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Annual Workshop of the European Union Reference Laboratory for chemical elements in food of animal origin

Istituto Superiore di Sanità
Rome, September 28, 2016

ABSTRACT BOOK

Edited by
A. Sorbo, M. D'Amato, G. Fornari Luswergh and L. Ciaralli

ISTITUTO SUPERIORE DI SANITÀ

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Department of Food Safety and Veterinary Public Health

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This volume collects the abstracts of the contributions presented at the Workshop organized by the European Union Reference Laboratory for chemical elements in food of animal origin (EURL-CEFAO) hosted by the Department of Food Safety and Veterinary Public Health of the Italian National Institute of Health. The volume provides an overview of the activities carried out by both the EURL-CEFAO and the National Reference Laboratories of the EU Member States working in the field of chemical elements in food of animal origin. In particular, it is focused on proficiency testings, analytical topics and regulatory issues.

Key words: Chemical elements, Proficiency Testings, European Union Reference Laboratory.

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Questo volume raccoglie i riassunti dei contributi presentati durante il Workshop organizzato dal Laboratorio Europeo di Riferimento per gli elementi chimici nelle matrici di origine animale (EURL-CEFAO) che ha sede presso il Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare dell'Istituto Superiore di Sanità. Il volume offre una panoramica sulle attività svolte dall'EURL-CEFAO e dai Laboratori Nazioni di Riferimento degli Stati Membri dell'Unione Europea operanti nell'ambito degli elementi chimici nelle matrici alimentari di origine animale. In particolare, esso è incentrato sui circuiti interlaboratorio, su argomenti analitici e su problemi regolatori.

Parole chiave: Elementi chimici, Circuiti interlaboratorio, Laboratorio di Riferimento dell'Unione Europea.

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Workshop Chairperson

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NOTE FOR THE READER

This volume gathers all the contributions presented at the workshop. Abstracts are divided into oral and poster presentations. For easy consultation, oral presentations are listed in the order of the programme.

Posters are listed after the oral presentations. The poster abstracts are numbered with a code including the letter “P” followed by a progressive number.

At the end of the volume, the authors’ index is provided for the reader’s convenience.

PREFACE

Over the past few decades, the protection of consumer health has become a top priority for the European Union which established and strengthened the control systems to ensure food safety.

A key role in this complex organization is played by the European Union Reference Laboratories (EURLs) and the National Reference Laboratories of the EU Member States (EU-NRLs) whose synergistic action is aimed at ensuring that the analytical results, obtained in the various fields of expertise, are uniform and that outcome of foodstuffs compliance statement is harmonized.

The interaction between the EURLs and their respective EU-NRLs is a key to ensuring an efficient food safety assurance system. The organization of meetings, one of the main tasks of the EURLs, is an excellent way to support this interaction. As for the annual workshop of the European Union Reference Laboratory for Chemical Elements in Foods of Animal Origin (EURL-CEFAO), it was held in Rome on 28th September 2016.

During the meeting, issues of common interest were discussed. Moreover, the participants were updated by a representative of the European Commission on the most important changes in legislation relevant to food safety.

One of the key topics of the EURL-CEFAO workshop is to present and discuss the results of proficiency tests (PTs) that are organized on annual basis.

The participation in these exercises is an effective tool to evaluate and harmonize the performance of the analytical methods used by the EU-NRLs. For this reason, in the last decade, several PTs have been carried out by the EURL-CEFAO that has invested a lot of resources and expertise in this specific activity. The PTs are usually planned on the basis of matrices/elements combinations that are of particular interest to the network, including combinations that could be incorporated in new or revised pertinent regulations.

Recently, some NRLs have started to use the EURL-CEFAO PT materials to organize exercises for the benefit of their official laboratories. This activity is extremely important as the main task of the NRLs is to increase the quality, accuracy and comparability of the results produced by official control laboratories. During the meeting the outcome of PTs were discussed by both the EURL-CEFAO and one of the EU-NRLs that had conducted the exercise. The analytical activity was a further topic on which the workshop was focused: the determination of inorganic arsenic in fresh mussel tissue and the analysis of mercury in food matrices were detailed and discussed by EURL-CEFAO experts.

The participants were involved in a training on selection and Use of Reference Materials performed by a TrainMic trainer as well.

An important part of the workshop was dedicated to discuss the applicability of Commission Decision 657/2002 to the analysis of chemical elements in food of animal origin in the view of revising the actual regulations.

The relevance of the EURL-CEFAO annual workshop as a real opportunity for strengthening cooperation, sharing experiences and training, is confirmed by the increasingly active participation of NRLs as well as by the participation of experts from third countries. Throughout the years the NRLs have given a positive response enriching the meeting with talks and posters based on their experiences. These topics represent an important focus for discussions and collaborations so pushing the EURL to dedicate even more effort and attention in the organization of this meeting.

This volume gathers the abstracts of the contributions presented at the *"Annual Workshop of the European Union Reference Laboratory for chemical elements in food of animal origin 2016"* organized at the Istituto Superiore di Sanità, Rome, on 28th September 2016.

Laura Ciaralli
Workshop chairperson
EURL-CEFAO Director

Oral session 1

Chairperson
Laura Ciaralli

DO WE NEED FRESH SAMPLES FOR PROFICIENCY TESTING? REPORT ON TWO PTs WITH PIG LIVER

Timo Kapp
Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany

Two PTs were performed with pig liver: The first exercise was done in 2011 with a lyophilised sample, while in 2016 a deep-frozen fresh material was used. While the first material was easy to store and to work with, the second one can be assumed to be more representative for routine work.

The fresh material was prepared at the NRL from liver of four individual animals. For some elements (Cd, Cu, Hg, Mn, Mo, Se, Zn) the natural contents were left unchanged, while for other elements (Ag, As, Co, Pb, Tl, U) spiking was necessary to achieve analytically reasonable values. The resulting concentrations were between 0.020 mg/kg for U and 42.7 mg/kg for Zn.

Twenty-two German official laboratories took part in this PT. As only As, Cd, Hg and Pb were mandatory analytes there were between 16 (for Ag) and 22 (for Hg and Cd) data sets for statistical evaluation. Reporting was done according to ISO13528 using the participants' data for calculating the robust mean and the robust standard deviation.

The correlation with the standard deviation calculated from the Horwitz equation (modified by Thompson) showed HorRat-values between 0.28 for cobalt and 0.62 for zinc. The overall rate receiving a satisfying score was nearly 95%, with no laboratory producing clear outlier results.

