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Proficiency test on pesticide residues in olive oil in 2020

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AMBIENTE E SALUTE

ISTITUTO SUPERIORE DI SANITÀ

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Dipartimento Ambiente e Salute

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2021, v, 41 p. Rapporti ISTISAN 21/17

In 2020, 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 in olive oil named COIPT-20. 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 olive oil sample, chosen from a target list of twenty-eight compounds. Thirty-five participating laboratories submitted results; twenty-seven participants analysed all the seven spiked compounds. The majority of participants obtained a satisfactory performance (z-score) for all tested pesticides.
Key words: National Reference Laboratory; International Olive Council; Pesticide residues; Proficiency Test; Olive oil

Istituto Superiore di Sanità
Risultati del circuito interlaboratorio su residui di antiparassitari in olio di oliva nel 2020.
Tiziana Generali, Patrizia Stefanelli, Valentina Picardo, Silvana Girolimetti, Danilo Attard Barbini
2021, v, 41 p. Rapporti ISTISAN 21/17 (in inglese)

Nel 2020, 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 test di competenza in olio d'oliva chiamato COIPT-20. 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 d'oliva, scelti da una lista prestabilita di ventotto composti. Trentacinque laboratori partecipanti hanno fornito risultati; ventisette 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

L'organizzazione di questo PT è stata realizzata grazie al contributo di un progetto di collaborazione con il Ministero della Salute, Direzione Generale per l'Igiene e la Sicurezza degli alimenti e la nutrizione

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TABLE OF CONTENTS

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

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/12682/2019 (3)

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

GENERAL CONSIDERATION ON MAXIMUM RESIDUE LEVEL IN OLIVE OIL

The olive tree is one of the most important and ancient crops in the Mediterranean area where 95% of the olive oil in the world is produced. Olive oil is one of the major 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 (4).

Spain, Italy, Portugal and Greece are the most representative olive oil exporters from the European Union to other countries. They cover around 70% of global olive oil exports (5).

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 (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 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 Commission website.

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

- 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 (6) 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 0.01mg/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 European Union (7), it is declared that each Member States are requested to report the processing factors used to analyse virgin olive oil samples. Currently in Italy this processing factor is equal to 5.

PROFICIENCY TEST ON OLIVE OIL: THE COIPT-20

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 2020 on olive oil was named COIPT-20.

The exercise consisted in the determination of six different pesticides in an olive oil sample spiked with a definite range of concentration (0.050-0.350 mg/kg). These pesticides were chosen from a list of twenty-eight compounds presented in COIPT-20. Announcement that was sent to participant on 6 October 2020. 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-five laboratories agreed to participate in this PT: four NRLs, thirteen official control laboratories and eighteen private laboratories. To assess the performance of the participating laboratories, z-scores are used following the norms of the International Laboratory Accreditation Cooperation (ILAC) and the International Organization for Standardization (ISO) (8, 9).

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 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: Boscalid, Diazinon, Kresoxim-methyl, Phosalone, Procymidone and Trifluralin. Aliquots of 50 g of this spiked oil named COIPT-20 SPIKED OIL were transferred into dark glass bottles as well as aliquots of 50 g of the blank oil named COIPT-20 BLANK OIL. Samples were sealed and stored at ambient temperature before the shipment to participants. Each participant received one COIPT-20 SPIKED OIL sample and one COIPT-20 BLANK OIL sample. The current MRLs for these six pesticides are showed in Table 1 (10-15).

Table 1. Current MRLs for the six pesticides spiked in the blank oil

Compounds	Current EU Regulation	MRL on olive for oil production (mg/kg)
Boscalid	Regulation (EU) 2021/590 Applicable from: 03/05/2021	**0.01***
Diazinon	Regulation (EU) 834/2013 Applicable from: 26/04/2013	**0.02***
Kresoxim-methyl	Regulation (EU) 2020/856 Applicable from: 9/07/2020	**0.2**
Phosalone	Regulation (EU) 2020/1633 Applicable from: 25/05/2021	**0.02***
Procymidone	Regulation (EU) 1096/2014 Applicable from: 7/05/2015	**0.02***
Trifluralin	Regulation (EU) 2015/552 Applicable from: 28/10/2015	**0.01***

***** Limit of analytical determination

Homogeneity and stability test

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

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

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

– Day 1: during the shipment of the samples on 26th November 2020;
– Day 2: after one month by the deadline for reporting results on 23rd January 2021.

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 the target standard deviation used for proficiency assessment. This test demonstrated that any significant decrease in the pesticide levels was showed for the duration of the PT. The individual results are indicated in Table 2.

Table 2. COIPT-20: data (mg/kg) of the stability test

Pesticide	Concentration mg/kg				
	Mean 1 (M1) n=6	**Mean 2 (M2) n=6**	**|M1-M2|**	**σ**	**0.3xσ**
Boscalid	0.315	0.333	-0.018	0.077	0.023
Diazinon	0.271	0.256	0.015	0.059	0.018
Kresoxim-methyl	0.121	0.135	-0.014	0.029	0.009
Phosalone	0.257	0.273	-0.015	0.055	0.017
Procymidone	0.207	0.221	-0.015	0.049	0.015
Trifluralin	0.075	0.080	-0.005	0.016	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| < 0.3xσ**

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

Table 3. Homogeneity results (mg/kg) for COIPT-20

Sample number	Boscalid	Diazinon	Kresoxim-methyl	Phosalone	Procymidone	Trifluralin
75	0.329	0.279	0.150	0.280	0.225	0.074
92	0.321	0.244	0.136	0.257	0.222	0.078
93	0.328	0.247	0.147	0.290	0.213	0.070
94	0.312	0.250	0.142	0.280	0.218	0.072
108	0.323	0.263	0.140	0.290	0.228	0.071
112	0.346	0.270	0.150	0.279	0.220	0.085
115	0.318	0.238	0.158	0.280	0.207	0.074
118	0.320	0.268	0.155	0.282	0.217	0.076
119	0.322	0.290	0.150	0.280	0.217	0.080
121	0.322	0.239	0.135	0.292	0.221	0.076
Mean	0.325	0.259	0.146	0.281	0.219	0.076
SD	0.009	0.018	0.008	0.010	0.006	0.005
σ_{EUPT}	0.077	0.059	0.029	0.055	0.049	0.016
SD/σ_{EUPT}	0.320	0.303	0.269	0.179	0.122	0.283
Critical value	0.3	0.3	0.3	0.3	0.3	0.3
$SD/\sigma_{EUPT} \leq 0.3$	yes	yes	yes	yes	yes	yes

SD Standard Deviation
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

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 either by e-mail or fax along with the details of methodology used.

The samples were sent to participants between 23-27th November 2020. The deadline for results was 13th January 2021.

The final report was dispatched to all participant at the end of March 2021.

Statistical evaluation of results

The organiser of this PT decided to use the z-score parameter to evaluate the laboratory by the formula performance for each compound using the same model of the PTs carried out by the European Reference Laboratories (EURLs) (16, 17) 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:

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

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 (18) 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:

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

The formula is the sum of the z-score value, multiplied by itself [ω (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.

In this PT, participants were asked to provide voluntary information on their own measurement uncertainty (MU). In particular, about the combined standard uncertainty u based on its own within-laboratory data, the applied coverage factor k and finally the approach to estimate the MU. Only few laboratories answered these requests and in the Tables 4 and 5 are summarized their response.

Table 4. COIPT-20: voluntary information on measurement uncertainty general approaches

Lab code	Measurement uncertainty (MU)	coverage factor *k*
2	Bottom-up approach 41%	2
18	Top-down approach	2
19	Calculated with Horwitz approach	2
21	50% (Sante document)	2
28	Bottom-up approach	2
34	50% (Sante document)	2

Table 5. COIPT-20: voluntary information on measurement uncertainty individual compound data

Lab code	Results (mg/kg)	Measurement uncertainty (MU) (mg/kg)	coverage factor *k*
09			
Boscalid	0.298	21.5	2
Diazinon	0.284	16.9	2
Kresoxim-methyl	0.129	22.5	2
Phosalone	0.225	22.2	2
Procymidone	0.225	21.3	2
Trifluralin	0.078	26.9	2

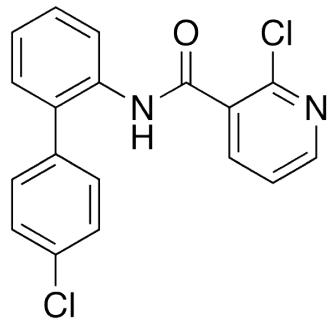
COIPT-20: RESULTS

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). In addition, frequency histograms and Kernel density plots were used to check graphically for normal distribution and to identify multi-modality in the data distributions. All the compound data sets were not normally distributed except for Phosalone. 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.

