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REPORT OF THE SCIENTIFIC ADVISORY BOARD'S WORKSHOP ON CHEMICAL WARFARE AGENT TOXICITY, EMERGENCY RESPONSE AND MEDICAL COUNTERMEASURES

1. EXECUTIVE SUMMARY

1.1 The Organisation for the Prohibition of Chemical Weapons (OPCW) Scientific Advisory Board (SAB) in cooperation with the Secrétariat Général de la Défense et de la Sécurité Nationale (SGDSN) held a workshop on "Chemical Warfare Agents: Toxicity, Emergency Response and Medical Countermeasures" from 26 to 27 September 2016 in Paris, France.[1] The workshop was the second in a series intended to inform the report of the SAB on developments in science and technology to the Fourth Review Conference[2] of the Chemical Weapons Convention, which is to be held in 2018.

1.2 Effective emergency response and medical treatment form a frontline defence against the use of chemical agents. The more effective detection and alarm systems, protective equipment, decontamination equipment, medical antidotes and treatments become; the less effective are chemical weapons. Staying abreast of developments in science and technology related to the toxicology of chemical warfare agents (CWAs), clinical detection of exposure and medical response (both short- and long-term) is of vital importance. This importance is underscored by current events in the Syrian Arab Republic[3] and growing concerns over the potential for the use of chemicals by terrorists. In this regard, understanding the molecular biological mechanisms and the chemistry[4] through which chemical agents exert their toxic effects is critical for the development of more effective medical countermeasures and for the long-term treatment of survivors of exposure.

1.3 This workshop brought together experts from relevant scientific fields and stakeholders in chemical security to discuss and review current knowledge and

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1 Funding for the workshop was provided through the generous support of the SGDSN and also project III (Science and Technology: Assessment of Developments in Science and Technology) of EU Council Decision (CFSP) 2015/259 dated 17 February 2015. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2015.043.01.0014.01.ENG

2 Fourth Special Session of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention.

3 Third report of the Organizsation for the Prohibition of Chemical Weapons-United Nations Joint Investigative Mechanism; (United Nations, S/2016/738, dated 24 August 2016). Available at: http://www.un.org/ga/search/view_doc.asp?symbol=s/2016/738

4 D. Ajami, J. Rebek, Jr.; Chemical approaches for detection and destruction of nerve agents; *Org. Biomol. Chem.*, 2013, *11*, 3936-3942.

practices in toxicology and emergency response to CWA exposure. This workshop follows advice from the SAB in its report on the Convergence of Chemistry and Biology, recommending the monitoring of scientific developments with capabilities for assistance and protection (especially decontaminants),[5] and complements SAB reports reviewing currently available medical countermeasures and treatments for CWA exposure[6,7] as well as a recently published guide for first responders to a chemical weapons attack.[8] The information contained within the report has direct relevance to the OPCW's assistance and protection programmes[9] and the recently established rapid reaction and assistance mission.[10]

1.4 The workshop was chaired by Dr Christopher Timperley (OPCW SAB Chairperson). Dr Augustin Baulig (SGDSN), Dr Jonathan Forman (OPCW Science Policy Adviser and Secretary to the SAB) and Dr Timperley opened the workshop, welcoming the participants and providing an overview of the programme and its intended outcomes.

1.5 From the workshop discussions, the following outcomes are submitted for consideration by the SAB at its Twenty-Fourth Session in October 2016:

(a) There has never been a greater need to find fast and efficient means to diagnose and treat people who have been exposed to toxic chemicals. Research into more effective methods continues and many gaps still exist. Developments across the fields should be regularly monitored and efforts made to bring experts working in both civilian and governmental organisations together to share best practices.

(b) New approaches to address old and continuing problems must also be considered. For example, sulfur mustard was first used as a weapon of war almost 100 years ago, yet to this day the precise mechanism by which it produces blisters is not understood. Only when it is, will it be possible to rationally design drugs that could be used to reduce or prevent blistering.

(c) Treatment of exposure to toxic chemicals requires medical countermeasures and decontamination procedures (for victims of exposure, infrastructure and

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5 Convergence of Chemistry and Biology: Report of the Scientific Advisory Board's Temporary Working Group (SAB/REP/1/14, dated 27 June 2014). Available at: http://ow.ly/Oz8J304SOey

6 Response to the Director-General's Request to the Scientific Advisory Board to Provide Further Advice on Assistance and Protection (SAB-21/WP.7, dated 29 April 2014)). Available at: www.opcw.org/fileadmin/OPCW/SAB/en/sab-21-wp07_e_.pdf

7 Response to the Director-General's Request to the Scientific Advisory Board to Provide Further Advice on Assistance and Protection (SAB-22/WP.2/Rev.1, dated 10 June 2015). Available at: https://www.opcw.org/fileadmin/OPCW/SAB/en/sab-22-wp02_e_.pdf

8 *Practical Guide for Medical Management of Chemical Warfare Casualties* (OPCW, 2016). Available at:www.opcw.org/fileadmin/OPCW/ICA/APB/Practical_Guide_for_Medical_Management_of_Chemical_Warfare_Casualties_-_web.pdf

9 Organisation for the Prohibition of Chemical Weapons Fact Sheet 8: Assistance and Protection Against Attack with Chemical Weapons. Available at: https://www.opcw.org/documents-reports/fact-sheets/.

10 Establishment of a Rapid Response Assistance Team (S/1381/2016, dated 10 May 2016). Available at: www.opcw.org/fileadmin/OPCW/S_series/2016/en/s-1381-2016_e_.pdf

the environment). Appropriate consideration must be given to both aspects when evaluating procedures for response to chemical incidents.

(d) Much literature and information exists on how to respond to chemical incidents. There is little standardisation internationally (even to the point that some responders are not allowed to deploy countermeasures that are stockpiled for use in emergencies by others). The procedures are in many cases specifically tailored to certain groups (e.g. military, emergency responder, etc.) and for a general civilian population may not represent best practices. A compilation of information categorised by who it is meant to apply to might be considered as a reference collection.

(e) The collation and consolidation of information that can aid responders to incidents involving chemical and/or biological agents could be valuable to ensure access to pertinent information. The Technical Secretariat in collaboration with the SAB may wish to consider updating and reissuing relevant technical documents (toxin fact sheets, for example).

(f) On-site and point-of-care detection methods to identify exposure and trigger response to chemical agent exposure represent a first line of defence. Given the time-critical nature of treatment, a thorough review and evaluation of existing tools would be valuable for verification and investigative purposes.

(g) Biosensors are valuable tools for point-of-care detection of chemicals. There may be opportunities to make better use of plant biomarkers for measuring and detecting (possibly in real time) toxic chemical exposure. A literature review and/or proof-of-concept research project (funded through international cooperation and/or other suitable funding opportunities) might be considered.

(h) There are many overlapping methods and technical dimensions in response to chemical agents and biological toxins, another example of the convergence of chemistry and biology. Efforts should be made to build networks that bridge communities of medical and emergency responders to share best practices and experience. These networks could be used to help maintain strong links between the SAB and the BTWC community.

2. AGENDA ITEM TWO – Adoption of the Agenda

The workshop adopted the following agenda:

1. Welcome and opening remarks
2. Adoption of the agenda
3. *Tour de Table* to introduce workshop participants
4. Establishment of a drafting committee
5. Introductory lecture from Professor Matthew Meselson
6. Chemical weapon toxicity and mechanisms of action

(a) Toxicity of nerve agents

(b) Toxicity of vesicants

(c) Long-term health effects of nerve agents and vesicants

(d) Human toxicological values of chemical warfare agents

(e) Toxicity of ricin

7. Emergency response to a chemical terrorist attack

(a) The French concept of emergency response to a chemical incident

(b) Biological markers of exposure to chemical warfare agents

(c) Clinical detection of nerve agents and vesicant exposure

(d) Field detection of chemical warfare agents

8. Medical countermeasures and decontamination against chemical warfare agents

(a) Existing and future countermeasures against nerve agents and vesicants

(b) New strategies for medical countermeasures against cyanide

(c) Current and emerging strategies for organophosphate decontamination

9. Adoption of the report

10. Closure of the workshop

3. AGENDA ITEM THREE – *Tour de Table* to Introduce Workshop Participants

A *tour de table* was undertaken to introduce the workshop participants. A complete list of participants is contained in Annex 1 of this report.

4. AGENDA ITEM FOUR – Establishment of a Drafting Committee

A drafting committee of members of the SAB was formed to prepare the report of the workshop.

5. AGENDA ITEM FIVE – Introductory Lecture

5.1 An introductory lecture providing a perspective on the successes and challenges to chemical disarmament was presented by Professor Matthew Meselson (Harvard University and co-director of the Harvard Sussex Programme on CBW Armament and Arms Limitation). Professor Meselson praised the scientists who advised and negotiated the Chemical Weapons Convention itself, discussed the norms of the Convention and his own involvement (beginning in 1963) as an adviser on biological and chemical weapons defence and arms control to numerous government agencies.

5.2 Professor Meselson pointed to the proficiency testing mechanism of the OPCW and its designated laboratories[11,12] as one of the great successes of the Convention. The mechanism serves as a demonstration of the ability to obtain credible and reliable analytical results that can provide decision makers with sound scientific information on the use and presence of chemical agents. He then cautioned that we must be careful not to get caught up in hype that distracts from realistic threat assessments and in the worst case might inspire malicious acts.

