Chemical agents of warfare and their detection in biomedical samples

Agents chimiques de guerre et leur détection dans les échantillons biomédicaux

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Abstract

For centuries, toxic chemicals have been used in wars, conflicts and more recently, even in terrorist attacks. Although nowadays there is greater awareness of its danger, the threat continues to exist. For this reason, it is necessary to continue developing preventive strategies and other measures aimed at mitigating damage in case of exposure to chemical warfare agents. Toxidrome can be a tool for medical responders in cases where a specific treatment is available and cannot wait for confirmation from the laboratory. Assessing and verifying exposure to these chemical weapons is an excellent capability. Not only would it guide accurate medical diagnosis and treatment of victims, but it would also provide solid evidence in case of violation of the international norms agreed upon the Chemical Weapons Convention (CWC). However, only highly specialized and qualified analytical laboratories, such as those designated by the Organisation for the Prohibition of Chemical Weapons (OPCW), can provide unequivocal results regarding the exposure to chemical warfare agents.
Keywords: Biological sample, chemical warfare agent, Chemical Weapons Convention

Résumé

Pendant des siècles, des produits chimiques toxiques ont été utilisés lors de guerres, de conflits et, plus récemment, même lors d'attaques terroristes. Malgré une meilleure prise de conscience du danger, la menace persiste. C'est pourquoi il est nécessaire de continuer à développer des stratégies préventives et d'autres mesures visant à atténuer les dommages en cas d'exposition à des agents de guerre chimique. Le toxidrome peut être un outil pour les intervenants médicaux dans les cas où un traitement spécifique est disponible et ne peut attendre la confirmation du laboratoire. L'évaluation et la vérification de l'exposition à ces armes chimiques constituent une excellente capacité. Elle permettrait non seulement d'établir un diagnostic médical précis et de traiter les victimes, mais aussi de fournir des preuves solides en cas de violation des normes internationales convenues dans le cadre de la convention sur les armes chimiques (CAC). Cependant, seuls des laboratoires d'analyse hautement spécialisés et qualifiés, tels que ceux désignés par l'Organisation pour l'interdiction des armes chimiques (OIAC), peuvent fournir des résultats sans équivoque concernant l'exposition à des agents de guerre chimique.
Mots clés : Échantillon biologique, agent de guerre chimique, Convention sur les Armes Chimiques

Introduction

For centuries, toxic chemicals have been used in wars, conflicts and more recently, even in terrorist attacks. The effectiveness of these weapons is based on the toxicity of their active principles which are capable to cause death, temporary incapacitation, or permanent harm. Although nowadays there is greater awareness of its danger, the threat continues to exist. For this reason, it is necessary to continue developing preventive strategies and other measures aimed at mitigating damage in case of exposure to chemical warfare agents (CWA). This paper presents a brief overview of key points related to its historical use, classification, toxicity, antidotes, and detection in biological samples.

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Main part

Definitions

NATO´s Chemical Biological Radiological and Nuclear (CBRN) Medical Support Doctrine defines a "chemical agent" as a "*chemical substance which is intended for use in military operations to kill, seriously injure, or incapacitate personnel through its physiological effects"* (1). Similarly, the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and their Destruction (Chemical Weapons Convention, CWC) uses the term "toxic chemical" to refer to *"any chemical which, through its chemical action on life processes, can cause death, temporary incapacitation or permanent harm to humans or animals"*, while the term "chemical weapon" is used not only to talk about toxic chemicals and their precursors, but also it refers to munitions, devices and equipment specifically designed to weaponize or use directly in connection with toxic chemicals (2).

