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Dipartimento Ambiente e Salute

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2022, v, 37 p. Rapporti ISTISAN 22/14

In 2021, the Italian National Reference Laboratory for pesticide residues in products of Animal Origin and commodities with high fat content (NRL-AO), thanks to the contribution of a collaboration project with the Italian Ministry of Health, organized a new Proficiency Test (PT) in sunflower seed oil named AO-PT1. Laboratories invited to participate in this PT are laboratories involved in the National and European monitoring programs for pesticide residues in food (NRLs, official control laboratories and private laboratories). The exercise consisted in the determination of unknown five different pesticides in a spiked sunflower seed oil sample, chosen from a target list of twenty-eight compounds. Thirty-three participating laboratories submitted results: twenty-three participants analysed all the five spiked compounds. The majority of participants obtained a satisfactory performance (z-score) for all tested pesticides.

Key words: National Reference Laboratory; Pesticide residues; Proficiency Test; Sunflower seed oil

Istituto Superiore di Sanità
Risultati del circuito interlaboratorio su residui di antiparassitari in olio di semi di girasole.
Tiziana Generali, Patrizia Stefanelli, Valentina Picardo, Silvana Girolimetti, Danilo Attard Barbini
2022, v, 37 p. Rapporti ISTISAN 22/14 (in inglese)

Nel 2021, il Laboratorio Nazionale di Riferimento italiano per i residui di pesticidi nei prodotti di origine animale e alimenti 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 grazie al contributo di un progetto di collaborazione con il Ministero della Salute un nuovo test di competenza in olio di semi di girasole chiamato AO-PT1. I laboratori invitati a partecipare in questo circuito interlaboratorio sono laboratori europei coinvolti nei programmi di monitoraggio nazionali ed europei per i residui di pesticidi negli alimenti (NRL, laboratori di controllo ufficiali e laboratori privati). L'esercizio consisteva nella determinazione di cinque diversi pesticidi sconosciuti in un campione di olio di semi di girasole, scelti da una lista prestabilita di ventotto composti. Trentatré laboratori partecipanti hanno fornito risultati; ventitré hanno analizzato tutti i composti addizionati. La maggior parte dei partecipanti ha ottenuto una soddisfacente prestazione (*z-score*) per tutti gli antiparassitari oggetto del test.

Parole chiave: Laboratorio Nazionale di Riferimento; Residui di antiparassitari; Circuito interlaboratorio; Olio di semi di girasole

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

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

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Redazione*: Sandra Salinetti*
La responsabilità dei dati scientifici e tecnici è dei singoli autori, che dichiarano di non avere conflitti di interesse.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
AZ^2	Average of the Squared z-scores
CAS	Chemical Abstract Service
EC	European Commission
EU	European Union
EUPT	European Union Proficiency Test
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 established a network of National Reference Laboratories (NRLs) and official control laboratories. The overall purpose is to improve the quality, accuracy and comparability of the analytical results regarding the determination of pesticide residues in food.

Current European legislation on pesticides in and on food requires the official laboratory participation in specific proficiency tests (PTs), particularly those organized by the NRLs. Regular participation in 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 European Union (EU) coordinated control pesticide residue monitoring programs, follow the same European analytical quality control technical guidance document SANTE/12682/2019 (3).

During the 2020, the EU Reference Laboratory for pesticides in food of Animal Origin and commodities with high fat content (EURL-AO) has organized a PT on a plant rape seed oil, similar to sunflower seed oil.

In 2021, the Italian NRL for pesticide residues in products of Animal Origin and products with a high fat content (NRL-AO) organized a PT on sunflower seed oil thanks to a collaboration project with the Italian Ministry of Health.

GENERAL CONSIDERATION ON MAXIMUM RESIDUE LEVEL IN SUNFLOWER SEED OIL

Sunflower seed oil is extracted from the seeds of the sunflower plant. The main feature of this oil is the high content of fatty acids. Most of them, including vitamin E, have antioxidant properties (4). Sunflower seed oil is currently one of the most vegetable oils used in cooking and is becoming increasingly popular, also in Italy. In addition to not only is it used for frying but also for condiments, substituting the most expensive and precious olive oil, especially in fried foods.

Sunflower seed oil can also be used for cosmetic purposes; for instance, being rich in antioxidants, it is an excellent anti-wrinkle.

Ukraine and Russia remain the leading sunflower seed producers in the world. They produce nearly half of the sunflower seeds in the world. Ukraine produced 11 million tons of sunflower seeds representing 24.8% of world production, while Russia produced 10.6 million tons in 2013 accounting for 23.7% of world production. Argentina, China, Romania, Bulgaria and Tanzania are also the main sunflower seed producers in the world. In 2013, the world wild sunflower seed production was 44.5 million tons (5)

The sunflower plant is vulnerable to various pest attacks, so certain Plant Protection Products (PPPs) are used to avoid losses of the seed crop. The use of PPPs may lead to contamination of sunflower seeds and consequently of sunflower oil.

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

- 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 MRL is then established in sunflower seed (as for all crops) by the

Regulation (EC) 396/2005 (6) and amendments. For those pesticides not allowed in/on sunflower seed 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 this value at 0.01 mg/kg. To calculate MRL values in processed products such as sunflower seed 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, is declared that each Member State is requested to report the processing factors used to analyse sunflower seed oil samples. Nowadays, the Italian processing factor is set to the figure of 2.5.

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PROFICIENCY TEST ON SUNFLOWER SEED OIL: AO-PT1

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.

In 2021, the Italian NRL-AO organized a PT on sunflower seed oil thanks to the contribution of a collaboration project with the Italian Ministry of Health named AO-PT1.

The exercise consisted in the determination of five different pesticides in a sunflower seed oil sample spiked with a definite range of concentration (0.025-0.300 mg/kg). These pesticides were chosen from a list of twenty-eight compounds presented in AO-PT1 Announcement that was sent to participant on 17th March 2021. The possible list of compounds includes mainly those considered in the official control plans, with spiked concentration levels around their reference values set in the European Regulations.

Thirty-six laboratories agreed to participate in this PT and thirty-three submitted results.

To assess the performance of the participating laboratories, z-scores are used following the criteria of the International Laboratory Accreditation Cooperation (ILAC) and the International Organization for Standardization (ISO) (7, 8).

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 sunflower seed 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

An amount equal to 4.2 kg of sunflower seed oil available in one of the Italian supermarkets was used to prepare samples. All the 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: Chlorpyrifos, Imidacloprid, Kresoxim-methyl, Terbuthylazine, and Trifluralin.

Aliquots of 50 g of this spiked oil named AO-PT1 SPIKED OIL were transferred into dark glass bottles as well as aliquots of 50 g of the blank oil named AO-PT1 BLANK OIL. The current MRLs for these five pesticides are showed in Table 1 (9-13).

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

Compounds	Current EU Regulation	MRL on sunflower seed (mg/kg)
Chlorpyrifos	Regulation (EU) 2020/1085 Applicable from: 3/11/2020	**0.01***
Imidacloprid	Regulation (EU) 491/2014 Applicable from: 5/06/2014	**0.1**
Kresoxim-methyl	Regulation (EU) 2020/856 Applicable from: 9/07/2020	**0.05***
Terbuthylazine	Regulation (EU) 2021/618 Applicable from: 6/11/2021	**0.1**
Trifluralin	Regulation (EU) 2015/552 Applicable from: 28/10/2015	**0.01***

***** Limit of analytical determination

Homogeneity and stability tests

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 12th May 2021;

– Day 2: after one month by the deadline for reporting results on 18th June 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 no significant decrease in the pesticide levels occurred during the PT. The individual results are presented in Table 2.

