.008	0.013	0.012
Spiking level	**0.098**	**0.183**	**0.320**	**0.181**	**0.168**	**0.201**	**0.182**
(Δ_M/M_1)%*	**-11**	**0**	**-7**	**-4**	**-1**	**-2**	**1**

*$\Delta_M = M_2-M_1$

All the seven compounds passed the homogeneity test and the related data are shown in Table 3 and Table 4.

Table 3. Homogeneity results (mg/kg) for lambda-Cyhalothrin, Diazinon, alpha-Endosulfan and beta-Endosulfan tested by International Protocol

Sample number	lambda-Cyhalothrin		Diazinon		alpha-Endosulfan		beta-Endosulfan	
	a*	***b****	***a****	***b****	***a****	***b****	***a****	***b****
33	0.081	0.080	0.171	0.166	0.322	0.305	0.169	0.162
51	0.094	0.104	0.180	0.191	0.272	0.319	0.180	0.197
52	0.080	0.079	0.169	0.161	0.314	0.305	0.169	0.165
54	0.083	0.097	0.170	0.195	0.280	0.350	0.164	0.187
67	0.092	0.101	0.169	0.184	0.237	0.302	0.167	0.187
74	0.081	0.086	0.157	0.191	0.239	0.343	0.146	0.185
76	0.096	0.097	0.182	0.188	0.304	0.306	0.185	0.186
77	0.088	0.091	0.170	0.181	0.322	0.336	0.175	0.180
97	0.097	0.089	0.179	0,170	0.289	0.276	0.180	0.169
111	0.083	0.086	0.156	0.186	0.240	0.335	0.148	0.182
Mean	0.089		0.176		0.300		0.174	
SD	0.008		0.012		0.012		0.013	
σ_{EUPT} **	0.023		0.041		0.060		0.038	
SD/ σ_{EUPT}	0.300		0.169		0.294		0.224	
Critical value ***	0.3		0.3		0.3		0.3	
SD/σ_{EUPT} ≤0.3 ****	Yes		Yes		Yes		Yes	

SD Standard Deviation
* a, b = replicates of the same sample
** σ_{EUPT} = Standard Deviation *target*
*** Critical value = critical value according to ISO 13528:2015
**** SD/σ_{EUPT} ≤0.3 = If SD/σ_{EUPT} ≤0.3 the material has sufficient homogeneity

Table 4. Homogeneity results (mg/kg) for Phosalone, Kresoxim-methyl and Trifloxystrobin tested by International Protocol

Sample number	Phosalone		Kresoxim-methyl		Trifloxystrobin	
	a*	***b****	***a****	***b****	***a****	***b****
33	0.165	0.156	0.191	0.199	0.161	0.162
51	0.165	0.175	0.220	0.227	0.176	0.189
52	0.166	0.161	0.196	0.189	0.161	0.164
54	0.164	0.177	0.201	0.223	0.173	0.190
67	0.164	0.174	0.214	0.217	0.174	0.183
74	0.160	0.179	0.191	0.218	0.170	0.193
76	0.166	0.173	0.216	0.217	0.181	0.183
77	0.174	0.178	0.204	0.211	0.180	0.182
97	0.166	0.153	0.211	0.196	0.178	0.160
111	0.160	0.175	0.193	0.212	0.165	0.186
Mean	0.168		0.207		0.176	
SD	0.008		0.012		0.011	
σ_{EUPT}**	0.040		0.049		0.043	
SD/ σ_{EUPT}	0.122		0.200		0.191	
Critical value ***	0.3		0.3		0.3	
SD/σ_{EUPT} ≤0.3 ****	Yes		Yes		Yes	

* a, b = replicates of the same sample
** σ_{EUPT} = Standard Deviation *target*
*** Critical value = critical value according to ISO 13528:2015
**** SD/σ_{EUPT} ≤0.3 = If SD/σ_{EUPT} ≤0.3 the material has sufficient homogeneity y

Distribution of samples and instructions to participants

Two dark glass bottles containing 50 g of blank oil and 50 g of spiked oil respectively were sent to the participating laboratories. Because olive oil usually is disposable at ambient temperature samples were shipped without refrigeration.

An information message was sent out by e-mail before shipment so that laboratories could make their own arrangements for the reception of the package.

The participants (Appendix A) were asked:

– to treat the test material as if it were a sample for their routine analysis:
– to report results in the appropriate form and sent to the organizer either by e-mail or fax along with the details of methodology used.

The samples were sent to participants between 25-29 May 2015.

The deadline for results was 3 July 2015.

The final report was dispatched to all participant at the end of December 2015.

Statistical evaluation of results

The organiser of this PT decided to use the z-score parameter to evaluate the laboratory performance for each compound using the same model of the PTs carried out by the European Reference Laboratories (EURLs) (15, 16) for the statistical treatment of the initial results.

The median value and the robust mean (according to algorithm A) were calculated. The median is a simple and highly outlier resistant estimator of the population mean for symmetric distributions. The algorithm A minimises the influence of outlying results and provides good estimations of the standard deviation. In comparison with the median, the robust mean is less influenced by deviating results and for this reason at the end the *robust mean* was used as consensus value calculated in accordance with the algorithm A as explained in the Annex C.3.1 of ISO 13528:2015 document (Appendix B).

The z-score has been calculated by the formula:

$$z_{EUPT} - \text{score} = \frac{(\text{x} - \text{X})}{\sigma_{EUPT}}$$

where x is the laboratory mean, X is the *consensus* value (the robust mean), σ_{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 and –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 unacceptable.

In this exercise any z-score values of z > 5 have been reported as 5* and z-score values were calculated for false negative results using:

- the Reporting Limit (RL) of 0.05 mg/kg (value set by the organiser for all compounds) where the RL of the laboratory was higher than, or equal to RL of 0.05 mg/kg;
- the RL of the laboratory in cases where the RL of the lab was lower than the RL of 0.05 mg/kg.

No z-score has been calculated for false positive result.

The spread of the results for each compound was evaluated performing some statistical tests (asymmetry test, normality tests by using the SPSS software).

Furthermore, the global performance (17) of each participating laboratory was assessed by calculating the Average of the Squared z-scores (AZ^2).

The global performance of each participating laboratory has been assessed only for laboratories which have achieved the *sufficient scope.* The $|\ AZ^2\ |$ is estimated using the following formula:

$$AZ^2 = \frac{\sum_{i=1}^{n} |Z_i| \omega(Z_i)}{\text{n}}$$

The formula is the sum of the z-score value, multiplied by itself [$\omega\ (Z_i) = Z_i$] and divided by the number of z-scores (n) including those from false negatives.

The AZ^2 was used to evaluate the global performance of each laboratory with three sub-classifications:

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

When the assigned value is derived as a robust mean, the standard uncertainty (u, mg/kg) of the assigned value X may be estimated using the following formula, where s* is the robust standard deviation and n is the total number of results:

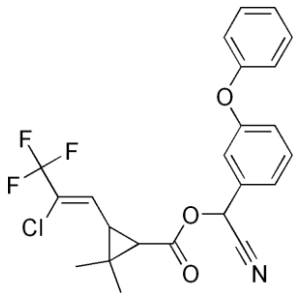
$$u = 1.25 \text{ x } \frac{s*}{\sqrt{n}}$$

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.

COIPT-15: RESULTS

Description and statistical evaluation of the results are presented for each compound separately and as final comments.

lambda-Cyhalothrin



Common name
lambda-Cyhalothrin or lambda-cyhalothrine

Structure formula $C_{23}H_{19}ClF_3NO_3$

CAS number 91465-08-6

EC no. 415-130-7

The technical product is a mixture of equal quantities of (1S, 3S) and (1R, 3R) isomers with molecular weight of 449. This compound belong to pyrethroids class with good solubility in organic solvents and stable to light and stable on storage for more than 6 months at 15-25°C.
Non-systemic insectide that acts on the nervous system of insects.
Insecticide authorized on olive tree with a MRL value of 1 mg/kg on olive as established by the Regulation (EC) 396/2005.

In Italy four lambda-Cyhalothrin plant protection products are authorized: two are granular formulations and two micro-emulsion formulations.

Figure 1 shows the results of lambda-Cyhalothrin (mg/kg) submitted by all laboratories in the COIPT-15. The distribution of the results is clearly not symmetric.