Hence, the fresh material obviously did not pose problems to the participants. The ratios of repeatability to reproducibility were between 1.9 (for copper) and 3.8 (for silver). These unobtrusive ratios do not indicate any significant differences between the participants' ways of working.

It is often stated that lyophilised materials are easier to handle than fresh materials. Hence, we compared the recent data to the data generated with lyophilised pig liver in the same network in 2011. The data were normalised to lyophilised liver and compared: For lead and selenium, the contents were nearly identical. For lead, the laboratory performance expressed in terms of the reported standard deviation was comparable, while for selenium the recent PT showed a significantly lower standard deviation (8.5% *vs* 16.3%). Even though for arsenic, cadmium, mercury and zinc the concentrations in the recent PT were significantly lower, the standard deviation was nearly the same (for arsenic) or even lower. The only element with a higher content in the recent PT was copper. There the standard deviation was lower with the higher content.

Even though some participants stated more difficulties working with fresh material, the data do not suggest a higher level of difficulty compared to lyophilised material. Therefore, the use of lyophilised materials as reference material is to be preferred because of the better long-term stability. However, fresh material could be a good alternative in cases of matrices without stable lyophilisates, *e.g.* fruit materials.

24th PROFICIENCY TESTING ON HONEY: OVERVIEW OF STATISTICAL ANALYSIS AND COMMENTS ON RESULTS

Angela Sorbo
EURL-CEFAO, European Union Reference Laboratory for Chemical Elements in Food of Animal Origin, Istituto Superiore di Sanità, Rome, Italy

The content of chemical elements in honey has sparked a growing interest not only amongst researchers, but also amongst the EU competent authorities dealing with the protection of the public health and the functioning of the internal market. The monitoring performed annually at national level on the content of chemical elements in different categories of food has highlighted the presence of Pb in honey, sometimes at rather high concentrations. As a result, some Member States of the European Union (EU-MSs) were steered to define their own action levels, which turned out to fall in a wide concentration range. In order to ensure the protection of the EU market on one hand and the health of the consumers on the other, the European Commission published Commission Regulation (EU) 2015/1005, which sets a harmonized Maximum Level (ML) for Pb in honey.

The introduction of this new analyte/matrix combination in the EU legislation forces the official control laboratories to have adequate analytical methods. Moreover, the methods performance needs to be satisfactory and as uniform as possible amongst the laboratories.

In view of providing suitable means for assessing the performance of these methods and of assisting laboratories in their improvement, the EURL-CEFAO based the 24th Proficiency Testing (PT) on the determination of cadmium (Cd), total mercury (Hg) and lead (Pb) in honey. The analytes included in the exercise had been selected on the basis of the regulation in force, the possible inclusion in new or existing regulations and analytical considerations. All the National Reference Laboratories (NRLs) belonging to the EURL-CEFAO network joined the PT together with three extra-network participants.

The statistical evaluation of the results was performed in accordance with ISO 13528:2015. The standard deviations for proficiency assessment (σ_{pt}) were established by using algorithms that allow the EURL-CEFAO to obtain values lower than Horwitz/Thompson equation (17% *vs* 22% for Cd; 12% *vs* 22% for Pb; 13% *vs* 22% for Hg). As for Cd and Pb, the statistical analysis was straightforward: data distribution was roughly symmetric and unimodal without significant differences between results obtained by different analytical techniques. Robust mean was set as the assigned value for both analytes (0.0072 mg/kg and 0.140 mg/kg for Cd and Pb, respectively).

Whereas, as regards Hg, the criteria on robust standard deviation and uncertainty of the assigned value were not fulfilled. This was due to the data dispersion that resulted greater than the σpt set a priori, whose enlargement (from 13% to 16%) was necessary to release the assigned value and to provide the participants with a z-score.

The general performance of the network was extremely satisfactory: almost all NRLs obtained a z-score lower than 2 for all analytes.

An overview of the statistical analysis of the results will be given and the rationale of the enlargement of σpt for Hg will be discussed. Additionally, participants were requested to state the sample compliance for Pb so the outcome will be presented. Finally, the performances of the current PT and the 19th PT will be compared.

PLANNING OF 2017 PROFICIENCY TESTINGS

Laura Ciaralli
EURL-CEFAO, European Union Reference Laboratory for Chemical Elements in Food of Animal Origin, Istituto Superiore di Sanità , Rome, Italy

The conduction of Proficiency Testings (PTs) is an integral part of the activity performed by the European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO). Each exercise is carefully planned to be as fruitful as possible for the National Reference Laboratories (NRLs) and the monitoring of long-term performance is one of the criteria adopted to choose the matrix/analytes combination on which the PT will be based. Therefore, some combinations are proposed more than once to allow NRLs to improve their performances or to undertake corrective actions.

The 2017 PTs have been planned with this specific view. In particular, the 26th PT will be based on the determination of cadmium (Cd), copper (Cu), lead (Pb) and total mercury (Hg) in freeze dried meat. The needs of confirming the observed dependence of the participants performance on the physical state of the sample as well as the needs of providing NRLs with extra-samples accounts for the preparation of PT material in freeze-dried form. In order to check and harmonize the step of the compliance statement, the sample conformity will be requested for Cd and Pb as well.

The 27th PT will be based on the determination of total arsenic (As), Cd and Pb in powdered and liquid Infant Formula (IF) based on animal proteins. This choice was influenced by the publication of “Commission Regulation (EU) 2015/1005, amending Regulation (EC) No 1881/2006, as regards maximum levels (MLs) of lead in certain foodstuffs”, entered in force on 1 January 2016.

Two different MLs in IF are established depending on the physical state in which it is marketed (liquid 0.010 mg/kg or powder 0.050 mg/kg). Cadmium will be also included in the exercise to check if the good performance of the network obtained in the 22nd PT is confirmed. Furthermore, also for Cd two different MLs in IF have been set in Commission Regulation (Eu) No 488/2014 (0.010 mg/kg for powder and 0.005 mg/kg for liquid).

Due to the introduction of different MLs for powdered and liquid IF, some problems could occur when the sample compliance is to be stated. Therefore, the 27th PT is conceived not only to check the capability of the NRLs to face analytical problems related to these two physical states but also to evaluate if sample compliance for Pb and Cd is correctly stated.

PT materials will be produced by the EURL-CEFAO according to well-established internal procedures.