Table 2. AO-PT1: data (mg/kg) of the stability test

Pesticide	Concentration mg/kg				
	Mean 1 (M1) n=6	**Mean 2 (M2) n=6**	**|M1-M2|**	**σ_{EUPT}**	**0.3xσ_{EUPT}**
Chlorpyrifos	0.161	0.170	-0.009	0.035	0.010
Imidacloprid	0.244	0.258	-0.014	0.064	0.019
Kresoxim-methyl	0.210	0.219	-0.009	0.057	0.017
Terbuthylazine	0.122	0.129	-0.008	0.027	0.008
Trifluralin	0.046	0.049	-0.003	0.010	0.003

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_{EUPT} = target standard deviation
The acceptance criterion of the stability test is = **$|M1-M2| < 0.3x\ \sigma_{EUPT}$**

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

Table 3. Homogeneity results (mg/kg) for AO-PT1

Sample number	Chlorpyrifos	Imidacloprid	Kresoximmmethyl	Terbuthylazine	Trifluralin
71	0.152	0.222	0.200	0.121	0.050
77	0.163		0.193	0.131	0.050
84	0.152	0.228	0.202	0.119	0.050
90	0.162	0.253	0.233	0.120	0.048
100	0.156	0.237	0.207	0.118	0.047
106	0.169	0.254	0.210	0.127	0.048
107	0.165		0.189	0.135	0.056
109	0.161	0.221	0.200	0.119	0.050
111	0.178	0.268	0.215	0.141	0.052
114	0.174		0.215	0.130	0.048
Mean	0.163	0.240	0.206	0.126	0.050
SD	0.009	0.018	0.013	0.008	0.003
σ_{EUPT}	0.035	0.064	0.057	0.027	0.010
SD/σ_{EUPT}	0.251	0.283	0.223	0.295	0.260
Critical value	0.3	0.3	0.3	0.3	0.3
$SD/\sigma_{EUPT} \leq 0.3$	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 sunflower seed 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 10-14th May 2021.

The deadline for results was 11th June 2021.

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

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) (14, 15) 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 by the formula:

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

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 0.025 mg/kg (value set by the organiser for all compounds) where the RL of the laboratory was higher than, or equal to RL of 0.025 mg/kg;
- the RL of the laboratory in cases where the RL of the lab was lower than the RL of 0.025 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 to be included in the interpretation of the results of the proficiency testing.

Furthermore, the global performance (16) of each participating laboratory was assessed by calculating the Average of the Squared z-scores (AZ^2).

The global performance 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 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.

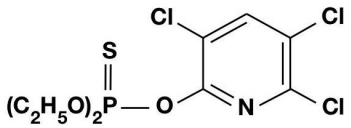
AO-PT1: RESULTS

Description and statistical evaluation of the results are reported 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 Trifluralin. In any case, the Kernel density plots displayed one main mode indicating homogeneous data populations for all compounds.

The frequency histogram reports also the Gaussian curve.

Chlorpyrifos



Common name
chlorpyriphos-ethyl, chlorpyriphos, chlorpyrifos

Structure formula $C_9H_{11}Cl3NO_3PS$

CAS number 2921-88-2

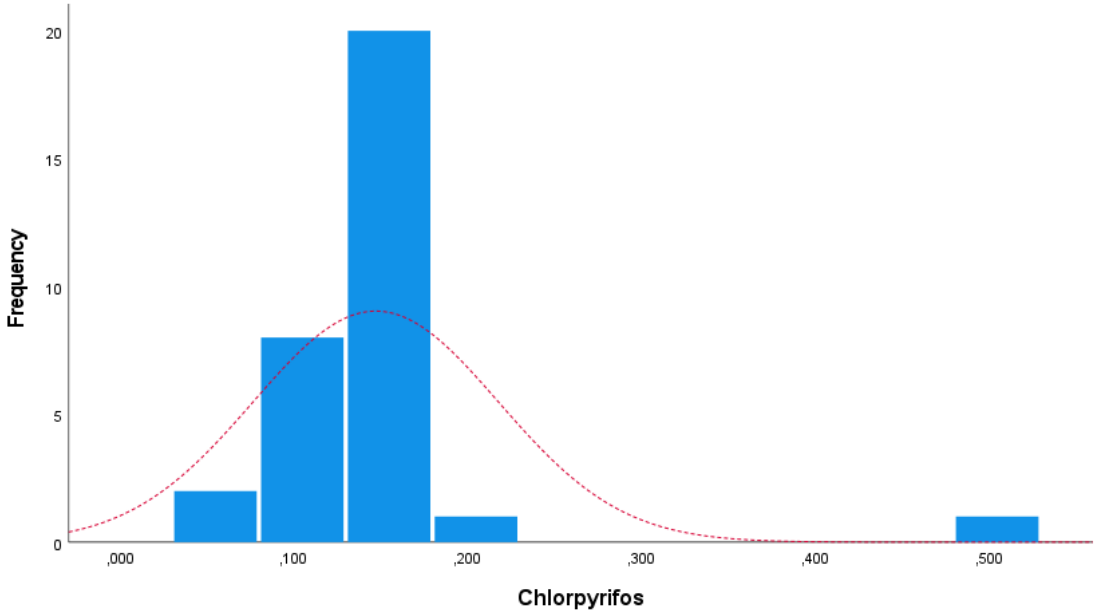
EC no. 220-864-4

Its physical form consists of colourless crystals with a mild mercaptan odour Its weight molecular is of 350.6 g/mol. This compound has good solubility in organic solvents and the rate of hydrolysis increase with pH and in the presence of copper. It is a non-systemic insecticide commercially introduce in 1965.
Not authorized in Italy on sunflower plant with a MRL value of 0.01 mg/kg on sunflower seed as established by the Regulation (EC) 396/2005 that correspond at limit of analytical determination.
It could be present in sunflower seed oil as contaminant as consequence of his liphofilic properties.

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

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

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 Chlorpyrifos. The Robust Relative Standard Deviation (Robust RSD%) and the uncertainty of the assigned values *u* for Chlorpyrifos resulted acceptable.



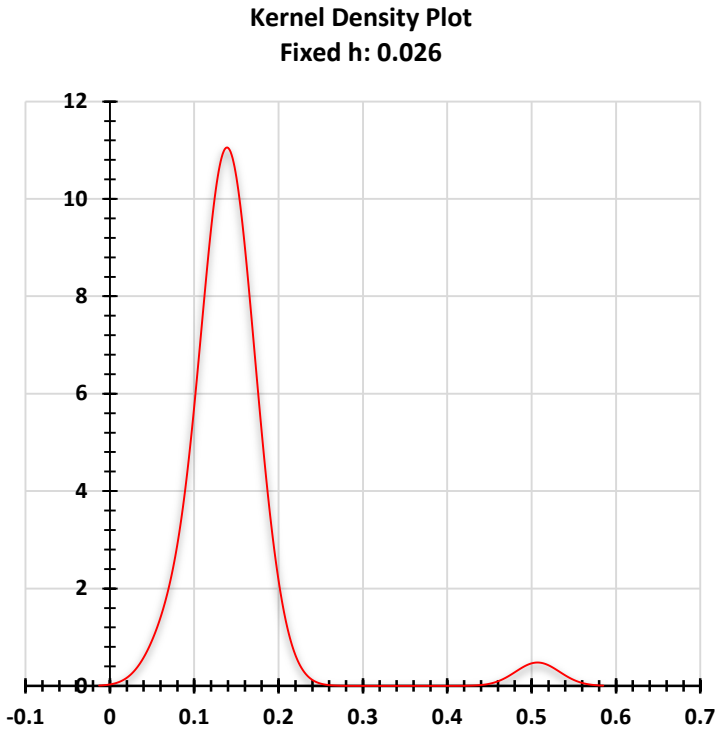


Figure 1. CHLORPYRIFOS: frequency histogram of the results (mg/kg) and Kernel density plot

Table 4. Statistical parameters (mg/kg) of Chlrpyrifos

Parameter	Value
Spiked value	0.168
Mean	0.147
Median	0.140
Robust mean or Assigned value (mg/kg)	0.139
s*	0.025
σ_{EUPT}	0.035
Uncertainty (u) (mg/kg)	0.006
u/σ_{EUPT} *	0.171
FFP RSD (%)	25
Robust RSD (%)	18

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

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

Lab Code	z-score	Recovery %
1	-0.3	86
2	1.4	100
3	-0.5	64
4	0.0	105
5	0.9	STD-add
6	-0.5	87
7	0.2	90
8	0.7	84
9	-0.1	100
10	0.7	85
11	-1.8	75
12	0.7	97
13	5*	-
14	-1.1	75
15	-2.1	70
17	-0.3	90
18	0.1	80
19	0.0	92
20	-0.4	96
21	-0.1	81
22	0.9	98
23	-0.8	95
25	-1.0	74
26	-0.4	84
27	-0.2	89
28	0.1	87
30	0.1	98
32	0.7	96
33	0.0	90
34	-0.1	100
35	0.1	102
36	0.1	92

All z-score values with recoveries supplied by participants are presented in Table 5 and as graphical representation in Figure 2.

