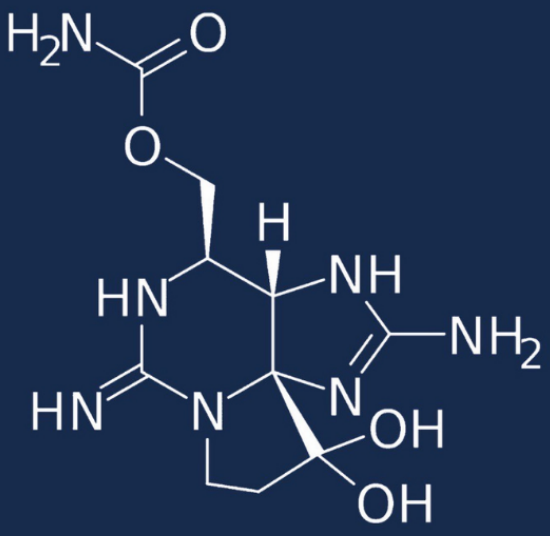
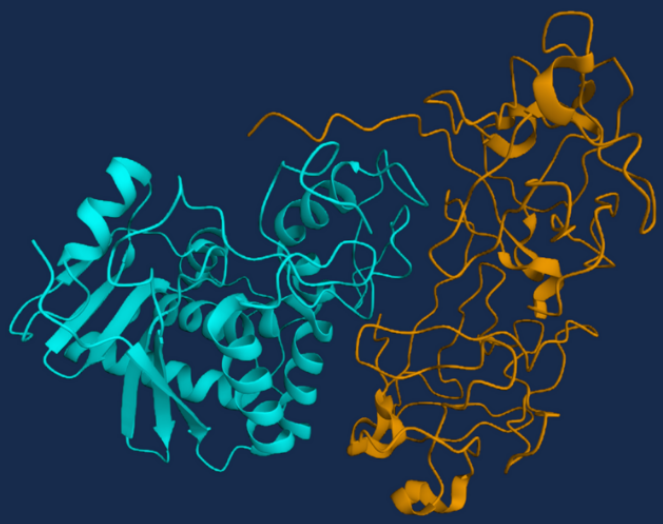
Analysis of Biotoxins

Report of the Scientific Advisory Board's Temporary Working Group



Saxitoxin



Ricin

SAB/REP/1/23
April 2023

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EXECUTIVE SUMMARY

1. Biotoxins are toxic chemicals usually obtained from biological source materials. Two specific biotoxins are listed in Schedule 1 of the Annex on Chemicals to the Chemical Weapons Convention (hereinafter "the Convention"): saxitoxin, which is a small molecule, and ricin, which is a large protein. These two examples illustrate well that biotoxins vary widely in properties such as structure, size and mechanisms of toxicity. The misuse of any biotoxin, regardless of whether it is specifically listed in the Annex on Chemicals to the Convention (the three schedules of chemicals), is prohibited under the General Purpose Criterion, a central provision of the Convention.

2. The previous Temporary Working Group (TWG) on Investigative Science and Technology evaluated methods for sampling and analysis of chemicals that are of relevance to the Convention in the context of non-routine missions.[1] The TWG found that analysis of biotoxins differs appreciably from analysis of the well-known chemical warfare agents, such as the nerve and mustard agents. The TWG found that few laboratories are skilled in both high molecular weight (HMW) and low molecular weight (LMW) biotoxin analysis, making it unlikely that a single network of laboratories could be designated for analysis of a broader range of both LMW and HMW biotoxins.

3. The TWG on Investigative Science and Technology recommended that the Director-General "consider establishing a TWG to advise on how to ensure that the Secretariat has access to required capabilities for the analysis of relevant biological toxins" (Recommendation 30). The Director-General accepted this recommendation and directed the new TWG to review the science and technology relevant to the analysis of biotoxins and considerations that need to be taken into account in investigations of their alleged use. This review also recognises that the United Nations Secretary-General's Mechanism (UNSGM) for investigating Alleged Use of Chemical and Biological Weapons also may be called on to investigate alleged use of biotoxins.

4. The TWG on the Analysis of Biotoxins was organised into subgroups, focused on five groups of questions posed by the Director-General.

5. Subgroup 1 identified possible underlying requirements for analysis of biotoxins. It outlined the overall requirements for an investigation of an incident of alleged use of a biotoxin as a weapon. This included the importance for field detection and the potential value of strengthening early clinical diagnosis of biotoxin exposure. Subgroup 1 noted that biotoxins are naturally occurring and stressed the need to determine whether or not exposure was due to deliberate use, rather than natural exposure. It also noted that, given the wide range of biotoxins that might be encountered and the differing analytical methods these could involve, information from field screening and medical diagnosis of suspected

[1] The report of the TWG on Investigative Science and Technology (SAB/REP/1/19, dated December 2019) is available at: https://bit.ly/TWGIST.

victims are important to facilitate choice of appropriate laboratory methods for unambiguous identification.

6. Subgroup 2 identified a number of biotoxins that are most likely to be relevant in investigations of alleged use, based on a series of criteria that the subgroup developed. Among the criteria are historical use, availability, toxicity/activity, and stability. The list contains nine biotoxins or biotoxin families deemed most relevant, with a wide range of toxicological effects, and includes both LMW and HMW biotoxins. The subgroup noted that it is not practical for the OPCW to develop an independent capability for analysis of every biotoxin considered "most relevant" and recommended that the OPCW plan to draw on sophisticated biotoxin analysis capabilities that exist in other fields. The TWG recommends that the OPCW sponsor a workshop, with participation of outside experts, to assist in identifying likely sources of analytical expertise.

7. Subgroup 3 reviewed technical requirements for analysis of biotoxins. It stressed the need for the OPCW to take fully into account that requirements differ widely between LMW and HMW biotoxins. For LMW biotoxins, the OPCW should generally rely on traditional mass spectrometry-based techniques, such as liquid chromatography-mass spectrometry. For HMW biotoxins, the OPCW should employ a combination of mass spectrometry-based techniques and orthogonal techniques, such as immunological methods and biotoxin activity assays. Subgroup 3 also highlighted a need to review and modify identification and reporting criteria as the analysis of biotoxins differs from that of traditional chemical warfare agents. The current approach is not entirely suitable as it does not take into account methods currently accepted as common practice for HMW biotoxins and/or does not evaluate these methods correctly.

8. Subgroup 3 stressed that standardisation of methods for biotoxins analysis is extremely challenging given the wide range of biotoxins, matrices, and analytical methods. The TWG recommends the application of a more flexible approach to establish best practices. Laboratories involved should work under an overarching quality management system, employing methods that have been published in a peer-reviewed journal and/or demonstrated to be effective in international analytical exercises. In view of the value for investigations of alleged deliberate use, reporting of sample analyses should also include the presence of inactivated biotoxins and presence of chemicals that are characteristic of biotoxin preparation and/or source. The subgroup also noted that biotoxins are likely to be present in biomedical samples from suspected victims only at extremely low concentrations and that the OPCW should develop a capability to analyse biotoxins in such samples.

9. Subgroup 4 addressed cooperation between the OPCW and other international efforts for biotoxin analysis. It noted that the focus of the OPCW is expected to remain on the two biotoxins on Schedule 1, saxitoxin and ricin, for the foreseeable future. In this connection, the TWG noted that the UNSGM deals more broadly with a range of HMW biotoxins. To ensure that analytical practices are consistent between the OPCW and UNSGM, and available to either investigation mechanism, a process to harmonise biotoxin analysis-related activities is necessary. The TWG recommends that the OPCW and UN cooperate and assist each other in strengthening international capabilities for biotoxin analysis, drawing on the relationship agreement for cooperation between the two organisations.

Common guidelines and best practices should be developed for use by both. To facilitate building international capabilities for forensic analysis of biotoxins, the OPCW should work closely with the United Nations (UN) and other potential partners, to establish an informal network of biotoxin analysis laboratories. Responsibility for coordinating such a network should be shared between the OPCW and the UN.

10. The TWG also noted that multiple efforts to develop analytical capabilities for biotoxins exist and analytical exercises are being conducted by the OPCW and the RefBio project,[2] which is linked to the UNSGM. To help ensure that results are broadly applicable, unnecessary duplication is minimised, and gaps are filled, the TWG recommends that the OPCW should invite other organisations conducting biotoxin analysis programmes to meet informally as soon as possible, and periodically thereafter.

11. Subgroup 5 examined what institutional or legal measures need to be established to facilitate cooperation between the OPCW and other organisations working on development of capabilities for analysis of biotoxins. The TWG recommends that in developing mechanisms for international cooperation, the OPCW should emphasise sustainability and utilise existing structures, such as the relationship agreement between the OPCW and the UN, possibly supplemented by a relatively simple document that provides flexible terms of reference. There is no need for new formal, legal agreements in order to create the cooperation and coordination mechanisms recommended by the TWG.

12. The recommendations of the TWG are listed in full below. They have been grouped thematically and numbered accordingly. These recommendations are subsequently repeated in their respective subgroup sections, where they will not necessarily appear in ascending numerical order. Note that of the 23 recommendations listed, while the TWG considers all of these to be important, there are 9 specifically that it feels warrant prioritised consideration and are thus marked as ‘strong’ recommendations.

RECOMMENDATIONS

In-field detection and identification (to include clinical diagnosis)

13. **Strong recommendation 1:** The OPCW should compile and disseminate information on the diagnosis and treatment of biotoxin exposure, including through convening a technical workshop on this topic involving clinicians and veterinarians with relevant experience, Technical Secretariat (hereinafter “the Secretariat”) staff, and representatives from the TWG on the Analysis of Biotoxins. Not only would early clinical diagnosis assist in identification of the agent used, but dissemination of information on diagnosis and treatment of biotoxin casualties would contribute to the OPCW’s efforts on assistance and protection.

[2] More information on RefBio is available at: https://bit.ly/RKIRefBio.

14. **Recommendation 2:** The Secretariat should become familiar with currently available methods for field detection of biotoxins and monitor developments in this area and disseminate this information as appropriate. It should evaluate what commercial off-the-shelf (COTS) products or other validated detection devices could be acquired within the short timeframes required during an investigation and whether any field detection devices for biotoxins should be kept in the OPCW's inventory of approved equipment.

Most relevant biotoxins to consider

15. **Strong recommendation 3:** Based on the factors outlined by the TWG, the OPCW's efforts to develop its capabilities for investigation of alleged biotoxin use should focus on the nine "most relevant" biotoxins listed below. Recognising that seven of these nine biotoxins are not listed on Schedule 1 in the Annex on Chemicals to the Convention, the OPCW should plan to draw on sophisticated biotoxin analysis capabilities that may exist in other fields. The "most relevant" biotoxins are:

(a) abrin;

(b) aflatoxins;

(c) botulinum toxins;

(d) epsilon toxin;

(e) ricin;

(f) saxitoxin;

(g) *Staphylococcus aureus* enterotoxins;

(h) T-2 toxin; and

(i) tetrodotoxin.

16. **Strong recommendation 4:** The OPCW should, in the near term, survey existing literature and recognised experts in biotoxin analysis to identify laboratories that possess specialised capabilities for analysis of each of the "most relevant" biotoxins. The OPCW should consider convening a workshop as part of this effort.

17. **Recommendation 5:** The OPCW should continue to monitor developments in the field for the potential further modification of the list of "most relevant" biotoxins presented in this report. In assessing which biotoxins are the most relevant, the OPCW should continue to take into account the weighted rating criteria provided to the Secretariat. The criteria include factors such as historical use, availability, toxicity/activity, and stability.

18. **Recommendation 6:** The OPCW should continue to monitor developments on compounds of biological origin, in the field of bioregulators in particular, for indications of increased risk of misuse as weapons.

Forensic considerations

19. **Strong recommendation 7:** The OPCW should adopt a comprehensive forensic approach to every investigation of alleged use of biotoxins (e.g., determining naturally occurring versus deliberate release, recombinant production, and sample provenance or batch matching via a comprehensive molecular analysis of the sample).

20. **Recommendation 8:** The OPCW should continue to support activities that aid international capability development with respect to the identification of the provenance of a biotoxin. This may include exercises involving the "batch matching" or linking of samples collected during an investigation.

21. **Recommendation 9:** For authentic biotoxin samples, the OPCW should also include reporting on the presence of chemicals that are characteristic of biotoxin preparations and may assist in identifying the source and purity of a biotoxin preparation, such as ricinine in ricin-related samples. Other examples include extraction solvents, as well as lipids, peptides, and proteins specific to the source organism.

Laboratory analysis and best practices

22. **Strong recommendation 10:** In its activities related to analysis of biotoxins, the OPCW should take fully into account that the technical requirements for analysis differ widely between LMW and HMW biotoxins.

    (a) For LMW biotoxins, the OPCW should generally rely on traditional mass spectrometry-based techniques, such as liquid chromatography-mass spectrometry.

    (b) For HMW biotoxins, the OPCW should employ a combination of mass spectrometry-based techniques and orthogonal techniques, such as immunological methods and biotoxin activity assays. For HMW biotoxins present in samples at a very low level (nanogram/millilitre or below), the combination of immunoaffinity enrichment-based methods and functional methods (such as biotoxin activity assays) may be the only combination of methods with sufficient sensitivity for the analysis. Both approaches should be used, as long as enough sample material is available.

23. **Strong recommendation 11:** The OPCW should document and disseminate best practices for the unambiguous identification of specific biotoxins included in analysis exercise programmes to support the further development of analytical capability among laboratories.

24. **Strong recommendation 12:** The OPCW should develop minimum specification requirements for performance criteria of immunological and activity assays for the analysis of HMW biotoxins. This should include minimum specification for the immunological components (antibodies) as well as the overall immunoassay and activity assay performance criteria. It is strongly recommended that this is conducted in partnership with the UNSGM laboratory network.

25. **Recommendation 13:** The TWG recommends that:

(a) Laboratories involved in an international investigation should work under an overarching quality management system ensuring regular quality management measures (e.g., pipette calibration, lot documentation, appropriate calibration and documentation of methods, and regular error analysis).

(b) The exact procedures used in an international investigation should be technically robust and should have been published in a peer-reviewed international journal and/or their performance demonstrated in international analytical exercises.

(c) Accreditation of the specific method to be applied in an investigation is not absolutely necessary as long as the laboratory works under an overarching quality management system for biotoxin analysis. This will ensure that the performance criteria of the assays are established and their limitations understood. This approach can help assure the OPCW of laboratories' capability to deal with emerging biotoxins and/or to apply new technologies, if required. Finally, innovative analytical approaches should be considered for use when only a small quantity of sample is available and/or the biotoxin is in a challenging matrix (e.g., blood, environmental). Intelligence and situational awareness might help with sample triage and directing the type of laboratory analysis required.

26. **Recommendation 14:** In view of its value for investigations of alleged use, the OPCW should consider both active and inactive biotoxins within its verification regime.

27. **Recommendation 15:** The OPCW should develop a capability to analyse biotoxins at a clinically relevant range (nanogram/millilitre - picogram/millilitre range) that are likely to be present in biomedical samples from suspected victims, working closely with laboratories that are interested in and technically capable of developing and improving such capabilities.

28. **Recommendation 16:** To better understand possible international technical and forensic legal requirements for biotoxin analysis, the OPCW should make further efforts to identify and compile specific national and international standards (e.g., ISO/IEC 17025:2017) and guidelines for biotoxin analysis (e.g., VERIFIN Blue Book[3]), as well as forensic requirements relating to the use of technical evidence in legal proceedings.

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[3] Vanninen, Paula. "Recommended operating procedures for analysis in the verification of chemical disarmament." The Ministry for Foreign Affairs of Finland, University of Helsinki (2011): 163.

Reporting criteria and testing

29. **Strong recommendation 17:** The OPCW should consider a proficiency test regime for biotoxin analysis that enables a laboratory to seek separate designation for the analysis of saxitoxin or of ricin.

30. **Recommendation 18:** The OPCW should consider reviewing the reporting criteria for the analysis of HMW biotoxins together with representatives of OPCW Designated Laboratories and UNSGM-affiliated laboratories. The modified reporting system should incorporate immunological or functional methods that are relevant for the unambiguous identification of HMW biotoxins. Furthermore, consideration should be given to modifying the current requirements for mass spectrometric analysis taking into account the accepted reporting scheme in analogous scientific fields (e.g., proteomics). This would necessitate a change in the scoring system associated with the analytical exercises.

International cooperation and coordination

31. **Strong recommendation 19:** The OPCW should work closely with the UN, drawing on the relationship agreement for cooperation between the two organisations (EC-MXI/DEC.1, dated 1 September 2000), along with any other interested organisations and laboratories from different sectors (e.g., food safety) to establish an informal network for biotoxin analysis to facilitate building international capabilities for forensic analysis of biotoxins, including in such areas as:

    (a) common guidelines and best practices for biotoxin analysis to be used by the OPCW and the UN in international investigations;

    (b) coordination of requirements for quality assurance management systems for acceptance of biotoxin analysis data in investigations;

    (c) development of a reporting format acceptable for OPCW and UNSGM missions for reporting results of biotoxin analysis, including definition of performance and acceptance criteria for a range of relevant methods; and

    (d) coordination of efforts to minimise gaps and unproductive duplication, including analysis exercises and proficiency testing.

32. **Recommendation 20:** The OPCW should work closely with the informal network of biotoxin analysis laboratories, discussed in the section on "Measures for international cooperation" by subgroup 5. This will develop partnerships with external laboratories with demonstrated expertise in the analysis of specific "most relevant" biotoxins (other than saxitoxin and ricin) to the standard required for an OPCW investigation, and who are willing to provide analytical services to the OPCW on request.

33. **Recommendation 21:** Since the OPCW and the UN would be key partners in the proposed informal network of biotoxin analysis laboratories, the responsibility for coordinating the

network should be shared. The OPCW and the UN should each designate a staff member to act as co-facilitators. The OPCW should consider designating a laboratory staff member for this part-time function.

