

**ANNEX II**

**ACTION**





MINISTERIO  
DE SANIDAD, SERVICIOS SOCIALES  
E IGUALDAD

**aecosan**  
agencia española  
de consumo,  
seguridad alimentaria y nutrición



# EUROPEAN REFERENCE LABORATORY FOR MARINE BIOTOXINS

WORK PROGRAMME FOR THE EUROPEAN REFERENCE LABORATORY FOR  
MARINE BIOTOXINS

January-December (2016 and 2017)

Signed: Dr. Ana Gago-Martínez  
EURLMB Director

European Union Reference Laboratory for Marine Biotoxins (EURLMB)  
Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN)  
CITEXVI Fonte das Abelleiras s/n  
Campus Universitario de Vigo  
36310-Vigo SPAIN  
E-mail: [eurlmb@msssi.es](mailto:eurlmb@msssi.es)  
<http://www.aesan.msssi.gob.es/en/CRLMB/web/home.shtml>

## **Work Programme for the EU Reference Laboratory for Marine Biotoxins**

### **Objectives and work plan for the period January-December 2016**

The legal functions and duties of the Community Reference Laboratories for feed and food are described in Article 32(1) of Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

According to the functions and duties above mentioned the EURLMB presents this *Work Programme* compiling all the activities required with the aim of accomplishing the responsibility as European Reference Laboratory.

The activities of the EURLMB for 2016 and 2017 are presented here and described as follows:

#### **1. Coordination of activities of NRL network and provision of technical assistance and training**

##### **1.1. Organization of the XIX and XX Workshops of the European NRL network of marine Biotoxins**

Dates: to be determined (Oct. - Nov. 2016 and 2017)

Place: to be agreed during the XVIII and XIX meetings

Duration: 1,5-2 days

The EURLMB will organise and coordinate these workshops emphasizing the participation of the NRLs which will be requested to present a summary of their activities as NRLs.

The EURLMB will edit the minutes and conclusions of the meetings which will be distributed to the participants and published at the EURLMB Website. All the presentations and information of interest provided in the meeting will be also posted at the EURLMB Website.

The participation of invited experts in these workshops could be contemplated in case there are issues of particular interest where input from external experts could provide an added value to the conclusions and decisions to make

##### **1.2. Supply information to EU MS and third countries on analytical methods for marine biotoxins**

Duration: ongoing

In addition to on-going assistance, the EURLMB website is available at:  
<http://www.aesan.msssi.gob.es/en/CRLMB/web/home.shtml>.

Issues related with marine toxins legislation, methodologies, standard operating procedures, validation reports, seminars, meetings, minutes, conclusions and recommendations, as well as the activities of the EURLMB will continue to be published and updated at the EURLMB Website.

All the private information of the Network will be published in a restricted area of the EURLMB Website to which only NRLs can access using an specific code.

Additional information might be also supplied by email or any other possible source when requested.

### **1.3. Assist NRLs on the implementation, validation, accreditation of EU Reference methods for the analysis of marine biotoxins**

Support on issues related with analytical methods (Standard Operation Procedures, etc.) for marine biotoxins and their application will be facilitated. This support could be provided electronically, although visits of staff from NRLs to the EURLMB and viceversa when necessary could be also contemplated.

Duration: ongoing.

EURLMB will also assist NRLs on the implementation and validation of alternative analytical methods of interest, by providing them with technical information, standards and contaminated materials if available,, etc., at their request. Moreover, protocols for the analysis of the different groups of marine biotoxins regulated in the EU will be published in the EURLMB website, although any specific information or support related with the application of these protocols will be also directly provided to the NRLs when necessary.

### **1.4. Technical assistance to Third Countries on analytical methods**

EURLMB will assist Third countries with technical support on analytical methodologies or any other useful information about the analysis and control of marine biotoxins (analytical methods, legislation, etc.), The EURLMB might also provide samples from Proficiency Testing which can be used as secondary reference materials for own testing on methods performance. This will be useful for the validation and accreditation of methods for marine biotoxins detection. The provision of samples will depend on availability. Participation in Proficiency Testing could be also contemplated upon request and according to availability. Priority should be given to Third Countries with limited resources and involved in commercial trades with the EU . Laboratories from Third countries will be responsible for shipping expenses or any other additional fees.

### **1.5. Training activities for the staff of NRL MS and Third countries**

Trainings on methodological advances on Marine Toxins will be organised according to the NRLs needs. These needs will be discussed and identified at the 2015 annual NRLs Workshop that will take place in Cessenatico, Italy , kindly hosted by the Italian NRL in October 2015 . Two full trainings per year will be considered as the maximum number that EURLMB can offer.

