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# HYBRID APPROACH TO ASSESSING THE UNCERTAINTY OF MEASURING INDICATORS OF BIOLOGICAL SAFETY OF AGRICULTURAL RAW MATERIALS

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**Abstract.** The article analyzes the current state of the concept of safety of agricultural raw materials and products, the consequences of harmonizing national requirements in accordance with international standards, and a different approach to evaluating the results of laboratory analysis of raw materials. 147 consecutive routine measurements of the concentration of the genetically modified soybean line MON 40-3-2 in CRM were analyzed using different evaluation methods and the content of genetically modified organisms was determined using real-time polymerase chain reaction (PCR-RT) and a hybrid approach to assessing the uncertainty of the results of measuring biological safety indicators. A model hybrid approach to assessing measurement uncertainty according to type A was tested by estimating the standard uncertainty of precision based on the results of retrospective analysis of routine measurements of certified reference material under conditions of intra-laboratory reproducibility in combination with the standard uncertainty of error based on the results of interlaboratory comparison with the participation of an accredited provider. The results obtained using four "classical" and hybrid approaches to the assessment of measurement uncertainty were compared, considering the requirements for using expanded uncertainty in decision-making regarding the safety of food and feed agricultural raw materials. It was found that most modern approaches proposed by international regulatory documents are focused on more stable systems, for example, in calibration or chemical analysis, which have several advantages and disadvantages, which significantly affects the quality of the calculated interval. The approach to evaluating the results is based on the arithmetic mean of the sample, returns a narrow interval and cannot sufficiently consider the error. The method is highly dependent on the sample size and the presence of outliers among the measurement results. The arithmetic mean is not always the optimal measure of central tendency in the conditions of methods for analyzing biological indicators. The use of a sample of measurement results of certified reference material allows for a balanced assessment of uncertainty, considering the contributions of the precision and error components. However, this approach cannot always consider the influence of the sample preparation procedure, which often includes steps such as homogenization, release or extraction of the analyte, uniform distribution of the measurement object and other influencing factors. The approach, which is based on the results of interlaboratory comparisons with the participation of an accredited provider, provides a significant evidence base, but considers the conditions of measurement of all laboratories participating in the comparison. This forms the distance of the obtained interval from the aspects of the implementation of the analytical procedure in the conditions of a particular laboratory, and the obtained intervals may significantly exceed the practical uncertainty of this particular laboratory. Differences between the intervals obtained using different approaches can have a significant impact on decision-making regarding product safety, and the uncertainty has significant variability over time. Despite the absence of a direct requirement to update information on uncertainty, which is already established for a test method during the relevant study, there is a need for periodic reassessment of its interval. The need to develop a model approach to assessing measurement uncertainty has been established, which will allow considering as many influential sources of uncertainty as possible that may affect the result of the analysis of biological safety indicators.

**Keywords:** quality control, measurement uncertainty, biological safety indicators.

## Introduction. Formulation of the problem

The current state of the concept of safety of agricultural raw materials and products has undergone significant changes during the last decade. As a result of the active harmonization of national requirements in accordance with international standards, not only the list of characteristics that must be established, but also the approach to decision-making, which is based on the results of laboratory analysis, has changed [1]. Current guidelines require a well-founded and risk-oriented approach to decision-making regarding product compliance with established criteria. One of the most important such requirements is the need to take measurement uncertainty into account. Mutual recognition of the results of measurements obtained by laboratories, which is based on accreditation by bodies that have an international MRA (The Mutual Recognition Arrangement), requires the presence of a procedure for assessing the uncertainty of measurements. However, in the case of measuring biological safety indicators, the development of an optimal approach to uncertainty analysis faces difficulties that lie in the peculiarities of biosystems [2]. One of these features is the natural variability of the analyte and the matrix, which may not depend on external factors and may increase under their influence. Most of the modern approaches offered by international regulatory documents are oriented towards more stable systems, for example, in calibration or chemical analysis. However, each of these approaches has a number of both advantages and disadvantages, which can significantly affect the quality of the calculated interval.

## Analysis of recent research and publications

One of the most important aspects of the safety of food and fodder agricultural raw materials is compliance with national and international requirements. According to the current conditions of international trade relations, the normative documents provide a wide list of safety indicators, which include both biological and chemical analytes [3,4]. However, in addition to the established list of indicators, the modern concept of safety requires a well-founded and risk-oriented approach to decision-making regarding the compliance of the product with the established criteria. In particular, such a decision should take into account the uncertainty of the analysis result provided by the laboratory (in accordance with ILAC G17:01 2021).

