OPCW

Scientific Advisory Board

Thirty-Seventh Session
28 August – 1 September 2023

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# REPORT OF THE SCIENTIFIC ADVISORY BOARD AT ITS THIRTY-SEVENTH SESSION

## 1. AGENDA ITEM ONE – Opening of the session

1.1 The Scientific Advisory Board (SAB) met for its Thirty-Seventh Session from 28 August to 1 September 2023. The session was chaired by Mr Günter Povoden, with Dr Andrea Leisewitz serving as Vice-Chairperson.

1.2 Mr Povoden opened the Thirty-Seventh Session of the SAB by welcoming all Board members and especially the three newly elected members who joined the Board in January 2023. He recalled that the SAB is unique among disarmament treaties and that its success is inspiring States Parties to other treaties to consider establishing similar advisory mechanisms. Mr Povoden noted that 2023 had been an exciting year for the SAB, with the publication of both the Board's Scientific Report to the Fifth Review Conference[1] on developments in science and technology (RC-5/DG.1, dated 22 February 2023) and the end-of-mandate report of the Temporary Working Group (TWG) on the Analysis of Biotoxins.[2] The SAB Chairperson also thanked the five Board members who will leave the SAB at the end of 2023.

**Executive summary**

1.3 The Board met in person at OPCW Headquarters and those members unable to travel were able to participate virtually via the Organisation's videoconferencing equipment.

1.4 The SAB received six briefings from external speakers on topics including 3D bioprinting, medical countermeasures and therapeutics, high-throughput experimentation, and biomarkers. In addition, the methodologies and results of the OPCW's Plant Biomarker Challenge were presented to the SAB by representatives from five of the six funded projects. The Board also heard from staff members of the Technical Secretariat (hereinafter "the Secretariat"), and received updates from the SAB Secretary, the Office of Strategy and Policy, the Public Affairs Branch, the OPCW Laboratory, the Inspectorate Division, and the OPCW Fact-Finding Mission in Syria (FFM). Several Board members also presented their own research and provided updates on recent activities of interest to the SAB. In addition, the Board was able to take a guided tour of the OPCW Centre for Chemistry and Technology (ChemTech Centre), which it had previously visited during its Thirty-Sixth Session when it was still under construction. The Board was impressed with the facility and the capabilities it offers.

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1 Review Conference = Special Session of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention.

2 SAB/REP/1/23 (dated April 2023), attached as Annex 2 to this report.

1.5 Based on deliberations at its Thirty-Seventh Session, the Board recommends to the Director-General through this report that:

(a) a new TWG be established. The Board suggests the following three different topics for consideration:

(i) the provenancing of chemical samples relevant to the Chemical Weapons Convention (hereinafter "the Convention"), including methods of chemical forensic profiling;

(ii) scientific and technical considerations regarding chemicals acting on the central nervous system (CNS); and

(iii) the long-term degradation and effects of abandoned and old chemical weapons and associated chemicals;

(b) consideration be given to convening additional topical workshops that may not require a full TWG. Suggested topics are:

(i) hazard mitigation of chemical warfare agents to establish current best practice in the area of medical countermeasures, detection, protection, and decontamination of new and emerging threat chemicals. Assessments of OPCW-deployable hazard mitigation capabilities highlight potentially significant shortfalls in field operatives' ability to work safely and effectively in operational environments. There is therefore an urgent need to better understand the severity of this capability gap, particularly in relation to newly scheduled and non-scheduled chemicals such as CNS-acting chemicals and biotoxins. The outputs of this workshop can be used to inform procurement options for more fit-for-purpose capabilities in the Secretariat;

(ii) new approaches in medical countermeasures and their development, particularly given the potential misuse of non-scheduled chemicals such as CNS-acting chemicals and biotoxins. The Board feels there are significant developments in ongoing and potential approaches to developing medical countermeasures, and a dedicated workshop on this topic would be beneficial to the Secretariat;

(iii) emerging alternatives for the replacement of highly toxic chemicals in industry and healthcare; and

(iv) the ability of plants, insects, and other materials to act as sensors, sentinels, and evidence of exposure to chemical warfare agents. The Plant Biomarker Challenge has highlighted the potential for plants to assist in identifying whether exposure to toxic chemicals has occurred. Likewise, recent research in how materials interact with reactive chemicals, like chlorine, can be useful in investigations;

(c) the Director-General consider in full, and the Secretariat implement, to the extent possible, the recommendations provided in the final report by the TWG on the Analysis of Biotoxins, but in particular the call for increased harmonisation with the United Nations Secretary-General's Mechanism for Investigation of Alleged Use of Chemical and Biological Weapons (UNSGM) and the establishment of a network of laboratories for the analysis of biotoxins through proficiency testing; and

(d) the Secretariat evaluate the possibility of joint OPCW-International Union of Pure and Applied Chemistry (IUPAC) initiatives and projects to benefit from the important partnership and the breadth of expertise of IUPAC.

## 2. AGENDA ITEM TWO – Adoption of the agenda

The SAB adopted the following agenda for its Thirty-Seventh Session:

1. Opening of the session
2. Adoption of the agenda
3. *Tour de table*
4. Establishment of a drafting committee
5. Welcome address by Mr Marcin Wroblewski
6. Update on the activities of the Technical Secretariat and the Scientific Advisory Board
7. Presentation of the end-of-mandate report of the Temporary Working Group on the Analysis of Biotoxins
8. The evolution of chemical high-throughput experimentation and high-throughput analysis at MSD
9. Updates from the IUPAC World Chemistry Congress
10. Updates from the OPCW Advisory Board on Education and Outreach
11. Plants as sensors: Early detection of plant response to chlorine gas exposure
12. Presentation on the OPCW Public Affairs Branch
13. A mobile application and biomarkers-based detection of cyanide contamination in the environment using sunflower plants
14. New medical countermeasures against percutaneous intoxication by low-volatility (persistent) organophosphorus nerve agents
15. Technical countermeasures using artificial intelligence technology and quantum chemistry calculations for the threat of novel chemical agents
16. SAB discussion
17. Influence of chlorinating agents on the formation of stable biomarkers in hair for the retrospective verification of exposure
18. Sentinel plants for the surveillance of chemical risk: Natural plant-based sentinel systems to detect acetylcholinesterase-inhibiting pesticides in the environment

19. Plant biomarkers as evidence of chemical warfare agent exposure

20. Therapeutics innovation of pseudo-natural peptides and neobiologics

21. Bioprinting and advanced biomaterial 3D printing in healthcare: State of the technology and state of the industry

22. Hazardous chemical substitution in products and processes

23. Plant metabolomics for revealing chemical exposure biomarkers and portable vis/NIR spectrometry as a hotzone detector of plant poisoning

24. Lessons learned from the illness of students in Iranian schools: Investigating claims of chemical poisoning from reality to allegation

25. Updates from the OPCW Laboratory

26. OPCW deployment capabilities: A retrospective and prospective glimpse

27. Recap of the Fifth Review Conference

28. Updates on the OPCW Fact-Finding Mission in Syria

29. SAB Chairperson and Vice-Chairperson election

30. Any other business and final remarks

31. Adoption of the report

32. Closure of the session

## 3. AGENDA ITEM THREE – *Tour de table*

All participants in the session were invited to introduce themselves to their colleagues.

## 4. AGENDA ITEM FOUR – Establishment of a drafting committee

The Chairperson asked volunteers who wished to be part of the drafting committee to notify the SAB Chairperson, Vice-Chairperson, or Secretary, accordingly. Report preparation would revert to the pre-pandemic practice of drafting and adopting the report during the session.

## 5. AGENDA ITEM FIVE – Welcome address by Mr Marcin Wroblewski

5.1 The Director of the Office of Strategy and Policy, Mr Marcin Wroblewski, welcomed everyone to the Thirty-Seventh Session of the Board. He commended the Board on its excellent Scientific Report to the Fifth Review Conference (RC-5/DG.1), highlighting the importance of scientific advice in decision making regarding the implementation and operation of the Convention.

5.2 While acknowledging that the destruction of declared stockpiles is now complete, Mr Wroblewski emphasised that the threat of re-emergence of chemical weapons is real and the OPCW cannot afford to be complacent. The recent thorough and comprehensive review of the Convention clearly demonstrated States Parties' ongoing commitment to its implementation and generated a number of ideas and proposals that may assist the Organisation with its increased focus on preventing re-emergence.

5.3 Mr Wroblewski outlined some of the main priorities of the OPCW and underscored the fundamental role that the SAB plays in ensuring that the Organisation's activities are responding to the opportunities and challenges presented by scientific and technological advances.

5.4 Finally, the Director of the Office of Strategy and Policy expressed his gratitude to the TWG on the Analysis of Biotoxins for its comprehensive assessment which was particularly well-received by the Director-General. Mr Wroblewski requested that the Board consider and identify additional topics warranting such an in-depth study and to set out its recommendations in this regard in this session's report.

5.5 In conclusion, Mr Wroblewski thanked the Board members for their assistance and assured them of his Office's continuing commitment to facilitating their work.

## 6. AGENDA ITEM SIX – Update on the activities of the Technical Secretariat and the Scientific Advisory Board

6.1 The SAB Secretary provided an overview of the SAB's preparatory work for its recent Scientific Report to the Fifth Review Conference (RC-5/DG.1) and encouraged Board members to start considering their overall approach, relevant topics, and potential topical workshop ideas, as the monitoring process had begun for the Sixth Review Conference, the next such conference which was likely to take place in 2028. He also highlighted that the SAB's engagement with the scientific expert community plays an important role in accessing the widest possible network of additional expertise, and he highlighted some recent and upcoming conferences in this regard.

6.2 The SAB Secretary described the objectives of the OPCW's recently completed Plant Biomarker Challenge, funded by the European Union, and introduced the projects that would be presented during the session. He noted the work of the TWG on the Analysis of Biotoxins, also funded by the European Union, and recalled that the TWG's end-of-mandate report would be considered and adopted by the Board during this session. Furthermore, he highlighted that additional European Union funds should be available to organise future TWGs. Two topics had already been proposed by the Board: provenancing of chemical samples (recommended in the final report of the TWG on Investigative Science and Technology) and current developments concerning CNS-acting chemicals (recommended in the Scientific Report to the Fifth Review Conference (RC-5/DG.1)). However, the SAB Secretary encouraged members to consider any other topics that would benefit from such an in-depth analysis.

## 7. AGENDA ITEM SEVEN – Presentation of the end-of-mandate report of the Temporary Working Group on the Analysis of Biotoxins

7.1 Dr Crister Åstot, the Chairperson of the TWG on the Analysis of Biotoxins, provided the Board with an overview of the Group's mandate and work. He summarised the TWG's composition and expertise (including external invited speakers), its division of labour into five subgroups, and the seven specific questions that the Director-General had requested the Group to address. During its two-year mandate, the TWG met a total of seven times, with four virtual and three in-person meetings, in addition to numerous intersessional virtual meetings. Dr Åstot noted the SAB's appreciation to the European Union for providing funding for the TWG.

7.2 The TWG's end-of-mandate report (SAB/REP/1/23) was submitted to the SAB for consideration in April 2023 (the full final report is attached as Annex 2 to this report). In addition to the TWG's detailed scientific findings on the analysis of biotoxins, the report sets out a total of 23 thematically grouped recommendations. While the TWG views all 23 recommendations as important, it deemed that nine warrant prioritised consideration and are thus marked as "strong" recommendations. Dr Åstot briefed the SAB on these nine strong recommendations.

7.3 The TWG identified a list of nine biotoxins it deemed most relevant to consider when building capabilities for investigations of alleged use. Recognising that seven of these nine biotoxins are not listed on Schedule 1 in the Annex on Chemicals to the Convention (hereinafter "the Annex on Chemicals"), the OPCW should plan to draw on sophisticated biotoxin analysis capabilities that may exist in other fields. The UNSGM network was identified as such a capability for the analysis of high molecular weight (HMW) biotoxins and the TWG recommended the OPCW work closely with the United Nations in order to facilitate the harmonisation of procedures, methods, and laboratory exercises between the organisations.

7.4 The SAB posed comments and questions related to the difference in analytical capabilities and techniques required to unambiguously identify low molecular weight (LMW) biotoxins, such as saxitoxin, and HMW biotoxins, such as ricin. There was support for a proficiency test regime for biotoxin analysis that would enable a laboratory to seek separate designation for the analysis of saxitoxin or ricin. It was acknowledged that, for the analysis of HMW biotoxins, knowledge beyond the network of traditional designated laboratories needed to be leveraged, and the lack of publicly available information on laboratories with these capabilities (for HMW biotoxins other than ricin) made this challenging. In addition, the Board discussed the need for validated field tests, effective sampling strategies, clinical approaches to confirming exposure to biotoxins, laboratory accreditation, and ongoing discussions between the OPCW and the UNSGM to avoid any duplication of effort.

## 8. AGENDA ITEM EIGHT – The evolution of chemical high-throughput experimentation and high-throughput analysis at MSD

8.1 High-throughput experimentation (HTE) is an approach that allows a larger number of experiments to be performed while doing less work per experiment. Subsequent high-throughput analysis also ensures that all the generated samples are expeditiously assessed. HTE is revolutionising the pharmaceutical industry, as well as other sectors

where functional molecules are synthesised, and it is commonplace for pharmaceutical companies to have dedicated HTE groups. Dr Michael Shevlin briefed the SAB on the evolution of HTE at MSD, a large research-intensive biopharmaceutical company with research sites in the United States of America and the United Kingdom of Great Britain and Northern Ireland.

