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Dipartimento Ambiente e salute

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2024, v, 39 Rapporti ISTISAN 24/23

In December 2022, as every year, the Italian National Reference Laboratory for pesticide residues in products of Animal Origin and commodities with high fat content (NRL-AO) organized in cooperation with the IOC (International Olive Council) a new Proficiency Test (PT) for the determination of pesticide residues in olive oil named COIPT-22. Laboratories invited to participate in these PTs are Mediterranean laboratories of IOC and European laboratories (NRLs, official control laboratories and private laboratories), involved in the National and European monitoring programs for pesticide residues in food. The exercise consisted in the determination of unknown six different pesticides in a spiked extra virgin olive oil sample, chosen from a target list of forty compounds. Forty laboratories participated and provided results with twenty-six participants analyzing all 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
Risultati del circuito interlaboratoriosu residui di antiparassitari in olio di oliva nel 2022.
Tiziana Generali, Patrizia Stefanelli, Silvana Girolimetti, Danilo Attard Barbini
2024, v, 39 p. Rapporti ISTISAN 24/23 (in inglese)

Nel dicembre 2022, come ogni anno, il Laboratorio Nazionale di Riferimento italiano per i residui di pesticidi nei prodotti di origine animale e materie prime ad alto contenuto di grasso (*National Reference Laboratory for pesticide residues in products of Animal Origin and commodities with high fat content*, NRL-AO) ha organizzato in collaborazione con il Consiglio Oleicolo Internazionale (COI) un nuovo circuito interlaboratorio (*Proficiency Test*, PT) per la determinazione di residui di pesticidi in olio d'oliva chiamato COIPT-22. I laboratori invitati a partecipare in questi circuiti interlaboratorio sono laboratori mediterranei del COI e laboratori europei (NRL, laboratori di controllo ufficiali e laboratori privati), coinvolti nei programmi di monitoraggio nazionali ed europei per i residui di pesticidi negli alimenti. L'esercizio consisteva nella determinazione di sei diversi pesticidi sconosciuti in un campione di olio extravergine di oliva, scelti da una lista prestabilita di trentotto composti. Quaranta laboratori hanno partecipato e fornito risultati con ventisei partecipanti che 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
AZ²	Average of the Squared z-scores
CAS	Chemical Abstract Service
EC	European Commission
EU	European Union
EUPT	European Union Proficiency Test
EURLs	European, Reference Laboratories
FFP	Fitness for Purpose
GAP	Good Agricultural Practice
GC	Gas Chromatography
ILAC	International Laboratory Accreditation Cooperation
ISO	International Organization for Standardization
LC	Liquid Chromatography
LOQ	Limit of Quantification
MRL	Maximum Residue Limit
MS	Mass Spectrometry
MU	Measurement Uncertainty
NRL-AO	National Reference Laboratory - Animal Origin
NRLs	National Reference Laboratories
PPP	Plant Protection Product
PT	Proficiency Test
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RL	Reporting Limit
RSD	Relative Standard Deviation
SD	Standard Deviation

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 (PTs), particularly those organized by the NRLs. Regular participation in PTs programs is considered a suitable external quality control system for assessing reliability of their results (1).

Furthermore, in accordance with article 37 of Regulation (EU) 2017/625, the laboratories designated for official control have to adopt the general quality criteria for testing laboratories laid down in ISO/IEC 17025 (2).

In particular, all the official laboratories involved in the European Union (EU) coordinated control pesticide residue monitoring programs, follow the same European analytical quality control technical guidance document SANTE/11312/2021 (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 (IOC), 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 of the Mediterranean:

According to official data of the IOC (year 2021-2022) relating to the production of olive oil area the 94% of the olive oil in the world is produced by Mediterranean countries (4) with 70% of the olive oil provided by Spain, Italy, Greece and Portugal (5).

Olive oil is one of the great components in the Mediterranean diet and as consequence of the high content of monounsaturated fats, the consumption of virgin olive oil prevents the onset of the coronary heart diseases, tumours, diabetes, neurodegenerative diseases and autoimmune and immuno-inflammatory diseases (6).

The olive tree is vulnerable to several pest attacks, flattening the production curve even in term of quality of the crop and the processed product there of. 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 considering that the olives are also harvested with the beating technique from tents placed on the ground, a contamination of the olives and therefore of the olive oil is possible.

The pesticides arising as a result of use in plant protection products, in veterinary medicine and as a biocide are defined “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 (EC) has established MRLs in or on food and feed of plant and animal origin, and these MRLs for all crops and all pesticides can be found in the MRL database on the Commission website.

The EC 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 EC website.

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

- directions of use of a PPP in/on the crop (GAP) – e.g., number of treatments, quantity of the active ingredient, frequency of the treatments, growth stage of the plant, Pre Harvest Interval (PHI, days from the last treatment and the harvest);
- experimental data on the expected residues when the pesticide is applied according to the 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 daily intake does not exceed the toxicological values, then the GAP can be considered “safe” for the proposed use; the MRLs is then established in olives (as for all crops) by the Regulation (EC) 396/2005 (7) and amendments. For those pesticides not allowed in/on olive and for pesticides that do not cause any quantifiable residue in olive fruit, the MRL can be set by default at the lowest quantification value (Limit Of Quantification, LOQ).

The Regulation (EC) 396/2005 set at 0.01mg/kg this value. To calculate MRL values in processed products such as olive oil, it is necessary to use processing factors. Pending the publication of annex VI of the Regulation (EC) 396/2005 containing the list of processing factors of processed products, in coordinated multiannual control programmes of the EU (8), is declared that each Member States are requested to report the processing factors used to analyse virgin olive oil samples (9). Currently in Italy this processing factor is equal to 5.

PROFICIENCY TEST ON OLIVE OIL: THE COIPT-22

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 IOC, 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 2022 on olive oil was named COIPT-22.

The exercise consisted in the determination of six different pesticides in an extra virgin olive oil sample spiked with a definite range of concentration (0.050-0.350 mg/kg). These pesticides were chosen from a list of forty compounds presented in COIPT-22. The announcement was sent to participant on 16 December 2022. 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 laboratories agreed to participate in this PT: three NRLs, sixteen official control laboratories and twenty-one private laboratories. To assess the performance of the participating laboratories, z-scores are used following the norms of the International Organization for Standardization (ISO) (10, 11).

To investigate the impact on the analytical results of different testing procedures, detailed information of the methodologies was requested to the whole participants as well. 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 Mass Spectrometry (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.

Test materials

The test materials consisted of 4.5 kg of olive oil from an olive oil company. 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 2.2 kg of the blank oil, was spiked with the following pesticides: Aldrin, Azoxystrobin, lambda-Cyhalothrin, Cypermethrin, Difenoconazole, and Vinclozolin. Aliquots of 50 g of this spiked oil named COIPT-22 SPIKED OIL were transferred into dark glass bottles as well as aliquots of 50 g of the blank oil named COIPT-22 BLANK OIL. Samples were sealed and stored at ambient temperature before the shipment to participants. Each participant received one COIPT-22 SPIKED OIL sample and one COIPT-22 BLANK OIL sample. The current MRLs for these six pesticides are showed in Table 1 (12-17).

Table 1. COIPT-22: current MRLs for the six pesticides spiked in the blank oil

Compounds	Current EU Regulation	MRL on olive for oil production (mg/kg)
Aldrin	Regulation (EC) 839/2008 Applicable from: 01/09/2008	**0.01*** Aldrin and dieldrin combined expressed as dieldrin
Azoxystrobin	Regulation (EU) 2023/129 Applicable from: 26/02/2023	**0.01***
lambda-Cyhalothrin	Regulation (EU) 2021/590 Applicable from: 03/05/2021	**0.5** Lambda-cyhalothrin (includes gamma-cyhalothrin) (sum of R,S and S,R isomers)
Cypermethrin	Regulation (EU) 2017/626 Applicable from: 27/04/2017	**0.05*** Cypermethrin (cypermethrin including other mixtures of constituent isomers, sum of isomers)
Difenoconazole	Regulation (EU) 2019/552 Applicable from: 25/04/2019	**2**
Vinclozolin	Regulation (EU) 2015/868 Applicable from: 30/12/2015	**0.02***

* Limit of analytical determination

Homogeneity and stability tests

Homogeneity and stability were tested according to ISO 13528:2015 (11).

Regarding the homogeneity test ten bottles of the spiked oil samples were randomly chosen and analysed in duplicate. A pesticide was considered to be adequately homogeneus if $SD/\sigma_{EUPT} \leq 0.3$ where SD is the Standard Deviation and σ_{EUPT} is the target standard deviation used for proficiency assessment. All results are presented in Table 2.

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

– Day 1: during the shipment of the samples on 6 February 2023;
– Day 2: after one month by the deadline for reporting results on 7 March 2023.

A pesticide was considered to be adequately stable if $|x_i - y_i| \leq 0.3 \times \sigma_{EUPT}$, where x_i is the mean value of the first stability test, y_i the mean value of the last stability test and σ_{EUPT} the target standard deviation used for proficiency assessment. The individual results are indicated in Table 3.

All the six spiked compounds passed the homogeneity and stability tests.