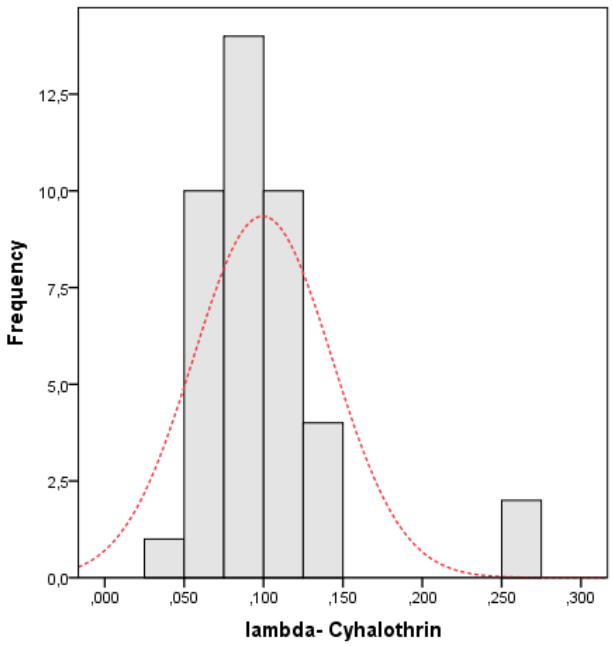


Figure 1. LAMBDA-CYHALOTHRIN: frequency histogram of the results (mg/kg)

Statistical evaluation of the lambda-Cyhalothrin results are presented in Tables 5 and 6.

Table 5. Statistical parameters (mg/kg) of lambda-Cyhalothrin

Parameter	Value
Spiked value	0.098
Mean	0.098
Median	0.091
Robust mean	0.092
s*	0.026
σ_{EUPT}	0.023

s*= robust standard deviation

Table 6. Assigned value, uncertainty and % RSD for lambda-Cyhalothrin

Parameter	Value
Assigned value (mg/kg)	0.092
Uncertainty (u) (mg/kg)	0.005
u/σ_{EUPT} *	0.22
FFP RSD (%)	25
Robust RSD (%)	28

* $u/\sigma_{EUPT} \leq 0.3$; RSD: Relative Standard Deviation

From a statistical point of view, the results can be considered satisfactory, since the data used for the assigned value produced median and robust mean that are practically almost the same for lambda-Cyhalothrin. The *median* and the *robust mean* in Table 5 differ only from 1 unit and are close to spiked value of 0.098 mg/kg. The Robust Relative Standard Deviation (Robust RSD) and the uncertainty of the assigned values *u* for lambda-Cyhalothrin resulted acceptable.

All z-score values are presented in graphical form in Figure 2.

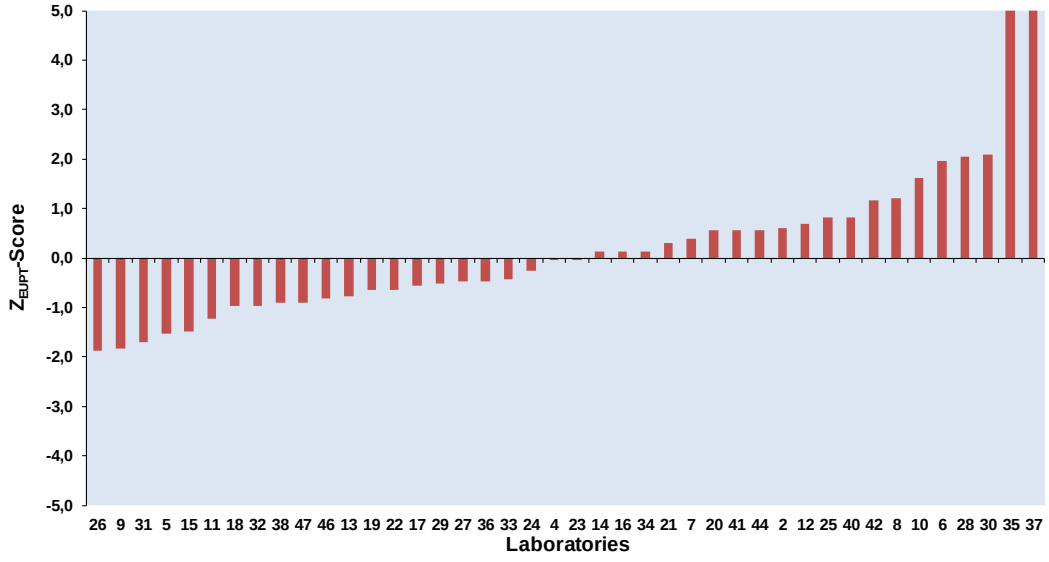


Figure 2. LAMBDA-CYHALOTHRIN: z-score values (spiked value = 0.098 mg/kg)

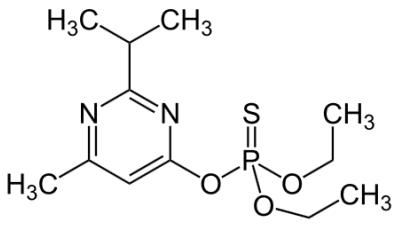
Z_{EUPT}-scores of lambda-Cyhalothrin for all participants are presented in Table 7 with recoveries estimated as numerical values.

Table 7. LAMBDA-CYHALOTHRIN: z_{EUPT}-score and recovery (%)

Lab Code	Recovery	z_{EUPT}-score
2	108	0.6
3	-	-
4	91	0.0
5	80	-1.5
6	70	2.0
7	75	0.4
8	101	1.2
9	-	-1.8
10	70	1.6
11	99	-1.2
12	92	0.7
13	95	-0.8
14	90	0.1
15	-	-1.5
16	**30**	0.1
17	128	-0.6
18	85	-1.0
19	97	-0.7
20	85	0.6
21	115	0.3
22	72	-0.7
23	81	0.0
24	82	-0.3
25	77	0.8
26	79	-1.9
27	92	-0.5
28	67	2.0
29	77	-0.5
30	65	2.1
31	**48**	-1.7
32	90	-1.0
33	101	-0.4
34	85	0.1
35	90	5*
36	96	-0.5
37	71	5*
38	62	-0.9
39	-	-
40	103	0.8
41	-	0.6
42	98	1.2
43	-	-
44	104	0.6
46	72	-0.8
47	120	-0.9

Fourty-one laboratories submitted results for lambda-Cyhalothrin and two z-scores > 5 were calculated for Lab 35 and Lab 37 respectively and one false negative was presented by Lab 9. The poor performance of the laboratories 35 and 37 is not connected with recoveries presented that are acceptable.

Diazinon



Common name
Diazinon

Structure formula $C_{12}H_{21}N_2O_3PS$

CAS number 333-41-5

EC no. 206-373-8

It is a colourless liquid and in technical form a yellow liquid with weight molecular of 304.3.
Non-systemic insecticide and acaricide most used in the past and belongs to organophosphate compounds. This compound has good solubility in organic solvents and is stable in neutral media but is susceptible to oxidation above 100°C.
Not authorized on olive tree with a MRL value of 0.02 mg/kg on olive as established by the Regulation (EC) 396/2005 that corresponds at limit of analytical determination.
It could be present in olive oil as contaminant as consequence of his liphofilic properties.

In Figure 3 results of Diazinon (mg/kg) submitted by all laboratories are expressed as frequency histogram. The distribution of the results presented is definitely symmetric.

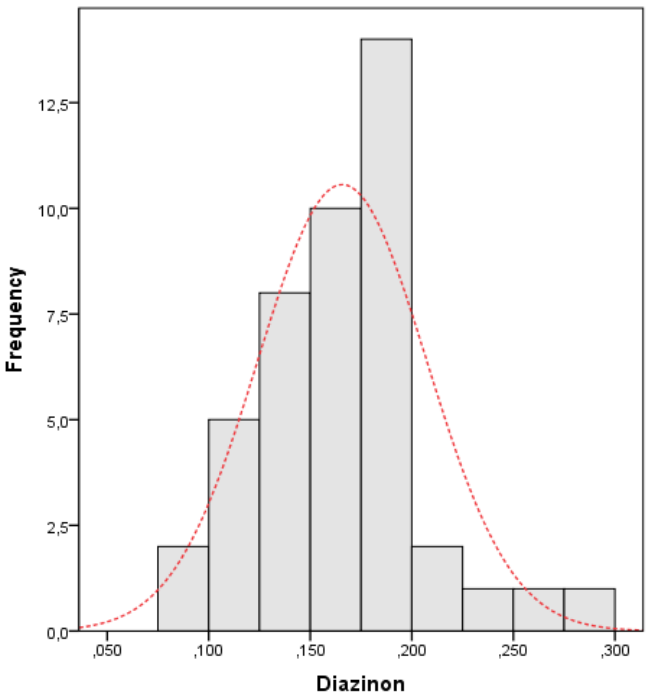


Figure 3. DIAZINON: frequency histogram of the results (mg/kg)

Statistical evaluation of the Diazinon results are presented in Tables 8 and 9.

