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Review Article

Reliability of Pesticide Residue Data Used for Estimation of Consumers Exposure

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Abstract

The safety of pesticide residues should be evaluated based on the residues remained in treated crops. The accuracy and precision of residue data used for exposure assessment are affected by the performance of steps of pesticide residue analysis. Analysts usually simply pay attention to the detection of the residues in spiked test portions and report the performance parameters based on these measurements which may only have a minor influence on the true accuracy and reproducibility of the results.

The objective of this paper is to draw the attention to the importance of obtaining representative samples, the thorough comminution of the edible portion of food commodities, testing the stability of residues during the whole process and selection of residue components to be analysed. The potential bias and combined uncertainty of the steps of the whole procedure shall be estimated and considered in assessing the reliability of calculated daily intakes. The useful references given provide details for the correct performance of the whole processes.

Keywords: *Pesticide Residues Analysis; Reliability if Results; Quality Control; Consumer's Exposure; Dietary Intake Assessment*

Abbreviations

ADI: Acceptable Daily Intake; ARfD: Acute Reference Dose; bw: Bodyweight [kg]; CAC: Codex Alimentarius Commission; EC: European Commission; EDI: Estimated Daily Intake; EFSA: European Food Safety Authority; EPC: European Parliament and Council; ESTI: Estimate of Short-Term Intake; EU: European Union; FAO: Food and Agriculture Organization of United Nations; GAP: Good Agricultural Practice; JMPR: FAO/WHO Joint Meeting on Pesticide Residues; MRL: Maximum Residue Limit [mg/kg]; P: Primary SamplesQC: Quality Control; TP: Test Portion Withdrawn from the Processed Analytical Sample for Extraction; USA: United States of America; WHO, World Health Organization

Introduction

The use of pesticides is inevitable for providing sufficient and safe food for the continuously growing population of the World. The increasingly popular 'bio products' can only contribute with a minor proportion to the food supply required. The active ingredients of pesticides are toxic compounds having chronic and in certain cases acute toxicity of greatly different magnitude. For instance, the acceptable long -term intakes (ADI) evaluated by the 2019 JMPR ranged between 0.001 - 4 mg residue/kg(bw) per day and 0.003 mg/kg(bw) day, while the lowest acute reference dose (ARfD) was 0.003 mg/kgbw per day and there were compounds for which establishing ARfD was not necessary [1]. These ranges practically cover all pesticides for which Codex MRLs have been established [2].

The authorisation of pesticides is a complex process. As the first steps the biological efficacy, human and environmental toxicities of the active ingredients are tested. If the tests gave satisfactory results, the optimal use conditions (dosage, frequency of application, pre-harvest intervals etc.) are determined and published in the registration documents including the authorised use patterns, considered as Good Agricultural Practice (GAP), and the permissible maximum residue limits. To facilitate international trade the Codex Alimentarius Commission establish the Codex MRLs [2] based on the toxicological and residue evaluation of the Joint FAO/WHO expert meeting on pesticide residues (JMPR) [3]. The MRLs reflect the highest residues expected following the maximum recommended use of pesticides, which must lead to consumer exposure lower than the corresponding ADI or ARfD values. Consequently, the national or Codex MRLs are not safety limits, but residues at the MRLs must be toxicologically acceptable [4].

To assure safe and efficient use of pesticides and to verify compliance with MRLs large number of samples are analysed for pesticide residues annually in many countries [5-7]. For instance, during 2015-17 over 84000 samples were analysed every year within the EU Co-ordinated Monitoring Programmes, and the residues complied with the MRLs were over 95-98% of the samples [8-10]. This programme is complemented with the national residue control activities amounting to several thousand samples in each year in many countries.

Notwithstanding the complex efficacy and safety evaluations of pesticides before their use is authorised and the extensive monitoring programmes indicated high level of compliance, 30 - 49% of the population are concerned about pesticide residues in the majority of member states of the European Union, while their proportion is > 50% in 9 countries [11].

