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Review Article

Environmental exposure to organophosphorus nerve agents

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ABSTRACT

Exposure to organophosphorus nerve agents, the most deadly chemical warfare agents, is possible in a variety of situations, such as destruction of chemical warfare agents, terrorist attacks, armed conflicts or accidents in research laboratories and storage facilities. Hundreds of thousands of tons of chemical munitions were disposed of at the sea in the post World War II period, with European, Russian, Japanese and US coasts being the most affected. Sulfur mustard, Lewisite and nerve agents appear to be the most frequently chemical warfare agents disposed of at the sea. Addressing the overall environmental risk, it has been one of the priorities of the world community since that time. Aside from confirming exposure to nerve agents in the alleged use for forensic purposes, the detection and identification of biological markers of exposure are also needed for the diagnosis and treatment of poisoning, in addition to occupational health monitoring for specific profiles of workers. When estimating detrimental effects of acute or potential chronic sub-lethal doses of organophosphorus nerve agents, released accidentally or intentionally into the environment, it is necessary to understand the wide spectra of physical, chemical and toxicological properties of these agents, and predict their ultimate fate in environmental systems.

1. Introduction

Bearing in mind that over 100 dumping sites in the Baltic region (Gotland Deep, Bornholm Deep, Little Belt, Skagerrak Strait) were used for depositing chemical warfare agents (CWAs) between 1945 and 1970, as well as over 60 sites in the Gulf of Mexico, the coasts of Japan and the oceans on both coasts of the USA – which has been confirmed in the CWA exposure reports by hundreds of fishermen who have caught dangerous cargo via their nets – this method of exposure is not to be underestimated. Moreover, at several places all over the world, e.g. in the US, China, and Europe, old chemical munition has been lost or buried and could be accidently released as it is shown in Fig. 1 (CHEMSEA, 2013; Vučinić et al., 2014).

While a wide variety of chemical warfare agents including also sulfur mustard, Lewisite, have been dumped or buried, this manuscript deals with problems that arise from nerve agents exposure. The aim of this review is to raise the attention of environmental exposure to NAs that could persist in different media long after initial exposure, producing a wide range of toxic effects. Better understanding of the time frame for detection of biological markers of exposure to NAs is critical for diagnosis and treatment in uncertain cases of exposure.

After the first confirmed use of nerve agents (NAs) in the Iran-Iraq War (1980–1988), when the Iraqi army used tabun and sarin against the Iranian forces (Majnoon Island) and the civilian population

