OPCW **Scientific Advisory Board**

Thirty-Third Session
15 – 18 November 2021

SAB-33/WP.2
14 February 2022
ENGLISH only

SUMMARY OF THE THIRD MEETING OF THE SCIENTIFIC ADVISORY BOARD'S TEMPORARY WORKING GROUP ON THE ANALYSIS OF BIOTOXINS

1. AGENDA ITEM ONE – Opening of the meeting

1.1 The Temporary Working Group (TWG) on the Analysis of Biotoxins of the Scientific Advisory Board (SAB) held its Third Meeting from 10 – to 12 November 2021 in a virtual format. The meeting was chaired by Dr Daan Noort on behalf of the SAB, with Dr Suzy Kalb as Vice-Chairperson.

1.2 The TWG Chairperson opened the session welcoming TWG members to the third official meeting of the Group

2. AGENDA ITEM TWO – Adoption of the agenda

The TWG adopted the following agenda for its third meeting:

1. Opening of the meeting (*TWG Chairperson*)
2. Adoption of the agenda (*All*)
3. *Tour de table* (*All*)
4. Subgroup Readouts / Updates (*All*)
5. Analysis of plant toxins in food supplements: a multi-target LC-MS/MS method (*Dr Els Van Pamel*)
6. Recent and ongoing exercises on biotoxins under different frameworks (*Dr Brigitte Dorner*)
7. Combining affinity-based enrichment methods with LC-MS analysis (*Dr Thomas Bergstrom*)
8. Quality assurance for high molecular weight toxins relevant in the food chain (*Dr Jacques-Antoine Hennekinne*)
9. United Nations Secretary-General's Mechanism (UNSGM) (*Dr Christine Uhlenhaut*)
10. Toxins in Food (*Dr Arjen Gerssen*)
11. Subgroup breakout sessions (*All*)
12. Final discussion, comments and next steps (*All*)
13. Closure of the meeting (*TWG Chairperson*)

3. AGENDA ITEM THREE – *Tour de Table*

Given the participation of several external speakers, it was decided that each TWG member, guest speaker, and Technical Secretariat staff member should introduce themselves and provide a short background.

4. AGENDA ITEM FOUR – Subgroup Readouts / Updates

4.1 Before the subgroup leads provided updates on their ongoing work, the SAB Secretary noted that the report from the TWG's Second Meeting had been finalised and could be found on the OPCW's website.[1] The Chairperson then invited each of the subgroup leads to provide an update on their progress to date, noting not just their progress but also any outstanding work and any challenges they have. Subgroups 1, 2, and 3 noted they had progress to report and the floor was given to them each in turn. The overarching questions being considered by each subgroup are:

(a) What are the underlying requirements for the analysis of biological toxins in order to investigate alleged use of toxic chemicals as weapons? (subgroup 1)

(b) What classes of biological toxins are most likely to be relevant in investigations of alleged use? (subgroup 2)

(c) Are there other relevant compounds of biological origin that should also be considered based on their potential for misuse or technological change associated with them? (subgroup 2)

(d) What are the technical requirements for analysis of the most relevant types of biological toxins? (subgroup 3)

(e) What are the analytical standards and requirements of other international and national investigative authorities and how do these compare and/or factor into OPCW considerations and operations? (subgroup 4)

(f) How can programmes of analytical exercises conducted by different networks of laboratories be coordinated or harmonised to minimise duplication, promote consistent practices, and develop a comprehensive picture of laboratory capabilities? (subgroup 4)

(g) What institutional or legal measures need to be established to facilitate cooperation between the OPCW and other organisations working on the development of capabilities for the analysis of biological toxins? (subgroup 5)

Subgroup 1

4.2 Dr Clark (subgroup lead) recalled the task of subgroup 1 (Question 5a from the Terms of Reference) noting that the subgroup had met several times during the intersessional period. They focused their discussions on what would be required by the OPCW, in an end-to-end process, to investigate an alleged use of biotoxins, starting from the alleged

[1] Summary of the Second Meeting of the Scientific Advisory Board's Temporary Working Group on the Analysis of Biotoxins (SAB-33/WP.1, dated 15 October 2021). Accessible at https://www.opcw.org/sites/default/files/documents/2021/11/sab-33-wp01%28e%29.pdf.

incident and arriving at the scene all the way through to reporting to the Director-General. The subgroup started mapping the process out and prepared and circulated a preliminary draft report within the subgroup.

4.3 Dr Clark went through some of the main points outlined in the draft report. The group is dividing the process of investigation of the alleged use of a biotoxin into four stages: initial in-field sampling and collection activities, screening of samples, unequivocal analysis and providing provenance. He noted that wider discussion with and input from the entire TWG is needed on some of the elements. In particular, he emphasised that the subgroup is seeking guidance on which key elements of biotoxins (or their alleged use) should be considered and/or prioritised?

4.4 Finally, Dr Clark gave an overview of a summary table that provided a high-level definition to the entire process of an investigation into alleged use of a biotoxin. For each part of the process they are considering implementing a Green-Amber-Red (GAR) system to indicate the maturity of existing capabilities that are available. The floor was then opened to questions and comments.

4.5 It was asked whether both environmental and biomedical samples were considered when understanding the state of in-field detection and analysis. Dr Clark answered that indeed the subgroup considered both types of samples, with the importance of biomedical samples being considered early on in their discussions. It was further noted that clinical presentation is an important piece of data as it may prompt treating medical staff to order further laboratory analysis on suspicion of biotoxin exposure. Dr Clark also noted that the decision on how far to go in terms of clinical diagnosis will also depend on the TWG's decision on which biotoxins it will consider for this work.

4.6 Another point raised was that of the discrepancy between analysis times for different techniques that are reported in the literature with what are often longer times in practice. Dr Clark acknowledged that it was important to note the entire operational timeframe of an analysis, to include collection, screening and analysis, and that realistic analysis times need to be understood and used similarly throughout the five subgroups. Of note is analysis of any unknown sample or of an unforeseen biotoxin – total analysis times may vary drastically in these cases and may be much longer than in less complex cases.

4.7 The potential for the analysis of an unknown toxin raised additional discussion. A question was raised whether it would be advisable to consider other types of signs or circumstantial evidence that might point to toxin use, such as dead animals. It was decided that subgroup 1 should consider the role of potential tangential evidence.

4.8 Dr Clark then mentioned a few of the next steps of the subgroup. He singled out the question of provenance as one that requires more consideration if the TWG wants to include this topic in its final report. He suggested having a separate discussion or a break-out session on this issue specifically. He also noted that once the TWG decides on the list of biotoxins of concern, the subgroup will return to its initial report to see whether any points should be reconsidered.

Subgroup 2

4.9 Dr Bossée (subgroup lead) recalled the task of subgroup 2 (Terms of Reference questions 5b and 5c) and reported that the group had consulted open literature to

complete a draft summary table for different biotoxin families capturing relevant criteria, including presence on a control list, ease of production, historical misuse, toxicity, stability, ease of detection, etc., noting that they are still collecting data for a few of the toxin families. She noted that all appropriate literature references are included. The summary table will allow for a prioritisation of biotoxins for consideration by the TWG based on different variables and factors. The subgroup met several times during the intersessional period in order to discuss and agree on the methodology for prioritisation of biotoxins relevant for alleged of use, which had been proposed by Dr Ghanei.

