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Hakme, Elena; Herrmann, Susan Strange; Poulsen, Mette Erecius

Published in:
Food Control

Link to article, DOI:
10.1016/j.foodcont.2023.109867

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

Food Control 152 (2023) 109867

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

European Union Proficiency Tests for pesticide residues in cereals and feedstuff, from 2007 to 2022- Data collection experience

Elena Hakme *, Susan Strange Herrmann, Mette Erecius Poulsen

National Food Institute, Technical University of Denmark, Kongens Lyngby, Denmark

ARTICLE INFO

Keywords:
Pesticide residues
European laboratory
Z score
False positive
False negative

ABSTRACT

The European Union Reference Laboratory for pesticide residues in cereals and feeding stuff (EURL-CF) has, for 16 consecutive years since 2007, organized European Union Proficiency Tests (EUPTs) for analysis of multiple pesticide residues in various cereals and feedstuff commodities. The EUPTs are directed to National Reference Laboratories (NRLs), and official laboratories (OfLs) in European member (EU) states and in European Free Trade Association (EFTA) countries. The aim of the EUPTs is to gain information regarding the quality, accuracy and comparability of pesticide residue data in cereals and feedstuff reported by different EU and EFTA laboratories, to help evaluate and improve their performance. This article summarizes 16 years' experience in processing and analyzing results for EUPTs for pesticide residues in cereals and feedstuff. It provides an overview of the performance and development of as many as 160 laboratories throughout the years. Results are highlighted in terms of the number of participants, analytical scope, z scores, false positive and false negative reported results, and classification of laboratories. The performance of laboratories in the EUPTs has improved over time. A notable increase was observed in NRLs and OfLs classified as category A, indicating satisfactory overall performance and scope coverage for the majority of participating laboratories.

1. Introduction

The concept of European Union Reference Laboratories (EURLs) is delineated in the regulation (EC) No 882/2004 of the European Parliament and of the Council on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules (European Commission, 2004). According to Article 32 of Regulation 882/2004, the EURLs are responsible for developing and validating new analytical methods and providing National Reference laboratories (NRLs) with details of analytical methods. EURLs are also responsible for the organization of European Union Proficiency tests (EUPTs). On the behalf of the European Commission, the EURL for pesticide residues in cereals and feeding stuff (EURL-CF) annually organize proficiency tests to evaluate the performance of NRLs and OfLs regarding the analysis of pesticide residues in cereals and feedstuff. Laboratories from the European Free Trade Association (EFTA) and from non-EU countries can participate in these EUPTs. The EUPT results provide the EURLs and the European Commission with an overview of the performance of NRLs and official laboratories (OfLs), thus aiding in improving the quality, accuracy and comparability of the results. Proficiency tests are key externals quality controls in monitoring of pesticide residues in food. Proficiency tests analysis allow to evalute the quality and uniformity of analytical results, which can prevent products conform to the food safety regulations being rejected, and products non-conform to the regulations being released to the EU market.

EUPTs also allow laboratories to verify their individual performance and assess the suitability of their methods. If a laboratory fails a proficiency test, corrective actions should be taken, starting by understanding the cause of the poor performance. Moreover, EURL representatives visit every year two of the NRLs underperforming for two consecutive years, to discuss the reasons of the underperformance, to give the laboratories the possibility to express their challenges, to provide scientific recommendations, and to agree on corrective actions for improvement. Evaluating the performance of these laboratories is critical to ensure the highest possible quality of European pesticide residue control and monitoring, to which the NRLs and OfLs contribute. The results of pesticide residue control are used not only for compliance checks with current MRLs but also for ongoing pesticide residue exposure and risk assessment. The quality of the exposure and risk assessment is thus

* Corresponding author.
E-mail address: elehak@food.dtu.dk (E. Hakme).

https://doi.org/10.1016/j.foodcont.2023.109867
Received 9 February 2023; Received in revised form 3 May 2023; Accepted 13 May 2023
Available online 30 May 2023

Table 1
General information on the EUPT-CFs organized during the period 2007–2022.

Year	EUPT number	Test Item	EU and EFTA participant laboratories		Non-European participant laboratories	
			Registered laboratories	Percentage of laboratories that submitted results	Registered laboratories	Percentage of laboratories that submitted results
2007	EUPT-C1/ SRM2	Wheat	63	98%		
2008	EUPT-C2	Wheat	74	99%		
2009	EUPT-C3/ SRM4	Oats	111	93%		
2010	EUPT-C4	Rye	118	98%		
2011	EUPT-C5/ SRM6	Rice	155	97%	21	90%
2012	EUPT-C6	Barley	149	94%	22	86%
2013	EUPT-CF7	Cereal based feed	106	97%	14	71%
2014	EUPT-CF8	Wheat	141	96%	22	68%
2015	EUPT-CF9	Maize	147	97%	16	69%
2016	EUPT-CF10	Rye	160	97%	18	78%
2017	EUPT-CF11	Oats	149	97%	22	91%
2018	EUPT-CF12	Hay	119	93%	11	91%
2019	EUPT-CF13	Rye kernels	152	98%	6	100%
2020	EUPT-CF14	Rice kernels	156	96%	8	100%
2021	EUPT-CF15	Rapeseed cake	129	95%	8	100%
2022	EUPT-CF16	Barley kernels	151	97%	9	100%

directly associated with the quality of residue control, i.e., the performance of the contributing laboratories.

This article summarizes the results and development of EUPTs on cereals and feedstuff over a period of 16 years, from 2007 to 2022. Since 2007, the EURL-CF has organized 16 proficiency tests, one each year, on cereals or feed commodities. The results of each EUPT have been published in reports generated by the EURL-CF (Poulsen, Anastassiades, Kostelac, Christiensen, & Herrmann, 2007, 2008, 2010, 2011, 2013, 2014, 2015, 2016; Poulsen, Herrmann, & Rindom, 2017, 2018, 2019, 2021, 2022; Poulsen & Christensen, 2012; Poulsen & Hakme, 2020; Poulsen, Michelangelo, et al., 2009). The results of EUPTs are collected and evaluated in accordance with the General Protocol for EU Proficiency tests on Pesticide Residues in Food and Feed (General protocol, 2023). Herein, we describe the preparation of test material, the reporting webtool, and how the data and results were interpreted in terms of the number of participants, target list, scope of coverage, standard deviation of assigned values, z score and overall z score, and laboratory classification.

