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Quality Control of Pesticide Residue Measurements and Evaluation of Their Results

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Abstract: Pesticide residues are monitored in many countries around the world. The main aims of the programs are to provide data for dietary exposure assessment of consumers to pesticide residues and for verifying the compliance of the residue concentrations in food with the national or international maximum residue limits. Accurate residue data are required to reach valid conclusions in both cases. The validity of the analytical results can be achieved by the implementation of suitable quality control protocols during sampling and determination of pesticide residues. To enable the evaluation of the reliability of the results, it is not sufficient to test and report the recovery, linearity of calibration, the limit of detection/quantification, and MS detection conditions. The analysts should also pay attention to and possibly report the selection of the portion of sample material extracted and the residue components according to the purpose of the work, quality of calibration, accuracy of standard solutions, and reproducibility of the entire laboratory phase of the determination of pesticide residues. The sources of errors potentially affecting the measured residue values and the methods for controlling them are considered in this article.

Keywords: pesticide residues; quality control procedures; sources of errors of residue analyses; reproducibility of results

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1. Introduction

A sufficient amount of safe food cannot be provided for the continuously growing population of the world without the use of pesticides at the current technological level. The global demand for, and the production as well as the use of pesticides have increased steadily during the past decades and are projected to continue growing [1,2]. Pesticides are chemical substances with various degrees of toxicity and modes of action [3,4]. To control the target pests certain concentrations of pesticide residues must remain in/on the treated species. Consumers are generally concerned about the toxic chemicals in their food. According to the survey conducted by the European Food Safety Authority, pesticide residues in food (40%) and antibiotic, hormone, or steroid residues in meat (39%) are the main food safety-related concerns among Europeans [5].

To protect consumers and the environment, the national authorities authorize the use of pesticides only after the critical evaluation of their toxicity, biological efficacy and residues remaining in/on food as well as in the environment [6–11]. The OECD Guidelines for Testing of Chemicals are a collection of the most relevant internationally agreed testing methods used by government, industry, and independent laboratories [12]. They are intended to enhance the validity and international acceptance of test data and reduce unnecessarily repeated tests [13–15]. Many non-OECD member countries adopt the same principles [12] or give permission for use only after [16–18] a pesticide active ingredient has been authorized by countries having an advanced registration system [8,10,11]. To facilitate

international trade and assist the national registration authorities to establish their own limits, the CODEX maximum residue limits, MRLs, are elaborated by the FAO/WHO Joint Meeting on Pesticide Residues, JMPR, [19], further considered in a stepwise procedure by the Codex Committee on Pesticide Residues, and approved by the Codex Alimentarius Commission [20,21].

To control the safe and efficient use of pesticides, their residues are regularly monitored in food and environmental samples in many countries according to risk-based sampling plans [22–30] or targeted surveillance with limited scope and sampling targets. For example, the world-wide activities are demonstrated with some selected publications from Argentina to Vietnam [31–46]. In the European Union the largest number of residues tested within the EU-coordinated and national pesticide residue monitoring programs were reported in 2020 by Luxembourg (659), Malta (643), Germany (626), France (619) and Belgium (617) [47]. Concerning all 30 countries reporting their monitoring results to EFSA, multiple residues were detected in 27.2% of the samples, and 30%, 22.3%, 4.1%, 0.5%, and 0.02% of samples contained 0, 1, 5, 10 and 15 different residues, respectively. However, in extreme cases 18 and 31 residues were detected in single strawberry [47] and honeysuckle samples [48]. These results underlined the importance of applying screening methods of the widest possible scope with low limit of detection/limit of quantification (LOD/LOQ) values. For this purpose, good progress has been made in expanding the scope of the methods [49–51].

Most publications referenced above [30–50] mainly reported minor modifications in sample preparation procedures of the original QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method [52] and often provided details of the conditions of the MS mass spectrometry (MS) detection. Other authors reported various combinations of sample preparation [53–61]. The authors typically stated the recoveries, linearity, LOD and LOQ values, matrix effects and compared them to the acceptance criteria specified in the major guidance documents [62–64]. On the other hand, none of them provided information on the details of sampling, efficiency of subsampling and comminution affecting the reproducibility of the results, or accuracy of reference standard solutions, albeit these steps can be major hidden sources of random and systematic errors [65–70].

Drawing realistic conclusions and making appropriate corrective actions can only be done if the monitoring results are accurate and derived from the analyses of samples taken according to the specific objectives of the program. That can only be achieved by implementing rigorous internal quality control of the whole process of the determination of pesticide residues. The basic quality requirements for the monitoring results are defined in five major guidance documents [62–64,70,71]. However, several potential hidden errors are not explicitly addressed in these documents. Although over the last two decades several scientific publications have highlighted the effect of these errors on the accuracy and uncertainty of the measurement results [65,67,68,72–75], the actions for limiting them have rarely been reported in the monitoring studies. Therefore, the reliability of these study results cannot be assessed. Table 1 summarizes the main steps of residue analyses and gives examples for the sources of potential errors.

Every laboratory should introduce and implement appropriate quality control procedures to assure that the results of the analyses are as accurate as possible, and that their uncertainties are kept as low as practical. The random error indicated by the combined relative uncertainty of the results (CV_R) is influenced by four main factors (Equation (1)): sampling (S), laboratory sample handling including subsampling of large crops (CV_{SS}), comminution (CV_{Sp}), test portion selection and analyses of sample extracts (CV_A) [66].

$$CV_R = \sqrt{CV_S^2 + CV_{SS}^2 + CV_{Sp}^2 + CV_A^2} \quad (1)$$

The CV_R incorporates the relative precision of all steps of the determination of pesticide residues including sampling.

Table 1. Examples for the sources of errors in the results of pesticide residues determination.

Operation	Potential Sources of Errors Random	Systematic
Sampling	Sample size; heterogeneous distribution of analyte; varying temperature during shipping and storage	Sampling target selection; sampling plan and method; degradation, evaporation of analyte; contamination of the sample; mislabeling
Selection of the portion of commodity to be analyzed	Inconsistent preparation of sample portion	Wrong part of the sample selected for extraction,
Sample size reduction, subsampling	Subsample does not represent the composition of the laboratory sample	primary samples are not proportionally represented
Comminution of selected sample portions	Particle size distribution in the homogenate; varying temperature and duration of comminution	Decomposition, evaporation of analytes
Test portion selection	Test portion does not represent the comminuted sample matrix	
Extraction	Varying intensity and temperature of extraction	Efficiency of extraction
Clean-up	Variation in the composition (e.g., water, fat, and sugar content) of sample materials;	Loss of analyte
Qualitative/quantitative determination of residues	Changing the retention time—shifting mass acquisition window; linearity and confidence intervals of calibration	Deviation from residue definition; missing analytes present in targeted or non-targeted analyses; high LOD; inaccurate standard solutions; matrix effect

The analysts usually only report the within-laboratory repeatability/reproducibility of steps from the extraction of the test portions (CV_A). On the other hand, the reproducibility (CV_L) is the parameter that realistically characterizes the laboratory measurements including all steps from subsampling to the quantitative determination of residues.

$$CV_L = \sqrt{CV_{SS}^2 + CV_{Sp}^2 + CV_A^2} \quad (2)$$

The analyses phase can be further subdivided into extraction (Ex), clean-up (Cl), evaporation (Ev), and chromatographic determination (Ch):

$$CV_A = \sqrt{CV_{Ex}^2 + CV_{Cl}^2 + CV_{Ev}^2 + CV_{Ch}^2} \quad (3)$$

However, the individual quantification of the contributions of the steps affecting CV_A can only be done in practice with applying isotope labelled compounds in specialized laboratory conditions with specific detection instruments [74]. Therefore, their combined effect should be determined in practice with repeated recovery tests (CV_A) performed at the concentration range that is expected to occur in the samples. Such tests reflect only the effect of operations carried out after spiking the test portions. If the tests are carried out on different days by different analysts the calculated relative standard deviation of the results will only indicate an interim reproducibility of the analyses step, but it is not equivalent to CV_L as defined by Equation (2). The results of recovery tests can be used to characterize the within-laboratory reproducibility (CV_L) only if they are performed with samples containing incurred residues derived from the prior application of a pesticide [65,72,74].

Our objectives are to call attention to the hidden errors in the analyses of pesticide residues that can significantly affect the accuracy and reliability of the results. Without aiming for a full review of the vast amount of published data, we describe some practical

options for the quality control actions that the program managers can get implemented by the laboratory staff to obtain accurate results with quantified uncertainty.

2. Methods

2.1. Sampling

The main objectives of the monitoring program, and in general the analyses of samples, are to obtain correct information with known uncertainty on the pesticide residue levels in the sampling targets and not only in the sample. It is generally recognized that the accuracy and validity of analytical results cannot be better than that of the samples analyzed. The sampling designs and methods are widely described in the scientific literature. Their coverage is beyond the scope of this paper. Briefly, for the monitoring of the pesticide residues in plant commodities and soil, stratified random sampling is the best choice. The sampling target (the area from where the samples are to be collected) can be stratified for instance according to crop, cultivation mode, growing season, soil type, etc. Random samples should be separately taken from each stratum. The minimum number of primary samples to be collected for one composite sample (sample size) depends on the objectives of the program. For instance, the provisions of the Codex sampling standard [70] for the minimum number of primary samples and total mass of a composite laboratory sample should be satisfied where the compliance with MRLs is assessed in goods offered for sale. It is not sufficient to collect [76], for instance, 4 pieces of head cabbages or Chinese cabbages (instead of the minimum five specified in the Codex GL) even though their total mass may be well over 10 kg and 4–5 kg, respectively, which are much larger than the specified minimum of 2 kg. A larger number of primary samples may be collected than the minimum, provided that their representative part can be effectively comminuted with the available laboratory equipment.

The sampling uncertainty is inversely proportional to the sample size (n, the number of primary samples) and depends on the variability of residues in crop units or in single sample increments (CV_1):

$$CV_S = \frac{CV_1}{n} \tag{4}$$

The variability of residues in individual crop units derived from a single field (called within field variability) is close to 80–100-fold [77,78], therefore increasing 'n' will decrease the uncertainty of the results and improve the accuracy of the estimated average residue in the sample. Under typical growing conditions the relative uncertainty of sampling is in the range of 25–40% for samples of size 10 and 5, respectively [79,80]. These uncertainties shall be considered when a product is tested before export.

2.2. Selection of Portion of Sample to Be Analysed

For testing compliance with MRLs, the portion of commodities specified in the Codex CAC/GL-41-1993 standard should be considered [81]. However, for providing data to estimate the dietary exposure of consumers, the edible portion of commodities should be analyzed. Since the edible portion varies and for instance depends on the variety, maturity of the crop, and local practices for its consumption. Consequently, the specific way of selecting the edible portions should be precisely described in the publications to enable the comparison of the results with other studies.

Most of the operations required for the preparation of the test portions depend very much on the actual condition of the test item and cannot be generally standardized. The laboratory assistants should be well trained on the principles enabling them to perform the tasks properly. Inconsistent operation may lead to high, uncontrollable variability (unquantifiable uncertainty) of the results. For instance, the way of removing adhering soil from root vegetables or outer, withered leaves from leafy vegetables can substantially influence the residues measured. The outer leaves usually contain much higher residues than the inner leaves. Therefore, only the loose leaves should be removed (Figure 1)

otherwise the measured residues will not correctly reflect the residue content and may lead to dispute if the lot is repeatedly sampled along the commercial chain.

(**a**) (**b**) (**c**)

Figure 1. Phases of preparation of head cabbage for analysis: (**a**) collecting head cabbage; (**b**) removing outer leaves; (**c**) obtaining portion of commodity to be analysed after cutting off the stalk.

To prepare samples for the analyses of the edible portion the peeling of fruits with inedible peel should be made in a way that the edible part is not cross contaminated by the residues being on the peel. Large fruits (e.g., watermelon, pumpkin, jackfruit) should be cut into wedge-shaped sections and the flesh part removed with proper spoons as shown in Figure 2. It should be noted that for checking compliance with MRLs, the whole fruit shall be comminuted and further processed. It is recommended that one section from each of the five large crops making up one composite sample according to the Codex sampling standard [70] is used for determining the residues in edible portions and a second set of five sections is comminuted for determination of residues in/on whole fruits.

Figure 2. Processing of jackfruit: top left: cutting wedge-shaped section from jackfruit; top right: comminution of the whole fruit; bottom: peel remaining after removal of edible part.

2.3. Subsampling and Comminution of Selected Sample Portions

Because of the usually very large difference in the concentration of residues in individual crop units [78], the whole laboratory sample or representative part of each primary sample (crop unit) must be processed to obtain accurate information on the average residue

in the laboratory sample. Omeroglu [82] and Ambrus [83] provided detailed graphical illustrations for obtaining representative subsamples and calculation of CV_L.

The distribution of residues within the natural crop units is also uneven. For instance, the residues concentrate on the lower part of fruits hanging on the trees or vines due to the runoff of the sprays. Therefore, slices should never be cut from crop units (Figure 3).