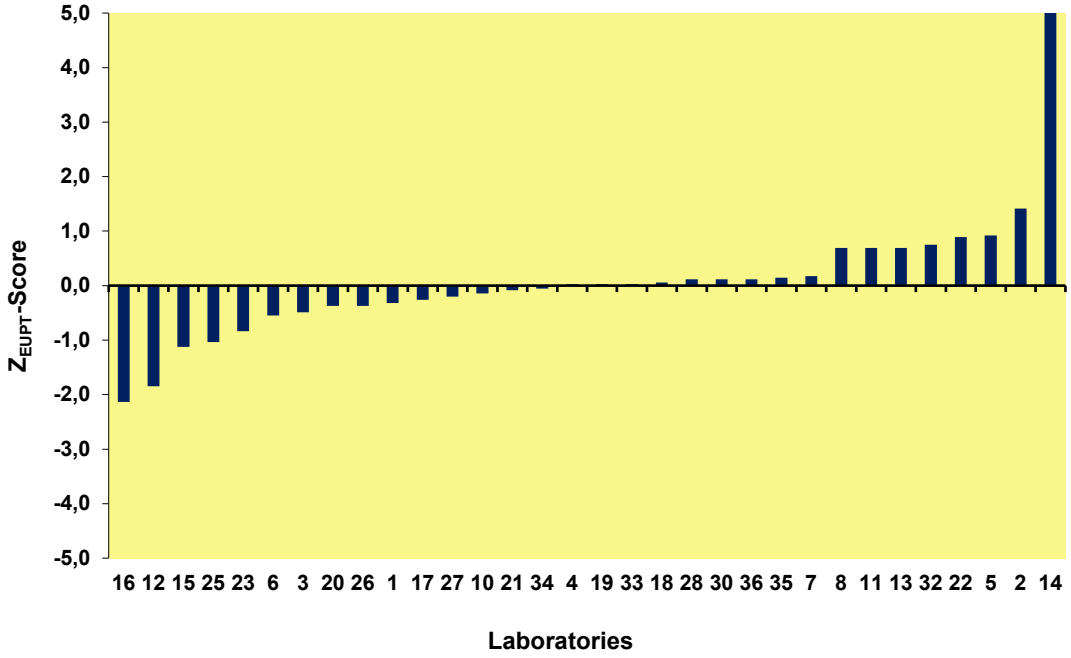
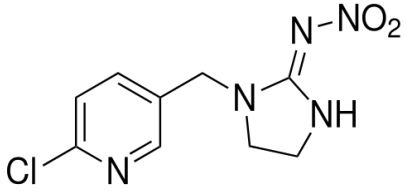


Figure 2. Chlorpyrifos: z-score values (spiked value = 0.168 mg/kg)

Thirty-two laboratories submitted results for Chlorpyrifos: thirty-one had excellent z-score values between 0.0 and 2.1 as absolute values; only one participant has obtained a false negative value of z-score > of 5 and highlighted as indicate in the *Statistical evaluation of results* paragraph as 5*.

Imidacloprid



Common name
imidacloprid or imidaclopride

Structure formula $C_9H_{10}ClN_5O_2$

CAS number 138261-41-3

Originally defined as a mixture of (E)- and (Z)-isomers but in 2007 the material was actually the (E)-isomer.
It is a neonicotinoid, constituted by colourless crystal with a weak characteristic odour and a weight molecular of 255.7 g/mol. It is a systemic insecticide with translaminar activity and with contact and stomach action.
This compound is highly soluble in organic solvents and stable to hydrolysis at pH 5-11.
Not authorized in Italy on sunflower plant with a MRL value of 0.1 mg/kg on sunflower seed as established by the Regulation (EC) 396/2005.

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

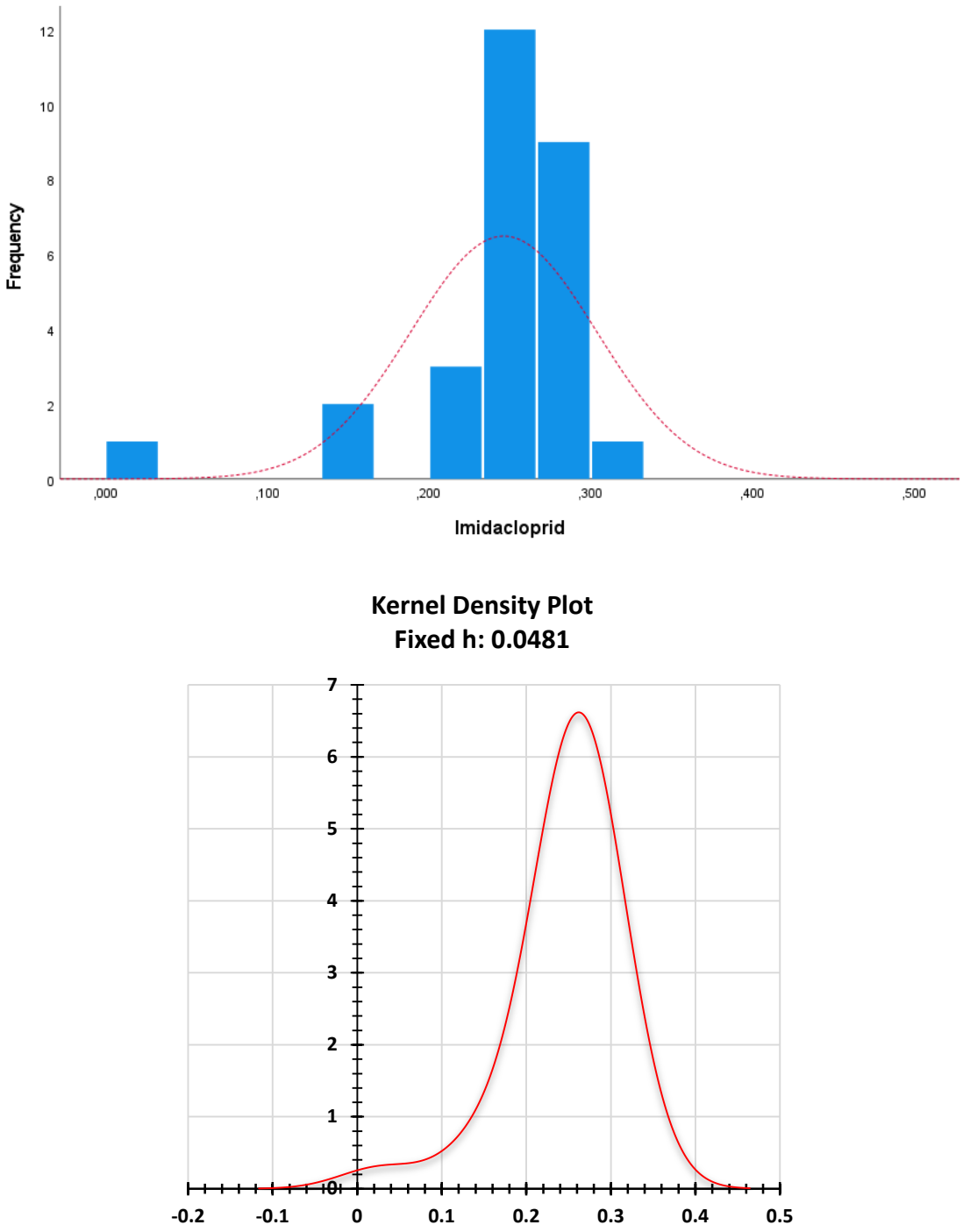


Figure 3. IMIDACLOPRID: frequency histogram of the results (mg/kg) and Kernel density plot

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

Table 6. Statistical parameters (mg/kg) of Imidacloprid

Parameter	Value
Spiked value	0.258
Mean	0.246
Median	0.262
Robust mean or Assigned value (mg/kg)	0.257
s*	0.032
σ_{EUPT}	0.064
Uncertainty (u) (mg/kg)	0.007
u/σ_{EUPT} *	0.109
FFP RSD (%)	25
Robust RSD (%)	12

s*= robust standard deviation
* $u/\sigma_{EUPT} \leq 0.3$; 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-score values with recoveries are presented in Table 7 while in Figure 4 are showed the same data in graphical form. For Imidacloprid twenty-eight laboratories supplied results with excellent calculated z-score values in the range 0.0-1.7 as absolute value except in the case of Lab 23 that has obtained an unacceptable z-score value of -3.6.