2. Materials and methods

2.1. EUPTs methodology

2.1.1. Preparation of the test items

All test items used have been based on cereal/feedstuff grown and treated in the field with a mixture of pesticide formulations. Untreated crops were grown together with sprayed crops and used as blank material. The raw materials except for two EUPTs were grown in Denmark and sprayed in the field by the Faculty of Agricultural Sciences, University of Aarhus. The exceptions were EUPT-C5 and EUPT-C14, wherein the test item was rice and thus was not suitable for cultivation in Denmark. For EUPT-C5, the rice was grown in Brazil. And the spraying was performed by the Universidade Estadual de Ponta Grossa, Brazil, in collaboration with the Federal University of Santa Maria, Brazil. Likewise, for EUPT-CF14, the rice was grown and sprayed in India in collaboration with InDepth Management India Pvt. Ltd. and the EURL for Residues of Pesticides–Single Residue Methods (EURL-SRM). EUPT-C1, C4 and C5 were organized in collaboration with the EURL-SRM. EURL-SRM is responsible for providing EUPTs covering pesticides that are not amenable to multiple residue methods or are difficult to analyze, thus requiring single residue methods. The test items (EUPT-C1, C4 and C5) were therefore sprayed with pesticides amenable to multiple residue methods as well as pesticides requiring analysis with a single residue method.

2.1.2. EUPTs selected pesticide formulations

The crops were sprayed with different pesticide formulations, and when the spraying was performed in Denmark, primarily pesticides authorized there were used. The doses used for spraying were approximately two to three times the standard application rate, to ensure residues higher than 0.030 mg/kg in the test material. The test items were typically also treated postharvest in the laboratory. Postharvest treatment was applied in cases in which relevant formulations were not approved for use in Denmark or the application of combined formulations resulted in damage to the crop. In some cases, in the laboratory, additional pesticides were spiked in if the residues obtained as a result of spraying in the field were lower than the lowest reporting level for the pesticide in question. Primarily insecticides and fungicides were included in the PTs, but herbicides were also included. Pesticides that are widely used within the EU were prioritized to be included in the EUPTs. A total of 121 pesticides were included in one or more of the 16 EUPT test items. Often, one pesticide from the previous year was required to be included in the test item of the next EUPT. Azoxystrobin was included in all EUPTs. Carbendazim was included in the test items of 13 EUPTs. Four pesticides were included in eight to ten PTs (pyraclostrobin, epoxiconazole, boscalid and tebuconazole). Forty-five pesticides were included in two to five PTs, and 70 pesticides were included once. The minimum number of pesticides included in the test item was six (EUPT-C1), and the maximum number of pesticides included in the test item was 23 (EUPT-CF7).

2.1.3. EUPTs selected commodity

Table 1 shows the 16 EUPTs, organized from 2007 to 2022, with the EUPT number name, the commodity, the number of participating laboratories from the EU, EFTA, and non-European countries, as well as the number of laboratories that submitted results. The EUPTs covered wheat (EUPT-C1, EUPT-C2 and EUPT-C8), rye and rye kernels (EUPT-C4, EUPT-CF10 and EUPT-CF13), oats (EUPT-C3 and EUPT-CF11), rice and rice kernels (EUPT-C5 and EUPT-CF14), barley and barley kernels (EUPT-C6 and EUPT-CF16), and feed (cereal based EUPT-CF7, maize EUPT-CF9, hay EUPT-CF12 and rapeseed cake EUPT-CF15). The EUPTs were organized to cover different matrices regarding the item composition and the complexity of analysis, e.g., wheat and rye with low amount of fats; and oats, rice and barley, with relatively high amounts of

fat, and for which analysis of pesticide residues might be more challenging. The same type of commodities was tested again after an interval of several years, to assess the development in performance of the laboratories.

2.1.4. Homogeneity and stability tests of the test items

Field and laboratory treated test items were homogenized. Approximately, 100 g portions were weighed into screw-capped polyethylene plastic bottles, sealed, numbered and stored in a freezer at approximately −20 °C until shipment to the participants. A blank sample was included in the package, until 2020. Starting with EUPT-CF14, blank material was no longer provided, because having the laboratories find their own appropriate blank samples was similar to the real-world situation in which laboratories receive and analyze real samples for which a blank sample is not available. This new approach therefore provides a better picture of the performance of the laboratories in routine analysis.

The test item was verified for homogeneity before shipment to the participants. Eleven bottles of the test item were randomly chosen and analyzed in duplicate. The statistical evaluation was performed according to the International Harmonized Protocols published by IUPAC, ISO and AOAC (Thompson, Ellison, & Wood, 2006). The acceptance criteria for sufficient homogeneity for the proficiency test was $S_S^2 < c$, where Ss is the between-bottle sample standard deviation, and $c = F_{1\times\sigma_{all}^2}+F_{1\times s_{an}^2}$, where F1 and F2 are constants with values of 1.83 and 0.93, respectively, from the 11 samples taken, $\sigma_{all}^2 = 0.3 \times$ FFP RSD (25%) $\times$ the analytical sampling mean for all pesticides, and s_{an} is the estimate of the analytical standard deviation. A test item was considered homogeneous when all pesticides passed the homogeneity test.

The test item was also verified for stability according to ISO 13528 (ISO 13528, 2015). The stability of the pesticides in the treated test Item was verified three times during the period from shipment to the results submission deadline. Two storage temperatures were tested: room temperature and −18 °C. Six sub-samples (analytical portions) were analyzed on each test day. A pesticide was considered adequately stable if $|x_1–y_i| \leq 0.3 \times \sigma$, where x_1 is the mean value of the first stability test, y_i is the mean value of the last stability test, and σ is the standard deviation used for proficiency assessment (25% of the assigned value).

2.1.5. Communication and data collection policy

An invitation in the form of an announcement letter was sent out each year to all NRLs and OfLs at least 3 months before the planned day of shipment. Together with the announcement, a calendar was published containing important dates, e.g., the deadline for registration, shipment of samples, and deadline for result submission. Additionally, a pesticide target list enumerating the names of the pesticides that should be targeted and the minimum required reporting levels (MRRLs) that the participants were requested to meet were published. All documents were made available on the EURL website (EURL-CF, 2023) and the CIRCA BC platform (*CIRCA, 2023*). The participants were requested to register with the online registration link and to provide information on contact persons, delivery and invoice addresses. A scientific protocol for each EUPT is published on the EURL-CF website including information on the handling of storage of the test item.

