OPCW

Scientific Advisory Board

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SUMMARY OF THE SIXTH 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 sixth meeting from 18 to 20 October 2022 in The Hague. The meeting was chaired by Dr Crister Åstot on behalf of the SAB, with Dr Suzy Kalb as Vice-Chairperson.

1.2 The TWG Chairperson opened the session welcoming everyone to the sixth official meeting of the TWG. He noted that this sixth meeting was the last opportunity for the group to consider information from external experts before putting its full attention on finalising its views and recommendations and drafting the end-of-mandate report.

2. AGENDA ITEM TWO – Adoption of the agenda

The TWG adopted the following agenda for its sixth meeting:

1. Opening of the meeting (TWG Chairperson)
2. Adoption of the agenda (All)
3. Microbial investigation processing interlaboratory exchange (Dr Cindi Corbett)
4. The use of antibodies in toxin detection (Dr Stéphanie Simon and François Becher)
5. Method development for biotoxin forensics (Dr David Wunschel)
6. The United Nations Secretary-General's Mechanism Designated Laboratories Workshop series: coordinating networks to increase standardisation while retaining methodological flexibility (Dr Cédric Invernizzi)
7. Vision and anticipated activities for the Network of ASEAN CBR Defence Experts (Dr Loke Weng Keong and Ms Chen Hsiao Ying)
8. Challenge to legal expertise and outbreak management in case of detection of bacterial toxins involved in foodborne illness (Mr Alexandre Leclercq)
9. Clinical diagnosis of toxin exposure (Dr Ziad Kazzi)
10. OPCW biotoxin exercises (Dr Daan Noort)

11. Discussion on cooperation with the United Nations Secretary-General's Mechanism (All with Dr Christine Uhlenhaut)

12. Any other business and next steps (All)

13. Closure of the meeting (TWG Chairperson)

3. AGENDA ITEM THREE – Microbial investigation processing interlaboratory exchange

3.1 Dr Cindi Corbett, Public Health Agency of Canada, began by providing an overview of the National Microbiology Laboratory and its Health Security and Response Division. The Health Security and Response Division plays an important role in Canada's National CBRNE[1] Team and its ability to respond to incidents.

3.2 The microbial investigation processing interlaboratory exchange (MI-PIE) was established to identify best practices in laboratory process during a biocrime/bioterrorism investigation through interlaboratory exercises and the development of recommendations. A recent MI-PIE exercise involved the participation of 11 laboratories in different countries—six of which participated fully—that had to detect and identify bacterial agents on an unknown panel of five samples. The National Microbiology Laboratory scored the submissions and provided a laboratory report on the findings of each participating laboratory. After the exercise, an assessment of the supporting laboratory processes utilised within the laboratory was then conducted by a panel of subject matter experts coordinated by the Irregular Warfare Support Directorate. The panel of experts reviewed the legal aspects on, inter alia, chain of custody records, communication records, laboratory notes, scientific personnel qualifications, issuance of reports, standard operating procedures (SOPs), and quality assurance programmes. The panel aggregated the feedback and were able to provide a list of relative strengths in place in the microbiology laboratories, as well as opportunities for improvement to support law enforcement investigations. Dr Corbett concluded by noting some considerations their laboratory is considering moving forward to further improve support for legal investigations that require microbial analysis.

3.3 The group asked whether the SOPs used by the laboratories were acceptable in all situations, such as when an unknown sample needs to be analysed. It was noted in the following discussion that there always needs to be a modicum of flexibility in SOPs as one will never know a priori all information about an unknown sample or situation at the onset of analysis, and this is accepted.

4. AGENDA ITEM FOUR – The use of antibodies in toxin detection

4.1 Dr Stéphanie Simon and Dr François Becher, of the French Alternative Energies and Atomic Energy Commission (CEA), gave an overview of the work their research teams conduct on producing and using antibodies for the detection and diagnosis of biotoxins. Dr Simon first provided context to the work of their unit and the different types of work they perform.