TrainMiC - SELECTION AND USE OF REFERENCE MATERIALS

Marina Patriarca*
Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

TrainMiC (Training in Metrology in Chemistry) started as a European Union funded programme in support of accession countries in 2001 and successfully developed as a shared initiative to involve most countries in Europe and beyond. TrainMiC is a platform for advanced professional training on generic issues related to the measurement science in chemical measurements. It aims to promote and provide a European wide harmonised training in metrology in chemistry and related analytical sciences, via a network of national providers sharing resources, to improve and harmonise the quality of analytical results in Europe. The structure of this initiative is based on national TrainMiC teams sharing common training materials (lectures, examples, exercises) and in turn contributing to its production. TrainMiC material covers all aspects of the technical requirements of ISO/IEC 17025 and provide an overview of the criticalities involved and reference to further reading.

Reference materials play a key role in assuring the quality of analytical results. Over the last few years, a process of revision, update and extension of the guidance from ISO/REMCO on reference materials has been in place, aiming to cover quality control materials prepared in-house as well as setting the basis for formal accreditation of reference material producers. However, using a reference material does not automatically provides better results! It is of the utmost importance that the criticalities associated with appropriate selection and use of reference materials are fully understood by all those involved.

To this aim, the reasons why reference materials are required will be reviewed, leading to the definitions of different types of reference materials and to stress the key-role of certified reference materials to achieve the traceability of measurement results. An overview of the processes involved in the production of reference materials will demonstrate what is the level of effort that is put into them and what should be expected from the supplier in order to trust the reference materials. The issues to be considered when choosing a reference material as part of a specific measurement will be highlighted as well as the importance of correct handling and monitoring of the reference materials once they enter the laboratory.

**Authorised TrainMiC Trainer*

CRITICAL POINTS IN MERCURY DETERMINATION BY DIRECT MERCURY ANALYSIS

Maria Ciprotti
EURL-CEFAO, European Union Reference Laboratory for Chemical Elements in Food of Animal Origin, Istituto Superiore di Sanità, Rome, Italy

Mercury determination presents several problems due to the intrinsic peculiarity of this chemical element. High volatility, absorption on containers walls, absorption on particle suspended and colloids, inclusion in stable complexes and amalgams make the determination of this metal quite tricky.

The most common conventional methods for the measurement of total mercury include: Neutron Activation (NAA); Cold Vapour Atomic Absorption Spectrometry (CVAAS) and Cold Vapour Atomic Fluorescence Spectrometry (CVAFS). However, the solid sampling has recently become one of the most popular analytical technique. In fact, it permits interference-free analysis of solid and liquid samples performing an automatic sample combustion carried out approximately at 800°C under oxygen flow. After the combustion of the sample and the catalytic conversion of the gases, the elemental mercury is selectively concentrated by amalgam formation and then quantified by Atomic-Absorption Spectrometry (AAS).

The main advantage of using the Direct Mercury Analyser technique (DMA) is the determination of mercury directly on the sample without any pre-treatment or dilution. This leads to a sensible time reduction of the analysis and also permits to lower the limits of detection and quantification.

Although the technique and the software seem to be very simple, some aspects need to be carefully considered in order not to undervalue the technical critical points such as memory effect and instability of mercury standard solutions.

Several mercury analyses in different food matrices were performed by DMA during the analytical activity of the EURL-CEFAO allowing the laboratory to increase its expertise on problems related to mercury determination: In particular, a lot of experience on key aspects, from the stability and storage of solutions containing a low level of mercury up to the study of different thermic profiles for different matrices with different carbon content, has been gathered.

This presentation is focused on a short description of the analytical technique characteristics including suggestions to get profitable results. The main advantages of direct mercury analysis, namely the possibility of using an equipment that allows the analyst to obtain results in short time as well as the low impact to the environment due to small use of chemical reagents, will be discussed. Finally, the application of the technique to the analysis of the honey sample of the 24th PT to assess the material homogeneity is examined emphasizing the achievement of the thermic profile of decomposition.

Oral session 2

Chairperson
Laura Ciaralli

METROFOOD-RI: A NEW PAN-EU RESEARCH INFRASTRUCTURE FOR PROMOTING METROLOGY IN FOOD AND NUTRITION

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METROFOOD-RI "*Infrastructure for promoting Metrology in Food and Nutrition*" is a European Research Infrastructure project listed as "Emerging" on the **2016 ESFRI Roadmap.** METROFOOD-RI is a new, distributed Research Infrastructure of Global Interest, by means of which it will be possible to carry out different activities supporting data collection and measurement reliability, as well as basic and frontier research in food and nutrition.

METROFOOD-RI aims at providing high quality metrology services in food and nutrition, comprising an important cross-section of highly inter-disciplinary and inter-connected fields throughout the food value chain, including agro-food, sustainable development, food safety, quality, traceability and authenticity, environmental safety, and human health. Its general objective is to enhance scientific cooperation and encourage interaction between the various stakeholders, as well as the creation of a common and shared base of data, information and knowledge. METROFOOD-RI is also characterized by several activities: collection, dissemination and sharing of information on principles, terminology, tools and metrological needs in food and nutrition; harmonization and integration of food composition databases; development of new standardized tools for food quality, safety & authenticity.

A network of plants, laboratories and experimental fields/farms will be realized (*Physical*-RI) and an *e*-RI will be developed. The *Physical*-RI will enable to carry out different research activities supporting data collection and measurement reliability; quality & safety and traceability of food production, as well as basic and frontier research in food and nutrition. The *e*-RI will provide a new useful, free access web platform to share and integrate information and data on availability of metrological tools for food analysis. It will deal with integration of existing database on food, focusing on emerging needs and collection of data on food composition, nutritional contents and levels of contaminants in foods produced in different geographic regions by applying to different technologies.

Organised as a distributed RI structured on the basis of a *hub & nodes* model, METROFOOD-RI is supported by the economic endorsement of 3 Member States and the political endorsement of 13 Countries. Currently, 35 partners from 17 Countries, together with an international partner (FAO) are involved. Each partner brings its wide and consolidated network of international collaboration, which will ensure a very broad range of action, open also to developing countries and new markets and able to meet the needs of the scientific community and all stakeholders at a global level.

UPDATE FROM THE EURL-CEFAO

Andrea Colabucci
EURL-CEFAO, European Union Reference Laboratory for Chemical Elements in Food of Animal Origin, Istituto Superiore di Sanità, Rome, italy

Some important news on the ongoing activity of European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO), relevant to the interaction with its National Reference Laboratories (NRLs) network, is object of this presentation.

