

# **WORK PROGRAMME FOR THE EU REFERENCE LABORATORY FOR PESTICIDES IN CEREALS AND FEEDINGSTUFF**

(Version 20 October 2014)

## **January-December 2015**

### **LEGAL FUNCTIONS AND DUTIES**

The functions and duties of the Reference Laboratory are described in Article 32 of the EC Regulation No 882/2004.

#### **1. OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2015**

- A. General tasks**
- B. Development and validation of analytical methods**
- C. Quality assurance and quality control including the organisation and implementation of proficiency tests.**
- D. Technical and scientific support to NRLs/ EU Official labs, Commission and third countries**

#### **2. WORKING PLAN FOR THE PERIOD JANUARY - DECEMBER 2015**

##### **A. General Tasks**

*Covering the operational objectives:*

- (3) To ensure the availability of scientific and technical assistance provided by the EU-RLs,*
- (4) To ensure a sound and efficient management of EU-RL funding cycle.*

1. Maintenance of the common web portal <http://www.crl-pesticides.eu> for the pesticide EURLs (horizontal task – see description in AWP for EURL-SRM). The communication platform supports the coordination activities of the EURLs and serves as contact, reference and service points for the National Reference laboratories and official pesticide residue laboratories in Europe. The website for Cereal and Feeding stuff will be maintained in coordination with the other EURLs and the following items will be updated: Presentation of the EURL, information on proficiency test, training courses, workshop, annual work programme, validation data and analytical methods. The website is accessible for everybody. Additional also input to the development of the Datapool, especially the EUPT-Archive

Maintenance of the common CIRCA database (horizontal task – see AWP for EURL-AO). The CIRCA database is only accessible for a limited number of persons, mainly persons employed in NRLs or OfLs. Consequently, information can be uploaded to this platform if it has a more confidential content. It could e.g. be data that could be published in scientific papers. However, the platform can also send emails when new documents are uploaded. Therefore, information put on the open web portal will also be uploaded to the CIRCA platform.

Maintenance of the EURL/NRL/OfL network for laboratories performing analysis of cereals and feed. A record with information on the NRLs and OfL in the field of pesticide residues in cereals and feeding stuff will be updated, with contact name, addresses, email addresses and number of official samples analysed by the lab. The task is performed in cooperation with EURL-SRM.  
FF.R&D.1

2. Maintenance and further development of the online registration, result database and web tool for proficiency tests.

Three annual proficiency tests (PTs) organised by EURL-CF, EURL-AO and EURL-SRM use the Oracle database programs and server facilities located at the premises at DTU in Copenhagen. Although many hours of work have been used to develop these programs, they still need adjustments for each single PT. Many of these task demand edition in text on main pages, subpages and forms, and also changes in programs and database tables, views, procedures, sequences and triggers. Backup of the data is produced regularly, and all changes are stored in archive-files.

Before the new rounds of PTs the four EURLs agree on the changes to be made, both purely maintenance and new features.

*Maintenance before the PT:*

Registration database and web tool: The webpage is changed to fit the individual PT. This will be e.g. PT name, specific information needed to ship

test items from different countries. We must also maintain a list of laboratories for which participation is mandatory.

Furthermore, the text on confirmation emails, assignment of usernames and passwords, organizer access to forms and online reports so they can supervise the registration.

The result submission database and web tool: Before the database is opened for submission of test results the web pages and data forms must be edited. A list of the pesticides in the actual PT is loaded into the database. In the main database table a row for each participant and each pesticide in the test is inserted (150 participants and 150 pesticides makes 15,000 rows in the database table). The main webpage and subpages are changed to fit the individual PT. The forms for result submission must be edited (PT name, test item name, the list of target pesticides, method information needed. Forms and reports must be prepared for the organizing EURL, so they can supervise the data submission. Before the link to the webpage is activated, the system is tested and a guide to enter results and information is prepared.

*Maintenance during the PT:*

During the registration period the process is supervised to take care of double registration and missing emails etc. When the PT is running and the result submission is likewise supervised to be able to correct any errors. After deadline for result submission, links to the web-pages must be de-activated and data must be extracted from the database for statistical processing.