The participants received a link to the DTU webtool as well as login credentials, and were asked to enter the webtool and select the scope of pesticides for which they wanted to be evaluated. Before the laboratories receive the EUPT material, they are asked to register their scope, i.e., which of the pesticides included on the scope list for the specific EUPT they intend to analyze and submit results for. The laboratories were asked to verify the state of the sample on receipt and to report in the webtool whether they would accept or reject the test item. The participants also submitted their results via the webtool. All participants had access to the result-submission website from several days after shipment until the result-submission deadline. Participants were asked not only to report their analytical results but also to provide additional information regarding accreditation status, reporting limits and details regarding the applied methods. In 2018, starting with EUPT-CF12, adding method information on the webtool became mandatory.

2.2. Statistical and other evaluation methods

2.2.1. False positives and negatives

False positives are the results above the MRRL that show the apparent presence of pesticides in the Target Pesticide List that were: (i) not detected by the organizer, even after repeated analysis, and (ii) not detected by the overwhelming majority of the participating laboratories (e.g., 95% of the laboratories) that targeted the specific pesticide. False negatives are results for pesticides reported by the laboratories as analyzed but for which quantitative values were not reported, although they were used by the organizer to treat the test item, and they were detected by the organizer as well as most participants that had targeted these specific pesticides at or above the respective MRRLs (EU Reference Laboratories for Residues of Pesticides, 2019).

2.2.2. Assigned values and target standard deviation

Various methods and calculations can be used to achieve the assigned value (Medina-Pastor, Fernández-Alba, Andersson, & Rodríguez-Torreblanca, 2010); however, for a test item where the residue concentrations are unknown due to the field treatment, a consensus value is used. Because the number of participants was quite high (approximately 150), the assigned values were calculated from all reported results. For EUPTs before EUPT-CF7, the median was used as the assigned value. For EUPT-CF7 and later PTs, the assigned values were calculated as the algorithm A mean, including the reported results submitted by laboratories from EU and EFTA countries (Poulsen, Andersen, & Herrmann, 2014). The assigned values for the residues in the test items were between 0.030 and 11.6 mg/kg. Because earlier experience indicated significantly biased results from laboratories not adding water to the sample prior to extraction (or using a mixture of water and extraction solvent), these results were not included in the calculation of the algorithm A mean (Poulsen, Christensen, et al., 2009).

The target standard deviation was a fixed FFP-RSD value of 25%, according to experience from the earlier EUPT-FV (Medina-Pastor et al., 2010). In parallel, the algorithm A standard deviation (Alg A-RSD) was calculated for informative purposes only. The uncertainty of the assigned values is calculated according ISO 13528 (ISO 13528, 2015) as $\mu = s^*/\sqrt{n}$, where s^* is the robust standard deviation estimate, and $\sqrt{n}$ is the number of data points equal to the number of results used to calculate the assigned value.

2.2.3. Z score calculation

A z score for each laboratory/pesticide combination was calculated according to the following equation: $z_i = (x_i - x_{pt})/(FFP - \sigma_{pt})$, where x_i is the value reported by the laboratory, x_{pt} is the assigned value, and $FFP - \sigma_{pt}$ is the standard deviation.

Z scores were rounded to one decimal place. Z scores are interpreted in the following way as set in ISO 13728:2022 (ISO 13528:2022, 2022); | z 2 ≤ | acceptable; 2 < | z 3 < | questionable; and | z 3 ≥ | unacceptable.

2.2.4. Combined z score and category classification

Most pesticide residues are analyzed with a so-called multimethod covering many commodities and pesticides. For evaluating the overall performance of the laboratories, a combined z score was introduced from EUPT-C1, on the basis of the principle described by Rosario, Martínez, and Silván (2008). However, the calculation was changed slightly in EUPT-CF7 (Poulsen, Andersen, & Lykkeverg, 2013), wherein an average of the squared z scores (AZ^2 score) was introduced.

However, the combined z scores are relevant only for laboratories that analyze and detect sufficient numbers of pesticides. Consequently, the laboratories are classified in categories A or B. Laboratories that a)

Table 2
Number of compulsory and voluntary pesticides in the target list, and number and assigned values of pesticides in the test item.

Year	EUPT number	Number of compulsory pesticides in the target list	Number of voluntary pesticides in the target list	Number of pesticides present in the test item	Minimum and maximum assigned values (mg/kg)
2007	EUPT-C1/SRM2	33		6	0.078; 0.353
2008	EUPT-C2	53		12	0.038; 0.570
2009	EUPT-C3/SRM4	65		11	0.011; 1.23
2010	EUPT-C4	71		16	0.078; 3.87
2011	EUPT-C5/SRM6	112		16	0012; 0.813
2012	EUPT-C6	107		18	0.083; 0.943
2013	EUPT-CF7	116		23	0.057; 0.333
2014	EUPT-CF8	111		17	0.040; 0.766
2015	EUPT-CF9	117		18	0.044; 0.429
2016	EUPT-CF10	134	7	16	0.032; 0.347
2017	EUPT-CF11	153	9	17	0.050; 0.706
2018	EUPT-CF12	155	9	7	0.799; 11.6
2019	EUPT-CF13	160	32	15	0.025; 0.334
2020	EUPT-CF14	164	38	16	0.010; 0.308
2021	EUPT-CF15	172	43	12	0.012; 0.567
2022	EUPT-CF16	169	53	15	0.0016; 4.395

can analyze at least 90% of the compulsory pesticides in the target pesticide list, b) have correctly detected and quantified a sufficiently high percentage of the pesticides present in the test item (at least 90%) and c) report no false positives, have sufficient scope to be classified in category A. For these laboratories, the AZ^2 score is calculated as follows: $AZ^2 = (\sum_{i=1}^{n} Z_i^2)/n$, where n is the number of z scores that were considered in this formula. For the calculation, any z score >5 is set at 5. On the basis of the AZ^2 achieved, the laboratories are classified as follows:

$AZ^2 \leq 2$: good; $2 < AZ^2 < 3$: satisfactory; and $AZ^2 \geq 3$: unsatisfactory.

Laboratories that do not meet the criteria described above are classified in category B.

3. Results

3.1. Participants

The number of EU and EFTA laboratories participating in the EUPTs on cereals, as shown in Table 1, increased steadily over the years, from 63 in 2007 to 156 in 2020. The highest number of participants (160) was observed with EUPT-CF10, in 2016, on rye. Fewer laboratories participated in the EUPTs organized for feed; 106 participated in EUPT-CF7 on cereal-based feed, 119 participated in EUPT-CF12 on hay, and 129 participated in EUPT-CF15 on rapeseed cake. The reason for this lower number of participants is that not all laboratories have feed in their scope and therefore are not obliged to participate.

