2nd Edition

Approved: November 2010

GENERAL PROTOCOL

for EU Proficiency Tests for Pesticide Residues in Food and Feed

Introduction

This protocol contains general procedures valid for all European Union Proficiency Tests (EUPTs) organised on behalf of 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) in the EU Member States. Laboratories outside 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 the National Food or Feed Authorities based on the provisions of Regulation 882/2004/EC, whereas OfLs are laboratories that are actively involved in

[1] DG-SANCO = European Union, 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

official controls in the sense of Article 26 of Regulation 396/2004/EC (e.g. by conducting pesticide residue analyses within the frame of national and/or EU control programmes).

According to Article 28 (3) of Regulation 396/2005/EC[4] all laboratories analysing samples for the official controls on pesticide residues shall participate in the EUPT(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. 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 cooperation with one another.

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

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

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

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

[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.

The OT and the SC (AG and QCG) together form the **EUPT-Panel**. The role of the SC is to help the OT in making decisions concerning the design of the EUPT: 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 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 material and the concentration levels at which they should be present in the test material.

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

EUPT Participants

Eligible, and at the same time legally obliged, to participate in EUPTs are all NRLs covering the same area as the organising EURL as well as all OfLs, the scope of which overlaps with that of the EUPT. The list of eligible labs will be generated using the Lab-Network Database within the EURL-Data Pool and based on the entries concerning the commodity scope of each lab. This list will be communicated to all relevant parties before each EUPT.

NRLs are responsible to check whether all relevant OfLs within their network are included in the list of eligible laboratories and whether the contact information is correct.

OfLs are responsible for keeping their profiles within the EURL-DataPool up-to-date, especially their commodity and pesticide scopes as well as their contact information.

DG-SANCO expects from each eligible lab not intending to participate in a given EUPT to explain the reasons of non-participation. This also applies to initially participating laboratories that do not deliver results.

In special cases the Organisers upon consultation with DG-SANCO will allow laboratories outside of the EURL/NRL/OfL-Network to participate in EUPTs.

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Confidentiality:

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

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

As laid down in Regulation 882/2004, NRLs are responsible for evaluating and improving their OfL network. For this reason, the EURLs will confide the laboratory codes of OfLs to their NRLs together with the final report. This will allow the NRLs to obtain the correlation between the laboratories within their network and their performance. The EURLs furthermore reserve the right to share the EUPT-results and codes among them, for example for the purpose of evaluating the 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 material is distributed to the laboratories. The announcement will be published on the EURL portal and additionally distributed via 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 additional related documents, and 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 pesticides, metabolites and residue definitions to be tested as well as the Minimum Required Reporting Levels (MRRLs) valid for the EUPT in question. The MRRLs are basically based upon the lowest MRLs of Regulation 396/2005/EC or the Commission Directive 2006/125/EC (Baby Food Directive).

The residue definitions listed in the Target Pesticide List of each EUPT are to be followed. In certain justified cases these residue definitions may differ from the legal ones.

Specific Protocol

For each EUPT a Specific Protocol will be published at least 2 weeks before the test material is distributed to the laboratories. This protocol will contain all the information included in the invitation in its final version, information on payment for delivery service and/or participation. Furthermore, it will also include instructions on how to handle the test material upon receipt, on how to submit results, and other relevant information.

General Procedures for Reporting Results

Laboratories are responsible for reporting their results to the Organiser within the stipulated deadlines. Each laboratory must only report one result for each of the analytes present in the test material, using the analytical procedure(s) that they would routinely use for each compound for monitoring purposes although more than one method may be used to cover all the compounds to be sought. The results (residue levels of the pesticides detected) are expressed in mg/kg and in some cases of food of animal origin in µg/kg. The laboratories will be requested to not only report individual pesticides and metabolites but also to express the residue as stated in the residue definition according to the Target Pesticide List.

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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 of the range 70-120%, with good precision). Therefore if residues 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 rate used. No recovery data is required where recovery adjustments resulted from using the 'standard additions' approach, or from the use of isotopically labelled internal standards (in both cases with spiking of the test material at the beginning of the extraction procedures). In these cases, the laboratories should report the technique used for calculation of the results instead of the recovery.

Evaluation of the Results

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

- ***False Positives***

These are the results above the MRRLs that show the apparent presence of pesticides that were listed in the Target Pesticide List, but which were: (i) not detected by the organiser, even after repeated analysis, and (ii) not detected by the overwhelming majority of the participating laboratories (e.g. 95% of the laboratories) that have targeted the specific pesticide. However, in certain instances case-by-case decisions by the EUPT-Panel will 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 material and were detected by the Organiser and the majority of the participants that have targeted this specific pesticide, at or above the MRRL. However, in certain instances case-by-case decisions by the EUPT-Panel will be necessary.

Where the assigned value is smaller than 4 times the MRRL, false negatives will not be assigned as this is statistically not justifiable.

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

The "true" concentration (assigned value) will be typically estimated using the robust 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. using 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. 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" particularly where summed z-scores of many pesticides are calculated (see SWZ and SZ2 below).

z-Scores will be interpreted in the following way:

$\|z\| \leq 2$	Acceptable
$2 < \|z\| \leq 3$	Questionable
$\|z\| > 3$	Unacceptable

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.

However, a z-score will not be calculated for any false positive result.

– ***Category A and B Classification***

The EUPT-Panel will decide in each EUPT whether to classify the laboratories in two groups, A and B. Laboratories that detect a sufficiently high percentage of the pesticides present in the test material (e.g. at least 90%) and reported no false positives will have

demonstrated 'sufficient scope' and will therefore be classified in 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%	Min. number 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	

- ***Combined z-scores***

  - For evaluation of the overall performance of the laboratories within Category A, two formulas will be used.

i. Sum of Weighted z-Scores (SWZ)

The sum of weighted z-scores formula uses the z-scores with a fixed maximum value of 5 for individual z-scores, using the following formula:

$$|SWZ = \frac{\sum_{Zi=0}^{|Zi|\leq 2} |z_i| \cdot 1 + \sum_{|Zi|>2}^{|Zi|\leq 3} |z_i| \cdot 3 + \sum_{|Zi|>3}^{\infty} |z_i| \cdot 5}{n}$$

n = number of detected results

ii. Sum of Squared z-Scores (SZ^2)

The sum of squared z-scores formula multiplies each z-score by itself and not by an arbitrary number, using the following formula:

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

The SWZ and the SZ^2 have the following classification similar to the z-score:

Formula	Good	Satisfactory	Unsatisfactory
SWZ	≤ 2	2 < SWZ ≤ 3	SWZ > 3
SZ^2	≤ 2	2 < SZ^2 ≤ 3	SZ^2 > 3

Both, SWZ and SZ^2 are considered to be of lesser importance than the individual z-scores. The EUPT-Panel retains the right not to use them if they are considered not useful.

- Laboratories in Category B will be ranked according to the percentage of pesticides detected from the total number of pesticides present in the sample. The number of acceptable z-score achieved will be recall too.

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Publication of Results

The preliminary results from the EUPTs will be reported to the participants within 2 months from the deadline for result submission.

The final report will be published shortly 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 by the EURLs each year, the final report may be published up to 8 months after the deadline for results submission.

Follow-up activities

Laboratories are expected to undertake activities towards tracing back the sources of erroneous or strongly deviating results including all false positives and false negatives as well as results with |z|>2.

Upon request the corresponding NRL or EURL of a lab are to be informed about the outcome of these traceability activities.

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 a 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 a re-evaluation and will give an explanation.