History of Chemical Warfare Agents

Chemical weapons have a long history. Its origin is associated with prehistorical methods of hunting, fishing, and fumigating, such as the use of poisoned arrows or the toxic combustion of products (3). These strategies were later applied in ancient wars. For instance, in 256 C.E. a jar of bitumen and Sulphur crystals was used on a siege of a Roman garrison by Persians in the city of Dura-Europos, Syria (4) and in the 7th century Greeks used "Greek fire", a

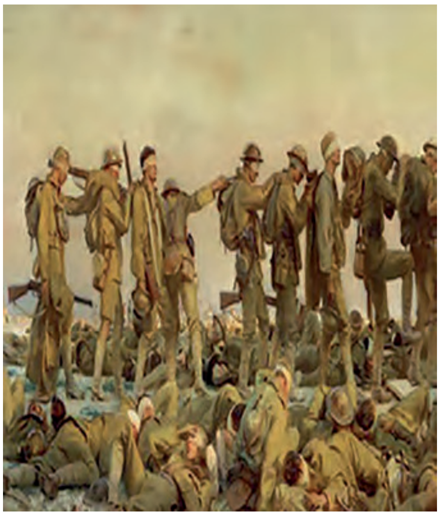

Fig 1. Soldiers temporarily blinded following exposure to sulphur mustard in 1917

toxic and incendiary mixture of Sulphur and naphtha.

Modern use of CWA dates to World War One when chlorine was used in Ypres on 22 April 1915 causing over 1.100 deaths. This dense gas reacts with water in lungs to form hydrochloric acid which has a permanent damaging effect in lung tissue (5). Two years later, Sulphur mustard (commonly known as mustard gas) (6) was introduced in this war conflict. This strong vesicant agent caused temporal blindness and chemical burns following its exposure. The lack of ammunition and the development of the chemical industry promoted research into this type of agents.

Public opinion refused the use of CWA and condemnation by the international community followed. However, chemical weapons continued to evolve, developing the organophosphates in the lates 1930s. These inhibitors of acetylcholinesterase (AChE) made all other chemical agents virtually obsolete due to their high toxicity and versatility in tactical use (7). Since then, other compounds have been emerging and many of them have been used (VX, R-33) (8). During the 1960s, both the US and the former Soviet Union were actively developing psychochemical warfare agents, including the anticholinergic 3-quinuclidinyl benzilate (BZ). In March 1984 it was confirmed that Iraq was using chemical weapons on a large scale in the Iran-Iraq war (9).

Chemical weapons have also been used on a small scale by non-State actors, being the most notorious of these the 1995 sarin attack in the Tokyo subway by the Aum Shinrikyo religious cult, where 13 civilians were killed and 6,226 people filed an injury report to the police (10).

The Chemical Weapons Convention came into effect on 29 April 1997. The organization in charge of implementing the provisions of this convention is the Organization for the Prohibition of Chemical Weapons (OPCW). After the CWC entered into force, other chemical attacks have occurred (i.e., Dubrovka in 2002 with the implication of carfentanyl (11), Syria in 2013 with sarin (12) or Salisbury poisoning in 2018 where Novichok was used (13)). All these examples serve to highlight the crucial role of qualified and specialized analytical laboratories in dealing with violations of the CWC. Up to the present time, 193 countries have participated in the Convention. The last chemical weapon from stockpiles declared by all States Parties to the Chemical Weapons Convention was destroyed on 7 July 2023 (14). However, the threat continues to exist given that there are non-stockpiled chemical weapons and some countries that have not joined the CWC yet.

Classification, toxidrome and antidotes

NATO´s CBRN Medical Support Doctrine classifies chemical agents into 5 groups depending on their main effect: nerve agents (organophosphorus), blister agents (vesicants), pulmonary agents (lung-damaging agents), chemical asphyxiants (including cyanogen agents) and incapacitating agents (mental incapacitants and physical incapacitants) (1). Attacks with these types of agents require a rapid response (15). In this sense, identifying each agent class of toxidrome (pattern of signs and symptoms that are characteristic) can guide diagnosis, reducing mortality and decreasing the risk for first responders (16).

Toxicity caused by some nerve agents, chemical asphyxiants, psychochemicals or central nervous system acting chemicals may be reversed by antidote administration. For example: sarin can reverse its toxicity with atropine and pralidoxime; cyanide with hydroxicobalamine or sodium thiosulfate and sodium nitrite; BZ with physostigmine and carfentanyl with naloxone. For other agents, the only countermeasures that can be applied are decontamination and supportive care (17).