*Development:*

For each PT round new wishes for the web tool and database are implemented as far as possible. The new features will be agreed upon during a coordination meeting between the four EURLs

FF.PT.1

3. Administrative duties e.g. budget management, preparation of work programme, budgets will be accomplished as well as compilation of annual technical and financial reports.

## **B. Development and Validation of Analytical Methods**

*Covering the operational objectives*

- (1) *To ensure the development and use of high quality analytical methods across the EU-RL framework,*
- (2) *To maintain appropriate level of proficiency testing ensuring efficiency of control analysis methods.*

To provide validated method for use by EFSA, the NRLs and OfLs, analytical scientific work can include the improvement and enhancement of existing approved multi-methods and the development or implementation of new multi-methods and detection techniques.

The development and validation with mainly focus on the following areas.

4. Extension of the pesticide profile for cereals multi methods using LC/MS/MS or GC/MS/MS

Validation experiment with the QuEChERS or Sweet method with additional 20 pesticides will be performed. The pesticides will mainly be included from the EFSA list on progress on MRL reviews. This could be some of the following pesticides; 6-Benzyladenine, 8-Hydroxyquinoline, Amisulbrom, Bispyribac, Carbetamide, Cyflufenamid, Difenacoum, Fenbutatin oxide, Fenpyrazamine, Fenpyroximate, Metam, Novaluron, Penoxsulam, Phosphane, Profoxydim, Propaquizafop, Pyridalyl, Pyriofenone, Quizalofop-P, Sedaxane, Spinetoram, Spirotetramat, Tembotrione, Thiencarbazone, Triazoxide and Triflusaluron. However, the a final decision on precisely which pesticides to include will be taken based on last minute information on the most relevant pesticides to cover in 2015. The validation will enable the EURL to contribute to the Art. 12 process by establishing LOQ for the pesticides in question. Also other pesticides used worldwide in cereal production and/or new pesticides included in the coordinated control program will be considered. The validation will include minimum three cereal matrices. The validation data will be generated, converted in the specific format and uploaded to the common database. This information is important for the EFSA and the official laboratories.

FF.ANA.1, FF.PT.5, FF.PT.6

Validation experiments on cereal based babyfood with the QuEChERS or SweeE method will be performed for 20 pesticides. The pesticides included will be selected mainly among pesticides with MRLs below 0.01 mg/kg or pesticides that are considered to be toxicological concern. The validation could include the following pesticides taking into account the residue definitions of Reg. 396/2005: 3-Hydroxy-carbofuran, Aldrin, Cadusafos, demeton-S-methyl sulfone, Demeton-S-methyl/demeton-S-methyl, Diazinon, Dichlorvos, Dieldrin Dimethoate, Disulfoton, Endrin, Ethoprophos, Fensulfothion, Fipronil, Haloxyfop, Heptachlor, Hexachlorobenzene, Nitrofen, Omethoate, Terbufos Triazoxide. However, other pesticides could be included if it is considered to be more valuable. The validation aim to achieve LOQs at 0.001 mg/kg.

FF.ANA.1, FF.PT.5, FF.PT.6

5. Construction of database with exact masses of pesticide fragments for use in screening methods on GC-Q-TOF-MS (cooperation with EURL-FV)

GC-Q-TOF-MS is a high resolution technique based on accurate masses. Those techniques can provide high improvements in identification of pesticides included or not in the current validated methods of the laboratory and additionally a very important analytical tool complementary to confirm positive detections by other common MS techniques. However, unlike LC-MS, compounds analysed with GC-MS are commonly fragmented in the ion source and the molecular ion is rarely seen or only seen in low abundance. Consequently, it is necessary to look for the fragments and to assign the correct exact mass for each one. These data are currently not available. In 2014 the EURL-FV and EURL-CF started to calculate exact masses for pesticide fragments, with focus on pesticides that are not amendable for LC, in order to construct a database. This work will be continued.

FF.ANA.1, FF.ANA.QI

6. Development and validation of High Resolution Mass Spectrometer screening methods for analysis of cereals

In recent years, cost for High Resolution Mass Spectrometer (HRMS) instruments have decreased and are becoming affordable for NRL and OfL. Screening methods on HRMS has several advantages and will be a powerful tool in combination with MS-MS instruments.

