Conference paper

Elena Fischer, Marc-Michael Blum, Wesam S. Alwan[a] and Jonathan E. Forman*

Sampling and analysis of organophosphorus nerve agents: analytical chemistry in international chemical disarmament

DOI 10.1515/pac-2016-0902

Abstract: Chemistry is a science that contributes to all aspects of our everyday lives and our professions. There are clear examples in law enforcement (forensics) and public health and perhaps less clear (but equally important) uses of chemicals in applications that include automobile manufacturing, electronics, packaging materials, currency printing, and even waste management (recycling and value-added products from garbage). Chemistry can also influence international diplomacy – an area that is likely to be unfamiliar to many chemistry professionals. Take for example the United Nations led investigation into the alleged use of chemical weapons in Syria in August of 2013. Environmental and biomedical samples were collected and analyzed, and they undisputedly confirmed the use of the nerve agent sarin. The results were published in a report by the United Nations Secretary-General and were one of the many influences leading to the accession of The Syrian Arab Republic to the Chemical Weapons Convention (an international treaty prohibiting chemical weapons) and the declaration and dismantlement of a chemical weapons programme. Using this investigation as an example, we highlight some of the chemistry that influenced decision making in a high visibility international event.

Keywords: acetylcholinesterase; chemical weapons; nerve agent; Organisation for the Prohibition of Chemical Weapons; organophosphorus; sarin; 2016 Spring ConfChem; Syria; United Nations.

Introduction

This issue of *Pure and Applied Chemistry* looks at the contributions of the Organisation for the Prohibition of Chemical Weapons (OPCW) that were presented in a recent ConfChem [1], including papers describing the importance of outreach in achieving the goals of universal chemical disarmament [2], the role of responsible science and ethics in chemistry education [3–5], the use of sensors as educational tools for supporting scientific cooperation (a norm of the Chemical Weapons Convention) [6], and the chemistry and history of riot control agents [7]. These topics touch upon many important dimensions of the OPCW and its mission, yet they are certainly not what immediately comes to mind when most people think about the OPCW. Rather,

Article note: A collection of invited papers based on presentations at the Open Access Online Conference "Science, Disarmament, and Diplomacy in Chemical Education: The Example of the Organisation for the Prohibition of Chemical Weapons", which was held from 2nd May till 20th June 2016.

[a]Current affiliation: Monash Institute of Pharmaceutical Sciences, Parkville, Victoria, Australia.
***Corresponding author: Jonathan E. Forman,** Office of Strategy and Policy, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands, e-mail: jonathan.forman@opcw.org
Elena Fischer and Wesam S. Alwan: Office of Strategy and Policy, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands
Marc-Michael Blum: OPCW Laboratory, Organisation for the Prohibition of Chemical Weapons, Rijswijk, The Netherlands

the OPCW is likely to be familiar for receiving the Nobel Peace Prize in 2013[1] and for its role in international events related to the conflict in the Syrian Arab Republic.[2] In this paper, we look at some of the higher profile activities of the OPCW through describing chemistry that has contributed to international security affairs.

Allegations of alleged use of chemical weapons in Syria

Allegations of the use of chemical weapons in the Syrian civil war began surfacing in 2012, yet independent disarmament experts were unable to judge whether or not these claims were true given the evidence available to them [8, 9].

In March of 2013, the Deputy Prime Minister of the Syrian Arab Republic requested that the United Nations Secretary-General conduct a specialised, impartial, and independent investigation of one of the alleged incidents, while the governments of France and the United Kingdom of Great Britain and Northern Ireland requested additional incidents be investigated [10]. Negotiations on the modalities of the investigations followed, and it was only in August of 2013 that inspectors, led by the United Nations with staff from the OPCW and the World Health Organisation (WHO), entered Syria. Their mission was "…to ascertain the facts related to the allegations of the use of chemical weapons, and to gather relevant data and to undertake the necessary analyses for this purpose…" [10]. The mandate was to determine whether chemical weapons had been used. It was not to identify perpetrators.

Inspectors arrived in Damascus on 18 August for what was to be a 2-week mission. Three days later on 21 August, a flurry of videos, camera phone images, and social media posts began reporting a chemical attack in the Damascus suburb of Ghouta [11] where as many as 1400 people are thought to have died. Given their presence in the country, inspectors were able to access the site from 26 to 29 August, only a few days after the reported incident. They conducted interviews with survivors and medical responders, and collected both biomedical (blood, urine, and hair) and environmental (soil, fragments, surface wipes and scrapings, and clothes) samples. Analysis of the samples confirmed the use of the nerve agent sarin [10]. This is where chemistry enters the story.

Organophosphorus nerve agents

Nerve agents are amide or ester derivatives of phosphonic acid (Fig. 1) [12]. These chemicals are highly potent inhibitors of the enzyme acetylcholinesterase (AChE). The inhibition results in cholinergic hyper-stimulation and a continual transmission of nerve impulses that prevents contracting muscles from relaxing (Fig. 2) [14]. Clinical presentation of nerve agent poisoning includes hypersecretion and miosis, which can lead to seizures, respiratory failure, and ultimately paralysis and death. Videos posted online of victims of the Ghouta incident supported the suspicion of exposure to nerve agents [11].

