Working Paper
Version 1

Experience of Medical Forensics in Poisoning Cases and Possible Relevance for CBW Investigative Mechanisms

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Presented at:
International Society of Military Sciences (ISMS)
Annual Conference (2015) '*(R)evolution of War*'
Working Group 3: Military Technology
National Defence University
13-15 October 2015
Helsinki, Finland

*The views expressed are the authors' and do not necessarily reflect those of SIPRI or the Bulent Ecevit University (Zonguldak).

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Abbreviations and acronyms

ADP	advanced data processing
AG	Australia Group
AMDIS	Automated Mass spectral Deconfolution and Identification System
ARDS	acute respiratory distress syndrome
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
BAL	bronchial alveolar lavage
CAM	chemical agent monitor
CBRN	chemical, biological, radiological, nuclear
CBRNe	chemical, biological, radiological, nuclear, explosive
CBW	chemical and/or biological warfare/weapon
CCMC	Central Crisis Management Cell
CFSP	common foreign and security policy
CIJA	Commission for International Justice and Accountability
Cl	chlorine
CN	cyanide
CSA	coordination and support action
CW	chemical warfare/chemical weapon
CWA	chemical warfare agent
CWC	Chemical Weapons Convention
DAT	Declaration Assessment Team
DG	Director-General
DNA	deoxyribonucleic acid
DOC/PSF	discrete organic chemical [that may contain] phosphorus, sulphur, fluorine
EC	Executive Council
EDA	European Defence Agency
EU	European Union
eNOS	endothelial nitric oxide synthase
GC/AED	gas chromatography with atomic emission detection
GC/FPD	gas chromatography—flame photometric detector
GC/FTIR	gas chromatography—infrared spectroscopy
GC/MS	gas chromatography—mass spectrometry
HCl	hydrochloric acid
HPMEC	human pulmonary microvascular endothelial cells
ICC	International Criminal Court
ICSR	International Centre for the Study of Radicalisation and Political Violence
IED	improvised explosive device

IMS	ion mobility spectrometry
iNOS	isoform nitric oxide synthase
IR	infrared
IS	Islamic State [Daesh]
ISAF	International Security Assistance Force
KDP	Kurdish Democratic Party
KRSC	Kurdistan Region Security Council
LC/HRMS	liquid chromatography—high resolution mass spectrometry
LC/MS	liquid chromatography—mass spectrometry
LD	lethal dose
MSF	Medecins Sans Frontières
NATO	North Atlantic Treaty Organization
NC3A	NATO Consultation, Command and Control Agency
NMR	nuclear magnetic resonance
OCAD	OPCW Central Analytical Database
OCl	hypochlorate
OPCW	Organisation for the Prohibition of Chemical Weapons
PE	point-of-exposure
PPE	personal protective equipment
PPM	parts per million
RADS	reactive airways dysfunction syndrome
RIA	regulatory impact analysis
ROS	reactive oxygen species
SAB	Scientific Advisory Board
SAMS	Syrian American Medical Society
SME	small and medium-size enterprise
SOP	standard operating procedure
TIC	toxic industrial chemical
TIH	toxic inhalation hazard
TUNEL [assay]	terminal deoxynucleotidyl transferase dUTP nick end labeling
TRL	technology readiness level
TRP	transient receptor potential [channel]
TWG	temporary working group
UK	United Kingdom
UN	United Nations
VILE	ventilation induced lung injury
VPA	valproic acid
WADA	World Anti-Doping Agency
WHO	World Health Organization
WI	work instruction

Summary

Physiological and morphological aspects associated with toxic chemical exposure from a mainly forensic pathology perspective are presented. Selected arms control, and security and defence sector, and international peace and security implications are then reviewed. Our purpose is to attempt to assist the further consideration of policy and technical aspects for determining the nature of and attributing responsibility for the use of toxic chemicals (mainly chlorine) as a method of warfare where inhalation is the principal route of exposure.

Chlorine-related suspected cases are one of the most difficult problems in forensic pathology for at least four major reasons. First, animal studies show that there are different LD_{50} values for different species. It has been shown that rats survive exposure to chlorine gas up to 1500 ppm for more than two hours. It has also been shown that in industrial accidents and in armed conflict that acute high dosages of chlorine can kill immediately. According to some calculations, the chlorine gas level can be as high as 400 ppm in industrial accidents. Reliable information on the lethal doses in humans may be more limited. Second, considerable research on chlorine gas exposure has been done on animals (e.g., rats). However, these studies appear to have been mainly conducted to research chronic effects or (at best) sub-acute effects. Third, acute chlorine gas exposure may cause mainly pulmonary respiratory effects, such as edema of the respiratory tract, acute inflammation of the trachea and bronchi. Acute exposure deaths are the result of pulmonary edema, and respiratory and cardiovascular failure. In most autopsies, the cause of death is determined to be asphyxia. However, the precise mechanism of death is unknown or not determined. Fourth specific biomarkers for acute and chronic exposure to chlorine gas are lacking. So there is limited possibility to provide clear evidence of chlorine exposure in vivo or post-mortem.

Recommendations

The following *scientific and technical* points might provide a useful basis for consultation and clarification vis-à-vis their relevance for the detection, treatment and attribution of responsibility for the use of toxic chemicals and their precursors as a method of warfare.

-Systematic linkage of state-of-the-art human body sample type indicative of chemical compound (by class or agent) exposure vis-à-vis best forensics and medical documentation practice and treatment; CW alleged use standard operating procedures (SOPs) and work instructions (WIs); and legal investigations and prosecutions frameworks.

-Bronchial alveolar lavage (BAL) methodologies and best practice, including their relevance for the taking of samples for analysis of possible CW agent use.

-To what extent can the lung toxicity effects typology of i.) Type 1 and 2 cell effects, ii.) neuronal cell effects, and iii.) endothelial/epithelial cell effects be employed to achieve a better understanding of in vivo?[1] For example, c-FOS expression can be used as a marker for neuronal activation.

-Understandings and research directions regarding Adult Respiratory Distress Syndrome (ARDS).

-Role of TRP channels in the study of lung toxicity should be further considered for CW verification relevance. For example, what analytical techniques exist for the understanding of TRP channel function in the lung in vivo?

-Role of TICs (e.g., phosgene) on glutamate metabolism. What analytical techniques exist for the understanding of TIC effects on metabolic processes in general and glutamate in particular that can assist with the elucidation of exposure by chemical class or type?

-Epigenetic modifications can be associated with longterm lung tissue damage with variation in damage according to type of chemical compound. The role of DNA repair mechanisms (in general) and according to chemical class or type could perhaps be further elucidated in this regard.

-To what extent can ion channel complex formation and structure be elucidated in vivo? What are the consequent CW verification-related implications?

-The use of steroids for therapeutic effect against inhaled TICs and the possible resulting implications for sampling and analysis for CW agents.

-With respect to sudden death in cases of acute Cl exposure, further research is required in the following areas: i.) which type of cell death is more common in Cl exposure, anoikis, necroptosis, and apoptosis? ii.) Acute cardiac toxicity should be further investigated in view of the fact that cardiotoxic effects may be the cause of death, and iii.) more specific medical pathology protocols should be evaluated for possible relevant to CW use investigations protocols on the basis of Cl exposure in particular.

Methodology

We have sought to make this paper relevant (i.e., multidisciplinary) to interested persons or relevant bodies. We attempt to review succinctly the activities and interests of many of the most interested actors and relevant institutions, while simultaneously taking into account the practice of forensic medicine and providing a sizeable cross-section of the English-language scientific and technical literature.

Specific actions taken in the literature review include searches of academic databases (e.g., PubMed and Scopus for English-language articles for the

[1] Typology from xx here.

period 1991-2015 regarding the health effects of exposure to chlorine gas, and similar searches on recent research of cyanide exposure and forensics practice for cases of suspected toxic chemicals exposure). We have not attempted to reflect, much less duplicate, the substantial work conducted by national defence establishments, NATO, and UN-type bodies in the context of sampling and analysis procedures as they relate, in general, to investigations of alleged chemical warfare agent (CWA) use and, in particular, to the Syria case. Much of this work remains restricted and is therefore not accessible to us. We nevertheless suspect that such work might benefit from (further) forensics pathology and medicine perspectives. Individuals and institutions engaged in humanitarian and human rights work should also possess relevant information and understandings.

We have received—and continue to seek—expert opinion from forensic pathologists and others. One of us (Dr Toprak) is supervising the collection of data from animal exposure study trials being performed at Bulent Ecevit University (Zonguldak) and Istanbul University under relevant codes of ethical conduct and standards.

This paper has not been peer-reviewed. A second iteration will be presented at the *3rd International Symposium on Development of CBRN Defence Capabilities* (Oct. 2015, Berlin). This second version will, in principle, reflect some external review comments.

Any conclusions or ‘lessons’ that might be derived from this working paper should be validated in consultation with relevant authorities and checked against the relevant regulations and guidelines.

We thank inter alia Bulent Ecevit University colleagues and the SIPRI Library for their interest and support.

1.0 Introduction

Numerous legal, moral, political and technical questions have arisen among a wide range of actors and institutions in connection with the investigation of allegations of alleged chemical weapons (CW) use in the ongoing Syrian civil war and adjacent armed conflict areas. Some cases have been proven, while other instances remain probable or uncertain.[2] The use of toxic chemicals as a method of warfare in the Syrian conflict has technical, policy and political implications for international peace and security, humanitarian and criminal law, and the national security and defence sectors (e.g., with respect to capabilities and 'reach back' support).

1.1. Background

International engagement with Syria on its CW programme and allegations of CW use in its civil war have been ongoing for more than three years.[3] This year there have been strong indications that IS forces have employed toxic chemicals as a method of warfare (i.e., chlorine and sulphur mustard).

A hybrid cooperative arrangement evolved in 2013 first as an inspection team headed by Dr Åke Sellström under the authority of the UN Secretary-General's authority to investigate alleged chemical and/or biological weapons (CBW) use through the UN's Office for Disarmament Affairs (UNODA).[4] This team was composed largely of Organisation for the Prohibition of Chemical Weapons (OPCW) inspectors and was co-headed by a CW expert from the World Health Organization (WHO). This team was based in Cyprus and was unable to enter Syria due to disagreements over the inspection mandate (mainly among and between the Government of Syria and several members of the UN Security Council). The political dynamic changed on 21 August 2013 when it was widely and credibly reported that more than 1000 people had been affected by an unknown chemical agent with symptoms similar to organophosphate poisoning.

Dual-track processes in the UN in New York and the OPCW in The Hague resulted in coordinated agreement among interested states with a variety of ancillary activities and consultations being synchronized in parallel. On 27 September the UN Security Council passed resolution 2118 (2013) which

[2] UN General Assembly and Security Council, Report of the United Nations Mission to Investigate Allegations of the Use of Chemical Weapons in the Syrian Arab Republic on the alleged use of chemical weapons in the Ghouta area of Damascus on 21 August 2013, A/67/997-S/2013/553, 16 Sep. 2014; and UN General Assembly and Security Council, United Nations Mission to Investigate Allegations of Use of Chemical Weapons in the Syrian Arab Republic, Final Report, annex to A/68/663-S/2013/735, 13 Dec. 2013.

[3] Rosman, Y., et al., 'Lessons learned from the Syrian attack: evaluation of a clinical syndrome through social media', *Annals of Internal Medicine*, vol. 160 (2014), pp. 644-648.

[4] The UN Secretary-General's authority is partly based on UN General Assembly Resolution A/RES/42/37, 30 Nov. 1987; and UN Security Council Resolution 620, 26 Aug. 1988.

encourages UN member states 'to provide support, including personnel, technical expertise, information, equipment, and financial and other resources and assistance'. On 14 September 2013 Syria joined the CWC, and the OPCW Executive Council agreed a destruction plan that called for Syria to destroy its stockpile by 30 June 2014 and which envisaged the destruction of toxic chemicals on board a specially equipped US vessel in the Mediterranean Sea. The inspection team was then formally transformed on 16 October 2013 into an OPCW–UN Joint Mission headed by Sigrid Kaag of the Netherlands in her capacity as Special Coordinator of the OPCW–UN Joint Mission.[5] The maritime destruction operation completed its work on schedule.[6]

In addition, in 2013 France, the UK and the United States reportedly provided the UN with 'a trove of evidence, including multiple blood, tissue and soil samples' that the United States maintained 'proves that Syrian troops used' sarin 'on the battlefield'.[7] In June the French Government stated it had conclusive evidence that sarin had been used in Syria.[8] On 4 June the French Minister of Foreign Affairs publicized the results of analysis that 'demonstrated the presence of sarin in a number of samples in our possession, collected, namely, following attacks in Jobar between 12 and 14 April and in Saraqeb on 29 April'.[9] This information was provided to Sellström's team. The UK produced a similar assessment.[10] Russia has also conducted technical assessments.

