3rd Edition

Approved: January 2012

GENERAL PROTOCOL

for EU Proficiency Tests on Pesticide Residues in Food and Feed

Introduction

This protocol contains general procedures valid for all European Union Proficiency Tests (EUPTs) organised on behalf of the European Commission, DG-SANCO[1] by the four European Union Reference Laboratories (EURLs) for pesticide residues in food and feed. These EUPTs are directed at all National Reference Laboratories (NRLs) and Official Laboratories (OfLs) within the EU Member States. Laboratories outside of this EURL/NRL/OfL-Network[2] may be permitted to participate on a case-by-case basis after consultation with DG-SANCO.

The following four EURLs for pesticide residues were appointed by DG-SANCO based on regulation 882/2004/EC[3]:

- EURL for Fruits and Vegetables (EURL-FV)
- EURL for Cereals and Feedingstuff (EURL-CF)
- EURL for Food of Animal Origin and Commodities with High Fat Content (EURL-AO) and
- EURL for Single Residue Methods (EURL-SRM)

NRLs are appointed by Member State based on the provisions of Regulation 882/2004/EC, whereas OfLs are laboratories that are actively involved in official controls following Article 26 of Regulation 396/2004/EC (e.g. by conducting pesticide residue analyses within the framework of national and/or EU-controlled programmes).

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[1] DG-SANCO = European Commission, Health and Consumer Protection Directorate-General

[2] For more information about the EURL/NRL/OfL-Network please refer to the EURL-Web-portal under: http://www.eurl-pesticides.eu

[3] Regulation (EC) No 882/2004 of the European Parliament and of the Council on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Published at OJ of the EU L191 of 28.05.2004

According to Article 28 (3) of Regulation 396/2005/EC[4], all laboratories analysing samples for the official control of pesticide residues shall participate in the European Union Proficiency Test(s) organised by the European Union. The aim of these EUPTs is to obtain information regarding the quality, accuracy and comparability of the pesticide residue data in food and feed sent to the European Union within the framework of the national control programmes and the co-ordinated multiannual community control programme[5]. Participating laboratories will be provided with an assessment of their analytical performance and the reliability of their data – compared to the other participating laboratories.

EUPT-Panel

EUPTs are organised by individual EURLs or by more than one EURL in joint cooperation.

An **Organising Team** is appointed from the EURL(s) in charge. This team is responsible for all administrative and technical matters concerning the organisation of the PT, e.g. PT-announcement; Test Item production; undertaking the homogeneity and stability tests; packing and shipment of Test Item, as well as the handling and first assessment of participants' results.

Approved by DG SANCO, expert scientists with long-term experience in pesticide residue analysis will be chosen as members of a joint **EUPT-Scientific Committee** (SC). This Committee is made up of the following two subgroups:

a) An independent **Quality Control Group** (QCG) and

b) An **Advisory Group** (AG)

The SC's role is to help the organisers make decisions regarding the EUPT design: the selection of pesticides to be included in the Target Pesticide List (see below); the establishment of the Minimum Required Reporting Levels (MRRLs); the evaluation and statistical treatment of the results and the drafting of the protocol and final report. The

[4] Regulation (EC) No 396/2005, published at OJ of the EU L70 of 16.03.2005, as last amended by Regulation 839/2008 published at OJ of the EU L234 of 30.08.2008.

[5] European Commission Proficiency Tests for Pesticide Residues in Fruits and Vegetables, Trends in Analytical Chemistry, 2010, 29 (1), 70-83.

QCG has the additional function of supervising the quality of the EUPT and to assist the EURL in confidential aspects such as the choice of the pesticides to be present in the Test Item and the concentration levels at which they should be present in the Test Item.

The EUPT-Organising Team and the EUPT-Scientific Committee (the AG and the QCG) together form the **EUPT-Panel**.

The present EUPT General Protocol was drafted by the EUPT-Panel and was approved by DG-SANCO.