Nerve agents inhibit AChE through attachment to a serine residue within the active site of the enzyme, blocking enzymatic activity (Fig. 2b) [13]. The inhibited serine can undergo a second process referred to as "aging", releasing an alkoxy group from the phosphorus atom and leaving an anionic phosphonate residue (Fig. 2b) [13]. The aged protein adduct is not reversible; its normal function does not return [13]. The kinetics of inhibition and ageing are influenced by the substituents and leaving groups on the phosphorus atom. Significant differences can actually be observed with stereoisomers of nerve agent [15]. Before ageing occurs, the primary protein adduct can undergo hydrolysis resulting in a reactivated enzyme and a phosphonic acid

1 The 2013 Nobel Peace Prize was awarded to Organisation for the Prohibition of Chemical Weapons "for its extensive efforts to eliminate chemical weapons" as described at: https://www.nobelprize.org/nobel_prizes/peace/laureates/2013/.

2 More information and links to official documents of these missions can be found at: www.opcw.org/special-sections/syria-and-the-opcw/.

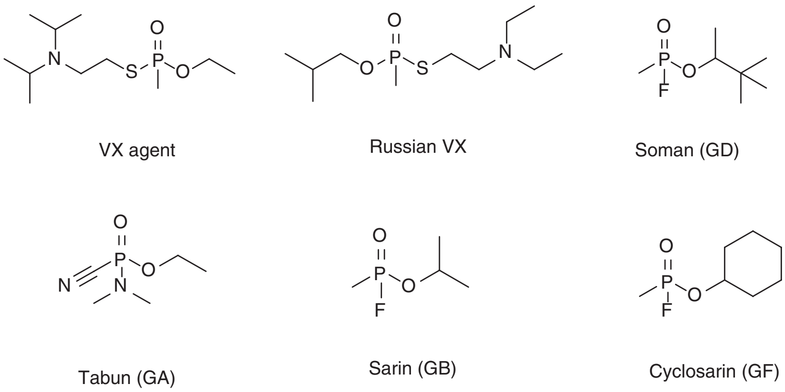

Fig. 1: Representative organophosphorus nerve agents.

