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Dipartimento Ambiente e Salute

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In 2019, 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-19. 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-six compounds. Forty participating laboratories submitted results thirty-five of which analysed all the six 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 2019.
Tiziana Generali, Patrizia Stefanelli, Silvana Girolimetti, Danilo Attard Barbini
2020, v, 39 p. Rapporti ISTISAN 20/32 (in inglese)

Nel 2019, 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 (NRL-AO) ha organizzato in collaborazione con il Consiglio Oleicolo Internazionale (COI) un nuovo test di competenza per i residui di pesticidi nell'olio d'oliva, chiamato COIPT-19. 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 ventisei composti. Quaranta laboratori partecipanti, trentacinque dei quali hanno analizzato tutti i composti addizionati, hanno fornito risultati. La maggior parte dei partecipanti ha ottenuto una soddisfacente prestazione (z-score) per tutti gli antiparassitari oggetto del test.

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

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ABBREVIATIONS

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
AZ²	Average of the Squared z-scores
CAS	Chemical Abstract Service
EC	European Commission
EU	European Union
EUPT	European Union Proficiency Test
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
UHPLC	Ultra-High Performance Liquid Chromatography

Symbols

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

PREFACE

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

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

Furthermore, in accordance with article 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).

In European Union, Italy, Spain and Greece are the three main olive oil market stakeholders, as reported in the newest data of the International Olive Council. They account for about 70% of the global olive oil production (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 for this reason a contamination of olive fruit and olive oil is likely. 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.

To set any MRL for pesticides applicants – e.g., producers of plant protection products, farmers – 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 experimental data on residues, the daily intake of a certain pesticide through all food is calculated and then compared with:

- 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 transformation factors of processed products, in coordinated multiannual

control programmes of the European Union, 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-19

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 2019 on olive oil was named COIPT-19.

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-six compounds presented in COIPT-19. Announcement that was sent to participant on 19 April 2019. The possible list of compounds includes mainly those considered in the official control plans, with spiked concentration levels around their reference values set in the European Regulations.

Forty laboratories agreed to participate in this PT: six NRLs, eighteen official control laboratories (fourteen were Italian laboratories) and sixteen 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 of different testing procedures on the analytical results, 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 4.8 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 2.5 kg of the blank oil, was spiked with the following pesticides: Buprofezin, Cypermethrin, Dimethoate, Metidathion, Phosalone, Tebuconazole. Aliquots of 50 g of this spiked oil named COIPT-19 SPIKED OIL were transferred into dark glass bottles as well as aliquots of 50 g of the blank oil named COIPT-19 BLANK OIL. Samples were sealed and stored at ambient temperature before the shipment to participants. Each participant received one COIPT-19 SPIKED OIL sample and one COIPT-19 BLANK OIL sample. The current MRLs for these six pesticides are showed in Table 1 (10-14).

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

Compounds	Current EU Regulation	MRL on olive for oil production (mg/kg)
Buprofezin	Regulation (EU) 2019/91 Applicable from: 13/08/2019	**0.01***
Cypermethrin	Regulation (EU) 2017/626 Applicable from: 27/04/2017	**0.05***
Dimethoate	Regulation (EU) 2017/1135 Applicable from: 16/01/2018	**3.0**
Methidathion	Regulation (EU) 310/2011 Applicable from: 21/10/2011	**0.02***
Phosalone	Regulation (EU) 899/2012 Applicable from: 26/04/2013	**0.02***
Tebuconazole	Regulation (EU) 2018/1514 Applicable from: 01/11/2018	**0.5**

***** 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 17th June 2019;
– Day 2: after one month by the deadline for reporting results on 17th September 2019.

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 no 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-19: data (mg/kg) of the stability test in

Pesticide	Concentration mg/kg				
	Mean 1 (M1) n=6	**Mean 2 (M2) n=6**	**|M1-M2|**	**σ**	**0.3xσ**
Buprofezin	0.124	0.120	0.004	0.030	0.009
Cypermethrin	0.247	0.247	0.000	0.058	0.017
Dimethoate	0.231	0.215	0.015	0.050	0.015
Methidathion	0.302	0.324	0.022	0.073	0.022
Phosalone	0.087	0.091	0.004	0.020	0.006
Tebuconazole	0.121	0.130	0.010	0.039	0.012

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-19

Sample number	Buprofezin	Cypermethrin	Dimethoate	Methidathion	Phosalone	Tebuconazole
82	0.117	0.256	0.202	0.304	0.086	0.139
83	0.120	0.248	0.219	0.308	0.086	0.130
86	0.119	0.259	0.200	0.310	0.086	0.150
87	0.120	0.244	0.227	0.318	0.093	0.123
89	0.133	0.230	0.200	0.260	0.080	0.132
90	0.122	0.250	0.206	0.346	0.096	0.139
102	0.143	0.259	0.232	0.304	0.080	0.151
104	0.120	0.254	0.222	0.304	0.083	0.149
107	0.123	0.289	0.217	0.317	0.085	0.158
110	0.124	0.274	0.213	0.308	0.078	0.153
Mean	0.124	0.256	0.214	0.308	0.085	0.142
SD	0.008	0.016	0.012	0.021	0.006	0.012
σ_{EUPT}	0.030	0.058	0.050	0.073	0.020	0.039
SD/σ_{EUPT}	0.265	0.279	0.230	0.288	0.284	0.295
Critical value	0.3	0.3	0.3	0.3	0.3	0.3
SD/σ_{EUPT} ≤0.3	yes	yes	yes	yes	yes	yes

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

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 03-06th June 2019.

The deadline for results was 8th July 2019.

The final report was dispatched to all participant at the end of January 2020.

Statistical evaluation of results

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

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

The z-score has been calculated by the formula:

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

where x is the laboratory mean, X is the *consensus* value (the robust mean), σ_{EUPT} is a fit-for-purpose relative target standard deviation (FFP RSD) corresponding at the 25% of the robust mean value.

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

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

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

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

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

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

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

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

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.

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-19: voluntary information on measurement uncertainty general approaches

Lab code	Measurement uncertainty (MU)	coverage factor *k*
2	Bottom-up approach 41%	2
14	50% in the range 0.010-0.050 mg/kg (SANTE document) 30% > 0.050 mg/kg Bottom-up approach	2
19	Horwitz approach	2
23	SANTE document	2
28	Bottom-up approach	
33	50% (SANTE document)	2
34	50% (SANTE document)	2

Table 5. COIPT-19: voluntary information on measurement uncertainty individual compound data

Lab code	Results (mg/kg)	Measurement uncertainty (MU) (mg/kg)	coverage factor *k*
09			
Buprofezin	0.099	0.024	2
Cypermethrin	0.262	0.046	2
Dimethoate	0.180	0.046	2
Methidathion	0.282	0.029	2
Phosalone	0.082	0.021	2
Tebuconazole	0.129	0.064	2
20			
Buprofezin	0.148	0.063	2
Cypermethrin	0.315	0.120	2
Dimethoate	0.208	0.084	2
Methidathion	0.296	0.114	2
Phosalone	0.088	0.039	2
Tebuconazole	0.183	0.076	2

COIPT-19: RESULTS

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

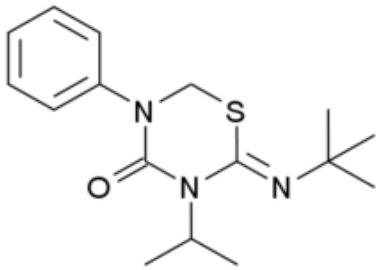
All data for each compound, were analysed for normal distribution by applying the Shapiro – Wilk test (α=0.05).

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.

Most of the compound data sets were not normally distributed except for Buprofezin and Cypermethrin. 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 and extrapolation curves.

Buprofezin



Common name
buprofézine or buprofezin

Structure formula $C_{16}H_{23}N_3OS$

CAS number 953030-84-7

Its physical form consists of white crystals and in technical form a white or pale yellow crystalline powder with weight molecular of 305.4. Persistent insecticide and acaricide with contact and stomach action. This compound has good solubility in organic solvents and it is stable in acidic and alkaline media and stable to heat and light.
Not authorized on olive tree with a MRL value of 0.01mg/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 Buprofezin (mg/kg) submitted by all laboratories with the Kernel density plot. The distribution of the results is symmetric.