4.10 Dr Ghanei, a subgroup 2 member, then provided on overview of the developed methodology they are applying to the prioritisation of toxins. The method gives each criterion a weighting factor corresponding to its relative importance in whether a particular toxin would be, or could be, used with malicious intent. The total score for each biotoxin reflecting its risk of misuse is then calculated from these weighting factors and scores. Then prioritisation will be done through sorting these scores. The decision on which biotoxins are relevant or not should be made by all members of the TWG together. It is important to first make sure that the scores attributed for different families under each (sub)criterion are valid for all TWG members. The floor was then opened for questions and comments.

4.11 The SAB Secretary referred to the methodology for prioritisation asking how the subgroup determined the relative weight for each factor, and if this was based on their group expertise or literature or other known prioritisation methods. He also asked if the whole TWG is requested to provide feedback and if there needed to be any adjustments.

4.12 Dr Ghanei explained that in deciding on the criteria and scoring values they weighted these from the standpoint of a potential user of a biotoxin. For example, if it was simple to detect it received a low value, and if it was easy to produce it received a high value. He concluded that the chosen families, the methodology used, and final scores should be accepted by consensus of the entire TWG.

4.13 Another point raised was whether to include entire groups of toxins on the list or just the most likely one; for example, to include all staphylococcal enterotoxins and not only Staphylococcal enterotoxin B (SEB). Dr Bossée replied that only a family leader is presented in the table for the sake of simplification of work. However, she agreed that it is important to discuss within the TWG whether to have the whole family or just one (a family leader).

4.14 Dr Bossée announced that the first list of relevant biotoxins for consideration by the Group, with related summary tables and attributed scores with the presented methodology, were being finalised by the subgroup with the aim of sharing with all TWG members for their comments before the end of 2021.

Subgroup 3

4.15 Dr Kalb (subgroup co-lead with Dr Åstot) reported on the discussions the subgroup had in the intersessional period. In regard to analytical approaches required for the unambiguous identification of both low and high molecular weight biotoxins, it was determined that, for protein toxins, digestion of proteins and tandem mass spectrometry (MS/MS) analysis of tryptic fragments is well established, but this technique requires

a relatively high level of toxin and an absence of high matrix samples that may be a challenge in certain situations (e.g., biomedical samples). Other useful analytical approaches include enzyme-linked immunosorbent assay (ELISA), antibody-affinity, gel, 2D gel, DNA sequencing, functional assays, etc., but none of them should stand alone as unambiguous identification. Alternative approaches to mass spectrometry need good characterisation of reagents (antibodies) and information on relevant control experiments and how the overall analysis was done. As for antibody affinity experiments, there are still a lot of unknowns and more information is needed.

4.16 Dr Åstot continued the presentation, highlighting some of the questions related to the issue of instrumentation and/or procedures to be standardised across laboratories, noting that the process of standardisation may limit the speed of development and also presents financial burden on laboratories. Examples of good approaches include those applied in traditional chemical warfare agents, in particular environmental samples, such as: recommended methods and highly characterised reagents; development of minimum data sets on antibodies acceptable for verifying the presence of biotoxins; sticking to requirements-based reporting; and performance-based or standardisation of reporting (data vs. method required). He also underlined that it is hard to have one laboratory with expertise over the entire range of toxins (laboratories good at high molecular weight measurements are not necessarily good for low molecular weight measurements and vice-versa). A good option could be to provide standard access to technologies with everyone able to do at least one technique as a baseline.

4.17 Dr Åstot further highlighted some of the questions raised in relation to the analytical criteria that should be in place to match forensic evidence, including what the expectation on geographic or production attribution is (matching of toxin samples to deduce a common origin; geographical source attribution; toxin purification method attribution). There is limited research on other molecules that may be present in a sample of toxin. Because no synthesis takes place for biotoxins, looking at the impurity profile might be informative. He reiterated that laboratories good at analysis of high molecular weight toxins will probably not be good at analysis of low molecular weight contaminants that may be present and vice-versa, which presents an interesting challenge. It may be important in an investigation to link samples to the scene or to detect differences in the preparation methods of toxins; the preparation of small molecule toxins is very different from the preparation of large toxins. These differences would result in very different markers with different techniques required to detect them.

4.18 Moving to the role and utility of degradation products and other markers, Dr Åstot noted that in the context of biotoxins, degradation could mean loss in size, or also degradation through toxin inactivation. Inactivated toxin could pose different challenges vs. typical chemical warfare agents, such as what to do if a batch of inactivated toxin is found. If there is no biological activity in a discovered use of toxin, then the immediate public health threat is far lower than it might otherwise be. However, the possibility of a public health threat is separate from the intent to commit a crime. In comparison, most nerve agents have two stereoisomers, with one often considerably more toxic than the other, but both may pose a public health threat and are equally regulated.

4.19 Lastly, Dr Kalb summarised the discussion on the role of markers in biomedical samples. It is believed that toxins will not react with human proteins to form adducts as with classical chemical warfare agents, and only the identification of the intact toxin or

detection of toxin activity is possible. The biggest challenge is that toxin levels in biomedical samples are often very low (especially with high molecular weight toxins) and often too low to use traditional MS/MS techniques for detection. For the success of a case, detection of toxin, and especially toxin activity in biomedical samples, is valuable, powerful information. She pointed out several differences between high molecular weight toxins and traditional chemical warfare agents, including the often-delayed onset of symptoms after exposure with large biotoxins and the fact that large biotoxins are not excreted through urine and remain in the body for longer.

4.20 Dr Hennekinne shared the point of view of the food sector on the issue of standardisation. Standardisation is a long process, but in the food sector it is considered mandatory. In the European Union (EU) regulations on food microbiological criteria, for each food type there is a standard to be achieved (International Organization for Standardization (ISO) standard). In the food sector it is important to have a clear standardisation process whatever the type of bacteria or toxins. Currently there are different available standards for bacterial toxins within the European Commission. Standards are also currently being developed by different groups for other toxins, including, for example, liquid chromatography–mass spectrometry (LC-MS) based on methods for staphylococcal enterotoxin confirmation analysis. There is also a committee that works on how to develop and validate methods for bacterial toxin analysis in food samples in order to fulfil EU regulations.

4.21 Dr Åstot remarked that the standardisation in the food industry seems to be more quantitative (concentration levels are important). Dr Hennekinne explained that it depends on regulations on food safety criteria. For pathogens or toxins in some cases there is a concentration threshold (e.g., for Cereulide), but for others a qualitative method is sufficient. It is important to strike a balance between benefits for consumers and benefits for food manufacturers to be sure that you put on the market a safe product.

4.22 To conclude the discussion, it was noted that when it comes to natural toxins, there is a challenge to prove that there was really an intent of intoxication. In that respect, quantitation could be important.

5. AGENDA ITEM FIVE – Analysis of plant toxins in food supplements: a multi-target LC-MS/MS method

5.1 Dr Els Van Pamel (Flanders Research Institute for Agriculture, Fisheries and Food, Belgium) presented on the analytical method developed for the detection of plant toxins in food supplements developed in the framework of the ANAPLANTOX research project financed by the Belgian federal public service Health, Food Chain Safety and Environment.[2,3]

5.2 After the presentation the floor was opened to questions. On being asked about the biggest challenge to overcome on this project, Dr Van Pamel replied that it was finding information on toxicity, which is connected to the lack of pure standards available. She

[2] Van Pamel, E.; Henrottin, J.; et al. “Multi-Class UHPLC-MS/MS Method for Plant Toxins and Cyanotoxins in Food Supplements and Application for Belgian Market Samples.” *Planta Med* 2021 Oct; 87(12-13). doi: 10.1055/a-1517-5828.