8.2 HTE is a time-effective approach that allows experimentation to proceed more quickly, making it a powerful way to conduct scientific research. Breakthroughs in the pharmaceutical sector require enormous amounts of experimentation, and HTE enables orders of magnitude more experiments to be performed over traditional techniques. Employing an approach similar to an assembly line, tools are used to parallelise common operations, reagents are dispensed as stock solutions, and pre-dispensed "libraries" of common chemicals, such as catalysts, can be used.[3] Through miniaturisation, more experiments can be performed using limited quantities of reagents, thus saving raw materials. Cost savings have also been achieved through the development of new catalysts, enabling precious metals to be replaced with cheaper and more widely available transition metals. HTE was originally developed for use in biology; Dr Shevlin and his colleagues have adapted these biological tools for use in synthetic chemistry. Custom sealable multiwell plates comprising glass vials lined with chemically resistant fluoropolymer have been developed and disposable pipette tips, commonly used in the biological sciences, have been found to be compatible with virtually all reagents and solvents for organic synthesis, with a few exceptions such as trifluoroacetic acid. Effective agitation approaches have been developed, along with pressure vessels for reactive gases, and equipment for photochemical and electrochemical reactions.

8.3 Dr Shevlin also reported developments in high-throughput analysis. Analysis of 1,536 reactions from a single microplate has been successfully reduced from three days using serial liquid chromatography-mass spectrometry (LC-MS) to just 2.5 hours using pooled MISER—multiple injections in a single experimental run—chromatography. Although this approach trades some accuracy for speed, this is acceptable in discovery chemistry. Beyond chromatography, and with a similar trade off, MALDI-TOF MS—matrix-assisted laser desorption/ionisation–time-of-flight mass spectroscopy—is extremely fast, enabling 1,536 samples to be analysed in as little as eight minutes.[4]

8.4 Collaboration and commercialisation of the HTE devices developed at MSD have been key to increasing the impact of chemical research, and Dr Shevlin noted the growing numbers of academic and industrial groups using HTE.

8.5 Dr Shevlin's briefing demonstrated that HTE had been successfully applied to the synthesis of small organic molecules, often with high enantiomeric selectivity and well-defined stereochemical properties. Board members were keen to understand whether this approach could also be applied to the synthesis of polymers. While polymers can be synthesised using this approach, water-sensitive polymerisations are particularly challenging as it is difficult to ensure the complete absence of moisture in

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3 Buitrago Santanilla A., Regalado E. L., et al. Nanomole-scale high-throughput chemistry for the synthesis of complex molecules. Science (2014), Vol 347, 49-53.

4 Lin S., Dikler S., et al. Mapping the dark space of chemical reactions with extended nanomole synthesis and MALDI-TOF MS. Science (2018), Vol 361.

such small-scale equipment. Reactions in multiwell plates may be carried out under an inert atmosphere in a glove box, and this handling technique is especially useful for ensuring the longevity of the pre-dispensed libraries of catalysts.

8.6 The Board was interested in reaction scaling and mass transfer effects. Dr Shevlin noted that homogenous reactions usually have fewer mass transfer problems and are easily scaled up: heterogenous reactions tend to be more challenging.

8.7 Questions were raised relating to the software used and skillsets needed to conduct the research described. Dr Shevlin mentioned that while the majority of the workforce comprises traditional scientists and engineers, a larger proportion of the newer hires bring additional traits, such as coding experience. He stressed that as important as automation and computer-aided processes and decision making are, the human element is still critical in the whole process.

8.8 It was difficult to say whether HTE tools reduce the cost of drug discovery, but they have certainly enabled the development of a particular product which would not otherwise have been brought to market.

## 9. AGENDA ITEM NINE – Updates from the IUPAC World Chemistry Congress

9.1 IUPAC recently held its 52nd IUPAC General Assembly (18 – 25 August 2023) and the 49th IUPAC World Chemistry Congress, combined with the 11th edition of CHAINS, the largest chemistry congress from the Netherlands (20 – 25 August 2023). While this biennial conference is hosted all over the world, in 2023 it was held at the World Forum in The Hague, next to the OPCW Headquarters, providing a unique opportunity for the OPCW and SAB members to participate.

9.2 The SAB Secretary provided a brief overview of the OPCW's involvement at the conference, noting that multiple Secretariat staff had attended, including some as panel speakers. He also noted the presence of an OPCW booth in the exhibition area where conference attendees could learn more about the Organisation, its mission, and potential career opportunities, and the offering of a tour of the Headquarters building and of the ChemTech Centre for interested conference attendees. He added that there had been generally a lot of interest in the OPCW, with the tours being particularly well attended.

9.3 Several SAB members then provided updates based on their involvement at the conference. Prof Elisa Orth both participated in a panel session focused on meeting the modern challenges of chemical weapons and moderated a panel session, supported by the SAB, on women in chemical security and safety. An additional focus session, co-organised by the SAB and IUPAC, featured Dr Matteo Guidotti as the moderator and Dr Andrea Leisewitz as a panel speaker. The panel focused on ethics and dual-use considerations in chemistry where "responsibility" was discussed, along with the need to generate awareness on dual use and to educate younger generations.

9.4 The possibility of proposing projects jointly funded by IUPAC and the OPCW on topics of mutual interest was discussed, with SAB members suggesting some specific topics for consideration.

## 10. AGENDA ITEM TEN – Updates from the OPCW Advisory Board on Education and Outreach

10.1 Dr Richard Guthrie, a member of the Advisory Board on Education and Outreach (ABEO), introduced the Board's current members and noted that the majority of the ABEO had been elected only recently in 2022. He presented the work of some ABEO members in the area of education and outreach. This includes the efforts to compile an inventory of international organisations of relevance to the work and mandate of the OPCW, as well as the development of ethical perspectives in light of scientific and technological developments. He further noted the development of a set of online educational modules by The King's University, Canada, which he hoped to see expanded to include a greater number of universities and translations.

10.2 Dr Guthrie went on to discuss the strategic plan priorities of the ABEO for its work in the next intersessional period. These include a review of its strategic plan of 2021 and the adaptation of that plan in light of both the future opportunities afforded by the inauguration of the ChemTech Centre and the complete destruction of all declared chemical weapons.

10.3 Members of the SAB inquired about the online dual-use chemistry modules and asked how these modules will be used or disseminated in the future. Further questions focused on the modules' content, whether they target academic or industrial audiences, and whether national authorities were aware of them. The Board proposed that national learned and scientific societies could be leveraged to disseminate relevant information, and Dr Guthrie called on the SAB members to share any contacts they may have in these societies for this very purpose. Finally, Dr Guthrie confirmed that, as a result of the Fifth Review Conference, the ABEO would also be looking at the issue of staff representation at the OPCW (specifically gender balance) and identifying an improved outreach strategy to better inform States Parties of the assistance available to them under Articles X and XI of the Convention.

## 11. AGENDA ITEM ELEVEN – Plants as sensors: Early detection of plant response to chlorine gas exposure

11.1 Dr Veronica Borrett (La Trobe Institute of Sustainable Agriculture and Food) presented one of the projects funded as part of the OPCW's Plant Biomarker Challenge. After introducing the project team and detailing the wide range of facilities available to them at the institute (including plant phenomics, bioinformatics, and analytical, biochemical, and microbiology facilities), Dr Borrett presented her team's approach for the proof-of-concept project.

11.2 *Arabidopsis thaliana* was selected for this work as it is a well characterised plant model with extensive data available, and is ubiquitous worldwide. The plants were grown under highly controlled conditions in a greenhouse and exposed to chlorine gas (generated in situ from bleach via the addition of hydrochloric acid droplets) in a fume hood. The effects of exposure were determined by non-invasive imaging (red-green-blue (RGB) and fluorescence microscopy), metabolomics analysis (mass spectrometry (MS) and nuclear magnetic resonance (NMR)), and DNA methylation pattern changes. The initial chlorine challenge concentration was found to be too high, immediately affecting photosynthesis and ultimately resulting in plant death. Lowering the concentration enabled the measurement of observable effects, as well as the survival and subsequent recovery of the plants.

11.3 While some changes attributable to these lower concentration exposures could be observed by fluorescence imaging, no visual changes could be observed using the RGB camera. Furthermore, Dr Borrett noted a lack of statistically significant difference in DNA methylation in chlorine-treated plants. This was unexpected, and she suggested that a more in-depth study with a different, longer-living plant would be required to probe this further. Plant tissue was collected on the day of exposure and then on days 1, 2, and 7 post-exposure, and a total of 26 samples were analysed by MS. Using data deconvolution, a total of 1,165 chlorinated compounds were found to be present in the 26 samples. A total of 50 upregulated chlorinated compounds were identified as potential biomarkers, including 26 metabolites with annotation. One of these upregulated metabolites is 3-chlorotyrosine, which has been reported as a biomarker in the literature.

11.4 Dr Borrett concluded that the plant imaging results demonstrated a powerful technique for an early indicator of chemical exposure, with knock-on effects for determining sampling hotspots and building situational awareness post-incident.

11.5 The main topics raised during the questions session concerned the experimental set-up (including the exposure method and chlorine concentration), plant growth conditions, the inherent antioxidant defence system which is not specific to chlorine exposure, future perspectives, and the outputs identified during the experiments. Dr Borrett confirmed that the concentration of chlorine was not monitored, that the growth conditions could be modified with fine control over temperature and humidity, and that multiple chlorination products and chlorinated lipids had been observed.

## 12. AGENDA ITEM TWELVE – Presentation on the OPCW Public Affairs Branch

12.1 Ms Elisabeth Waechter, Head of the Public Affairs Branch, presented an update on the work of the Branch and its relation to the work of the SAB. The Public Affairs Branch performs a wide variety of duties in terms of outreach and representing the Organisation before the public. Ms Waechter described her long-term advocacy and practice of a specific strategy of outreach involving a wide variety of audiences and channels. A hallmark of this approach had been the creation of public videos on the function of the Organisation in conjunction with its recently redesigned website. Two more of these videos are in production: one on the recent destruction of all declared stockpiles of chemical weapons, and another on the Organisation’s new ChemTech Centre. Ms Waechter also detailed the relevance of the OPCW’s social media presence in an increasingly fragmented social media landscape.

12.2 The approach of the Public Affairs Branch to public outreach includes a strong emphasis on scientific communication. A primary challenge in this area is conveying key scientific ideas in an effective and layperson-friendly manner without simplifying those same ideas to the point of inaccuracy, a task the Public Affairs Branch puts a great deal of emphasis on achieving. Another key element in the Branch’s approach to scientific communication is emphasising the work of the SAB, and how it can serve as a role model for other international organisations. The SAB lends a great deal of credibility to the OPCW in terms of combating disinformation and establishing the Organisation as a reliable source for credible scientific information in the public eye. When asked about effectively combating disinformation campaigns related to the mission of the OPCW, Ms Waechter commented on the vital importance of this task,

stating that it was crucial that accurate information be appropriately disseminated to create an inhospitable environment for disinformation. In the fight against disinformation, however, she emphasised the importance of moderation when speaking directly on behalf of the Organisation, owing to the requirements of the Organisation's mandate and the desires of States Parties.

## 13. AGENDA ITEM THIRTEEN – A mobile application and biomarkers-based detection of cyanide contamination in the environment using sunflower plants

13.1 The second Plant Biomarker Challenge project was presented by Dr Abubakar Gumi (Usmanu Danfodiyo University). His team chose to focus their work on cyanide, as it is a particularly prevalent pollutant in Nigeria as a result of its use in gold mining and poses serious environmental and health concerns. Given their ability to act as a hyper-accumulator of contaminants such as heavy metals and pesticides, sunflower plants (*Helianthus annus L.*) were selected as the plant model for the study. Additionally, sunflower plants have an inbuilt detoxification pathway which converts hydrogen cyanide and cysteine to β-cyanoalanine, which is then converted to asparagine.

13.2 A total of 60 sunflower plants were grown under controlled conditions in a greenhouse free from cyanide contamination, and were exposed to a variety of concentrations of cyanide, applied to the plant pot as an aqueous sodium cyanide solution. The plants were then observed and analysed over a 10-week period. The plants underwent non-destructive analysis using a leaf spectrophotometer to measure plant stress and a range of pigment indicators. Biomass was determined to examine growth response and elemental analysis was performed. The data was analysed using statistical packages and confirmed a differential response of the plants to the varying cyanide concentrations. The plants were also seen to develop a tolerance to cyanide exposure over time, and visible changes included changes in leaf colouration and root hair loss. The data collected will be used to develop a mobile application using machine learning algorithms, which would be able to confirm a plant's exposure to cyanide from a photo, allowing for in-field detection. This application was still under development.

13.3 In response to questions from the Board, Dr Gumi confirmed that the pH of the soil was neutral and was not varied during the study. While sunflower plants have not been exploited for remediating cyanide-contaminated soil, they have been used to detoxify soil polluted with lead and oil. The plants are usually destroyed in a high temperature incinerator in accordance with local environmental regulations. The applicability of this soil detoxification method to Lewisite-contaminated soil was considered.