Statistical evaluation of the Buprofezin results is presented in Tables 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 Buprofezin. The Robust Relative Standard Deviation (Robust RSD) and the uncertainty of the assigned values *u* for Buprofezin 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-seven laboratories submitted results for Buprofezin with excellent z-score values between 0 and 2.3 as absolute values.

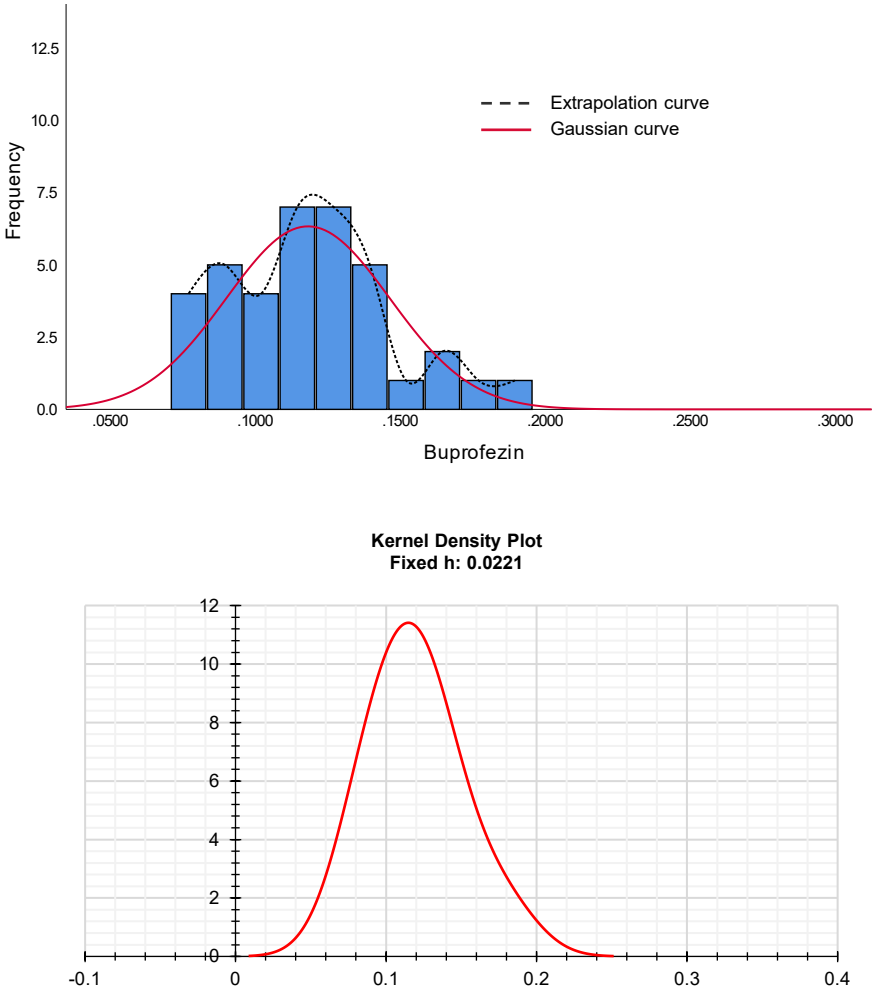


Figure 1. BUPROFEZIN: frequency histogram of the results (mg/kg) and Kernel density plot

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

Parameter	Value
Spiked value	0.143
Mean	0.120
Median	0.119
Robust mean or Assigned value (mg/kg)	0.118
s*	0.028
σ_{EUPT}	0.030
Uncertainty (u) (mg/kg)	0.006
u/σ_{EUPT} *	0.200
FFP RSD (%)	25
Robust RSD (%)	24

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

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

Lab Code	z_{EUPT}-score	Recovery %
1	-0.3	83
2	0.2	89
3	-0.1	70
5	-0.2	-
6	-0.9	71
7	-0.5	80
8	-1.1	-
9	-0.6	105
10	1.8	73
11	-1.2	56
12	0.0	86
13	0.2	83
14	0.3	90
15	-1.2	56
16	-1.0	-
17	-0.1	80
18	0.5	87
19	0.2	72
20	1.0	94
21	0.1	82
22	0.7	91
23	-0.5	80
24	-0.8	70
25	-0.4	95
26	1.0	73
27	2.3	84
28	0.6	97
31	-0.8	66
32	0.2	80
33	0.7	91
34	-1.4	50
35	1.7	-
36	-0.2	71
37	0.8	-
38	0.2	89
39	-1.5	85
40	2.2	-

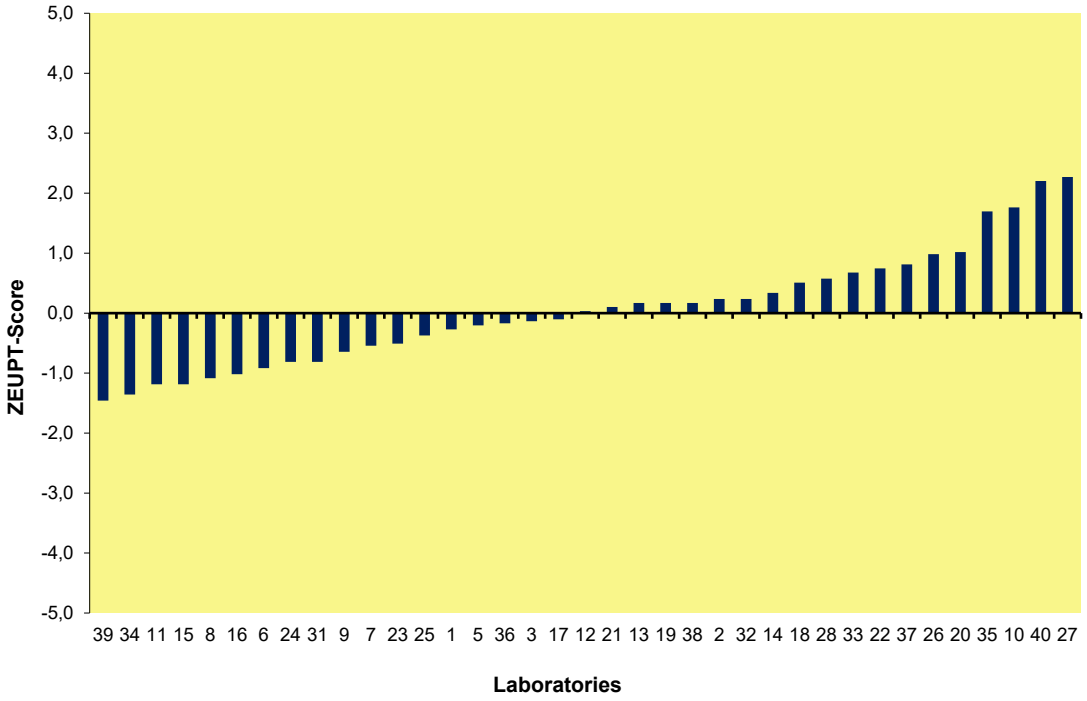
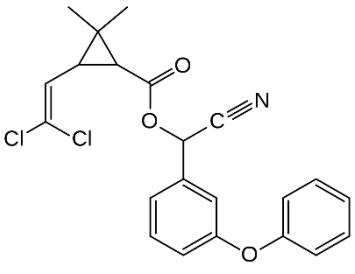


Figure 2. BUPROFEZIN: z-score values (spiked value = 0.143 mg/kg)

Cypermethrin



Common name
cyperméthrine or cypermethrin

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

CAS number 52315-078

Its physical form consists of odourless crystals and in technical form of a yellow-brown viscous semi-solid at ambient temperature with weight molecular of 416.3.
Non-systemic insecticide with contact and stomach action belongs to pyrethroid group.
This compound has good solubility in organic solvents and is relatively stable in neutral and weakly acidic media with optimum stability pH 4 while it is hydrolysed in alkaline media
Authorized in Italy on olive tree with a MRL value of 0.05 mg/kg on olive as established by the Regulation (EC) 396/2005 that corresponds at limit of analytical determination.
About fifteen formulation of PPP type EC (Emulsifiable Concentrate) containing Cypermethrin are authorized in Italy.