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

Lab Code	z-score	Recovery %
3	0.6	102
4	-0.6	111
5	-0.2	STD-add
7	-0.6	92
10	0.1	93
11	0.3	93
12	-0.2	114
14	0.1	97
15	-0.1	100
16	-1.7	77
17	-0.3	90
18	0.5	101
19	-0.2	97
20	0.5	90
21	0.4	97
22	0.1	99
23	-3.6	96
24	0.0	95
25	-0.5	107
26	0.1	88
27	0.4	104
28	0.1	103
30	0.3	105
32	0.4	97
33	1.0	85
34	-1.7	94
35	-0.2	100
36	0.2	90

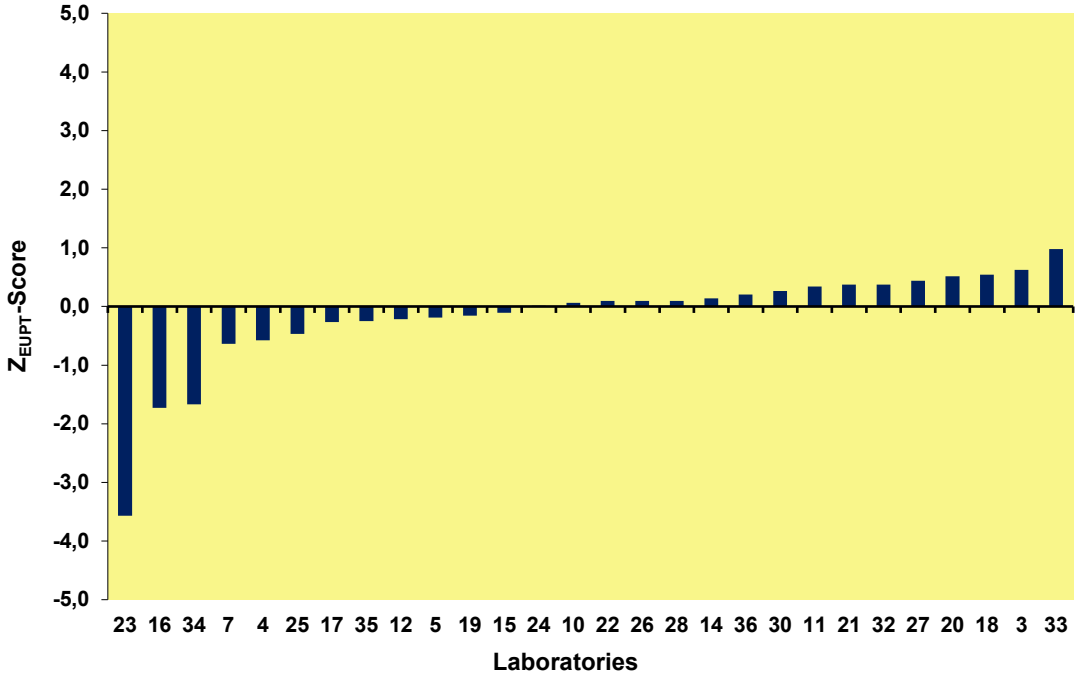
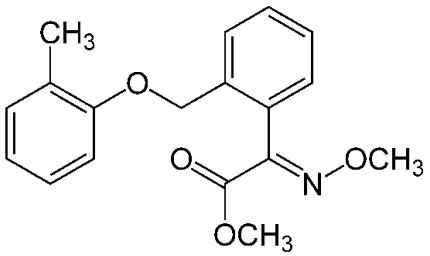


Figure 4. IMIDACLOPRID: z-score values (spiked value = 0.258 mg/kg)

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 solubulity in organic solvent and it is relatively stable at pH 5, but it hydrolyses in alkaline media.
Not authorized on sunflower plant with a MRL value of 0.05 mg/kg on sunflower seed as established by the Regulation (EC) 396/2005 that correspond at limit of analytical determination.
It could be present in sunflower seed oil as contaminant as consequence of his liphofilic properties.

Figure 5 shows the results of Kresoxim-methyl (mg/kg) submitted by all laboratories in the AO-PT1. The distribution of the results is not symmetric.

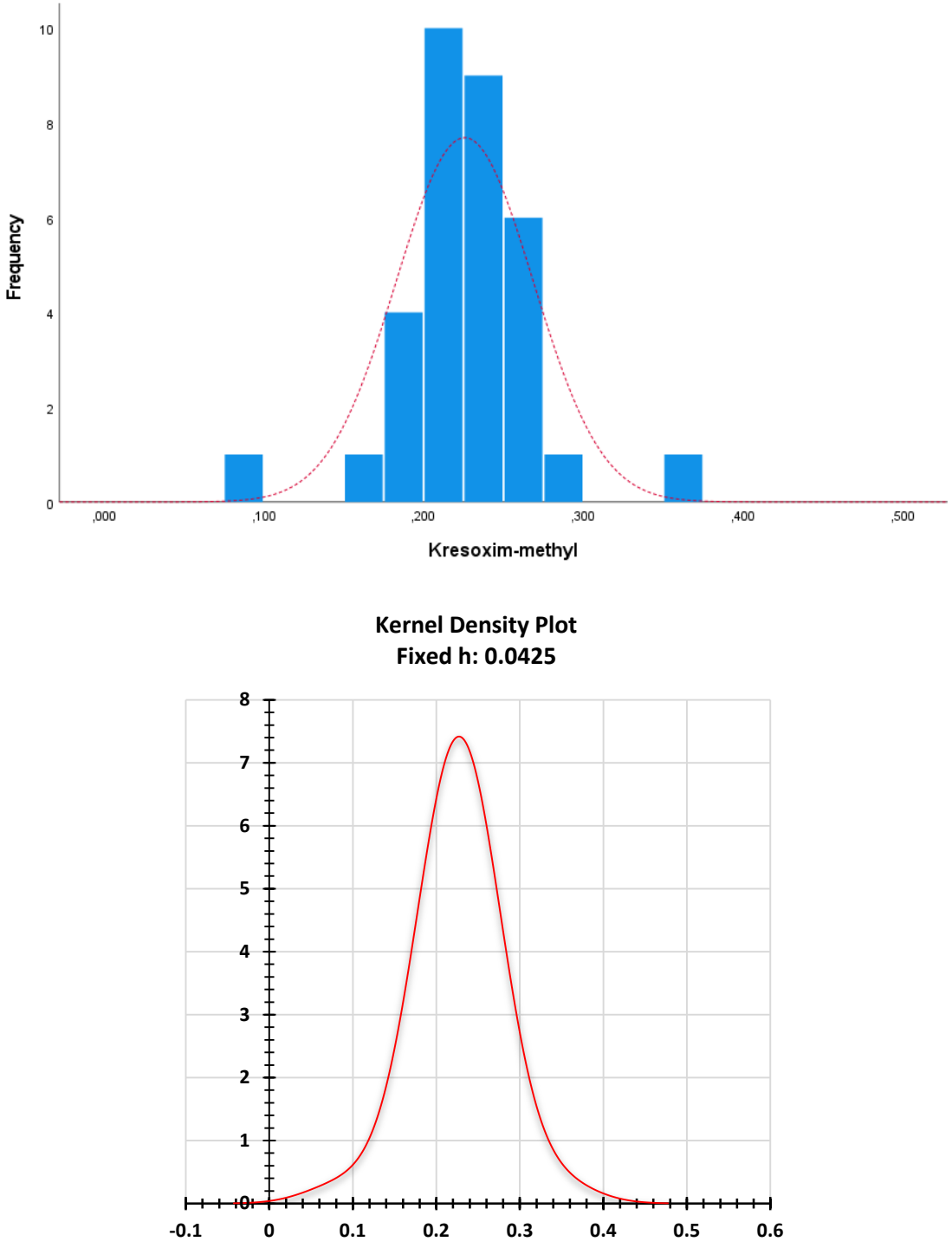


Figure 5. KRESOXIM-METHYL: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of the Kresoxim-methyl results is presented in Table 8.

Table 8. Statistical parameters (mg/kg) of Kresoxim-methyl

Parameter	Value
Spiked value	0.238
Mean	0.226
Median	0.225
Robust mean or Assigned value (mg/kg)	0.227
s*	0.031
σ_{EUPT}	0.057
Uncertainty (u) (mg/kg)	0.007
u/σ_{EUPT} *	0.123
FFP RSD (%)	25
Robust RSD (%)	14

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

The supplied results for Kresoxim-methyl can be considered good, since the data used for the assigned value produced median and robust mean that are similar as absolute value. The Robust Relative Standard Deviation (Robust RSD%) also is good with a value of 14% together with the uncertainty value of 0.007. All z-score values with corresponding recoveries are showed in Table 9 and as graphical representation in Figure 6.