5.3 Touching on the future challenges to the Convention, Professor Meselson pointed to central nervous system (CNS) acting agents as a potential threat to preventing the re-emergence of chemical weapons. He noted that while this topic frequently focuses on opioid receptor binding substances (e.g. fentanyls), there are potentially thousands of targets for which chemical agents could be designed (these include organ specific receptors and enzymes, biological pathways associated with cognition, cardiovascular activity, and much more). He asked whether a norm against the development of such agents could be established, noting that the issue will not be solved by chemistry alone; it requires the efforts of scientists and policy makers alike.

5.4 Concluding his remarks Professor Meselson stressed the need to be forward looking as the science of today may impact the society of tomorrow, stressing that we have a responsibility to be careful about using chemistry to manipulate what it means to be human.

6. AGENDA ITEM SIX – Chemical weapon toxicity and mechanisms of action

6.1 Ms Farhat Waqar moderated the session on chemical weapon toxicity and mechanisms of action

Subitem 6(a): Toxicity of nerve agents

6.2 Colonel Fréderic Dorandeu (IRBA) provided an overview of inhalation toxicity mechanisms of organophosphorus (OP) nerve agents (NAs). He described experimental models and reviewed current issues in the study of NA toxicity.[13,14,15]

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11 For more information on the most recent OPCW Proficiency Test, see Evaluation of the Results of the Thirty-Ninth Official OPCW Proficiency Test (S/1409/2016, dated 23 August 2016). Available at: www.opcw.org/fileadmin/OPCW/S_series/2016/en/s-1409-2016_e_.pdf

12 Status of Laboratories Designated for the Analysis of Authentic Samples (S/1408/2016, dated 23 August 2016). Available at: www.opcw.org/fileadmin/OPCW/S_series/2016/en/s-1408-2016_e_.pdf

13 M. P. Nambiar, B. S. Wright, P. F. Rezk, K. B. Smith, R. K. Gordon, T. S. Moran, S. M. Richards, A. M. Sciuto; Development of a microinstillation model of inhalation exposure to assess lung injury following exposure to toxic chemicals and nerve agents in guinea pigs; *Toxicol. Mech. Methods*, 2006, *16(6)*, 295-306. DOI: 10.1080/15376510600748760.

14 M. W. Perkins, B. Wong, A. Rodriguez, J. L. Devorak, D. A. Alves, G. Murphy, A. M. Sciuto; Inhalation toxicity of soman vapor in non-anesthetized rats: a preliminary assessment of inhaled bronchodilator or steroid therapy; *Chem. Biol. Interact.*, 2013, *206(3)*, 452-461. DOI: 10.1016/j.cbi.2013.07.009. Epub 2013 Jul 23.

15 X. Peng, M. W. Perkins, J. Simons, A. M. Witriol A. M. Rodriguez B. M. Benjamin, J. Devorak A. M. Sciuto; Acute pulmonary toxicity following inhalation exposure to aerosolized VX in anesthetized rats; *Inhal. Toxicol.*, 2014, *26(7)*, 371-379. DOI: 10.3109/08958378.2014.899410. Epub 2014 Apr 25.

6.3 OP agents, whether designed as a chemical weapon (e.g. NAs) or as a pesticide, represent a serious risk to human health. Their main mechanism of toxicity is a potent inhibition of acetylcholinesterase (AChE), the enzyme that degrades the neurotransmitter acetylcholine (ACh) at different sites of the body. Inhibition leads to an accumulation of ACh in the synaptic cleft (neuromuscular junction) and prolonged stimulation of muscarinic and nicotinic receptors. This stimulation triggers various signs and symptoms such as widespread hypersecretions, contraction of smooth muscles (bronchoconstriction), miosis, cardiovascular changes and convulsive seizures. There are additional biochemical effects resulting from the impact of AChE inhibition. Respiratory effects can arise from a combination of peripheral changes (e.g. bronchoconstriction and airway over-secretion, diaphragm paralysis) or central effects (alteration of the respiratory drive) and can lead to death. Pre-eminence of one toxicity mechanism over another has been difficult to establish and may depend on the route of exposure and toxicokinetics of the agent. NAs can be dispersed in the form of liquid aerosols or vapours that will penetrate unprotected respiratory tract or mucosa. The less volatile NAs can penetrate the body through the respiratory tract after having been aerosolised, percutaneous poisoning is also possible.

6.4 The inhalation toxicity briefing was followed with a review of percutaneous toxicity mechanisms of NAs by Dr Helen Rice (Dstl). She explained that low volatility, persistent NAs, like VX, can readily penetrate the skin.[16] This route of poisoning is particularly difficult to treat because it results in a slower onset of poisoning and an extended exposure to the toxic agent. This has implications for the efficacy of treatment with medical countermeasures. It is necessary to understand the factors that affect the responses of different species to poisoning by the percutaneous route and therapeutic interventions, so that experimental work in appropriate animal species can inform the development and optimisation of therapeutic interventions.[17,18]

6.5 It is highly likely that casualties exposed to a NA via the percutaneous route, whether military personnel or civilian victims of a terrorist attack, will require further supportive therapeutic interventions and medical management following first aid therapy.[19] Recent proof of principle work has demonstrated the benefit from extended duration infusion of medical countermeasures and supplementary therapy with the bioscavenger human butyrylcholinesterase (BChE) following percutaneous VX

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16 C. H. Dalton, I. J. Hattersley, S. J. Rutter, R. P. Chilcott; Absorption of the nerve agent VX (*O*-ethyl-*S*-[2(di-isopropylamino)ethyl] methyl phosphonothioate) through pig, human and guinea pig skin *in vitro*, *Toxicology in Vitro*, 2006, *20*, 1532-1536.

17 G. Reiter, S. Müller, I. Hill, K. Weatherby, H. Thiermann, F. Worek, J. Mikler, *In vitro* and *in vivo* toxicological studies of V nerve agents: molecular and stereoselective aspects, *Toxicology Letters*, 2015, *232*, 438- 448.

18 H. Mumford, C. J. Docx, M. E. Price, A. C. Green, J. E. H. Tattersall, S. J. Armstrong, Human plasma-derived BuChE as a stoichiometric bioscavenger for treatment of nerve agent poisoning, *Chemico-Biological Interactions*, 2013, *203*, 160-166.

19 M. J. A. Joosen, M. J. van der Schans, H. P. M. van Helden, Percutaneous exposure to the nerve agent VX: Efficacy of combined atropine, obidoxime and diazepam treatment, *Chemico-Biological Interactions*, 2010, *188*, 255-263.

poisoning.[20,21] This work will inform strategies for the integration and optimisation of therapeutic interventions in the military casualty management chain.

6.6 In the subsequent discussion on these two presentations, the following points were raised:

(a) Controlling contamination is critical in casualty care. Decontamination must also be considered when deploying medical treatment due to the potential for secondary exposure resulting from contact with contaminated surfaces.

(b) Countermeasures should be treated as a continuum: there is a need to recognise appropriate measures across the timelines of exposure and treatment. The effect on brain cholinesterase by OP agents, for example, is most significantly observed after inhibition of blood cholinesterase. Temporal availability of administered therapeutic drugs must be considered in treatment and response. Effective treatment and response requires specially trained personnel.

(c) The mechanisms of dispersion determine the route of exposure. They can impact the ability to recognise if there has been any contamination (for example when it is transported by the weather[22]) and delayed effects might occur due to untreated and undetected low levels of exposure. Low volatility agents can be of particular concern since droplets on exposed skin may result in severe toxicity after a delayed period. Toxicity that is slow to manifest can extend beyond the short lived protection offered by the therapeutic drugs in autoinjectors, necessitating repeat dosing.

(d) Median lethal dose (LD_{50}) data determined through rapid exposure under controlled conditions may not translate into representative long term LD_{50} values.

(e) For inhalation studies, as observed in studies of inhaled and nasal delivery of pharmaceuticals, dose determination can be complex, as there can be difficulties in determining the exact amount of material delivered to the lung in animal toxicology studies.[23]

Subitem 6(b): Toxicity of vesicants

6.7 Dr John Jenner (Dstl) described the acute toxicity mechanisms of vesicants (blister agents), beginning with a brief overview of the development of vesicants as chemical weapons. The need to bypass early protective measures of the respiratory tract which

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20 H. Rice, T. M. Mann, S. J. Armstrong, M. E. Price, A. C. Green, J. E. H. Tattersall, The potential role of bioscavenger in the medical management of nerve-agent poisoned casualties, *Chem.Biol.Interact.*, in press (2016) Available online 1 May 2016. DOI: 10.1016/j.cbi.2016.04.038

21 H. Mumford, C. J. Docx, M. E. Price, A. C. Green, J. E. H. Tattersall, S. J. Armstrong, Human plasma-derived BuChE as a stoichiometric bioscavenger for treatment of nerve agent poisoning, *Chemico-Biological Interactions*, 2013, *203*, 160-166.

22 J. Tuite, R. W. Haley; *Neuroepidemiology*, 2013, *40*, 160–177. DOI: 10.1159/000345123

23 R. K. Wolff; Toxicology studies for inhaled and nasal delivery; *Mol. Pharmaceutics*, 2015, *12(8)*, 2688–2696. DOI: 10.1021/acs.molpharmaceut.5b00146

reduced the effects of suffocating gasses initially used in World War I led to the development of vesicant CWAs. The "lead" agent in this class, sulfur mustard (SM), was first used in 1917 and resulted in a large number of casualties that did not die soon after exposure and required prolonged nursing care.[24] In the years since 1917 a number of other vesicants have been identified and developed as toxic agents, including the nitrogen mustards (HN), Lewisite (L) and phosgene oxime (CX). SM remains the most utilised vesicant CWA and since its first use as a CWA, it has been used for little else other than to wage war. It has also been the subject of a large amount of research to define its mechanism of action and though we have a good knowledge of what it does, the precise biochemical event that initiates injury remains elusive.[25,26,27] SM is a bifunctional alkylating agent and the HN compounds share this property. Lewisite contains an arsenic atom that is linked to its activity as a vesicant. The interactions of the vesicants have been well characterised and the mustards alkylate key tissue components (DNA, proteins lipids etc.). Similarly the arsenic atom in lewisite combines with thiol groups, inhibiting several of key enzymes.