34. **Recommendation 22:** To help ensure that results are broadly applicable, unnecessary duplication is minimised, and gaps are filled, the OPCW should invite other organisations conducting biotoxin analysis exercise programmes to meet informally as soon as possible, and periodically thereafter. The purpose should be to exchange information on exercises being planned or under consideration, with a view to coordinating the various efforts. This will minimise the burden for laboratories of participating in multiple exercises and to help ensure that the exercise programmes collectively provide a broad picture of the capabilities available internationally for biotoxin analysis.

35. **Recommendation 23:** In developing mechanisms for international cooperation, the OPCW should emphasise sustainability and utilise existing structures, such as the relationship agreement for cooperation between the OPCW and the UN (EC-MXI/DEC.1, dated 1 September 2000), or base them on a relatively simple document that provides flexible terms of reference. The TWG believes there is no need for new formal, legal agreements in order to create the mechanisms recommended in this report.

BACKGROUND

Formation and objectives of the Temporary Working Group on the Analysis of Biotoxins

36. For more than a decade, OPCW inspectors have participated in a number of non-routine missions, notably in the Syrian Arab Republic. Recognising that the non-routine mission portfolio posed new challenges for the Secretariat and required the OPCW to develop new capabilities, the Director-General established the Scientific Advisory Board's (SAB) TWG on Investigative Science and Technology to review science and technology relevant to investigations.

37. During the course of its work, the TWG on Investigative Science and Technology evaluated methods for sampling and analysis of chemicals that are of relevance to the Convention. The TWG found that analysis of biotoxins – toxic chemicals normally obtained from biological source organisms – differs appreciably from analysis of the well-known chemical warfare agents, such as the nerve and mustard agents. Not only do biotoxins vary widely in chemical structure, from small molecules such as saxitoxin to large protein biotoxins such as ricin and botulinum toxins, but very different analytical techniques may be needed in their analysis. Detection of HMW protein-based biotoxins requires very different technologies, instrumentation, and expertise compared to that of LMW biotoxins. The LMW biotoxins are amenable to classic chemical analytical methods, while analysis of ricin and other HMW biotoxins involves methods more characteristic of laboratories that carry out analyses of biological organisms or tissues.

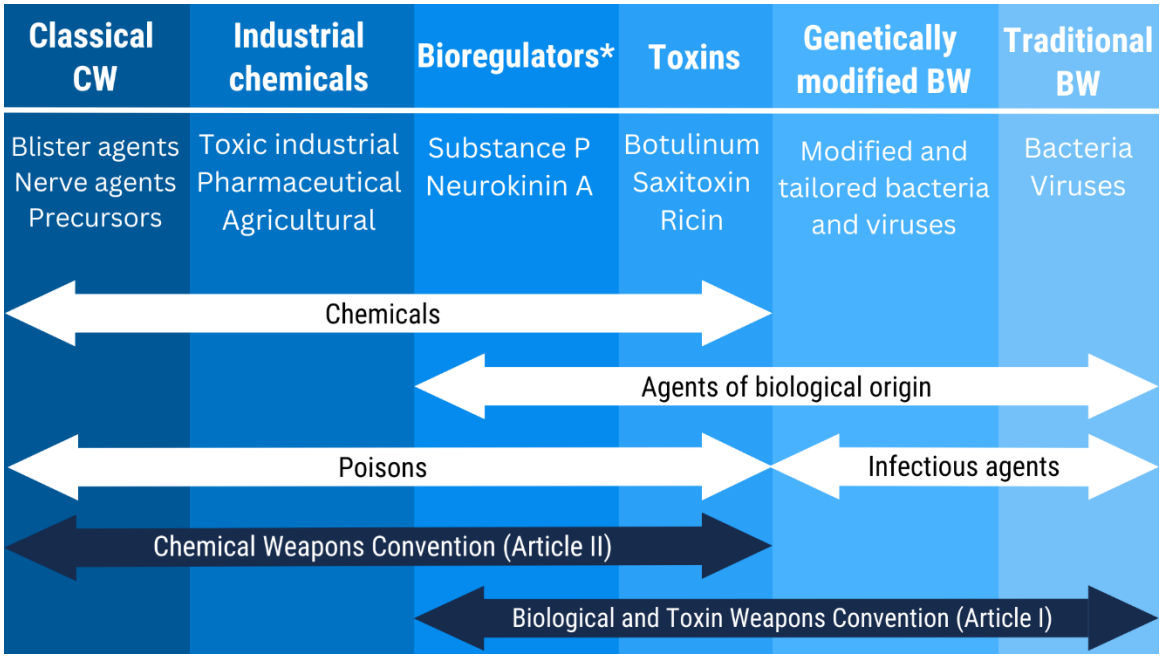
38. The TWG found that few laboratories are skilled in both HMW and LMW biotoxin analysis and given the diversity of molecules within both classes, specialisation on specific groups of biotoxins further separates laboratory capability. In particular, laboratories that analyse chemical warfare agents may not be equipped for the analysis of the broad variety of HMW biotoxins. Also, laboratories that are skilled in HMW biotoxins may not have expertise in analysis of LMW biotoxins. This makes it unlikely that a single network of laboratories could be developed for analysis of both LMW and HMW biotoxins.

39. Consequently, the TWG recommended (Recommendation 30 in SAB/REP/1/19, dated December 2019) that the Director-General "consider establishing a TWG to advise on how to ensure that the Secretariat has access to required capabilities for the analysis of relevant biological toxins." The TWG suggested that the "discussions should bring together SAB members, representatives of Designated Laboratories, and other experts in biological toxin analysis." The TWG also noted that "given the broad diversity of techniques required for toxin analysis, understanding the capabilities of a wider group of laboratories that perform analyses of toxins, in particular, High Molecular Weight (HMW) toxins, would be critical should toxin analysis be required for an investigation. An approach to overcoming capability limitations could be to rely on outside proficiency testing exercises to identify those laboratories experienced in the analysis of HMW toxins specifically, highly toxic protein toxins. Laboratories supporting the United Nations Secretary-General's Mechanism (UNSGM) have experience with analysis of HMW toxins, and could, likewise, potentially seek laboratory and other support from OPCW Designated Laboratories that are experienced in analysis of low molecular weight (LMW) toxins".

40. This recommendation was endorsed by the SAB and forwarded to the Director-General. After careful consideration, the Director-General decided to establish a new SAB TWG on the Analysis of Biotoxins. The objective of this new TWG is to review the science and technology relevant to the analysis of biotoxins and considerations that need to be taken into account in investigations of their alleged use. The terms of reference (TOR) for the TWG are in Annex 3. Dr. Daan Noort was appointed as the Chairperson of the Group. He was succeeded in June 2022 by Dr. Crister Åstot. Dr. Suzy Kalb was appointed Vice-Chairperson.

41. In the TOR, the Director-General noted that the use of any biotoxin as a weapon is prohibited both under the Convention and the Biological and Toxin Weapons Convention (BTWC) (see Figure 1). Thus, the capability to detect, identify, and characterise biotoxins that may be present in samples taken during investigations is essential for the OPCW. The UNSGM for Investigating Alleged Use of Chemical and Biological Weapons also has a mandate to investigate the misuse of biotoxins and provides guidance and assistance in this respect. As such, it is also imperative that the OPCW and the UNSGM work cohesively to share information and minimise duplication of effort, since either might be called on to conduct an investigation of alleged use of a biotoxin.

Figure 1: Visual representation of the spectrum of agents covered by the Convention and the BTWC[4]



42. The TWG held seven meetings during its mandate. Due to the COVID-19 pandemic, the first four meetings could only be held in a virtual format. The remaining three meetings were held in a hybrid format where the majority of participants attended in person, but TWG members and external speakers who could not travel were still able to participate virtually. This approach worked well and ensured maximum participation during the meetings. During the course of its work, the TWG received briefings from 19 invited experts. These briefings supplemented the expertise of the TWG members and included insights on: various analytical methods and considerations for many different types of biotoxins; diagnoses of biotoxin exposures; approaches to provenancing biotoxin samples; discussions on additional compounds of biological origin to consider; updates on UNSGM and other international coordination groups that consider biotoxins; investigations; confidence-building exercises related to biotoxin analysis; and the importance of chain of custody and reporting, among others. Lists of the TWG members and guest speakers who helped inform their deliberations are provided in Annexes 5 and 6 of this report.

FINDINGS OF THE TEMPORARY WORKING GROUP ON THE ANALYSIS OF BIOTOXINS

43. Five subgroups were established to implement the programme of work. The questions in the TOR that the TWG was asked to address were grouped into five sets of thematic topics

[4] Adapted from: Pearson, Graham. ASA Newsletter, February 1990, 90-91; and from: Mathews, Robert. "CBW Overlap/Convergence: A Brief History." Presentation, TWG on Convergence, November 15, 2011. *Differing views exist regarding whether or not bioregulators are covered by the BTWC.

and each set assigned to one subgroup as indicated in Table 1. This summary of findings is organised according to the work of each subgroup.

Table 1: Subgroups of the TWG and their areas of consideration

Subgroup	TOR subparagraph	Question
1	5(a)	What are the underlying requirements for the analysis of biological toxins in order to investigate alleged use of toxic chemicals as weapons?
2	5(b)	What classes of biological toxins are most likely to be relevant in investigations of alleged use?
	5(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?
3	5(d)	What are the technical requirements for analysis of the most relevant types of biological toxins?
4	5(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?
	5(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?
5	5(g)	What institutional or legal measures need to be established to facilitate cooperation between the OPCW and other organisations working on development of capabilities for analysis of biological toxins?

44. In addition, recommendations are made, as appropriate, throughout the text. In some cases, the recommendations are based on considerations from several areas of the text and will appear after the last of those considerations. If necessary, reference is made to earlier text.

Subgroup 1: Underlying requirements for analysis

45. Subgroup 1 addressed question 5 (a): “What are the underlying requirements for the analysis of biological toxins in order to investigate alleged use of toxic chemicals as weapons?”.

46. This subgroup considered the overall process that will be required in order to effectively investigate the alleged use of a biotoxin.[5] More specifically, this section highlights similarities in approaches with existing scheduled chemicals under the Convention and key differences in the requirements for biotoxins that should be considered.

Process of investigating the use of a biotoxin

47. There are effectively five stages to the process of sampling and analysis in the investigation of the alleged use of a biotoxin. All procedures need to conform to forensic standards. Forensics include the application of scientific analysis to support an investigation, including rigorous documentation, such as using a chain of custody and conducting the work under the auspices of a quality management system. The five stages are:

(a) stage 1: sample collection (which may include in-field detection and analysis to collect relevant samples or evidence);

(b) stage 2: initial screening of samples within an appropriate facility (safety triage and corroborating any in-field test results);

(c) stage 3: analysis and unambiguous identification of the presence of a biotoxin;

(d) stage 4: comprehensive molecular profiling of the entire sample; and

(e) stage 5: reporting to the OPCW.

48. Each of these elements forms the basis for how this initial question in the TOR is considered.

Stage 1: Sample collection and in-field detection and analysis

49. One of the principal differences between biotoxins and traditional chemical warfare agents listed in Schedule 1 of the Convention is the difficulty detecting and identifying their presence in the field. There is no universal instrument or detector that can be used for wide-area monitoring of biotoxins. Furthermore, the matrices within which biotoxins might be present are many and varied, including environmental sources (liquid, solid, aerosol), contaminated food and water, and clinical samples. Biotoxins can be found naturally, for example within a castor plant or in contaminated shellfish. In contrast, traditional chemical warfare agents (such as nerve agents) and their precursors have no natural reservoirs. Therefore, the investigation into the alleged use of a biological toxin will need to consider the situation, source of the biotoxin, and if there are indeed valid medical or biotechnological uses for the substance. In general, there are no specific requirements

[5] The criteria for triggering an OPCW investigation were *not* covered by subgroup 1, whose focus was on the underlying requirements to carry out analysis of a biotoxin after an investigation had been initiated. In addition, defining the criteria or a framework as to what areas or facilities should be investigated was also not considered. For example, defining what represents legitimate production and/or use of biotoxins (e.g., medical, research purposes) was deemed out of subgroup 1's scope.

unique to biotoxins with respect to sample quantities, storage, and transportation (C-I/DEC.47, dated 16 May 1997). The collection of blank samples as background control reference material and preservation of the chain of custody of all samples remain essential.

50. With regards to the personal protective equipment (PPE) required by personnel investigating the alleged release of a biotoxin, there are no additional specific recommendations or requirements that should be employed beyond the standard contact and respiratory protection recommended for chemical warfare agents.

51. The principal mode of in-field detection for biotoxins would be either a suite of lateral flow assays (LFA) specific for particular biotoxins or emerging automated systems using proprietary biotoxin detection equipment (see Table 2). A number of COTS or other validated LFA devices are available for a limited range of biotoxins (including the scheduled biotoxins ricin and saxitoxin) in order to fulfil the function of basic in-field analysis. A recent summary of in-field detection assays and equipment for environmental samples has been undertaken and reported by the aforementioned TWG on Investigative Science and Technology (for more information, see SAB/REP/1/19, dated December 2019) and therefore this will not be repeated here. The report highlights that one limitation of the large number of COTS enzyme-linked immunosorbent assays (ELISA) is that most if not all cannot distinguish between a biotoxin and its analogues, such as between ricin and the antigenically related, non-toxic ricin agglutinin; or saxitoxin and its paralytic shellfish poisoning analogues. With respect to LFAs, it was judged that these assays also have significant limitations, such as false positives or, more of a concern, false negatives, and are often only validated for a specific purpose or matrix. Therefore, it should not be assumed that a LFA designed for the analysis of clinical samples (e.g., blood, urine) can be employed for the screening of environmental samples.

52. Nevertheless, from the perspective of ease of use and minimal operator burden, particularly for first responders, a suite of LFAs for different biotoxins may represent a valuable tool during the initial phase of an investigation into alleged use of a biotoxin. An important caveat is that such assays are only indicative of the presence of a biotoxin, rather than definitive proof, with further in-depth laboratory-based analysis required in order to corroborate a positive in-field result. On the other side, a negative result does not prove the absence of a biotoxin, so in-field results should in any case be corroborated in a specialised laboratory. Indirect methods for the detection of biotoxins include identifying the presence of DNA from the original source organism being carried through to identification within a fieldable polymerase chain reaction (PCR) or with whole genome sequencing platform technologies (e.g., MinION). The presence of contaminating DNA in a biotoxin preparation is more likely from low-tech, improvised biotoxin preparation methods rather than from a high-end, state-sponsored facility. Nonetheless, a positive result for the source organism for a biotoxin can represent a further corroborating piece of evidence.

Table 2: Summary of the process of analysis during an investigation of alleged use of biotoxins

	Increasing time from event →				
	Immediate aftermath	**In-field**	**Lab screening (< 2 h)**	**Lab analysis (hours – days)**	**Provenance (weeks – months)**
Technical considerations	Clinical presentation /symptomology Pathology (animal/human)	LFA COTS automated platforms based upon ELISA DNA detection	LFA COTS automated platforms Luminex PCR	Sample prep (e.g., immunoprecipitation) LC/HRMS, LC-MS/MS ELISA *In vitro* cell assays Mouse bioassay Cell-free (substrate cleavage/enzymatic, colorimetric MS) Indirect biomarkers of exposure	DNA sequencing LC/HRMS, LC-MS/MS Elemental analysis Nuclear magnetic resonance Characteristic sample components Peptidic ratio comparison for isoforms (e.g., ricin isoforms)
Other requirements	Trained clinicians and veterinarians to identify biotoxin exposures Appropriate environmental sampling and storage of samples (for downstream analysis)	Trained first responder with appropriate PPE Agreed collection/storage conditions Maintain chain of custody	Mixed hazard screening capability Maintain chain of custody	Different sample preparations depending on matrix (e.g., blood, urine, tissue, food, environmental) Two independent laboratories Two orthogonal validated techniques Activity/toxicity demonstrated Maintain chain of custody	Biotoxin repository Certified reference materials Maintain chain of custody

	Increasing time from event →				
	Immediate aftermath	**In-field**	**Lab screening (< 2 h)**	**Lab analysis (hours – days)**	**Provenance (weeks – months)**
Accreditation or quality standard	N/A	Some COTS products may be available for a highly defined purpose (e.g., biomedical samples only) Accreditation of COTS products (at discretion of manufacturer)	Potential for some screening techniques to be validated and/or accredited	Two orthogonal techniques Both validated for unambiguous identification Analytical laboratory has accredited quality management system Demonstrated proficiency in external quality assurance (EQA) exercises Methods employed have been published in a peer-reviewed journal or used in international proficiency tests	Analytical laboratory has quality management system Demonstrated proficiency in EQA exercises Methods employed have been published in a peer-reviewed journal or used in international proficiency tests
Confidence in result (report to Director-General at the OPCW)	Low	Low (Yes; indicative positive)	Medium (Yes; presumptive positive)	High (Yes; unambiguous positive)	Case by case (Yes; with demonstrable supporting evidence for methods used)

53. **Recommendation 2:** *The Secretariat should become familiar with currently available methods for field detection of biotoxins and monitor developments in this area and disseminate this information as appropriate. It should evaluate what COTS products or other validated detection devices could be acquired within the short timeframes required during an investigation and whether any field detection devices for biotoxins should be kept in the OPCW's inventory.*

54. Depending on the specific type of biotoxin, the symptoms of exposure can appear from within 12 hours to up to five days later. Therefore, a clinical diagnosis, increased prevalence of ill health in a population (signs, symptoms), or even posthumous pathological identification may represent the first indication of an intentional release of biotoxin. Similarly, an increased incidence of ill health or death in the animal population may be a further indicator of the release of a biotoxin, meaning the monitoring of animal health could be valuable. For example, tissue samples from these animals for subsequent analysis in downstream laboratories may aid in the identification of the presence of biotoxin in the environment.

