Sampling and Analysis of Organophosphorus Nerve Agents:
Analytical Chemistry in International Chemical Disarmament

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Abstract:

Chemistry is an extremely important field of science, one that contributes to applications across the broad range of sectors that intersect with our everyday lives. There are clear examples in law enforcement (forensics) and public health; less clear, but equally important uses of chemicals might include automobile manufacture, electronics, packaging materials, currency printing, or waste management (recycling and value-added products from garbage). Chemistry can also influence world events and international diplomacy.

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; 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.

Paper:

Introduction

Looking back at the past six weeks of this Organisation for the Prohibition of Chemical Weapons (OPCW) focused ConfChem, we have discussed papers describing the importance of outreach and awareness raising in achieving the goals of universal chemical disarmament,[1] the role of responsible science and ethics in chemistry education,[2-4] the use of sensors as educational tools for supporting scientific cooperation (a norm of the Chemical Weapons Convention),[5] and the chemistry and history of riot control agents.[6] 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, the OPCW is likely to be most familiar for receiving the Nobel Peace Prize in 2013[7] and for its role in international events related to the conflict in the Syrian Arab Republic.[8] In this paper we seek to bring a perspective on some of the higher profile activities of the OPCW through a look at 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.[9, 10]

In March of 2013, the Deputy Prime Minister of the Syrian Arab Republic requested the United Nations Secretary-General to conduct a specialized, 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.[11] 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 Organization (WHO), entered Syria; their mission "…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…".[12] The mandate was to confirm if chemical weapons had been used, it was not to identify perpetrators.

Inspectors arrived in Damascus on 18 August for what was to be a two-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;[13] an incident, 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; conducting interviews with survivors and medical responders, and collecting both biomedical (blood, urine, hair) and environmental (soil, fragments, surface wipes and scrapings, clothes) samples.

Analysis of the samples confirmed the use of the nerve agent sarin.[14] And this is where chemistry enters the story.

Organophosphorus Nerve Agents

Nerve agents are amide or ester derivatives of phosphonic acid (Figure 1).[15] These chemicals are highly potent inhibitors of the enzyme acetylcholinesterase (AChE), inhibition of which results in cholinergic hyper-stimulation (and a continual transmission of nerve impulses that prevents contracting muscles from relaxing; Figure 2).[16] Clinical presentation of nerve agent poisoning includes hypersecretion and miosis which can lead to seizures, respiratory failure, and death; observations supporting the exposure to nerve agents were seen in videos posted online of victims of the Ghouta incident.[13]

