CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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CODEX COMMITTEE ON PESTICIDE RESIDUES

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PROPOSED DRAFT REVISION OF THE GUIDELINES ON THE ESTIMATION OF UNCERTAINTY OF RESULTS FOR THE DETERMINATION OF PESTICIDE RESIDUES
(Appendix to the Guidelines on Estimation of Uncertainty of Results CAC/GL 59-2006)

(AT STEP 3)

(Prepared by Australia and China)[1]

Governments and interested international organizations wishing to submit comments on the revised Appendix to Guidelines on the Estimation of Uncertainty of Results for the Determination of Pesticides (see Annex) are invited to do so in writing to: Ms. Duang Lifang, Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA), P.R China, Fax: +86-10-59194252, Email: ccpr@agri.gov.cn with a copy to: Secretariat, Codex Alimentarius Commission, Joint WHO/FAO Food Standards Programme, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy, by Email codex@fao.org or Fax: +39-06-5705-4593 by 15 March 2011.

BACKGROUND

1. At the 42nd Session of the Codex Committee on Pesticide Residues, the Committee agreed to return the proposed draft Guidelines on the Estimation of Uncertainty of Results for the Determination of Pesticide Residues to Step 3 for comments and consideration by an Electronic Working Group chaired by Australia and co-chaired by China, open to all Codex Members and Observers and working in English only, which would prepare a revised version for consideration by the next session of the Committee (ALINORM 10/33/24 Appendix XIII)[2]. Codex members and observers were invited by Circular letter (CL 2010/11-PR) to submit comments on the proposed draft Guidelines as well as formally nominate their interest in participating in the electronic Working Group (eWG).

2. Based on the input from member states and the eWG, the following document has been prepared for further comment and discussion at 43rd Session of the Committee.

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1 with assistance from Argentina, Chile, Ecuador, Ethiopia, EUMS, India, Japan, Malaysia, New Zealand, Thailand, Uruguay, Croplife, CIAA (EU) and ICGMA.

2 ALININORM 10/33/24, paras. 119-123.

ANNEX

PROPOSED DRAFT REVISION OF THE GUIDELINES ON THE ESTIMATION OF UNCERTAINTY OF RESULTS FOR THE DETERMINATION OF PESTICIDE RESIDUES
(Appendix to the Guidelines on Estimation of Uncertainty of Results CAC/GL 59-2006)

Introductory notes

1. As noted in the guideline document CAC/GL 59-2006, the estimation of measurement uncertainty associated with analytical data is a requirement for laboratories accredited under ISO/IEC 17025 and an expectation for all laboratories operating under GLP in Pesticide Residue analysis. Decisions in regard to compliance of food whether for domestic or international standards for chemical residues and contaminants need to take into consideration the uncertainty associated with the test results reported by laboratories for analysis of specific lots or consignments.

2. It is not uncommon for laboratories to report widely different estimates of MU in Proficiency Tests (PT) despite the fact that they employ very similar test methods for analysis. This evidence suggests that the estimation of MU still appears to be a developing science for a number of food laboratories. This annex is intended to describe some of the options laboratories might employ in estimating measurement uncertainty, particularly the use of in-house method validation, quality control and long-term precision data for multi-residue pesticide methods. It is also anticipated that a more harmonised approach to the estimation of MU for pesticide residue results will minimise possible disputes in compliance decisions for residue levels near MRLs.

3. There are broadly two approaches commonly employed for the determination of MU; the so-called GUM (*Guide to the Expression of Uncertainty in Measurement*) or 'bottom up' approach and the 'top-down' procedures based around application of analytical precision and bias.

4. The GUM approach is based on a rigorous analysis of all the individual components of an analytical process and the estimation of random and systematic errors assigned to these steps. This process, whilst initially very laborious, requires the analyst to have or develop a detailed understanding of the analytical steps on the process and identify the critical control points in the method. Unless all steps are considered in the process, it is possible to underestimate the MU. On the other hand, some operational errors may cancel out which, if ignored, could provide an overestimate of the uncertainty. It is generally acknowledged that the bottom-up approach is more suited to physical metrology than to analytical chemistry activities and, in particular, to the more complex multi-pesticide residue methods.