[3] See https://pureportal.ilvo.be/en/projects/ontwikkeling-van-een-multi-targetmethode-ter-analyse-van-plantent.

also underlined the high diversity of different toxins, noting the need for having more dedicated methods for different classes to gain sensitivity.

5.3 It was noted that one would expect to need different analysis conditions based on the varying degrees of polarity and a question was posed whether they were able to cover most alkaloids on their list, and—if the method they used did not perform well on some of those—were those generally the polar ones; did this impact how many of the toxins they ultimately screened and performed analyses of?

5.4 Dr Van Pamel replied that from the first list obtained, because of the lack of standard availability, they had to leave out some of the toxins from the methodology. The goal was to have one methodology, or at least a few methodologies, but this was challenging if a toxin required very specific analysis conditions. For some compounds they could go quite low (low ppb levels), but for others it was very difficult.

5.5 It was then asked, when it comes to developing a screening tool, how did they strike a balance in the different detection techniques and approaches? Dr Van Pamel commented that this is always a challenge; you need sensitivity to measure at relevant regulatory limits, but you also want good peak shape, good separation, etc. It is relatively easy when only looking at two compounds, but with more than 25 compounds, compromises had to be made. Luckily, they had colleagues from various sectors advising them. She noted there are also nontechnical limitations, like time and budget, that need to be considered.

5.6 Dr Van Pamel was then asked whether they considered focusing more on method development for smaller groups of toxins instead of the more generic method they developed. She commented that it would be more efficient if looking into a specific class or toxin family. However, given the original project call and the varying degrees of prevalence for different toxins, a more all-encompassing approach was aimed at. She concluded that it may of course still be worth developing methodologies for specific toxins.

5.7 It was also noted that the study focused only on smaller molecules and peptides and it was asked if they would expect to have the same generic approach if they incorporated protein toxins.

5.8 Dr Van Pamel explained that one of her colleagues, a protein specialist, came up with a methodology for the lectin jacalin. She noted that when looking into proteins where a standard is not available, one can develop a methodology if the protein sequence is known by doing an *in-silico* digestion to see which specific peptides can be generated. It is then possible to analyse real samples on high-resolution mass spectrometers and if you have your dedicated sequences, you can go to LC-MS/MS to gain sensitivity.

6. AGENDA ITEM SIX – Recent and ongoing exercises on biotoxins under different frameworks

6.1 Dr Brigitte Dorner (TWG member, Robert Koch Institute, Germany) spoke about recent and ongoing exercises on biotoxins under different frameworks, concentrating on two projects in which Robert Koch Institute has been involved—EuroBioTox (coordinated

by Dr Dorner) and RefBio.[4] The testing activities started in 2012 with EQuATox, an EU project that is currently continued under EuroBioTox and that will run until the end of 2022. With both projects there is close cooperation on quality assurance, and exercises. In addition, there is close coordination with the United Nations Secretary-General's Mechanism (UNSGM) to ensure that information and results can be shared with and used by UNSGM roster laboratories.

6.2 An initial project, EQuATox (2012-2014), involved 35 participants from the security, verification, health and food sectors.[5] Proficiency tests (PT) started with the generation and characterisation of toxin reference material combining the expertise from EU laboratories and setting up PT schemes including real sample materials and evaluation of results. Dr Dorner provided an example of the first ricin PT conducted under the project. It turned out that those laboratories that had combined approaches (immunological, MS-based and functional) were more successful in their results. Depending on the concentration of ricin (high vs. low), different analysis methods were more or less successful. The results, along with the analysis methods, were put in a table and these results published in a dedicated issue of *Toxins*.[6] The EQuATox project thus gave a snapshot on biological toxin detection capabilities and helped identify gaps and technical limitations.

6.3 With those results they were able to start the ongoing EuroBioTox project that gathers 63 institutions from 23 countries with an objective to increase detection capabilities to an advanced technical level and establish a Pan-European network of competence. The specific goals include establishment of a comprehensive mechanism to increase quality assurance in the field (provide certified reference materials, repository of tools, training courses, PTs); evaluation and refinement of measurement procedures; enabling replacement of animal experiments; and coordination of laboratories, industrial partners and end-users working on biological toxins in the EU to spread know-how from experts to practitioners.

6.4 When it comes to the status of certified reference materials, whose development requires 30% of the funds, Dr Dorner noted the development and production of candidate certified reference materials (CRMs) for ricin, BoNT/A, BoNT/B and SEB (purified from natural sources and/or produced from recombinant techniques), noting some of the challenges involved in the process including, among others, security/safety concerns and the need for comprehensive characterisation to demonstrate high purity and high functional activity. In terms of characterisation, Dr Dorner noted various methods were combined for identification/quantification of impurities; value assignment (concentration and biological activity), and homogeneity/stability of samples being critical.

6.5 Dr Dorner further provided an overview of exercises and PTs on biotoxins organised since 2012, including OPCW exercises on ricin, abrin and Shiga toxin (Stx), and the ongoing tests under EuroBioTox and RefBio. She noted that there is an overlap between laboratories participating in the exercises organised under the two projects and those that are OPCW designated laboratories, and that therefore the RefBio project wants to

---

4 For more information on EuroBioTox see https://eurobiotox.eu/#. For more information on RefBio see https://www.rki.de/EN/Content/Institute/International/Biological_Security/RefBio.html.

5 See http://www.equatox.eu/.

6 See https://www.mdpi.com/journal/toxins/special_issues/detect-identi-toxins?view=abstract&listby=date.

offer exercises that are meaningful for new laboratories while also providing a new challenge for those laboratories that participated in EuroBioTox or OPCW exercises.

6.6 The RefBio project (2017-2024) is funded by the German Federal Foreign Office and seeks to strengthen the bioanalytical reference laboratories in the UNSGM. As the project continues, there are an increasing number of participating laboratories with broader geographical representation. So far, the exercises conducted under the project included bacteria, viruses, toxins, and even SARS-CoV-2 (as of 2020). Dr Dorner provided a more detailed overview of the 2019 External Quality Assurance Exercise (EQAE), in which 16 participating laboratories were presented with a fictious scenario and asked to detect and identify ricin-positive samples. Under the UNSGM exercise regimen, individual samples always come in a sample set of three (one negative control and two unknown samples: one that definitely contains a bioagent and one that definitely does not). The laboratories were each provided with three sets of three samples (containing clinical, food and environmental samples) with different concentrations and given four days to report initial results and three weeks for confirmation results. They were also provided with a number of additional challenging scientific questions to evaluate technical capabilities. Qualitative results after four days were above 90%, whereas after three weeks, they varied depending on the analyte to be detected. As for the quantitative results after three weeks, 75% of laboratories provided a good quantitative value for all the three types of samples. Out of 16 laboratories, only five were already accredited (nationally or internationally) for environmental/food samples and one for clinical samples. All the participating laboratories correctly identified the ricin samples as expected, however the more challenging the task, the less success seen.

6.7 Annual workshops are also organised involving laboratories and external experts to share experiences and best practices, planning and evaluation of laboratory proficiency tests according to the EQAE, with a goal of building a trusted network of microbiological analytical laboratories.

6.8 In conclusion, Dr Dorner noted that, within these projects, a comprehensive mechanism to increase quality assurance in the field has been successfully established based not only on exercises but also on CRMs, PTs, repository of tools and training courses.

