EUROPEAN UNION REFERENCE LABORATORIES IN THE FIELD OF VETERINARY PUBLIC HEALTH WITHIN THE EUROPEAN UNION

EURL for residues

RIKILT Wageningen UR

at Wageningen, NL

Work programme

January 1st, 2016 – December 31st, 2017

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WORK PROGRAMME FOR THE EUROPEAN UNION REFERENCE LABORATORY FOR RESIDUES, RIKILT, Wageningen, the Netherlands

HORMONAL GROWTH PROMOTING COMPOUNDS, SEDATIVES AND MYCOTOXINS

January 2016 – December 2017

LEGAL FUNCTIONS AND DUTIES

The functions and duties of the Reference Laboratory are described in Article 32 of Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 (Official Journal of the European Union L 165, 30.04.2005, pp 1-141, corrected and republished in Official Journal of the European Union L 191, 28.05.2005, pp 1-52.

The general objective of the Commission for the period 2015-2016 is "to contribute to a high level of protection for consumers and the environment while favouring competiveness and the creation of jobs[1]". This general objective is elaborated in four operation objectives which are the foundation of the EURL workprogramme for 2016-2017.

The EURL workprogramme is divided in 4 parts, linked to the four operation objectives. For each operational objective individual tasks have been formulated which are described in more detail for the two year period.

Operational objectives	EURL activities
To ensure the development and use of high quality analytical methods across the EU-RL framework.	1. Maintenance or extension of existing analytical methods 2. New analytical methods 3. Development of knowledge system for identification of injection sites. 4. Studies to detect abuse of (semi-)natural hormones 5. Identification of new compounds
To maintain an appropriate level of inter laboratory comparative testing ensuring efficiency of control analysis methods	6. Maintenance of in-house QA/QC activities 7. Organisation of proficiency tests 8. Production of incurred sample material
To ensure the availability of scientific and technical assistance provided by the EU-RLs	9. Evaluation of the draft Annual National Residue Plans of Member States 10. Analytical support and training. 11. Missions to NRLs and dissemination of information 12. Technical assistance to the Commission and international, organisations

[1] Commission implementing decision of 24.7.2015 on the adoption of the work programme of the Commission for the years 2015 and 2016 and on the financing of the Union contribution to the European Union Reference Laboratories

	13. Provision of standard substances 14. Analyses of official samples in case of technical, problems or arbitration 15. Organisation of annual workshop 16. Documentation and information services
To ensure a sound and efficient management of the EU-RL funding cycle	17. Meeting 4 EURLs, EURLs for residues management 18. Compilation of annual report and cost-statement

Operational objective: To ensure the development and use of high quality analytical methods across the EU-RL framework.

Development and validation of state of the art analytical methods is one of the major tasks of the EU-RL. New analytes, or metabolites of compounds, will have to be included on a regular basis and new technologies will have to be implemented. Based on the results of research activities within the EU-RL-NRL network, methods will have to be re-evaluated on specific aspects. Adequately validated methods for all analyte-matrix combinations included in the list of Recommended Concentrations for Control (CRL Guidance paper)[2], MRPLs or the RPA-values to be set will be maintained and made available on request. Priorities are set on the basis in input by the Commission, discussions with the NRLs, e.g. during annual workshops and the EURLs view on important scientific and technical trends and innovations.

New methods will be developed for new classes of compounds, not yet included in the CRL-guidance paper, or for analytes for which methods have proven to be inadequate. Developing and implementing efficient strategies for the control on natural hormones will remain an important research topic during the coming years. This includes four different classes of compounds: the classic natural hormones, the so-called minor androgens that can be present or formed in biological samples, protein hormones and substances that can be foodborne or stress related (e.g. corticosteroids). This part of the work programme is based on the EURL Reflection paper[3].