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

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

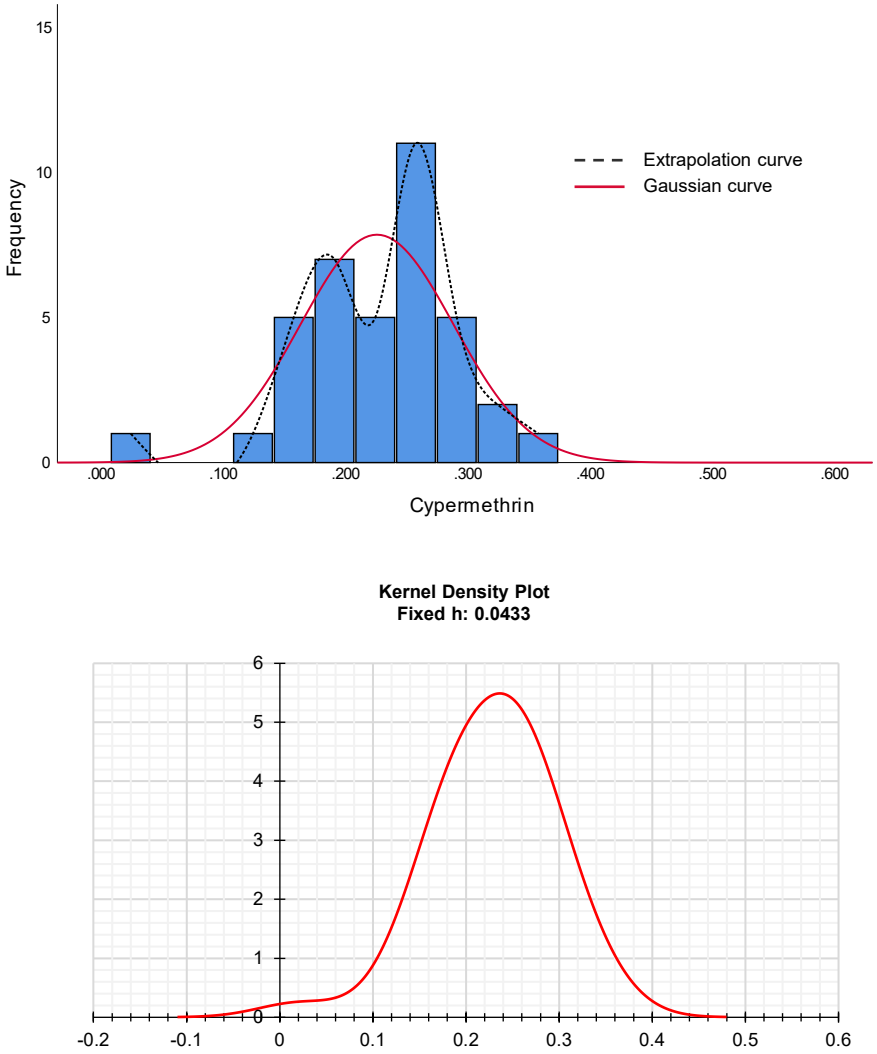


Figure 3. CYPERMETHRIN: frequency histogram of the results (mg/kg) and Kernel density plot

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

Parameter	Value
Spiked value	0.265
Mean	0.228
Median	0.242
Robust mean or Assigned value (mg/kg)	0.231
s*	0.058
σ_{EUPT}	0.058
Uncertainty (u) (mg/kg)	0.010
u/σ_{EUPT} *	0.172
FFP RSD (%)	25
Robust RSD (%)	25

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

In this case also 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. CYPERMETHRIN: z_{EUPT}-score and recovery (%)

Lab Code	z_{EUPT}-score	Recovery%
1	-0.3	80
2	-0.5	92
3	0.4	84
4	-0.3	93
5	-1.2	73
6	-0.4	93
7	-0.9	100
8	0.6	-
9	0.5	90
10	1.1	100
11	-0.7	85
12	-0.9	96
13	-0.9	106
14	0.5	90
16	-0.9	-
17	0.3	100
18	-0.3	86
19	2.1	80
20	1.4	105
21	0.3	95
22	0.8	88
23	-0.5	78
24	0.5	77
26	0.8	82
27	-1.9	82
28	1.2	118
29	0.5	74
30	0.4	94
31	-1.2	75
32	0.3	101
33	-3.6	
34	0.1	88
35	0.2	-
36	-1.0	60
37	1.6	-
38	0.2	102
39	-1.2	115
40	1.1	-

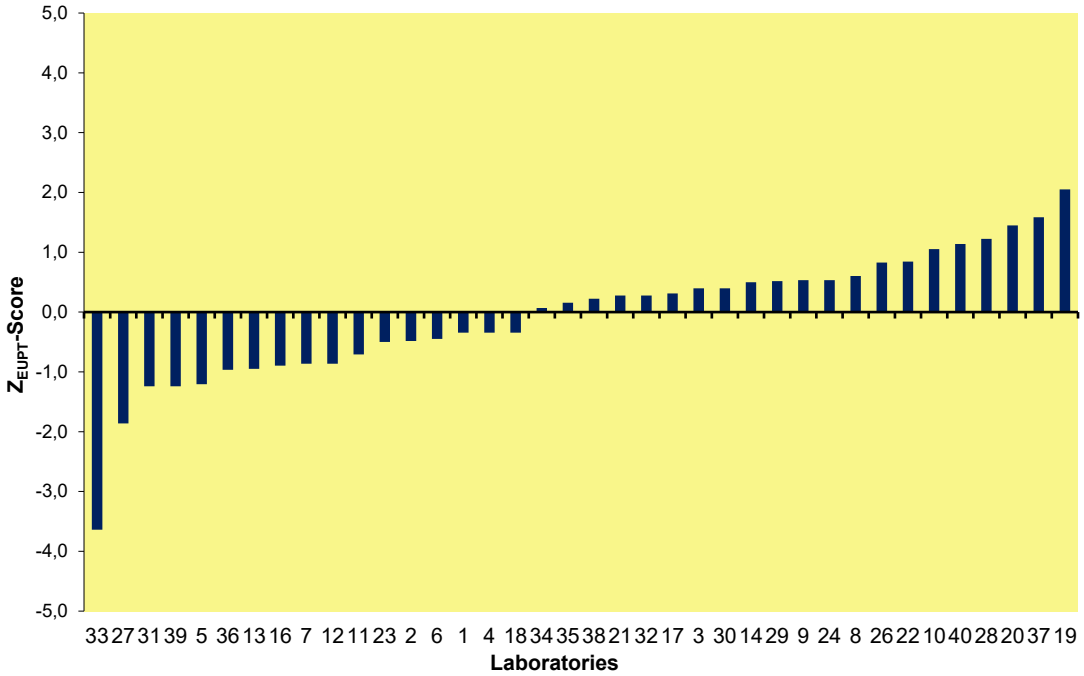
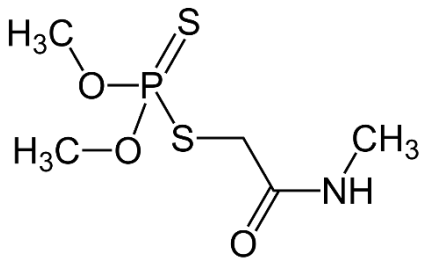


Figure 4. CYPERMETHRIN: z-score values (spiked value = 0.265 mg/kg)

Thirty-seven laboratories supplied results with excellent calculated z-score values in the range 0-2.1, except for a false negative value of -3.6 for Lab 33.