For this reason, it is of extreme importance for medical responders to recognize the possible clinical presentations of each class of agent and to be familiarized with specific triage algorithms to accurately assign onsite status codes to victims of mass casualties (18). Ideally, these algorithms should rapidly identify those toxidromes that can be effectively treated with antidotes. Different approaches and recommendations have been published in the last decade (17,19–21). Although the use of toxidrome recognition to approximate the causative agent is a widely accepted by first responders, it is important to highlight that the identity of the substance causing the poisoning can only be determined with the highest certainty degree by analytical laboratories.

As the potential of CWA is devastating, armies around the world are committed to improving preparedness for any chemical incident. Regarding the Spanish Armed Forces, 23 beds of the special NBC-Unit of the Central Defence Hospital, are offered to OPCW for the care of these type of patients in case of need.

Fig 2. The Spanish Defence Institute of Toxicology, Central Defence Hospital Gómez Ulla, Madrid, Spain

In this sense, the antidote production capacity of the Spanish Defence Miliary Pharmacy Center also stands out.

Detection

The Chemical Weapons Convention allows investigations in cases of alleged chemical weapons use. The main objective of these investigations, described in articles IX and X of the cited Convention, is to determine whether chemical weapons have been used. Members of the inspection team may collect chemical, environmental, and biomedical samples for analysis on-site or offsite at OPCW-designated laboratories (22). From the collection of the sample through the analytical and testing procedures to its final reporting, custody chain must be ensured in order to prove the reliability of the results. In addition, transport is critical and must be specifically designed to ensure samples traceability and integrity.

The analysis of environmental samples aims to detect any chemical contained in the schedules of the CWC or their degradation products (hydrolysis products). Matrices commonly analyzed are bulk solid, soil, water, liquid, and wipe samples. The concentration of possible CWA present in this type of samples could be at the parts per billion level (23).

However, biomedical samples typically pose a greater challenge for an analytical laboratory (24). Among the main causes that justify this statement, the complexity of biological matrices (such as blood, plasma, tissue, or urine), the low concentration of analytes likely to be encountered and the toxicokinetic processes can be highlighted.

Regarding the analytic strategy in biological samples, direct and indirect biomarkers (i.e., AChE or BChE activity) could be used. Direct biomarkers include original agents, degradation products, metabolites, or covalently bound residues to macromolecules, which provide unequivocal evidence of exposure to CWA. In addition to the utility of this type of samples to conduct a verification analysis, they may be used for diagnostic purposes allowing the choice of the best treatment available and monitoring the exposure (25).

Nevertheless, intact agents and free metabolites in blood are relatively rapidly excreted from the body. Ideally, indicators should remain in the organism for much longer in order to enable retrospective detection of exposure up to several weeks. Some toxic chemicals, such as vesicants, nerve agents or phosgene, are reactive electrophiles that react with nucleophile domains present on macromolecules including DNA and proteins. Products resulting from these reactions are called adducts and they are the main long-live biological indicators of exposure (26).

The majority of the analytical methods used for the qualitative analysis of free metabolites and adducts are based on Gas or Liquid Chromatography coupled to Mass Spectrometry. Due to the complexity of these biological matrices, sophisticated extraction procedures are often required to isolate, clean, liberate adducts, and concentrate the markers of interest from their specimens suitable for detection (27,28). To solve these challenges, advanced technology and highly specialized teams are required.

The OPCW has established a network of 19 designated laboratories from 14 countries experts in biological samples analysis (29). Criteria that laboratories must satisfy for designation include having implemented a quality assurance system in accordance with internationally recognized standards (for example, ISO/IEC 17025 or equivalent), having obtained accreditation by an internationally recognized accreditation body for the analysis of aggressive chemicals in biological samples and having successfully passed the last two proficiency tests conducted by the OPCW.

In this sense, in Spain, the Toxicological Institute of the Defense (*Instituto de Toxicología de la Defensa*) is the reference laboratory for the analysis of CWA in biological samples. This center is working to enter the select group of designated laboratories of OPCW.

Conclusion

Toxidrome can be a tool for medical responders for cases in which a specific treatment is available and cannot wait for confirmation from the laboratory. However, only highly specialized and qualified analytical laboratories, such as those designated by the OPCW, can provide unequivocal results regarding the exposure to chemical warfare agents in order to prove possible violations of the convention and serve for an accurate medical diagnosis.