Table 2. COIPT-22: homogeneity results (mg/kg)

Sample number	Aldrin	Azoxystrobin	lambda-Cyhalothrin	Cypermethrin	Difenoconazole	Vinclozolin
81	0.266	0.108	0.358	0.124	0.182	0.074
85	0.263	0.114	0.322	0.116	0.210	0.066
86	0.260	0.121	0.346	0.128	0.196	0.083
92	0.264	0.118	0.373	0.134	0.207	0.066
95	0.267	0.118	0.383	0.135	0.195	0.077
106	0.265	0.105	0.390	0.121	0.173	0.066
107	0.270	0.108	0.347	0.118	0.189	0.073
114	0.267	0.106	0.349	0.115	0.171	0.075
118	0.266	0.112	0.337	0.114	0.182	0.073
120	0.263	0.120	0.374	0.134	0.190	0.077
Mean	0.265	0.113	0.358	0.124	0.190	0.073
SD	0.003	0.006	0.022	0.008	0.013	0.006
σ_{EUPT}	0.055	0.028	0.080	0.028	0.050	0.019
SD/σ_{EUPT}	0.050	0.216	0.270	0.298	0.260	0.296
Critical value	0.3	0.3	0.3	0.3	0.3	0.3
SD/σ_{EUPT}≤0.3	yes	yes	yes	yes	yes	yes

SD Standard Deviation
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 3. COIPT-22: data (mg/kg) of the stability test

Pesticide	Concentration mg/kg				
	Mean 1 (M1) n=6	Mean 2 (M2) n=6	(M1-M2)	σ_{EUPT}	0.3xσ_{EUPT}
Aldrin	0.269	0.268	0.001	0.055	0.017
Azoxystrobin	0.111	0.104	0.008	0.028	0.008
L-Cyhalothrin	0.380	0.362	0.018	0.080	0.024
Cypermethrin	0.134	0.127	0.008	0.028	0.008
Difenoconazole	0.204	0.189	0.015	0.050	0.015
Vinclozolin	0.080	0.073	0.006	0.019	0.006

M1 = mean of duplicates of three bottles analysed in the first day
M2 = mean of duplicates of three bottles analysed in the second day
σ = target standard deviation
The acceptance criterion of the stability test is = |M1-M2| < 0.3xσ_{EUPT}

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 (*see* 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 by e-mail along with the details of methodology used.

The samples were sent out on 31 January 2023. The deadline for results was 3 March 2023.

The final report was dispatched to all participant at the end of June 2023.

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) (18, 19) 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 means 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:

$$\mathrm{Z-score} = \frac{(\mathrm{x} - \mathrm{X})}{\sigma_{EUPT}}$$

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).

When the assigned value is derived as a robust mean, the standard uncertainty (u, mg/kg) of the consensus 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.

Furthermore, the global performance (20) 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:

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

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* $|AZ^2| \geq 3.0$

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 (11). However, the AZ^2 parameter is normally used in the evaluation of a multiresidue method for the analysis of pesticides residues in food.

COIPT-22: RESULTS

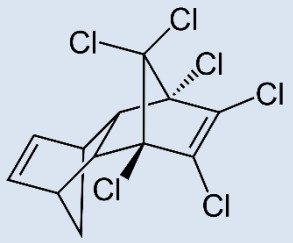
Description and statistical evaluation of the results are presented for each compound separately and as final comments.

All data for each compound were analysed for normal distribution by applying the Shapiro-Wilk test (α=0.05). The distribution of the concentrations of the pesticides reported by the laboratories has been plotted as histograms with a bandwidth of 0.75 σ where σ represent the target standard deviation.

In addition, Kernel density plots were used to identify multi-modality in the data distributions All the compound data sets were normally distributed except for Aldrin and lambda-Cyhalothrin. In any case, the Kernel density plots displayed one main mode indicating homogeneous data populations for all compounds.

The frequency histograms report also the Gaussian curve.

Aldrin



Common name
HHDN, aldrin, aldrine

Structure formula $C_{12}H_8Cl_6$

CAS number 309-00-2

Its physical form consists of colourless needles with weight molecular of 364.93. This compound is moderately soluble in aromatic solvents,esters, ketones, haologenated solvents and paraffins. Sparingly soluble in alcohols.
It is an organochlorine insecticide.
Not authorized on olive tree with a MRL value of 0.01 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.

Figure 1 shows the results of Aldrin (mg/kg) submitted by all laboratories with the Kernel density plot. The distribution of the results is not symmetric.

Statistical evaluation of the Aldrin results is presented in Table 4.

In the case of Aldrin, submitted results can be considered good, with Robust RSD% and uncertainty of the assigned values *u* acceptable.

All z-score values with recoveries estimated as numerical values are presented in Table 5. Furthermore, the z-score values are presented in graphical form in Figure 2.

Aldrin was analysed by thirty-five out of forty laboratories with a questionable z-score value of -2.7 for Lab 27 and a false negative value of -3.8 calculated in the case of Lab 05.

It was noted that the majority of recoveries was under the 100% with some data under 60%. Some laboratories declared a correction of recovery value for Aldrin.

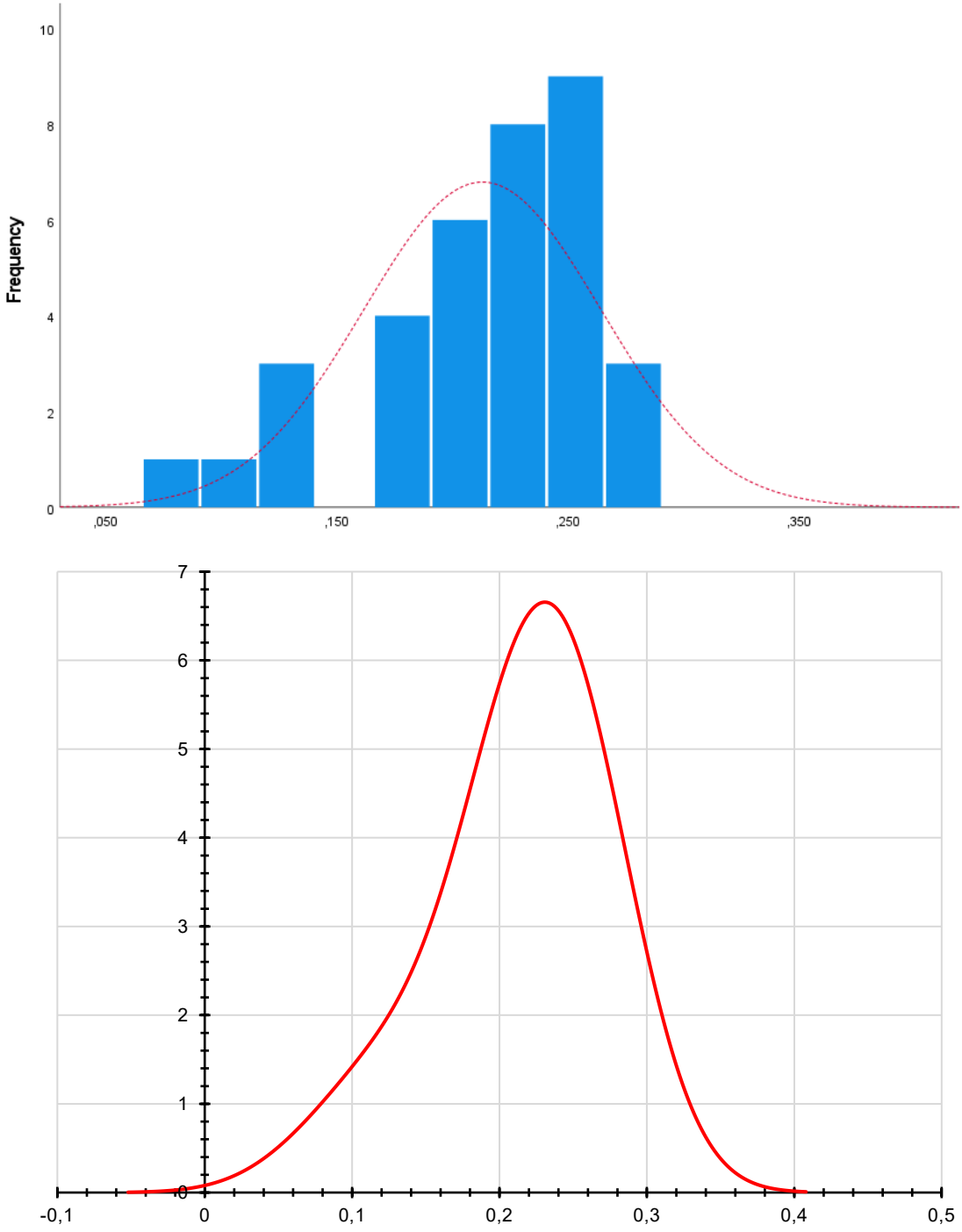


Figure 1. ALDRIN: frequency histogram of the results (mg/kg) and Kernel density plot

Table 4. ALDRIN: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.263
Mean	0.213
Median	0.223
Robust mean or Assigned value (mg/kg)	0.219
s*	0.045
σ_{EUPT}	0.055
Uncertainty (u) (mg/kg)	0.009
u/σ_{EUPT} *	0.164
FFP RSD (%)	25
Robust RSD (%)	20

s*= robust standard deviation
* $u/\sigma_{EUPT} \leq 0.3$; RSD: Relative Standard Deviation

Table 5. ALDRIN: z-score and recovery (%) values

Lab Code	z_{EUPT}-score	Recovery %
1	-0.2	65
2	0.2	49*
3	0.5	44
4	-1.8	117
5	-3.8	-
6	-1.6	93
8	-0.9	91
9	0.8	104
10	0.0	84
11	-0.4	54*
12	0.6	82
14	0.4	90
16	-2.1	88
17	0.5	50*
18	-0.7	89
19	-0.5	116
20	0.7	102
21	0.9	100
22	0.8	97
23	0.2	30
24	0.3	38
25	0.3	37
26	-0.3	88
27	-2.7	-
28	1.2	100
29	-0.2	80
30	-0.3	70
31	-0.8	62
33	-0.6	35*
34	-0.2	80
35	0.4	Std add
36	0.8	105
37	-1.7	83
38	0.8	105
39	0.1	50
40	0.0	40*