Boscalid



Common name
boscalid or boscalide

Structure formula $C_{18}H_{12}Cl_2N_2O$

CAS number 188425-85-6

EC no. 606-143-0

Its physical form consists of odourless white crystals with weight molecular of 343.2 g/mol. This compound has good solubility in organic solvents and it is stable to acqueous photolysis and to hydrolysis at pH 4, 5, 7 and 9.
It is a foliar fungicide, with translaminar and acropetal movement within the plant leaf for preventing and curative action.
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.

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

Statistical evaluation of the Boscalid results is presented in Table 6.

From a statistical point of view, the results can be considered satisfactory, since the data used for the assigned value produced median and robust mean that are practically almost the same for Boscalid. The Robust Relative Standard Deviation (Robust RSD) and the uncertainty of the assigned values *u* for Boscalid resulted acceptable.

All z_{EUPT}-score values with recoveries estimated as numerical values are presented in Table 7 and as graphical representation in Figure 2.

Thirty-one laboratories submitted results for Boscalid with good z-score values between 0.1 and 2.0 as absolute values except for the false negative value of -3.8. for lab 31.

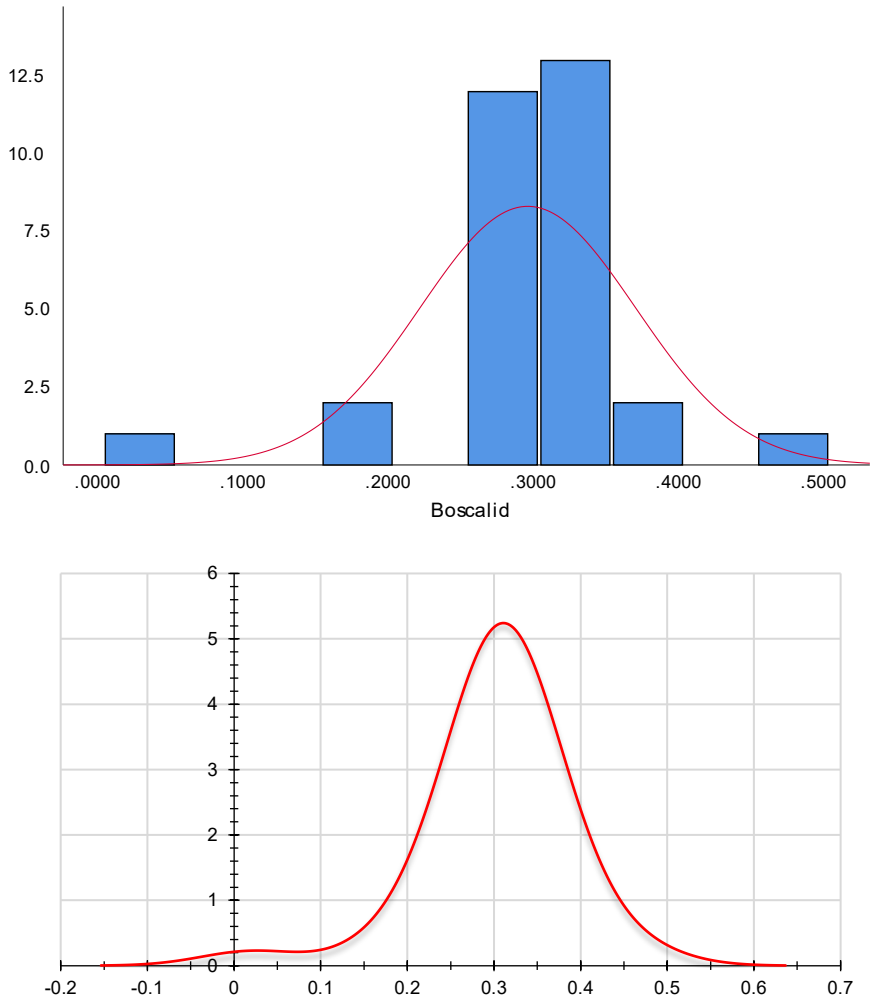


Figure 1. BOSCALID: frequency histogram of the results (mg/kg) and Kernel density plot

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

Parameter	Value
Spiked value	0.312
Mean	0.302
Median	0.313
Robust mean or Assigned value (mg/kg)	0.309
s*	0.044
σ_{EUPT}	0.077
Uncertainty (u) (mg/kg)	0.010
u/σ_{EUPT} *	0.130
FFP RSD (%)	25
Robust RSD (%)	14

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

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

Lab Code	z_{EUPT}-score	Recovery %
1	-0.1	81
2	0.5	105
3	0.1	96
4	0.6	115
5	-0.4	-
6	-0.6	94
7	0.8	111
8	-0.2	-
9	-0.1	99
10	0.6	105
11	1.2	104
12	0.1	107
13	0.6	101
14	0.4	90
15	0.1	70
17	-0.2	93
18	-1.4	105
19	0.1	85
20	-0.5	102
21	0.3	99
22	-1.7	91
23	-0.6	82
24	2.0	84
25	-0.2	88
26	0.3	84
28	-0.2	87
31	-3.8	-
32	0.2	100
33	-0.5	90
34	-0.1	89
35	0.1	-

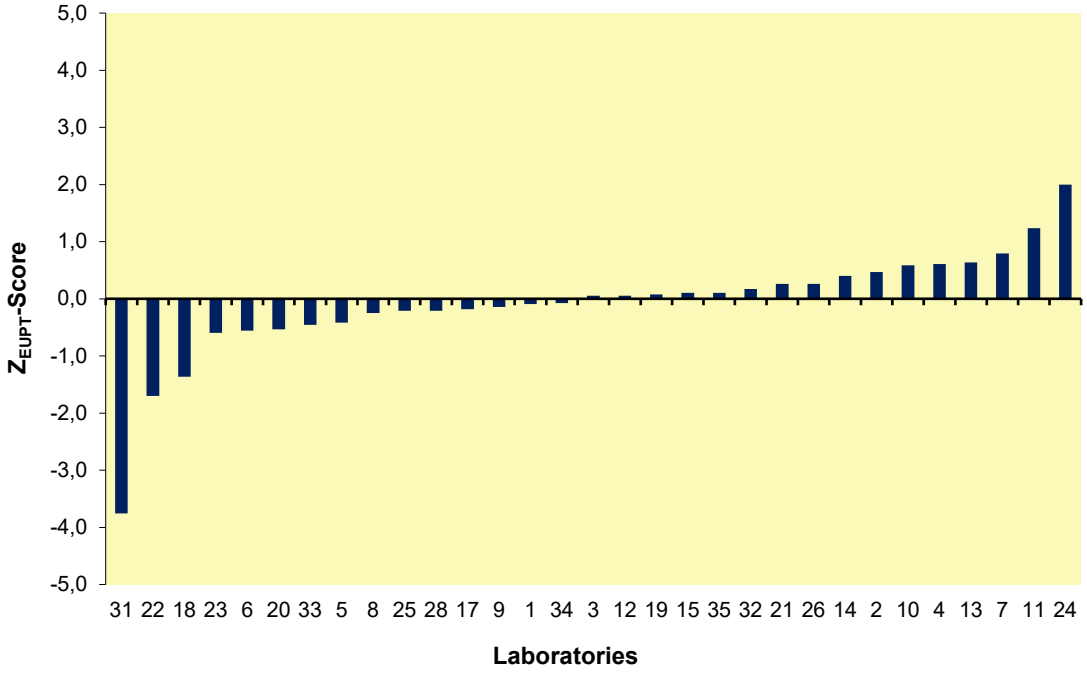
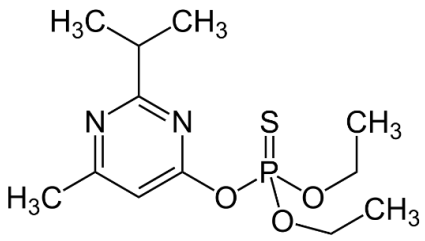


Figure 2. BOSCALID: z-score values (spiked value = 0.312 mg/kg)

Diazinon



Common name
diazinon or dimpylate

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

CAS number 333-41-5

EC no. 206-373-8

It is a thiophosphoric acid ester, faint and colourless to yellow-dark brown liquid with weight molecular of 304.34 g/mol. It is a non-systemic organophosphate insecticide and acaricide with contact, stomach and respiratory action.
This compound is highly soluble in organic solvents and stable only in neutral media, but it is susceptible to oxidation above 100°C and decomposes above 120°C.
Not authorized on olive tree with a MRL value of 0.02 mg/kg on olive as established by the Regulation (EC) 396/2005 that corresponds at limit of analytical determination.
It could be present in olive oil as contaminant as consequence of his liphofilic properties.

In the case of Diazinon also the distribution of submitted data resulted not symmetric as indicated in Figure 3.

Statistical evaluation of the Diazinon results is presented in Table 8.