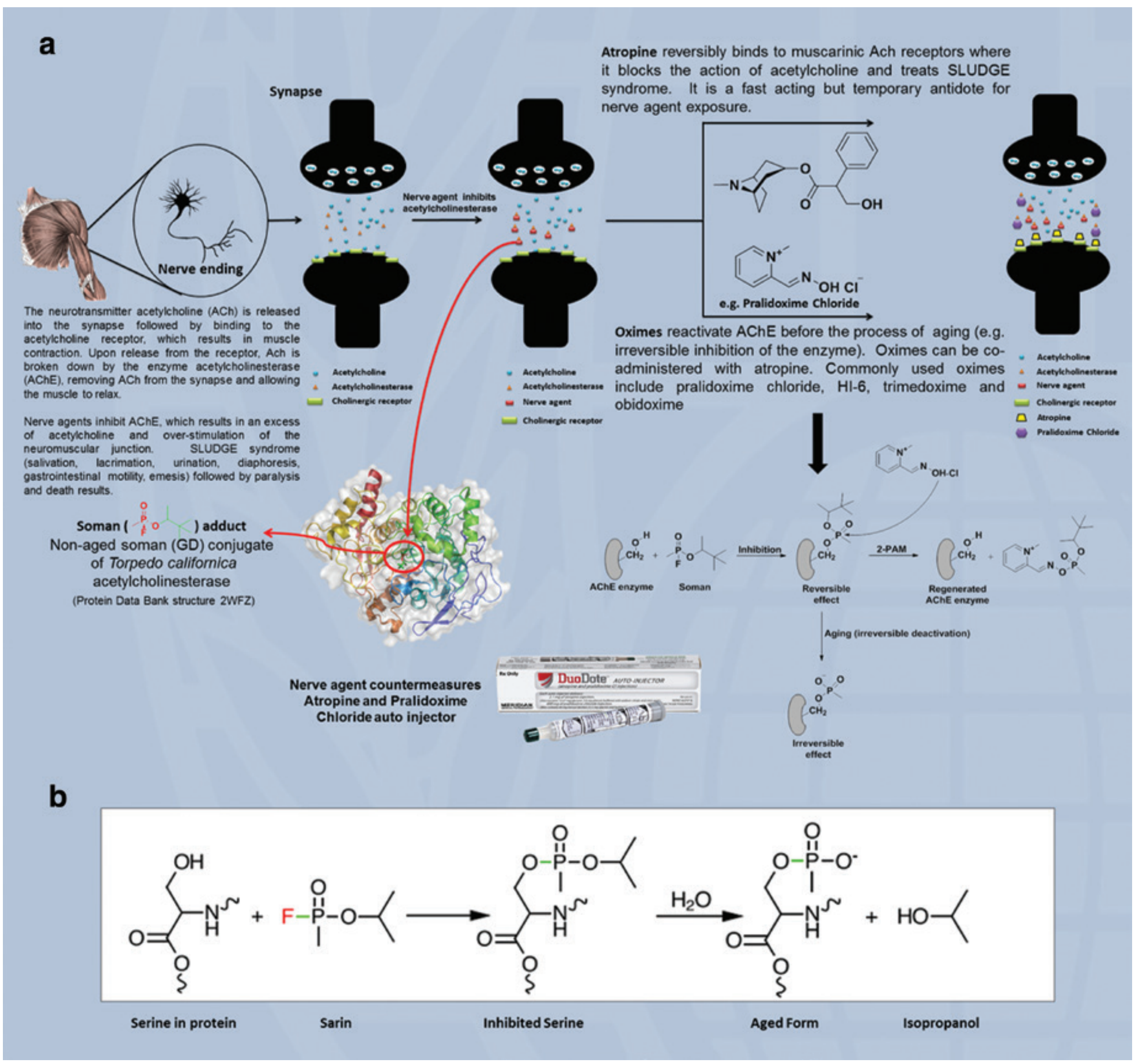

Fig. 2: (a) Method of action of nerve agent countermeasures used in treatment/prevention of toxic effects. (b) Chemistry of nerve agent (sarin) inhibition of AChE and ageing of the enzyme-inhibitor adduct [13].

(isopropyl methylphosphonic acid, IMPA, in the case of sarin). This is however a slow process for the highly toxic nerve agents [13]. Reactivation with nucleophilic compounds such as quaternary oximes can be used, in some cases, to reactivate the inhibited AChE and restore normal function. Oximes, such as 2-PAM (Pralidoxime) as shown in Fig. 2a, are commonly used as countermeasures to nerve agent poisoning [16]. Treatment of nerve agent exposure involves combinations of muscarinic receptor binding compounds (atropine, Fig. 2a) to block the action of enhanced ACh concentrations, and oximes for reactivation of inhibited AChE [16].

Nerve agents as chemical weapons would typically be delivered as aerosolized material, such that the respiratory system is the primary portal of entry into the body. However, nerve agents can also be absorbed through skin, which can result in exposure to unprotected emergency responders while treating a casualty (as well as requiring adequate decontamination for environmental presence of agents). Figure 3 illustrates comparative toxicities of a variety of chemical warfare agents, the position on the *y*-axis indicating median lethal concentration (LCt_{50} in mg min/m^3 for inhalation) and the position on the *x*-axis indicating median lethal dose (LD_{50} in mg/kg bodyweight for dermal exposure). The nerve agents, being in the lower left corner of the plot, display the greatest toxicity of all the agents.

Sampling and analysis

Inspectors conducting an investigation gather a lot of information before any chemistry is actually performed. This information helps determine what type of samples might be best retrieved for analysis and what precautions may be required. In an incident such as the one that occurred at Ghouta, events on the