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[1] CBRNE = chemical, biological, radiological, nuclear, and explosives.

4.2 Dr Simon then focused on the immuno-analysis work of the unit. It takes them nine to 10 months to go from antigen production all the way through test development and validation. Lateral flow immunoassays are developed and used in the field while ELISA[2] and mass spectrometry methods are used for confirmation in the laboratory. They have developed monoclonal antibodies for a range of biotoxins, to include ricin, *Clostridium perfringens* enterotoxin, *Clostridium perfringens* Epsilon toxin (ETX), *botulinum* neurotoxins (BoNT), and staphylococcal enterotoxins (SEs). She detailed the extensive work the team has conducted on developing different lateral flow immunoassays and ELISAs for detecting the various aforementioned biotoxins, to include the recent development of a CE marked test for in vitro diagnosis of ricin intoxication. A multiplex lateral flow immunoassay for BoNT (A,B,E), ricin, and staphylococcal enterotoxin B was developed at CEA and is commercially available.

4.3 Dr Becher then presented on the mass spectrometry-based biotoxin identification and detection work the unit conducts. They have developed methods to detect ricin, abrin, BoNT, SEs, and ETX. They often use antibodies grafted to magnetic beads to capture biotoxins for subsequent analysis using tandem mass spectrometry, both via targeted proteomics as well as activity-based assays. They use both bottom-up methods (where the biotoxins are digested and signature peptides are detected), as well as sometimes top-down methods (where they detect the intact protein, as in the case of staphylococcal enterotoxin A).

4.4 The group was impressed with the wide range of high molecular weight biotoxins CEA has expertise in and noted the challenges involved with staying proficient in so many different biotoxins.

5. AGENDA ITEM FIVE – Method development for biotoxin forensics

5.1 Dr David Wunschel, from Pacific Northwest National Laboratory in the United States of America, shared his experience in developing methods for biotoxin forensics. He began by noting all the different common forensic questions that may be asked about a sample containing a biotoxin, such as which toxin is present, or what production method was used when making and/or isolating the biotoxin present. Dr Wunschel then noted some different methods developed for the characterisation of several different biotoxins.

5.2 Questions were raised as to whether it can be determined if a ricin-containing sample had been intentionally purified, and if so, what details about this preparation can be determined. Proteomics-based approaches for protein identification can be valuable. In addition, it is worth understanding the different isolation approaches one might take to isolate ricin from castor beans. This can yield a list of potential related chemicals or metabolite markers that may have detectable signatures. Understanding these signatures can then indicate a specific production route or purification method that was used.

5.3 A forensic analysis of botulinum toxin-containing samples can also be done, although the approach is very different. Complex protein ratios can be determined depending on train and sample fraction, but the analysis can be complicated. Peptide toxins can also be analysed. The field of peptidomics has developed a number of useful approaches for peptide identification; however, challenges exist for the identification of unknowns.

5.4 Dr Wunschel was asked how he would approach analysing a truly unknown sample. He replied that he would start with a comprehensive extraction using the methods they have

[2] ELISA = enzyme-linked immunosorbent assay.

in place and would then screen for masses and fragmentation patterns. He could subsequently use existing databases to do a comprehensive proteomics analysis, but the challenge was knowing which databases to use.

5.5 When asked about the maturity of the field for determining the provenance of biotoxin samples, Dr Wunschel indicated that it is still in its infancy. Most work done to date has been on crude samples that provide a lot of information because they have not been purified. Not much work has been done on understanding signatures of purified samples. Additionally, it is unknown how any signatures that are present in the samples age with the biotoxin itself.