Dimethoate



Common name
dimethoate or fosfamid

Structure formula $C_5H_{12}NO_3PS_2$

CAS number 60-51-5

EC no. 200-480-3

Its physical form consists of colourless crystals and in technical form of white solid pellets with weight molecular of 229.3
This compound has good solubility in organic solvents and is relatively stable in aqueous media at pH 2-7 and is hydrolysed in alkaline solutions.
Systemic insecticide and acaricide belongs to organophosphate compounds.
Authorized on olive tree from 16 January 2018 until 16 December 2020, with a MRL value of 3 mg/kg on table olives and olive for oil production as established by the Regulation (EC) 396/2005.
In Italy, Dimethoate has been authorized in derogation for phytosanitary emergency situations on olive trees for the period 1 July - 28 October as indicated by Art. 52 of Regulation (EC) 1107/2009

Figure 5 shows the results of Dimethoate (mg/kg) submitted by all laboratories in the COIPT-19. The distribution of the results is clearly not symmetric. Statistical evaluation of the Dimethoate results is presented in Table 10.

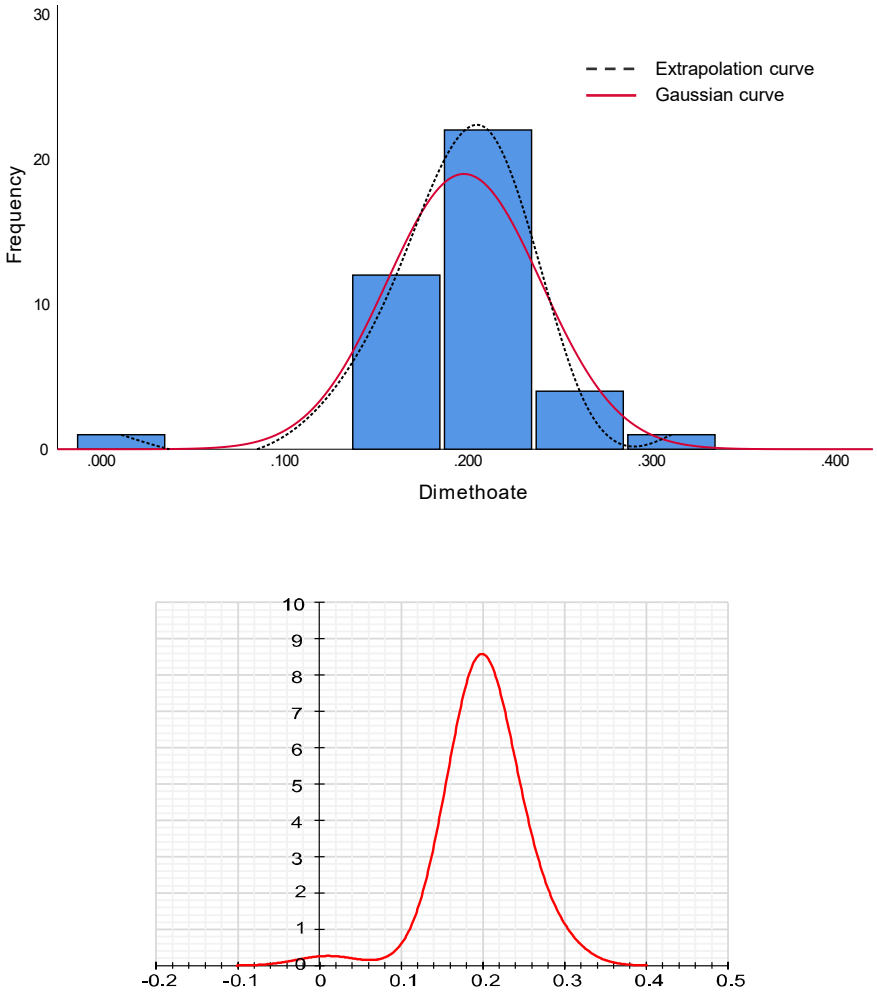


Figure 5. DIMETHOATE: frequency histogram of the results (mg/kg) and Kernel density plot

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

Parameter	Value
Spiked value	0.209
Mean	0.199
Median	0.200
Robust mean or Assigned value (mg/kg)	0.200
s*	0.022
σ_{EUPT}	0.050
Uncertainty (u) (mg/kg)	0.004
u/σ_{EUPT} *	0.080
FFP RSD (%)	25
Robust RSD (%)	11

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

The supplied results for Dimethoate can be considered good, since the data used for the assigned value produced median and robust mean that have the same value. The Robust Relative Standard Deviation (Robust RSD) also is good with a value of 11% 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.

Dimethoate was the most analysed compound from all the laboratories with excellent calculated z-score values in the range 0-1.8, except for a false negative value of -3.8 for Lab 27.

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

Lab Code	z_{EUPT}-score	Recovery%
1	0.3	*84*
2	-0.9	88
3	1.2	109
4	0.1	109
5	0.8	79
6	-0.4	88
7	-0.3	99
8	-0.3	-
9	-0.4	102
10	0.3	97
11	-0.4	91
12	0.0	107
13	-0.1	102
14	0.0	90
15	-0.5	80
16	0.0	-
17	0.1	95
18	1.2	115
19	0.4	88
20	0.2	102
21	0.0	97
22	0.0	91
23	-0.1	92
24	0.0	**122**
25	0.0	94
26	0.3	95
27	-3.8	
28	0.0	91
29	0.4	103
30	1.5	130
31	-0.7	75
32	0.1	101
33	-0.3	87
34	-0.3	102
35	0.2	-
36	-1.0	68
37	-0.3	-
38	0.3	102
39	-0.1	115
40	1.8	-

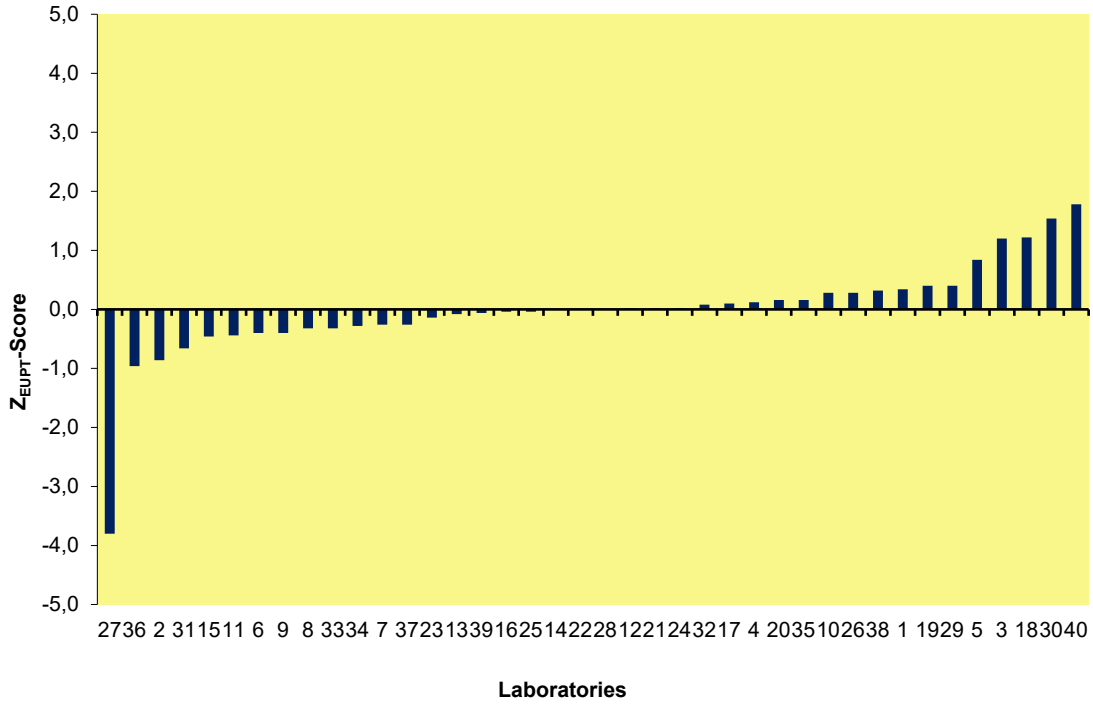
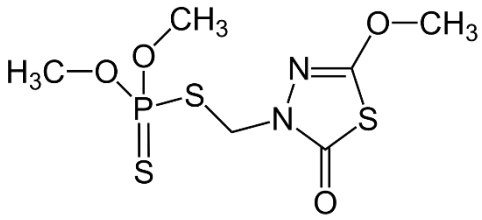


Figure 6. DIMETHOATE: z-score values (spiked value = 0.209 mg/kg)

Methidathion



Common name
methidathion or DMTP

Structure formula $C_6H_{11}N_2O_4PS_3$

CAS number 950-37-8

EC no. 213-449-4

Its physical form consists of colourless crystals with weight molecular of 302.3.
Non-systemic insecticide and acaricide belongs to organophosphate compounds. This compound has good solubility in organic solvents and is relatively but is rapidly hydrolysed inn alkaline and strongly acidic media.
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 corresponds 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 Dimethoate (mg/kg). As in the case of dimethoate the distribution of the results is not symmetric.