Figure 3. Distribution of residues in/on crop units: Left: Residues concentrate on the bottom part of fruits hanging on the trees; Right: Cutting the middle of cucumber leads to biased result. Slices should never be cut for subsampling as emphasized by the crossing red lines.

Obtaining representative portion of the large crop units (e.g., cabbage, watermelon, papaya, etc.) requires special attention making sure that each crop unit is proportionally represented in the subsample to be comminuted. Figure 4 illustrates the subsampling of large fruits.

The efficiency of cutting, blending of the sample materials may vary from day-to-day and sample-to-sample because of the changing physical properties and textural composition of crops depending on the variety and maturity. Moreover, it is strongly influenced by the sharpness of cutting blades. The fundamental sampling error defined by Gy [84] can be applied for characterizing the relative variance of the residues in comminuted materials [85].

$$CV_{Sp}^2 = \frac{C \times d^3}{w} \quad (5)$$

In Equation (5), the C is the sampling constant depending on the nature of the homogenized material, d is the diameter of the 95th percentile of the comminuted particles, and w is the mass of the test portion. Though CV_{Sp} cannot be calculated for plant materials applying Gy's theory, Equation (5) clearly indicates the importance of particle size (d^3) distribution. Reducing the particle size in a comminuted laboratory sample considerably reduces CV_{Sp} and consequently CV_L (Equation (2)). Therefore, the proper homogeneity of the comminuted materials should be checked for each sample.

A very quick and convenient method for this purpose is the 'Petri dish' test, in which a small portion of the comminuted material is spread on the glass surface and the particle size distribution is visually checked. If the particles are smaller than 2 mm, the homogeneity would be generally sufficient to keep CV_{Sp} smaller than 10–12% if 10–15 g test portions are taken for extraction [73,86]. Otherwise, the comminutions should be continued preferably by adding a further portion of dry ice [75,87]. Figure 5 provides some examples. Much smaller particles can be obtained, and considerably reduced test portions can be used when liquid nitrogen is used for cryogenic processing [88–90]. The two-stage sample processing can also be used in the combination of pre-homogenization of a large sample with proper choppers (C_L), then transferring its representative 100–150 g portion into a Waring laboratory blender (or a baker if Ultra Turrax is used for fine cutting), and adding about 10% known amount of distilled water for fine comminution (C_F) [83].

Figure 4. Cutting wedge-shaped portions of large fruits. Top left: papaya fruit; Top right: papaya subsamples; Bottom left: winter squash; Bottom right: sections of winter squash. Note: that the cut portions from each fruit should be kept in separate groups until taking the required number of pieces from each group for comminution.

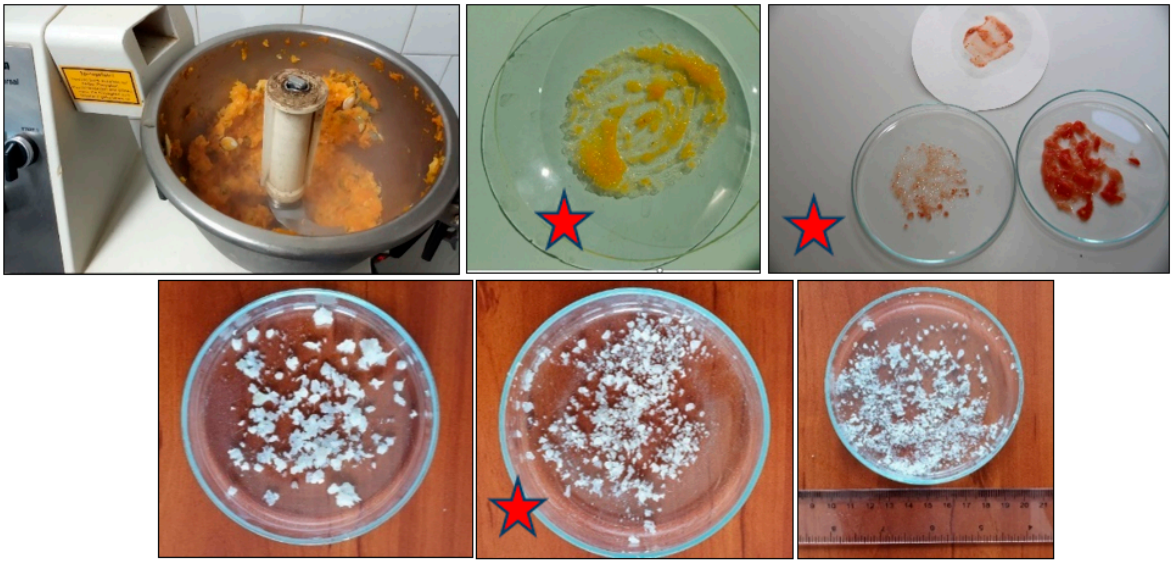

Figure 5. Top row left Blending winter squash; top center and right: tomato homogenates on filter paper and Petri dish; second row: cabbage leaves homogenized to different particle sizes. The star marks the acceptable particle size distribution.

It is generally recommended to add a small portion of water to dry materials to improve the efficiency of comminution [62,63]. The exact amount of added water shall be

accounted for in reporting the residue concentrations. The portions for further processing should be taken without delay in small increments (preferably > 10) of the test material from various positions of the blender to obtain representative test portion and avoid segregation.

The CV_{Sp} will be determined by the combined effects of the two comminution steps.

$$CV_{Sp} = \sqrt{\frac{C_L}{w_L} + \frac{C_F}{w_F}} \quad (6)$$

Equation (6) should also be applied for estimation of sample processing uncertainty in case of two-stage processing with liquid nitrogen [88,89]. The CV_{Sp} will depend on the C_L/w_L ratio. It is misleading to report the repeatability/reproducibility based on the analyses of spiked portions taken after fine comminution with liquid nitrogen.

The size of test portion significantly affects the reproducibility of the measurements. Based on Gy's sampling theory the relationship between the mass of the comminuted laboratory sample (m_L), the test portion (m_{TP}), and the CV_{Sp} can be described as [85]:

$$CV_{Sp}^2 = Cd^3\left(\frac{1}{m_{Tp}} - \frac{1}{m_L}\right) \quad (7)$$

Table 2 shows the change of CV_{Sp} depending on the test portion size taken from the same comminuted material.

Table 2. Change of CV_{Sp} as a function of the test portion mass.

	CV_{Sp}	
m_L [g]	>5000	1000
T_p [g]		
1	0.387	0.387
2	0.274	0.274
5	0.173	0.173
10	0.122	0.122
15	0.100	0.099
25	0.077	0.076

Table 2 indicates that reducing the test portion size from 15 g to 1 g will increase the CV_{Sp} by about 3.2 times. For instance, if the CV_{Sp} is 12.2% when a 10 g test portion containing incurred residues is extracted, and then one gramme portions are also taken from the same comminuted matrix, the theoretically expected CV_{Sp} would be about 38.7%. Naturally, the measurable CV_L will depend on the combined contribution of CV_{Sp} and CV_A according to Equation (2) (CV_{SS} is zero in this case). Provided that the CV_A from recovery tests is 10%, and the CV_{Sp}-s from Table 2 are 12.2% and 38.7%, the corresponding CV_L would be 15.8% and 40%, respectively, if 10 g and 1 g test portions were extracted from the same comminuted material. This significant effect remains unnoticed when the recoveries are determined with spiking the test portions. Therefore, making use of the high sensitivity of the recent MS systems and extracting 1–2 g test portions should only be done after careful checking of the reproducibility of the method with incurred residues, otherwise the real variability of the results may not be reflected [91–93]. A practical solution is to extract 5–10 g portions and dilute the extracts to utilize the sensitive detection and reducing the matrix effect [45,49].

2.4. Definition of Residues

Where the toxic metabolites or degradation products are present in a treated commodity in toxicologically significant proportion, they should be considered for the determination of the dietary exposure of consumers to pesticide residues. The principles are explained, for instance, in the FAO/WHO JMPR Manual [93]. The analyses of polar metabolites that

are often present in conjugated form requires specific procedures and cannot be determined with the usual multi-residue methods. To facilitate testing the compliance with MRLs carried out in large number of samples, the regulatory authorities often establish different definitions of residues for monitoring and risk assessment purposes. The JMPR emphasized that the definition of residues for enforcement purposes should be as practical as possible and preferably based on a single residue component (the parent compound, a metabolite, or a derivative produced in an analytical procedure) as an indicator of the total significant residue, and it should be determinable with a multi-residue procedure whenever possible [93]. Some examples for the different residue definitions are highlighted in Tables 3 and 4 [94,95].

Table 3. Different residue definitions for flupyradifurone.

Flupyradifurone [4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan2(5H)-one	
definition of the residue (for compliance with MRLs) for plant commodities	flupyradifurone
definition of the residue (for dietary risk assessment) for plant commodities	sum of flupyradifurone, difluoroacetic acid and 6-chloronicotinic acid, expressed as parent equivalents

Table 4. Different residue definitions for fluxapyroxad.

Fluxapyroxad [3-(difluoromethyl)-1-methyl-N-(3′,4′,5′-trifluoro [1,1′-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide]	
definition of the residue (for compliance with the MRL for plant and animal commodities)	fluxapyroxad
definition of the residue for estimation of dietary intake for plant commodities	sum of fluxapyroxad and 3-(difluoromethyl)-N-(3′,4′,5′-trifluoro[1,1′-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide (M700F008) and 3-(difluoromethyl)- 1-(ß-D-glucopyranosyl)-N-(3′,4′,5′-triflurobipheny-2-yl)-1Hpyrzaole-4-carboxamide (M700F048) and expressed as parent equivalents
for estimation of dietary intake for animal commodities	sum of fluxapyroxad and 3-(difluoromethyl)-N-(3′,4′,5′-trifluoro[1,1′-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide (M700F008) expressed as parent equivalents; the residue is fat soluble

The definition of residues in commodities of animal origin is often much more complex. The list of Codex MRLs indicates the residues to be tested for checking compliance with MRLs and for risk assessment purposes [21]. The latest recommendations of the JMPR can be found in the JMPR reports [96]. Alternately, the proper composition of residues can be accessed from the websites of the national registration authorities [10,97].

The examples above underline the importance of adhering to the residue definition that fits for the objectives of the study in order to obtain accurate results. Due to the inclusion of metabolites the total residue for risk assessment purposes can be much higher than that for monitoring purposes. In such cases, the calculation of estimated daily intake (EDI) based on the residues defined for monitoring purposes will underestimate the real exposure of consumers and result in wrong conclusions.

2.5. Extraction of Residues and Cleanup of Extracts

The selection of solvents and adjusting the pH to obtain acceptable recoveries have been extensively studied, providing sufficient information for the optimization of the procedures for various matrix-analyte combinations. A detailed guidance document for testing the efficiency of extraction [98] provides the basis, if followed, for obtaining accurate results. The efficiency of extraction should always be tested with incurred residues in all kinds of samples.

2.6. Accuracy of Standard Solutions

It is evident for every analyst that the accuracy of standard solutions is one of the very basic pre-conditions for the correct quantification of the residues. We cannot assume that the analytical standard prepared in our laboratory is accurate unless it is verified. To assist the laboratories participating in EU proficiency tests to find out the reasons for unsatisfactory results, the EU Reference Laboratory for Pesticide Residues in Fruits and Vegetables organized a ring test for the determination of the concentrations of certified pesticide analytical standards provided in a mixture. Forty official and national reference laboratories from 20 countries took part in the tests [99]. The summary of results is given in Table 5. The accuracy and uncertainty of the analytical standards may be affected by their storage and handling conditions.

Table 5. Summary of results of EU-RT-FV-17 [a].

	Bupirimate	**Carbendazim**	**Diazinon**	**Difenoconazole**	**Diflubenzuron**	**Methoxyfenozide**	**Pendimethalin**	**Permethrin**	**Spinosad**	**Thiabendazole**	**Trifloxystrobin**
Certified conc. mg/L	5.00	5.00	5.04	18.99	18.96	14.95	4.97	15.05	15.03	19.04	19.00
No. Lab	33	31	36	34	25	30	35	32	30	32	33
Accurate	2	1	0	0	0	0	0	0	0	0	0
Rel dif.% Min	−74.2	−86.6	−41.5	−43.7	−40.9	−51.5	−59.8	−54.1	−36.1	−32.5	−36.0
Rel dif.% Max [b]	40.0	164	202	36.9	129	107	28.0	73.4	91.0	116	118
No ≥ 10%	17	23	18	19	16	18	19	23	19	16	19

[a]: Courtesy of Carmen Ferrer Amate; [b]: rounded to 3 digits; No. Number of laboratories: reported result; Accurate: Certified = reported; No ≥ 10%: number of laboratories reported >10% rel. difference.