An OPCW Fact-Finding Mission (FFM) later concluded in 2014 'with a high degree of confidence' that chlorine had been employed as a weapon. Further instances of chlorine use have been reported this year. In March 2015 the UN Security Council adopted resolution 2209 condemning the use of any toxic chemical in Syria (a party to the 1993 Chemical Weapons Convention, CWC). While it did not attribute responsibility, this resolution in principle authorizes the use of force under Chapter VII of the UN charter if such weapons are employed again.

[5] OPCW–UN Joint Mission, <http://opcw.unmissions.org/Default.aspx?tabid=6576&error=Object+reference+not+set+to+an+instance+of+an+object.>.

[6] Hart, J., 'The Maritime Component of the Syrian Chemical Disarmament Operation: Lessons for Maritime Security Cooperation'. Paper presented at *SIPRI Maritime Security Forum, 2014*; 9-11 July 2014; Stockholm, Sweden.

[7] Lynch, C. and Warrick, J., 'In Syrian chemical weapons claim, criticism about lack of transparency', *Washington Post*, 21 June 2013.

[8] Erlanger, S. and Cumming-Bruce, N., 'France offers evidence of "multiple" uses of nerve gas in Syria', *International New York Times*, 4 June 2013; and 'Syrie—Armes chimiques—Déclaration de Laurent Fabius (4 juin 2013)' [Syria-chemical-weapons declaration of Laurent Fabius (4 June 2013)], 4 June 2013, <http://www.diplomatie.gouv.fr/fr/dossiers-pays/syrie/la-france-et-la-syrie/evenements-4439/article/syrie-armes-chimiques-declaration>. See also France, 'Synthèse Nationale de Renseignement Déclassifiè: Programme Chimique Syrien, Cas d'emploi passes d'agents chimiques par le régime, Attaque chimique conduit par le regime le 21 août 2013', <http://www.gouvernement.fr/sites/default/files/fichiers_joints/syrie_synthese_nationale_de_renseignement_declassifie_02_09_2013.pdf>.

[9] OPCW, 'France: response to the statement by the Director-General of the OPCW on the matter of chemical weapons in Syria', EC-73/NAT.13, 16 July 2013, p. 1.

[10] British Joint Intelligence Organisation, 'Syria: reported chemical weapons use', 29 Aug. 2013, <https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/235094/Jp_115_JD_PM_Syria_Reported_Chemical_Weapon_Use_with_annex.pdf>.

On 7 August the UN Security Council unanimously adopted resolution 2235 (2015) which establishes for one year a joint UN-OPCW investigative mechanism which is mandated to seek to identify those responsible for perpetrating, organizing, or sponsoring or who are otherwise involved in the use of chemicals as weapons in Syria.[11] The UN Secretary General in consultation with the OPCW Director-General were requested to submit to the UN Security Council within 20 days recommendations for the establishment of this body which will also cooperate with the FFM (please see below).

1.2. Syria's CW declaration(s)

The basis for the OPCW verification regime was a decision taken by the Executive Council on 27 September 2013 in consultation with UN officials and others.[12] A joint Russian–US framework understanding specified that Syria must provide a comprehensive listing of its CW within one week; equipment for the production, mixing and filling of CW must be destroyed by November 2013; and Syria's CW materials and equipment must be destroyed during the first half of 2014. As previously mentioned, on 16 October 2013 the joint UN–OPCW mission was established to accomplish these goals.

Syria declared to the OPCW on 19 September and 4 October 2013 the possession of sulphur mustard, sarin and VX.[13] It declared having 41 facilities at 23 sites, 18 chemical weapon production facilities, 12 chemical weapon storage facilities, 8 mobile filling units, 3 CW-related facilities, 1000 tonnes of Category 1 chemicals (mainly precursors), 290 tonnes of Category 2 chemicals, 1230 unfilled munitions, 2 cylinders not claimed by the Syrian Government and possibly filled with CW and site diagrams for CW storage facilities.[14] Major elements of the inspection and verification effort (which was hindered by insecurity within the country) were to ensure the completeness and correctness of the declarations, to complete the destruction of Category 3 CW in 2013, and to make arrangements for the trans-shipment of toxic chemicals to a shipboard hydrolysis unit in the Mediterranean Sea. Denmark and Norway agreed to transfer the toxic chemicals from the Syrian port of Latakia, while the United States provided a ship specially fitted with a hydrolysis unit. Hydrolysates are to be used for peaceful purposes by private companies awarded public tenders circulated by the OPCW. Verification activity was also partly hindered by delays in the transfer of overpack material by Lebanese customs.[15]

[11] For an opinion regarding the effect of the formation of this joint mission on the likelihood of attributing responsibility and follow-on criminal proceedings, see Kersten, M., 'Laying the blame: justice in Syria just got a bit more likely', Justiceinconflict.org, 24 Sep. 2015, <http://justiceinconflict.org/2015/09/24/laying-the-blame-justice-in-syria-just-got-a-bit-more-likely/>.

[12] OPCW, 'Destruction of Syrian chemical weapons', Decision, EC-M-33-/DEC.1, 27 Sep. 2013.

[13] OPCW, 'Note by the Director-General: progress in the elimination of the Syrian chemical weapons programme', EC-M-34/DG.1, 25 Oct. 2013.

[14] 'Note by the Director-General, progress in the elimination of the Syrian chemical weapons programme', OPCW document EC-M-34/DG.1, 25 Oct. 2013.

[15] Personal communication.

Syria initially declared 26 CWPFs (of which 12 were hangars or tunnels).[16] It subsequently declared a ricin CWPF (Syria had stated initially that its ricin activity was suspended and was basic research medical-type work).[17] Syria unsuccessfully requested the OPCW Executive Council to approve conversion of the 12 hangars and tunnels for non-prohibited purposes.[18]

On 29 April 2014 the OPCW Director-General established the FFM to establish the facts connected to allegations of the use of toxic chemicals in the Syrian civil war. The FFM issued reports in June, September and December 2014.[19] All three reports concerned attacks in the opposition controlled villages of Talmenes, Al Tamanah and Kafr Zita in the April-May 2014 timeframe.[20] In August 2014 the FFM interviewed 37 victims, eye witnesses and medical personnel in a neighbouring country.[21] The FFM also collected more than 100 documents and videos.[22] Eyewitness testimony 'invariably connected the devices to helicopters flying overhead'.[23] At least 56 states parties to the CWC signed a statement of support that endorsed the work of the FFM and (at least broadly) its conclusions.[24]

In April 2014 the OPCW Director-General established a Declaration Assessment Team (DAT) which then traveled to Syria 6 times that year.[25] Syria did not wish to declare the Scientific Studies and Research Centre at Barzah.[26] The United States also noted that Syria has provided little (if any)

[16] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[17] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[18] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[19] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 4, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[20] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 4, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[21] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[22] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[23] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[24] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[25] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[26] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

documentation on his CW programme.[27] The EU has also noted with concern the lack of documentation at EC meetings.[28]

1.3. Recent developments

Chlorine has been explosively disseminated against coalition occupation forces in Iraq.[29] There have also been continued allegations of the use of chlorine and sulphur mustard (typically associated with artillery shells and mortars) fired from IS-held positions against Kurdish positions in Iraq and Syria.[30]

According to Syrian doctors (working outside Syrian Government controlled areas) who contacted Doctors Without Borders (Medicins Sans Frontières, MSF), a chlorine attack involving the use of helicopters occurred on 16 March 2015 in the vicinity of the town of Sarmin (Idlib region) killing 6 and injuring 70.[31] A hospital director in the region was quoted as stating '…the air smelt of cleaning products and [the clothing of the victims] had the distinctive smell of chlorine'.[32] A total of 20 patients in serious condition were described as 'agitated, foaming at the mouth and showing skin rashes'.[33] Patients were treated to induce bronchial dilation and provided O_2 therapy.

Information concerning the circumstances of a shell fired on 21 August 2015 at 19:30 into a family home in Marea and the symptoms and treatment of family members were recently reported by MSF and the media.[34] The family members comprised the two parents, a 3-year old girl and a 5-day old girl.[35] An odor filled the room smelling of 'rotten eggs or rotten garlic, something

[27] *Compliance with the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction Condition 10(c) Report* (US Dept. of State: Washington, DC, 15 Apr. 2015), p. 5, <http://www.state.gov/t/avc/rls/rpt/2015/243245.htm>.

[28] Luxembourg, 'Luxembourg statement on behalf of the European Union delivered by H. E. Ambassador Pierre-Louis Lorenz, Permanent Representative of Luxembourg to the OPCW at the seventy-ninth of the Executive Council', OPCW document EC-79/NAT.6, 7 July 2015, p. 2.

[29] Wehling, F., 'A Toxic Cloud of Mystery: Lessons from Iraq for Deterring CBRN Terrorism', pp. 273-298 in Eds. Andreas Wenger and Alex Wilner, *Deterring Terrorism: Theory and Practice* (Stanford University Press: Stanford, 2012).

[30] Anonymous, 'IS militants "used mustard gas" in north Iraq', Radio Free Europe/Radio Liberty, 9 Oct. 2015, <http://www.rferl.org/content/iraq-islamic-state-mustard-gas/27293807.html>; and Tarini, G., 'Bad chemistry: ISIS and mustard agents', *Bulletin of the Atomic Scientists* (9 Oct. 2015), <http://thebulletin.org/bad-chemistry-isis-and-mustard-agents8793>.

[31] MSF, 'Syria: use of chlorine in an attack on a village in Northern Syria', Press Statement, 18 Mar. 2015, <http://www.msf.org/article/syria-use-chlorine-attack-village-northern-syria>.

[32] MSF, 'Syria: use of chlorine in an attack on a village in Northern Syria', Press Statement, 18 Mar. 2015, <http://www.msf.org/article/syria-use-chlorine-attack-village-northern-syria>.

[33] MSF, 'Syria: use of chlorine in an attack on a village in Northern Syria', Press Statement, 18 Mar. 2015, <http://www.msf.org/article/syria-use-chlorine-attack-village-northern-syria>.

[34] MSF, 'Syria: MSF treats patients with symptoms of exposure to chemical agents', Press Statement, 25 Aug. 2015, <http://www.msf.org/article/syria-msf-treats-patients-symptoms-exposure-chemical-agents>; and Chivers, C. J., 'What an ISIS chemical strike did to one family', *New York Times*, 6 Oct. 2015, <http://www.nytimes.com/2015/10/07/world/middleeast/syrian-familys-agony-raises-specter-of-chemical-warfare.html?_r=1>.

[35] MSF, 'Syria: MSF treats patients with symptoms of exposure to chemical agents', Press Statement, 25 Aug. 2015, <http://www.msf.org/article/syria-msf-treats-patients-symptoms-exposure-chemical-agents>.

rotten'.[36] The family members were examined by first aid personnel in the field, then transferred to a MSF-operated facility and then retransferred to a second facility for further observation and specialized treatment.[37] The family members were treated at a hospital in Tel Rifaat (it is unclear if this is the MSF-operated hospital).[38] The family arrived at the MSF hospital 1 hour after the attack.[39] The members displayed respiratory distress, inflammation of the skin, reddened eyes and conjunctivitis.[40]

The MSF programme manager in Syria stated that his organization possesses no laboratory evidence to confirm the cause the symptoms. He elaborated 'However, the patients' clinical symptoms, the way these symptoms changed over time, and the patients' testimony about the circumstances of the poisoning all point to exposure to a chemical agent'.[41] The baby died on 4 September. A photograph showed the baby to have lost her hair.[42] One family member experiences continuing shortness of breath, emits phlegm, and wears sunglasses indoors due to light sensitivity.[43] A lesson from this experience is that those who are decontaminated or washed down while naked must exchange their clothes for a fresh, non-exposed set.

Some of the footage and photos of those burned (e.g., of Kurdish fighters) show the 'classic' raised sulphur mustard blisters (specifically the presence of epidermolysis bullosa).[44] Other photos indicate skin burns, including possible streaking patterns that could be associated with being exposed to liquid(s).

[36] Chivers, C. J., 'What an ISIS chemical strike did to one family', *New York Times*, 6 Oct. 2015, <http://www.nytimes.com/2015/10/07/world/middleeast/syrian-familys-agony-raises-specter-of-chemical-warfare.html?_r=1>.