6.8 SM is absorbed rapidly by all biological surfaces it contacts as a vapour or a liquid and produces injury. SM and L are highly potent in producing effects on the skin, eyes and lungs, with microgram quantities required to be absorbed to produce inflammation. The skin shows an initial inflammation which progress in a dose dependent manner to more severe oedema. Pinhead vesicles form and coalesce to form large pendulous fluid-filled blisters. The effect on the eyes is conjunctivitis of increasing dose dependent severity until the eyelids go into spasm to prevent further injury (blepharospasm).[28,29] Though possible, the incidence of permanent loss of sight is low, though a delayed keratosis of the cornea has been recorded. Effects on the lungs are the most lethal of the effects of vesicants. The lining of the lungs is damaged or destroyed with the inevitable irritancy and pulmonary oedema. After a few days a pseudomembrane can be formed lining the major airways that can slough off and block the airways leading to a chronic obstructive pulmonary disease (COPD) type syndrome.

6.9 The massive inflammatory reaction that characterises poisoning by vesicants in general, and SM in particular, is followed by destruction of the affected tissue and further inflammation during the healing process. The release of many mediators of inflammation have been demonstrated to change during the development and healing

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24 J. L. Willems; Clinical management of mustard gas casualties; *Annales Medicinae Militaris*, 1989, 3, 1-61.

25 B. Papirmeister, A. J. Feister, S. I. Robinson, R. D. Ford; Molecular mechanisms of cytotoxicity, in: *Medical defence against mustard gas: toxic mechamisms and pharmacological implications*; B. Papirmeister et al (Eds); Boco Raton, CRC Press, 1991, 155-210.

26 J. Jenner; Toxicology of vesicants, in: *Chemical Warfare Toxicology*; F. Worek, J. Jenner, H. Thiermann (Eds); Cambridge, UK.; Royal society of Chemistry, 2016, 29-80.

27 J. Jenner; Human exposures to sulfur mustard, in: *Chemical Warfare Toxicology*; F. Worek, J. Jenner, H. Thiermann (Eds); Cambridge, UK.; Royal society of Chemistry, 2016, 154-178.

28 J. S. Anderson; The effect of mustard gas vapour on eyes under Indian hot weather conditions., CDRE India Report 241, UK National Archive Number WO 189/3234, 1942.

29 T. Kadar, J. Turetz, E. Fishbine, R. Sahar, S. Chapman, A. Amir; Characterization of acute and delayed ocular lesions induced by sulfur mustard in rabbits, *Current Eye Research*, 2001, *22(1)*, 42-53.

of a mustard lesion and in cultured cells treated with mustards.[30,31] These include histamine, prostaglandin E2, interleukins 1β and 6, and tumour necrosis factor α. The involvement of proteases in mustard and lewisite lesions has also been implicated with an increase in the activity of matrix metalloprotease, collagenase and caspase enzymes. The increase in caspase enzyme activity is indicative of apoptotic cell death which has been demonstrated in vitro and in vivo, though the necrotic pathway clearly dominates from an early stage in vivo making apoptotic bodies difficult to observe. Many of the observations in vitro and in vivo made over the years are now being confirmed by measurement of gene expression changes after exposure to SM.[32,33] Changes in the expression of genes controlling cell adhesion, cytoskeletal structure, cell cycle and inflammatory response, though not unexpected, confirm the involvement of these systems in the early stages of SM injury.

6.10 In the subsequent discussion, the following points were raised:

(a) Reducing the effect of acute injury to SM provides the best defence against long term effects. The reduction of acute toxicity remains the most effective way to avoid long term consequences.

(b) There is a continuing need to identify early biochemical events to better understand the mechanisms that lead to vesicant injury. In this regard OMICS technologies and *in vitro* assay formats have shown great promise.

Subitem 6(c): Long-Term Health Effects of Nerve Agents and Vesicants

6.11 Professor Mohammad Abdollahi described the potential delayed toxic effects that can manifest upon acute and chronic exposure to SM[34] and/or NAs. The clinical signs and symptoms of delayed effects are very diverse, and hard for physicians to diagnose and differentiate their pathogenesis. Long term complications[35,36,37] reported in survivors

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30 R. M. Black, R. W. Read; Biological fate of sulphur mustard, 1,1'-thiobis(2-chloroethane): identification of beta-lyase metabolites and hydrolysis products in human urine. *Xenobiotica*, 1995, *25(2)*, 167-173.

31 J. L. Hambrook, J. M. Harrison, D. J. Howells, C. Schock; Biological fate of sulphur mustard [1,1' -thio-bis(2-chloroethane)]: urinary and faecal excretion of ^{35}S by rat after injection or cutaneous application of 35S-labelled sulphur mustard; *Xenobiotica*, 1992, *22(1)*, 65-75.

32 J. F. Dillman, A. I. Hege, C. S. Phillips, L. D. Orzolek, A. J. Sylvester, C. Bossone, C. Henemyre-Harris, R. C. Kiser, Y. W. Choi, J. J. Schlager, C. L. Sabourn; Microarray analysis of mouse ear tissue exposed to bis-(2-chloroethyl) sulfide: Gene expression profiles correlate with treatment efficacy and an established clinical endpoint; *Journal of Pharmacology and Experimental Therapeutics*, 2006, *317(1)*, 76-87.

33 B. J. Jugg, H. Hoard-Fruchey, C. Rothwell, J. F. Dillman, J. David, J. Jenner, A. M. Sciuto; Acute Gene expression profile of lung tissue following sulfur mustard inhalation exposure in large anesthetized swine; *Chem. Res. Toxicol*., 2016. DOI: 10.1021/acs.chemrestox.6b00069

34 M. Balali-Mood, M. Hefazi; Comparison of early and late toxic effects of sulfur mustard in Iranian veterans." Basic & clinical pharmacology & toxicology; 2006, *99(4)*, 273-282. DOI: 10.1111/j.1742-7843.2006.pto_429.x

35 S. M. Razavi, P. Salamati, S. Masoud, M. Abdollahi. A review on delayed toxic effects of sulfur mustard in Iranian veterans. *DARU Journal of Pharmaceutical Sciences,* 2012, *20*, 51. Available at: http://www.darujps.com/content/20/1/51.

36 M. Ghanei, A. Amini Harandi; Long term consequences from exposure to sulfur mustard: a review; *Inhalation Toxicology*, 2007, *19(5),* 451-456.

of SM exposure include: respiratory,[38] reproductive, ocular,[39] dermatological,[40] hematologic,[41] neurological, immunological,[42] psychological complications and cancers.[43] In the case of NAs, reported long-term effects include neurological, hematologic, cutaneous, cardiovascular and psychological complications; as well as pulmonary diseases and lung damage. Professor Abdollahi recommended that relevant information and materials be made available to physicians and health professionals, so that they could become familiar with the delayed effects of these toxicants and medical management of their long-term health effects.

6.12 In the subsequent discussion, the following points were raised:

(a) Understanding the long-term health effects from OP compound exposure is broader than CWAs as pesticides can accumulate across the food chain and their effects may impact aging populations after years of low level chronic exposure.

(b) While many studies have been performed, the data are incomplete and patients exposed many years prior to these studies may be suffering additional related and unrelated health issues whose cause may be difficult to determine unambiguously.

(c) It was noted that for survivors of chemical attacks in the Iran-Iraq war, many had been exposed to both SM and NAs,[44] further complicating the origins of some of the long-term health effects observed amongst groups of veterans.

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37 M. Balali-Mood, S. H. Mousavi, B. Balali-Mood; Chronic health effects of sulphur mustard exposure with special reference to Iranian veterans; *Emerg. Health Threats J.*, 2008, *1*, e7. DOI: 10.3134/ehtj.08.007

38 M. Balali-Mood, R. Afshari, R. Zojaji, H. Kahrom, M. Kamrani, D. Attaran, S. Mousavi et al.; Delayed toxic effects of sulfur mustard on respiratory tract of Iranian veterans; *Human & Experimental Toxicology*, 2011, *30(9)*, 1141-1149. DOI: 10.1177/0960327110389501

39 M. Etezad-Razavi, M. Mahmoudi, M. Hefazi, M. Balali-Mood; Delayed ocular complications of mustard gas poisoning and the relationship with respiratory and cutaneous complications; *Clinical & Experimental Ophthalmology*, 2006, *34(4)*, 342-346. DOI: 10.1111/j.1442-9071.2006.01220.x

40 M. Hefazi, M. Maleki, M. Mahmoudi, A. Tabatabaee, M. Balali-Mood; Delayed complications of sulfur mustard poisoning in the skin and the immune system of Iranian veterans 16-20 years after exposure; *International Journal of Dermatology*, 2006, *45(9)*, 1025-3101. DOI: 10.1111/j.1365-4632.2006.03020.x

41 M. R. Keramati, M. Balali-Mood, S. R. Mousavi Seyed, M. Sadeghi, B. Riahi-Zanjani; Biochemical and hematological findings of Khorasan veterans 23 years after sulfur mustard exposure; *Journal of Research in Medical Sciences*, 2013, *18(10)*, 855-859.