As announced in 2015 EURL-CEFAO annual workshop, the PT results submission has changed. As for the 24th PT on honey, an intermediate procedure has been chosen: participants were required to submit the official results by e-mail, as usual, but also to test the online submission system. Starting from the 25th PT on milk, results can be submitted only in the on-line restricted area. Participants' feedback and advices have been collected to be discussed during the workshop.

The second issue is related to changes in the European legislation. In fact, the entering into force of the Commission Regulation (CR) 582/2016 amending CR 333/2007 has changed the definitions of Limit of Detection and Limit of Quantification and modified the Table 5 reporting the minimum analytical method requirements. An overview of the information related to the methods employed by the NRLs, as submitted in the 2016 EURL-CEFAO PTs result form, is given to verify whether all the laboratories have acknowledged the new requirements set in this amendment.

Furthermore, the ongoing annual evaluation of the National Residue Monitoring Plans (NRMPs) for group B3c for 2016 is a key point to be analysed.

The discussion is focused on the different action levels (ALs) adopted by the EU Member States (MSs) to state sample compliance, for those matrix/element combinations not included in the Commission Regulation 1881/2006.

In particular, one of the main issues to be highlighted is that not all the EU MSs take into account the Maximum Residue Limits set for Mercury and Copper in the pesticides legislation (Regulation 396/2005).

NRMPs results for 2015 is also part of the discussion. The high number of non-compliant samples found in some specific matrix/element combinations (*e.g.* cadmium in kidney) is highlighted as well as the relevant follow-up action reported by the MSs in the "Questionnaire on the actions taken as a consequence of non-compliant results". Finally, the representatives of the NRLs are reminded of the opportunity to include arsenic and nickel in their 2016-2018 NRCPs at least for the matrices prescribed in the Commission Recommendations 1381/2015 and 1111/2016.

APPLICABILITY OF THE CD 657/2002 TO THE ANALYSIS OF CHEMICAL ELEMENTS IN FOOD OF ANIMAL ORIGIN

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The urgent need for a review of the Commission Decision (CD) 2002/657/EC was highlighted in the expert committee on residues of veterinary medicinal products meeting, held in June 2015.

For this purpose, the four European Union Reference Laboratories (EURLs) for residues (BVL, RIKILT, ANSES, and ISS) were asked by the European Commission for technical assistance including the revision of CD 2002/657/EC in their work programmes for 2016/2017.

In July 2015, a questionnaire was prepared by the three “organic residue” EURLs (BVL, ANSES and RIKILT) asking their National Reference Laboratories network to rate for each article of the Decision the need for changing from 0 (no changes required) to 5 (changes urgently needed).

Aside from this, article 1 of CD 657/2002 and the specifications of the document SANCO/2726/2004 state that the provision of the Decision are not applicable for those substances for which a more specific regulation is set. For group B3c (chemical elements), the field of competence of the EURL at the ISS-Chemical Elements in Food of Animal Origin (CEFAO), more specific provisions are reported in the Commission Regulation (CR) 333/2007 that, however, does not indicate any mandatory document to be preferred for method validation.

The EURL-CEFAO, reckoning that some laboratories may have chosen the same validation procedures for both organic and inorganic analytes based on the CD/657/2002 provisions, sent a questionnaire to the NRLs of its network asking them whether and how this decision is applied.

The NRLs applying any part of the Decision were asked to fill in the relevant fields in the questionnaire prepared by the “organic” residue EURLs.

The number of NRLs of the EURL-CEFAO network employing at least a part of CD/2002/657/EC in their validation procedure is not negligible (12 out of 28; 43%).

In the EURL-CEFAO intention, the questionnaire was thought to be complementary to the one prepared by the “organic” residues EURLs. The aim was to obtain information on the specific need for chemical elements but, in most cases, the EURL-CEFAO received information more related to the organic issues rather than the inorganic ones.

One of the further step in the legislation revision was to include specific topics of interest in the workshop organized by the four EURLs.

The “Applicability of the CD 657/2002 to the analysis of chemical elements in food of animal origin” will be the issue to be discussed in the EURL-CEFAO workshop.

A questionnaire on validation procedures adopted by the laboratories was sent in July 2016; the outcome will be presented at the annual workshop as the starting point for fruitful discussions.

DETERMINATION OF INORGANIC ARSENIC IN MUSSEL TISSUES BY HPLC-ICP-MS

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Analytical methods for arsenic speciation in food of animal origin can still be demanding to be implemented as a part of the routine activities of those laboratories not primarily focused on research. Most works reported in the literature deal with the identification of the variety of arsenic species typically found in marine samples, whereas the interest in developing robust analytical methods focusing on inorganic arsenic (i-As), as the most toxic fraction, has increased only recently. Therefore, the implementation of a relatively cheap, fast, and easy-to-use method directly applicable to incurred samples can represent an added value for routine laboratories.

Due to the rising interest in arsenic speciation at the level of control laboratories and in compliance with its tasks, the European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO) has developed a method for i-As determination in fresh mussels using water bath extraction and anion exchange chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS).

This method might be applicable to incurred samples of animal origin. In particular, an effective procedure for extracting, separating and quantifying i-As was developed in order to reduce sample preparation, *e.g.* avoiding freeze-drying.

The proposed method has to be considered only as a general guideline for inorganic arsenic determination in fresh mussels and as a support to NRLs when approaching analytical issues related to i-As speciation.

During the method development, the optimization of an HPLC-ICP-MS method for selective i-As determination was investigated and the results obtained with anion-exchange chromatography by using different extraction mixtures and columns were compared and evaluated. The method consists in the extraction with a HNO_3/H_2O_2 mixture in a shaking water bath and elution with ammonium phosphate and ammonium nitrate in 2% methanol (pH=5.5) on Hamilton PRPX-100 column. Following the good preliminary results obtained, the method was considered for validation.

The method was validated using samples of Mediterranean mussels containing roughly 3.8 mg/kg of total As. With the proposed extraction procedure using HNO_3/H_2O_2 mixture, the complete oxidation of As(III) into As(V) was achieved, which resulted in a well-separated peak in the anion exchange HPLC-ICP-MS chromatogram. Then, the i-As was easily quantified as As(V).

In the validation procedure LoD, LoQ, repeatability, within-laboratory reproducibility and accuracy were assessed. A provisional value of expanded uncertainty was also estimated.

Finally, an improvement of the proposed method with lower amount of extraction reagents was considered.