Since 2011, countries outside Europe were invited to participate in the PTs organized by the EURL-CF for the first time. Laboratories from 30 non-European countries participated in one or more of the 16 EUPTs. Laboratories from Brazil, India, Argentina, Singapore, China and Thailand participated in more than eight EUPTs. The highest numbers of participating laboratories from non-European countries were in 2012 for EUPT-CF6 on barley and in 2017 for EUPT-CF11 on oats. As shown in Table 1, some registered laboratories did not submit results because to the test item did not reach the laboratory as a result of difficulties in custom clearance. Other reasons for EU, EFTA or non-European laboratories not submitting results included instrument failure or other analytical errors.

3.2. Pesticides target list and laboratories' scope

The selection of pesticides included in the target list is based on pesticides included in the scope of the EU multi-annual coordinated control program (*EU Multiannual Monitoring Programme, 2023*), the EURL working document (*EURL-CF Working Documents, 2023*), and pesticides found to be relevant to feed and/or cereal production in Europe and in other parts of the world from which substantial quantities of feed and cereals are imported. Table 2 shows the number of compulsory pesticides included in the target list and the number of voluntary pesticides, as well as the number of pesticides present in the test item for each EUPT. The number of compulsory pesticides included

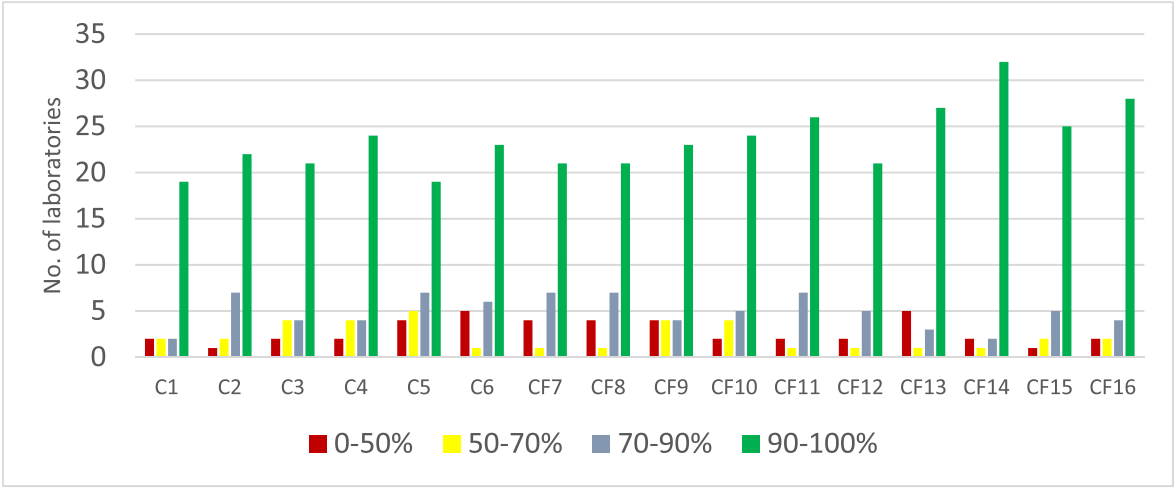

Fig. 1. Average scope of coverage by NRLs.

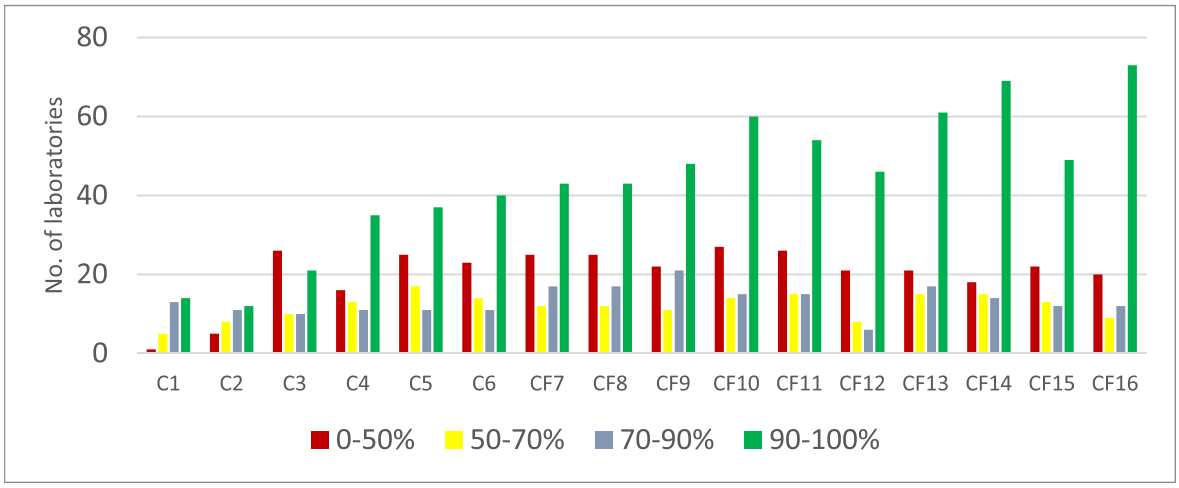

Fig. 2. Average scope of coverage by OfLs.

in the target list increased from 33 in 2007 to 172 in 2021, owing to a demand by the EU commission and member states to increase the scope of analysis. In 2016, several voluntary pesticides were included in the target list for the first time. The number of voluntary pesticides increased from seven in 2016 to 53 in 2022. In 2022, the number of compulsory and voluntary pesticides decreased, owing to the changes in the residue definition for enforcement of some compounds, e.g., spirotetramate. The residue definition of spirotetramat included spirotetramat metabolite enol-glucoside, spirotetramat-enol, spirotetramat-ketohydroxy and spirotetramat-monohydroxy. In 2020, the residue definition was changed to the sum of spirotetramat and spirotetramat-enol only, expressed as spirotetramat (Abdourahime et al., 2019; European Commission, 2022, pp. 68–107). Accordingly, the target list was updated, and the three other metabolites of spirotetramat were removed.