The EURL has developed and validated a screening method for cereals by LC-QTOF during 2012-2014. The method is based on QuEChERS extraction and clean-up and is currently validated for around 100 pesticides and additional 50 pesticides will be validated before end of 2014. For 2015 the method will be optimised for different parameters to lower the detection limit and make the method more robust. The parameters to be optimized could be extract dilution, injection volume, flow, and instrument state at 4 GHz instead of 2 GHz extended dynamic range. This information is also relevant for updating EU QC Guidelines where the MS based identification parameters are established. Furthermore, in cooperation with EURL-FV, work will be initiated to prepare the method for CEN.

The EURL has developed and validated a screening method for cereals by GC-QTOF during 2013 for 36 pesticides. The method is based on QuEChERS extraction and clean-up. Validation will be performed of additional 25 pesticides in 2014. The method will in 2015 in cooperation with EURL-FV be validated for additional 25 pesticides. The pesticides will be chosen from the compounds where exact masses is established see point 5. Like the LC-QTOF method, the GC-QTOF method will be optimised for relevant parameters.

FF.ANA.1, FF.ANA.QI

7. Study of the efficiency of extensive clean-ups using high speed centrifugation or specific sorbents based on Zr or others.  
During extraction of cereals a wide range of plant compound are coextracted, e.g. fatty acids or sterols. These compounds co-elute with the pesticides during analysis on LC and GC instruments and can also result in various matrix effects and deterioration of the chromatography. Extensive clean-up experience for

cereals using high speed centrifugation or specific sorbents based on Zr will be performed in cooperation with EURL-FV. The EUPT cereal test materials from earlier PTs will be used for this study.

FF.ANA.1,

8. Study on milling of cereals to enhance the extraction efficiency.  
EURL-CF during 2013 made experiment on milling of cereal kernels showing that the particle size in the flour is important for the extractability of the incurred pesticides. The smaller particle the higher pesticide residue is found. But also the temperature during the milling is important. This has resulted in a recommendation to obtain particle size below 1 mm.

Cryogenic milling could be a way to obtain small particle without heating the cereals and clog the sieves and other part of the mill. Equipment for cryogenic milling is now commercially available however, the cost are quite high. The EURL will explore the possibility perform cryogenic milling with other and cheaper equipment, like shakers produces for other purpose.

Furthermore, to get an overview of the situation in EU laboratories, a survey on milling/particle size will be performed by the EURL-CF. Samples of wheat kernels with incurred pesticides will be shipped to the NRLs. The samples will leftover from earlier PTs. The NRLs will mill the samples with their routine method and send back the flour to the EURL-CF. The EURL-CF will measure the particle size and analyse for content of pesticides. By this we will be able to estimate any possible underestimation of the intake of pesticides from cereals.

FF.ANA.1,

9. Evaluation of extract backgrounds for MRM-pesticides (QuECHERS or SweEt) in cereals.

The evaluation of the background of relevant commodities affecting the performance of the analytical method applied will be developed as a powerful tool to help OfLs to avoid matrix effect e.g. ion suppression effects. The project will be performed in cooperation with EURL-FV that has software packages to extract matrix information from chromatograms achieved with LC-QTOF instrument. EURL-CF will analyse samples of cereal using QTOF. The resulting data (chromatograms) will be evaluated at EURL-FV to get information of the number of the matrix components being present through the whole chromatogram. This information will be used for interpretation of matrix effects and for improvement of the clean-up with the aim to reduce matrix effects in GC- and LC-MS for a better quantification.

FF.ANA.1,

### **C. Quality Assurance and Quality Control**

#### ***Covering the operational objectives***

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- (2) *To maintain appropriate level of proficiency testing ensuring efficiency of control analysis methods,*
- (3) *To ensure the availability of scientific and technical assistance provided by the EU-RLs.*

10. Proficiency test PT-CF9 on maize.

A proficiency test will be organized in 2015. The proficiency test will cover pesticides analysed by multi methods. The target pesticides will includes approximately 120 pesticides, as agree on by the Advisory Group. The test item maize will be produced by a consultant. After production the test item will be homogenized and portions will weighed out into screw-capped polyethylene plastic bottles, sealed, numbered, and stored in a freezer at about -20 °C prior to homogeneity and stability testing. Before shipment of the test items, 20 homogeneity experiments will be performed (double determinations of 10 randomly selected test items). Stability test will be performed on several occasions during the PT periods, in total 50 experiments.

