Bundesamt für Verbraucherschutz und Lebensmittelsicherheit

European Union
Reference Laboratory
supported by

European Commission

EUROPEAN UNION REFERENCE LABORATORY FOR RESIDUES OF VETERINARY MEDICINES AND CONTAMINANTS IN FOOD OF ANIMAL ORIGIN

(Groups of substances: A5-B2a-B2b-B2e)

AT THE

FEDERAL OFFICE OF CONSUMER PROTECTION AND FOOD SAFETY (BVL)

BERLIN, GERMANY

WORK PROGRAMME PROPOSAL

2016 / 2017

I LEGAL FUNCTIONS AND DUTIES

The functions and duties of the European Union Reference Laboratories 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, 30.04.2004, pp. 1 – 141, corrected and republished in the Official Journal of the European Union L 191, 28.05.2004, pp. 1 - 52).

II WORK PROGRAMME FOR THE PERIOD JANUARY 2016 – DECEMBER 2017

ACTIVITIES

1. Meeting 4 EURLs

4 EURLs for residue management

As a consequence of the EURL evaluation, the Commission stated that EURLs with overlapping or similar responsibilities should agree upon their work more closely. The agreement with the Commission is also indispensable. For this reason at least one meeting of the 4 EURLs for residues and a representative of the European Commission is necessary per year.

Output: internal documents

(Contribution to operational objectives 1 and 4 of the Commission work programme)

2. EU/EURL-related EU and internal bodies; co-operation with international organisations

a) Ongoing tasks

Technical and scientific support will be provided to the Commission institutions DG SANTE, DG JRC (IRMM), EMA and EFSA. The cooperation with international organisations is an ongoing task and will be intensified as far as possible. At the moment the EURL is cooperating in ISO working groups for standardisation, in CEN working groups for standardisation, in the Codex Alimentarius Committees CCRVDF and CCMAS, supporting IAEA (training, method data base) and working in the CCQM working group OAWG of the CIPM.

b) “Special” task (Revision of Commission Decision 2002/657/EC)

At the meeting of the Expert Committee on Residues of Veterinary Medicinal Products in June 2015, the MS indicated that they considered a review of Commission Decision (CD) 2002/657/EC as necessary. Subsequently the EURLs were asked to support DG SANTE in this process. In September 2015 the EURLs carried out a survey among the NRLs on their view on required changes in this Decision. The evaluation of this survey will be done and the outcome will be discussed with the NRLs in order to start preparing a draft revision of the document.

Output: working documents

(Contribution to operational objectives 1 and 3 of the Commission work programme)

3. Reports, cost estimate, documentation

Several reports will be issued, e.g. reports on proficiency tests, evaluations of the NRCPs of the Member States, the technical and financial reports on the EURL working periods 2015, 2016 and 2017, reports on the EURL performance (performance indicators) as well as the cost estimate and work programme for 2018. Other reports will be provided upon request.

Output: reports as described above

(Contribution to operational objective 4 of the Commission work programme)

4. Validation of a multi-residue method for antiparasitics by LC-MS/MS

The substance group of endoparasiticides comprises the groups of anthelmintics and anticoccidials (B2a/B2b). The NRLs keep expressing a lot of interest in the use of substance-group-comprehensive methods, but at present only separate validated multi-residue methods for the respective groups exist at the EURL Berlin. By implementing new technical developments and possibilities, a substance-group-comprehensive method for the matrix muscle was developed and validated during the 2014/2015 work period.

This method shall be transferred and validated for two additional matrices (e.g. liver, milk) and, where required, also to new instruments. The validation of the adopted and optimised method will be performed and method descriptions will be provided.

Output: validated methods; validation reports; method descriptions

(Contribution to operational objective 1 of the Commission work programme)

5. Re-validation of the methods for nitroimidazoles in milk, plasma and muscle

The validated methods for the determination of nitroimidazoles in milk, plasma and muscle are, meanwhile, several years old and need to be revised, updated and transferred to new LC-MS systems. Optimisation potential for sample preparation is checked, the analyte list will be enhanced and lower decision limits should be achieved due to more sensitive instruments.