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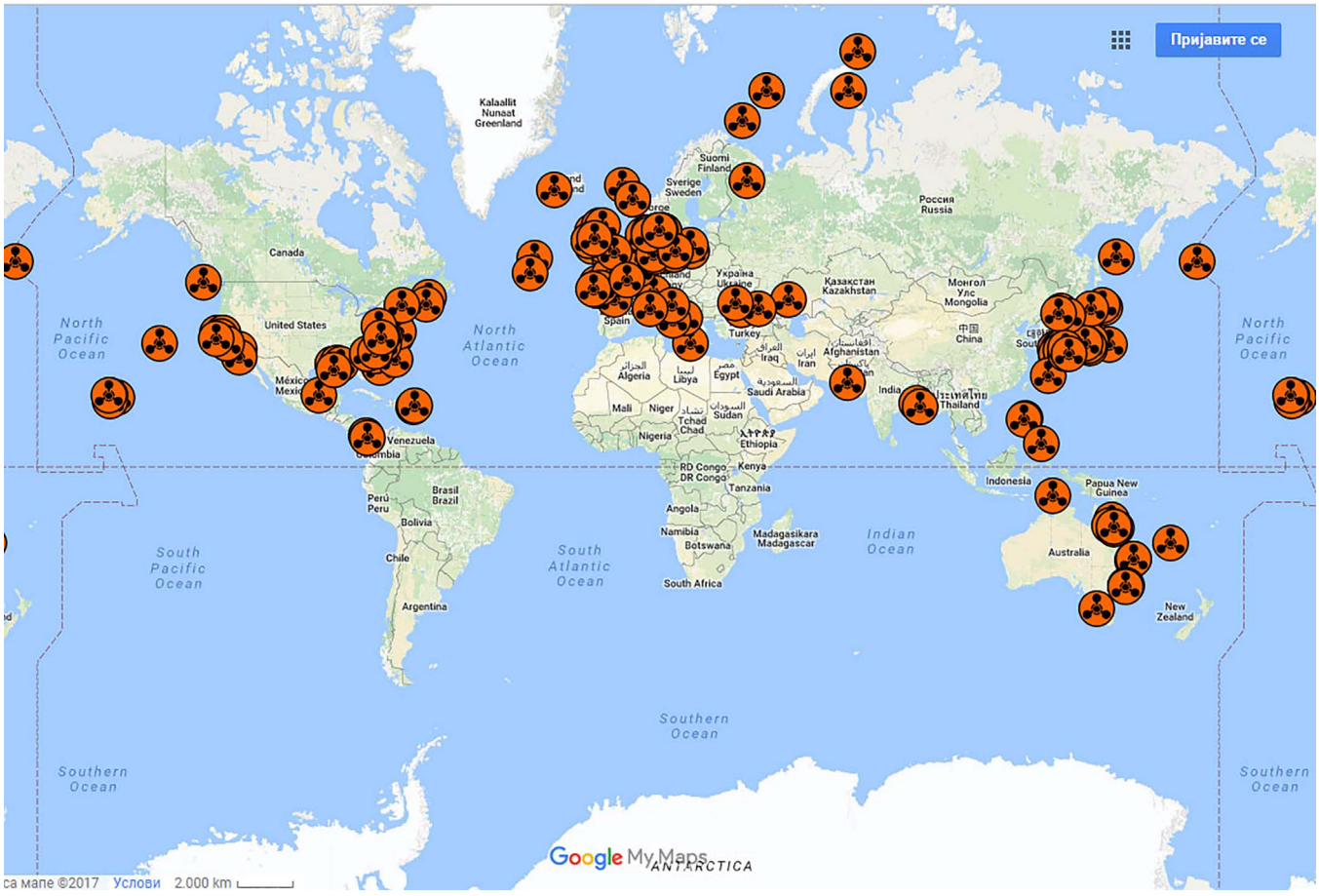

Fig. 1. Chemical Weapon Munitions Dumped at Sea – Available at: www.nonproliferation.org/chemical-weapon-munitions-dumped-at-sea, August 1, 2017.

(Halabjah) (Balali-Mood and Balali-Mood, 2008), these deadly agents were used for the terrorist attacks in Japan. The terrorist attacks in Matsumoto in 1994, which claimed the lives of 7 people (Nakajima et al., 1999) and Tokyo in 1995, with over 5500 injured and 13 dead (Nagao et al., 1997; Murata et al., 1997), showed the necessity for development of specific diagnostic methodology for identifying nerve agents, which had not existed until then (Salem et al., 2008a,b).

The importance of the engagement of the Organisation for the Prohibition of Chemical Weapons (OPCW) and the chain-of-custody procedures had became obvious after the UN mission in the Syrian Arab Republic, performed by the OPCW and WHO teams which had investigated the Ghouta and other sites of attacks. Difficulties in establishing a credible epidemiological pattern through interviews with first responders, victims and medical personnel had confirmed the importance of designated laboratories (Pita and Domingo, 2014).

The core objective of the OPCW is the promotion and adherence to the Chemical Weapons Convention (CWC). Among other activities, the OPCW performs inspections of facilities for destroying CWAs, industry inspections, challenge inspections and investigations of alleged use, in order to verify destruction and non-proliferation of chemical agents. The OPCW technical secretary provides off-site analysis through a network of designated laboratories proficient in NAs analysis and their degradation products at concentrations greater than 1 ppm in environmental and man-made samples (OPCW, 1994; Black and Read, 2013). Since 2016 the OPCW has had 17 designated laboratories for analysis of biological samples. Some of them were already involved in the verification of poisoning induced by CWAs during the recent events in Syria.