According to the fact that the true value of the indicator is a hypothetical value that is fundamentally impossible to obtain during measurement, the result of quantitative analysis is an interval that with a certain probability contains the real value of the indicator (in accordance with ISO IEC Guide 98-1 2009, BIPM JCGM 104:2009). Accordingly, deciding without considering the interval that forms the measurement uncertainty cannot satisfy the need to find a balance between the error of the first (false positive result, consumer risk) and second (false negative result, producer risk) kind (in accordance with ASME B89.7.3.1 – 2001 R2019).

Given this, among all analytes included in the list of safety indicators, the greatest difficulties arise when working with biological safety factors. In the case of analytical methods that estimate the parameters of biological objects, an effective Type B uncertainty assessment with the calculation of individual source contributions may be limited in applicability or unable to provide the necessary granularity of assessment. This is due to the high variability inherent in almost all characteristics of experimental biological samples from the matrix to the analyte and their systemic interaction. In such a case, a direct determination of the combined uncertainty, considering the influence of all or most of the type A sources, may be most appropriate [5-7]

However, even though this approach is mathematically simpler to implement, it requires a deep understanding of the process and a careful approach to the design of the experiment to ensure that the influence of as many sources of uncertainty as possible and their states are considered. In addition, it should be considered that a detailed evaluation of all or most sources of uncertainty, including enough sampling and duplicate measurements, can lead to significant material costs. In this regard, the use of retrospective data can have a positive effect on both the quality and the economic component of the type A uncertainty assessment procedure. Such data can include the accumulated results of a routine measurement of a certified reference material or an intralaboratory test of a blind sample, a chain of interlaboratory comparisons with the participation of an accredited provider, etc. However, if such data are used, evidence should be provided that they can be objectively applied, taking into account changes that may have affected the characteristics of the method over time since acquisition [5]. Our previous studies have also demonstrated the high potential of using retrospective data obtained during internal quality control, the stability of which is confirmed by a combination of standard score charts and Shewhart control charts [8].

Additional difficulties arise when choosing a method for calculating the uncertainty interval. Most of the modern general approaches for estimating the combined standard uncertainty are based on four models (in accordance with ISO/IEC Guide 98-3:2008/Supp 2:2011, BIPM JCGM 101:2008) [5,9,10]:

– estimation of the combined standard uncertainty using the standard deviation of the sample, calculated relative to the arithmetic mean of a series of measurements performed under reproducibility conditions;
– assessment of the combined standard uncertainty using the standard deviation of the sample, calculated relative to the reference value of the

certified reference material (CRM) taking into account the error;
- assessment of the combined standard uncertainty using the standard deviation of the general population, calculated by an accredited provider based on the results of an interlaboratory comparison;
- assessment of the combined standard uncertainty using the standard deviation of the general population, calculated by an accredited provider based on the results of an interlaboratory comparison, taking into account the laboratory error.

Industry standards and guidelines that provide guidelines for evaluating the uncertainty of measurements of methods of analysis of biological indicators are based on general recommendations emphasizing individual features that must be implemented in the design of the experiment according to the nature of the analyte and the analytical method (in accordance with EPPO Standard Diagnostics. PM 7/98 (5), ISO 19036:2019) [11]. However, despite the practical suitability, each of the specified methods of calculating the uncertainty interval has certain limitations and disadvantages.

Regardless of the ability to create a high-quality design that can consider all or most sources, an estimation method based on the arithmetic mean of the sample leads to the formation of narrow uncertainty intervals that cannot adequately account for error and are highly dependent on sample size and random deviations. In addition, it should be noted that the arithmetic mean is not always the best indicator of central tendency in the analysis of biological indicators.

The use of a chain of results of routine measurements of certified reference material allows to consider both precision contributions and errors. Nevertheless, this approach may not consider such an important source of uncertainty as the sample preparation procedure, including the stages of homogenization, extraction, and uniform distribution of the measurement object.

The method based on the result of interlaboratory comparisons has a significant evidence base, but the precision component considers the measurement conditions of all laboratories participating in the comparison. This leads to a distance from the peculiarities of the implementation of the measurement in a certain laboratory, mostly in the direction of overstating the real indicator.

In addition, the uncertainty of the measurement of biological indicators can be characterized by significant fluctuations in the interval, which may not be considered in the process of method validation. This variability demonstrates the need for periodic updating of measurement uncertainty assessment to support the ability to make informed decisions regarding biological safety indicators of food and feed agricultural raw materials. However, periodic update of the uncertainty interval may not be economically feasible and may not allow to cover the maximum indicators.

An alternative to experimental actualization is a retrospective analysis, of the results of measuring the SRM. Many test methods aimed at establishing indicators of biological safety of food and fodder agricultural raw materials require the use of certified reference material in the process of routine research. Examples of these methods can be quantitative molecular genetic methods based on PCR, enzyme immunoassay methods using measurements of optical density, measurement of the content of mycotoxins, etc.