*Adjusted for recovery

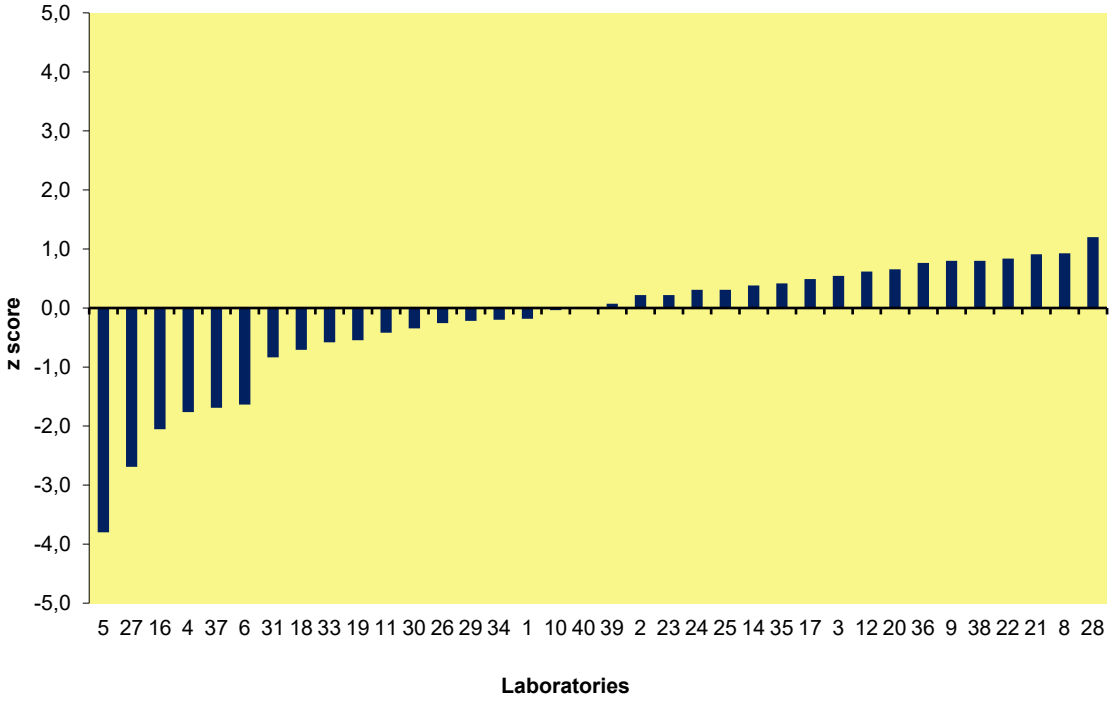
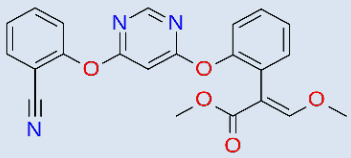


Figure 2. ALDRIN: z-score values (spiked value = 0.263 mg/kg

Azoxystrobin



Common name
Azoxystrobine, azoxystrobin

Structure formula $C_{22}H_{17}N_3O_5$

CAS number 131860-33-8

The physical form consist of white crystalline powderwith weight molecular of 403.4 g/mol. It is fungicide with protectant, curative, eradican5t, translaminar and systemic properties.
This compound is highly soluble in polar organic solvents as acetonitrile and dichloromethane.
This fungicide is authorized on olive tree with two PPP as SC (Suspension Concentrates) formulation.
The MRL value is 0.01 mg/kg on olive for oil production as established by the Regulation (EC) 396/2005 that corresponds at the limit of analytical determination.

In the case of Azoxystrobin the distribution of submitted data resulted symmetric as indicated in Figure 3.

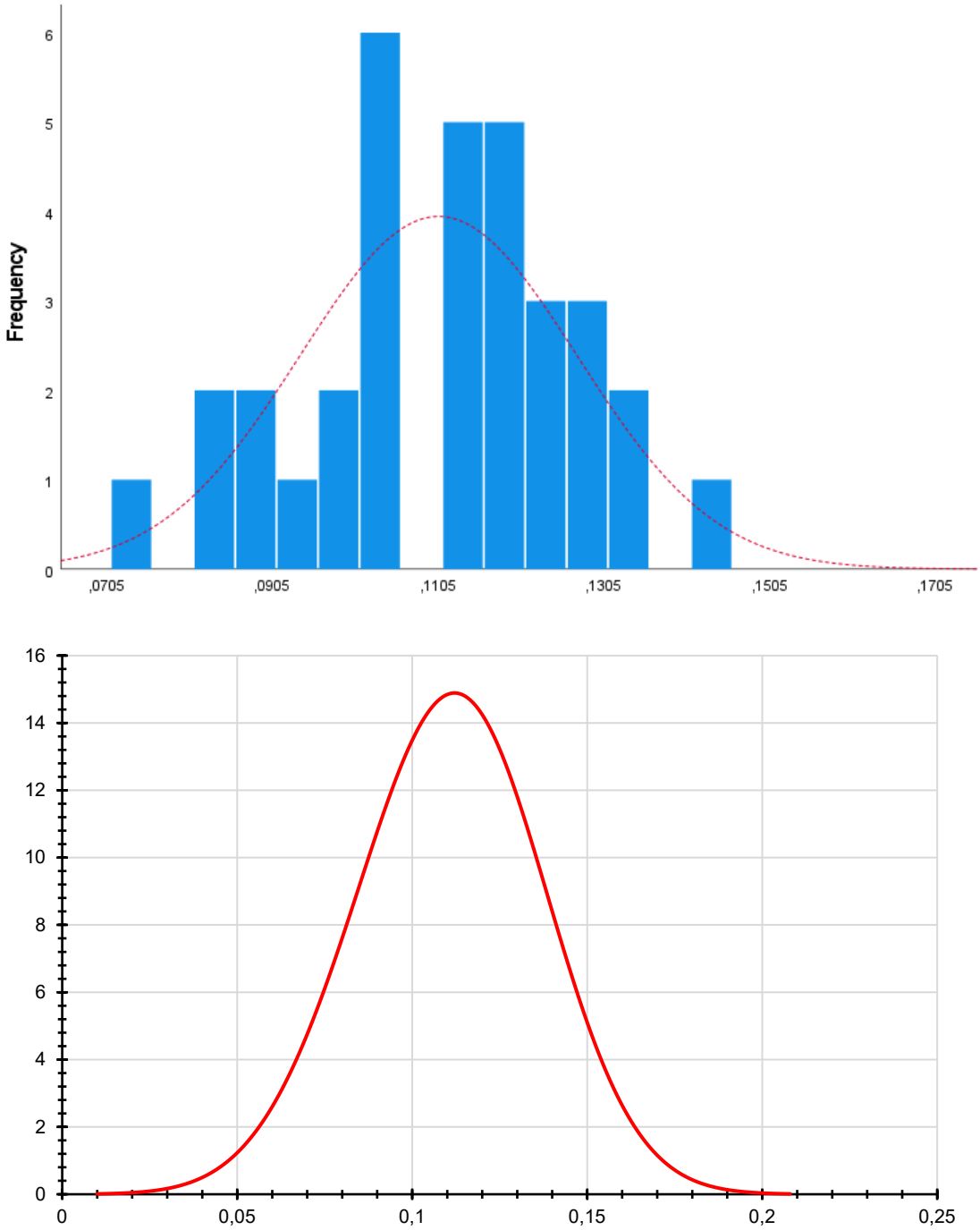


Figure 3. AZOXYSTROBIN: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of the Azoxystrobin results is presented in Table 6. The supplied results for Azoxystrobin can be considered good, with Robust RSD% of 16 and uncertainty value of 0.004 mg/kg.

Table 6. AZOXYSTROBIN: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.117
Mean	0.111
Median	0.114
Robust mean or Assigned value (mg/kg)	0.111
s*	0.017
σ_{EUPT}	0.028
Uncertainty (u) (mg/kg)	0.004
u/σ_{EUPT} *	0.143
FFP RSD (%)	25
Robust RSD (%)	16

s*= robust standard deviation
* $u/\sigma_{EUPT} \leq 0.3$; RSD: Relative Standard Deviation

All z-score values with recoveries estimated as numerical values are presented in Table 7 with z-score showed as graphical representation in Figure 4.