55. **Strong recommendation 1:** *The OPCW should compile and disseminate information on the diagnosis and treatment of biotoxin exposure, including through convening a technical workshop on this topic involving clinicians and veterinarians with relevant experience, Secretariat staff, and representatives from the TWG on the Analysis of Biotoxins. Not only would early clinical diagnosis assist in identification of the agent used, but dissemination of information on diagnosis and treatment of biotoxin casualties would contribute to the OPCW's efforts on assistance and protection.*

56. Finally, the optimal method of sample collection and storage will be determined on a case-by-case basis with respect to the type of biotoxin and the circumstances of the potential release. In broad terms, samples should be collected into individual, sterile, single-use containers, packaged for transport as per the International Air Transport Association regulations, while maintaining a cold chain to preserve sample integrity (in accordance with C-I/DEC.47, dated 16 May 1997).

57. Other considerations relate to the importance of maintaining chain of custody from the very outset of the investigation. From the point that a sample is first taken, all the way through to reporting unambiguous analytical results from analytical techniques to the OPCW, a robust procedure for maintaining chain of custody will be crucial to both the investigation and to identifying the provenance of the biotoxin present within a sample.

Stage 2: Initial screening of samples within an appropriate facility (safety triage and corroborating any in-field identification)

58. Consistent with sampling procedures for traditional chemical agents, sub-samples should be sent by the OPCW to appropriate international laboratories for corroboration of the in-field result. This corroboration would involve in-depth analysis techniques and may include a repeat of the in-field detection method in a more controlled laboratory setting. For safety reasons, these samples should be screened for the presence of any other chemical, biological, or radiological material. The procedures potentially required during

a biotoxin investigation are summarised in Table 2 and also covered in depth later in this report.

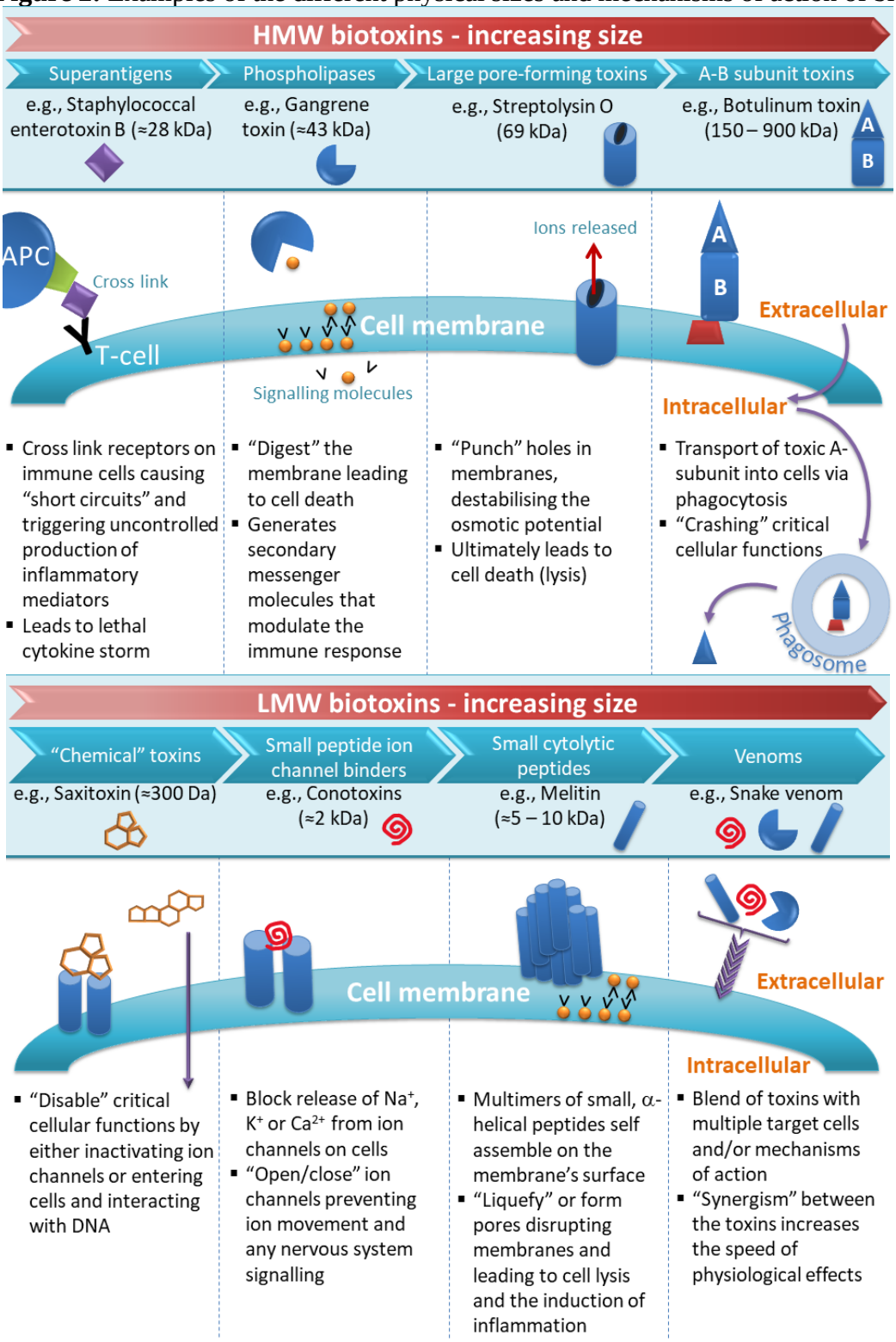
Stage 3: Analysis and unambiguous identification of the presence of a biotoxin, if possible taking into account clinical information

59. Given the wide range of biotoxins that might be encountered and the differing analytical methods these may require, information from field screening and medical diagnosis of suspected victims will be important for tentative identification to facilitate an appropriate method of analysis.

60. As for traditional chemical warfare agents, a minimum of two independent laboratories are required to analyse the samples generated from an investigation. These laboratories should either be existing OPCW Designated Laboratories or internationally recognised laboratories (e.g., academic, small to medium-sized enterprises with specialist expertise, defence and security, and public and/or animal health) that have demonstrated their expertise in relevant analyses through research in the field of biotoxins and/or participation in relevant external quality assessments (e.g., proficiency test) involving the identification of biotoxins in sample matrices. A minimum of two orthogonal techniques is required to unambiguously confirm the presence of a biotoxin in a sample. It is recognised that even in laboratory-based techniques there is a significant disparity between the different detection and identification technologies with biological recognition techniques, such as ELISA, being several orders of magnitude more sensitive than some other techniques, such as mass spectrometry.

61. As stated previously, biotoxins can vary markedly in terms of their composition and biological properties (see Figure 2). Therefore, unlike traditional chemical warfare agents, it is unrealistic to expect laboratories to have the skills or expertise to identify the chemical and biological properties for an extended range of biotoxins (these techniques are described in detail by subgroup 3 later in the report). For example, it may be that sub-samples need to be sent to an analytical laboratory for confirmation of the presence of a biotoxin through traditional analytical techniques (such as mass spectrometry) whilst other expert laboratories would be required to provide bespoke services for protein biotoxin analysis, such as toxicity assays or immunological methods.

Figure 2: Examples of the different physical sizes and mechanisms of action of biotoxins[6]



6 Adapted from: Clark, Graeme C., *et al.* "Friends or foes? Emerging impacts of biological toxins." Trends in Biochemical Sciences, 44, no. 4 (2019): 365-379. https://doi.org/10.1016/j.tibs.2018.12.004.

Stage 4: Comprehensive molecular profiling

62. The unambiguous identification of the presence of a biotoxin within an authentic sample represents a key step during an investigation by the OPCW. However, there may also be questions regarding the source and/or provenance of the biotoxin material. These are particularly important questions for the OPCW to answer since exposures to biotoxins can occur naturally (e.g., food poisoning cases), unlike those to traditional chemical warfare agents, which do not occur in nature. Therefore, establishing whether an incident has occurred as a consequence of a natural occurrence of the biotoxin or following nefarious release will be an essential further step required by the OPCW. To assist in making this judgement, the OPCW will require access to a capability able to undertake molecular profiling. This includes the identification and estimation of relative abundance of relevant compounds in the sample, such as small molecules, lipids, proteins, nucleic acids, and chemical contaminants. This may provide information on route of production, level of purity, genetic content, geographical origin, or linking different samples ("batch matching"), all of which could aid the investigation. Such techniques could also assist in identifying those involved in deliberate use, consistent with past OPCW decisions that "those responsible must be held accountable".

63. **Strong recommendation 7:** *The OPCW should adopt a comprehensive forensic approach to every investigation of alleged use of biotoxins (e.g., determining naturally occurring versus deliberate release, recombinant production, and sample provenance or batch matching via a comprehensive molecular analysis of the sample).*

64. Such an approach would include providing standardised guidance with respect to maintaining chain of custody of samples from in-field collection through the analytical process and highlighting the importance of documenting processes and procedures during an investigation. These practices would be particularly relevant when conducting investigations of biotoxins not covered by the Convention schedules and involve laboratories beyond the network of OPCW Designated Laboratories. A molecular profiling approach could also include the search for markers of biotoxin purification.

65. **Recommendation 8:** *The OPCW should continue to support activities that aid international capability development with respect to the identification of the provenance of a biotoxin. This may include exercises involving the "batch matching" or linking of samples collected during an investigation.*

Stage 5: Reporting to the OPCW

66. In terms of reporting results from investigations of the alleged use of biotoxins to the OPCW, some unique technical reporting requirements will need to be met. These are covered in more detail by subgroups 3 and 4. Overall, from the perspective of the Director-General, the level of confidence in the result will depend on the availability of techniques and laboratories to undertake the analysis of samples collected at the scene (Table 2). The reporting of in-field screening and/or identification of a biotoxin by an international analytical laboratory will come with the appropriate caveats outlining the limitations of the approach taken (e.g., limits of detection, accreditation of procedures, utilisation of an

overarching quality system, technical approach that has been previously peer reviewed or used in national/international quality assurance exercises, and the availability of orthogonal techniques).

Subgroup 2: Most relevant biotoxins

67. Subgroup 2 addressed two questions, and these are discussed separately below:

(a) question 5 (b): "What classes of biological toxins are most likely to be relevant in investigations of alleged use?"; and

(b) question 5 (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?".

Question 5 (b): "What classes of biotoxins are most likely to be relevant in investigations of alleged use?"

68. Recognising that the misuse of any biotoxin is prohibited by the Convention under the General Purpose Criterion,[7] the subgroup evaluated the risk of malicious use of a wide range of biotoxins using a methodology that takes into account relevant criteria, appropriately weighted, and yields a composite score. A similar approach has been used for risk assessment of pathogens and toxins for public health purposes by such governmental agencies as the United States Centers for Disease Control and Prevention (CDC).[8]

69. A large list of biotoxins was compiled, taking into account several existing lists from: Schedule 1 in the Annex on Chemicals of the Convention, the Australia Group, CDC, and the European Union (EU). The relevant biotoxins are presented in Annex 1, which summarises the type of chemical structure; the toxic mechanism; for some, the specific biotoxin chosen as representative of a large group of closely related biotoxins (the "family leader"); and, if it exists, the name of the disease caused. A more detailed set of biotoxin tables has been submitted separately, as an addendum, to the Secretariat via the SAB.

70. Relevant criteria were identified and open literature data on those selected criteria were sought for each biotoxin family or specific biotoxin. The criteria included factors such as historical use, availability, toxicity/activity, and stability.

71. To shorten the list and identify biotoxins, and biotoxin classes, representative of those considered most relevant for the OPCW in investigations of alleged use, the subgroup gave each criterion a weighting factor corresponding to its importance in the risk analysis and assigned scoring guidance for subcriteria. Full details about this process have been provided separately to the OPCW Secretariat via the SAB.

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7 Any chemical intended for chemical weapons purposes, regardless of whether it is specifically listed in the Convention or its Annexes (including the three schedules of chemicals) is considered a chemical weapon.

8 https://emergency.cdc.gov/agent/agentlist-category.asp.

72. Furthermore, a threshold was created to shorten the list. To assess the process, the nerve agent VX was used to ensure the validity of this approach.

73. The TWG identified the following list of nine biotoxins and biotoxin families with various properties that should be considered "most relevant" in the context of the OPCW (see Figures 3 and 4). These are listed in alphabetical order:

(a) abrin;

(b) aflatoxins;

(c) botulinum toxins;

(d) epsilon toxin;

(e) ricin;

(f) saxitoxin;[9]

(g) *Staphylococcus aureus* enterotoxins;

(h) T-2 toxin; and

(i) tetrodotoxin.

74. It is important to note that this list of relevant biotoxins was established using criteria adapted to the question that was posed to the TWG in the TOR (i.e., relevance for possible use) and not with criteria based on public health relevance. Nevertheless, this list resembles the lists developed by internationally known and respected public health agencies. These biotoxins can be considered materials of concern from an OPCW perspective. As indicated below, the TWG was briefed that the focus of the OPCW will remain on the two scheduled biotoxins for the foreseeable future.

75. This prioritised list includes six individual biotoxins and three biotoxin families. Five are HMW biotoxins and four are LMW biotoxins. It should be noted that these biotoxins are sufficiently different in terms of properties such as polarity, molecular weight, and activity that not all can be addressed by the same laboratory. Furthermore, this prioritised list implies that several confidence-building exercises based on the different properties of the

[9] Saxitoxin (STX) is one biotoxin from the paralytic shellfish poison biotoxins which include a very broad group of compounds (about 60 known analogues of saxitoxin belonging to gonyautoxin and saxitoxin families). During the metabolism of microalgae (and also humans), saxitoxin is transformed into mono-sulfated, di-sulfated, decarbamoylated and other compounds including gonyautoxins, neoSTX, dcSTX, etc. Reverse transformations also take place. The number of known analogues is increasing and will continue to increase in the future. This is not due to the appearance of new metabolites, but to an increase in interest in the problem and an increase in analytical capabilities. Furthermore, STX is usually not extracted alone but with other congeners from which neoSTX, GTX1 and GTX4 are the analogues with a similar toxicity as STX.

biotoxins and on the requirements for their analysis would be necessary. A minimum of five separate exercises on: ricin and abrin; botulinum toxins; aflatoxins and T-2 toxin; saxitoxin and tetrodotoxin; and *Staphylococcus aureus* enterotoxins and epsilon toxin would be required. The routine coordination of this number of analytical exercises, given the resources and breadth of expertise needed, is unrealistic for the OPCW. Each analytical exercise is estimated to cost at least 100,000 euros, likely requiring a minimum of six months of work of one OPCW Secretariat scientist.[10] Additionally, significant resources and time would be needed for any individual laboratory to participate. Furthermore, it is estimated that establishing the appropriate capability for any of the biotoxins starting from scratch would take 5 to 10 years. The issue of proficiency exercises for specific biotoxins is further considered in section 5(d)(v).

[10] For scenario establishment and stability studies, the shipment of samples to 25 participants and 2-3 months for evaluating the analytical reports was estimated.

Figure 3: Some of the properties of the nine "most relevant" biotoxins and (representatives of) families of biotoxins for the OPCW