To enable drawing realistic conclusions on the safety of our food, the residue analytical measurements should be reliable and accurate. There is no information on the performance of the residue laboratories during the monitoring programmes, but certain quality assurance quality control programmes must be in place as these laboratories should be accredited according to ISO 17025 Standard [12]. However, the proficiency test results indicate the capabilities of the laboratories, which can be expected when samples are analysed with full attention. Participating in proficiency tests is one of the basic requirements of accreditation according to ISO 17025 standard and it is compulsory for the European Reference and Official Laboratories. As an example, the performance of the laboratories in the EU, the results of a proficiency test are shown in figure 1. Hundred forty-five laboratories participated in the test. They received a list of potential pesticide residues (162) of which actually 18 compounds were present and had to be identified and quantified.

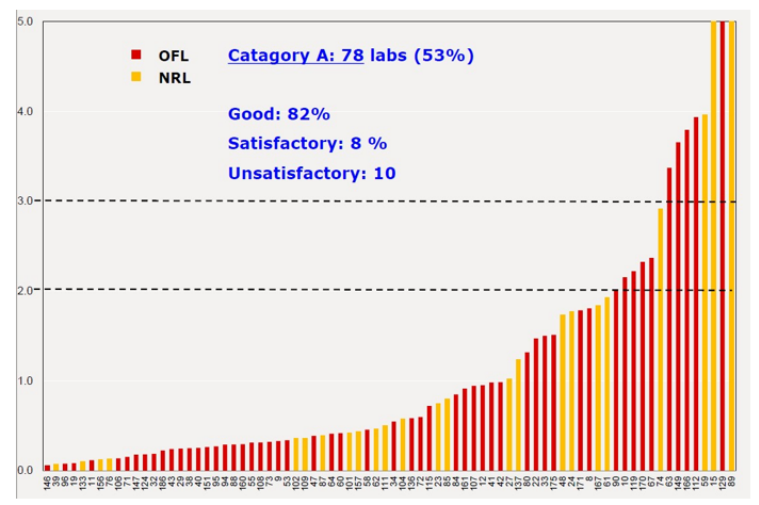

Figure 1: *Results of European proficiency test for multi pesticides in cereal grains. (NRL: National Reference Laboratory, OFL: Official Laboratory.*

The average Z-score is used in the EU proficiency test programmes for evaluation of the performance of participating laboratories [13].

$$AZ^2 = \frac{\sum_{i=1}^{n} Z^2}{n} \quad (1)$$

where Z is the standard normal variate calculated with typical standard deviation obtained with robust statistical methods, n is the number of quantified analytes reported. If the average Z-score is ≤ 2.0, or between 2 - 3, the performance of the laboratory is good and satisfactory, respectively.

The main steps of determination of pesticide residues are illustrated in figure 2. The definitions of terms [14] used in figure 2 follow:

- Consignment may consist of several lots or part of a lot.
- Sample preparation: The first of two processes which may be required to convert the laboratory sample into the test sample. The removal of parts that are not to be analyzed [15], if required.
- Sample processing: The second of two processes which may be required to convert the laboratory sample into the test sample. The process of homogenization, comminution, mixing, etc., if required.
- Test sample: The laboratory sample after removal of any parts that are not to be analyzed, e.g. bones, adhering soil. It may or may not be comminuted and mixed before withdrawing test portions.
- Test portion: A representative sub-sample of the test sample, i.e. the portion which is to be analyzed.