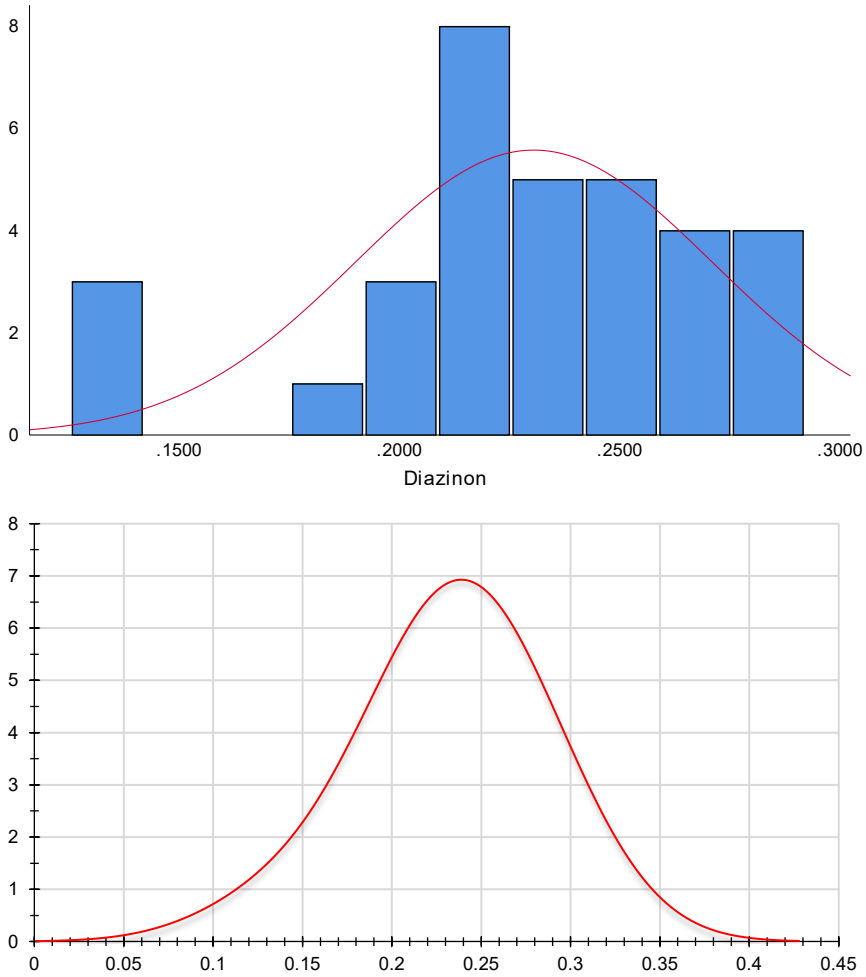


Figure 3. DIAZINON: frequency histogram of the results (mg/kg) and Kernel density plot

Table 8. DIAZINON: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.271
Mean	0.232
Median	0.233
Robust mean or Assigned value (mg/kg)	0.236
s*	0.036
σ_{EUPT}	0.059
Uncertainty (u) (mg/kg)	0.010
u/σ_{EUPT} *	0.170
FFP RSD (%)	25
Robust RSD (%)	15

s*= robust standard deviation
* u/σ_{EUPT}≤ 0.3; RSD: Relative Standard Deviation

Also in this case, submitted results can be considered satisfactory, with Robust Relative Standard Deviation (Robust RSD) and uncertainty of the assigned values *u* acceptable.

All z_{EUPT}-score values with recoveries estimated as numerical values are presented in Table 9 while in Figure 4 are presented in graphical form the z_{EUPT}-scores values listed in the table above.

Table 9. DIAZINON: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
1	-0.2	89
2	0.0	70
3	0.4	95
5	0.2	-
6	-0.5	83
7	-0.4	81
8	0.8	-
9	0.8	95
10	0.5	93
11	0.4	95
12	0.5	90
13	0.0	95
14	0.7	90
15	-1.7	75
16	-0.5	86
17	-0.2	88
18	-1.7	86
19	-0.2	95
20	0.9	103
21	-0.3	84
22	-0.9	74
23	-0.1	88
24	1	81
25	-0.4	89
26	-0.4	83
27	-0.2	90
28	-0.1	85
29	-0.3	81
30	0.3	80
31	-1.6	-
32	0.6	100
33	0.0	80
34	0.1	84
35	0.4	-

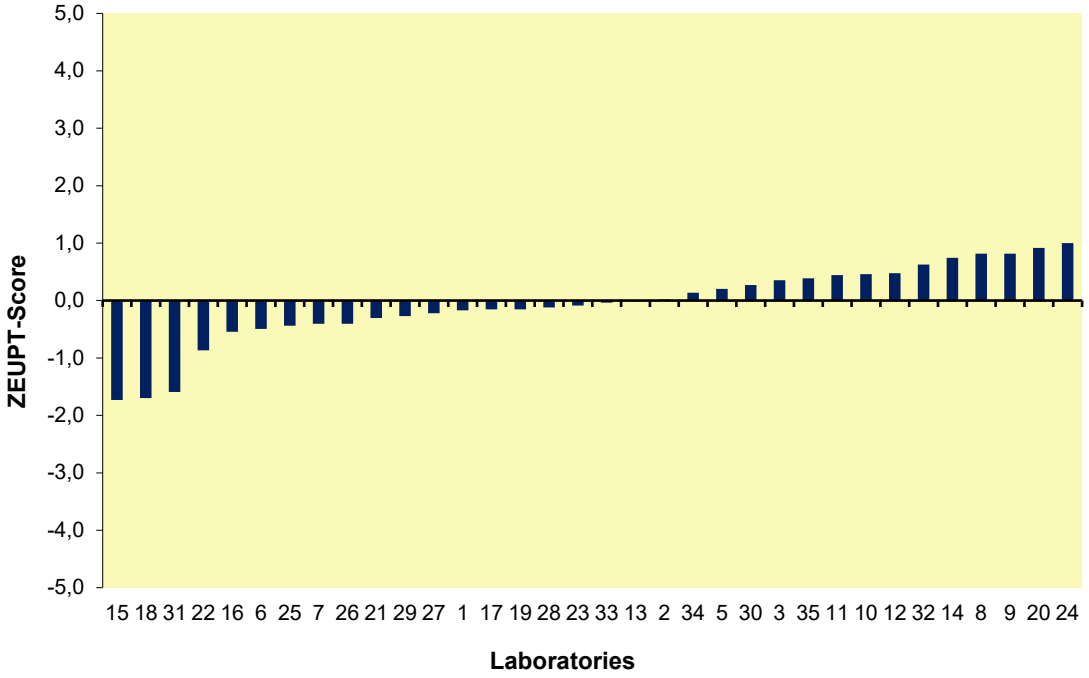
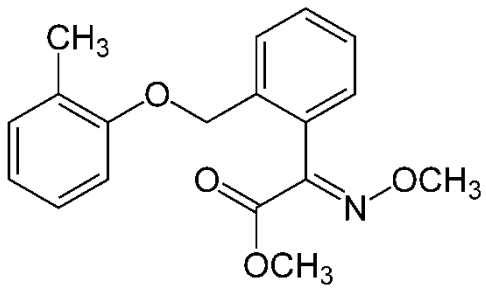


Figure 4. Diazinon: z-score values (spiked value = 0.271 mg/kg)

For Diazinon thirty-four laboratories supplied results with excellent calculated z-score values in the range 0.1-1.7 as absolute values.

Kresoxim-methyl



Common name
kresoxim-methyl or krésoxim-méthyle

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

CAS number 143390-89-0

EC no. 417-880-0

Its physical form consists of odorless or mildly aromatic, white or colourless solid crystals with weight molecular of 313.4 g/mol. It is a carboxylic ester with the function of long lasting, protective, curative fungicide through the inhibition of mithocondrial respiration. It has good solubility in organic solvent and it is relatively stable at pH 5, but it hydrolyses in alkaline media.

Authorized on olive tree with a MRL value of 0.2 mg/kg on olive as established by the Regulation (EC) 396/2005.

Four formulations of PPP type WG (Water dispersible Granules) containing Kresoxim-methyl are authorized in Italy.

Figure 5 shows the results of Kresoxim-methyl (mg/kg) submitted by all laboratories in the COIPT-20. The distribution of the results is clearly not symmetric.