The EURLMB is considering to propose a training on LC-MS/MS, mainly focused in dealing with specific method troubleshooting, extension to other matrices, calculations and expression of results etc. This training will contribute to harmonize practical concepts and to deal *in situ* with relevant issues of importance to ensure method performance. This will also help NRLs to deal with method accreditation with the aim of harmonizing criteria among EU NRLs. The same initiative could be also extended to LC-FLD methods for PSTs if this is considered an issue of interest among the EU NRLs Network.

Participants from Third Countries or from other non EU-NRLs laboratories interested in these trainings could be admitted, upon request and according to availability. These participants will be responsible for their own expenses

Training activities will be supported by the research team of the Analytical Chemistry and Food Department at the University of Vigo under the Contract signed between the AECOSAN and University of Vigo.

The participation of external experts might be also contemplated depending on the training activity and the specific issues involved in such training.

### **1.6. Meetings**

Meetings dealing with the above mentioned methodological advances on Marine Toxins could be also organised after the identification of the needs which will be also discussed at the 2015 annual EU/NRLs Workshop. These meetings will be organised and the participation of experts will be considered when appropriate.

The EURLMB has created an Advisory Board in which experts from the most active NRLs have been included. This Advisory Board will meet when necessary to discuss specific issues of particular relevance in the field, these issues could be considered for further discussion among WG or among the whole NRLs Network.

Priority will be given to meetings dealing with important issues on the analytical aspects of marine biotoxins including emerging toxins such as Ciguatoxins or Palytoxins, which are becoming an important concern in certain areas of the EU.

Collaborations with research groups with experience in the field, including potential visits of experts to the EURL (to attend seminars or meetings), or even visits of EURL staff to expert laboratories to collaborate on methods setting up or implementations, will be considered as a priority in the EURLMB working plan.

The possibility of organizing trainings and meetings on alternative methods of particular interest for the analysis of marine biotoxins might be also contemplated.

#### **1.6.1. Working groups (WG)**

##### **a) Working group focused on issues related with the application of analytical methodologies**

**a.1)** Particular issues related with the application of existing and implemented methodologies: new matrices, new validations as well as issues related with expression of results, uncertainty, accreditation etc, will be discussed among the WG. These issues might come as a result of previous discussions among the Working Group of bivalve molluscs organize by DGSANTE or as a result of discussions among the EURLMB/NRL Network. The named issues will be presented at the Annual Workshop (Cessenatico 2015) and the EURLMB might organize WGs according to their relevance which will be previously discussed among the EURLMB Advisory Board.

##### **a.2) LC-MS (for LPTs)**

The WG on LC-MS/MS for Lipophilic toxins will meet to continue with the work initiated in 2015 to establish criteria for an interlaboratory validation of an implemented LC-MS/MS method for LPTs extended to Domoic Acid, and Cyclic Imines

The validation will be designed among the WG and after a previous ring trial laboratories with adequate performance will be selected to participate in this validation.

An additional WG could be also organized depending on the progress of the validation.

As it was above mentioned the organization of any additional WG will be dependent on the interest expressed by NRLs during the 2015 Annual Workshop.

##### **b) Working Group on Phytoplankton control**

The Working Group on Phytoplankton control has initiated its activities in 2015 and after a first meeting in Brussels, an electronic Working Group has been created to work on the definition of particular issues.

The activity of this WG is considered an ongoing activity and the evaluation and conclusions of this initial work will be carried out on a physical Working Group meeting in 2016 and further meetings will be scheduled according to the conclusions of this meeting.

The electronic WG will be active during 2016 and the possibility of a second physical WG meeting, scheduled at the end of 2016 could be contemplated depending on the achievements of the electronic WG. The same approach will be followed in 2017

The participation of external experts in these WGs could be also contemplated. To progress in harmonisation of sampling protocols, pilot studies based on *in situ* measurements may be set up to test and compare the performance of the different methodologies proposed for discussion to the WG. This activity might be initiated by the end of 2016 and will continue along 2017.

## **2. Scientific advice and support**

### **2.1. Provide scientific assistance to DGSANCO**

The EURLMB will assist DGSANCO and FVO if it is required on issues related with the control of marine biotoxins in molluscs.

Any other scientific assistance of interest for DGSANCO must be also provided when requested.