## 14. AGENDA ITEM FOURTEEN – New medical countermeasures against percutaneous intoxication by low-volatility (persistent) organophosphorus nerve agents

14.1 In the case of low-volatility nerve agents such as VX, dermal exposure is the dominant route of intoxication. Following accumulation of a persistent agent in the upper layers of the skin, a reservoir of agent forms in the epidermis, releasing agent to the bloodstream over several hours. Using so-called "catch-up therapy",[5] a second chemical (a "scavenger") may be applied to and absorbed by the skin, neutralising the

[5] Chilcott R. P., et. al. Evaluation of a Barrier Cream against the Chemical Warfare Agent VX using the Domestic White Pig. Basic & Clinical Pharmacology & Toxicology (2005) Vol 97, 35-38.

dermal reservoir before the toxic chemical reaches the bloodstream, thus preventing systemic absorption. Dr Nissan Ashkenazi (Israel Institute for Biological Research) presented his team's work on developing a catch-up therapy lotion.

14.2 Development started with the identification of nucleophilic active pharmaceutical ingredients (APIs) capable of degrading nerve agents, which had already been approved by the United States Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA). Not only did these APIs need regulatory approval for medical (and preferably dermal) use, but they also needed to decompose nerve agents quickly and have a $pK_a$ below 12 so that the resultant lotion would not be too basic for the skin. A review of common pharmaceutical databases identified 11 APIs that were suitable, and reactivity testing using nitrophenyl phosphate revealed acetohydroxamic acid (AHA) as the most effective of this group. Further experimentation determined potassium and diethylammonium cations to be the most effective counterions, and polyethylene glycol methyl ether (mPEG) and dimethylsulfoxide (DMSO) were found to be the most effective drug vehicles (solvents) for API transportation to the nerve agent reservoir.

14.3 Skin permeation studies performed in vitro showed that the potassium salt of AHA penetrates the skin more effectively than Reactive Skin Decontamination Lotion (RSDL),[6] the current FDA-approved and in-service broad-spectrum decontamination lotion for chemical warfare agents. In addition, these studies showed that DMSO (with water, 1:4 v/v) was a better permeation-enhancing vehicle than mPEG (with water, 1:9 v/v). While the diethylammonium counterion increased the permeability of the scavenger more than the potassium cation, an interaction between the former cation and the nerve agent was observed, causing under-skin burns. The interaction was not investigated further but use of the diethylammonium salt was discontinued.

14.4 In a series of in vivo tests, swine were exposed to a lethal percutaneous dose of an organophosphorus nerve agent. To ensure a realistic scenario, treatment (either classical antidote[7] only or antidote plus lotion) was delayed and only administered when the swine began to display obvious signs of exposure. Information relating to survival rate, recovery, and cholinesterase activity was collected.

14.5 Exposure to VX showed that the combined treatment of antidotes plus lotion was more effective than antidotes alone.[8] Furthermore, both recovery and cholinesterase reactivation were significantly faster for the combined treatment with the lotion. The survival rate for both treatment regimens was 100%. Conversely, following exposure to a phosphorus-based newly scheduled chemical, treatment with the classical antidote alone yielded a 0% survival rate. The combined treatment, however, led to a 100% survival rate.

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6 Nahum V., Nili U., et al. Towards catch-up therapy: evaluation of nucleophilic active pharmaceutical ingredients for the treatment of percutaneous VX poisoning, in-vial and in-vitro studies. International Journal of Pharmaceutics (2021), Vol 603, 120689.

7 Immediate treatment comprises a combination of TMB-4, atropine, and benactyzine. Additional treatment with atropine, toxogonin, and midazolam may be required if toxic signs recur.

8 Bloch-Shilderman E., Nili U., et al. "Catch-up" therapy: combining antidotal treatment with dermal application of AHA following percutaneous VX poisoning in the domestic swine. Archives of Toxicology (2023) in press.

14.6 As the confirmation of a chemical's identification may not be readily and quickly available following an exposure, the lotion provides an effective generic treatment for intoxication by any persistent organophosphorus nerve agent. It is recommended that approval for use as an emergency treatment be acquired pending permanent regulatory approval.

14.7 As hydroxamic acids are particularly nucleophilic, a question was raised by the SAB regarding the possibility of other reactions occurring because of nucleophilic attack at the carbon atom rather than the phosphorus. Dr Ashkenazi confirmed that no such reactions had been observed. The SAB was also interested in the timelines for application of the lotion. Dr Ashkenazi stated that the lotion should be applied at the onset of clinical signs of intoxication, but depending on the skin on the part of the body affected, in addition to variation in the individual themselves, this could range from 30 minutes to 36 hours. In response to a question from the SAB, Dr Ashkenazi confirmed that he was familiar with human ex vivo skin models (such as Genoskin), but considering the need to assess physiological effects in addition to dermal effects, swine were necessary in this case.

## 15. AGENDA ITEM FIFTEEN – Technical countermeasures using artificial intelligence technology and quantum chemistry calculations for the threat of novel chemical agents

15.1 In 2019 four new entries were added to Schedule 1.A of the Annex on Chemicals. These "newly scheduled chemicals" comprise both phosphorus-containing and carbamate-based nerve agents. While the decision to add entries to the Annex on Chemicals by consensus demonstrates a commitment by States Parties to ensure that the Convention adapts as needed over time, the SAB notes the continued lack of available technical data concerning these compounds. As highlighted in its recent Scientific Report to the Fifth Review Conference (RC-5/DG.1), many types of technical data are still not known to the Secretariat, presenting challenges to its mission readiness. Dr Keunhong Jeong (Korean Military Academy) shared with the SAB some of his work related to predicting spectra and technical data associated with the newly scheduled chemicals.

15.2 Three of the four new entries to the Annex on Chemicals are phosphorus-based nerve agents, and these have been the focus of Dr Jeong's research efforts. Two of these entries are families of chemicals, where many different analogues are covered by the Convention. While this approach ensures that the Convention covers all the potentially synthesisable compounds in these families, it poses a challenge in trying to collect and curate all the associated spectral and chemical and physical property data. These data are crucial to ensuring proper detection and identification of these compounds, as well as in informing safe handling and storage procedures and the appropriate physical protective equipment and medical countermeasures that are effective. Synthesising all the discrete compounds covered in the families is not practical, so machine learning and quantum chemical calculations were used to predict some of the compounds' properties and spectral data.[9]

15.3 While these predictive approaches can be powerful, they all still rely on the utility and accuracy of the data fed into them. Therefore, while predictions can be made on toxicity and vapour pressure, these are limited and not totally accurate as not enough verifiable

[9] Jeong K., Lee J-Y., et al. Vapor Pressure and Toxicity Prediction for Novichok Agent Candidates Using Machine Learning Model: Preparation for Unascertained Nerve Agents after Chemical Weapons Convention Schedule 1 Update. Chemical Research in Toxicology (2022) Vol 35, 774-781.

experimental data on the newly scheduled chemicals is available to use to train the models. Still, the predictions can give an indication of properties in a relative sense, and these may still be useful.

15.4 In addition to properties, predicting spectra of Raman, infrared (IR), NMR, and MS would be incredibly useful in ensuring detection and analysis equipment is able to accurately detect and identify one of these compounds in a real-life scenario. Quantum chemical calculations have allowed them to populate and train deep learning algorithms to predict NMR and MS spectra,[10] and they are also currently working on similar approaches for Raman and IR spectra.

15.5 Lastly, Dr Jeong shared some recent work his team has conducted on predicting hydrolysis rates for some of these newly scheduled chemicals.[11] Hydrolysis is a common procedure used to break down nerve agents to ensure their destruction, and rate of hydrolysis is an important metric to consider when developing new decontaminants and medical countermeasures. They used density functional theory (DFT) and the electrophilicity index (EI) of various A-, V-, and G-series nerve agents to predict hydrolysis rates that correlate well with experimental rates in the cases of V- and G-series agents. The hydrolysis rates of these compounds are much slower than for traditional nerve agents. They are then able to predict hydrolysis rates of other analogous compounds based on the developed model.

15.6 The Board queried Dr Jeong about whether he thought his research could be misused, given that he is generating a lot of information about very toxic compounds and their potential utility as weapons. He indicated that he and his colleagues are careful not to provide all of the information on how they generate their property predictions in their publications. He also noted that the generated data itself is currently not very accurate—so this would not be of much benefit to someone looking to do harm.

15.7 Regarding the NMR shift predictions, Dr Jeong was asked whether this takes into account solvent effects. It does, and his team was very careful to incorporate previous work done in the field regarding the impact of solvent effects on DFT calculations of NMR shifts for different compounds.

15.8 There was a discussion regarding the use of the term toxicity and what in fact was being computed. While a predicted "degree of toxicity" for different nerve agents was shown, the Board queried whether it would be more accurate to further define what is meant by this and to call this a degree of lethality, given what was actually being predicted was the relative $LD_{50}$ number and not effective dose, which is different. Dr Jeong agreed that what was being measured was in fact lethal dose.

## 16. AGENDA ITEM SIXTEEN – SAB discussion

The SAB held an internal discussion on topics of relevance to the Board.

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10 Jeong K., Ryu T. I., et al. Precisely predicting the $^{1}H$ and $^{13}C$ NMR chemical shifts in new types of nerve agents and building spectra database. Scientific Reports (2022) Vol 12, 20288.

11 Rashid M. A. M., Lee B., et al. Theoretical prediction on the hydrolysis rate of the new types of nerve agents: A density functional study. Toxicology Reports (2023) Vol 10, 27-31.

## 17. AGENDA ITEM SEVENTEEN – Influence of chlorinating agents on the formation of stable biomarkers in hair for the retrospective verification of exposure

17.1 Dr Christophe Curty opened his presentation with a brief overview of the work and organisational structure of the Spiez Laboratory, the Swiss Federal Institute for nuclear, biological, and chemical (NBC) protection. With a broad range of facilities, the Spiez Laboratory is an OPCW designated laboratory for the analysis of environmental samples and a Schedule 1 facility for protective purposes. Recent work at the Spiez Laboratory has focused on identifying biomarkers for the verification of chlorine exposure.[12]

17.2 Chlorine is a classic dual-use chemical with a number of legitimate, peaceful uses, and a history of use as a chemical weapon, not only in World War I, but also in the recent past. The retrospective confirmation of the use of chlorine as a chemical weapon remains challenging because of its chemical properties—it dissipates quickly, degrades in contact with moist air, and only a few environmental markers are known.

17.3 Dr Curty and his co-workers have been developing a new methodology to confirm the use of chlorine as a chemical weapon, with the ultimate aim of identifying a biomarker that would be specific to chlorine exposure. Hair was selected as the sample matrix. Hair is readily accessible, almost entirely composed of keratin, and already an established biological matrix in testing for illicit substances and for drug doping in sports. It was crucial that the sample could be prepared quickly and analysed easily.

17.4 Hair was exposed to eight sources of chlorine[13] in the form of gases, vapours, and liquids. The exposed hair was then hydrolysed under strongly acidic conditions to amino acids, the resultant hydrolysate derivatised and then analysed by LC-MS. Mono- and di-chlorinated tyrosine compounds were identified and subsequently verified by the analysis of reference materials for exposure to chlorine, bleach, and sulfuryl chloride: these tyrosine compounds were not specific to chlorine. It was observed that the chlorinated water from swimming pools had no effect on swimmers' hair as analysis revealed the absence of chlorotyrosines.

17.5 In their quest to identify a chlorine-specific biomarker, three other amino acids were explored: cysteine, methionine, and tryptophan. Chlorine converts cysteine to cysteic acid, and methionine to methionine sulfoxide and sulfone. However, it was observed that exposure to all chlorine sources produced the same products, in addition to their being present in unexposed hair. They were therefore considered unsuitable markers. Similarly, tryptophan products were also unsuitable as they decomposed during the acidic treatment of sample preparation.

17.6 In collaboration with colleagues in the biological laboratory, an untargeted approach using principal component analysis was employed. Using the raw data, it was possible to differentiate between the different sources of chlorine.

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12 Martz S. V., Wittwer M., et al. Influence of Chlorinating Agents on the Formation of Stable Biomarkers in Hair for the Retrospective Verification of Exposure. Analytical Chemistry (2022) Vol 94, 16579-16586.

13 Chlorine gas, hydrogen chloride, phosgene, sulfuryl chloride, bleach, oxalyl chloride, thionyl chloride, and chloropicrin.

17.7 Dr Curty concluded that chlorotyrosines were found to be stable biomarkers for chlorine gas, sulfuryl chloride, and bleach, and remained stable in hair for 10 months post-exposure. The untargeted approach showed that chlorotyrosines are the main discerning features of chlorine-exposed samples and that further differentiation is possible. They plan to continue to search for some specific ions (presence or absence) that clearly explain the differentiation observed with the chlorine untargeted approach data.

17.8 Board members had a number of questions relating to other factors that may affect the composition of the hair, including hair dye and similar products, and natural hair colour and type. Regarding hair dye, Dr Curty had confirmed that in Switzerland there are no hair dyes that contain chlorinated products, only oxidising chemicals, but that he did not have a global view. Natural hair colour is not related to keratin but to a pigment, so the methodology should be applicable to all hair types and colours. Further work will look at chemically treated hair and will also subject hair to a range of concentrations of chlorinating agents to determine the limits of detection.