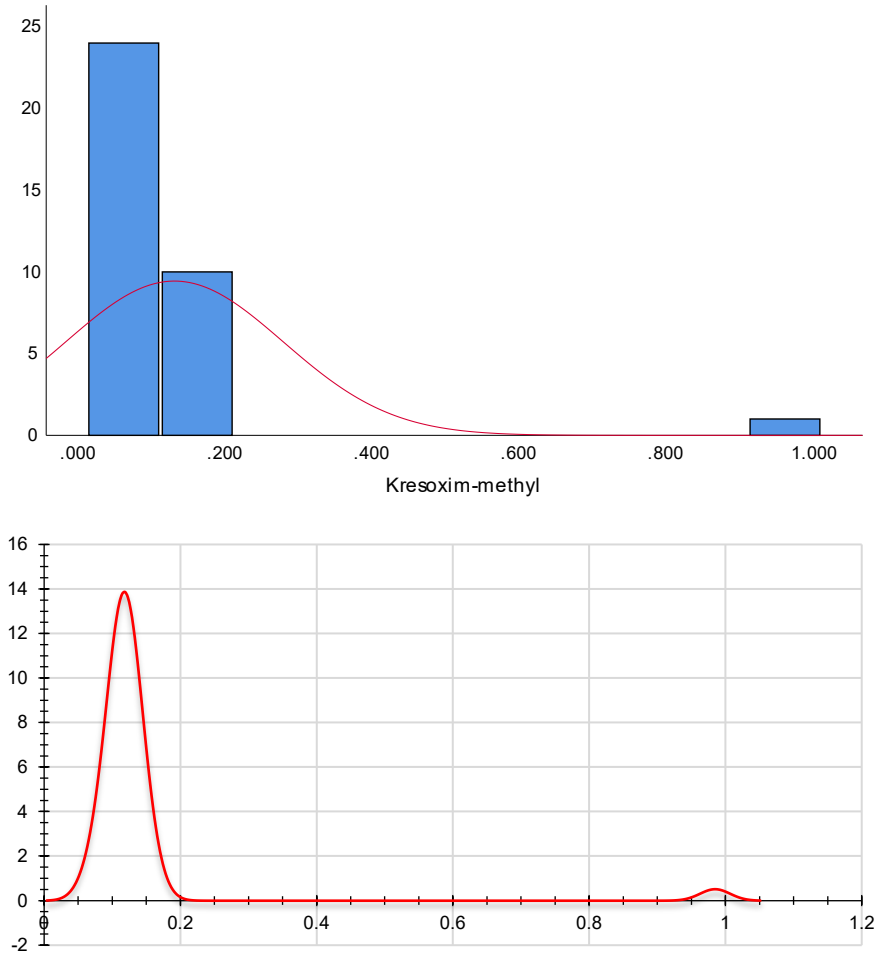


Figure 5. KRESOXIM-METHYL: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of the Kresoxim-methyl results is presented in Table 10.

Table 10. KRESOXIM-METHYL: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.119
Mean	0.141
Median	0.120
Robust mean or Assigned value (mg/kg)	0.118
s*	0.017
σ_{EUPT}	0.029
Uncertainty (u) (mg/kg)	0.004
u/σ_{EUPT} *	0.140
FFP RSD (%)	105
Robust RSD (%)	15

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

The supplied results for Kresoxim-methyl can be considered good with a Robust Relative Standard Deviation (Robust RSD) value of 15% together with the uncertainty value of 0.004.

All z_{EUPT}-score values with recoveries estimated as numerical values are presented in Table 11 and as graphical representation in Figure 6.

Table 11. KRESOXIM-METHYL: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
1	0.1	95
2	0.3	107
3	0.0	103
4	0.8	115
5	-0.6	-
6	-0.4	101
7	0.0	101
8	-0.3	-
9	0.4	99
10	0.7	95
11	0.6	120
12	0.2	93
13	0.6	95
14	-0.3	90
15	-1.3	87
16	1.3	100
17	-0.4	102
18	-1.2	112
19	1.0	98
20	0.1	102
21	0.4	105
22	-0.9	88
23	-0.1	83
24	-0.3	91
25	-0.6	104
26	0.1	-
27	-0.1	94
28	0.1	90
29	-1.7	89
30	-0.2	83
31	5.0*	-
32	-0.3	100
33	-0.5	89
34	0.1	94
35	0.1	-

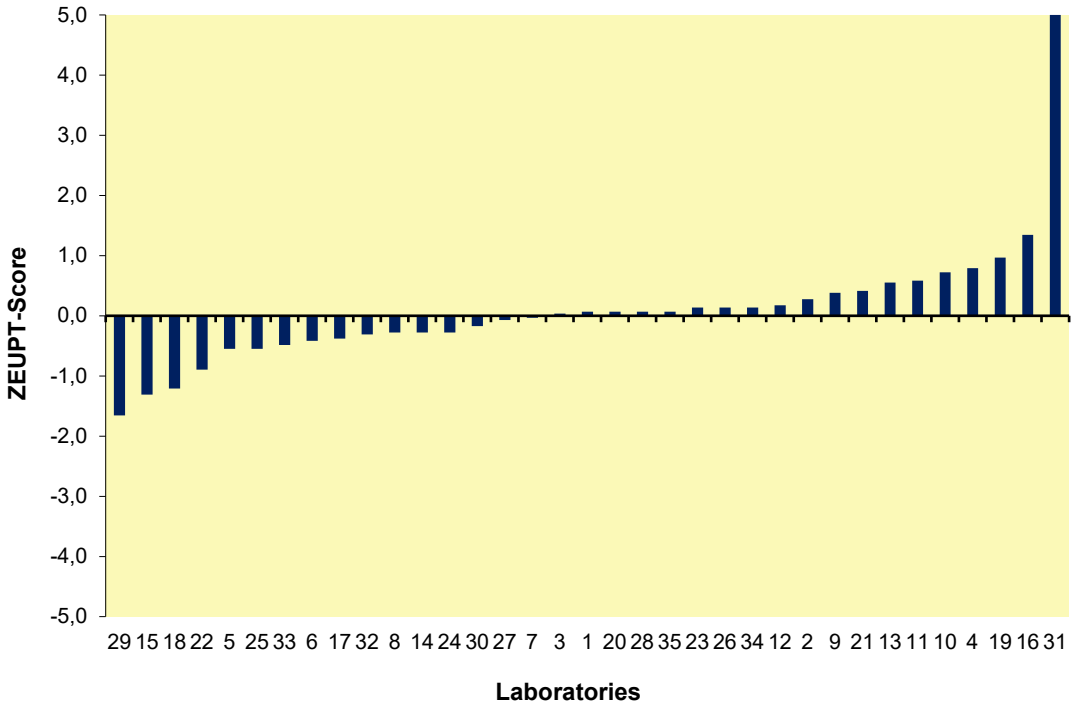
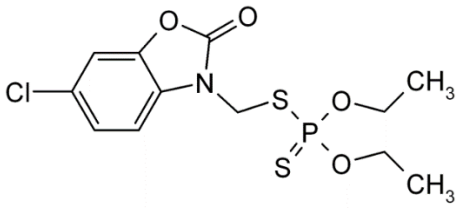


Figure 6. KRESOXIM-METHYL: z-score values (spiked value = 0.119 mg/kg)

Kresoxim-methyl was the most analysed compound with excellent calculated z-score values in the range 0.0-1.7 as absolute value, except for Laboratory 31 with an unacceptable z-score value of 5.

Phosalone



Common name
phosalone or benzofos

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

CAS number 2310-17-0

EC no. 218-996-2

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

Figure 7 shows the results as frequency histogram together with the kernel density plot of Phosalone (mg/kg). In the case of Phosalone the distribution of the results is symmetric. Statistical evaluation of Phosalone results is presented in Table 12.

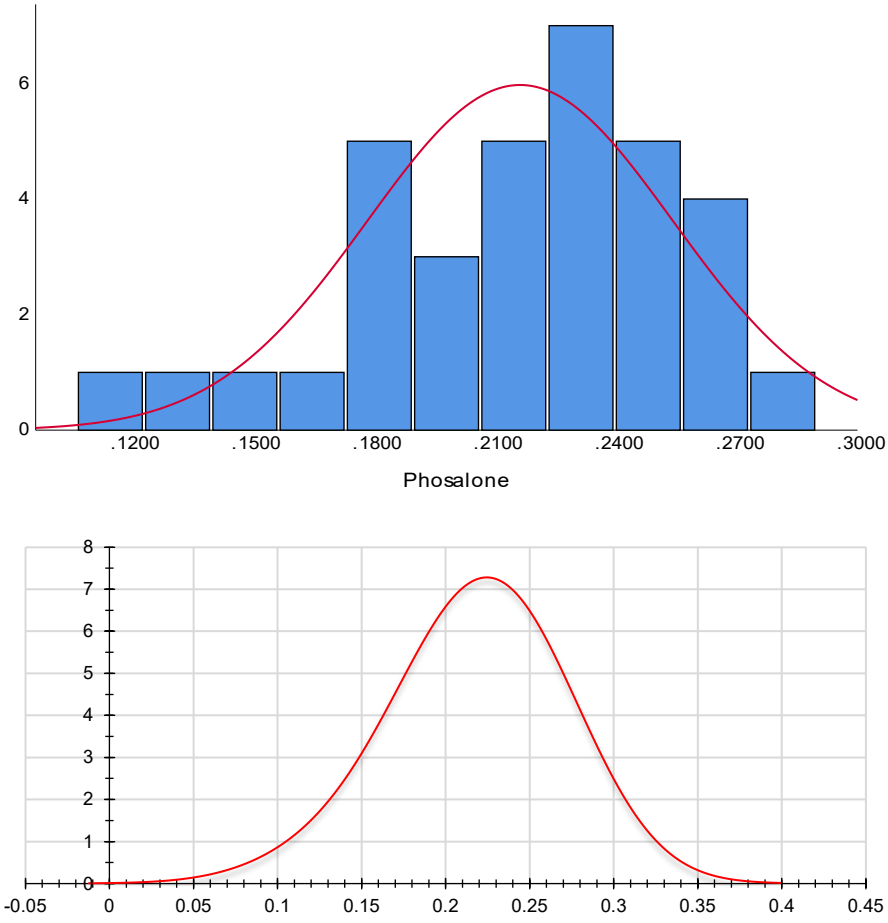


Figure 7. PHOSALONE: frequency histogram of the results (mg/kg) and Kernel density plot

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

Parameter	Value
Spiked value	0.254
Mean	0.218
Median	0.225
Robust mean or Assigned value (mg/kg)	0.221
s*	0.037
σ_{EUPT}	0.055
Uncertainty (u) (mg/kg)	0.008
u/σ_{EUPT} *	0.145
FFP RSD (%)	17
Robust RSD (%)	17

s*= robust standard deviation
* u/σ_{EUPT}≤ 0.3; RSD: Relative Standard Deviation

Statistically results for Phosalone can be considered satisfactory.

