RAPPORTI ISTISAN 23|17

ISSN: 1123-3117 (cartaceo) • 2384-8936 (online)

Results of the proficiency test on pesticide residues in olive oil in 2021

T. Generali, P. Stefanelli, V. Picardo,
S. Girolimetti, D. Attard Barbini

AMBIENTE
E SALUTE

ISTITUTO SUPERIORE DI SANITÀ

Results of the proficiency test on pesticide residues in olive oil in 2021

Tiziana Generali, Patrizia Stefanelli, Valentina Picardo,
Silvana Girolimetti, Danilo Attard Barbini
Dipartimento Ambiente e Salute

ISSN: 1123-3117 (cartaceo) • 2384-8936 (online)

Rapporti ISTISAN
23/17

Istituto Superiore di Sanità
Results of the proficiency test on pesticide residues in olive oil in 2021.
Tiziana Generali, Patrizia Stefanelli, Valentina Picardo, Silvana Girolimetti, Danilo Attard Barbini
2023, v, 41 p. Rapporti ISTISAN 23/17

In 2021, 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) in olive oil, named COIPT-21. 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 thirty-eight compounds. Thirty-seven participating laboratories submitted results; twenty-three participants analysed all the 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 interlaboratorio su residui di antiparassitari in olio di oliva nel 2021.
A cura di Tiziana Generali, Patrizia Stefanelli, Valentina Picardo, Silvana Girolimetti e Danilo Attard Barbini
2013, v, 41 p. Rapporti ISTISAN 13/17 (in inglese)

Nel 2021, come ogni anno, il Laboratorio Nazionale di Riferimento (LNR) 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) in olio d'oliva chiamato COIPT-21. I laboratori invitati a partecipare a questi test di competenza sono laboratori mediterranei del COI e laboratori europei (LNR, 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. Trentasette laboratori partecipanti hanno fornito risultati; ventitré 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

Per informazioni su questo documento scrivere a: tiziana.generali@iss.it

Il rapporto è accessibile online dal sito di questo Istituto: www.iss.it

Citare questo documento come segue:

Generali T, Stefanelli P, Picardo V, Girolimetti S, Attard Barbini D. *Results of the proficiency test on pesticide residues in olive oil in 2021.* Roma: Istituto Superiore di Sanità; 2023. (Rapporti ISTISAN 23/17).

Legale rappresentante dell'Istituto Superiore di Sanità: *Silvio Brusaferro*
Registro della Stampa - Tribunale di Roma n. 114 (cartaceo) e n. 115 (online) del 16 maggio 2014

Direttore responsabile della serie: *Paola De Castro*
Redazione*: Sandra Salinetti*
La responsabilità dei dati scientifici e tecnici è dei singoli autori, che dichiarano di non avere conflitti di interesse.

viale Regina Elena, 299 – 00161 Roma

TABLE OF CONTENTS

Abbreviations iii

Preface v

General consideration on maximum residue level in olive oil 1

Proficiency test on olive oil: the COIPT-21 3
Rationale 3
Test materials 3
Homogeneity and stability tests 4
Distribution of samples and instructions to participants 6
Statistical evaluation of results 6

COIPT-21: results 8
Carbendazim 8
Chlorpyrifos 10
Beta-Endosulfan 13
Oxyfluorfen 16
Tebuconazole 19
Trifloxystrobin 22

COIPT-21: final consideration 26

Comparison results between COIPT-21 and AO-PT1 28

Conclusions 30

References 31

Appendix A
List of participants 35

Appendix B
Robust analysis: algorithm A 39

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
EURL	European, Reference Laboratory
FFP	Fitness for Purpose
GAP	Good Agricultural Practice
GC	Gas Chromatography
ILAC	International Laboratory Accreditation Cooperation
ISO	International Organization for Standardization
LC	Liquid Chromatography
LOD	Default Lowest Limit
MRL	Maximum Residue Limit
MS	Mass Spectrometry
MU	Measurement Uncertainty
NRL-AO	National Reference Laboratory - Animal Origin
NRL	National reference Laboratory
PPP	Plant Protection Product
PT	Proficiency Test
RL	Reporting Limit
RSD	Relative Standard Deviation
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
SD	Standard Deviation
Std add	Standard addition

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 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 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, 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 International Olive Council (year 2020-2021) relating to the production of olive oil area the 92% of the olive oil in the world is produced by Mediterranean countries (4) with 70% of the olive oil provided by Spain, Greece and Italy (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 thereof. Most Plant Protection Products (PPPs) 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 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 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 European Commission website.

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

To set any MRL for pesticides applicants, producers of plant protection products, farmers, importers, EU (European Union) 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.

The Regulation (EC) 396/2005 set at 0.01 mg/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 European Union (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-21

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 2021 on olive oil was named COIPT-21.

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 thirty-eight compounds presented in COIPT-21 Announcement that was sent to participant on 7 October 2021. 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.

Thirty-seven laboratories agreed to participate in this PT: three NRLs, eighteen official control laboratories and sixteen 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 4.2 kg 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 2.1 kg of the blank oil, was spiked with the following pesticides: Carbendazim, Chlorpyrifos, beta-Endosulfan, Oxyfluorfen, Tebuconazole and Trifloxystrobin. Aliquots of 50 g of this spiked oil named COIPT-21 SPIKED OIL were transferred into dark glass bottles as well as aliquots of 50 g of the blank oil named COIPT-21 BLANK OIL. Samples were sealed and stored at ambient temperature before the shipment to participants. Each participant received one COIPT-21 SPIKED OIL sample and one COIPT-21 BLANK OIL sample. The current MRLs for these six pesticides are showed in Table 1 (12-17).

Table 1. COIPT-21: current MRLs for the six pesticides spiked in the blank oil

Compounds	Current EU Regulation	MRL on olive for oil production (mg/kg)
Carbendazim	Regulation (EU) 559/2011 Applicable from: 01/01/2012	**0.1*** on olive as sum of benomyl and carbendazim expressed as carbendazim
Chlorpyrifos	Regulation (EU) 2020/1085 Applicable from: 13/11/2020	**0.01***
beta-Endosulfan	Regulation (EU) 310/2011 Applicable from: 21/10/2011	**0.05*** on olive as sum of alpha and beta-isomers and endosulfan-sulphate expresses as endosulfan
Oxyfluorfen	Regulation (EU) 2022/1321 Applicable from: 21/02/2023	**1**
Tebuconazole	Regulation (EU) 2018/1514 Applicable from: 01/11/2018	**0.5**
Trifloxystrobin	Regulation (EU) 2019/1791 Applicable from: 18/11/2019	**0.3**

* Limit of analytical determination

Homogeneity and stability tests

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

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 homogeneous 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 7th December 2021;
- Day 2: after one month by the deadline for reporting results on 7rd February 2022.

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.

Of the six spiked compounds, only Carbendazim failed the stability test as well as the homogeneity test

Table 2. COIPT-21: homogeneity results (mg/kg) for COIPT-21

Sample number	Carbendazim	Chlorpyrifos	beta-Endosulfan	Oxyfluorfen	Tebuconazole	Trifloxystrobin
70	0.132	0.223	0.297	0.178	0.249	0.208
73	0.123	0.223	0.285	0.213	0.252	0.208
76	0.095	0.212	0.304	0.178	0.242	0.202
79	0.122	0.220	0.306	0.215	0.263	0.208
82	0.100	0.216	0.292	0.184	0.250	0.208
85	0.105	0.217	0.305	0.206	0.241	0.204
86	0.123	0.220	0.306	0.208	0.263	0.211
88	0.111	0.221	0.303	0.186	0.269	0.209
90	0.125	0.221	0.302	0.195	0.257	0.216
121	-	0.219	0.313	0.214	0.240	0.203
Mean	0.115	0.219	0.301	0.198	0.253	0.208
SD	0.013	0.003	0.008	0.015	0.010	0.004
σ_{EUPT}	0.024	0.051	0.072	0.051	0.058	0.047
SD/σ_{EUPT}	0.533	0.067	0.111	0.298	0.175	0.087
Critical value	0.3	0.3	0.3	0.3	0.3	0.3
$SD/\sigma_{EUPT} \leq 0.3$	no	yes	yes	yes	yes	yes

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

Table 3. COIPT-21: data (mg/kg) of the stability test for COIPT-21

Pesticide	Concentration mg/kg				
	Mean 1 (M1) n=6	Mean 2 (M2) n=6	(M1-M2)	σ_{EUPT}	$0.3x\sigma_{EUPT}$
Carbendazim	0.113	0.127	-0.014	0.024	0.007
Chlorpyrifos	0.208	0.222	-0.014	0.051	0.015
beta-Endosulfan	0.296	0.296	0.000	0.072	0.022
Oxyfluorfen	0.210	0.197	0.014	0.051	0.015
Tebuconazole	0.237	0.252	-0.016	0.058	0.017
Trifloxystrobin	0.209	0.211	-0.002	0.047	0.005

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 = $|M1-M2| < 0.3x\sigma_{EUPT}$

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 to participants between 23-30th November 2021. The deadline for results was 11thJanuary 2022.

The final report was dispatched to all participant at the end of March 2022.

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:

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

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).

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

COIPT-21: 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.

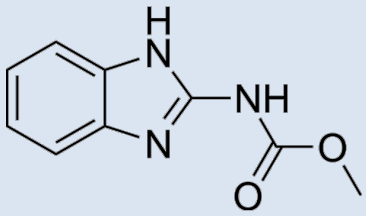
In addition, Kernel density plots were used to identify multi-modality in the data distributions.

All the compound data sets were normally distributed except for Chlorpyrifos and Tebuconazole.

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.

Carbendazim



Common name
carbendazim, carbendazime and carbendazol

Structure formula	$C_9H_9N_3O_2$
CAS number	10605-21-7
EC no.	234-232-0

Its physical form consists of crystalline powder with weight molecular of 191.2 g/mol.
This compound has good solubility in water
and it is stable in acids, forming water-soluble salts.
It is a systemic fungicide, with protective and curative action.
Not authorized on olive tree with a MRL value of 0.1 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 Carbendazim (mg/kg) submitted by all laboratories with the Kernel density plot. The distribution of the results is symmetric.