6.9 A question was asked about the different types of techniques required to show that a toxin is present and asked how many laboratories actually have the capabilities to do it all. Dr Dorner replied that many are specialised laboratories with a particular focus (e.g., on food or chemical weapons analysis). Out of the 63 laboratories under EuroBioTox, only three or four work on all the toxins. She underlined the need for having common reporting for all the exercises, rendering it irrelevant as to who is requesting an investigation (one reporting format acceptable to all potential stakeholders who could request an investigation).

6.10 A reference was made to the recommended or standard operating procedures and if anything is in pipeline following the recent project. Dr Dorner confirmed that they

would be interested in publishing this information but would need to think of an appropriate format (e.g., VERIFIN Blue Book).[7]

7. AGENDA ITEM SEVEN – Combining affinity-based enrichment methods with LC-MS analysis

7.1 Dr Thomas Bergström (FOI – Swedish Defence Research Agency, Sweden) shared some of the toxin activities conducted at the FOI including broad research activities (detection, identification, activity measurements, modifications, production, toxicity, etc., collaborations and international projects (EuroBioTox, UNSGM, OPCW), and providing national experts an analytical resource (support to the Armed Forces and Civil Defence)). As such, they perform broad screening of suspicious samples with different CBRN methods.

7.2 FOI started developing an affinity enrichment method for detection of toxins back in 2005 using in-house packed columns with galactose ligands and taking advantage of galactose affinity for type-2 ribosome inactivating proteins, like ricin. Matrix tests were done with 1 µg ricin/mL, with matrices including water, beverages, and wipe samples. They were then given the chance to test their method when the Swedish police seized a homemade ricin/abrin mixture. The samples were successfully analysed using the described affinity columns – starting with UV detection indicating galactose binding proteins, followed by enzymatic digestion and LC-MS/MS analysis on all present peptides.

7.3 FOI then wanted to make their system more stable in relation to different matrices and decided to try magnetic beads incorporating the same galactose ligands. When evaluating different binding chemistry and ligand modifications they saw promising results but initially had large variation in recovery and linearity. They are currently looking at different multi-galactose supramolecules, like dendrimers and dendrons, but more development and evaluation is needed both on the ligand chemistry and on the enrichment protocol. They hope to have the validated method up and running in the future to complement their other methods.

7.4 Recently, they have used magnetic beads with coupled antibodies (within the EuroBioTox project) with various matrices including, for example, powder milk and human serum. They analysed accuracy and precision of the method and short-term stability. This method was applied in the first EQAE on biological toxins as part of RefBio. One interesting question they considered was if the obtained results could indicate if the toxin detected in the different matrixes originated from the same source. They started with ELISA where they correctly identified the positive samples, but could not distinguish between ricin D and ricin E using ELISA only. Additional methods and techniques also could not verifiably provide the needed information, so the answer was no.

7.5 In conclusion, Dr Bergström highlighted that FOI's approach to toxin analysis roughly corresponds to: screening for multiple toxins (with lateral flow assays); verification with LC-MS/MS multi MRM, after galactose and/or ab-based enrichment methods (in parallel they try to do ELISA for suspected toxin); quantification, if requested

[7] See https://www2.helsinki.fi/en/verifin-finnish-institute-for-verification-of-the-chemical-weapons-convention/information/blue-book.

(with ELISA and nowadays with LC-MS/MS), and detailed analysis looking into general protein content and background matrices. For different samples they go to different levels and sometimes just stop at verification depending on the question asked. The floor was then opened for questions.

7.6 A question was asked how FOI decides which method to use – galactose binding or using known antibodies that they already have. Dr Bergström explained that it depends on what they know about the sample. There could be some indication from a crime scene as to what kind of sample it may be (e.g., traces of ricin preparation). Sometimes they need to screen the sample for general protein content to see what it is. For example, if it is bacterial then it gets sent to a bacterial polymerase chain reaction (PCR) analysis group. It is different for different samples. As for immunoaffinity, they have it only for ricin, abrin and SEB, but not BoNT toxins.

7.7 It was then noted that the extraction with galactose is a very good approach for lectins, but perhaps less so for larger toxins, like BoNT. In case where he would have access to high-affinity monoclonal antibodies, would he still prefer a galactose binding approach over the antibody binding approach.

7.8 Dr Bergström concurred that the method is not perfect for BoNT and would not be used as a stand-alone method (antibodies would be used), but the approach could be used for toxin enrichment. However, methods can be used complementary—one set of galactose beads and one set of beads with antibodies. Personal experience in working with magnetic beads with galactosyl ligands was shared and it was agreed that two methods should complement one another and should both be available in any given laboratory's "toolbox."

8. AGENDA ITEM EIGHT – Quality assurance for high molecular weight toxins relevant in the food chain

8.1 Dr Jacques-Antoine Hennekinne (Deputy Director of the Food Safety Laboratory– Anses, France) opened his presentation with a case study on a food poisoning outbreak in five schools in Guam islands, when 295 people presented symptoms consistent with a large staphylococcal food poisoning outbreak. The local authorities (Guam National Guard) performed sampling and detection with available (non-validated) methods that indicated SEB, leading to activation of Guam's Emergency Response Center in response to a potential act of bioterrorism. In parallel, samples were sent for confirmation to a laboratory in the state of Hawaii (in the United States of America) and a United States Food and Drug Administration district laboratory which used dedicated and validated methods to screen staphylococcal enterotoxin content in foods. They demonstrated that only Staphylococcus Enterotoxin D (SED), without any SEB, was present in the contaminated food source. The initial positive SEB result was due to a cross reaction of the methods used. This example demonstrates a crucial need for using properly validated methods in all types of food matrices to avoid misinterpretation.

8.2 Turning to the relevant legislation, Dr Hennekinne recalled Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority (EFSA) and laying down procedures in matters of food safety,

including the EU Reference Laboratory (EURL) network in charge of making sure that Member States properly apply relevant regulations, in particular those related to microbiological criteria for foodstuffs.[8] The latter lays down the microbiological criteria for certain microorganisms and the rules that food businesses must follow to ensure that the food they handle, supply or process complies with these criteria, which are very detailed.

8.3 For each criterion, the EC decided to nominate a EURL in charge of making sure that these regulations are properly applied by Member States. EURLs tasks for food and feed include developing and validating analytical methods targeting microbiological criteria according to ISO standards; providing national reference laboratories (NRLs) with details of analytical methods, including reference methods and/or materials (CRMs, etc.); providing training for NRLs; coordinating NRL activities (e.g., organising PTs); performing confirmatory analysis (official control, food-borne outbreak, disputes between Member States); providing scientific and technical assistance to the Commission and collaborating with official laboratories nominated by third-party countries. Each Member State has to nominate one or more NRLs for each EURL to check if the EU regulation is properly applied. NRLs have to collaborate with the EURL, coordinate, for their area of competence, the activities of official laboratories (confirmatory analysis, PT organisation, etc.), provide scientific and technical assistance to competent authorities and, if more than one NRL is nominated, ensure coordination. All EURL/NRLs must be accredited according to EN ISO/IEC 17025:2017.[9] All the methods used by the laboratory must be accredited.

8.4 In case of a notification of a food poisoning event in France, there are two types of parallel enquiries launched: an epidemiological one lead by the Ministry of Health and one by the Ministry of Agriculture. The two investigations corroborate in order to combine the results of their enquiries. In France, positive results must be sent for confirmatory analysis to the NRL. The Rapid Alert System for Food and Feed (RASFF) can be activated by a Member State to advise other Member States in case of a food poisoning event due to unsafe product that has been exported. In 2019 (last available data), 27 Member States reported a total of 5,175 food-borne outbreaks, with almost 1,000 being attributed to bacterial toxins (51% Bacillus cerus, 24.7% Clostridium spp and 24.3% Staphylococcus aureus). Classification is based on both strong and weak evidence based on the availability of data on symptoms, etc.