Table 8. Statistical parameters (mg/kg) of Diazinon

Parameter	Value
Spiked value	0.183
Mean	0.166
Median	0.164
Robust mean	0.164
s*	0.039
σ_{EUPT}	0.041

s*= robust standard deviation

Table 9. Assigned value, uncertainty and % RSD for Diazinon

Parameter	Value
Assigned value (mg/kg)	0.164
Uncertainty (u) (mg/kg)	0.007
u/σ_{EUPT} *	0.17
FFP RSD (%)	25
Robust RSD (%)	24

* $u/\sigma_{EUPT} \leq 0.3$

Results submitted for Diazinon are really good with the same value of 0.164 mg/kg for *median* and *robust mean*. The Robust RSD and the uncertainty of the assigned values *u* for Diazinon resulted acceptable with a value of 24%and 25% respectively.
All z-score values are presented in graphical form in Figure 4.

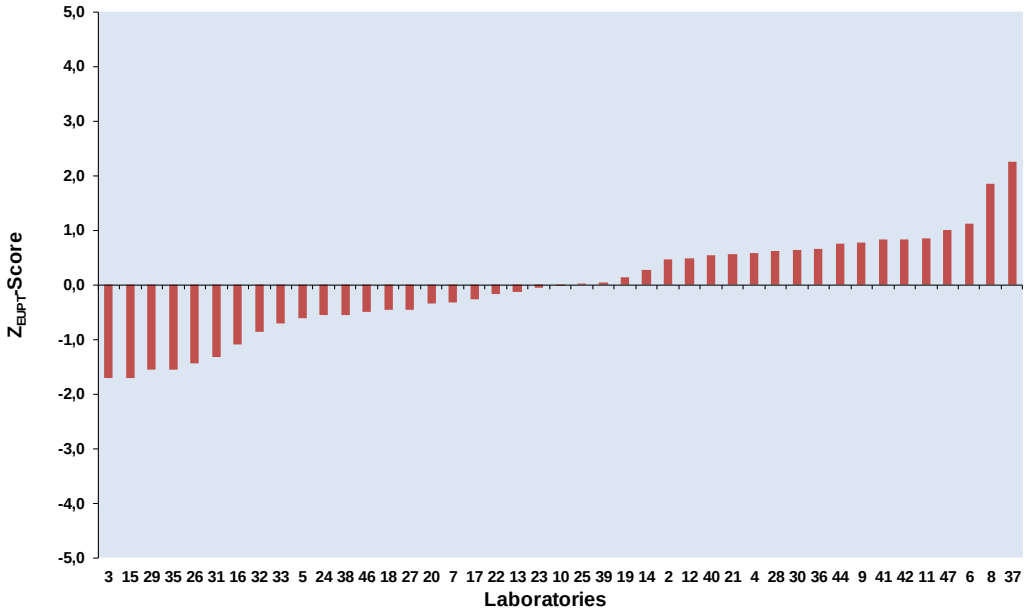


Figure 4. DIAZINON: z-score values (spiked value = 0.183 mg/kg)

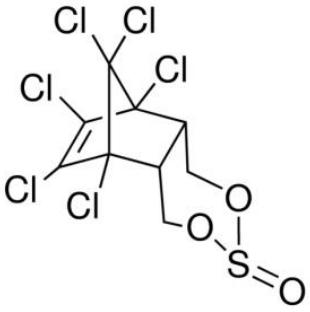
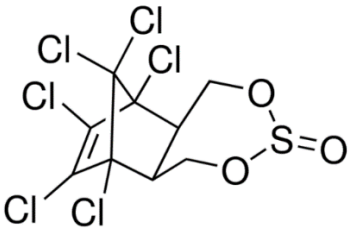
In Table 10 are listed the z_{EUPT}-scores of Diazinon for all participants with the corresponding recovery values.

Table 10. z_{EUPT}-score and recovery (%) results for Diazinon

Lab Code	Recovery	z_{EUPT}-score
2	100	0.5
3	-	-1.7
4	98	0.6
5	75	-0.6
6	92	1.1
7	76	-0.3
8	99	1.9
9	107	0.8
10	97	0.0
11	102	0.9
12	91	0.5
13	97	-0.1
14	90	0.3
15	115	-1.7
16	74	-1.1
17	77	-0.3
18	83	-0.5
19	110	0.1
20	87	-0.3
21	114	0.6
22	90	-0.2
23	93	0.0
24	96	-0.6
25	75	0.0
26	76	-1.4
27	90	-0.5
28	85	0.6
29	70	-1.6
30	88	0.6
31	63	-1.3
32	110	-0.9
33	91	-0.7
34	80	2.9
35	89	-1.6
36	99	0.7
37	88	2.2
38	68	-0.6
39	87	0.0
40	103	0.5
41	-	0.8
42	92	0.8
43	-	-
44	115	0.8
46	93	-0.5
47	120	1.0

Fourty-five laboratories submitted results for Diazinon that was the compound most analyzed in the COIPT-15 with an excellent performance because all calculated z-scores obtained were resulted acceptable in the range ± 2.

alpha and beta-Endosulfan

Common name alpha-Endosulfan**,** Endosulfan I **CAS number** 959-98-8	**Common name** beta-Endosulfan, Endosulfan II **CAS number** 33213-65-9
	
Structure formula $C_9H_6Cl_6O_3S$	
alpha and beta-Endosulfan are two stereoisomers of the same molecule with molecular weight of 406.9. The technical product is a mixture of the two isomers composed of beige crystals. It belongs to cyclodiene organochlorine with good solubility in organic solvents and stable to light. Non-systemic insecticide and acaricide with contact and stomach action. Not authorized on olive tree with a MRL value of 0.05 mg/kg on olive as sum of alpha- and beta-isomers and endosulfan-sulphate expresses as endosulfan established by the Regulation (EC) 396/2005. This value of MRL correspond at limit of analytical determination. It could be present in olive oil as contaminant as consequence of his liphofilic properties.	

Results are presented in Figures 5 and 6 as frequency histograms. The distribution for alpha-Endosulfan is definitely symmetric whereas for beta-Endosulfan asymmetric.

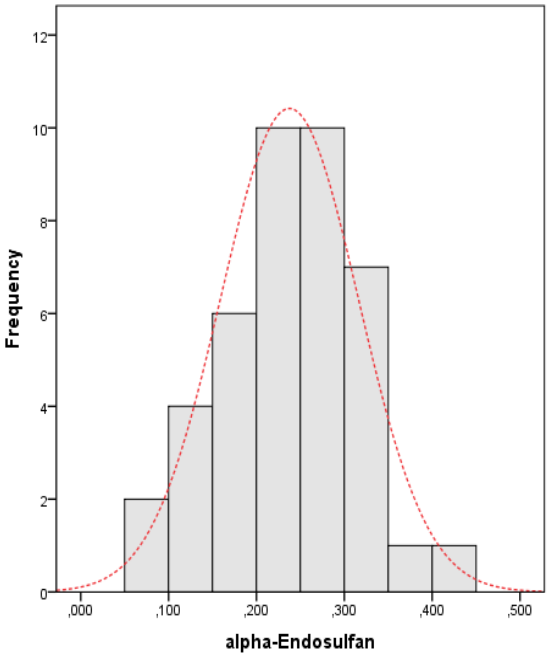


Figure 5. ALPHA-ENDOSULFAN: frequency histograms of the results

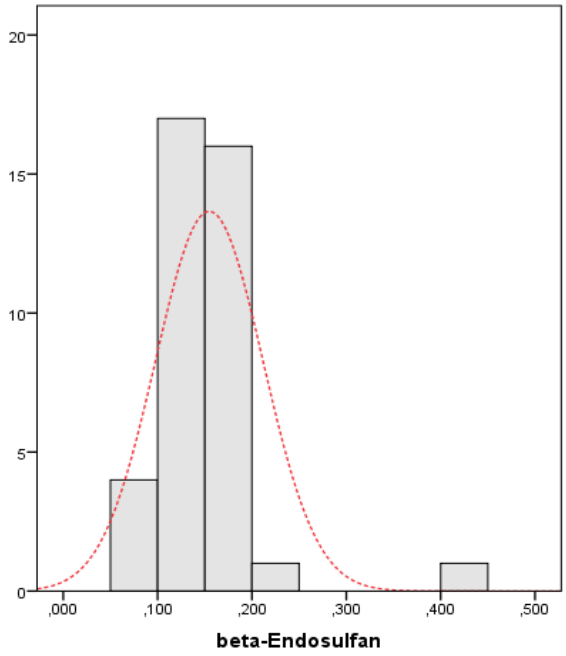


Figure 6. BETA-ENDOSULFAN: frequency histograms of the results

Statistical evaluation of the alpha and beta-Endosulfan results are presented in Tables 11 and 12.