Carbendazim, as previously mentioned, did not pass the stability and homogeneity tests and as consequence it was decided not to assign z-score values for this compound.

In fact, the unsatisfactory Robust RSD% value of 31 is indicative of the dispersion of results.

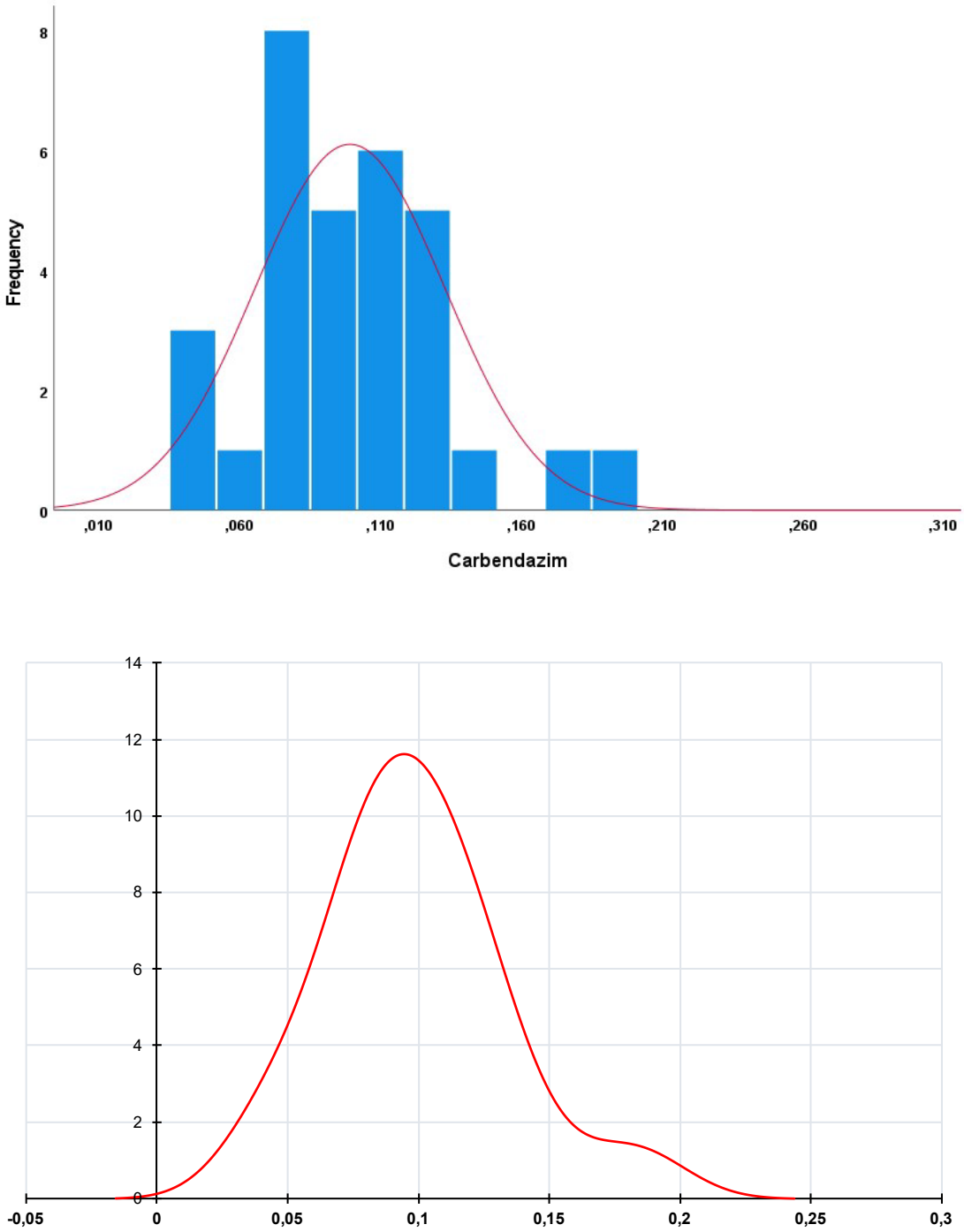


Figure 1. CARBENDAZIM: frequency histogram of the results (mg/kg) and Kernel density plot

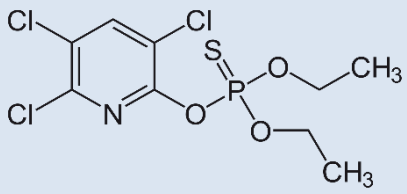
Statistical evaluation of the Carbendazim results is presented in Table 4.

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

Parameter	Value
Spiked value	0.162
Mean	0.099
Median	0.099
Robust mean or Assigned value (mg/kg)	0.097
s*	0.031
σ_{EUPT}	0.024
Uncertainty (u) (mg/kg)	0.007
u/σ_{EUPT} *	0.292
FFP RSD (%)	25
Robust RSD (%)	31

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

Chlorpyrifos



Common name
chlorpyriphos-éthyl, chlorpyriphos,chlorpyrifos

Structure formula $C_9H_{11}Cl_3NO_3PS$

CAS number 2921-88-2

EC no. 220-864-4

The physical form consist of colourless crystals, with a mild mercaptan odour with weight molecular of 350.6 g/mol. It is a non-systemic organophosphate insecticide with contact, stomach and respiratory action.
This compound is highly soluble in organic solvents and decomposes above 160°C.
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 as consequence of his liphofilic properties.

In the case of Chlorpyrifos the distribution of submitted data resulted not symmetric as indicated in Figure 2.

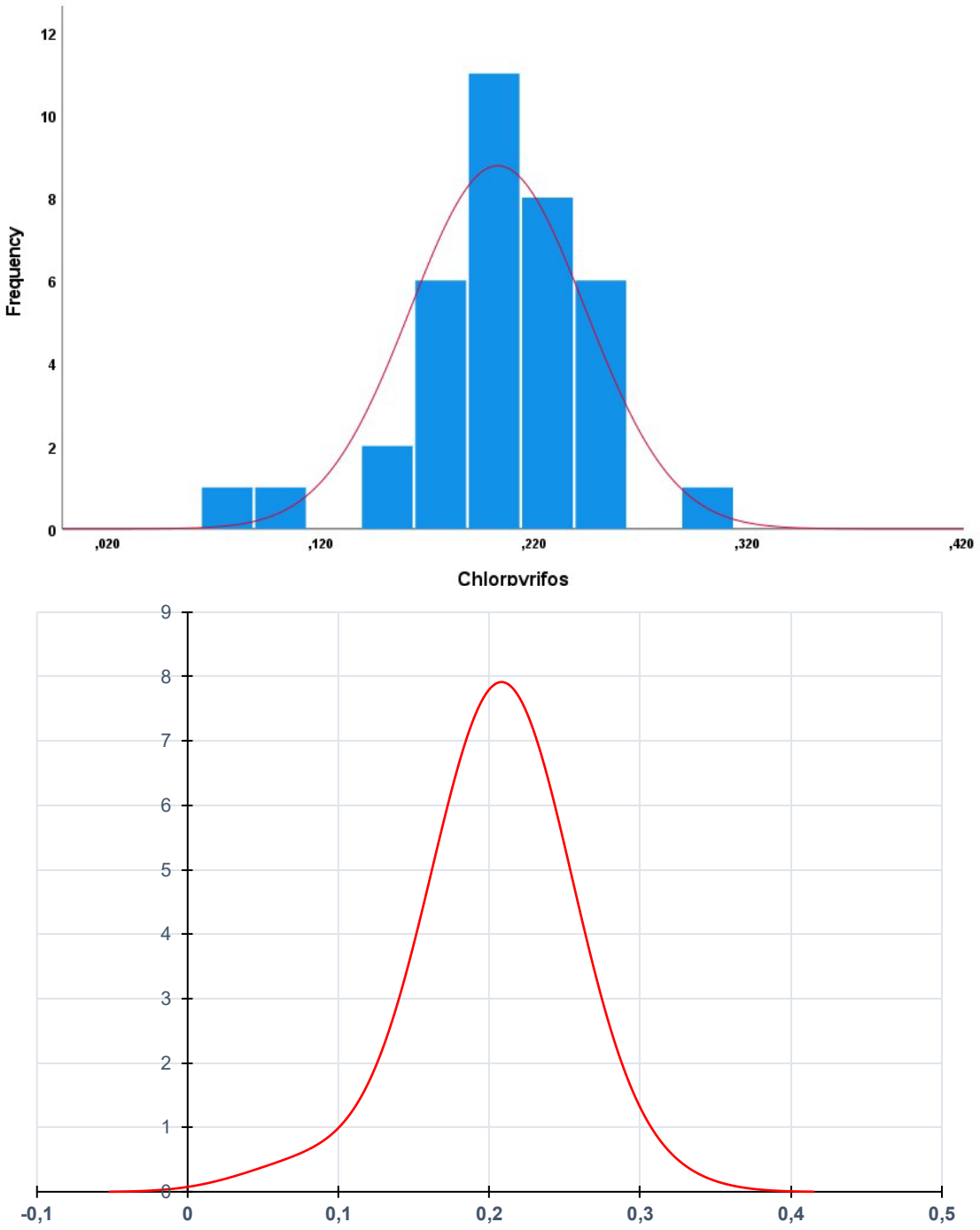


Figure 2. CHLORPYRIFOS: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of the Chlorpyrifos results is presented in Table 5. In this case submitted results can be considered good, with Robust RSD% and uncertainty of the assigned values *u* acceptable.