Aside from confirming exposure to NAs in the alleged use for forensic purposes, the detection and identification of biological markers of exposure is also needed for the diagnosis and therapy of intentional or accidental poisoning, in addition to occupational health monitoring for specific worker profiles (Black and Read, 2013).

2. Nerve agents and toxicity

Chemical weapons, including OP NAs, have been used for decades in armed conflicts and terrorist attacks, but accidental leakage from industrial and storage facilities, laboratories, dumping and chemical weapon incineration sites, has also led to health issues and even deaths in those exposed. Although the scientific literature on the NAs is extensive (PubMed search listed 54.400 articles), less than 10% of articles deal with the environmental exposure to OP Nas (Vučinić et al., 2014; Davisson et al., 2005; Kingery and Allen, 1995; Lionetto, 2013; USACHPPM/ORNL, 1999).

Published articles refer mainly to the significant alterations in ecosystems with CWA dumpsites, where the solubility and hydrolysis rates of CWAs' innate toxicity had been assessed for marine environment risk prediction. There is indeed evidence of chronic toxicity, though studies in marine organisms have not exhibited concerning amount in tissues of these agents and their by-products. Acute exposure to an agent presents the major human health risk, either by accidental recovery of a CWA on a fishing vessel or by munitions washed ashore onto beaches (CHEMSEA, 2013).

In order to provide successful medical protection it is essential to understand the toxicity of NAs (VX vapor is the most toxic with LCt_{50} of 10 mg/min/m^3, and tabun is the least toxic with LCt_{50} of 400 mg/min/m^3), but also the relevant physicochemical properties of NAs such as: volatility (for sarin 22,000 mg/m^3 and VX 10.5 mg/m^3), vapor pressure showing how quickly an NA will evaporate (for sarin at 20 °C

Table 1
Physical, chemical and toxicological properties of OP Nas.

Property	Tabun (GA)	Sarin (GB)	Soman (GD)	VX
Mol. weight/(Da)	162.3	140.1	182.2	267.4
Boiling point/(°C)	230	158	198	298
Melting point/(°C)	−49	−56	−80	−20
Vapor pressure/ mmHg	0.036 (20 °C)	2.1 (20 °C)	0.27 (20 °C)	0.00044 (20 °C)
Vapor Density	5.6	4.9	6.3	9.2
Liquid Density (g/ml) (25 °C)	1.8	1.10	1.02	1.01
Volatility (mg/m^3) (25 °C)	610	22000	3900	10.5
Solubility in water	9.8 g per 100 g	Miscible	2.1 g per 100 g	Miscible
Peristency on soil	1–1.5 day (half-life)	2–24 h (5–20 °C)	Relatively persistent	2–6 days
LCt_{50} in humans/ (mg min m^3)	400	100	50	10
LD_{50} in humans/(mg *per* 70 kg) percutaneous	1000	1700	100	6–10
Ageing half-life	13 h	3 h	40 s–10 min	36 h

2.1 mmHg, while VX evaporates 2000 times slower), vapor density (VX has a high vapor density so it accumulates in low grounds), stability and persistence on soil and in water. While nerve agents are toxic by all routes of exposure, due to mentioned properties, G agents act primarily via inhalation, whereas the skin penetration is most commonly the route of exposure for V agents. Ingestion of NAs (from contaminated water) is highly unlikely, considering the persistence of NAs, the effect of hydrolysis, dilution and water treatment processes (Salem et al., 2008a,b). Environmental persistence of agents such as tabun can be found in water from one (at 20 °C) to six days (at 5 °C); sarin evaporates as fast as the water; soman and cyclosarin are less volatile and more persistent, lingering up to two days, and both pose a significant threat if inhaled or if they reach the skin. The colorless, relatively non-volatile liquid thiophosphonate NA "venomous agent X" (VX) can stay in water for weeks to months due to its slow evaporation (approximately 2000 times slower than sarin) (Augerson, 2000; Kikilo, 2001; Capacio et al., 2008; Fatz, 2004; National Research Council of the National Academies, 2003; Agency for Toxic Substances & Disease Registry, 2014; Carnes and Watson, 1989; Petrakis et al., 2017) (Table 1).

