WORK PROGRAMME OF THE
EUROPEAN UNION REFERENCE LABORATORY FOR
CHEMICAL ELEMENTS IN FOOD OF ANIMAL ORIGIN
AT THE
ISTITUTO SUPERIORE DI SANITÀ
ROME, ITALY
LEGAL FUNCTIONS AND DUTIES

1. OBJECTIVES FOR THE PERIOD 01 JANUARY 2014 - 31 DECEMBER 2014

A. General tasks
   Article 32, paragraph 1 (e)
B. Development and validation of analytical methods
   Article 32, paragraph 1 (a, c)
C. Quality Assurance and Quality Control, including the organisation of proficiency tests and the development of test material.
   Article 32, paragraph 1 (b)
D. Technical and scientific support to NRLs and Third countries
   Article 32, paragraph 1 (a, d, e, f)

2. WORKING PLAN FOR THE PERIOD 01 JANUARY 2014 - 31 DECEMBER 2014

A. General Tasks
   Article 32, paragraph 1 (e)

1. Meeting of all EURLs
   An EURL-CEFAO representative will participate in the coordination meetings of the EURLs.

   Objective: participation in at least 1 meeting of EURLs.

   Expected Output: sharing of the outcome with EURL staff to meet the European Commission expectations and suggestions.

2. Technical and scientific support to the Commission, co-operation with international organisations and participation in international congresses
   The EURL-CEFAO is available to offer its professional expertise to provide technical and scientific support to the European Commission and related Institutes upon request.
   The EURL is also available to co-operate with international organisations.
   The EURL carefully selects the scientific conferences to attend either as speaker or simply as a participant considering the interest and pertinence of the topics, since participation, not being a routine activity, can be considered really useful only if these topics are strictly connected with the activity of the EURL or can provide new issues for the future work. Therefore the EURL is willing to participate in at least one international congress.

   Objective: evaluation of the National Residues Control Plans (NRCP) of the EU Member States to be performed in due time. The assessment will take into consideration the scope of testing, the pertinence of analytical methodologies, the fulfilment of the requirements regarding relevant regulations, the suitability and adequacy of the methods adopted by each Member State. As for the evaluation it will also contemplate whether the scope of the testing is exhaustive within the relevant
substance group. According to the evaluation of the NCRPs, the EURL CEFAO will give specific suggestions, when necessary. Spread of the EURL activity and up-dating of topics of interest getting in touch with experts of other countries by participating in international congresses.

**Expected Output:** editing of the Report regarding the evaluation of NCRPs and dispatch to the European Commission. Providing information for any other request (e-mail/letters/documents) by European Commission related to the Institutes and International Organizations within the EURL CEFAO’s duties. Presentation of at least one lecture or poster in an international congress.

### 3. Compilation of annual reports
The reports on the activities carried out for the relevant contract period will be regularly issued both for EC and NRLs.

**Objective:** Drawing up of Reports for European Commission within the due date.

**Expected Output:** Releasing of: Interim Annual Report, Final Activity Report, reports on Workshop and NRLs visits to constantly inform the EC regarding the EURL-CEFAO activity.

### 4. Documentation services, interchange of information
As usual, the EURL-CEFAO website will implement and maintain the possibility to exchange information and update the documentation services. In the EURL web site a section dedicated to the EURL-CEFAO publications will be included so that the EC and NRLs can obtain a more detailed and thorough understanding of the activity of the EURL. Upon request, information on EU legislation and analytical support will be given to NRLs and to Official Laboratories of Third Countries. The collaboration to reach an agreement on common strategies with the EURL for Feed and Food (Geel) and interchange of information with the EU Reference Laboratory for pesticides requiring Single Residues Methods (EURL-SRM) will be continued.

**Objective:** Continuous updating of the restricted area regarding indicators related to the performance of NRLs in PTs; reports of workshop, analytical methods, EU legislation.

**Expected Output:** Reports related to PTs; issuing of updated z-scores Control Charts of each NRL; updating of Handbook of Analytical Methods of the NRLs.; list of Official Methods and bibliography for the network.

