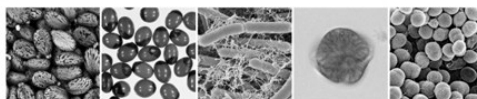




General Public

Annual newsletter 1

EuroBioTox



YEAR 1, N° 1

FEBRUARY 2018

EUROBIOTOX UPDATES

EuroBioTox successfully launched

It is a great joy for EuroBioTox partners to publish the very first EuroBioTox annual newsletter, aiming at describing the project and its progress, the different meetings and forthcoming events, and to shed light on different analytical techniques, participating countries, toxins and so on.

The need of a new project emerged after EQuATox results in 2015, whose objective was to evaluate the preparedness of European countries to bioterrorist threats. The findings of the EQuATox project showed that there is need to strengthen preparedness to biotoxin incidents. This can be achieved by supplying common tools for quality control, method validation, standardised procedures and refer-

ence materials. Within the frame of the European Commission call Horizon 2020 Framework Programme our EuroBioTox project has been granted to overcome these issues. Thirteen institutions from 7 countries are responsible as core members for this project.



Additionally the EuroBioTox network involves a total of 60 institutions from 23 countries.

Focus will be on detection and identification of 5 biological

toxins: staphylococcal enterotoxin B, botulinum neurotoxins A and B, ricin, abrin and saxitoxin.

The objective is to complete this project within 5 years starting in June 2017 and ending in May 2022. Within the project certified reference materials, reagents and tools will be produced, practical trainings and subsequent proficiency tests will be organised. *In-situ* detection and forensic procedures will be developed and validated, as well as alternative methods to replace the animal test for BoNT by a more ethical method.

We wish all of the participants great success in their involvement!

Let's kick it off!



The kick-off meeting held at Robert Koch Institut (RKI) in Berlin, Germany last June, had a great success. All 60 organisations were represented during this 2-day meeting, followed by another 2-day meeting gathering the core members. All aspects of the project were introduced by the coordinator of the project Brigitte Dorner from RKI as well as by the work package leaders of the project. Partners got the chance to meet and discuss with their peers in a casual but conscientious atmosphere.

7 countries, 13 organizations

RKI in Germany

The RKI is the central federal institution responsible for disease control and prevention in Germany and acts as reference institution for applied, response-orientated research and for the Public Health Sector. The Biological Toxins division is appointed as German consultant laboratory for neurotoxin-producing clostridia (botulism, tetanus). The tasks include diagnostics of toxins of microbial and plant origin with relevance to bioterrorism using immunological, cell-based, mass spectrometric and genetic engineering techniques. The division is accredited for diagnostics of ricin and botulinum neurotoxins. The group provides reference materials, diagnostic tools (antibodies), SOPs and organizes proficiency tests in order to introduce quality assurance measures in diagnostic assays. Additionally, strategies for decontamination of relevant toxins are developed. A main research focus is the pathogenesis of toxin-induced diseases.

Toxogen in Germany

Toxogen GmbH, a privately owned small enterprise, offers world-wide diagnostics services and contract research in the field of botulinum and tetanus neurotoxins, e.g. i) Determination of the potency of BoNT solutions according to European Pharmacopoeia, ii) Determination of potency of BoNT antitoxin according to European Pharmacopoeia, iii) Determination of neutralising anti-BoNT antibodies in patients sera following GCP regulations, iv) Detection of BoNT/A-H in clinical matrices (serum) and selected food/ environmental samples.

EC-JRC in Belgium

The Joint Research Center of the European Commission, Directorate F – Health, Consumers, and Reference Materials has the mission to promote a common European measurement system in support of European policies. JRC-F is one of the world's largest reference material producers, provides international measurement evaluation, training programs and is assisting the National Reference Laboratories in the EU Member States to maintain a high level of measurement quality. JRC-F's Unit for Reference Materials is accredited for the production of a large range of certified reference materials and various testing capabilities. The institute has a long-standing expertise in collaborative research, the organisation of international inter-comparisons and method evaluation campaigns.