**2.1.1.** Provide technical assistance in cases where the results of analysis are contested between Member States and/or Member States and Third Countries (when requested)

**2.1.2.** Technical assistance for CODEX Alimentarius, (participation in Working Groups, meetings, etc.) will be provided. Any other technical support requested by DGSANCO will be also facilitated

**2.1.3** The EURLMB participates on an ongoing activity of DG-SANTE through a Working Group on bivalve molluscs in which specific issues of interest related to their control (microbiological, biotoxins) are discussed.



The issues affecting marine biotoxins might require the debate through an specific Working group of experts among the EU NRLs. Examples of this are the issue of potential PTX de-regulation, analysis of processed sample for LPTs, Uncertainty, etc.

These Working Group meetings will be therefore scheduled during 2016-2017 according to the needs.

### **3. Proficiency Testing**

Proficiency Testing organised by the EURLMB for the NRLs are aimed at evaluating the ability of the NRLs to apply efficiently the recognised testing methods for marine biotoxins, for the purpose of Regulations (EC) Nos. 853/2004 and 854/2004 stated in Annex III of Commission Regulation (EC) N° 2074/2005. At the same time, the equivalence of the methods applied for the different toxin groups included in the EU Regulation is also evaluated. The EURLMB will organise trials for each of the regulated marine biotoxins group and the methodologies included in these PTs will be the ones officially included in the EU Legislation.

#### **3.1. EURLMB 2016 and 2017 Proficiency Testing for PSP toxins determination)**

EURLMB 2016 and 2017 Proficiency Testing for LPTs determination EURLMB 2016 and 2017 Proficiency Testing for ASP toxins determination

The schedule for the Proficiency Testing (registration form , samples dispatch, results submission and PT report) will be agreed by the EU-NRLs network and published at the “EU EURLMB/NRLs timetable of activities for 2016” ( the same for 2017) which will be circulated to the network in the first quarter of 2016 and 2017.

NRLs will receive the Programme and the application form to register on the PT trials. Information about the foreseen dates for samples dispatch and results submission and all the required documentation for the studies will be also provided.

Laboratories will follow the EURLMB Instructions and recommendations for the Tests and will confirm the reception of all the documentation. Results of the Proficiency Tests will be submitted to the EURLMB on the Reporting Files taking into account the established deadline.

The results on EURLMB--Proficiency Testing will be presented by the EURLMB during the Annual Workshop of the European NRL network of marine biotoxins, which will be scheduled in October (2016 and 2017), and discussed by the group of participants.

A final and detailed report including all the information about participants, aim of the study, schedule and instructions, materials preparation, methods used, evaluation of the results, etc. will be elaborated and edited by the EURLMB for each PT, and after circulation to the participants in the study, for comments and

revision, this report will be published at the EURLMB website on the restricted area for NRLs.

Follow-up actions will be taken with the aim of analysing the reasons for Laboratories underperformance when required. These follow-up actions will also help Laboratories to solve the problems encountered.

A follow-up report on the management, in cases of underperformance in the Proficiency Testing by NRLs, will be submitted to DG SANCO together with the EURLMB technical reports for 2015 and 2016 (planned for March 2016, 2017).

#### 4. Missions

- 4.1. Assist DGSANCO on working groups and meetings related to marine biotoxins. (ongoing participation in the Codex Alimentarius, FVO, USA Equivalence Project, etc)
- 4.2. Visit NRLs when necessary, in order to solve problems related with the application and performance of methodologies for marine toxins, or other related issues of interest (results after PT trials, **validation studies**, etc.) (see points 3 and 5). These visits could be also related with Phytoplankton control issues
- 4.3. Participate when requested in activities related to the involvement on the Working Group 5 ('Biotoxins') of CEN/TC 275 Technical Committee "Food Analysis–Horizontal methods" (European Committee for Standardization). The present activity of this Working Group is focused on two HPLC methods (one for ASP toxins and one for PSP toxins) which have been recently proposed to be considered as active work items. This Working Group is active at present and a meeting has been recently scheduled for the end of 2015. Further meetings could be also scheduled for 2016 and 2017 in which the participation of the EURLMB could be contemplated depending on the issues included in the Agenda.
- 4.4. Assist Third countries on technical issues upon request is considered and ongoing activity.
- 4.5. Participation in International Forums of discussion and scientific events (conferences, seminars, meetings, etc.) related with the advances on the field of Phytoplankton, Marine Biotoxins and their Analysis. The EURLMB will participate in meetings of special interest for the marine toxin field (AOAC, RAFA, Gordon Conferences, etc) . The participation on these meetings or any other scientific forum during 2016-2017 will be decided depending of the relevance of the issues and their interest for the advances in the control of marine biotoxins.