[37] MSF, 'Syria: MSF treats patients with symptoms of exposure to chemical agents', Press Statement, 25 Aug. 2015, <http://www.msf.org/article/syria-msf-treats-patients-symptoms-exposure-chemical-agents>.

[38] Chivers, C. J., 'What an ISIS chemical strike did to one family', *New York Times*, 6 Oct. 2015, <http://www.nytimes.com/2015/10/07/world/middleeast/syrian-familys-agony-raises-specter-of-chemical-warfare.html?_r=1>.

[39] MSF, 'Syria: MSF treats patients with symptoms of exposure to chemical agents', Press Statement, 25 Aug. 2015, <http://www.msf.org/article/syria-msf-treats-patients-symptoms-exposure-chemical-agents>.

[40] MSF, 'Syria: MSF treats patients with symptoms of exposure to chemical agents', Press Statement, 25 Aug. 2015, <http://www.msf.org/article/syria-msf-treats-patients-symptoms-exposure-chemical-agents>.

[41] MSF, 'Syria: MSF treats patients with symptoms of exposure to chemical agents', Press Statement, 25 Aug. 2015, <http://www.msf.org/article/syria-msf-treats-patients-symptoms-exposure-chemical-agents>.

[42] Chivers, C. J., 'What an ISIS chemical strike did to one family', *New York Times*, 6 Oct. 2015, <http://www.nytimes.com/2015/10/07/world/middleeast/syrian-familys-agony-raises-specter-of-chemical-warfare.html?_r=1>.

[43] Chivers, C. J., 'What an ISIS chemical strike did to one family', *New York Times*, 6 Oct. 2015, <http://www.nytimes.com/2015/10/07/world/middleeast/syrian-familys-agony-raises-specter-of-chemical-warfare.html?_r=1>.

[44] Photos of the 'classic' H/HD blistering are shown in Chulov, M., '"My body was burning": survivors recall horror of Isis mustard gas attack', *Guardian*, 2 Sep. 2015, <http://www.theguardian.com/world/2015/sep/02/syria-mustard-gas-attack-my-body-was-burning>. We thank Milton Leitenberg and his colleague for drawing our attention to this article.

On 6 October 2015 MSF delivered 15 chemical protection suits to Peshmerga fighters in Makhmour (Black Tiger Camp) under the command of Mustafa Barzani who heads the Kurdish Democratic Party (KDP).[45]

IS (or similar) have manufactured CW, there are leftovers from the Syrian Government holdings.

In September 2015 the Kurdistan Region Security Council (KRSC) reported that IS had used chemical weapons against Peshmerga in four instances.[46]The same month it was reported that UK special forces accompanied by US CW specialists had been deployed to destroy an underground 'chlorine warehouse' approximately 80 km from the Iraq border.[47] The operation was reportedly launched partly on information provided by Kurdish groups. An anonymous source stated 'We believe ISIS have other chemical weapons which they stole from Assad's forces when they captured military bases across Syria'.[48] Some media reports (e.g., in connection with the Marea attack) suggest that IS has manufactured sulphur mustard.

The start of the UN-OPCW joint mission's work has been delayed partly due to concerns expressed by Russia on the modalities of its work.[49] On 9 September UN Secretary-General Ban Ki-moon assured Russia's Permanent Representative to the UN Ambassador Vitaly Churkin that the UN would 'expeditiously consult' with the Syrian Government on how the joint mission will operate and that this mission would have 'reasonable grounds' for requesting access as it proceeds in implementing its UN Security Council mandate.[50]

With respect to international verification efforts, a variety of actors have an interest in or have understandings that can contribute to the further clarification of alleged CW use in the Syrian conflict and adjacent areas.

1.3.1. Commission for International Justice and Accountability

The Commission for International Justice and Accountability (CIJA), which is comprised of legal experts and investigators with prior experience working for

[45] Nagl, K., 'MSF gives Peshmerga anti-chemical weapons gear', Rudaw.net, 8 Oct. 2015, <http://rudaw.net/english/kurdistan/071020151>.

[46] Khalid, H., 'Masrour Barzani: four IS chemical weapons attacks on Peshmerga', *Basnews*, 22 Sep. 2015, <http://www.basnews.com/en/news/2015/09/22/masrour-barzani-four-is-chemical-weapons-attacks-on-peshmerga/>.

[47] Anonymous, 'British special forces hunt for IS chemical weapons stock in Syria: report', *i24 News*, 27 Sep. 2015, <http://www.i24news.tv/en/news/international/middle-east/87142-150927-british-special-forces-hunt-for-is-chemical-weapons-stock-in-syria-report>; and Giannangeli, M., 'SAS V ISIS: UK special forces race to destroy terrorist gas plant that could kill MILLIONS', *Express*, 28 Sep. 2015, <http://www.express.co.uk/news/world/608263/SAS-race-destroy-ISIS-poison-gas-plant>.

[48] Anonymous, 'British special forces hunt for IS chemical weapons stock in Syria: report', *i24 News*, 27 Sep. 2015, <http://www.i24news.tv/en/news/international/middle-east/87142-150927-british-special-forces-hunt-for-is-chemical-weapons-stock-in-syria-report>;

[49] Charbonneau, L., 'After Russian delay, UN council okays probe of Syria gas attacks', 10 Sep. 2015, Reuters, <http://www.reuters.com/article/2015/09/10/us-mideast-crisis-syria-chemicalweapons-idUSKCN0RA24D20150910>.

[50] Charbonneau, L., 'After Russian delay, UN council okays probe of Syria gas attacks', 10 Sep. 2015, Reuters, <http://www.reuters.com/article/2015/09/10/us-mideast-crisis-syria-chemicalweapons-idUSKCN0RA24D20150910>.

the war crimes tribunals of the former Yugoslavia and Rwanda, as well as for the International Criminal Court (ICC), reportedly posses approximately 500.000 pages of orders and reports within and by the Syrian Government's Central Crisis Management Cell (CCMC).[51] These documents have been smuggled out of Syria in a 3-year operation involving some 50 couriers headed by a person code-named Adel.[52] The CIJA is funded by Canada, Denmark, the EU, Germany, Norway, Switzerland, the UK and the United States.[53] The CIJA has reportedly collected more than 470.000 videos and testimony.[54]

1.3.2. Syrian American Medical Society

The Syrian American Medical Society (SAMS) operates more than 90 medical facilities in the Syrian armed conflict region.[55] One doctor stated that he was present at Kafr Zeta when helicopters flew overhead and that he had provided to Western embassies blood samples, urine samples, soil samples and the remnants of a canister.[56] Mohammed Tennari is a doctor who has testified to the UN Security Council and to members of the US political establishment.[57] He is based in a SAMS office in Turkey.

The International Centre for the Study of Radicalisation and Political Violence (ICSR) produces reports of the fighting in the Middle East, including summaries of interviews of IS defectors. Some of this information and analysis could inform CW-related analyses and discussions.[58]

Dr Khaled Almilaji and Hazem Alhalabi (members of a Chemical Biological Response Network (CBRN) Task Force [affiliation unknown]) have reportedly obtained forensic evidence that chlorine was used in the Syrian conflict.[59] On 7

[51] Borger, J., 'Smuggled Syrian documents enough to indict Bashar al-Assad, say investigators', *Guardian*, 12 May 2015, <http://www.theguardian.com/world/2015/may/12/smuggled-syrian-documents-indict-assad-investigators>.

[52] Borger, J., 'Smuggled Syrian documents enough to indict Bashar al-Assad, say investigators', *Guardian*, 12 May 2015, <http://www.theguardian.com/world/2015/may/12/smuggled-syrian-documents-indict-assad-investigators>.

[53] Borger, J., 'Smuggled Syrian documents enough to indict Bashar al-Assad, say investigators', *Guardian*, 12 May 2015, <http://www.theguardian.com/world/2015/may/12/smuggled-syrian-documents-indict-assad-investigators>.

[54] Borger, J., 'Smuggled Syrian documents enough to indict Bashar al-Assad, say investigators', *Guardian*, 12 May 2015, <http://www.theguardian.com/world/2015/may/12/smuggled-syrian-documents-indict-assad-investigators>.

[55] Syrian American Medical Society (SAMS), 'SAMS Foundation', <https://www.sams-usa.net/foundation/>; and Meuse, A. and Amos, D., 'In Syria, chlorine attacks continue to take a toll', National Public Radio, 25 July 2015, <http://www.npr.org/sections/parallels/2015/07/25/425898852/in-syria-chlorine-attacks-continue-to-take-a-toll>.

[56] Meuse, A. and Amos, D., 'In Syria, chlorine attacks continue to take a toll', National Public Radio, 25 July 2015, <http://www.npr.org/sections/parallels/2015/07/25/425898852/in-syria-chlorine-attacks-continue-to-take-a-toll>.

[57] Meuse, A. and Amos, D., 'In Syria, chlorine attacks continue to take a toll', National Public Radio, 25 July 2015, <http://www.npr.org/sections/parallels/2015/07/25/425898852/in-syria-chlorine-attacks-continue-to-take-a-toll>.

[58] ICSR, <http://icsr.info/about-us-2/>.

[59] Sparrow, A., 'Syria: death from Assad's chlorine', *New York Review of Books*, 7 May 2015, <http://www.nybooks.com/articles/archives/2015/may/07/syria-death-assads-chlorine/>.

May 2015 it was reported that civilians in Kansafra saw a barrel bomb fall from a helicopter which did not explode.[60] A SAMS-supported hospital treated 25 people who exhibited signs of chemical exposure.[61] Finally, sources of information that can, in principle, inform such investigations include the group *Raqqa is Being Slaughtered Silently*.[62]

2.0 Pulmonary effects of TICs

Various definitions and guidelines have been developed for mapping the pulmonary effects of toxic inhalation hazards (TIHs) in general and toxic industrial chemicals (TICs) in particular. Pulmonary toxicity can be caused by inter alia medicinal effects, radiotherapy, chemicals, and/or particulates. Ideally differentiation strategies should be developed or further validated according to the various TICs or CWAs that induce serious pulmonary injuries according to at least four main factors: 1. acute effects, 2. chronic effects, 3. *in vivo* and 4. post-mortem.

Lung system effects may be divided according to: (*a*) type I and II cell effects, (*b*) neuronal effects and (*c*) endothelial/epithelial effects.[63] Lung injury can be assessed by pathologists using the dry/wet ratio. Physiological exposure effects can, in general, include TRP channel effects. Intervention or treatment strategies include lung ventilation. The air pressure applied in such cases must not exceed prescribed levels otherwise ventilation induced lung injury (VILE) may occur.

Relevant literature exists for exposure to inter alia ammonia, chlorine, phosgene, cyanide compounds, and perfluroisobutylene (PFIB).[64] PFIB can cause the more generalized symptoms of polymer fume fever. Inhalation of chlorine gas may cause respiratory system effects, neurological effects, immunological and lymphoreticular effects and systemic effects. Phosgene is a common industrial chemical that prompts generalized TIC-type pulmonary effects. It can be used by the chemical industry as a chlorinating agent. As such chlorine and phosgene exposure effects might be confused or both sets of

[60] SAMS, 'Press release: chlorine attacks and barrel bombs devastate Idlib on Thursday', Press Release, 8 May 2015, <https://www.sams-usa.net/foundation/index.php/component/content/article/2-uncategorised/166-press-release-chlorine-attacks-and-barrel-bombs-devastate-idlib-on-thursday>.

[61] SAMS, 'Press release: chlorine attacks and barrel bombs devastate Idlib on Thursday', Press Release, 8 May 2015, <https://www.sams-usa.net/foundation/index.php/component/content/article/2-uncategorised/166-press-release-chlorine-attacks-and-barrel-bombs-devastate-idlib-on-thursday>.

[62] Sly, L., 'Inside an undercover network trying to expose Islamic State's atrocities', *Washington Post*, 9 June 2015, <http://www.washingtonpost.com/world/middle_east/islamic-state-learned-to-exploit-the-internet-activists-are-turning-the-tables/2015/06/09/8d3e490a-0964-11e5-951e-8e15090d64ae_story.html?hpid=z5>.

[63] Insert xx.