References

1. North Atlantic Treaty Organization. NATO Standard AJMedP-7, Allied Joint Cchemical, Biological, Radiological and Nuclear (CBRN) Medical Support Doctrine, Ed. B v. 1, Jan 2022, p 2-3. Available from: https://www.coemed.org/files/stanags/02_AJMEDP/AJMedP-7_EDB_V1_E_2596.pdf.
2. OPCW [Internet]. [cited 2023 Oct 25]. Article II – Definitions and Criteria. Available from: https://www.opcw.org/chemical-weapons-convention/articles/article-ii-definitions-and-criteria
3. Pitschmann V. Overall View of Chemical and Biochemical Weapons. Toxins (Basel). 2014 Jun 4;6(6):1761–84.
4. Smoked romans. Science. 2009 Jan 30;323(5914):565–565.
5. Cevik Y, Onay M, Akmaz I, Sezigen S. Mass casualties from acute inhalation of chlorine gas. South Med J. 2009 Dec;102(12):1209–13.
6. Wattana M, Bey T. Mustard gas or sulfur mustard: an old chemical agent as a new terrorist threat. Prehosp Disaster Med. 2009;24(1):19–29; discussion 30-31.
7. Pohanka M. Cholinesterases, a target of pharmacology and toxicology. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2011 Sep;155(3):219–29.
8. Pampalakis G, Kostoudi S. Chemical, Physical, and Toxicological Properties of V-Agents. Int J Mol Sci. 2023 May 11;24(10):8600.
9. Marshall E. Iraq's Chemical Warfare: Case Proved. Science. 1984 Apr 13;224(4645):130–2.
10. Sugiyama A, Matsuoka T, Sakamune K, Akita T, Makita R, Kimura S, et al. The Tokyo subway sarin attack has long-term effects on survivors: A 10-year study started 5 years after the terrorist incident. PLoS One. 2020 Jun 23;15(6):e0234967.
11. Riches JR, Read RW, Black RM, Cooper NJ, Timperley CM. Analysis of clothing and urine from Moscow theatre siege casualties reveals carfentanil and remifentanil use. J Anal Toxicol. 2012;36(9):647–56.
12. John H, van der Schans MJ, Koller M, Spruit HET, Worek F, Thiermann H, et al. Fatal sarin poisoning in Syria 2013: forensic verification within an international laboratory network. Forensic Toxicol. 2018;36(1):61–71.
13. Mark Peplow. Nerve agent attack used 'Novichok' poison. C&EN Global Enterp. 2018 Mar 19;96(12):3–3.
14. OPCW [Internet]. [cited 2023 Oct 15]. OPCW confirms: All declared chemical weapons stockpiles verified as irreversibly destroyed. Available from: https://www.opcw.org/media-centre/news/2023/07/opcw-confirms-all-declared-chemical-weapons-stockpiles-verified
15. Kales SN, Christiani DC. Acute chemical emergencies. N Engl J Med. 2004 Feb 19;350(8):800–8.
16. Rosman Y, Eisenkraft A, Milk N, Shiyovich A, Ophir N, Shrot S, et al. Lessons learned from the Syrian sarin attack: evaluation of a clinical syndrome through social media. Ann Intern Med. 2014 May 6;160(9):644–8.
17. Ciottone GR. Toxidrome Recognition in Chemical-Weapons Attacks. New England Journal of Medicine. 2018 Apr 26;378(17):1611–20.
18. Culley JM, Svendsen E. A review of the literature on the validity of mass casualty triage systems with a focus on chemical exposures. Am J Disaster Med. 2014;9(2):137–50.
19. Madsen J. Chemical terrorism: Rapid recognition and initial medical management. UpToDate [Internet]. [cited 2023 Oct 25]; Available from: https://www.uptodate.com/contents/chemical-terrorism-rapid-recognition-and-initial-medical-management
20. Lavonas EJ, Akpunonu PD, Arens AM, Babu KM, Cao D, Hoffman RS, et al. 2023 American Heart Association Focused Update on the Management of Patients With Cardiac Arrest or Life-Threatening Toxicity Due to Poisoning: An Update to the American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2023 Oct 17;148(16):e149–84.
21. Khoshnevis MA, Panahi Y, Ghanei M, Borna H, Sahebkar A, Aslani J. A Triage Model for Chemical Warfare Casualties. Trauma Mon. 2015 Aug;20(3):e16211.
22. OPCW [Internet]. [cited 2023 Oct 13]. Responding to the Use of Chemical Weapons. Available from: https://www.opcw.org/our-work/responding-use-chemical-weapons
23. Konopski L, Liu P, Wuryani W, Sliwakowski M. OPCW Proficiency Test: a practical approach also for interlaboratory test on detection and identification of pesticides in environmental matrices. ScientificWorldJournal. 2014;2014:542357.
24. Noort D, Benschop HP, Black RM. Biomonitoring of exposure to chemical warfare agents: a review. Toxicol Appl Pharmacol. 2002 Oct 15;184(2):116–26.
25. Nakajima T, Sasaki K, Ozawa H, Sekjima Y, Morita H, Fukushima Y, et al. Urinary metabolites of sarin in a patient of the Matsumoto sarin incident. Arch Toxicol. 1998 Sep;72(9):601–3.