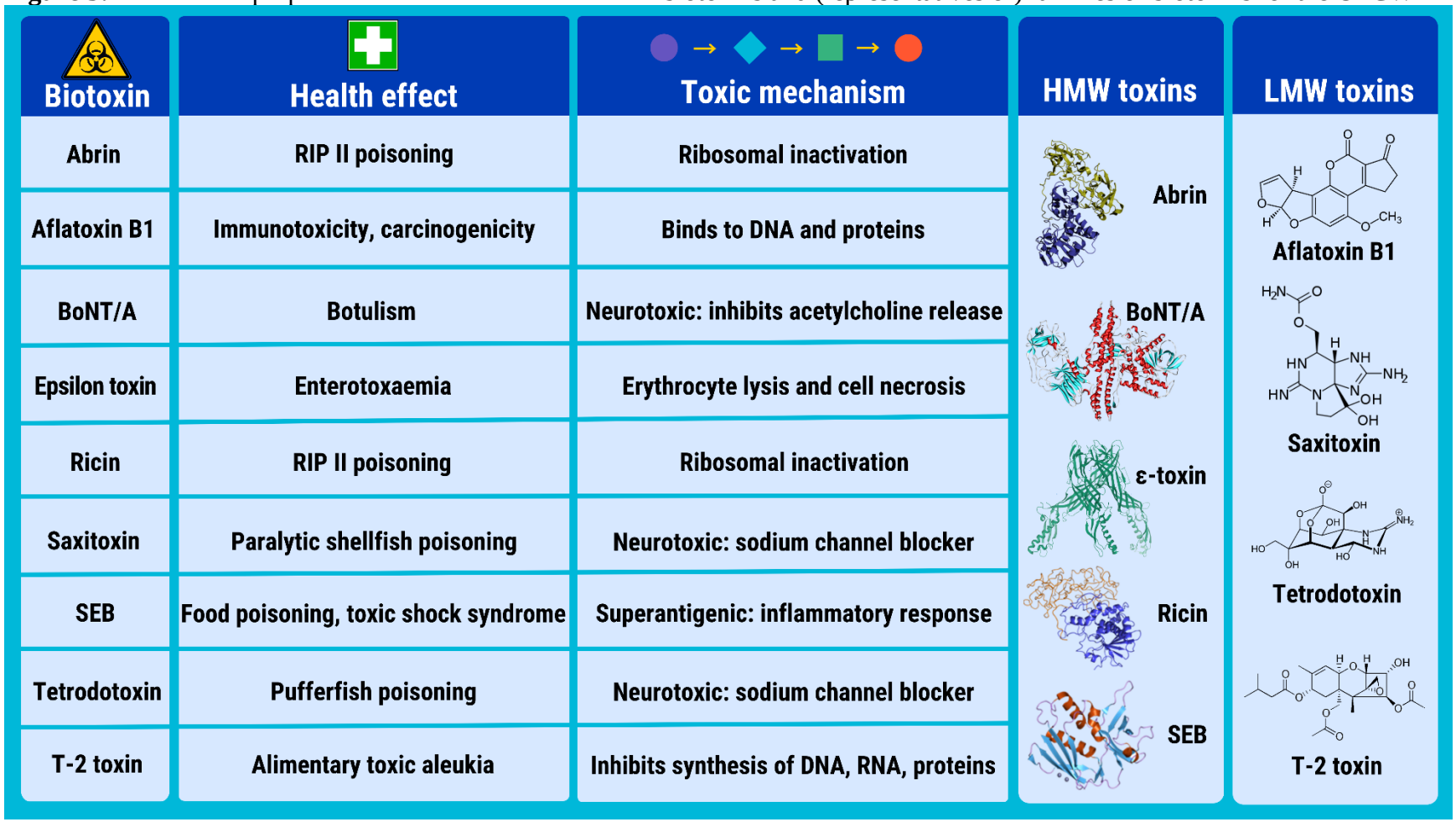
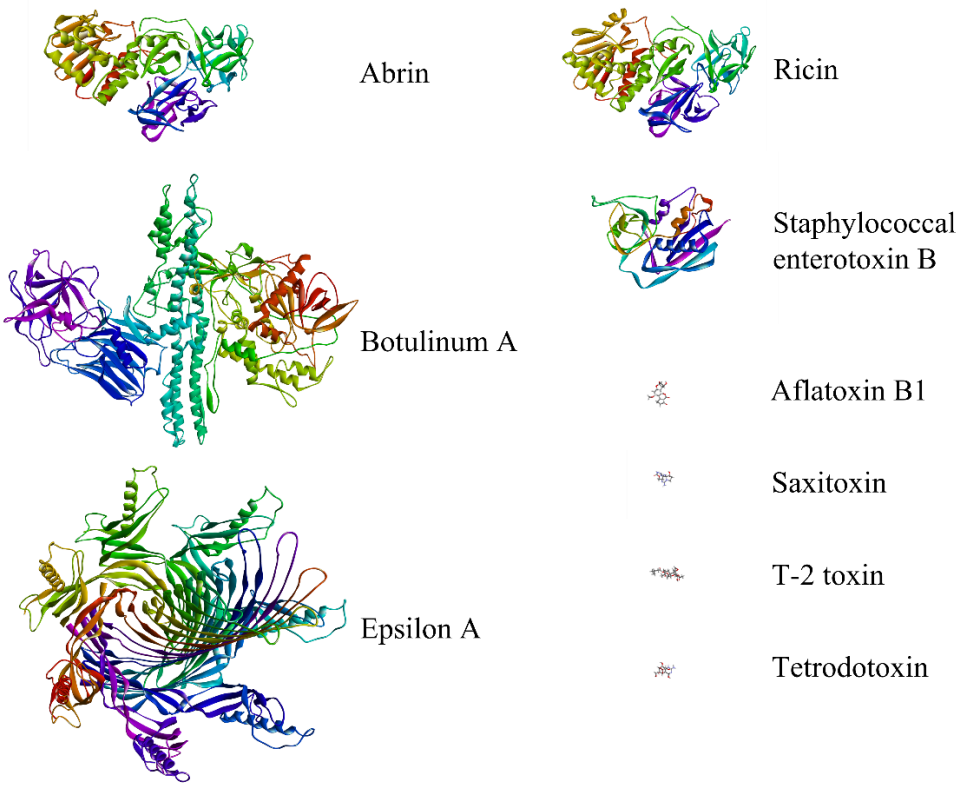


Figure 4: Chemical structures, to relative scale, of the most relevant biotoxins for the OPCW. For biotoxins where there are multiple analogues, one was taken as a token representation. Protein structures were taken from RCSB Protein Data Bank[11] (abrin A[12], 5Z3J; botulinum neurotoxin A[13], 3BTA; epsilon A[14], 6RB9; ricin[15], 7KBI; staphylococcal enterotoxin B[16], 3SEB). Structures for aflatoxin B1, saxitoxin, tetrodotoxin and T-2 toxin may be viewed in full in Figure 3.



11 www.rcsb.org.

12 Bansia, Harsh, Shradha Bagaria, Anjali Anoop Karande, and Suryanarayanarao Ramakumar. "Structural basis for neutralization of cytotoxic abrin by monoclonal antibody D6F10." *The FEBS Journal* 286, no. 5 (2019): 1003-1029. https://doi.org/10.1111/febs.14716. PMID: 30521151.

13 Lacy, D. Borden, William Tepp, Alona C. Cohen, Bibhuti R. DasGupta, and Raymond C. Stevens. "Crystal structure of botulinum neurotoxin type A and implications for toxicity." Nature structural biology 5, no. 10 (1998): 898-902. https://doi.org/10.1038/2338. PMID: 9783750.

14 Savva, Christos G., Alice R. Clark, Claire E. Naylor, Michel R. Popoff, David S. Moss, Ajit K. Basak, Richard W. Titball, and Monika Bokori-Brown. "The pore structure of Clostridium perfringens epsilon toxin." Nature communications 10, no. 1 (2019): 2641. https://doi.org/10.1038/s41467-019-10645-8. PMID: 31201325; PMCID: PMC6572795.

15 Rudolph, Michael J., Amanda Y. Poon, Simona Kavaliauskiene, Anne Grethe Myrann, Claire Reynolds-Peterson, Simon A. Davis, Kirsten Sandvig, David J. Vance, and Nicholas J. Mantis. "Structural analysis of toxin-neutralizing, single-domain antibodies that bridge ricin's AB subunit interface." Journal of Molecular Biology 433, no. 15 (2021): 167086. https://doi.org/10.1016/j.jmb.2021.167086. PMID: 34089718.

16 Papageorgiou, Anastassios C., Howard S. Tranter, and K. Ravi Acharya. "Crystal structure of microbial superantigen staphylococcal enterotoxin B at 1.5 Å resolution: implications for superantigen recognition by MHC class II molecules and T-cell receptors." Journal of Molecular Biology 277, no. 1 (1998): 61-79. https://doi.org/10.1006/jmbi.1997.1577. PMID: 9514739.

76. **Recommendation 5:** *The OPCW should continue to monitor developments in the field for the potential further modification of the list of "most relevant" biotoxins presented in this report. In assessing which biotoxins are the most relevant, the OPCW should continue to take into account the weighted rating criteria provided to the Secretariat. The criteria include factors such as: historical use, availability, toxicity/activity, and stability.*

77. **Strong recommendation 3:** *Based on the factors outlined by the TWG, the OPCW's efforts to develop its capabilities for investigations of alleged biotoxin use should focus on the nine "most relevant" biotoxins listed below. Recognising that seven of these nine biotoxins are not listed in Schedule 1 in the Annex on Chemicals to the Convention, the OPCW should plan to draw on sophisticated biotoxin analysis capabilities that may exist in other fields. The "most relevant" biotoxins are:*

(a) *abrin;*

(b) *aflatoxins;*

(c) *botulinum toxins;*

(d) *epsilon toxin;*

(e) *ricin;*

(f) *saxitoxin ;*

(g) *Staphylococcus aureus enterotoxins;*

(h) *T-2 toxin; and*

(i) *tetrodotoxin.*

Challenges encountered by subgroup 2

78. In the case of mycotoxins, it is very hard to obtain pure samples of a specific mycotoxin. Usually, a sample contains a mixture of mycotoxins and such a mixture may lead to synergistic effects from different chemicals present. Also, no data on acute toxicity of mycotoxins were found: only data on chronic toxicity are available.

Question 5 (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?"

79. Although the subgroup concentrated its effort on biotoxins, it also reviewed the open literature on potential weaponisation of bioregulators, which is relevant to question 5(c) in the TOR, regarding other potentially relevant compounds of biological origin.

80. Bioregulators are small molecules, peptides, or proteins that are naturally produced within an organism and are utilised in biological processes through adaptation in order to regulate

particular equilibrium balances (homeostatic systems). These naturally produced small molecules are present in nearly all systems throughout the human body, and can be multiorgan, target organ, and even cell-specific in action, effect, and release. The human body utilises bioregulators to maintain homeostasis in overarching systems like sleep cycle (circadian rhythm), hunger, blood pressure, and higher brain functions as well as others. Dysregulation of bioregulators can lead to adverse and potentially lethal effects and are common contributors to many debilitating diseases some of which do not yet have a cure.

81. No such chemicals appear to pose a risk comparable to that from the biotoxins listed, although information is sparse despite the subgroup's in-depth review of the publicly available literature. A table of some of the bioregulators considered can be found in Annex 2.

82. **Recommendation 6:** *The OPCW should continue to monitor developments on compounds of biological origin, in the field of bioregulators in particular, for indications of increased risk of misuse as weapons.*

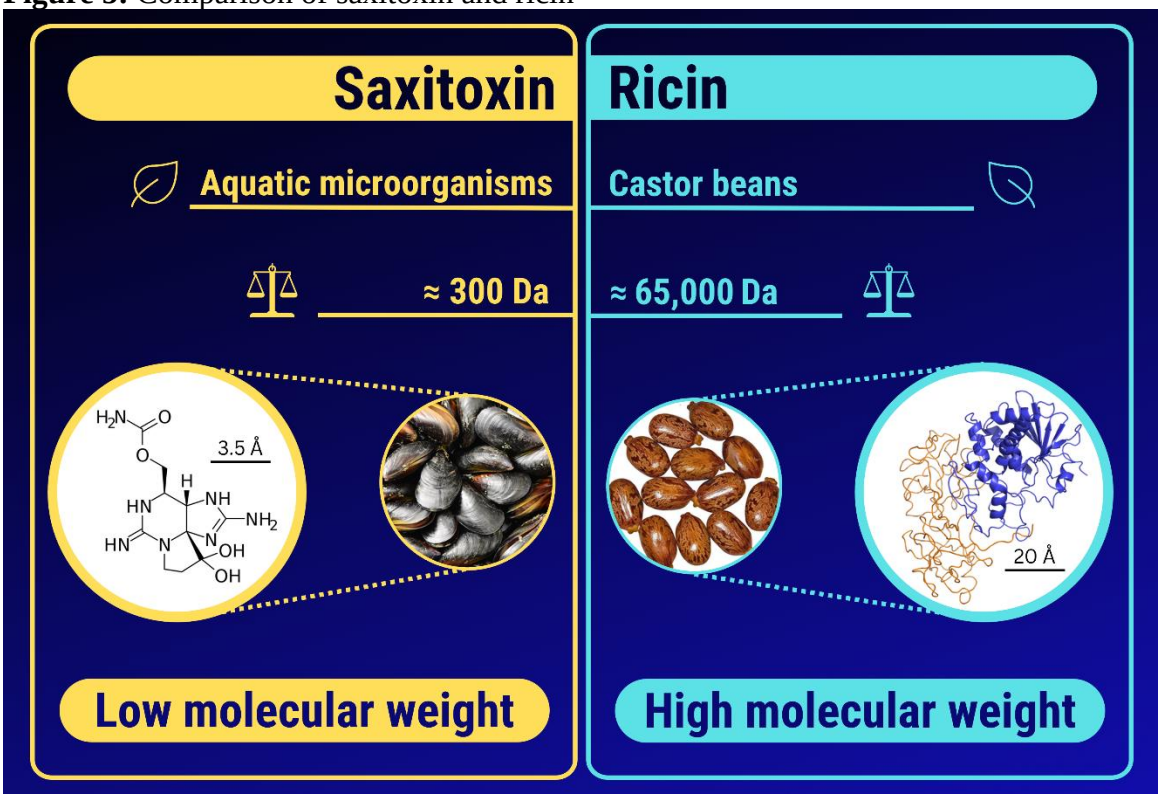
Subgroup 3: Technical requirements for analysis

83. Subgroup 3 addressed question 5 (d): "What are the technical requirements for analysis of the most relevant types of biological toxins?" and its sub-questions.

Sub-question 5 (d)(i): "Analytical approaches needed for unambiguous identification of both low and high molecular weight biotoxins"

84. The field of biotoxins exhibits great structural diversity. Biotoxins can loosely be divided into LMW biotoxins, such as saxitoxin, and HMW biotoxins, such as ricin (Figure 5). All are toxic and have a biological organism as their origin, but beyond that, they are extremely different in terms of size, and chemical properties like stability and polarity. Low molecular weight biotoxins are small organic molecules acting, for example, as inhibitors for human enzymes or certain channel blockers, thereby mediating their toxicity. In contrast, the HMW biotoxins are all proteins and their toxicity is often linked to enzymatic activity.

Figure 5: Comparison of saxitoxin and ricin

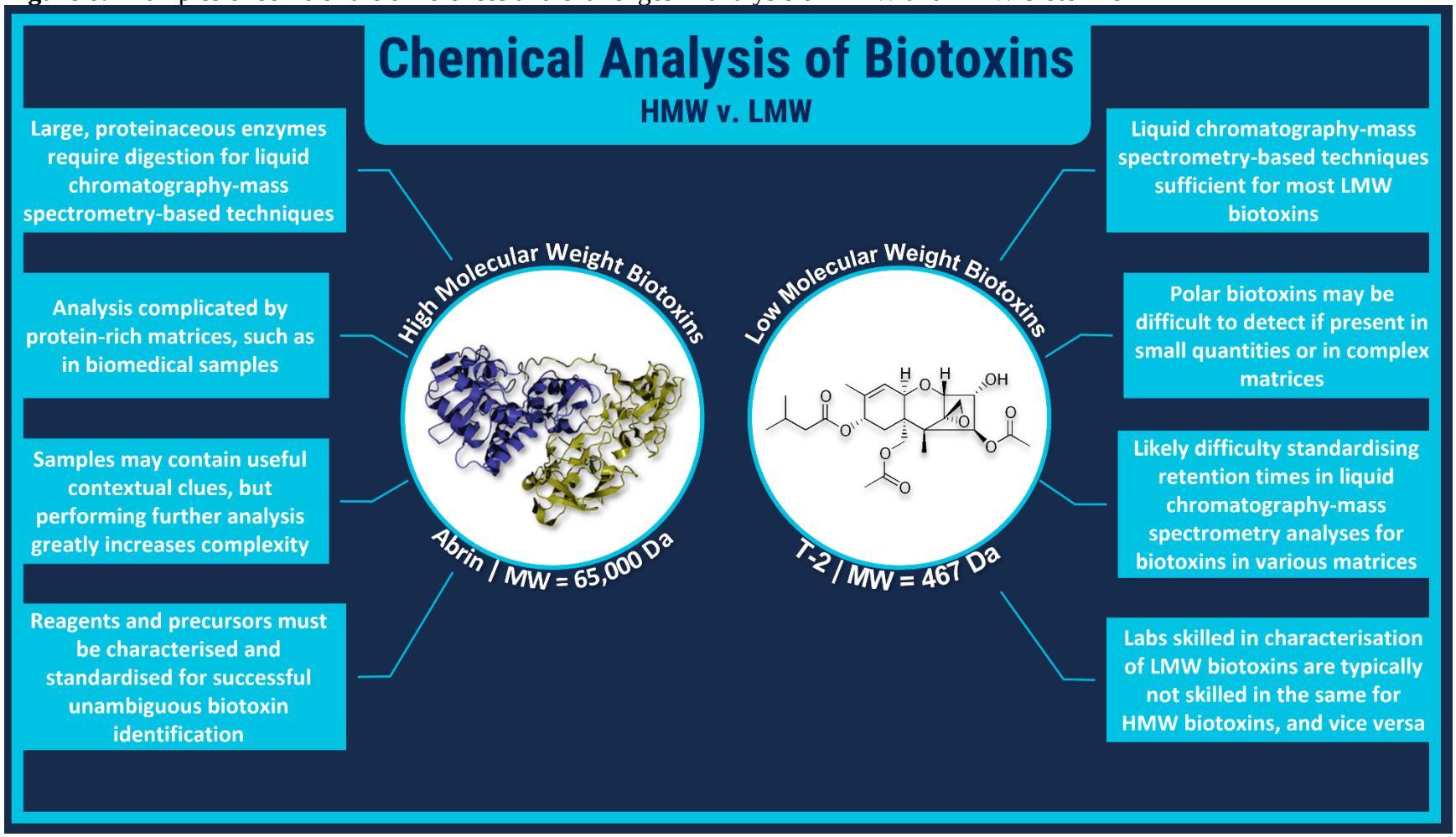


85. A wide range of biochemical processes in the human body may be inhibited or, on the contrary, over-stimulated by the activity of a biotoxin. Consequently, parameters such as the rapidity of disease onset, clinically relevant concentrations, and symptoms of exposure will differ between classes of biotoxins. Since biotoxins include very different types of chemical molecules, there is not one overall analytical technique which can be applied universally to all biotoxins. Especially when demonstrating functional activity of biotoxins, well-targeted assays need to be applied.

86. For many LMW biotoxins, traditional liquid chromatographic mass spectrometry methods (LC-MS) for identification are likely to be useful, with some consideration given to solubility and polarity of the biotoxin being analysed. The molecular masses of LMW biotoxins (100 – 10,000 Da) are often within the mass range of modern mass spectrometers and selective LC-MS/MS methods can be used for biotoxin identification. However, this technique is susceptible to signal suppression by the sample matrix and polar biotoxins such as saxitoxin can be difficult to detect if present at low levels in samples of complex matrices (e.g., food). The chemical analysis of HMW biotoxins (20,000 – 500,000 Da) by mass spectrometry is based on digestion of proteins to peptides and MS/MS analysis of the digests. Tryptic fragments of proteins are well-characterised and peptide sequences can be matched to protein sequence databases covering a large part of the biotoxin-producing organisms of the world. However, this technique requires a high enough level of biotoxin and a relatively low level of protein matrix, which may be a challenge in certain situations, such as in biomedical samples.

87. In the case of HMW biotoxins, potential orthogonal approaches to mass spectrometry include immunological methods (such as ELISA), acrylamide gel electrophoresis, DNA detection and analysis (such as polymerase chain reaction, or sequence analysis) and functional assays including *in vitro* toxin activity assays (such as endopeptidase assays or adenine release assay) and *in vivo* toxicity assays in animal models. However, none of these individual assays should stand alone as unambiguous identification, and a combination of different approaches is necessary for accurate biotoxin identification. The use of some of these alternative approaches should take into consideration that rigorous characterisation of reagents such as antibodies is necessary for accurate biotoxin identification (Figure 6).