[64] Of these chemicals all but ammonia have been considered within a chemical warfare security and defence context. Early pathology or forensic medicine literature on cause(s) of injury or death due to exposure to toxic chemicals provide and possible continued relevance. E.g., on detection and quantification of cyanide in human tissue, see Gettler, A. O. and Baine, J. O., 'The Toxicology of Cyanide', *American Journal of Medical Sciences*, no. 2, vol. 195 (1938), pp. 182-98. For an up-to-date comprehensive overview, see US Environmental Protection Agency (EPA), *Toxicological Review of Hydrogen Cyanide and Cyanide Salts* (EPA: Sep. 2010, Washington, DC), <http://www.epa.gov/iris/toxreviews/0060tr.pdf>.

effects could be present. Chlorine and phosgene were used in World War I as CW agents (they are not today considered particularly effective for state-to-state CW armed conflict and for states to openly employ such weapons would be 'beyond the pale' of acceptable international norms and standards). The focus in this paper is on chlorine effects from a mainly forensic pathologist perspective.

Detection methods should ideally serve to document the nature and type of TIC exposure. Treatment strategies and time delays may complicate or prevent such documentation efforts.

2.1. Role of TRP channels in the study of lung toxicity

Generalized lung exposure effects of TICs or TIHs include: (*a*) lipid oxidation, (*b*) reactive oxygen species (ROS), and (*c*) DNA damage. Chemosensation in the body could suggest TIH targets for post-exposure treatment strategies.

Transient Receptor Potential (TRP) type receptors, acid-sensitive receptor channel group are an important basis for the consideration of post-exposure physiological effects. TRP channels play an important role in the stimulation of the sensory neural pathways in the lung. TRPV4 is from this family sub-group that can show vanilloid stimulation, particularly in mechanical and osmotic stress; stimulation has the ability and is also important in muscle contraction.[65] It is known that chlorine induces TRPA1 sensory channels.[66] TRPV4 was shown to be induced by extracellular hypotonia. This and similar aqp5 is a process parallel to the already well AQP5 function.[67] TRPV4 sensitive to extracellular calcium, and recent study has shown that the effect of reducing the AQP5, a calcium-free environment seems to have disappeared. A decrease in TRPV4 inhibition of pathological changes caused by the chlorine gas, which runs in parallel with the expression of aquaporins and other AQP5 suggests that changes in chlorine poisoning has occurred.

3.0 Chlorine

Chlorine and cyanide-related deaths are typically the result of household and industrial accidents. A significant amount of medical forensic experience and expertise has been developed in dealing with such cases, including by creating and applying chain-of-evidence for legal inquiries and criminal investigations (e.g., by conducting interviews according to templates and specialized procedures). In addition to the physiological manifestations of exposure, such

[65] Holzer, P., 'Acid-sensitive ion channels and receptors', *Handbook of Experimental Pharmacology*, (2009), pp. 283-332. fix

[66] Bessac, B. F., and Jordt, S. E., 'Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control', *Physiology* (2008), pp. 360-370. fix

[67] Sidhaye, V. K., Guler, A. D., Schweitzer, K. S., D'Alessio, F., Caterina, M. J., and King, L. S., 'Transient receptor potential vanilloid 4 regulates aquaporin-5 abundance under hypotonic conditions', *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 12 (2006), pp. 4747-4752.

deaths are of special importance in legal investigations because the use of such toxic chemicals can be classed as a war crime or be subject to investigation under the authority of the UN Secretary-General's investigation mechanism for determining possible uses of CBW and to investigations of alleged chemical weapons use carried out by the OPCW.

Short and longer-term effects of chlorine exposure may be manifested among victims. Difficulties for post-mortem analysis of chlorine and cyanide-related deaths remain challenging obstacles for forensic medicine. In chlorine-related deaths, in particular, no reliable traces (i.e., biomarkers) of the exposure may be found. Although not specific to chlorine exposure, some histopathological changes have been recently identified.[68] Gene expression research on similar deaths is promising.[69] No specific autopsy guidelines appear to exist for chlorine and cyanide-related deaths.

Inhalation of chlorine gas may cause respiratory system effects, neurological effects, immunological and lymphoreticular effects and systemic effects. However in this paper, mainly respiratory system effects are considered.

Chlorine is a toxic gas and it is one the top ten chemicals produced (by gross weight) in following industries; plastic production, pulp and paper production, solvent production, water purification and the like.[70] Deaths and injuries have occurred due to releases of chlorine gas into the environment (e.g., in railroad accidents).[71] The effects of high concentrations of chlorine on plant life have also been studied.[72] Finally, chlorine exposure may occur in household accidents due to mixing of sodium hypochlorite with any acid-containing item, including swimming pool sanitation chemicals.

Chlorine was used as a chemical weapon in World War I. Chlorine has been used in Iraq and Syrian war as a chemical weapon.[73]

The effect of chlorine gas ranges from mild irritation of mucous membranes to death.[74] Although the exact mechanism is unknown, acute inhalation of chlorine causes respiratory dysfunction.[75] Low-level exposure may cause

[68] 3 SWE Cl marker articles + implications.

[69] Insert xx.

[70] Evans, R. B., 'Chlorine: state of the art', *Lung*, vol. 183, no. 3 (May-June 2005), pp. 151-67.

[71] Agency for Toxic Substances and Disease Registry (ATSDR), *Toxicological Profile for Chlorine*, (US Department of Health and Human Services, Public Health Service: Atlanta, GA, 2010), pp. 26-29; and Duncan, M. A., et al., 'Follow-up assessment of [the] health consequences after a chlorine release from a train derailment—Graniteville, SC, 2005', *Journal of Medical Toxicology*, vol. 7 (2011), pp. 85-91.

[72] E.g., Schreuder, M. D. J. and Brewer, C. A., 'Effects of short-term, high exposure to chlorine gas on morphology and physiology of *Pinus ponderosa* and *Pseudotsuga menziessi*', *Annals of Botany*, vol. 88 (25 June 2001), pp. 187-195. discuss linkage to CW investigation SOPs and WIs & documentation procedures.

[73] Jones, R., Wills, B., Kang, C., 'Chlorine gas: an evolving hazardous material threat and unconventional weapon', *Western Journal of Emerging Medicine*, vol. 11, no. 2 (May 2010), pp. 151–156; and OPCW, <http://www.opcw.org/news/article/opcw-fact-finding-mission-compelling-confirmation-that-chlorine-gas-used-as-weapon-in-syria>.

[74] Evans, R. B., 'Chlorine: state of the art', *Lung*, vol. 183, no. 3 (May-June 2005), pp. 151-67.

[75] Massa, C. B., Scott, P., Abramova, E., Gardner, C., Laskin, D. L., Gow, A. J., 'Acute chlorine gas exposure produces transient inflammation and a progressive alteration in surfactant composition with accompanying mechanical dysfunction', *Toxicology and Applied Pharmacology*, vol. 278, no. 1 (July 2014), pp. 53-64.

deteriorating, but transient, effect on FEV1, peak flow, airways resistance, functional residual capacity and total lung capacity.[76]

The long-term consequences are described as reactive airways dysfunction syndrome (RADS), bronchiectasis, decline in lung volumes and increased airway resistance.[77]

The severity of pathological changes in chlorine gas toxicity vis-à-vis individual and genetic factors has been significantly elucidated in animal studies and asthmatic or atopic patients (i.e., patients who display allergic hypersensitivity). Both of these clinical and morphological changes; as well as the relationship between the dose and duration of exposure gene expressions that accompany these changes has been the subject of various studies. On the other hand, the factors (and their underlying reasons) that determine the rate of death have not been elucidated sufficiently.[78]

One of the important consequences of the resulting high-dose inhaled chlorine made on the mechanism of alveolar damage, which is available in several studies. When chlorine gas comes into contact with the liquid in the alveolar surface, liquid hydrochloric acid (HCl), as well as hypochlorite (OCl) and hypochlorous acid (HOCl) radicals are formed. HCl buffered with bicarbonate, HOCl and OCl cause oxidative damage on cells. The damage created at the alveolar level is expressed by the oxidant effect. In the alveoli, resulting in early cytokines collected neutrophils, and myeloperoxidase activity has been suggested as responsible for further increase oxidant formation.[79]

Hydrochloric and hypochlorous acids stimulate neutrophils (the most common type of white blood cells) and macrophages leads to increased local

[76] Rotman, H. H., et al., 'Effects of low concentrations of chlorine on pulmonary function in humans', *Journal of Applied Physiology*, vol. 54 (1983), pp. 1120-24.

[77] Jonasson, Sofia, Koch, Bo, and Bucht, Anders, 'Inhalation of chlorine causes long-standing lung inflammation and airway hyperresponsiveness in a murine model of chemical-induced lung injury', *Toxicology*, vol. 303 (2013), pp. 34–42.

[78] Demnati, R., Fraser, R., Ghezzo, H., Martin, J. G., Plaa, G., & Malo, J. L., 'Time-course of functional and pathological changes after a single high acute inhalation of chlorine in rats', *European Respiratory Journal*, vol. 11, no. 4 (1998), pp. 922-928; D'Alessandro, A., Kuschner, W., Wong, H., Boushey, H. A., and Blanc, P. D., 'Exaggerated responses to chlorine inhalation among persons with nonspecific airway hyperreactivity', *Chest*, vol. 109, no. 2 (1996), pp. 331-337; Mo, Y., Chen, J., Schlueter, C. F., and Hoyle, G. W., 'Differential susceptibility of inbred mouse strains to chlorine-induced airway fibrosis', *American Journal of Physiology: Lung Cellular and Molecular Physiology*, vol. 304, no. 2 (2013), pp. L92-L102; Leikauf, G. D., Pope-Varsalona, H., Concel, V. J., Liu, P., Bein, K., Berndt, A., Fabisiak, J. P., 'Integrative assessment of chlorine-induced acute lung injury in mice', *American Journal of Respiratory Cell and Molecular Biology*, vol. 47, no. 2 (2012), pp. 234-244; and Leikauf, G. D., Pope-Varsalona, H., Concel, V. J., Liu, P., Bein, K., Brant, K. A., Prows, D. R., 'Functional genomics of chlorine-induced acute lung injury in mice', *Proceedings of the American Thoracic Society*, vol. 7, no. 4 (2010), pp. 294-296.

[79] McGovern, T. K., Goldberger, M., Allard, B., Farahnak, S., Hamamoto, Y., O'Sullivan, M., and Martin, J. G., 'Neutrophils mediate airway hyperresponsiveness after chlorine-induced airway injury in the mouse', *American Journal of Respiratory Cell and Molecular Biology*, vol. 52, no. 4 (2015), pp. 513-522; Martin, J. G., Campbell, H. R., Iijima, H., Gautrin, D., Malo, J. L., Eidelman, D. H., and Maghni, K., 'Chlorine-induced injury to the airways in mice', *American Journal of Respiratory and Critical Care Medicine*, vol. 168, no. 5 (2003), pp. 568-574.

nitric oxide concentration.[80] Under normal circumstances, the airways' physiological functions that regulate the amount of nitric oxide is quite low, but display a sharp rise in pathological conditions.[81] Honavar et al. showed that chlorine gas exposure inhibits endothelial nitric oxide synthase (eNOS) activity which is important for regulating local and systemic vascular physiological function. On the other hand, chlorine gas exposure induces nitric oxide synthase (iNOS) which plays an important role in pathological conditions.[82] These processes cause immediate direct oxidative damage to the epithelium. Progressive damage to the epithelium caused by proinflammatory cytokines (TNF-α, IL-1, IL-6, IL-8) early expression, neutrophils and macrophages, such as inflammatory cell activation, migration into tissue, released from these cells have a role oxidant and proteolytic enzymes.[83] These products can damage lung endothelium, epithelial basement membrane, cell organelles, including mitochondria and DNA.[84] Jonasson et al. showed that 2-6 hours after exposure inflammatory mediators, particularly macrophages and monocytes expressed the early phase of activation in pathologic conditions, can be found in serum and bronho-alveoler lavage.[85]

In addition, the chemotactic factors secreted from these cells, stimulate activated neutrophils to migrate to the airway.[86] Chemotactic factors, such as CXCL1 and TNF-α are for neutrophils, whereas CCL11 and CCL5 are for eosinophils, promote epithelial damage with release of proteolytic enzymes and reactive oxygen species.[87] On the other hand, activation of neutrophils release myeloperoxidase plays a role in pulmonary and systemic endothelial

[80] White, C. W., and Martin, J. G., 'Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models', *Proceedings of the American Thoracic Society*, vol. 7, no. 4 (2010), pp. 257-263.

[81] Olin, A. C., Ljungkvist, G., Bake, B., Hagberg, S., Henriksson, L., and Toren, K., 'Exhaled nitric oxide among pulpmill workers reporting gassing incidents involving ozone and chlorine dioxide', *European Respiratory Journal*, vol. 14, no. 4 (1999), pp. 828-831.