Work plan per activity

1**) Maintenance or extension of existing analytical methods**, inclusive the validation status. It is foreseen that updated procedures of existing analytical methods for the following analyte-matrix combinations will become available in 2015 and 2016, to be validated in 2016 and 2017. The individual studies are prioritised, see numbering below.

[2] CRL-guidance paper (7 December 2007). CRLs view on the state of the art analytical methods for national residue control plans.

[3] EURL Reflection paper; Natural growth promoting substances in biological samples. EURL, RIKILT, Wageningen, 14th May 2014.

1. Validation of a multi method for steroids (A3) in meat.

2. Validation of quantitative method for prednisolone, metabolites and precursors in liver and urine

3. Extension of IGF-1 method with different forms.

4. Validation confirmatory IRMS method for Boldenone and Nortestosterone

5. Extension of the rbST method. Exchanging the animal experiment obtained antibodies by animal friendly new binders like affimers. This will enable European wide implementation of the developed method.

2) **New analytical methods**:

Development of a profiling analytical method for screening of thiouracil in bovine urine and/or serum. Thiouracil is a compound which can be formed in the intestine of the bovine and porcine animal. To distinguish thiouracil abuse from endogenously formed thiouracil a screening method in which thyroid hormones, thyreostatics and e.g. Thyroid stimulation hormone (TSH) will be analysed. Statistical models will be studied to develop a strategy that can be used to distinguish abuse from formation

Research into the use of new state of the art full scan high resolution GC-mass spectrometry for multi analyte steroid detection and untargeted analysis. Preliminary studies into the feasibility of this technique for steroid analysis

3) Development of a tool for identification of injection sites.

Knowledge on recognition of injection sites in slaughterhouses is not in all Member States available. RIKILT has a lot of knowledge and documentation including pictures of injection sites. To instruct Member States on this subject in 2013 an expert system was built where information can be found on detection of illegal growth promoters in cattle. The focus was on changes that can be observed in living animals and in the carcass, especially injection sites. In 2014 additional information on morphological and histological changes in the target tissues was selected. This work was continued in 2015. In 2016 the knowledge system comprising the histological information will be further supplemented with picture information. This expanded prototype of the knowledge system will after these additions be validated. In 2016 an in 2015 selected platform for on site use in slaughterhouses will be build eg. App or other android iOS platform. In 2017 this on site application will be tested in real life in slaughterhouses and in several memberstates this platform will be made available.

4) Studies to detect abuse of (semi-)natural hormones.

Based on the methods and models developed within the EU-RL, selected populations of samples were analysed for their steroid profiles (precursors, physiological active compounds and their metabolites). For the models to be implemented effectively, it is necessary to (1) improve the quality of the reference datasets of well described and untreated animals and (2) the development of a confirmatory method on the basis of GC-c-IRMS.

In 2014 this strategy was implemented in the Dutch Annual residue Control Plan. The GC-c-IRMS methods developed for confirmation of testosterone and estradiol will be extended and used to confirm exogenous administration in the cases screening analysis turns out suspect.

Based on the EURL Reflection Paper, priorities for research were set. One of the priority topics identified was : "Detection of steroidesters in hair and serum". The detection of steroid esters offers possibilities for detecting both synthetic endogenous (Testosterone and Oestradiol) and exogenous hormones. The analyses of serum is especially of interest for on-site (farm) testing whereas hair analyses has great potential for testing after prolonged periods of time. Until now, several studies have been published showing promising results. However, a systematic approach and harmonized outcome are not yet available. The work programme for 2016 includes extending the scope and analytical validation of the methods for serum and hair and biological validation of the method on samples to be obtained from an animal experiment in which bovine animals (veal calves) will be treated with selected steroid esters. The detailed work programme will be discussed within the EURL working groups "Minor Androgens" and "Natural Hormones".

5) Identification of new compounds.

Identification of new and unknown compounds illegally used for growth promoting purposes. On the basis of sample materials received (biological samples, cocktails or animal feed) or information obtained through other sources, studies will be undertaken to identify individual compounds. When necessary, based on *in vitro* studies, the metabolism will be studied. Special attention will be given to the use of e.g. pro-hormones.