However, for some methods of quantitative analysis of biological analytes, there are no control samples. An example of this can be methods of microbiological research based on counting the number of colonies, membrane filtration or the most probable number. Obtaining a reference value for such methods is a difficult task, which is traditionally solved by using the mathematical expectation described by the arithmetic mean of the sample.

According to this, the most effective can be a hybrid approach, which is based on a retrospective analysis of the chain of results of routine measurement of certified reference material, the statistical controllability of which is confirmed by combined control charts, with an assessment of the deviation of the measurement result from the reference value provided by the accredited provider. Such an uncertainty assessment model can allow for both the effect of variability inherent in laboratory-specific reproducibility conditions, covering the effect over a significant period, and the contribution of error. Thanks to this, it is possible to obtain an economic, reasonable characterization of laboratory uncertainty, based on evidence, and the possibility of balanced control of the probability of $\alpha$ and $\beta$ errors when making conclusions about the compliance of food and fodder agricultural raw materials with modern national and international requirements of biological safety can be formed.

**The purpose** of this study was to analyze the suitability of a hybrid approach to assessing the uncertainty of the results of measuring biological safety indicators for intended use.

To achieve the goal, the following **tasks** have been formulated:
- to test the model hybrid approach of estimating the uncertainty of measurement according to type A, using the estimation of the standard uncertainty of precision, based on the results of a retrospective analysis of the routine measurement of a certified reference material under conditions of intra-laboratory reproducibility, in combination with the standard uncertainty of the error, based on the results of an inter-laboratory comparison with the participation of an accredited provider.
- to compare the results obtained using four "classical" and hybrid approaches to the assessment of measurement uncertainty, considering the

requirements of using extended uncertainty in decision-making regarding the safety of food and fodder agricultural raw materials.

## Research materials and methods

As a model for practical testing of the researched approach, the procedure for determining the content of genetically modified organisms using real-time polymerase chain reaction (PCR-RT) was used. The use of this model is justified by the fact that this method contains in its procedure a significant number of operational stages carried out by different operators, requires the use of certified reference material during routine measurement, has relatively low limits of qualitative and quantitative determination of the analyte, and returns the result with an accuracy of one thousandth.

The research material was a sample consisting of the results of 147 consecutive routine measurements of the concentration of the genetically modified soybean line MON 40-3-2 in SRM. These measurements were carried out under conditions of intra-laboratory reproducibility in the period from 04.02.2019 to 21.11.2022 at the testing center of the SP "SGS Ukraine" using two batches of SRM manufactured by ERM (European Reference Material), catalogue number ERM-BF410dp. Compliance with the requirements of ISO 17034 is confirmed by quality certificates. The certified attributed concentration value of the reference material was 10.0 g/kg, which was equal to 0.1% of the GM content of the material, with an expanded uncertainty of each batch of ±0.6 g/kg.

In addition, the results of the participation of the testing center of the SP "SGS Ukraine" in two rounds of interlaboratory comparisons with the participation of an accredited provider, by the method of quantitative determination of the content of genetically modified soybeans by the PCR-RT method, with the participation of the provider "Bipea" (ISO 9001 accreditation certificate No. 9912427), certificate accreditation ISO 17043 No. 1-1495) throughout 2019.

Using the above data, two subgroups of data were formed that characterized the first and second half of 2019 based on the subgroup of SRM measurements 1-83 and subgroup 84-147, respectively. The two interlaboratory comparisons are similarly distributed.

The identification of sources of uncertainty that exerted or could exert an influence on the measurement result was performed using the method of root cause analysis with graphical display by the method of Ishikawa diagrams (Cause and Effect Diagram) (in accordance with ISO 31000:2018, IEC 31010:2019).

The normality of the distribution of the research sample was checked using the Shapiro-Wilk test (in accordance with ISO 5479:1997).

The assessment of the state of statistical controllability of the measurement method was carried out using the analysis of the standardized assessment and quantitative control charts of Schuhart according to the distribution of ranges without a given reference value and the distribution of individual values with a given standard value (in accordance with ISO 7870-1:2019, ISO 7870-2:2023). As a reference value when calculating the standardized assessment, the SRM value was used. The $3\sigma$ indicator was chosen as the criterion of conformity of the indicators of the standardized assessment, which corresponds to the consideration of 99.7% of the values.

To establish statistical controllability using Shewhart's quantitative maps, the total sample with 147 measurements was divided into 5 subsamples with 25 measurements and 1 subsample with 22 measurements. Violation of the calculated upper or lower control limits is accepted as the compliance criterion for assessing statistical controllability using Shuhart's quantitative control charts, using the reference value of SRM. The criterion of stability, which was used both for the map with the specified and for the map without the specified parameter, was the interpretation of the patterns of distribution of values according to the Westgard rules [12], [13] and the criteria of the ISO 7870-2 standard (in accordance with ISO 7870-2:2023). Admissible values based on a 20% deviation were accepted as additional evaluation criteria when using Shewhart's quantitative maps. Thus, for the swing control chart, the maximum allowable value was 0.2 points, which formed the suitability interval from 0.0 to 0.2 points, and for the control charts of individual values, the minimum and maximum allowable value formed the suitability interval from 0.8 up to 1.2 points.