All laboratory equipment used for the preparation of analytical standards have their own inherent uncertainty of the nominal volume that is combined with the variability of filling them to mark depending on the daily performance of the analysts. Various manufacturers provide volumetric glassware of different grades. The relative uncertainty of the measured volume can be calculated from their specified tolerance (e.g., 50 ± 0.05 mL) assuming triangular distribution [100],

$$u = 0.05/\sqrt{6} = 0.02 \text{ mL}, CV = 0.02/50 = 4.08 \times 10^{-4}$$

The combined uncertainty (CV_{exp}) of volumetric measurements can be calculated from the tolerance of the glassware (CV_T) and the variability of filling them to mark (CV_{fil}):

$$CV_{exp} = \sqrt{CV_{fil}^2 + CV_T^2} \quad (8)$$

Involving our technicians making most accurately the volumetric measurements based on prior tests, we determined the relative uncertainties of filling in the volumetric glassware [100]. An example of the results is given in Table 6.

Table 6. Example of reproducibility of filling A-grade volumetric flasks.

Vol. Flasks	**Specification**	**CV_T**	**CV_{Rfil}**	**CV_{Rexp}**
25 mL	±0.03 mL	4.899×10^{-4}	7.30×10^{-3}	7.32×10^{-3}
50 mL	±0.05 mL	4.082×10^{-4}	7.59×10^{-4}	8.61×10^{-4}

Using our five-digit analytical balances, the weighing relative uncertainty of 25 mL water is 1.6×10^{-6}. It is three magnitudes lower than the volumetric measurement (Table 6). Therefore, the diluted standard solutions should be prepared based on weighing except the last step where an A-grade $\geq$ 25 mL volumetric flask should be used to obtain the standard concentration in mass/volume (e.g., µg/mL) [65].

We tested the reproducibility of the preparation of diluted standard solutions with the combination of weighing and volumetric measurements according to the regular practice in two of our laboratories [101]. The relative differences were calculated for the nominal concentrations. Some of the results are summarized in Table 7.

Table 7. Example for the reproducibility of preparation of analytical standard solutions.

C_0 mg/mL	CV_R	Effective Concentration			Deviation [1] from C_0 [%]		
		C_{min}	C_{ave}	C_{max}	Min	Average	Max
1	0.0079	0.9840	1.0044	1.0084	−1.6%	0.44	0.84
0.005	0.0086	0.0050	0.0052	0.0053	0%	4.00	6.00
0.001	0.0103	0.0010	0.0011	0.0011	0%	10.00	10.0

[1]: Deviation of the effective concentration from the nominal concentration [C_0].

The results indicated that the analytical standards can occasionally deviate by 10% from the nominal concentration even with the most careful and precise preparation. The relative uncertainty of nominal concentration (CV_{Rep}) increases with the increasing dilution of the standard solutions. These findings underline the importance of verifying the accuracy of analytical standard solutions. As a minimum, two new solutions should be prepared independently and their average chromatographic responses from minimum 5 replicate injections should be compared. If their relative difference is less than 10%, the two solutions can be combined for use. If the difference is larger, then a 3rd solution should be prepared, and the two closest ones can be combined.

The same procedure can be used for comparing the old standard solution with the new one. The SANTE Guidance document suggests accepting the two solutions (old and new or two new ones) if their relative difference is less than 10% [62]. It is pointed out that the *t*-test comparing the mean values cannot be used for this purpose, because it is designed to prove that the two mean values are not significantly different. Instead, the two-sample *t*-test (TOST) should be used to correctly verify that the relative difference between the two mean values is $\leq$10% [102]. As an alternative to the relatively complicated calculations, Figure 6 can be used for visually testing that the two standard solutions are within the targeted range ($\Delta_{rd} \leq 10\%$). Based on a minimum of 5 replicate injections of both standard solutions the relative difference Δ_{rd} is calculated as

$$\Delta_{rd} = \overline{C}_{diff}\% = 100 \times \frac{\overline{C}_{new} - \overline{C}_{old}}{C_{new}} \quad (9)$$

If the pooled relative standard deviation of the responses is above the critical decision line we cannot state with 95% probability that the relative difference is within the acceptance criterion (10%). Further on, the figure indicates that $|\Delta_{rd}|$ is inversely proportional to CV_p. The closer the $|\Delta_{rd}|$ to 10% the smaller the CV_p must be to verify compliance with the $\leq$10% criterion.

Since the typical repeatability CV of replicate injections into LC-MS/MS is about 2–2.5%, the maximum difference between the two standard solutions which can be stated 'equivalent' is about 7.67% and 7.09%, respectively. The calculation with Equation (10) is shown in Table 8.

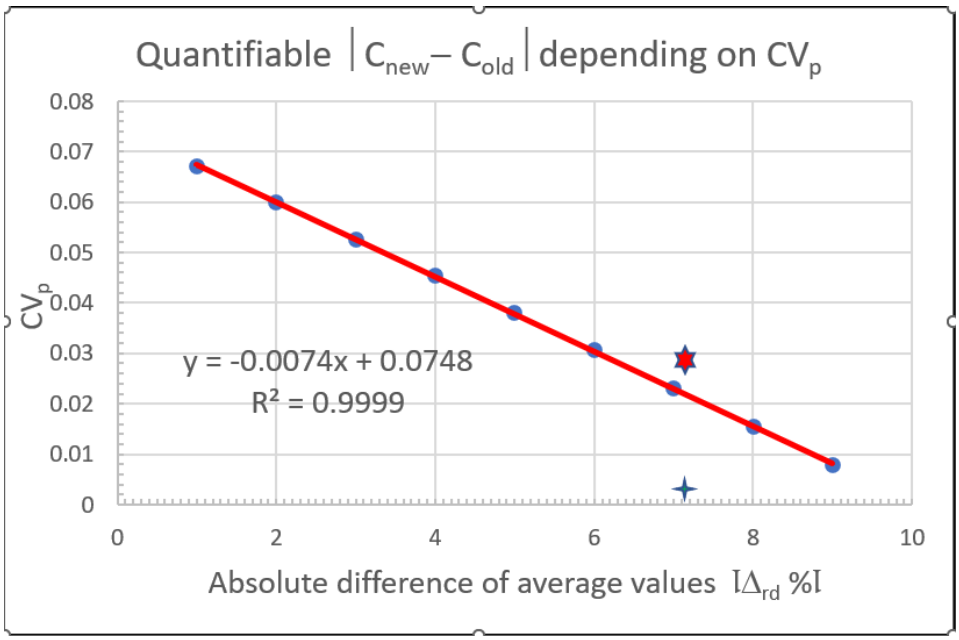

Figure 6. Relation of the absolute value of Δrd and maximum CV_p that can be used to verify that Δrd ≤ Θ = 10% at 95% probability level if $n_1 = n_2 = 5$. The symbols ✦ and ✸ indicate that the acceptance criterion (Δrd ≤ 10%) can or cannot be confirmed, respectively. Taken with permission from [103].

Table 8. Testing the difference in nominal concentrations of analytical standard solution.

	Standard A1	Standard A2	Standard B1	Standard B2
	121315	112823	123453	114811.3
	121525	112813	131282	122092.3
	121310	113000	123456	114814.1
	121401	113121	124356	115651.1
	121392	112802	123451	114809.4
Ave	121388.6	112911.8	125199.6	116435.6
Δrd	7.2%		7.3%	
CV	0.000718	0.001262	0.027337	0.027337
CVp	0.001027		0.027337	
	✦		✸	

The symbols ✦ and ✸ indicate that the acceptance criterion ($\Delta_{rd} \leq 10\%$) can or cannot be confirmed, respectively.

The pooled CV_P is calculated as:

$$uCV_P = \sqrt{\left(CV_{A1}^2 + CV_{A2}^2\right)/2} \quad (10)$$

The corresponding Δ_{rd} and CV_P values are plotted on Figure 6. It can be seen that for the B standard solutions the CV_p is above the critical line. Consequently, according to the TOST calculation, we cannot state with 95% probability that the difference between B1 and B2 standard solution is ≤10%.

2.7. Stability of Analytes

The pH of the plant fluids, enzymes released during the cutting, chopping of sample material can decompose sensitive analytes [87]. The analytes remaining in the final extract are also influenced by their physical–chemical properties, the temperature of comminution and mass of the laboratory sample. The disappearance of captan and dithiocarbamate

residues during sample comminution was already observed in the middle of the 70s [104]. Hill reported the decomposition of chlorothalonil and phthalimide type of compounds especially in lettuce and onion [86]. The procedure for the determination of the stability of analytes was reported in the case of tomato, lettuce and maize [105]. It was found that buprofezin and chlorpyrifos did not decompose in the tested matrices at ambient temperature either. Their recoveries were well reproducible and close to 100%, therefore they can be used as reference compounds for assessing the stability of other analytes by comparing their residue concentrations surviving after comminution. Since the recovery tests performed with spiked test portions before extraction do not reveal any information on the stability of analytes, the stability tests should be executed as part of the method validation or performance verification for the new analyte matrix combinations with surface-treated sample material.

The stability test is practically the same as the procedural recovery. Its performance briefly described hereunder:

(a) Take about two or more kg of the crop in which the stability of analytes will be tested.
(b) Prepare the portion of the commodity to be analyzed according to Codex CAC/GL 41-1993 [81] from the whole laboratory sample.
(c) Use approximately half of the sample matrix for the stability test and the remaining part for the recovery tests performed with spiked test portions as usual.
(d) Prepare analytical standard mixture of exactly known concentration of compounds to be tested together with buprofezin (Bu) and chlorpyrifos (Ch) at well detectable concentrations keeping in mind the total mass of the sub-sample to be processed. The number of pesticides or metabolites included in the mixture is limited only by the capability of the chromatographic separation and detection system.
(e) Take about 1/3 of the part of the laboratory sample (e.g., 3–4 units out of 10 fruits) obtained in step 3;
(f) Treat the surface of the selected portion applying either Hamilton syringe for carefully spreading the standard mixture (step 4) on the surface of the crops or injecting the standard solution into the flesh of the fruit [106]. Use liquid dispenser to treat leafy vegetables or small-size crops. Perform the treatment in a fume cupboard over a tray with filter paper which can absorb the runoff. The exact amount of standard mixture that remains on the crop surface need not be known as the concentration ratios of the reference and test compounds will be calculated.
(g) Steps for the treatment of the surface of tomatoes:
    i. Place the surface-treated portions into the chopper together with the remaining 2/3 part of the sample and comminute the whole matrix. By this way you represent a potentially worst-case scenario for testing the efficiency of sample processing and determination of CV_L at the same time as testing the stability of analytes. The test may be performed both at ambient temperature and under cryogenic conditions applying dry ice or liquid nitrogen following the normal procedure applied in the laboratory.
    ii. Verify the efficiency of comminution with a Petri dish test (See Section 2.3). Continue the process until an acceptable particle size distribution is obtained. Note that a lengthy process may increase the decomposition.
    iii. Remove test portions from the comminuted matrix according to the normal procedure of the laboratory, but preferably from $\geq$ 10 different positions.
(h) Using the remaining part of the test material, perform the recovery test as usual by spiking the selected test portion with the standard mixture. Calculate the recovery for each compound.
(i) Determine the concentration of survived residues from the surface-treated material and their recovery from spike test portions with the method to be applied.
(j) Perform the test in $\geq$5 replicates.

Calculation of the Stability of Test Compounds

The measured concentrations of survived reference compounds are C_{ch} and C_{Bu}. Average recoveries of chlorpyrifos and buprofezin from spiked test portion are denoted as $\overline{\sigma}_{Ch}$ and: $\overline{\sigma}_{Bu}$, respectively. The measured concentrations of the 'ith' test compound from surface treatment is C_i. Average recovery of compound 'i' from the spiked test portion: $\overline{\sigma}_i$. Surviving residues are calculated with the average recoveries for each replicate test portion separately because the concentrations present in the test portions are different due to the inhomogeneity of the comminuted material (the efficiency of comminution). The survived portion of compound 'i' is calculated from the first test portion as:

$$\varphi_{iCh1} = \frac{C_{i1} \times \overline{\rho}_{Ch}}{C_{Ch1} \times \overline{\rho}_i}; \ \varphi_{iBu1} = \frac{C_{i1} \times \overline{\rho}_{Bu}}{C_{Ch1} \times \overline{\rho}_i} \tag{11}$$

The first estimate of the survived portion ($\overline{\varphi}_{i1}$) is calculated as the average obtained from the comparison with chlorpyrifos (Φ_{iCh1}) and buprofezin (Φ_{iBu1}). The stability of an analyte is characterized with the estimated grand average of survived portions of the 'ith' compound obtained from the n replicate measurements:

$$\overline{\overline{\varphi}}_i = \frac{\sum_1^n \overline{\varphi}_i}{n} \tag{12}$$

The numerical calculations are illustrated in Tables 9–11. The surviving proportions were calculated with Equations (11) and (12). It is pointed out that each test portion was analyzed by different analysts taking part in one of our international training workshops. The test mixture used contained 17 pesticide active substances. The participants got acquainted with the QuEChERS method during the workshop and they had not used it before. Consequently, better reproducibility can be expected with the staff having experience with the method and working in their own laboratory. The results indicated the within-laboratory reproducibility of the analyses phase (CV_A) and whole process of determination of pesticide residues (CV_L). The summary of results is given in Table 9.