Table 5. CHLORPYRIFOS: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.239
Mean	0.203
Median	0.209
Robust mean or Assigned value (mg/kg)	0.206
s*	0.031
σ_{EUPT}	0.051
Uncertainty (u) (mg/kg)	0.010
u/σ_{EUPT} *	0.196
FFP RSD (%)	25
Robust RSD (%)	15

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

All z_{EUPT}-score values with recoveries estimated as numerical values are presented in Table 6.

Table 6. CHLORPYRIFOS: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
1	0.0	70
2	0.1	80
3	-0.3	80
4	0.0	94
5	-2.0	74
6	-0.6	75
7	-0.5	85
8	0.7	97
9	0.2	94
10	0.2	99
11	-0.9	65
12	0.1	106
13	-0.4	82
14	0.7	90
15	1.8	51
16	-0.7	86
17	-0.3	90
18	-2.8	102
20	0.5	102
21	0.2	100
22	0.7	85
23	0.4	72
24	0.2	85
25	-0.2	82
26	-0.2	72
27	0.5	70
28	-0.5	84
29	-0.6	88
30	0.9	91
31	-0.9	76
32	0.0	-
33	0.7	102
34	0.3	89
35	0.7	Std add
36	-0.2	76
37	0.1	97

Furthermore, in Figure 3 the z_{EUPT}-score values are presented in graphical form.

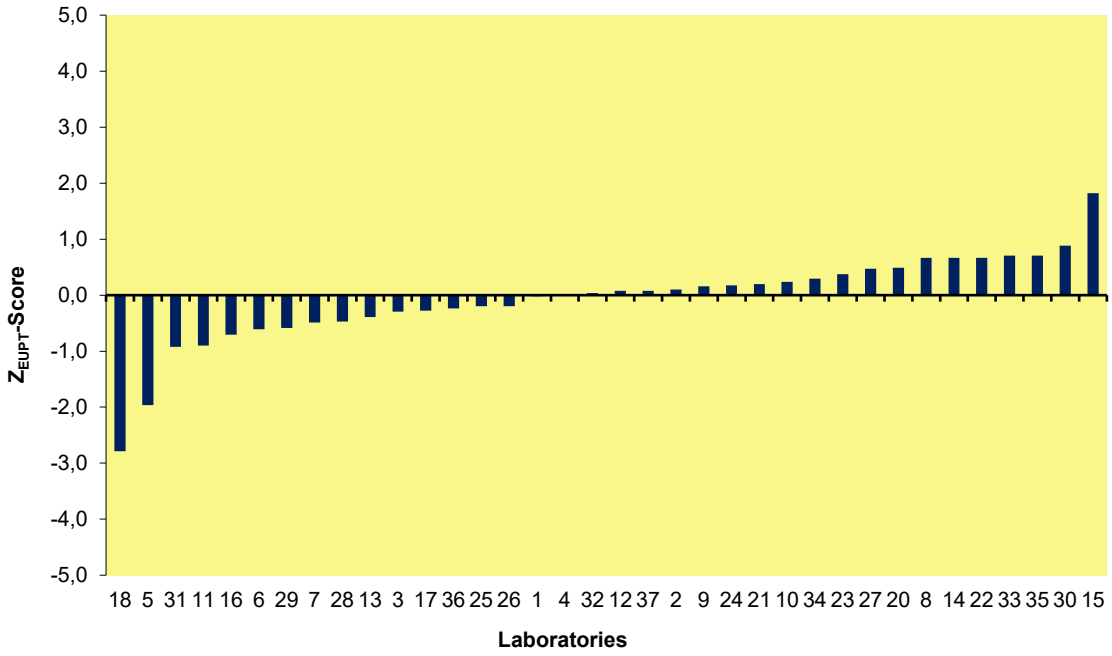
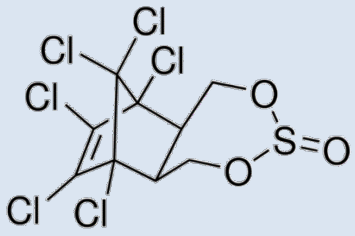


Figure 3. CHLORPYRIFOS: z-score values (spiked value = 0.239 mg/kg)

In the case of Chlorpyrifos thirty-six laboratories supplied results with good calculated z-score values in the range 0.1-2.0 as absolute values except for Lab 18 with a questionable z-score of 2.8.

beta-Endosulfan



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.

Figure 4 shows the results of beta-Endosulfan (mg/kg) submitted by all laboratories in the COIPT-21. The distribution of the results is clearly symmetric.

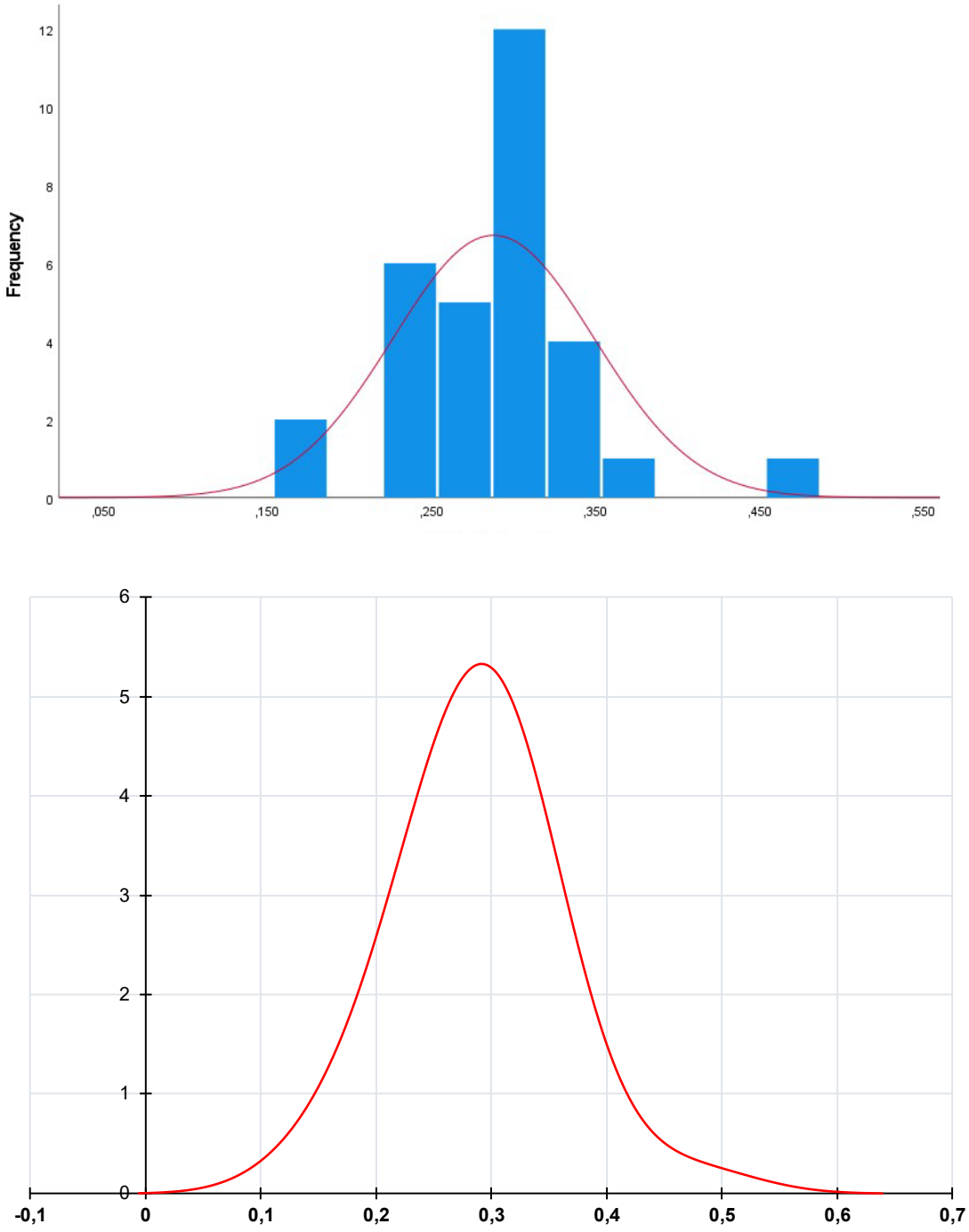


Figure 4. BETA-ENDOSULFAN: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of the beta-Endosulfan results is presented in Table 7.

Table 7. BETA-ENDOSULFAN: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.339
Mean	0.288
Median	0.300
Robust mean or Assigned value (mg/kg)	0.287
s*	0.050
σ_{EUPT}	0.072
Uncertainty (u) (mg/kg)	0.011
u/σ_{EUPT} *	0.153
FFP RSD (%)	25
Robust RSD (%)	17

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

The supplied results for beta-Endosulfan can be considered satisfactory with a Robust RSD% value of 17 together with the uncertainty value of 0.011 mg/kg.

Table 8. BETA-ENDOSULFAN: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
2	0.0	74
3	-0.4	73
4	-0.7	85
5	-0.6	91
6	-0.6	66
7	-1.8	89
8	-3.8	-
9	2.7	94
10	0.4	82
11	0.2	61
12	0.2	60
14	0.5	90
16	-0.8	60
17	0.2	92
18	-0.4	84
20	0.6	98
21	0.5	100
22	-1.7	60
23	0.3	60
24	0.3	85
25	-0.4	84
26	0.1	80
27	-0.9	56
28	0.1	85
30	1.3	94
31	-0.9	78
32	0.4	-
33	0.8	80
34	0.2	80
35	0.4	Std add
36	-0.4	82
37	0.8	91

All z_{EUPT}-score values with recoveries estimated as numerical values are presented in Table 8 with z_{EUPT}-score showed as graphical representation in Figure 5.