5. Proponents of the top-down approach note that laboratory data collected from in-house validation, long-term precision and analytical quality control (QC) is likely to provide more reliable information on MU. Where available, PT data can also be used to estimate MU, either as the sole basis for estimates or more often in combination with in-house data. The inter-laboratory reproducibility data from PT studies can also provide a useful 'benchmark' for single laboratory estimates.

6. All options should be considered in the estimation of MU. The initial aim should be to obtain the best possible estimate using the information available. Initial laboratory estimates should be verified by comparison with alternative methods, literature reports and comparisons from PT studies. Furthermore professional judgement has an important role when estimating and verifying measurement uncertainty. Estimates should be reviewed as more precision data becomes available, for example, within-batch QC data routinely generated during the course of an analytical program.

7. This Appendix focuses on the estimation of MU using the top-down approach, based on data obtained from different sources.

1. Applying a default value for MU for pesticide residues in foods

8. EU member states have recently adopted a MU 'default' value of +/- 50% for pesticide residues in food consignments entering the EU. The default value is based around the statistical results of a number of EU-based PT studies involving competent residue laboratories participating in a number of multi-residue studies on fruit and vegetables. The mean relative standard deviations reported from a number of these studies have ranged between 20 to 25% providing a MU approximating at 50%.

9. In the absence of other statistical data, an export laboratory could presumably adopt a default MU of 50% provided it could establish its analytical proficiency through participation in EU or similar PT studies and/or it can demonstrate acceptable long-term precision and bias associated with its test results. It the longer term however, it should be incumbent on the laboratory to verify its adoption of the default MU by independently estimating MU based on in-house precision and validation data.

2. Precision data derived from the use of the Horwitz relationship

10. In the absence of data from inter-laboratory studies on a particular method, the reproducibility standard deviation, and hence MU, may be determined from an equation reported by Horwitz which correlates reproducibility standard deviation with analyte concentration. The Horwitz relationship between coefficient of variation (CV) and analyte concentration is based on the results from a large number of food-based collaborative studies reported in the literature. The Horwitz equation is also a helpful tool to compare in-house MU estimates against the expected value derived from published inter-laboratory studies.

3. Precision data derived from Inter-laboratory studies (Collaborative Studies and PT Studies)

11. The results reported for inter-laboratory studies are subject to both imprecision and bias. If such studies involve a sufficient number of laboratories and are designed to cover real test conditions (range of analytes and matrices), the reproducibility standard deviations obtained will reflect the typical errors likely to be encountered in practice. PT study data therefore may be used to provide reasonable estimates of measurement uncertainty.

12. Collaborative studies on methods are generally well defined with well documented instructions on the analytical process and usually only involve expert laboratories with reputable experience in residue analysis. Under these conditions the analytical variance is likely to be the best achievable when applying the method under reproducibility conditions, particularly as error contributions from sample in-homogeneity are likely to be negligible. Providing a laboratory can demonstrate an ability to achieve the analytical performance associated with a particular collaborative study, the reproducibility standard deviation obtained for the study will be a good basis for estimating MU. A competent laboratory however, should be able to improve on the inter-laboratory method precision when conducting the method under within-laboratory reproducibility conditions, and hence reduce the MU.

13. If certified reference materials (CRMs) are employed in collaborative studies, the study report should provide an estimate of the bias of the method against the 'certified' value and this will need to be taken into consideration when estimating the MU.

14. In PT studies, it is normal for laboratories to employ their own test method for analysis. The method may be a standard method, a modified standard method or a method developed and validated in-house. Furthermore, there is generally greater variability in the analytical competence of the participating laboratories than is the case for collaborative studies. Because of these factors, the reproducibility standard deviation obtained for PT studies is likely to be larger than that anticipated from a method-based collaborative study. MU based on such data may be larger than the estimates reported by many participant laboratories. Nevertheless, an estimate of MU based on a PT study involving laboratories with a range of expertise using a variety of methods may be more pragmatic and useful for judging compliance of food commodities with respect to pesticide residues in international trade. The 50% default MU applied by the EU member states is based on PT data for a range of pesticides and food matrices.