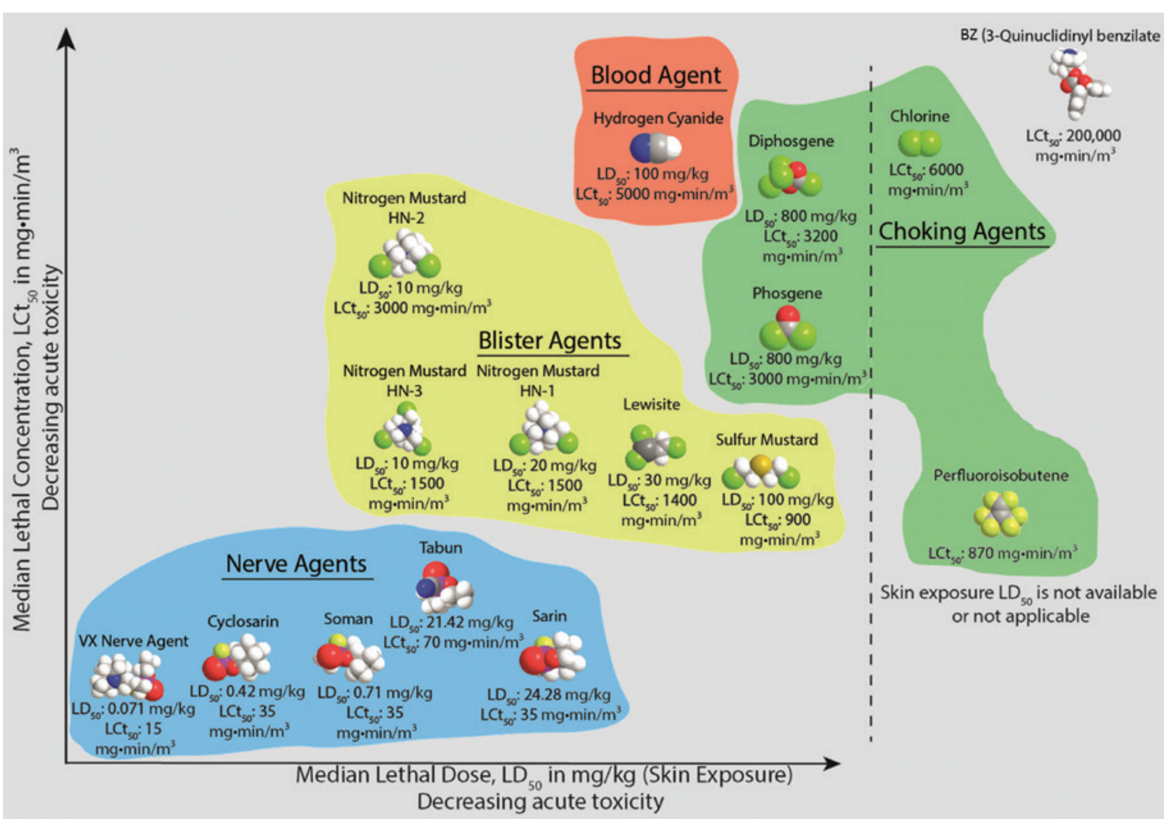

Fig. 3: Relative toxicity of representative chemical warfare agents. The compounds are arranged on a vertical scale that ranks the median lethal concentration (LCt_{50} in mg min/m^3 for inhalation) and a horizontal scale that ranks the median lethal dose (LD_{50} in mg/kg bodyweight for dermal exposure) [17–24].

ground are subject to unexpected change and take place in an uncertain and potentially dangerous security environment.

In the absence of verifiable information, inspectors must proceed with caution and have the necessary protective equipment to avoid chemical agent exposure. Hand-held detectors [25], such as Flame Photometric Detection (FPD) and ion mobility spectrometry (IMS) chemical agent monitors (Fig. 4), are typically employed as early warning devices. The FPD device will give signals when exposed to vapours containing phosphorus or sulfur compounds, and IMS devices will give signals when mass values of molecules contained within the instruments library are detected. These devices will aid inspectors in making decisions on where to take samples, however, these tools can also generate many false positives. More robust detection devices are necessary to confirm the presence of chemical agents. Such tools are not typically easily portable (especially in high risk environments), requiring inspectors to collect samples and send them to laboratories capable of more detailed chemical analysis.

The sample collection itself becomes a subject of scrutiny as ensuring samples do not decompose before analysis and chain of custody of the samples in transit to the analysis laboratory is critical for impartial reporting. (The Report of the UN Secretary-General contains many details on the measures taken to ensure chain of custody for those interested). As a further means to insure impartiality and quality, samples are split and sent to two different laboratories with a track record of high-performance in OPCW Laboratory Proficiency Tests [26–29]. One of the laboratories that received environmental samples from Ghouta was the Spiez Laboratory in Switzerland. An account of the work done there was published in their 2013 Annual Report [30]. Analysts were instructed to look for sarin or similar compounds (e.g. other nerve agents) as these chemicals would be consistent with the symptoms observed in the victims of exposure [30].

Environmental samples from materials that are found to be contaminated with a chemical agent help to prove the use of the agent. Samples contaminated with the degradation products of the chemical agent provide additional evidence of the use or presence of chemical agents (degradation products may actually be detected without detection of unreacted chemicals agent). Inspectors collected munition fragments; soil samples, and materials such as textiles, rubber, and hair (Fig. 5). They also took wipe samples from surfaces believed to have been exposed. Environmental samples exposed to chemical agents typically have concentrations of characteristic molecules at parts per million or higher. At these concentrations, a survey analysis is possible, and analytes can be extracted from the samples and detected using gas chromatography-mass spectrometry (GC/MS) and/or liquid chromatography-mass spectrometry (LC/MS) [31].

The chemicals detected in these samples depend upon the agent that may have been used and its persistence in the environment. Sarin and other G-type nerve agents, have relatively high vapour pressures (about 0.1–1.0 mm Hg) and hydrolysis rates (about 0.03–0.1 h^{-1} at neutral pH) [31]. Thus depending on temperature, humidity and moisture content of the environment, the ability to detect the non-degraded agent may be time limited [31–33]. The hydrolysis of sarin to phosphonic acid degradation products is illustrated in

Fig. 4: Flame photometric detection (FPD) and ion mobility spectrometry (IMS) chemical agent monitors.

Fig. 5: Environmental samples sent to the Spiez Laboratory in Switzerland [Adapted from Ref. 30].

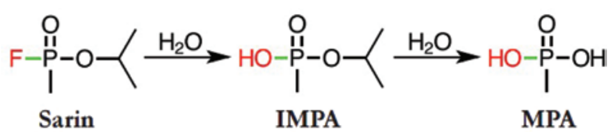

Fig. 6: Degradation pathway of the nerve agent sarin in water and the environment [13].

Fig. 6 [13, 32, 33]. Isopropyl methylphosphonic acid (IMPA), the first degradation product is stable for several months in water, in soil, and on other surfaces (however, this is pH dependent) and points directly to the previous presence of sarin. At neutral pH, IMPA hydrolyses slowly into the second degradation product, methylphosphonic acid (MPA). IMPA is the degradation product that would be indicative of sarin use, as MPA could also come from other organophosphonates (including other nerve agents, their precursors, and a variety of pesticides) [13]. The presence of the key marker IMPA was detected in 20 of 42 reported environmental samples from Ghouta, as recorded in the final report to the UN Secretary-General [34].