The average scope coverage for participating NRLs and OfLs is presented in Figs. 1 and 2, respectively. On average, 70% of NRLs and 48% of OfLs covered more than 90% of the pesticides on the compulsory target list. The number of NRLs covering more than 90% the target list increased from 19 for EUPT-C1 to 32 for EUPT-CF14, and then decreased to 28 for EUPT-CF16. The number of OfLs covering more than 90% of the target list increased from 14 laboratories for EUPT-C1 to 73 for EUPT-CF16. Massive developments were observed in the performance of OfLs, with the target list increasing every year.

Enlarging the scope usually requires purchase of reference standards or certificate materials, optimizing MRM transitions for each new pesticide on the GC- or LC-MS/MS instruments and, most importantly, validating the method for these new pesticides in different food commodities according to SANTE guidelines (P.Tuija, A.R. Fernández-Alba, C.F. Amate, M.E. Poulsen, B.Hardebusch, 2021) and/or ISO 17025. Thus, expanding the analytical scope may be difficult for some laboratories, owing to a lack of financial resources or staff. However, the laboratories' striving to cover the target list is critical.

3.3. Methods of analysis

Participating laboratories are instructed to use the analytical procedure(s) that they would routinely employ in official control activities.

3.3.1. Sample preparation procedures

The standardized European method for the analysis of pesticide residues in cereals, EN 15662:2018, consists of acetonitrile extraction and clean-up by dispersive SPE followed by analysis by GC-MS/MS and LC-MS/MS (EN15662, 2008). Some modifications have been implemented in many laboratories, depending on the matrix commodity. Experience has indicated that the two most important steps in the analysis of dry matrices such as cereals are the addition of water before extraction and the freezing out step before d-SPE clean-up. Other modifications may be implemented depending on the need for additional clean-up. For instance, a different ratio of acetonitrile/water, or different SPE sorbents or combinations of sorbents, may be implemented according to the type of cereal or feedstuff under analysis.

On the basis of the results from EUPT-CF2 on wheat, the EURL-CF recommended the addition of water before extraction to improve the extraction efficiency of pesticide residues from low moisture containing commodities such as cereals (Poulsen, Anastassiades, et al., 2009). The recommendation was introduced to the guidance document on analytical quality control and validation procedures for pesticide residue analysis in food and feed, SANTE 11945/2015. After this, more than 90% of the laboratories reporting information on their analytical methods added water to the sample before extraction. For CF12, 13 and 14, more than 96% of the laboratories reported that they added water before extraction.

Citrate buffer QuEChERS (EN15662) was the extraction method used by 60% of the laboratories before 2017. Later, with EUPT-CF12, 13, 14, 15 and 16, more than 70% of the laboratories used this method. The mini-Luke method was used by 6–7% of the laboratories in 2015–2016, but during the past 3 years, this number has decreased to 2–3%. The percentage of laboratories using the original QuEChERS version method J. AOAC 86, 2003 (7%) (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003), QuEChERS buffered (AOAC official method 2007.01) (AOAC, 2007) (7%) and SweET method (CEN/TS 17743, 2022) (4%) did not change over the years.

When citrate buffer QuEChERS (EN15662) was applied, it was combined with a clean-up step using dispersive-SPE consisting of a mix of sorbents. The sorbent PSA/$MgSO_4$ was used by an average of 60% of the laboratories, and in the last PT (EUPT-CF16), 71% of the laboratories used this sorbent combination. Other sorbents, such as ODS and $MgSO_4$, are also used. Until EUPT-CF12 (2018), the freezing-out step was used as an additional clean-up step by less than 20% of the laboratories that provided information on their analytical method. From EUPT-CF13 until the latest PT, more than 25% of the laboratories used the freezing-out step, and the highest percentage of laboratories used the freezing-out step (32%) for EUPT-CF15 on rapeseed. The freezing-out step allows for the precipitation of poorly soluble matrix co-extractive components, thus decreasing matrix interference. Moreover, cleaner extracts result in less need for maintenance of the instrument (liner changes, column cuts and ion source cleaning).

Table 3
Number of false positive results in the period 2007–2022 and the detection technique used to report the results.

Year	EUPT number	Commodities	Number of false positive results	Number of laboratories reporting one false positive result	Number of laboratories reporting two false positive results	Number of laboratories reporting more than two false positive results	Number of false positives reported with MS/MS	Number of false positives reported with other detection (MS, MSD, ion trap or ECD)	Number of false positive reported with HRMS (Q-ToF. Q-Orbitrap)
2007	EUPT-C1/ SRM2	Wheat	1	1	0	0			
2008	EUPT-C2	Wheat	2	2	0	0			
2009	EUPT-C3/ SRM4	Oats	4	1	0	1			
2010	EUPT-C4	Rye	17	8	1	2			
2011	EUPT-C5/ SRM6	Rice	10	7	0	1	4	5	
2012	EUPT-C6	Barley	2	2	0	0	1		
2013	EUPT-CF7	Feed	12	8	2	0	5	6	
2014	EUPT-CF8	Wheat	4	4	0	0	2	1	
2015	EUPT-CF9	Maize	9	7	1	0	7	2	
2016	EUPT-CF10	Rye	0	0	0	0			
2017	EUPT-CF11	Oats	19	16	0	1	14	4	
2018	EUPT-CF12	Hay	7	7	0	0			
2019	EUPT-CF13	Rye kernels	3	1	1	0	3		
2020	EUPT-CF14	Rice kernels	14	12	1	0	10	4	
2021	EUPT-CF15	Rapeseed cake	9	5	2	0	6	3	
2022	EUPT-CF16	Barley kernels	25	19	3	0	19		2

3.3.2. Analytical instruments

Quantitative pesticide residue analysis is usually performed by unit mass resolution, GC-MS/MS and LC-MS/MS, which offer good selectivity and sensitivity. According to the SANTE guidelines, the requirements for identification of a pesticide in a given sample are a minimum number of two product ions; a signal to noise ratio >3; overlapping analyte peaks from both product ions in the extracted ion chromatograms; and an ion ratio from sample extracts within ±30% (relative) of the average of calibration standards from same sequence (P. Tuija, A.R. Fernández-Alba, C.F. Amate, M.E. Poulsen, B.Hardebusch, 2021). Other chromatographic and detection techniques may be used, as long as satisfactory performance can be documented. New trends include the use of high-resolution mass spectrometry (ToF, qTOF, Orbitrap and Q-Orbitrap-MS) and data processing by target screening or non-target screening against in-house or commercial databases containing more than 500 pesticides.