15. Whether or not a laboratory uses PT data to estimate MU, the information from PT studies is useful to compare and verify estimates based on data such as in-house validation or quality control experiments.

4. MU derived from In-house validation and quality control data

16. There is general consensus amongst chemical metrologists that the best source of uncertainty data on the analytical process is derived from the laboratory's method validation/verification studies and long-term quality control data. This is based on the assumption that the laboratory has undertaken validation an/or verification studies and has sufficient experience to have built up long-term bias and reproducibility data on suitable quality control (QC) samples, CRMs, reference materials (RMs) or matrix spikes.

17. The limited availability of CRMs for pesticide residues in food matrices usually requires laboratories to focus on spiked samples or other suitably characterised samples for internal quality control. The use of matrix-based QC samples such as samples with incurred residues, left-over PT study samples or spiked residue-free laboratory samples provides laboratories with a capability to monitor and control method (and analyst) performance while gathering information on both bias and precision. Control charts are excellent tools for evaluating long-term precision and monitoring statistical control of the analytical process.

18. Bias, where significant, and the uncertainty of bias should be considered when estimating MU. This is illustrated in the example discussed under paragraph 5.4.

19. Bias can best be determined from the use of CRMs. However given the paucity of CRMs for pesticides in food and the large number of pesticides normally incorporated into a multi-residue screen, it is generally necessary to rely on the recoveries of spiked matrix samples to provide information on method bias.

20. The performance of laboratories in PT studies can further provide a useful indication of the bias of individual laboratories against the consensus values and, in some instances, the spiking level of the PT samples. However, bias should be based on or confirmed by the results from a number of PT studies before it is used as an input in the estimation of MU.

21. The following worked examples describe acceptable procedures for estimating MU based on different combinations of in-house validation data, in-house precision data and inter-laboratory data. The Horwitz equation and results from PT studies further provide useful benchmarks for comparison with in-house MU estimates.

5. Worked Examples

The following worked examples use hypothetical data and draw heavily on examples presented in Eurolab Technical report No 1/2007 [1] and the Nordtest Report TR537 [2].

5.1 Estimating MU using the Horwitz Equation

The Horwitz equation expresses reproducibility standard deviation as a function of analyte concentration.

$$u' = 2^{1-0.5 \log c}$$

where u' = *relative reproducibility standard deviation*

c = *concentration of analyte (in g/g).*

The relative expanded MU, U' *(at 95% confidence level) may then be estimated by*

U' = 2u'.

Since the Horwitz equation is a function of analyte concentration, it will provide a range of MU values depending on pesticide concentration as noted in the following table:

Concentration (mg/kg)	u'*(%)*	U' *(%)*
1.0	*16*	*32*
0.1	*22.6*	*45*
0.01	*32*	*64*

Example 1:

A laboratory measures 0.40 mg/kg chlorpyrifos in a sample of tomato.

The Horwitz Equation predicts a relative reproducibility standard deviation of 18.4% at a concentration of 0.40 mg/kg.

u' = *18.4 %*

U' = 2u' = *37%*

The laboratory would therefore report the result as 0.40 ± 0.15 mg/kg

The laboratory report should state that the reported uncertainty was an expanded uncertainty with a coverage factor of 2 to give a level of confidence of approximately 95%. Unless stated otherwise, this is generally assumed for results reported with expanded uncertainties.

In the absence of supporting data, the Horwitz equation should be used with some caution and only as an indicator of the likely uncertainty associated with test results. Advances in analytical methodologies, particularly instrumental techniques, have provided the capability to achieve very low limits of quantitation with much less uncertainty then predicted by the Horwitz Equation. Thompson and Lowthian [3] have reported that laboratories tend to out-perform the Horwitz function at low concentrations.

5.2 Estimating MU by application of the EU default value of 50%

Before applying a default MU, laboratories should ensure that they are able to routinely achieve uncertainties not greater than the default value.

Example 2:

A laboratory measures 0.40 mg/kg chlorpyrifos in a sample of tomatoes. An agreed default value of ± 50% is to be applied to the measured result.