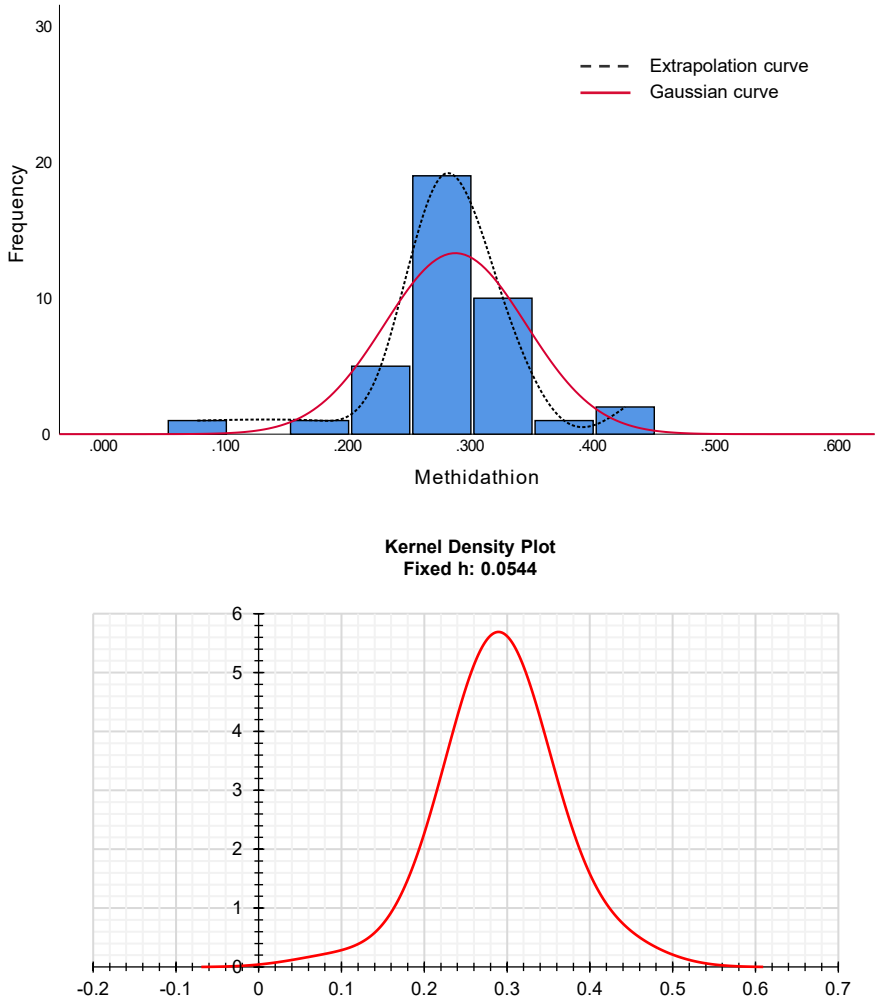


Figure 7. METHIDATHION: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of Methidathion results is presented in Table 12.

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

Parameter	Value
Spiked value	0.305
Mean	0.290
Median	0.296
Robust mean or Assigned value (mg/kg)	0.290
s*	0.039
σ_{EUPT}	0.073
Uncertainty (u) (mg/kg)	0.008
u/σ_{EUPT} *	0.110
FFP RSD (%)	25
Robust RSD (%)	14

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

Statistically results for Methidathion can be considered satisfactory.

The median and the robust mean in Table 12 are similar with a good value for Robust RSD of 14% 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.

Methidathion was analysed by 39 laboratories out of 40 with good calculated z-score values all in the range ± 3.

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

Lab Code	z_{EUPT}-score	Recovery%
1	0.3	82
2	-0.6	92
3	0.4	99
4	-0.1	101
5	0.5	93
6	-0.4	84
7	0.0	94
8	-0.6	-
9	-0.1	100
10	0.1	87
11	-0.5	90
12	0.3	102
13	0.7	100
14	0.0	90
15	-1.3	93
16	-0.4	-
17	0.2	94
18	1.8	110
19	0.1	88
20	0.1	96
21	-0.2	95
22	0.1	101
23	-0.2	90
24	-0.7	100
25	-0.1	96
26	0.4	79
27	-2.7	94
28	0.1	81
29	-0.2	81
30	0.3	91
31	-0.6	74
32	0.2	81
33	0.2	66
34	1.3	90
35	0.5	-
36	-1.0	68
37	-0.5	-
38	0.6	105
40	2.1	-

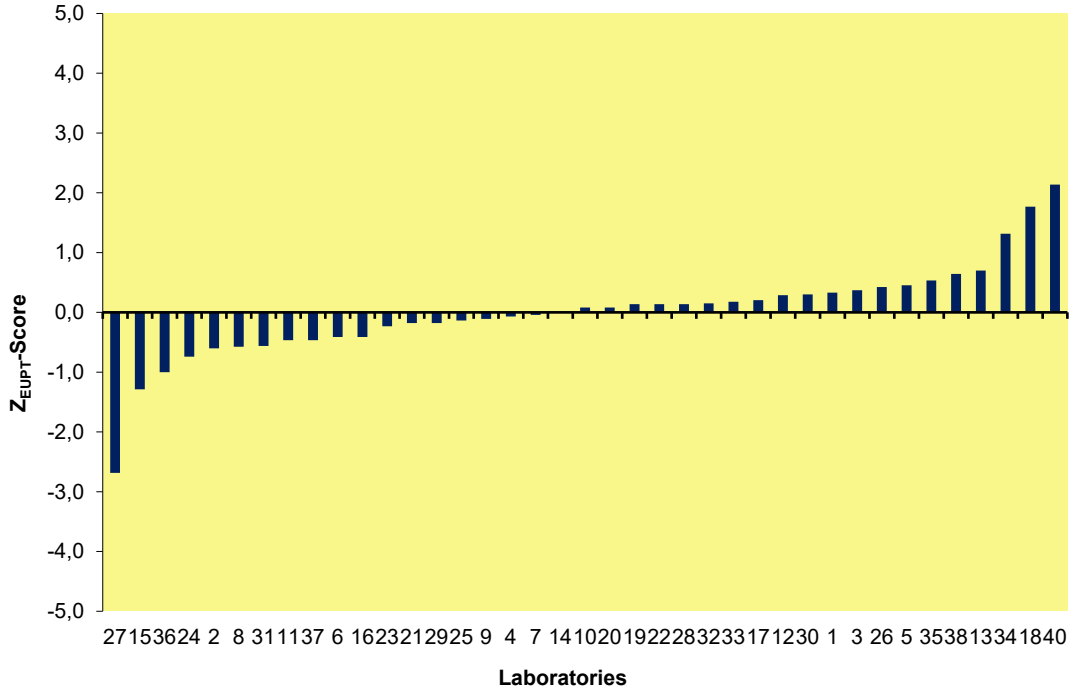
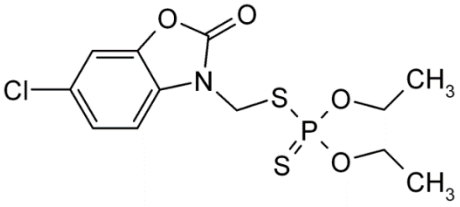