Output: validated methods; validation reports; lowered decision limits, method descriptions

(Contribution to operational objective 1 of the Commission work programme)

6. Collaborative study for the validation of a confirmatory method for the determination of basic and acidic NSAIDs in milk

In the 2015 work programme methods for the determination of acidic NSAIDs in plasma, basic NSAIDs in muscle as well as acidic and basic NSAIDs in milk were revalidated and transferred to a new and more sensitive mass spectrometer. The method for milk was completely revised and the substance spectrum was enhanced to basic and acidic compounds according to the results of the pre-tests for the development of a multi-method for basic and acidic NSAIDs (work programme 2015).

Due to the interest of several NRLs in the implementation of this method, in addition to the in-house validation, the milk method shall be validated in a collaborative study based on an orthogonal experimental design plan. The concept of this way of validation was developed by BVL and Quo data and was tested on the national level. It shall now be transferred to and evaluated on an international level.

Prior to this validation study an introduction into the method and the validation approach is planned in form of a one day-training for interested participants from the NRLs (limited number of participants). The expected benefit is multiple: for the participating NRLs a complete in-house validation study is performed, and for the method itself robust method performance characteristics are determined; this means that for residue control in the EU a contribution to a harmonised control in form of a comprehensive multi-method for NSAIDs is achieved.

Output: (in-house) validated methods in the participating NRLs; laboratory-comprehensive validated method; validation report; method description

(Contribution to operational objective 1 of the Commission work programme)

7. Pre-tests for the transfer of the multi-method approach for basic and acidic NSAIDs in milk to additional matrices

Following requests from NRLs for the development of a joint multi-method for basic and acidic NSAIDs in milk, this method was developed, finalised and validated in 2015. The transferability to additional matrices, e.g. plasma, of the approach with a joint sample preparation and measurement of basic and acidic NSAIDs is checked in pre-tests. The results of the pre-tests shall be used to decide whether a complete new method development is promising and a subsequent

validation is sensible. Depending on the outcome of the results, a new method shall be validated or the old method for basic NSAIDs in plasma shall be re-validated.

Output: decision whether or not to develop a joint multi-method; validation report; method description

(Contribution to operational objective 1 of the Commission work programme)

8. Re-validation of the methods for beta-agonists

The methods for the determination of beta-agonists were validated on an instrument which proved to be not sufficiently reliable. Hence the instrument was substituted in the end of 2014. Accordingly, the methods for the different relevant matrices had to be revalidated. In a first step, the methods for beta agonists in liver and urine were reviewed and analyte lists were enhanced if required. In a second step the methods for beta agonists in hair and muscle will be revalidated. Furthermore the methods shall be transferred to an additional highly sensitive LC-MS/MS system (different supplier). The transferability of the method to additional matrices (lung, gut) will be checked.

Output: validated methods; validation report; lowered decision limits; method descriptions

(Contribution to operational objective 1 of the Commission work programme)

9. Tests on the transferability of confirmatory methods to HRMS instruments

High-resolution mass-spectrometers (HRMS instruments like QTOF and Orbitrap) are increasingly used by the laboratories in addition to the classical LC-MS/MS instruments. Since these instruments are already being used in other areas, e.g. pesticides, the transferability to drug residue analysis shall be tested and will be checked for selected examples (e.g. beta-agonists) of the present confirmatory methods.

Output: validated methods; validation report; method descriptions

(Contribution to operational objective 1 of the Commission work programme)

10. Investigation of fundamental questions:

a) Influence of matrix components on signal intensity of different veterinary drugs by LC-MS/MS

b) Enzymatic hydrolysis of different incurred materials in stock

c) Use of calibration curve data as a QA tool for the improvement of the reliability of measurement results

a) In the course of the establishment of the LC-MSn technique, which has meanwhile become the most frequently applied technique in the field of residue analysis, the problem of matrix influences on the intensity of the signal is observed more and more often. The phenomenon is difficult to describe, and its causes have still not been entirely understood. The results of the studies carried out in 2014 showed that it looked promising to use two approaches for the estimation of potential matrix effects: a post-column infusion approach and the fortification of extracts of blank samples after the last step of the sample preparation procedure. These approaches were checked as part of selected validation studies in 2015, where the latter approach seemed to be the preferable one. Due to its simplicity and suitability for routine analysis, this approach will be followed in the planned validation studies. The resulting data will be summarised and evaluated. Recommendations for the implementation in validation routines will be given.