Poster contributions

P01 24th PT ON HONEY: COMPARISON OF THE PERFORMANCE OF GERMAN RFLS WITH THE NRL NETWORK

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In parallel to the NRL network 15 German official routine laboratories took part in a national PT on the same material. As for the NRLs, the task was to determine cadmium, lead and mercury. All laboratories delivered quantitative results for all parameters. However, some reported a quantification limit above their results. This was especially true for the low contents of cadmium and mercury.

The PT parameters (robust mean and standard deviation) were determined based on the participants' data according to ISO13528. The robust means were comparable to the ones calculated by the NRL network (cadmium 0.0072 (national) *vs* 0.0072 mg/kg (NRL network); lead 0.1409 *vs* 0.140 mg/kg and mercury 0.0161 *vs* 0.0156 mg/kg). The reported robust standard deviations were not directly comparable. However, they were lower than the standard deviation predefined by the EURL CEFAO. The correlation to the standard deviation calculated by means of the Horwitz equation (modified by Thompson) showed HorRat-values between 0.25 for lead and 0.51 for mercury.

As the data were rather homogenous, the evaluation by z_U-scores revealed only one participant with an unsatisfying score (for mercury).

The assessment of compliance comparing their own lead result to the Maximum Level (ML) laid down for lead in Reg.1881/2006 was carried out correctly by most participants. The clearly non-compliant sample was truly assessed as “non-compliant” by most participants. However, one laboratory claimed the sample “non-compliant” and its measurement uncertainty overlapped with the ML. Furthermore, two participants assessed this sample to be “compliant”, even though their results lowered by their expanded uncertainty was clearly above 0.10 mg/kg.

Some participants seemed to have problems to estimate their measurement uncertainty. Compared to their precision, the reported uncertainties were too low. Some laboratories even reported uncertainties much lower than the uncertainty of the mean of the honey material. This might be due to a lack of experience with this matrix as PT sample.

Despite these practical aspects with room for improvement, the network of the national routine laboratories has worked in a comparable manner to the NRL network for the second time. This shows that the European reference laboratory system works well and comparability between the different network levels is achievable.

P02 DOUBLE PHASE-REFERENCE MATERIALS: A NEW OPPORTUNITY IN FOOD ANALYSIS

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ENEA R&DT activities on Reference Materials are focused - among others and in addition to the production of “conventional” RMs - on the possibility to introduce elements of innovation in the RM preparation procedures in order to develop “innovative” RMs, useful to cover different phases of the measurement process and make RMs more representative, easier in use and less subject to alterations.

Double Phase-RMs (DP-RMs) are RMs split, during the preparation procedure, into their two liquid (aqueous) and anhydrous components and supplied in their two separated components (liquid phase and solid phase) to be re-combined before use.

The use of an industrial rotary evaporator (V=100 L) gives the possibility to prepare such kind of RMs obtaining the two components (concentrate + vapour phase) and, after a further lyophilisation step on the concentrate the two final components are obtained: lyophilsed concentrate + condensed extract. The dry powder is bottled and the condensed phase is sealed in glass vials. It is necessary at firs to set up the operative conditions (sample weight, pressure, temperature, time) for both vacuum evaporation and lyophilisation; then, taking into account the weight losses due to the vacuum evaporation and lyophilisation, the exact amount of both condensed extract and water to be added for obtaining the reconstituted matrixes can be established.

DP-RMs permit to have highly representative RMs thanks to the possibility to store and use for the lyophilized matrix reconstitution not only pure water, but the actual (natural) solvent itself. In addition DP-RMs could represent a good solution also in terms of stability, especially when the parameter of interest is a volatile or degradable substance (*e.g.*: for food flavour analysis).

Feasibility studies have been conducted on different matrixes of both animal and vegetal origin, like whole and partly skimmed milk and strawberry fruit. In order to evaluate the feasibility and demonstrate the potentiality and the effectiveness of such kind of RMs (especially in terms of representativeness and stability), after having set up the preparation procedures, chemical analyses have been carried out for characterizing and comparing the condensed extracts and the matrixes reconstituted both only with pure water and with the (pre-dosed) condensed extract and the appropriate amount of pure water.

P03 EXPOSURE ASSESSMENT OF DIETARY CHEMICALS: FOCUS ON TOTAL DIET STUDIES

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Dietary exposure assessment is quantitative evaluation of the intake of chemical substances (including nutrients) via food at large, i.e. including beverages, drinking-water and food supplements. It encompasses different types of surveys, from Duplicate Diets Studies (for estimating dietary intakes at the individual level) to Total Diet Studies (TDSs). The latter represent the gold standard for calculating population dietary exposure and assessing potential impact on public health, and have been the subject of a joint guidance by EFSA, FAO and WHO in 2011.

A TDS consists of selecting, collecting and analysing commonly consumed food purchased at retail level, processing the food as for consumption, pooling the prepared food items into representative food groups, homogenising the pooled samples, and analysing them for harmful and beneficial chemical substances. TDSs are designed to cover the whole diet and to measure the amount of each chemical substance ingested by the population living in a country, ideally using average and high-level consumption data for final exposure calculations. In Italy, ISS steered the 2012-14 TDS that assessed the intake of both toxic (aluminium, inorganic arsenic, cadmium, lead, methyl-mercury, inorganic mercury, uranium) and essential trace elements (e.g., iron, zinc, copper, selenium, iodine) of the Italian population. The Italian national TDS also assessed the exposure to a number of other food contaminants, i.e., radionuclides (^{40}K, ^{134}Cs, ^{137}Cs, ^{90}Sr) mycotoxins (e.g. aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, fumonisins, T-2 and HT-2 toxins), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (DL-PCBs), and non-dioxin-like polychlorinated biphenyls (NDL-PCBs).

Even though the key aspects of a TDS are well defined, there are many methodological differences in the way this type of study is performed at the national level that limit the possibility to compare exposure of different populations across Europe and worldwide. The European project TDS-Exposure was launched in February 2012 with the aim of creating an EU-wide network of TDS-Centres using common tools like databases and modelling software. With 26 participants from 19 countries, and strong links with European and international organisations like WHO, FAO or EFSA, TDS-Exposure aimed at harmonizing the TDS methodology and ensuring data collected in the future can be compared across countries. The project was concluded in January 2016. ISS contributed to TDS-Exposure, both in the development and harmonization of the TDS methodology and in providing training for those countries where TDS have not yet been carried out.

P04 RESULTS OF A PROFICIENCY TEST FOR Cd, Cu, Hg AND Pb IN PORK MEAT

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Following an initiative of the EURL-CEFAO and to support the Italian official control laboratories, the Italian National Reference Laboratory for Heavy Metals in Food (NRL-HM) organized a proficiency testing (PT) for the content of cadmium (Cd), copper (Cu), mercury (Hg) and lead (Pb) in pork meat.