- 4.6. Visits of EURL staff to expert labs to implement methodologies for marine toxins (if required)

## **5. Research activities on the development of analytical and detection methods**

The research activities considered relevant for 2016-2017 are indicated below. These activities will be carried out with the direct support of the research team of the Analytical Chemistry and Food Department of the University of Vigo directly involved in the work carried out by the EURLMB under the Contract signed between the AECOSAN and the University of Vigo.

The activities indicated below are scheduled for 2016-2017, priority is given to research topics related with implementation of existing methodologies or other issues of relevance in the EU Legislation for marine biotoxins.

At this point is necessary to remark that the EURLMB will emphasize activities in accord with the agreements achieved by the different CODEX Committees involved in the control and regulation for marine biotoxins in seafood.

The transition from Biological to Chemical methods for the control of marine biotoxins prompted the need for investing additional R&D efforts in accommodating the chemical methods not only to the whole spectrum of seafood matrices where marine toxins could be present, but also to the toxin profiles relevant for the EU.

Emphasis is going to be made on an attempt to make available alternative and reliable analytical methodologies capable to detect and quantify the toxins present in these contaminated matrices as well as to develop methodologies for new and emerging toxins in the EU.

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According to this the R&D activities of the EURLMB during 2016-2017 will be focus on:

- 5.1. Setting up the Pre-trial for the International Validation of the extension of the LC-MS/MS methodology for multitoxin analysis and further work for the International validation set-up.
- 5.2. Development and optimization of alternative sample pretreatment protocols for the analysis of different groups of marine biotoxins in complex matrices.
- 5.3. Ongoing activities on the setting up, implementation and application of alternative assays (biochemical, biological, etc.) of particular interest for the analysis of Emerging toxins (in particular for Ciguatoxins, considered as priority emerging toxins in the EU), work carried out in collaboration with experts worldwide (USA, Japan) and other EU laboratories.
- 5.4. Continuation with ongoing activities related with the implementation of methodologies based on LC tandem mass spectrometry (LC-MS/MS) for the determination of Emerging toxins in the EU (Cyclic Imines, Ciguatoxins, Palytoxins). Collaboration with experts worldwide (Japan, other EU laboratories)

5.5. Setting up of LC-MS/MS for PSP Toxins

5.6. Continuation with tasks related with the Implementation of the HPLC-FLD post-column method (AOAC 2011.02 OMA) for the determination of PSP toxins to extend its applicability to additional PSTs profiles, such as *Gymnodinium Catenatum* profiles, typically present in some coastal areas of the EU (Spain and Portugal)

**6. Implementation of DG SANCO “Protocol for the management of underperformance in comparative testing and/or lack of collaboration of National Reference Laboratories (NRLs) with EURLs activities”**

6.1. Underperformance (i.e. failure in proficiency test). See Point 3. The EURLMB will carry out a follow-up of those NRLs that fails in the EURLMB-Proficiency Testing (2016-2017) for PSP, Lipophilic (LPT) and ASP toxins determination in order to identify the origin of this underperformance.

6.2. Re-enforcement of the collaboration between NRLs and EURLMB.

According to DG SANCO protocol, the EURLMB will emphasize the contact with NRLs by requesting information about NRLs activities also emphasizing their communication with their own Competent Authorities in particular about EURLMB discussions and activities.

The EURLMB will take special and individual follow-up actions with those NRLs for which a lack of collaboration is detected.

As it was above mentioned the EURLMB will follow the activities of the different EU NRLs by asking them to present their activities during the Annual Workshop of the European NRL network of marine Biotoxins. This will facilitate the EURLMB follow up and will also facilitate the interaction among NRLs and Competent Authorities since all these presentations will be posted at the EURLMB Website.

DG SANCO will be informed by EURLMB about all the issues and follow up actions taken as a result of the lack of performance or lack of collaboration of NRLs.

Note: This Work Program contents all the activities that are planned for 2016 and 2017, while activities such as Proficiency Testing, scientific advice and support are clearly defined according to the functions of the EURLMB, some of the activities included for 2017 are still tentative and will be dependent on the situation of marine biotoxins control at the end of 2016. Some new activities or modifications could be included for 2017 which will be notified to the EU Commission with the adequate explanation and justification.