[82] Honavar, J., Samal, A. A., Bradley, K. M., Brandon, A., Balanay, J., Squadrito, G. L., and Patel, R. P., 'Chlorine gas exposure causes systemic endothelial dysfunction by inhibiting endothelial nitric oxide synthase-dependent signaling', *American Journal of Respiratory Cell and Molecular Biology*, vol. 45, no. 2 (2011), pp. 419-425.

[83] Evans, R. B., 'Chlorine: state of the art', *Lung*, vol. 183, no. 3 (2005), pp. 151-167.

[84] Tuck, Stephanie A., Ramos-Barbón, David, Campbell, Holly, McGovern, Toby, Karmouty-Quintana, Harry, and Martin, James G., 'Time course of airway remodelling after an acute chlorine gas exposure in mice', *Respiratory Research*, vol. 9 (2008) p. 61; Jonasson, S., Koch, B., and Bucht, A., 'Inhalation of chlorine causes long-standing lung inflammation and airway hyperresponsiveness in a murine model of chemical-induced lung injury', *Toxicology*, vol. 303 (2013), pp. 34-42; Honavar, J., Bradley, E., Bradley, K., Oh, J. Y., Vallejo, M. O., Kelley, E. E., and Patel, R. P., 'Chlorine gas exposure disrupts nitric oxide homeostasis in the pulmonary vasculature', *Toxicology*, vol. 321 (2014), pp. 96-102; and White, C. W., and Martin, J. G., 'Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models', *Proceedings of the American Thoracic Society*, vol. 7, no. 4 (2010), pp. 257-263.

[85] Jonasson, S., Wigenstam, E., Koch, B., and Bucht, A., 'Early treatment of chlorine-induced airway hyperresponsiveness and inflammation with corticosteroids', *Toxicology and Applied Pharmacology*, vol. 271, no. 2 (2013), pp. 168-174.

[86] Jonasson, S., Wigenstam, E., Koch, B., and Bucht, A., 'Early treatment of chlorine-induced airway hyperresponsiveness and inflammation with corticosteroids', *Toxicology and Applied Pharmacology*, vol. 271, no. 2 (2013), pp. 168-174.

[87] Jonasson, S., Wigenstam, E., Koch, B., and Bucht, A., 'Early treatment of chlorine-induced airway hyperresponsiveness and inflammation with corticosteroids', *Toxicology and Applied Pharmacology*, vol. 271, no. 2 (2013), pp. 168-174.

damage in chlorine gas exposure.[88] The resulting reactive intermediates, the nitration of aromatic amino acid, causing chlorination and dimerization constitute loss of structure and function.[89] Due to the high reactivity of chlorine, acute symptoms begin within minutes and hours, these effects can be significantly mitigated by strategies to protect or treat exposed tissue.[90] Acute symptoms in the respiratory tract are pulmonary edema, tracheobronchitis temporary airway dysfunction and acute respiratory distress syndrome (ARDS), reactive airways dysfunction syndrome (RADS) is defined as observed in the long-term consequence.[91] RADS developing against substances such as chlorine feature a high degree of irritative and non-immunological hypersensitivity is a condition characterized by airway obstruction.[92] As a result, the chlorine gas exposure on lung tissue can cause oxidative damage, antioxidant capacity reduction, the loss of surfactant, inflammation, alveolar epithelial ion transport and dysfunction, and activation of sensory neurons. All these changes lead to epithelial cell damage, destruction of the alveolar-capillary barrier, airway hyperreactivity, pulmonary inflammation and edema.

Apart from oxidant effects, other targets of chlorine are amino acids which are found in environment and tissue. Chlorine reacts with amino acid side chains and terminal amino group and this leads to the formation of chloramines and chloro-tyrosine compounds. These compounds can have harmful effects locally and are cardiotoxic.

On the other hand, studies showed that pneumocytes damage did not occur at the early stages, usually evident within 12-24 hours. In general, apoptosis is not prevalent at the early stages, probably dominated by necrosis, apoptosis increased after 24 hours, during which the regenerative changes were reported in subsequent days. To summarize several of the related studies: 1500 ppm for five minutes at a dose of inhalation causes damage in the bronchial epithelium 24 hours after exposure in rats has been reported that decomposition and necrosis were observed. After 24 hours, regeneration starts and regeneration is active up to seven days. The third day was found to be traceable to smooth

[88] Jurkuvenaite, A., Benavides, G. A., Komarova, S., Doran, S. F., Johnson, M., Aggarwal, S., and Matalon, S., 'Upregulation of autophagy decreases chlorine-induced mitochondrial injury and lung inflammation', *Free Radical Biology and Medicine*, vol. 85 (2015), pp. 83-94.

[89] White, C. W., and Martin, J. G., 'Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models', *Proceedings of the American Thoracic Society*, vol. 7, no. 4 (2010) pp. 257-263.

[90] Jonasson, S., Koch, B., and Bucht, A., 'Inhalation of chlorine causes long-standing lung inflammation and airway hyperresponsiveness in a murine model of chemical-induced lung injury', *Toxicology*, vol. 303 (2013), pp. 34-42.

[91] Jonasson, S., Koch, B., and Bucht, A., 'Inhalation of chlorine causes long-standing lung inflammation and airway hyperresponsiveness in a murine model of chemical-induced lung injury', *Toxicology*, vol. 303 (2013), pp. 34-42.

[92] Jonasson, S., Koch, B., and Bucht, A., 'Inhalation of chlorine causes long-standing lung inflammation and airway hyperresponsiveness in a murine model of chemical-induced lung injury', *Toxicology*, vol. 303 (2013), pp. 34-42.

muscle proliferation.[93] Tuck, et al (2008) showed that epithelial basement membrane separation can be observed 12 hours after exposure to 800 ppm.[94] Another study demonstrated that basal cells are responsible for regenerative activity in the high dose exposures.[95]

Biological effects that cause damage to the epithelium, is associated with the formation mechanism of the injury. There are different pathways of cell death: necrosis, apoptosis, anoikis and necroptosis.[96] The mechanism of death in necroptosis is between necrosis and apoptosis. After exogenous TNF-α stimulation and mitochondrial damage due to intracellular calcium are the first steps of necroptosis, and it is similar to apoptosis at the first stages. Since TNF-α is the most important activator of necroptosis and there is no increase in plasma levels of TNF-α, it is believed that chlorine inhalation related cell death, especially at the first stages, is not dominated by necroptosis.[97]

3.1. Mechanism of death in Cl exposure

According to autopsy reports, the cause of death from high levels of chlorine gas exposure is issued as asphyxia.[98] At least three possible mechanisms can explain asphyxia in those cases. First, acute high dose chlorine exposure may replace oxygen in lungs. Indeed nontoxic gases, such as carbon dioxide, can be asphyxiating if too high a concentration is present in an enclosed space and rapid collapse of lung function may occur on entering such an environment.[99] Since chlorine is more than twice as dense as air, it tends to 'settle' near where it is released and its leads to locally very high concentrations.[100]

[93] Demnati, R., Fraser, R., Ghezzo, H., Martin, J. G., Plaa, G., and Malo, J. L., 'Time-course of functional and pathological changes after a single high acute inhalation of chlorine in rats', *European Respiratory Journal*, vol. 11, no. 4 (1998), pp. 922-928.

[94] Tuck, S. A., Ramos-Barbón, D., Campbell, H., McGovern, T., Karmouty-Quintana, H., and Martin, J. G., 'Time course of airway remodelling after an acute chlorine gas exposure in mice', *Respiratory Research*, vol. 9 (2008), p. 61.

[95] Musah, S., Chen, J., and Hoyle, G. W., 'Repair of tracheal epithelium by basal cells after chlorine-induced injury', *Respiratory Research*, vol. 13 (2012), p. 107.

[96] Skenderi, Faruk, Vranic, Semir, and Damjanov, Ivan, 'Regulated cell death in diagnostic histopathology', *International Journal of Developmental Biology*, vol. 59 (2015), pp. 149-158.

[97] Honavar, J., Samal, A. A., Bradley, K. M., Brandon, A., Balanay, J., Squadrito, G. L., and Patel, R. P., 'Chlorine gas exposure causes systemic endothelial dysfunction by inhibiting endothelial nitric oxide synthase-dependent signaling', *American Journal of Respiratory Cell and Molecular Biology*, vol. 45, no. 2 (2011), pp. 419-425; and Mo, Y., Chen, J., Humphrey, D. M., Jr., Fodah, R. A., Warawa, J. M., and Hoyle, G. W., 'Abnormal epithelial structure and chronic lung inflammation after repair of chlorine-induced airway injury', *American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 308, no. 2 (2015), pp. L168-78.

[98] Van Sickle D., Wenck, M. A., Belflower, A., Drociuk, D., Ferdinands, J., Holguin, F., Svendsen, E., Bretous, L., Jankelevich, S., Gibson, J. J., Garbe, P., Moolenaar, R. L., 'Acute health effects after exposure to chlorine gas released after a train derailment', *American Journal of Emergency Medicine*, vol. 27, no. (Jan. 2009), pp. 1-7. See also Balte, P. P., et al., 'The immediate pulmonary disease pattern following exposure to high concentrations of chlorine gas', *Pulmonary Medicine*, (2013), <http://dx.doi.org/10.1155/2013/325869>.

[99] Eds J. Payne-James, R. W., Byard, T. S. Corey, C. Henderson, *Encyclopedia of Forensic and Legal Medicine* (Elsevier: Oxford, 2005), p. 152.

[100] White, C. W., and Martin, J. G., 'Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models,' *Proceedings of the American Thoracic Society*, vol. 7, no. 4 (July 2010), pp. 257-63.

A second possible mechanism may be direct oxidative injury to the airway epithelium and further damage to migration and activation of inflammatory cells within the airway epithelium.[101] Another point is that chlorine can lead to post-exposure extrapulmonary vascular endothelial dysfunction and inflammation characterized by the loss of eNOS-derived signaling and increased iNOS expression that can cause acute cardiovascular events.[102]

A third possible mechanism may be cardiotoxic effect of chlorine. Chlorine and its reactants can cause to cardiac toxicity and this may lead cardiac dysfunction and potentially contributing to mortality.[103]

3.2. Possible biomarkers in Cl exposure

There appears to be no histopathologic indicator for chlorine exposure. However, in one study, a loss of cilia in the trachea was shown in Fisher-344 rats sub-chronically exposed to 0, 0.5, 1.5, or 5.0 ppm of chlorine.[104] In another study, 3-Chlorotyrosine and 3,5-Dichlorotyrosine were shown as good biomarkers of environmental exposure to chlorinating chemicals. These biomarkers were found in the respiratory and transitional epithelium versus the olfactory epithelium of the nasal cavity. However, both methods are not currently applicable to humans.[105] To summarize, specific biomarkers for acute and chronic exposures to chlorine gas are currently lacking.[106]

3.3. New technologies in studying Cl exposure

Genome sequencing technics permit a more comprehensive approach of studying molecular and cellular dynamics. These new molecular techniques permit higher throughput studies and are well suited to elucidating the

[101] White, C. W., and Martin, J. G., 'Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models,' *Proceedings of the American Thoracic Society*, vol. 7, no. 4 (July 2010), pp. 257-63.

[102] Honavar, J., Samal, A. A., Bradley, K. M., Brandon, A, Balanay, J., Squadrito, G. L., MohanKumar K., Maheshwari, A., Postlethwait, E. M., Matalon, S., and Patel, R. P., 'Chlorine gas exposure causes systemic endothelial dysfunction by inhibiting endothelial nitric oxide synthase-dependent signaling', *American Journal of Respiratory Cell and Molecular Biology*, vol. 45, no. 2 (Aug. 2011), pp. 419-25.

[103] Zaky, A., Bradley, W. E., Lazrak, A., Zafar, I., Doran S, Ahmad A, White CW, Dell'Italia, L. J., Matalon, S., and Ahmad, S., 'Chlorine inhalation-induced myocardial depression and failure', *Physiological Reports*, vol. 3, no. 6 (June 2015), <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4510636/>.

[104] Kutzman, R. S., A study of Fisher-344 rats subchronically exposed to 0, 0.5, 1.5, or 5.0 ppm chlorine. (Upton, NY: Brookhaven National Laboratory: Upton, New York, 1983). Cited in Agency for Toxic Substances and Disease Registry, *Toxicological Profile for Chlorine* (US Department of Healtth and Human Services: Nov. 2010). See also Sochaski, M. A., et al., '3-Chlorotyrosine and 3,5-Dichlorotyrosine as biomarkers of respiratory tract exposure to chlorine gas', *Journal of Analytical Toxicology*, vol. 32 (Jan./Feb. 2008). Expand and discuss 2008 art.