The test material, prepared by the EURL-CEFAO, was a pork meat sample homogenized and spiked with Cd, Cu, Hg and Pb, previously distributed to the NRLs as part of the EURL-CEFAO proficiency testing activities. Assigned values for all elements were determined as the consensus from the results provided by the NRLs that can be considered expert laboratories in the network of official control laboratories. The test items were stored at -80°C and shipped frozen under dry ice.

Thirteen laboratories participated to this PT, using methods of their choice. Analytical performance was assessed by z-scores and the standard deviation for proficiency assessment (σ_p) was derived from the Horwitz equation. All laboratories obtained a satisfactory z score for Cd and Pb (out of one for Cd) confirming the capability of the participating laboratories to reliably assess the compliance of meat samples to the requirement of Regulation (EC) 1881/2006 about maximum levels set in this matrix. In addition all laboratories obtained a satisfactory result for Cu and only one laboratory obtained a questionable result for Hg.

PT results were used also for some other evaluations. For each laboratory, the zeta - score was calculated in order to evaluate the suitability of the reported measurement uncertainty values. For Cd and Pb it was verified if the limits of detection (LoD) and quantification (LoQ) of the methods applied by the laboratories were in compliance to the new requisite set by the Regulation (UE) 582/2016 amending Regulation (EC) 333/2007.

P05 DETERMINATION OF TITANIUM IN SWEETS

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Artificially manufactured TiO_2 is a common additive in the food industry. Especially in sweets it is used as the whitest and brightest known pigment. The additive (E171) is permitted in food processing with no maximum level specified. However, recent knowledge about nanoparticles has opened the discussion about the toxicology of nanoparticle TiO_2. A risk assessment has not been published yet because of the complexity of the nanoparticles (mineral forms, coatings, size, shape, …), the lack of toxicity data and the analytical difficulties for measuring these particles in relevant food matrices. Measuring total Ti can be used as screening method before further analysis on nanoparticle TiO_2.

Total Ti concentration can be determined after chemical digestion but dissolving Ti needs the use of concentrated sulfuric acid or hydrofluoric acid (HF). The use of S is not optimal for ICP-MS analysis, because several possible interferences with S. A digestion method was optimized using concentrated HNO_3 and diluted HF.

The determination by ICP-MS of Ti is not an evidence due to several interferences by different spectral overlaps. Several collision/reaction gasses were tested using an Agilent 8800 ICP-MS-MS instrument. Solutions containing possible interfering ions (Ca, Cr, S, P, Cl or C) were injected to check if interferences could be detected. The signal was monitored in no-gas, He, O_2 and NH_3 mode at all relevant masses. Both the quadrupoles are used. In no gas and He mode, the same mass is selected on the quadrupoles. The use of O_2 or NH_3 causes a reaction with Ti, so the element is measured with a mass shift of respectively 16 (O_2) or 102 (6 x NH_3) on the second quadrupole. The concentration of possible interfering ions was high enough to predict a worst-case scenario.

Based on the isotopic abundance of the different masses, the results of this interference test, preliminary tests and literature data, measurements were performed in He mode at mass 47-47. In this mode at m/z 47-47 no interferences were detected caused by Ca, Cr, S, P, Cl or C. The abundance of Ti at this mass is relatively low (7.32 %), but LOQ values were similar compared with more abundant isotopes.

The method was validated for different kind of sweets, with a broad application domain (0.16 mg Ti/kg - 390 mg Ti/kg) and resulted in an expanded measurement uncertainty of 20.2%.

P06 A FIT-FOR-PURPOSE FACILITY FOR THE CHARACTERIZATION AND ANALYTICAL DETERMINATION OF NANOMATERIALS: A POWERFUL TOOL FOR RISK ASSESSMENT OF NANOTECHNOLOGY APPLICATIONS IN THE AGRI-FOOD SECTOR

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Adequate characterization of nanomaterials, encompassing chemical identity and physico-chemical forms, is essential for safety assessment. The physico-chemical parameters of a nanomaterial change in various environments and the characterization has to target a specific stage of the life of the material, its intended use and potential interaction with biological systems. In the case of, *e.g.*, food/feed applications, the characterization strategy should be applicable to different scenarios/requirements: the nanomaterial as manufactured (in the pristine state), as delivered for use in food/feed products, as present in the food/feed matrix, as used in toxicity testing, or as present in biological fluids and tissues.

In order to deal with the multifaceted challenges posed by the characterization of nanomaterials and their analytical determination in complex matrixes such as food and biological samples, a fit-for-purpose facility has been established at our department. The core of the facility is a trace element-free clean room laboratory. The laboratory is composed of one dressing room and two workspaces, i.e., the instrument and the sample preparation room. The sample preparation room has the highest cleanliness (ISO 6 class), achieved through a positive pressure gradient obtained through HEPA-filtered air. The facility is completed by an ordinary laboratory for trace analysis equipped with a laminar flow work bench for clean sample manipulation.

The facility features a complementary analytical platform based on state-of-the-art ICP-MS(/MS) instruments for analysis of inorganic nanoparticles along with a variety of other techniques, either present in the facility or made available by partner laboratories, including single particle-ICP-MS, Asymmetric Flow Field Flow Fractionation (AF4)-UV-MALS-ICP-MS, HDC-ICP-MS, HPLC-ICP-MS, DLS, ELS (Electrophoretic Light Scattering), APC (Analytical Photo-Centrifugation), BET, and SEM/TEM/ESEM-(EDX).

With regard to nanoparticle detection and characterization in liquid suspension and complex matrices, activities carried out in the facility mainly focus on (i) characterization of nanomaterials in terms of size, size distribution, mass/number concentration, chemical composition, (ii) determination of minor inorganic components and impurities which might have toxicological significance, (iii) development of dispersion protocols.

The other main area of work consists in advanced sample preparation methods for undertaking studies on the fate of nanomaterials in the food chains, namely: (i) forms and fate of nanoparticles in biological systems, (ii) their stability and dissolution in synthetic and biologically relevant media, (iii) *in vitro* simulated gastrointestinal digestion of nanoparticles and nanoparticle-containing food matrices, (iv) discrimination of soluble and particulate forms in biological tissues and fluids after *in vivo* nanoparticle administration, (v) chemical speciation of the soluble fraction originating from dissolution of inorganic nanoparticles, and (vi) biodistribution/ADME studies.