Figure 8. METHIDATHION: z-score values (spiked value = 0.305 mg/kg)

Phosalone



Common name
phosalone or benzofos

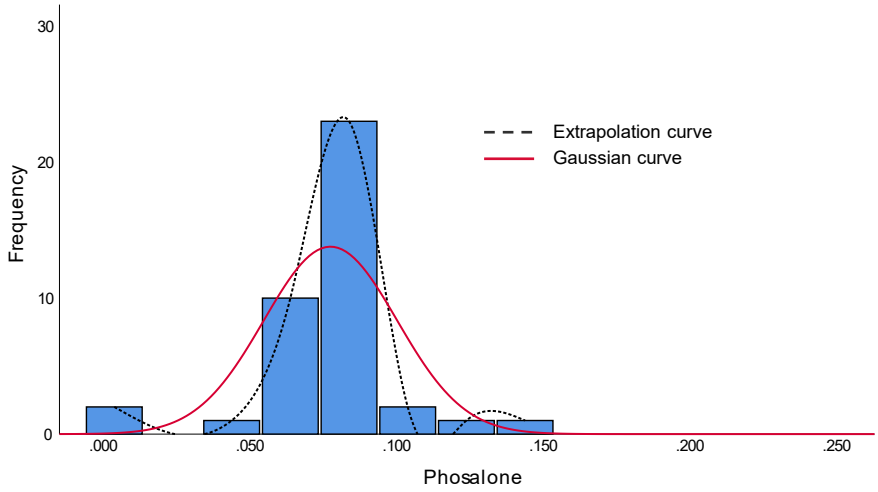
Structure formula $C_{12}H_{15}ClNO_4PS_2$

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



Kernel Density Plot
Fixed h: 0.0148

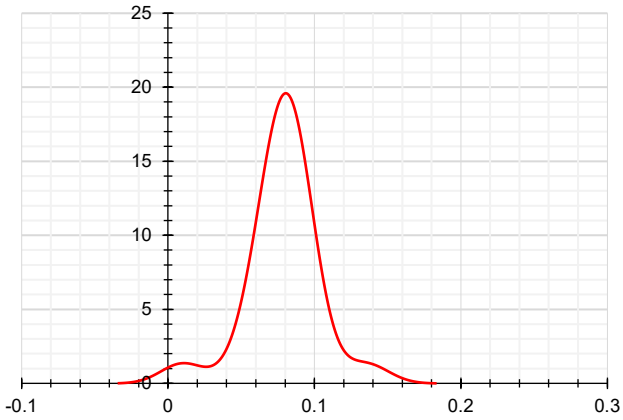


Figure 9. PHOSALONE: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of Phosalone results is presented in Table 14.

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

Parameter	Value
Spiked value	0.087
Mean	0.078
Median	0.081
Robust mean or Assigned value (mg/kg)	0.079
s*	0.013
σ_{EUPT}	0.020
Uncertainty (u) (mg/kg)	0.003
u/σ_{EUPT} *	0.150
FFP RSD (%)	25
Robust RSD (%)	16

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 15 with the corresponding graphical representation in Figure 10.

Phosalone was determined by 37 laboratories out of 40 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.

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

Lab Code	z_{EUPT}-score	Recovery%
1	-0.2	76
2	-0.6	91
3	0.5	90
4	0.1	110
5	0.3	81
6	-0.9	78
7	0.0	102
8	0.1	-
9	0.2	92
10	0.7	91
11	-0.3	105
12	0.8	112
13	0.6	90
14	-0.2	90
15	-1.0	93
16	-0.8	-
17	0.5	94
18	0.4	90
19	0.6	85
20	0.5	94
21	-0.3	95
22	0.1	91
23	-0.1	78
24	-0.5	97
25	0.1	99
26	0.4	82
27	-3.5	
28	0.2	92
29	-3.5	
30	0.1	118
31	-0.5	79
32	0.7	94
33	0.3	74
34	-0.5	69
35	1.0	-
36	-0.8	63
37	3.0	
38	0.4	104
39	2.7	109
40	3.0	-

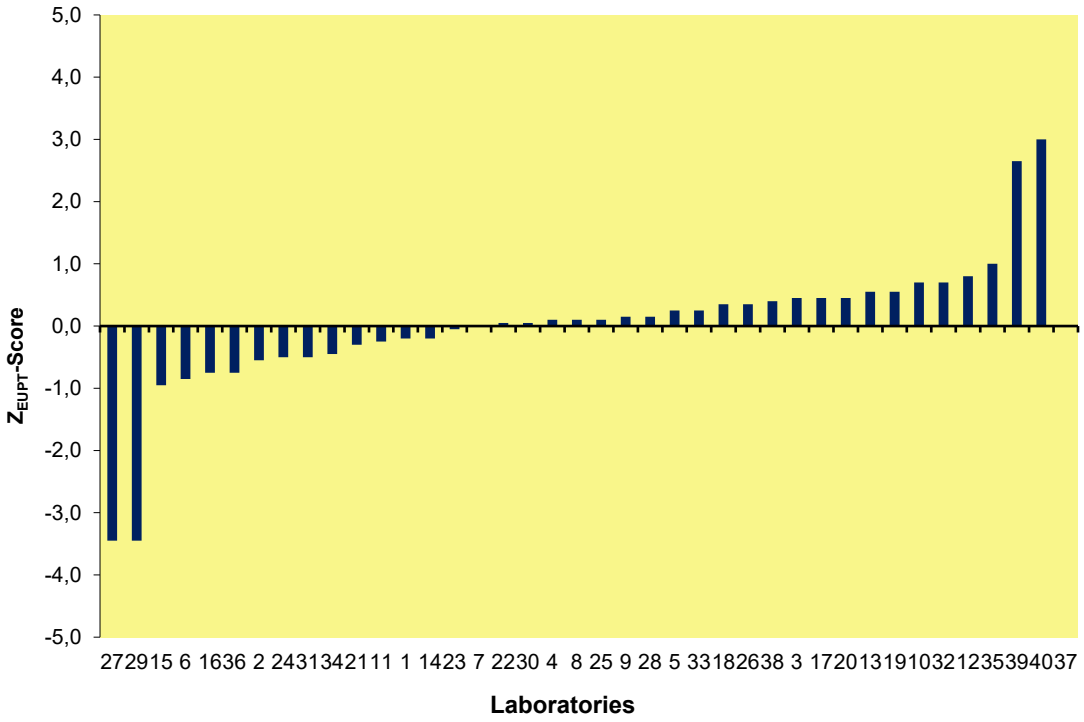
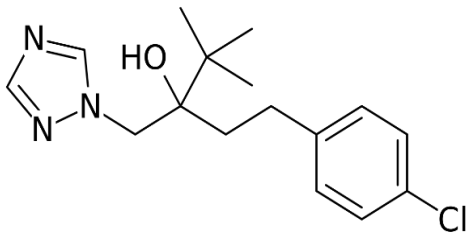


Figure 10. PHOSALONE: z-score values (spiked value = 0.087 mg/kg)