Table 9. KRESOXIM-METHYL: z-score and recovery (%)

Lab Code	z-score	Recovery %
1	-0.6	93
2	0.5	100
3	0.2	102
4	-0.3	110
5	-0.6	STD-add
6	0.0	90
7	-0.1	80
8	-0.1	94
10	-0.1	102
11	0.0	90
12	-0.8	113
13	0.5	103
14	1.0	126
15	-0.1	90
16	-1.1	87
17	-0.3	90
18	0.4	114
19	0.1	85
20	0.8	101
21	0.1	102
22	0.7	98
23	-2.5	86
24	-0.1	81
25	-0.4	91
26	-0.2	96
27	0.3	106
28	0.2	100
30	0.1	103
32	0.4	99
33	2.2	92
34	-0.8	90
35	0.2	102
36	-0.4	96

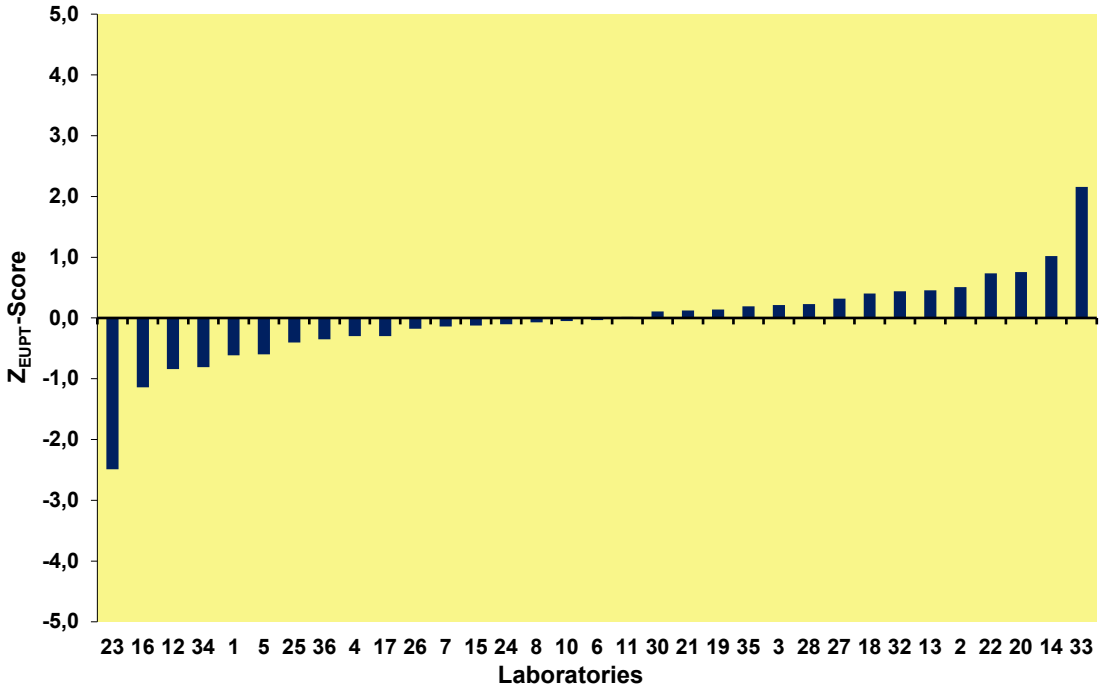
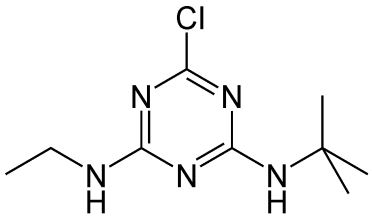


Figure 6. KRESOXIM-METHYL: z-score values (spiked value = 0.238 mg/kg)

Kresoxim-methyl was analysed by all participants with excellent z-score values in the range 0.0-0.8 as absolute value and two questionable z-score values of 2.2 and 2.5.

Terbuthylazine

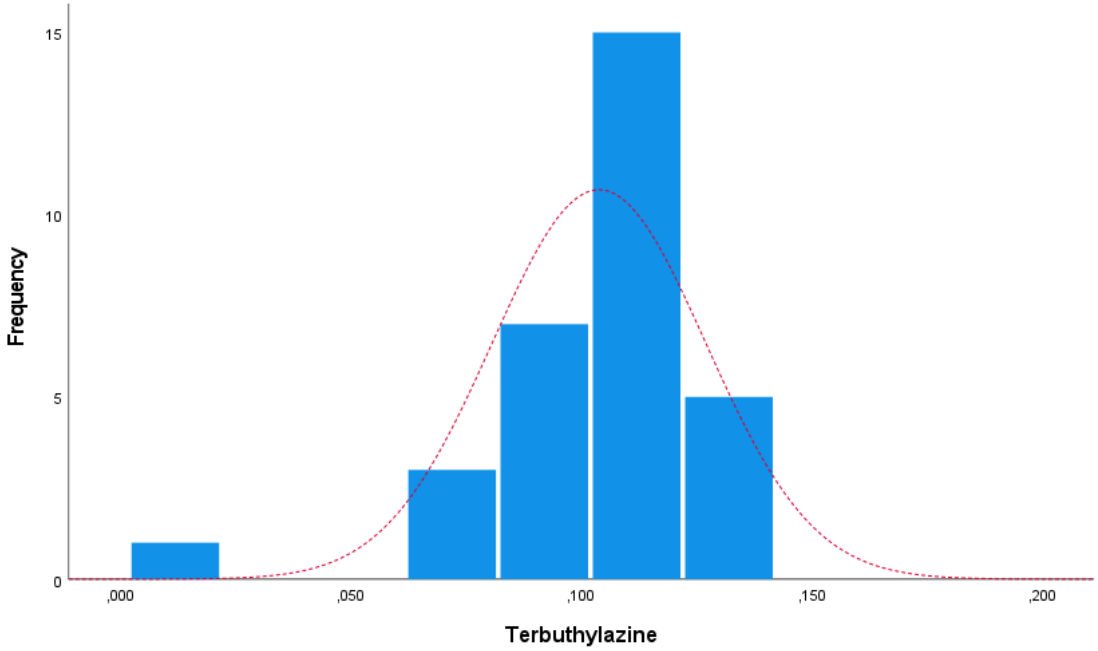


Common name
terbuthylazine

Structure formula	$C_9H_{16}ClNO_5$
CAS number	5914-41-3
EC no.	227-637-9

Its physical form consists in colourless powder with weight molecular of 229.7.
Herbicide at broad-spectrum used in pre- or post-emergence weed control in many crops. This compound has good solubility in organic solvents and good stability.
Not authorized in Italy on sunflower plant with a MRL value of 0.1 mg/kg on sunflower seed as established by the Regulation (EC) 396/2005.
It could be present in sunflower seed oil as contaminant as consequence of his liphofilic properties.

Figure 7 shows the results as frequency histogram together with the Kernel density plot of Terbuthylazine (mg/kg). Also in this case the distribution of supplied data resulted not symmetric.



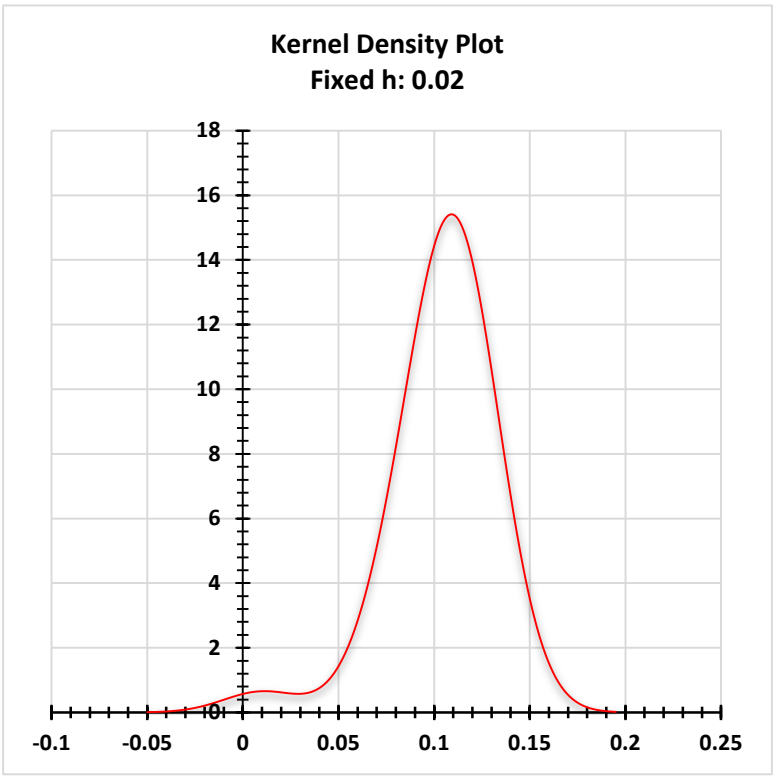


Figure 7. TERBUTHYLAZINE: frequency histogram of the results (mg/kg) and Kernel density plot

Statistical evaluation of Terbuthylazine results is presented in Table 10.