[105] Sochaski, M. A., et al., '3-Chlorotyrosine and 3,5-dichlorotyrosine as biomarkers of respiratory tract exposure to chlorine gas', *Journal of Analytical Toxicology*, vol. 32, no. 1 (2008), pp. 99-105.

[106] White, C. W., Martin, J. G., 'Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models', *Proceedings of the American Thoracic Society*, vol. 7, no. 4 (July 2010), pp. 257-63.

mechanisms of cellular responses to toxicants such as CWAs.[107] In order to demonstrate the effects of sulfur mustard and phosgene exposure at the studying molecular and cellular, microarray analysis was performed.[108] A similar analysis was done for chlorine exposure.[109] However this study focused on low level exposure (i.e., 45 ppm 24 hours). Hence the acute effects of chlorine exposure at the cellular level is still uncertain or largely unknown.

4.0 Cyanide

Cyanides are poisonous chemicals that widely exist in nature and industrial processes as well as accidental fires potentially causing death within minutes.[110] Cyanide compounds (e.g., cydrocyanic (prussic) acid) have been known since ancient times, were evaluated for CW purposes during both world wars and remain a mainly safety concern.[111] The principal route of cyanide exposure in humans is from plants such as bamboo, cassava and sorghum.[112] Cyanide may also be produced by some algae, bacteria and fungi.[113] Manmade cyanide hazards include fire inhalation, automobile emissions and cigarette smoke.[114] Al Qaeda affiliate interest in cyanide has been reported.[115]

Cyanide is life-threatening preventing cellular respiration by inhibiting cytochrome c oxidase, resulting in cardiopulmonary failure, hypoxic brain injury, and death within minutes or it can produce a chronic delayed-onset

[107] Sekowski, J. W., and Dillman, J. F., 'Application of genomic, proteomic, and metabolomic technologies to the development of countermeasures against chemical warfare agents', in Eds. J. A. Romano, B. J. Lukey, and H. Salem, *Chemical Warfare Agents: Chemistry, Pharmacology, Toxicology, and Therapeutics* (CRC Press: Boca Raton, Florida, 2008), p. 125.

[108] Sciuto, A. M., Phillips, C. S., Orzolek, L. D., Hege, A. I., Moran, T. S., Dillman, J. F., 'Genomic analysis of murine pulmonary tissue following carbonyl chloride inhalation', *Chemical Research in Toxicology*, vol. 18 (2005), pp. 1654–60; and Dillman, J. F., Phillips, C. S., Dorsch, L. M., Croxton, M. D., Hege, A. I., Sylvester, A. J., Moran, T. S., and Sciuto, A. M., 'Genomic analysis of rodent pulmonary tissue following bis-(2-chloroethyl) sulfide exposure', *Chemical Research in Toxicology*, vol. 18 (2005), pp. 28–34.

[109] Leikauf, G. D., Pope-Varsalona, H., Concel, V. J., Liu, P., Bein, K., Berndt, A., Martin, T. M., Ganguly, K., Jang, A. S., Brant, K. A., Dopico, R. A. Jr, Upadhyay, S., Di, Y. P., Li Q., Hu, Z., Vuga, L. J., Medvedovic M., Kaminski, N., You, M., Alexander, D. C., McDunn, J. E., Prows, D. R., Knoell, D. L., and Fabisiak, J. P., 'Integrative assessment of chlorine-induced acute lung injury in mice', *American Journal of Respiratory Cell and Molecular Biology*, vol. 47, no. 2 (Aug. 2012), pp. 234-44.

[110] Zhang, Q., Maddukuri, N., and Gong, M. A. 'Direct and rapid method to determine cyanide in urine by capillary electrophoresis', *Journal of Chromatography A* (2015), pp. 158-62.

[111] Szinicz, L., 'History of chemical and biological warfare agents', *Toxicology* (2005), pp. 167-81.

[112] Vinnakota, C. V., et al., 'Comparison of cyanide exposure markers in the biofluids of smokers of and non-smokers', *Biomarkers*, vol. 17, no. 7 (2012), p. 625.

[113] Vinnakota, C. V., et al., 'Comparison of cyanide exposure markers in the biofluids of smokers of and non-smokers', *Biomarkers*, vol. 17, no. 7 (2012), p. 625.

[114] Vinnakota, C. V., et al., 'Comparison of cyanide exposure markers in the biofluids of smokers of and non-smokers', *Biomarkers*, vol. 17, no. 7 (2012), p. 625; and Augustine, J. and Walsh, D. W., 'Smoke associated cyanide exposure: the importance of prompt recognition and protocols for prehospital treatment', *Fire Engineering*, vol. 159, no. 8 (Aug. 2006), pp. 15-19.

[115] Hilmas, E. and Hilmas, C. J., 'Medical management of chemical toxicity in pediatrics', p. 944 in Ed. Ramesh C. Gupta, *Handbook of Toxicology of Chemical Warfare Agents* (Elsevier: London, 2009).

neurological syndrome that includes symptoms of Parkinson's Disease.[116] The lethal capacity of cyanide has been exploited for mass murder in several infamous cases.[117] For example, cyanide was used for the collective suicide-murder of hundreds of people in the Jim Jones sect in the 1970s. In 1982 cyanide-laced Tylenol† capsules were placed in six stores in the Chicago area, and resulted in eight reported deaths.[118]

4.1. Chemical identities and physico-chemical properties

The gaseous form of cyanide, hydrogen cyanide (HCN) smells of bitter almonds having a boiling point of 26^0 C (i.e., highly volatile). The liquid form of cyanide is known as hydrocyanic acid and is colourless The common solid form usually comprises sodium and potassium salts (i.e., a white colour forming compound).[119]

4.2. Biological mechanisms of acute toxicity

Cyanide inhibits mitochondrial cytochrome oxidase with disruption of the ability of cells to use oxygen. In other words it causes histotoxic hypoxia.[120] When cytochrome oxidase's activity is blocked, oxidative phosphorylation ceases and the cell must then switch to anaerobic metabolism of glucose to generate ATP.[121] The broad-ranging clinical manifestations of acute cyanide poisoning mainly reflect the nonspecific effects of histotoxic hypoxia.[122] The heart and brain require a large, continuous supply of oxygen and ATP for normal function and are therefore, in principle, most susceptible.[123] Inhibition of the respiratory centre leads to transitory hyperventilation followed by respiratory depression while myocardial depression causes further hypoxia with decreased cardiac output and shock, coma, and death.[124]

[116] Zhang, D., Lee, B., Nutter, A., Song, P., Dolatabadi, N., Parker, J., Sanz-Blasco, S., Newmeyer, T., Ambasudhan, R., McKercher, S. R., Masliah, E., and Lipton, S. A., 'Protection from cyanide-induced brain injury by the Nrf2 transcriptional activator carnosic acid', *Journal of Neurochemistry*, vol. 133, no. 6 (June 2015), pp. 898-908.

[117] Baud, F. J., 'Cyanide: critical issues in diagnosis and treatment', *Human & Experimental Toxicology*, vol. 26, no. 3 (Mar. 2007), pp. 191-201.

[118] Baud, F. J., 'Cyanide: critical issues in diagnosis and treatment', *Human & Experimental Toxicology*, vol. 26, no. 3 (Mar. 2007), pp. 191-201.

[119] Renklidag, T., Karaman, A. G., Siyanur, Zehirlenmesi, STED, vol. 12, no. 9 (2003), p. 353. check

[120] Prieto, I., Pujol, I., Santiuste, C., Poyo-Guerrero, R., and Diego, A., 'Acute cyanide poisoning by subcutaneous injection', *Emergency Medicine Journal* (2005), pp. 389-90.

[121] <http://www.uptodate.com/contents/cyanide-poisoning#H4>, 03.10.2015. fix

[122] Nelson, L., 'Acute cyanide toxicity: mechanisms and manifestations', *Journal of Emerging Nursing* (2006) S8-11. Fix pp no.

[123] Nelson, L., 'Acute cyanide toxicity: mechanisms and manifestations', *Journal of Emerging Nursing* (2006) S8-11. Fix pp.

[124] Prieto, I., Pujol, I., Santiuste, C., Poyo-Guerrero, R., and Diego, A., 'Acute cyanide poisoning by subcutaneous injection. *Emergency Medicine Journal* (2005), pp. 389-90.

4.3. Metabolism and detoxification of cyanide

The primary endogenous mechanism of cyanide detoxification is metabolism in the liver by rhodanese to thiocyanate, which is a nontoxic compound excreted in the urine.[125] However, small amounts cyanide can be excreted unmetabolized in breath, urine, and sweat and it causes a bitter almond-like odor in the breath or in gastric contents urine.[126]

4.4. Determinants for cyanide toxicity

Rapid and accurate determination of cyanide exposure would facilitate forensic investigation, medical diagnosis, and chronic cyanide monitoring.[127] Confirmation of cyanide exposure is difficult because, in vivo, cyanide quickly breaks down by a number of pathways, including the formation of both free and protein-bound thiocyanate.[128]

4.5. Acute toxicity of cyanides

Cyanide can cause death due to respiratory arrest immediately following poisoning.[129] The principal targets of acute high-level inhalation exposure are the respiratory, central nervous, and cardiovascular systems.[130]

Short-term exposures to lower concentrations (~2.7 mg/m3) may induce symptoms and signs that include headache, dizziness, confusion, nausea, and vomiting.[131] Low dose HCN may also cause headache, anxiety, nausea, and a metallic taste. On the other cyanogen chloride (CNCl) exposure predominantly results in eye and mucous membrane irritation and then pulmonary symptoms, namely bronchorrhea, coughing, and dyspnea.[132]

[125] Nelson, L., 'Acute cyanide toxicity: mechanisms and manifestations', *Journal of Emerging Nursing* (2006) S8-11. Fix pp.

[126] Nelson, L., 'Acute cyanide toxicity: mechanisms and manifestations', *Journal of Emerging Nursing* (2006) S8-11. Fix pp.

[127] Zhang, Q., Maddukuri, N., and Gong, M., 'A direct and rapid method to determine cyanide in urine by capillary electrophoresis', *Journal of Chromatography A* (2015), pp. 158-62.

[128] Youso, S. L., Rockwood, G. A., Lee, J. P., and Logue, B. A., 'Determination of cyanide exposure by gas chromatography-mass spectrometry analysis of cyanide-exposed plasma proteins', *Analytica Chimica Acta*, vol. 677, no. 1 (Sep. 2010), pp. 24-28. Ch ref.

[129] Ed. E. Hodgson, *A Textbook Of Modern Toxicology*, 3rd edtn (John Wiley & Sons, Inc., Hoboken, New Jersey, 2004) p. 224. Ch ref.

[130] Watson, A., Dolislager, F., Hall, L., Raber, E., Hauschild, V. D., and Love, A. H., 'Developing Health-Based Pre-Planning Clearance Goals for Airport Remediation Following a Chemical Terrorist Attack: Decision Criteria for Multipathway Exposure Routes', *Human and Ecological Risk Assessment*, vol. 17 (2011), pp. 57-121, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3046627/pdf/bher17-57.pdf>.

[131] Watson, A., Dolislager, F., Hall, L., Raber, E., Hauschild, V. D., and Love, A. H., 'Developing Health-Based Pre-Planning Clearance Goals for Airport Remediation Following a Chemical Terrorist Attack: Decision Criteria for Multipathway Exposure Routes', *Human and Ecological Risk Assessment*, vol. 17 (2011), pp. 57-121, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3046627/pdf/bher17-57.pdf>.