17.9 A similar, untargeted approach can also be used to reveal exposure to phosgene. Some biomarker candidates appear to be specific to this agent only, but they need to be identified and confirmed.

17.10 The Board finished with a short discussion about the various research approaches that are happening with the goal of finding a way to unambiguously identify when chlorine gas was used in a chemical weapons attack. These include looking for signs or markers in fingernails, hair, plants, and human plasma. While each individual approach appears to have some limitations relating to full specificity in comparison with other chlorine sources, using multiple approaches in combination may provide a path forward.

## 18. AGENDA ITEM EIGHTEEN – Sentinel plants for the surveillance of chemical risk: Natural plant-based sentinel systems to detect acetylcholinesterase-inhibiting pesticides in the environment

18.1 Organophosphate compounds are a common constituent in different types of pesticides, as well as nerve agents. They are considered safe for application in agriculture owing to their rapid rate of degradation. However, when chronic or accidental exposure occurs, they can produce toxicity in humans, plants, and animals. They inhibit acetylcholinesterase (AChE), which leads to respiratory, reproductive, nervous, hepatic, and renal disorders in people and animals. Their accumulation in food and in the environment is becoming more and more of a concern globally. In particular, chlorpyrifos and dimethoate pesticides are among those AChE inhibitor pesticides with acute deleterious effects on plants and other photosynthetic organisms. Prof Graciela González shared the results of the research conducted by the University of Buenos Aires under the auspices of the OPCW Plant Biomarker Challenge, where researchers looked to understand how different plant species may be used to detect the environmental presence of chlorpyrifos and dimethoate.

18.2 Five different plant species, two terrestrial and three aquatic, were used in the experiments: *Cichorium intybus* (chicory), *Glycine max* (soybean), *Salvinia molesta* (giant salvinia), *Vallisneria* (eelgrass), and *Spathiphyllium wallisii* (peace lily). Each were tested as potential sentinel plants for the presence of pesticides in the environment. Chlorpyrifos concentrations from 0.105 to 52.5 g/L were sprayed on *Cichorium intybus*

and from 0.105 to 21.0 g/L on G*lycine max*. Additionally, dimethoate solutions with concentrations ranging from 0.375 to 7.5 g/L were sprayed on *Cichorium intybus*. Regarding the aquatic plants, the pesticide treatment was carried out by adding the pesticide, either individually or as a mix of the two, to the aquatic media where the plant floated. Pesticide concentrations ranged from 0.5 to 1000 ppm for experiments with the aquatic plants. Spectroscopical and optical properties of plant leaves (fast transient of the variable chlorophyll-a fluorescence, also called the OJIP curve; Kautsky kinetics of variable chlorophyll-a fluorescence; spectral distribution of the initial chlorophyll-a fluorescence; reflectance and RGB images) were collected and analysed. The most sensitive parameters for the presence of pesticides were derived from the fast chlorophyll-a fluorescence transient, which was recorded in vivo. It was noted that other stress factors affecting the plant may lead to false positive results using chlorophyll-a fluorescence.

18.3 In addition, the research team wanted to better understand the AChE activity in the leaves of the pesticide-treated plants in comparison with control plants. To do this they focused on detecting thiocholine as a marker of AChE activity. An electrochemical system was designed using disposable, screen-printed graphite electrodes with silver nanoparticles incorporated. Thiocholine could then be detected by measuring its capture at the electrode surface and observing the changes produced either in the electron transfer process or in the capacitance by electrochemical impedance spectroscopy (EIS) of the surface.

18.4 The presentation ended with a notional protocol of use for the developed detection and analysis methods. This involves establishing an objective, choosing the appropriate plant species, use of a non-destructive biomonitoring technique for potential presence of chosen pesticide, and monitoring alert levels based on a predetermined threshold level. If needed, physical sampling can then be done to verify the presence of pesticide.

## 19. AGENDA ITEM NINETEEN – Plant biomarkers as evidence of chemical warfare agent exposure

19.1 The work conducted by the Netherlands Organisation for Applied Scientific Research (TNO) as part of the OPCW's Plant Biomarker Challenge was presented by Ms Mirjam de Bruin-Hoegée. She introduced TNO's interest in forensic research and outlined the challenges associated with evidence collection relating to chemical warfare agents. While there are proven indicators of exposure to chemical warfare agents in biomedical samples, there may be ethical, cultural, and safety aspects to consider. Using plants as alternative indicators of exposure could negate these issues, with the added advantages that plants are abundant and easy to collect.

19.2 In the first part of the study, three plants common to either tropical or temperate climates were selected: bay laurel, basil, and stinging nettle. The high protein content of stinging nettle plants was also relevant to its selection. The plants were exposed to vapours of sarin, chlorine, sulfur mustard, and one of the newly scheduled chemicals. Following exposure, the leaves were simply cut into small pieces, washed with acetone and dried, digested with pronase, and then analysed by LC-MS/MS and/or LC-MS/HRMS.[14]

[14] HRMS = high-resolution mass spectrometry.

19.3 Biomarkers for sarin, sulfur mustard, and "A-234" (a Schedule 1.A.14 chemical) were identified and subsequently verified with reference samples. In the cases of sarin and sulfur mustard, a colour change could be observed in the leaves post-exposure and the biomarkers identified were found to be stable for at least three months. Similarly, the mono- and dichlorotyrosine biomarkers identified for chlorine exposure were also found to be stable for at least three months.[15] This method could be a powerful tool to investigate the alleged use of chemical weapons. The stability of the biomarkers provides an excellent window of opportunity for an investigation, and as these biomarkers are the same as for biomedical samples, the same analytical methods can be used.

19.4 As biomarkers identified post-chlorine exposure were not chlorine-specific, further work was carried out to attempt to differentiate between chlorine and bleach. A slightly different set of three plants was used: stinging nettle, feathergrass, and green spire. The plants were exposed to different types of bleach and concentrations of chlorine gas, and some useful visual results were observed. All biomarker compounds found underwent principal component analysis and linear discriminant analysis. The amino acid adducts chlorophenylalanine, chlorotyrosine, and dichlorotyrosine, in addition to the DNA adduct chlorocytosine, were biomarkers found mainly in the samples exposed to chlorine gas. The biomarker found mainly in bleach samples was identified as the DNA adduct chloroadenosine. Biomarker identification was verified by analysis with reference standards. Chloro-, dichloro-, and trichlorodopamine were identified exclusively in the plants exposed to chlorine gas. Dopamine promotes the growth of plants in stressful environments but interestingly was found only in samples exposed to chlorine gas.

19.5 The dopamine result provided an interesting point of discussion for the Board. Ms De Bruin-Hoegée confirmed that there were no chlorodopamines present in the control samples and reiterated that they were present in all chlorine gas-exposed samples. The presence of dopamine was unexplained and had not been investigated. A question was raised regarding field sampling, storage, and transport of the samples. There is no requirement to store the sample at low temperature (that is, below 4° C)—a leaf can simply be sealed in a vial and left to dry slowly. It can easily be analysed at a later date.

## 20. AGENDA ITEM TWENTY – Therapeutics innovation of pseudo-natural peptides and neobiologics

20.1 Prof Hiroaki Suga (University of Tokyo) delivered a lecture on his team's development of pseudo-natural peptides and neobiologics for innovative therapeutics discovery and development. Compounds can have a wide range of molecular weights—from the small size of a few hundred daltons all the way up to 150,000 daltons or more seen in antibodies. Initial drug discovery and development focused on small molecular weight compounds that could be synthesised according to the Lipinski Rule of 5. However, given the complexity of human biological processes, many complex diseases are hard to treat or cure with small molecules. Pharmaceutical companies over time have focused more and more on higher molecular weight drugs.

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15 De Bruin-Hoegée M., Lamriti L., et al. Verification of exposure to chemical warfare agents through analysis of persistent biomarkers in plants. Analytical Methods (2023).

20.2 Prof Suga's team have focused on designing and developing macrocyclic peptides that are chiral.[16] They show efficacies to treat complex diseases while maintaining synthetic simplicity and delivery associated with small molecule drugs. This approach is quite useful to pharmaceutical companies, not just because of its novelty and potential to enable rapid discovery and design of new therapeutics, but also because of the simplicity and subsequent cost and time savings—a powerful driver.

20.3 There are platforms that can be utilised in the discovery of such pseudo-natural peptides such as genetic code reprogramming enabled by Flexizyme (FIT system) and RaPID platform technology.[17] These platforms mostly depend on Flexizyme, which is a manipulated form of a naturally available part of protein synthesis machinery of the cells known as tRNA. It is used as a catalyst for the main expression procedure of pseudo-natural peptides. The technology allows for differentiated and high-scale production of the pseudo-natural macrocyclic peptides in one go. Also, the production process requires low volumes and is very cheap. The platform has great advantages in synthesis and commercialisation of the ligands, including high screening ability, high versatility, high performance, low labour for protein preparations, high reliability, high affinity of produced ligands, and long shelf life of the products. All of the positive aspects of this technique can also be incorporated with non-standard amino acids to increase the stability and protease resistance of the peptides. These small peptides can also be incorporated into the structure of some antibodies and specific stable proteins to increase their blood availability and their permeability through the blood-brain barrier. Additionally, specific surface-binding proteins of viruses can be engineered and replaced with these peptides that could only interact with specific types of cells. Prof Suga showed that the peptides have a high stability and high affinity and specificity for the tasks for which they were designed (such as enzyme activation of signalling pathways).

20.4 The Board was curious about the possibility of the technology and peptides to detoxify or clean poisons from the human body. Prof Suga mentioned that this would likely be possible given the low side effects of the technology on humans. The peptides could compete to bind the toxic chemicals. He mentioned that low molecular weight peptides do not trigger an immune response. Furthermore, in the case of the antibody (Fc) incorporated peptide, the Fc masks the peptide's impact on the immune system and prohibits the immune system from recognition of the peptide.

20.5 The SAB inquired whether the technologies could be used to treat chronic or systematic inflammatory diseases. Prof Suga responded that, owing to the safe nature of the technology, it could be done but had not yet been carried out. The Board also asked about the differences between this technology and nanobodies. Prof Suga mentioned that the concept was the same but in case of nanobodies, the molecular weight is higher than peptides, meaning that they could elicit a human immune response unless properly engineered. He mentioned that there were several companies working on this issue and that a number of drugs of this type were in initial phases of clinical trials.

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16 Peacock H. and Suga H., Discovery of De Novo Macrocyclic Peptides by Messenger RNA Display. Trends in Pharmacological Sciences (2021).

17 Goto Y. and Suga H., The RaPID Platform for the Discovery of Pseudo-Natural Macrocyclic Peptides. Accounts of Chemical Research (2021) Vol 54, 3604-3617.

## 21. AGENDA ITEM TWENTY-ONE – Bioprinting and advanced biomaterial 3D printing in healthcare: State of the technology and state of the industry

21.1 Dr Adam Jakus provided an overview of the work of his company, Dimension Inx, which is concerned with tissue therapeutics. It designs, develops, and manufactures therapeutic products in order to restore the function of damaged tissue or organs, using advanced biomaterial design and bioprinting. To do this, they engineer and manufacture 3D microenvironments to direct cell and tissue behaviour. There is a wide variety of 3D printing types which use different materials, processes, and post-processing techniques, but all produce a tangible final object. Extrusion is the most common type of 3D printing applied to the process of bioprinting. The final product is created out of a slurry or paste, which is extruded and solidified.

21.2 Bioprinting is the application of 3D printing in conjunction with living or non-living biological components and/or for the primary purpose of eliciting a specific biological response. Bioprinting may have a compositional definition, where the "bioink" contains living cells. Alternatively, it may have an applicational definition where the "bioink" does not contain living cells but elicits a biological response (such as exhibiting scaffolding suitability for bone growth). Put simply, bioprinting can be expressed as 3D printing with a bioink. Dr Jakus discussed the hardware, software, and materials (that is, bioinks) utilised in this process. Bioinks are the most important component of bioprinting and have a variety of compositions. There are efforts to create 3D suspensions that contain a variety of differently composed bioink. Many companies are involved in developing these inks and the structures made from them, including Dimension Inx.

21.3 So, why use bioprinting? Crucially, biological systems are very complex, and control is key. Bioink can relate to complex compositions and chemistry. 3D printing can relate to complex forms and interdependencies, and bioprinting ultimately relates to both of these.

21.4 A wide variety of technological aspects are under consideration when dealing with bioprinting. The key considerations relating to bioinks are printing (such as extrudable form), structure (such as biocompatibility), and cell encapsulation (sterility). They need to have a form that can be printed, is self-supporting, and will not be rejected by the body. The platform Dimension Inx uses is distinct: it features rapid room-temperature extrusion and instant solidifications without additional necessary processes nor additional material wastage. The drying rate and the kinetics of the material also allow complex designs to be created.