Figure 6: Examples of some of the differences and challenges in analysis of HMW and LMW biotoxins



88. For routine analysis in public health laboratories, a single assay composed of a combination of characteristics or procedures of orthogonal techniques could be considered for unambiguous identification of a HMW biotoxin for clinical diagnosis. One example would be an assay that uses an affinity technique to isolate the biotoxin, followed by an enzymatic reaction to measure the specific enzymatic activity of the biotoxin. Such an assay takes into consideration specificity from both the affinity capture of the biotoxin and the functionality of the biotoxin, acquiring information on two different aspects of the biotoxin within a single assay. Another example would be an assay which uses an affinity capture of the biotoxin followed by enzymatic digestion and MS/MS of the resultant peptides. This singular assay attains the necessary specificity by combining the affinity of the biotoxin with the amino acid sequence of the biotoxin.

89. **Strong recommendation 10:** *In its activities related to analysis of biotoxins, the OPCW should take fully into account that the technical requirements for analysis differ widely between LMW and HMW biotoxins.*

(a) *For LMW biotoxins, the OPCW should generally rely on traditional mass spectrometry-based techniques, such as liquid chromatography-mass spectrometry.*

(b) *For HMW biotoxins, the OPCW should employ a combination of mass spectrometry-based techniques and orthogonal techniques, such as immunological methods and biotoxin activity assays. For HMW biotoxins present in samples at a very low level (nanogram/millilitre or below), the combination of immunoaffinity enrichment-based methods and functional methods (such as biotoxin activity assays) may be the only combination of methods with sufficient sensitivity for the analysis. Both approaches should be used, as long as enough material is available.*

Sub-question 5 (d)(ii): "Instrumentation and/or procedures that should be standardised across labs to ensure reproducible and consensus results"

90. Standardisation of instrumentation and/or procedures presents some difficulties in the field of biotoxin analysis. In addition, standardisation of instrumentation and/or procedures has not been a process applied to chemical analysis of traditional chemical warfare agents by the Designated Laboratories, which instead rely on best practices suggested by the OPCW and in published recommended operating procedures, such as the VERIFIN 'Blue Book'.[17] The process of standardisation may limit the speed of development, and standardisation of methods may prevent modernisation with improved techniques. Instrumentation standardisation requires funding and some laboratories may not be able to afford the purchase of specific equipment, thereby limiting the overall number of laboratories able to analyse biotoxins. The overall number of laboratories with biotoxin analysis capabilities will already be limited by the extreme difficulty of having one laboratory with expertise over the entire range of biotoxins to analyse; labs which are good at HMW measurements are not typically good at LMW measurements, and vice versa. The broad diversity of biotoxins also presents difficulties in standardising methods. For example, the

[17] For more information, see: https://www.helsinki.fi/en/verifin/about-verifin/blue-book.

chromatography involved in LC-MS/MS analysis of HMW biotoxin-derived peptides is fairly easy to standardise. However, for LMW biotoxins, it is more difficult to have reproducible retention times for a biotoxin in a complex matrix since the matrix often influences the precise retention time.

91. Many assays for HMW biotoxins utilise antibodies, either directly for analysis or indirectly as a method of concentration and purification of the biotoxin prior to some other type of analysis (e.g., mass spectrometry). However, not all antibodies are created equal, and there are many different types, from a variety of sources, used for different purposes. These important reagents need to be characterised in terms of sensitivity, quantification, limit of detection, and specificity to better understand their limitations.

92. Currently, the OPCW conducts analytical exercises for the two biotoxins listed in Schedule 1 of the Convention. The documentation and dissemination of methods used by laboratories that have proven successful in these exercises as "best practices" would assist other laboratories in developing and strengthening their capabilities.

93. **Strong recommendation 11:** *The OPCW should document and disseminate best practices for the unambiguous identification of specific biotoxins included in analysis exercise programmes to support the further development of analytical capability among laboratories.*

94. **Strong recommendation 12:** *The OPCW should develop minimum specification requirements for performance criteria of immunological and activity assays for the analysis of HMW biotoxins. This should include minimum specification for the immunological components (antibodies) as well as the overall immunoassay and activity assay performance criteria. It is strongly recommended that this is conducted in partnership with the UNSGM laboratory network.*

95. Besides standardisation of instrumentation and/or procedures, other approaches might be to recommend methods which are accredited (ISO or similar) and validated and make highly characterised reagents (including antibodies) available for all laboratories, to develop a minimum data set for analysis of biotoxins that would be acceptable, and to utilise requirements-reporting, performance-based reporting, or standardisation of reporting. One option would be to provide standard access to technologies with all laboratories able to perform at least one technique as a baseline. Relying on orthogonal approaches and providing stringent reporting criteria would result in a robust testing algorithm without the traditional "standardisation" of methods, although standardisation is recommended where possible.

96. Another area where HMW toxins should differ from traditional chemical warfare agents is in the scoring system for reported analytical results. Just as the scoring system considers information from LC-MS/MS measurements to be more specific than LC-MS measurements, information obtained from an immunoaffinity assay can differ in importance based on the reagents used. An immunoaffinity assay that uses high affinity antibodies which are known to have no cross-reactivity to related proteins yields more specific, important information than an immunoaffinity assay using low specificity

reagents which cross-react to related proteins. The scoring system should differentiate between immunoaffinity measurements performed with highly characterised, highly specific reagents versus those using poorer quality reagents.

97. Additionally, proteomics[18] has become the gold standard for protein identification as it gives information about the amino acid sequence of the biotoxin. Here too, there are different levels of specificity from proteomic analyses, and the scoring system should take into account the level of specificity obtained from proteomic analyses. A protein biotoxin identification obtained by peptide mass fingerprinting (MS of a mixture of peptides) should not have the same score as a protein biotoxin identification obtained by high resolution MS/HRMS.

98. **Recommendation 18:** *The OPCW should consider reviewing the reporting criteria for the analysis of HMW biotoxins together with representatives of OPCW Designated Laboratories and UNSGM-affiliated laboratories. The modified reporting system should incorporate immunological or functional methods that are relevant for the unambiguous identification of HMW biotoxins. Furthermore, consideration should be given to modifying the current requirements for mass spectrometric analysis taking into account the accepted reporting scheme in analogous scientific fields (e.g., proteomics). This would necessitate a change in the scoring system associated with the analytical exercises.*

99. To make best use of the expertise developed in the different networks, the laboratories involved should, at a minimum, work under coherent quality assurance regimens. Based on the overview from the indicated networks and exercises, currently only a minority of laboratories are accredited under international standards for biotoxin analyses (some 10 – 35% of the expert laboratories involved in the activities, depending on the biotoxin targeted). The following international standards were mentioned by the expert laboratories involved in biotoxin analysis:

(a) Standard ISO/IEC 17025 defining the requirements for competence of testing and calibrating laboratories (applicable to environmental sample analysis, for example); and

(b) Standard ISO 15189 defining the requirements for competence of medical laboratories (applicable to clinical sample analysis, for example).

100. Beyond the requirements defined for the analytical laboratories, there is also a relevant standard for laboratories which offer interlaboratory exercises:

(a) ISO/IEC 17043 on conformity assessment defining the requirements for proficiency testing.

101. Generally, accreditation according to national and international standards is important to document the analytical performance since only comprehensively validated methods can be used under an overarching quality management system. Parameters such as target

[18] Digestion of a protein into smaller peptides, followed by mass spectral analysis of the peptides and protein identification through database searching of the mass spectral data.

specificity, sensitivity, precision, robustness, and reliability of experimental data are defined for accredited methods and build the basis for the credibility of laboratory results in a political context such as an OPCW or UNSGM investigation or in a legal context for domestic criminal prosecution.

102. It is unlikely that an individual laboratory will have tailored and accredited methods for a broad range of biotoxins. This is certainly also related to the diversity of biotoxin structures, functions, and properties, which would require a comprehensive suite of tools, methods and instrumentation (see TOR question 5(d)). Consequently, setting the guidelines for analytical laboratories in an international investigation too restrictively could be counterproductive, especially when new or emerging biotoxin threats are suspected in an attack. Furthermore, if the quality assurance requirements are set too narrowly, this could make it difficult to apply new or improved approaches in the case of an alleged use of a (novel) biotoxin. The development of a new targeted method, validation of the method, and finally accreditation of the method, is a process usually lasting several years.

103. **Recommendation 13:** *The TWG recommends that:*

(a) *Laboratories involved in an international investigation should work under an overarching quality management system ensuring regular quality management measures (e.g., pipette calibration, lot documentation, appropriate calibration and documentation of methods, and regular error analysis).*

(b) *The exact procedures used in an international investigation should be technically robust and should have been published in a peer-reviewed international journal and/or their performance demonstrated in international analytical exercises.*

(c) *Accreditation of the specific method to be applied in an investigation is not absolutely necessary as long as the laboratory works under an overarching quality management system for biotoxin analysis and the performance criteria of the assays utilised and their limitations are understood. This approach ensures the laboratories' capability to deal with emerging issues and to apply new technologies, if required. Also, innovative approaches could be helpful to analyse limited or difficult sample materials, taking into consideration the intelligence and situational awareness that might help sample triage.*

Sub-question 5 (d)(iii): "Analytical criteria that should be in place in order to match forensic requirements"

104. The first step is to understand the goal of forensic requirements. Forensics relate to the application of scientific methods and techniques to an investigation. This includes conducting and documenting the scientific analysis to withstand legal and political scrutiny. Therefore, any biological toxin analysis investigation will utilise forensic protocols (chain of custody, conducting analysis within a quality management system, robust documentation, as well as utilisation of valid analytical processes for sample characterisation).

105. There are multiple options to extract analytical information of biotoxin samples in order to generate information of relevance for a forensic investigation of an incident including biotoxin use. Information on how a biotoxin has been purified from the biological source organism can be very valuable and support an investigation of alleged misuse. Analysis of the sample quality can indicate the technical competence and resources of the perpetrator. Comparative analysis of biotoxin samples (i.e., of their molecular profiles, including small molecules, lipids proteins, nucleic acid, and chemical contaminants) may help match the samples (e.g., batch-matching) or provide more information related to their origin (e.g., sample provenance). For traditional chemical warfare agents, comprehensive analysis of the sample helps to identify the organic synthesis route. In the case of biotoxins, production is commonly based on purification techniques from the source organisms. It should also be considered that generation of biological toxins can be conducted utilising recombinant methods. Therefore, analysis of the molecular profile of the sample may enable matching to a source sample, elucidating the sophistication of the perpetrator, and clearly helping to distinguish that the presence of the biotoxin is not due to natural factors; this is a fundamental difference from the area of traditional chemical warfare agents.

106. A typical biotoxin source tissue, for example seeds or a cell suspension of biotoxin-producing bacteria or algae, will contain a low level of biotoxin (<1%). In order to produce a threat, agent-different purification processes that include chemical reagents and equipment will be used to enrich the biotoxin. Thus, an impurity profile of a biotoxin sample will contain both traces of endogenous substances from the source material and remains of the chemicals used in the purification process. Besides providing information on how the biotoxin was produced, such impurity profiles may also be used to match different samples with a suspected common origin (i.e., batch matching). The batch matching of HMW biotoxins may also be based on intrinsic markers as biotoxin sequence (i.e., ecotype sequence variation), or the sequence of other specific peptides or proteins of the source organism (e.g., *Ricinus communis* biomarker peptides).

107. The preparation of LMW biotoxins is very different from the preparation of HMW biotoxins. These would give very different markers, with different techniques required to detect them. Adding to the complexity of this situation is the fact that laboratories that excel at analysis of HMW biotoxins are not likely competent at analysis of LMW contaminants which might originate from biotoxin preparation methods.

Sub-question 5 (d)(iv): "The role and utility of degradation products and other markers and/or compounds"

108. In the context of biotoxins, degradation can mean different things. It could mean loss in size, similar to hydrolysis of small molecule compounds; however, in the case of HMW biotoxins, inactivation can occur without a change in size (e.g., by modification of the protein fold or denaturation). The concept of inactivated biotoxin brings new challenges to unambiguous biotoxin identification. However, identification of inactivated biotoxin has great importance as it may corroborate the intent to commit a crime.

109. The issue of active and inactivate biotoxins draws a close parallel with the accepted role of stereoisomers for nerve agents. Most nerve agents have two or more stereoisomers, with

one stereoisomer being much more toxic than the other. However, all stereoisomers are regulated equally. Based on this rationale, an inactive biotoxin should be considered as important as an active biotoxin.

110. **Recommendation 14:** *In view of its value for investigations of alleged use, the OPCW should consider both active and inactive biotoxins within its verification regime.*

111. **Recommendation 9:** *For authentic biotoxin samples, the OPCW should also include reporting on the presence of chemicals that are characteristic of biotoxin preparations and may assist in identifying the source and purity of a biotoxin preparation, such as ricinine in ricin-related samples. Other examples include extraction solvents, as well as lipids, peptides, and proteins specific to the source organism.*

Sub-question 5 (d)(v): "The role of biomarkers and biomedical samples"

112. This area of consideration is perhaps the one that differs most in comparison to traditional chemical warfare agents. Unlike traditional chemical warfare agents, biotoxins are not known to form stable chemical adducts with human proteins. Indeed, there is a huge knowledge gap about the effects of biotoxins as they enter and react with the human body. Currently, the role of biomarkers in the identification of biotoxin exposure is limited and only the direct detection of the biotoxin or its activity in biomedical samples are the available methods to verify biotoxin exposure.

113. The biggest challenge with biotoxin analysis in biomedical samples is that biotoxin levels are often quite low (especially with HMW biotoxins) and often too low to use traditional LC-MS/MS techniques for detection and identification. Yet, the role of biomedical samples is perhaps more important with biotoxins than traditional chemical warfare agents. This is due to the delayed onset of symptoms after exposure to large biotoxins and the fact that some biotoxins are not excreted through urine and remain in the body longer than traditional chemical warfare agents. Still, the time window of detection of HMW biotoxins in clinical matrices is limited. That means that samples need to be collected immediately after an alleged attack. For some of the large biotoxins, LMW marker molecules also present in the producing organism can be detected as a proxy for the biotoxin itself (e.g., ricinine instead of ricin), but this only works for crude biotoxin preparations most likely linked with attacks by non-State actors.

114. Most importantly, the precise detection and identification of biotoxins are hampered by the fact that they occur naturally in multiple isoforms or variants that may or may not vary in terms of toxicokinetics, toxicodynamics, structure and function (e.g., more than 50 different saxitoxin analogues and more than 40 different botulinum neurotoxins). Consequently, a recurrent theme for biotoxins is that they represent a larger group of related molecules that are challenging to detect, differentiate, and quantify. Finally, it is noted that for the success of an investigation, detection of biotoxins, and especially detection of biotoxin activity in biomedical samples, is very valuable, powerful information.

115. **Recommendation 15:** *The OPCW should develop a capability to analyse biotoxins at a clinically relevant range (nanogram/millilitre-picogram/millilitre-range) that are likely to*

be present in biomedical samples from suspected victims, working closely with laboratories that are interested in and technically capable of developing and improving such capabilities.

Considerations regarding analysis of specific biotoxins

116. As noted earlier, it is impractical for the OPCW to attempt to develop an independent capability for analysis of all nine of the "most relevant" biotoxins identified (Figure 3). Under these circumstances it is logical for the OPCW to prioritise development of a designated laboratory network for the analysis of the two biotoxins listed in Schedule 1. The TWG was briefed that this, in fact, is the Secretariat's intention and that training analysis exercises for saxitoxin and for ricin are already being conducted.

117. A flexible regime would allow a laboratory that is highly specialised in one of the two scheduled biotoxins to seek designation, thereby helping to ensure that the OPCW has the analytical capabilities that it needs and strengthening the OPCW designated laboratory network. Biotoxin analysis exercises and proficiency tests should involve realistic concentrations and matrices, including biomedical samples.

118. **Strong recommendation 17:** *The OPCW should consider a proficiency test regime for biotoxin analysis that enables a laboratory to seek separate designation for the analysis of saxitoxin or of ricin.*

119. If the OPCW develops a capability for analysis of saxitoxin and ricin within its network of designated laboratories, it will still need a capability to analyse any of the other "most relevant" biotoxins. This analysis capacity will mainly be obtained from non-OPCW sources. The need to conduct an investigation involving one of those biotoxins could arise at any time. The OPCW needs to promptly develop at least a general understanding of which laboratories are experienced in analysing each of the most relevant biotoxins, since the OPCW may need to draw on their expertise for its own investigations.

120. **Strong recommendation 4:** *The OPCW should, in the near term, survey existing literature and recognised experts in biotoxin analysis to identify laboratories that possess specialised capabilities for analysis of each of the "most relevant" biotoxins. The OPCW should consider convening a workshop as part of this effort.*

121. In the medium term, the OPCW will need to build working relationships with laboratories that possess specialised analytical capabilities for biotoxins that may be needed for an OPCW investigation.

Recommendation 20: *The OPCW should work closely with the informal network of biotoxin analysis laboratories, discussed in the section on "Measures for international cooperation" by subgroup 5. This will develop partnerships with external laboratories with demonstrated expertise in the analysis of specific "most relevant" biotoxins (other than saxitoxin and ricin) to the standard required for an OPCW investigation, and willing to provide analytical services to the OPCW on request.*

Subgroup 4: Cooperation between the OPCW and other international efforts for biotoxin analysis

122. Subgroup 4 addressed two questions, and these are discussed separately below:

(a) question 5 (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?"; and

(b) question 5 (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? Please consider:

(i) the quality system requirements for the laboratories that should be in place (e.g., consideration of ISO 17025 for OPCW Designated Labs); and

(ii) how the analytical exercises can be harmonised yet remain flexible to address new or emerging biotoxin threats.".

Question 5 (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?"