8.5 Turning to virulence of bacteria-producing toxin, Dr Hennekinne noted that pathogenic potential depends on species and strains, ranging from non-hazardous to lethal. He submitted that food poisoning characterisation is a tricky exercise as symptoms can be very similar. Bacterial identification in food with classical microbiological methods is not a reliable indicator of intoxication risk. There is a lack of tools and methods to detect/quantify toxins and/or putative virulence factors. Food matrices are really challenging. Therefore, there is need to develop a "toolbox" strategy using complementary approaches by using ISO standards or by developing in-house methods to provide relevant and reliable results to decision makers.

---

[8] Regulation (EC) No 178/2002 is accessible at https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32002R0178.

[9] For more information on ISO/IEC 17025:2017 see https://www.iso.org/standard/66912.html.

8.6 To manage these drawbacks, focus should not only be on the toxins but also on the producing microorganism, especially at the DNA/RNA levels (genomics, transcriptomics). It is therefore necessary to incorporate complementary skills to cover classical microbiology (behaviour of the microorganisms during the food process), skills in molecular biology (to evaluate mutations, to develop internal standards), biochemistry (especially in the development of ELISA based methods), analytical chemistry (to optimise all the parameters, from toxin extraction to identification of specific peptides), and toxicology (to propose relevant thresholds). The standardisation process is critical to know how to properly develop/validate methods. With regard to the development of complementary skills, Dr Hennekinne gave several examples of the work they have done as a EURL for Coagulase Positive Staphylococci (CPS), including: dose-response modelling for SEs using outbreak data in order to determine performance characteristics to be achieved by antibodies; development of ISO 19020:2017 for SE screening in matrices;[10] development of a genomic tool named NAuRA to evaluate the number of variants depending on the type of SE strain, which has been used as one of the main sources of SE genes in the SE genome.[11] They have also developed a highly sensitive and specific ELISA-based method for SEs.[12] Dr Hennekinne also provided a brief overview of the involvement in standardisation activities on the French, EU and ISO levels.

8.7 He also briefly touched upon the possible (though not definitively determined) use of SEB as a bioweapon, including first possible use in World War II (in North Africa) and during the Cuban missile crisis (Operation "Zapata" before the Bay of Pigs Invasion).

8.8 Lastly, Dr Hennekinne presented three examples of several real-life cases of SE poisoning events, the approaches taken and related findings, including the first confirmation of Staphylococcal Enterotoxin H (SHE) in a food-borne outbreak by using LC-MS.

8.9 In conclusion, Dr Hennekinne underlined that detecting bacterial toxins in foods is like looking for a needle in a haystack (focusing on very small molecules in a very large batch of other types of proteins). It needs to be considered that bacterial toxins are encoded by living microorganisms, leading to a possibility of mutations. For that reason, there is a clear need for a toolbox with complementary approaches to characterise food-borne outbreaks (FBO) due to bacterial toxins. Finally, access to fully characterised calibrants and/or CRM is needed to perform quantitation to contribute to risk assessment. The floor was then opened for questions.

8.10 It was asked how reporting works in the food industry—what is the level of detail if they have a positive outbreak, if they need to provide primary data (or is it just a check box), and if they can be sure that the results reported and collected over Europe are accurate and correct.

8.11 Dr Hennekinne explained that each Member State has its own reporting protocols on food-borne outbreaks. Many poisoning events are due to bacillus cereus and clostridium perfringens, and more than 85% of these events are reported by France because it has

---

[10] For more info on ISO 19020:2017 see https://www.iso.org/obp/ui/#iso:std:iso:19020:ed-1:v1:en.

[11] See https://pubmed.ncbi.nlm.nih.gov/32714310/.

[12] Tarisse, C. F.; Goulard-Huet, C.; et al. 'Highly Sensitive and Specific Detection of Staphylococcal Enterotoxins SEA, SEG, SEH, and SEI by Immunoassay.' *Toxins* 2021 Feb 9; 13(2). doi: 10.3390/toxins13020130.

been mandatory to report food outbreaks in France since 1979. But each country is different. It happens sometimes that Member States send samples for analysis but do not file notifications on outbreaks. Currently there are attempts to harmonise different approaches in order to "educate" member states on how to report food poisoning outbreaks at an EU level. As for the reporting format, he explained that there is a template in the EU, but it is not mandatory to use. In addition, each country has different classification in terms of the strength of evidence.

8.12 The task of the TWG and the need for high-credibility data in any case of alleged biotoxin use was recalled. In addition to any OPCW investigation there may always be a UNSGM-led investigation. It was suggested that an acceptable reporting format that would be suitable for use in performing analyses under both regimes could be ideal.

8.13 Dr Hennekinne was then asked if the genome sequencing approach they use is looking to amplify specific toxin genes or it just looks for the presence of the organism. He noted that they currently perform whole genome sequencing (WGS) analysis and not metagenomics. They locate some strains and analyse the DNA content of those strains. They have designed a fully automated approach to focus only on SE genes because there are a lot of variants, especially in the enterotoxin gene cluster. As there is no standard to perform quantitation or develop MS approaches, they need to target a very specific peptide and to take into account all the possible variants. Using WGS they can map the DNA sequence of all the strains (currently they have sequenced more than 500 strains coming from all over Europe). With this approach they can also focus on dedicated genes to develop their own standards (no commercially available standards).

8.14 When queried if they are using an algorithmic approach based on clinical symptoms, Dr Hennekinne explained that the national reference laboratory is used in the food sector whereas it is the national reference centre used for human exposure analysis. He noted that the two laboratories are in frequent contact. Sometimes they have to compare results between the food samples and the stomach content after an autopsy. As for the epidemiological analysis, he noted that it is not their duty, but the duty of medical doctors to perform such analysis to highlight the type of food to focus on. It falls in the remit of the Ministry of Health and not the Ministry of Agriculture.

9. AGENDA ITEM NINE – United Nations Secretary-General's Mechanism (UNSGM)

9.1 Dr Christine Uhlenhaut (Office for Disarmament Affairs in Geneva, Switzerland) opened her presentation by providing an overview on the establishment and mandate of the UNSGM going back to the 1925 Geneva Protocol and leading up to the UNSGM Resolution (A/42/37 C) in 1987.[13] The establishment of the UNSGM was reaffirmed by the United Nations Security Council in 1988 by resolution 620.[14] She noted that though the OPCW was established to implement the Chemical Weapons Convention (CWC), the UNSGM can still be launched to investigate alleged use of chemical weapons by non-States Parties to the CWC. The Biological Weapons Convention (BWC), on the other hand, has no implementing body or verification mechanism. The UNSGM is therefore the only international mechanism to investigate the alleged use of biological weapons. Dr Uhlenhaut reminded the Group of the cooperation agreement

[13] https://undocs.org/a/res/42/37.
[14] https://undocs.org/s/res/620(1988).

between the United Nations and OPCW that stipulates how the two bodies should work together.[15] Additionally, in the UNSGM: Guidelines and Procedures (A/44/561), paragraph 18 states: "As early as the convention on prohibition of chemical weapons enters into force, the Secretary-General should co-operate, as appropriate, with the organs provided for in the convention, in carrying out investigations in accordance with these guidelines and procedures and the relevant provisions of the chemical weapons convention".[16] The UNSGM guidelines and procedures were developed by a group of experts in 1989 (A/44/561) and they cover the preparation and conduct of a UNSGM investigation: technical procedures; roles of qualified experts, expert consultants, and laboratories; and drafting and content of report to the United Nations Secretary-General.[17] The technical appendices were updated in 2007 to account for technical developments in biology, with Appendix V containing the list of diagnostic and analytical laboratory specialisations.