Table 11. Statistical parameters (mg/kg) of alpha and beta-Endosulfan

Parameter	Value	
	alpha- Endosulfan	**beta- Endosulfan**
Spiked value	0.320	0.181
Mean	0.237	0.151
Median	0.240	0.145
Robust mean	0.239	0.150
s*	0.085	0.040
σ_{EUPT}	0.060	0.038

s*= robust standard deviation

Table 12. Assigned value, uncertainty and % RSD for alpha and beta-Endosulfan

Parameter	Value	
	alpha- Endosulfan	**beta- Endosulfan**
Assigned value (mg/kg)	0.239	0.15
Uncertainty (u) (mg/kg)	0.017	0.008
u/σ_{EUPT} *	0.28	0.21
FFP RSD (%)	25	25
Robust RSD (%)	36	27

* $u/\sigma_{EUPT} \leq 0.3$; RSD: Relative Standard Deviation

Statistically results for alpha and beta-Endosulfan can be considered satisfactory. The *median* and the *robust mean* in Table 11 are similar for both compounds. The Robust RSD value for beta-Endosulfan is equal at 27% while is higher for alpha-Endosulfan with a value of 36%.

All z-score values are presented in graphical form in Figures 7 and 8.

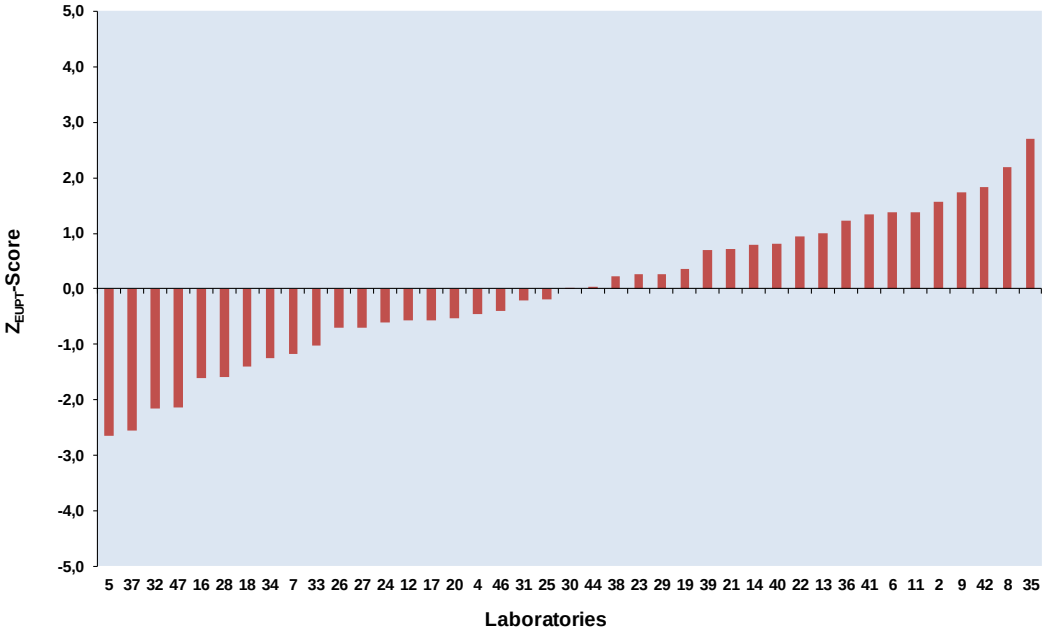


Figure 7. ALPHA-ENDOSULFAN: z-score values (spiked value = 0.320 mg/kg)

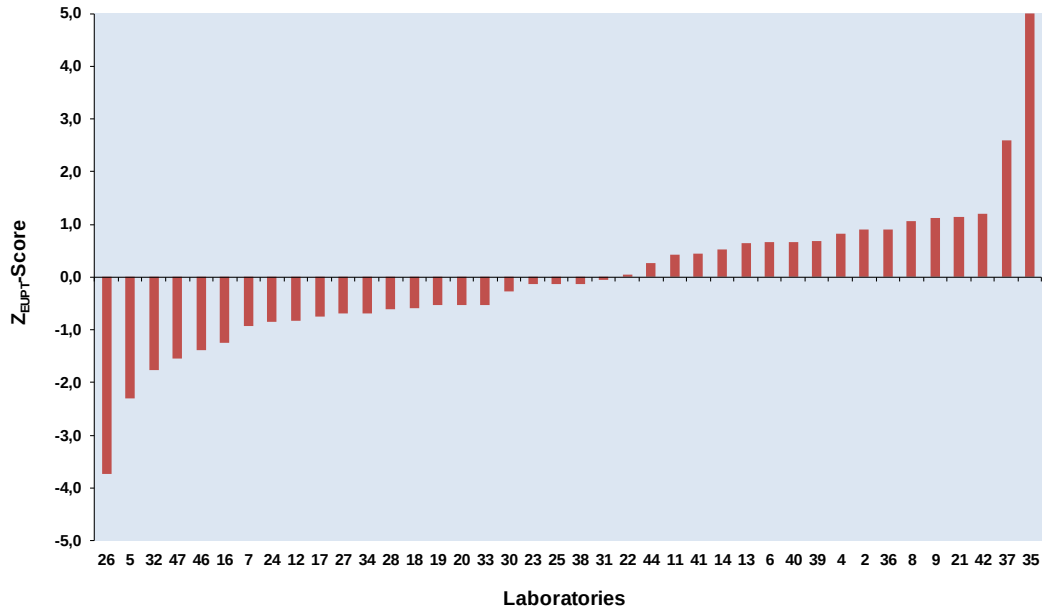


Figure 8. BETA-ENDOSULFAN: z-score values (spiked value = 0.181 mg/kg)

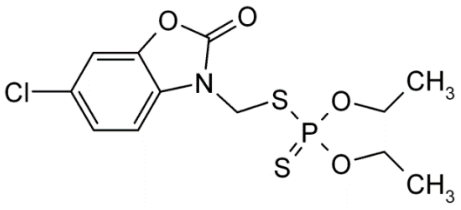
In Table 13 are showed the z_{EUPT}-scores of alpha and beta-Endosulfan calculated for all participants with the corresponding recovery data.

Table 13. z_{EUPT}-score and recovery (%) results for alpha and beta-Endosulfan

Lab Code	alpha-Endosulfan		beta-Endosulfan	
	Recovery	z_{EUPT}-score	Recovery	z_{EUPT}-score
2	104	1.6	102	0.9
3	-	-	-	-
4	78	-0.5	116	0.8
5	85	-2.6	85	-2.3
6	83	1.4	108	0.7
7	70	-1.2	73	-0.9
8	101	2.2	101	1.1
9	100	1.7	101	1.1
10	-	-	-	-
11	91	1.4	93	0.4
12	72	-0.6	75	-0.8
13	89	1.0	96	0.6
14	85	0.8	85	0.5
15	-	-	-	-
16	-	-1.6	-	-1.3
17	98	-0.6	110	-0.7
18	65	-1.4	69	-0.6
19	95	0.4	96	-0.5
20	75	-0.5	85	-0.5
21	117	0.7	117	1.1
22	91	0.9	86	0.1
23	75	0.3	69	-0.1
24	86	-0.6	88	-0.9
25	73	-0.2	70	-0.1
26	90	-0.7	-	-3.7
27	71	-0.7	80	-0.7
28	78	-1.6	88	-0.6
29	60	0.3	-	-
30	68	0.0	76	-0.3
31	65	-0.2	75	-0.1
32	70	-2.2	70	-1.8
33	60	-1.0	87	-0.5
34	60	-1.3	62	-0.7
35	101	2.7	101	5*
36	109	1.2	94	0.9
37	80	-2.6	80	2.6
38	75	0.2	72	-0.1
39	70	0.7	75	0.7
40	76	0.8	91	0.7
41	-	1.3	-	0.5
42	104	1.8	97	1.2
43	-	-	-	-
44	72	0.0	88	0.3
46	81	-0.4	86	-1.4
47	108	-2.1	120	-1.5

Fourty-one laboratories submitted results for alpha-Endosulfan and fourty for beta-Endosulfan. In the case of beta-Endosulfan one false negative value was calculated for Lab 26 and one z-score >5 was presented by Lab 35 that appear not be consistent with the corresponding recovery presented.