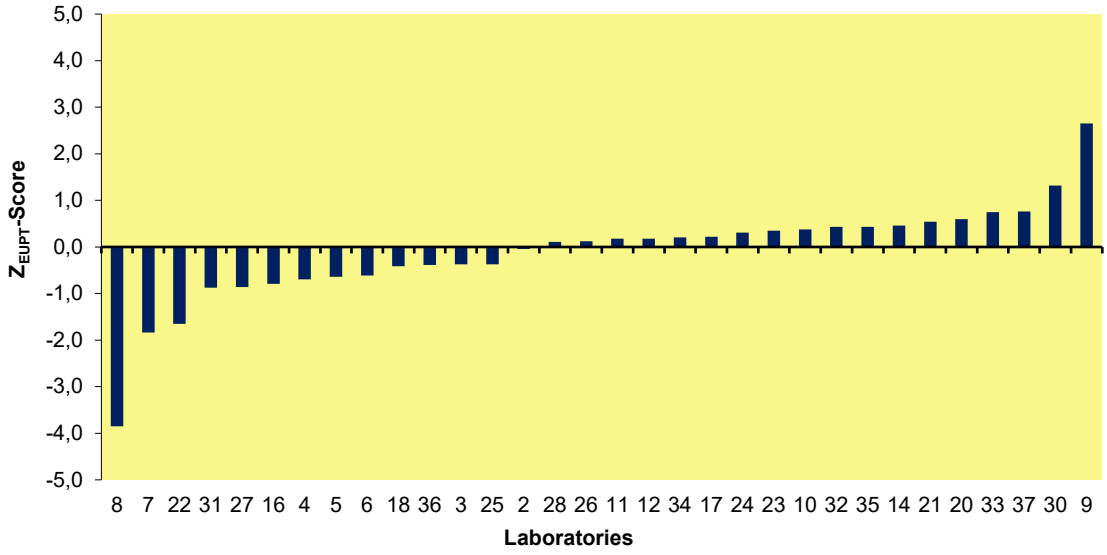
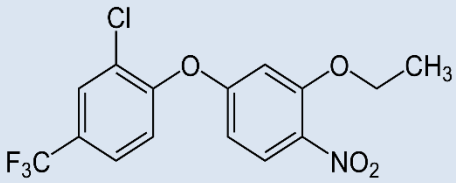


Figure 5. BETA-ENDOSULFAN: z-score values (spiked value = 0.339 mg/kg)

Beta-Endosulfan was analysed by thirty-two out of thirty-seven laboratories with a questionable z-score value of 2.7 for Lab 9 and a false negative value of -3.8 calculated in the case of Lab 08.

Oxyfluorfen



Common name
oxyfluorfène, oxyfluorfen

CAS number	42874-03-3
EC no.	255-983-0
Structure formula	$C_{15}H_{11}ClF_3NO_4$

Its physical form consists of orange crystalline solid with weight molecular of 361.7
This pesticide has a good solubility in organic solvents and good stability up to 50°C. Decomposed rapidly by UV irradiation
Selective contact herbicide authorized in Italy on olive tree with four PPP as EC (emulsifiable concentrates)
and four PPP as SC (aqueous suspension concentrates) formulations. The MRL value is 1.0 mg/kg on olive as established by the Regulation (EC) 396/2005.

Also in the case of Oxyfluorfen the distribution of submitted data resulted symmetric as indicated in Figure 6.

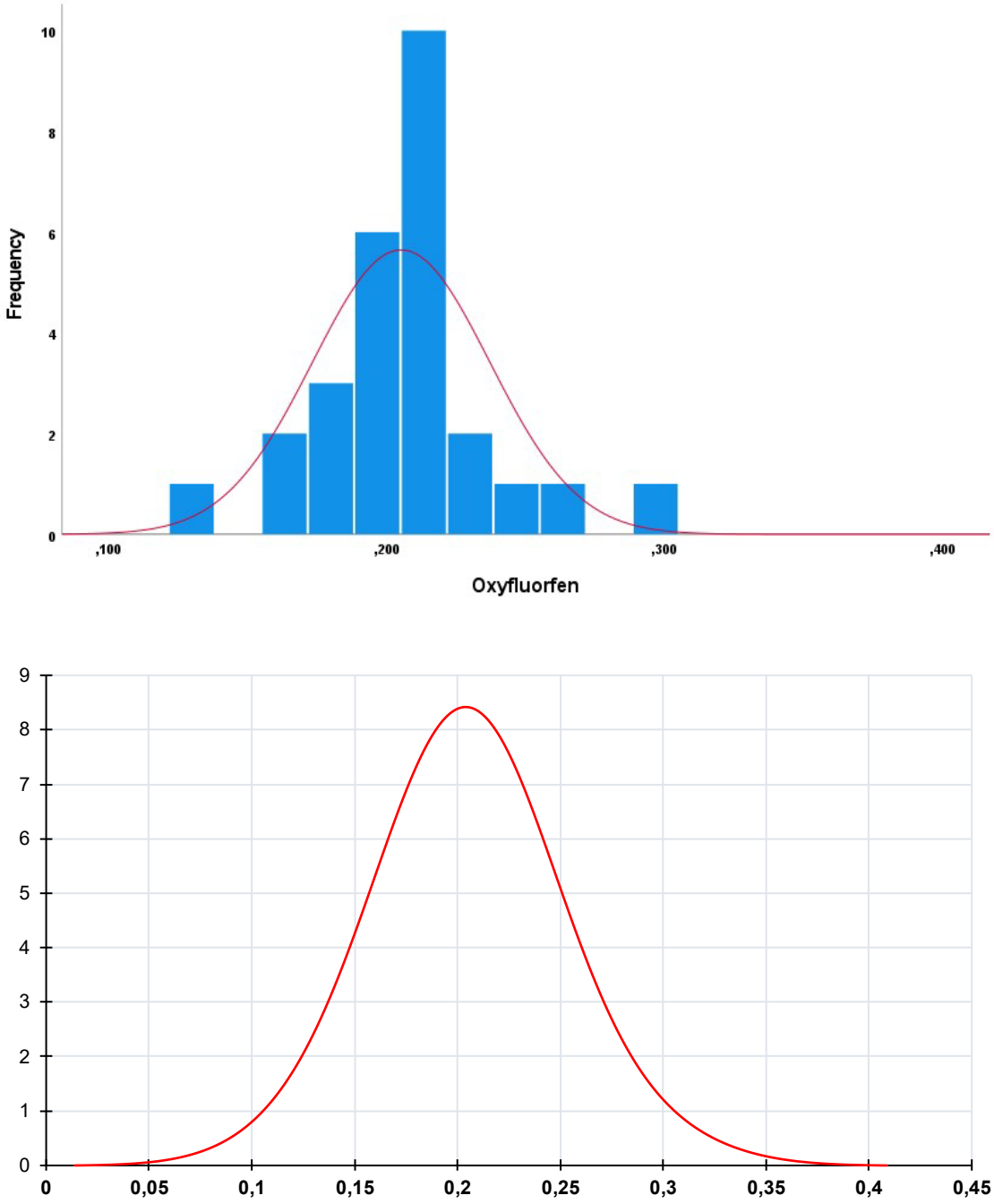


Figure 6. OXYFLUORFEN: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of the Oxyfluorfen results is presented in Table 9.

Table 9. OXYFLUORFEN: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.219
Mean	0.205
Median	0.205
Robust mean or Assigned value (mg/kg)	0.204
s*	0.026
σ_{EUPT}	0.051
Uncertainty (u) (mg/kg)	0.006
u/σ_{EUPT} *	0.118
FFP RSD (%)	25
Robust RSD (%)	13

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

Regarding Oxyfluorfen data, the obtained performance can be considered good with a Robust RSD% value of 13 and an uncertainty value of 0.006 mg/kg.

All z_{EUPT}-score values with recoveries estimated as numerical values are presented in Table 10 graphical representation of z_{EUPT}-score is showed in Figure 7.

Oxyfluorfen was the less analysed compound with twenty-seven laboratories out of thirty-seven that supplied results. The calculated z-score values obtained were good, all in the range 0.0-2.0.

Table 10. OXYFLUORFEN: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
2	0.4	91
3	-0.3	85
4	-1.5	103
6	-0.3	79
8	0.3	97
10	0.3	108
12	-0.1	120
14	0.3	90
15	0.1	57
16	1.1	100
17	0.2	100
20	0.2	87
21	-0.3	100
22	-0.6	95
23	-0.6	75
24	0.0	89
26	0.0	88
27	-0.3	89
28	0.5	92
30	0.1	87
31	-0.7	69
32	1.8	-
33	-0.1	91
34	0.0	74
35	-0.9	Std add
36	0.8	79
37	0.2	107

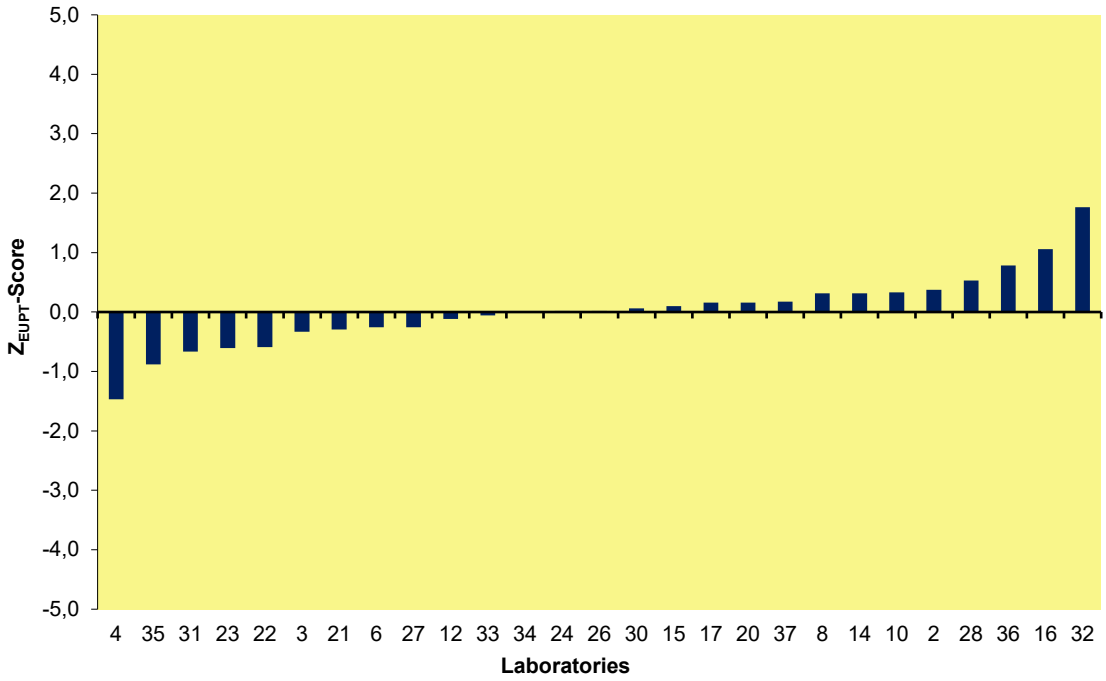
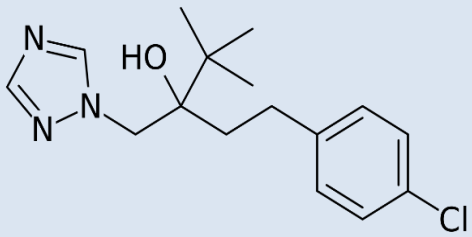