Table 10. Statistical parameters (mg/kg) of Terbuthylazine

Parameter	Value
Spiked value	0.118
Mean	0.104
Median	0.109
Robust mean or Assigned value (mg/kg)	0.107
s*	0.017
σ_{EUPT}	0.027
Uncertainty (u) (mg/kg)	0.004
u/σ_{EUPT} *	0.148
FFP RSD (%)	25
Robust RSD (%)	16

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

Statistically results for Terbuthylazine can be considered satisfactory.

The median and the robust mean in Table 10 are similar with a good value for Robust RSD of 16% as the uncertainty equal to 0.004 mg/kg.

All z-score values with recoveries are presented in Table 11 and as graphical representation in Figure 8.

Table 11. Terbuthylazine z-score and recovery (%)

Lab Code	z-score	Recovery %
1	-0.8	76
2	1.0	100
3	0.3	71
4	-0.3	106
5	0.3	STD-add
7	0.2	90
8	-3.6	89
10	-0.3	100
11	0.4	76
12	-0.6	102
13	0.6	100
14	0.0	-
15	-0.7	74
16	-1.4	70
17	0.1	90
18	-0.1	97
19	0.0	104
20	0.4	97
21	0.4	101
22	0.6	96
24	-0.3	70
25	-1.0	78
26	0.4	104
27	0.1	96
28	0.1	88
30	-0.3	86
32	0.7	98
33	0.9	98
34	-1.1	101
35	0.1	101
35	0.1	95

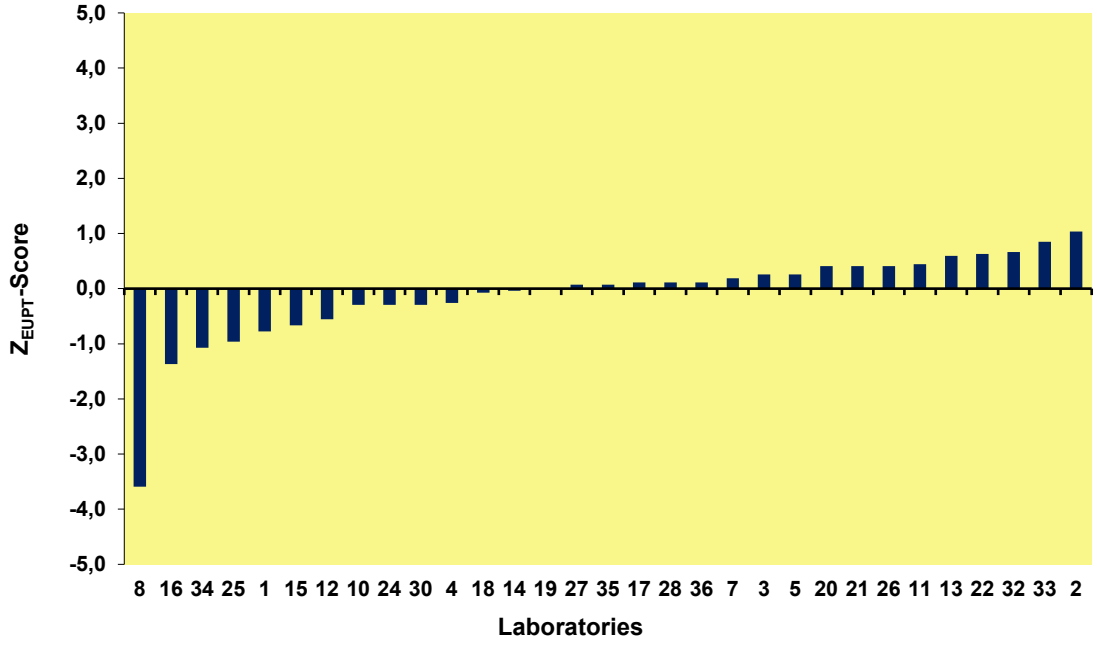
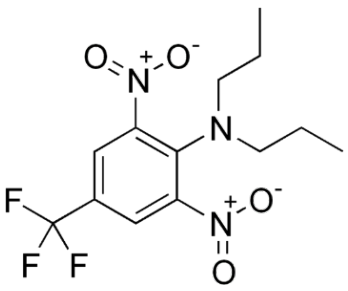


Figure 8. Terbuthylazine: z-score values (spiked value = 0.118 mg/kg)

Terbuthylazine was analysed by thirty-one laboratories out of thirty-three with excellent calculated z-score values all in the range 0.0-0.9 except for Lab 8 with an unacceptable z-score value of -3.6.

Trifluralin



Common name
trifluralin or trifluraline

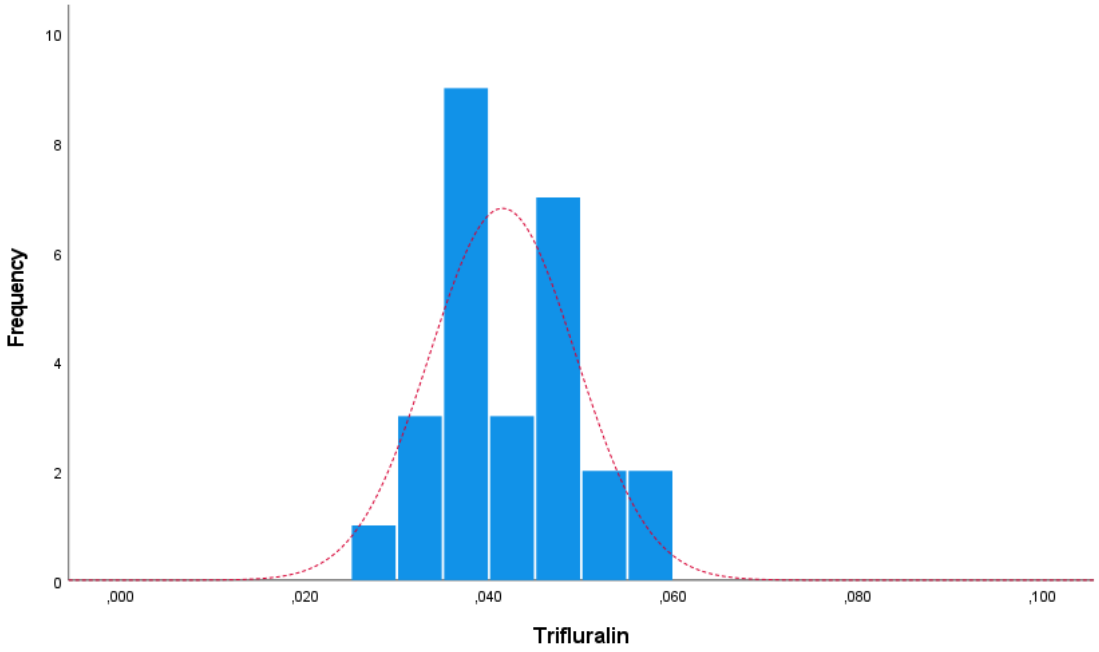
Structure formula $C_{16}H_{22}ClN_3O$

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 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. It is a selective soil-herbicide which acts by entering the seedling in the hypocotyl region and also inhibts root development.
Not authorized in Italy on sunflower plant with a MRL value of 0.01 mg/kg on sunflower seed as established by the Regulation (EC) 396/2005 that correspond at limit of analytical determination.
It could be present in sunflower seed oil as contaminant as consequence of his liphofilic properties.

Figure 9 shows the results of Trifluralin (mg/kg) submitted by all laboratories expressed as frequency histogram. Only for this compound the distribution of data resulted symmetric.