EUPT Participants

All NRLs operating in the same area as the organising EURL are legally obliged to participate in EUPTs - as well as all OfLs whose scope overlaps with that of the EUPT. The four EURLs will be annually issuing and distributing via the EURL website, a joint list of all OfLs that shall participate in all EUPTs to be conducted within a given year. The "list of obliged labs" is to be considered as tentative as it will be only based on information submitted by OfLs concerning their commodity scope and status. The legal obligation of NRLs and OfLs to participate in EUPTs arises from:

- Art. 28 of Reg. 396/2005/EC (for all OfLs analyzing for pesticide residues within the framework of official controls in food or feed)
- Art. 33 of Reg. 882/2004/EC (for all NRLs)

If necessary the "list of obliged labs" will be updated within the same year to take account of any changes in the lab profiles.

NRLs are responsible for checking whether all relevant OfLs within their network are included in the list of obliged laboratories and whether the contact information is correct.

The NRLs should further make arrangements to urge all relevant OfLs within their network to participate in all EUPT relevant to them.

OfLs are urged to keep their own profiles within the EURL-DataPool up-to-date, especially their commodity and pesticide scopes and their contact information.

Any OfL not intending to participate in a given EUPT will have to explain to the EURL its reasons for non-participation without prejudice of any legal action taken against it for not

participating. This also applies to initially participating laboratories that do not deliver results.

Official labs from EFTA countries and EU-candidate countries are also welcome to participate in the EUPTs. In special cases, the Organisers, upon consultation with DG-SANCO, will also allow laboratories outside of the EURL/NRL/OfL-Network to participate in EUPTs.

Confidentiality

The proprietor of all EUPT data is DG-SANCO and thus has access to all information.

In each EUPT, the laboratories are given a unique code, initially only known to themselves and the Organisers. In the final EUPT-Report, the list of participating laboratories will not be linked to their laboratory codes. It should be noted that the organisers, at the request of DG-SANCO, may present the EUPT-results to the Standing Committee on the Food Chain and Animal Health on a country-by-country basis. It is therefore possible that a link between codes and laboratories could be made, especially for those countries where only one laboratory has participated.

As laid down in Regulation 882/2004, NRLs are responsible for evaluating and improving their own OfL network. For this reason, the EURLs will provide the OfL laboratory codes to their NRLs together with the final report. This will allow NRLs to correlate the laboratories within their network and their performance. Furthermore, the EURLs reserve the right to share EUPT results and codes among themselves: for example, for the purpose of evaluating overall lab performance as requested by DG-SANCO.

Communication

The official language used in all EUPTs is English.

Communication between participating laboratories during the test on matters concerning this PT exercise is not permitted.

Announcement / Invitation Letter

The announcement of the individual EUPT will be issued at least 3 months before the Test Item is distributed to the laboratories. The announcement will be published on the EURL portal and additionally distributed via e-mail to the NRL/OfL mailing list available to the EURLs. The announcement will contain an invitation letter, details on how to register and where to find additionally-related documents, as well as some preliminary information on the specific protocol such as the tentative calendar, the name of the commodity expected to be used, and the tentative Target Pesticide List.

Target Pesticide List

This list contains all analytes (pesticides and metabolites) to be tested, along with the Minimum Required Reporting Levels (MRRLs) valid for the specific EUPT. The MRRLs are based upon the lowest MRLs found either in Regulation 396/2005/EC or Commission Directive 2006/125/EC (Baby Food Directive).

In some cases, that will be clearly marked, results calculated according to the pesticide residue definition may be requested with those residue definitions differing from the legal ones in certain cases.

Specific Protocol

For each EUPT a Specific Protocol will be published at least 2 weeks before the Test Item is distributed to the laboratories. This protocol will contain all the information previously included in the Invitation Letter but in its final version, in addition to information on payment for delivery service and/or participation. It will furthermore include instructions on how to handle the Test Item upon receipt, on how to submit results, and any other relevant information.

General procedures for reporting results

Laboratories are responsible for reporting their results to the Organiser within the stipulated deadlines. Any pesticide that was targeted by a participating laboratory should be reported as “analysed”. Each laboratory must report only one result for each of the analytes detected in the Test Items, using the analytical procedure(s) that they

would routinely use for each compound for monitoring purposes. The residue levels of the pesticides detected should be expressed in mg/kg and in some cases for products of animal origin in µg/kg fat.

One Test Item is intentionally treated with pesticides and one is not. Both Test Items have to be analysed by the laboratories and any pesticide detected in them shall be reported.