26. Grigoriu N, Epure G, Ginghina R, Mosteanu D. An Overview Of The OPCW's Programme For Biomedical Samples Analysis. International conference KNOWLEDGE-BASED ORGANIZATION. 2015 Jun 1;21(3):815–20.
27. John H, Worek F, Thiermann H. LC-MS-based procedures for monitoring of toxic organophosphorus compounds and verification of pesticide and nerve agent poisoning. Anal Bioanal Chem. 2008 May;391(1):97–116.
28. John H, Thiermann H. Poisoning by organophosphorus nerve agents and pesticides: An overview of the principle strategies and current progress of mass spectrometry-based procedures for verification. J Mass Spectrom Adv Clin Lab. 2021 Jan;19:20–31.
29. Pawale P. Note by the Director-General: Status of Laboratories Designated for the Analysis of Authentic Biomedical Samples.

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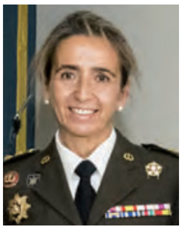

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24th LoAC Course 2024

General Information

The **24th ICMM Specialized Course for Military Medical Personnel on the Law of Armed Conflict (LoAC)** will take place from **07-11 April 2024**. As in the previous years, it will be organised by the Medical Services Directorate of the Swiss Armed Forces. The **venue** of the course has changed and it will now take place in the facilities of the **Swiss Federal Institute of Sport in Magglingen**, Switzerland. The venue offers both modern hotel facilities as well as an up-to-date teaching infrastructure.
The LoAC course will be offered both as an **on-site course in Magglingen** and as a **hybrid course** as well.


Course Program

- **The provisional Course program can be downloaded** on the website (https://www.melac.ch/courses-workshops/loac-courses/loac-hybrid-2024). Changes to the program may occur.

If you have questions, please contact mme-loac.lba@vtg.admin.ch

Application

Please use the **application form** provided at https://www.melac.ch/apply/loac-mme-2024
For **questions** regarding the course or the registration, please contact mme-loac.lba@vtg.admin.ch

Language

The course will be held in English and French language. Separate syndicates will be available in both languages and plenary lectures are translated simultaneously. Please note: online participation is only possible in English language.

Course Fee

On-site-participation

- A **fee of 1'100.00 CHF** will be charged and **has to be paid by course participants in advance.** Details about the payment modalities will be communicated to confirmed participants.
  – It includes
  – Accommodation in the hotel, all meals, non-alcoholic beverages for the course duration.
- Extensive course documents, cultural events and excursions with transfers are free of charge for participants and will be paid by the Swiss Confederation.
- Participants shall bear their travelling costs to and from Switzerland.

Online-particpation (English only)

- The online course can be attended free of charge.
- A **motivation letter** is required and the online class will be limited to 15 participants in order to allow for discussions and excange among the participants.

Please use the **application form** provided at https://www.melac.ch/apply/loac-mme-2024