General Public

Annual newsletter 2

EUROBIOTOX UPDATES

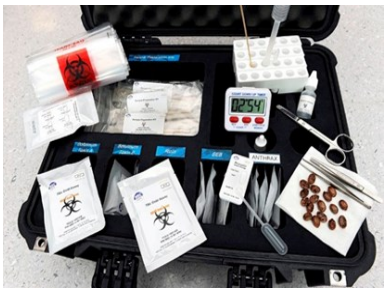
EuroBioTox

APRIL 2019

Eurobiotox project is progressing well

It is a great joy for all EuroBioTox partners to publish the second EuroBioTox annual newsletter describing the progression of the project, the past meetings and forthcoming events and highlighting a specific toxin: saxitoxin.

Biological agents can be misused as potential threat agents and recent incidents in Paris and Cologne showed that the threat posed by biological toxins is a burning issue. That is the reason why, within the frame of the European Commission call Horizon 2020 Framework Programme, our EuroBioTox project has been granted to improve the robustness in European preparedness for biotoxin incidents.



@CEA

Thirteen institutions from seven countries are responsible as core members for implementing the project and a total of 60 institutions from 23 countries are involved in the EuroBioTox network participating in different project activities.

The project was launched in June 2017 and will end in May 2022. So far, significant progress was made during the first year of the project.

Indeed, no relevant delay was noticed concerning tasks of any work package and each member is working hard to make this project a success.

We hope for the same success in the next years!

Face-to-face meetings

In order to increase and manage the progression of the project, to foster cross-fertilization of ideas and to make exchanges between inner partners easier, several face-to-face meetings have been set up at different locations.
First, a meeting between all thirteen core partners was organized by Queen's University of Belfast in May 2018. This meeting was very useful for all participants to discuss in detail on issues concerning each work package, the further proceedings and to make decisions for the future.

Meeting in Belfast *@EuroBioTox consortium*

The second face-to-face meeting with all 13 core partners will be organized this May at FOI (Sweden) to update all partners on the progress and next critical steps ahead the road.

Technical meetings to push forward the project

Meeting in Geel, Belgium *@EuroBioTox consortium*

Meeting in Umeå, Sweden *@EuroBioTox consortium*

Meeting in Saclay, France *@EuroBioTox consortium*

Meeting in Berlin, Germany *@EuroBioTox consortium*

Three additional small technical meetings were organized with different core partners in the first reporting period to foster technical exchange.

During the first one, EC-JRC, CEA, Anses, Toxogen, VBS-LS and RKI (six inner partners) met at EC-JRC in Belgium in September 2017 for three days. At this meeting, significant decisions were made regarding production and filling of reference material, proficiency tests and training course organisation.

Concerning the second meeting in Umeå, members of CEA, RKI and FOI met for three days in March 2018. Main discussions focussed on the characterisation of reference materials, the preparation of consolidated validation protocols for mass spectrometry (MS) and enzyme-linked immunosorbent assay (ELISA) methods, proficiency tests, training courses and in-situ detection.

The next meeting took place in Saclay at CEA in November 2018. Members of CEA, FOI and Verifin made significant progress on planning work package 6 that involves in-situ detection and validation of forensic procedures. After visiting the first responders of CEA, the organization and the content of a workshop for first responders was discussed; this workshop will be an important activity involving external partners and is scheduled for March 2020.

In January 2019, a meeting focussing on all activities on botulinum neurotoxins (BoNT) in the project took place in Berlin between the core partners CEA, Pasteur, Sciensano (former WIV-ISP), toxogen, ChemStat and RKI. An important part of this meeting was to plan and discuss on work package 7, which will evaluate different animal replacement methods for BoNT detection.

Training courses

In the framework of EuroBioTox, practical training courses arranged by expert laboratories are intended to strengthen the laboratories' technical capabilities for the analysis of biological toxins. The courses will enable networking and exchange of know-how, improvement of technical capacities and foster harmonization of analytical methods.