Uncertainty assessment was carried out according to type A, with the help of mathematical processing of the results obtained by multiple measurements of a certified reference sample under conditions of intra-laboratory reproducibility and the results of participation in inter-laboratory comparisons (in accordance with ISO/IEC Guide 99:2007, BIPM JCGM 200 2012).

The initial concept of the calculation of the combined standard measurement uncertainty (Combined standard measurement uncertainty, *uc*), which includes all or most of the important sources, is an equation consisting of the square root of the sum of the squares of the standard uncertainty of precision and error.

To estimate the standard uncertainty, which is based on the arithmetic mean of repeated measurements, the test sample was taken regardless of the reference value of the certified reference material. The combined standard uncertainty was represented by the standard deviation divided by the square root of the sample [5].

The combined uncertainty based on the variance relative to the reference value was calculated using the standard precision uncertainty, which is represented by the standard deviation of the sample of measurements

relative to the assigned CPM value. The error component was calculated as a combination of the average arithmetic difference between the reference value and the measurement results and the standard deviation of the difference data [9, 10].

Two approaches were used to estimate the uncertainty based on the results of the interlaboratory comparison. In the first approach, as an indicator of combined uncertainty, the indicator of the standard deviation of the general population, which is determined by the provider, was taken. In this way, the category of methods that cannot rely on the value of the certified reference material was modelled, with the corresponding calculation of the error contribution to the combined uncertainty.

The combined uncertainty based on non-interlaboratory comparison results was calculated using the standard precision uncertainty, which is the difference between the reference value of the interlaboratory comparison provided by the provider and the measurement result obtained by the laboratory [9, 10]. In this calculation, to exclude the influence of the error of other laboratories, the determination of the standard uncertainty of the error was performed using the laboratory result of only one target laboratory. Thus, the standard deviation of the differences between the reference value and the measurement result was equal to 0, and the standard uncertainty of the error took the value of the relative difference between the reference value and the measurement result of the target laboratory.

The expanded measurement uncertainty was calculated by multiplying the combined uncertainty by the coverage factor, which was taken to be equal to 2, which corresponds to p 0.05 and provides consideration of approximately 95% of the distribution of values near the normalized mean (in accordance with ISO/IEC Guide 98-3:2008/Suppl 2:2011, BIPM JCGM 101:2008).

The calculation of the protective interval for visualizing the influence of the results of uncertainty assessment on decision-making was carried out using the multiplication of the calculated indicators of standard uncertainty by a factor of 1.65, with subsequent use as a factor for expanding and narrowing the limits of suitability (in accordance with ILAC G8 09 2019).

## Results of the research and their discussion

Based on the identification of sources of uncertainty that affect or may affect the result of measuring the content of genetically modified soybeans using polymerase chain reaction in real time, six basic categories of sources were identified.

To assess human factor drift, the sample preparation procedure was performed by six operators, the DNA extraction procedure was performed by six operators, and the polymerase chain reaction procedure was performed by five operators, according to which the measurement results were recorded. The total number of operators who could or did influence the result of the test, considered in the retrospective analysis, was eleven laboratory employees. In the process of accumulating retrospective data, regular changes were made in the sphere of influence of some operators, while other operators were fixed on the performance of a certain operation. According to the requirements of the analytical method, each operator performed only one operation during the work shift.

Considering the influence of time in the process of data accumulation was carried out within ten months from February to December 2019.

The measurement procedure was carried out under the conditions of daily control of environmental indicators within the limits stipulated by the technical documentation of the equipment and consumables and regulatory documentation (in accordance with ISO 21571:2005/Amd 1:2013, ISO 21569:2005/Amd 1:2013, ISO/IEC 17025:2017) [14].

The influence of the sample on the measurement result was evaluated using two batches of certified reference material and two samples provided by an accredited interlaboratory comparison provider.

To account for the impact of the equipment, alternative equipment performing the same function was used. The list of alternative equipment included changes between three amplifiers, four thermostats, three centrifuges, three balances, and ten variable volume dispensers. The measuring equipment used during the data collection period was subject to annual calibration and assessment of statistical controllability in the inter-calibration interval, in accordance with modern regulatory requirements (in accordance with ISO/IEC 17025:2017).

Consumables included commercial extraction and amplification kits, replaceable disposable dispenser tips, disposable microtubes, and amplification plates, which were renewed as needed during the retrospective analysis period.

The operational procedure is accepted as a permanent non-alternative influence factor that has not undergone modifications according to the annual update of the basic regulatory documents.