Output: summary report on results; recommendations for implementation in validation routines

b) Numerous substances are present in the animal's body in a metabolised form, mostly protein-bound or as glucuronide. It is not known for all substances to which extent they are present in a bound form in the tissue to be examined, and thus escape detection. For this reason the EURL Berlin carried out investigations of selected incurred materials from former animal studies. These

few tests showed that conjugated residues may play a significant role in different matrices and for different analytes. Discussions with NRLs showed that there is considerable uncertainty about how to deal with conjugated residues, e.g. in the evaluation of the compliance of MRL-compounds. Another aspect is the practicability of enzymatic digestion by means of enzymes like protease and glucuronidase/sulfatase in multi – substance group screening or confirmatory methods.

In order to enhance the data base for the evaluation of the relevance of bound residues, additional incurred materials will be checked, materials in stock as well as materials from planned animal studies. Discussions with the NRLs (and EURLs) will be continued with the aim to sensitise them to this problem, to provide possible solutions and to promote a harmonised approach of the laboratories.

Output: reports on results; position paper on how to deal with conjugated residues

c) Commission Decision 2002/657/EC requires a calibration curve for each analytical series. In most cases the information from these calibrations is used only within the respective analytical series, but not, e. g. in the form of control charts, in order to implement preventive measures before questionable results are produced. A new control chart for the recording of quality control data was developed and tested. The approach proved to be suitable in the tested examples. The data collection will be continued and evaluated, and additional studies under simulated conditions will be conducted in order to provide more substantiated information on the potential of this approach.

Output: summary of results; template of a control chart; recommendations for QA measures

(Contribution to operational objective 1 of the Commission work programme)

11. Testing and evaluation of appropriate procedures for the development of a draft guideline for purity testing

Accreditation bodies require the traceability of measurements to SI units, hence also the knowledge of the purity of standard substances. This knowledge is a prerequisite for reliable testing as well as for a correct estimation of the measurement uncertainty. Experience showed that the values indicated on the bottles or certificates of commercial providers are not always correct. Thus the EURL Berlin decided to start a project on purity testing of selected standard substances. Criteria for the selection were: particularly important substances, substances often used but for which no purity is given, substances for which irregularities have been observed, as well as isotopically labelled compounds (deuterated, 13C-labelled, 15N-labelled), in particular of forbidden compounds like ß-agonists, where unlabelled impurities may generate false positive results.

For purity testing new types of instruments were to be purchased and established, like HCN analyser and Karl-Fischer-titration. Recently a TGA (coupled to GC/MS) and an ELSD coupled to a QTOF-MS have been put into operation. In addition classical chromatographic techniques are applied for the characterisation of organic impurities. The purity figures are and will be spread among the relevant NRLs and routine laboratories together with the respective standard substances to support their QA systems and to enhance the reliability of measurements.

Based on the experiences started in 2013 and still running, possible general approaches on how to do these highly labour-intensive purity studies systematically and efficiently, were developed and tested. The studies are to be continued, and a draft guideline for purity testing as a basis for discussions with the EURLs and NRLs will be produced.

Output: report(s) on purity; draft guideline for purity testing

(Contribution to operational objective 1 of the Commission work programme)

12. Follow-up on treatment of hens with nitroimidazoles: testing of eggs and additional matrices with respect to the post-treatment detectability of residues

In 2015 laying hens were treated with nitroimidazoles to produce residues in egg. During the study also additional materials (feathers, preen oil samples) were collected. Furthermore, egg samples from different withdrawal periods of the medication were taken.

The additional materials will be analysed in order to estimate how long after the end of medication residues may be detected, and to contribute to the clarification of the uptake mechanism of residues in feathers.

Output: scientific paper; recommendations for residue control of nitroimidazoles

(Contribution to operational objective 1 of the Commission work programme)

13. Stability studies for all substance groups

The stability testing of analytes in solution and in matrix is required by CD 2002/657/EC. It was agreed that it is not necessary for each individual laboratory to carry out these investigations themselves, but that they can use stability data provided by the EURLs. Therefore and for the production of proficiency test material and in-house reference material as well as for the EURL's own needs, stability studies are and will be carried out for all analytes we are responsible for in several incurred matrices and in solutions. For the improved evaluation of stability data an Excel-based template will be developed and tested.