In summary, there is a threat to both military and civilian population from the environmental NAs persistency. While sarin and tabun are very evaporable, which makes them more dangerous when inhaled, soman and cyclosarin are less evaporable and more persistent, making them dangerous with both inhalation and dermal exposure. However, soman might be more persistent by a "thickening agent" such as polymethymethacrylate. Sarin hydrolysis to isopropyl methylphosphonic acid and hydrofluoric acid depends on pH; at pH levels between 6.5 and 14, hydrolysis is mediated by hydroxide-ion catalysis, while at pH levels between 4 and 6.5, reaction occurs between sarin and water molecules. Temperature also effects hydrolysis, so at 25 °C under alkaline conditions, the half-life of sarin is 5.4 h. A principal hydrolysis product of soman is hydrofluoric acid, however soman is more stable in water than sarin. Efficient hydrolysis of tabun in water after 10 min of heating at 95 °C, allows using this water for technical purposes. VX is not easily evaporable, it is persistent and dangerous when dermally absorbed or inhaled as aerosol. Other important factors that are correlating with temperature and ground persistency are vapor pressure and vapor density (Augerson, 2000) (Table 2).

In the different environmental conditions VX can have significant behavior variation: at pH 5, the half-life of VX is ≈100 days, whereas at pH 8 the half-life is 9 days. The hydrolysis rates depend on temperature as well. In seawater, the half-life of VX was about 5–14 days ate 25 °C, but at 4 °C it may be several years. At pH 5, ethyl methylphosphonic acid (EMPA) and methylphosphonic acid (MPA), that show low toxicity, are the most abundant degradation products; but at pH 8, the most abundant degradation product is *S*-(2-diisopropylaminoethyl)methylphosphonic acid (which is considerably less toxic than VX). The fate of VX at trace-levels and whether adsorption on soil surfaces or complexation with natural organic matter may affect its degradation rate is also not fully clarified. It is thought that VX is more likely to persist in an adsorbed or complexed form, meaning that there is still potential for secondary release and exposure (Augerson, 2000; Fatz, 2004; Agency for Toxic Substances & Disease Registry, 2014; US EPA, 1995; Breyer et al., 2010).

Table 2
Persistence of NAs.

Agent	Persistence
Tabun	Heavily splashed liquid – 1–2 days. In water 1 day (20 °C) and 6 days (5 °C)
Sarin	Evaporates as fast as water
Soman	Heavily splashed liquid – 1–2 days. Thickeners can extend.
Cyclosarin	Heavily splashed liquid – 1–2 days.
VX	Splashed liquid can persist for weeks or months (evaporates 2000 times slower than sarin)

3. Confirmation of exposure

There are different requirements for confirmation of NAs exposure in certain scenarios. Hence, a comprehensive assessment of clinical picture, biological and environmental samples as well as reliable witness reports is crucial. Environmental samples are used to prove not only the identity, but possibly even the origin of NA. There are different portable detectors for rapid on-site detection and identification of CWAs and technologies of identification of agent such as ion mobility spectrometry, flamme photometry, photoionisation, Raman spectroscopy etc. (Black and Read, 2013; Augerson, 2000; National Research Council of the National Academies, 2003; Breyer et al., 2010; van der Schans et al., 2008).