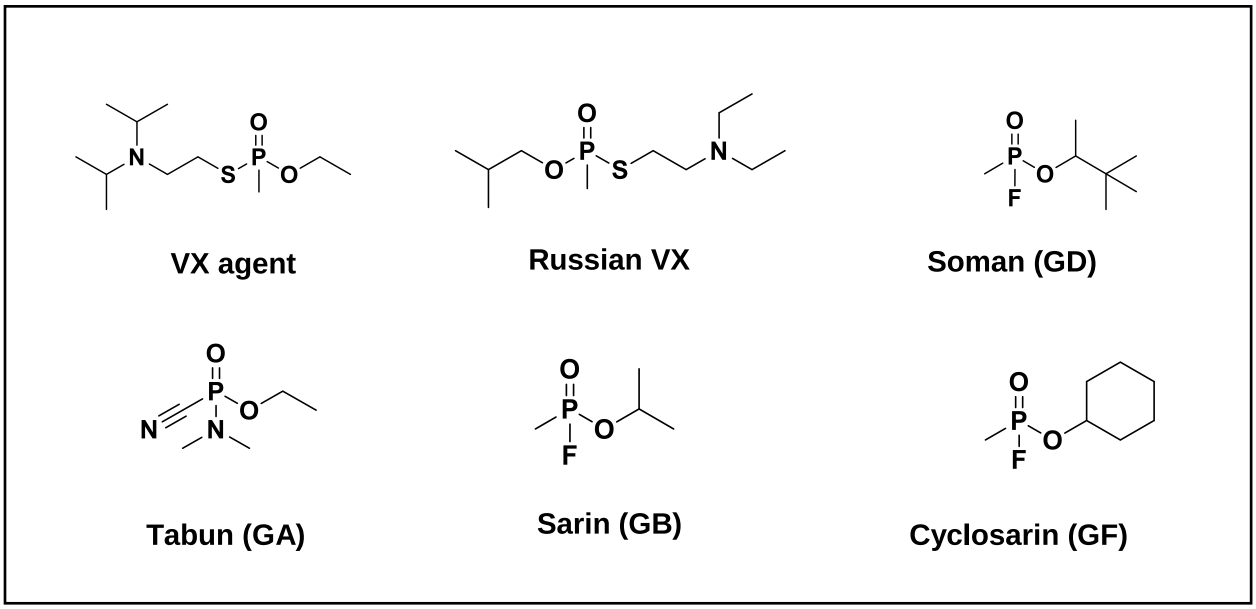


Figure 1: Representative Organophosphorus Nerve Agents

Nerve agents inhibit AChE through attachment to a serine residue within the active site of the enzyme, blocking enzyme activity (Figure 2B).[17] The inhibited serine can undergo a second process referred to as “aging”, releasing an alkoxy group from the phosphorus and leaving a anionic phosphonate residue (Figure 2B).[17] The aged protein adduct is not reversible; its normal function does not return.[17] The kinetics of inhibition and aging are influenced by the substituents and leaving groups on the phosphorous atom, significant differences can actually be observed with stereoisomers of nerve agent.[18] Before ageing occurs, the primary protein adduct can undergo hydrolysis resulting in a reactivated enzyme and a phosphonic acid (isopropylmethylphosphonic acid, IMPA, in the case of sarin), this is however a slow process for the highly toxic nerve agents.[17] Reactivation with nucleophilic compounds such as quaternary oximes can be used, in some cases, to reactivate the AChE and restore its normal function. Oximes, for example 2-PAM (Pralidoxime) as shown in Figure 2A are commonly used as countermeasures to nerve agent poisoning.[19] Treatment of nerve agent exposure involves combinations of acetylcholine (ACh) receptor binding compounds (atropine, Figure 2A) to block the action of enhanced ACh levels and oximes for reactivation.[19]

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. 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 the chemical warfare agents, the position on the *y*-axis indicating median lethal concentration (LC_{t50} 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.

