

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

(Version 15 October 2015)

January 2016 - December 2017

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.*

A1 Ongoing tasks.

Maintenance of the common web portal <http://www.eurl-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

A2 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 Roskilde. Each year adjustments for each single PT have to be made. Many of these task demand change in the 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 as archive-files.

Before each new round of PTs the four EURLs agree on the changes that needs to be made, both purely for maintenance and for new features.

Developments:

The method information page will be updated in order to optimize the performance of the database while PTs are running. The changes will aim at improving how the fields are displayed allowing the participant to get a better overview of their submitted data. Presently, the participant's needs to scroll to the right with the risk of losing track of the pesticide. In future all the method information for a specific pesticide will be visible at the screen.

Maintenance before the PT:

Registration database and web tool: The webpage is changed to fit the individual PTs. This will be e.g. PT name, specific information needed to ship the test items from different countries. A list of laboratories for which participation is mandatory also needs to be maintained. Furthermore, the text on confirmation emails, assignment of usernames and passwords, organizer access to forms and online reports so they can supervise the registration are things that needs to be changed for each PT.

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 PT in question 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 22500 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.

A3 Administrative duties e.g. budget management, preparation of work programme, budgets will be accomplished as well as compilation of annual technical and financial report.

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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.*

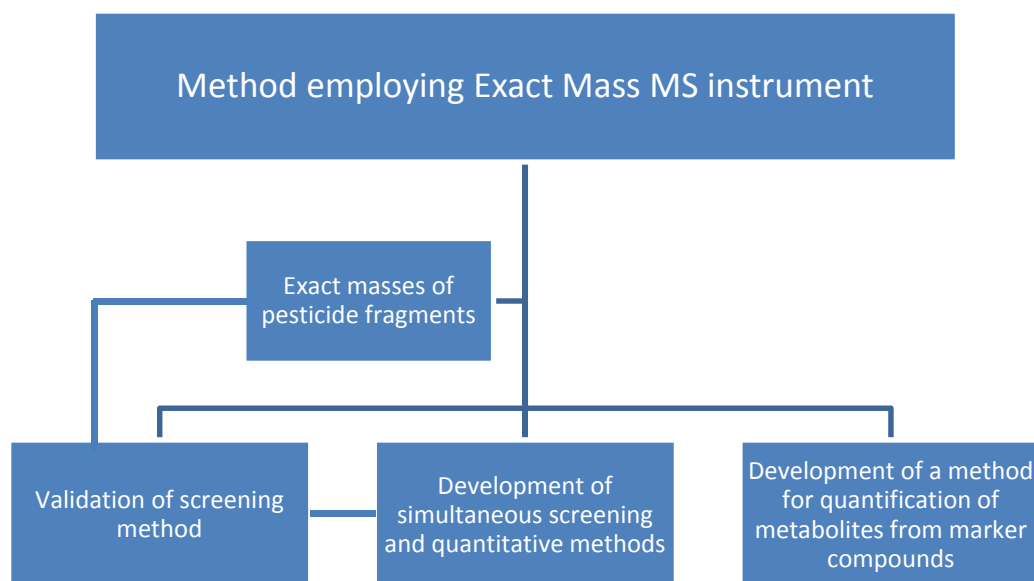
In order to provide robust validated methods for use by the NRLs and OfLs, analytical scientific work include improvement and enhancement of existing approved multi-methods and the development or implementation of new multi-methods and detection techniques.

The development and validation will in the next period mainly focus on the following areas:

B1 Method development employing Accurate Mass Spectrometry

In recent years, the cost for Accurate Mass Spectrometers has decreased hence is becoming affordable for NRL and OfL. Screening methods based on Accurate Mass Spectrometry add several new possibilities to current GC/LC-MS/MS methods and is a powerful tool in combination with MS-MS that allow significant expansion of the number of pesticide/metabolites sought for. Additionally, as Accurate Mass Spectrometry has proven its stability the methodology can readily be used for identification, and quantitation within the same run. Finally, having the accurate (exact) mass offers the possibility for tentative identification of compounds, e.g. metabolites of pesticides where standards are not available or not affordable. For risk assessment it is not sufficient to identify a compound, but also to quantify the level. Therefore, a documented (validated) procedure to quantify the levels of pesticides/metabolites in cases where analytical standards are not available will be of significant value for the risk assessment. Furthermore, this procedure will also allow estimating the levels of unexpected pesticides in screenings analysis where the relevant standards were not included.