42 M. Mahmoudi, M. Hefazi, M. Rastin, M. Balali-Mood; Long-term hematological and immunological complications of sulfur mustard poisoning in Iranian veterans; *International Immunopharmacology*, 2005, *5(9)*, 1479-1485. DOI: 10.1016/j.intimp.2005.04.003

43 S. M. Razavi, M. Abdollahi, P. Salamati; Cancer events after acute or chronic exposure to sulfur mustard: a review of the literature; *Int. J. Prev. Med.*, 2016, *7*, 76. DOI: 10.4103/2008-7802.182733

44 E. Darchini-Maragheh, H. Nemati-Karimooy, H. Hasanabadi, M. Balali-Mood; Delayed neurological complications of sulphur mustard and tabun poisoning in 43 Iranian veterans; *Basic & Clinical Pharmacology & Toxicology*, 2012, *111(6)*, 426-432. DOI: 10.1111/j.1742-7843.2012.00922.x

Subitem 6(d): Human toxicological values of chemical warfare agents

6.13 Dr Marie-Laure Cointot (DGA-MNRBC) reviewed current knowledge and discussed future prospects in human toxicology of CWAs. Substances that can be used as CWAs are of interest to the Department of CBRN Risk Management of the French Ministry of Defence. Nerve and blister agents are the major focus. Researchers in toxicology are involved in designing equipment for determination of exposure thresholds (toxicological reference values) for a better understanding of how to protect human health (especially decontamination options and protective equipment). This information is useful in the assessment of health risks of people exposed to chemical agents accidentally (emergency response) or during their professional activities (occupational exposure).

(a) Exposure can occur in a variety of complex ways involving both inhalation and dermal penetration; both contributions must be evaluated and integrated into the risk assessment. The CBRN risk management department is working on the determination of toxicological values at atmospheric concentrations, taking into account possible dermal exposure and skin penetration of G agents (e.g.. sarin or soman) using in vivo and in vitro approaches. Studies on surface contamination are also necessary for the design of effective protective equipment and decontamination. The presentation provided reference materials for guidance on how to use toxicity information in risk assessments, including: acute exposure guidelines for chemical inhalation,[45,46] characterisation of dose response for human health,[47] and military publications,[48,49,50]

6.14 In the subsequent discussion, the following points were noted:

(a) Actionable information requires that both in vitro and in vivo toxicological data be extrapolated to humans for assessing timely health impacts.

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45 Chemical Priority Lists for Acute Exposure Guideline Levels (AEGLs); United States Environmental Protection Agency. Available at: https://www.epa.gov/aegl/chemical-priority-lists-acute-exposure-guideline-levels-aegls

46 Good practices for the use of acute toxicity thresholds inhalation under regulatory studies (INERIS, DRC27 - Opération e, dated 30 November 2010). Available (in French) at: http://www.ineris.fr/centredoc/drc27-bonnes-pratiques-v6web-1413910609.pdf

47 Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health (European Chemicals Agency, ECHA-2010-G-19-EN, dated November 2012). Available at: https://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258

48 Potential Military Chemical/Biological Agents and Compounds (The United States Army Chemical School, dated November 2005). Available at: http://fas.org/irp/doddir/army/fm3-11-9.pdf

49 Environmental Health Risk Assessment and Chemical Exposure Guidelines for Deployed Military Personnel (Army Public Health Center, Technical Guide 230, 2013 Revision). Available at: https://phc.amedd.army.mil/PHC%20Resource%20Library/TG230.pdf

50 Development of Exposure Guidelines for Chronic Health Effects Following Acute Exposures to Toxic Industrial Chemicals – A Toxidrome-Based Approach (Army Public Health Center, PHIP No. 39-04-0116, dated March 2016). Available at: https://phc.amedd.army.mil/PHC%20Resource%20Library/PHIP-39-04-0116-AECE-ToxidromeBasedApproach-March2016.pdf

(b) While reference values for making decisions are available, given the complexity of toxicity mechanisms and consequences of the use of CWA (there other processes that occur in addition to AChE inhibition) risk assessments have high degrees of uncertainties. The best suited emergency response strategies will situation dependent.

Subitem 6(e): Toxicity of ricin

6.15 The toxicity of ricin was reviewed by Dr Brigitte Dorner (Robert Koch Institute, RKI). Ricin is produced by the plant *Ricinus communis* and is one of the most toxic plant toxins known. It belongs to the family of type II ribosome-inactivating proteins. As a prototype AB toxin ricin consists of a sugar-binding B chain (~34 kDa) linked via a disulfide bond to the catalytically active A chain (~32 kDa) which acts as an RNA *N*-glycosidase resulting in a holotoxin of about 65 kDa. Cell binding through the B chain involves different oligosaccharide residues on the cell surface. After internalisation, the A-B heterodimer undergoes retrograde transport via the Golgi network to the endoplasmic reticulum where the heterodimer is reduced and separated into the two subchains. The A chain is then transported into the cytosol and binds to the ribosome where it removes a single adenine from the 28S rRNA, thus preventing further binding of elongation factors, inhibiting protein biosynthesis and finally leading to cell death.

6.16 *Ricinus communis* is grown worldwide on an industrial scale for the production of castor oil. As a by-product of castor oil production, ricin is mass produced in a quantity above one million tons per year. On the basis of its availability, toxicity, ease of preparation and the current lack of medical countermeasures, ricin has gained attention as a potential toxic agent. In the past, the toxin has been explored for potential military use by different nations. It was included in weapons programmes during World War II (codename: Compound W), and weaponised material was produced until the 1980s. The use of ricin as a weapon is prohibited under both the Chemical Weapons Convention, (where it is listed on Schedule 1 and the Biological and Toxins Weapons Convention (BTWC). In the last decade, the focus fell on this toxin for criminal use and various attempted acts of bioterrorism.[51] To provide a few examples, ricin was found in threat letters to members of the US Senate, the White House and the US President in 2003/2004 and 2013; *Ricinus communis* seeds and means for the preparation of ricin have been discovered during a raid against terrorists in London in 2002. In a larger number of cases worldwide the production and possession of ricin has been well documented.

6.17 Accidental and intended *Ricinus communis* intoxications in humans and animals have been known for centuries. A literature search for cases of intoxication since 1888 has shown that – among all plant poisonings reported – human cases of ricin poisoning are rare. Of those attributed to ricin/*Ricinus communis*, 1.5% of the accidental oral intoxications ended fatally. However, of the intentional intoxications (suicide attempts) 45.5% of the cases resulted in death, all of them after injection of *Ricinus.*

51 B. G. Dorner, R. Zeleny, K. Harju, J. A. Hennekinne, P. Vanninen, H. Schimmel, A. Rummel; Biological toxins of potential bioterrorism risk: current status of detection and identification technology. *Trends in Analytical Chemistry* (in press), 2016, DOI: 10.1016/j.trac.2016.05.024

communis extracts.[52] This finding is in line with the different toxicity figures which have been estimated based on experiments in rodents and non-human primates. The human LD_{50} after oral uptake is estimated to be in the range of 1-20 mg/kg, and approximately 3 µg/kg after injection or inhalation.

6.18 Generally, independent of the uptake route (oral or parenteral injection/inhalation) the symptoms induced by ricin/*Ricinus communis* were quite similar, and the severity increases with the amount of toxin incorporated. Symptoms arise after three to twenty hours after ingestion or injection. Physical symptoms can include abdominal pain, emesis, diarrhoea (with or without blood), muscular pain, cramped limbs, circulatory collapse, dyspnoea and dehydration. Muscular pain and circulatory collapse are more commonly observed with injected ricin, as well as pain at the injection site. Biochemical analyses often reveal an increase in white blood cells, blood urea nitrogen (BUN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), indicating dysfunction of the liver and kidneys. Autopsy in fatal cases show haemorrhagic necrosis in intestines and the heart and oedema in the lungs. Since ricin is taken up in many different cell types after incorporation, the main mechanism of toxicity is induction of cell death and tissue damage by an arrest of protein biosynthesis, membrane damage, inflammation and apoptosis. Due to the rather unspecific symptoms induced by ricin, an intentional release of the toxin would be challenging to detect rapidly.

6.19 The RKI has been involved in the analysis of several attempted and successful suicides involving *Ricin communis* extracts. In 6 out of 8 cases, ricin intoxication could be documented based on immunological and spectrometric methods. The analysis of suicide (attempts) has provided valuable information on suitable sample materials,[53] detection methods[54,55,56] and the kinetics of human intoxication.[57]

6.20 In the subsequent discussion, the following points were raised:

(a) There is a lack of inhalation toxicity data for ricin in the open literature.

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52 S. Worbs, K. Köhler, D. Pauly, M. A. Avondet, M. Schaer, M. B. Dorner, B. G. Dorner; *Ricinus communis* intoxications in human and veterinary medicine - a summary of real cases. *Toxins*, 2011, *3*, 1332-1372.

53 S. Worbs, M. Skiba, M. Söderström, M. L. Rapinoja, R. Zeleny, H. Russmann, H. Schimmel, P. Vanninen, S.-A. Fredriksson, B. G. Dorner; Characterization of ricin and *R. communis* agglutinin reference materials. *Toxins*, 2015, *7 (12)*, 4906-4934.

54 S. Simon, S. Worbs, M. A. Avondet, D. M. Tracz, J. Dano, L. Schmidt, H. Volland, B. G. Dorner, C. R. Corbett; Recommended immunological assays to screen for ricin-containing samples; *Toxins*, 2015, *7(12)*, 4967-4986.

55 S. Worbs, M. Skiba, J. Bender, R. Zeleny, H. Schimmel, W. Luginbühl, B. G. Dorner; An international proficiency test to detect, identify and quantify ricin in complex matrices; *Toxins*, 2015, *7(12)*, 4987-5010.

