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Organisation of proficiency testing and other activities of the European Union Reference Laboratory for chemical elements in food of animal origin (EURL-CEFAO)

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Abstract

The EURL-CEFAO, hosted by the Istituto Superiore di Sanità of Rome (ISS), is one of the European Union Reference Laboratories (EURLs) designated by Council Directive 96/23/EC responsible for chemical elements (group B3c) in food of animal origin.

The tasks of EURLs are listed in Article 32 of Regulation 882/2004. In particular, the EURL-CEFAO focuses its activity on preparation of *ad hoc* reference materials for inter-laboratory comparisons for its network of National Reference Laboratories (NRLs) and validation of easy-to-use methods based on the most widely used techniques in its field of competence. In addition, other activities include organisation of a yearly workshop, assistance to EU-NRLs, and organisation of training courses for NRLs of candidate EU Member States and non-EU countries.

Keywords

- Chemical elements
- European Union
- Food
- Laboratory proficiency testing
- Organisation

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Introduction

In order to ensure the safety of food products and guarantee public health, the European Commission has nominated European Union Reference Laboratories (EURLs) to contribute to the standardisation of analytical methods and the harmonisation of performance among the EU Member State National Reference Laboratories (NRLs). In this way, analytical data should have the same level of quality and reliability in all member states. The tasks of both the EURLs and the NRLs are listed in Regulation (EC) No 882/2004 of the European Parliament and of the Council "on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules". In particular, Article 32 of this Regulation defines the EURLs' general tasks that are mainly focused on technical support to the European Commission in their field of competence and assistance to the NRLs. Therefore, the EURLs act as an interface between the European Commission and the NRLs.

The European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO) is housed in the Department of Veterinary Public Health and Food Safety (ISS) and includes four researchers and three technicians. It is responsible for the substances listed in Annex I, Group B3(c) in Council Directive 96/23/EC of 29 April 1996, *i.e.* Chemical Elements in Food of Animal Origin. The presence of chemical elements in these matrices is not the result of intentional use for animal treatments, but is rather related to environmental contamination as well as contamination of feed and dietary supplements used in animal feeding. The inclusion of these elements in a European Commission Directive regarding residues in live animals and animal products was mainly due to the fact that some legal limits for these substances, considered potentially toxic in humans, had already been established in order to guarantee maximum protection of EU consumers.

The EURL-CEFAO acts to ensure that the National Reference Laboratories belonging to its network uniformly apply Regulation (EC) No 1881/2006 and subsequent amendments, which set maximum levels (MLs) for certain chemical elements in foodstuffs. Close interactions

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between the EURL and the NRLs are a key to improving consistent and homogeneous application, especially since the main task of the NRLs is to increase the quality, accuracy and comparability of the results produced by official control laboratories.

In compliance with the duties listed in Article 32, the EURL-CEFAO carries out a wide range of different activities, but over the years it has focused its work on: method validation based on the most widely used techniques in its field of competence, organisation of proficiency testing (PT) intended for NRLs, implementation of follow-up actions for under-performing laboratories, assistance to the EC and NRLs, and organisation of workshops and training courses. Some aspects of these activities are detailed in this article, pointing out their relevance in food safety policy.

Accreditation Status

The EURL-CEFAO has developed its own quality system on the basis of which it has been accredited as a testing laboratory according to ISO/IEC 17025 since 2005. In order to improve the qualification of its work and promptly face the needs arising from the update of regulations relevant to MLs for food of animal origin, the EURL-CEFAO applied for and obtained the flexible scope of accreditation. Furthermore, the laboratory has been accredited as a proficiency testing provider (PTP) according to international standards since 2010. In particular, the first accreditation as PTP was in accordance with ISO Guide 43-1 and subsequently, on 9 January 2012, the EURL-CEFAO was the second laboratory in Italy to be accredited according to ISO/IEC 17043:2010. This status enables the EURL-CEFAO to provide participants with exercises of recognised high quality.