Most laboratories used GC and LC-MS/MS for analytical determination in all EUPTs. The use of GC-MS/MS and LC-MS/MS increased from 61% in EUPT-C5 to 92% in EUPT-CF15. It then decreased to 90% in EUPT-CF16, because some laboratories implemented high resolution mass spectrometry (HRMS) for quantitative pesticide analysis. The use of HRMS in the analysis of PT samples started as early as 2012 with EUPT-C6, wherein two laboratories reported some results based on GC-ToF-MS and LC-Orbitrap-MS. In the period of 2015–2021 (C8 to CF15), five to six laboratories used HRMS (GC-ToF, LC-ToF, LC-Q-ToF, LC-Orbitrap and/or LC-Q-ToF-Orbitrap). In 2022, eight laboratories reported results based on HRMS. Among all HRMS methods, GC-Orbitrap was not reported to be used. The use of single quadrupole and ion trap LC-MS and GC-MS decreased significantly from 2011 (19%) to 2016 (10%). In the last six PTs, an average of 5% of laboratories used these detectors. The percentage of laboratories using electron capture detectors (ECDs) also decreased from 18% in 2011 to 1% in 2022.

Thus, laboratories use diverse methods to monitor pesticide residues in cereals and feedstuff. Regardless of the method chosen by laboratories, validation in accordance with ISO 17025:2017 is required, and comparable results should therefore be achievable across laboratories and methods.

3.3.3. Quantification approaches

Signal suppression and enhancement are frequently observed in LC-MS/MS and GC-MS/MS. However, these effects can largely be compensated for by the use of matrix-matched calibrations, wherein standard solutions are diluted in an extract of a control sample commonly referred to as matrix. This approach is used by most laboratories. However, other compensatory approaches can also be used, such as procedural standard calibration, in which the calibration standards undergo the full analytical method, or standard addition, either to the sample or to the extract.

3.4. False positives and negatives

Table 3 shows the total number of false positives reported by all the laboratories with each EUPT. The table also indicates the number of laboratories reporting less than one, two, and more than two false positives. The number of false positives reported with the use of MS/MS, HRMS, and other detectors is also shown in Table 3. The highest number

Table 4
Number and percentage of false negative results and number of laboratories reporting false negative results with each EUPT.

Year	EUPT number	Number of EU and EFTA laboratories submitting results	Number of pesticides present	Maximum number of possible determinations	Number of false negatives	Percentage of false negatives	Number of laboratories reporting false negative results	Number of laboratories reporting one false negative result	Number of laboratories reporting more than one false negative result
2007	EUPT-C1/SRM2	62	6	323	8	2.48%	7	6	1
2008	EUPT-C2	73	12	574	17	2.96%	12	7	5
2009	EUPT-C3/SRM4	103	11	961	28	2.91%	14	8	6
2010	EUPT-C4	116	16	1467	51	3.48%	35	26	9
2011	EUPT-C5/SRM6	151	16	1690	51	3.02%	27	23	4
2012	EUPT-C6	140	18	1741	20	1.15%	15	11	4
2013	EUPT-CF7	103	23	1932	50	2.59%	22	9	13
2014	EUPT-CF8	135	17	1893	42	2.22%	27	22	5
2015	EUPT-CF9	143	18	2012	42	2.09%	27	17	10
2016	EUPT-CF10	155	16	2100	25	1.19%	17	12	5
2017	EUPT-CF11	145	17	2172	114	5.25%	49	26	23
2018	EUPT-CF12	111	7	733	7	0.95%	4	2	2
2019	EUPT-CF13	149	15	2007	47	2.34%	23	16	7
2020	EUPT-CF14	150	16	2298	79	3.44%	49	34	15
2021	EUPT-CF15	122	12	1315	25	1.90%	19	16	3
2022	EUPT-CF16	155	15	2091	92	4.40%	53	31	22

of false positives was observed for EUPT-CF16 on barley kernels with 19 laboratories reporting one false positive result and three laboratories reporting more than one false positive results. There could be multiple factors that contributed to the false positive results, such as sample preparation, analytical method, and contamination during analysis. In the EUPT-CF11 on oats, 16 laboratories reported one false positive result and one laboratory reported more than one false positive results. These findings were probably because oats are a difficult matrix to analyze. Oats are the cereal type containing the highest amount of fat. The free fatty acids can interfere with the co-eluting pesticides, thus resulting in distorted peaks, shifted retention times and ion suppression or enhancement. Moreover, the oats used in EUPT-CF11 were not freshly milled. Samples of oat flour stored for several months require more extensive cleanup than freshly milled samples (Herrmann & Poulsen,

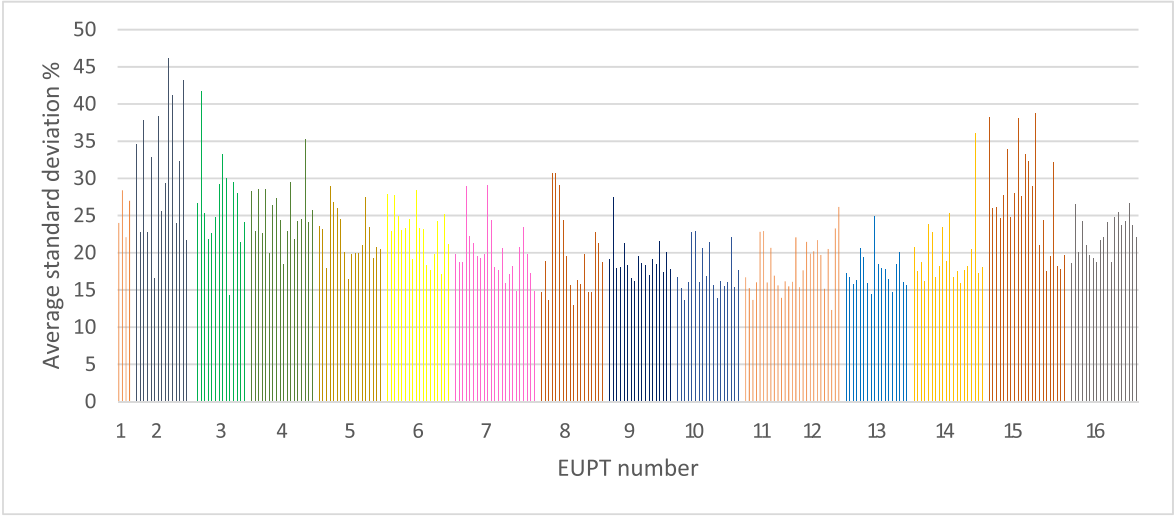

Fig. 3. Robust average standard deviations obtained with each pesticide residue in all EUPTs.