123. From an international legal standpoint, the misuse of any biotoxin, regardless of whether it is specifically listed in the Annex on Chemicals to the Convention, is prohibited under the General Purpose Criterion. In addition, ricin and saxitoxin are singled out for special monitoring as Schedule 1 compounds, based on their history in biotoxin weapon programmes. Furthermore, all biotoxins are covered as toxin weapons under the BTWC. Under either treaty, the development, production or use of a biotoxin as a weapon would be a violation. Thus, the possibility arises that an investigation of prohibited development, production, or use of a biotoxin as a weapon could be conducted under either the Convention or the BTWC.

124. Under the Convention, an investigation related to the use of biotoxin weapons in a state party to the Convention would be conducted by the OPCW which oversees implementation of the global ban on chemical weapons. Samples would be analysed in "designated laboratories" that have been selected because of capabilities they have demonstrated in regular analytical exercises. With respect to biotoxins, the current focus of OPCW exercises is on ricin and saxitoxin (and their analogues) as Schedule 1 compounds, while other biotoxins that might be relevant are not explicitly addressed. The TWG was briefed that the OPCW's focus is expected to remain on these specific biotoxins for the foreseeable future. In investigations of alleged biotoxin use, the mandate would focus on whether or not a biotoxin had been used as a weapon, not on who was responsible.

125. Under the BTWC, the situation is more complicated since the treaty does not have a comparable implementing body. However, a 1987 UN General Assembly resolution authorises the UN Secretary-General to carry out an investigation of the possible use of

toxin weapons, if requested by a UN Member State (General Assembly Resolution A/RES/42/37C, dated 30 November 1987; Security Council Resolution 620, dated 26 August 1988; and General Assembly Document A/44/561 Annex I, dated 4 October 1989).[19] This mechanism is commonly called "the UNSGM". The scope of the UNSGM concerns the possible use of chemical, bacteriological and toxin weapons that may constitute a violation of the 1925 Geneva Protocol. The purpose of the UNSGM is to ascertain the facts of the matter and to report promptly the results of any such investigation to all Member States. The mandate would include attribution to a user only if this was included in the request for an investigation and the UN Secretary-General agreed.

126. The UNSGM is essentially based on three pillars:

(a) expert consultants (subject matter experts chosen by the Secretary-General to advise and assist in an ongoing investigation);

(b) qualified experts (subject matter experts chosen by the Secretary-General to conduct the on-site mission); and

(c) analytical laboratories (qualified laboratories chosen by the Secretary-General to perform sample analysis).

127. For sample analysis, the UN Secretary-General would draw from a roster of analytical laboratories, nominated by UN Member States. Currently, a major challenge is that it is unclear on what basis analytical laboratories would be selected to analyse samples for a particular UNSGM investigation. Since there are no required criteria for laboratory nominations to the roster, it is critical to implement common exercises for relevant laboratories to ensure the validity and accuracy of their analysis (see paragraph 141 for details on the RefBio project). Because of the overlap on the topic of biotoxins among the UNSGM exercise laboratories and the OPCW Designated Laboratories, it is important to gain a better understanding of the requirements for analysis of biotoxins in an international investigation under auspices of both treaties, and to develop realistic ideas for an international framework for future work. Under the UNSGM, cooperation with other international organisations has been increasingly expanded, including supplementary agreements and joint training with the OPCW, and Memoranda of Understanding with WHO[20] and WOAH[21].

128. The analytical capabilities needed for an international investigation regarding biotoxins pose distinct technical challenges that stem from unique characteristics of biotoxins (see subgroup 2, question 5(b) and subgroup 3, question 5(d)). Efforts to develop these capabilities are currently underway through several laboratory networks (see question 5(f) in this section). In contrast to the OPCW's focus on the two Schedule 1 biotoxins, these efforts deal more broadly with a range of HMW protein biotoxins. To ensure these analytical practices are consistent, rather than divergent, and available to either

19 Available at: https://bit.ly/UNSGMDocs.

20 World Health Organization (WHO).

21 World Organisation for Animal Health (WOAH, formerly OIE).

investigation mechanism, a process to harmonise biotoxin analysis-related activities is necessary.

129. The UNSGM guidelines and procedures given in the above cited General Assembly document A/44/561, dated 4 October 1989, stipulate, *inter alia*, for analytical laboratories that it is their task to:

(a) identify any chemical, biological and toxin (CBT) agent;

(b) determine characteristic impurities and degradation products;

(c) validate preliminary analyses;

(d) elucidate the nature of unknown CBT agents;

(e) timely prepare and transmit a report of the results to the Secretary-General;

(f) participate in interlaboratory calibration studies; and

(g) note any information that might permit the identification of the origin of any CBT agent.

130. Since these guidelines overlap with OPCW's procedures for an international investigation on the alleged use of biotoxins, it would therefore be important to:

(a) identify the scope of biotoxins most relevant for each investigative mechanism;

(b) identify appropriate and common standards and procedures of laboratory analysis for biotoxins considering their identity, biological activity, and quantity;

(c) define and harmonise reporting criteria and reporting formats so they are acceptable under both mechanisms, taking into account that biotoxins are different from traditional chemical agents; and

(d) specify guidelines for selecting laboratories to conduct analyses of biotoxins under the OPCW and the UNSGM that are acceptable to both to ensure that results can be used under the two regimens.

131. **Strong recommendation 19:** *The OPCW should work closely with the UN, drawing on the relationship agreement for cooperation between the two organisations (EC-MXI/DEC.1, dated 1 September 2000), along with any other interested organisations and laboratories from different sectors (e.g., food safety) to establish an informal network for biotoxin analysis to facilitate building international capabilities for forensic analysis of biotoxins, including in such areas as:*

(a) *common guidelines and best practices for biotoxin analysis to be used by the OPCW and the UN in international investigations;*

(b) *coordination of requirements for quality assurance management systems for acceptance of biotoxin analysis data in investigations;*

(c) *development of a reporting format acceptable for OPCW and UNSGM missions for reporting of results of biotoxin analysis, including definition of performance and acceptance criteria for a range of relevant methods; and*

(d) *coordination of efforts to minimise gaps and unproductive duplication, including analysis exercises and proficiency testing.*

132. Potential partners include laboratories already affiliated with the OPCW and the UN, laboratories participating in biotoxin analysis exercises, and food analysis laboratories. The goal should be availability of the capabilities of the laboratories in the informal network as a resource for the OPCW, the UNSGM, and other international and national organisations conducting investigations of alleged use of biotoxins as weapons.

133. **Recommendation 21:** *Since the OPCW and the UN would be key partners in the proposed informal network of biotoxin analysis laboratories, the responsibility for coordinating the network should be shared. The OPCW and the UN should each designate a staff member to act as co-facilitators. The OPCW should consider designating a laboratory staff member for this part-time function.*

134. As noted above, the OPCW has focused on developing an analytical capability for the two biotoxins (ricin and saxitoxin) listed in Schedule 1 of the Convention. As a practical matter, this means that unless it commits to developing its own capability, the OPCW will need to draw on other sources, such as the UNSGM-related efforts, for analytical capabilities for other toxins on the list of "most relevant" biotoxins discussed above in the subgroup 2 section under question 5 (b). Seeking to establish an independent capability would require the OPCW to commit substantial additional personnel and financial resources over an extended period, as outlined above in paragraph 75. For biotoxins that are less commonly studied (for example, epsilon toxin), the resource requirements would be disproportionately high.

135. Apart from the OPCW and UNSGM, there are no other international mechanisms with a broad investigative mandate covering biotoxins. Regional networks exist and include the surveillance of toxins in food and feed for public or animal health purposes within the EU. However, only a few biotoxins identified under question 5 (b) might be covered by such existing regional networks (e.g., aflatoxins, *S. aureus* enterotoxins, saxitoxin) and often the laboratories involved are mandated to analyse only selected matrices, such as food.

136. The general analytical requirements and standards relevant in the context of biotoxin analysis have been discussed under subgroup 3 question 5 (d)(ii). The analytical methods and forensic standards established in regional networks could be relevant, nonetheless, to an OPCW or UNSGM investigation. Furthermore, the analytical capabilities of individual laboratories in these networks could be very useful to the OPCW and UNSGM, even if their experience with the biotoxins and matrices of particular concern is limited. In particular, it would be useful to compile additional information on laboratories that are

specialised in analysis for one or more of the biotoxins of special concern. Since neither the OPCW nor the UNSGM currently has its own capabilities for these biotoxins, such laboratories would be an important analytical resource for the OPCW.

137. To what extent national standards exist for biotoxin analysis is unclear. It seems likely that some national authorities may have established analytical standards for particular biotoxins of public or animal health concern, such as saxitoxins or aflatoxins. For example, the U.S. Department of Agriculture operates a laboratory approval programme for aflatoxin analysis and an international standard method also exists (ISO 16050:2003). Equally as important are the forensic standards established in a number of countries for admissibility of technical evidence in prosecution of criminal cases. In the United States, for example, the so-called Daubert Standard is applied.[22] Under this standard, technical evidence must meet criteria of relevance, reliability, and a sound technical foundation.

138. If an OPCW or UNSGM investigation is linked to a domestic criminal investigation, as might well be the case in a suspected bioterrorism event, knowledge and implementation of the relevant forensic evidentiary standard would be essential. Furthermore, the political credibility of the findings from an OPCW or UNSGM investigation will depend on meeting such high evidentiary standards, even if this is not required in a strictly legal sense.

139. **Recommendation 16:** *To better understand possible international technical and forensic legal requirements for biotoxin analysis, the OPCW should make further efforts to identify and compile specific national and international standards and guidelines for biotoxin analysis, as well as forensic requirements relating to the use of technical evidence in legal proceedings.*

Sub-question 5 (f) (i): "Quality system requirements for the laboratories that should be in place (e.g., consideration of ISO 17025 for OPCW Designated Labs)"

140. The issues related to this sub-question are covered under question 5 (d)(ii) above.

Sub-question 5 (f) (ii): "How analytical exercises can be harmonised yet remain flexible to address new or emerging biotoxin threats"

141. Since 2012, there have been several international efforts to develop analytical capabilities in the field of biotoxin analysis through different OPCW and other laboratory networks, flanked by annual UNSGM workshops[23] organised by Spiez Laboratory, Switzerland:

(a) activities driven by the OPCW Laboratory to develop technical capacities for Schedule 1 biotoxins:

(i) since 2017: seven exercises on ricin, abrin and saxitoxin;

22 Daubert v. Merrell-Dow Pharmaceuticals, Inc., 509 U.S. 579 (1993).

23 https://www.spiezlab.admin.ch/en/kontrolle/unsgm.html.

(b) activities driven by the RefBio project funded by the German Federal Foreign Office integrating nominated roster laboratories supporting the UNSGM:[24]

(i) since 2019: three exercises on ricin and clostridial neurotoxins (BoNT/A, B, E, F, and tetanus neurotoxin (TeNT));

(c) activities driven by the EU Research and Innovation programme, specifically two consecutive projects focusing on quality assurance measures for biotoxin analysis in large EU networks:

(i) EQuATox, 2012 – 2014:[25] four exercises on ricin, saxitoxin, botulinum neurotoxins and *Staphylococcus aureus* enterotoxins; and

(ii) EuroBioTox, 2017 – 2023:[26] 11 exercises on ricin, abrin, saxitoxin, botulinum neurotoxins (including sero- and subtypes pathogenic to humans) and *Staphylococcus aureus* enterotoxins as well as dedicated laboratory-based and on-site detection exercises.

142. The networks integrate different participating laboratories (e.g., focusing on OPCW Designated Laboratories, on UNSGM roster labs or on expert laboratories in the security, health and food sector) with a limited number taking part in all activities. They have a related, but not identical, focus in terms of biotoxins targeted (Schedule 1 and/or beyond), scope of analysis (qualitative/quantitative and/or forensic analysis), biotoxin concentrations tested (µg/mL to pg/mL range), laboratory-based and/or on-site detection, result reporting (e.g., adherence to pre-set reporting criteria) and geographical representation (e.g., EU laboratories and/or laboratories worldwide). For a comparison of the different existing test/exercise regimes related to biotoxins see Figure 7.

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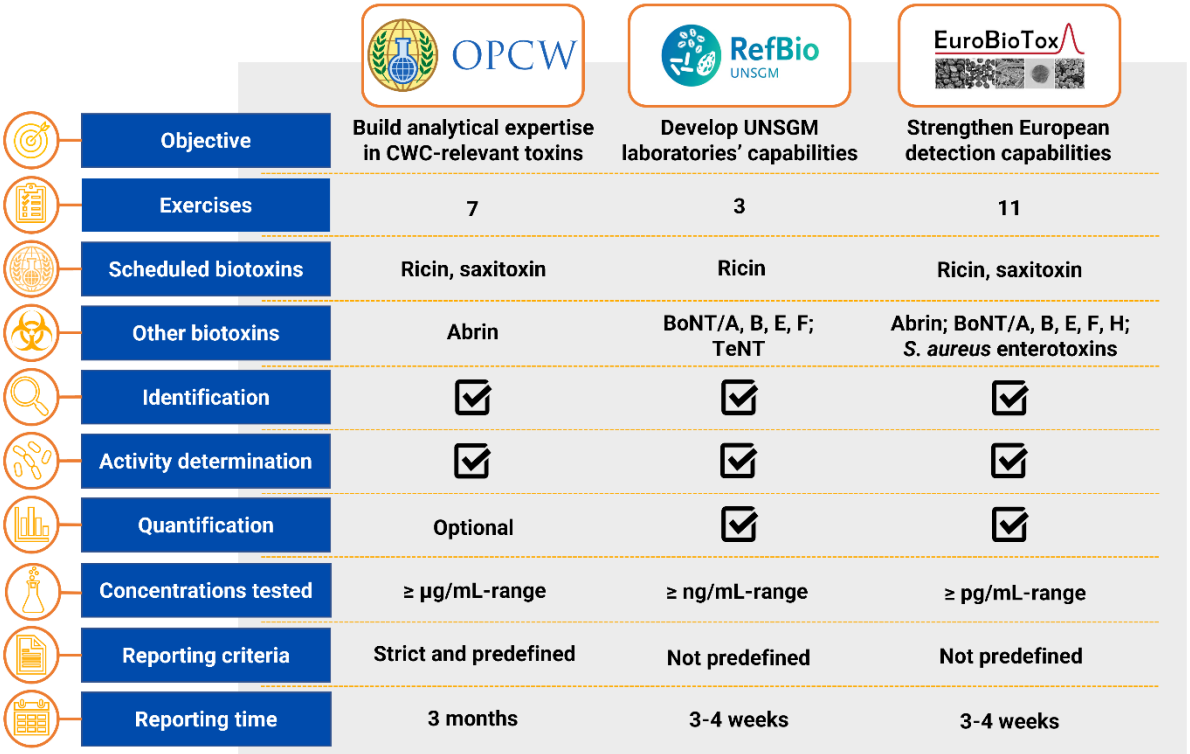
24 RefBio project: "Germany's contribution to strengthen the reference laboratories Bio in the UNSGM"; funded by the German Federal Foreign Office 2017 – 2024; more information available at: https://bit.ly/RKIRefBio and in Appelt, Sandra, Anna-Maria Rohleder, Cédric Invernizzi, Robert Mikulak, Annika Brinkmann, Andreas Nitsche, Maren Krüger et al. "Strengthening the United Nations Secretary-General's Mechanism to an alleged use of bioweapons through a quality-assured laboratory response." Nature Communications 12, no. 1 (2021): 3078. https://doi.org/10.1038/s41467-021-23296-5.

25 The EQuATox Consortium. "Establishment of Quality Assurance for the Detection of Biological Toxins of Potential Bioterrorism Risk". EU-project funded by the EU's seventh framework programme from 2012 to 2014; http://www.equatox.eu; Special Issue of Toxins: "Detection and identification of biological toxins in international proficiency tests". https://www.mdpi.com/journal/toxins/special_issues/detect-identi-toxins.

26 The EuroBioTox Consortium. "European Programme for the Establishment of Validated Procedures for the Detection and Identification of Biological Toxins". EU-project funded by the EU's Horizon 2020 programme from 2017 to 2023: https://www.eurobiotox.eu.

Figure 7*:* Summary of recent quality assurance measures on biotoxins undertaken in different international networks



143. With respect to future international collaboration, the EU project EuroBioTox develops certified reference materials for different relevant biotoxins, among them the Schedule 1 compound ricin, that will be accessible to authorised expert laboratories worldwide, thus strengthening quality assurance measures internationally, in a sustainable way.

144. With respect to sub-question 5 (f) (ii)—*how the analytical exercises can be harmonised yet remain flexible to address new or emerging biotoxin threats*—the TWG notes that in the past biotoxin analytical exercises have been conducted for three general purposes:

(a) building capacity for investigations under the Convention of possible use of biotoxins listed in Schedule 1;

(b) building capacity for investigations under the UNSGM of possible use of a broad range of biotoxins; and

(c) harmonising approaches to biotoxin analysis among laboratories in EU countries.

145. The TWG expects that this pattern will continue in the future and that additional exercise programmes may also be initiated for other purposes. Thus, the exercise landscape will be complex, with a variety of funding and implementing organisations, different schedules and timeframes for planning and conducting exercises, different goals, varying but overlapping lists of participants, and differing willingness to disclose results.

146. Overall, the goal should be for the various exercises to complement and supplement each other, in order to build capabilities for forensic analysis of a broad range of biotoxins, both LMW and HMW, that can be utilised by the OPCW, the UNSGM, and other international and national investigative authorities.

147. **Recommendation 22:** *To help ensure that results are broadly applicable, unnecessary duplication is minimised, and gaps are filled, the OPCW should invite other organisations conducting biotoxin analysis exercise programmes to meet informally as soon as possible, and periodically thereafter. The purpose should be to exchange information on exercises being planned or under consideration, with a view to coordinating the various efforts. This will minimise the burden for laboratories of participating in multiple exercises and to help ensure that the exercise programmes collectively provide a broad picture of the capabilities available internationally for biotoxin analysis.*

148. Ideally, this picture would help the OPCW and the UN assess what laboratories are most skilled in analysis of a particular biotoxin and with what limitations (for example, some laboratories are highly specialised in analysis of food samples), as well as identifying where important gaps exist for which adequate capabilities need to be developed.