6. AGENDA ITEM SIX – The United Nations Secretary-General's Mechanism Designated Laboratories Workshop series: coordinating networks to increase standardisation while retaining methodological flexibility

6.1 Dr Cédric Invernizzi, from Spiez Laboratory in Switzerland, provided an overview of the United Nations Secretary-General's Mechanism (UNSGM). He started by giving a very detailed overview of the guidelines and procedures associated with the UNSGM. These were then summarised in a table and compared to the processes involved in the Biological and Chemical Weapons Conventions. An investigation of alleged use was used as an example to show the similarities and differences between the different investigatory mechanisms. Dr Invernizzi then highlighted some of the recent activities and laboratory exercises associated with ensuring readiness of the UNSGM laboratory network. These include support through workshops, the organisation of laboratory exercises, and other supplementary products and activities. He concluded by noting that further coordination and cooperation among existing networks, notably between the OPCW designated laboratories and the laboratories participating in UNSGM-affiliated exercises, will further strengthen biotoxin analysis efforts.

6.2 Dr Invernizzi was asked whether funding is available to sponsor participation in the UNSGM meetings from countries with limited resources. He responded that while there are some opportunities for sponsored participation, in general it is the responsibility of each Member State to sponsor their own participation.

7. AGENDA ITEM SEVEN – Vision and anticipated activities for the Network of ASEAN CBR Defence Experts

7.1 Dr Loke Weng Keong, from DSO National Laboratories (DSO) in Singapore, gave an overview of recent activities of the Network of ASEAN CBR Defence Experts with a focus on current efforts to establish an ASEAN biotoxin laboratory network. He noted the current threat from non-State actors in the region and highlighted some foiled CBRN terror plots from the recent past. He noted that the ASEAN laboratory network is a fairly new concept, having had its inaugural meeting in November 2019. He listed the objectives of the network, highlighting their commitment to ensuring a cooperative, regional approach to threat analysis and response in the event of a CBR incident. He then recounted network activities over the past few years—including several in partnership with the OPCW—and described some of the upcoming activities they plan to pursue over the next few years. Dr Loke noted the ASEAN network was looking to develop a hub and spoke model for CBR verification and forensics in southeast Asia, where DSO could act as the hub for other laboratories in the network.

7.2 Ms Chen Hsiao Ying, also from DSO, then joined Dr Loke and shared some specific work on biotoxins that DSO has been conducting lately. In particular, DSO recently developed an 11-in-one biotoxin ELISA detection test kit. She emphasised the quality control steps and adherence to ISO[3] standards that DSO adheres to.

7.3 The group asked how many active laboratories are in the ASEAN network. Dr Loke replied that there are currently 10 active laboratories, but they are still in the early stages of setting up the network and intend to reach out to more laboratories moving forward. Dr Loke reminded the group that there is a large spectrum of capability, available resources, and interest associated with laboratories in the region. Many of the laboratories involved are either military laboratories or public health laboratories. They would like to ensure that at least most of the interested laboratories can participate in activities related to field diagnostics and identification. Dr Loke continued to indicate that the primary focus for the ASEAN countries is to organise themselves into a robust network.

7.4 The group also asked whether the ELISA for the detection of 11 biotoxins that DSO has developed is commercially available. Dr Loke answered that it was not yet available as their first and primary customer is the military, and any subsequent use would require the appropriate permissions. However, they are not against commercialising the product if there is suitable interest.

8. AGENDA ITEM EIGHT – Challenge to legal expertise and outbreak management in case of detection of bacterial toxins involved in foodborne illness

8.1 Mr Alexandre Leclercq, from the Institut Pasteur in France, discussed the important aspects of any investigation related to biotoxin exposure. He gave an overview of his expertise and extensive experience as a technical expert in legal cases of food poisoning outbreaks. He gave an overview of all the elements involved in any investigation and subsequent legal proceedings, from initial outbreak and management thereof, through sampling to analysis, and then the role of technical experts and litigation. Some critical aspects were then highlighted through several case studies.

8.2 Dr Leclercq described a number of lessons learned from all the cases he has been involved in, touching on many aspects important to an investigation of a food poisoning outbreak. Of utmost importance is to always remain impartial and objective, noting any conflicts of interest. One should be fully transparent about one's expertise and experience in any legal case. In addition, as an expert witness, it is important to ensure all accreditations are up to date, that one maintains relevant trainings, and hostile questions and situations are handled appropriately.