To assess normality using the Shapiro-Wilk W test, the total sample was divided into five subgroups of 50 measurements. Descriptive statistics characterizing the total sample and the results of assessing the normality of the corresponding subgroups are shown in Table 1.

Considering the guidelines EA-4/02 M:2022, it was concluded that the data of the subgroups and the total sample were removed from the normal distribution and are suitable for further analysis.

According to the results of the assessment of the stability of the measurement process using standardized assessment cards, no contradictory values were obtained, which would indicate the influence of special factors on the results. Violations of the

established requirements for compliance were not detected. As a result of the comparison of two approaches to the formation of this type of maps, the same geometric pattern of distribution of results relative to the control limits was revealed. However, the standardized score, which is based on the reference value, produces more accurate data, and the Z-values obtained from the reference value have a more centered value, compared to the standardized score from the arithmetic mean. Descriptive statistics of the standardized score are shown in Table 2.

**Table 1 – Results of testing the normality of the sample distribution using the Shapiro-Wilk test**

| Descriptive statistics of the sample | | | |
|---|---|---|---|
| Total sample size | 147 | | |
| Minimum value | 0.081 | | |
| Maximum value | 0.118 | | |
| Average value | 0.01 | | |
| Median | 0.102 | | |
| Moda | 0.102 | | |
| Swing | 0,037 | | |
| Standard deviation | 0.007610645 | | |
| Shapiro-Wilk test | | | |
| Subgroup | 1-50 | 51-100 | 101-147 |
| $W$ *is experimental* | 0.982512 | 0.957438 | 0.942133 |
| $W$ *critical (p 0.05, n 50)* | 0.954017 | | |
| Normality | + | + | - |

**Table 2 – Descriptive statistics of the two approaches to calculating the standardized score**

| Statistical indicator | Z from the reference value | Z from the average value |
|---|---|---|
| Minimum value | -2.497 | -2.678 |
| Maximum value | 2.365 | 2.184 |
| Average value | 0.263 | 0.081 |
| Median | 0.263 | 0.081 |
| Moda | 0.182 | 0.000 |
| Swing | 4.862 | 4.862 |

According to descriptive statistics, despite the fact that both approaches take into account the same measure of range, location relative to the central line, and, accordingly, estimates of central tendency, when evaluating Z relative to the mean, show a bias of about 0.2 standard deviations toward overestimation indicator This can be a significant error in the case of methods of research of biological indicators that have a high sensitivity to external factors and for samples with a small volume, in which the mathematical expectation can differ significantly from the real state of the indicator.

According to the result of the evaluation of the process using the quantitative maps of Shuhart, the established maximum permissible value of the span was not violated. A comparative analysis of the geometric patterns revealed that the 26-50 subgroup swing map exhibits relatively shifted control limits. This is because the average range of measurements for this sample was 0.003 percent, which is the lowest value among all subgroups, while the median value was 0.007 percent. However, this displacement does not exclude the possibility of using the limits as a geometric reference for evaluating the stability of the results. This case emphasizes the need to use not only the largest possible sample, but also the joint analysis of grouped data. More important for this subgroup is the comparable decrease in the average range of the measurement results, since considering the results of this subgroup can significantly affect the result of the uncertainty assessment, leading to a narrowing of the final interval. Since the factor that led to such a decrease in the average span had an impact only within the subgroup 26–50, it can be recommended to perceive this set of measurements as a drop, with the subsequent exclusion of its indicators from the calculation of the uncertainty interval. Alternatively, it may be recommended to evaluate this subgroup for the presence of outliers in the middle of the data, for example by calculating the Cochrane test or the Grubbs test (in accordance with ISO 5725-2:2019).

In addition, it should be emphasized the difficulties that arise when using a single list of patterns to make a conclusion about the exit of the process from the state of stability. The rules of interpretation of geometric patterns of distribution of values relative to control limits on statistical maps should not be used as a single and indisputable evaluation criterion. To carry out an effective analysis, it is necessary to focus on the characteristics of the process that are formed in certain conditions of its implementation, which also demonstrates the prospects of processing retrospective data.

According to the result of evaluating the process using Shuhart's quantitative maps, for the analysis of the dynamics of individual measurement results, no signs of exit from the state of controllability were found. As a result of the assessment, it was found that the upper and lower control limits, which were calculated using the standard calculation method, do not allow forming sufficiently strict conditions, which are necessary to ensure the assessment of compliance with the established requirements. In this regard, they can only be used to interpret the geometric pattern of the distribution of results. The integrated minimum and maximum limits of compliance of the individual measurement were not violated.