All the accreditation processes were completed by the Swedish Board of Accreditation (Swedac) but, as a consequence of Regulation (EC) No 765/2008 of the European Parliament (Articles 4 and 39), in July 2012, the EURL-CEFAO successfully transitioned to the national accreditation body ACCREDIA for both ISO 17025 and ISO 17043.

Coordination of the NRL network

The EURL-CEFAO manages a network of 28 NRLs designated by the competent authorities of the relevant EU Member States.

An important tool to coordinate the network and to share information among the laboratories is the EURL website (**http://www.iss.it/lcdr/**). It consists of two sections: an open-access area and a restricted area where it is possible to access confidential information (*e.g.* outcomes and follow-up of PTs, handbooks of NRL analytical methods, etc.). As of 2016, a specific section will be included in the restricted area to better manage proficiency testing. In fact, this new dedicated section will enable the NRLs to confirm their participation in the PT and directly upload their results to the website.

One of an EURL's duties is to organise an annual workshop. The EURL-CEFAO pays special attention to this meeting because it is an important opportunity to share technical experience and to disseminate information regarding analytical methods and PTs. Furthermore, during the workshop a representative of the European Commission provides updates on changes in regulations.

In addition to the NRL network, several laboratories from other countries such as Brazil, Denmark, FYROM, Luxembourg, Norway and Russia participate in the inter-laboratory compari-

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son and some of them attend the annual workshop.

This is evidence of the increasing interest of many countries in the activities of the EURL-CEFAO.

Cooperation with other EURLs and organisations

The EURL-CEFAO has been collaborating with the EURL for Heavy Metals in feed and food (EURL-HM) for many years. The objective of this collaboration is to exchange views and proposals about topics of interest to NRLs operating in the field of chemical elements in food. Furthermore, the EURL-CEFAO is in contact with the EURL for Single Residue Methods (EURL-SRM) located in Stuttgart, Germany operating in the field of pesticide residues. In particular, cooperation is focused on organising proficiency tests on the determination of mercury and copper in meat.

The EURL-CEFAO's director is also a member of the EURACHEM PT working group. Moreover, the EURL's experts provide their technical opinion to the Italian Ministry of Health so as to support the Italian position when the revision of maximum levels for some matrix/element combinations is discussed at the European Commission level.

Activity as a proficiency testing provider

The EURLs play a key role in guaranteeing that analytical results produced by the NRLs are as reliable and uniform as possible. The EURL-CEFAO is aware of how valuable PTs are in checking and harmonising laboratory performance. To this end, the laboratory has invested significant energy and resources in the organisation of inter-laboratory comparisons in order to reach the required quality and uniformity of analytical results produced within the European Union.

In particular, to better qualify this activity, the laboratory has been accredited as a PTP since 2010 and over the years it has developed and optimised many procedures to produce materials suitable for its tests. Except for a few specific processes (*i.e.* sterilisation and lyophilisation) performed by qualified suppliers, all steps of PT sample preparation are carried out by the EURL staff in the laboratory's facilities. Furthermore, EURL staff performs the statistical evaluation of the participants' results under a scheme based on the consensus approach. Therefore, thorough knowledge of every aspect of PT organisation allows the EURL-CEFAO to know the drawbacks of each phase and the best ways to overcome them.

As far as the programme is concerned, the number of PTs to be conducted each year as well as the matrix/analyte combinations on which they are based are to be set in the annual work-programme submitted by the EURL to the European Commission. In this schedule, both the outcome of the previous rounds and the specific needs of NRLs are carefully evaluated.

The PT material is usually prepared starting from commonly consumed food of animal origin purchased at retail stores. The concentration levels of analytes of interest (*e.g.* As, Cd, Pb, Hg, Mo, Cu) are often adjusted so as to be around the MLs set in the relevant regulations. As for the physical state of the matrix, considerable efforts have been made over the years to distribute fresh test items (*e.g.* frozen meat, frozen fish, frozen offal) or liquid test items (*e.g.* milk) [Ciprotti *et al.*, 2013] instead of freeze-dried samples [Colabucci *et al.*, 2015]. In fact, although lyophilised matrices are easier to handle and store, the fresh or liquid items are more representative of the real samples that laboratories are called on to analyse during their routine work. Moreover, sample compliance has to be stated on fresh foodstuffs for which MLs

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are set in the pertinent legislation. Therefore, the inclusion in the EURL-CEFAO scheme of matrices in this physical state is particularly useful to the NRLs.