Output: after the respective time period (in general after one year), a report on the detected stability of the analyte/matrix sample is issued; Excel-based template

(Contribution to operational objective 1 and 2 of the Commission work programme)

14. Research and identification of unknown compounds

It is an ongoing task to investigate possible new veterinary drugs, their metabolisation or degradation products as well as adequate internal – preferably isotopically labelled – standards.

Output: cooperation with synthesis laboratories, synthesis of new standards and/or literature reviews on new substances

(Contribution to operational objective 1 of the Commission work programme)

15. Proficiency tests on beta-agonists in liver, nitroimidazoles in eggs, NSAIDs in milk and coccidiostats in muscle

Comprehensive reports on the 2015 proficiency tests will be finalised. The following new proficiency tests (depending on the availability of appropriate material with sufficient concentration levels) are planned:

For 2016:
- a proficiency test on beta-agonists in liver
- a proficiency test on nitroimidazoles in egg

For 2017:
- a proficiency test on NSAIDs in milk (follow-up on PT 2015 / laboratory-comprehensive validation)
- a proficiency test on coccidiostats in sheep/goat in muscle or liver

It is planned to produce matrix samples with different analytes spread over 3 to 4 samples (including a blank sample).

Furthermore it will be checked whether the traceability to SI units can be provided to the participants of the PTs for single analytes/methods based on the CMC (calibration and measurement capabilities) entries of the EURL Berlin.

Output: final reports on the 2015 and 2016 PTs, short reports on proficiency tests 2016 and 2017, assessment of the performance of the NRLs, assignment of values to the reference materials

(Contribution to operational objective 2 of the Commission work programme)

16. Participation in PTs by commercial providers

In order to document and to prove our proficiency not only in the framework of our own proficiency tests and in order to fulfil the requirements of EA and of the German accreditation body (DAkkS), it is necessary to participate in commercially offered PTs, as well. Furthermore, this way, PT providers can be checked for quality. Participation depends on the range of PTs offered by commercial providers. So far the programmes for 2016/17 have been published only in parts, so that we cannot state yet in how many and in which PTs we will participate.

Output: certificates by the PT providers

(Contribution to operational objective 1 of the Commission work programme)

17. Production of incurred "raw" sample material – animal studies with cattle, minor species and horse

On the basis of the following animal experiments, different incurred matrix materials shall be produced (exclusively production, animal treatment, matrix collection, pre-testing, storage):

- Cows will be treated with NSAIDs and nitroimidazoles to produce residues in milk.
- In addition cows will be treated with beta-agonists in order to produce incurred hair samples.
- Minor species (sheep or goat) will be treated with coccidiostats to produce residues in muscle and liver. The animals will be slaughtered and incurred materials, e.g. muscle samples, will be collected.
- A horse will be treated with one or two NSAIDs to produce residues in plasma and muscle. The animal will be slaughtered and incurred materials, e.g. muscle samples, will be collected.
- Aquaculture (trouts) will be treated with avermectins, and, if feasible, also with a nitroimidazole. The substances are not authorized for aquaculture. Muscle/skin reference material shall be produced.

These materials are the basis for the future production of new reference materials in order to organise proficiency tests and to provide incurred material for scientific purposes (internally and to the NRLs).

Output: pre-tested incurred matrix material for proficiency tests and for scientific purposes

(Contribution to operational objectives 1 and 2 of the Commission work programme)

18. Production of reference materials from "raw" incurred sample material

Reference materials for the proficiency tests in the 2016/17 working period will be produced. The production covers the following steps: dilution of the material if necessary, homogenisation of the material, aliquotation and packaging of test portions; tests on homogeneity and stability (short-term and mid-term). The following materials will be produced and characterised:

- Beta-agonists in liver (the old material has been distributed to the NRLs and RFLs for QA-purposes and is meanwhile out of stock)
- Additional materials for nitroimidazoles in eggs
- NSAIDs in milk
- Coccidiostats in muscle or liver

Depending on the required efforts to transfer the existing methods for beta-agonists to the matrices lung and gut, also for these matrices reference materials may be produced.