WIV-ISP in Belgium

Scientific Institute of Public Health (WIV-ISP) is the Belgian federal reference center for scientific expertise on public health aspects, food safety and environmental safety. It conducts bottom-up research (detection, standardisation etc.) as well as surveillance and monitoring activities. Two accredited units are involved in the project and work complementary. Unit 1 "Chemical Residues and Contaminants" acts as the National reference Laboratory for chemical analyses of Toxins and Unit 2 "Foodborne pathogens" plays a role in the National /International monitoring and reference laboratories network for the pathogens transmitted to humans through food and the collection of data in case of food poisoning.

ANSES in France

The French Agency for Food, Environmental and Occupational Health & Safety undertakes monitoring, expert assessment, research and reference activities in a broad range of topics that encompass human health, animal health and well-being, food safety and plant health. SBCL unit deals with three bacterial hazards: Staphylococci, Bacillus and Clostridia, covering the entire biological spectrum from bacterial isolation/enumeration to toxin production including molecular characterization of bacterial strains. Tool box used in the unit includes various methodologies such as classical microbiology, typing methods (MALDI-ToF, PCR, RT PCR, PFGE, WGS) and immune enzymatic as well as mass spectrometry based methods for toxin characterisation.

CEA in France

The French Alternative Energies and Atomic Energy Commission is a key player in research, development and innovation in four main areas: defense and security, nuclear, technological research for industry and fundamental research in the physical and life sciences. The Pharmacology and Immunoanalysis (SPI) unit has an expertise in analytical methods based on immunoanalysis and mass spectrometry, especially for toxins and pathogens (handled in a BSL3 laboratory) of the CBRN risk, and in the implementation of analytical methods to support drug development, biomarker discovery and CBRN detection. One field of activity is the use of MS-based approaches to quantify protein biomarkers, recombinant proteins or toxins/

pathogens in complex matrices.

Institut Pasteur in France

The Institut Pasteur (IP) is a well-known international institution involved in basic research in biology, notably in microbiology and infectious diseases, and has a key role in teaching, training, as well as in public health, medicine, and translational research. The Anaerobic bacteria and toxins Unit in Pasteur Institute is involved in research projects in *C. botulinum* (regulation of the toxinogenesis in *C. botulinum*, passage of the botulinum neurotoxin through the intestinal barrier). One part of the laboratory called National Reference Center for anaerobic bacteria and botulism is in charge of diagnosis of human and animal botulism in France and of development of diagnosis methods.

VERIFIN in Finland

VERIFIN is an international institute in analysis of chemical warfare agents at University of Helsinki, which is the oldest and largest institution of academic education in Finland, with 40,000 students and researchers. In international university rankings, the UH typically ranks among the top 100. VERIFIN is performing research on sample preparation, development of reference chemicals, and analytical methods. VERIFIN is strongly focusing on analysis of environmental and biomedical samples to develop tools for verification of the Chemical Weapons Convention. Other areas of activities of VERIFIN include training. VERIFIN is an accredited testing laboratory with the flexible scope "Environmental/ Material and product/Clinical testing, Chemistry, Verification of chemical weapons". VERIFIN is one of the designated laboratories of the OPCW for both environmental and biomedical samples. VERIFIN is coordinating international work to create "The Recommended Operating Proce-

dures for analysis in the verification of chemical disarmament".

FOI in Sweden

The Swedish Defence Research Agency, FOI, is an assignment-based authority under the Ministry of Defence. Core activities are research and development of technology for the benefit of defence, security and safety. The Division of CBRN Defence and Security focuses on CBRN issues and has a broad base of interdisciplinary competence in the field. The Analytical Chemistry Group has extensive experience in characterisation and identification of organic compounds, including Chemical Warfare Agents, toxic chemicals, pesticides, biomolecules and biological toxins. The laboratory is an OPCW designated laboratory.