The final proof of poisoning, however, is only possible through confirmation with forensic investigation of samples (blood, tissue, urine) from the victim. Such forensic analysis needs advanced analytical methods and complex technical equipment (Hernández et al., 2014; Kavvalakis and Tsatsakis, 2012; Tsatsakis et al., 2009).

4. Clinical picture and field treatment

Based on their lipophilic qualities, the NAs permeate the skin, lungs and gastrointestinal tract easily and reach circulation whereupon they distribute to organs and tissues (Balali-Mood and Balali-Mood, 2008; van der Schans et al., 2008; David, 2005). On the whole, the acute effects of exposure to organophosphorus compounds is well described and can be predominantly characterized by the cholinergic crisis (muscarinic, nicotinic and CNS effects), with the occurrence of the signs and symptoms, as well as the time course of poisoning and severity based on the dosage, route and length of exposure (Kikilo et al., 2001; Senanayake and Karalliedde, 1987; Crook et al., 1983; Romano et al., 2001; Dabisch et al., 2001; Moshiri et al., 2012). Military services and several special units are trained in skills necessary to manage Chemical, Biological, Radiological and Nuclear (CBRN) events. In detail, they may even be equipped with drugs for self and buddy aid. Such medical equipment is generally not available for civilian population and drug administration is the task of the medical service. In most countries, especially the administration of drugs, e.g. antidotes is the responsibility of a physician. Hence, the following recommendations on drug administration and dosing are directed to physicians. Fig. 2 shows health effects of sarin, cyclosarin and VX.

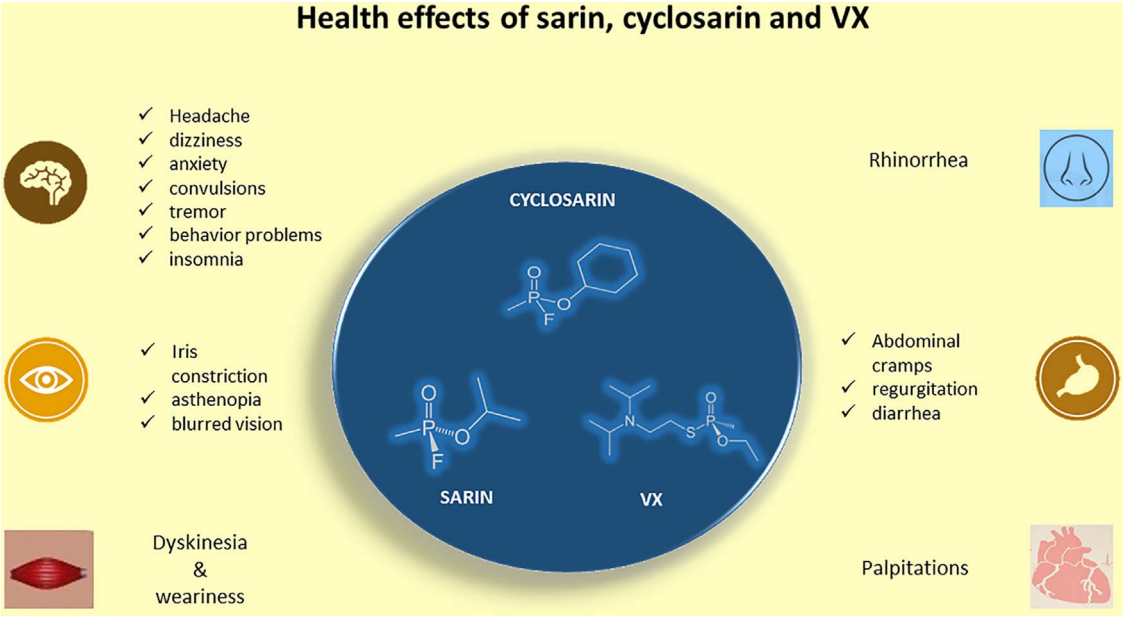

Fig. 2. Health effects of sarin, cyclosarin and VX.