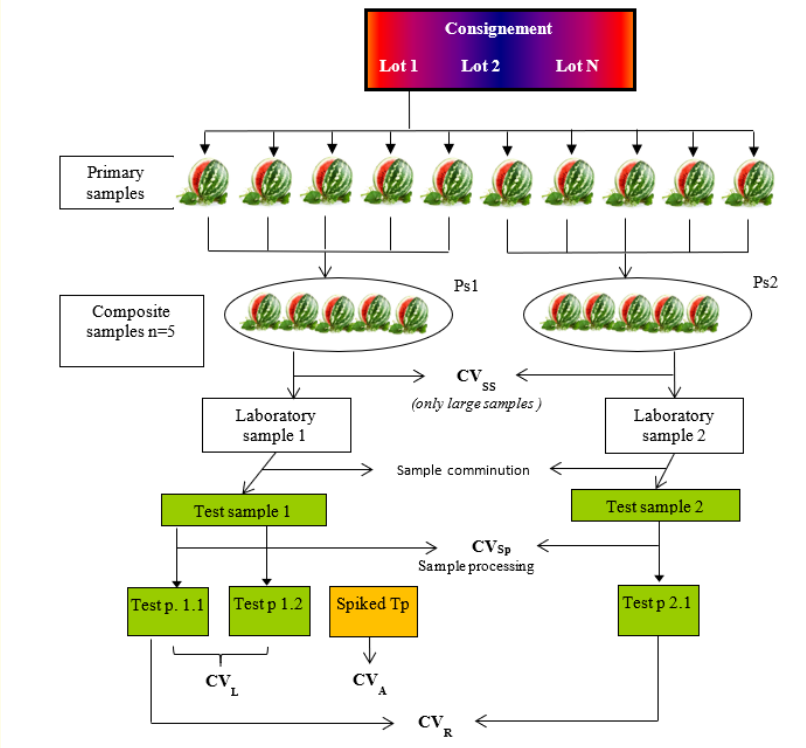

Figure 2: *Illustration of the steps of pesticide residue analysis (adapted with permission from Farkas Zs. Optimization of Sampling Procedures for Verifying Compliance of Commodities with Maximum Residue Limits of Pesticides, Doctoral Thesis St. István University Budapest, 2017).*

The results of method development and validation are most frequently published based on spiking the test portions withdrawn from the comminuted test sample. The contribution of sample mass reduction of large crops and disintegration of the parts of analytical sample to the accuracy and uncertainty of the results are usually not considered or reported in these publications.

The combined uncertainty of the results (CV_R) including sampling [16] and the laboratory phase (sample mass reduction, sample processing and analysis) (CV_L) shall be considered in interpreting the measurement data for dietary exposure assessment [17-19] and testing compliance with legal limits which refer to the average residues in the samples specified by the corresponding sampling guidance documents [20]. Examples for the calculation of uncertainty of measurement results are given by Farkas., *et al* [21].

The objective of this short paper is to call the attention of analysts to typical errors in the determination of pesticide residues to facilitate obtaining accurate, fit for the purpose results.

Selection of residue components to be determined

The chronic and acute exposure of consumers are calculated with probabilistic methods in the USA [22] and with deterministic method applied in many other countries [23,24]. For dietary exposure assessment all residue components shall be determined in the edible portions of food items which are included in the definition of residues for risk assessment purposes. While for testing the compliance with MRLs, the residues defined for enforcement purposes should be determined in the portion of commodity to which the MRLs refer to [15]. The definition for enforcement is made as simple as possible to facilitate the official control of pesticide residues in large number samples [25]. The definition of residues may also be different for different foods depending on the significant metabolites present in them. A good example is [26,27] cyantraniliprole [3-bromo-1-(3-chloro-2-pyridyl)-4′-cyano-2′-methyl-6′-(methylcarbamoyl)pyrazole-5-carboxanilide]. Four different definitions were proposed by the JMPR for this compound depending on the type of food:

1. Definition of residue for compliance with MRL for both animal and plant commodities: cyantraniliprole.
2. Definition of residue for estimation of dietary intake for unprocessed plant commodities: cyantraniliprole.
3. Definition of residue for estimation of dietary intake for processed plant commodities: sum of cyantraniliprole and IN –J9Z38, expressed as cyantraniliprole.
4. Definition of residue for estimation of dietary intake for animal commodities: sum of cyantraniliprole, 2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1H-pyrazol-5-yl]-3,4-dihydro3,8-dimethyl-4-oxo-6-quinazolinecarbonitrile [IN-J9Z38], 2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1Hpyrazol-5-yl]-1,4-dihydro-8-methyl-4-oxo-6-quinazolinecarbonitrile [IN-MLA84], 3-Bromo-1-(3-chloro-2-pyridinyl)-N-[4-cyano-2-(hydroxymethyl)-6-[(methylamino)carbonyl]phenyl]-1H-pyrazole5-carboxamide [IN- N7B69] and 3-Bromo-1-(3-chloro-2-pyridinyl)-N-[4-cyano2[[(hydroxymethyl)amino]carbonyl]-6-methylphenyl]-1H-pyrazole-5-carboxamide [IN-MYX98], expressed a cyantraniliprole.