[132] <http://www.uptodate.com/contents/cyanide-poisoning#H4>, access date: 03.10.2015 fix

4.6. Chronic toxicity of cyanides

Chronic low dose cyanide exposure can result in Parkinson's Disease-like syndromes, confusion, and intellectual deterioration.[133] It is suggested that the wide-spread pathologic condition of tropic ataxic neuropathy is associated with the intense consumption of cassava with high CN-Glycoside content.[134]

Chronic effects of low exposure to cyanide include conjunctival irritation from direct contact and skin ulceration.[135] Thyroid gland enlargement has been described, particularly in areas where iodine intake is low.[136] Residual symptoms after chronic exposure include a bitter almond taste in the mouth and headache.[137]

Long-term exposure to cyanide and/or its main metabolite, thiocyanate, has been associated with goiter, pancreatic diabetes and several neurological disorders.[138]

4.7. Forensic pathology aspects

In forensic practice, the gas form of cyanide (hydrogen cyanide) appears most commonly in persons exposed to fires. Cyanide will be gradually disappear in specimens stored for prolonged periods for two reasons; volatilization and inactivation by tissues. On the other hand cyanide can be produced in blood and other specimens by the action of cyanogenic bacteria. For this reason specimens should be analyzed immediately, otherwise they should stored at 60^0 C until the time for analysis.[139]

[133] Petrikovics, I., Budai, M., Kovacs, K., and Thompson, D. E., 'Past, present and future of cyanide antagonism research: From the early remedies to the current therapies', *World Journal of Methodology*, vol. 5, no. 2 (26 June 2015), pp. 88-100.

[134] Petrikovics, I., Budai, M., Kovacs, K., and Thompson, D. E., 'Past, present and future of cyanide antagonism research: From the early remedies to the current therapies', *World Journal of Methodology*, vol. 5, no. 2 (26 June 2015), pp. 88-100.

[135] Emara, A. M., Abbas, A. B., Sabry, D., and Manawil, M., 'Some health effects due to chronıc occupatıonal exposure to cyanıde', *Egyptian Journal of Occupational Medicine* (2014), pp. 141-15.

[136] Emara, A. M., Abbas, A. B., Sabry, D., and Manawil, M., 'Some health effects due to chronıc occupatıonal exposure to cyanıde', *Egyptian Journal of Occupational Medicine* (2014), pp. 141-15.

[137] Emara, A. M., Abbas, A. B., Sabry, D., and Manawil, M., 'Some health effects due to chronıc occupatıonal exposure to cyanıde', *Egyptian Journal of Occupational Medicine* (2014), pp. 141-15.

[138] Sousa, A. B., Soto-Blanco, B., Guerra, J. L., Kimura, E. T., and Górniak, S.L., 'Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity?' *Toxicology* (2002), pp. 87-95.

[139] Eds J. Payne-James, R. W. Byard, T. S. Corey, and C. Henderson, *Encyclopedia of Forensic and Legal Medicine* (Elsevier: Oxford, 2005), p. 218. For historical context on the preparation and analytical methods employed and evaluated by Alexander Gettler, an Austro-Hungarian Empire-born toxicologist with the Office of [the] Chief Medical Examiner of the City of New York, see Gettler, A. O. and Baine, J. O., 'The toxicology of cyanide', *American Journal of Medical Sciences*, no. 2, vol. 195 (1938), pp. 182-98.

5.0 Forensic pathology

The term 'forensic pathology' may be understood as the application of 'what is known about disease, along with all medical science, to legal problems'.[140] The principal task of the forensic pathologist is to determine the cause and manner of death.[141] The manner of death in many jurisdictions is limited to one of five categories: (*a*) homicide, (*b*) suicide, (*c*) accident, (*d*) natural and (*e*) undetermined.[142]

5.1. Investigative procedures

There are at least two main internationally-accepted standard autopsy protocol, the Minnesota Protocol, a detailed set of international guidelines providing instructions for conducting forensic autopsies and analysis of skeletal remains.[143] The Minnesota Protocol was adopted by the UN the Economic and Social Council in its resolution 1989/65 on 24 May 1989. A second widely employed international standard are the European harmonization of medico-legal autopsy rules.[144]

Autopsy protocols are useful tools for forensics or medical investigations in most cases. However, chlorine related deaths leave almost no trace and only some non-specific findings can be found. These nonspecific findings can assist to eliminate most of possible causes of death. For this reason chlorine exposure and related deaths cannot in principle be proven on the basis of autopsy findings as yet.

As there is no conclusive evidence from autopsy, crime scene investigation, processing of the evidence and personal testimonies acquire greater importance.

6.0 International verification frameworks and capacity

The CWC is the main legal basis for the international prohibition against chemical warfare. Two major multilateral frameworks for verifying the use of toxic chemicals and their precursors as a method of warfare are the OPCW

[140] Johnson, D. G., 'Forensic pathology: separating fact from fiction', *Medical Laboratory Observer* (Aug. 2003), p. 28. Cf medical history tome.

[141] Johnson, D. G., 'Forensic pathology: separating fact from fiction', *Medical Laboratory Observer* (Aug. 2003), p. 30. On the cooperation between the medical examiner staff and the New York City homicide investigators of the early 20th century, see Carey, A. A., *On the Track of Murder* (JARROLDS Publishers: London, 1931), p. 122; and Gettler, A. O. and Baine, J. O., 'The toxicology of cyanide', *American Journal of Medical Sciences*, no. 2, vol. 195 (1938), pp. 182-98.

[142] Johnson, D. G., 'Forensic pathology: separating fact from fiction', *Medical Laboratory Observer* (Aug. 2003), p. 30.

[143] Centre for Social Development and Humanitarian Affairs, *Manual on the Effective Prevention and Investigation of Extra-Legal, Arbitrary and Summary Executions* (United Nations: New York, 1991).

[144] B. Brinkmann, 'Harmonization of medico-legal autopsy rules', *International Journal of Legal Medicine*, vol. 113 (1999), Letter to the Editor, <http://link.springer.com/article/10.1007%2Fs004140050271?LI=true#page-1>.

and the UN Secretary-General's mechanism to investigate allegations of CBW use.

Biomedical sample matrices comprise blood, urine and tissue. Environmental sample *versus* biomedical sample issues. The taking of samples from corpses is not part of the OPCW normal operating procedure.[145] The WHO does have some relevant experience, including with respect to methodologies for anonomyzing samples. In principle, it is possible for the OPCW representatives to witness the taking of samples. So long as chain-of-custody is preserved and authoritatively documented, this is a possible way to go with the taking of samples from corpses.

There have been five OPCW biomedical sample exercises in 2010-2015. The target matrices included synthetic urine and human blood plasma. The OPCW will start biomedical proficiency tests in 2016. Fluoride reactivation of blood samples collected by UN Secretary-General team has been carried out. Biomedical sampling can or should use EU and World Anti-Doping Agency (WADA) criteria.

As previously mentioned, samples may be taken from air, soil or water. Each requires a specific set of techniques to extract and prepare for analysis.

The updated UN Secretary General's technical guidelines and procedures outlines the principal areas in which laboratories should have expertise in chemistry, microbiology and toxicology including:

(*a*) identification, in all types of sample, of known chemical warfare agents, as well as their impurities and their degradation productions (and evaluation of quantities);

(*b*) identification and elucidation, in all the types of sample, of the structure of toxic agents, including those present in trace quantities (and evaluation of quantities);

(*c*) identification and characterization, in different kinds of samples, including clinical and environmental samples, of biological warfare agents (bacteria, viruses, others) and/or toxins;

(*d*) identification and characterization, in different kinds of samples, including clinical and environmental samples, of biological agents (bacteria, viruses, others) and/or toxins,

(*e*) evaluation of the effects of biological warfare agents and toxins, including epidemiological and ecological modeling;

(*f*) pathological and biochemical examination of organs and tissue taken from victims of CBT weapons, and where possible identification of the agent concerned;

(*g*) expertise in investigation and diagnosis of animal or plant diseases, which may include toxicology, pathology, microbiology, and epidemiology; and

[145] The OPCW is constrained from taking samples from corpses. The CWC negotiators generally that such sampling should be avoided and (if done) it would be an unusual practice and special permission must be granted.

(*h*) examination and evaluation of munitions, munition components, and other military delivery devices, including all their technical specifications, [and] analysis of explosives.[146]

The UN Secretary General's procedures for sample collection, handling, storage, transport and analysis are:

(*a*) neat agent, munitions, remnants of munitions, other military delivery devices;

(*b*) NBC clothing and respirator canisters;

(*c*) environmental samples;

(*d*) food and drinking water;

(*e*) biomedical samples from human or animal source; and

(*f*) any affected crops and other vegetation.[147]

The CWC states: 'If the inspection team collects through, inter alia, identification of any impurities or other substances during laboratory analysis of samples taken, any information in the course of its investigation that might serve to identify the origin of any chemical weapons used, that information shall be included in the report'.[148]

Ideally, the sample size needs to be big enough to permit the body conducting the analysis to do so at least twice. This means that a portion of the sample should be placed into storage in case the initial analysis is challenged.

All chemical and biological agent detection systems have trade-offs, including size versus specificity and detection limits. Thus field detection systems tend to have higher (i.e., worse) detection limits, more false positive and negative readings and less specificity for reliably detecting the target agent. Field systems may tend to operate faster, while older laboratory techniques (such as culturing) can take longer. One should also be aware possible reasons for false and negative and positive readings. Biomedical samples (e.g., blood and urine) are major areas of focus, including in the context of the conflict in Syria. Environmental sampling was arguably more important in the case of Syria. Finally, it should be noted that epidemiology, medical case histories, statistical analysis of health treatment and the like are also important.

The OPCW has a standard equipment 'suite' for in-field sampling and analysis of chemical warfare agents and their possible degradation products. In principle the DOC/PSF category covers all organic compounds except polymers and hydrocarbons under the routine verification system (i.e., those cases where allegations of non-compliance with the CWC have not been made).[149]

[146] 'Appendix V, List of diagnostic and analytical laboratory specializations', http://www.un.org/disarmament/WMD/Secretary-General_Mechanism/appendicies/V/ (accessed 2 Jan. 2011).

[147] 'Appendix VII, Procedures for sample collection, handling, storage, transport, and analysis', http://www.un.org/disarmament/WMD/Secretary-General_Mechanism/appendicies/VII/ (accessed 2 Jan. 2011).

[148] CWC, Verification Annex, Part XI, para. 26.

[149] OPCW briefing at sampling and analysis demonstration exercise, 22 Aug. 2012, The Hague.

The OPCW standard in-field configuration consists of approximately 20 items, can be packed in a space occupying approximately 3 cubic meters and weighs approximately 1 tonne.[150]

OPCW inspection equipment includes the following. CALID-3 detection paper that changes colour in the presence of G agents (orange), V agents (dark green) and sulphur mustard (bright red). The OPCW also uses ORI-217 chemical detection kit. This is a tube based system that is used to draw air samples for several minutes. When ready for analysis the second end is broken. The OPCW also uses CAMs (an IMS unit) which measures ion mobility and uses computer-based algorithms to identify the presence of a range of chemical agents.[151] Finally the OPCW uses the handheld AP2C flame spectrometry detector system which detects the presence of sulphur- and phosphorus-based chemicals for both liquid and air samples.[152] The AP2C flame spectrometry detector system detects colour changes which is suitable for use on the battlefield. The dectector contains hydrogen gas stored in palladium.[153]

OPCW inspectors use a protective ensemble with level 8 offering the maximum level of protection. OPCW inspectors fill out a special form to help ensure chain of custody. Information on the form include: an identifying number of the sample, whether the sample is authentic or a blank sample code, the type of sample, the weather conditions, whether and how the sample is split, a description of the circumstances under which the sample was taken, and the dates and times for analysis.[154] Sample preparation may include some or all of the following steps: 1. add buffers, 2. derivitization, 3. solubility using various solvents, and 4. specific procedures according to type of agent. The OPCW takes 8 fractions of the sample. One is passed to the inspected State Party (2 for in-country analysis and 5 splits for off-site analysis).[155]

The OPCW Central Analytical Database (OCAD), updated in 2013, does not include all degradation products of 'standard' CW agents.

The OPCW requires two complementary methods for confirming the detection of a CW agent or known-CW degradation product. One of these methods should ideally be spectrometric.[156]

The OCAD contains mostly retention indices and mass spectrometry data.[157] If permission is granted, the OPCW is authorized to use commercial

[150] OPCW briefing at sampling and analysis demonstration exercise, 22 Aug. 2012, The Hague.

[151] The algorithm compares the time-of-flight and only works if it has been validated. Sulphur mustard and organophosphorus nerve agent ions have opposite polarity.

[152] This system entails rubbing an absorbent pad on a surface of the sample point. The pad is then heated and the suspected CW agent desorbed.

[153] Some parties to the CWC object to OPCW inspection equipment that contains a radioactive source.

[154] OPCW briefing at sampling and analysis demonstration exercise, 22 Aug. 2012, The Hague.

[155] OPCW briefing at sampling and analysis demonstration exercise, 22 Aug. 2012, The Hague.

[156] The preceding text is partly based on Hart, J. D., *The Analysis of Competing Hypotheses (ACH) in the Assessment of Chemical Warfare Activities* (NDU: Helsinki, 2014), <http://www.doria.fi/bitstream/handle/10024/102142/Hart%20%28NetFinal%29.pdf?sequence=2>.