4.1. Minimal exposure

Minimal vapor exposure to NAs includes miosis, blurred vision, eye pain and rhinorrhea. Vapors and fumes can cause toxic effects within minutes. In contrast, when exposure arises from vapor or liquids via skin without inhalation, it may take hours for signs and symptoms to manifest. For the treatment of ocular symptoms, topical atropine or homatropine can be administered. First systemic sign might be tachycardia due to initial stimulation of sympathetic nicotinic receptors. As long as heart rate is markedly increase, any systemic atropine administration needs specific care (Balali-Mood and Balali-Mood, 2008; Agency for Toxic Substances & Disease Registry, 2014; Kassa, 2005).

4.2. Light exposure

In case of miosis, rhinorrhea, light dyspnea, nausea and vomiting, systemic effects can be diagnosed. In this case, atropine and an oxime should be administered. A starting dose of 2 mg atropine is generally recommended. Such a dose should be sufficient after light exposure (otherwise, see below). However, atropine only antagonises symptomatically NA induced effects. In contrast, oximes are used to restore NA inhibited acetylcholinesterase (AChE) thereby resolving the effects of the NA and coincidentally metabolising it. Therefore, military services and several special forces are equipped with autoinjectors. They are filled either with atropine alone (most commonly with 2 mg) or with a combination of atropine (2 mg) and an oxime, e.g. pralidoxime or obidoxime. Two prominent devices are the Mark I kit – atropine (2 mg, 0.7 ml) and 2-PAM (600 mg) or the ATOX II consisted of atropine (2 mg, 0.7 ml) and obidoxime (220 mg). Other types of autoinjectors are commercially available. At present, the most effective strategy is the use of a combination of atropine (2 mg) and obidoxime (220–250 mg). Using this regimen, effective concentrations for substantial reactivation of AChE inhibited by a relative broad spectrum of NAs may be adjusted. At any case, autoinjectors can be used as soon as systemic signs and symptoms arise. However, young children are quite sensitive to atropine and administration needs caution. Apart from undressing, followed by showering (at best by using soapy water), no specific decontamination is required if the poisoning was caused by vapours. However, after exposure to liquids, thorough spot decontamination with reactive skin decontamination lotion (RSDL), followed by showering, is strongly recommended. In the case of dermal exposure, localized sweating and fasciculations are expected and one autoinjector containing atropine and an oxime should be administered.

4.3. Moderate exposure

Moderate exposure is followed by miosis, rhinorrhea, moderately severe to severe dyspnea, nausea and vomiting. If the patient is attended by medical service personnel, in the first 5 min, 2 autoinjectors and diazepam should be administered. Following immediate atropinization, further doses of atropine should be titrated according to signs and symptoms. If a liquid NA exposure is suspected, extended observation for at least 18 h is necessary. During such a potential latency period, repetitive determination of red blood cell AChE (RBC-AChE) activity may be extremely helpful to estimate the course of poisoning. At the best, effective oximes can be administered while AChE activity is decreasing and the signs and symptoms are not yet detectable. With such a strategy, even symptomatic poisoning could be prevented.

4.4. Severe exposure

Generally it has to be mentioned that self protection of medical personnel and facilities is mandatory. The life-threatening signs of severe exposure are loss of consciousness, respiratory depression, apnea, cardiovascular failure, convulsions, and flaccid paralysis. Three autoinjectors [e.g. 3× MARK I (2 mg atropine and 600 mg pralidoxime, each) or one ATOX II (220 mg obidoxime and 2 mg atropine) followed by two AtroPen (2 mg atropine)] should be given immediately, if available. Otherwise, fast atropinisation is recommended according to the schedule mentioned above and oxime treatment should be started. Alternatively, treatment has to be initiated as an initial dose of atropine of 6 mg, followed by 2–4 mg i.v. and oxime (if no autoinjector is available, e.g. 250 mg obidoxime i.m or better i.v.). Additionally, diazepam (e.g. 10 mg) should be administered for amelioration of central effects. Ventilatory support is of vital importance for survival (Fig. 3).