56 D. Stern, D. Pauly, M. Zydek, C. Mueller, M. A. Avondet, S. Worbs, F. Lisdat, M. B. Dorner, B. G. Dorner; Simultaneous differentiation and quantification of ricin and agglutinin by an antibody-sandwich surface plasmon resonance sensor; *Biosens Bioelectron.*, 2016, *15(78)*, 111-117.

57 D. Pauly, S. Worbs, S. Kirchner, O. Shatohina, M. B. Dorner, B. G. Dorner; Real-time cytotoxicity assay for rapid and sensitive detection of ricin from complex matrices, *PLoS One*, 2012, *7*, e35360. DOI: 10.1371/journal.pone.0035360

(b) Dr Dorner's presentation contained valuable reference information on ricin. Updates to the SABs previous fact sheet on this toxin,[58] and/or the production of infographic reference materials by the OPCW were suggested.

(c) The SAB noted that the OPCW Laboratory is preparing to conduct a ricin proficiency test[59] (work that supports the recommendation of the SABs Temporary Working Group on Verification[60]).

7. AGENDA ITEM SEVEN – Emergency Response to a Chemical Terrorist Attack

7.1 Professor Mohammad Abdollahi moderated the session on emergency response to a chemical terrorist attack.

Subitem 7(a): The French concept of emergency response to a chemical incident

7.2 Dr Lionel Lachenaud (SGDSN) described the French mechanism for emergency response in the case of a chemical incident. The presentation began with an overview of the organisation, tools and methods in response to a major crisis. Dr Lachenaud continued with an overview of the use of rescue and medical care resources in case of a terrorist action involving chemicals. Here the main objective is to save and preserve human lives. Situations of concern, danger zones resulting from chemical release, on-scene handling of casualties, and the intervention and organisation of emergency responders, were reviewed.

7.3 In the subsequent discussion, it was noted that information exchanged on the French mechanism could be valuable to assist others to formulate their own emergency response procedures against terrorist incidents.

Subitem 7(b): Biological markers of exposure to chemical warfare agents

7.4 Dr Franz Worek (Bundeswehr Institute of Pharmacology and Toxicology) briefed the workshop on biomedical verification of exposure to CWAs. Although clinical diagnosis of poisoning by such agents is the primary tool, on-site laboratory analysis is important for confirmation of clinical diagnosis and as an early indicator in case of low-level exposure or delayed onset of signs. At present, on-site assays are available for determination of cholinesterase activity in whole blood, a key parameter for detecting exposure to NAs and OP pesticides, and for skin detection of SM.

7.5 Unequivocal proof of exposure to CWAs can be achieved by analysis of biomedical samples which requires sophisticated, laborious and expensive off-site methods. These methods provided some of the first confirmations of the use of sarin in

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58 Ricin Fact Sheet (SAB-21/WP.5, dated 28 February 2014). Available at: www.opcw.org/fileadmin/OPCW/SAB/en/sab-21-wp05_e_.pdf

59 Call for Nominations for an Exercise on Analysis of Ricin (S/1422/2016, dated 16 September 2016). Available at: www.opcw.org/fileadmin/OPCW/S_series/2016/en/s-1422-2016_e_.pdf

60 Verification, Report of the Scientific Advisory Board's Temporary Working Group (SAB/REP/1/15, dated June 2015). Available at: www.opcw.org/fileadmin/OPCW/SAB/en/Final_Report_of_SAB_TWG_on_Verification_-_as_presented_to_SAB.pdf

August 2013[61] with samples taken from Syrian casualties; and of the use of SM in September 2015.[62]

7.6 Despite long-lasting research, on-site laboratory diagnosis and off-site biomedical verification is only available for selected CWAs and toxic industrial chemicals. Further, intensified efforts are needed to counter the ongoing threat of potential use of toxic chemicals by terrorists.

7.7 In the subsequent discussion, the following points were raised:

(a) Biosensors represent the first point of detection and are valuable for point-of-care diagnostic tests. Positive results could trigger administration of countermeasures, decontamination procedures, and/or evacuation.

(b) AChE activity is a valuable metric to recognise early signs of exposure.

(c) The analysis of biomedical samples has focused on adducts of SM[63] and NAs. There is a need to understand if there are markers suited for industrial chemicals (e.g. chlorine[64]). There may also be opportunities for the use of biomarkers present in vegetation.

Subitem 7(c): Clinical detection of nerve agents and vesicant exposure

7.8 Dr Florian Nachon (IRBA) briefed the workshop on clinical recognition of NA exposure. He explained that diagnosis of NA poisoning is initially based on the evaluation of typical signs of cholinergic crisis, including central nervous system, respiratory, cardiovascular, gastrointestinal, musculoskeletal, skin and mucous symptoms (e.g. headache, convulsions, coma, rhinorrhea, bronchorrhea, nausea, diarrhea, muscle weakness, fasciculation, sweating, and/or lacrimation). The clinical diagnosis can be confirmed through measurement of cholinesterase activity.[65,66] However, intra- and inter-individual variation can limit the reliability of such measurements (inhibition levels can vary by more than >20% between measurements).

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61 United Nations Mission to Investigate Allegations of the Use of Chemical Weapons in the Syrian Arab Republic, New York, USA (2013). Available at: https://unoda-web.s3.amazonaws.com/wp-content/uploads/2013/12/report.pdf

62 Report of the OPCW Fact-Finding Mission in Syria Regarding the Alleged Incidents in Marea, Syrian Arab Republic. S/1320/2015, dated 29 October 2015. Available at: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20151029-Marea.pdf

63 H. John, M. Siegert, F. Gandor, M. Gawlik, A. Kranawetvogl, K. Karaghiosoff, H. Thiermann; Optimized verification method for detection of an albumin-sulfur mustard adduct at Cys(34) using a hybrid quadrupole time-of-flight tandem mass spectrometer after direct plasma proteolysis; *Toxicol Lett.*, 2016, *244*, 103-111. DOI 10.1016/j.toxlet.2015.09.027

64 P. Hemström, A. Larsson, L. Elfsmark, C. Åstot; L-α-phosphatidylglycerol chlorohydrins as potential biomarkers for chlorine gas exposure; *Anal. Chem.*, 2016. DOI: 10.1021/acs.analchem.6b01896

65 F. Worek, P. Eyer, H. Thiermann; Determination of acetylcholinesterase activity by the Ellman assay: a versatile tool for in vitro research on medical countermeasures against organophosphate poisoning; *Drug testing and analysis*, 2012, *4 (3-4)*, 282-291. DOI: 10.1002/dta.337

66 D. Du, J. Wang, L. Wang, D. Lu, Y. Lin; Integrated Lateral Flow Test Strip with Electrochemical Sensor for Quantification of phosphorylated cholinesterase: biomarker of exposure to organophosphorus agents; *Anal. Chem.*, 2012, *84(3)*, 1380–1385. DOI: 10.1021/ac202391w

7.9 Biomarkers of NAs (sarin, cyclosarin, soman, tabun, VX, RVX) fall into two main groups, free metabolites and adducts to proteins.[67,68,69] The metabolites, e.g. alkyl methylphosphonic acids, are detectable for a short period in urine (several days in case of moderate exposure) and have a shorter lifetime in the blood. Analytical methods, based on gas chromatography (GC) or liquid chromatography (LC) combined with tandem mass spectrometry (MS-MS) or high resolution mass spectrometry (HRMS) are required for confirmation of exposure. The most sensitive analytical methods are based on LC–MS-MS of the non-derivatised metabolite or GC–MS–MS of pentafluorobenzyl esters. These methods have limits of detection (LOD) at or below 0.1 ng/ml. Protein adducts derived from phosphylation of the serine residue in the active sites of plasma BChE offer longer lived biomarkers of exposure (about 2 weeks) at the ng/ml concentration. A simple approach, fluoride regeneration, to identify an adduct, consists of displacing the bound NA from the enzyme with fluoride ion, and generating a phosphono- or phosphoro-fluoridate, that can be analysed by GC–MS or GC–MS–MS after extraction. A limitation to fluoride regeneration is that it cannot be applied to aged enzyme, which for a NA like soman can occur in a few minutes. A more sophisticated detection approach, requiring greater expertise and more sensitive instrumentation, isolates the enzyme and identifies a specific phosphylated peptide by LC–MS–MS after enzymatic digestion. The specificity is lost upon ageing preventing the identification of the agent.

7.10 Adducts of agents can also form with a specific tyrosine residue of the albumin proteins that are abundant in plasma. In the case of VX, albumin adducts form only at high concentrations due to its lower chemical reactivity. Albumin adducts can be identified by the same approaches as AChE and BChE adducts, and also by antibody-mediated molecular recognition of the specific phosphylated tyrosine of albumin. Despite their lower concentrations, albumin tyrosine adducts present an advantage of longevity over cholinesterase adducts, particularly if oxime therapy has been administered.

7.11 In the subsequent discussion it was noted that metabolites identifying exposure to chemical agents may also be found in hair and nail clippings.[70,71]

7.12 Complementing the previous presentation, Professor Mahdi Balali-Mood (Mashhad University of Medical Sciences) described clinical recognition of vesicant exposure.

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67 J. P. Langenberg, M. J. van der Schans, D. Noort; Assessment of nerve agent exposure: existing and emerging methods; *Bioanalysis*, 2009, *1*, 729–739.

68 L.M. Schopfer, O. Lockridge; Analytical approaches for monitoring exposure to organophosphorus and carbamate agents through analysis of protein adducts; *Drug Testing and Analysis*, 2012, *4*, 246-261.

69 R. M. Black, R. W. Read; Biological markers of exposure to organophosphorus nerve agents. *Arch Toxicol.*, 2013, *87(3)*, 421-437.