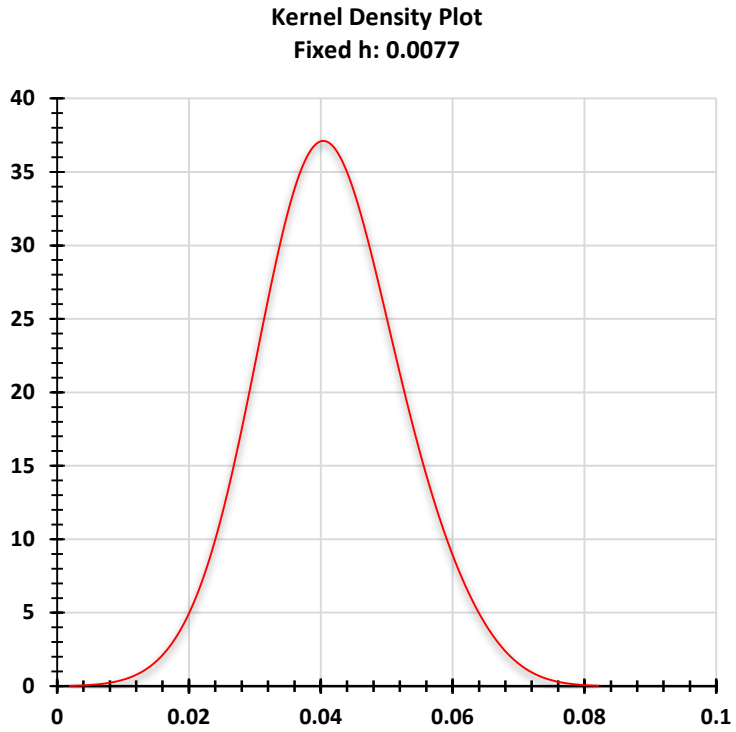


Figure 9. TRIFLURALIN: frequency histogram of the results (mg/kg) and Kernel density plot

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

Table 12. Statistical parameters (mg/kg) of Trifluralin

Parameter	Value
Spiked value	0.048
Mean	0.041
Median	0.040
Robust mean or Assigned value (mg/kg)	0.041
s*	0.008
σ_{EUPT}	0.010
Uncertainty (u) (mg/kg)	0.002
u/σ_{EUPT} *	0.200
FFP RSD (%)	25
Robust RSD (%)	19

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

Statistically results can be considered satisfactory with Media and Robust Mean that have a very similar value. The Robust RSD% and u are good with values of 19% and 0.002 mg/kg respectively

Table 13 reports the calculated z-scores that are presented in graphical form in Figure 10.

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

Lab Code	z-score	Recovery %
2	1.8	100
3	0.1	102
4	-0.9	67
5	-0.5	STD-add
6	-0.7	85
7	0.4	93
8	0.5	92
10	-0.2	100
11	1.2	77
12	-1.6	80
13	-0.4	94
14	-0.4	-
15	1.6	70
17	-0.6	90
20	-0.3	77
21	0.4	85
22	0.6	85
25	1.1	70
26	-0.1	108
27	-0.2	98
28	-0.4	91
30	-0.2	97
32	0.4	89
33	-1.1	100
34	0.5	87
35	0.4	94
36	-0.1	97

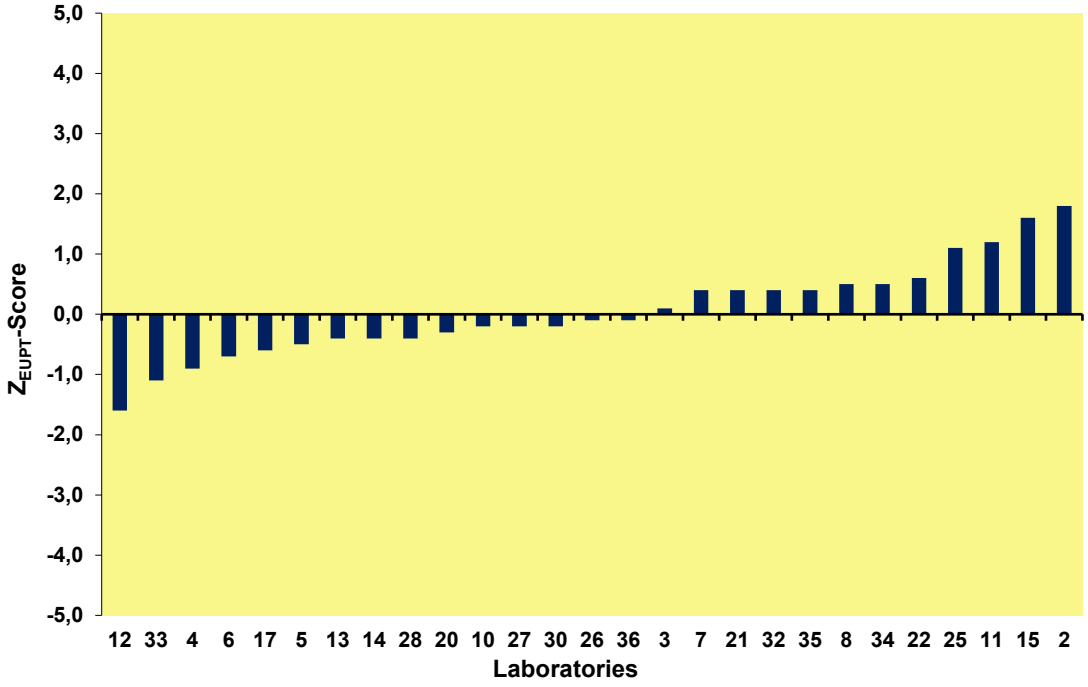


Figure 10. TRIFLURALIN: z-score values (spiked value = 0.048 mg/kg)

Twenty-seven laboratories supplied results for Trifluralin with excellent calculated z-score values in the range 0.1-1.8 but two laboratories 19 and 24 presented methods not consistent with the indicate level of compounds range concentration equal to 0.025-0.300 mg/kg.

AO-PT1: FINAL COMMENTS

From a statistical point of view, the results for all the five 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 (17).

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 12 to 19 % for the five compounds while the range of *u* was from 0.002 to 0.070.

Thirty-three laboratories submitted results and twenty-three (equal to 70%) analysed all compounds with Kresoxim-methyl that was the most analysed compound.

One false negative value was calculated in the case of Lab 14 for Chlorpyrifos.

Regarding Trifluralin two laboratories 19 and 24 have presented a method not consistent with the indicate level of compounds range concentration equal to 0.025-0.300 mg/kg. 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 11 was an accurate representation of the results of the AZ^2.

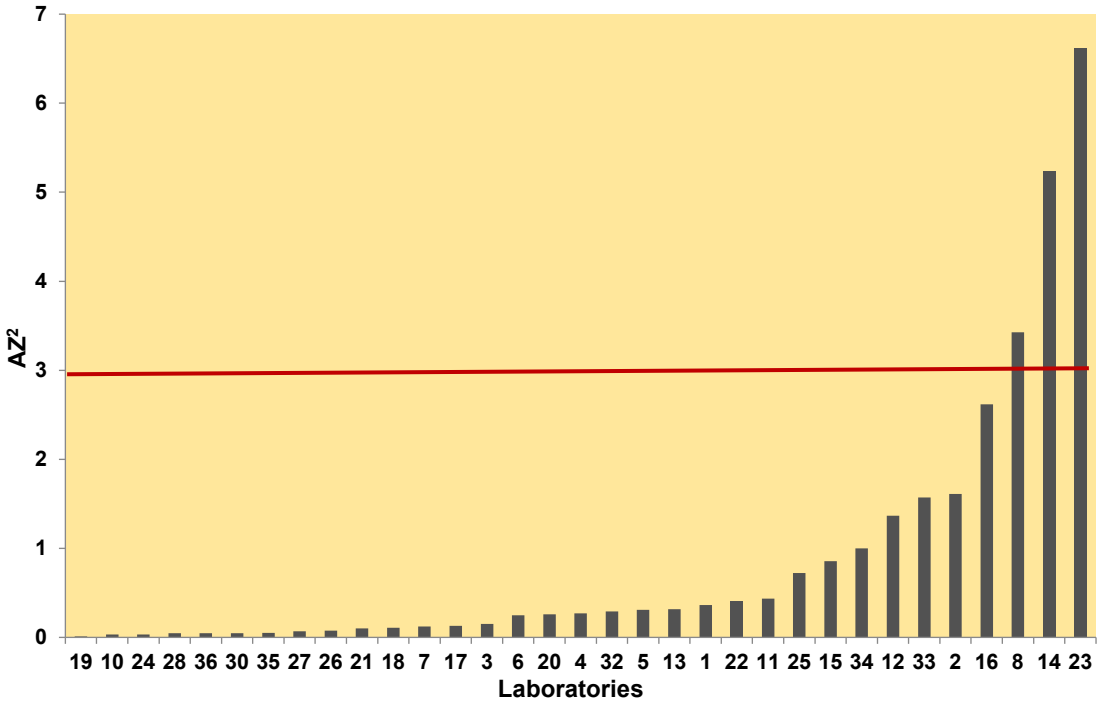


Figure 11. Global performance of laboratories: AZ^2 values

Respect to the analytical methods applied by participants, the majority of laboratories corresponding to nineteen out of thirty-three used the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) methodology or methods based on QuEChERS (18).