9.2 Dr Uhlenhaut moved on to describe the steps involved in considering whether and how to launch an investigation following a report of alleged use of chemical or biological weapons. Any Member State can report a suspicion of the possible use of a chemical, bacteriological (biological) and/or toxin (CBT) weapon to the Secretary-General. The Secretary-General can then decide on their own (like was the case with Syria), or ask for expert advice, on whether to launch an investigation and to report the results of the investigation to all Member States. Unlike the OPCW, the UNSGM is not a standing investigative body. Instead, Member States nominate expert consultants, qualified experts and analytical laboratories which are then listed in a roster and may be called upon to support a UNSGM investigation in accordance with the UNSGM Guidelines and Procedures.

9.3 The United Nations Office for Disarmament Affairs (UNODA) serves as the custodian of the UNSGM, which includes ensuring the operational readiness to carry out a mission in response to reports from Member States; maintaining the roster of nominated experts and laboratories; coordinating on training activities with partners, including Member States, laboratories and international organisations (including the OPCW); conducting outreach activities and coordinating the United Nations Internal Task Force (UNITF) of points of contact within United Nations departments and agencies that would support a UNSGM mission and training.

9.4 As for the roster, UNODA sends a note verbale to all United Nations Member States once a year requesting nominations for the UNSGM roster, though Member States can submit new nominations or update their nominations at any time. To test responses and make sure the roster is up to date, UNODA also conducts annual call-out exercises. Currently, there are 494 qualified experts (including 99 with chemical expertise), 39 expert consultants (3 with chemical expertise), and 83 analytical laboratories.

9.5 Qualified experts can be dispatched to the field to investigate the alleged use of weapons. Examples include: providing the Secretary-General with an estimate of the number of possible CBT victims in the course of investigation, as well as a description of the types of injuries; observing and taking part in the analysis in the designated

---

[15] Relationship Agreement Between the United Nations and the OPCW. (EC-MXI/DEC.1, dated 1 September 2000), assessable at https://www.opcw.org/sites/default/files/documents/EC/11/ECMXIDEC1_e_.pdf.

[16] https://undocs.org/pdf?symbol=en/A/44/561

[17] https://www.un.org/disarmament/wmd/secretary-general-mechanism-old/appendices.

laboratories of samples gathered and acquainting themselves with the results of the analyses for use in drawing up the report of the team for Secretary-General; and participating in training activities. Expert consultants advise and assist in the overall conduct and operation of the UNSGM, from planning, deployment, operation and reporting. Examples include: advising and assisting the Secretary-General in a consultative capacity in various fields (legal, scientific, military, logistical and other) for the successful preparation and conduct of an investigation; evaluating any report made by a Member State concerning the alleged use of CBT weapons and assisting the Secretary-General in conducting the investigation. Analytical laboratories provide services to test for the presence of CBT agents. Examples include: identifying CBT agents, their characteristic impurities, and degradation products, unexploded munitions which may be related to the possible use of CBT weapons; validating the preliminary analyses; elucidating the nature of unknown CBT agents; preparing and transmitting a report of the details and results of their analyses to the Secretary-General; and participating in inter-laboratory calibration studies to establish the validity and accuracy of their analytical methods.

9.6 The operating environment for a UNSGM mission is likely to be time-sensitive, multi-faceted (various expertise needed), politically complex and hazardous, involving a diverse team whose members are not familiar with each other. Although Member States nominate experts, the UNSGM still insists on training to ensure the operability of any potential UNSGM mission. Specialised training is offered/donated by Members States and organised by UNODA. So far, there have been 16 courses with focus on core skills training since 2009, one course on Hazardous Environment Awareness Training (HEAT) and several additional workshops and table-top exercises.

9.7 EQAEs are also offered and funded by Member States and coordinated with UNODA with a view to demonstrating competence of the analytical laboratories for the detection and identification of known CBT agents, evaluating the capability to detect the presence of other toxic substances unknown to the laboratory in biomedical and environmental samples, and demonstrating the level of competence represented by the laboratories collectively. Based on the results of these interlaboratory calibration studies, the expert consultants should develop an assessment of the competence of the individual laboratories as well as of the laboratories collectively. Results of EQAEs are shared with UNODA.

9.8 Finally, Dr Uhlenhaut provided a brief overview of past UNSGM investigations in 1992 in Mozambique, where it was not possible to determine if a chemical weapon has been used by rebel troops, and in 1992 in Azerbaijan where no evidence of the use of chemical weapons by Armenian armed forces in the context of the Nagorno-Karabakh war was presented to the team. An overview was also given on the mission sent to the Syrian Arab Republic in 2013 where it was determined that chemical weapons were used. Unlike the other previous investigations, the UNSGM investigation in the Syrian Arab Republic was carried out with the support of the OPCW. The floor was then opened for questions.

9.9 Dr Uhlenhaut was first asked how the UNSGM decides which laboratory to pick from its roster in any given investigation, and if certain criteria are used (geographical representation, quality of laboratory measurements or performance in exercises).

9.10 Dr Uhlenhaut noted that, when it comes to chemical weapons, a good example is the Syrian investigation which was conducted in close coordination with the OPCW and OPCW designated laboratories. For investigation into a biological weapon incident, there is a lot of weight placed on technical performance and ability, though of course geographical representation would need to be considered.

9.11 It was then asked what kind of approach would be taken in case of a botulinum release (as opposed to ricin or saxitoxin as CWC Schedule 1 chemicals). Dr Uhlenhaut replied that it would depend partially on what is alleged in the report, and this would be a factor in any advice given to the United Nations Secretary-General on how to proceed (whether in conjunction with OPCW, only with UNSGM biological laboratories, or some combination thereof). It was then noted that this precise situation is one of the issues to be tackled by the TWG. Although these are rare events one has to be prepared. It is believed that biological UNSGM reference laboratories can still offer approaches that have historically not been the focus of OPCW designated laboratories, such as more highly sensitive, biological analysis methods. This again underscores the potential benefits of having common reporting mechanisms, insofar as possible.

9.12 Dr Uhlenhaut expressed her full agreement noting that the reporting should be agreed on before, and not after, or during, an incident. On the other hand, some flexibility is needed, staying away from SOPs and stringent procedures (not knowing what an incident may be) and instead promoting recommended procedures could be beneficial.

9.13 An example of another tricky situation was given where the causative agent is not known, but there is reason to suspect from the clinical presentation that there is a certain toxin involved. Expert consultants would play a very important role in helping the Secretary-General decide what kind of team to field. However, the selection is never just technical—there are always geographic and political considerations as well. Dr Uhlenhaut opined that Member States are invited to nominate whichever laboratory they think they want; there is no entry test, or pre-qualification of any kind. For the UNSGM it can be a simple process. In case of an unknown outbreak, the UNSGM likely would not be the first response mechanism. She also stressed that the UNSGM does not seek to answer questions related to attribution in an investigation, though this can be added to the mandate. Its main role is to distinguish between a natural occurring incident and use of a weapon.