70 A. S. Appel, H. McDonough, J. D. McMonagle, B. A. Logue; Analysis of Nerve Agent Metabolites from Hair for Long-Term Verification of Nerve Agent Exposure; *Anal. Chem.*, 2016, *88(12)*, 6523–6530. DOI: 10.1021/acs.analchem.6b01274

71 A. S. Appel, B. A. Logue; Analysis of nerve agent metabolites from nail clippings by liquid chromatography tandem mass spectrometry; *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 2016, *1031*, 116-122.. DOI: 10.1016/j.jchromb.2016.07.034

He presented clinical data on victims of SM exposure in the Iran-Iraq war[72] and described his own experiences as a doctor treating injured soldiers.

7.13 In the subsequent discussion, it was noted that reports of several human accidental exposures to SM had been recently published.[73,74,75]

Subitem 7(d): Field detection of chemical warfare agents

7.14 Lt Col Ivana Moravcová (JCBRN Defence COE) provided an overview of methods used for field detection of CWAs. Lt Col Ivana Moravcová discussed detection methods, available instruments (including fieldable portable mass spectrometers, electrochemical and surface acoustic wave devices) and the requirements for fieldability. She further highlighted the advantages and limitations of each system.

7.15 In the subsequent discussion, the following points were raised.

(a) Further information is available from published reviews on hand-held CWA detection devices.[76] Understanding the limitations and the ability to recognise false positive and negative signals is required to use these devices most effectively. For optimal safety, a combination of detectors can be used to maximise the chances of detecting chemical weapons agents during reconnaissance operations in the field, in some cases to assist the taking of samples for subsequent analysis by OCPW designated laboratories.

(b) There are several point-of-care detection options for toxins, often lateral flow and other simple types of devices. Paper based devices have also been reported for CWAs.[77] A review would be of value as a reference to those personnel operating in the field, especially OPCW inspectors.

8. AGENDA ITEM EIGHT – Medical Countermeasures and Decontamination Against Chemical Warfare Agents

8.1 Dr Zrinka Kovarik moderated the session on medical countermeasures and decontamination against chemical warfare agents.

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72 M. Balali-Mood, B. Balali-Mood; Sulphur mustard poisoning and its complications in Iranian veterans ; *Iran. J. Med. Sci.*, 2009; *34(3)*, 155-171.

73 J. R. Barr, C. L. Pierce, J. R. Smith; B. R. Capacio, A. R. Woolfitt, M. I. Solano, J. V. Wooten, S. W. Lemire, J. D. Thomas, D. H. Ash, D. L. Ashley; Analysis of Urinary Metabolites of Sulfur Mustard in Two Individuals after Accidental Exposure, *J Anal Toxicol,*, 2008, *32(1)*, 10-16.

74 J. R. Smith, B. R. Capacio, W. D. Korte, A. R. Woolfitt, J. R. Barr; Analysis for plasma protein biomarkers following an accidental human exposure to sulfur mustard; , *J Anal Toxicol,*, 2008, *32(1)*, 17-24.

75 D. Steinritz, E. Striepling, K.-D. Rudolf, C. Schröder-Kraft, K. Püschele, A. Hullard-Pulstinger, M. Koller, H. Thiermann, F. Gandor, M. Gawlik, H. John; Medical documentation, bioanalytical evidence of an accidental human exposure to sulfur mustard and general therapy recommendations; *Toxicology Lett.*, 2016, *244*, 112-120.

76 A.-B. Gerber; Testing of hand-held detectors for chemical warfare agents; *Spiez Laboratory Annual Report 2015*, 38-39. Available at: http://www.labor-spiez.ch/en/dok/ge/pdf/88_003_e_laborspiez_jahresbericht_2015_web.pdf

77 D. Pardasani, V. Tak, A. K. Purohit, D. K. Dubey; μ-PADs for detection of chemical warfare agents; *Analyst*, 2012, *37(23)*, 5648-5653. DOI: 10.1039/c2an36273b

Subitem 8(a): Existing and future countermeasures against nerve agents and vesicants

8.2 Dr Horst Thiermann (Bundeswehr Institute of Pharmacology and Toxicology) reviewed existing countermeasures and new strategies for medical protection against NAs. NAs belong to the large group of OP compounds and exert their life threatening toxicity by inhibition of the pivotal enzyme AChE. As a result of this inhibition, ACh accumulates in cholinergic synaptic clefts and overstimulates muscarinic and nicotinic receptors leading to cholinergic crises. Although this toxic mechanism is typical for the highly toxic NAs and hundreds of pesticides, the course of poisoning, e.g. time to onset and duration of signs and symptoms may vary significantly due to different chemical and physical properties of the single compounds. Moreover, highly toxic NAs require self-protection of medical personnel in order to avoid life threatening secondary contamination.

8.3 For decades, treatment of NA poisoning was performed through general administration of a muscarinic antagonist (e.g. atropine), a reactivator of inhibited AChE (e.g. an oxime), and a benzodiazepine. Additionally, further medical management - e.g. support of ventilation and cardiovascular stabilisation - complete treatment. Recent findings from research and re-evaluation of the scientific literature support an optimised treatment protocol with more aggressive atropinisation, adequate dosing of oximes and benzodiazepines, as well as enhanced ventilatory support.

8.4 Moreover, as especially in certain circumstances the effectiveness of oximes is limited, alternative therapeutic approaches are being investigated. Small molecule scavengers, e.g. cyclodextrins, are expected to enhance elimination of NAs from the circulatory system, thereby preventing or relieving poisoning. First results clearly show that selected cyclodextrins are able to stereoselectively scavenge NAs in vitro and in vivo. Unfortunately, a cyclodextrin showing the required effectiveness against critical V-agents or a broader spectrum of NAs has not yet been identified. An alternative approach consists of modulation of nicotinic receptors to restore their function in spite of ACh overflow. The first encouraging results revealed that a bispyridinium non-oxime enabled survival after soman poisoning in an animal model.[78] Single compounds of diverse bispyridinium non-oxime structures show different binding properties towards nicotinic receptors[79] and are able to antagonise desensitisation of nicotinic receptors at high transmitter concentration.

8.5 In conclusion, improvement of existing therapeutic protocols by more specific treatments should be possible. New and encouraging approaches are under investigation which may improve treatment in the long term.

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78 C. M. Timperley, M. Bird, C. Green, M. E. Price, J. E. Chad, S. R. Turner, J. E. H. Tattersall; 1,1′-(Propane-1,3-diyl)bis(4-tert-butylpyridinium) di(methanesulfonate) protects guinea pigs from soman poisoning when used as part of a combined therapy; *Med. Chem. Commun.*, 2012, *3*, 352-356. DOI: 10.1039/C2MD00258B

79 K. V. Niessen, T. Seeger, J. E. Tattersall, C. M. Timperley, M. Bird, C. Green, H. Thiermann, F. Worek; Affinities of bispyridinium non-oxime compounds to [(3)H]epibatidine binding sites of Torpedo californica nicotinic acetylcholine receptors depend on linker length; *Chem Biol Interact.*, 2013, *206(3)*, 545-554. DOI: 10.1016/j.cbi.2013.10.012

8.6 Professor Pierre-Yves Renard (Université de Rouen) briefed the workshop on new oximes, compounds designed to reactivate inhibited AChE. The acute toxic effect of OPNAs are based on the irreversible inhibition of AChE via the formation of a covalent P-O bond at a hydroxyl group of a serine moiety located within the active site of the enzyme. As AChE is responsible for the breakdown of the neurotransmitter ACh at the neuronal synapses and neuromuscular junctions, its irreversible inhibition leads to an accumulation of the neurotransmitter in the synaptic cleft, causing the over-stimulation of cholinergic receptors, seizures, respiratory arrest and death. The current treatment of OPNA poisoning is a combination of an antimuscarinic drug (e.g. atropine), an anticonvulsant drug (e.g. diazepam), and an AChE reactivator from the pyridinium aldoxime family (pralidoxime, trimedoxime, obidoxime, HI-6, HLö-7). The high nucleophilicity of these oximes allows displacement of the phosphyl group from the catalytic serine, thus inducing the recovery of the catalytic activity. Over the past 50 years of research in the reactivator field, numerous structural modifications on monopyridinium oximes and bispyridinium oximes have been carried out. In the past decade, medicinal chemists have focused their research on bispyridinium reactivators that are potentially more efficient than currently used monopyridinium compounds.[80,81]

8.7 Existing reactivators described in the literature have a number of drawbacks. Due to their permanent positive charge, they poorly cross the blood brain barrier (BBB) and thus do not readily reactivate AChE in the central nervous system. They have unequal efficiencies against AChE inhibited by different types of OPNA, and cannot reactivate "aged" AChE. Professor Renard summarised recent strategies for the development of effective oxime-based AChE reactivators, focusing on those able to cross the BBB. Improvement of BBB permeability of clinically used quaternized pyridine aldoximes can be managed by nanoparticulate transport, as well as by modulation of the BBB through inhibition of P-glycoprotein efflux pumps. Modification of the chemical structure of pyridinium aldoximes to increase the lipophilicity by substitution of hydrogen by fluorine atoms, and replacement of the pyridyl ring by the dihydropyridyl moiety was also shown to enhance the BBB permeability. Glycosylation of a pyridine aldoxime can also increase BBB penetration due to facilitative diffusion by the GLUT-1 transporting system. To date, one of the most promising and attractive strategies to surmount the BBB is the development of novel uncharged reactivators. Broad spectrum oximes targeting BChE for potential use when co-administered with pseudocatalytic bioscavengers was also discussed.[82]

8.8 The final presentation on NA countermeasures was provided by Professor Patrick Masson (Kazan Federal University) covering the emergence of catalytic

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80 G. Mercey, T. Verdelet, J. Renou, M. Kliachyna, R. Baati, F. Nachon, L. Jean, P.-Y. Renard; Reactivators of Poisoned Acetylcholinesterase by Organophosphorus Nerve Agents; *Acc Chem Res*, 2012, *45*, 756-766.