The median and the robust mean in Table 12 are similar with a good value for Robust RSD of 17% as the uncertainty equal to 0.008 mg/kg.

All z_{EUPT}-score values with recoveries estimated as numerical values are presented in Table 13 and as graphical representation in Figure 8.

Table 13. PHOSALONE: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
1	-0.1	76
2	1.0	103
3	0.3	97
4	0.7	107
5	-0.6	81
6	-0.2	99
7	0.1	98
8	-0.6	-
9	0.1	101
10	0.7	104
11	0.0	54
12	0.2	106
13	0.8	97
14	0.5	90
15	-1.2	93
16	-0.3	79
17	0.1	96
18	-0.1	106
19	0.7	98
20	0.3	88
21	-0.6	84
22	-0.7	85
23	-0.5	81
24	0.4	86
25	0.6	99
26	0.1	87
27	-2.0	84
28	-0.4	87
29	-1.5	85
30	0.4	92
31	0.9	-
33	-0.1	80
34	0.2	85
35	-0.2	-

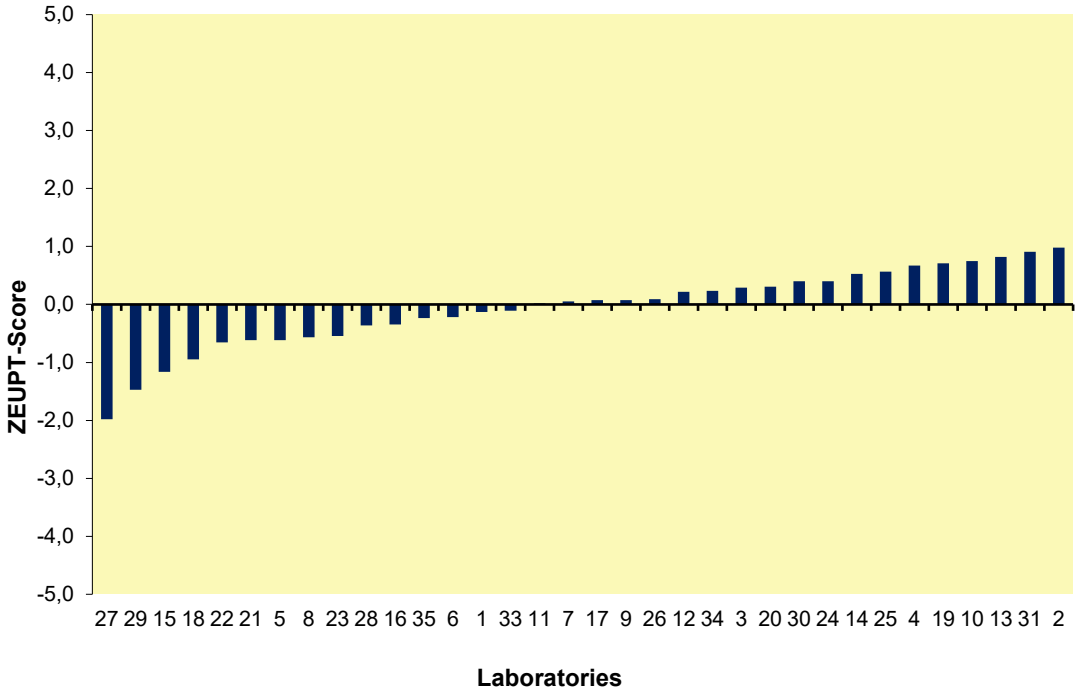
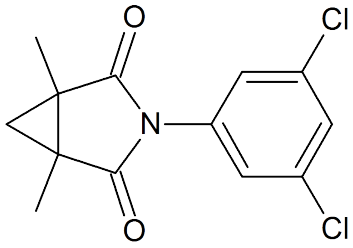


Figure 8. PHOSALONE: z-score values (spiked value = 0.254 mg/kg)

Phosalone was analysed by thirty-four laboratories out of thirty-five with excellent calculated z-score values all in the range 0.0-2.0.

Procymidone



Common name
procymidone

Structure formula $C_{13}H_{11}Cl_2NO_2$

CAS number 32809-16-8

EC no. 251-233-1

This compound presents colourless crystals or a light brown solid with weight molecular of 284.14 g/mol.
It is a moderate systemic fungicide and an endocrine distruptor, soluble in organic solvents and stable both under normal storage conditions and to light, heat and moisture.
Not authorized in Italy on olive tree with a MRL value of 0.02 mg/kg on olive as established by the Regulation (EC) 396/2005 that correspond at limit of analytical determination.
It could be present in olive oil as contaminant as consequence of his liphofilic properties.

Figure 9 shows the results of Procymidone (mg/kg) submitted by all laboratories expressed as frequency histogram. The distribution of data resulted not symmetric.

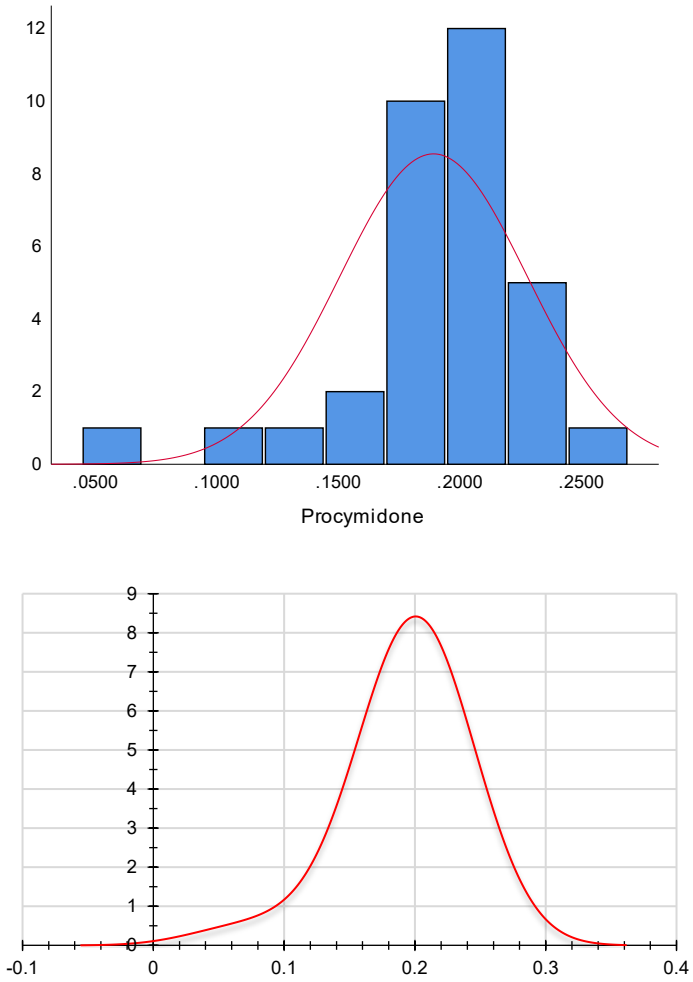


Figure 9. PROCYMIDONE: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of Procymidone results is presented in Table 14.

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

Parameter	Value
Spiked value	0.215
Mean	0.193
Median	0.201
Robust mean or Assigned value (mg/kg)	0.198
s*	0.028
σ_{EUPT}	0.049
Uncertainty (u) (mg/kg)	0.006
u/σ_{EUPT} *	0.122
FFP RSD (%)	20
Robust RSD (%)	14

s*= robust standard deviation
* u/σ_{EUPT}≤ 0.3; RSD: Relative Standard Deviation

Also with regard to Procymidone, the results obtained statistically can be considered satisfactory.

The median and the robust mean in Table 14 are similar with a good value for Robust RSD of 14% as the uncertainty equal to 0.006 mg/kg.

All z_{EUPT}-score values with recoveries estimated as numerical values are presented in Table 15 with the corresponding graphical representation in Figure 10.