ChemStat in Switzerland

ChemStat is a privately owned small firm that offers consulting in the field of applied statistics and chemometrics, including training for scientists, planning and design of experiments, stochastic modelling and data analysis, statistical quality control and support in publication of scientific work involving statistics. ChemStat evaluates Proficiency Tests on a regular basis for Agroscope (CH) and was responsible for most of the statistical work in the EU FP 7 project EQuATox.

Spiez laboratory in Switzerland

Spiez Laboratory, the Swiss Federal Institute for NBC-Protection, is a division of the Federal Office for Civil Protection that provides services relating to arms control, protection measures, health and incident management for international organisations, authorities and the general population. It contributes scientifically to peace support and to the safety of humans and the environment.

ZHAW in Switzerland

The Centre for Biochemistry and Bioanalytics at the Institute of Chemistry and Biotechnology of Zurich University of Applied Sciences (ZHAW) has been demonstrating profound knowledge in bioanalytical methods for the detailed characterisation of glycoproteins, with a variety of HPLC and capillary electrophoresis methods. In particular, methods for the detailed characterisation of glycoproteins and glycans with LC-MS (ESI-Q-TOF) are established for industrial partners, academic collaborators and Swiss government organisations. For over 10 years the group has been collaborating with the Spiez Laboratory on the bioanalytics of toxins.

QUB in United Kingdom

Queen's University of Belfast, a member of the Russell group - a group of 24 leading UK teaching and research intensive universities, is ranked No.1 in the UK for Agriculture, Veterinary and Food science for research intensity. The Institute for Global Food Security (IGFS) is the core unit of Agriculture, Veterinary and Food science research at the university. One mission of the IGFS is to nurture world's leading scientists in food security, and to build a food-fortress, ensuring the Integrity of the Food Supply Chain throughout Europe and beyond to be 100% safe, nutritious and authentic. The institute hosts a state of the art ASSET (Assured Safe Traceable Food) Technology Centre comprising state of the art analytical and bioanalytical tools for the monitoring and analysis of chemical and biological contaminants pertinent to human and animal health through contamination in soil, water or food.



toxins

Toxins, EQuATox special issue

The special issue "Detection and identification of biological toxins in international proficiency tests" published in *Toxins* (2015), an open access toxinology journal, highlighted the results obtained in the EQuATox project, i.e. the status of toxin detection focusing on ricin, saxitoxin, SEB and BoNT.

For each of the four toxins, a series of manuscripts describes the reference materials produced in the project, as well as the results of dedicated international proficiency tests taking into

account the balance between information sharing and confidential issues. Additionally, for each toxin different articles provide information on good analytical practices identified in the exercises. In this context, the data presented provides a solid basis and a starting point for future advancement in quality assurance efforts.

EQuATox gathered expert laboratories from the security, verification, health and food sector - 35 laboratories from 20 countries

worldwide. Ten author organisations carried on with EuroBioTox as inner partners: RKI, Toxogen, EC-JRC, ChemStat, CEA, FOI, WIV-ISP, Spiez laboratory, VERIFIN and ANSES.

Also, CDC (Atlanta, USA), NIBSC (UK), Public Health Agency and University of Manitoba (Winnipeg, Canada), Ghent University (Belgium), Marine Institute (Ireland) and Finnish Environment Institute (Finland) published in this issue.

"EuroBioTox website will soon be accessible with an open-access to general public."

So far, so good!

Our labs have been working hard to actively contribute to the project. Our consortium agreement is now drawn up and aspects related to quality and information restriction are outlined. Main information given during the kick-off meeting about the project design is available in the minutes. Core members responsible for the delivery of reference

materials are in the production phase or about to finalise the production of some of them. The production of monoclonal antibodies as toxins immunodetection tools is still on-going. Contents and schedules of the available training courses were disseminated to the outer partners who started booking their places and some of the courses are

already fully booked. Basic training courses on immunological and chromatographic detection methods of saxitoxin were given in January 2018; basic training courses on SEB immunodetection will be given in March. EuroBioTox website will be accessible mid-February with an open-access to general public (<https://eurobiotox.eu>).