In order to exploit the possibilities given by Accurate Mass Spectrometry, the following important method development tasks illustrated and described below have been defined.



B1-1 Building a database with exact masses of pesticide fragments for use in screening methods on GC-QTOF-MS (cooperation with EURL-FV) (2016 and 2017)

GC-Q-TOF-MS is an accurate GC-MS/MS technique that is based on high resolution time-of-flight mass spectrometry. As mentioned above, the accurate mass provides a significant improvement in identification of pesticides and can therefore be a valuable tool for confirmation of positive detections by other common MS techniques. However, unlike in LC-MS, compounds analysed by GC-MS are commonly fragmented in the ion source and the molecular ion is rarely detected or only detected in low abundance. Using GC-QTOF-MS it is therefore necessary to use the fragments for identification and therefore also to assign the correct exact mass for all relevant fragments. These data are currently not available in standard libraries. In 2014 the EURL-FV and EURL-CF started to calculate exact masses for pesticide fragments in order to build a database. In 2015 EURL-CF updated the database with 53 new pesticides and their fragments. In 2016 and 2017 the database will in corporation with EURL-FV be continuously updated with at least 100 more pesticides and their fragments. The focus for EURL-CF will be pesticides not included in MCPA. FF.ANA.1, FF.ANA.QI

B1-2 Development and validation of Accurate Mass Spectrometry screening methods for analysis of cereals (LC-QTOF-MS and GC-QTOF-MS) (Preferable in 2016)

The EURL has developed and validated a screening method for pesticides in cereals by LC-QTOF-MS during 2012-2015. The method is based on QuEChERS extraction and clean-up, and is to day validated for around 500 pesticides during 2012-2015. In cooperation with EURL-FV, work was initiated to draft the method for CEN and this work will be continued.

The EURL has equally developed and validated a screening method for cereals by GC-QTOF-MS during 2013 for 36 pesticides and during 2015 the method will be validated for additionally 75 pesticides. The method is based on QuEChERS extraction and clean-up. The method will be validated for additionally 50 pesticides in 2016-17 and be optimized by introducing retention time locking. The 50 pesticides will be chosen among the compounds for which exact masses was established in the task described above.

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FF.ANA.1, FF.ANA.QI

B1-3 Development of simultaneous screening and quantitative methods for analysis of pesticides residues in cereals using Accurate Mass Spectrometry and evaluation of its applicability to routine analysis (Preferable in 2016)

Accurate Mass Spectrometers have significantly improved the performance to allow much more reliable quantification over a wider dynamic range. A method that combines identification *and* quantification of a number of frequently found pesticides, while screening for a much large number of other pesticides, would be of great value for the pesticide control laboratories. The screening methods on LC-QTOF-MS and/or GC-QTOF-MS already developed (B.4) will be further developed to include quantitation for 50 frequently found pesticides. Validation will be performed for minimum 3 cereal matrices.

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FF.ANA.1

B1-4 Development of a method for quantification of metabolites from marker compounds using Accurate Mass Spectrometry (Planned in 2017)

In some cases analytic standards are not available and that is a relatively common scenario for metabolites of the pesticides included in the residue definitions. Many of these metabolites are either very expensive to purchase as analytical standards or are not commercially available.

Work has been initiated in other fields of food safety with developing methods for the quantification of analytes in case where standards are not available. The core idea is developing LC-MS responses model that rely on data to be normalized against relevant similar compounds. Thereby the chromatographic conditions and ion source settings can be optimised, such that a large group of analytes in similar concentration, can be expected to generate a response that are similar to response generated by a marker compound at the same concentration.