According to the conducted research, the suitability of combined statistical maps to satisfy the requirements for the formation of objective evidence of the suitability of retrospective data for the assessment of measurement uncertainty has been demonstrated. Objective evidence of the statistical controllability of the analytical method in the specified time was obtained, based on the evaluation of retrospective data obtained because of routine measurement of SRM. The obtained results indicate the stability and compliance of the data with the established requirements. A special case among the results is the subgroup of dimensions

26–50. Despite the absence of violations of the established requirements, the geometric pattern of data stability of this subgroup shows a significant difference from other subgroups. According to the descriptive statistics, which characterize the range between the measurement result and the reference value of the certified reference material of this data set, there is a possibility of narrowing the uncertainty interval when they are included in the evaluation.

Considering the practical use of the uncertainty interval and the above conclusions regarding the suitability of "classical" methods for its assessment, a model of the hybrid approach was formed. Since the combined standard uncertainty is based on the combination of the standard uncertainty of the precision and the standard uncertainty of the error, the most reasonable sources of input data for each should be chosen in such a way as to ensure that the influence of the largest number of factors that shape the uncertainty in the measurement result is estimated.

An objective means of considering the precision is the data of the retrospective analysis of the routine measurement of SRM, which was carried out under conditions of intra-laboratory reproducibility and characterizes the dispersion of the measurement results from the reference value obtained under certain conditions. This makes it possible to evaluate the factors inherent in the implementation of laboratory activities in the conditions of a certain laboratory.

Considering the error component can be implemented by including the results of interlaboratory comparison, namely, the range between the reference value of the sample provided by the accredited provider and the measurement result obtained under the conditions of a certain laboratory.

The calculation of the combined standard uncertainty by the hybrid approach compensates for many of the shortcomings inherent in the "classical" approaches and provides an evidence base for justifying the expanded uncertainty interval, which is calculated using the formula:

$$u_C = \sqrt{\left(\frac{Std_X RL * 100}{X_{ave}}\right)^2 + \left(\frac{\Delta_i IC \times 100}{X_i IC}\right)^2} \quad (1)$$

$u_c$ – combined standard uncertainty;

$Std_xRL$ – standard deviation obtained because of a series of measurements of a certified reference material;

$X_{ave}$ – is the average value of the certified reference material;

$\Delta_{iIC}$ – the difference between the reference value of the interlaboratory comparison and the measurement result obtained by the laboratory;

$X_i$ – is the measurement value obtained by the laboratory in an interlaboratory comparison.

Thus, the use of a retrospective analysis of the chain of results of routine measurement of SRM, which have evidence of statistical controllability, obtained using combined control charts, makes it possible to take into account the impact of the variability of the conditions of the activity, which are inherent in a certain laboratory, taking into account their variability over time. It should be noted that to calculate the standard uncertainty of precision, the variance relative to the mean of the measurement is used, since this component of the expanded uncertainty reflects the distance between parallel measurements, and not between the measurement and the reference value. The use of a certified reference sample is necessary, first of all, precisely for the formation of an evidence base for the stability of the statistical controllability of the process, and the reference value can be used in additional studies of the characteristics of the method.

Accounting for the standard error uncertainty, using an estimate provided by an accredited provider, allows for a reasonable estimate of the precision contribution to the combined uncertainty, taking into account sources of uncertainty that cannot be captured by the measurement analysis of the certified reference sample.

Thus, the proposed hybrid evaluation method makes it possible to obtain a balanced and justified interval of extended uncertainty with the help of economically feasible procedures that allow covering the widest range of factors that influence or can influence the measurement result.

The comparative results of the five uncertainty estimation approaches are shown in Figure 1.

The obtained results demonstrate a very close position of the intervals obtained by the hybrid method to those calculated using the combined uncertainty assessment based on the chain of SPM measurements. However, unlike the method that relies entirely on data obtained in conditions of intralaboratory reproducibility, the hybrid method harmonizes the assessment result with the dynamics observed in the error recognized by the results of interlaboratory comparisons.

To visualize the impact of the uncertainty of not deciding, a diagram of the use of protective intervals was created, which was calculated based on the highest indicators that were obtained in the previous calculation, namely the data characterizing the second half of 2019. As a model, a control limit of 0.9% was used, which corresponds to the current European requirements of Regulation 1829/2003 regarding the labelling of agricultural raw materials as GMO in case of exceeding this level [15]. The resulting safety intervals established using each uncertainty estimation method are described in Table 3.

The gray intervals, which are postponed in the direction of increasing the limit of suitability, serve to ensure the reliability of the conclusion regarding compliance, and the black intervals, which are postponed in the direction of decreasing the limit of suitability, are the non-compliance of the product to be analyzed. According to this, the use of the hybrid method of uncertainty assessment forms limits of suitability almost two times narrower than those

resulting from the results of interlaboratory comparison, which, together with the validity of the assessment of source contributions and agreement with the risk-oriented orientation of the interpretation of the results of the analysis, can provide the possibility of a more balanced control of the probability occurrence of α and β errors.