Environmental samples testing positive for nerve agents and/or their degradation products would indicate previous presence of the agents. To verify human exposure requires testing biomedical samples, such as urine or blood samples taken from casualties (exposed persons). Clinical samples are more difficult to analyze than environmental samples as the chemical agent, its adducts, and metabolites degrade and are excreted from the body. This gives a limited time window to collect and analyze samples. Additionally, concentrations in these samples are likely to be in the parts per billion range, requiring a targeted rather than a survey approach to the analysis (highlighting the importance of collecting as much information as possible to guide the analysts in selecting the most appropriate method and target to screen for) [35].

In the case of sarin exposure, metabolites such as the hydrolysis product IMPA, can be found in urine or blood, and blood samples can be analyzed for protein adducts. Free metabolites are typically eliminated from the body within a few days, while protein adducts may persist for several weeks. Nerve agents also react with proteins other than AChE, for example butyrylcholinesterase (BChE). BChE has an active site similar to that of AChE and a comparable molecular mechanism of inhibition when exposed to nerve agents [36]. However, unlike the membrane bound AChE, BChE is found in blood serum [36].

In the presence of excess sodium fluoride, non-aged sarin-protein adducts can be regenerated to release the intact agent, which can be detected by mass spectrometry [13, 37]. This method, called fluoride reactivation (Fig. 7), will not work with aged protein adducts. With other nerve agent adducts, the original chemical

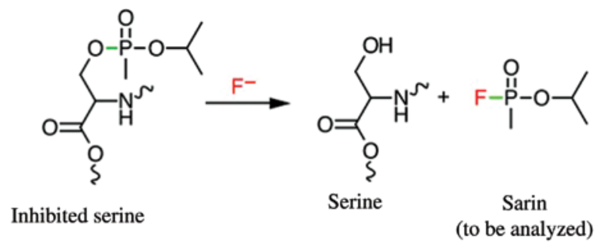

Fig. 7: Fluoride reactivation of sarin-protein adduct [13].

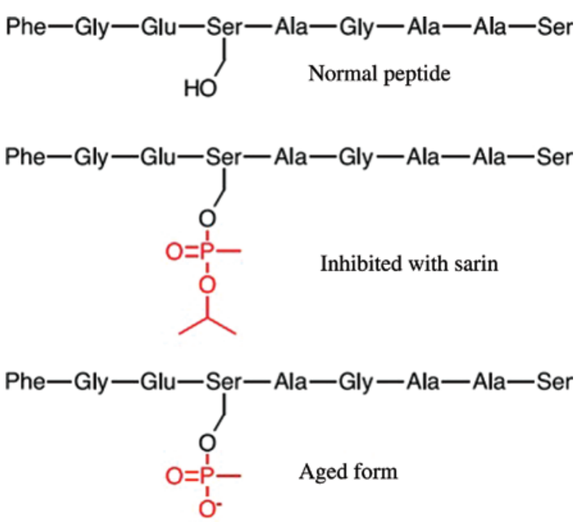

Fig. 8: Peptides indicative of sarin exposure [13].

agent may not be produced by fluoride reactivation. For example in the case of tabun, fluorotabun is produced, and in case of VX, ethylsarin results [13].

Direct analysis of protein-adducts is also possible (Fig. 8) using procedures that rely on BChE in human blood plasma. Instead of using the intact protein (consisting of 574 amino acids), the protein is digested using the enzyme pepsin, followed by separation with liquid chromatography and analysis by tandem mass spectrometry (LC-MS/MS) [13]. The characteristic peptide contains nine amino acids with a serine residue bonded to the nerve agent residue [13]. As the leaving group of the agent is lost when binding to AChE or BChE (with further loss of identifying alkoxy groups during the ageing process), this analysis cannot reveal the absolute identity of the nerve agent, but it does indicate human exposure. Protein adducts of serum albumin can also form with nerve agents [38] (and mustard agents [39]), providing other characteristic biomarkers of exposure.

Reporting

An interim report of the investigations confirming sarin in both environmental and biomedical samples was published on 16 September 2013 [40], and a final report (covering more investigated areas than Ghouta) was published on 12 December 2013 [10, 41]. The chemical analysis, supported by interviews of witnesses, clearly established the use of sarin. Here is where the chemistry steps aside and diplomacy moves forward.