Table 9. Results of stability test performed with 1 g test portions at ambient temperature.

	Recovery Tests with 0.2 mg/kg Spike Residues Measured [mg/kg]					**Survived Residues [mg/kg]**			
	Bu	Ch	Etri	Etox		Bu	Ch	Etri	Etox
	0.177	0.165	0.137	0.176		0.161	0.157	0.122	0.139
	0.186	0.182	0.156	0.176		0.173	0.180	0.117	0.165
	0.204	0.183	0.151	0.153		0.129	0.116	0.100	0.133
	0.178	0.152	0.164	0.170		0.142	0.128	0.108	0.132
	0.169	0.169	0.150	0.164		0.135	0.137	0.106	0.125
$\overline{\rho}$	0.913	0.852	0.758	0.840	$\overline{\rho}$	0.207	0.231	0.176	0.217
CV_A	0.015	0.015	0.013	0.011	CV_L	0.698	0.937	0.908	0.885

Notes: Ch: chlorpyrifos, Etri: etridiazole; Etox: etoxazole; $\overline{\rho}$: average recovery. The Table shows rounded values, but the calculations were performed with four-digit numbers.

Comparing the CV_L values obtained with the analyses of 1 g and 10 g test portions clearly indicates the effect of test portion size on the reproducibility of the results which is about two times higher for the 1 g portion than the 10 g portion. The corresponding CV_A values (0.015 and 0.014) for buprofezin and chlorpyrifos do not show any dependence from the test portion size. It is not surprising because they reflect the repeatability of the procedure from the point of spiking of the test portions. The CV_{Sp} values, calculated with the rearranged Equation (2):

$$CV_{Sp} = \sqrt{CV_L^2 - CV_A^2} \tag{13}$$

are practically the same as the CV_L because the CV_A is much smaller. The average CV_{Sp1}:CV_{Sp10} ratio is about two which is smaller than that predicted with Equation (7)

indicating that the latter provides only an approximate tendency. These results underline the importance of regular testing the reproducibility of the whole pesticide residue determination process that can be done most conveniently with the reanalysis of retained test portions described hereunder.

Table 10. Proportion of survived residues based on 1 g test portion [1].

	Etridiazole with Bu	Etridiazole with Ch	Etoxazole with Bu	Etoxazole with Ch
	0.523	0.502	0.662	0.636
	0.469	0.420	0.731	0.655
	0.533	0.555	0.788	0.819
	0.528	0.547	0.711	0.737
	0.542	0.500	0.709	0.654
φ	0.519	0.505	0.720	0.700
$\bar{\bar{\varphi}}$	0.512		0.710	

Note: [1]: The proportions of survived residues were calculated applying both buprofezin (Bu) and chlorpyrifos (Ch) as stable reference compounds.

Table 11. Summary of recoveries, survived residues, CV_A and CV_L values obtained with the tests performed at ambient temperature during the training workshop.

Parameter	Bu	Ch	Etri	Etox
Spiking 1 g test portion (5 replicates)				
Average recovery	0.913	0.852	0.758	0.840
CV_A	0.015	0.015	0.013	0.011
Spiking 10 g test portions (5 replicates)				
Average recovery	0.970	0.949	0.815	0.957
CV_A	0.014	0.014	0.027	0.017
Extracting 1 g portion from surface-treated tomato				
Average survived [mg/kg]	0.148	0.143	0.111	0.139
CV_L	0.123	0.175	0.080	0.110
CV_{Sp}	0.122	0.175		
Extracting 10 g portion from surface-treated tomato				
Average survived [mg/kg]	0.146	0.146	0.135	0.145
CV_L	0.055	0.098	0.082	0.125
CV_{Sp}	0.053	0.097		

2.8. Determination and Demonstration of within-Laboratory Reproducibility

According to various guidance documents [62–64] the precision of the analysis steps (CV_A) should be determined with recovery tests. The individual recoveries are affected by the random and systematic errors. The sum of systematic errors is indicated by the average recovery, and the standard deviation of individual recovery values reflects the uncertainty (precision) of the measurements. Where the individual recoveries are within the 60–140% default range [62] and the average recoveries obtained for individual analyte sample matrix combinations are statistically not different (e.g., based on Grubb's outlier test [102] the average recovery and the pooled CV_A (CV_{AP}) can be calculated [107] and used for describing initially the performance of the method.

$$CV_{AP} = \sqrt{\frac{\sum df_i CV_{Ai}^2}{\sum df_i}} \quad (14)$$

Point to note: when the Grubb's test is applied: there are several websites offering the critical values for the test. To obtain a correct outcome the critical values should be selected

for a two-sided test, as given by ISO 5725 [102]. The initial estimate of method performance, based on a limited number of tests, should be verified, or refined, if necessary, during the ongoing performance verification that requires testing the recovery in each analytical batch. Keeping in mind the wide scope of the methods, covering often over 300 residues, it would not be practical to include all of them in every batch. It is recommended to test at least 10% of analytes (minimum five) included in the scope of the method in the rolling program together with various representative commodities from different commodity groups [62]. Consequently, hundreds of recovery values are generally generated in a laboratory monitoring pesticide residues. Each recovery value obtained on different days provides one estimate for the precision (relative uncertainty) of the results under within-laboratory reproducibility conditions. It is usually assumed that the random error of analytical results conforms to normal distribution because the total error is made up of the combination of small independent random errors arising at the various stages of an analytical procedure [108].

Assuming normal distribution, we can expect that the individual recovery values vary around the average (μ). Provided that the determination process is under "statistical control" 95% and 99.7% of the recovery values should be within the average (μ) ± 1.96sd and μ ± 3sd intervals (sd = standard deviation). Consequently, the control chart for individual recoveries is constructed based on the initial method validation data. The upper (UWL) and lower (LWL) warning limits encompass the μ ± 2sd range, whereas the corresponding action limits (UAL, LAL) are at μ ± 3sd. Since the probability for falling outside one of the action limits is very small (0.15%) such a situation would require immediate action by the operators. ISO 17025-2017 recommends preparing control charts to record the results in such a way that trends are detectable [109].

The original QuEChERS method has been used [52] with no or minor modifications in combination with GG-MS/MS and LC-MS/MS detection in one of our laboratories. The initial in-house validation of the method with representative analytes and sample materials resulted in an average recovery of 91.7% with a 'within-laboratory reproducibility' CV_{AR} = 9.6%. As part of the regular internal quality control 2354 recovery tests were performed at 0.01 mg/kg and 0.05 mg/kg levels altogether with 302 pesticide residues during the previous four months. The sample matrices included fruits and vegetables of high-water content such as apple, carrot, cucumber, eggplant, dragon fruit, grape, longan, mango, onion, orange, and sweet and chili pepper. Control charts were constructed for the selected groups of pesticides that were tested together. One example is shown in Figure 7, indicating only the results of the first 15 testing days with a limited number of pesticide residues to enable the graphical presentation and visual evaluation of the data.

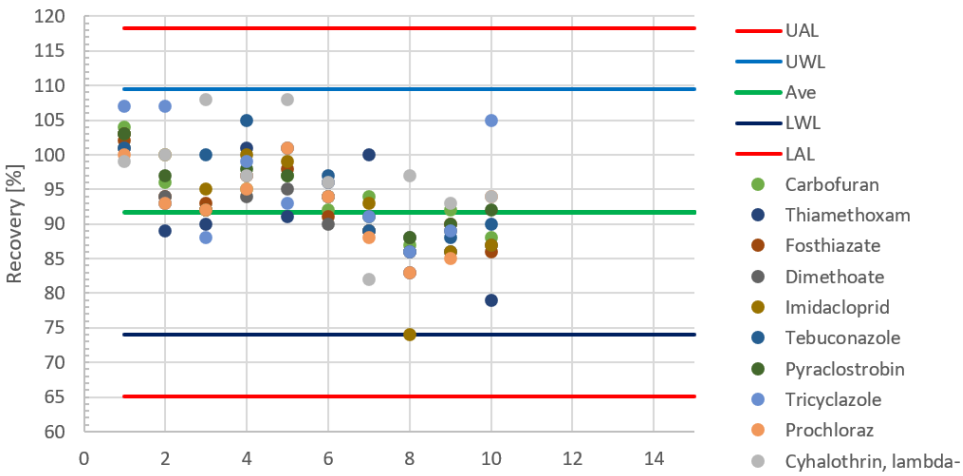

Figure 7. Example for the control chart demonstrating the within-laboratory reproducibility of the analyses phase of the determination of ten pesticide residues. Action (AL) and warning limits (WL) are indicated with red and blue lines, and the green line shows the average recovery.

Figure 8 shows that the recoveries were within the warning limits (74 and 109) of the randomly selected pesticides, though their distribution is not symmetrical, without displaying any clear tendency. In view of the size limitations of control charts, the periodic evaluation of a great number of recovery data (e.g., 2354 recoveries for the tested 302 compounds) can be better done based on their relative frequency diagram shown in Figure 9. The calculation can be easily done with Excel and has no size limitations.

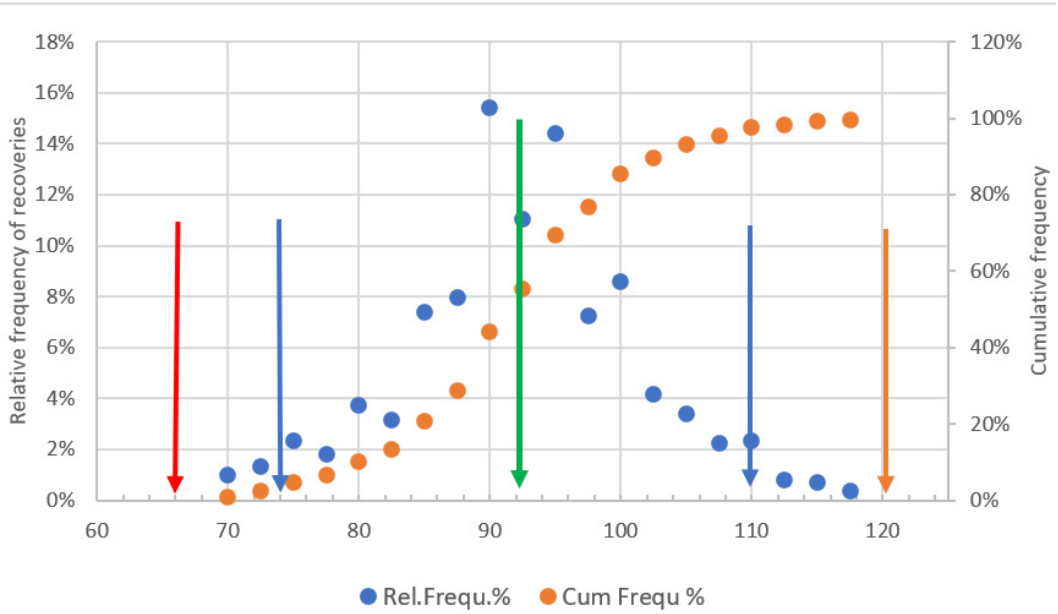

Figure 8. Relative and cumulative frequency distribution of 2354 recovery data obtained with 302 pesticide residues in commodities of high-water content during a four-month period. Red and blue arrows indicate the action and warning limits, respectively. Green arrow indicates the average recovery.

Figure 8 indicates that the highest recovery (117.5%) is within the upper action limit (UAL) (120%) and the lowest recovery is 70% well above the 65% lower action limit. Moreover, the mean recovery (91.7%) determined during the validation of the method is encompassed by the most frequently occurring 90% and 95% recoveries within the warning limits. The results of the 2354 recovery tests confirm that the tested 302 substances can be determined in fruits and vegetable samples with the typical performance parameters established during the validation of the method.

The long-term within-laboratory reproducibility (CV_L) of the residue determination process, which incorporates the contribution of subsampling, sample homogenization and analyses (Table 2), can be most conveniently determined [65] with the reanalyzes of the retained test portion that is also recommended by ISO17025:2017 as an internal quality control action [109]. The retained test portions must be obtained from samples containing incurred residues to demonstrate the efficiency of comminution. Analysts should be aware that only the CV_L can indicate the performance of the whole determination process and not the CV_A. Therefore, CV_L should be determined regularly for each type of commodity as part of the internal quality control plan of the laboratory. For performing the reanalyzes of retained test portions, prepare 10–15 test portions from each sample. If residues are detected, keep the test portions for further analyses. If no residue is detected a few test portions may be kept for preparing matrix-matched calibration solutions. The remaining test portions can be discarded. In due course of the regular analyses of various samples, a retained test portion should be included in the analytical batch and blindly reanalyzed. The results should be recorded in the format shown as an example in Table 12.

Table 12. Results of the analyses of retained test portions (example).