Output: reference materials for beta-agonists in liver, NSAIDs in milk, nitroimidazoles in egg and coccidiostats in muscle or liver as support to NRLs/RFLs and for scientific purposes

(Contribution to operational objective 2 of the Commission work programme)

19. Technical, scientific support and training

Technical and scientific support and training will be provided on request to NRLs and official routine laboratories as well as to official laboratories of Third Countries. The support via internet (FIS-VL), where all relevant information is available on validated methods, standard substances, reference materials, reports and many more, will be continued. E-mail and telephone support will be provided.

Output: provision of reference materials and of information on analytical methods; scientific support via e-mail or telephone; training courses

(Contribution to operational objective 1 of the Commission work programme)

20. Follow-up of PT

Follow-up measures will be carried out if necessary in compliance with the Commission draft "Protocol for management of underperformance […]" guideline of 2007. An overview of the performances per laboratory and MS in the past few years was established in 2013/2014 and is up-dated regularly.

Output: certificates on successful participation; questionnaire of the EURL sent to all participants, asking the failing laboratories what kind of support they need (substances, materials, methods, training); provision of additional PT test material on request; preparation of test material for bi-/tri-/x-lateral comparisons on request (as alternative to comprehensive PTs); diagrams on the trend and development of the performance of the NRLs; report to COM on underperformance (if applicable)

(Contribution to operational objective 2 of the Commission work programme)

21. QA measures, provision of standard substances incl. procuring, storage, administration, documentation, shipment

The QM system according to ISO 17025 is continuously maintained, developed further and extended to an accreditation as proficiency test provider according to ISO 17043. Costs (including fees for annual visits of the accreditation body) are not explicitly included.

As a service to official control laboratories, small amounts of standard substances will be provided on request.

Output: certificates on accreditation; provision of standard substances and corresponding certificates and material safety data sheets; annually updated substance lists and lists of suppliers

(Contribution to operational objectives 1, 2 and 3 of the Commission work programme)

22. Analysis of official samples

Official samples will be analysed on request in case of disputes between MS.

Output: analytical reports and definite results

(Contribution to operational objective 1 of the Commission work programme)

23. Visit to NRLs and NRLs of Third Countries

a) In general one to two European MS NRLs per year are visited after consultation with the Commission on necessity. Scientific information and technical support in the form of methods, SOPs etc. and/or a specific training (practical or theoretical) will be provided, and specific problems like QA, QC, validation, legislation etc. will be discussed.

b) The Veterinary Public Health Laboratory (VPHL), Bureau of Quality Control of Livestock Products (BQCLP) and Department of Livestock Development (DLD) in Thailand has been

appointed as ASEAN Food Reference Laboratory for Veterinary Drug Residues and regularly organises trainings/workshop to ASEAN MS (in the same way as the EURLs do). In earlier years these activities were supported by the EU, but are meanwhile organised by the ASEAN RL itself with the budget from the Royal Thai Government. The VPHL asked the EURL Berlin for possibilities of support. It will be supported on request by a visit to their workshop in order to transfer knowledge and to promote scientific exchange. Scientific information and technical support in the form of methods, SOPs etc. and/or a specific training (practical or theoretical) will be provided, and specific problems like QA, QC, validation, legislation etc. will be discussed.

Output: support of the respective NRL, support of the ASEAN-RL network (lectures, training), reports on the visits

(Contribution to operational objective 1 of the Commission work programme)

24. Organisation and performance of a workshop

Annual EURL-workshops will be organised in 2016 and 2017. The following subjects (among others) will be covered:

- Discussions on necessary changes to Decision 2002/657/EC
- Validation of multi-residue/multi-substance-group methods
- New instruments and method developments
- Importance of conjugated residues
- Evaluation of Proficiency Tests and follow-ups
- Stability testing in matrix and in solution
- QA-measures
- NRCP evaluation and information on substance groups
- Presentations by the NRLs
- Practical Training
- Suggestions of NRLs (collected in surveys conducted at the end of the workshops, or specific topics asked for in additional queries)

The evaluation of the annual EURL work programme as well as the forthcoming work programme will be treated and further specific questions will be discussed depending on the needs of the participants.

Output: several days' workshop

(Contribution to operational objectives 1 and 2 of the Commission work programme)