Table 15. PROCYMIDONE: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
1	-1.2	80
2	0.8	103
3	-0.3	86
4	-0.3	104
5	-0.3	85
6	0.4	97
7	-0.7	80
8	1.1	-
9	0.6	96
10	0.3	102
11	0.9	59
12	-0.3	85
14	0.4	90
15	-2.9	105
17	-0.3	90
18	-0.4	109
19	-0.2	105
20	0.4	102
21	0.2	95
22	-0.7	81
23	0.2	84
24	0.0	82
25	0.1	102
26	0.1	78
27	0.1	93
28	0.1	93
29	-2.0	85
30	-0.1	78
31	-0.5	
32	0.5	100
33	0.3	86
34	-0.3	84
35	0.6	-

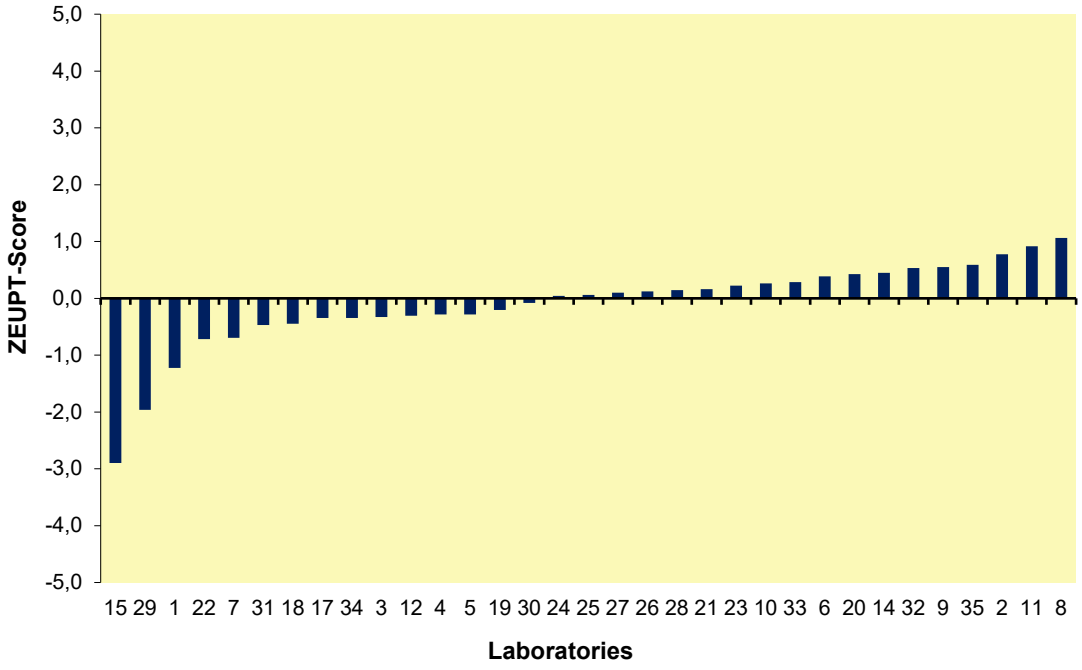
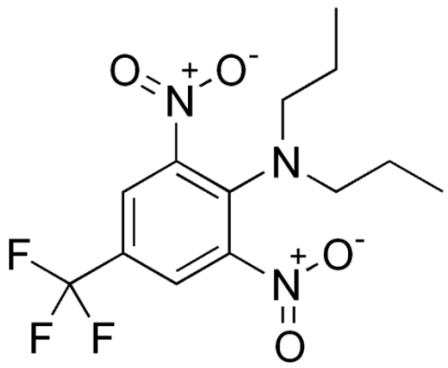


Figure 10. PROCYMIDONE: z-score values (spiked value = 0.087 mg/kg)

Procymidone was determined by thirty-three laboratories out of thirty-five with a range of z-score acceptable values between 0.2-2.0. Only laboratory 15 has obtained a questionable z-score of 2.9.

Trifluralin



Common name
trifluralin or trifluraline

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

CAS number 1582-09-8

EC no. 216-428-8

This compound is a pre-emergence and controller herbicide. Its physical form consists of yellow-orange crystalline solids with no appreciable odor and with weight molecular of 335.3 g/mol. It has a very good solubility in organic solvents; it is stable at 52°C and to hydrolysis at pH 3.6 and 9, but decomposes by uv irradiation.
Not authorized in Italy on olive tree with a MRL value of 0.01 mg/kg on olive as established by the Regulation (EC) 396/2005 that correspond at limit of analytical determination.
It could be present in olive oil as contaminant as consequence of his liphofilic properties.

Figure 11 shows the results of Trifluralin (mg/kg) submitted by all laboratories expressed as frequency histogram. Also for this compound the distribution of data resulted not symmetric.

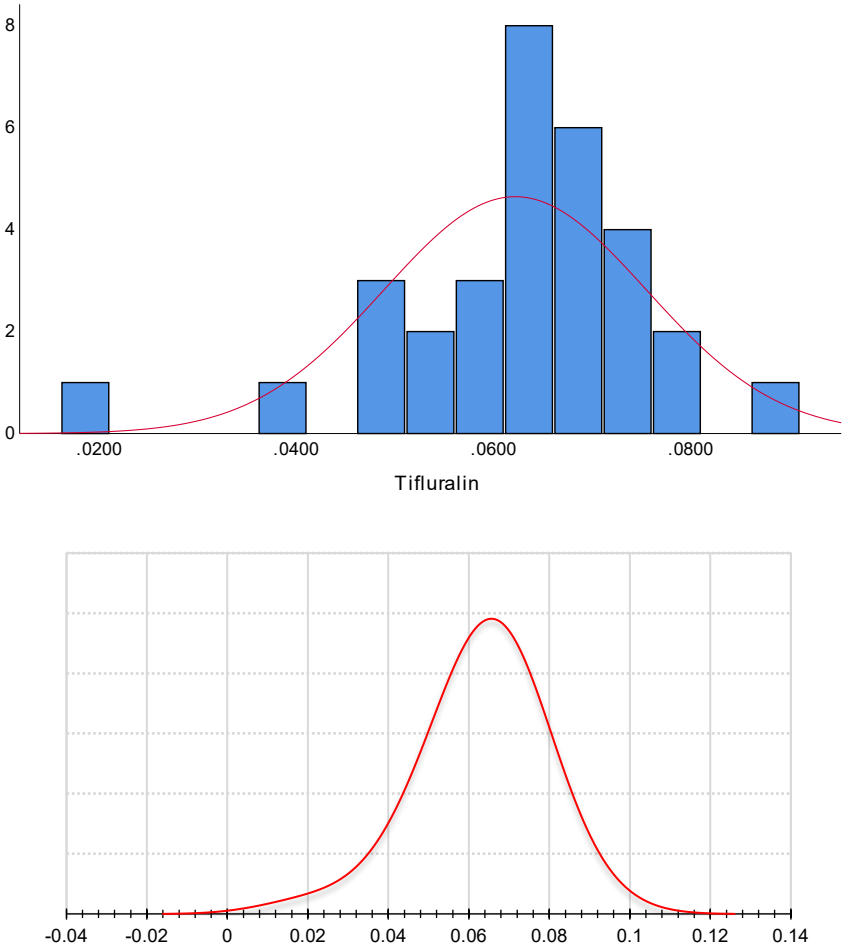


Figure 11. TRIFLURALIN: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of Trifluralin results are presented in Table 16 while in Table 17 are listed all z_{EUPT}-score values with corresponding recoveries estimated.

Table 16. TRIFLURALIN: statistical parameters (mg/kg)

Parameter	Value
Spiked value	0.072
Mean	0.063
Median	0.064
Robust mean or Assigned value (mg/kg)	0.064
s*	0.011
σ_{EUPT}	0.016
Uncertainty (u) (mg/kg)	0.002
u/σ_{EUPT} *	0.125
FFP RSD (%)	21
Robust RSD (%)	17

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

Statistically results for Trifluralin can be considered satisfactory considering that the median and the robust mean presented in Table 16 have the same numerical value.

The Robust RSD of 17% as the uncertainty equal to 0.002 mg/kg are both good.

Table 17. TRIFLURALIN: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery %
2	-0.1	86
3	-0.1	87
4	-1.0	99
5	0.4	-
6	-0.7	88
7	-0.8	80
8	0.7	-
9	0.9	112
10	0.4	94
11	0.6	48
12	-0.1	105
14	0.1	90
17	1.6	91
18	-1.1	83
19	-0.3	102
20	0.8	94
21	-0.3	85
22	-1.6	64
23	0.4	81
24	-0.2	80
25	0.2	98
26	0.3	98
27	0.4	92
28	0.6	87
29	-0.9	73
30	0.8	96
31	-2.8	-
32	0.0	100
33	-0.1	82
34	0.1	82
35	-0.1	-

In Figure 12 are presented in graphical form the z_{EUPT}-scores values of Trifluralin listed in the table above.