Correction of results for recovery

According to the Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, (Document SANCO), it is common practice that pesticide analysis results are not corrected for recovery, but may be corrected if the average recovery is significantly different from 100% (typically if outside the 70-120% range with good precision), therefore, if residue data are adjusted for recovery, then this must be indicated on the specific field of the 'reporting result form'. Laboratories are required to report whether their results were adjusted for recovery and, if this was the case, the recovery (as percentage) used should be also reported. No recovery data are required where correction for recovery results automatically from using the 'standard addition(s)' approach, or isotopically-labelled internal standards (in both cases with spiking of the Test Item at the beginning of the extraction procedures). In these cases, the laboratories should report the calculation technique used for the results instead of the recovery data.

Methodology information

All laboratories are requested to provide information on the analytical method(s) they have used. If no sufficient information on the methodology used is provided, the Organiser reserves the right not to accept the analytical results reported by the participants concerned.

Results evaluation

The procedures used for the treatment and assessment of results are described below.

- ***False Positives***

These are results reported above the MRRLs that suggest the presence of pesticides that were listed in the Target Pesticide List, but which were: (i) not detected by the Organiser, even after repeated analyses, and/or (ii) not detected by the overwhelming majority (e.g. 95%) of the participating laboratories that had targeted the specific pesticide. However, in certain instances, case-by-case decisions by the EUPT-Panel may be necessary.

Any results reported that are lower than the MRRL will not be considered as false positives, even though these results should not have been reported.

- ***False Negatives***

These are results for pesticides reported by the laboratories as “analysed” but without reporting numerical values although they were used by the Organiser to treat the Test Item and were detected by the Organiser and the majority of the participants that had targeted these specific pesticides, at or above the MRRL. Results reported as <RL (RL= Reporting Limit of the laboratory) will be considered as not detected and will be judged as false negatives. However, in certain instances, case-by-case decisions by the EUPT-Panel may be necessary.

In cases of the assigned value being less than a factor of 4 times the MRRL, false negatives will not be assigned as this is not statistically justifiable.

- ***Estimation of the true concentration (μ)***

The “true” concentration (assigned value) will be typically estimated using the median of all the results. In special justifiable cases, the EUPT-Panel may decide to use only part of the population of results to establish the median (e.g. only results with z-scores ≤ 5.0, or by excluding results generated by a method that demonstrably generates significantly biased results, e.g. due to incomplete extraction).

– ***Standard deviation of the assigned value (target standard deviation)***

The target standard deviation (δ) of the assigned value will be calculated using a Fit-For-Purpose Relative Standard Deviation (FFP-RSD) approach, as follows:

$$\delta = b_i * \mu_i \qquad \text{with } b_i = 0.25 \text{ (25\% FFP-RSD)}$$

The percentage FFP-RSD is set at 25% based on experience from previous EUPTs[6]. The EUPT-Panel reserves the right to also employ other approaches on a case-by-case basis considering analytical difficulties and experience gained from previous proficiency tests.

– ***z-scores***

This parameter is calculated using the following formula:

$$z_i = (x_i - \mu_i) / \delta_i$$

Where: x_i is the value reported by the laboratory, μ_i the assigned value, and δ_i the standard deviation at that level for each pesticide (i).

Any z-scores of > 5 will be reported as >5 and where combined z-scores are calculated a value of "5" will be used.

z-Scores will be interpreted in the following way:

$\lvert z\rvert \leq 2$	Acceptable
$2 < \lvert z\rvert \leq 3$	Questionable
$\lvert z\rvert > 3$	Unacceptable

[6] Comparative Study of the Main Top-down Approaches for the Estimation of Measurement Uncertainty in Multiresidue Analysis of Pesticides in Fruits and Vegetables. J. Agric. Food Chem., 2011, 59(14), 7609-7619.

For results that are considered to be false negatives, z-scores will be calculated using the MRRL or RL (the laboratory's Reporting Limit) if the RL < MRRL.

The EUPT-Panel will consider whether, or not, these values should appear in the z-score histograms.

z-Scores will not be calculated for any false positive result.

– ***Category A and B classification***

The EUPT-Panel will decide whether to classify the laboratories into two groups - A or B. Laboratories that detect a sufficiently high percentage of the pesticides present in the Test Item (e.g. at least 90%) and reported no false positives will have demonstrated 'sufficient scope' and will therefore be classified into Category A. The 90% criterion will be applied following Table 1.