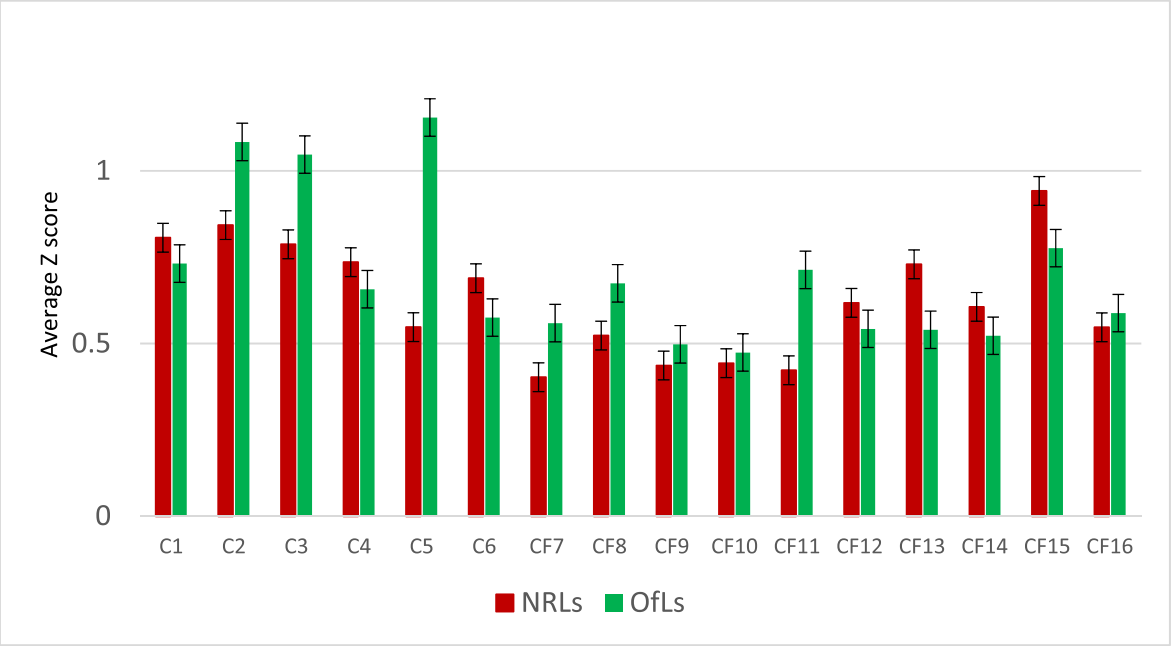

Fig. 4. Average of absolute z scores obtained with each EUTP for NRLs and OfLs.

2015). Thus, in this case, an adequate amount of sorbent material, e.g., 150 mg of PSA/ml instead of the standard amount of 25 mg/ml, is required for the removal of fatty acids; this information was later shared with all participants. The only time when no false positives were reported was in 2016 for EUPT-CF10 on rye.

Table 4 summarizes the false negative results for each EUPT. The maximum number of possible determinations was calculated by multiplying the number of pesticides present in the test item by the number of participating EU and EFTA laboratories. The highest number of false negatives was observed in EUPT-CF11 on oats (5.25%), EUPT-CF16 on barley kernels (4.40%), EUPT-C4 on rye (3.48%), and EUPT-CF14 on rice kernels (3.44%). Although, on the basis of many years of experience, the percentage of false negatives was expected to be lower in 2020 and 2022, a decrease was not observed, mainly because in the last years, no blank was sent out with the PT-test item; thus, laboratories used their own blank that could have interfered with the analysis. False negative results can also be due to maintenance problems, low instrument sensitivity, and low extraction efficiency.

The highest number of false negative and positive findings was observed with the QuEChERS–citrate buffered method (EN 151662), which was also the most used extraction method. Most false positives reported were detected by gas chromatography and by MS/MS. Moreover, two false positives were reported in 2022, for barley kernels, by Q-Orbitrap-MS. HRMS tends to provide higher specificity and therefore is expected to be less prone to matrix interference. However, when screening against an in-house or commercial databases, criteria such as retention time should be carefully updated to avoid any false positives or false negatives.

3.5. Assigned values and target standard deviation

The standard deviation calculated for each pesticide included in each EUPT is shown in Fig. 3. A high average standard deviation between 27% and 30% was observed with the first three EUPTs, C1, C2 and C3, for which laboratories had not yet developed sufficient experience in the analysis of pesticide residues in cereals. A lower average standard deviation, between 20% and 25%, was observed in the subsequent years with EUPTs, C4, C5, C6, CF7 and CF8. An average deviation of less than 20% was observed for the last five EUPTs, except for EUPT-CF15 on rapeseed, for which an average deviation of 20% was obtained. Rapeseed is a commodity with a high fat content (up to 20%), and this can pose analytical difficulties when testing for pesticides. The fat content can make it difficult to extract the pesticides from the sample and can lead to variations in the results. In addition, different laboratories may use different quantification approaches when analyzing the samples, such as matrix-matched calibration using different matrices, standard addition, and others, which can contribute to the observed high standard deviation in the results. Some compounds such as pyraclostrobin and azoxystrobin showed a high standard deviation in EUPT-CF16, although the test item was a matrix (barley) relatively amenable to analysis. Because the test item contained residues of high concentrations of these two compounds, for quantification purposes, the sample required dilution—a step that might have been omitted by some laboratories.

Carbendazim was one of the pesticides included in 13 of the PTs. The results showed a high standard deviation (>25%) in the earlier PTs (C1, C2, C3, C4, C6, CF7, CF8 and CF9), whereas a lower standard deviation was observed in PT-C5 and later PTs (CF10, CF11, CF13 and CF14). This finding reflects the improvements in the procedures for the preparation of stock solutions of this pesticide by the participating laboratories. Carbendazim has low solubility in organic solvents, such as ethyl acetate (0.135 mg/ml) (British Crop Production Council, 2022). Therefore, preparing a fresh stock solution, and verifying and ensuring that carbendazim is actually solubilized before withdrawal of an aliquot for preparation of a calibration solution are critical. The issue with low solubility of carbendazim and the risk of overestimating residue content was communicated to the participants in several reports and oral presentations at workshops.

3.6. Z score calculation

The average absolute z score obtained for each EUPT for NRLs and OfLs is shown in Fig. 4. Results submitted from non-EU countries were not included in the final z score results. The trend indicated that both NRLs and OfLs had similar performance. The average z score declined from EUPT-C3 to EUPT-CF14, thus demonstrating an improvement in the performance of the laboratories.