The availability of reference standards and conjugated reference standards hampers confirmation sometimes in practice. Preliminary studies into the possibility of using the fungus *Cunninghamella elegans* to produce metabolite standards will be undertaken.

Operational objective 2: To maintain an appropriate level of inter laboratory comparative testing ensuring efficiency of control analysis methods

Quality assurance is considered an essential part of the activities of both Reference and Official Laboratories. The EURL provides assistance, mainly through the organisation of advances proficiency tests. Advances means that the tests are based on incurred sample materials, obtained through treatment of food producing animals with relevant growth promotors, sedatives of mycotoxins.

6) Maintenance of in-house QA/QC activities

Activities necessary to maintain all national accreditations will be undertaken. Since these activities are part of the overall efforts of RIKILT Wageningen UR necessary to maintain its status as Chemical Food Safety Reference laboratory, these activities are not charged to the EURL.

7) Organisation of proficiency tests.

Topics to be determined during annual workshops. Proficiency tests are organized on a regular basis, on average 3 tests per period of 2 years. Priorities are set on an annual basis, after consultation of the NRLs, amongst others during the workshops. As a rule, the proficiency tests

are based on incurred materials, obtained during a controlled animal experiment. It is then objective to prepare a preliminary report within 2 months after the results have been received, a full report within 6 months.

The following proficiency tests are foreseen during the period 2016 – 2017

(1) Steroid esters in hair.

(2) Group A growth promoters in Urine; Two PTs, 2016 and 2017.

(3) To be determined (EURL workshop Spring 2016); Two PTs, 2016 and 2017.

8) Production of incurred sample material.

An animal study in preparation of future proficiency tests are scheduled for 2016 and 2017. Priorities will be set during the 2016 and 2017 annual workshop.

Operational objective 3: To ensure the availability of scientific and technical assistance provided by the EURLs

The EURL provides assistance to both the Commission and the NRLs. Next to these, also International organisations are assisted on request.

9) Evaluation of the draft Annual national Residue Control plans

An evaluation of the draft Annual National Residue Plans of 2016 and 2017 will be produced. A list of matrix/method combinations which was prepared by the EU-RLs (Guidance paper of December 2007), has been distributed as a reference that remains the basis for further evaluations. When necessary, specific suggestions for improvement will be included in the report. In 2016 and 2017 the plans for both bovine and porcine animals will be evaluated. Other relevant species will be evaluated in more general terms.

10) Analytical support and training of NRs.

Analytical support, both by means of advice or training, will be given to NRLs upon their request. Organisation of an additional training for NRLs on analyses of growthpromoters. This training will be organised if a minimum number of 5 participants is reached.

11) Missions to NRLs and dissemination of scientific information.

Missions will be undertaken to specific NRLs on the basis of their individual needs, e.g. in order to discuss and evaluate the results of a proficiency test. Analytical support. The choice for 2016 and 2017 will be based on the current progress in the NRLs in the newer EU-Member States, and in consultation with the other EU-RLs for Residues.

12) Technical and scientific support to the Commission and other International organisations

Upon request, technical assistance will be given to the European Commission and its Offices and its related institutes like the Joint Research Centre (JRC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA).

Specific: EC/EU-RL related co-operation with International Bodies (e.g. AOAC international, Eurachem, Codex, CVMP, TAIEX, EMA, EFSA, JRCs, IRMM, FVO and IAEA) on method validation, analytical methodology, reference materials and performance quality criteria (communication, co-ordination, and harmonisation).

The process of revising this Decision will start in 2016. Assistance will be provided to the Commission and the NRLs will be requested to provide input.

13) Provision of standard substances

Provision of standard substances including storage, administration, documentation and shipment. When necessary and possible, selected compounds will be purchased or (custom) synthesised.

14) Analyses of official samples.