Prior to the release of the interim report, following pressure from the international community, the Syrian Arab Republic signed the Chemical Weapons Convention, officially becoming its 190th State Party in October 2013 [14]. Subsequently, a OPCW-UN Joint Mission facilitated the removal and destruction of Syria's declared chemical weapons, along with destruction of chemical weapons production and storage facilities and mixing and filling equipment.[3] The removal of chemicals involved maritime cooperation among several governments [42], and the delivery of industrial chemicals and neutralisation effluents to disposal facilities in four countries (including the United States of America) was a truly international effort [43]. The story however, has continued with new allegations and questions regarding declarations [44], additional fact-finding missions [45–50], and the establishment of a Joint Investigative Mechanism to identify individuals or entities responsible for use of chemical weapons in the Syrian civil war [51, 52]. For a perspective on current issues in chemical security, we refer readers to a speech by the OPCW Director-General to the 19th International Chemical Weapons Demilitarisation Conference held recently in London.[4]

Chemistry alone certainly will not resolve political issues, but its application and the evidence base it supplies can play an important role in informing diplomatic negotiations and enabling political decisions on a complicated international stage.

References

[1] *2016 Spring ConfChem: Science, Disarmament, and Diplomacy in Chemical Education: The Example of the Organisation for the Prohibition of Chemical Weapons*. Published online at: http://confchem.ccce.divched.org/2016SpringConfChem.

[2] J. Ballard, J. E. Forman in *2016 Spring ConfChem: Science, Disarmament, and Diplomacy in Chemical Education: The Example of the Organisation for the Prohibition of Chemical Weapons*, Paper 1 (2016). Published online at: http://confchem.ccce.divched.org/2016SpringConfChemP1.

[3] A. G. Suárez in *2016 Spring ConfChem: Science, Disarmament, and Diplomacy in Chemical Education: The Example of the Organisation for the Prohibition of Chemical Weapons*, Paper 2 (2016). Published online at: http://confchem.ccce.divched.org/2016SpringConfChemP2.

[4] T. Engida in *2016 Spring ConfChem: Science, Disarmament, and Diplomacy in Chemical Education: The Example of the Organisation for the Prohibition of Chemical Weapons*, Paper 3 (2016). Published online at: http://confchem.ccce.divched.org/2016SpringConfChemP3.

[5] J. Apotheker in *2016 Spring ConfChem: Science, Disarmament, and Diplomacy in Chemical Education: The Example of the Organisation for the Prohibition of Chemical Weapons*, Paper 4 (2016). Published online at: http://confchem.ccce.divched.org/2016SpringConfChemP4.

[6] P. G. Mahaffy, K. J. Ooms, A. F. Tappenden, J. E. Forman, U. Mans, J. Sabou in *2016 Spring ConfChem: Science, Disarmament, and Diplomacy in Chemical Education: The Example of the Organisation for the Prohibition of Chemical Weapons*, Paper 5 (2016). Published online at: http://confchem.ccce.divched.org/2016SpringConfChemP5.

[7] C. Green, F. B. Hopkins, C. D. Lindsay, J. R. Riches, C. M. Timperley in *2016 Spring ConfChem: Science, Disarmament, and Diplomacy in Chemical Education: The Example of the Organisation for the Prohibition of Chemical Weapons*, Paper 6 (2016). Published online at: http://confchem.ccce.divched.org/2016SpringConfChemP6.

[8] J. P. Robinson, *Harvard Sussex Program Occasional Paper: Alleged Use of Chemical Weapons in Syria*, **4** (2013). Available on at: http://www.sussex.ac.uk/Units/spru/hsp/occasional%20papers/HSPOP_4.pdf.

[9] Complicating impartial information gathering from Syria is that much of the publicly accessible information has and continues to come from social media. See for example: M. Lynch, D. Freelon, S. Aday; *Syria's Socially Mediated Civil War*, United States Institute for Peace (2014). Available online at: http://www.usip.org/publications/syria-s-socially-mediated-civil-war.

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

[11] Y. Rosman, A. Eisenkraft; N. Milk, A. Shiyovich, N. Ophir, S. Shrot, MD; Y. Kreiss, M. Kassirer. *Ann. Intern. Med.* **160**, 644 (2014).

[12] S. W. Wiener, R. S. Hoffman. *J. Intensive Care Med.* **19**, 22 (2004).

[13] M.-M. Blum, M. Mamidanna, H. Gregg. *Conducting Analysis of Biomedical Samples to Assess Exposure to Organophosphorus Nerve Agents*; Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands (2014). Available online at: www.opcw.org/fileadmin/OPCW/Science_Technology/Diplomats_Programme/S_T_Biomed_Analysis_Poster.pdf.

3 More information and links to official documents of these missions can be found at: https://opcw.unmissions.org/.

4 This speech can be viewed at www.opcw.org/news/article/director-general-declares-opcws-readiness-to-help-states-counter-chemical-terrorism/.

[14] *Practical Guide for Medical Management of Chemical Warfare Casualties*; Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands (2016). Available online at: www.opcw.org/fileadmin/OPCW/ICA/APB/Practical_Guide_for_Medical_Management_of_Chemical_Warfare_Casualties_-_web.pdf.

[15] OPCW. *Response to the Director-General's Request to the Scientific Advisory Board to Provide Further Advice on Scheduled Chemicals*; SAB-23/WP.1, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands, paragraph 5.2 (2016). Available online at: www.opcw.org/fileadmin/OPCW/SAB/en/sab-23-wp01_e_.pdf.