The time for initial response should be spared before proceeding with atropine dosing, and it is deemed wise to wait, before further atropine administration, until the secretion decreases and the breathing improves. The critical parameter is actually the restoration of oxygenation. In the case of dermal exposure, if similar, though delayed, signs as severe vapor exposure occur and the same treatment should be applied (Balali-Mood and Balali-Mood, 2008; Moshiri et al., 2012; Kassa,

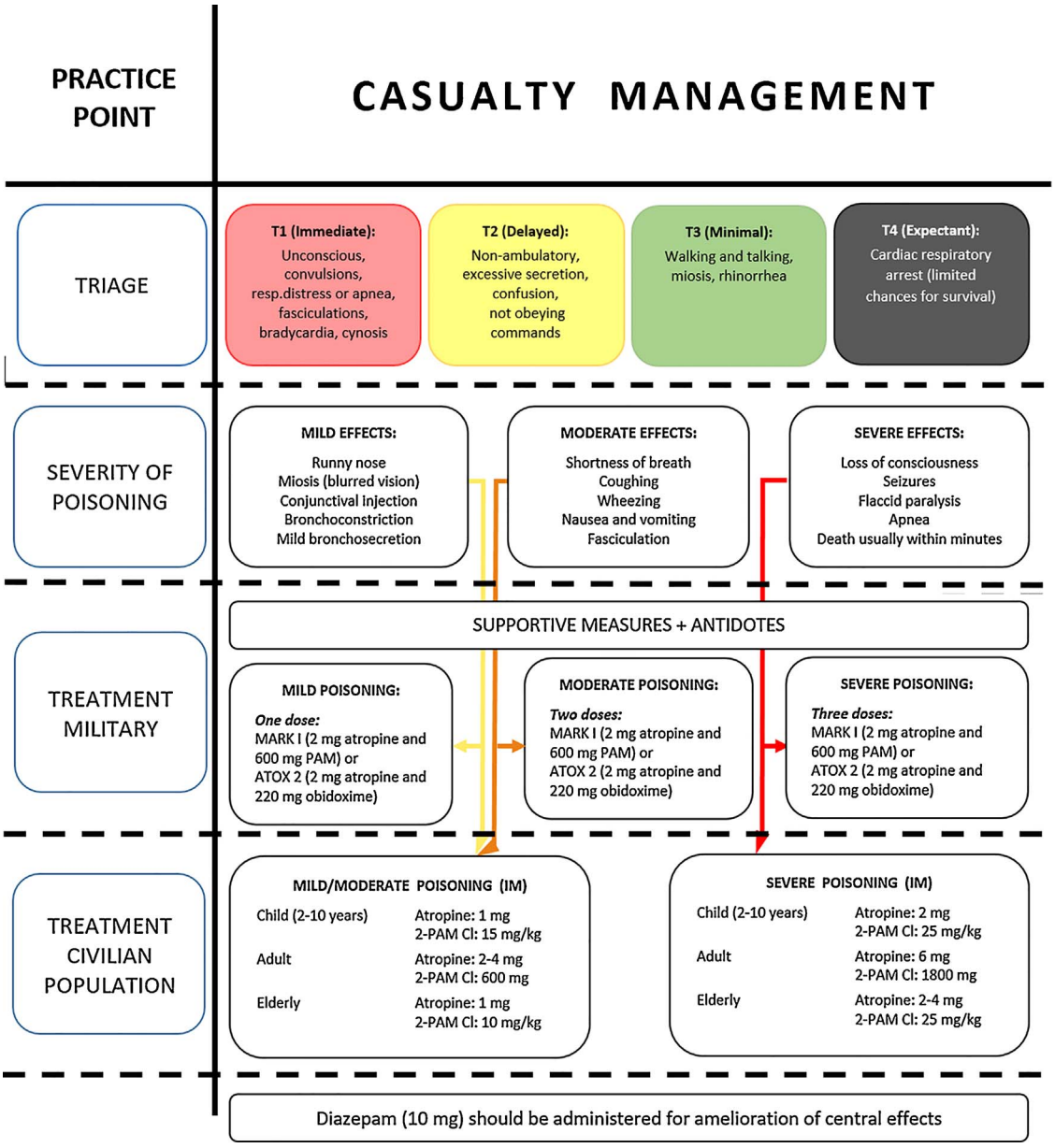

Fig. 3. CBRN Emergency Medical Treatment.

2005).

Suspected exposure to NAs can sometimes occur in an environment where NAs can be found as liquids, albeit this could be still described as unconfirmed exposure, where perhaps not even the afflicted are cognizant that they were exposed to a harmful chemical (Claborn, 2004). Following skin contact, the onset of signs and symptoms of NA poisoning may also be delayed up to 18 h. In such scenarios, repetitive determination of AChE activity might be helpful. The longer the interval between the exposure and the symptoms, the less severe will the latter be – which does not imply that medical attention is not necessary (Agency for Toxic Substances & Disease Registry, 2014).

In any case of severe poisoning, especially with persisting agents such as VX, prolonged treatment (several days) may be necessary. Dosing of atropine should be performed according to signs and symptoms at best using an i.v. line. However, over-atropinisation should be avoided. As long as reactivation of inhibited AChE can be expected, sufficient oxime doses (e.g. 750 mg obidoxime per 24 h) should be administered via continuous infusion. Simultaneously, necessary symptomatic intensive care measures have to be applied.

Especially in scenarios with NA mass casualties, oximes are expected to be of enormous help: they may enable survival on the spot when the resources for artificial ventilation are not available or run short, and they may contribute to the reduction of the period of necessity for intensive care treatment when the units are overwhelmed with patients.