Table 1. No. of pesticides needed to be detected to have sufficient scope.

No. of Pesticides Present in the Sample (N)	90%	No. of Pesticides needed to be detected to have sufficient scope (n)	n
3	2.7	3	N
4	3.6	4	
5	4.5	4	N - 1
6	5.4	5	
7	6.3	6	
8	7.2	7	
9	8.1	8	
10	9.0	9	
11	9.9	10	
12	10.8	11	
13	11.7	12	
14	12.6	13	
15	13.5	13	N - 2
16	14.4	14	
17	15.3	15	
18	16.2	16	
19	17.1	17	
20	18.0	18	
21	18.9	19	
22	19.8	20	
23	20.7	21	
24	21.6	22	
25	22.5	22	N - 3
26	23.4	23	

For evaluation of the overall performance of laboratories within Category A, the Average of the Squared z-Score (AZ^2)[7,8] will be used.

Laboratories within Category B will be ranked according to the total number of pesticides present in the sample. The number of acceptable z-scores achieved will be presented too. The EURL-Panel retains the right to calculate combined z-scores (see below) also for Category B labs, e.g. for informative purposes, provided that a minimum number of results (z-scores) is available.

– ***Combined z-scores***

For evaluation of the overall performance, the Average of the Squared z-Score (AZ^2) will be used. The AZ^2 is calculated as follows:

$$AZ^2 = \frac{\sum_{i=1}^{n} |Z_i||Z_i|}{n}$$

This formula multiplies each z-score by itself and not by an arbitrary number. Based on the AZ^2 achieved, the laboratories are classified as follows:

Formula	Good	Satisfactory	Unsatisfactory
AZ^2	≤ 2	$2 < AZ^2 \leq 3$	$AZ^2 > 3$

Combined z-scores are considered to be of lesser importance than the individual z-scores. The EUPT-Panel retains the right not to calculate AZ^2 if it is considered as not being useful. In the case of EUPT-SRMs, where only few results per lab are available,

[7] Formerly named "Sum of squared z-scores (SZ^2)"

[8] Laboratory assessment by combined z-score values in proficiency tests: experience gained through the EUPT for pesticide residues in fruits and vegetables. Anal. Bioanal. Chem., 2010, 397, 3061–3070.

the Average of the Absolute z-scores (AAZ) will be calculated for informative purposes, but only for labs within Category A and as long as 5 or more z-scores are available.

Publication of results

The EURLs will publish a preliminary report, containing tentative medians and z-score values for all pesticides present in the test sample, within 2 months from the deadline for result submission.

The Final Report will be published after the EUPT-Panel has discussed the results. Taking into account that the EUPT-Panel meets normally only once a year to discuss the results of all EUPTs organised annually by the EURLs in the running year, the final report may be published up to 8 months after the deadline for results submission.

Certificates of participation

Along with the Final Report, the EURL Organiser will deliver a Certificate of Participation to each participating laboratory with the z-score achieved for each pesticide and the combined z-scores calculated (if any) together with the classification into Category A and B.

Feedback

After the distribution of the final report of an EUPT, participating laboratories will be given the opportunity to give their feedback to the Organiser and make suggestions for future improvements.

Follow-up activities

Laboratories are expected to undertake follow-up activities to trace back to the source of any erroneous or (strongly) deviating results - including all false positives and false negatives, along with results with $|z|>2$.

Upon request, the laboratory's corresponding NRL, or EURL, are to be informed of the outcome of these traceability activities.

According to instructions by DG-SANCO, the "Protocol for management of underperformance in comparative testing and/or lack of collaboration of National Reference Laboratories (NRLs) with EU Reference Laboratories (EURLs) activities" will be followed for NRLs.

Disclaimer

The EUPT-Panel retains the right to change any parts of this EUPT – General Protocol based on new scientific or technical information. Any changes will be communicated in due course.

Laboratory Rights

After the Final Report has been sent, the laboratories will have the right to communicate the nonconformity of their result evaluation in written form. Any detected errors in the preliminary report should also be reported to the Organiser. The Organiser, assisted by the Scientific Committee, will decide upon any re-evaluation and will give a corresponding explanation.