The aim of this task is to develop an LC-MS method that will allow quantification of pesticide metabolites included in the pesticide residue definitions, where standards are not available (or outside the reach of the laboratories as they are too expensive to purchase)

The method will be developed and tested using pesticide metabolites that are commercially available.

48 % financed by DTU

FF.ANA.1

B2 Extension of the pesticide profile for cereals/feed multi methods using LC-MS/MS or GC-MS/MS

Inclusion of more pesticides in the quantitative LC-MS/MS and GC-MS/MS methods is a constant request. New pesticides are authorised for use in EU, the number of pesticides included in the MACP are generally expanded the pesticides relevant in regard to directive 669 is changing etc. The work on validation of pesticides will therefore be continued.

B2-1 Validation experiments on cereals using the QuEChERS or Sweet method for additional 20 pesticides will be performed, both in 2016 and 2017. The pesticides chosen will mainly be from the EFSA Progress report MRL review (10/06/2013), MACP, the working document and new authorisations. The final decision on which pesticides to include will be based on last minute information on the most relevant pesticides to cover. However, the following pesticides would be of interest: 1,4-dimethylnaphthalene, 1-naphthyl acetic acid, chitosan hydrochloride, acequinocyl, amisulbrom, benalaxyl-M, disodium phosphate, flubendiamide, flumetralin, flupyradifurone, halauxifen, ipconazole, mandestrobin, pyriofenone, pyroxsulam, s-abscisic acid, sedaxane, valifenalate. The validation will additionally enable the EURL to contribute to the Art. 12 process by establishing LOQ for the pesticides in question. 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 EFSA and the official laboratories.

33 % financed by DTU

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

B2-2 Validation experiments on a feed variety of maize using the QuEChERS or SweEt method will be performed for 60 pesticides. The pesticides included will be selected among pesticides relevant for feed and possible pesticide residues in commodities of animal origin, also including pesticides in the coordinated control program. Pesticides of interest could be: acrinathrin, bitertanol, bromopropylate, bromuconazole, cadusafos, chlorfenapyr, chlorobenzilate, clofentezine, dicloran, diphenylamine, EPN, fenamiphos, fenarimol, fenpropathrin, fenthion, flufenoxuron, fosthiazate, indoxacarb, isofenphos-methyl, mepanipyrim, methidathion, myclobutanil, oxadixyl, parathion-methyl, pencycuron, phenthoate, phosalone, profenofos, propyzamide, prothiofos, tau-fluvalinate, tefluthrin, tetraconazole, tetradifon, tolclofos-methyl. (Preferable in 2016)

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

B2-3 Validation experiments on feed for farmed fish using the QuEChERS or SweEt method will be performed for 20 pesticides. The pesticides included will be selected among pesticides relevant for feed and possible residues in fish, including pesticides in the coordinated control program, Directive 2002/32 on

undesirable substance in animal feed and the widely used antioxidant/pesticide ethoxyquin. The validation will include one feed stuff matrix e.g. composite feed for fish. Pesticides of interest could be: ethoxyquin, aldrin, chlordane cis-, chlordane oxy-, chlordane trans-, dieldrin, endrin, heptachlor, heptachlorepoxyd-cis, heptachlorepoxyd-trans, azoxystrobin, boscalid, chlorpyrifos, chlorpyrifos-methyl, cypermethrin, deltamethrin, dichlorvos, fenvalerate, pirimiphos-methyl, pyraclostrobin, tebuconazole (Preferable in 2016)
FF.ANA.1, FF.PT.5, FF.PT.6

B3 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 and is a continuation of the test started in 2015. In 2015 extract of wheat, rye, oat, rice and maize, with and without PSA clean-up will be analysed. In 2016 focus will be on oat extract cleaned up by different means, e.g. Z-sep, PSA, C18, freezing out, dilution and ultra-centrifugation. EURL-CF will extract samples and perform the clean-up EURL-FV will analyse on Accurate Mass Spectrometer and evaluate the resulting data (chromatograms) 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. (Preferable in 2016)
FF.ANA.1

B4 Pesticide residues in feed and in the animals receiving the feed

Pesticides residues in feed will be transferred to the animals. However, for most feed/pesticide/animal combinations the transfer will not result in measurable pesticide residue levels in the animals. However, pesticide residues in eggs and in fish could be of interest. The following two task focus on this.