Figure 7. OXYFLUORFEN: z-score values (spiked value = 0.219 mg/kg)

Tebuconazole



Common name
tebuconazole

Structure formula $C_{16}H_{22}ClN_3O$

CAS number 107534-96-3

EC no. 403-640-2

This compound belongs to the triazole family.
Its physical form consists of colourless crystals and in technical form of colourless to light brown powder with weight molecular of 307.8
This pesticide has a good solubility in organic solvents and good stability at pH 5.
Fungicide authorized in Italy on olive tree with a PPP as WG (Water Dispersible Granule) formulationon.
The MRL value is 0.5 mg/kg on olive as established by the Regulation (EC) 396/2005.

Figure 8 shows the results of Tebuconazole (mg/kg) submitted by all laboratories in the COIPT-21. The distribution of the results was not symmetric.

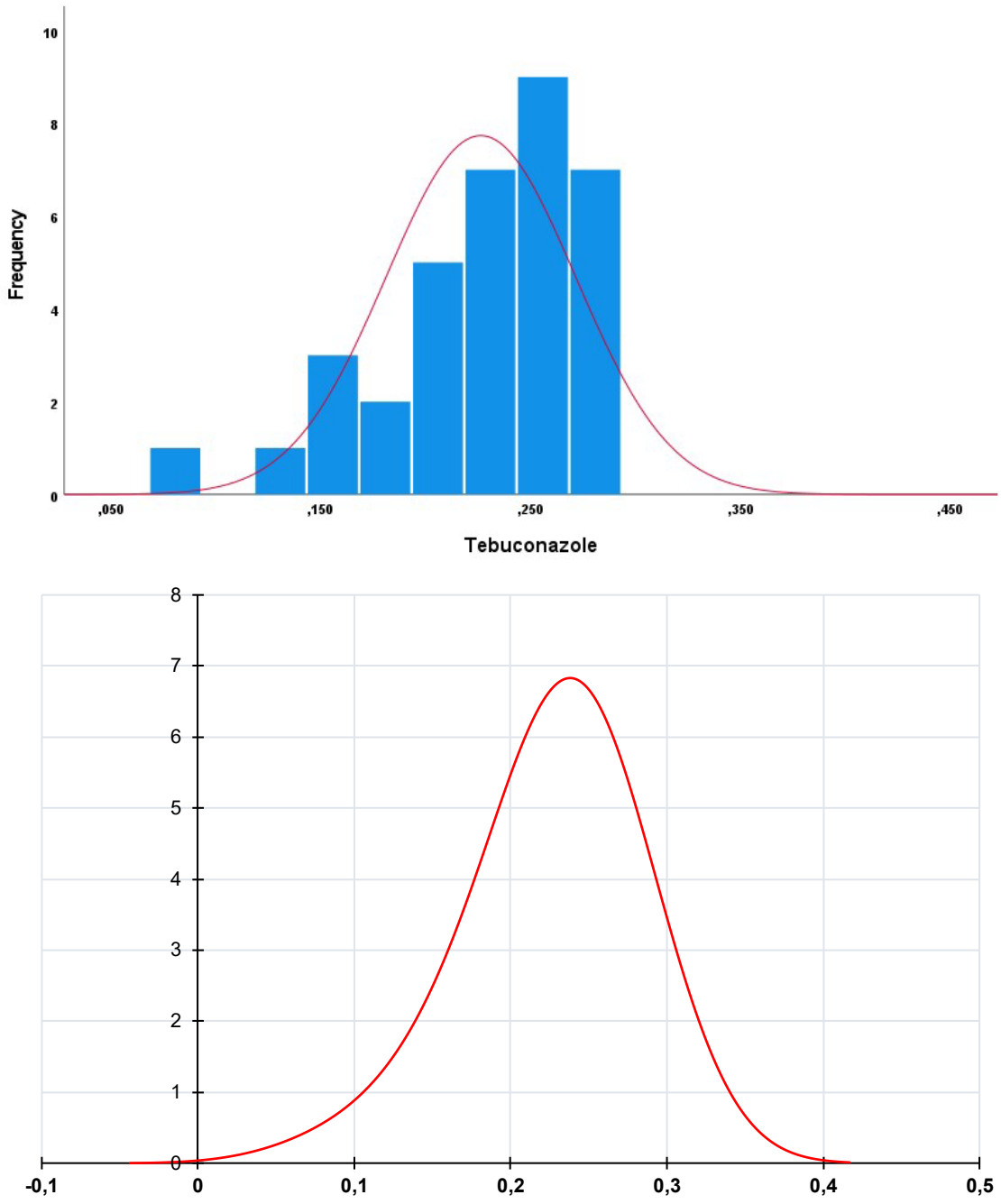


Figure 8. TEBUCONAZOLE: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of Tebuconazole results is presented in Table 11, while in Table 12 are listed all z_{EUPT}-score values with corresponding recoveries estimated.

Statistically results for Tebuconazole can be considered satisfactory. The median and the robust mean are similar with a good value for Robust RSD% of 18 as the uncertainty equal to 0.009 mg/kg.

Table 11. TEBUCONAZOLE: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.250
Mean	0.226
Median	0.232
Robust mean or Assigned value (mg/kg)	0.230
s*	0.041
σ_{EUPT}	0.058
Uncertainty (u) (mg/kg)	0.009
u/σ_{EUPT} *	0.155
FFP RSD (%)	25
Robust RSD (%)	18

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

Table 12. TEBUCONAZOLE: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
1	0.0	116
2	0.3	89
3	0.0	89
4	-0.5	74
5	-1.2	114
6	-0.9	64
7	0.3	99
8	0.3	98
9	-0.3	-
10	0.5	101
11	-0.2	102
12	0.2	78
13	0.4	97
14	0.7	90
15	0.3	72
17	-0.3	86
18	-2.5	99
19	-1.2	112
20	0.3	102
21	0.0	93
22	0.9	100
23	-0.2	82
24	0.3	75
25	0.1	101
26	1.0	87
27	-0.7	58
28	-0.1	83
30	0.0	96
31	-1.1	70
32	0.9	-
33	0.7	88
34	-0.3	98
35	0.8	Std add
36	-1.7	84
37	0.7	107

The z_{EUPT}-score values presented in Table 12 are represented as graphical form in Figure 9.

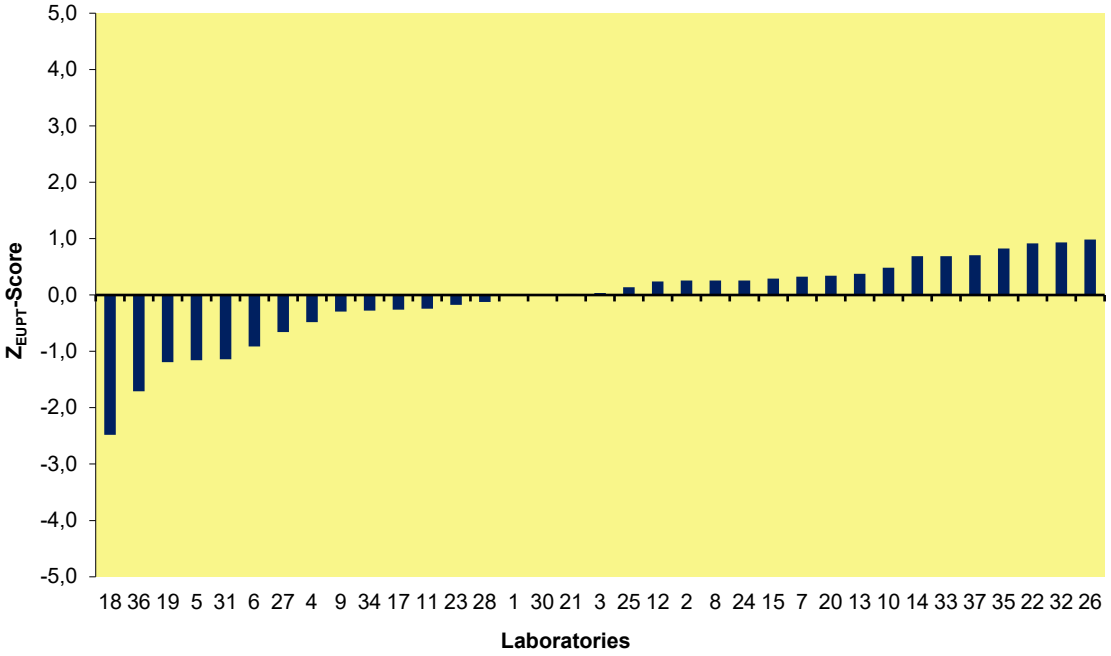
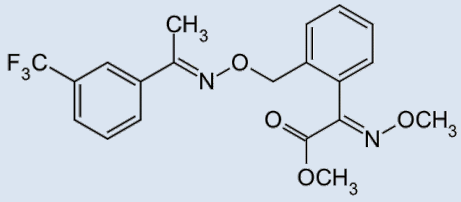


Figure 9. TEBUCONAZOLE: z-score values (spiked value = 0.250 mg/kg)

In the case of Tebuconazole thirty-five laboratories supplied results with good calculated z-score values in the range 0.1-2.0 as absolute values except for Lab 18 with a questinable z-score of 2.5

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 in Italy on olive tree with a PPP as WG (Water dispersible Granule) formulation.
The MRL value is 3.0 mg/kg on olive as established by the Regulation (EC) 396/2005.