21.5 It is important to remember that cells are smart—they recognise their surroundings, and the microstructure is just as important as the composition when it comes to materials that will be put into the body. Dimension Inx has created four such microstructures, and their platform enables a pipeline of therapeutic products to assist in rebuilding healthy tissues and restoring organ functions. One such microstructure the company produces for hard tissues is its Hyperelastic Bone®, which emulates bone composition and microstructure and turns into actual bony tissue over time once within the body. It was recently cleared by the United States FDA for usage in the mandible. A second example is its targeted Tissue Papers™, which are made from decellularised organs and tissues.

21.6 The Board discussed 3D printing and personalised medicine, and Dr Jakus indicated that future developments in the field could include patient-matched implants that also incorporate drugs specific to that patient.

## 22. AGENDA ITEM TWENTY-TWO – Hazardous chemical substitution in products and processes

22.1 Board member Prof Syeda Sultana Razia (Bangladesh University of Engineering and Technology) introduced the principle of hazardous chemical substitution. More than 95% of all manufactured goods rely on an industrial chemical process and, consequently, everyday items may contain known or unknown harmful chemicals. There are regulatory measures and legislation in place to reduce the use of hazardous chemicals, and hazardous chemical substitution can be an effective strategy to make products safer. Despite the existence of guidelines and tools to implement chemical substitution, there are gaps in existing frameworks, which tend to be limited by specific goals and applications and focus on individual substances rather than products more generally.

22.2 As part of a collaborative project, Prof Razia has developed a (not publicly available) chemical substitution database, ChemSub, which consists of three knowledge sections: (1) chemical product classes; (2) physical, chemical, and toxicological properties; and (3) hazard data. Products are stored under different classes and subclasses along with data including chemical identity, compositions, and chemical functions. Chemical property data are divided into pure component property, functional pure component property, functional mixture property, and phase equilibrium-related properties. Hazards are divided into physical, environmental, and health.

22.3 Prof Razia explained the six-step workflow for harmful chemical substitution and highlighted that each step involved its own specific computational methods and tools. She then provided two case studies: N,N-diethyl-meta-toluamide (DEET) in mosquito repellent and formaldehyde in the manufacture of textiles.

22.4 However, for sustainability, manufacturing safer products may not be enough. Every product or chemical is related to its manufacturing process and life cycle assessment of a product may indicate the need for substitution of hazardous chemicals used in processing. For substitution in processes, computer-aided tools for chemical substitution need to be integrated with process simulators to generate necessary information on process parameters.

22.5 As for future work, even though over 910,000 chemicals are listed in the ChemSub database, measured property data exist for less than 10% of these, and there is therefore a need to establish integrated, accessible databases based on reliable experimental data and accompanying property models to fill these gaps.

22.6 The SAB discussed the economic viability and effectiveness of the proposed substituents. In addition, the importance of understanding hazard properties of materials depending on their structure or formulation was also noted. Regarding nanosized or nanostructured chemicals whose properties differ from the related bulk materials, Prof Razia stated that these will likely also be considered in the future.

## 23. AGENDA ITEM TWENTY-THREE – Plant metabolomics for revealing chemical exposure biomarkers and portable vis/NIR spectrometry as a hotzone detector of plant poisoning

23.1 Dr Ljubodrag Vujisić (University of Belgrade) presented his team's research results from the Plant Biomarker Challenge. He started by providing an overview of the Center for Instrumental Analysis at the University of Belgrade. He detailed the various activities his Center had been a part of over the years, highlighting its chemical detection and chemical warfare agent-related work, especially in association with the OPCW and some of its designated laboratories. In addition, he has personally been involved in creating and teaching a chemical weapons course at the university to engage students on the topic and promote better safety and security in chemistry.

23.2 The overall objective of the research was to develop a cutting-edge tool for fast and accurate detection of chemical threats by lead sensing. Two species of plant, *Triticum aestivum* (wheat) and *Plantago lanceolata* (ribwort plantain), were selected. The researchers then set out to understand the chemistry of healthy leaves and observe changes upon chemical exposure, to determine markers of exposure by high-throughput metabolomics, and to test in-field, non-destructive detection devices and software models for their efficacy.

23.3 They decided to use malathion, an organophosphate insecticide, and arsenic trichloride, a precursor of Lewisite, as toxic chemicals with which to expose the plants. They used exposure concentrations of 10 ppm and 100 ppm (malathion) and 0.2 ppm and 50 ppm (arsenic trichloride) to mimic both exposure from low-level environmental contamination as well as a more acute event, such as an industrial accident. Plants were grown in a soilless system with vermiculite. Morphological studies provided information related to leaf area and biomass content.

23.4 A suite of analytical techniques was used to characterise the plant leaves pre- and post-exposure to the chemicals. These included proton NMR, LC/HRMS, ATR-IR,[18] GC/MS, and ICP-OES.[19] A method for reproducible sampling and extraction (using either phosphate buffer in $D_2O$:$CD_3OD$ or $CH_3OH$:$H_2O$) of the plant leaves was developed to have appropriate samples for analysis, depending on the instrument. With some machine learning models, principal component analysis and PLS-DA,[20] the researchers were able to identify numerous differences between control plants and those subjected to the two toxic chemicals.

23.5 Non-destructive techniques were also considered and tested. A SpectraVue 710S leaf spectrometer was purchased, which allows for in-field non-destructive analysis. The hand-held spectrometer measures transmission, absorption, and reflection, along with many other plant stress and pigment indicators using built-in indices. The team identified the wavelengths for each of the modes that provide the best opportunities for detection of stress in the plants owing to the chemical exposure.

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18 ATR-IR = Attenuated total reflection-infrared.

19 ICP-OES = Inductively coupled plasma-optical emission spectrometry.

20 PLS-DA = Partial least squares-discriminant analysis.

23.6 Lastly, Dr Vujisić highlighted some of the outreach work his team had conducted to disseminate the results of the research. This included setting up a project website,[21] presenting the work and its importance to the public, and being active on social media.

23.7 The Board asked whether not using soil in the growth of the plants affected the uptake of chemicals by the plants—specifically, whether the clay used instead of soil could act as a buffer against the intake of the arsenic chloride. Dr Vujisić did not think this was a factor, noting that the experiments used relatively small amounts of chemicals and allowed the plants to take up the chemicals in question directly. However, further studies would be necessary to confirm this.

## 24. AGENDA ITEM TWENTY-FOUR – Lessons learned from the illness of students in Iranian schools: Investigating claims of chemical poisoning from reality to allegation

24.1 Board member Prof Mostafa Ghanei (Baqiyatallah University of Medical Sciences) described his investigation into the reported mass illness of Iranian schoolchildren. He assessed the demographics of the patients, including gender and age, as well as their reported symptoms. He concluded that, based on the clinical and laboratory approach, examination of the reported symptoms, the social media spread of the phenomenon, and the incredibly narrow demographic range of affected patients, there were no chemical agents used, and that the event was in fact a mass psychogenic illness, where mass hysteria causes people to believe themselves ill, which leads to a manifestation of physical symptoms.

24.2 He listed a series of "lessons learned," including that: mass casualty management becomes important following accidents because of the problematic nature of states of anxiety; having a centre to guide the health personnel is mandatory; "control of social media" and the "mental management" of the community during a crisis is the first step; managing health infrastructure during a crisis should be decided precisely; and social assurance by experts and well-known specialists plays an important role. He added that, in general, the fear of toxic chemicals, even in the absence of a chemical, can be highly detrimental, that physicians should be trained in differentiating between mass hysteria and a real chemical event based on clinical evidence, and that documentation of best practices and knowledge transfer in different ways should be part of management.

## 25. AGENDA ITEM TWENTY-FIVE – Updates from the OPCW Laboratory

25.1 Mr Daan Noort (Head of the OPCW Laboratory) presented the Laboratory's purpose and function, ongoing and planned research, and discussed the move from the former facility to the new ChemTech Centre. He estimated that the operational capacity of the new facility was already at 90% and that it was anticipated that the microsynthesis facility would be operational by the end of the year. An overview of the OPCW's network of designated laboratories was provided to include an update on the proficiency testing programmes for 2022 and 2023. While, overall, there was a good geographical distribution of designated laboratories, he hoped that the first one in Africa would soon be established. He emphasised the high level of skills and expertise possessed by the designated laboratories, and the stringent requirements they must meet to achieve and maintain designated status.

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21 http://chem.bg.ac.rs/projekti/151/index-en.html.

25.2 On the topic of biotoxins, he presented details of the Seventh Exercise on the Analysis of Biotoxins. A proficiency test for biotoxins would soon be established for the scheduled biotoxins—saxitoxin and ricin—with a trial proficiency test for saxitoxin scheduled for September 2024. The OPCW intended to strengthen its relationship with the UNSGM in this regard, considering the expertise on biotoxins available through its roster of laboratories. Lastly, the Head of the Laboratory mentioned that the biotoxin work to be carried out at the ChemTech Centre would be funded by the United Kingdom of Great Britain and Northern Ireland through its participation in the Global Partnership Against the Spread of Weapons and Materials of Mass Destruction.

25.3 Another significant project at the ChemTech Centre would focus on chemical forensics, enabling further development of chemical forensic analytic methods. There was a need for scientifically sound, peer-reviewed analytical techniques in this area, enhancing the utility of chemical fingerprints for verification activities. A number of activities were planned to include developing a methodology to create simulated chemical warfare agent-contaminated environmental samples using the new microsynthesis facility, validating sample extraction and analytical protocols for HRMS, and conducting sample and synthesis product extraction and analysis after varying degrees of ageing and degradation.

25.4 Questions and further discussion from the Board related to the microsynthesis facility, the impurity profiles expected from microsynthesis compared to macrosynthesis, and training opportunities at the ChemTech Centre.

## 26. AGENDA ITEM TWENTY-SIX – OPCW deployment capabilities: A retrospective and prospective glimpse

26.1 The OPCW's deployable capabilities are a combination of training, equipment, and procedures. The presentation, delivered by Mr Davin Carter and Mr Ardalan Zargham (members of the Inspectorate Division), discussed the current pool of approved equipment, processes in place to acquire it, and efforts to respond to recent decisions on expanding the list of Schedule 1 chemicals, as well as keeping pace with the threats and potentials posed by advancements in science and technology.

26.2 The equipment that inspectors can use for inspections is strictly governed by decision C-I/DEC.71* (dated 30 November 2010) of the Conference of the States Parties, which sets out the technical specifications. The second and last updated version of this decision dates back to November 2010, the consequence of which is that the technical specifications have not been updated since that date.

26.3 A new test and validation programme had been commenced by the Technology and Training Hub at the ChemTech Centre to ensure that the Organisation can continually track, review, and benefit from scientific advances so that the Secretariat will be better equipped to identify new technologies that could potentially be integrated into the equipment pool. The test and validation programme begins with a needs assessment and market research to select candidates for laboratory, field, and live-agent testing. The results of testing could potentially inform the Secretariat's recommendations to States Parties to update the list of approved equipment. The project would benefit from inputs and recommendations from the SAB.

26.4 The Board recognised that a flexible approach and technical capability to ensure Secretariat personnel have the most up-to-date equipment was an absolute must for the Secretariat—inspectors and other personnel cannot properly carry out their technical mission work if they are not absolutely safe and using the needed, evaluated technology and equipment. The Board discussed some considerations in trying to impress the importance of this upon States Parties.

26.5 There needs to be a paradigm shift in thinking about how equipment and technology needs to be assessed and procured for the ongoing work of the Secretariat. The fast pace of technology advancements nowadays requires re-evaluation more often (and more flexibly) than every 10 years.

## 27. AGENDA ITEM TWENTY-SEVEN – Recap of the Fifth Review Conference

27.1 Mr Szymon Bochenski (Senior Policy Officer in the Secretariat) made a presentation on the outcomes of the review process of the Convention, which had concluded in May 2023. He recognised the important contributions of the SAB to the preparatory process leading up to the Fifth Review Conference and at the Conference itself. Specifically, the importance of the SAB's Scientific Report to the Fifth Review Conference (RC-5/DG.1) and the SAB Chairperson's presentations to the Review Conference and the Open-Ended Working Group for the Preparations of the Fifth Review Conference were highlighted. Mr Bochenski commented on the outcomes of the Fifth Review Conference, and emphasised that, although consensus on the final report had not been reached, the review process allowed for a thorough and comprehensive review of the Convention, reaffirmed collective understandings related to its implementation, and generated many ideas on future activities of the Organisation. A common ground had been forged on several issues, which were identified by States Parties during the 103rd Session of the Executive Council in July 2023. Follow-up discussions had already started on some of the issues. This would not have been possible without an enabling role of the review process.

27.2 The Board inquired about the algorithmic selection used for facility/country inspections and whether this could be modified to be more appropriate for the current verification needs. Mr Bochenski responded that the algorithm can be changed, and had been in the past, and this would be explored in ongoing meetings of the Industry Cluster.

## 28. AGENDA ITEM TWENTY-EIGHT – Updates on the OPCW Fact-Finding Mission in Syria

28.1 Mr Sami Barrek, Head of the FFM, provided the SAB with updates on the FFM's activities since the last presentation to the Board. Given the fact that new members had recently joined the SAB, Mr Barrek started his presentation with an overview on the establishment, mandate, and work of the FFM since its inception. Describing the field activities and the challenges that the FFM had faced during its investigations, Mr Barrek informed the members of the SAB that, to date, the FFM had issued 20 reports covering 73 allegations; 20 events of likely or confirmed use of chemical weapons had been reported (14 for chlorine, 3 for sulfur mustard, and 3 for sarin).