Starting from 2006, the EURL-CEFAO focused its activity as a PTP on improving and harmonising the performance of its network on the analysis of chemical elements in matrices for which MLs are established in Commission Regulation (EC) No 1881/2006 and subsequent amendments.

After this initial period, as the network reached an extremely satisfactory and steady level of performance, the EURL-CEFAO focussed on other matrix/element combinations, in particular those included in the National Residues Control Plan that each Member State submits annually for approval to the EC. This plan contains information such as the species to be controlled, the analytes to be determined, the number of samples, the sampling procedures, the requirements for laboratories performing the analyses, and the actions to be carried out if samples are not compliant.

TABLE 1 / Proficiency tests organised by the EURL-CEFAO over the period 2006-2015. Analyte/matrix combination and physical state of the samples.

Year	EURL-CEFAO's PTs		
2006	Meat Cd, Pb Freeze-dried	Milk Cd, Pb Freeze-dried	
2007	Meat Cd, Pb Freeze-dried	Milk Cd, Pb Freeze-dried	Fish Cd, Pb, Total Hg Freeze-dried
2008	Milk Cd, Pb Liquid	Liver Cd, Pb Freeze-dried	Meat Cd, Pb Freeze-dried
2009	Fish Cd, Pb, Total Hg Freeze-dried	Milk Cd, Pb Liquid	Fish Cd, Pb, Total Hg Frozen
2010	Meat Cd, Pb Frozen	Milk Cd, Pb Liquid	
2011	Liver Cd, Pb Frozen	Meat Cd, Pb Freeze-dried	
2012	Milk Cd, Pb Liquid	Infant formula Cd, Pb Powdered	
2013	Meat Cd, Cu, Pb, Total Hg Frozen	Honey Cd, Pb	
2014	Kidney Cd, Cu, Pb, Total Hg Frozen	Fish Cd, Pb, Total Hg Freeze-dried	
2015	Infant formula Cd, Pb, Mo Powdered	Mussels Total As, Cd, Pb, Total Hg Freeze-dried	

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This strategy has enabled the EURL-CEFAO to gain a clear picture of the matrices of increasing interest, such as honey [Ciaralli *et al.*, 2015] and infant formula [Sorbo *et al.*, 2015], so as to organise exercises before the relevant MLs become applicable. This gives the participants the chance to address analytical issues related to these matrices in advance. The PTs organised in the ten-year period 2006-2015 are summarised in table 1.

In addition, the inter-laboratory comparisons are designed as part of a long-term programme pursuing the objective of providing the participants with a scheme useful for checking and improving the performance of their analytical methods as well as verifying the effectiveness of any corrective actions in case of under-performance. This objective is met by repeating PTs on the same or similar matrices and/or by producing additional samples to be distributed to the network.

According to international standards, the performance of participants is assessed in terms of z-scores expressed as $z = \frac{(x_i - x_{pt})}{\sigma_{pt}}$ where x_i is the laboratory result, x_{pt} is the assigned value and σ_{pt} is the standard deviation for proficiency assessment. In the food sector the σ_{pt} commonly used is based on the Horwitz/Thompson equation, but, considering that the performance expected from the NRLs should be better than that of routine control laboratories, adequate lower values of standard deviation were set for the proficiency assessment (σ_{pt}). These values are calculated according to some specific equations developed by the EURL-CEFAO.

Over the period from 2005 until now, the general performance of the network has been satisfactory on the whole and in some cases it has improved over the years, even when new Member States entered the network.