Accordingly, the laboratory would report the result as 0.40 ± 0.20 mg/kg.

5.3 Estimating MU based on Intra-laboratory QC and data from PT Studies

5.3.1 Using the assigned (or consensus) value from PT studies

U' = 2u' *Equation 1*

$$u' = \sqrt{u'(Rw)^2 + u'(bias)^2}$$ *Equation 2*

where U' = *expanded relative uncertainty*

u' = *combined relative standard uncertainty*

u'(R_w) = *relative standard uncertainty due to within-laboratory imprecision (relative intra-laboratory reproducibility standard deviation)*

u'(bias) = *relative standard uncertainty component due to bias*

Example 3:

In this example, u'(R_w) *is obtained from within-laboratory QC data, preferably long-term QC data and* u'(*bias) is estimated from PT data.*

Laboratory result for chlorpyrifos in tomato = 0.40 mg/kg

Relative standard deviation from analysis of in-batch QC samples of tomato spiked at 0.5 mg/kg with chlorpyrifos (one spiked sample per week for previous 3 months) = 15%.

The laboratory has participated in 6 PT studies where the analytes have included chlorpyrifos in different vegetables and fruit matrices. For these studies, the relative differences between the laboratory's result and the assigned value were -15%, 5%, -2%, 7%, -20% and -12%. An average of 16 laboratories participated in each of the PT studies. The average relative reproducibility standard deviation (S_R) reported for chlorpyrifos in the six studies was 25%.

$$u'(bias) = \sqrt{RMS'_{bias}{}^2 + u'_{(C\,ref)}{}^2} \qquad \textit{Equation 3}$$

where RMS'_{bias} = *root mean square of relative bias value*

$u'(C_{ref})$ = *average relative uncertainty of the assigned values for chlorpyrifos in the six studies.*

$$RMS'_{bias} = \sqrt{\frac{\Sigma(bias)^2}{n}} \qquad (n = \text{Number of PT studies}) \qquad \textit{Equation 4}$$

$$= = \sqrt{\frac{(-15)^2 + (5)^2 + (-2)^2 + (7)^2 + (-20)^2 + (-12)^2}{6}}$$

$$= 11.9\%$$

$$u'(C_{ref}) = \frac{S_R}{\sqrt{m}}$$ *where* S_R = *average relative standard deviation for chlorpyrifos from the six studies*

m = *average number of participants per study*

$$= \frac{25}{\sqrt{16}}$$

$$= 6.3\%$$

So, $u'(bias) = \sqrt{(11.9)^2 + (6.3)^2} = 13.5\%$

From Equation 2,

$$u' = \sqrt{(15)^2 + (13.5)^2} = 20\%$$

From Equation 1, the expanded relative uncertainty (95% confidence) = 40%

The Laboratory should report the result as 0.40 ± 0.16 mg/kg

Notes:

1. The *RMS'bias* value accounts for both bias and the uncertainty of bias.
2. The calculated MU is a best estimate only since the PT data is for different matrices and different concentrations of chlorpyrifos.
3. If possible, MU should be calculated based on data generated at or near the most critical concentration, for example the Codex MRL.

5.3.2 PT Studies with Certified Reference Materials (CRMs)

If a suitable CRM containing chlorpyrifos is distributed as a sample in a PT study, then there would be no need to calculate $u'(C_{ref})$ *from the PT results.*

In this case , $u'(C_{ref})$ *would be the uncertainty stated for the certified concentration, converted to a relative standard deviation.*

For example, if the 95% confidence range for the certified value for chlopyrifos in the CRM was 0.489 ± 0.031 mg/kg, then:

$u(C_{ref})$ *(standard deviation)* $= \frac{0.031}{2} = 0.0155$ *mg/kg, and*

$u'(C_{ref})$ *(relative standard deviation)* $= \frac{0.0155 \times 100}{0.489} = 3.17\%$

In the unlikely event that several CRMs containing chlorpyrifos were distributed in different rounds of the PT studies, then the mean $u_{(C\,ref)}$ *would be used to calculate* U.