28.2 The Head of the FFM presented an overview of the fields of science and technology that had been and were currently used during FFM investigations, and which contributed to confidently reaching all its conclusions. These included identification, profiling, toxicology, epidemiology, damage evaluation and modelling, and plume dispersion studies in relation to the agents.

28.3 During his presentation, Mr Barrek highlighted the significance of developments in science and technology in supporting any investigation activities. In this regard, he stressed the need to continue research activities and to further support the work that had been already performed in relation to the use of chlorine as a weapon. He also emphasised that it was important that the scientific community prospectively engage in similar studies with other toxic chemicals which, like chlorine, could be used as a chemical warfare agent.

28.4 Finally, Mr Barrek stressed the significance of the continuous training of the FFM team and inspectors on conducting investigation activities with the perspective of implementing novel findings in research and technology pertaining to the work of the FFM, such as in detection, documentation, decontamination, identification, and modelling.

28.5 The SAB appreciated the efforts of the FFM team, noting its indispensable nature to ensuring the proper implementation of the Convention. The Board and Mr Barrek had a discussion on various topics regarding the FFM's work, including mission preparation and flexibility, on-site sampling, the potential to incorporate new approaches and technologies into their work, and the importance of the designated laboratories in that work. Mr Barrek thanked the Board for its consideration of the FFM's work and looked forward to further suggestions from the Board on how the FFM's work could be further optimised.

## 29. AGENDA ITEM TWENTY-NINE – SAB Chairperson and Vice-Chairperson election

The Board, with the assistance of the SAB Secretary, held a private meeting to elect its Chairperson and Vice-Chairperson for 2023. No interpretation was provided, and only Board members physically present were in attendance. Mr Günter Povoden was re-elected as Chairperson of the SAB and Dr Imee Su Martinez was elected as Vice-Chairperson, by consensus, to serve in the next year. The Board thanked Dr Andrea Leisewitz, who voluntarily stepped down from her position, for her efforts as Vice-Chairperson from 2021 through 2023.

## 30. AGENDA ITEM THIRTY – Any other business and final remarks

30.1 The SAB Chairperson thanked the staff of the Secretariat and the interpretation team for their support at this successful session and noted that he was looking forward to the next in-person meeting. He then thanked all of the members for their participation and contributions to the Thirty-Seventh Session of the SAB.

30.2 The SAB is grateful to all States Parties, organisations, and institutions that have financially assisted the work of the Board. In particular, it thanks the European Union, whose funding made possible the work of the TWG on the Analysis of Biotoxins.

## 31. AGENDA ITEM THIRTY-ONE – Adoption of the report

The Board adopted the report of the session by consensus.

## 32. AGENDA ITEM THIRTY-ONE – Closure of the session

The Chairperson closed the Thirty-Seventh Session of the SAB at 17:17 on 1 September 2023.

Annexes:

Annex 1:

List of Participants in the Thirty-Seventh Session of the Scientific Advisory Board

Annex 2 (English only):

Analysis of Biotoxins – Report of the Scientific Advisory Board's Temporary Working Group

**Annex 1**

**LIST OF PARTICIPANTS IN THE THIRTY-SEVENTH SESSION OF THE SCIENTIFIC ADVISORY BOARD**

| | Participant | Institution |
|---|---|---|
| **Members of the Scientific Advisory Board** | | |
| 1. | Dr Crister Åstot | Swedish Defence Research Agency (FOI), Sweden |
| 2. | Dr Khaldoun Bachari | Algerian Public Scientific and Technical Research Centre in the Physico-Chemical-CRAPC, Algeria |
| 3. | Dr Karim Ben Ali | Tunisian Military Research Center, Tunisia |
| 4. | Dr Elma Biscotti | Scientific and Technical Research Institute for Defense, Argentina |
| 5. | Dr Anne Bossée | DGA CBRN Défense, France |
| 6. | Prof Vladimir Dimitrov | Institute of Organic Chemistry with the Centre of Phytochemistry, Bulgarian Academy of Sciences, Bulgaria |
| 7. | Mr Raza Ellahi | Defence Science & Technology Organization (DESTO), Pakistan |
| 8. | Prof Mostafa Ghanei, MD | Baqiyatallah University of Medical Sciences, Islamic Republic of Iran |
| 9. | Dr Norman Govan | Defence Science and Technology Laboratory, United Kingdom of Great Britain and Northern Ireland |
| 10. | Dr Matteo Guidotti | Institute of Chemical Sciences and Technologies (SCITEC) of the Italian National Research Council, Italy |
| 11. | Mr Wilford Jwalshik | Institute of Chartered Chemists, Nigeria |
| 12. | Dr Andrea Leisewitz (Vice-Chairperson) | Universidad San Sebastián, Chile |
| 13. | Dr Imee Su Martinez | University of the Philippines-Diliman, Philippines |
| 14. | Dr Catharina Müller-Buschbaum | Accenture, Germany |
| 15. | Prof Elisa Souza Orth | Federal University of Paraná, Brazil |
| 16. | Dr Meehir Palit | Defence Research and Development Organisation, India |
| 17. | Mr Günter Povoden (Chairperson) | CBRN Defence Centre, Ministry of Defence, Austria |
| 18. | Prof Ines Primožič | University of Zagreb, Croatia |
| 19. | Prof Syeda Sultana Razia | Bangladesh University of Engineering and Technology (BUET), Bangladesh |
| 20. | Prof Ahmed E. M. Saeed | Sudan University of Science and Technology, Sudan |
| 21. | Prof Fengxia Sun | Hebei University of Science and Technology, China |

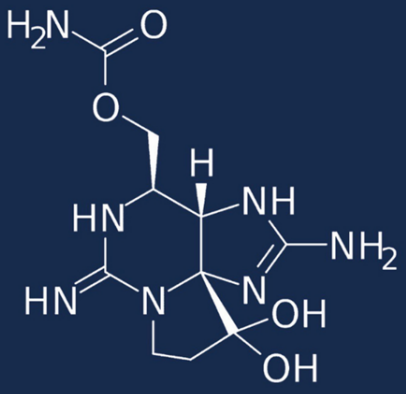
| | | |
|---|---|---|
| **Invited Participants** | | |
| 22. | Dr Nissan Ashkenazi | Israel Institute for Biological Research, Israel |
| 23. | Dr Sami Barrek | Organisation for the Prohibition of Chemical Weapons, Netherlands |
| 24. | Mr Szymon Bochenski | Organisation for the Prohibition of Chemical Weapons, Netherlands |
| 25. | Dr Veronica Borrett | La Trobe Institute of Sustainable Agriculture and Food, Australia |
| 26. | Dr Davin Carter | Organisation for the Prohibition of Chemical Weapons, Netherlands |
| 27. | Dr Christophe Curty | Spiez Laboratory, Switzerland |
| 28. | Ms Mirjam de Bruin-Hoegée | TNO, Netherlands |
| 29. | Prof Graciela González | University of Buenos Aires, Argentina |
| 30. | Dr Abubakar Gumi | Usmanu Danfodiyo University, Nigeria |
| 31. | Dr Richard Guthrie | Member of the OPCW Advisory Board on Education and Outreach |
| 32. | Dr Adam Jakus | Dimension Inx, United States of America |
| 33. | Dr Keunhong Jeong | Korean Military Academy, Republic of Korea |
| 34. | Dr Daan Noort | Organisation for the Prohibition of Chemical Weapons, Netherlands |
| 35. | Dr Michael Shevlin | MSD, United States of America |
| 36. | Prof Hiroaki Suga | University of Tokyo, Japan |
| 37. | Dr Ljubodrag Vujisić | University of Belgrade, Serbia |
| 38. | Ms Elisabeth Waechter | Organisation for the Prohibition of Chemical Weapons, Netherlands |
| 39. | Dr Ardalan Zargham | Organisation for the Prohibition of Chemical Weapons, Netherlands |
| **Secretary to the Scientific Advisory Board** | | |
| 40. | Dr Peter Hotchkiss | Organisation for the Prohibition of Chemical Weapons, Netherlands |

**Annex 2**

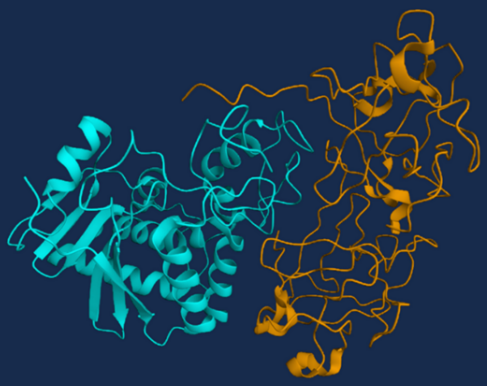
**ANALYSIS OF BIOTOXINS
REPORT OF THE SCIENTIFIC ADVISORY BOARD'S
TEMPORARY WORKING GROUP**

# 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.[22] 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 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

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

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,[23] 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.

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.

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23 More information on RefBio is available at: https://bit.ly/RKIRefBio.

### 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[24]), as well as forensic requirements relating to the use of technical evidence in legal proceedings.

### 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.

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[24] 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.

## 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.

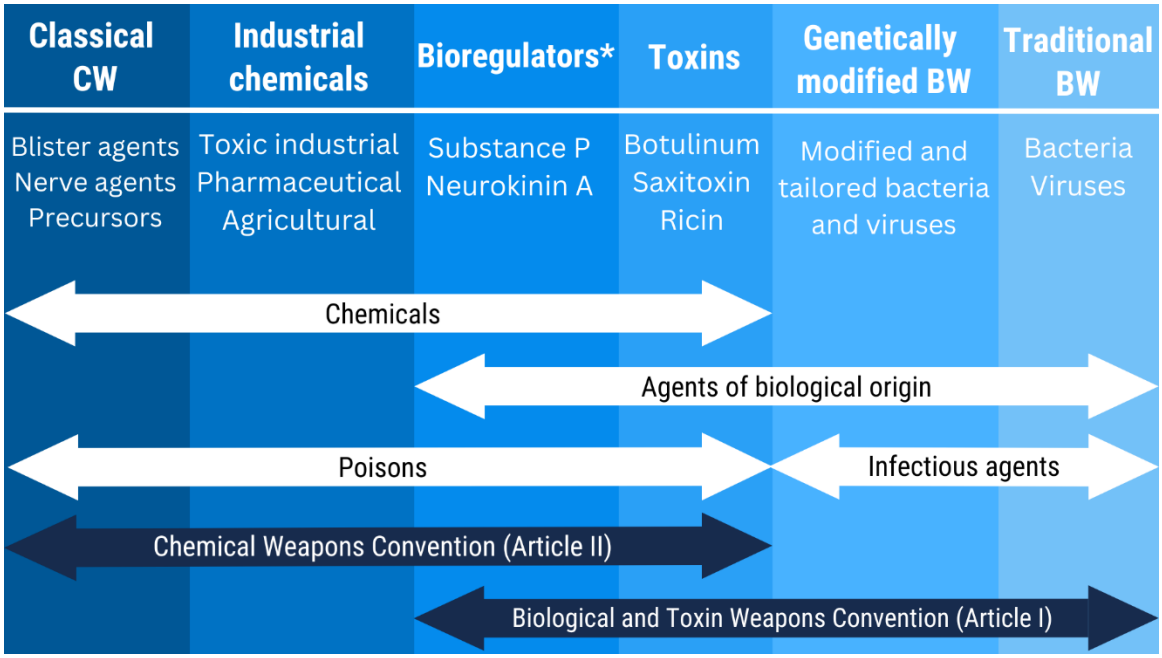
39. Consequently, the TWG recommended (Recommendation 30 in SAB/REP/1/19, dated December 2019) that the Director-General "consider establishing a TWG to advise on how to ensure that the Secretariat has access to required capabilities for the analysis of relevant biological toxins." The TWG suggested 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[25]




25 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.

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 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? |

| Subgroup | TOR subparagraph | 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? |
| **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.[26] 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);

26 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.

(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 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<br>Pathology (animal/human) | LFA<br>COTS automated platforms based upon ELISA<br>DNA detection | LFA<br>COTS automated platforms<br>Luminex<br>PCR | Sample prep (e.g., immunoprecipitation)<br>LC/HRMS, LC-MS/MS<br>ELISA<br>*In vitro* cell assays<br>Mouse bioassay<br>Cell-free (substrate cleavage/enzymatic, colorimetric MS)<br>Indirect biomarkers of exposure | DNA sequencing<br>LC/HRMS, LC-MS/MS<br>Elemental analysis<br>Nuclear magnetic resonance<br>Characteristic sample components<br>Peptidic ratio comparison for isoforms (e.g., ricin isoforms) |
| **Other requirements** | Trained clinicians and veterinarians to identify biotoxin exposures<br>Appropriate environmental sampling and storage of samples (for downstream analysis) | Trained first responder with appropriate PPE<br>Agreed collection/storage conditions<br>Maintain chain of custody | Mixed hazard screening capability<br>Maintain chain of custody | Different sample preparations depending on matrix (e.g., blood, urine, tissue, food, environmental)<br>Two independent laboratories<br>Two orthogonal validated techniques<br>Activity/toxicity demonstrated<br>Maintain chain of custody | Biotoxin repository<br>Certified reference materials<br>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)<br>Accreditation of COTS products (at discretion of manufacturer) | Potential for some screening techniques to be validated and/or accredited | Two orthogonal techniques<br>Both validated for unambiguous identification<br>Analytical laboratory has accredited quality management system<br>Demonstrated proficiency in external quality assurance (EQA) exercises<br>Methods employed have been published in a peer-reviewed journal or used in international proficiency tests | Analytical laboratory has quality management system<br>Demonstrated proficiency in EQA exercises<br>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<br>(Yes; indicative positive) | Medium<br>(Yes; presumptive positive) | High<br>(Yes; unambiguous positive) | Case by case<br>(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.