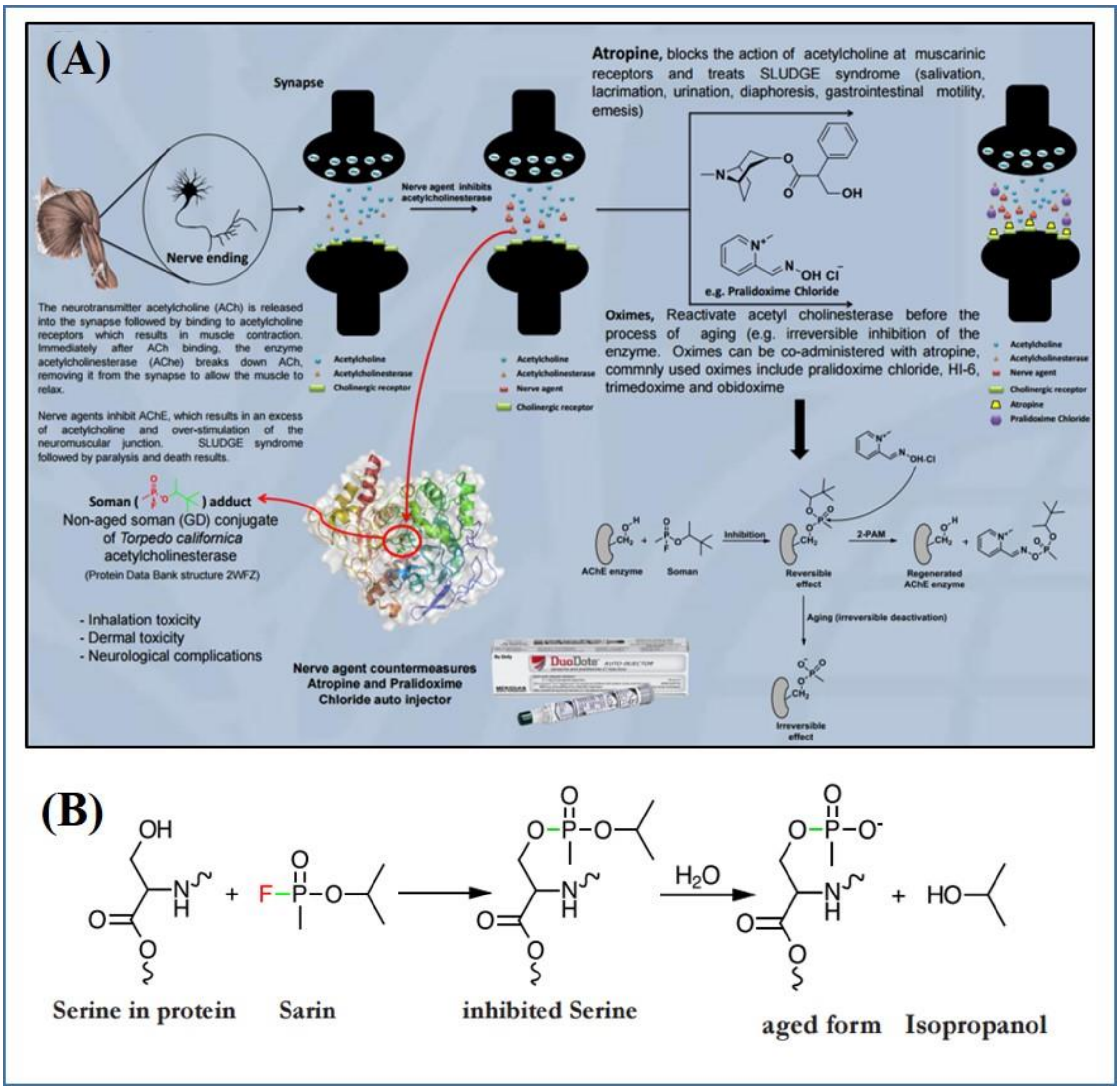


Figure 2: (A) Method of action of nerve agents countermeasures used in treatment/prevention of toxic effects. **(B)** Chemistry of inhibition and aging illustrated with sarin.[17]