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

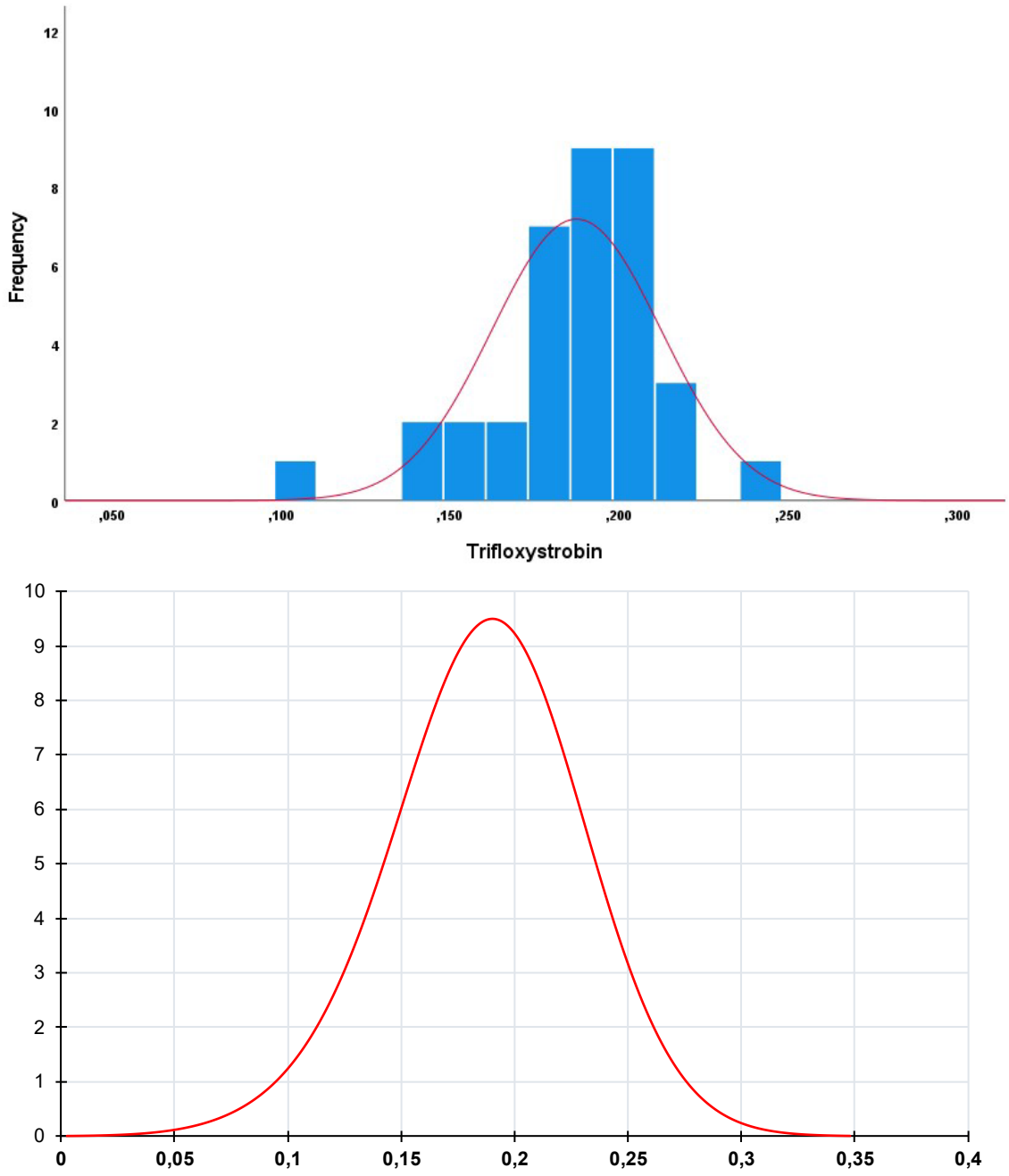


Figure 10. TRIFLOXYSTROBIN: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of Trifloxystrobin results is presented in Table 13.

The supplied results for Trifloxystrobin can be considered excellent with a Robust RSD% value of 11 together with the uncertainty value of 0.004 mg/kg.

All z_{EUPT}-score values with recoveries estimated as numerical values are presented in Table 14, while in Figure 11 the z_{EUPT}-score are represented.

Table 13. TRIFLOXYSTROBIN: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.194
Mean	0.187
Median	0.193
Robust mean or Assigned value (mg/kg)	0.189
s*	0.021
σ_{EUPT}	0.047
Uncertainty (u) (mg/kg)	0.004
u/σ_{EUPT} *	0.085
FFP RSD (%)	25
Robust RSD (%)	11

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

Table 14. TRIFLOXYSTROBIN: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
1	0.5	86
2	0.3	97
3	-0.2	88
4	0.1	100
5	0.5	78
6	-0.2	84
7	0.6	80
8	0.4	97
9	0.2	98
10	0.4	103
11	0.0	97
12	-0.3	97
13	0.2	105
14	0.4	90
15	-0.6	103
16	-0.3	103
17	0.1	105
18	-1.7	97
19	-0.7	112
20	0.1	96
21	-0.1	97
22	-1.1	76
23	0.0	80
24	-0.7	100
25	0.1	97
26	0.1	95
27	1.1	113
28	-0.2	89
30	0.0	98
31	-0.9	91
32	0.2	-
33	-0.1	85
34	0.3	83
35	-0.4	Std add
36	0.2	95
37	0.3	100

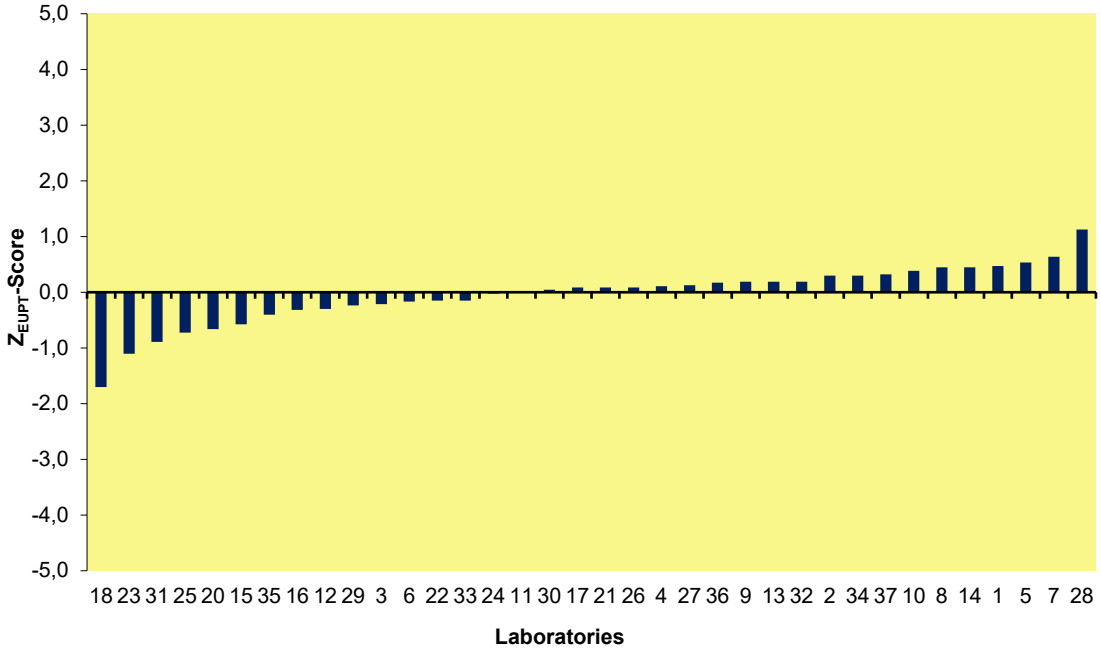


Figure 11. Trifloxystrobin: z-score values (spiked value = 0.194 mg/kg)

Trifloxystrobin was the most analysed compound together with Chlorpyrifos with excellent calculated z-score values in the range 0.0-1.7 as absolute value.

COIPT-21: FINAL CONSIDERATION

From a statistical point of view, with the exception of Carbendazim, the results for the other five compounds object of the COIPT-21 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 very good from 11 to 18% (except for Carbendazim of 31%) while the range of u was from 0.004 to 0.011 mg/kg.

All laboratories submitted results and twenty-three (equal to 62%) analysed all compounds with Chlorpyrifos and Tebuconazole that resulted the most analysed compounds.

A false negative value was calculated in the case of Lab 08 for beta-Endosulfan.

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 is an accurate representation of the results of the AZ^2.