Test No [1]	Original Test Portion				Retained Test Portion		
	Sample code	Date of anal. [2]	Residue/commodity Name	[mg/kg]	Test portion Code [3]	Date of anal.	Residue [mg/kg]
1	M261	22 August 2022	Bupirimate/ orange	0.205	M261/1	29 August 2022	0.216
2					M261/2	6 September 2022	0.210
3					M261/3	14 September 2022	0.195
4	M283	15 September 2022	Lufenuron/ pepper	0.52	M283/1	22 September 2022	0.75
5					M283/2	26 September 2022	0.45
6					M283/3	3 October 2022	0.50
7					M283/4	10 October 2022	0.68

Notes: [1]: The repeated tests can be performed at various time intervals after the first analysis. [2]: Date of the first analysis of the sample. [3]: Test portions retained form the sample at the time of the first analyses.

The results of the reanalyzes of retained test portions may be evaluated based on the standard deviation of the difference of the two measurements made on "closely similar materials containing residues fairly close in amount present" [110]. The number of sample portion pairs analyzed should be ≥5 to obtain a realistic estimate for the CV_L. Since the average residues of the original and retained test portions are different, their relative difference should be used for the estimation of CV_L.

$$CV_L = \sqrt{\frac{\sum R_{\Delta i}^2}{2n}} \tag{15}$$

where $R_{\Delta I} = 2(R_{i1} - R_{i2})/(R_{i1} + R_{i2})$, R_{i1} and R_{i2} are the residues obtained from the analyses of the ith test portions and n is the number of test portion pairs. Assuming that only random error affects the duplicate measurements, their average must be zero, thus the degree of freedom is equal to 'n', the number of measurement pairs. Alternately, the range statistics [111] can be used for the estimation of CV_L that does not assume the above-mentioned preconditions specified by Youden. For the ith measurement pairs the CV_{Ri} is calculated with Equation (16). The d_2 for two replicate measurements is 1.128.

$$CV_{Ri} = \frac{R_{max} - R_{min}}{\overline{R} \times d_2} \tag{16}$$

The CV_L is calculated from 'n' test option pairs with pooling the CV_{Ri} values [112]):

$$CV_L = \sqrt{\frac{\sum CV_{Ri}^2}{n}} \tag{17}$$

The degree of freedom for the corresponding standard deviations [sd = $CV_L \times R$] of the measured residues (R) is equal to 'n'. The two estimates of CV_L with Equations (15) (0.1283) and (17) (0.1608) are slightly, but statistically not significantly, different. We recommend using the larger CV_L to avoid underestimating the long term within-laboratory reproducibility of the residue determination process.

2.9. Chromatographic Determination of Residues

The gas and liquid chromatographic separation and MS detection conditions are generally well described in the publications often following the guidance given by SANTE/11312/2021, SANTE/2020/12830, USFDA documents [62–64]. However, there are a few points that should be considered when the chromatographic conditions are characterized.

The reported LOD values or reporting limits should always be checked at the beginning and at the end of the analytical batch of sample extracts for all targeted analytes preferably in blank sample extract, because loading the column with coextracted materials may change the resolution of the column and or shift the retention times as illustrated in

Figures 9 and 10. This is especially important in the case of screening methods for unknown pesticide residues in monitoring programs.

The inertness and satisfactory operating conditions of gas chromatographic columns can be improved by applying the so-called analyte protectants [112,113] A critical review and re-assessment of analyte protectants in gas chromatography was published by Rodríguez-Ramos [114].

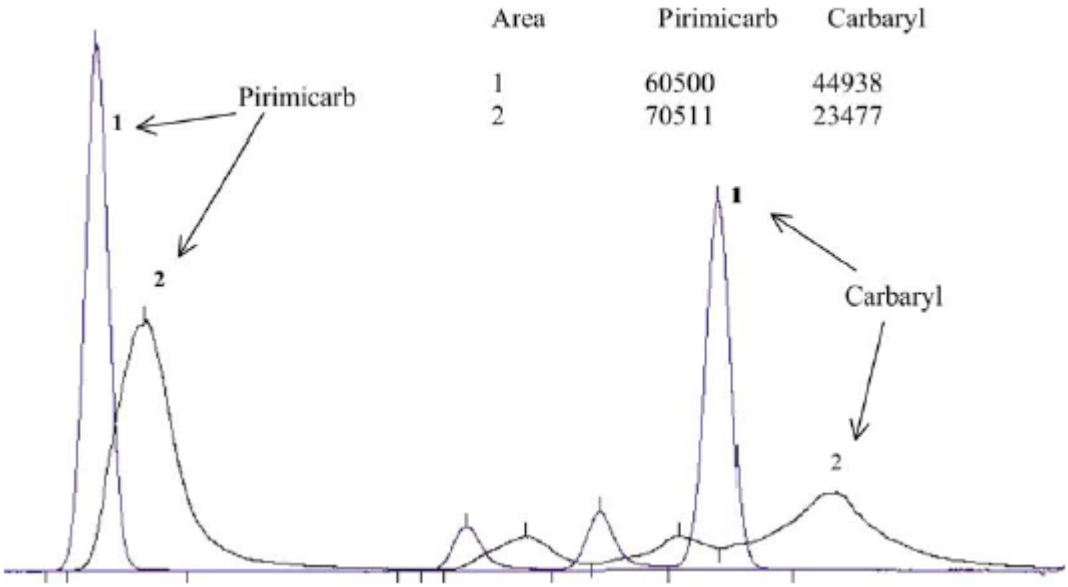

Figure 9. Deterioration of chromatographic peak shape after injecting 40 sample extracts (5 g sample/mL). Taken with permission form [115].

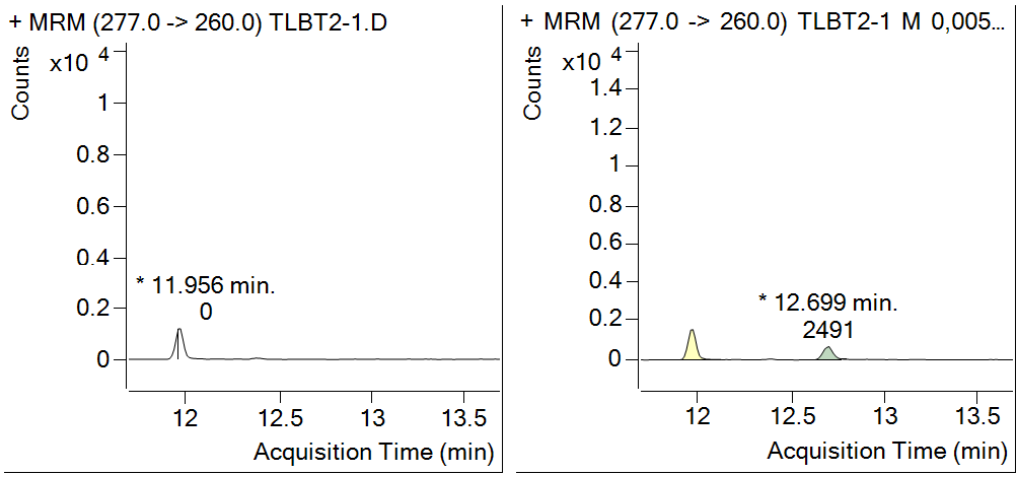

Figure 10. Shifting the retention time from 11.956 to 12.699 and changing the shape of the response of fenitrothion at the beginning and at the end of the analytical batch. Note the shapes of peaks obtained after injection of 0.005 µg/mL considered to be the LOD. MRM: multi reaction monitoring mode, TLBT2-1: sample identifier.

The data analyses reports provided by the software should not be viewed as a 'black box' and accept it without verification of its correctness. The modern data analyzers (e.g., Aglient Mass Hunter) usually offer six different curve fit types (linear, quadratic, power, first order ln, second order ln, and average of response factors), four possible choices for the origin (ignore, include, force, blank offset), and seven for weighing (none, 1/x, 1/x, 1/y, $1/y^2$, Log, $1/sd^2$). The reported results can be quite different depending on which integration options are selected. Attention is also required to assess the number

of disabled points and the reported confidence limits of the slope and intercept of the regression equations. For instance, where three out of six calibration points are disabled the predicted analyte concentration should be critically considered, and possibly additional calibration injections should be made.

Chromatograms must be inspected by the analyst and the actual baseline fit examined and adjusted, if necessary. The response of the suspected peaks should always be checked to verify that the ion(s) acquisition includes the whole peek(s) and their integration is correct (Figure 11).

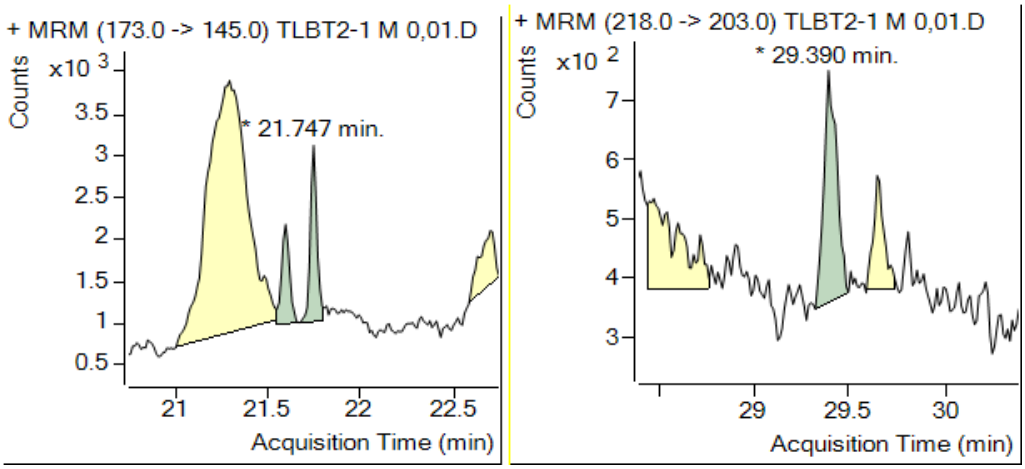

Figure 11. Examples for verification of correct identification of suspected peaks of propiconazole and indoxacarb in left and right pictures. MRM: multi reaction monitoring; TLBT2-1: sample identifier.

For multi-level calibration the standard concentrations should be equidistantly distributed over the calibrated range. Figure 12 illustrates a frequently applied questionable practice where four calibration points [ng/mL] were in the first 1/10 part of the calibrated range (0.05, 0.1, 0.5, 1, 5, 10 µg/kg).

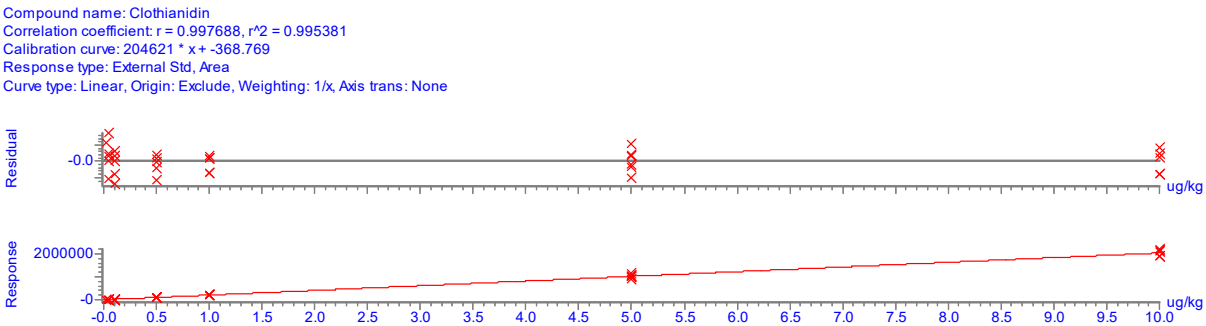

Figure 12. Improper selection of calibration points that should be equidistantly distributed. ∗ indicates the position of the response obtained with the injection of standard solutions.

Such a calibration program type is only justified where analytes potentially present at low concentrations are looked for in screening analyses.

It should be recognized that the correlation coefficient (r) or the coefficient of determination (R^2) provides information only on the linearity of the calibration but does not characterize the quality of the calibration. It can be assessed based on confidence intervals, calculated by those of the data processing software for the slope and intercept of the regression line or from the standard deviation of the relative residuals. The latter parameters should also be reported together with 'r' or R^2.

Figures 13 and 14 show calibration charts with confidence and tolerance intervals around the linear regression line obtained with 1/x weighting [116]. Note that the R^2 values indicating the linear fit are practically the same, but the standard deviation of

relative residuals (Sd_{rr}) indicating the scatter of the responses around the regression line as well as the width of the confidence and tolerance intervals are substantially different. The confidence intervals around the regression line are strongly influenced by the number of standard injections (not shown in the figure).