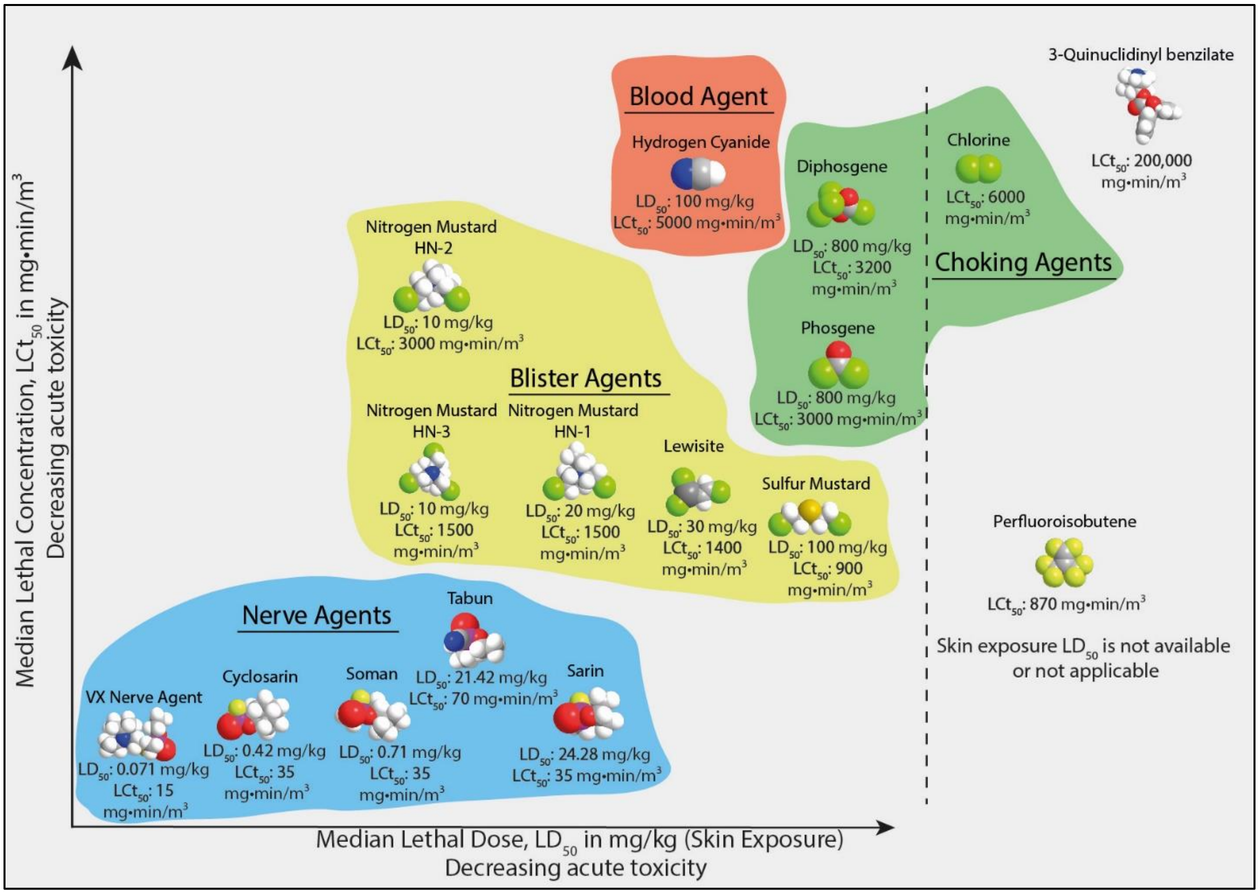


Figure 3: Relative toxicity of representative chemical warfare agents. The compounds are arranged on a vertical scale that ranks the median lethal concentration (LC_{t50} in mg min/m^3 for inhalation) and a horizontal scale that ranks the median lethal dose (LD_{50} in mg/kg bodyweight for dermal exposure).[20]

Sampling and Analysis

Inspectors conducting an investigation gather a number of types of information before any chemistry is actually performed – much of this is not chemical analysis, but the information helps determine what type of samples might be best retrieved for analysis and what pre-cautions may be required. In an incident such as the one that occurred at Ghouta, events on the ground are fluid, subject to unexpected change and take place in an uncertain and potentially dangerous security environment.

In the absence of verifiable information, inspectors must be diligent about protective equipment and proceeding with caution, to keep themselves safe from chemical agent exposure should it be present. Hand-held detectors,[21] such as Flame Photometric Detection (FPD) and Ion Mobility Spectrometry (IMS) chemical agent monitors (Figure 4), are typically employed as early warning devices. The FPD device will give signals when exposed to vapors containing phosphorus or sulfur compounds, and IMS devices will give signals when mass values of molecules in its library are detected – while valuable for warning systems and to inform on where to take samples, these tools can also generate many false positives. More robust methods of detection are necessary to confirm the presence of chemical agents. However such tools are not typically easily portable (especially in high risk environments), requiring that inspectors collect samples and send them to laboratories capable of more detailed chemical analysis.

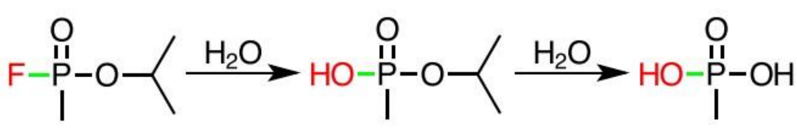
Figure 4: Flame Photometric Detection (FPD) and Ion Mobility Spectrometry (IMS) chemical agent monitors.

The sample collection itself becomes a subject of scrutiny as sample integrity and ensuring 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). Samples are split and provided to two different laboratories with a track record of high-performance in OPCW Laboratory Proficiency Tests,[22] as a further means to insure impartiality and quality. One of the laboratories that received environmental samples from Ghouta was the Spiez Laboratory in Switzerland, which published an account of this work in their 2013 Annual Report.[23] Analysts were instructed to look for sarin or similar compounds as these agents would be consistent with the symptoms observed in victims of exposure.[23]

Environmental samples from materials that have been in direct contact with chemical agent or contain high concentration of agent (or its associated degradation products) provide strong evidence of the use or presence of chemical agents. Inspectors collected munition fragments; soil samples, and materials such as textiles, rubber, and hair (Figure 5). They may also take wipe samples from surfaces believed to have been exposed. Environmental samples exposed to chemical agents typically have concentrations of characteristic molecules in parts per million or higher. At these concentrations, a survey analysis is possible, and analytes can be extracted from the samples and detected by the techniques of gas chromatography-mass spectrometry (GC/MS) and/or liquid chromatography-mass spectrometry (LC/MS).[24]

Figure 5: Environmental samples sent to the Spiez Laboratory in Switzerland.[23]