Table 7. AZOXYSTROBIN: z-score and recovery (%) values

Lab Code	z_{EUPT}-score	Recovery %
1	0.5	88
2	0.7	120
3	0.0	96
4	0.3	109
5	-0.2	78
6	-0.6	98
7	0.6	110
8	1.2	99
9	0.4	99
10	0.2	89
11	-0.5	64*
12	0.1	88
13	0.1	104
14	0.2	90
15	-0.4	89
17	0.5	109
18	-1.4	96
19	-1.0	115
20	-1.0	97
21	0.4	102
22	-0.9	67
23	0.7	100
24	-0.9	99
25	0.1	99
26	-0.3	87
28	0.1	90
29	0.3	75
30	-0.2	94
33	-0.2	95
35	-0.3	Std add
37	0.9	95
39	0.3	91
40	-0.4	89*

*Adjusted for recovery

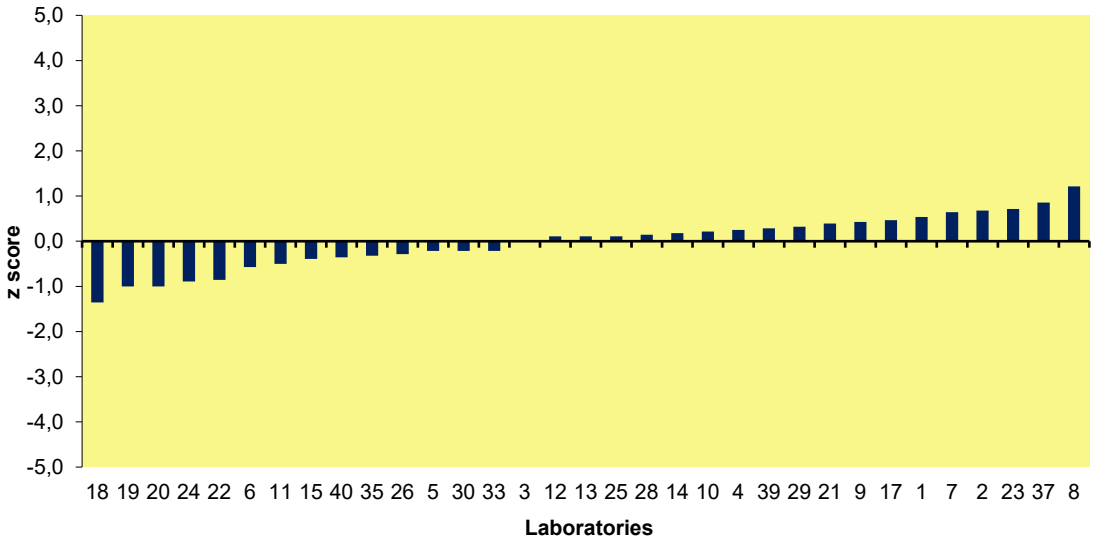
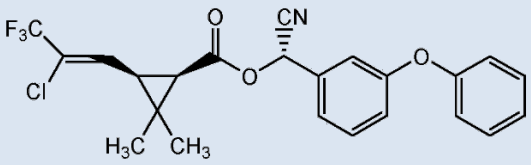


Figure 4. AZOXYSTROBIN: z-score values (spiked value = 0.117 mg/kg)

In the case of Azoxystrobin thirty-three laboratories supplied results with good calculated z-score values in the range 0.1-2.0 as absolute values.

lambda-Cyhalothrin



Common name
lambda-cyhalothrin, lambda-cyhalothrine

CAS number 91465-08-6

EC no. 415-130-7

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

This compound belongs to the pyrethroid family.
Its phusycal form consiste of colourless solid with a molecular weight of 449.9.
The technical product is a mixture of the equal quantities of (1S,3S) abd (1R,3R) isomers.
Non-systemic insecticide with contact and stomach action and repellent properties. This pesticide has a good solubility in organic solvents and it is stable to light.
This pesticide is authorized in Italy on olive tree with four PPP as WG (Water Dispersible Granules) and two PPP as RP (Ready-to-use Bait) used in organic farming.
The MRL value for lambda-Cyhalothrin on olive for oil production is 0.5 mg/kg includes gamma-cyhalothrin and sum of R,S and S,R isomers, as established by the Regulation (EC) 396/2005.

Figure 5 shows the results of lambda-Cyhalothrin (mg/kg) submitted by all laboratories in the COIPT-22. The distribution of the results is not symmetric.

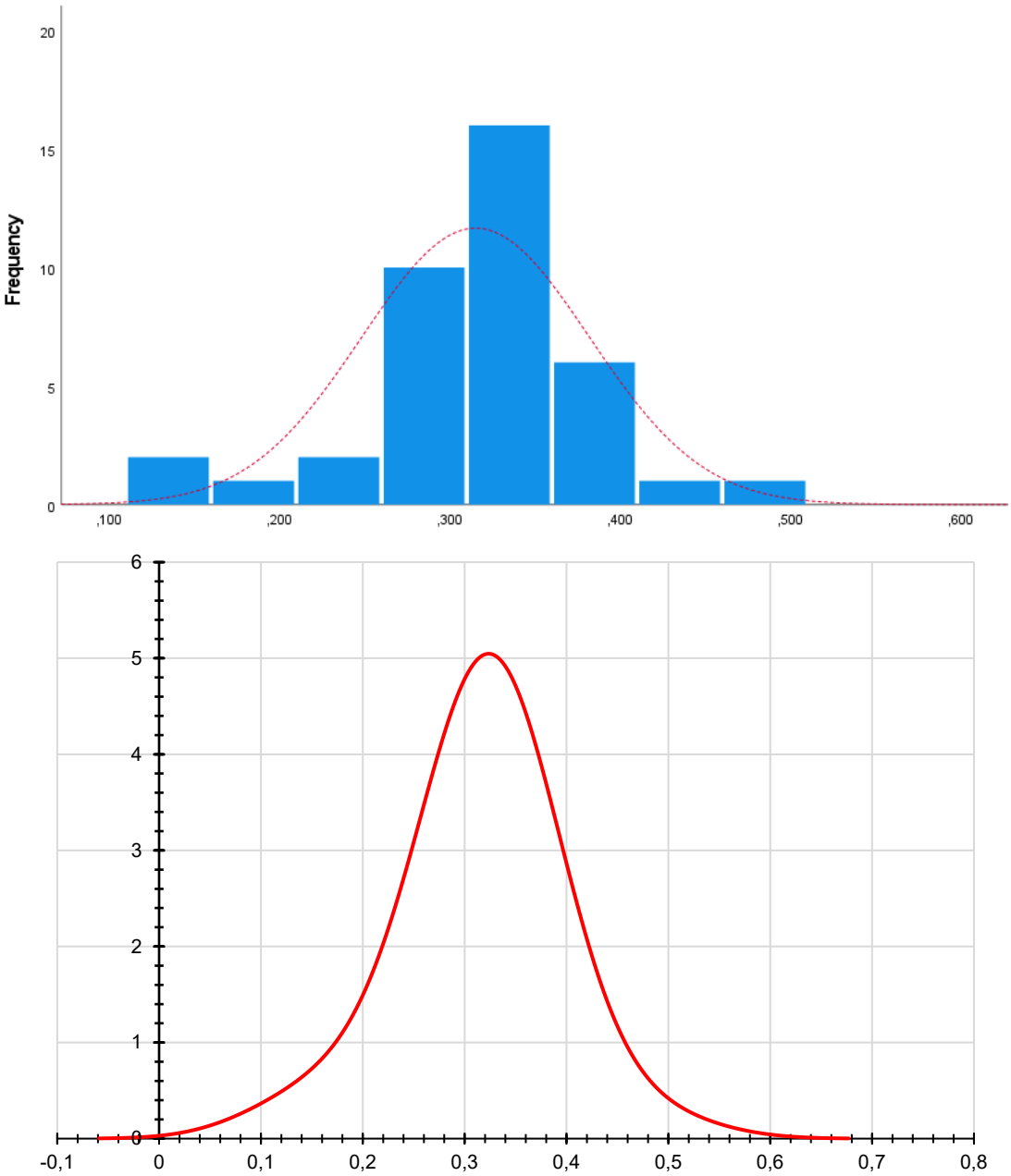


Figure 4. LAMBDA-CYHALOTHRIN: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of the lambda-Cyhalothrin results is presented in Table 8. Regarding lambda-Cyhalothrin data the obtained performance can be considered good with a Robust RSD% value of 14 and an uncertainty value of 0.009 mg/kg.

All z-score values with recoveries estimated as numerical values are presented in Table 9.

Table 8. LAMBDA-CYHALOTHRIN: statistical parameters (mg/kg) -

Parameter	Value
Spiked value	0.348
Mean	0.315
Median	0.319
Robust mean or Assigned value (mg/kg)	0.320
s*	0.046
σ_{EUPT}	0.080
Uncertainty (u) (mg/kg)	0.009
u/σ_{EUPT} *	0.113
FFP RSD (%)	25
Robust RSD (%)	14

s*= robust standard deviation
* $u/\sigma_{EUPT} \leq 0.3$; RSD: Relative Standard Deviation

Table 9. LAMBDA-CYHALOTHRIN: z-score and recovery (%) values

Lab Code	z_{EUPT}-score	Recovery %
1	0.6	90
2	0.7	112
3	0.0	85
4	-0.3	110
5	-0.2	73
6	-2.5	80
7	0.6	82
8	0.7	99
9	0.2	100
10	0.7	104
11	-1.2	81
12	-0.1	89
13	0.1	97
14	-0.5	90
15	0.0	70
16	-1.5	87
17	0.2	102
18	-0.1	94
20	-0.1	89
21	0.2	100
22	-0.3	90
23	-0.1	95
24	-1.0	90
25	0.3	105
26	2.2	94
27	0.1	–
28	0.6	82
29	-0.3	86
30	0.3	85
31	-2.1	61
32	0.2	88
33	-0.5	71
34	-0.1	100
35	-0.3	Std add
36	-0.7	95
37	1.4	102
38	0.3	105
39	0.4	64
40	-0.3	85*

*Adjusted for recovery

Furthermore, the z-score values are presented in graphical form in Figure 5.