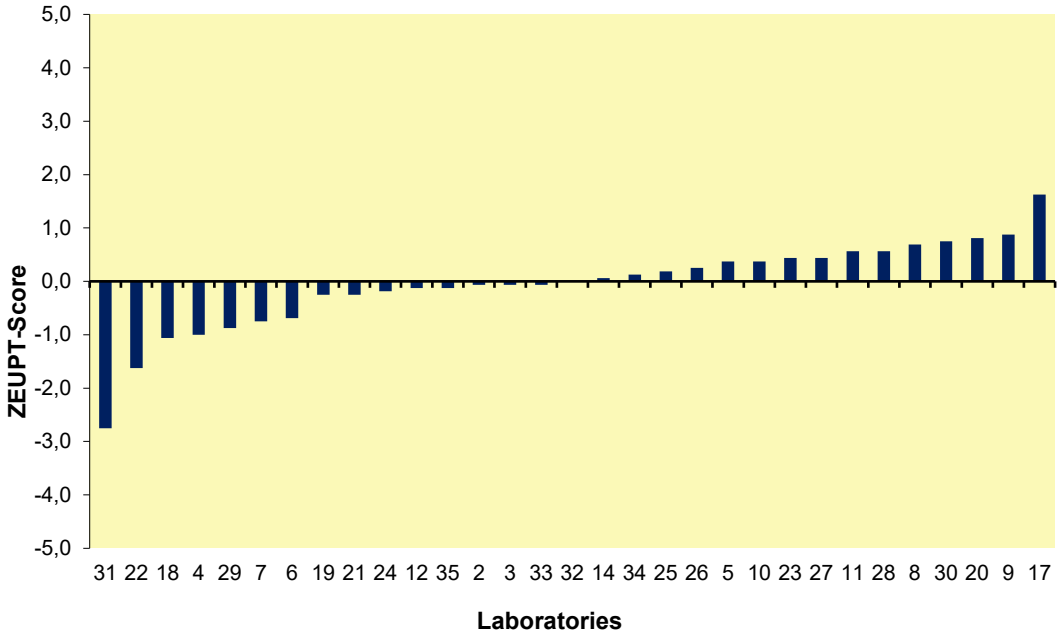


Figure 12. TRIFLURALIN: z-score values (spiked value = 0.072 mg/kg)

Thirty-one laboratories supplied results for Trifluralin with good calculated z-score values in the range 0-1.6 with a questionable z-score of -2.8 calculated for laboratory 31.

CASE STUDY: PHOSALONE

Some considerations must be made regarding the pesticide Phosalone tested in COIPT-19 and COIPT-20. In COIPT-19 this compound was analysed by 37 laboratories out of 40 participants while 3 laboratories, despite having declared it in the method, did not find it in the spiked sample, consequently obtaining 3 false negative values of z-score. For this reason, it was decided to test the pesticide Phosalone also in COIPT-20.

Figure 13 shows the comparison of z-score values in both PTs. For some laboratories, the performance has improved in the COIPT-20. This is a confirmation of the importance for laboratories to participate in these PTs on a regular basis, to improve their performances in the analysis of pesticide residues in olive oil.

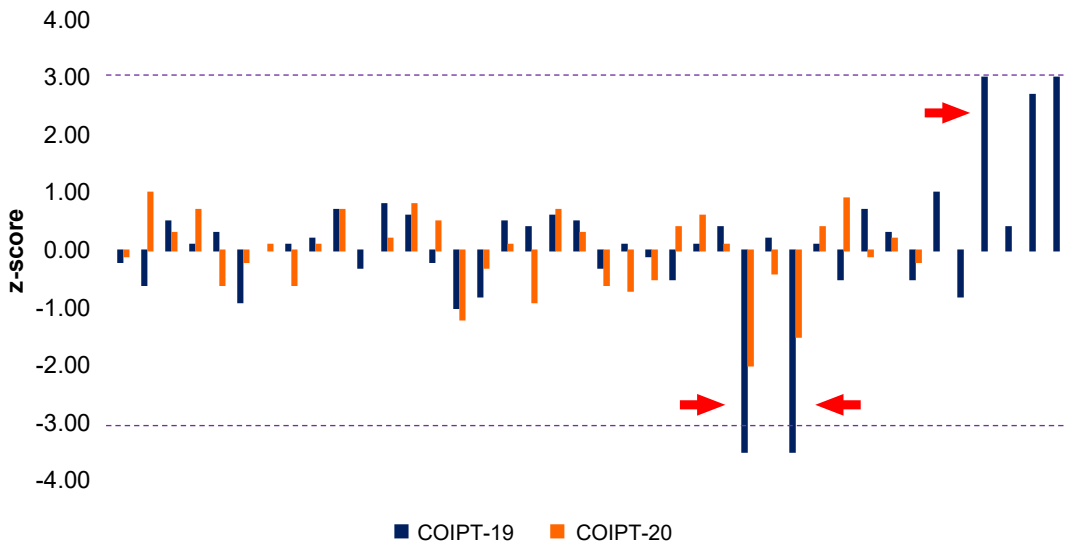


Figure 13. Comparison of Phosalone z-scores data

In general, the negative performance, highlighted in COIPT 19, might be concerned with the limitations in the degree of identification of the employed instrumental techniques, for example selective detectors such as FPD and/or ECD. Nowadays, these selective detectors are less widely used as they offer only limited specificity.

COIPT-20: FINAL COMMENTS

From a statistical point of view, the results for all the six compounds presented can be considered satisfactory, since the data used for the assigned value produced *median and robust mean* that are practically almost the same for each analyte (19).

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

Further the Robust RSD and the uncertainty of the assigned values *u* were presented for all pesticides. The range of Robust RSD values was very good from 14 to 17 % for the six compounds while the range of *u* was from 0.002 to 0.010.

All laboratories submitted results and twenty-six (equal to 74%) analysed all compounds with Kresoxim-methyl that was the compound analysed by all laboratories.

Two false negative values were calculated: both in case of Lab 31 for Boscalid and Trifluralin. 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 13 was an accurate representation of the results of the AZ^2.

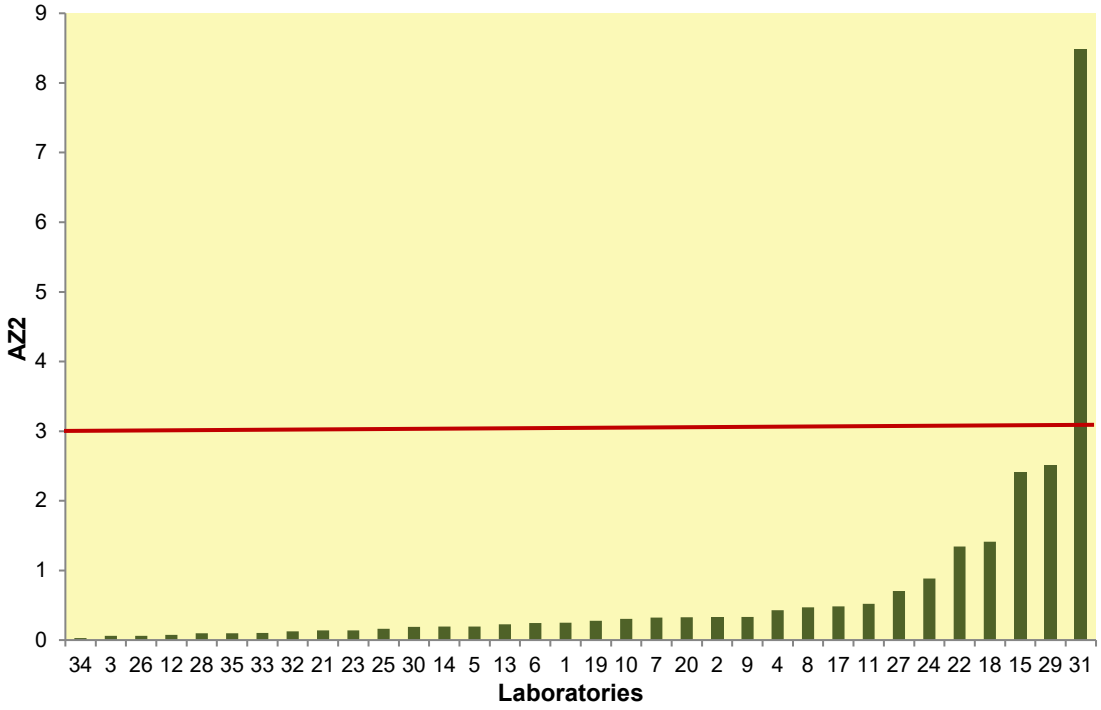


Figure 13. Global performance of laboratories: AZ^2 values

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 (20).

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).

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

One laboratory 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} as SPE 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. Seven laboratories used instead the solvent calibration and two laboratories performed the standard addition procedure.

Figure 14 reports the overall recoveries data submitted by the participants as a control chart.