Saxitoxin detection

Basic training courses for the detection of saxitoxin via immunological and chromatographic methods were organised by the Institute for Global Food Security at Queen's University Belfast from 15-19th January 2018. Participants from seven scientific institutions from Denmark, Finland, Germany, Portugal, Slovakia, Sweden and United Kingdom attended the courses. The aim of the training course was to give the participants hands-on experience using commercially available kits that could be easily integrated into their own laboratories. Additionally, the high pressure liquid chromatography-fluorescence detection (HPLC-FLD) method was trained including information on sample preparation, data analysis and assay validation.

The Institute for Global Food Security and Verifin led the training course. The programme involved lectures on the theory behind the ELISA, Lateral Flow Device and HPLC-FLD methods and the sample preparation that was required for each method. The theory was followed by a practical demonstration and workshop which allowed the participants to prepare and test samples followed by analysis of results and interpretation. Following the workshop, an introduction was given on the proficiency test that was planned and organised by Queen's University in May / June 2018 for the detection of saxitoxin and information was provided for the participants on how to get involved. Based on this practical course on saxitoxin detection, all but one of the participants involved in the training took part in the proficiency test using the ELISA method trained during this course.

The results returned later from the proficiency test demonstrated that the participants were able to successfully introduce the trained methods in their laboratories – a nice success for participants and trainers!

SEB detection

A training course on basic immunological methods to detect staphylococcus aureus enterotoxin B (SEB) was organised by CEA and ANSES at CEA Saclay on the 13-14th and 15-16th of March 2018. Six scientific institutions were represented from Italy, Sweden, Poland, Slovakia and the Netherlands. The aims of this training were to give the participants an understanding of the different immunological methods to analyse SE containing samples and to implement this approach in their own laboratories as part of an overall analysis strategy. This training involved safety and security lectures as well as theoretical lectures on SEB and antibody production, immunological methods and an overview of qualitative methods for SE detection. The focus was laid on how to set up sandwich ELISA starting from monoclonal antibodies commercially available or from the EuroBioTox repository. Two different ELISA protocols and one lateral flow immunoassay (LFA) were trained. Tips on interpretation of results were then given to the participants. The feedback from the trainees was very positive.

Ricin/Abrin detection

Another training course for basic immunological methods to detect ricin and abrin was organised by RKI and CEA at RKI Berlin from 20 to 22 November 2018. Five scientific institutions were represented from Sweden, Norway, Finland and the Netherlands. The aims of the training course were, as mentioned before, to teach the participants how to perform the analysis of ricin and abrin toxins in complex matrices based on different immunological methods and to implement these approaches in their own laboratories as part of an overall analysis strategy. The training involved safety and security issues, theoretical lectures on ricin and abrin, advantages and limitations of monoclonal and polyclonal antibodies and their production as well as different immunological methods and their value in the context of other methods such as functional and spectrometric approaches. The focus was laid on how to set up sandwich ELISA starting from monoclonal antibodies available from the EuroBioTox repository, biotinylation of antibodies and how to analyse and interpret results. As special focus was laid on assay controls important to recognize matrix effects. Two different ELISA protocols, one from CEA and one from RKI, were introduced to the participants. After the training, which was highly appreciated by the participants, all of the trainees now plan to use the trained methods in the next proficiency test organized on ricin and abrin detection (scheduled for September 2019).

About EuroBioTox website

EuroBioTox partners are happy to announce that the EuroBioTox website is accessible with an open access to general public since January 2018. This website provides background information on the EuroBioTox project, on the thirteen core project partners, on the science of the toxins including scientific publications, related events, and hyperlinks to related projects. Come and visit this website regularly to learn more about the progression of our project !

Interim report

All network activities, results obtained and materials generated in the different work packages were described in an interim report and evaluated by the European Commission. A review meeting took place in Brussels in September 2018 where the consortium presented all the activities carried out so far including the deliverables submitted, the project management and use of resources, ethics issues as well as dissemination and communication activities.

Thanks to an excellent coordination by RKI and very active contributions by the core partners, the reviewers and experts from the Commission were highly satisfied with the progress made in the first 12 months. All scheduled tasks have been completed on time!

Proficiency tests

The very first proficiency test under EuroBioTox focussed on saxitoxin and was conducted in May/ June 2018, organized by QUB, UK. Ten samples (comprising shellfish, urine and standard solution) containing different concentrations of saxitoxin were analyzed by 17 laboratories. Individual evaluation reports were sent to all participants. Qualitative and quantitative results provided by participants were relevant to highlight the status quo and potential for technical improvement. Furthermore, information on good analytical practices, available tools, reagents and protocols were collected.