The number of exercises carried out in the period 2005-2015 (*i.e.* 24 PTs), as well as the huge amount of samples produced (*i.e.* about 3200 samples) make the organisation of PTs a core activity of the EURL-CEFAO. Furthermore, the high value of the exercises proposed is confirmed by the fact that requests from NRLs not belonging to the EURL-CEFAO network to participate in the PTs have increased over the years (Figure 1).

FIGURE 1 / Increase in the number of participants in EURL-CEFAO proficiency tests organised from 2005 to 2015.

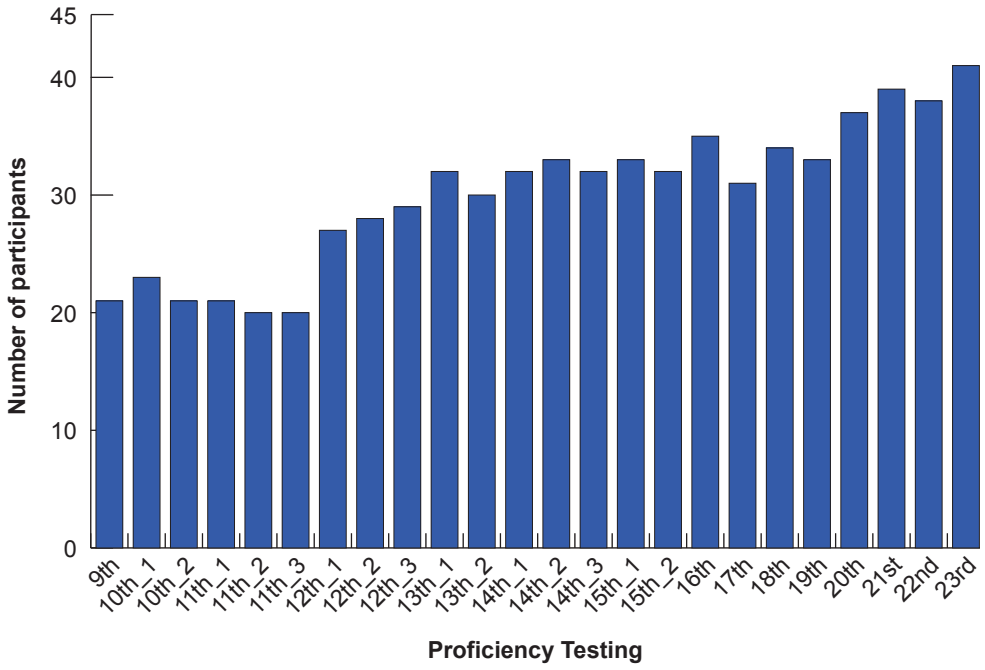

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FIGURE 2a / Percentage of incorrect, not stated and correct assessments of sample compliance with respect to each proficiency test.
Sample compliance for Cd

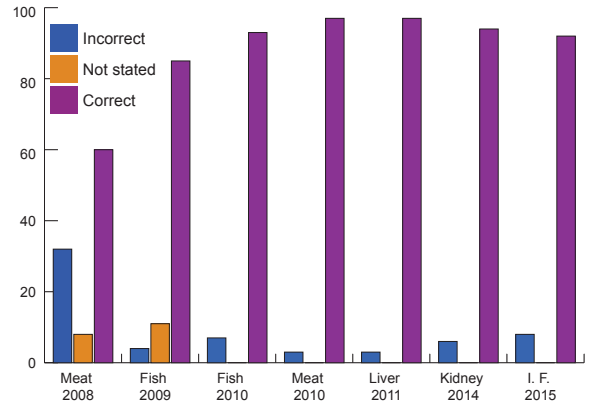
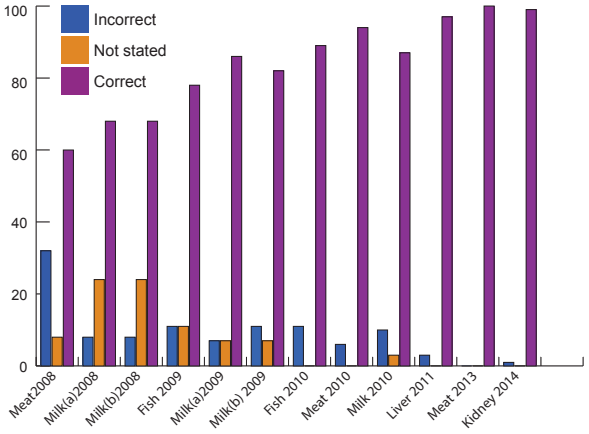