149. This coordination effort could take the form of an informal working group that includes experts from all the different networks that are planning exercises and assessing them. The group might meet once or twice a year to exchange information on planned future exercises, lessons learned, and assessment of needs for biotoxin analysis capabilities. Each network would be free to pursue the approach that best serves its needs, but the group might also find it useful to coordinate activities in some areas.

150. Topics on which coordination might be considered in order to ensure that results are broadly applicable include the following:

(a) ensuring that report formats are compatible, appropriate for biotoxin analysis, and information is presented in a form that can withstand forensic scrutiny;

(b) providing sufficient information for an outside expert to assess the capabilities of the laboratory for future taskings;

(c) setting common standards for quality assurance – ISO/IEC 17025 (applicable to analytical laboratories for environmental and biomedical samples) and/or ISO 15189 (applicable to biomedical laboratories for clinical samples); and

(d) providing transparency about analytical procedures used, including measurement guidelines.

Subgroup 5: Measures for international cooperation

151. Subgroup 5 addressed question 5 (g): “What institutional or legal measures need to be established to facilitate cooperation between the OPCW and other organisations working on development of capabilities for analysis of toxins?”.

152. In the prior section addressing the work of subgroup 4, the TWG makes several recommendations regarding increased cooperation between the OPCW and other organisations on the development of biotoxin analytical capabilities.

153. Broadly speaking, the goal should be to establish an informal network of laboratories whose members are highly skilled in biotoxin analysis, are working to further develop their capabilities, and also could be available to assist an international investigation conducted by the OPCW or the UNSGM.

154. To reap the benefits of this cooperation, such a network would need to continue its work over an extended period of time. It needs to be sustainable. Thus, a highly informal network based on cooperation and understandings among key individuals would not be sufficient since it might be disrupted if key experts are no longer involved for some reason. On the other hand, the network should maintain flexibility to adapt the participation and activities to the needs and interests of the OPCW, the UNSGM, and other relevant organisations. The OPCW, together with the UNSGM, could readily use the existing UN-OPCW cooperation agreement agreed in October 2000 (EC-MXI/DEC.1, dated 1 September 2000) as the overall legal basis for cooperation regarding biotoxin analysis. In this agreement, the two organisations recognise "the need to work jointly to achieve mutual objectives" and, with a view to facilitate the effective exercise of their responsibilities, "agree to cooperate closely within their respective mandates and to consult on matters of mutual interest and concern".

155. To facilitate biotoxin analysis cooperation, a flexible structure for a biotoxin analysis network could be based on a relatively simple document that provides the terms of reference, spelling out: the goal for the network; the activities to be undertaken (e.g., exchange of information, coordination of activities, and harmonisation of quality management practices); participation (e.g., OPCW, UNODA, and individual laboratories); and organisational matters (e.g., staffing and officers). Membership in the network could be established simply by an exchange of letters between the network and the organisation. It might also be desirable for laboratories to re-confirm their membership in the network every few years.

156. Since such a network would rely on active participation by members, the funding and staffing requirements would be minimal and borne largely by the participating organisations. Experience from analogous laboratory networks has shown, however, that having a coordinator, housed at one of the participating organisations would be highly beneficial. In this case, it would make most sense to have a part-time coordinator on the staff of the OPCW Laboratory.

157. **Recommendation 23:** *In developing mechanisms for international cooperation, the OPCW should emphasise sustainability and utilise existing structures, such as the relationship agreement for cooperation between the OPCW and the UN (EC-MXI/DEC.1, dated 1 September 2000), or base them on a relatively simple document that provides flexible terms of reference. The TWG believes there is no need for new formal, legal agreements in order to create the mechanisms recommended in this report.*

ACKNOWLEDGEMENTS

158. The TWG on the Analysis of Biotoxins expresses deep appreciation to the Director-General for his interest in, and support of, this work. It also expresses its gratitude to the European Union who funded the work of the group. The TWG acknowledges all the guest speakers and observers listed in Annex 6 of this report who contributed to its deliberations. The TWG also wishes to acknowledge the many members of the Secretariat who participated in its meetings and discussions: In particular the TWG thanks Ernesa Ademagic for her tireless support of all the meetings of the TWG.

GLOSSARY

Abbreviation or term	Definition
15-ADON	15-Acetyldeoxynivalenol
2D	Two dimensional
3-ADON	3-Acetyldeoxynivalenol
5-HT	5-Hydroxytryptamine
ADP	Adenosine diphosphate
AF	Aflatoxins
ATP	Adenosine triphosphate
ATS	Anatoxin-a(s)
ATX	Anatoxin-a
AZA	Azaspiracid
BnTX	Bungarotoxin
BoNT	Botulinum neurotoxin
BTWC	Biological and Toxin Weapons Convention
BTX	Batrachotoxin
CaC	Calcicludine
CaS	Calciseptine
CBRN	Chemical, biological, radiological, and nuclear
CBT	Chemical, biological, and toxin
CDC	Centres for Disease Control and Prevention
CgTX	Ciguatoxin
COTS	Commercial off-the-shelf
CTx	Cholera toxin
CTX	Cardiotoxin
CW	Chemical weapon
CWC	Chemical Weapons Convention
CYN	Cylindrospermopsin
Da	Dalton
DA	Domoic acid
DAS	Diacetoxyscirpenol

Abbreviation or term	Definition
dcSTX	Decarbomoylsaxitoxin
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
DpTX	Dinophysistoxin
DTX	Dendrotoxin
EHEC	Enterohaemorrhagic *Escherichia coli*
ELISA	Enzyme-linked immunosorbent assay
EQA	External quality assurance
ET-1	Endothelin
ETX	Epsilon toxin
EU	European Union
G protein	Guanine nucleotide binding protein
GPCR	G-protein-coupled receptor
GRP	Gastrin releasing peptide
GTX	Gonyautoxin
h	Hour
Hl	Hemolysin
hERG	Human ether-à-go-go related gene
HMW	High molecular weight
HT-2	HT-2 toxin
IBD	Inflammatory bowel disease
IEC	International Electrotechnical Commission
IFN-γ	Interferon γ
IL	Interleukin
ISO	International Organization for Standardization
Lab	Laboratory
LC	Liquid chromatography
LFA	Lateral flow assay
LMW	Low molecular weight
LSD	Lysergic acid diethylamide

Abbreviation or term	Definition
LT	Leukotriene
MC	Microcystin
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MTX	Maitotoxin
nAChR	Nicotinic acetylcholine receptor
NEOS	Neosolaniol
neoSTX	Neosaxitoxin
NIV	Nivalenol
NOD	Nodularin
OA	Okadaic acid
OPCW	Organisation for Prohibition of Chemical Weapons
PbTx	Brevetoxin
PCR	Polymerase chain reaction
PGE2	Prostaglandin E2
PlTX	Palytoxin
PPE	Personal protective equipment
PT	Pertussis toxin
PTX	Pectenotoxin
RIP II	Ribosome-inactivating protein type II
RNA	Ribonucleic acid
RTX	Repeats-in-toxin
SAB	Scientific Advisory Board
SCN	Short-chain neurotoxin
SE	*Staphylococcus aureus* enterotoxins
SK channel	Small conductance calcium-activated potassium channel
STX	Saxitoxin
Stx	Shigatoxin
T-2	T-2 toxin
TCT	Trichothecene

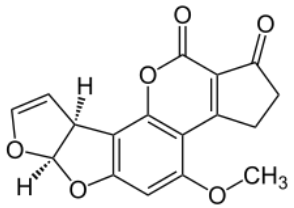
Abbreviation or term	Definition
TeNT	Tetanus neurotoxin
TNF-α	Tumour necrosis factor α
TOR	Terms of reference
TTX	Tetrodotoxin
TWG	Temporary Working Group
Tx	Thromboxane
UN	United Nations
UNODA	United Nations Office for Disarmament Affairs
UNSGM	United Nation Secretary-General's Mechanism
WHO	World Health Organization
WOAH	World Organisation for Animal Health
YTX	Yessotoxin

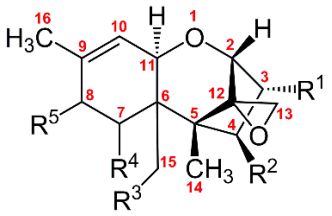
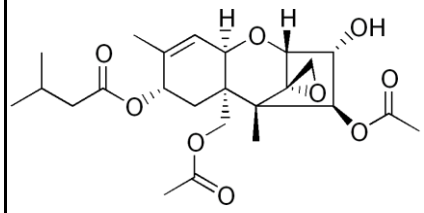
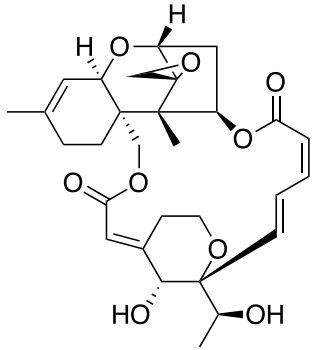
ANNEX 1

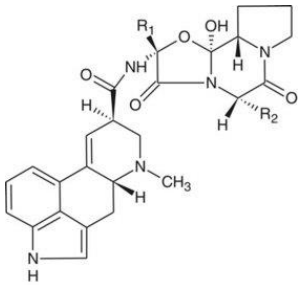
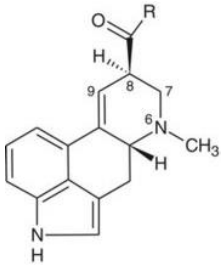
GROUPS OF BIOTOXINS, THEIR CHEMICAL PROPERTIES AND GENERAL MECHANISMS OF TOXICITIES

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Microalgae toxins (phycotoxins) - *Cyanobacteria*				
Saxitoxins (STXs) (STX, neoSTX, dcSTX, dcneoSTX)	Paralytic shellfish poisoning	Tricyclic guanidine alkaloids	Neurotoxic, block sodium channels along nerve cells	STX
Gonyautoxins (GTXs) (GTX-1 –GTX-8, etc)		Alkaloids, sulfate homologues of STXs	Neurotoxic, block sodium channels	GTX-1, GTX-4
Anatoxin-a (ATX) (ATX-a, homo-ATX-a)	-	Amine alkaloids	Neurotoxic, acetylcholine antagonist, blocking acetylcholinesterase activity	ATX-a
Anatoxin-a(s) (ATS)	-	Organophosphate	Neurotoxic, inhibits the active site of acetylcholinesterase	–
Microcystins (MCs) (MC-LR, MC-RR, MC-LW, MC-LF, etc)	-	Cyclic peptides	Hepatotoxic, inhibit the activity of phosphatases 1 and 2A	MC-LR
Nodularins (NODs) (NOD-R, NOD-V, NOD-Har, etc)	-	Cyclic peptides	Hepatotoxic, inhibit the activity of phosphatases 1 and 2A	NOD-R

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Cylindrospermopsins (CYNs) (CYN, 7-epi-CYN, 7-deoxy-CYN, 7-deoxy-desulfo-CYN, 7-deoxy-desulfo-12-acetyl-CYN)	-	Tricyclic alkaloids	Hepatotoxic, cytotoxic, neurotoxic, inhibit cellular serine/threonine protein phosphatase 1 and 2A.	CYN
Microalgae toxins (phycotoxins) – *Dinoflagellates*				
Saxitoxins (STXs) (STX, neoSTX, dcSTX, dcneoSTX)	Paralytic shellfish poisoning	Tricyclic guanidine alkaloids	Neurotoxic, block sodium channels along nerve cells	STX
Gonyautoxins (GTXs) (GTX-1 to GTX-8)		Alkaloids, sulfate homologues of STXs	Neurotoxic, block sodium channels	GTX-1, GTX-4
Brevetoxins (PbTxs) (PbTx-1 to PbTx-10)	Neurotoxic shellfish poisoning	Cyclic polyethers	Neurotoxic, cause excessive opening of sodium channels	PbTx-2
Ciguatoxins (CgTXs) (CgTX-1, CgTX-2, etc)	Ciguatera fish poisoning	Cyclic polyethers	Neurotoxic, cause excessive opening of sodium channels	CgTX-1
Maitotoxins (MTXs) (MTX, MTX-2, MTX-3, MTX4)		Cyclic polyethers	Neurotoxic, stimulate calcium influx into the cells	MTX
Okadaic acid (OA) and its derivatives dinophysistoxins (DpTX-1, DpTX-2, etc)	Diarrhoetic shellfish poisoning	Polyethers with spiro keto ring	Neurotoxic, phosphatase inhibitor	OA

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Pectenotoxins (PTXs) (PTX-1, PTX-2 to PTX-15)		Polyether-lactones	Hepatotoxic, cytotoxic, alter actin-based structures	PTX-2
Yessotoxin and its derivatives (YTX, homo-YTX, nor-YTX, hydroxyl-YTX, etc)		Disulfated polycyclic polyethers	Hepatotoxic, cardiotoxic, neurotoxic, alter calcium homeostasis	YTX
Azaspiracids (AZAs) (AZA-1to AZA-41)	Azaspiracid shellfish poisoning	Nitrogen-containing polyethers	Hepatotoxic, neurotoxic, immuno-toxic, cardiotoxic, inhibit hERG voltage-gated potassium channels	AZA-1, AZA-2, AZA-3
Palytoxins (PlTXs) (PlTX, homo-PlTX, bishomo-PlTX, neo-PlTX, deoxy-PlTX, etc)	-	Polyhydroxylated and partially unsaturated compounds (8 double bonds)	Neurotoxic, inhibit Na^+/K^+-ATPase	PlTX
Microalgae toxins (phycotoxins) – *Diatoms*				
Domoic acid (DA) and derivative (epi-DA)	Amnesic shellfish poisoning	Cyclic amino acids with 3 carboxylic acid groups	Neurotoxic, acts on ionotropic glutamate receptors	DA
Mycotoxins (fungal biotoxins)				
Aflatoxins (AFs) (AFB1, AFB2, AFG1, AFG2, AFM1, AFM2, etc)	Carcinogenicity, impaired development, immunotoxicity	 AFB1 structure	Covalent DNA and protein binding that can lead to DNA mutations and cytotoxicity	AFB1

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Trichothecenes (TCTs) class B Nivalenol (NIV), deoxynivalenol (DON), 3-acetylDON (3-ADON),15-acetylDON (15-ADON)	Alimentary toxic aleukia: inflammation of gastric and intestinal mucosa, leukopenia, granulopenia, progressive lymphocytosis, a red rash on the skin of the body, haemorrhage of skin and mucosa	Class of sesquiterpenes 	Inhibition of protein synthesis	DON NIV
Trichothecenes (TCTs) class A T-2 toxin (T2), HT-2 toxin (HT2), neosolaniol (NEOS), diacetoxyscirpenol (DAS)	Alimentary toxic aleukia	 T-2 structure	Inhibition of protein synthesis, DNA and RNA synthesis, etc.	T-2
Stachybotryotoxins from *Stachybotrys chartarum* Satratoxins F, G, and H, verrucarin J, roridin E, and trichoverrols A and B	Dermal toxicity, respiratory distress (asthma), epistaxis, eye irritation, neurocognitive dysfunction, mucous membrane irritation, and immune disorders	 Satratoxin H structure	Apoptosis of olfactory sensory neurons	Satratoxin H

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Ergot alkaloids from *Claviceps purpurea* fungus 6,8-dimethylergoline derivatives	Vasoconstriction: skin discoloration and gangrene of hands or feet, caused by constriction of blood vessels called St. Anthony's Fire	Amino acid alkaloids 	Agonists and antagonists at adrenergic, dopaminergic, and tryptaminergic (also called serotonin or serotonergic, e.g., 5-hydroxytryptamine (5-HT)) receptors	Ergotamine
Ergot alkaloids from *Claviceps purpurea* fungus Lysergic acid derivatives	Depersonalisation or hallucinations and may produce toxic psychosis; mydriasis, increased blood pressure, tachycardia, elevated body temperature, tremors, and hyperreflexia	Amine alkaloids 	Perturbations of serotonergic neurotransmission mediated by the activation of 5-HT receptors; catecholaminergic stimulation	Lysergic acid diethylamide (LSD)
Bacterial toxins				
***Clostridium botulinum* toxins** (BoNT/A, B, E, F, H)	Human botulism	Proteins with AB structure, Zn^{2+} dependent metalloprotease MW = 150 kDa	Neurotoxic, acetylcholine release blockage, resulting in flaccid paralysis	BoNT/A
***Clostridium botulinum* toxins** (BoNT/C, D)	Animal botulism			

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Cholera toxin (CTx)	Cholera	Protein with AB_5 structure (1 A unit and 5 B units), MW = 83 kDa	Catalyses the ADP-ribosylation of G proteins, then unable to inhibit adenylate cyclase activity leading to several systemic effects	–
Tetanus neurotoxin (TeNT)	Tetanus	Protein with AB structure, Zn^{2+} dependent metalloprotease MW = 150 kDa	Neurotoxic: inhibits neurotransmission of inhibitory interneurons, causing spastic paralysis	–
Clostridium perfringens epsilon toxin (ETX)	Enterotoxaemia, necrotic enteritis, and gas gangrene	Protein, MW = 29 kDa	Erythrocyte lysis and cell necrosis	–
C. perfringens alpha toxin		Protein, MW = 35 – 43 kDa	Phospholipase C and sphingomyelinase	–
C. perfringens beta-1 and beta-2 toxins		Proteins, MW = 35 – 43 kDa	Pore-forming toxins	–
C. perfringens iota toxin		Protein, MW = 35 – 43 kDa	ADP-ribosylating toxin and modifies G-actin	–
Staphylococcus aureus **enterotoxins (SEs)** (SEA, SEB, SEC, SED, SEE, TSST-1, etc)	Food SE poisoning, toxic shock syndrome	Proteins, MW = 25 – 35 kDa	Major inflammatory response via superantigenic properties	SEB
Hemolysin (Hl) toxins (α, β,γ) from *Staphylococcus aureus* (RTX toxins)	Haemolytic anaemia, pneumonia	Proteins, MW = 33 kDa	Erythrocyte lysis	α-Hl