Phosalone



Common name
Phosalone other name benzphos

Structure formula $C_{12}H_{15}ClNO_4PS_2$

CAS number 2310-17-0

EC no. 218-996-2

Its physical form consists of colourless crystals with an odour of garlic with weight molecular of 367.8.
Non-systemic insecticide and acaricide belongs to organophosphate compounds. This compound has good solubility in organic solvents and good stability.
Not authorized on olive tree with a MRL value of 0.02 mg/kg on olive as established by the Regulation (EC) 396/2005 that correspond at limit of analytical determination.
It could be present in olive oil as contaminant as consequence of his liphofilic properties.

Figure 9 shows the results of Phosalone (mg/kg) submitted by all laboratories are expressed as frequency histogram. It is evident the symmetric distribution of results presented for Phosalone.

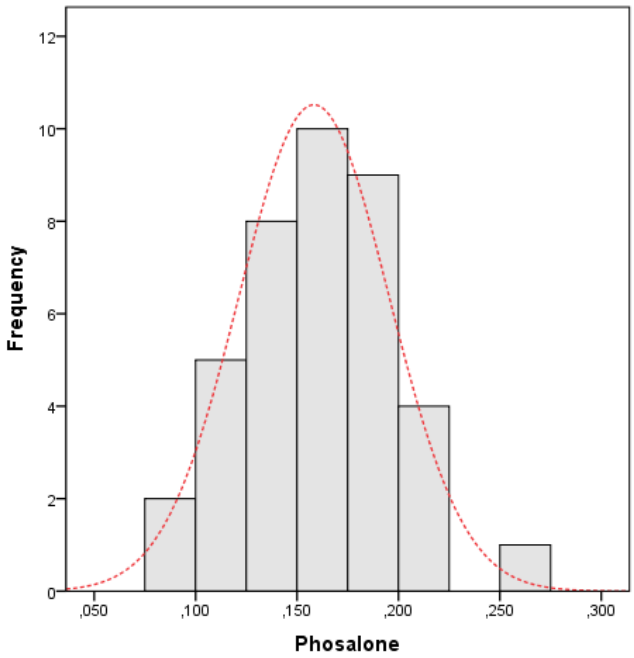


Figure 9. PHOSALONE: frequency histogram of the results (mg/kg)

Statistical evaluation of the Phosalone data are presented in Tables 14 and 15.

Table 14. Statistical parameters (mg/kg) of Phosalone

Parameter	Value
Spiked value	0.168
Mean	0.155
Median	0.158
Robust mean	0.158
s*	0.035
σ_{EUPT}	0.040

s*= robust standard deviation

Table 15. Assigned value, uncertainty and % RSD for Phosalone

Parameter	Value
Assigned value (mg/kg)	0.158
Uncertainty (u) (mg/kg)	0.007
u/σ_{EUPT} *	0.18
FFP RSD (%)	25
Robust RSD (%)	22

* $u/\sigma_{EUPT} \leq 0.3$

As in the case of Diazinon, results for Phosalone are really good with the same value of 0.158 mg/kg for *median* and *robust mean*. The Robust RSD and the uncertainty of the assigned values *u* resulted acceptable with a value of 22%and 25% respectively.

All z-score values are showed in graphical form in Figure 10.

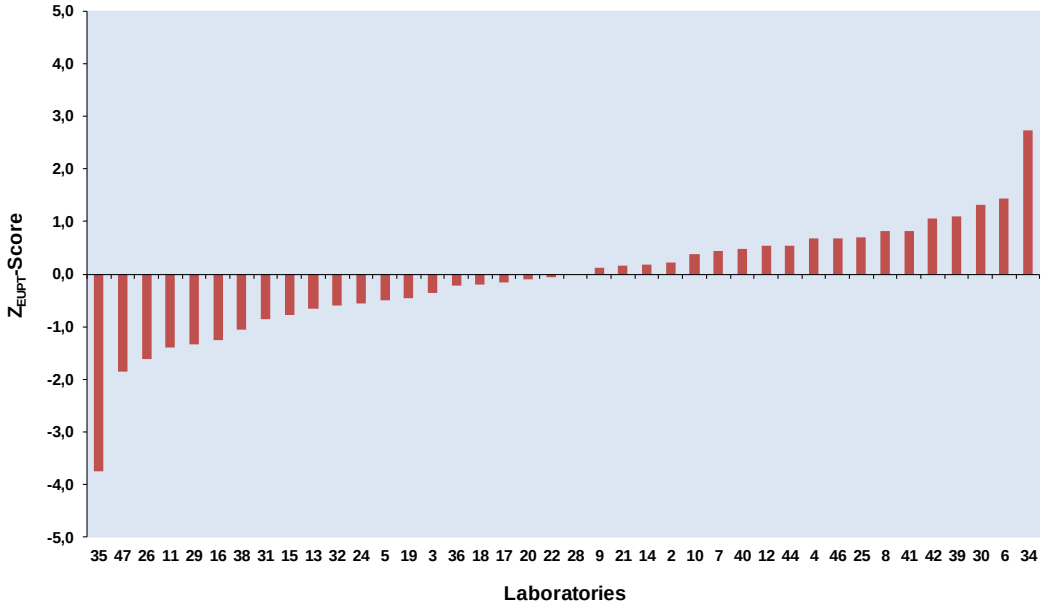


Figure 10. PHOSALONE: z-score values (spiked value = 0.168 mg/kg)

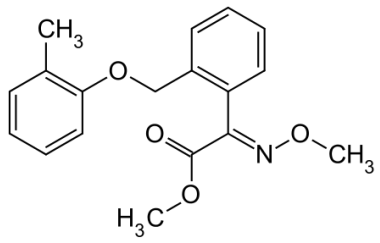
In Table 16 are listed the z_{EUPT}-scores of Phosalone for all participants with the corresponding recovery values.

Table 16. z_{EUPT}-score and recovery (%) results for Phosalone

Lab Code	Recovery	z_{EUPT}-score
2	99	0.2
3	88	-0.4
4	119	0.7
5	75	-0.5
6	95	1.4
7	75	0.4
8	101	0.8
9	100	0.1
10	102	0.4
11	88	-1.4
12	96	0.5
13	91	-0.7
14	90	0.2
15	93	-0.8
16	71	-1.3
17	84	-0.2
18	91	-0.2
19	87	-0.5
20	72	-0.1
21	112	0.2
22	84	-0.1
23	-	-
24	97	-0.6
25	68	0.7
26	82	-1.6
27	-	-
28	96	0.0
29	75	-1.3
30	86	1.3
31	93	-0.9
32	110	-0.6
33	-	-
34	85	2.7
35		-3.7
36	96	-0.2
37	-	-
38	77	-1.1
39	86	1.1
40	100	0.5
41	-	0.8
42	97	1.1
43	-	-
44	111	0.5
46	75	0.7
47	116	-1.8

Thirty-nine laboratories submitted results for Phosalone with a good performance. In fact, the majority of laboratories obtained z-scores acceptable in the range ± 2 except for one value of 2.7 (Lab 34) and for one false negative value calculated for Lab 35 equal to 3.7.

Kresoxim-methyl



Common name
Kresoxim-methyl or krésoxim méthyle

Structure formula $C_{18}H_{19}NO_4$

CAS number 143390-89-0

EC no. 417-880-0

This compound belongs to the strobilurin family with a physical form of white, mildly aromatic crystals and a weight molecular of 313.4.
Fungicide that inhibits spore germination with good solubility in organic solvents and it is relatively stable at pH 5.
Fungicide authorized on olive tree with a MRL value of 0.2 mg/kg on olive as established by the Regulation (EC) 396/2005.

In Italy any plant protection product of Kresoxim methyl is authorized.

In Figure 11 all results of Kresoxim-methyl (mg/kg) are showed as frequency histogram. The distribution of results for kresoxim-methyl is clearly symmetric.

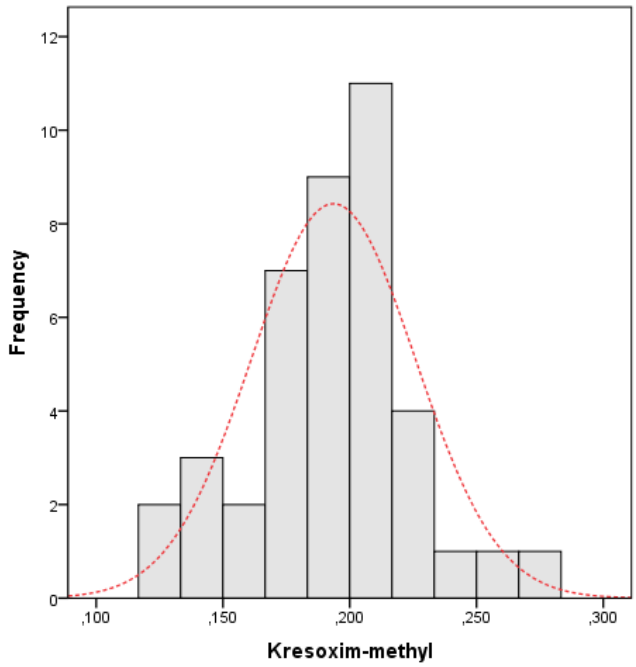


Figure 11. KRESOXIM-METHYL: frequency histogram of the results (mg/kg)

Statistical evaluation of the Kresoxim-methyl results is showed in Tables 17 and 18.