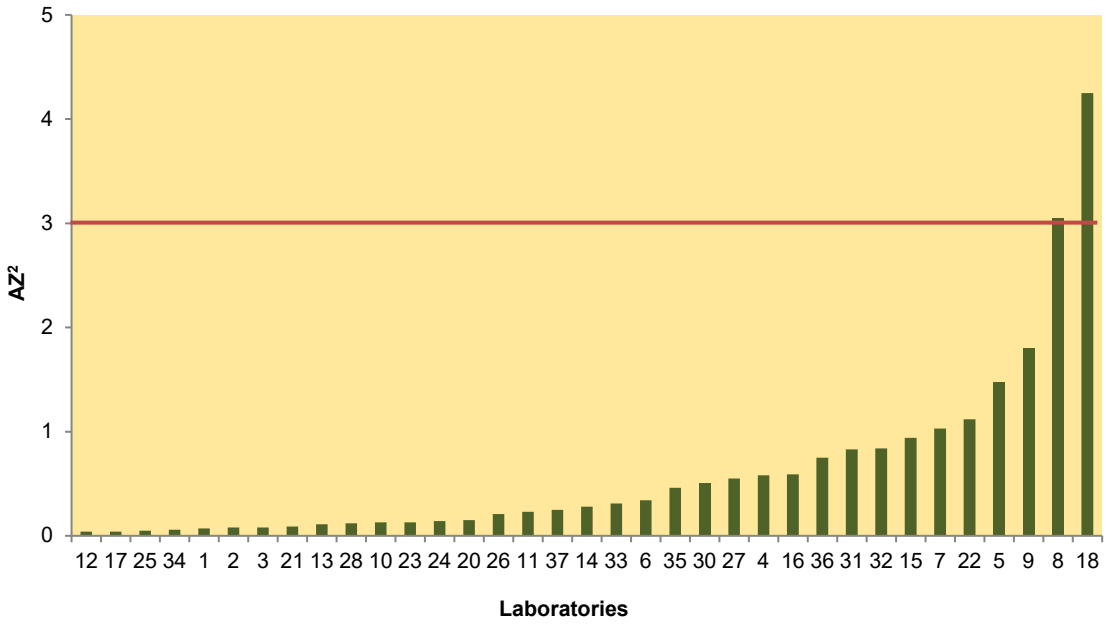


Figure 12. Global performance of laboratories: AZ^2 values

Respect to the analytical methods applied by participants, the majority of laboratories corresponding to twenty-three participants out of thirty-seven used the QuEChERS methodology or methods based on QuEChERS (21).

The QuEChERS method is a streamlined approach that makes 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).

Eleven 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, alumina cartridge or using combination of different materials as extrelut + silica+C_{18} or PSA+GCB+C_{18} or freezing technique. The amount of the sample test was in the range 0.2-10 g while the final analysis volume was between 0.15 and 10 ml.

In the analysis of pesticide residues, the laboratories use multiresidue method because of the large number of analytes enclosed in official plans.

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

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

COMPARISON RESULTS BETWEEN COIPT-21 AND AO-PT1

The regular participation to PTs is an effective tool for laboratories to monitor their performances in the pesticide residue analysis

Therefore, it was recognized interesting to compare the results of the COIPT-21 with another PT organized in 2021 on sunflower seed oil named AO-PT1 organized within the financial project of the Italian Ministry of Health and described in the *Rapporto ISTISAN* 22/14 (23)

Analysis of pesticide residues in food is usually carried out by using Multi-Residue Methods (MRMs) (24-26). This is a consequence of the large number of compounds enclosed in the pesticide target list of the official controls.

Generally, the performance of the participating laboratories is focused on their z-scores as reported by Cortex *et al*. and Andin *et al.* (27, 28).

The full evaluation of the participants performance in the two PTs is discussed in Figures 13 and 14.

Figure 13 concerns the z-score values obtained by both COIPT-21 and AO-PT1 participants distributed following the z-score classification. The 97% of the z-score values obtained were classified as acceptable in the range of +2 to –2 (see Statistical evaluation of results).

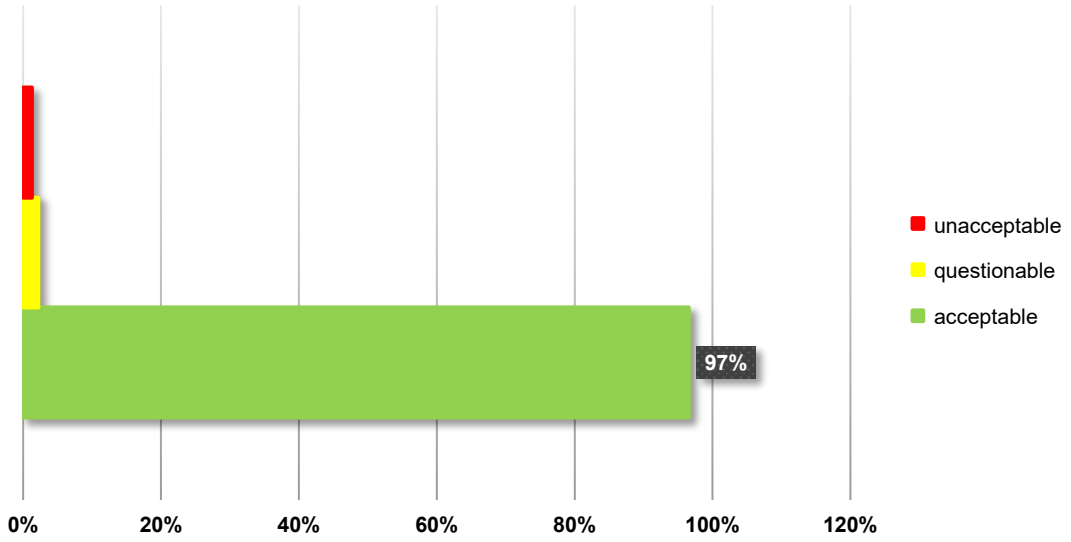


Figure 13. z-scores values of COIPT-21 and AO-PT1 clustered according z-score Classificaton

The same z-score values presented in Figure 13 are shown as single values in a control chart with the corresponding frequency histogram in Figure 14.

In both graphical representations is shown the good performance obtained by the laboratories, considering that out of 317 total z-score values the percentage of 88% resulted in the range of +1 to –1 as absolute value.

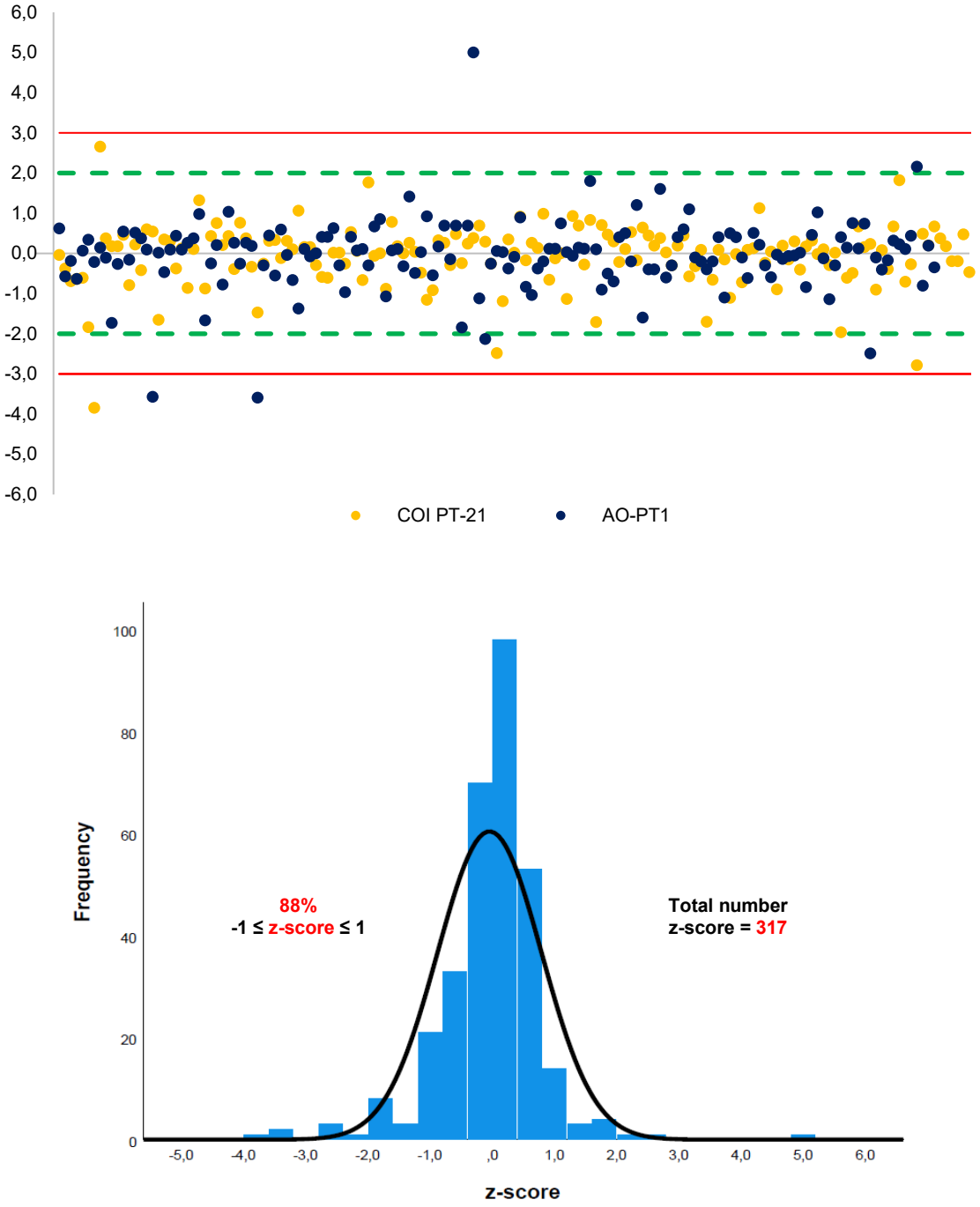


Figure 14. Control chart an frequency histogram of z-scores supplied by participants in COIPT-21 and AO-PT1

CONCLUSIONS

The outcome of the COIPT-21 can be considered satisfactory from several point of view.

One is the good participation of laboratories. Thirty-seven laboratories: three NRLs, eighteen official control laboratories and sixteen 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.