It is foreseen that around 120 EU NRL and EU OfL will participate in the PT and additionally 10-20 official laboratories from Third Country.  
FF.PT.1, FF.PT.2, FF.PT.3, FF.PT.4, FF.ORG.1, FF.ORG.2, FF.ORG.3

11. Preparation of test items for proficiency test 2016, EUPT-CF10

If necessary, field spraying of cereals performed by a consultant will take place to prepare for EUPT-CF10. The proficiency test will focus on crops agreed upon by the Advisory Group. The NRLs will be involved as well in the selection of test item, during the 2014 workshop.  
FF.PT.1

12. Contribution to the revision of “Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed” (Document N° SANCO/12751/2013

In order to continue the process of achieving complete harmonisation measures for pesticide residue analysis within the EU, the SANCO document “Analytical quality control and method validation procedures for pesticide residues analysis in food and feed” (SANCO 12571/2013) needs to be revised and updated on continuous basis, especially when difficulties arise. This is a collaboration between the four EURL and headed by EURL-FV.  
FF.R&D.1

13. Maintenance of in-house QA/QC activities in according to ISO 17025 accreditation of all analytical work done within the EURL and ISO 17043 in relation to the proficiency test organized by the EURL.  
FF.PT.QI

## **D. Technical and Scientific Support to NRLs/EU official labs, the Commission and Third Countries**

### ***Covering the operational objectives***

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#### **14. Workshop on Pesticide Residue Analysis in cereals**

A workshop coordinated between the EURL-FV, EURL-SRM and EURL-AO will be organized and held in Stuttgart, Germany. The workshop will be arranged for NRLs, but OfL laboratories will also be invited. The agenda for the workshop will include results from proficiency test, discussion on coordinated monitoring programme and analytical challenges and other relevant issues decided by the EURLs. The main organizer will be EURL-SRM  
FF.NRL.1, FF.NRL.2, FF.NRL.3

#### **15. Training**

A 1-2 days training course will be organised at the premises of the EURL in collaboration with EURL-FV on HRMS and screening methods. EURL-FV will invite up to four NRLs and EURL-CF up to four NRLs from the area of cereals will be invited. However, it is a requirement that the invited NRLs have HRMS instruments available for pesticide residue analysis in their laboratories. The training course will also aim to introduce modifications in the SANCO/12571/2013 document of “Analytical quality control and method validation procedures for pesticide Residues analysis in food and feed” as regard the validation procedures for HRMS.

FF.NRL.5, FF.NRL.6, FF.NRL.QI, FF.ORG.QI

#### **16. Webinars (horizontal task – see AWP for EURL-FV)**

One webinars intended to disseminate information to the NRLs and official laboratories in a cost effective but still interactive way will be organised. The system from EURL-FV will be used.

FF.R&D.1

#### **17. Technical and scientific support to the Commission and its offices, as requested by the Commission.**

Support to the COM for the drafting of the EU coordinated control programme and Article 12. Coordination of Article 12 is shared with EURL-SRM.

Support in Analysis of official samples, counter analysis will be performed on request by the Commission.

FF.COM.1, FF.COM.2, FF.COM.QI



18. Involvement in the EFSA residue evaluation to give its opinion on LOQs, residue definitions and routine validated methods for cereals and feeds, especially on article 12 for up to 50 compounds.  
FF.COM.2
  
19. Missions  
Participation in up to 2 coordinating meetings between EURLs for pesticide residues in order to coordinate AWP, PTs, web portals, Sanco Document and/or online result submission.  
Participation up to two Standing Commission meeting and/or EFSA pesticide network group meetings.  
Participation in advisory groups organized by the EURL for fruit and vegetables (horizontal task organised by EURL-FV, see AWP for EURL-FV).  
Participation in RAFA 2015, Prague, 3-6 November 2015  
Pre-approval from the Commission will be obtained for all non-planned missions.  
FF.COM.2
  
20. One NRL visit will be conducted to a laboratory selected in agreement with the COM, where the EUPTs results have been problematic over the last years. The task could be performed in collaboration with other EURLs.  
FF.NRL.4