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Diphtheria toxin	Diphtheria	Protein with AB structure, MW = 70 kDa	Inhibiting protein synthesis	–
Shigatoxins (Stx (from *Shigella dysenteriae*); Stx1, Stx2 (from *E. coli*))	Shigellosis, enterohaemorrhagic *Escherichia coli*	Proteins with AB_5 structure (1 A unit and 5 B units), MW = 55 kDa	Ribosomal inactivation (N-glycosidase activity): protein synthesis inhibition, disruption of cell membranes	Stx
Pertussis toxin (PT) from *Bordetella pertussis*	Whooping cough	Protein with AB_5 structure (1 A unit and 5 B units), MW = 105 kDa	Catalyses the ADP-ribosylation of G proteins, then unable to inhibit adenylate cyclase activity leading to several systemic effects	–
Plant toxins				
Ricin	RIP II poisoning	Protein with AB structure, MW = 65 kDa	Ribosomal inactivation (N-glycosidase activity): protein synthesis inhibition, cell death	–
Abrin and other RIP II (nigrin, winter aconite lectin, ebulin, modeccin, viscumin, volkensin)	RIP II poisoning	Proteins, MW = 65 kDa (abrin) MW = 57 kDa (modeccin) MW = 62 kDa (volkensin) MW = 115 kDa (viscumin)	Ribosomal inactivation (N-glycosidase activity): protein synthesis inhibition, cell death	Abrin
Alkaloids (strychnine, atropine, coniines)	Nervous and muscular paralysis	Terpene (strychnine) Hyoscyamine (atropine) Piperidine analogues (coniines)	Block muscarinic (strychnine, atropine), nicotinic acetylcholine receptors (coniines)	Strychnine, atropine, γ-coniine

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Fish toxin				
Tetrodotoxin (TTX)	Tetrodotoxin poisoning	Guanidinium-containing 2,4-dioxaadamantane-like compound	Neurotoxic: binding to the voltage-gated sodium channels in nerve cell membranes	–

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Marine cone snail toxins				
α-**Conotoxins**	Nervous and muscular paralysis	Disulfide bond-containing peptides (12–30 residues) Their disulfide bond frameworks stabilise compact loop structures that often contain protein-like secondary motifs such as α helices, β turns, and β sheets	Neurotoxic: antagonists of nicotinic acetylcholine receptors (nAChR)	GI
γ- Conotoxins			Neurotoxic: neuronal pacemaker cation currents (inward cation current)	PnVIIA, TxVIIA
δ-Conotoxins			Neurotoxic: voltage-gated sodium channels (agonist, delay inactivation)	TxVIA
ε- Conotoxins			Neurotoxic: Presynaptic Ca channel or G protein-coupled presynaptic receptors	TxVA
ι- Conotoxins			Neurotoxic: voltage-gated sodium channels (agonist, no delayed inactivation)	RXIA
κ- Conotoxins			Voltage-gated K channels (blocker)	PVIIA
μ- Conotoxins			Voltage-gated sodium channels (antagonist, blocker)	GIIIA
ρ- Conotoxins			Alpha1-adrenoceptors (GPCR)	TIA
τ- Conotoxins			Somatostatine receptors	CnVA
σ- Conotoxins			Serotonin-gated ion channels (GPCR)	GVIIIA
χ- Conotoxins			Neuronal noradrenaline transporter	MrIA, CMRVIA
ω- Conotoxins			Voltage-gated calcium channels (blocker)	GVIA

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Spider toxin				
α-Latrotoxin	Latrodectism	130 kDa dimeric or tetrameric protein	Neurotoxic: acts presynaptically to release neurotransmitters (acetylcholine)	–
Snake toxins				
α-**Bungarotoxin** (α-BnTX), γ-Bungarotoxin (γ-BnTX)	*Bungarus multicinctus* poisoning	Disulfide bond-containing peptides	Neurotoxic: paralytic, irreversible, competitive inhibitor of postsynaptic nicotinic acetylcholine receptors of skeletal muscles and brain	α-BnTX
β -Bungarotoxins (β-BnTXs) β_1-BnTX to β_5-BnTX		Disulfide bond-containing peptides – larger ~15 kDa catalytic subunit with Ca^{2+}-activated phospholipase A2 activity, 1–2 other smaller peptides	Neurotoxic: hydrolyses membranes of nerves and muscles	β-BnTX
Cardiotoxins (CTX I to CTX V)	Cobra snake poisoning	Disulfide bond-containing SCNs 60-mer peptides	Neurotoxic: lethal cytotoxin, induces apoptosis via release of cytochrome C	CTX I, CTX-2

Group	Disease	Chemical structure	Toxic mechanism	Family leader
Calciseptine (CaS), **FS2**, and c**alcicludine** (CaC)	Mamba snake poisoning	Disulfide bond-containing SCNs 60-mer peptides	Neurotoxic: block L-, N-, and P-type high-voltage-gated calcium channels	CaC
Dendrotoxins (δ-DTX, DTX-I, DTX-J, DTX-K, BPTI)		Disulfide bond-containing SCNs 57–60-mer peptides	Neurotoxic: block voltage-gated potassium channels in neurons to release acetylcholine, results in hyperexcitability, convulsive	α-DTX
Amphibian toxins				
Batrachotoxins	*Dendrobates* and *Phyllobates* poisoning	Steroidal alkaloids	Neurotoxic: activates sodium ion channels, digitalis (digitoxin)-like cardiotoxin, causes ventricular fibrillation and cardiac arrest	BTX
Insect toxin				
Apamin	Bee and wasp venom poisoning	18 amino acid peptide	Neurotoxic: selectively blocks SK channels, a type of Ca^{2+} activated K^{+} channel expressed in the central nervous system	–

ANNEX 2

BIOREGULATORS AND DISEASES ASSOCIATED WITH THEIR DYSREGULATION

The bioregulators considered by subgroup 2 are reported in the table below. They are grouped according to their linked common traits and the diseases associated with bioregulator dysregulation are also listed. Unless marked with an asterisk, the diseases listed are linked with upregulation or overactivation with their associated bioregulator. *Indicates a downregulation or decrease in efficacy of the associated bioregulator. Most diseases are considered associated with chronic dysregulation. Some associations are based around causal relationships and observed changes of during disease progression and may not be the causal factor behind the diseases.

Linked traits	Bioregulator name	Associated model diseases
Cytokines	IL-6	Rheumatoid arthritis, Cattleman's disease, cardiac myxomas, lymphocytopenia
	IL-1	Stroke, myocardial infarction, kidney failure, liver disease, inflammatory diseases
	TNF-α	Rheumatoid arthritis, inflammatory diseases, cytokine storms
	IFN-γ	Systemic lupus erythematosus
Inflammatory agents	Histamine releasing factors	Asthma, atopic dermatitis, food allergy, chronic idiopathic urticaria, pulmonary arterial hypertension
Eicosanoids	Thromboxane: TxA2	Myocardial infarction, atherosclerosis, Prinzmetal angina, asthma, nephritis, hepatic injury, rhinitis, atopic dermatitis, angiogenesis of cancer
	Prostaglandins: PGE2	Arthritis, thermo-dysregulation, nephritis, hepatic injury
	Leukotrienes: LTB, LTC, LTD, LTE	Asthma, nephritis, hepatic injury
Endocrine	Gastrin releasing peptide (GRP) (Bombesin)	Parkinson's disease*, Alzheimer's disease*, autism (deletion/mutation), schizophrenia*, eating disorders*, human glioma, anorexia
	Somatostatin	Heart failure
	Oxytocin	Chronic depression
	Catecholamines	Parkinson's disease
	Insulin	Hypermetabolic states, dumping syndrome

Linked traits	Bioregulator name	Associated model diseases
Neurotransmitters	Endorphins (α, β, δ), Enkephalins, Dynorphins	"Runner's high"
	Neurokinin-1 (A and B), Substance P, Tachykinins	Inflammatory bowel diseases (IBDs), Crohn's disease, ulcerative colitis, pseudomembranous colitis, appendicitis, cholecystitis
	Neuropeptide Y	Alzheimer's disease, Machado-Joseph disease, Parkinson's disease, Huntington's disease
	Neurotensin	Parkinson's disease or schizophrenia (possible link)
	Orexin (hypocretins)	Type I narcolepsy*, chronic insomnia
Coagulation	Thrombin (coagulation cascade)	Thrombophilia, venous thromboembolism, antiphospholipid syndrome, anti-cardiolipin antibodies, anti-β2-glycoprotein 1 antibodies
Blood pressure	Endothelin (ET-1)	Pre-eclampsia, post-menopausal hypertension, pulmonary hypertension, hyperglycaemia
	Bradykinin (kinin-9), Kallidin, Kallikrein	Angioedema
	Angiotensin (I and II)	Hypertension, cardiac failure
	Vasopressin	Hypervolemia, hyponatremia

ANNEX 3

TERMS OF REFERENCE

1. The use of biological toxins as weapons is prohibited both under the Chemical Weapons Convention (CWC) and the Biological and Toxin Weapons Convention (BTWC). In the past, several biological toxins were weaponised, leading to the inclusion of both saxitoxin and ricin in Schedule I of the Annex on Chemicals to the CWC. Further, there are some biological toxins that are of interest to non-state actors. Accordingly, the capability to detect, identify, and characterise biological toxins that may be present in samples taken during investigations is essential for the OPCW. Internationally, there are other stakeholders with a mandate related to biotoxins; the UN Secretary-General's Mechanism for Investigating Alleged Use of Chemical and Biological Weapons (UNSGM) also provides guidance and assistance related to misuse of biotoxins. As such, it is also imperative that the OPCW and the UNSGM work cohesively to share information and minimise duplication of effort, since either might be called on to conduct an investigation of alleged use of a biological toxin.

2. An in-depth review of the methods and technologies used in the analysis of biotoxins would be useful and would be relevant to and augment the capacity of the Technical Secretariat. Further to his response to the report of the Twenty-Ninth Session of the SAB (SAB-29/1, dated 2 September 2020) and in accordance with paragraph 9 of the terms of reference of the SAB (Annex to C-II/DEC.10/Rev.1, dated 2 December 2004), the Director-General has therefore decided to establish a Temporary Working Group (TWG) on the Analysis of Biotoxins and has appointed Dr Daan Noort as the Chairperson of the Group.

3. The objective of the TWG is to review the science and technology relevant to the analysis of biotoxins and considerations that need to be taken into account in investigations of their alleged use. Considerations should be given to the work and recommendations from the SAB's previous TWG on Investigative Science and Technology (SAB/REP/1/19, dated 1 December 2019). The work of this TWG is intended to identify capabilities, skill sets, and equipment that would augment and strengthen the Technical Secretariat's capabilities. The findings will be considered by the SAB and recommendations will be provided to the Director-General.

4. The TWG will consist of individuals who have expertise in the theory and practice of biotoxin analysis, including but not limited to laboratory techniques, low and high molecular weight biotoxins, investigational analysis, evidence collection, forensic sciences, informatics, toxicology, or experience of implementation of the Chemical Weapons Convention. The TWG will be comprised of qualified members of the SAB as well as representatives from relevant scientific and international organisations. Guest speakers will be invited regularly to assist the TWG in its collection of data and information and the formulation of advice. The TWG may also, when necessary, draw upon the expertise of the Technical Secretariat, in particular the OPCW Laboratory, Inspectorate, non-routine missions and the Assistance and Protection Branch.

5. The TWG will report to the SAB, and will consider the following questions, in particular:

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

b. What classes of biological toxins are most likely to be relevant in investigations of alleged use?

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?

d. What are the technical requirements for analysis of the most relevant types of biological toxins? Please consider:

   i. analytical approaches needed for unambiguous identification of both low and high molecular weight biotoxins;

   ii. instrumentation and/or procedures that should be standardised across labs to ensure reproducible and consensus results;

   iii. analytical criteria that should be in place in order to match forensic requirements; and

   iv. the role and utility of degradation products and other markers and/or compounds; and

   v. the role of biomarkers and biomedical samples.

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?

f. How can programs 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? Please consider:

   i. the quality system requirements for the laboratories that should be in place (e.g., consideration of ISO 17025 for OPCW Designated Labs); and

   ii. how the analytical exercises can be harmonised yet remain flexible to address new or emerging biotoxin threats.

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

6. In addition, the TWG will provide advice, as requested, on Technical Secretariat proposals for methodologies, procedures, technologies, and equipment for the analysis of biotoxins.

7. The Director-General might pose other relevant questions to the TWG, through the SAB.

8. The TWG will exist for a period of two years from the date of this memo. Thereafter, its work will be reviewed by the SAB and the Director-General, and a decision will be made

as to whether it should continue its work and, if so, whether these terms of reference should be revised.

ANNEX 4

REPORTS AND BRIEFINGS OF THE TEMPORARY WORKING GROUP

Date issued	Document	Available at
6 May 2021	"Summary of the First Meeting of the Scientific Advisory Board's Temporary Working Group on the Analysis of Biotoxins" (SAB-32/WP.1)	https://bit.ly/TWGAB1
15 October 2021	"Summary of the Second Meeting of the Scientific Advisory Board's Temporary Working Group on the Analysis of Biotoxins" (SAB-33/WP.1)	https://bit.ly/TWGAB2
14 July 2022	"Summary of the Third Meeting of the Scientific Advisory Board's Temporary Working Group on the Analysis of Biotoxins" (SAB-33/WP.2)	https://bit.ly/TWGAB3
29 July 2022	"Summary of the Fourth Meeting of the Scientific Advisory Board's Temporary Working Group on the Analysis of Biotoxins" (SAB-36/WP.1)	https://bit.ly/TWGAB4
17 November 2022	"Summary of the Fifth Meeting of the Scientific Advisory Board's Temporary Working Group on the Analysis of Biotoxins" (SAB-36/WP.2)	https://bit.ly/TWGAB5
17 April 2023	"Summary of the Sixth Meeting of the Scientific Advisory Board's Temporary Working Group on the Analysis of Biotoxins" (SAB-37/WP.1)	https://bit.ly/TWGAB6

ANNEX 5

MEMBERS OF THE TEMPORARY WORKING GROUP

No.	Name	Affiliation
1	Dr Crister Åstot*	Swedish Defence Research Agency (FOI), Umeå, Sweden
2	Dr Anne Bossée	Directorate General of Armaments (DGA) CBRN Defence, France
3	Dr Graeme Clark	Defence Science and Technology Laboratory (Dstl), CBR Division, Porton Down, United Kingdom
4	Dr Cindi Corbett	National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada
5	Dr Christophe Curty	Spiez Laboratory, Switzerland
6	Dr Brigitte Dorner	Centre for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany
7	Prof Mostafa Ghanei	Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Islamic Republic of Iran
8	Dr Suzanne Kalb	Centers for Disease Control and Prevention, National Center for Environmental Health, Atlanta, USA
9	Dr Zrinka Kovarik	Institute for Medical Research and Occupational Health, Zagreb, Croatia
10	Dr Andrea Leisewitz	Integrity, Safety and Ethics in Research at the Universidad San Sebastián, Chile
11	Dr Robert Mikulak	Department of State, Washington DC, United States of America
12	Dr Daan Noort**	TNO, Netherlands
13	Dr Isel Pascual Alonso	Center for Protein Studies, Faculty of Biology, University of Havana, Cuba
14	Dr Yulya Polyak	Russian Academy of Sciences, Moscow, Russian Federation
15	Mr Günter Povoden***	CBRN Defence Centre, Austrian Armed Forces, Vienna, Austria
16	Dr Fengxia Sun	College of Chemical and Pharmaceutical Engineering, Hebei University of Science and Technology, Shijiazhuang, China

*Chairperson of the TWG
** TWG Chairperson from January 2021 until April 2022, resigned from TWG in May 2022
*** Joined TWG starting in 2022 upon beginning duties as SAB Chairperson

ANNEX 6

GUEST SPEAKERS AT MEETINGS OF THE TEMPORARY WORKING GROUP

No.	Speaker	Affiliation
Third Meeting		
1	Dr Thomas Bergstrom	Swedish Defence Research Agency (FOI), Sweden
2	Dr Arjen Gerssen	Wageningen Food Safety Research (WFS), The Netherlands
3	Dr Jacques-Antoine Hennekinne	Agency for Food, Environmental and Occupational Health and Safety, France
4	Dr Els Van Pamel	Flanders Research Institute for Agriculture, Fisheries and Food, Belgium
5	Dr Christine Uhlenhaut	Office for Disarmament Affairs, United Nations
Fourth Meeting		
6	Dr Robert Bull	Federal Bureau of Investigation, United States of America
Fifth Meeting		
7	Dr Michael Crowley	University of Bradford, United Kingdom
8	Prof Malcolm Dando	University of Bradford, United Kingdom
9	Mr David Frisby	Metropolitan Police, London, United Kingdom
10	Dr Isabelle Oswald	National Research Institute for Agriculture, Food and Environment (INRAE), France
Sixth Meeting		
11	Dr Cédric Invernizzi	Spiez Laboratory, Switzerland
12	Dr Ziad Kazzi	Emory University, United States of America
13	Dr Alexandre LeClercq	National Reference Center & WHO-CC Listeria, Institut Pasteur, France
14	Dr Weng Keong Loke	DSO National Laboratories, Singapore
15	Dr Stéphanie Simon	Atomic Energy and Alternative Energies Commission (CEA), France
16	Dr François Bécher	Atomic Energy and Alternative Energies Commission (CEA), France
17	Dr Christine Uhlenhaut	Office for Disarmament Affairs, Switzerland
18	Dr David Wunschel	Pacific Northwest National Laboratory, United States of America

No.	Speaker	Affiliation
19	Ms Chen Hsiao Ying	DSO National Laboratories, Singapore

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