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

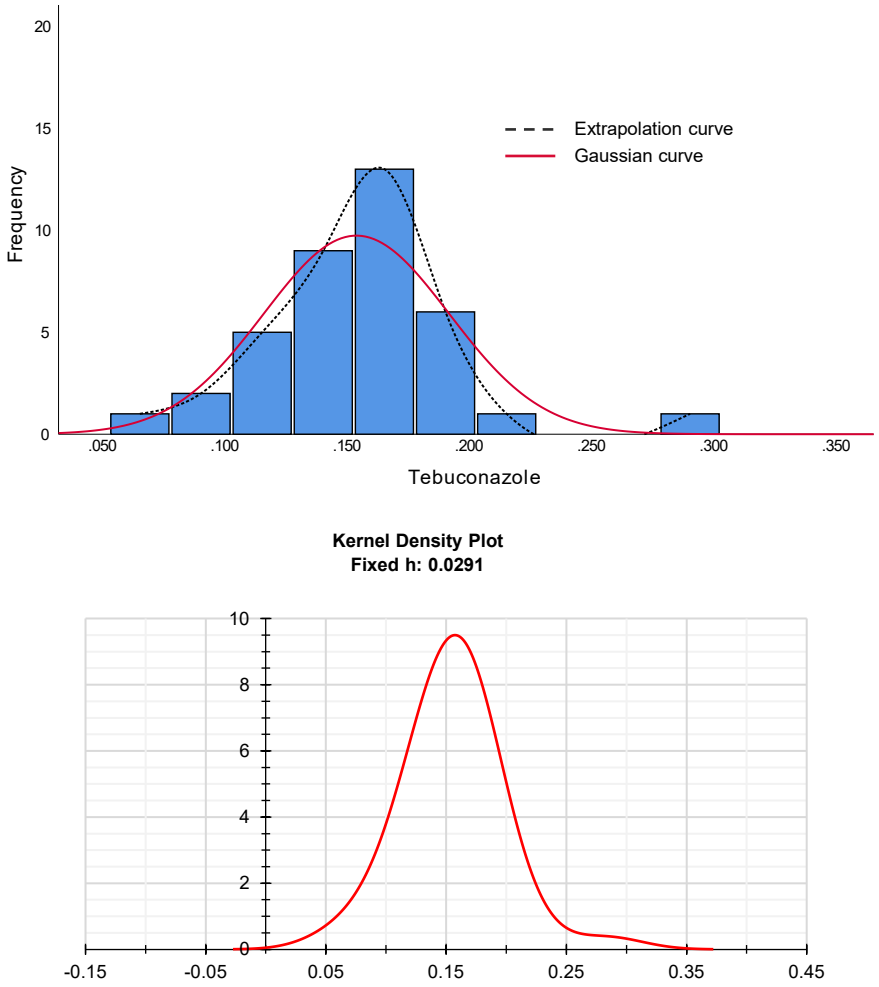


Figure 11. TEBUCONAZOLE: frequency histogram of the results (mg/kg) and Kernel density plot

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

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

Parameter	Value
Spiked value	0.169
Mean	0.155
Median	0.160
Robust mean or Assigned value (mg/kg)	0.155
s*	0.029
σ_{EUPT}	0.039
Uncertainty (u) (mg/kg)	0.006
u/σ_{EUPT} *	0.154
FFP RSD (%)	25
Robust RSD (%)	19

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

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

Lab Code	z_{EUPT}-score	Recovery%
1	0.5	77
2	-0.6	88
3	0.6	113
5	0.3	-
6	-0.6	86
7	0.1	87
8	-2.4	-
9	-0.7	87
10	0.9	79
11	-0.6	89
12	0.4	106
13	0.3	110
14	-0.1	90
15	0.2	72
16	-0.3	-
17	-0.2	79
18	1.0	98
19	0.4	86
20	0.7	101
21	0.3	94
22	0.4	96
23	-0.8	76
24	-1.8	74
25	0.3	102
26	0.2	79
27	0.8	94
28	0.0	96
30	1.6	125
31	-0.5	63
32	-0.8	125
33	-0.2	95
34	-1.4	66
35	0.6	-
36	-0.9	69
37	3.0	-
38	-0.1	103
39	-1.0	99
40	3.3	-

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

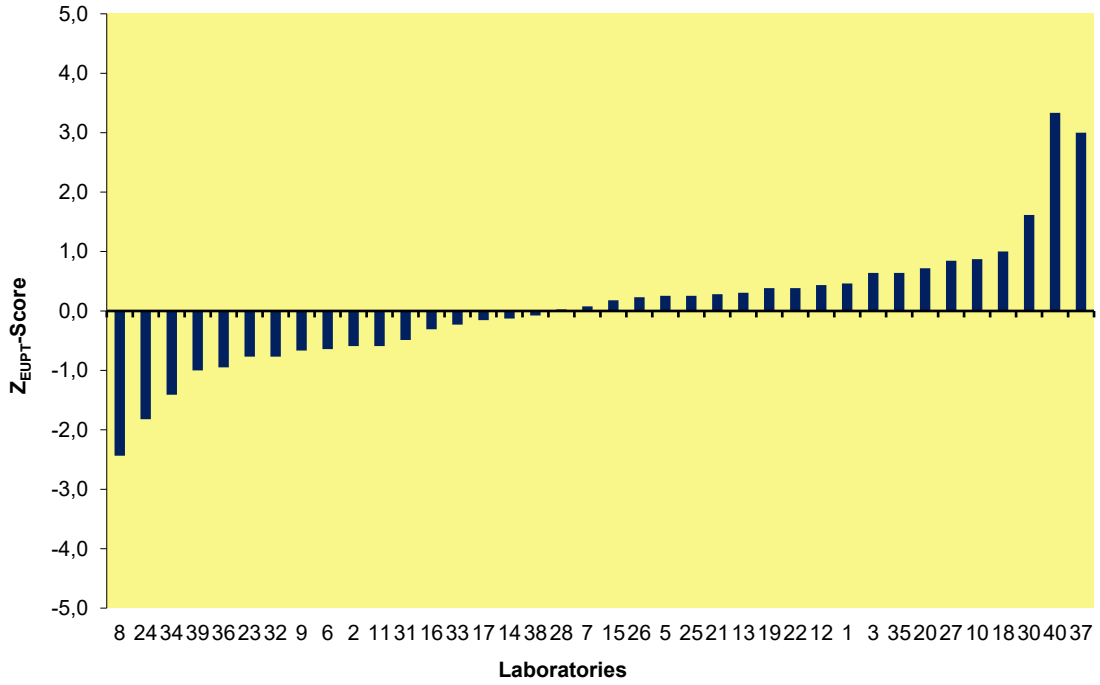


Figure 12. TEBUCONAZOLE: z-score values (spiked value = 0.169 mg/kg)

Thirty-eight laboratories supplied results for tebuconazole with good calculated z-score values in the range ± 2 except for Lab 37 and 40 with two values of 3.0 and 3.3 respectively.

COIPT-19: FINAL COMMENTS

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

As in the case of Dimethoate, 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 good from 13 to 25 % for six compounds while the range of u was from 0.003 to 0.010.

All laboratories submitted results and thirty-five (equal to 88%) analysed all compounds with Dimethoate that was the most analysed compound.

Five false negative values were calculated: one in the case of Lab 33 for Cypermethrin and Lab 27 for Dimethoate and three values regarding for Phosalone (Lab 27, 29, 37). 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.