Proficiency tests

Eleven proficiency tests (PT) will be organised under EuroBioTox within five years by eight organisers. This scheme is composed of two exercises per toxin, as well as one exercise focusing on on-site detection. The individual PTs will focus on the analysis of toxins and will target both qualitative and quantitative detection. Toxins will be spiked at different concentrations in

buffer as well as in selected clinical, food and environmental samples. The statistical analysis of anonymous result data will subsequently be performed according to internationally accepted standards. The results of the different technologies will be used as indicators for the quality of the different approaches. Also, results of the first PT round will guide on the contents of both the

subsequent PT and the subsequent training contents to make sure that good analytical practices will be spread and trained. All together these measures aim at further improving technical capabilities of the laboratories.

The very first PT will start on saxitoxin in June 2018, organised by QUB, EC-JRC and VERIFIN. We wish good luck to all participants!

Trainings

The former EQuATox consortium highlighted the need for a laboratory network to spread and share the information about available methods and reagents. Also, some of the laboratories that participated in the study had difficulties in reporting results and sample preparations and some applied techniques lacking sensitivity and/or specificity. Therefore, practical training courses arranged by expert laboratories are intended to increase the technical capabilities

of the laboratories for the analysis of biotoxin samples through networking and exchange of know-how, improvement of technical capacities and harmonization of analytical methods. Selected biological toxins have distinct features and analytical methods vary greatly, with technical challenges notably due to the potential high number of isoforms. Therefore, EuroBioTox is organizing basic and advanced training courses that will concentrate on teaching of

the various methods for qualitative and quantitative analysis. The training courses are offered on different immunological, mass spectrometric, chromatographic and functional analysis methods together with sample preparation techniques. Practical issues such as safety and security issues as well as assay validation will also be addressed. The first training on saxitoxin was given in January, the next one will be given in March 2018 on SEB.

National/International events

Biotox-Piratox network seminar

The french national Biotox-Piratox laboratory network, as part of the national CBRN-E plan, aims at supplying authorities with proper analytical tools and procedures in case of any kind of nuclear, radiological, biological, chemical or explosive threats.

In this context and as part of this network, J-A Hennekinne from ANSES and M-R Popoff from Institut Pasteur presented the contributions of their labs in the EuroBioTox project, during the 12th seminar of the network that took place at Institut Pasteur in Paris, last September. Both labs are involved in the preparation of reference materials and organisation of proficiency testing trials (toxin-spiked biological and environmental samples), where ANSES deals with recombinant staphylococcal enterotoxin B (SEB) while Institut Pasteur deals with Botulinum toxin (BoNT). Representative types and sub-types of BoNT will be prepared followed by mouse bioassay calibration; replacement of mouse bioassay by *in vitro* tests will also be assessed.

Security symposium

The 11th symposium of the interdisciplinary workshop on global security was held at the University Pierre and Marie Curie in Paris last September. Different sessions highlighted subjects such as detection and identification tools, needs and inputs of basic research, presentation of on-going European calls. CEA was represented by Laurent Olmedo.

IBRCC 2017

The Interagency Botulism Research Coordinating Committee (IBRCC) meeting is an annual forum for presenting state-of-the-art research on botulinum toxin, whose fundamental purpose is to provide coordination between federal and non-federal agencies to improve public health responses and medical counter-measures. It was held in San Francisco last October where RKI, Institut Pasteur, Toxogen and WIV-ISP were present. IBRCC 2017 gathered about 90 attendees from US, Japan, Germany, France, UK, Finland, Belgium, Italy, Sweden, and included 8 sessions from