B4-1 Pesticide residues in feed for laying hens and resulting pesticide residues in eggs (Preferable in 2016)

The EURL-AO and EURL-CFs initiated in 2011 a study with the aim to study the occurrence of pesticide residues in eggs when known levels of pesticides are present in the feed provided for the laying hens. However, the feeding study was terminated after few days due to a high weight loss for the hens. This was probably due to high concentrations of especially organochlorines (aldrin and lindane). A repetition of the feeding study on laying hens with feed containing lower levels of pesticides will be organised. EURL-CF will produce the feed and EURL-AO will organize the feeding experiments and analyse the resulting residues in the eggs. The study will enable the estimation of the risk for consumers in relation to pesticide residues in eggs.

B4-2 Pesticide residues in feed for farmed fish and pesticide residues in fish (Preferable in 2017)

So far only few data on pesticide residues in feed for farmed fish and in fish exist. Fish feed consist mainly of cereals and fish meal. Both constituent may contain pesticide residues. To elucidate the correlations between pesticides residues in the feed and in the fish, the EURL-AO and EURL-FV will cooperate on a feeding study with known pesticides residues in the feed. EURL-CF will produce the feed and EURL-AO will organize the feeding experiments and analyse the resulting residues in the fish. The study will enable the estimation of the risk for consumers in relation to pesticide residues in fish.

B4-3 Survey on pesticide residues in the feed for farmed fish in EU (Preferable in 2016)

To monitor the pesticide residues in feed for farmed fish in EU, the EURL-CF will analyse samples collected in the Member States with significant production of farmed fish. The NRLs will be asked to collect two samples and ship it to the EURL-CF for analysis. The samples will be analysed by validated multi methods and screening methods.

B5 Processing factors for cereals, bran versus flour

Bran, mainly from wheat, is widely used in manufacture of feed. However, it is also increasingly being used to enhance the fibre content of various foods such as breads and breakfast cereals. MRLs are fixed for the whole grains inclusive the bran parts. Processing factors are necessary for carrying out the risk assessment of bran product that is either used directly or added products to increase the fibre content. There are studies showing how pesticides are distributed between the bran and the remaining part of the kernels for some specific substances but far from for all pesticides/cereals. By using the left over from cereal kernels that originally was produced to prepare PT reference materials and that consequently are treated with pesticides in experimental fields, it is possible to establish additional processing factors for the pesticides included in the treatment. The processing factors will add to the existing literature.

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C. Quality Assurance and Quality Control

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,*
- (3) To ensure the availability of scientific and technical assistance provided by the EU-RLs.*

C1 Proficiency test PT-CF10 on rye.

A proficiency test will be organized in 2016. The proficiency test will cover pesticides analysed by multi methods. The target pesticides will include approximately 120 pesticides, as agreed on by the Advisory Group. The rye that will be used as test item, was produced by a consultant in 2015. After reception of the rye, it will be homogenized and portions will be 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 period, in total 50 experiments. *21% financed by DTU and third countries participation fees to cover the participation of third countries*

FF.PT.1, FF.PT.2, FF.PT.3, FF.PT.4, FF.ORG.1, FF.ORG.2, FF.ORG.3

C2 Proficiency test PT-CF11.

A proficiency test will be organized in 2017. The proficiency test will cover pesticides analysed by multi methods. The target pesticides will include approximately 120 pesticides, as agreed on by the Advisory Group. The matrix to be tested will be decided in 2016 and if necessary produced by a consultant. After production the test material will be homogenized and portions will be 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 material, 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 NRLs and EU OfLs will participate in the PT and additionally 10-20 official laboratories from Third Countries. *21% financed by DTU and third countries participation fees to cover the participation of third countries*

FF.PT.1, FF.PT.2, FF.PT.3, FF.PT.4, FF.ORG.1, FF.ORG.2, FF.ORG.3

C3 Preparation of test items for proficiency test 2017, EUPT-CF11 (2016)

If necessary, field spraying of cereals performed by a consultant will take place to prepare for EUPT-CF11. 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 2016 workshop.