Table 17. Statistical parameters (mg/kg) of Kresoxim-methyl

Parameter	Value
Spiked value	0.201
Mean	0.189
Median	0.198
Robust mean	0.195
s*	0.026
σ_{EUPT}	0.049

s*= robust standard deviation

Table 18. Assigned value, uncertainty and % RSD for Kresoxim-methyl

Parameter	Value
Assigned value (mg/kg)	0.195
Uncertainty (u) (mg/kg)	0.005
u/σ_{EUPT} *	0.10
FFP RSD (%)	25
Robust RSD (%)	13

* $u/\sigma_{EUPT} \leq 0.3$

Statistically results for Kresoxim-methyl can be considered satisfactory.

The *median* and the *robust mean* in table 16 are similar with a really good value for Robust RSD of 13%.

All z-score values are presented in graphical form in Figure 12.

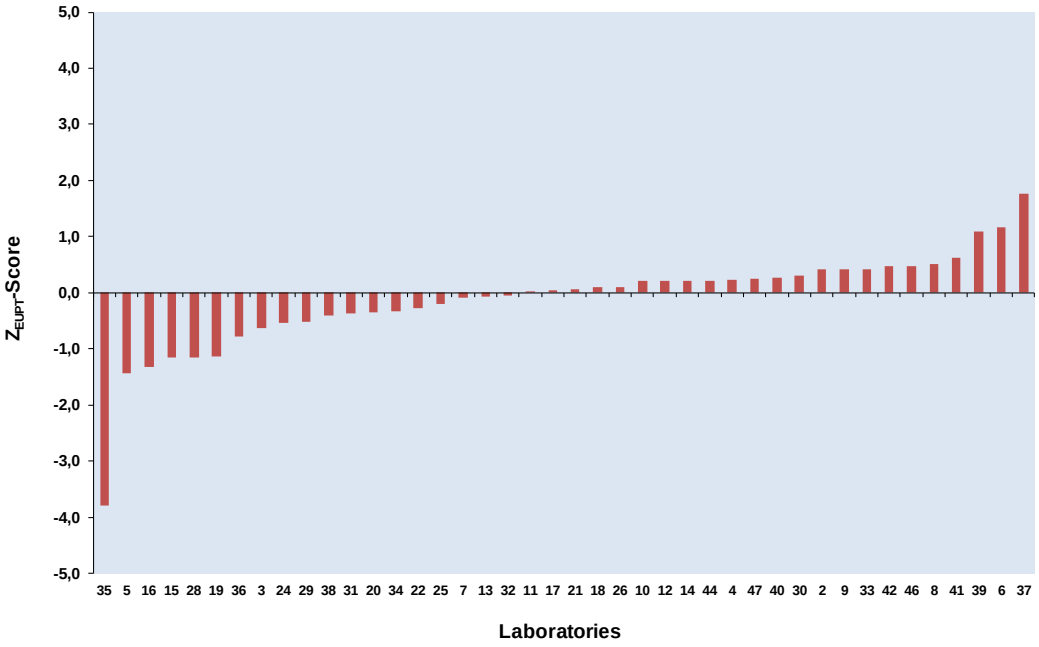


Figure 12. KRESOXIM-METHYL: z-score values (spiked value = 0.201 mg/kg)

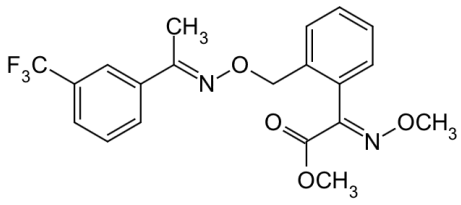
In Table 19 are presented the z_{EUPT}-scores of Kresoxim-methyl calculated for all participants with the corresponding recovery data.

Table 19. z_{EUPT}-score and recovery (%) results for Kresoxim-methyl

Lab Code	Recovery %	z_{EUPT}-score
2	107	0.4
3	94	-0.6
4	105	0.2
5	83	-1.4
6	100	1.2
7	83	-0.1
8	101	0.5
9	98	0.4
10	95	0.2
11	102	0.0
12	92	0.2
13	90	-0.1
14	90	0.2
15	116	-1.1
16	64	-1.3
17	90	0.0
18	92	0.1
19	91	-1.1
20	100	-0.3
21	131	0.1
22	90	-0.3
23	-	-
24	97	-0.5
25	72	-0.2
26	97	0.1
27	-	-
28	98	-1.1
29	84	-0.5
30	99	0.3
31	85	-0.4
32	120	0.0
33	105	0.4
34	92	-0.3
35	-	-3.8
36	101	-0.8
37	100	1.8
38	64	-0.4
39	70	1.1
40	110	0.3
41	-	0.6
42	91	0.5
43	-	-
44	118	0.2
46	102	0.5
47	84	0.2

Fourty-one laboratories submitted results for kresoxim-methyl and obtained a good performance. In fact, the majority of laboratories obtained z-scores acceptable in the range ± 1 (33 laboratories out of 41) and however all z-score were included in the range ± 1except for one false negative calculated for Lab 35 equal to 3.8.

Trifloxystrobin



Common name
Trifloxystrobin or Trifloxystrobine

Structure formula $C_{20}H_{19}F_3N_2O_4$

CAS number 141517-21-7

EC no. 417-880-0

This compound belongs to the strobilurin family as the previously compound Kresoxim-methyl Its physical form consists of odourless white solid with molecular weight of 408.4.

Mesostemic broad-spectrum fungicide with mainly preventive activity. This pesticide has a good solubility in organic solvents and a relatively stability at pH 5.

Fungicide authorized on olive tree with a MRL value of 0.3 mg/kg on olive as established by the Regulation (EC) 396/2005.

Formulation types of plant protection products containing Trifloxystrobin are EC (Emulsifiable Concentrate), FS (Flowable Concentrate), WG (Water Dispersible Granule) and SC (Suspension Concentrate).

In Italy one protection product containing Trifloxystrobin plus another pesticide is authorized in water dispersible granules as formulation (WG).

Figure 13 shows the results of Trifloxystrobin (mg/kg) submitted by all laboratories expressed as frequency histogram.

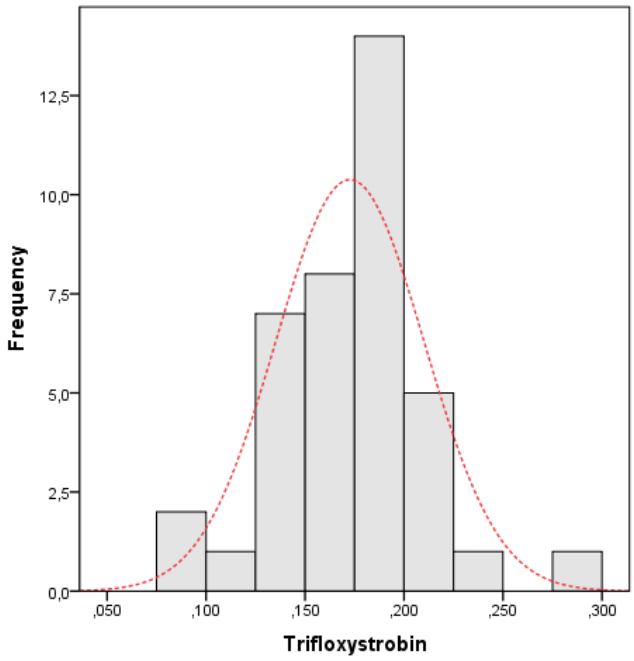


Figure 13. Trifloxystrobin: frequency histogram of the results (mg/kg)

Statistical evaluation of the Trifloxystrobin data are presented in Tables 19 and 20.

Table 20. Statistical parameters (mg/kg) of Trifloxystrobin

Parameter	Value
Spiked value	0.182
Mean	0.169
Median	0.178
Robust mean	0.173
s*	0.030
σ_{EUPT}	0.043

s*= robust standard deviation

Table 21. Assigned value, uncertainty and % RSD for Trifloxystrobin

Parameter	Value
Assigned value (mg/kg)	0.173
Uncertainty (u) (mg/kg)	0.006
u/σ_{EUPT} *	0.14
FFP RSD (%)	25
Robust RSD (%)	17

* u/σ_{EUPT}≤ 0.3

Statistically results for Trifloxystrobin are considered satisfactory. The *median* and the *robust mean* in Table 19 are similar with a really good value for Robust Standard Deviation (Robust RSD) of 17% (see Table 21).