8.3 Dr Leclercq was asked how a judge handles a situation when two experts have disparate technical views in a case. He replied that often the judge will then seek guidance via a third expert opinion to help resolve a situation where there is conflicting opinion.

9. AGENDA ITEM NINE – Clinical diagnosis of toxin exposure

9.1 Dr Ziad Kazzi, from Emory University in the United States of America, discussed his experience as a medical doctor who has clinically diagnosed poisonings by biotoxins in different situations and scenarios. He began by discussing some of the aspects of

[3] International Organization for Standardization.

biotoxin poisoning that make those cases challenging. For example, in ricin poisonings, clinical manifestations can depend on the route of exposure. In general, he noted that many biotoxins cause non-specific and overlapping clinical presentations, often resulting in exposures to them being either misdiagnosed and/or hard to diagnose. In addition, the onset of signs and symptoms of exposure can often be delayed, and there may be few resources available for testing, with laboratory testing potentially not even available depending on the biotoxin. He then presented examples of toxidromes associated with biotoxin poisoning.

9.2 Dr Kazzi then went into detail on several case studies—some of which he attended to personally—that included exposure to a biotoxin. Each of the studies highlighted the challenges and difficulties, with so many other potential causes, of diagnosing exposure to a biotoxin, as well as identifying the biotoxin in question so that the proper treatments could be administered.

9.3 After his presentation the group had a fruitful discussion on how physicians could better diagnose biotoxin exposure. Although it is often hard to diagnose exposure to a biotoxin in and of itself, let alone identifying the specific biotoxin, this is critical in order to provide the appropriate treatment. Dr Kazzi remarked that having a user-friendly tool, via a smart phone app, where one could enter symptoms and clinical manifestation that could assist a user in identifying the biotoxin involved would be very helpful, especially for frontline clinicians and those in a pre-hospital field testing setting as well. The challenge would be to make it freely available in different languages, but also to carefully consider what would prompt someone to use this tool and what conclusions could be reached.

10. AGENDA ITEM TEN – OPCW biotoxin exercises

10.1 Dr Daan Noort, Head of the OPCW Laboratory, briefed the TWG on the status of the OPCW biotoxin exercises. He started by describing the work of the OPCW Laboratory and the importance of its off-site analysis via the designated laboratory network. There are currently two types of designated laboratories: those for environmental sample analysis and those for biomedical sample analysis. There are currently 25 designated laboratories in 21 countries for environmental sample analysis, and 20 designated laboratories in 14 countries for biomedical sample analysis.

10.2 Turning to the biotoxin exercises, Dr Noort then gave an overview of their status. Six exercises have been organised since 2017 with the purpose of building expertise in the analysis of samples of ricin and saxitoxin—the two biotoxins listed in the Annex on Chemicals to the Chemical Weapons Convention. There have been at least 20 participating laboratories in each of the biotoxin exercises. Each exercise has a specific scenario and a scope of analysis associated with it for participating laboratories to take into account. The scoring scheme was then described.

10.3 The next logical step is to create a new biotoxin proficiency test so that there would then be a third type of designated laboratory. In order to reach this final step, there is still a lot more work to do, such as ensuring the willingness among potential participating laboratories, updating various documentation, and ensuring proper adherence to the existing, appropriate ISO standards. Dr Noort concluded by noting that the work and recommendations of the TWG will be helpful to the OPCW as it seeks to make the final push towards a proficiency test for biotoxins.

10.4 Dr Noort was asked whether it would be feasible for a biotoxin proficiency test to have different reporting criteria versus the current environmental and biomedical proficiency tests. He noted that the focus of exercises has largely been on mass spectrometry-based techniques, but that it would be good to also be able to assess bioactivity.

10.5 The group had a discussion centred on whether a future proficiency test should cover any biotoxins that are not scheduled. Dr Noort remarked that the immediate consideration is purely on ricin and saxitoxin as these are the two scheduled biotoxins. He added that it is certainly important to be aware of other laboratories that have proficiency in other biotoxins of interest, and this supports the impetus to strengthen the existing relationship with the UNSGM.