FF.PT.1

C4 Preparation of test items for proficiency test 2018, EUPT-CF12 (2017)

If necessary, field spraying of cereals performed by a consultant will take place to prepare for EUPT-CF11. 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 2016 workshop.

FF.PT.1

C5 Contribution to the revision of the 2015 version of the SANCO/12571/2013 document of “Analytical quality control and method validation procedures for pesticide Residues analysis in food and feed (SANCO Document) (2016 and 2017)

In order to continue the process of achieving complete harmonised measures for pesticide residue analysis within the EU, the SANCO document needs to be revised and updated on a continuous basis, especially when difficulties arise. This task will be performed in collaboration between all four EURLs and headed by EURL-FV.

FF.R&D.1

C6 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

(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,*

(3) *To ensure the availability of scientific and technical assistance provided by the EU-RLs.*

D1 EURL/NRL Workshop on Pesticide Residue Analysis in cereals

A workshop will be organised for the NRLs for cereals and feeding stuff. The workshop will be held in Copenhagen in the period September-December 2016. The agenda for the workshop will include results from proficiency test, discussion on coordinated monitoring programme, analytical challenges, accreditation issues and other relevant issues. One expert from each country will be reimbursed.

FF.NRL.1, FF.NRL.2, FF.NRL.3

D2 Joint EURL/NRL Workshop on Pesticide Residue Analysis

A joint workshop coordinated by the EURL-FV, EURL-SRM, EURL-AO and the EURL-CF will be organized and held in Freiburg, Germany, in the period September-December 2017. 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-AO
FF.NRL.1, FF.NRL.2, FF.NRL.3

D3 Training 2016

In 2016, a 1-day training will be organised, at the premises of the EURL on specific subjects from the SANCO document. This could be on issues like preparation and especially quality control of standard stock solutions and working standard solution.

D4 Training 2017

In 2017 a 1-2 days training course on Accurate Mass Spectrometry and screening methods will be organised by EURL-CF in collaboration with EURL-FV. The training course will take place in Almeria. Each EURL will invite up to four NRLs from their NRL-network. The NRLs will only be invited if they have Accurate Mass Spectrometer available for pesticide residue analysis in their laboratories. The training course will also aim to introduce modifications in the SANCO document as regard the identification and validation procedures for Accurate Mass Spectrometry.

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

D5 Webinars (horizontal task – see AWP for EURL-FV)

One webinars in 2016 and one in 2017 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

D6 Technical and scientific support to the Commission and its offices, as requested by the Commission.

- Support to the COM on the drafting of the EU coordinated control programme and Article 12. Coordination of Article 12 is shared with EURL-SRM.
- Establishment of a database that connect pesticides and information about available analytical methods. This task will be performed together with the EURLs AO, FV and SRM.
- Perform a survey on analytical capability for the substances in chapter 4 of the monitoring working document. This task will be performed together with the EURLs AO, FV and SRM.
- Support on Analysis of official samples, counter analysis will be performed on request by the Commission.

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

D7 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

D8 Missions

Participation in up to 2 coordinating meetings between EURLs for pesticide residues per year in order to coordinate AWP, PTs, web portals, web application, SANTE quality assurance document and/or online result submission.

Participation up to two Standing Commission meeting and/or EFSA pesticide network group meetings and/or monitoring expert group meetings.

Participation in advisory group meetings organized by the EURL for fruit and vegetables (horizontal task organised by EURL-FV, see AWP for EURL-FV).

Participation for two persons in EPRW 2016, 24-27 May 2016, Limassol, Cyprus.

Participation in RAFA 2016, Prague, Czech Republic (date not yet published)

Participation for up to two persons LAPRW 2017, Costa Rica (date not yet published)

Participation in 9th Workshop on Proficiency Testing in Analytical Chemistry, Microbiology and Laboratory Medicine. Place and date not yet published.

Pre-approval from the Commission will be obtained for all non-planned missions.

FF.COM.2

D9 One NRL visit per year 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