Z-score classification was not assigned for Carbendazim because this compound did not pass the stability and the homogeneity tests.

Moreover, the global performance (AZ^2 scores), assessed only for laboratories that achieved the *sufficient scope,* was proper.

By supplied data, thirty-three laboratories obtained a satisfactory performance for all tested compounds.

Regarding the methodologies used in this PT, the analyses 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.

REFERENCES

1. Europe. Regulation (EU) 2017/625 of the European Parliament and the Council of 15 march 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products. *Official Journal of the European Union* L95/1, 7 April 2017.

2. ISO/IEC 17025. *General requirements for the competence of testing and calibration laboratories.* Geneva: International Organization for Standardization; 2005.upgradetd 2018

3. European Commission - Directorate-General for Health and Food Safety. G*uidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.* Brussels: European Commission; 2021. (SANTE/11312/2021).

4. IOC. *Huiles d'olive – Olive oils*. *Mondial production.* Madrid: International Olive Council; 2022. Available from: https://www.internationaloliveoil.org/wp-content/uploads/2022/12/HO-W901-13-12-2022-P.pdf; last visited July 2023.

5. IOC. *Huiles d'olive – Olive oils*. *European production.* Madrid: International Olive Council; 2022. Available from: https://www.internationaloliveoil.org/wp-content/uploads/2022/12/HO-CE901-13-12-2022-P.pdf; last visited July 2023.

6. Fabiani R, Rosignoli P. Olio d'oliva e salute. In: Conte L, Servili M (Ed.). *Oleum. Qualità, tecnologia e sostenibilità degli oli da olive*. Milano: Edagricole 2022. p. 295-306

7. Europe. Regulation (EC) NO 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. *Official Journal of the European Union* L70/1, 16 March 2005.

8. Europe. Commission implementing Regulation (EU) 2020/585 of 28 April 2020 concerning a coordinated multiannual control program of the Union for 2021, 2022 and 2023 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin. *Official Journal of the European Union* L 135/1, 29 April 2020.

9. European Commission - Directorate-General for Health and Food Safety. *Information note on Article 20 of Regulation (EC) No 396/2005 as regards processing factors, processed and composite food and feed.* Brussels: European Commission (SANTE/ 10704/2021).

10. ISO 17043:2010 *Conformity Assessment – General requirements for proficiency testing* Geneva: International Organization for Standardization; 2010.

11. ISO 13528:2015 *Statistical methods for use in proficiency testing by interlaboratory comparison*. Geneva: International Organization for Standardization; 2015.

12. Europe. Commission Regulation (EU) No 559/2011 of 7 June 2011 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 captan, carbendazim, cyromazine, ethephon, fenamiphos, thiophanate-methyl, triasulfuron and triticonazole in or on certain products. *Official Journal of the European Union* L152/1, 11 November 2011.

13. Europe. Commission Regulation (EU) No 2020/1085 of 23 July 2020 amending Annexes II and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for chlorpyrifos and chlorpyrifos-methyl in or on certain products. *Official Journal of the European Union* L239/7, 24 July 2020.

14. Europe. Commission Regulation (EU) No 310/2011 of 28 March 2011 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 aldicarb, bromopropylate, chlorfenvinphos, endosulfan, EPTC, ethion, fenthion, fomesafen, methabenzthiazuron, methidathion, simazine, tetradifon and triforine in or on certain products. *Official Journal of the European Union* L 86/1, 1 April 2011.

15. Europe. Commission Regulation (EU) 2022/1321 of 25 July 2022 amending Annexes II, III, IV and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for fluoride ion, oxyfluorfen, pyroxsulam, quinmerac and sulfuryl fluoride in or on certain products. *Official Journal of the European Union* L 200/1, 29 July 2022.

16. Europe. Commission Regulation (EU) 2018/1514 of 10 October 2018 amending Annexes II, III and IV to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for abamectin, acibenzolar-S-methyl, clopyralid, emamectin, fenhexamid, fenpyrazamine, fluazifop-P, isofetamid, Pasteuria nishizawae Pn1, talc E553B and tebuconazole in or on certain products. *Official Journal of the European Union* L 256/8, 12 October 2018.

17. Europe. Commission Regulation (EU) 2019/1791 of 17 October 2019 amending Annexes II, III and IV to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 1-decanol, 2,4-D, ABE-IT 56, cyprodinil, dimethenamid, fatty alcohols, florpyrauxifen-benzyl, fludioxonil, fluopyram, mepiquat, pendimethalin, picolinafen, pyraflufen-ethyl, pyridaben, S-abscisic acid and trifloxystrobin in or on certain products. *Official Journal of the European Union* L 277/1, 29 October 2019.

18. European Reference Laboratories for Residues of Pesticides. *General protocol for EU Proficiency Tests on pesticide residues in food and feed*. Edition 9. Brussels: European Commission; 2019. Available from: http://www.eurl-pesticides.eu/library/docs/allcrl/General_Protocol_V9_2020.pdf.

19. Medina-Pastor P, Rodriguez-Torreblanca C, Andersson A, Fernandez-Alba AR. European Commission proficiency tests for pesticide residues in fruits and vegetables. *Trends Anal Chem* 2010;29(1):70-83.

20. Medina-Pastor P, Mezcua M, Rodriguez-Torreblanca C, Fernandez-Alba AR. Laboratory assessment by combined z-score values in proficiency tests: experience gained through the European Union proficiency tests for pesticide residues in fruits and vegetables. *Anal Bioanal Chem* 2010; 397:3061-70.

21. UNI EN 15662:2018. *Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method*. Milano: Ente Nazionale Italiano di Unificazione; 2018.

22. UNI CEN/TS 17062. *Foods of plant origin – Multimethod for the determination of pesticide residues in vegetable oils by LC-MS/MS (QuOil).* Milano: Ente Nazionale Italiano di Unificazione; 2019.

23. Generali T, Stefanelli P, Picardo V, Girolimetti S, Attard Barbini D. *Results of the proficiency test on pesticide residues in sunflower seed oil.* Roma: Istituto Superiore di Sanità; 2022. (Rapporti ISTISAN 22/14).

24. Motohashi N, Nagashima H, Párkányi C, Subrahmanyam B, Zhang GW. Official multiresidue methodsof pesticide analysis in vegetables, fruits and soil. *J Chromatogr A* 1996;754(1-2):333-46.

25. Stefanelli P, Santilio A, Cataldi L, Dommarco R. Multiresidue analysis of organochlorine and pyrethroid pesticides in ground beef meat by gas chromatography-mass spectrometry. *J Environ Sci Health, Part B* 2009;44:350-6.

26. Rajski Ł, Lozano A, Belmonte-Valles N, Uclés A, Uclés S, Mezcua M, Fernandez-Alba AR. Comparison of three multiresidue methods to analyse pesticides in green tea with liquid and gas chromatography/tandem mass spectrometry. *Analyst* 2013;138(3):921-31.

27. Cortez L, Duarte A, Hundewadt A, Schmidt A, Steffen B, Tholen D, Fostel H, Papadakis I, del Monte MG, Boley N, van Berkel PM. How to interpret information from proficiency test exercises concerning the relative performance of accredited laboratories. *Accred Qual Assur* 2003;8:511-3.

28. Antin L, Armishaw P. Aspects of proficiency testing studies of traces elements in environmental samples with a focus on laboratory performance. *Accred Qual Assur* 2010;15:467-71.

APPENDIX A
List of participants

The participants in COIPT-21 are listed below:

BELGIUM
Primoris Belgium (Zwijnaarde)
GERMANY
Eurofins Sofia GmbH (Berlin)
Eurofins Dr. Specht Laboratorien GmbH (Hamburg)
Institut Kirchoff Berlin GmbH (Berlin)
Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Lebensmittel und Veterinärinstitut Oldenburg (Oldenburg)
GREECE
CADMION (Kiato)
Chemicotecniki Lagouvardou-Spantidaki O.E. (Rethymno, Crete)
Food Allergens Laboratory (Nea Ionia)
General Chemical State Laboratory, Pesticide Residues Laboratory, D Chemical Division (Athens)
SKYLAB – Med S.A. (Athens)
IRELAND
Pesticide Control Laboratory, Department of Agriculture Food and Marine (Kildare)
ITALY
Agro.biolab Laboratory srl (Rutigliano, Bari)
Analytical srl (Firenze)
APPA Bolzano, Settore Laboratorio (Bolzano)
ARPA Emilia Romagna Area Fitofarmaci (Ferrara)
ARPA Friuli Venezia Giulia (Udine)
ARPAL La Spezia (La Spezia)
ARPA Puglia, Polo di Specializzazione “Alimenti” (Bari)
ATS Milano (Milano)
ATS Bergamo (Bergamo)
CHEMISERVICE srl (Monopoli, BA)
ICQRF, Laboratorio di Catania (Catania)
INNOVHUB-SSI, Divisione SSOG (Milano)
Istituto Superiore di Sanità, Dipartimento Ambiente e Salute (Roma)
IZS dell’Abruzzo e Molise (Teramo)
IZSLER (Brescia)
IZSLT (Roma)
IZS Piemonte, Liguria e Valle d’Aosta (Cuneo)
IZS della Sicilia (Palermo)
LABCAM srl (Albenga, Savona)
PH TUV SUD (Firenze)
USL Toscana Centro (Firenze)
Water e Life Lab srl (Bergamo)

POLAND
Voievodship Sanitary-Epidemiological Station in Rzeszow (Rzeszow)
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:

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

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

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:

$$\delta = 1.5\, s^* \qquad [3]$$

For each x_i ($i = 1, 2, \ldots p$), calculate:

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

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

$$x^* = \sum_{i=1}^{p} \frac{x_i^*}{p} \qquad [5]$$

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

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^*).

Serie Rapporti ISTISAN
numero di agosto 2023, 1° Suppl.

Stampato in proprio
Servizio Comunicazione Scientifica – Istituto Superiore di Sanità

Roma, settembre 2023