A second proficiency test on SEB detection was implemented in November 2018 by Anses, FR. Results obtained by the 22 participanting laboratories were already sent to the organiser and are currently under analysis.

Nine more proficiency tests will be organized within the next four years by eight organizers on the other toxins included in the project.

The next proficiency test is scheduled to be organized on ricin and abrin detection by RKI, DE, with sample dispatch in September 2019. We will inform all EuroBioTox partners before the summer break on the timeline.

WIV-ISP becomes Sciensano

On the first of April 2018, the Scientific Institute of Public Health (WIV-ISP) - which is one of our inner partners- and the Veterinary and Agrochemical Research Centre (CERVA-CODA) merged into a new federal research centre: Sciensano. The new institute counts more than 700 employees who work every day for a lifetime in good health!
Sciensano finds its foundation in the concept of 'One Health', whereby human health, animal health and the environment are intrinsically linked and in constant interaction. Sciensano draws its strength and specificity from the combination of different complementary and related scientific disciplines, and from its approach integrating several research angles.

The toxin units of both institutes are joined together at the Tervuren-site to strengthen the toxin research within Sciensano. Sciensano mission remains unchanged for the EuroBioTox project. We wish them a prosperous future !

sciensano

Ricin : Cologne case

@RKI/BKA

In June 2018 an Islamic extremist, Sief Allah H., has been arrested after a police raid which delivered, among others, large quantities of Ricinus communis seeds in his apartment. The suspect has indeed succeeded in manufacturing 83.4 mg ricin!

The suspect had bought more than three thousand castor beans and potential manufacturing devices to produce the toxic biological toxin. Investigators believe he was working with explosives to build an improvised explosive device containing ricin.

It is the first time in Europe that a suspected terrorist successfully produced ricin. Police found that this incident was possible because the suspect was following instructions from the internet and by contact to other persons. Along the same line, an investigation against two Egyptians in France in May 2018 led to the arrest of one of them for planning an attack on the French territory, and ricin was an option for this act.

More recently, in December 2018 another case involving plant toxins was reported: a German tourist bought a "pepper mixture" on a Tunisian street market and it turned out that some of the "peppers" were actually Abrus precatorius seeds .

In all three cases nobody was harmed but these incidents highlight the importance of our EuroBioTox activities: expert laboratories need to keep their vigilance high!

Ref :

https://www.generalbundesanwalt.de/de/showpress.php?themenid=20&newsid=788

http://www.ua-bw.de/pub/beitrag.asp?subid=2&Thema_ID=2&ID=2862&lang=DE&Pdf=No

Certified reference material

Significant progress was made in 2018 regarding the production of certified reference materials. The technical issues encountered have been resolved and three of the materials are far in progress. SEB and ricin reference materials are now already produced and filled. Concerning the BoNT/A the filling process is planned for the this spring. The characterisation of all three reference materials is ongoing.

We hope that the production of the next reference materials are running smoothly too !

Save the dates !

CBRNE Conference

The third international CBRNE conference will take place in Nantes (France) between the 20 to the 23 May 2019.

During these days you will have the opportunity to update your knowledge on future trends & new technical challenges on CBRNE! The first day will be dedicated to responders' feedback and expression of needs. The following days will focus on scientific updates. This will also include workshops and presentations of innovative materials and technologies. Several EuroBioTox partners will be represented at the conference with posters and presentations.

Workshop for first responders

A three-day workshop on biological toxins and on-site detection for first responders is planned between 03 to 05 March 2020.

During this event several important topics on in-situ analysis will be discussed. The first day will be focused on a general introduction to toxins and threat assessment. For the second day a practical demonstration of recommended procedures for first responders will take place. Then, the third day will be dedicated to presentations of innovative materials and technologies.

For all of you interested in the two topics, please save the dates and let's meet personally.