Examples are given concerning the detection of unlabelled metal and oxide nanomaterials (Ag, SiO_2, TiO_2) in food and biological samples; this problematic issue complicates the safety assessment of these widely used nanomaterials. Novel approaches using state-of-the-art analytical platforms are important for generating new data to support risk assessment of nanomaterials in food.

P07 BIVALVE MOLLUSC PRODUCTION IN PORTUGAL: MONITORING OF CONTAMINANT METALS

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Bivalve molluscs have an important role in the Portuguese socio-economy and are very much appreciated due to their intrinsic sensory attributes. As filter-feeding organisms they are able to concentrate in their soft tissues various chemical contaminants from ambient, water and sediments.

Due to the bioaccumulation process the consumption of these tissues could pose a risk to the consumer's health if they are contaminated.

Therefore, these species can only be commercially harvested from approved production areas, which are monitored to ensure they meet the chemical and microbiological criteria.

Under the IPMA regular activities bivalve molluscs producing areas are regularly monitored for levels of mercury (Hg), cadmium (Cd) and lead (Pb). Thus, the primary purpose of this work is to show the information collected over the last decade on these heavy metal concentrations in bivalves from the different producing areas. In addition, the levels of the three contaminants in live bivalves collected from several marketplaces were also verified.

The levels of Hg, Cd and Pb in all species of commercial bivalves, collected from the most producing areas, did not exceed the limits set by EU. As an exception the levels of Pb in peppery furrow (*Scrobicularia plana*) from Tagus estuary and oysters (*Crassostrea angulata*) from Sado estuary are noteworthy. Regarding live bivalves collected from seafood marketplaces, the percentage of contaminated samples is lower than 1 %.

Therefore, as for the consumption of shellfish harvested from Portuguese waters, there is no evidence that the chemical risk due to Hg, Cd and Pb is significant.

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P08 EURACHEM: A FOCUS FOR ANALYTICAL CHEMISTRY IN EUROPE

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Eurachem (www.eurachem.org) is a network of organisations within Europe designed to: a) establish a system for the international traceability of chemical measurements; b) promote good quality practices in analytical sciences.

Currently represented in 32 European countries, Eurachem aims to provide a forum for analytical scientists, laboratory staff and those interested in using the results of analytical measurements to discuss common problems and develop informed and considered approaches to both technical and policy issues.

Eurachem members and stakeholders meet once a year at the Eurachem General Assembly. An Executive Committee and several topical Working Groups pursue the organisation’s stated goals throughout the year, often in cooperation with other organisations. Participation is open and channeled through national representatives.

Eurachem’s main output is authoritative guidance documents, promoted through dedicated events which are also designed to provide opportunities for collecting feedback. Beside the guides, Eurachem publishes information leaflets, i.e. short briefing documents on specific topics usually intended to inform a wide audience, including laboratory staff, managers and laboratory customers. This poster aims to summarise current Eurachem activities, inform readers about the available guidance and attract active participation.

**Members of the Eurachem Executive Committee*

P09 STATE-OF-THE-ART MASS SPECTROMETRIC TECHNIQUES FOR THE ANALYTICAL DETERMINATION OF INORGANIC NANOMATERIALS IN FOOD

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Inorganic nanomaterials (metals, their compounds, and oxides) - *e.g.* Ag, SiO2, TiO2, ZnO, TiN, Fe oxides/hydroxides, nanoclays - are a prominent class of materials with current or projected applications in the food sector ranging from food additives, to antibacterial agents, to food packaging. The assessment of their potential risks associated to dietary exposure requires analytical techniques that can be successfully employed in their identification and quantification in food products. It is thus essential to have analytical methods available to detect and characterize them as such, in food or - for food contact materials - food simulants (migration tests). Parameters such as diameter, volume, area, mass, surface charge, elemental composition, agglomeration and aggregation state play a major role in the bioavailability of iNMs in food and their potential adverse effects in humans.

State-of-the-art mass spectrometric techniques for the analytical determination of inorganic nanoparticles in dispersion and (after proper sample extraction) in complex matrices have recently become available. Being based on atomic mass spectrometric, they are element-specific (i.e. provide information on the chemical identity) and have the potential to measure size, size distribution, number and mass concentration of particles. Single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) is based on time resolved analysis of diluted nanoparticle dispersions using short dwell times (≤10 ms).

Each particle gives rise to a signal clearly distinguishable from random background noise and, by means of appropriate algorithms, signal frequency distributions are converted into size frequency distributions. In principle, the signal arising from ionic (i.e. soluble) forms of the element constituting the particles, if any, can be distinguished from that due to the presence of the particles themselves. Therefore, it is a particle-specific technique with sizing capability, presently having limitations mainly in the size detection limits (from ~10 to several tens nm, depending on the element). Another powerful technique is asymmetric flow field flow fractionation (AF4), which provides separation of particles according to their size, combined on-line with optical detectors for size determination (MALS, DLS, UV) and elemental detection/quantification by ICP-MS. With AF4-(UV-MALS)-ICP-MS particles having diameters down to 1 nm can be determined with the additional advantage of multi-detector capability.

Our laboratory was one of the few - on a worldwide level - that participated to the three interlaboratory studies promoted by the European Commission on the application of these techniques to food analysis, namely two interlaboratory studies on sp-ICP-MS determination of Ag nanoparticles in aqueous or ethanol dispersions and in chicken meat, respectively, and the first interlaboratory study on AF4-ICP-MS determination of Ag nanoparticles. However, far more challenging is the application of these techniques to the characterization of oxides such as SiO2 and TiO2 owing to a number of issues, the main being spectral interferences in the detection of Si and Ti. We developed a multi-method approach for the simultaneous determination of particle size and mass concentration of synthetic amorphous silica nanoparticles over the size range of 20-200 nm by AF4 coupled with online MALS and ICP-MS/MS detection. Ongoing work deals with sample preparation methods for nanosilica characterization in E551-containing food samples.

P10 APPLICATION OF FACTORIAL DESIGN FOR OPTIMIZATION OF A SIMPLE AND RAPID METHOD FOR TRACE ELEMENTS DETERMINATION IN RAW MILK BY ICP-MS

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Background. Milk is a widely consumed food, particularly by infants. For this reason, its safety and quality standards are a matter of great concern and close monitoring. International organizations have established very low limits for contaminants that can be present in trace levels in milk. Therefore, the development of a simple, rapid and sensible method for quantification of trace elements in milk is of critical importance. When developing new methods, factorial design can be a very powerful tool. Even with a small number of experiments, it can infer the variables that exert significant influence in the analytical method, the interaction between them, and their optimal value.