Consequently, careful planning and validation of targeted analytical methods are required before residue data are collected for assessment of consumer exposure. References and explanation for the definition of residues can be found in the FAO/WHO JMPR Evaluation of pesticide residues [28].

In many cases, the determination of all residue components included in the definition for dietary intake calculation is very difficult, may require analytical standards not readily available or several separate analytical procedures. The best estimate for the residue levels for intake calculations can be made by taking into account the residues derived from supervised trials where the two kinds of residue concentrations are reported separately. The EFSA publishes the reports on residue evaluations including the STMR and MRL values used as input for the long-and shorth term intake (if relevant) calculations [29]. The ratio of these input values can be used as first approximation to convert the residues, included in enforcement residue definition, measured in edible portions of food items. Alternately, the ratios of original residues data published by the JMPR or EFSA can be evaluated and a conversion factor calculated [30].

Distribution of pesticide residues

The pesticide residues are unevenly distributed in crop units on treated fields [31,32] and following the same pesticide treatment the field to field variation of average residue concentrations can be described with a typical CV value of 80% [33]. The pesticide residues are unevenly distributed within individual fruits or vegetables as well. Usually the peel contains higher residues then the pulp. The residues are generally much higher in outer leaves of leafy vegetables than the internal part. The great variability of residues within and among treated fields shall be taken into account in applying sampling protocols, designing sampling plans and processing samples.

Sampling

The sampling procedure elaborated by the Codex Committee on Pesticide Residues [15] for enforcement of MRLs is commonly accepted around the Globe. It is suitable for consumer's intake assessment, though collection of higher number of primary samples would be advantageous, provided that the larger sample mass can be reproducibly processed in the laboratory. Testing laboratories should pay attention to the quality of samples [34,35] received and reject those which do not comply with the minimum requirements (e.g. number of crop units being in the sample, minimum mass of sample) specified in the sampling standard or guidance documents.

The uncertainty of sampling for determination of pesticide residues was estimated based on the evaluation of over ten thousand samples taken from supervised trials or fields treated according to good agriculture practice [36]. The most comprehensive data on sampling uncertainty was published by Farkas., *et al.* [21] based on the evaluation of variability of residues in replicate samples derived from supervised trials. The compiled typical sampling uncertainties can be used for interpretation of the results in case of pre-market, pre-export control or dietary exposure assessment.

Sample preparation, sample processing

These terms have special meaning in pesticide residue analysis and should be used accordingly to avoid confusion.

The process of sample preparation cannot be validated, but the correct results can be assured by preparing detailed standard operation procedures (SOPs) and staff training.

One of the critical parts of sample processing is to reduce the mass of laboratory sample (e.g. 5 pcs watermelons or cabbage heads) to obtain a representative portion which can be homogenized with the available equipment. It can be achieved by cutting segments (and slices) from each crop and combine one or two segments from each crop unit to form the test sample [37].

Testing the homogeneity of test sample (also called analytical sample) is generally neglected by the analysts who concentrate on the analysis of test portions. A comprehensive review of the current practices was published by Lehotay and Jo [38]. The authors emphasised the importance of this step in obtaining accurate and reproducible results, especially in cases where the test portions are reduced to < 5 grams or even smaller portions are spiked and extracted. The samples should be comminuted together with dry ice at or below -20° [39,40] or preferably applying liquid nitrogen [41] for rapid cooling of the analytical sample to obtain thoroughly homogenised matrix and reduce the possibility of decomposition of pesticide residues.

Quality control

Regular, fit for the purpose quality control (QC) procedures are discussed in detail in a recent publication [42]. The laboratories should select those which are suitable for their conditions.

Conclusion

The laboratories analysing, pesticide residues or other contaminants in food should always pay attention to the quality of samples received, and include the whole process (sample mass reduction, comminution ("homogenization") of test sample, extraction, clean-up and instrumental determination) in the estimation of uncertainty of the results, and the repeatability, reproducibility of the method. For

dietary intake calculation the residues, defined for risk assessment, should be determined in the edible portion of food items. Alternately, the residue patterns derived from supervised trials should be evaluated and the ratio of the sum of residues for risk assessment and the residues for enforcement should be used to adjust the residue concentration including residue components defined for enforcement purposes.

Conflict of Interest

The authors declare no conflict of interest.

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