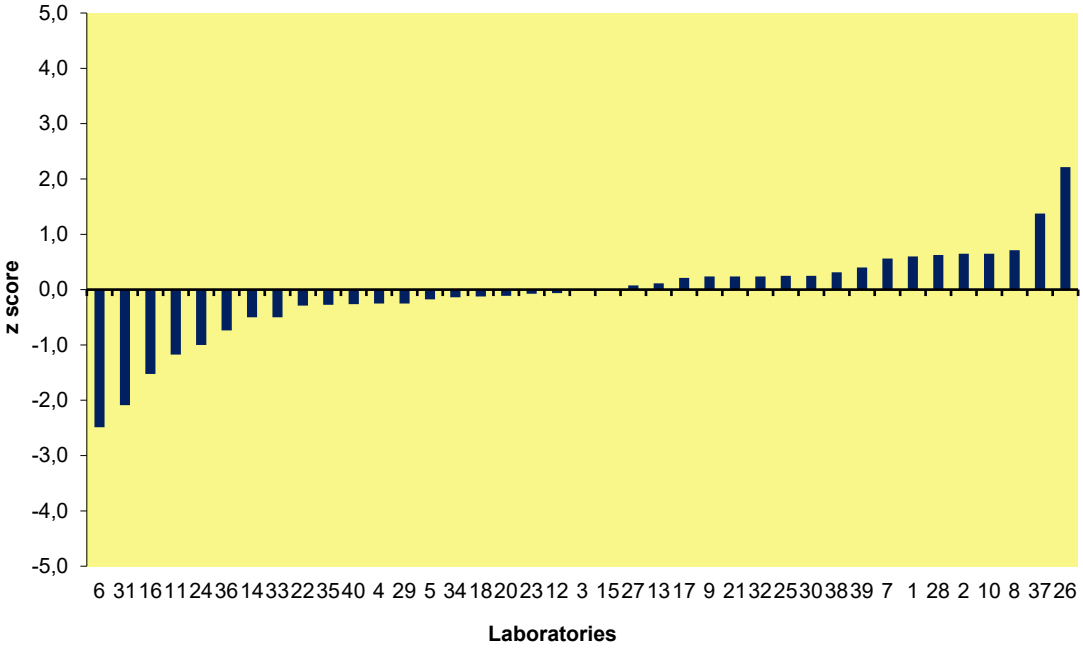
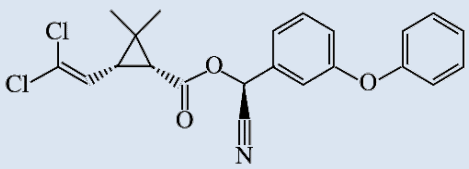


Figure 5. LAMBDA-CYHALOTHRIN: z-score values (spiked value = 0.348 mg/kg)

In the case of lambda-Cyhalothrin thirty-nine laboratories supplied results with good calculated z-score values in the range 0.1-2.0 as absolute values except for Lab 6, Lab 26 and Lab 31with questionable z-score of -2.5, 2.1 and 2.2 respectively.

Cypermethrin



Common name
cypermethrine,cypermethrin

CAS number 52315-07-8

Structure formula $C_{22}H_{19}Cl_2NO_3$

This compound belongs to the pyrethroid family as the lambda-cyhalothrin.
Its physical form consists of oudorless crystal with weight molecular of 416.3.
This pesticide has a good solubility in organic solvents and relatively stable to light in field situations.
Non-systemic insecticide with contact and stomach action.
This pesticide is authorized in Italy on olive tree with twenty PPP as EC (emulsifiable concentrates).
The MRL value for cypermethrin expressed as sum of isomers, is 0.05 mg/kg on olive as established by the Regulation (EC) 396/2005.

In the case of Cypermethrin too, the distribution of submitted data resulted symmetric as indicated in Figure 6.

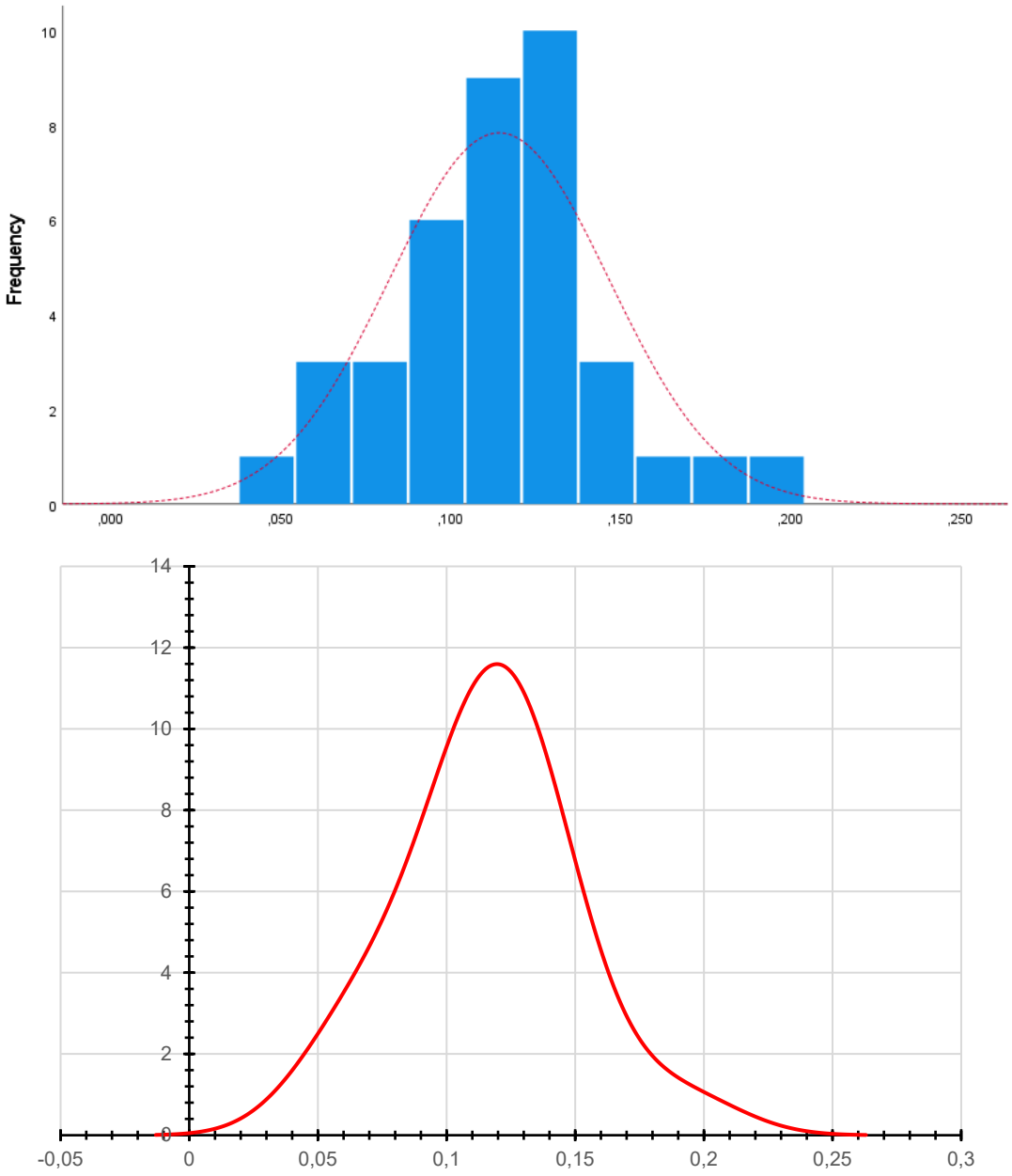


Figure 6. CYPERMETHRIN: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of the Cypermethrin results is presented in Table 10.

Regarding Cypermethrin data the obtained performance can be considered acceptable.

All z-score values with recoveries estimated as numerical values are presented in Table 11. Graphical representation of z-score is shown in Figure 7.

Table 10. CYPERMETHRIN: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.129
Mean	0.114
Median	0.115
Robust mean or Assigned value (mg/kg)	0.114
s*	0.030
σ_{EUPT}	0.028
Uncertainty (u) (mg/kg)	0.006
u/σ_{EUPT} *	0.214
FFP RSD (%)	25
Robust RSD (%)	27

s*= robust standard deviation
* $u/\sigma_{EUPT} \leq 0.3$; RSD: Relative Standard Deviation

Table 11. CYPERMETHRIN: z-score and recovery (%) values

Lab Code	z_{EUPT}-score	Recovery %
1	0.0	85
2	1.0	117
3	0.5	82
4	0.3	119
5	-3.7	-
6	-2.0	80
7	-0.4	81
8	0.8	92
9	2.5	118
10	0.5	97
11	-1.2	96
12	0.8	100
13	-0.4	98
14	-0.1	90
16	-1.4	83
17	1.0	92
18	0.6	111
19	-1.5	99
20	0.5	96
21	0.7	100
22	-0.7	67
23	-1.8	77
24	-0.5	84
25	0.6	93
26	0.0	82
27	-1.8	–
28	-0.1	81
29	-2.3	93
30	0.2	74
31	-0.9	61
32	1.6	110
33	0.0	80
34	-0.2	93
35	-0.4	Std add
36	1.2	96
37	3.1	105
38	0.6	103
39	-0.1	80
40	-0.3	80*