All z-score values are showed in graphical form in Figure 14.

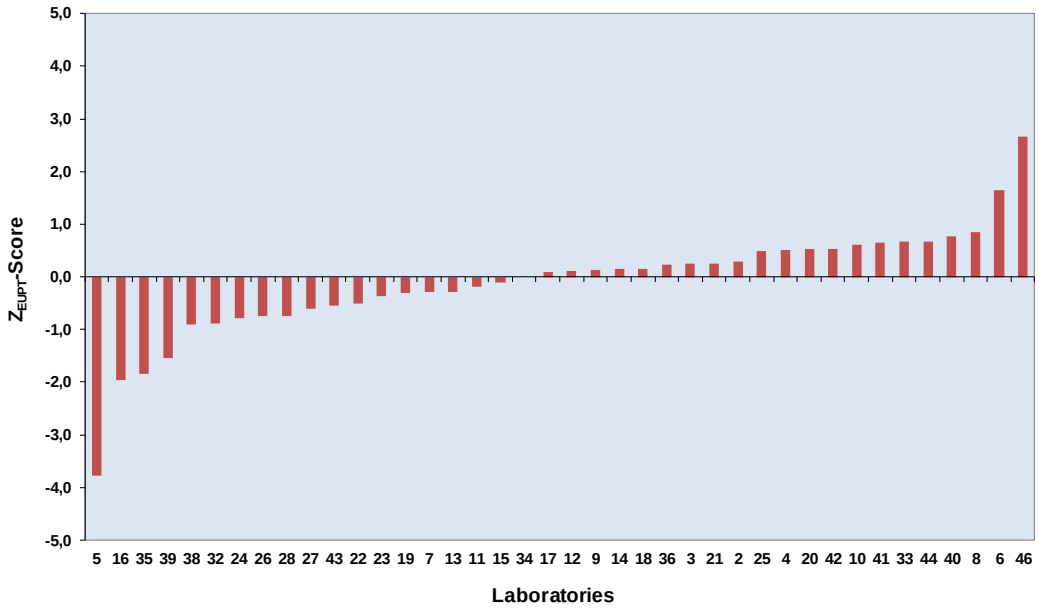


Figure 14. TRIFLOXYSTROBIN: z-score values (spiked value = 0.182 mg/kg)

Table 21 presents the z_{EUPT}-scores of Trifloxystrobin calculated for all participants with the corresponding recovery data.

Table 21. z-score and recovery (%) results for *Trifloxystrobin*

Lab Code	Recovery %	z_{EUPT}-score
2	102	0.3
3	90	0.3
4	117	0.5
5	-	-3.8
6	97	1.6
7	70	-0.3
8	99	0.9
9	100	0.1
10	114	0.6
11	97	-0.2
12	85	0.1
13	93	-0.3
14	90	0.2
15	106	-0.1
16	60	-2.0
17	88	0.1
18	101	0.2
19	103	-0.3
20	102	0.5
21	130	0.3
22	86	-0.5
23	-	-0.4
24	87	-0.8
25	75	0.5
26	92	-0.7
27	86	-0.6
28	102	-0.7
29	-	-
30	-	-
31	-	-
32	99	-0.9
33	117	0.7
34	95	0.0
35	101	-1.8
36	101	0.2
37	-	-
38	64	-0.9
39	98	-1.5
40	115	0.8
41	-	0.6
42	93	0.5
43	90	-0.6
44	112	0.7
46	88	2.7
47	-	-

Thirty-nine laboratories submitted results for Trifloxystrobin, the less analysed pesticide. The calculated z-score values were all in the range ± 2 except for one value of 2.7 (Lab 46) and one false negative presented by Lab 5.

COIPT-15: FINAL COMMENTS

From a statistical point of view, the results for all the six compounds presented can be considered satisfactory, since the data used for the assigned value produced *median and robust mean* that are practically almost the same for each analyte (18).

As in the case of Diazinon and Phosalone, the numerical values of *median and robust mean* are the same.

Further the Robust RSD and the uncertainty of the assigned values u were presented for all pesticides. The range of Robust RSD values was from 13 to 28 % for six compounds except alpha-Endosulfan with a value of 36% while the range of u was from 0.005 to 0.017.

Forty-five laboratories submitted results but thirty analysed all compounds with Diazinon analysed by the majority of laboratories on the contrary of Trifloxystrobin that resulted the less analysed pesticide. One laboratory (Lab 45) has not presented results because his poor scope (4 compounds analysed on 27 listed by the organiser).

Three z-scores > 5 were calculated: two for lambda-Cyhalothrin (Lab 35 and Lab 37) and one for beta-Endosulfan (Lab 35).

Five false negative results were found: one for lambda-Cyhalothrin (Lab 9), one for beta-Endosulfan (Lab 26), one for Phosalone (Lab 35), one for Kresoxim-methyl (Lab 35) and one for Trifloxystrobin (Lab 5).

One laboratory (Lab 15) reported quantitative results for one pesticide that was not present in the test material (false positive). No z-score has been calculated for this result.

The global performance of each participating laboratory has been assessed only for laboratories which have achieved the sufficient scope, by calculating the Average of the Squared z-scores (AZ_2). Figure 15 was an accurate representation of the results of the AZ_2.

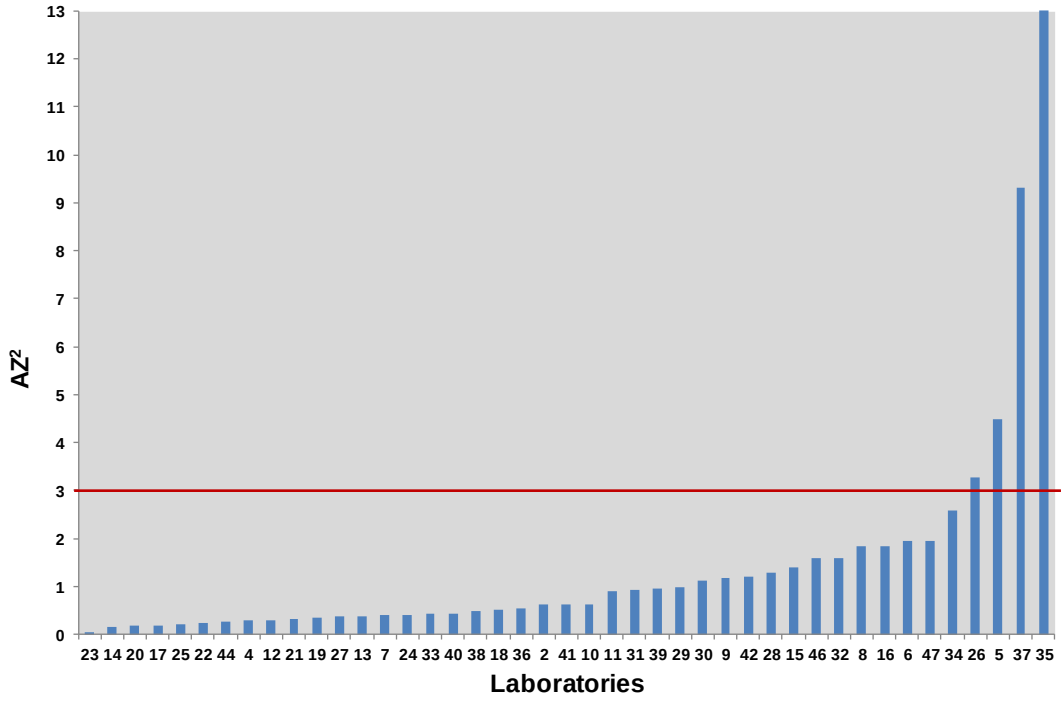


Figure 15. Global performance of laboratories: AZ^2 values

Respect to the analytical methods applied by participants, the majority of laboratories corresponding to twenty-eight participants out of forty-six used the QuEChERS methodology or methods based on QuEChERS (19).

The QuEChERS method is a streamlined approach that makes it easier and less expensive for analytical chemists to examine pesticide residues in food. The name is a portmanteau word formed from "Quick, Easy, Cheap, Effective, Rugged, and Safe". Since 2008 the QuEChERS method has been a standard procedure published by the European Committee for Standardization and transposed in Italy in 2009 (20).

Twelve laboratories used in house methods with an extraction step followed by a clean-up phase, but three out of twelve did not perform any purification.

In the above mentioned methods the purification was carried out using the GPC (Gel Permeation Chromatography) technique, alumina cartridge or using combination of different materials as extrelut + silica+C_{18} as SPE or PSA+GCB+C_{18}. The amount of the sample test was in the range 0.2-20 g.