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 to 1.0 mm Hg) and hydrolysis rates (about 0.03 to 0.1 hr^{-1} at neutral pH).[24] Thus depending on temperature, humidity and moisture content of the environment the ability to detect the non-degraded agent may be time limited.[24-26] The hydrolysis of sarin to a phosphonic acid degradation product is illustrated in Figure 6.[17, 25, 26] 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).[17] The presence of the key marker IMPA was detected in 20 of 42 reported environmental samples from Ghouta, as recorded the final report to the UN Secretary-General.[27]



Sarin IMPA MPA

Figure 6: pathway of the nerve agent sarin in water and the environment.[17]

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, concentration levels 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 needed to guide the analysts in selecting the most appropriate method and target to screen for).[28]

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 can 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.[29] However, unlike the membrane bound AChE, BChE is found in blood serum.[29]

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.[17, 30] This method, called fluoride reactivation (Figure 7), will not work with aged protein adducts. With other nerve agent adducts, the original chemical 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.[17]

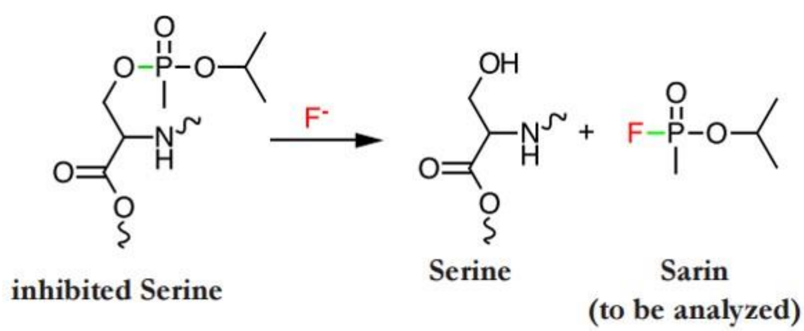


Figure 7: Fluoride reactivation of sarin-protein adduct.[17]

Direct analysis of protein-adducts is also possible (Figure 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).[17] The characteristic peptide contains nine amino-acids with a serine residue bonded to the nerve agent residue.[17] As the leaving group of the agent is lost when binding to AChE or BChE (with further loss of identifying alkoxy groups during the aging 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[31] (and mustard agents)[32] providing other characteristic biomarkers of exposure.

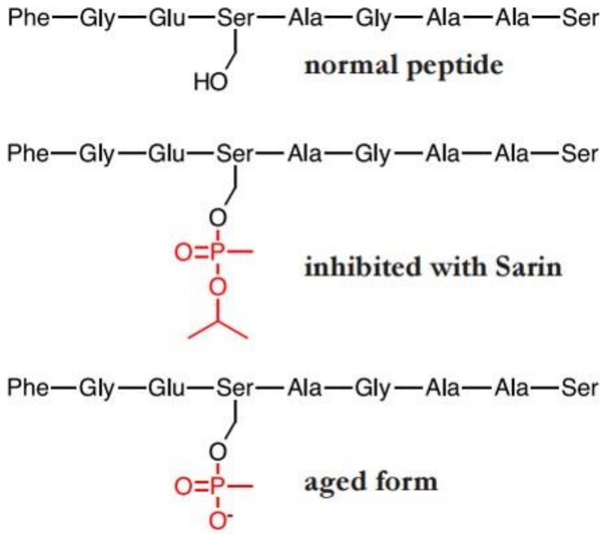


Figure 8: Peptides indicative of sarin exposure.[17]

Reporting

An interim report of the investigations confirming sarin in both environmental and biomedical samples was published on 16 September 2013[33] and a final report (covering more investigated areas than Ghouta) on 12 December 2013.[14] The chemical analysis, supported by interviews of witnesses, clearly established the use of sarin. And 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.[34] 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.[34,35] The removal of chemicals involved maritime cooperation amongst several governments,[36] and the delivery of industrial chemicals and neutralization effluents to disposal facilities in four countries (including the United States of America) – a truly international effort.[37] The story however, has continued[38] with new allegations,[8] additional fact-finding missions,[8] and the establishment of a Joint Investigative Mechanism to identify individuals or entities responsible for use of chemical weapons in the Syrian civil war.[39] For a perspective on current issues in chemical security, we refer readers to a recent speech by the OPCW Director-General to the 19th International Chemical Weapons Demilitarisation Conference, held recently in London.[40]

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

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