### B. Development and Validation of Analytical Methods

*Article 32, paragraph 1 (a, c)*

### 5. Analytical methods
The analytical activity of the EURL-CEFAO includes both the development of new analytical methods and the maintenance of those that are accredited/validated. As for new analytical methods, the development of procedures easy-to-use is one of the major EURL’s goals. In fact, the possibility of distributing new methods, developed using several analytical techniques that can be used by the NRLs, is an aspect of the strategy followed during the activity planning. More specifically, the studies of elements speciation are considered of particular difficulty, interest and relevance especially when procedures easy to apply are required.
5.1 Maintenance of analytical methods
The EURL-CEFAO is accredited according to ISO/IEC 17025:2005; its accreditation status has then been extended obtaining the flexible scope. Therefore, now the EURL can have accredited methods without submitting them in advance to the accreditation body. The flexible scope is often used by the EURL-CEFAO for the assessment of the sufficient homogeneity of the PT sample, when new matrices/elements are proposed to NRLs. Even if ISO/IEC 17043 does not impose the use of an accredited method for this scope, the EURL considers that the application of accredited methods can better qualify the PT scheme.

The maintenance of the accredited methods (included in the fixed and flexible scope) entails an important analytical activity including the updating/revision and validation assessment of these methods, when necessary.

**Objective:** evidence that the performance of the accredited analytical methods are steady.

**Expected Output:** confirmation of all methods included in the scopes of accreditation by the Accreditation Body.

5.2 Development of analytical methods

The determination of Mercury (Hg) by means of techniques that require sample treatment (e.g. Cold Vapour Atomic Absorption Spectroscopy CV-AAS, Hydride Generation Atomic Absorption Spectrometry HG-AAS) can imply some analytical difficulties when the concentration value of Hg in the matrices of interest is low or extremely low. This drawback can be overcome using a technique that does not involve any sample treatment. The Direct Mercury Analyzer (DMA) is a technique exclusively dedicated to the analysis of Hg with no need of sample treatment (i.e. microwave assisted digestion) and requiring very low amounts of sample to be weighed (usually, roughly ≤0.100 g). Therefore, it can also be retained as the best method to assess the material homogeneity at very low levels of Hg such as it can occur at natural levels of the element in some matrices. In fact, in this specific case the inhomogeneity becomes more evident since with this technique low amounts of sample undergo the analysis.

All this considered, a method based on DMA will be developed and validated using the flexible scope so as to be applied when PTs consist in preparation of material with basal content of Hg since the use of a method that requires a little quantity of sample to be weighed allows to be more confident of the material homogeneity.

Furthermore, the method developed by the EURL will be distributed to NRLs as a guideline containing the instrumental settings and the main validation parameters.

On the basis of the planned PTs 2014, in order to assess the sufficient homogeneity of PT test items, two methods for the determination of Copper (Cu) and Hg in frozen offal will be validate applying the flexible scope.

Two methods for the determination of Arsenic (As), Cadmium (Cd) and Lead (Pb) in lyophilised mussels will be developed and validated by means of both Inducted Coupled Plasma Mass Spectrometry (ICP-MS) and Atomic Absorption Spectroscopy (AAS) based techniques. As for the latter, the method is practically the sum of three methods, being AAS a monoelemental technique, and so a specific method for each analyte has to be developed.

Considering the recent “Scientific Opinion on Dietary Reference Values for molybdenum” by EFSA (EFSA Journal 2013; 11(8):333) in which the need to set Dietary Reference Values for these elements and the unavailability of data on molybdenum intakes and health outcomes is evidenced, a method on the determination of molybdenum in food matrices will be developed by EURL-CEFAO.
Since amounts of this element are present in nearly all foods, to develop and make an analytical method available for its quantification has been considered helpful to face this topic.

As for the speciation of inorganic arsenic in mussels by means of High Performance Liquid Chromatography-Inducted Coupled Plasma Mass Spectrometry (HPLC-ICP-MS), the work already on-going will be continued. At the state of the art, the method suffers from poor precision because of the complexity of both the extraction procedure and the chromatographic separation. The new step of this study will be focused on obtaining a satisfactory repeatability and reproducibility in order to start a complete validation.

Objective: development of analytical methods according to internal validation fulfilling the requirements related to their scope. Continuation of on-going study on inorganic arsenic speciation.

Expected Output: set up of 5 methods fit for specific purpose. Three of them (As, Cd and Pb in mussels by ICP-MS; As, Cd and Pb in mussels by AAS-based techniques; Hg by DMA) will also be distributed among the NRLs network as guidelines.