*Adjusted for recovery

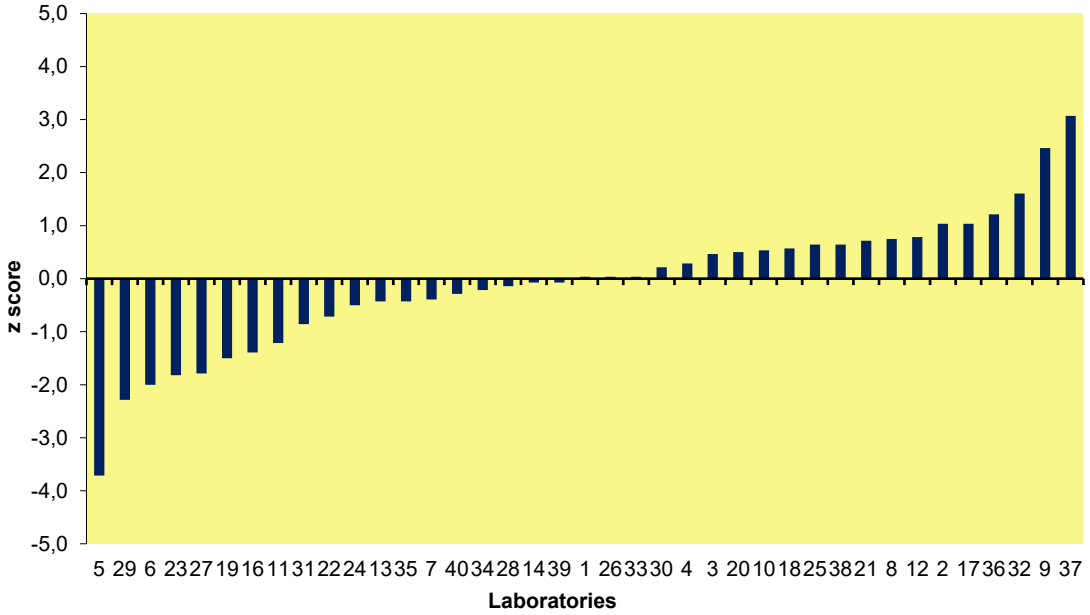
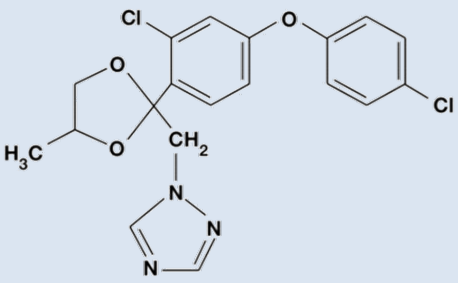


Figure 7. CYPERMETHRIN: z-score values (spiked value = 0.129 mg/kg)

Moreover, the Cypermethrin was analysed by thirty-nine laboratories supplied results with good calculated z-score values except for Lab 9 and Lab 29 with questionable z-score of 2.5 and -2.3 respectively, one unacceptable value of 3.1 for Lab 37 and one false negative z score value of -3.7 by Lab 5.

Difenoconazole



Common name
difenoconazole

Structure formula $C_{19}H_{17}Cl_2N_3O_3$

CAS number 119446-68-3

This compound belongs to the triazole family.
Its physical form consists of white to light beige crystals with weight molecular of 406.3
This pesticide has a good solubility in organic solvents and is hydrolytically stable.
Systemic fungicide with preventive and curative action.
This pesticide is authorized in Italy on olive tree with twelve PPP as EC (Emulsifiable Concentrates) formulationon and in association with other pesticides with three PPP: two SC(Suspension Concentrates) and one WG (Water Dispersible Granule).
The MRL value is 2.0 mg/kg on olive as established by the Regulation (EC) 396/2005.

Figure 8 shows the results of Difenoconazole (mg/kg) submitted by all laboratories in the COIPT-22. The distribution of the results was symmetric.

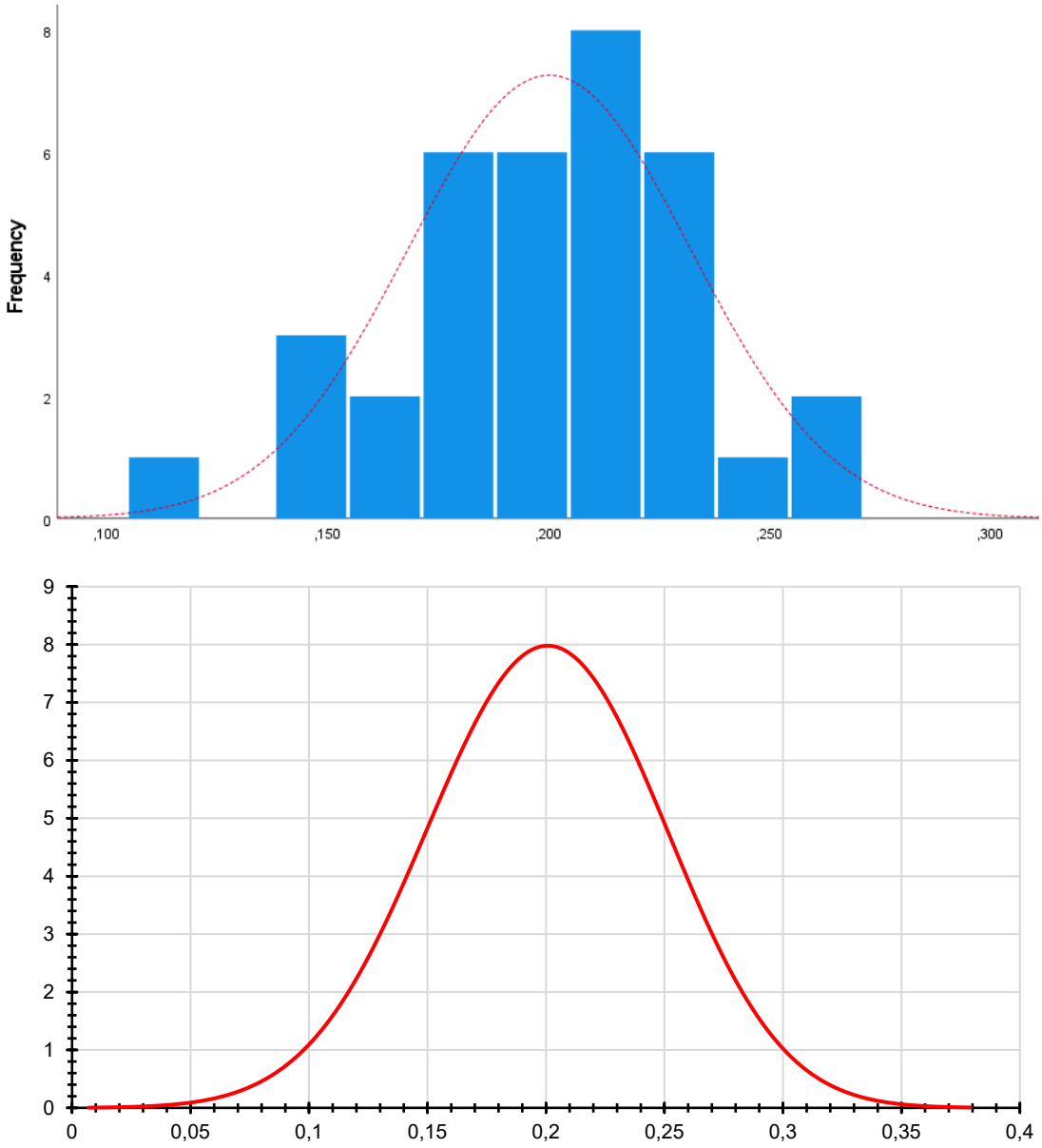


Figure 8. DIFENOCONAZOLE: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of Difenoconazole results is presented in Table 12 while in Table 13 all z-score values with corresponding recoveries estimated are listed.

Statistically results for Difenoconazole can be considered satisfactory. The median and the robust mean are similar with a good value for Robust RSD% of 16 as the uncertainty equal to 0.007 mg/kg.

Table 12. DIFENOCONAZOLE: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.197
Mean	0.200
Median	0.200
Robust mean or Assigned value (mg/kg)	0.201
s*	0.033
σ_{EUPT}	0.050
Uncertainty (u) (mg/kg)	0.007
u/σ_{EUPT} *	0.140
FFP RSD (%)	25
Robust RSD (%)	16

s*= robust standard deviation
* $u/\sigma_{EUPT} \leq 0.3$; RSD: Relative Standard Deviation

Table 13. DIFENOCONAZOLE: z-score and recovery (%) values

Lab Code	z_{EUPT}-score	Recovery %
1	0.5	82
2	0.6	118
3	0.1	74
4	-0.6	102
5	1.3	118
6	-1.0	81
7	0.4	99
8	0.7	99
9	0.8	101
10	0.3	88
11	-0.4	37*
12	-0.6	101
13	-0.2	96
14	-0.2	90
15	0.5	74
17	0.0	97
18	-1.6	91
19	-0.7	107
20	-0.4	97
21	-0.3	92
22	0.4	113
23	-0.5	86
24	-0.6	82
25	0.3	95
26	-1.0	89
28	-0.2	83
30	-0.1	102
33	-0.9	87
34	1.1	118
35	0.4	Std add
36	0.1	98
37	0.6	98
38	0.2	96
39	0.7	80
40	-0.1	88*

*Adjusted for recovery

The z-score values presented in Table 13 are represented as graphical form in Figure 9.