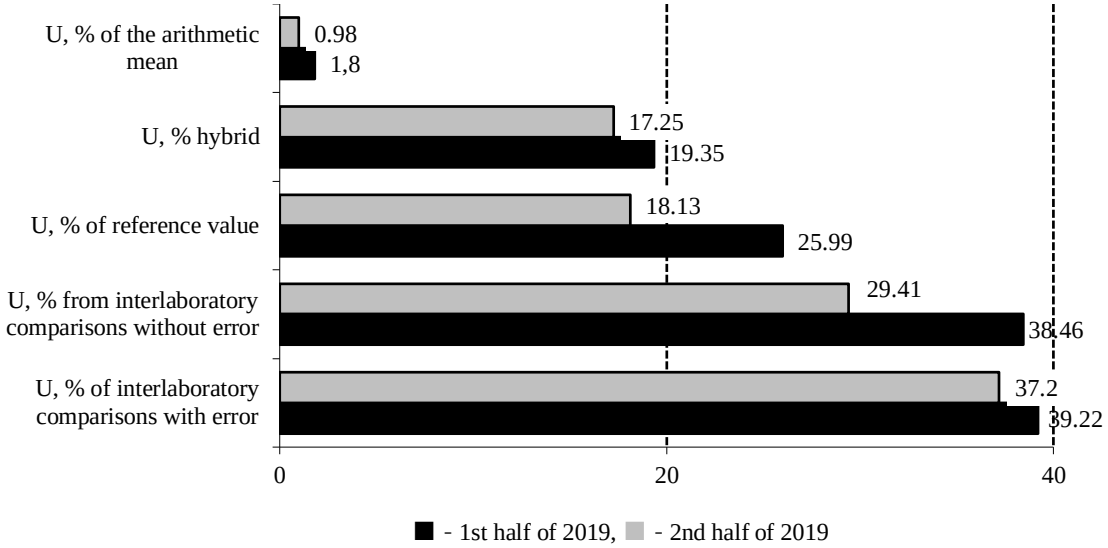


**Fig. 1. Graphic display of uncertainty assessment results obtained using four "classical" approaches and a hybrid approach**

**Table 3 – Safety intervals calculated using the four "classical" uncertainty estimation methods and using the hybrid method**

| Uncertainty assessment method: | U, % | u, % | w, % | W | Control limit + W | Control limit - W |
|---|---|---|---|---|---|---|
| From the average value ($U_{Xave}$), % | 1.801 | 0.901 | 1.486 | 0.013 | 0.913 | 0.887 |
| From the reference value ($U_{CRM}$), % | 19.347 | 9.674 | 15.961 | 0.144 | 1.044 | 0.756 |
| From interlaboratory comparison without error ($U_{IC}$), % | 25.989 | 12.994 | 21.441 | 0.193 | 1.093 | 0.707 |
| From interlaboratory comparison with error ($U_{IC}$+*bias*), % | 38.462 | 19.231 | 31.731 | 0.286 | 1.186 | 0.614 |
| Hybrid method ($U_H$), % | 39.223 | 19.612 | 32.359 | 0.291 | 1.191 | 0.609 |

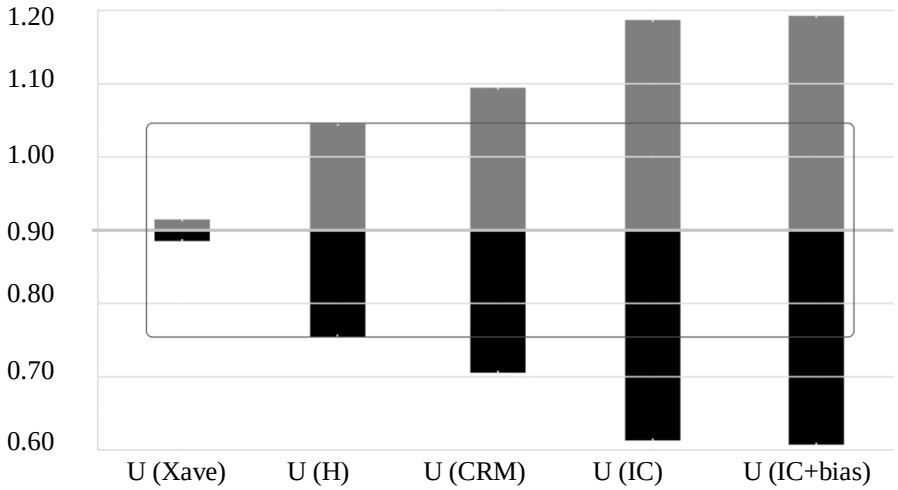


**Fig. 2. Graphic display of the use of protective intervals, calculated according to the uncertainty indicators estimated using different approaches**

## Conclusion

The combination of standardized assessment charts and Shewhart control charts with the integration of additional compliance limits to a set of control limits are highly promising in terms of providing objective evidence of the suitability of retrospective data for assessing measurement uncertainty. The use of combined maps allows you to make reasonable claims about the statistical controllability, stability and compliance of the measurement procedure in a specific period, based on the analysis of variability and average values of measurements.