C. Quality Assurance and Quality Control, including the organisation and implementation of proficiency tests

Article 32, paragraph 1 (b)

6. Maintenance of the QA/QC system
The EURL-CEFAO is accredited according to EN ISO/IEC 17025:2005, with the extension of the flexible scope, and according to ISO/IEC 17043:2010 as Proficiency Test (PT) Provider. The maintenance of the accreditation status, according to all the above mentioned regulations, implies the carrying out of analytical and documental activity requested by the QA/QC systems. To this end, the EURL-CEFAO participates in at least one commercial Proficiency Test in order to monitor and document the Quality Control. The choice and number of PTs depends on the availability and pertinence of analytes and matrices offered by commercial PT providers. Moreover, a surveillance audit by the Italian Accreditation Body ACCREDIA, to check the maintenance of each accreditation status, is regularly carried out.


Expected Output: maintenance of the accreditation status and successful outcome from the surveillance audits by the Accreditation Body.

7. Proficiency test
The activity of the EURL-CEFAO as PT Provider is mainly addressed to support the NRLs activity organizing exercises based on matrices and elements of their interest and that can be of help in their routine work and/or in facing emerging problems. Throughout the PTs an exercise on offal, in particular on liver, was proposed twice: 2008, in lyophilised state and 2011, in frozen state. The state of this latter was more similar to the routine sample allowing the laboratories to really practise on this matrix that is often considered in the NRCPs. The choice of producing offal in frozen state was very much appreciated by the NRLs and several laboratories requested the EURL to organise another PT on this matrix. Therefore the EURL-CEFAO has planned to carry out an exercise on the determination of Cd, Cu; Pb and Hg in frozen offal. As for the selection of the analytes, Cd and Pb were included in order to enable the laboratories
to check or improve the performance of their methods. Moreover, being the MLs for Cd and Pb set by the Commission Regulation (EC) No 1881:2006 and following amendments, NRLs shall have accredited methods for these determinations. All this considered, the participation in PTs is a useful tool, highly considered by the accreditation bodies, to show the fitness for purpose of the methods. The inclusion of Cu and Hg is not only strictly connected to the strategy of proposing new matrix/analytes combinations but also to what was evidenced during the evaluation of NRCPs. In fact, the 2013 NRCPs highlighted that the number of the analyses performed to quantify Cu and Hg in offal has increased. Furthermore, the interest of the network in the determination of Hg in matrices different from fish was demonstrated through the high number of NRLs that gave their results in the exercise carried out during the 14th PT (first round) and 18th PT, both on meat.

As for Cu, this element was proposed for the first time in meat (18th PT) and almost all laboratories submitted the relevant results. Therefore, taking into account the success of the combination meat/Cu, new to the network, the EURL-CEFAO decided to give the participants the possibility to exercise their methods for Cu in another matrix and to confirm their performances.

As the Commission Regulation (EC) No 1881:2006 and followings amendments, sets Maximum Levels (MLs) for Cd and Pb in mussels, the availability of a PT on this matrix/analytes combination is of particular interest. Based on both the shortcoming in commercial exercises and the requests made by the NRLs, the EURL-CEFAO has planned to organize the 21st PT on this matrix. As for the analytes, As and Hg will be considered together with Cd and Pb, as they are analytes of sure interest for NRLs.

The compliance of the sample for these two last elements (Cd, Pb) will be included in the PT. For both PTs NRLs will be requested to use their routine methods if in place. However, the EURL will provide them with three analytical procedures for the determination of As, Hg, Cd and Pb. The choice of preparing the material for the 21st PT in freeze-dried state allows the EURL-CEFAO to produce extra samples to be distributed to the NRLs for their internal scopes as well.

As for the other factors in the PT schemes, the sufficient homogeneity will be assessed according to ISO 13538:2005; the z-scores will be calculated using the EURLxp, as for the assigned value the internal procedure “Statistical Elaboration and Reporting of Results” could be improved by adding the possibility of setting the assigned value using the approach of “Consensus value from expert laboratories”.

This approach could be necessary when the difficulty of the exercise is high and the use of all laboratories’ data could lead towards a biased consensus value. This will imply the setting of several criteria such as the assessment of the expert laboratories, when to use this approach and how to perform it.

Details on procedures for sample preparation and measurement parameters used by NRLs will be recorded in the “Results Form” especially designed for this purpose. About forty days after the deadline of the PT, a “Short Report”, containing a summary statistics, the results and the z-scores, will be sent to the participants and published in the Restricted Area of the website. The “Final Report” including several issues (e.g. rationale of the round, appropriate statistics, homogeneity and stability data, comments) will be sent afterwards and it will replace the Short Report in the website. The Final Report will also be sent to the European Commission and to the NRLs not participating in that round.

**Objective:** Checking the network performance by means of a more complex matrix (mussels) and new elements/matrix combinations. Monitoring of performance on Cd and Pb in offal previously analysed in other EURL PTs.