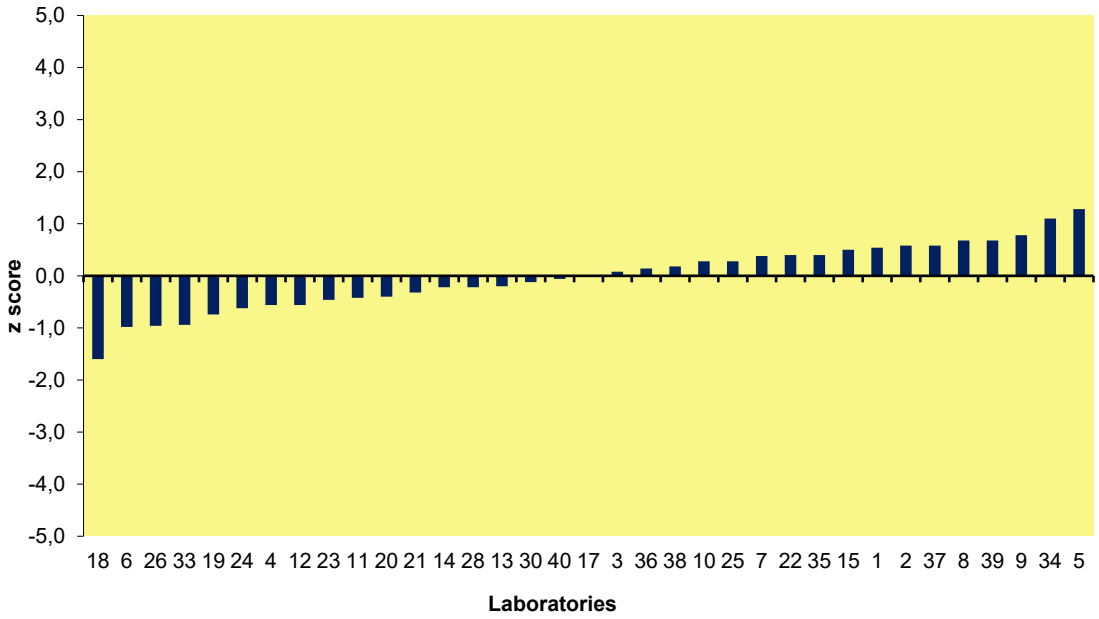
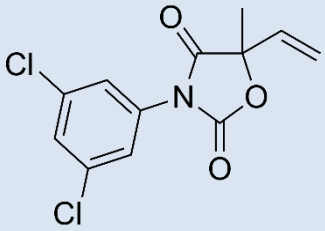


Figure 9. DIFENOCONAZOLE: z-score values (spiked value = 0.197 mg/kg)

In the case of Difenoconazole thirty-five laboratories supplied results with good calculated z-score values in the range 0.1-2.0 as absolute values.

Vinclozolin



Common name
Vinclozolin or vinclozoline

Structure formula $C_{12}H_9Cl_2NO_3$

CAS number 50471-44-8

This compound belongs to the dicarboximide family.
Its physical form consists of colourless crystals with a slight aromatic odour white molecular weight of 286.1.
Non-systemic fungicide with protective action.
This pesticide has a good solubility in organic solvents.
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.

Figure 10 shows the results as frequency histogram together with the kernel density plot of Vinclozolin (mg/kg). In the case of Vinclozolin the distribution of the results is symmetric.

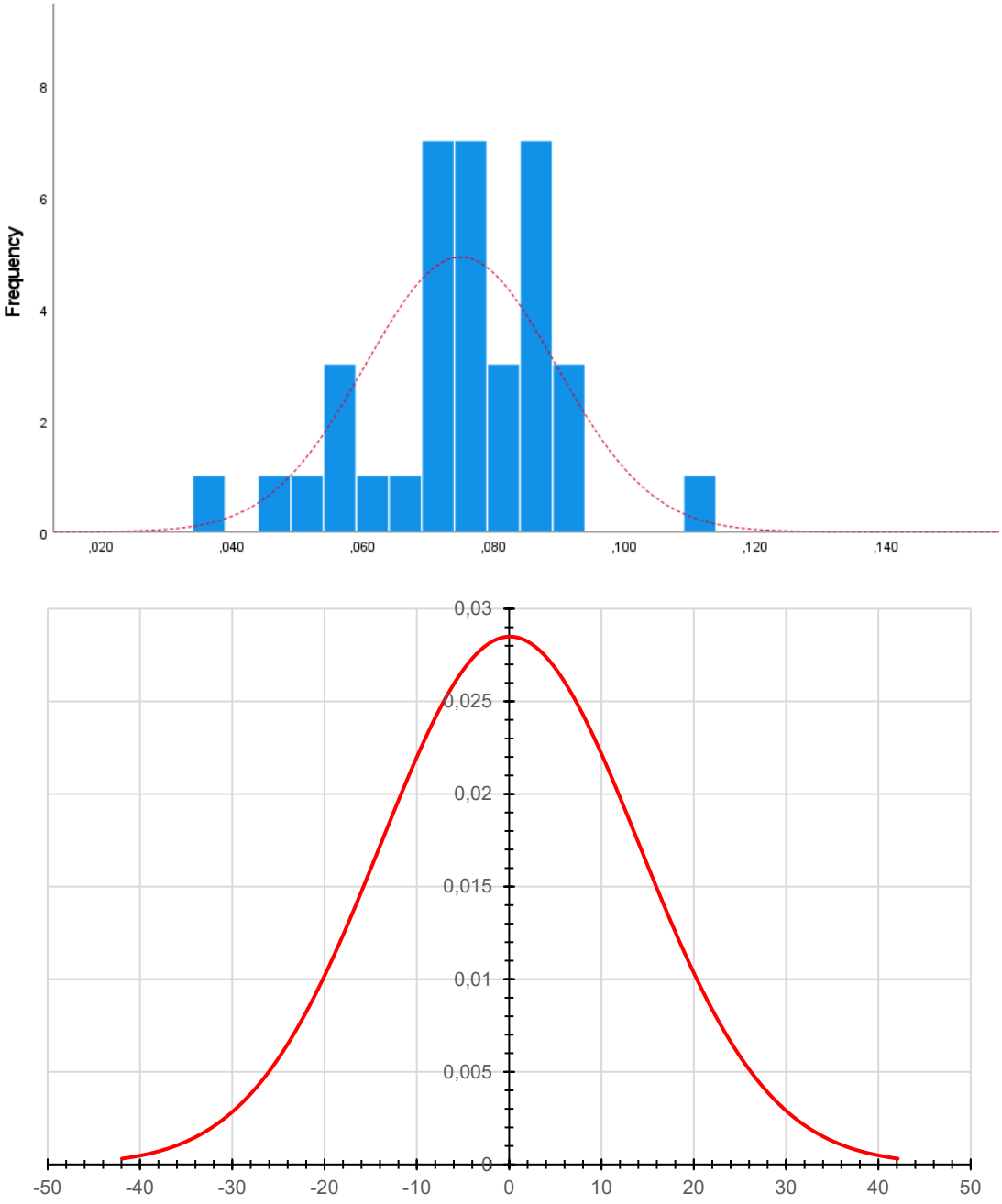


Figure 10. VINCLOZOLIN: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of Vinclozolin results is presented in Table 14. The supplied results for Vinclozolin can be considered satisfactory with a Robust RSD% value of 18 together with the uncertainty value of 0.003 mg/kg.

All z-score values with recoveries estimated as numerical values are presented in Table 15 while in Figure 11 are represented the z-score in graphical form.

Table 14. VINCLOZOLIN: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.074
Mean	0.075
Median	0.076
Robust mean or Assigned value (mg/kg)	0.075
s*	0.014
σ_{EUPT}	0.019
Uncertainty (u) (mg/kg)	0.003
u/σ_{EUPT} *	0.158
FFP RSD (%)	25
Robust RSD (%)	18

s*= robust standard deviation
* $u/\sigma_{EUPT} \leq 0.3$; RSD: Relative Standard Deviation

Table 15. VINCLOZOLIN: z-score and recovery (%) values

Lab Code	z_{EUPT}-score	Recovery %
1	1,8	87
2	0,4	115
3	-0,1	92
4	-1,2	128
5	-0,1	85
6	-1,9	80
7	0,7	73
8	0,7	99
9	0,2	97
10	0,5	110
11	-0,2	99
12	0,5	95
14	0.6	90
15	-3,9	-
16	-1,1	86
17	0,5	98
18	-0,9	98
19	-1,1	115
20	0,4	102
21	-0,1	100
22	-0,2	93
23	-0,8	82
24	-0,2	91
25	0,9	95
26	0,0	94
28	0,1	100
29	0,9	85
30	-0,5	108
31	0,1	90
33	-0,2	86
34	0,6	106
35	-1,6	Std add
36	0,7	103
37	0,1	93
38	0,4	104
39	-0,1	96
40	0,1	85*

*Adjusted for recovery

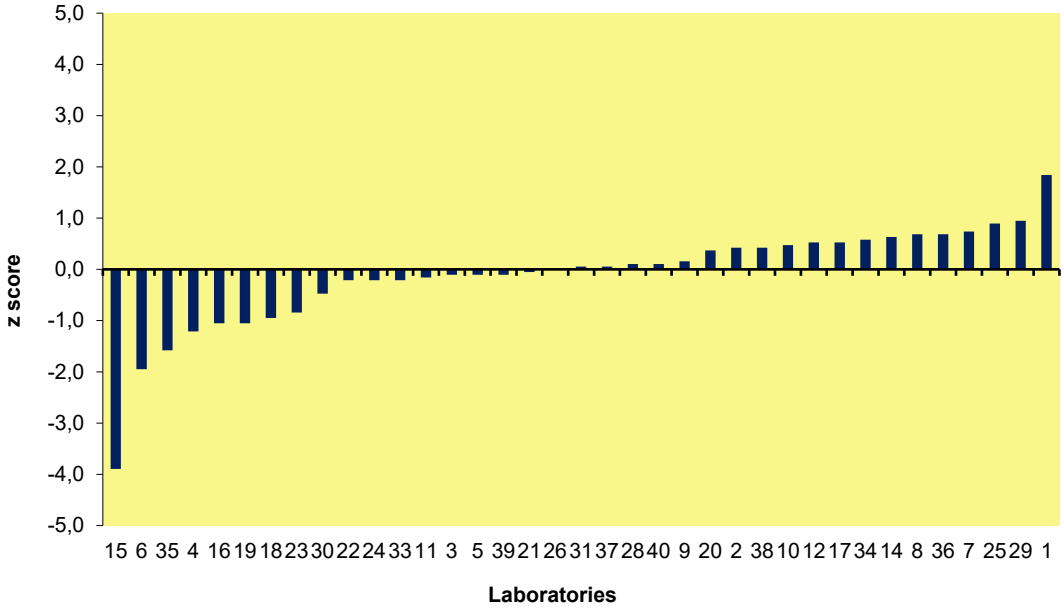


Figure 11. VINCLOZOLIN: z-score values (spiked value = 0.074 mg/kg)

Vinclozolin was analysed by thirty-seven out of forty laboratories with good calculated z-score values in the range 0.1-2.0 as absolute values except for a false negative value of -3.9 calculated in the case of Lab 15.