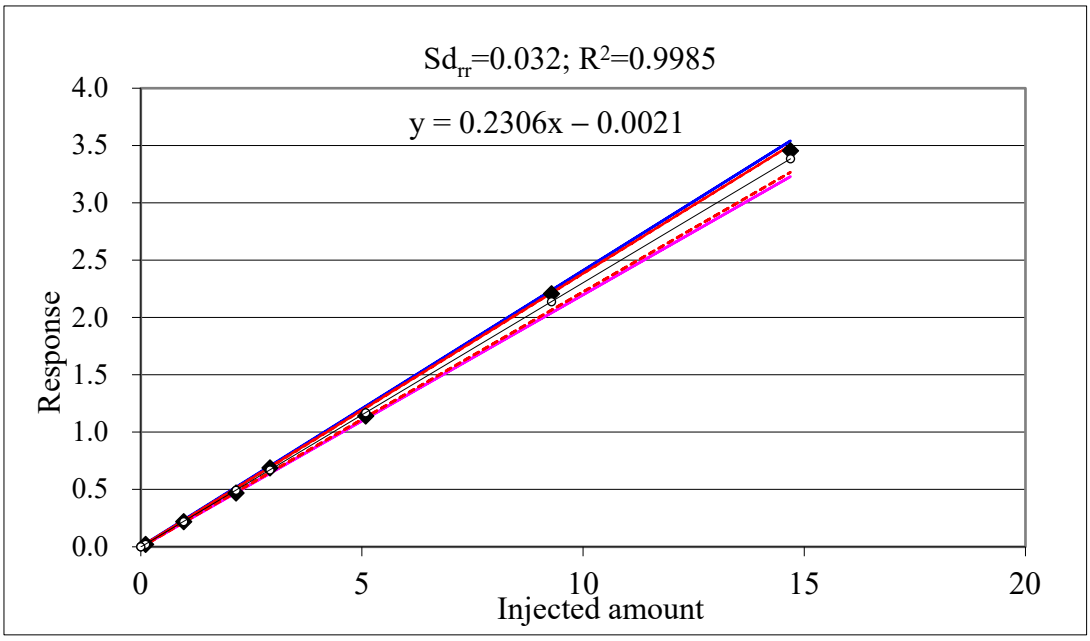

Figure 13. Terbuthylazine calibration charts. Blue lines indicate the confidence intervals, the red lines the tolerance intervals around the regression line that were calculated applying the approximation recommended by Miller and Miller [116].

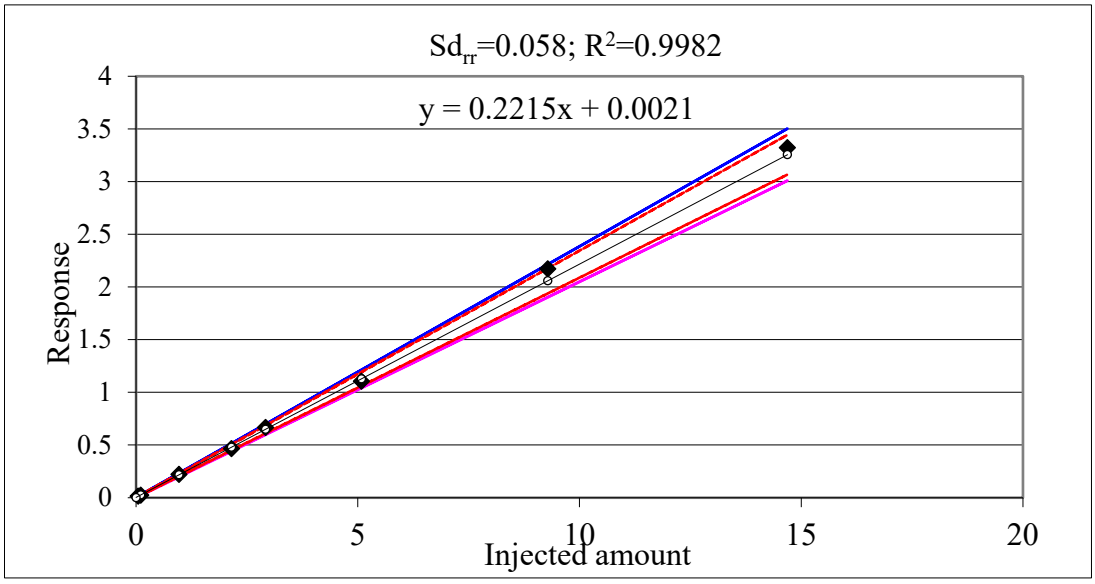

Figure 14. Terbuthylazine calibration charts. Blue lines indicate the confidence intervals, the red lines the tolerance intervals around the regression line that were calculated applying the approximation recommended by Miller and Miller [116].

The regression residual Δy_i describes the vertical distance of measured responses from the regression curve according to:

$$\Delta_{yi} = y_i - \hat{y}_i;\ \Delta_{yirel} = \frac{\Delta_{yi}}{\hat{y}_i} \tag{18}$$

The standard deviation of the relative residuals is calculated as:

$$Sd_{\Delta y/\hat{y}} = \sqrt{\frac{\left\langle \Delta_{yirel} - \overline{\Delta}_{rel}{}^2 \right\rangle}{n-2}} = Sd_{rr} \tag{19}$$

When each reference material is measured *k* times, the number of degrees of freedom is (nk–2).

Nonetheless the R^2 values are practically the same, the Sd_{rr} values indicating the large difference in the confidence/tolerance intervals in Figures 13 and 14. Table 13 shows further examples from our practice underlying the fact that the R^2 is not a proper indicator of the accuracy of the calibration [65]. Our experience suggests that for accurate calibration the Sd_{rr} should be <0.1 (10%). The Codex quality control guidelines suggest accepting a maximum of 20% relative residuals (30% near the instrument LOQ) [71].

Table 13. Examples for the corresponding Sd_{rr} and R^2 values.

Sd_{rr}	R^2
0.042	0.9937
0.061	0.9976
0.085	0.9988

3. Discussion

The monitoring programs are conducted around the world including large number of samples to provide data for carrying out:

- dietary exposure assessment of consumers;
- evaluating the residue levels and their compliance with national or international maximum residue limits or guidance values;
- assessing the contamination of the environment;
- providing the basis for the necessary corrective actions if the residues exceed the reasonably expectable levels in the treated crops.

Each analysis may have significant consequences. Therefore, the results should be representative and defendable even in legal proceedings. Analysts must be aware of their responsibilities and the fact that their credibility could be at stake. They should be able to verify the correctness of their measurements with documented evidence.

The international standards and guidelines provide the frame and acceptable performance criteria for performing the pesticide residue analytical measurements. They would facilitate obtaining accurate, defendable results only if the laboratory operations are performed by staff members (from the top manager, who has the key role, to each member) who are aware of their own responsibility and are working in coordination with each other.

It is not sufficient to validate our methods or test the performance of already validated methods once. The laboratories should establish their own internal quality control programs to be used daily for ensuring that their methods satisfy the specified performance characteristics when applied for instance to screen over several hundreds of analytes in samples of unknown origin or to test the residues in commodities before export.

The provisions of guidance documents should be fulfilled bearing in mind that the priorities of internal quality control are in order: (1) good analytical practice; (2) good science; (3) minimum bureaucracy; (4) facilitating reliability and (5) efficiency. The quality assurance/quality control (QA/QC)should only be an appropriate proportion of the activities related to the analyses of samples and reporting of the results.

Keeping in mind the above priorities, we emphasize that it is not sufficient to report the recoveries obtained with spiked test portions, the linearity of calibration, detection

conditions, and confirmation of the identity of substances. In addition, we propose checking and preferably briefly reporting, for instance, the validity of samples considering the parameters that can be verified in the laboratory, accuracy of analytical standards, stability of analytes during the laboratory operations, quality of calibration characterized with the relative residuals or their standard deviation, and the reproducibility relative standard deviation of the measured residues.

Moreover, the selection of the parts of samples and the composition of the residues to be determined should always be matched with the objectives of the work.

It is advisable to take part regularly in proficiency tests that provide a means of objectively evaluating and demonstrating the accuracy and reliability of our measurements. Critical review of the Z-scores and identification of the sources of the potential errors can help to improve the technical operation standard of the laboratory. However, participating in proficiency tests does not replace the regular and rigorous internal quality control actions.

Finally, reliable results on which regulatory decisions are based can be expected only from well-trained analysts whose knowledge should be regularly updated to fully utilize the advantages of the high-performance instruments and benefit from the rapidly expanding methodical experience gained by other laboratories through the analyses of a great variety of samples.

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
EPA	US Environmental Protection Agency
ADI	Acceptable Daily Intake
ANVISA	Brazil National Health Surveillance Agency
CAC	Codex Alimentarius Commission
CCPR	Codex Committee on Pesticide Residues
DAFF	Australian Government Department of Agriculture, Fisheries and Forestry
EC	European Commission
EFSA	European Food Safety Authority
EPC	European Parliament and Council
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
GAP	Good Agricultural Practice
JMH	Ministry of Health of Japan
JMPR	FAO/WHO Joint Meeting on Pesticide Residues
MRL	Maximum Residue Limit [mg/kg]

OECD	Organisation for Economic Cooperation and Development
USA	United States of America
US EPA	US Environmental Protection Agency
US FDA	US Food and Drug Administration

References

1. FAO/WHO. Synthesis Report on the Environmental and Health Impacts of Pesticides and Fertilizers and Ways to Minimize Them. 2022. Available online: www.fao.org/pest-and-pesticide-management/resources/en/ (accessed on 9 November 2022).
2. *National Research Council, 2020 Future Role of Pesticides in US Agriculture*; National Academies Press: Washington, DC, USA, 2020; p. 332. ISBN 0-309-50383-3.
3. Turner, A. Pesticide Manual, 19th ed.British Crop Production Council: Alton, UK, 2021; p. 1400. ISBN 978-1-9998966-5-2.
4. University of Hertfordshire (Hatfield, UK). Pesticide Property Database. Available online: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/3478.htm (accessed on 20 October 2022).
5. EFSA. Eurobarometer. 2022. Available online: https://www.efsa.europa.eu/sites/default/files/2022-09/EB97.2-food-safety-in-the-EU_report.pdf (accessed on 24 November 2022).
6. Australian Government. Agricultural and veterinary chemicals code, standards. 2022. Available online: https://www.legislation.gov.au/Details/F2022L00137 (accessed on 20 October 2022).
7. European Parliament and Council. Regulation (EC) no. 396/2005 of the European Parliament and of the Council of 23 February 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32005R0396 (accessed on 20 October 2022).
8. European Parliament and Council. Regulation (EC) no. 1107/2009 of the European Parliament and of the Council of 21 October 2009 Concerning the Placing of Plant Protection Products on the Market and Repealing Council Directives 79/117/EEC and 91/414/EEC. Available online: https://eur-lex.europa.eu/legal-content/HU/ALL/?uri=CELEX:32009R1107 (accessed on 20 October 2022).
9. EPA. Summary of the Federal Insecticide, Fungicide and Rodenticide Act. Available online: https://www.epa.gov/laws-regulations/summary-federal-insecticide-fungicide-and-rodenticide-act (accessed on 20 October 2022).
10. EPA. Regulation of Pesticide Residues on Food. 2022. Available online: https://www.epa.gov/pesticide-tolerances (accessed on 5 November 2022).
11. Japanese Ministry of Health, Labour and Welfare. Agricultural Chemical Residues in Foods. Available online: https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/shokuhin/zanryu/index_00016.htm (accessed on 5 November 2022).
12. Solecki, R.; Schumacher, D.M.; Pfeil, R.; Bhula, R. OECD Guidance documents and test guidelines. In *Food Safety Assessment of Pesticide Residues*; Ambrus, Á., Hamilton, D., Eds.; World Scientific: Hackensack, NJ, USA, 2017; pp. 507–510.
13. OECD. Test Guidelines for Chemicals. Available online: https://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm (accessed on 5 November 2022).
14. OECD. Work on Pesticides and Sustainable Pest Management. Vision for the Future. Available online: https://inergency.com/oecd-work-on-pesticides-and-sustainable-pest-management-vision-for-the-future (accessed on 5 November 2022).
15. OECD. Data Requirements for Pesticide Registration in OECD Member Countries: Survey Results. OECD/GD (94)47 Environment Monographs No. 77. Available online: https://www.oecd.org/env/ehs/pesticides-biocides/oecdguidancedocumentsforpesticideregistration.htm (accessed on 5 November 2022).
16. ASEAN. Maximum Residue Levels. Available online: https://asean.org/wp-content/uploads/images/2015/October/outreach-document/Edited%20MRLs-2.pdf (accessed on 5 November 2022).
17. Brazil Government. Biblioteca de Temas de Agrotóxicos. 2022. Available online: https://www.gov.br/anvisa/pt-br/assuntos/regulamentacao/legislacao/bibliotecas-tematicas/arquivos/agrotoxicos.pdf (accessed on 5 November 2022).
18. Vietnam. Ministry of Healthy Circular 50/2016 Regulations on MRL of Pesticide in Food. Available online: https://thuvienphapluat.vn/van-ban/The-thao-Y-te/Thong-tu-50-2016-TT-BYT-gioi-han-toi-da-du-luong-thuoc-bao-ve-thuc-vat-trong-thuc-pham-337490.aspx (accessed on 25 November 2022).
19. Hamilton, D.; Yoshida, M.; Wolterink, G.; Solecki, R. Evaluation of pesticide residues by FAO/WHO JMPR. In *Food Safety Assessment of Pesticide Residues*, 1st ed.; Ambrus, Á., Hamilton, D., Eds.; World Scientific Publishing: London, UK, 2017; pp. 113–196.
20. Yamada, Y. Importance of codex maximum residue limits for pesticides for the health of consumers and international trade. In *Food Safety Assessment of Pesticide Residues*, 1st ed.; Ambrus, Á., Hamilton, D., Eds.; World Scientific: Hackensack, NJ, USA, 2017; pp. 269–282.
21. Codex Alimentarius. Pesticide Index. Available online: http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticides/en/ (accessed on 5 November 2022).
22. Australian National Residue Survey. Available online: https://www.awe.gov.au/agriculture-land/farm-food-drought/food/nrs (accessed on 5 November 2022).
23. Austrian Agency for Health and Food Safety. National Pesticide Residue Control Programmes. Available online: https://www.ages.at/en/plant/pesticides/pesticide-residues#c5252 (accessed on 30 October 2022).