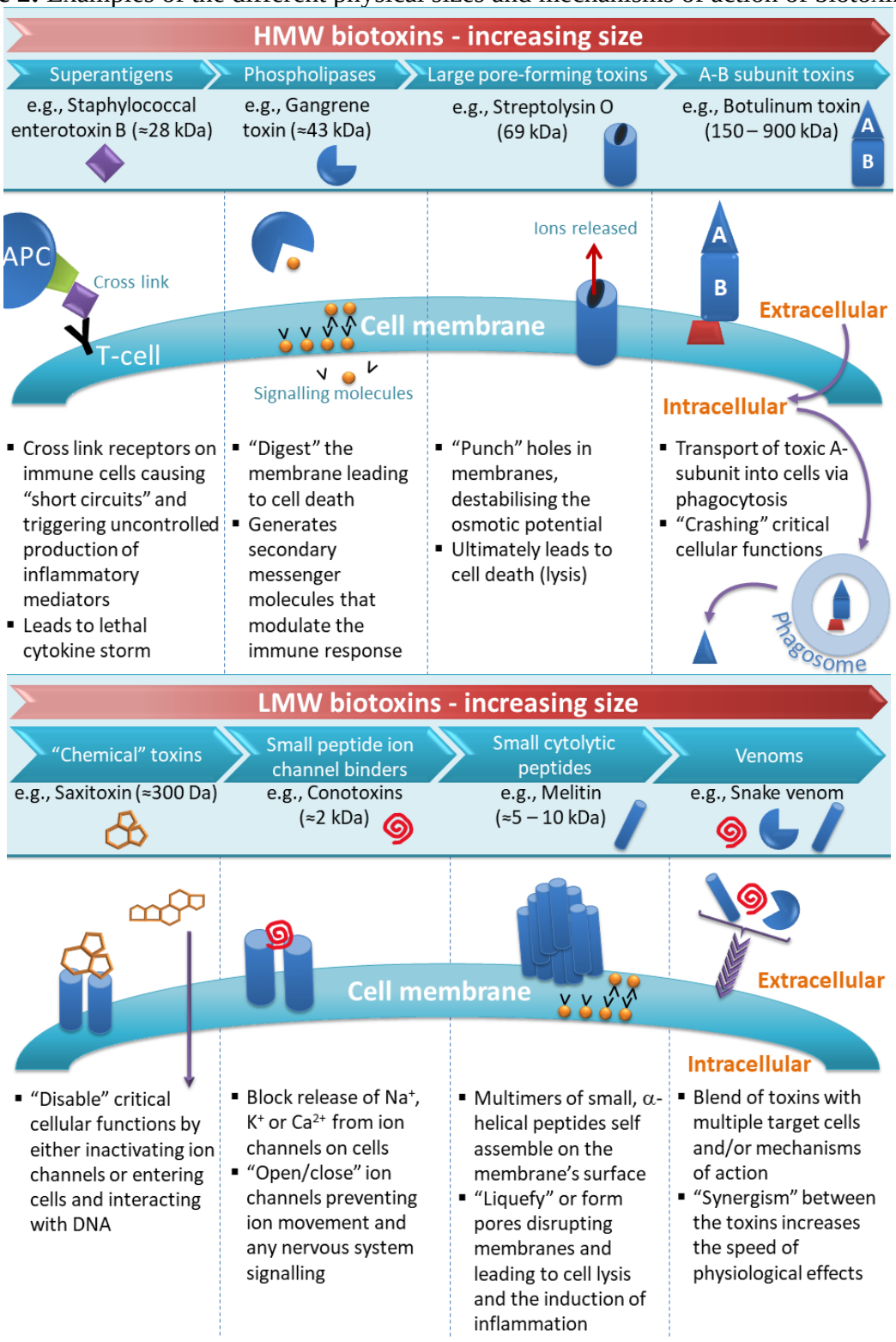
Stage 3: Analysis and unambiguous identification of the presence of a biotoxin, if possible taking into account clinical information

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) while 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[27]




27 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,[28] 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).[29]

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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28 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.

29 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;[30]

(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 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*

30 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.

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.[31] 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).

[31] 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

| Biotoxin | Health effect | Toxic mechanism |
|---|---|---|
| Abrin | RIP II poisoning | Ribosomal inactivation |
| Aflatoxin B1 | Immunotoxicity, carcinogenicity | Binds to DNA and proteins |
| BoNT/A | Botulism | Neurotoxic: inhibits acetylcholine release |
| Epsilon toxin | Enterotoxaemia | Erythrocyte lysis and cell necrosis |
| Ricin | RIP II poisoning | Ribosomal inactivation |
| Saxitoxin | Paralytic shellfish poisoning | Neurotoxic: sodium channel blocker |
| SEB | Food poisoning, toxic shock syndrome | Superantigenic: inflammatory response |
| Tetrodotoxin | Pufferfish poisoning | Neurotoxic: sodium channel blocker |
| T-2 toxin | Alimentary toxic aleukia | Inhibits synthesis of DNA, RNA, proteins |

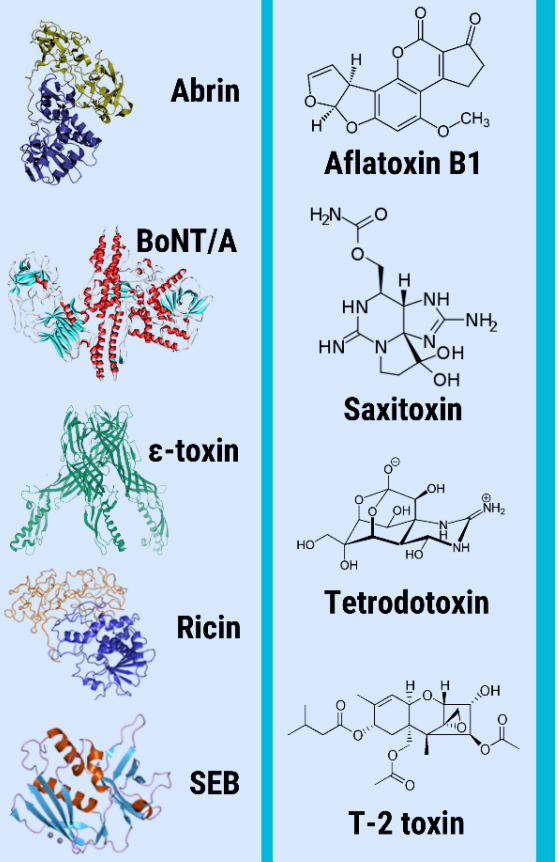
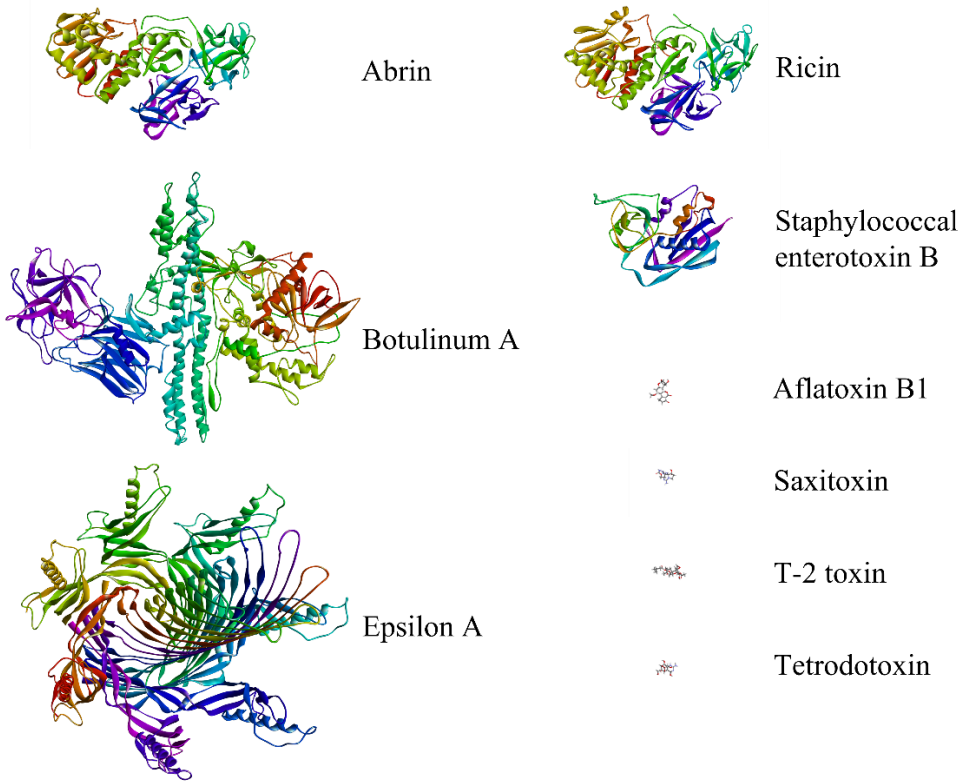


**Figure 4:** Chemical structures, to relative scale, of the most relevant biotoxins for the OPCW. For biotoxins where there are multiple analogues, one was taken as a token representation. Protein structures were taken from RCSB Protein Data Bank[32] (abrin A[33], 5Z3J; botulinum neurotoxin A[34], 3BTA; epsilon A[35], 6RB9; ricin[36], 7KBI; staphylococcal enterotoxin B[37], 3SEB). Structures for aflatoxin B1, saxitoxin, tetrodotoxin and T-2 toxin may be viewed in full in Figure 3.




32 www.rcsb.org.

33 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.

34 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.

35 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.

36 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.

37 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.*

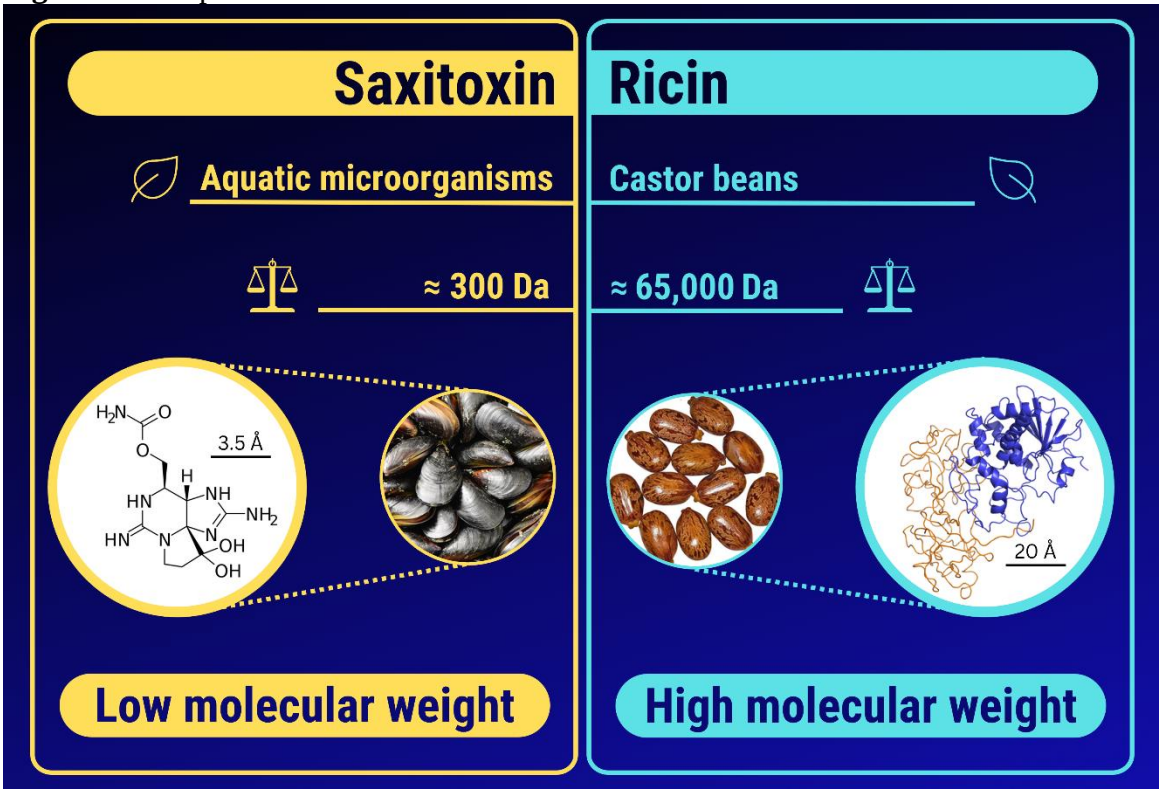
### Subgroup 3: Technical requirements for analysis