Objective. The aim of this study was to apply experimental design to find the optimal values for the main parameters of a simple dilute-and-shoot method for the determination of the trace elements arsenic (As), cadmium (Cd) and lead (Pb) in raw milk by inductively coupled plasma mass spectrometry (ICP-MS) after formic acid solubilization.

Method. One-milliliter aliquots of raw milk were spiked with As (50 µg L^{-1}), Cd (50 µg L^{-1}) and Pb (20 µg L^{-1}). Formic acid and an ultrasonic bath were used for the solubilization of the samples. Trace elements concentrations were determined by ICP-MS (Agilent’s 7700x). Experimental design: The variables “acid concentration” (AC) and “sonication time” (ST) were optimized using a central composite design ($2^{\wedge 2}$+1 center point +4 axial points). Acid concentration varied from 5.0% to 57.5% (v/v) and sonication time ranged from 9.5 minutes to 26.5 minutes. The desirability function was used to determine the optimal values of the variables for the three elements simultaneously. A recovery rate of 100% was considered the ideal desirability value (d=1).

Results. The variable AC had a significant effect on the recovery rate of the three elements. The optimal level of acid concentration for global maximum desirability was 1,125% and the desirability value was 0.88. The effect of the variable ST was not significant for any of the three elements within the studied limits. Therefore, for this variable, any value within the studied range can be adopted, and a value close to the lower limit (9.5 minutes) will be chosen.

Conclusion. The application of experimental design minimized reagent usage and allowed the development of a procedure that is less time-consuming, more economical and more accurate. The optimal values determined for the formic acid concentration and the sonication time will be used to validate a rapid and simple method for the official control of trace elements (As, Cd and Pb) in milk by the Brazilian Ministry of Agriculture, Livestock and Food Supply.

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P11 PREPARATION OF THE MATERIAL FOR THE 24th EURL-CEFAO PROFICIENCY TEST ON HONEY

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Reference Laboratories have been designed at EU level to contribute to the achievement of high quality and harmonization of analytical results among the National Laboratories of the Member States: the organization of Proficiency Testing schemes (PTs) is an effective tool to achieve this objective. Matrices, analytes and their mass fraction should therefore be properly chosen to accomplish with this task, bearing in mind the inputs from the EU legislation and the new emerging issues.

In this framework, the European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO) organized for its National Reference Laboratories network a PT on the determination of lead, cadmium and mercury in honey (24th PT). Honey is a primary animal product included in the list of commodities to be analyzed in the National Residue Monitoring Plans (NRMPs) according to the requirements of the Directive 96/23/EC. Lead and cadmium are the most frequently analyzed elements in this matrix, while mercury has been included in the PT as its determination in matrices other than fish is one of the emerging issue in the NRMPs.

As for the starting material, commercial wildflower honey was chosen for its low viscosity and easy availability on the market. Since preliminary analysis had showed a basal mass fraction for the three elements lower than the limit of quantifications, an aqueous solution spiked with the three analytes was added to provide participants with PT test items at suitable mass fraction values.

Honey as bought (jars, 750g each) was pre-heated as 40°C for 30 minutes to enhance its fluidity and therefore its miscibility with water. The material was then divided in 2 plastic beakers (5L capacity), added of the spiking solution and left under stirring for 30’.

The content of both the beakers was then poured in a granite machine and stirred again before being distributed in the PT containers.

Ten out of the 120 items produced were analysed in duplicate to verify the sufficient homogeneity of the sample. The analyses were carried out by direct determination atomic absorption techniques. In particular, after sample dissolution in an aqueous mixture of 2.5 % HNO_3 (v/v) and 12.5% H_2O_2 (v/v), cadmium and lead were analysed by graphite furnace absorption spectrometry by means of an-hoc method, while Hg was assayed by a Direct Mercury Analyser. The sufficient homogeneity of the material was evaluated using a standard deviation for proficiency assessment (10% for Cd, 13% for Pb and 12% for Hg) set a priori by the EURL.

The homogeneity test was passed and the mean of the results was consistent with the planned values.

P12 SURVEY ON THE OUTCOME OF PROFICIENCY TESTINGS ORGANIZED BY THE EUROPEAN UNION REFERENCE LABORATORY FOR CHEMICAL ELEMENTS IN FOOD OF ANIMAL ORIGIN

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When starting the organization of Proficiency Testings (PTs), the main objective of the European Union Reference Laboratory for Chemical Elements in Food of Animal origin (EURL-CEFAO) was to assist the National Reference Laboratories of the EU Member States (EU-NRLs) in: improving the performance of their analytical methods around Maximum Levels (MLs), harmonising the statement of sample compliance according to Commission Regulation (EC) No 333/2007, and reducing the level of disagreement amongst the results produced by the laboratories.

Since 2006, a number of exercises has been organized, with a special focus on those matrix/analyte combinations of interest to the network and subjected to regulation. The recurring repetition of PTs on similar matrices (i.e. milk; meat; fish and offal) has allowed the laboratories to reach satisfactory and steady performances. Although the standard deviations for proficiency assessment (σ_{pt}) and the concentration values of analytes have been often reduced over the PTs, nevertheless the general performance of the network is still improving. For instance, a significant improvement is evident in the analysis of lead in freeze-dried meat, where the percentage of $|\text{z-score}|\leq 2.0$ moved from 76% (10th PT; 2006) to 81% (15th PT; 2011), with a reduction of unsatisfactory results (24% *vs* 7%).

Furthermore, the data dispersion has become more and more narrow over the PTs, owing to the decrease of the standard deviation of the mean. In particular, since 2010 this indicator of data dispersion has resulted comparable to or even lower than the σ_{pt} and close to the robust standard deviation, making evident the higher degree of harmonization of results.

As for the sample compliance assessment, on the basis of the declared uncertainties, the percentage of laboratories that submitted a correct statement has reached 100% (24th PT).

The adequacy of the EURL-CEFAO PT materials is confirmed by the elevated number of EU-NRLs using these samples for quality control, especially in case of matrix/analyte combinations for which no adequate CRMs exist (*e.g.* Hg in meat; As in milk).

The introduction of new matrices (infant formula and honey) and, in general, the lowering of the proposed values for analytes concentration have led to an increase of truncated or suspected data. Therefore, the reduction of truncated/suspected results, by means of the promotion of lower LoD and LoQ values, can be regarded as a prominent tool for a further improvement of the network performance.

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