FIGURE 2b/ Percentage of incorrect, not stated and correct assessments of sample compliance with respect to each proficiency test.
Sample compliance for Pb



Considering that NRLs are often appointed as third parties in legal disputes, the exercises proposed by the EURL-CEFAO can be regarded as one of the most useful and proficient schemes available. In fact, other schemes on the market often do not cover matrices of interest, do not propose samples in a physical state similar to routine test items, and do not provide concentration levels suitable for testing methods around MLs. As an example of this deficit, the EURL-CEFAO recently organised a PT (22nd PT) on the determination of cadmium and lead in powdered infant formula and, since a similar exercise was not commercially available, the German NRL asked the EURL-CEFAO to provide them with some samples to be used for a PT involving national control laboratories. On this occasion, the performance of these official laboratories was evaluated on the basis of the assigned values set by the EURL-CEFAO, according to an internal procedure of statistical evaluation and derived from the results submitted by the 28 expert laboratories belonging to the EURL network. This kind of assistance, organised so as not to use EC funds, was also provided to the Italian and French NRLs and can be considered an important tool for harmonising the performance of EU laboratories, even at the local level.

Concerning the sample compliance statement, uniformity of assessment in different EU Member States is an important point to support the EU market. In fact, it is preferable that the same sample be considered in the same way (compliant or not compliant) in different EU Countries. Therefore, in order to ensure uniform assessment, starting from 2008, the EURL-CEFAO requested that participants state sample acceptance according to Commission Regulation (EC) No333/2007, considering this statement as an integral part of the exercise. Sample compliance is requested only for matrix/analyte combinations for which MLs have been established.

The general outcome showed that this issue needed to be addressed especially taking into account the key role played by NRLs. In fact, when this kind of exercise was proposed for the first time, only a few NRLs included the compliance statement for the sample in their results and incorrect assessments were common. The high number of PTs carried out over the last seven years and the fact that discussions on this issue have taken place several times during the workshops, has helped to overcome this problem although some errors still occur (Figure 2).

Evaluation of the long-term performance of NRLs and follow-up and training activities

The EURL-CEFAO monitors the performance of NRLs through a Shewart Control Chart of z-scores [Sorbo *et al.*, 2013], which are prepared for every laboratory, updated after each exercise, and published in the restricted area of the EURL-CEFAO website. Every graph is thoroughly evaluated in order to detect not only unsatisfactory results (|z|≥3) but also trends