COIPT-22: FINAL CONSIDERATION

From a statistical point of view the results for the six compounds object of the COIPT-22 can be considered satisfactory.

The Robust Standard Deviation (Robust RSD) and the uncertainty of the assigned values *u* (x_{pt}) were presented for all pesticides. The range of Robust RSD% values was good from 14 to 27 while the range of *u* was from 0.003 to 0.009 mg/kg.

All forty participants laboratories submitted results and twenty-six (equal to 65%) analysed all compounds with lambda-Cyhalothrin and Cypermethrin that resulted the most analysed compounds.

Three false negative values were calculated in the case of Lab 05 for Aldrin and Cypermethrin and in the case of Lab 15 for Vinclozolin. No false positive z-scores have been derived.

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 12 was an accurate representation of the results of the AZ^2.

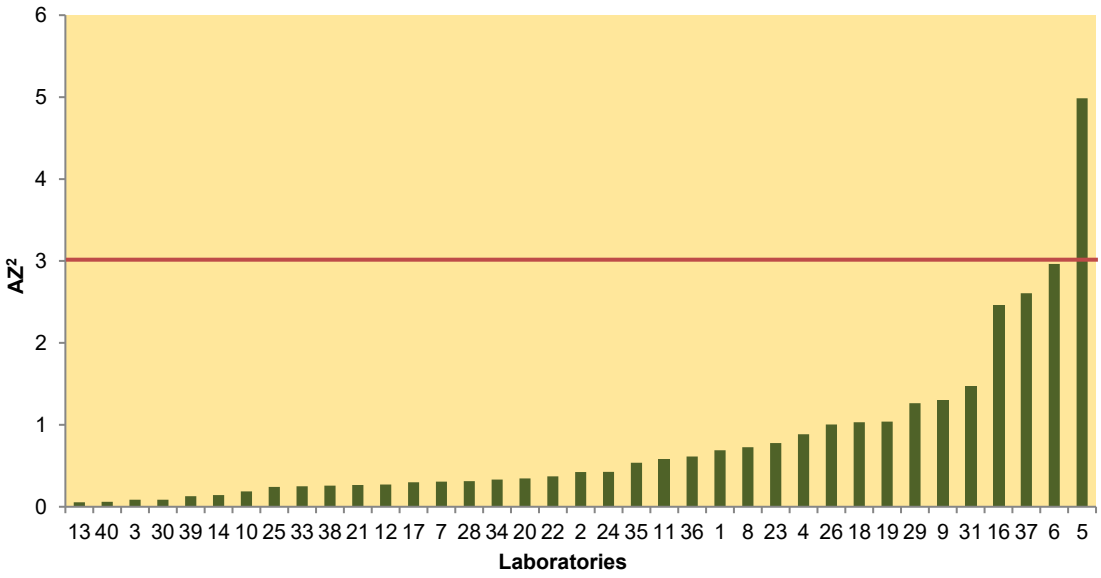


Figure 12. Global performance of laboratories: AZ^2 values

Respect to the analytical methods applied by participants, the majority of laboratories corresponding to twenty-six participants out of forty used the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) methodology or methods based on QuEChERS (21).

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 (21).

Twelve laboratories used in house methods with an extraction step followed by a clean-up phase; only one of them without any purification.

Two laboratories followed the method QuOil (22).

In the above-mentioned methods, the purification was carried out using the GPC (Gel Permeation Chromatography) technique, C_{18} or OASIS cartridges or using combination of different materials as PSA+C_{18} as SPE or PSA+GCB or freezing technique. The amount of the sample test was in the range 0.2-15 g while the final analysis volume was between 0.15 and 10 ml.

In the analysis of pesticide residues, the laboratories use Multi-Residue Methods (MRM) because of the large number of analytes enclosed in official control plans (23-25).

The majority of the laboratories as instrumental detection techniques have used GC (Gas Chromatography) or LC (Liquid Chromatography) coupled with MS/MS detector using two or three transitions.

In the large part of the cases the quantification has been carried out with matrix calibration at single or multiple levels. Five laboratories used instead the solvent calibration and six laboratories performed the standard addition procedure. Most laboratories used internal or process standards for quantification.

CONCLUSIONS

The outcome of the COIPT-22 can be considered satisfactory from several point of view.

One is the good participation of laboratories. Forty laboratories: three NRLs, sixteen official control laboratories and twenty-one private laboratories. The other regards the performance expressed in terms of z-score. The laboratory performance obtained for each tested pesticide was satisfactory by almost all participants.

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

Regarding the methodologies used in this PT, the analysis for the majority of laboratories were performed according QuEChERS method or QuEChERS based analytical methods with limited modifications.

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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residue levels for aclonifen, boscalid, cow milk, etofenprox, ferric pyrophosphate, L-cysteine, lambda-cyhalothrin, maleic hydrazide, mefentrifluconazole, sodium 5- nitroguaiacolate, sodium o-nitrophenolate, sodium p-nitrophenolate and triclopyr in or on certain products *Official Journal of the European Union* L 125/15, 13 April 2021.

15. Europe. Commission Regulation (EU) 2017/626 of 31 March 2017 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for acetamiprid, cyantraniliprole, cypermethrin, cyprodinil, difenoconazole, ethephon, fluopyram, flutriafol, fluxapyroxad, imazapic, imazapyr, lambda-cyhalothrin, mesotrione, profenofos, propiconazole, pyrimethanil, spirotetramat, tebuconazole, triazophos and trifloxystrobin in or on certain products. *Official Journal of the European Union* L 96/1, 7 April 2017.
16. Europe. Commission Regulation (EU) 2019/552 of 4 April 2019 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for azoxystrobin, bicyclopyrone, chlormequat, cyprodinil, difenoconazole, fenpropimorph, fenpyroximate, fluopyram, fosetyl, isoprothiolane, isopyrazam, oxamyl, prothioconazole, spinetoram, trifloxystrobin and triflumezopyrim in or on certain products *Official Journal of the European Union* L 96/6, 5 April 2019.
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APPENDIX A
List of participants

The participants in COIPT-22 are listed below.

BELGIUM
Primoris Belgium (Zwijnaarde)
GERMANY
Eurofins Sofia GmbH (Berlin)
Institut Kirchoff Berlin GmbH (Berlin)
Niedersaechsisches Landesamt Fuer Verbraucherschutz Und Lebensmittelsicherheit Lebensmittel Und Veterinaerinstitut Oldenburg (Oldenburg)
FRANCE
ITERG (Canejan)
GREECE
CADMION (Kiato)
Benaki Phytopathological Institute – Pesticide Residues Laboratory (Athens)
Chemicotecniki Lagouvardou-Spantidaki O.E. (Rethymno, Crete)
General Chemical State Laboratory, Pesticide Residues Laboratory, D Chemical Division (Athens)
Multichrom Lab Crete (Heraklion, Crete)
TUV Austria Food Allergens Labs (Attica)
TUV Austria Food Allergens Labs (Crete)
SKYLAB – Med S.A. (Athens)
ITALY
Agenzia delle Dogane e dei Monopoli VII - Direzionale Regionale per la Sicilia - Ufficio Antifrodi (Palermo)
Agro.biolab Laboratory srl (Rutigliano, BA)
Analytical srl (Firenze)
APPA Bolzano, Settore Laboratorio (Bolzano)
ARPA Emilia Romagna Area Fitofarmaci (Ferrara)
ARPA Friuli Venezia Giulia (Udine)
ARPA Lazio (Latina)
ARPA Puglia, Polo di Specializzazione “Alimenti” (Bari)
ATS Bergamo (Bergamo)
ATS Milano (Milano)
Cadir Lab (Alessandria)
CHEMISERVICE srl (Monopoli, BA)
ICQRF, Laboratorio di Catania (Catania)
INNOVHUB-SSI, Divisione SSOG (Milano)
Istituto Superiore di Sanità, Dipartimento Ambiente e Salute (Roma)
IZSLER (Brescia)
IZSLT (Roma)

IZS Piemonte, Liguria e Valle d'Aosta (Cuneo)
IZS della Puglia e Basilicata (Foggia)
LABCAM srl (Albenga, SV)
PH TUV SUD (Firenze)
USL Toscana Centro (Firenze)
Water e Life Lab srl (Bergamo)
SPAIN
Borges Agricultural & Industrial Edible Oils (Tàrrega, Lléida)
CNTA - National Center for technology and food Safety (San Adrian, Navarra)
Laboratorio Agroalimentario (Granada)

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 ($i = 1, 2, \ldots p$), 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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