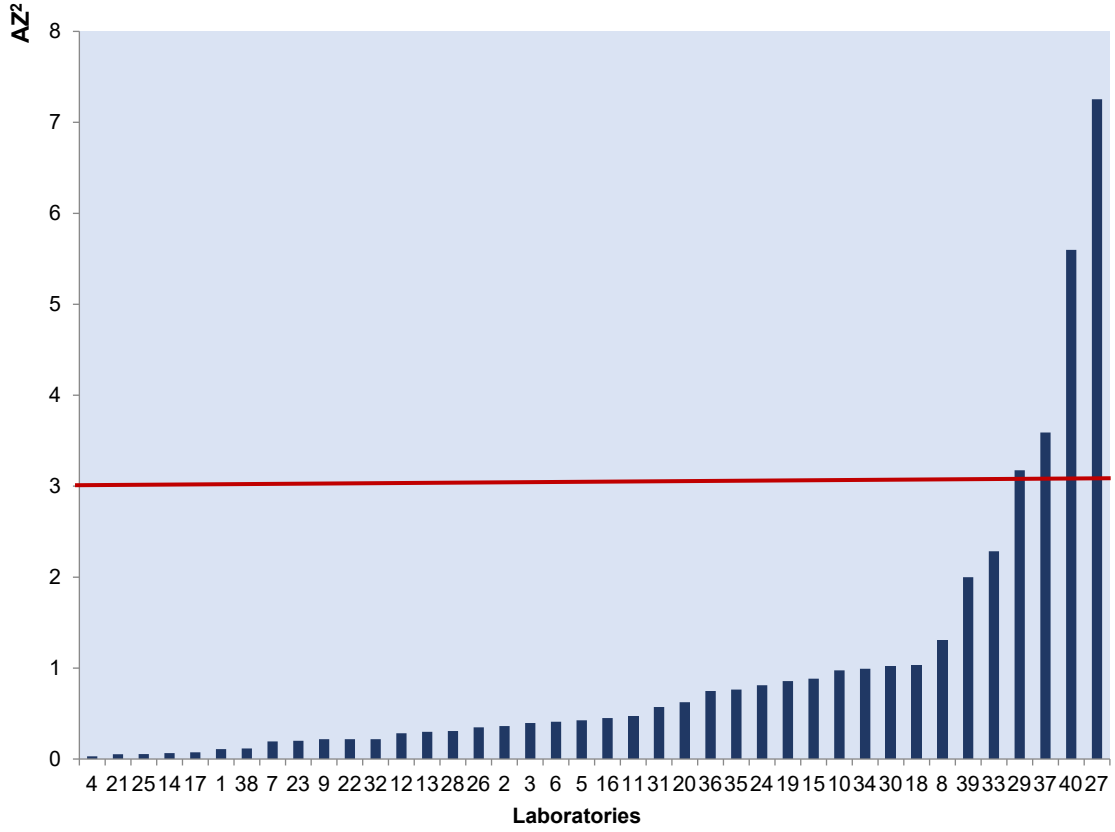


Figure 13. Global performance of laboratories: AZ^2 values (COIPT-19)

Respect to the analytical methods applied by participants, the majority of laboratories corresponding to twenty-five participants out of forty used the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) methodology or methods based on QuEChERS (19).

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

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

In the above-mentioned methods, the purification was carried out using the GPC (Gel Permeation Chromatography) technique, alumina cartridge or using combination of different materials as extrelut + silica+C_{18} as SPE or PSA+GCB+C_{18}or freezing technique. The amount of the sample test was in the range 0.2-10 g.

One laboratory has followed the method of Lentza Rizos (21), while another one the Method M6 by the European Union Reference Laboratory-Fruits and Vegetables in 2012 (22)

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

The instrumental detection techniques used by the majority of the laboratories were: GC (Gas Chromatography) coupled with Mass Spectrometry Detector (MSD), Mass Spectrometry Ion Trap Detector (MSITD), Time of Flight (TOF) MS detector, HRMS (High Resolution Mass Spectrometry) orbitrap detector, MS/MS detector; LC (Liquid chromatography) coupled with MS/MS detector or UHPLC (Ultra High-Pressure Liquid Chromatography) MS/MS.

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

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

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

In the large part of the cases the quantification has been carried out with matrix calibration at single or multiple levels. Four 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. For pesticide residues analysis in food and feed, acceptable limits for a single recovery result should normally be within the generalized range of 60-140 %, corresponding to the ± twice acceptance criterion value of the within – laboratory reproducibility (RSD ≤20%); the so-called warming limits are usually located at a distance corresponding to the absolute range 70-120% fixed as acceptance criteria of the mean recovery, in certain cases and typically with multi-residue methods, recoveries outside these range may be acceptable (3). A limited number of submitted recoveries did not respect these limits.

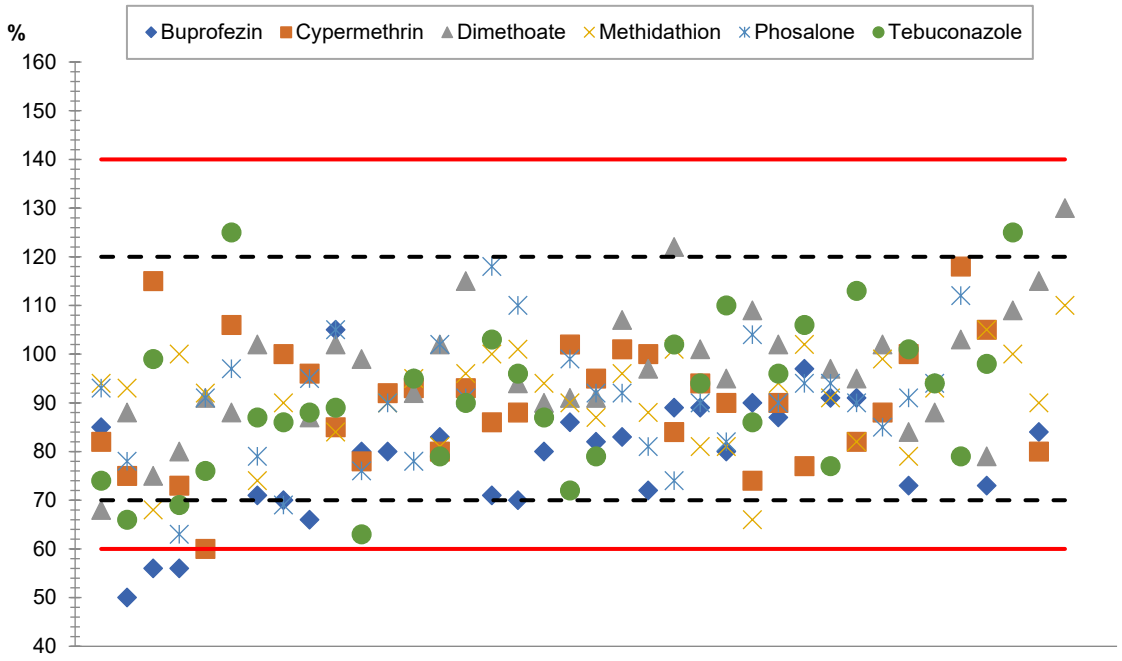


Figure 14. Control chart of the recoveries (%) submitted by the participants in COIPT-19

CONCLUSIONS

The outcome of the COI-PT19 can be considered satisfactory from several point of view.

One is the good participation of laboratories. Forty laboratories agreed to participate in this PT: six NRLs, eighteen Official control laboratories and sixteen 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-six out of forty laboratories obtained a satisfactory performance for all tested compounds.

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

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

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APPENDIX A
List of participants

The participants in COIPT-19 are listed below.

BELGIUM
Primoris Belgium (Zwijnaarde)
FRANCE
ITERG (Pessac)
Laboratori Du Scl De Montpellier (Montpellier)
GERMANY
Eurofin Dr. Specht Laboratorien GmbH (Hamburg)
Eurofin SOFIA GMBH (Berlin)
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)
Food Allergens (Nea Ionia)
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)
APPA Trento, Settore Laboratorio (Trento)
ARPA Emilia Romagna Area Fitofarmaci (Ferrara)
ARPA Friuli Venezia Giulia (Udine)
ARPA Lazio, Sezione di Latina (Latina)
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)
ICQRF, Laboratorio di Perugia (Perugia)
INNOVHUB-SSI, Divisione SSOG (Milano)
Istituto Superiore di Sanità, Dipartimento Ambiente e Connessa Prevenzione Primaria (Roma)
IZS dell’Abruzzo e del Molise (Teramo)
IZSLER Laboratorio Contaminanti Ambientali (Brescia)
IZSLT (Roma)
IZS SICILIA (Palermo)
LABCAM srl (Albenga, SV)
PH srl (Firenze)
USL Toscana Centro (Firenze)
Water e Life Lab srl (Bergamo)
POLAND
Voivodship Sanitary Epidemiological Station in Rzeszow (Rzeszow)
SPAIN
Aceites Borges Pont Sau (Tàrrega Lléida)
Laboratorio Agroalimentario (Granada)
Laboratorio Arbitral Agroalimentario (Madrid)

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:

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

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

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

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

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

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

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

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

where the summation is over i.

The robust estimates x^* and s^* may be derived by an iterative calculation, i.e. by updating the values of x^* and s^* several times using the modified data in equations 3 to 6, until the process converges. Convergence may be assumed when there is no change from one iteration to the next in the third significant figures of the robust mean and robust standard deviation (x^* and s^*).

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