[157] For a comprehensive, authoritative overview of the OCAD, see Mesilaakso, M., 'The OPCW Central Analytical Database', in *Chemical Weapons Convention Chemicals Analysis: Sample Collection,*

databases. The OPCW uses Automated Mass spectral Deconfolution and Identification System AMDIS (a software that identifies OCAD data).[158] AMDIS has low numbers of false positives. The OPCW also uses 8 special shipping units manufactured by Porton Down for Syrian samples. The OPCW uses seals and checks the weight of the containers to help ensure confidence that the samples have not been tampered with.

As of September 2015, there were 19 designated labs in 15 countries.[159] None of the labs are in Africa and Arabic speaking countries.

The OPCW's Scientific Advisory Board's (SAB) Temporary Working Group (TWG) on Verification stated that Dr Sellström believes that biomedical sampling and analysis should be 'augmented and institutionalised' and that environmental biosampling 'should be considered' for adoption by the OPCW.[160] Participants in this working group have also raised (but not agreed) a variety of points including: (*a*) results obtained from biomedical samples by the UN Secretary-General's investigative team in 2013 'were critical because they provided factual evidence of exposure to chemical warfare agents and corroborated evidence' for the use of such weapons, (*b*) DNA testing is a useful forensic tool for linking samples to individuals in this regard, (*c*) distinctions between procedures for an OPCW challenge inspection and investigation of alleged CW use respectively have been highlighted by OPCW proficiency testing protocols.[161]

This SAB TWG also discussed and considered inter alia:

(*a*) best practice for handling biomedical samples outside a regulated laboratory environment,

(*b*) providing sufficient information on the nature of the samples to the laboratories,

(*c*) points that should be communicated to the inspectors to ensure the are 'thorough in their sample and observation collection on-site',

(*d*) updating methods to permit all data to be considered and how to prioritize samples for analysis,

(*e*) communicating relevant analysis results to the teams in the field quickly,

(*f*) how to deal with data that was collected and which has value but which yield results that do not immediately 'lend themselves to immediate conclusions', and

Preparation and Analytical Methods (John Wiley & Sons, Ltd.: Chichester, 2005). See also Ed. Paula Vanninen, *Recommended Operating Procedures for Analysis of the Verification of Chemical Disarmament*, 2011 edtn. (Ministry for Foreign Affairs of Finland and University of Helsinki: Helsinki, 2011).

[158] CHEMDATA.NIST.GOV Mass Spectrometry Data Center, <http://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:amdis>.

[159] 'Note by the Director-General: status of laboratories designated for the analysis of authenticated samples', OPCW document S/1308/2015, 2 Sep. 2015.

[160] Scientific Advisory Board, 'Summary of the fourth meeting of the Scientific Advisory Board's temporary working group on verification' OPCW document SAB-22/WP.1, 1 Oct. 2014, para. 2.1(e), p. 3.

[161] Scientific Advisory Board, 'Summary of the fourth meeting of the Scientific Advisory Board's temporary working group on verification' OPCW document SAB-22/WP.1, 1 Oct. 2014, para. 2.2, p. 3.

(*g*) ways to more efficiently communicate information between the laboratory and the field.[162]

6.1. World Health Organization

In December 2012 the WHO carried out a table top CW attack response exercise. At about this time the WHO began to produce guidance literature related to CW, including on detection, decontamination and treatment. At about this time the WHO also formed an internal CW group. The WHO also stockpiled at least 1.2 million mg of atropine and pralidoxime. It also collected 400 sets of Level-C PPE for ambulance services and hospitals. The WHO also conducted a train the trainer course in Beirut and conducted outreach work in Iraq and Jordan. An inter-agency technical group convened by UN OCHA trained 140 staff in protective measures. Three WHO staff members contributed to Sellström's team. WHO collected biological samples under the Sellström team. A 'health cluster' for Syria is chaired by WHO.[163]

6.2. Security and defence networks

Defence establishment networks (e.g., having various institutional associations for lines of action such as the UNODA in the Syria context). Other mechanisms and processes are being pursued with a view towards documenting war crimes for eventual prosecution.

Forensics also plays a potential role in C-IED and CBRNe-related projects and field operation units. For example, the Joint IED Defeat Organisation organized its work according to three principles: (*a*) 'attack the network', (*b*) 'defeat the device', and (*c*) 'train the force'.[164]

6.2.1. European Defence Agency

The European Defence Agency (EDA) is implementing a number of programmes to strengthen C-IED and C-CBRNe capabilities in operations by European armed forces. This includes support for European-level actions to counter C-IED training and technology development options such as the acquisition of a C-IED Theatre Exploitation Laboratory, a Joint Deployable Exploitation and Analysis Laboratory, and the development of Manual Neutralization Techniques Courses and Exercise programmes. The EDA also plans to participate in training and research on CBRNe on forensics, CBRN detection and decontamination.[165]

[162] Scientific Advisory Board, 'Summary of the fourth meeting of the Scientific Advisory Board's temporary working group on verification' OPCW document SAB-22/WP.1, 1 Oct. 2014, para. 2.3, p. 4.

[163] WHO slides.

[164] Valpolini, P., 'Oh blast!', *Armada International*, no. 3 (2010), p. 24.

[165] Info. circular.

6.2.2. European Commission

The current EU five year budget cycle (Horisons 2020) comprises numerous and overlapping programmes to support research and development and capacity-building of direct and indirect relevance to the investigation of toxic chemicals exposure. Some of these programmes are internal to the EU (e.g., the programme of work oversee by Directorate-General (DG) Migration and Home Affairs), while others are external and consequently can be understood as supporting the EU's Common Security and Foreign Policy (CSFP). More specifically a number of relevant actions have been identified in the area of 'secure societies' for the period 2016-2017 which address the capacity of European actors to incidents of toxic chemicals exposure.[166] The total budget for 2016 actions (of all types) is €185.34 million. The total budget for 2017 actions (of all types) is €197.02 million.[167] These actions include:

(*a*) Disaster resilience (SEC-05-DRS-2016-2017) *Chemical, biological, radiological and nuclear (CBRN) cluster*,

(*b*) Fight against crime and terrorism (SEC-09-FCT-2016) *Forensics techniques on: a) trace qualification, and b) broadened use of DNA*,

(*c*) Fight against crime and terrorism (SEC-09-FCT-2017) *Toolkits integrating tools and techniques for forensic laboratories*,

(*d*) Fight against crime and terrorism (SEC-10-FCT-2017) *Integration of detection capabilities and data fusion with utility providers' networks*,

(*e*) Fight against crime and terrorism (SEC-11-FCT-2016) *Detection techniques on explosives: countering an explosive threat, across the timeline of a plot*, and

(*f*) Fight against crime and terrorism (SEC-12-FCT-2016-2017) *Technologies for [the] prevention, investigation, and mitigation in the context of [the] fight against crime and terrorism*.

Of these action lines, Project SEC-05-DRS-2016-2017 is perhaps of greatest relevance for CW forensics and attribution purposes.[168] The EU notes that technologies and innovations in the field of CBRN frequently address 'small niche markets', and that small and medium-sized enterprises (SMEs) may not possess the capabilities or strategic objective to enter international markets. This CBRN cluster is structured according to two types of actions: 1. A coordination and support action (CSA) shall gather in 2016 'the largest

[166] *Draft Horizon 2020 Work Programme 20162017 in the area of Secure societies—Protecting freedom and security of Europe and its citizens*, final version, <https://ec.europa.eu/programmes/horizon2020/sites/horizon2020/files/14.%20Secure%20societies_2016-2017_pre-publication.pdf>; accessed 1 Oct. 2015. The phrasing of EU documentation, including that generated by and for the Commission, reflects sensitivities and understandings that those without direct involvement cannot fully appreciate. A similar dynamic also exists within NATO.

[167] *Draft Horizon 2020 Work Programme 20162017 in the area of Secure societies—Protecting freedom and security of Europe and its citizens*, final version, <https://ec.europa.eu/programmes/horizon2020/sites/horizon2020/files/14.%20Secure%20societies_2016-2017_pre-publication.pdf>; p. 86.

[168] *Draft Horizon 2020 Work Programme 20162017 in the area of Secure societies—Protecting freedom and security of Europe and its citizens*, final version, <https://ec.europa.eu/programmes/horizon2020/sites/horizon2020/files/14.%20Secure%20societies_2016-2017_pre-publication.pdf>; pp. 20-21.

number of European companies capable and willing to market their products globally (e.g. companies producing integrated equipment for First Responder's, CBRN software systems, detectors, decontaminators, waste management and encapsulation equipment)'. CSA will identify technologies that are to be developed in order to integrate them into platforms such as (and possibly including) the inventory developed by the EDEN project.[169] In 2017 several regulatory impact analyses (RIAs) will be carried out on research and development (R&D) of novel CBRN technologies and innovations (each action undertaken will be headed by an SME). The amount of support for 2016 actions is expected to be € 2 million, while the amounts for 2017 actions at €3.5 million. Technology readiness levels (TRL) of 4-7 are specified.[170]

6.2.3. NATO

The NATO Consultation, Command and Control Agency (NC3A) turned its attention to C-IED problem in 2005.[171] NC3A provided has provided support for at least bases in Afghanistan: International Security Assistance Force (ISAF) headquarters, Kaia and the Kandahar airfield.[172] Many of the individuals who participate in IED action lines in EDA and NATO are the same.[173] As such these IED efforts have been described as 'well coordinated'.[174]

[169] The End-user driven Demo for cbrNe (EDEN) Framework Seven Security Demonstration Project was started on 1 Sep. 2013 and consists of 36 members in 15 countries. It is meant to address the full requirements and solutions spectrum of CBRNE. Its members are ainia, Astri Polska, Airbus Defence and Space, BAE Systems, Bruker UK, CBRNE Centre, CBRNE Ltd., ENEA, European Virtual Institute for Integrated Risk Management, Foundation pour la Recherche Stratégique (FRS), Fraunhofer (FhG EMI), Fraunhofer (FhG ICT), Fraunhofer (FhG INT), Hotzone Solutions BV, Indra Sistemas SA, INERIS, Interuniversity Chari in Law and the Human Genome, Instituo Affari International (IAI) LDIAMON, LDI Innovation O, The Main School of Fire Service (SGSP), MDA, Microfluidic, Norwegian Defence Research Establishment (FFI), Nucletudes, OMNIDATA SA, Przemysowy Instytut Atomotyki I Pomiartw PIAP, Robert Koch Institute (RKI), SAMU, SELEX ES, SICPA SA, Space Research Center, Tecnoalimenti SCpA, Universit Cattolicà del Sacro Cuore, Universite Catholique de Louvain (UCL) Center for Applied Molecular Technologies (CTMA), University of Reading, VTT, and Public Health England. See <https://www.eden-security-fp7.eu/>.

[170] *Draft Horizon 2020 Work Programme 20162017 in the area of Secure societies—Protecting freedom and security of Europe and its citizens*, final version, <https://ec.europa.eu/programmes/horizon2020/sites/horizon2020/files/14.%20Secure%20societies_2016-2017_pre-publication.pdf>; pp. 20-21. European Commission TRLs for 20142015 are: TRL 1-basic principles observed, TRL 2-technology concept formulated, TRL 3-experimental proof of concept, TRL 4technology validated in lab, TRL 5-technology validated in relevant environment (industrially relevant environment in the case of key enabling technologies), TRL 6-technology demonstrated in relevant environment (industrially relevant environment in the case of key enabling technologies), TRL 7-system prototype demonstration in operational environment, TRL 8-system complete and qualified, TRL 9-actual system proven in operational environment (competitive manufacturing in the case of key enabling technologies; or in space), <http://ec.europa.eu/research/participants/data/ref/h2020/wp/2014_2015/annexes/h2020-wp1415-annex-g-trl_en.pdf >, accessed 2 Oct. 2015.

[171] Valpolini, P., 'Oh blast!', *Armada International*, no. 3 (2010), p. 24.

[172] Valpolini, P., 'Oh blast!', *Armada International*, no. 3 (2010), p. 24.

[173] Valpolini, P., 'Oh blast!', *Armada International*, no. 3 (2010), p. 26.

[174] Valpolini, P., 'Oh blast!', *Armada International*, no. 3 (2010), p. 26.

7.0 International peace and security implications: arms control and security and defence aspects

Summary mapping to be provided in Version 2.
For recommendations, please see Summary.

End