**Expected Output:** from EURL point of view, the issuing of 2 Short reports within about forty days after the deadline of PT and issuing of 2 Final Reports including analytical and statistical comments, and the comparison with performance in previous PTs, when appropriate.
From NRLs point of view, the maintenance of the network performance in terms of z-score for consolidated analytes (Cd, Pb) and achievement of satisfactory z-scores for the new analytes/matrix combination.

8. Production of materials for PTs
Materials will be prepared in the EURL-CEFAO laboratories; only special processes such as lyophilisation and sterilization will be performed by accredited suppliers of services. As far as the production of frozen offal samples is concerned, the procedure, previously studied and applied by the EURL-CEFAO in other PTs, will be implemented and/or improved, if appropriate. The stability and homogeneity of Cd and Pb in this matrix is well known, whilst, for Cu and Hg, preliminary studies will be conducted. As for mussels, the starting material will be a sample available on the market. Since the basal content of the element of interest is strictly connected to the mussels origin and the process of production they underwent, the research of the suitable sample will be conducted by analysing many different products. The final concentration levels in the freeze-dried material will also be evaluated producing a preliminary batch on which studies to verify the homogeneity and stability of the material will be conducted.

Objective: production of stable and homogenous materials containing the elements of interest.

Expected Output: achievement of suitable materials such as not to cause inconveniences or non-conformities to the PT scheme.

D. Technical and Scientific Support to NRLs and Third Countries

Article 32, section 1 (a, d, e, f)

9. Analytical support and training
The EURL-CEFAO considers that monitoring the performance of NRLs through the control charts of z-scores assigned in the EURL-PTs is very helpful. For this reason the charts are updated and made available to NRLs after each PT. In this way the identification of laboratories that need analytical support becomes easier. The laboratory is contacted by telephone or e-mail, and a specific exercise is organised if convenient. During the “Annual Workshop of NRLs” a technical training is conducted by the EURL and/or international experts. The EURL gives its availability to welcome the representatives of the NRLs in its laboratories and, upon request, to organize training courses for them. In the same way, the EURL can also organize training courses for Official Laboratories of Candidates Member States and Third Countries, always upon request. Official analyses are performed, if necessary.

Objective: meet the needs of NRLs also by means of the organization of ad hoc training and providing proper analytical support.

Expected Output: general satisfaction through the questionnaire dedicated to participants.

10. Provision of reference materials
In producing the PT test items the EURL-CEFAO applies procedures allowing to obtain the highest number of samples without an excessive increase of expenses. In fact, the lyophilisation process has a fixed cost that is not directly connected to the amount of material obtained. Moreover, the EURL-CEFAO gained experience in treatment of different kinds of fresh samples reducing at minimum the quantity of waste material. All this considered, the
production of extra samples implies a low cost considering that NRLs will be provided with a stable reference material. So it will be possible to have some extra samples of mussels to distribute to NRLs. They will be available upon request of the laboratories to be used as reference material.

**Objective:** provision of extra samples of mussels useful for the NRLs analytical activity.

**Expected Output:** use of material by NRLs for their internal scopes.

11. **Visits to NRLs and international mission for scientific information**

The EURL-CEFAO selects the laboratories to be visited considering several aspects of their activity such as the results of the PTs, the evaluation of the control charts showing underperformance and analytical problems. They can be chosen also among those that have changed the analytical techniques and may need technical support.

Two NRLs are usually visited and each visit is organized to be as profitable as possible. During the visit analytical problems are discussed and the NRLs may be requested to prepare specific samples to be analysed. The EURL staff assists and trains the laboratory staff during the analysis giving them practical advice.

**Objective:** visits that may be useful for NRLs from a practical point of view and to ease up the relationship with the laboratories as well.

**Expected Output:** general satisfaction evidenced by the questionnaire dedicated to NRLs and by improvement of laboratory’s analytical performance.

12. **Organisation of the workshop.**

An “Annual NRLs–EURL Workshop” will be organised and specific topics of common interest will be proposed. The issues will also be based on outcome from previous workshops. The EURL-CEFAO representatives will show the outcome of the PTs carried out adding analytical comments on the difficulty or underperformance of the laboratories. Experts will be invited to present topics of common interest and/or to train the participants. The EURL-CEFAO will try to find ways of improving the interaction among the participants.

**Objective:** to organize a workshop with topics of interest for the NRLs. To create occasions to exchange ideas and points of view among participants.

**Expected Output:** general satisfaction through the questionnaire dedicated to NRLs and participation of the representatives of all Member States.