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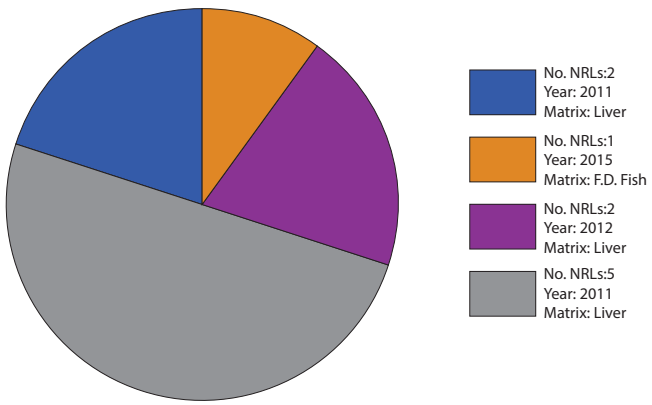
evident in the charts. The outcome of this investigation can prompt specific follow-up actions, which are performed by giving the laboratories suggestions or advice based on the EURL analytical background as well as on analytical information supplied by the participants (*e.g.* sample treatment, dilution factor, calibration points, LoD, LoQ, etc.). Furthermore, laboratories are contacted and requested to inform the EURL about the cause of their under-performance and/or if corrective actions have been undertaken. Should the participants require special analytical support, the EURL staff organises training courses at its facilities or includes this activity in the visits to NRL laboratories. In particular, the laboratories to be visited are selected on the basis of the following criteria: under-performance, new laboratories that entered the network or under-performing laboratories that have changed their analytical technique and laboratories already visited that still demonstrate the need for additional targeted training. In ten years of activity, EURL-CEFAO representatives have carried out 22 training sessions and visits to EU NRLs in order to provide the required support. Even though this specific activity is carried out to accomplish one of the EURL's tasks, the EURL-CEFAO pays a special attention to it, taking advantage of the strong analytical background of its staff. In fact, the possibility of making practical suggestions to NRLs on the way to overcome their analytical obstacles has been found to be the best approach to make the visit beneficial.

Similarly, the EURL organises training courses for official laboratories of candidate Member States and non-EU countries, upon request. Training courses for laboratories in the Philippines, Jordan, Brazil, and Macedonia have been carried out hosting about 15 scientists.

As a further way to address under-performance, the EURL-CEFAO is able to provide NRLs with *ad hoc* samples that laboratories are requested to analyse as if they were PT test items. The submitted results are evaluated by the EURL expert and an individual report containing z-scores and technical comments is sent to the participants. This activity is particularly useful to check the effectiveness of the corrective actions that under-performing laboratories have implemented. Until now, the EURL-CEFAO has organised 10 additional exercises to assist its network as shown in figure 3.

FIGURE 3 / Additional exercises organised by the EURL-CEFAO



Development of analytical methods

The development of new analytical methods is an important part of the EURL's activity [Sorbo *et al.*, 2014]. In particular, two objectives are pursued when the development of a new method is planned: provide the NRLs with analytical procedures suitable for the new matrix/analyte combinations on which EURL-CEFAO proficiency tests will be based and to develop new

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easy-to-use methods that can be required to address emerging topics. In both cases, the results of this activity are shared with the NRLs as these procedures are usually distributed to them or made available upon request. The possibility of developing methods using all the techniques available to the NRLs is one of the strong points of EURL-CEFAO analytical activity. In fact, two different methods are usually developed for the same analyte/matrix combination: one based on inductively coupled plasma-mass spectrometry (ICP-MS) and one based on graphite furnace atomic absorption spectrometry (GFAAS). Furthermore, EURL-CEFAO experts have validated methods for mercury determination based on both cold vapour atomic absorption spectrometry (CVAAS) and direct mercury analysis (DMA).

The methods always comply with the performance criteria required in Commission Regulation (EC) No 333/2007 and subsequent amendments. Over the years, 12 methods have been distributed to the NRLs, including a new method for inorganic arsenic determination in fresh mussels using water bath extraction and anion exchange chromatography-ICP-MS. This procedure is of particular interest to the laboratories as the concentration of inorganic arsenic in foodstuffs is a major concern for public health.

Conclusions

The strong connection between the EURL and NRLs enables easy sharing of analytical experience and development of expertise in the analytical sector within the European Union. The EURL-CEFAO carries out its activity with the aim of not only fulfilling all its duties but also being as useful as possible in the implementation and maintenance of high quality standards required by laboratories that have been appointed as NRLs.

Regulations on food safety control as well as analytical techniques are constantly evolving. As a result, the EURL-CEFAO needs to continuously update its technical knowledge. Over the years, this picture has led the laboratory to reach an ever-increasing level of specialisation in the activities carried out in order to be able to disseminate its experience among the NRLs. The activity performed by the EURL-CEFAO has received positive feedback from the EC and the NRL networks, confirming the suitability and effectiveness of the strategy that the laboratory follows in planning its work.

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