9.14 Dr Uhlenhaut was then asked about potential simultaneous investigations, for example where the OPCW is assisting the UNSGM while also being asked to assist a Member State via a technical assistance visit for the same incident. She replied that in case of such an event the UNSGM activities need to be isolated as it is a stand-alone investigation conducted under highest scientific but also political scrutiny. Those performing the investigation are not allowed to share the findings related to the investigation with anyone except the head of mission. The UNSGM investigation needs to be fully insulated from whatever other assistance is going on.

9.15 A question was then asked about the importance of data security during investigations and whether this is considered when picking a laboratory for analysis of sensitive samples. Dr Uhlenhaut noted that they have secure measures to communicate and they would be fully utilised in any investigation. Secure communications are crucial because

even a suspicion that evidence has been tampered with would certainly make it difficult to argue that an investigation is still intact. This is a continual concern and challenge.

10. AGENDA ITEM TEN – Toxins in food

10.1 Dr Arjen Gerssen (Wageningen Food Safety Research (WFS), the Netherlands) introduced the work of WFS, an independent research institute that works mainly for the Dutch Government. They are the official control laboratory for the Netherlands, conducting over two hundred thousand analyses per year, and serve as a national reference laboratory for all biochemical compounds and viruses in the EURL network and support other EU Member States in analysis and reference data associated with growth promoters and for plant and mycotoxins.

10.2 Referring to natural toxins in food, Dr Gerssen's group focuses on both plant toxins and algal toxins. Intoxication incidents with plant toxins are on the rise and getting a lot of attention from the European Commission, resulting in more relevant legislation. He then noted some real-life examples of plant toxins to which the WFS helped respond.

10.3 Algal toxins are released by some types of algae when they are present in large quantities (blooms) and decay or degrade. This is a natural process and occurs when high nutrient levels and warm temperatures often result in favourable conditions for algae blooms to form. Human intoxications are usually due to consumption of seafood contaminated by such algae and to some food supplements, e.g., those containing blue-green algae. Some of the algal toxins that the WFS monitor include Amnesic Shellfish Poisoning (ASP, caused by domoic acid produced by the diatom Pseudo-nitzschia algae), Paralytic Shellfish Poisoning (PSP, caused by saxitoxins), Tetrodoxins (TTX, origin in puffer fish, occurs naturally in (sub)tropical seas), Diarrheic shellfish poisoning (DSP, caused by okadaic acid and azaspiracid group toxins), and Ciguatera fish poisoning (caused by Gambierdiscus toxicus benthic algae).

10.4 Until recently, the official method for algal toxin analysis was the mouse bioassay. Due to some known drawbacks, this method was abandoned and a new official method based on using chemicals and cell-based methods was recently adopted. In the Netherlands, shellfish production sites are regularly tested and these results are combined with post-harvest (i.e., on the food market) testing to monitor regulated toxin levels. There is still a fair amount of research taking place in regard to enhancing the existing methods of analysis for all the different types of algal toxins. The case of palytoxins was noted. Because of the size of these toxins, oftentimes a lot of information is lost during analysis. To improve analysis by mass spectrometry, his group added lithium resulting in an increased sensitivity of the method. They also looked into fragmentation patterns to find out more about the structure of the compound and the type of palytoxin present in the sample.

10.5 WFS also searches for new toxins by using a combination of effect-based assays and analytical chemistry. They have created a library for over 1,100 algal toxins, but do not have standards for the majority of them so their unequivocal identification is tentative.

10.6 In conclusion, Dr Gerssen remarked that a large number of toxins might end up in the food chain. Monitoring of these substances is well organised and defined. A combination of both toxicology/in-vitro bioassays and analytical chemistry helps to identify unknowns. For most identified toxins, standards are lacking and isolation

and/or synthesis is very difficult, though some toxins are very easy to get, especially the water-soluble ones. The floor was then opened for questions.

10.7 Dr Gerssen was asked if he could provide numbers on cases of saxitoxin and PSP toxin intoxications. He responded that such incidents are not so prevalent in Europe or the United States of America due to strict monitoring. The incidents that he is aware of have been mainly registered in Latin America, with a few in Asia.

10.8 It was then posed whether it is known what determines presence or absence of marine biotoxins (climate, water temperature, nutrients). Dr Gerssen noted that this problem is under ongoing investigation. There is no simple answer, but it certainly depends on sunlight, wind, temperatures (in particular in oceanic systems) as well as on nutrients present (from heavy rainfalls or agriculture). It is predictable on the calendar, but the phenomena behind it is not yet understood.

10.9 It was asked what process was used when it is not that obvious what may have caused an intoxication in a patient. Dr Gerssen clarified that they do not analyse patient material often, but in cases of an animal intoxication they send out inspectors to take a large number of samples from the surroundings and do a lot of analysis with an affect-based assay to screen the origin and then go into depth on specific samples instead of running everything on high resolution mass spectrometers as it is very costly and time-consuming.

10.10 Dr Gerssen was then asked about the analysis techniques and capacity of the laboratory —do they have dedicated instruments for each toxin type? He replied that they have 45 LC-MS instruments and 10 high-resolution mass spectrometers. In accordance with the current legislation, they are still forced to often use triple quad mass spectrometer instruments for any molecular confirmation analyses. For some hydrophilic compounds (e.g., for hydrophilic pesticides), as well as for some groups of natural toxins, they have dedicated instruments.

10.11 Dr Gerssen explained that they do not always go all the way with identification but sometimes stop with a “tentative” identification as it is sufficient; full identification is more costly, requires isolation and more material, and is requested only in cases when an incident is so severe that they want to know what is causing it, especially in case of a new compound.

10.12 Dr Gerssen was then asked to comment on the prevalence of the use of tools like lateral flow assays across the different algal toxins. He explained that lateral flow assays are clearly useful tools but can be tricky in practice. For example, lipophilic toxins are completely different in structure and one needs five to six different lateral flow devices to tackle it, which then makes it less expensive to use other analysis tools instead. For the cellular systems, they cannot yet be taken easily into the field.

10.13 On being asked about availability of the libraries referred to throughout the presentation, Dr Gerssen explained that it is their own internally developed library, which they are happy to share with the TWG.

10.14 It was asked if the reference standards used in their laboratory come from a commercial source or if they were produced by the laboratory itself. Dr Gerssen noted that they tried to produce some standards, but it was too costly, so the majority of standards used are

either commercially available or available via relationships that they have with Japanese, American and EU scientists.

10.15 It was then asked if WFS conduct any on-site analyses, and if so what methods or instruments they use.

10.16 Dr Gerssen replied that they use different approaches, including antibody analysis with lateral flow devices, and they are investigating using portable and transportable mass spectrometers. When it comes to food safety, it is expected that in the future they will do more and more screening on-site and will only send suspect samples that require further investigation to the lab.

10.17 Dr Gerssen was then asked to elaborate more on how the potential for WFS' analyses to be used in legal proceedings and court impacts their analytical practices. Dr Gerssen provided an example of a toxin that was not explicitly listed or covered in existing legislation. Investigation of such a toxin could draw the attention of the competent authorities as this could cause production areas to be closed. Industry would be strongly impacted and would seek to go immediately to court where they would start arguing the validity of the laboratory results. Accreditation is therefore important so that toxins can continue to be added, decreasing these potential legal challenges. He stressed that they look to provide results and data and do not take actions – that is government's role. But they do sometimes need to prove to the government that a certain situation is a real threat that needs action.

11. AGENDA ITEM ELEVEN – Subgroup breakout sessions

The meeting participants split up to continue discussions by subgroups 2 and 3.