11. AGENDA ITEM ELEVEN – Discussion on cooperation with the United Nations Secretary-General's Mechanism

The TWG continued its deliberations related to the relationship between the OPCW and the United Nations Secretary-General's Mechanism (UNSGM) and how this may be further strengthened. Dr Christine Uhlenhaut, from the United Nations Office for Disarmament Affairs, was invited to partake in the discussion. The conversation touched upon the need for a reporting format that is exchangeable and acceptable to both entities before focusing on the steps that could be recommended by the group to further bolster the relationship between the OPCW and the UNSGM. The group discussed informal and formal working groups as well as the types of representatives, experts, and stakeholders, not only from the two entities but also from their respective laboratory networks, that need to be involved moving forward.

12. AGENDA ITEM TWELVE – Any other business and next steps

The Chairperson reminded the group that this is the last substantive meeting the TWG is able to hold, with a final drafting meeting in January 2023 to focus solely on finalising the text and recommendations in the end-of-mandate report.

13. AGENDA ITEM THIRTEEN – Closure of the meeting

The Chairperson ended the meeting at 16:06 on 20 October 2022.

1. ACKNOWLEDGEMENTS

The TWG members thank all the guest speakers for their informative presentations and participation in the meeting and 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 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 Sixth Meeting of the Scientific Advisory Board's Temporary Working Group on the Analysis of Biotoxins

Annex

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

	TWG Member	Institution
1.	Prof Isel Pascual Alonso	University of Havana, Cuba
2.	Dr Crister Åstot[*1]	Swedish Defence Research Agency (FOI), Umeå, Sweden
3.	Dr Anne Bossée[*]	DGA CBRN Defence, France
4.	Dr Graeme Clark	Defence Science and Technology Laboratory, Porton Down, Salisbury, United Kingdom of Great Britain and Northern Ireland
5.	Dr Cindi Corbett	National Microbiology Laboratory, Public Health Agency of Canada
6.	Dr Christophe Curty	Spiez Laboratory, Switzerland
7.	Dr Brigitte Dorner	Robert Koch Institute, Germany
8.	Prof Mostafa Ghanei[*]	Baqiyatallah University of Medical Sciences, Islamic Republic of Iran
9.	Dr Suzy Kalb[2]	Centers for Disease Control and Prevention, United States of America
10.	Prof Zrinka Kovarik	Institute for Medical Research and Occupational Health, Croatia
11.	Dr Andrea Leisewitz[*4]	Universidad San Sebastián, Chile
12.	Dr Robert Mikulak	Department of State, United States of America
13.	Mr Günter Povoden[*3]	CBRN Defence Centre, Ministry of Defence, Austria
14.	Dr Fengxia Sun[*]	Hebei University of Science and Technology, People's Republic of China
15.	**External Speakers**	**Institution**
16.	Dr François Becher	French Alternative Energies and Atomic Energy Commission, France
17.	Dr Cédric Invernizzi	Spiez Laboratory, Switzerland
18.	Dr Ziad Kazzi	Emory University, United States of America
19.	Mr Alexandre Leclercq	Institut Pasteur, France
20.	Dr Stéphanie Simon	French Alternative Energies and Atomic Energy Commission, France
21.	Dr Christine Uhlenhaut	United Nations Office for Disarmament Affairs
22.	Dr Loke Weng Keong	DSO National Laboratories, Singapore
23.	Dr David Wunschel	Pacific Northwest National Laboratory, United States of America
24.	Ms Chen Hsiao Ying	DSO National Laboratories, Singapore
25.	**Technical Secretariat Staff**	**Division**
26.	Dr Peter Hotchkiss	Office of Strategy and Policy
27.	Dr Daan Noort	OPCW Laboratory

[*] Member of the SAB
[1] Chairperson of the TWG
[2] Vice-Chairperson of the TWG
[3] Chairperson of the SAB
[4] Vice-Chairperson of the SAB

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