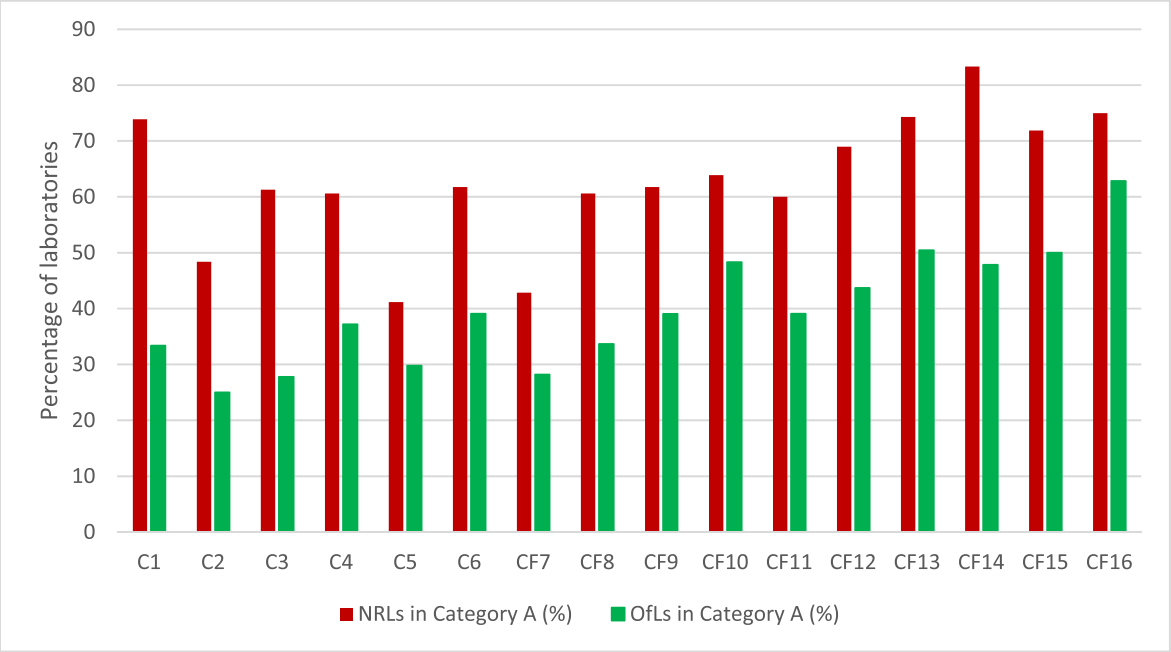

Fig. 5. Percentage of NRLs and OfLs classified in category A.

Fig. 6. Evaluation of category A NRLs as good, satisfactory or unsatisfactory, on the basis of combined z scores for each EUPT.

3.7. Combined z score and category classification

Fig. 5 shows the percentage of laboratories among NRLs and OfLs in category A. For NRLs, more than 60% of the laboratories are categorized as "A" laboratories. For OfLs, only approximately 40% of the laboratories are classified as "A" laboratories. A notable 11% increase in category A OfLs was observed with the last EUPT (CF16). Beyond the reporting of false positive results, some of the laboratories were classified as category B because of a low scope of coverage.

Fig. 6 shows the evaluation of category A NRLs as good, satisfactory or unsatisfactory for each EUPT. The percentage of laboratories classified as good was highest for EUPT-CF7 (feed), and CF13 (rye kernels). In the past 6 years, an increase in "good" laboratories was observed, except for CF15 (rapeseed). Fig. 7 shows the evaluation of category A OfLs as good, satisfactory or unsatisfactory for each EUPTs. The highest percentage of "good" laboratories was observed with PT-C6 (barley), CF7 (cereal based feed) and CF8 (wheat). Although a constant increase in the percentage of good laboratories was observed from CF11 to CF13, many factors have caused the figure to change with CF15, including the complexity of the matrix, the scope of analysis, and the COVID19 pandemic and resultant staff restrictions in the laboratories.

Fig. 7. Evaluation of category A OfLs as good, satisfactory or unsatisfactory, on the basis of combined z scores for each EUPT.

4. Conclusions

As many as 160 laboratories have participated in the EUPTs on cereals and feedstuff that were organized by the EURL-CF for 16 consecutive years. The target list was continually updated throughout the years to include more pesticides and the number increased from 33 compulsory pesticides in 2007 to 169 pesticides in 2022. The performance of the laboratories substantially improved after EUPT-C5, organized in 2011. The latest EUPT shows that 75% of NRLs and 63% of OfLs analyze for at least 90% of the compulsory pesticides in the target pesticide list, and correctly detect and quantify a sufficient percentage of the pesticides present in the test item without false positive results, and therefore classified in category A. However, false positives (25 compounds in the latest EUPT) and false negatives (4.40% in the latest EUPT) remain reported in EUPTs. The collection of EUPT data has generated valuable knowledge in the field of pesticide residue analysis in cereals and feedstuff. QuEChERS combined with GC-MS/MS and LC-MS/MS analysis is the most commonly employed approach for the analysis of pesticide residues in cereals and feedstuff. The EUPTs helped detect specific difficulties with the analytical methods used. Relevant information was shared with the laboratories to improve their performance, to limit the number of false positive and negatives, and to keep them abreast of new analytical techniques and the EU regulations, and in general improving their overall performance. Thus the results of the 16 EUPTs on cereal and feeding commodities clearly show that the comparability of the monitoring data reported to the EU Commission for use in the ongoing exposure and risk assessment of pesticide residues has improved from 2007 to 2022. Though there is still room for improvement and the EURLs therefore will continue to provide guidance and training to the NRLs and OfLs with the aim to improve their performance.

CRediT authorship contribution statement

Elena Hakme: Conceptualization, Writing – review & editing, All authors have performed writing, editing, reviewing, and conceptualization. **Susan Strange Herrmann:** Conceptualization, Writing – review & editing, All authors have performed writing, editing, reviewing, and conceptualization. **Mette Erecius Poulsen:** Conceptualization, Writing – review & editing, All authors have performed writing, editing, reviewing, and conceptualization.

Declaration of competing interest

No conflict of interest

Data availability

Data will be made available on request.

Acknowledgments

This work was funded by the European Union Reference Laboratory for pesticide residues in cereals and feedingstuffs (EURL-CF).

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