General overview

Saxitoxin (STX) is a biological toxin at the interface of classical biological and chemical agents - it is a prohibited substance under both the Chemical Weapons Convention (CWC, Schedule 1) and the Biological Weapons Convention (BWC). Furthermore, produced by marine algae and freshwater cyanobacteria, STX is among the toxins considered a potential biothreat.

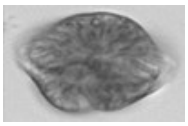
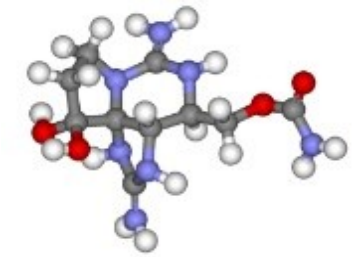


Image of a PST producing dinoflagellate
@Anke Kremp, Finnish Environment Institute

STX is produced by algae of the Alexandrium, Gymnodinium, and Pyrodinium genera; *A. tamarense, A. catenella and A. minutum* are among the most common algal producers of the toxin. Bivalve molluscs consume these dinoflagellates and accumulate paralytic shellfish toxins (PSTs) in their tissues. No known negative impacts of these compounds to shellfish occur, although consumption of contaminated shellfish by humans can lead to severe illness and death. PSTs block neuron transmission by binding to the voltage-gated sodium channels and inhibit sodium ions from entering cells, which prevents nerve cells from producing action potentials. This decrease in action potential can lead to perioral paresthesia, dizziness, paralysis, respiratory arrest, and death.

It may also contaminate drinking water, thus causing severe health problems. Numerous fatal cases of paralytic shellfish poisoning (PSP) have been reported globally, but the improved monitoring of microalgae and PSP toxins in shellfish has decreased the risks. STX is not living and does not have the ability to reproduce, it therefore shares many of the characteristics of classical chemical agents, such as stability under high heat and in acidic conditions. STX is a low molecular-weight biological toxin and is the most poisonous non-peptide marine toxin accumulating in seafood.

Chemical structure of STX



The best known Paralytic shellfish poisoning toxin is saxitoxin. At least 57 different analogues of saxitoxin divided into several structurally differing subclasses are listed in literature. Further 20 PSP toxins which have been isolated to date, show a high structural similarity to saxitoxin. PSP toxins are soluble in water, methanol and ethanol, but not in other organic solvents. They remain stable in slightly acidic environments, but tend to oxidise when exposed to alkaline conditions. The reference material is commercially available only for STX and some of its analogues making the analysis very complicated.

Strategies for Saxitoxin analysis

The mouse bioassay (MBA) was the internationally accepted method for the determination of paralytic shellfish toxin levels prior to commercialization of shellfish products for over 40 years and was used to provide a regulatory limit, 80 µg/ STXeq/100 g (EC Regulation 853/2004) in seafood but it could not be applied for detection of drinking water where the health alert level is calculated to be 3 µg/STXeq/one litre of water. The MBA has recently been replaced as the internationally accepted method by the pre-column oxidation LC method (AOAC Official Method℠ 2005.06).

While the MBA provides reliable information on the overall toxicity of a sample, no data as to the individual STX analogues contributing to the toxicity are available using this approach. Poor sensitivity and concerns over the number of live animals that are required for testing have been cited as the major reasons for the replacement of the MBA. The AOAC Official Method℠ 2005.06 method resolves the issues encountered with the MBA but is laborious and analytical standards are difficult to obtain. Oxidation of PSTs is required for HPLC with fluorescence detection because they lack a chromophore. Oxidation using both peroxide and periodate is required for the pre-column oxidation method resulting in the requirement for two analytical runs. Methods have now been established using mass spectrometry instrumentation and are undergoing validation studies which would allow for a faster, more efficient monitoring process. However, considering their high cost and the need for skilled scientists, physico-chemical methods are more suitable for confirmatory analysis than screening methods.

Screening prior to release of shellfish to markets require sensitive, cost-effective, rapid, simple methods accurate in the determination of PSTs. Rapid screening using immunological techniques have been developed to provide this service in the form of commercially available ELISA kits and Lateral Flow Devices, however, these methods should only be used in parallel with the confirmatory method and could not replace it.

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https://eurobiotox.eu/

https://cordis.europa.eu/project/rcn/209945/factsheet/de