[16] OPCW. *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, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands (2015). Available online at: www.opcw.org/fileadmin/OPCW/SAB/en/sab-22-wp02_e_.pdf.

[17] USAMRICS. *Medical management of chemical casualties Handbook*, US army medical research institute of chemical defense, chemical casualty care division; USAMRICD, Aberdeen Proving Ground, USA (2007).

[18] *Review of Acute Human Toxicity estimates of Selected Chemical Warfare Agents*, National Academy Press, Washington, DC, USA (1997).

[19] *Strategies to Protect the Health of Deployed U.S. Forces: Detecting, Characterizing, and Documenting Exposures*; T. E. McKone, B. M. Huey, E. Downing, L. M. Duffy (Eds.), National Academy Press, Washington, DC, USA (2000).

[20] M. Sharma, R. Vijayaraghavan, U. Pathak, K. Ganesan, *Def. Sci. J.* **59**, 512 (2009).

[21] A. Doro-on. *Risk Assessment for Water Infrastructure Safety and Security*, CRC Press, Boca Raton, Florida, USA (2011).

[22] *Toxnet, Toxicology data network*, Toxicology and Environmental Health Information Program (TEHIP) in the Division of Specialized Information Services (SIS) of the National Library of Medicine (NLM). USA. Accessible online at: https://toxnet.nlm.nih.gov/.

[23] H. P. van Helden van, D. van de Meent, J. P. Oostdijk, M. J. Joosen, J. H. van Esch, A. H. Hamme, R. V. Diemel. *Inhal. Toxicol.* **16**, 549 (2004).

[24] *Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 14*; National Academy Press, Washington, DC, USA (2013).

[25] A.-B. Gerber. *Spiez Laboratory Annual report 2015*; Pages 38–39 (2015). Available online at: http://www.labor-spiez.ch/en/dok/ge/pdf/88_003_e_laborspiez_jahresbericht_2015_web.pdf.

[26] OPCW. *Note by the Director-General: Status of Laboratories Designated for the Analysis of Authentic Samples*, S/1369/2016, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands (2016). Available online at: www.opcw.org/fileadmin/OPCW/S_series/2016/en/s-1369-2016_e_.pdf.

[27] OPCW. *Note by the Director-General: Status of the Laboratories Designated for the Analysis of Authentic Biomedical Samples*, S/1402/2016, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands (2016). Available online at: www.opcw.org/fileadmin/OPCW/S_series/2016/en/s-1402-2016_e_.pdf.

[28] OPCW. *Note by the Director-General: Evaluation of the Results of the Thirty-Eighth Official OPCW Proficiency Test*, S/1368/2016, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands (2016). Available online at: www.opcw.org/fileadmin/OPCW/S_series/2016/en/s-1368-2016_e_.pdf.

[29] OPCW. *Note by the Director-General: Evaluation of the Results of the First Official OPCW Biomedical Proficiency Test*, S/1401/2016, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands (2016). Available online at: www.opcw.org/fileadmin/OPCW/S_series/2016/en/s-1401-2016_e_.pdf.

[30] S. Mogl, P. Siegenthaler, B. Schmidt. *Spiez Laboratory, Annual report 2013*; 26–33 (2013). Available online at: http://www.labor-spiez.ch/en/dok/ge/pdf/88_003_e_laborspiez_jahresbericht_2013_web.pdf.

[31] M. L. Davisson, A. H. Love, A. Vance, J. G. Reynolds. *Environmental Fate of Organophosphorus Compounds Related to Chemical Weapons*, Lawrence Livermore National Laboratory (2005). Available online at https://e-reports-ext.llnl.gov/pdf/316349.pdf.

[32] N. B. Munro, S. S. Talmage, G. D. Griffin, L. C. Waters, A. P. Watson, J. F. King, V. Hauschild. *Environ. Health Perspect.* **107**, 933 (1999).

[33] OPCW. *Response to the Director-General's Request to the Scientific Advisory Board to Provide Further Advice on Chemical Weapons Sample Stability and Storage*, SAB-23/WP.2, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands (2016). Available online at: www.opcw.org/fileadmin/OPCW/SAB/en/sab-23-wp02_e_.pdf.

[34] United Nations Secretary-General. *United Nations Mission to Investigate Allegations of the Use of Chemical Weapons in the Syrian Arab Republic*, New York, USA, Appendix 5 (2013). Available online at: https://unoda-web.s3.amazonaws.com/wp-content/uploads/2013/12/report.pdf.

[35] N. D. Schulze, E. I. Hamelin Elizabeth, R. L. Shaner Rebecca, B. R. deCastro, B. G. Pantazides, J. D. Thomas; R. C. Johnson, W. R. Winkeljohn; B. J. Basden. *J. Anal. Toxicol.* **40**, 229 (2016).

[36] M. van der Schans, A. Fidder, D. van Oeveren, A. G. Hulst, D. Noort. *J. Anal. Toxicol.* **32**, 125 (2008).

[37] J. A. van der Meer, H. C. Trap, D. Noort, M. J. van der Schans; *J. Chromatogr. B* **878**, 1320 (2010).