81 R. Sharma, B. Gupta, N. Singh, J. R. Acharya, K. Musilek, K. Kuca, K. K. Ghosh; Development and structural modifications of cholinesterase reactivators against chemical warfare agents in last decade: a review; *Mini-Reviews in Medicinal Chemistry*, 2015, *15*, 58-72.

82 Z. Radić, T. Dale, Z. Kovarik, S. Berend, E. Garcia, L. Zhang, G. Amitai, C. Green, B. Radić, B. M. Duggan, D. Ajami, J. Rebek, P. Taylor; Catalytic detoxification of nerve agent and pesticide organophosphates by butyrylcholinesterase assisted with non-pyridinium oximes; *Biochem J.*, 2013, *450(1)*, 231-242.

bisocavengers.[83] Bioscavengers are natural or recombinant enzymes, reactive proteins, or antibodies that neutralise NAs before they reach physiological targets. They constitute an alternative approach for pre- and post-exposure treatments of NA poisoning.[84] They can be administered by injection or other methods (e.g. inhalation or gene delivery vectors). Their reaction with NAs in the bloodstream must be fast, thus preventing the action of these toxic molecules on their biological targets. Fieldable bioscavengers have to be produced at low cost and be stable to storage. They have to display a slow blood clearance and must not induce an immune response. Ideally, association of several bioscavengers could be used for prophylaxis against all NAs.

8.9 First generation bioscavengers were stoichiometric bioscavengers. Among them, human BChE has proven to be safe and effective in challenging multiple LD_{50}s of NAs. However, stoichiometric neutralisation of NAs needs administration of huge doses of extremely expensive biopharmaceuticals. Their use, even for the protection of first responders, is unrealistic given the limited capacity of current production biotechnology.

8.10 Second generation bioscavengers are catalytic bioscavengers.[85] They are capable of detoxifying NAs under first-order conditions with a high turnover. Thus, by virtue of a high bimolecular rate constant ($k_{cat}/K_m > 10^6$ M^{-1} min^{-1}), low enzyme doses are needed for rapid detoxification of NAs. The most promising catalytic bioscavengers are evolved phosphotriesterases (stable mutants of enzymes from bacteria, archaea, and mammalian paraoxonase-1),[86] displaying enantiomeric preference for the most toxic NA isomers, and conferring *in vivo* protection against NAs, including V agents. However, engineering of other enzymes - cholinesterases, carboxylesterases, prolidases, oxidases and catalytic antibodies - is being pursued. Research on novel catalytic bioscavengers[87] is based on computer re-design of known OP-reacting enzymes, implementation of combinational strategies, and HTP screening of libraries of mutated/evolved enzymes and natural enzymes from extreme biotopes.

8.11 Chemical modifications and/or encapsulation into nanoparticles make catalytic bioscavengers more stable upon storage and in the bloodstream, and prevent immune response. Research on artificial enzymes, i.e. functionalised β-cyclodextrins and hybrid molecules bearing nucleophilic groups, is promising. Certain small molecules have proven their effectiveness in detoxifying NAs at high rate both in vitro and in vivo. Lastly, association of cholinesterase mutants - not susceptible to ageing after

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83 P. Masson. S. V. Lushchekina; Emergence of catalytic bioscavengers against organophosphorus agents. *Chem. Biol. Interact.*, 2016, in press.

84 P. Masson; Nerve agents: catalytic scavengers, alternative approach for medical countermeasures, in: *Chemical Warfare Toxicology: Management of Poisoning;* F. Worek, J. Jenner, H. Thiermann, (Eds.), Royal Chemical Society, Cambridge, UK, 2016, 43-81.

85 F. Worek, H. Thiermann, T. Wille; Catalytic bioscavengers in nerve agent poisoning: a promising approach? *Toxicol. Lett*., 2016, *244*, 143-148.

86 Y. Ashani, H. Leader, N. Aggarwal, L. Silman, F. Worek, J. L. Sussman, M. Goldsmith; *In vitro* evaluation of the catalytic activity of paraoxonases and phosphotriesterases predicts the enzyme circulatory levels required for in vivo protection against organophosphate intoxications. *Chem. Biol. Interact.,* 2016, in press.

87 P. Masson; Novel approaches in pretreatment/prophylaxis and treatment of organophosphorus poisoning. *Phosphorus, Sulfur, and Silicon*, 2016, in press.

phosphylation - with novel fast-reactivating oximes leads to pseudo-catalytic bioscavenger systems.

8.12 In the near future, catalytic, pseudo-catalytic bioscavengers and artificial enzymes are expected to improve medical countermeasures in terms of efficacy, safety and cost. Nanomedicine technologies will make their implementation easier for long-lasting action against a large spectrum of OP agents. Preliminary results of transient in vivo production of catalytic bioscavengers, using short-induction vectors, are promising. However, safe and effective gene therapy is still a long way off.

8.13 Professor Slavica Vučinić (National Poison Control Centre, Military Medical Academy, Serbia) reviewed existing countermeasures and new strategies for medical protection against vesicants. Vesicants reduce soldiers' fighting capability through injury and force them to wear personal protective equipment; they can also be fatal following severe exposure. Historical facts and recent events confirm that they still present a hazard to the civilian population and the environment.[88] Clinical effects include a wide range of local and systemic damage (skin, eyes, lungs, respiratory and gastrointestinal tract, immune, hematopoietic and endocrine system)[89,90] with delayed complications and long-term effects.

8.14 Several antidotes are available against lewisite. For SM, however, there are no standardised or optimised treatment methods. Therapies aim to relieve symptoms, prevent infection and promote healing. Extensive research conducted over the past decade provides better insight into the complex and multifaceted mechanism of poisoning, leading to identification of potential pharmacological targets and pathways for novel therapeutic agents.[91] In addition to existing countermeasures, conventional medical treatments with different antioxidants, sodium thiosulfate and anti-inflammatory drugs have been used; many with limited efficacy. Poly ADP ribose polymerase (PARP) inhibitors, antimicrobial peptides, matrix metalloproteinase (MMP) inhibitors, herbal medicines and probably regulators of DNA damage repair are envisioned as new modalities for improved treatment.

8.15 In the subsequent discussion, the following points were raised:

(a) Response time for treatment is critical and should be characterised and understood for the choice of countermeasures. AChE activity monitoring can

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88 S. Vučinić, B. Djurović, B. Antonijević; Occupational and environmental exposure, prevention and Chemical Weapons Convention, in: *Basic and Clinical Toxicology of Mustard Compounds*; M. Balali-Mood, M. Abdollahi (Eds.); Springer International Publishing, Switzerland, 2015, 389-401. ISBN: 978-3-319-23873-9

89 A. Ghorani-Azam, M. Balali-Mood; Clinical Pharmacology and Toxicology of Mustard Compounds, in: *Basic and Clinical Toxicology of Mustard Compounds*, M. Balali-Mood, M. Abdollahi (Eds.); Springer International Publishing, Switzerland, 2015, 63-100. ISBN: 978-3-319-23873-9

90 M. Maleki, P. Layegh; Dermatologic aspects of sulfur mustard, in: *Basic and Clinical Toxicology of Mustard Compounds*, M. Balali-Mood, M. Abdollahi (Eds.); Springer International Publishing, Switzerland, 2015, 213-252. ISBN: 978-3-319-23873-9

91 K. Kahe, F. Balszuweit, J. Emmler, H. Kreppel, M. Jochum, H. Thiermann; Sulfur mustard research - strategies for the development of improved medical therapy. *Eplasty,* 2008, *8*, e32. Available at: www.ncbi.nlm.nih.gov/pmc/articles/PMC2431646

be of critical importance to recognise exposure in this regard, before signs and symptoms of poisoning intensify.

(b) Oximes are not universally effective against AChE inhibited by different CWAs – more work is required to better identify the most suitable oximes against specific NAs.

(c) Dosage must also be optimised for specific oximes due to the possibility of undesired side effects if they are administered in high dose.

(d) The use of adjunct treatments such as sodium bicarbonate was discussed.[92,93,94] Severe OP poisoning can lower the pH of blood and it can be useful to raise it back toward the physiological value of 7.4.

(e) Countermeasures for lewisite, such as British Anti-Lewisite (BAL) and the sodium salt of 2,3-dimercapto-1-propanesulfonic acid (DMPS), chelate and remove arsenic from the site of injury, reducing the vesicant effect. It was noted that BAL can transfer arsenic across the blood brain barrier because of the high lipophilicity of the BAL-Lewisite adduct. More hydrophilic chelating agents are preferable in cases of organoarsenical poisoning to prevent a similar penetration of the BBB from being able to occur.

(f) There are many manuals and published procedures on how to respond to chemical incidents, but there is little standardisation. Even certain commonly referred to medical countermeasure drugs are no longer used by some militaries (e.g. pralidoxime (2-PAM)). Response procedures are generally specifically tailored to certain groups (e.g. soldiers in a battlefield situation, emergency responders, etc.), and for a general civilian population, may not represent best practice. A compilation of information categorised by whom it applies best to, could usefully serve as a reference collection.