epidemiological/clinical aspects of botulism, countermeasures, detection/diagnostics, to basic science. Recent outbreaks of food borne botulism and infant botulism in the US were discussed. The diversity of botulinum toxin (BoNT) types and subtypes, as well as the properties of novel subtypes (A6, BoNT/X, BoNT/H) were shown. Counter-measures described novel strategies to produce neutralising antibodies against BoNTs using individual polyvalent or multiple engineered recombinant antigens for immunisation of horse or alpaca as well as small inhibitor molecules of BoNT enzymatic site. For example, an original method based on recombinant atoxic BoNT linked to a single chain antibody derived from an alpaca immunised with BoNT/A light chain was developed for the intraneural treatment of BoNT/A intoxication. Sensitive *in vitro* methods such as ELISA, lateral flow immunoassays, bioluminescent biosensor, biosensor derived from CANARY (cellular analysis of antigen risks and yields) technology, endoprotease assays and detection of cleaved substrates by ELISA or mass spectrometry

« The IBRCC meeting is an annual forum for presenting state-of-the-art research on botulinum toxin »

are developed in replacement of the mouse bioassay for the detection of BoNTs and diagnosis of botulism. An original endoprotease method allowing increased sensitivity consists in using recombinant multimeric substrates. A sensitive cell based assay accounting for all the steps of BoNT intoxication was described in SiMa cells with re-engineered VAMP molecules. The two sessions on basic science addressed various aspects of BoNTs and *C. botulinum*: intestinal absorption of BoNTs, interaction between BoNTs and associated proteins, characterisation of hemagglutinin negative progenitor toxins,

intracellular stability of BoNT light chain, spore germination, sporulation and toxinogenesis, regulation of BoNT synthesis at single cell level, *C. botulinum* engineering by CRISPR/Cas9 technology.

CWC 20th anniversary

In December 2017 VERIFIN organized the International Work-shop on Analysis of Chemical Warfare Agents to mark the 20th Anniversary of the CWC. During the meeting the new « Blue Book » was also announced. The Blue book, edition 2017 - Recommended Operating Procedures for Analysis in the Verification of Chemical Disarmament is an updated

version of the recommended operating procedures (ROPs) for the Chemical Weapons Convention-related analysis. Chapters were revised or newly written in collaboration with expert laboratories working in the field. The Blue Book 2017 version is available from VERIFIN as a hardcopy and pdf version (www.verifin.helsinki.fi).

Future events

Future events highlighting technical and scientific issues or solutions, regulations, security around CBRN will be held in 2018. A list of them is given below.

Save the dates

Event	Date	Place
Munich Security Conference 2018 ⁽¹⁾	16 - 18 February	Munich, Germany
UK Security Week ⁽²⁾	6 - 7 March	London, UK
Security and Defense 2018 ⁽³⁾	12 - 13 March	London, UK
CBRNe Summit Europe 2018 ⁽⁴⁾	17 - 19 April	Rome, Italy
Belfast Summit on Global Food Integrity ⁽⁵⁾	28 - 31 May	Belfast, UK
10TH SYMPOSIUM ON CBRNE THREATS ⁽⁶⁾	4 -7 June	Rovaniemi, Finland
Biodefense World Summit 2018 ⁽⁷⁾	27 - 29 June	Bethesda, MD, USA
CSCM 2018 world congress ⁽⁸⁾	2 -6 September	Cavtat, Croatia
World CBRN & Medical Congress (CEBIRAM) 2018 ⁽⁹⁾	17 - 19 October	Prague, Czech Rep
16th Medical Biodefense Conference ⁽¹⁰⁾	28 - 31 October	Munich, Germany

(1) <https://www.securityconference.de/en/>; (2) <https://www.uk-security-week.com/>; (3) <https://www.chathamhouse.org/security2018>; (4) <http://www.intelligence-sec.com/events/cbrne-summit-europe-2018>; (5) <https://www.qub.ac.uk/sites/ASSET2018Summit/>; (6) <http://nbc2018.org/?lang=en>; (7) <http://www.biodefenseworldsummit.com/> (8) <http://www.cscm-congress.org/cscm-2018>; (9) http://www.future-forces-forum.org/events/default/19_cebiram-2018?lang=en ; (10) <https://conference.instmikrobiobw.de/index.php?id=1>