12. AGENDA ITEM TWELVE – Final discussion, comments and next steps

12.1 The Chairperson invited the leads from subgroups 2 and 3 to present a short overview of the discussions held during the breakout session.

12.2 Dr Bossée remarked that subgroup 2 continued its discussions on the summary table they are working on. They still need some time to review the data compiled in the summary tables before sharing with all TWG members for their input, but do not think that the final prioritisation will change very much. They just want to make sure that the data is as correct and complete as possible.

12.3 Dr Bossée also noted that the subgroup is researching whether any bioregulators need to be considered. They are applying a similar set of criteria to see how some of these might be prioritised for consideration by the TWG.

12.4 Dr Kalb reported that the discussion in the subgroup 3 breakout session mainly revolved around the two topics they have focused on for the past few months: standardisation and reporting methods. On standardisation, they discussed how there seem to be more stringent criteria applied to mass spectrometry analysis as opposed to ELISA or some other methods. The question is how methods like ELISA can be more standardised without prescribing exact standard operating procedures for the laboratories. There was a lot of discussion regarding how the quality of results from any given laboratory can be surmised based on the techniques and criteria they are using. As for reporting, the

current reporting system in place is very prescribed, detailed and intense, likely because this is what has been done, successfully, for chemical warfare agents. However, this may not be the best way to go for biotoxins. There is a concern that some very good laboratories will not participate moving forward because the reporting criteria are too onerous.

12.5 It was noted that in previous experiences when laboratories were asked to provide very detailed and exact reporting, but were not used to the level of rigor required, it was best to ease the reporting requirements. This was done for laboratories during OPCW PTs, where the reporting required for biomedical samples was less strict at the beginning but made more so year after year as laboratories accustomed themselves to the reporting requirements. In fact, the level of last year's reporting on biomedical samples was very high, including from laboratories that did not have a long history of adhering to OPCW reporting requirements.

12.6 In providing final remarks, the Chairperson summarised some of the key points and questions raised during the Third Meeting of the TWG, including:

(a) Mass spectrometry databases should be more readily available to various countries.

(b) Availability of reference standards is crucial. This should be revisited after finalising the priority list of toxins from subgroup 2.

(c) An important question to consider is how to prove a case of alleged use of a biotoxin, considering ever-present natural background levels. This is especially relevant to food toxins. In this respect, quantification is important, which is not "standard" for OPCW-designated laboratories.

(d) The topic of quality assurance should not be forgotten and is crucial for OPCW/UNSGM.

(e) It is important to have the availability of complementary methods: immunological, mass spectrometry, functional; a "toolbox" approach.

(f) How can knowledge gained, and lessons learned from the various exercises to date be applied to the biotoxins that may appear on the list from subgroup 2?

(g) In the case of toxins that have not been widely considered before, can the TWG make a blueprint of activities necessary to better understand them or a potential case of misuse involving them?

(h) A mixture of complementary techniques is necessary; however, many laboratories are specialised in groups of toxins (e.g., food toxins) or certain chemical analysis techniques (e.g., many OPCW designated laboratories).

(i) UNSGM seems to be less stringent on reporting criteria. How does this translate to OPCW requirements?

(j) A uniform, agreed upon reporting format is very important. Every country seems to have their own, sometimes depending on the type of toxin.

(k) In a case where the causative agent is not known, how may this impact the laboratories that could or should be brought into the investigation? The UNSGM roster is flexible in that respect.

(l) The UNSGM and OPCW should continue the conversation around an agreed upon approach in any alleged use of a biotoxin.

12.7 Turning to the possible dates of the next TWG meeting, the Chairperson proposed meeting virtually again in the February/March 2022 timeframe. The SAB Chairperson, Dr Christophe Curty, also reminded TWG members that in case there is ever an urgent recommendation for the Director-General to consider, it can be passed along intersessionally to the SAB which can then forward it along; the TWG does not have to wait until its end-of-mandate report to provide all of its recommendations.

12.8 The TWG Chairperson applauded yet another productive TWG meeting filled with informative and inspirational presentations and thanked everyone for their participation.

13. AGENDA ITEM THIRTEEN – Closure of the meeting

The Chairperson ended the meeting at 17:05 on 12 November 2021.

ACKNOWLEDGEMENTS

The TWG members thank the guests and members of the Technical Secretariat who participated in discussions. The TWG also wishes to acknowledge Ms Ernesa Ademagić of the OPCW Office of Strategy and Policy for her support and contributions to the meeting and its preparations. Lastly, the TWG thanks the OPCW Director-General for his establishment and support of the TWG, and acknowledges the generous contribution of the European Union that helps to cover the costs of the Group's work.

Annex: List of Participants at the Third Meeting of the Scientific Advisory Board's Temporary Working Group on the Analysis of Biotoxins

Annex

LIST OF PARTICIPANTS AT THE THIRD MEETING OF THE SCIENTIFIC ADVISORY BOARD'S TEMPORARY WORKING GROUP ON THE ANALYSIS OF BIOTOXINS

	TWG Member	**Institution**
1.	Dr Isel Pascual Alonso*	University of Havana, Cuba
2.	Dr Crister Åstot	Swedish Defence Research Agency (FOI), Umeå, Sweden
3.	Dr Anne Bossée*	DGA CBRN Defense, France
4.	Dr Graeme Clark	Defence Science and Technology Laboratory, Porton Down, Salisbury, United Kingdom
5.	Dr Cindi Corbett	National Microbiology Laboratory, Public Health Agency of Canada
6.	Dr Christophe Curty*[18]	Spiez Laboratory, Switzerland
7.	Dr Brigitte Dorner	Robert Koch Institute, Germany
8.	Dr Mostafa Ghanei*	Baqiyatallah University of Medical Sciences, Islamic Republic of Iran
9.	Dr Suzy Kalb[19]	Centers for Disease Control and Prevention, United States of America
10.	Dr Zrinka Kovarik*	Institute for Medical Research and Occupational Health, Croatia
11.	Dr Andrea Leisewitz*[20]	Universidad San Sebastián, Chile
12.	Dr Robert Mikulak*	Department of State, Washington, D.C., United States of America
13.	Dr Daan Noort*[21]	TNO, Netherlands
14.	Dr Yulia Polyak	Russian Academy of Sciences, Russian Federation
15.	Dr Fengxia Sun*	Hebei University of Science and Technology, People's Republic of China
	External Speakers	**Institution**
16.	Dr Thomas Bergstrom	Swedish Defence Research Agency (FOI), Umeå, Sweden
17.	Dr Arjen Gerssen	Wageningen Food Safety Research, the Netherlands
18.	Dr Jacques-Antoine Hennekinne	French Agency for Food, Environmental and Occupational Health and Safety, France
19.	Dr Christine Uhlenhaut	Weapons of Mass Destruction Branch, Office for Disarmament Affairs, Switzerland
20.	Dr Els Van Pamel	Flanders Research Institute for Agriculture, Fisheries and Food, Belgium
	Technical Secretariat Staff	**Division**
21.	Dr Peter Hotchkiss[22]	Office of Strategy and Policy
22.	Mr Alexandre Bennet	OPCW Laboratory
23.	Dr Timothy Wood	OPCW Laboratory
24.	Mr Jakob Sax	Office of Strategy and Policy

* Member of the SAB

- - - o - - -

18 Chairperson of the SAB.

19 Vice-Chairperson of the TWG.

20 Vice-Chairperson of the SAB.

21 Chairperson of the TWG.

22 Secretary to the SAB.