24. European Commission. Commission Implementing Regulation (EU) 2021/601 of 13 April 2021 Concerning a Coordinated Multiannual Control Programme of the Union for 2022, 2023 and 2024 to Ensure Compliance with Maximum Residue Levels of Pesticides and to Assess the Consumer Exposure to Pesticide Residues in and on Food of Plant and Animal Origin. Available online: http://data.europa.eu/eli/reg_impl/2021/601/oj (accessed on 22 October 2022).
25. EFSA. The 2020 European Union Report on Pesticide Residues. Available online: https://multimedia.efsa.europa.eu/pesticides-report-2020/ (accessed on 5 November 2022).
26. Federal Office of Consumer Protection, Tables for the National Reporting of Pesticide Residues in Food 2020. Available online: https://www.bvl.bund.de/DE/Arbeitsbereiche/01_Lebensmittel/01_Aufgaben/02_AmtlicheLebensmittelueberwachung/07_PSMRueckstaende/01_nb_psm_2020_tabellen/nbpsm_2020_tabellen_node.html (accessed on 20 October 2022).
27. EPA 2015 Compliance Monitoring Strategy for Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)EC 2022a EU Pesticide Database. Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=search.as (accessed on 20 September 2022).
28. European Commission. EU Pesticide Database. Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=search.as (accessed on 20 October 2022).
29. USFDA. Pesticide Residue Monitoring Program Reports and Data. Available online: https://www.fda.gov/food/pesticides/pesticide-residue-monitoring-program-reports-and-data (accessed on 5 November 2022).
30. Zhang, M.; Zeiss, M.R.; Geng, S. Agricultural pesticide use and food safety: California's model. *J. Integr. Agric.* **2015**, *14*, 2340–2357. [CrossRef]
31. Mac Loughlin, T.M.; Peluso, L.; Etchegoyen, A.; Alonso, L.L.; de Castro, C.; Percudani, C.; Marino, D.J.G. Pesticide residues in fruits and vegetables of the Argentine domestic market: Occurrence and quality. *Food Control* **2018**, *93*, 129–138. [CrossRef]
32. Rahman, M.; Hoque, M.S.; Bhowmik, S.; Ferdousi, S.; Kabiraz, M.P.; van Brakel, M.L. Monitoring of pesticide residues from fish feed, fish and vegetables in Bangladesh by GC-MS using the QuEChERS method. *Heliyon* **2021**, *7*, e06390. [CrossRef] [PubMed]
33. Jardim, A.N.O.; Caldas, E.D. Brazilian monitoring programs for pesticide residues in food-results from 2001 to 2010. *Food Control* **2012**, *25*, 607–616. [CrossRef]
34. Galani, Y.J.H.; Houbraken, M.; Wumbei, A.; Djeugap, J.F.; Fotio, D.; Gong, Y.Y.; Spanoghe, P. Monitoring and dietary risk assessment of 81 pesticide residues in 11 local agricultural products from the 3 largest cities of Cameroon. *Food Control* **2020**, *118*, 107416. [CrossRef]
35. Calderon, R.; García-Hernández, J.; Palma, P.; Leyva-Morales, J.B.; Zambrano-Soria, M.; Bastidas-Bastidas, P.J.; Godoy, M. Assessment of pesticide residues in vegetables commonly consumed in Chile and Mexico: Potential impacts for public health. *J. Food Compos. Anal.* **2022**, *108*, 104420. [CrossRef]
36. Zhang, Y.; Wang, M.; Silipunyo, T.; Huang, H.; Yin, Q.; Han, B.; Wang, M. Risk Assessment of Triflumezopyrim and Imidacloprid in rice through an evaluation of residual data. *Molecules* **2022**, *27*, 5685. [CrossRef]
37. Gebremichael, S.; Birhanu, T.; Tessema, D.A. Analysis of organochlorine pesticide residues in human and cow's milk in the towns of Asendabo, Serbo and Jimma in South-Western Ethiopia. *Chemosphere* **2013**, *90*, 1652–1657. [CrossRef]
38. El Hawari, K.; Mokh, S.; Al Iskandarani, M.; Halloum, W.; Jaber, F. Pesticide residues in Lebanese apples and health risk assessment. *Food Addit. Contam. B* **2019**, *12*, 81–89. [CrossRef]
39. Kottadiyil, D.; Mehta, T.; Thasale, R.; Sivaperumal, P. Determination and dietary risk assessment of 52 pesticide residues in vegetable and fruit samples by GC-MS/MS and UHPLC-QTOF/MS from Gujarat, India. *J. Food Compos. Anal.* **2023**, *115*, 104957. [CrossRef]
40. Tripathy, V.; Sharma, K.K.; Sharma, K.; Gupta, R.; Yadav, R.; Singh, G.; Aggarwal, A.; Walia, S. Monitoring and dietary risk assessment of pesticide residues in brinjal, capsicum, tomato, and cucurbits grown in Northern and Western regions of India. *J. Food Compos. Anal.* **2022**, *110*, 104543. [CrossRef]
41. Philippe, V.; Neveen, A.; Marwa, A.; Ahmad Basel, A.-Y. Occurrence of pesticide residues in fruits and vegetables for the Eastern Mediterranean Region and potential impact on public health. *Food Control* **2021**, *119*, 107457. [CrossRef]
42. Ngabirano, H.; Birungi, G. Pesticide residues in vegetables produced in rural south-western Uganda. *Food Chem.* **2022**, *370*, 130972. [CrossRef] [PubMed]
43. Osaili, T.M.; Al Sallagi, M.S.; Dhanasekaran, D.K.; Bani Odeh, W.A.M.; Al Ali, H.J.; Al Ali, A.A.S.A.; Radwan, H.; Obaid, R.S.; Holley, R. Pesticide residues in fresh vegetables imported into the United Arab Emirates. *Food Control* **2022**, *133*, 108663. [CrossRef]
44. Ramadan, M.F.A.; Abdel-Hamid, M.M.A.; Altorgoman, M.M.F.; AlGaramah, H.A.; Alawi, M.A.; Shati, A.A.; Shweeta, H.A.; Awwad, N.S. Evaluation of pesticide residues in vegetables from the Asir Region Saudi Arabia. *Molecules* **2020**, *25*, 205. [CrossRef]
45. Vu-Duc, N.; Nguyen-Quang, T.; Le-Minh, T.; Nguyen-Thi, X.; Tran, T.M.; Vu, H.A.; Chu, D.B. Multiresidue pesticides analysis of vegetables in Vietnam by ultrahigh-performance liquid chromatography in combination with high-resolution mass spectrometry (UPLC-Orbitrap MS). *J. Anal. Methods Chem.* **2019**, *2019*, 3489634. [CrossRef]
46. Nguyen, N.T.; Huynh Nguyen, K.; Duong, T.; Tran, V.D.; Kim, K.H.; Pham, L.T. Evaluation of pesticide residues in vegetables from Mekong, Delta, Vietnam using LC-MS/MS. *Trop. J. Pharm. Res.* **2021**, *20*, 1497–1504. [CrossRef]
47. Carrasco Cabrera, L.; Medina Pastor, P. The 2020 European Union report on pesticide residues in food. *EFSA J.* **2022**, *20*, e07215. [CrossRef]

48. Wu, P.; Wang, P.; Gu, M.; Xue, J.; Wu, X. Human health risk assessment of pesticide residues in honeysuckle samples from different planting bases in China. *Sci. Total Environ.* **2021**, *759*, 142747. [CrossRef]
49. Pang, G.-F.; Fan, C.-L.; Liu, Y.-M.; Cao, Y.-Z.; Zhang, J.-J.; Fu, B.-L.; Li, X.-M.; Li, Z.Y.; Wu, Y.P. Multi-residue method for the determination of 450 pesticide residues in honey, fruit juice and wine by double-cartridge solid-phase extraction/gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. *Food Addit. Contam.* **2006**, *23*, 777–810. [CrossRef]
50. Pang, G.F.; Fan, C.L.; Chang, Q.Y.; Li, J.X.; Lu, J.K.L.M. Screening of 485 pesticide residues in fruits and vegetables by liquid chromatography-quadrupole-time-of-flight mass spectrometry based on TOF Accurate Mass Database and QTOF Spectrum Library. *J. AOAC Int.* **2018**, *101*, 1156–1182. [CrossRef] [PubMed]
51. Tran-Lam, T.-T.; Bui, M.Q.; Nguyen, H.Q.; Dao, Y.H.; Le, G.T. A combination of chromatography with tandem mass spectrometry systems (UPLC-MS/MS and GC-MS/MS), modified QuEChERS extraction and mixed-mode SPE clean-up method for the analysis of 656 pesticide residues in rice. *Foods* **2021**, *10*, 2455. [CrossRef]
52. Anastassiades, M.; Lehotay, S.J.; Štajnbaher, D.; Schenck, F.J. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and 'dispersive solid-phase extraction' for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412–431. [CrossRef] [PubMed]
53. Song, X.; Li, F.; Yan, T.; Tian, F.; Ren, L.; Jiang, C.; Wang, Q.; Zhang, S. Research progress in the sample pretreatment techniques and advanced quick detection methods of pesticide residues. *Process Saf. Environ. Prot.* **2022**, *165*, 610–622. [CrossRef]
54. Calza, P.; Guarino, B.; Bello, F.D.; Dioni, A.; Bergero, M.; Medana, C. Integrated approach for the analysis of neonicotinoids in fruits and food matrices. *Food Chem.* **2022**, *372*, 131153. [CrossRef]
55. Watanabe, E. Review of sample preparation methods for chromatographic analysis of neonicotinoids in agricultural and environmental matrices: From classical to state-of-the-art methods. *J. Chromatogr. A* **2021**, *1643*, 462042. [CrossRef]
56. Rutkowska, E.; Łozowicka, B.; Kaczyński, P. Three approaches to minimize matrix effects in residue analysis of multiclass pesticides in dried complex matrices using gas chromatography tandem mass spectrometry. *Food Chem.* **2018**, *279*, 20–29. [CrossRef]
57. Rutkowska, E.; Łozowicka, B.; Kaczyński, P. Compensation of matrix effects in seed matrices followed by gas chromatography-tandem mass spectrometry analysis of pesticide residues. *J. Chromatogr. A* **2019**, *1614*, 460738. [CrossRef]
58. Musarurwa, H.; Chimuka, L.; Pakade, V.E.; Tavengwa, N.T. Recent developments and applications of QuEChERS based techniques on food samples during pesticide analysis. *J. Food Compos. Anal.* **2019**, *84*, 103314. [CrossRef]
59. Xu, M.L.; Gao, Y.; Wang, X.; Han, X.X.; Zhao, B. Comprehensive strategy for sample preparation for the analysis of food contaminants and residues by GC–MS/MS: A review of recent research trends. *Foods* **2021**, *10*, 2473. [CrossRef]
60. García-Vara, M.; Postigo, C.; Palma, P.; José Bleda, M.; López de Alda, M. QuEChERS-based analytical methods developed for LC-MS/MS multiresidue determination of pesticides in representative crop fatty matrices: Olives and sunflower seeds. *Food Chem.* **2022**, *386*, 132558. [CrossRef] [PubMed]
61. Wang, F.; Li, S.; Feng, H.; Yang, Y.; Xiao, B.; Chen, D. An enhanced sensitivity and cleanup strategy for the nontargeted screening and targeted determination of pesticides in tea using modified dispersive solid-phase extraction and cold-induced acetonitrile aqueous two-phase systems coupled with liquid chromatography-high resolution mass spectrometry. *Food Chem.* **2019**, *275*, 530–538. [CrossRef] [PubMed]
62. European Commission. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed 11312/2021. Available online: https://food.ec.europa.eu/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf (accessed on 5 November 2022).
63. European Commission. Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-Approval Control and Monitoring Purposes SANTE/2020/12830, Rev. 1. Available online: https://food.ec.europa.eu/system/files/2021-02/pesticides_mrl_guidelines_2020-12830.pdf (accessed on 5 November 2022).
64. US FDA. *Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products*, 3rd ed.; Food and Drug Administration: Silver Spring, MD, USA, 2020. Available online: https://www.fda.gov/media/121751/download (accessed on 5 November 2022).
65. Ambrus, A. Reliability of measurements of pesticide residues in food. *Accredit. Qual. Assur.* **2004**, *9*, 288–304. [CrossRef]
66. Ambrus, Á.; Zentai, A.; Sali, J.; Ficzere, I. Hidden contributors to uncertainty and accuracy of results of residue analysis. *Accredit. Qual. Assur.* **2011**, *16*, 3–11. [CrossRef]
67. Lehotay, S.J.; Han, L.; Sapozhnikova, Y. Use of a quality control approach to assess measurement uncertainty in the comparison of sample processing techniques in the analysis of pesticide residues in fruits and vegetables. *Anal. Bioanal. Chem.* **2018**, *410*, 5465–5479. [CrossRef]
68. Lehotay, S.J.; Michlig, N.; Lightfield, A.R. Assessment of test portion sizes after sample comminution with liquid nitrogen in an improved high-throughput method for analysis of pesticide residues in fruits and vegetables. *J. Agric. Food Chem.* **2020**, *68*, 1468–1479. [CrossRef]
69. Ramsey, C.A.; Wagner, C. Sample quality criteria. *J. AOAC Int.* **2015**, *98*, 265–268. [CrossRef]
70. FAO. Recommended method of sampling for the determination of pesticide residues for compliance with mrls CXG33-1999. Available online: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B33-1999%252FCXG_033e.pdf (accessed on 5 November 2022).