[38] N. H. Williams, J. M. Harrison, R. W. Read, R. M. Black. *Arch. Toxicol.* **81**, 627 (2007).

[39] B. G. Pantazides, B. S. Crow, J. W. Garton, J. A. Quinones-Gonzalez, T. A. Blake, J. D. Thomas, R. C. Johnson. *Chem. Res. Toxicol.* **28**, 256 (2015).

[40] United Nations Secretary-General. *United Nations Mission to Investigate Allegations of the Use of Chemical Weapons in the Syrian Arab Republic, Report on the Alleged Use of Chemical Weapons in the Ghouta Area of Damascus on 21 August 2013*,

New York, USA (2013). Available online at: https://unoda-web.s3.amazonaws.com/wp-content/uploads/2013/12/report.pdf.

[41] UNODA. *The Secretary-General's Mechanism for Investigation of Alleged Use of Chemical, Bacteriological (Biological) or Toxin Weapons: A lessons-learned exercise for the United Nations Mission in the Syrian Arab Republic*, United Nations office of Disarmament Affairs, Geneva, Switzerland (2015). Available online at: https://www.un.org/disarmament/publications/more/syrian-ll-report.

[42] UNODA. *Workshop on the Lessons Learned from the International Maritime Operation to Remove and Transport the Syrian Chemical Materials in Furtherance of Security Council Resolution 2118 (2013) and Relevant OPCW Executive Council Decisions*, United Nations office of Disarmament Affairs, Geneva, Switzerland (2015). Available online at: https://unoda-web.s3-accelerate.amazonaws.com/wp-content/uploads/2015/05/proceedings-maritime-public.pdf.

[43] OPCW: *Removal and Destruction of Syrian Chemical Weapons*, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands (2014). Available online at: https://www.opcw.org/fileadmin/OPCW/files/Syra_Infographic.pdf.

[44] R. Trapp, Lessons Learned from the OPCW Mission in Syria", Chessenaz, France (2015). Available online at: https://www.opcw.org/fileadmin/OPCW/PDF/Lessons_learned_from_the_OPCW_Mission_in_Syria.pdf.

[45] OPCW. *Note by the Technical Secretariat: Summary report of the work of the OPCW Fact-Finding Mission in Syria covering the period from 3 to 31 May 2014*, Organisation for the Prohibition of Chemical Weapons, S/1191/2014, The Hague, The Netherlands (2014). Available online at: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20140616-1st-Chlorine-investigation-report.pdf.

[46] OPCW. *Note by the Technical Secretariat: Second report of the OPCW Fact-Finding Mission in Syria: Key findings,* Organisation for the Prohibition of Chemical Weapons, S/1221/2014, The Hague, The Netherlands (2014). Available online at: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20140910-2nd-Chlorine-investigation-report.pdf.

[47] OPCW. *Note by the Technical Secretariat: Third report of the OPCW Fact-Finding Mission in Syria,* Organisation for the Prohibition of Chemical Weapons, S/1230/2014, The Hague, The Netherlands (2014). Available online at: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20141218-3rd-Chlorine-investigation-report.pdf.

[48] OPCW. *Note by the Technical Secretariat: Report of the OPCW Fact-Finding Mission in Syria regarding the incidents described in communications from the Deputy Minister for Foreign Affairs and Expatriates and Head of the National Authority of the Syrian Arab Republic from 15 December 2014 to 15 June 2015,* Organisation for the Prohibition of Chemical Weapons, S/1318/2015/Rev.1, The Hague, The Netherlands (2015). Available online at: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20151217-Syria-request-Rev1.pdf.

[49] OPCW. *Note by the Technical Secretariat: Report of the OPCW Fact-Finding Mission in Syria regarding alleged incidents in the Idlib Governorate of the Syrian Arab Republic between 16 March and 20 May 2015*, Organisation for the Prohibition of Chemical Weapons, S/1319/2015, The Hague, The Netherlands (2015). Available online at: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20151029-Idlib-Governorate.pdf.

[50] OPCW. *Note by the Technical Secretariat: Report of the OPCW Fact-Finding Mission in Syria regarding alleged incidents in Marea, Syrian Arab Republic August 2015*, Organisation for the Prohibition of Chemical Weapons, S/1320/2015, The Hague, The Netherlands (2015). Available online at: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20151029-Marea.pdf.

[51] United Nations Secretary-General. *First report of the Organisation for the Prohibition of Chemical Weapons-United Nations Joint Investigative Mechanism*, S/2016/142, United Nations, New York, United States of America (2016). Available online at: http://www.securitycouncilreport.org/atf/cf/%7B65BFCF9B-6D27-4E9C-8CD3-CF6E4FF96FF9%7D/s_2016_142.pdf.

[52] United Nations Secretary-General. *Third report of the Organisation for the Prohibition of Chemical Weapons-United Nations Joint Investigative Mechanism*, S/2016/738, United Nations, New York, United States of America (2016). Available online at: http://www.un.org/ga/search/view_doc.asp?symbol=s/2016/738.