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 (19).

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

Three laboratories followed the method QuOil (20).

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 1-10 g while the final analysis volume was between 0.15 and 10 ml.

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

The majority of the laboratories as instrumental detection techniques have used GC (Gas Chromatography) or LC (Liquid Chromatography) coupled with MS/MS detector using two or three transitions. In some cases, selective detectors, as Electronic Capture Detector (ECD), Flame Photometric Detector (FPD) and thermionic Nitrogen Phosphorous Detector (NPD), coupled with GC were used and followed by a confirmation in GC-MS.

In the large part of the cases the quantification has been carried out with matrix calibration at single or multiple levels. Six laboratories used instead the solvent calibration and one laboratory performed the standard addition procedure.

Figure 11 reports the individual z-scores values relatively to QuEChERS method and other methods.

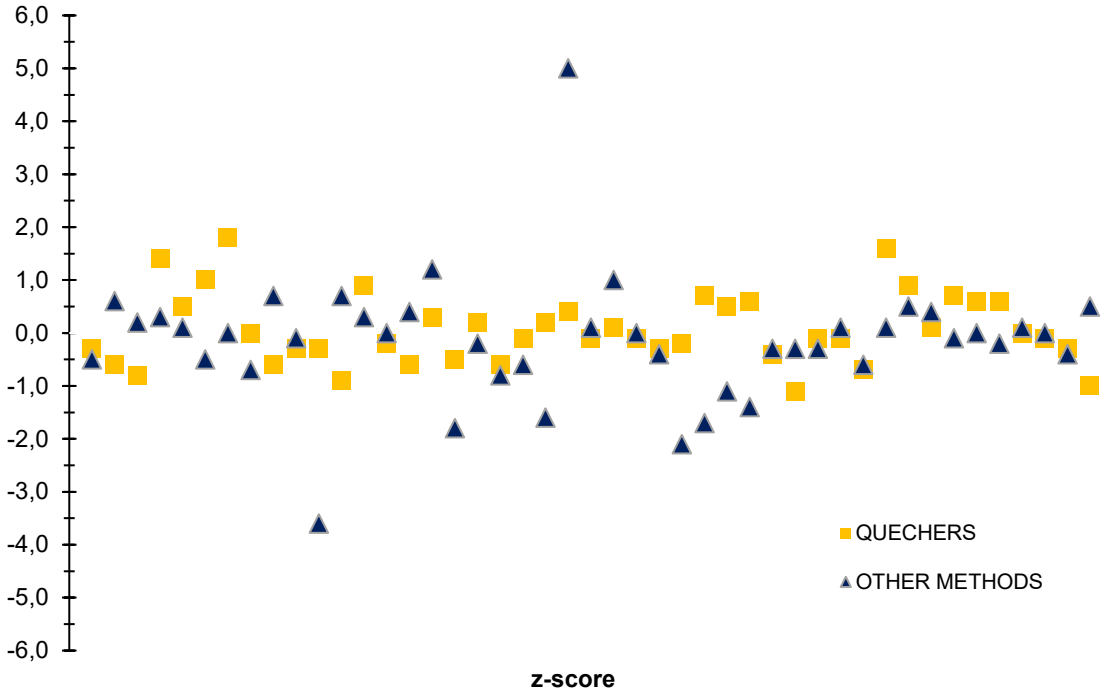


Figure 11. Values of z-score and methods used

In the case of the QuEChERS method the z-score obtained are good with values included in the range ±2 if compared with the other set of data regarding other methodologies where 2 values of z-score resulted unacceptable.

The overall recoveries data submitted by the participants as a control chart are showed in Figure 12.

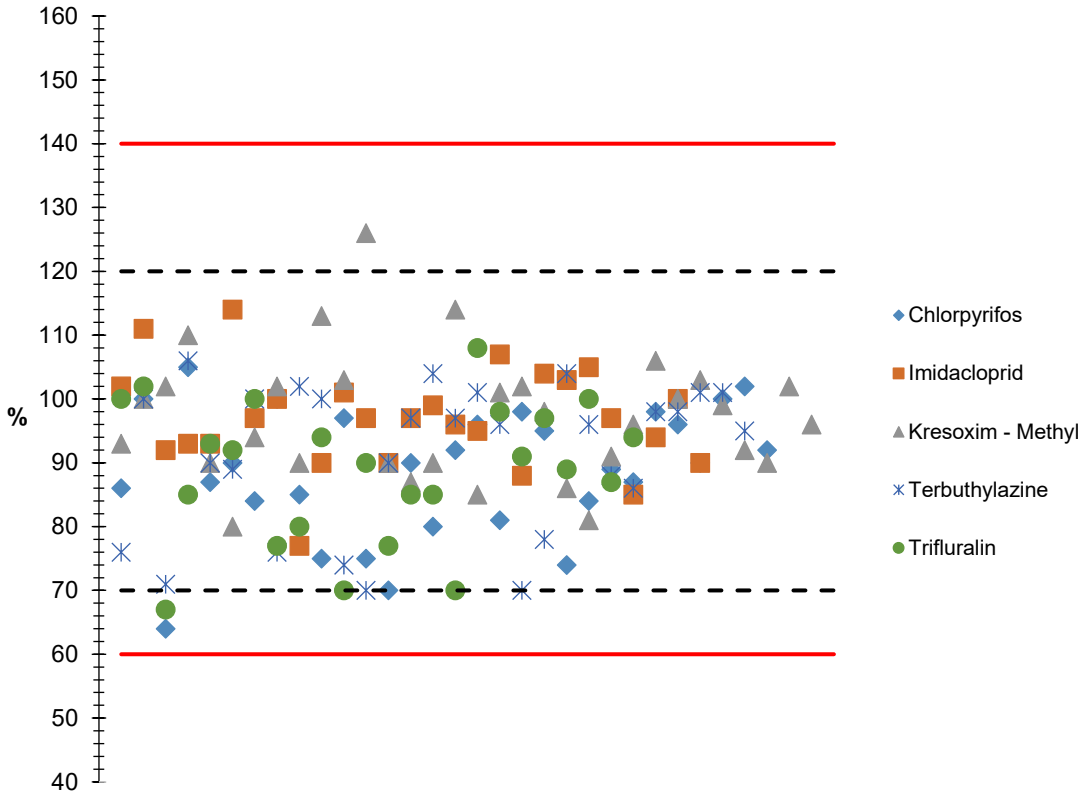


Figure 12. Control chart of the recoveries (%) submitted by the participants

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 recoveries presented are out of the range 70-120% but all submitted recoveries respect the generalized range of 60-140 %.

CONCLUSIONS

The outcome of the AO-PT1 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^2 scores) 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 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 sunflower seed oil.

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http://www.eurl-pesticides.eu/

APPENDIX A
List of participants

The participants in AO-PT1 in 2021 are listed below.

BELGIUM
Primoris Belgium (Zwijnaarde)
FRANCE
ITERG (Pessac)
SCL Montpellier (Montpellier)
GERMANY
Institut Kirchoff Berlin GmbH (Berlin)
GREECE
Benaki Phytopathological Institute, Pesticide Residue Laboratory (Kiphissia)
Chemicotecniki Lagouvardou-Spantidaki O.E. Dikonimou Mkri 1 (Rethymno-Crete)
General Chemical State Laboratory, Pesticide Residues Laboratory, D Chemical Division (Athens)
SKYLAB – Med S.A. (Athens)
UNIHER S.A (Iraklion-Crete)
IRELAND
Pesticide Control Laboratory, Department of Agriculture Food and Marine (Kildare)
ITALY
Agro.biolab Laboratory srl (Rutigliano, BA)
APPA Bolzano, Settore Laboratorio (Bolzano)
ARPA Emilia Romagna Area Fitofarmaci (Ferrara)
ARPA Friuli Venezia Giulia (Udine)
ARPAL La Spezia (La Spezia)
ARPA Lazio (Latina)
ARPA Puglia, Polo di Specializzazione “Alimenti” (Bari)
ATS Milano (Milano)
CADIR LAB srl (Alessandria)
CHEMISERVICE srl (Monopoli, BA)
INNOVHUB-SSI, Divisione SSOG (Milano)
Istituto Superiore di Sanità, Dipartimento Ambiente e Salute (Roma)
IZSLER (Brescia)
IZSLT (Roma)
IZS Piemonte, Liguria e Valle d’Aosta (Cuneo)
IZS del Mezzogiorno (Portici-Napoli)
IZS dell’Abruzzo e del Molise G Caporale (Teramo)
PH TUV sud (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 (Navarra)

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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