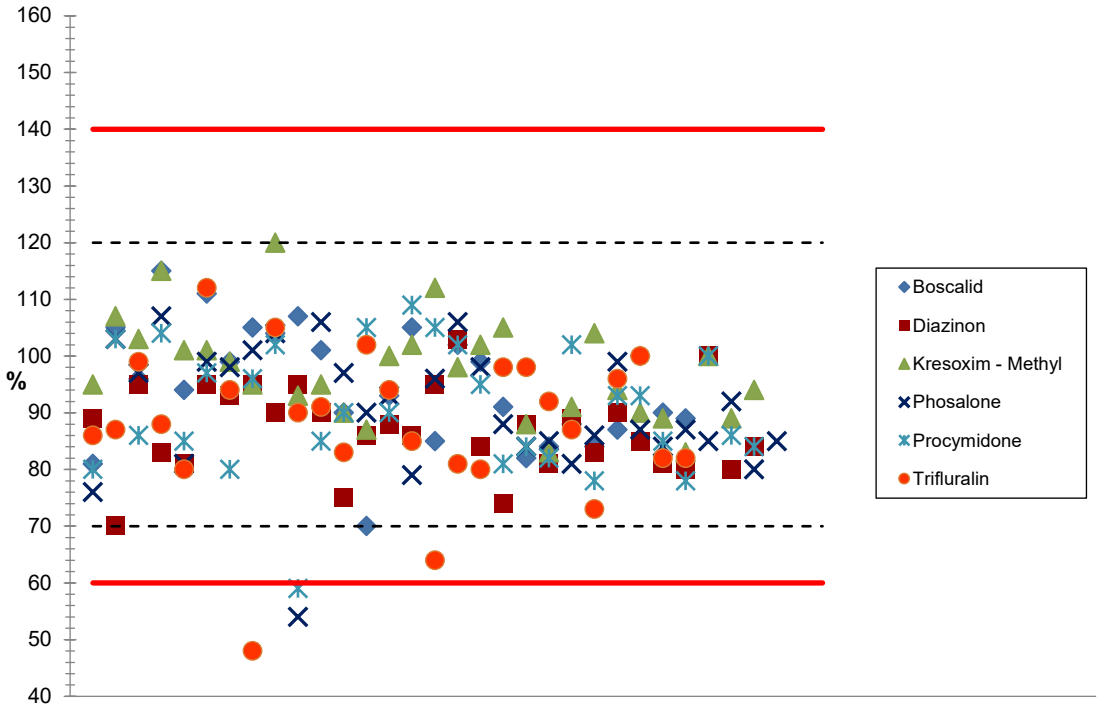


Figure 14. Control chart of the recoveries (%) submitted by the participants

For pesticide residues analysis in food and feed, acceptable limits for a single recovery result should normally be within the generalized range of 60-140%, corresponding to the ± twice acceptance criterion value of the within-laboratory reproducibility (RSD ≤20%); the so-called warming limits are usually located at a distance corresponding to the absolute range 70-120% fixed as acceptance criteria of the mean recovery, in certain cases and typically with multi-residue methods, recoveries outside these range may be acceptable (3). Only few submitted recoveries did not respect these limits.

CONCLUSIONS

The outcome of the COIPT-20 can be considered satisfactory from several point of view.

One is the good participation of laboratories. Thirty-five laboratories: four NRLs, thirteen official control laboratories and eighteen private laboratories. The other regards the performance expressed in terms of z-score.

In fact, the laboratory performance obtained for each tested pesticide was satisfactory by almost all participants.

Moreover, the global performance (AZ^2scores) assessed only for laboratories which 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 analysis for the majority of laboratories were performed according to UNI EN 15662: 2018 multiresidual and 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. Guidance document on analytical quality control and method validation procedures for pesticides residues and analysis in food and feed. Brussels: European Commission; 2019. (SANTE/12682/2019).
4. Gimeno E, Fitó M, Lamuela-Raventós RM, Castellote AI, Covas M, Farré M, de la Torre-Boronat MC, López-Sabater MC. Effect of ingestion of virgin olive oil on human low-density lipopreotein composition. *Eur J Clin Nutr* 2002; 56:114-20.
5. International Olive Council. *Export figures of olive oil in the European Union (EU-27)*. IOC; 07.10.2021.
6. 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.
7. Europe. Commission implementing Regulation (EU) 2019/533 of 28 March 2019 concerning a coordinated multiannual control program of the Union for 2020, 2021 and 2022 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 88/28, 29 March 2019.
8. Thompson M, Ellison SLR, Wood R. The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report). *Pure Appl Chem* 2006;78(1):145-96.
9. ISO 13528:2015. *Statistical methods for use in proficiency testing by interlaboratory comparison*. Geneva: International Organization for Standardization; 2015.
10. Europe. Commission Regulation (EU) 2021/590 of 12 April 2021 amending Annexes II and IV to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum 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 product. *Official Journal of the European Union* L125/15, 13 April 2021.
11. Europe. Commission Regulation (EU) No 834/2013 of 30 August 2013 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 acequinocyl, bixafen, diazinon, difenoconazole, etoxazole, fenhexamid, fludioxonil, isopyrazam, lambda-cyhalothrin, profenofos and prothioconazole in or on certain products. *Official Journal of the European Union* L233/11 del 31 August 2013.
12. Europe. Commission Regulation (EU) 2020/856 of 9 June 2020 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 cyantraniliprole, cyazofamid, cyprodinil, fenpyroximate, fludioxonil, fluxapyroxad, imazalil, isofetamid, kresoxim-methyl, lufenuron, mandipropamid, propamocarb, pyraclostrobin,

pyriofenone, pyriproxyfen and spinetoram in or on certain products. *Official Journal of the European Union* L 195/9 del 19 June 2020.

13. Europe. Commission Regulation (EU) 2020/1633 of 27 October 2020 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 azinphos-methyl, bentazone, dimethomorph, fludioxonil, flufenoxuron, oxadiazon, phosalone, pyraclostrobin, repellants: tall oil and teflubenzuron in or on certain products. *Official Journal of the European Union* L 367/1, 5 November 2020.
14. Europe. Commission Regulation (EU) No 1096/2014 of 15 October 2014 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for carbaryl, procymidone and profenofos in or on certain products *Official Journal of the European Union* L 300/5, 18 October 2014.
15. Europe. Commission Regulation (EU) 2015/552 of 7 April 2015 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 1,3-dichloropropene, bifenox, dimethenamid-P, prohexadione, tolylfluanid and trifluralin in or on certain products. *Official Journal of the European Union* L 92/20, 8 April 2015.
16. European Reference Laboratories for Residues of Pesticides. *General protocol for EU Proficiency Tests on pesticide residues in food and feed*. Edition 5. Brussels: European Commission; 2015. Available from: http://www.eurl-pesticides.eu/library/docs/allcrl/General_Protocol_4_ed_ revised.pdf; last accessed 31/11/17.
17. 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.
18. 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.
19. Ellison S, Barwick V, Duguid Farrant T. *Practical statistics for the analytical scientist: a bench guide.* 2nd Edition. Cambridge: RSC Publishing; 2009.
20. Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residue in products. *J AOAC Int* 2003;86 (2):412-31.
21. UNI EN 15662:2009. *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 - QuEChERS-method*. Milano: Ente Nazionale Italiano di Unificazione; 2009.
22. UNI CEN/TS 17062:2019. *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.

APPENDIX A
List of participants

The participants in COIPT-20 in 2020 are listed below.

BELGIUM
Primoris Belgium (Zwijnaarde)
FRANCE
ITERG (Pessac)
GERMANY
Institut Kirchoff Berlin GmbH (Berlin)
Niedersaechsisches Landesamt Fuer Verbraucherschutz Und Lebensmittelsicherheit Lebensmittel Und Veterinaerinstitut Oldenburg (Oldenburg)
GREECE
Benaki Phytopathological Institute, Pesticide Residue Laboratory (Kiphissia)
CADMION (Kiato Korinthia)
Chemicotecniki Lagouvardou-Spantidaki O.E. Dikonimou Mkri 1
General Chemical State Laboratory, Pesticide Residues Laboratory, D Chemical Division (Athens)
SKYLAB – Med S.A. (Athens)
UNIHER S.A (Iraklion)
IRELAND
Pesticide Control Laboratory, Department of Agriculture Food and Marine (Kildare)
ITALY
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)
ARPAL La Spezia (La Spezia)
ARPA Puglia, Polo di Specializzazione “Alimenti” (Bari)
ATS Milano (Milano)
ATS Bergamo (Bergamo)
CADIR LAB srl (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 Laboratorio Contaminanti Ambientali (Brescia)
IZSLT (Roma)
IZS Piemonte, Liguria e Valle d’Aosta (Cuneo)
LABCAM srl (Albenga, SV)
PH srl (Firenze)
USL Toscana Centro (Firenze)
Water e Life Lab srl (Bergamo)
SPAIN
Aceites Borges Pont Sau (Tàrrega Lléida)
Laboratorio Agroalimentario (Granada)
National Center for technology and food Safety (CNTA)

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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