Subitem 8(b): New strategies for medical countermeasures against cyanide

8.16 Professor Fréderic Baud (Assistance Publique - Hôpitaux de Paris) provided an overview of strategies for medical countermeasures against cyanide, summarising the 2013 report of the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) task force on the efficacy and safety of antidotes for acute poisoning by cyanides.[95] Antidotes were considered for direct poisonings with hydrocyanic acid or its salts, poisoning with cyanogenic compounds, smoke inhalation, and unknown substance poisoning where cyanide toxicity symptoms are found.

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92 M. Balali-Mood, M. H. Ayati, H. Ali-Akbarian Hassan; Effect of high doses of sodium bicarbonate in acute organophosphorous pesticide poisoning; *Clinical Toxicology*, 2005, *43(6)*, 571-574.

93 M. Balali-Mood, H. Saber; Recent advances in the treatment of organophosphorous poisonings; *Iran J. Med Sci.*, 2012, *37(2)*, 74–91.

94 M. Moshiri, E. Darchini-Maragheh, M. Balali-Mood; Advances in toxicology and medical treatment of chemical warfare nerve agents; *Daru*. 2012; *20(81)*. DOI: 10.1186/2008-2231-20-81

95 Efficacy and safety of antidotes for acute poisoning by cyanides (ECETOC, Technical Report 121, dated November 2013). Available at: http://www.ecetoc.org/publication/tr-121-efficacy-and-safety-of-antidotes-for-acute-poisoning-by-cyanides/

8.17 From the work of the task force, it was found that sodium thiosulfate acts rapidly, but when administered alone, is only effective in cases of moderate poisoning; amyl nitrite, when administered alone, can be effective in moderate to severe poisonings; and, hydroxocobalamin, when administered alone, was effective for severe poisonings. Combinations of sodium nitrite and sodium thiosulfate, with or without amyl nitrite, and of 4-dimethylaminophenol and sodium thiosulfate were also found to be at least partially effective. The methaemoglobin-forming agents all required combination with sodium thiosulfate for effective antidoting. Sodium thiosulfate can also be administered after administration of direct-acting antidotes in situations when a delayed formation of cyanides might occur. The report contains recommendations for different poisoning circumstances and severities.

8.18 In the subsequent discussion, the following points were raised:

(a) For stockpiling of supplies for emergency response, both mild and severe poisonings should be considered (to help reduce the cost involved in holding large stocks of reagents best suited to severe cases).

(b) In instances of smoke inhalation, there are hundreds of chemicals that can form from burning materials. Clinical recognition of symptoms for poisoning by specific chemicals remains critical to identify an appropriate medical countermeasure.

Subitem 8(c): Current and emerging strategies for organophosphate decontamination

8.19 Professor Eric Chabriére (Aix-Marseille University) briefed the workshop on both validated and emerging strategies for organophosphate decontamination.[96] He reviewed approaches that include chemical, physical, and biological methods, with special attention to the use of decontaminating enzymes. Considerable efforts have been dedicated during the past decade to the development of efficient OP degrading biocatalysts. Among these, the promising biocatalyst SsoPox isolated from the archaeon *Sulfolobus solfataricus* appears to be particularly attractive for external decontamination purposes with regard to both its catalytic and stability properties.

8.20 In the subsequent discussion it was noted that decontamination of people, equipment and the environment involves many types of materials and material properties. Best practices for use of decontamination solutions in contact with different surfaces and materials are important to understand.

9. AGENDA ITEM NINE – Adoption of the Report

The SAB members in attendance considered and adopted the report of the workshop on "Chemical Warfare Agents: Toxicity, Emergency Response and Medical Countermeasures".

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96 P. Jacquet, D. Daudé, J. Bzdrenga, P. Masson, M. Elias, E. Chabrière; Current and emerging strategies for organophosphate decontamination: special focus on hyperstable enzymes; *Environ Sci Pollut Res*, 2016, *23(9)*, 8200-8218. DOI 10.1007/s11356-016-6143-1

The SAB thanked the SGDSN and especially Brigadier General Thierry Carlier (Deputy Director International, Strategic and Technological Affairs, of the SGDSN) for their generosity in hosting this meeting and kind hospitality throughout the event.

10. AGENDA ITEM TEN – Closure of the Workshop

The Chairperson closed the workshop at 16:45 on 27 September 2016.

Annex: List of Participants at the Workshop on Chemical Warfare Agent Toxicity, Emergency Response and Medical Countermeasures.

Annex

**LIST OF PARTICIPANTS
AT THE WORKSHOP ON CHEMICAL WARFARE AGENT TOXICITY,
EMERGENCY RESPONSE AND MEDICAL COUNTERMEASURES**

	Participant	Institution
1.	Professor Mohammad Abdollahi*	Tehran University of Medical Sciences, the Islamic Republic of Iran
2.	Dr Abdullah Saeed Al-Amri*	Saudi Basic Industries Corporation, Riyadh, Saudi Arabia
3.	Profesor Isel Pascual Alonso*	University of Havana, Cuba
4.	Mr Dominique Anelli	Consultant, France
5.	Professor Mahdi Balali-Mood	Mashhad University of Medical Sciences, the Islamic Republic of Iran
6.	Professor Fréderic Baud	Assistance Publique - Hôpitaux de Paris, France
7.	Dr Augustin Baulig*	Secrétariat Général de la Défense et de la Sécurité Nationale (SGDSN), Paris, France
8.	Lt Col Gaétan Boireau	État-Major des Armées (EMA), Paris, France
9.	Professor Eric Chabriére	Aix-Marseille University, France
10.	Ms Nathalie Chaptal-Gradoz	Institut de Radioprotection et de Sûreté Nucléaire (IRSN), Paris, France
11.	Dr Marie-Laure Cointot	Direction Générale de l'Armement Maîtrise NRBC (DGA-MNRBC), France
12.	Dr David Daudé	Gene & Green TK, Marseille, France
13.	Dr Isidore Decostaire	Haut Fonctionnaire de Défense et de Sécurité-Ministère de l'Économie et des Finances (HFDS MINEFI), Paris, France
14.	Ms Claire Delessard	Ministère des Affaires Étrangères et du Dévelopement International, France
15.	Col Fréderic Dorandeu	Le Département de Toxicologie et Risques Chimiques de l'Institut de Recherche Biomédicale des Armées (IRBA), France
16.	Dr Brigitte Dorner	Robert Koch Institute, Berlin, Germany
17.	Dr Jonathan Forman	Organisation for the Prohibition of Chemical Weapons (OPCW)
18.	Dr John Jenner	Defence Science and Technology Laboratory (Dstl), Porton Down, United Kingdom of Great Britain and Northern Ireland
19.	Dr Zrinka Kovarik*	Institute of Medical Research and Occupational Health, Croatia
20.	Dr Lionel Lachenaud	Secrétariat Général de la Défense et de la Sécurité Nationale (SGDSN), Paris, France
21.	Col Xavier Liffrran	Direction Générale des Relations Internationales et de la Stratégie, France
22.	Mr Philippe Louvet	Direction Générale de l'Armement Maîtrise NRBC (DGA-MNRBC), France
23.	Professor Patrick Masson	Kazan Federal University, Russian Federation
24.	Professor Matthew Meselson	Harvard University, United States of America
25.	Lt Col Ivana Moravcová	JCBRN Defence COE, Czechia

	Participant	Institution
26.	ICA Yannick Morel	Direction Générale de l'Armement (DGA), France
27.	Dr Nicia Maria Fusaro Mourão*	Brazilian Chemical Industry, São Paulo, Brazil
28.	Dr Florian Nachon	Département de Toxicologie et Risques Chimiques, Institut de Recherche Biomédicale des Armées (IRBA), France
29.	Mr William Paccoud	Institut de Radioprotection et de Sûreté Nucléaire (IRSN), Paris, France
30.	Ms Marlene Payva	Organisation for the Prohibition of Chemical Weapons (OPCW)
31.	Dr Sandrine Perreira	Laboratoire Central de la Préfecture de Police, France
32.	Professor Pierre-Yves Renard	Université de Rouen, Bioorganic Chemistry Laboratory, France
33.	Dr Helen Rice	Defence Science and Technology Laboratory (Dstl), Porton Down, United Kingdom of Great Britain and Northern Ireland
34.	IPA Morgane Riou	Direction Générale de l'Armement Maîtrise NRBC (DGA-MNRBC), France
35.	Mr Didier Schneider	PROTEUS and ENERSENS, PCAS Group, France
36.	Ms Pauline Seon	Secrétariat Général de la Défense et de la Sécurité Nationale (SGDSN), Paris, France
37.	Dr Koji Takeuchi*	National Institute of Advanced Industrial Science and Technology, Japan
38.	Mr Cheng Tang*	Office for the Disposal of Japanese Abandoned Chemical Weapons, Ministry of National Defence, China
39.	Dr Laurent Taysse	Direction Générale de l'Armement Maîtrise NRBC (DGA-MNRBC), France
40.	Dr Horst Thiermann	Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany
41.	Dr Christopher Timperley*	Defence Science and Technology Laboratory (Dstl), Porton Down, United Kingdom of Great Britain and Northern Ireland
42.	Dr Ferruccio Trifirò*	Department of Industrial Chemistry, University of Bologna, Italy
43.	Mr Bruno Vanlerberghe	Laboratoire Central de la Préfecture de Police, France
44.	Professor Paula Vanninen*	VERIFIN, Department of Chemistry, Faculty of Science, University of Helsinki, Finland
45.	Dr Slavica Vučinić	National Poison Control Centre, Military Medical Academy, Belgrade, Serbia
46.	Ms Farhat Waqar*	Pakistan Atomic Energy Commission
47.	Dr Franz Worek	Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany
48.	Professor Mongia Said Zina*	Faculty of Sciences of Tunis, Tunisia

* Member of the OPCW SAB