The combination of a retrospective analysis of the sequence of results of a routine measurement of SRM, the statistical controllability of which is confirmed by combined control charts with an assessment of the deviation of the measurement result from the reference value provided by an accredited provider, allows to take into account the impact of variability characteristic of the conditions of reproducibility of a specific laboratory, taking into account their variability over time, so is the contribution of error. Thus, the hybrid method of uncertainty assessment provides a reasonable characterization of laboratory uncertainty, based on evidence, and forms the possibility of balanced control of the probability of α and β errors when making conclusions about the compliance of food and fodder agricultural raw materials with modern national and international requirements of biological safety.

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# ГІБРИДНИЙ ПІДХІД ДО ОЦІНЮВАННЯ НЕВИЗНАЧЕНОСТІ ВИМІРЮВАННЯ ПОКАЗНИКІВ БІОЛОГІЧНОЇ БЕЗПЕКИ СІЛЬСЬКОГОСПОДАРСЬКОЇ СИРОВИНИ

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**Анотація.** В статті проаналізовано сучасний стан концепції безпечності сільськогосподарської сировини та продукції, наслідки гармонізації національних вимог згідно з міжнародними нормативами, та різний підхід до оцінювання результатів лабораторного аналізу сировини. Різними методами оцінювання проаналізовано 147 послідовних рутинних вимірів концентрації лінії генетично модифікованої сої MON 40-3-2 у СРМ та визначено вміст генетично модифікованих організмів за допомогою полімеразної ланцюгової реакції в режимі реального часу (ПЛР-РЧ) та гібридного підходу до оцінювання невизначеності результатів вимірювання біологічних показників безпечності. Апробовано модельний гібридний підхід оцінювання невизначеності вимірювання по типу А, за допомогою оцінки стандартної невизначеності прецизійності, базованої на результатах ретроспективного аналізу рутинного вимірювання сертифікованого референсного матеріалу в умовах внутрішньолабораторної відтворюваності у комбінації зі стандартною невизначеністю похибки, базованої на результатах міжлабораторного порівняння з участю акредитованого провайдера. Порівняно результати отримані за використанням чотирьох «класичних» та гібридного підходу до оцінювання невизначеності вимірювання, з врахуванням вимог використання розширеної невизначеності у прийнятті рішень щодо безпечності харчової та кормової сільськогосподарської сировини. Встановлено, що більшість сучасних підходів, що пропонуються міжнародними нормативними документами орієнтовані на більш стабільні системи, наприклад, при калібруванні або хімічному аналізі, які мають низку переваг і недоліків, що суттєво впливає на якість розрахованого інтервалу. Підхід до оцінювання результатів спирається на показник середньої арифметичної вибірки, повертає вузький інтервал та не може у достатній мірі врахувати похибку. Метод є дуже залежним від обсягу вибірки і наявності випадів серед результатів вимірювання. Показник середньої арифметичної не завжди є оптимальною мірою центральної тенденції в умовах методів аналізу біологічних показників. Використання вибірки результатів вимірювання сертифікованого референсного матеріалу надає змогу збалансованого оцінювання невизначеності, враховуючи внески компонентів прецизійності і похибки. Однак, цей підхід не завжди може врахувати вплив процедури підготування зразків, що часто включає в себе такі етапи як гомогенізація, вивільнення чи екстракція аналіту, рівномірне розподілення об'єкту вимірювання та інші впливові фактори. Підхід, що спирається на результати міжлабораторних порівнянь з участю акредитованого провайдера, надає значну доказову базу, однак враховує умови здійснення вимірювань всіх лабораторій, що приймали участь у порівнянні. Це формує віддаленість отриманого інтервалу від аспектів реалізації аналітичної процедури в умовах певної лабораторії, а отримані інтервали можуть суттєво перевищувати практичну невизначеність даної конкретної лабораторії. Відмінності між інтервалами отриманими з використанням різних підходів здатні здійснювати істотний вплив на прийняття рішення відносно безпечності продукції, а невизначеність має значну варіабельність с плином часу. Незважаючи на відсутність прямої вимоги до оновлення інформації про невизначеність, яка вже є встановленою для методу випробування в ході відповідного дослідження, існує необхідність періодичного переоцінювання її інтервалу. Встановлена необхідність розробки модельного підходу до оцінювання невизначеності вимірювань, що надасть змогу врахувати якомога більшу кількість впливових джерел невизначеності, що можуть впливати на результат аналізу біологічних показників безпечності.

**Ключові слова:** контроль якості, невизначеність вимірювань, біологічні показники безпечності.