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



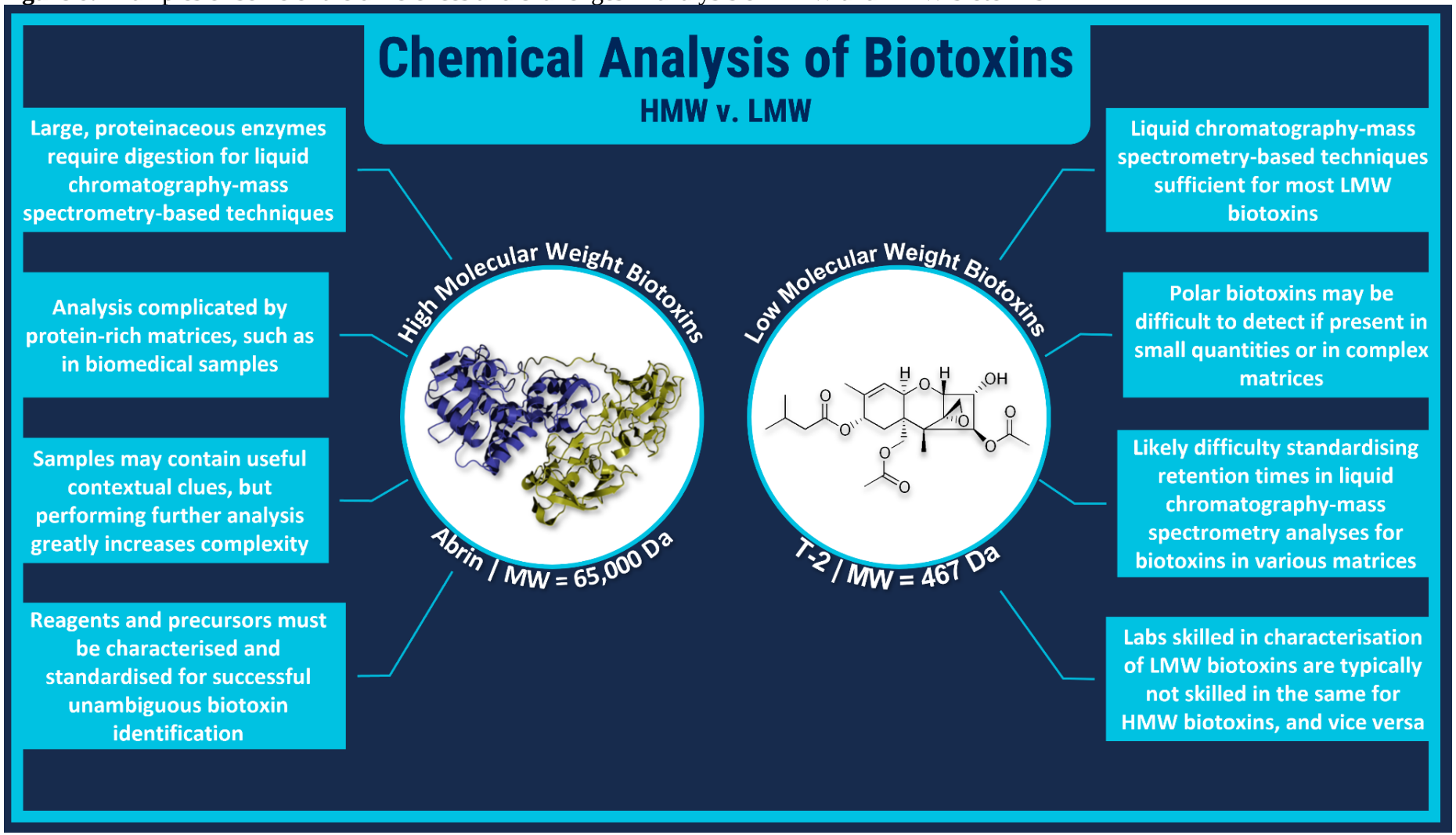

85. A wide range of biochemical processes in the human body may be inhibited or, on the contrary, over-stimulated by the activity of a biotoxin. Consequently, parameters such as the rapidity of disease onset, clinically relevant concentrations, and symptoms of exposure will differ between classes of 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'.[38] 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

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

difficulties in standardising methods. For example, the 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[39] 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 specificity, sensitivity, precision, robustness, and reliability of experimental data are defined for accredited methods and build the basis for the credibility of laboratory

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39 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.

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).[40] 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[41] and WOAH[42].

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

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

41 World Health Organization (WHO).

42 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.[43] 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[44] 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;

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

44 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:[45]

(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:[46] four exercises on ricin, saxitoxin, botulinum neurotoxins and *Staphylococcus aureus* enterotoxins; and

(ii) EuroBioTox, 2017 – 2023:[47] 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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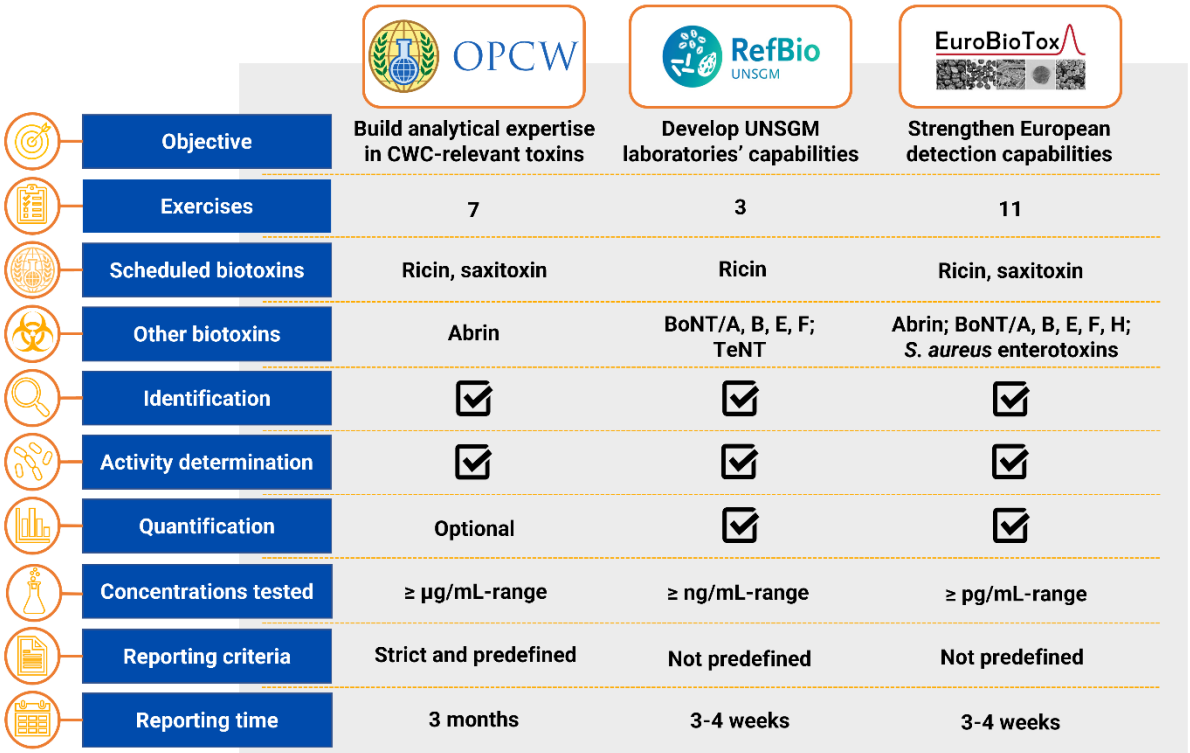
45 RefBio project: "Germany's contribution to strengthen the reference laboratories Bio in the UNSGM"; funded by the German Federal Foreign Office 2017 – 2024; more information available at: https://bit.ly/RKIRefBio and in Appelt, Sandra, Anna-Maria Rohleder, Cédric Invernizzi, Robert Mikulak, Annika 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.

46 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.

47 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 |
| dcSTX | Decarbomoylsaxitoxin |

| Abbreviation or term | Definition |
|---|---|
| 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 |
| LT | Leukotriene |
| MC | Microcystin |
| MS | Mass spectrometry |

| Abbreviation or term | Definition |
|---|---|
| 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 |
| TeNT | Tetanus neurotoxin |
| TNF-α | Tumour necrosis factor α |
| TOR | Terms of reference |
| TTX | Tetrodotoxin |
| TWG | Temporary Working Group |

| Abbreviation or term | Definition |
|---|---|
| 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 |

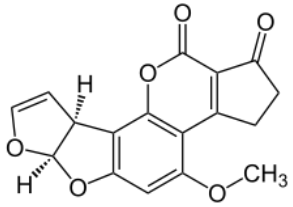
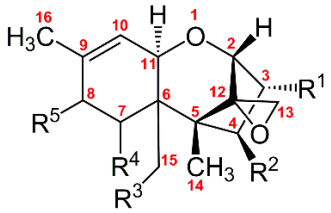
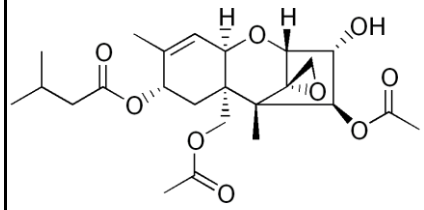
## ANNEX 1

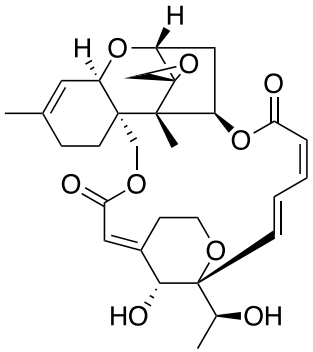
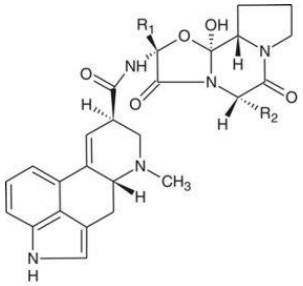
## GROUPS OF BIOTOXINS, THEIR CHEMICAL PROPERTIES AND GENERAL MECHANISMS OF TOXICITIES

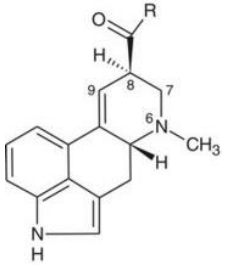
| Group | Disease | Chemical structure | Toxic mechanism | Family leader |
|---|---|---|---|---|
| **Microalgae toxins (phycotoxins) - *Cyanobacteria*** | | | | |
| **Saxitoxins** (STXs) (STX, neoSTX, dcSTX, dcneoSTX) | Paralytic shellfish poisoning | Tricyclic guanidine alkaloids | Neurotoxic, block 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 |

| Group | Disease | Chemical structure | Toxic mechanism | Family leader |
|---|---|---|---|---|
| **Okadaic acid** (OA) and its derivatives dinophysistoxins (DpTX-1, DpTX-2, etc) | Diarrhoetic shellfish poisoning | Polyethers with spiro keto ring | Neurotoxic, phosphatase inhibitor | OA |
| **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 |

| Group | Disease | Chemical structure | Toxic mechanism | Family leader |
|---|---|---|---|---|
| **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 |
| **Trichothecenes** (TCTs) class B<br><br>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<br>NIV |
| **Trichothecenes** (TCTs) class A<br><br>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 |

| Group | Disease | Chemical structure | Toxic mechanism | Family leader |
|---|---|---|---|---|
| **Stachybotryotoxins** from *Stachybotrys chartarum*<br><br>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 |  <br>Satratoxin H structure | Apoptosis of olfactory sensory neurons | Satratoxin H |
| **Ergot alkaloids** from *Claviceps purpurea* fungus<br><br>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<br>  | Agonists and antagonists at adrenergic, dopaminergic, and tryptaminergic (also called serotonin or serotonergic, e.g., 5-hydroxytryptamine (5-HT)) receptors | Ergotamine |

| Group | Disease | Chemical structure | Toxic mechanism | Family leader |
|---|---|---|---|---|
| **Ergot alkaloids** from *Claviceps purpurea* fungus<br><br>Lysergic acid derivatives | Depersonalisation or hallucinations and may produce toxic psychosis; mydriasis, increased blood pressure, tachycardia, elevated body temperature, tremors, and hyperreflexia | Amine alkaloids<br>  | 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 | | | |
| **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 | – |

| Group | Disease | Chemical structure | Toxic mechanism | Family leader |
|---|---|---|---|---|
| ***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 |
| **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 | Stx |

| Group | Disease | Chemical structure | Toxic mechanism | Family leader |
|---|---|---|---|---|
| | | | synthesis inhibition, disruption of cell membranes | |
| **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,<br>MW = 65 kDa (abrin)<br>MW = 57 kDa (modeccin)<br>MW = 62 kDa (volkensin)<br>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)<br>Hyoscyamine (atropine)<br>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)<br><br>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 |

| Group | Disease | Chemical structure | Toxic mechanism | Family leader |
|---|---|---|---|---|
| ω- Conotoxins | | | Voltage-gated calcium channels (blocker) | GVIA |
| **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) $\beta_1$-BnTX to $\beta_5$-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 |
| 19 | Ms Chen Hsiao Ying | DSO National Laboratories, Singapore |

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