In both cases, RMS'_{bias} *would be calculated using Equation 4.*

Example 4:

Study No.	*CRM*	*relative bias*	u' (C_{ref})
1	*A*	*-12%*	*2.3%*
2	*B*	*-15%*	*1.7%*
3	*C*	*-3%*	*2.0%*
4	*C*	*5%*	*2.0%*
5	*C*	*-20%*	*2.0%*
6	*A*	*0%*	*2.3%*

Mean u' (C_{ref}) = *2.05 %*

From Equation 4, RMS'_{bias} = *11.6 %*

From equation 3, u'(bias) = *11.8 %*

Note:

4. The relative uncertainty associated with CRMs is likely to be less than that associated with assigned or consensus values.

If the laboratory's relative standard uncertainty due to analytical imprecision u'(R_W) remained the same i.e.,15%, then from Equations 1 and 2.

u' = *19%*

U' = *38%*

The laboratory could report the result as 0.40 ± 0.15 mg/kg

5.4 Estimating MU using Intra – laboratory QC data

Example 5:

- Laboratory result for chlopyrifos in tomato = 0.40 mg/kg
- Stated purity of chlorpyrifos calibration material used to prepare the spiking solution = 95±2% (certificate of analysis)
- Fourteen recoveries (%) recorded for in-batch QC samples spiked at 0.5 mg/kg chlorpyrifos over the past 3 months; 90, 100, 87, 89, 91, 79, 75, 65, 80, 82, 115, 110, 65, 73 provided a mean recovery of 86 % and a relative standard deviation of 15 %.

Assuming the uncertainty stated for the reference material to be an expanded uncertainty U (95% confidence range),

$u'(C_{ref}) = \frac{2}{2}$ = *1%*

Note:

5. This assumes that the uncertainties associated with the preparation of the spiking solution and the spiking of the tomatoes are both insignificant. This is likely to be the case, but, if not, u' (C_{ref}) will nevertheless still be only a very minor contribution to the overall uncertainty.

u'(R_W) = 15% (relative intra-lab reproducibility standard deviation).

Using Equation 4, and taking bias to be 100 - % recovery,

RMS'_{bias} = *20%*

From Equation 3, u'(bias) = *20%*

From Equation 2, u' = *25%*

From Equation 1, U' = *50%*

The laboratory could report the result as 0.40 ± 0.20 mg/kg

Note:

6. This uncertainty would apply to results not corrected for recovery. If, at the end of the analytical program, the results were corrected for the average recovery achieved over the 3 month period of analysis, then u'(bias) need only reflect the uncertainty associated with the mean recovery. Then u'(bias) may be calculated as the relative standard uncertainty of the recovery factor applied (the relative uncertainty of the mean recovery) combined with the relative standard uncertainty of the spike concentration, u'(C_{ref}).

Relative Standard Uncertainty of mean recovery, $u'\overline{\mathrm{Re}\,c} = \frac{u'(Rw)}{\sqrt{n}}$ *, where*

n = the number of replicates from which the mean recovery is calculated.

$$u'\overline{\mathrm{Re}\,c} = \frac{15}{\sqrt{14}} = 4\%$$

$$u'(bias) = \sqrt{u'(\overline{\mathrm{Re}\,c})^2 + u'(C_{ref})^2}$$

thus $u'(bias) = \sqrt{(4)^2 + (1)^2} = 4.1\%$

Then, from Equation 2 and 1, using the u'(R_W) value of 15% calculated previously u' *= 15.5% and*

U' = *31%*

If results were corrected for recovery, the result should be reported as

0.40 ± 0.12 mg/kg

Note:

7. This example shows that if results are corrected for a mean recovery based on nine or more replicate recovery experiments conducted during the course of an analytical program, using a reference material for which the purity is with a high level of certainty, a reasonable estimate of measurement uncertainty may be calculated from solely the intra-lab reproducibility standard deviation.

References

[1] Eurolab (2007), 'Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation'. Technical Report 1/2007, www.eurolab.org

[2] Magnusson B., Naykki T., Hovind H. and Krysell M (2003), 'Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories', Nordtest Report TR537

[3] Thompson M and Lowthian PJ (1997), Journal of AOAC International, 80(3), 676-679.