Two laboratories have followed the method of Lentza Rizos (21), while three laboratories have used for the determination the procedure listed below:

- *Manual of Pesticide Residue Analysis* by the Deutsche Forschungsgemeinschaft in 1987 (22);
- Method M6 by the European Union Reference Laboratory-Fruits and Vegetables in 2012 (23)
- Method UNI EN 1528 parts 1-4 in 1997 (24-27).

In the analysis of pesticide residues the laboratories use multiresidue method, this is a consequence of the large number of analytes enclosed in official plans.

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.

Four laboratories did not performed confirmation with GC-MS/MS after the determination with selective detectors. But the use of selective detectors, even in combination with different polarity columns, does not provide unambiguous identification. Some unsatisfactory performance could be linked to the use of selective detectors.

A small number of laboratories routinely use liquid chromatography with mass spectrometry absolutely necessary for determining certain polar pesticides in complex matrices. The instrumental measurement was not the only factor affecting the final results (calibration procedure, reference material, use or not the internal standard).

In the large part of the cases (thirty-four laboratories out of forty-six) the quantification has been carried out with matrix calibration at single or multiple levels. Eight laboratories used instead the solvent calibration and four laboratories performed the standard addition procedure.

Figure 16 reports the overall recoveries data submitted by the participants as a control chart. For pesticide residues analysis in food and feed, acceptable limits for a single recovery result should normally be within the generalized range of 60-140 %, corresponding to the ± twice acceptance criterion value of the within – laboratory reproducibility (RSD ≤20%); the so-called warming limits are usually located at a distance corresponding to the absolute range 70-120% fixed as acceptance criteria of the mean recovery, in certain cases and typically with multi-residue

methods, recoveries outside these range may be acceptable (3). A limited number of submitted recoveries did not respect these limits.

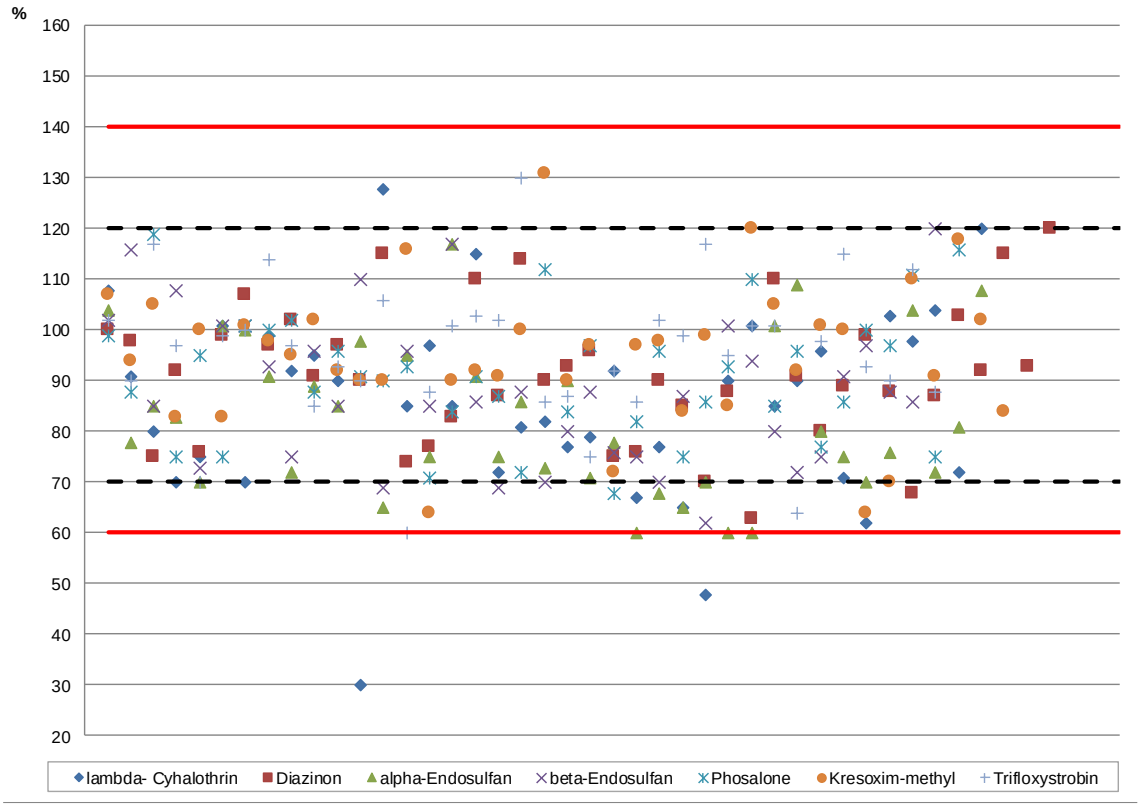


Figure 16. Control chart of the recoveries (%) submitted by the participants

CONCLUSIONS

The outcome of the COI-PT15 can be considered satisfactory from several point of view.

One is the good participation of laboratories. Forty – seven laboratories agreed to participate in this PT: six NRLs, twenty – three Official control laboratories and seventeen private laboratories.

The other regards the performance expressed in terms of z-score. In fact, the laboratory performance obtained for each tested pesticide was satisfactory by almost all participants reaching good results for two pesticides (Diazinon and alpha-Endosulfan).

Moreover, the global performance (AZ^2scores) assessed only for laboratories which achieved the *sufficient scope* was proper. By supplied data, thirty-nine out of forty-three laboratories obtained a satisfactory performance for all tested compounds.

Regarding the methodologies presented in this PT, the majority of participating laboratories used the QuEChERS methodology or QuEChERS variants.

It is important to consider that participation in these PTs on a routine basis is the only disposable tool for laboratories to monitor their competence in the pesticide residues analysis in olive oil.

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APPENDIX A
List of participants

The participants in COIPT-15 in 2015 are listed below.

BELGIUM
Primoris Belgium (Zwijnaarde)
FRANCE
ITERG (Pessac)
Laboratori Du Scl De Montpellier (Montpellier)
GERMANY
Bavarian Health and Food Safety Authority (Erlagen)
Eurofin SOFIA GMBH (Berlin)
Institut Kirchoff Berlin GMBH (Berlin)
Niedersaechsisches Landesamt Fuer Verbraucherschutz Und Lebensmittelsicherheit Lebensmittel Und Veterinaerinstitut Oldenburg (Oldenburg)
GREECE
Agricultural Cooperatives Union of Iraklion (Iraklion)
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)
Ministry of Rural Development and Food, Regional Center of Plant Protection and Quality Control of Piraeus (Athens)
Regional Centre of Plant Protection and Quality Control of Heraklion Laboratory of Pesticide Residues (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 Pordenone (Pordenone)
ARPA Lazio, Sezione di Latina (Latina)
ARPA Puglia, Polo di Specializzazione “Alimenti” (Bari)
ARPA Liguria, Dipartimento di La Spezia, UO Laboratorio (La Spezia)
ASL di Firenze (Firenze)
ASL Milano (Milano)
ASL Provincia di Bergamo (Bergamo)
CHEMISERVICE srl (Monopoli, BA)
INNOVHUB-SSI, Divisione SSOG (Milano)
Istituto Superiore di Sanità, Dipartimento Ambiente e Connessa Prevenzione Primaria (Roma)
IZS SICILIA “A. MIRRI” (Palermo)

IZSLER Laboratorio Pesticidi (Brescia)
IZSLT (Roma)
LABCAM srl (Albenga, SV)
MIPAAF-ICQRF, Laboratorio di Catania (Catania)
PH srl (Firenze)
PROMOFIRENZE, Div. Laboratorio Chimico merceologico (Firenze)
POLAND
Voivodship Sanitary Epidemiological Station in Warsaw Pesticide Residue Laboratory (Warsaw)
SPAIN
Aceites Borges Pont Sau (Tàrrega Lléida)
CNTA (San Adrian Navarra)
Laboratori Agroalimentari - DAAM (Generalitat De Catalunya) (Reus)
Laboratorio Agroalimentario (Granada)
Laboratorio Arbitral Agroalimentario (Madrid)
Laboratorio Regional De La CCAA (Logrono La Rioja)
TURKEY
A&G Pur Analiz Laboratuvarlari TIC.A.S (Izmir)
Egechelab Silliker, Food Analysis Laboratory (Izmir)

APPENDIX B
Robust analysis: algorithm A

This algorithm 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:

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

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

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

$$x_{(1)}, x_{(2)}, x_{(3)}, x_{(4)}, \ldots\ldots x_{(p)}$$

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:

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

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 and robust standard deviation (x^* and s^*).

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