71. FAO. CAC guidelines on performance criteria for methods of analysis for the determination of pesticide residues in food and feed CXG90-2017. Available online: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B90-2017%252FCXG_090e.pdf (accessed on 5 November 2022).
72. Bettencourt da Silva, R.J.N.; Santos, J.R.; Filomena, M.; Camoes, G.F.C. Evaluation of the analytical method performance for incurred samples. *Anal. Chim. Acta* **2003**, *485*, 241–252. [CrossRef]
73. Fussel, R.J.; Hetmanski, M.T.; MacArthur, R.; Findlay, D.; Smith, F.; Ambrus, Á.; Brodesser, P.J. Measurement uncertainty associated with sample processing of oranges and tomatoes for pesticide residue analysis. *J. Agric. Food Chem.* **2007**, *55*, 1062–1070. [CrossRef]
74. Suszter, K.G.; Ambrus, Á. Testing the efficiency of extraction of incurred residues from soil with optimized multi-residue method. *J. Env. Sci. Health B* **2017**, *52*, 547–556. [CrossRef]
75. Han, L.; Lehotay, S.J.; Sapozhnikova, Y. Use of an efficient measurement uncertainty approach to compare room temperature and cryogenic sample processing in the analysis of chemical contaminants in foods. *J. Agric. Food Chem.* **2017**, *66*, 4986–4996. [CrossRef] [PubMed]
76. Wang, W.; Cho, Y.-J.; Song, J.-W.; Kim, Y.-J.; Seo, J.-S.; Kim, J.-H. Residue behavior of methoxyfenozide and pymetrozine in Chinese cabbage and their health risk assessment. *Foods* **2022**, *11*, 2995. [CrossRef] [PubMed]
77. Ambrus, Á. Variability of pesticide residues in crop units. *Pest Manag. Sci.* **2006**, *62*, 693–714. [CrossRef] [PubMed]
78. Horváth, Z.; Ambrus, Á.; Mészáros, L.; Braun, S. Characterization of distribution of pesticide residues in crop units. *J. Environ. Sci. Health B* **2013**, *48*, 615–625. [CrossRef]
79. Ambrus, Á. Estimation of sampling uncertainty for determination of pesticide residues in plant commodities. *J. Env. Sci. Health B* **2009**, *44*, 627–639. [CrossRef]
80. Farkas, Z.; Cook, J.M.; Ambrus, Á. Estimation of uncertainty of measured residues and testing compliance with MRLs. In *Food Safety Assessment of Pesticide Residues*, 1st ed.; Ambrus, Á., Hamilton, D., Eds.; World Scientific: Hackensack, NJ, USA, 2017; pp. 404–466.
81. FAO. Portion of Commodities to Which Maximum Residue Limits Apply and Which is Analyzed CAC/GL 41-1993. Available online: www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B41-1993%252FCXG_041e.pdf (accessed on 5 November 2022).
82. Omeroglu, P.Y.; Ambrus, Á.; Boyacioglu, D.; Majzik, E.S. Uncertainty of the sample size reduction step in pesticide residue analysis of large-sized crops. *Food Addit. Contam. Part A* **2013**, *30*, 116–126. [CrossRef]
83. Ambrus, Á.; Szemánné-Dobrik, H.; Domák, Z.; Kerekes, K. Sources of random variation of pesticide residue analytical results. *J. AOAC Int.* **2021**, *104*, 526–538. [CrossRef]
84. Gy, P. *Sampling for Analytical Purposes*; John Wiley & Sons Ltd.: Chichester, UK, 1999; p. 71.
85. Minkkinen, P. Practical applications of sampling theory. *Chemometr. Intell. Lab. Syst.* **2004**, *74*, 85–94. [CrossRef]
86. Hill, A.R.C.; Harris, C.A.; Warburton, A.G. Effect of sample processing on pesticide residues in fruit and vegetables. In *Principles of Method Validation*; Fajgelj, A., Ambrus, Á., Eds.; Royal Society of Chemistry: Cambridge, UK, 2000; pp. 41–48.
87. Ambrus, Á.; Suszter, G. Quality Control and Quality Assurance. In *Analysis of Pesticides in Food and Environmental Samples*, 2nd ed.; Toledo, J., Ed.; CRC Press: Roca Baton, FL, USA, 2019; pp. 135–173.
88. Ritter, L.S.; Lynn, K.J.; Wujcik, C.E.; Buchholz, L.M. Interlaboratory assessment of cryomilling sample preparation for residue analysis. *J. Agric. Food Chem.* **2015**, *63*, 4405–4408. [CrossRef]
89. Ritter, L.S.; Wujcik, C.E. Novel two-stage fine milling enables high-throughput determination of glyphosate residues in raw agricultural commodities. *J. AOAC Internat.* **2018**, *101*, 867–875. [CrossRef]
90. Roussev, M.; Lehotay, S.J.; Pollaehne, J. Cryogenic sample processing with liquid nitrogen for effective and efficient monitoring of pesticide residues in foods and feeds. *J. Agric. Food Chem.* **2019**, *67*, 9203–9209. [CrossRef]
91. Thi Le, L.H.; Tran-Lam, T.T.; Cam, T.Q.; Nguyen, T.N.; Dao, Y.N. Pesticides in edible mushrooms in Vietnam. *Food Addit. Contam. B* **2021**, *14*, 139–148. [CrossRef]
92. Ly, T.-K.; Behra, P.; Nhu-Trang, T.-T. Quantification of 397 pesticide residues in different types of commercial teas: Validation of high accuracy methods and quality assessment. *Food Chem.* **2022**, *370*, 130986. [CrossRef] [PubMed]
93. FAO. Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed. In *FAO Plant Production and Protection paper 225*; FAO: Rome, Italy, 2016; p. 298.
94. FAO. Acibenzolar-S-methyl, benzovindiflupyr, flupyradifurone, penconazole, sulfoxaflor. In *Pesticide Residues in Food–2016 Evaluations*; FAO Plant Production and Protection Paper 231; FAO: Rome, Italy, 2017; p. 2403.
95. FAO. Ethephon, flutriafol, fluxapyroxad. In *Pesticide Residues in Food–2015 Evaluations*; FAO Plant Production and Protection Paper 226; FAO: Rome, Italy, 2016; p. 1629.
96. FAO. Pesticide Residues in Food—Reports. FAO Plant Production and Protection Papers Series. Available online: http://www.fao.org/agriculture/crops/core-themes/theme/pests/jmpr/jmpr-rep/en/ (accessed on 7 November 2022).
97. European Commission. Download MRLs Data. Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/mrls/download (accessed on 5 November 2022).
98. European Commission. Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods, SANTE/2017/10632 Rev. 4. 2022. Available online: https://food.ec.europa.eu/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2017-10632.pdf (accessed on 10 November 2022).

99. Ferrer, A.C. Personal communication 2022, EU-RT-FV17 Preliminary Results. 2022; *unpublished*.
100. Ellison, S.L.R.; Williams, A. (Eds.) *EURACHEM/CITAC Guide: Quantifying Uncertainty in Analytical Measurement*, 3rd ed.; CITAC: Kowloon Bay, Hong Kong, 2012; Available online: https://www.eurachem.org/index.php/publications/guides/quam (accessed on 5 November 2022).
101. Ambrus, Á.; Kamirán, Á.H.; Kötelesné-Suszter, G.; Németh, A.; Solymosné, M.E. Accuracy of analytical standard solutions and uncertainty in their nominal concentrations. *J. Food Investig.* **2017**, *63*, 1398–1421.
102. *ISO 5725-2:2019*; Accuracy (Trueness and Precision) of Measurement Methods and Results Part 2: Basic Method for the Determination of Repeatability and Reproducibility of a Standard Measurement Method. 2019. Available online: www.iso.org/standard/69419.html (accessed on 5 November 2022).
103. Ambrus, Á; Noonan, G.; Németh, A.; Kötelesné-Suszter, G.; Anastassiades, M.; Kamirán, Á.H.; Solymosné, M.E. Testing the accuracy of analytical standard solutions used for quantitative determination of pesticide residues. *J. AOAC Internat.* **2017**, *100*, 1–4. [CrossRef] [PubMed]
104. Ambrus, A.; Lantos, J.; Visi, E.; Csatlós, I.; Sárvári, L. General method for the determination of pesticide residues in samples of plant origin, soil and water, i extraction and cleanup. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 733–742. [CrossRef]
105. Ambrus, Á.; Buczkó, J.; Hamow, K.Á.; Juhász, V.; Solymosné Majzik, E.; Szemánné Dobrik, H.; Szitás, R. Contribution of sample processing to variability and accuracy of the results of pesticide residue analysis in plant commodities. *J. Agric. Food Chem.* **2016**, *64*, 6071–6081. [CrossRef] [PubMed]
106. Fussell, R.J.; Jackson Addie, K.; Reynolds, S.L.; Wilson, M.F. Assessment of the stability of pesticides during cryogenic sample processing. 1. Apples. *J. Agric. Food Chem.* **2002**, *50*, 441–448. [CrossRef]
107. Magnusson, B.; Örnemark, U. *Eurachem Guide: The Fitness for Purpose of Analytical Methods–a Laboratory Guide to Method Validation and Related Topics*, 2nd ed. EURACHEM. 2014. Available online: https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf (accessed on 5 November 2022). ISBN 978-91-87461-59-0.
108. Thompson, M.; Howarth, R.J. The frequency distribution of analytical error. *Analyst* **1980**, *105*, 1188–1195. [CrossRef]
109. *ISO/IEC 17025:2017*; General Requirements for the Competence of Testing and Calibration Laboratories. International Organization for Standardization: Geneva, Switzerland, 2018.
110. Youden, W.J. *Statistical Techniques for Collaborative Tests*; AOAC: Washington, DC, USA, 1967.
111. Anderson, L.R. *Practical Statistics for Analytical Chemists. Appendix D12*; Van Nostrand Reinhold Company: New York, NY, USA, 1987; ISBN 0442209738.
112. Anastassiades, M.; Maštovská, K.; Lehotay, S.J. Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides. *J. Chromatogr. A* **2003**, *1015*, 163–184. [CrossRef]
113. Maštovská, K.; Lehotay, S.J.; Anastassiades, M. Combination of analyte protectants to overcome matrix effects in routine GC analysis of pesticide residues in food matrixes. *Anal. Chem.* **2005**, *77*, 8129–8137. [CrossRef] [PubMed]
114. Rodríguez-Ramos, R.; Lehotay, S.J.; Michlig, N.; Socas-Rodríguez, B.; Rodríguez-Delgado, M.Á. Critical review and re-assessment of analyte protectants in gas chromatography. *J. Chromatogr. A* **2020**, *1632*, 461596. [CrossRef] [PubMed]
115. Soboleva, E.; Ambrus, Á. Application of a system suitability test for quality assurance and performance optimisation of a gas chromatographic system for pesticide residue analysis. *J. Chromatogr. A* **2004**, *1027*, 55–65. [CrossRef] [PubMed]
116. Miller, J.C.; Miller J., N. *Statistics and Chemometrics for Analytical Chemistry*, 6th ed.; Ellis Horwood PTR, Prentice Hall Pearson: Harlow, UK, 2010; p. 297. ISBN 978-0-273-73042-2.

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