Samples submitted by EU Member states in case of dispute between Member States or in case of analytical problems within a responsible NRL will be analysed.

15) Organisation of annual workshop on residue analysis.

The topic will be selected on the basis of a consultation of the NRLs during the 2015 and 2016 workshop.

16) Documentation and information services

Developments with respect to analytical methodology, (EU) legislation and the results of relevant scientific studies are constantly monitored. In addition, information on the use of new compounds or alternative approaches to improve the growth of livestock will be collected and used as input for future studies. Communication about issues of interest for NRLs will be through the annual workshop and the EU-RL website.

Specific. The EU-RL-website is maintained. The EU-RL website will be maintained with continued efforts to further implement its use within the EU-NRL/RFL network.

The database with EURL literature inclusive scientific reports, will be maintained as a source of information for EURLs and NRLs .

Extension and promoting use of EU-RL web forum for information exchange.

The Reflection paper with respect to the (semi)-natural occurrence of group A compounds prepared for discussion with the NRLs and the Commission in 2014 has generated research questions and control strategy approaches. The objective of this review was to provide both the NRLs and the Commission with tools for evaluating results of the NRPs and to provide general guidelines for enforcement. In 2014 four working-groups were established with an important role in initiating new research and evaluating new (published and unpublished) scientific information. In 2015 and an extension of the reflection paper with regard to Bovine Somatotropine was prepared. The revision of the document, is scheduled for 2016, and will also include pro-hormones.

Operational objective 4: To ensure a sound and efficient management of the EURL funding cycle

17) Meeting 4 EURLs, EURLs for residues management

Participation in annual co-ordination meeting and general EU-RL-management activities.

18) Compilation of annual report and cost-statement

Annual reports and cost statements with respect to the 2015 contract period will be prepared before 1st of April, 2016 and for the 2016 contract period before 1st of April, 2017 .

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Products to be delivered

Activity		Product 2016	Product 2017
1	Maintenance or extension of existing analytical methods		
	Validation mulit-method for steroids (A3) in meat	December	
	Validation of a quantitative method for prednisolone, metabolites and precursosrs in liver and urine		June
	Extension of IGF-1 method with different forms		June
	Validation of confirmatory IRMS method for boldenone and nortestosterone in bovine urine	June	
	Validation of confirmatory IRMS method for boldenone and nortestosterone in porcine urine		June
	Extension rBST method using affimers		December
2	New analytical methods		
	Profiling method for thyreostats		June
	GC-Orbitrap MS for A3 steroids	December	
3	Development of tool for identification of injection sites.		
	Addition of picture materials in knowledges system for histology	October	
	New platform for on-site testing protoype	December	
	On site use of prototype and dissemination in memberstates		December
4	Studies to detect abuse of (semi-)natural hormones		
	Extending the scoop of the steroid esters in hair method	December	
	Extending the scoop of the steroid esters in serum method		March
5	Identification of new compounds		
	Preliminary research into use of fungus for preparation of reference compounds	December	
6	Maintenance of in-house QA/QC activities	April	April
7	Organisation of two proficiency tests		
	Proficiency test for steroid esters in serum/plasma	June	
	Proficiency test Group A3 in urine		January
	To be determined subject for PT		June
8	Production of incurred sample material		
	Animal experiment	October	October
9	An evaluation of the draft Annual National Residue Plans	June	June 2
10	Analytical support and training.		

	Training	November	November 2017
11	Missions to NRLs and dissemination of information	November	November
12	Technical assistance to the Commission and international organisations		
	Revision of Commission Decision 2002/657/EC	December	December
	Organisation Euroresidue conference VIII	May	
13	Provision of standard substances	ongoing	ongoing
14	Analyses of official samples in case of technical, problems		
15	Organisation of annual workshop	May	May
16	Documentation and information services	ongoing	ongoing
17	Meeting 4 EURLs, EURLs for residues management		
18	Compilation of annual report and cost-statement	April	April