While in the times of Cold War, when troops of nations were facing each other, a certain level of protection was assumed, though at present, such scenarios have shifted towards asymmetric conflicts as well as terrorist attacks. Civilian population, unlike the army, was entirely unprotected against the exposure to NAs, as they did not possess personal protective equipment, autoinjectors and there was at least 30 min delay before they got the proper medical treatment.

While the acute effects of exposure are well documented, it has been only for the last twenty years that a upsurge of interest in the delayed effects of single-dose poisoning and chronic effects of exposure (continuous or intermittent) to NAs has been reported. Most published epidemiological studies are scarce, revealing exposure evaluation, using adequate methodology as main problem. Studies with military

personnel suffer from inaccurate exposure assessment and the clinical data are often insufficient. Over the last 15 years, studies with sarin attack survivors in Japan have provided additional data regarding the neurobehavioral effects and chronic decline of psychomotor skills and memory, along with neurophysiological function defects, which persisted for months and years after the exposure (Nakajima et al., 1999; Murata et al., 1997; Nagao, 1991; Yokoyama et al., 1998).

The kind of exposure represents a significant difference between the civilian and military population. In the case of civilians, the most possible ways of exposure are either single or repeated, whereas for the military population a dose of decreased level, results in immediate effects (mostly miosis), or reduced performance.

The long-term effects of NAs were thought to only be expected after sever intoxications but no consistent data exist on this subject of the repeated exposure to NAs. Thus, organophosphate-induced delayed neuropathy (OPIDN) has not been reported in Iranian chemical attack survivors. Asymptomatic sequelae of sarin poisoning in central and autonomic nerve system have been reported in Matsumoto and Tokyo attack survivors. However, it was concluded that they had no clinical relevance. It has been suspected for a while that there are chronic effects of exposure to low doses if OP compounds, but that has yet to be ascertained (Nakajima et al., 1999; Murata et al., 1997; Nagao et al., 1991; Yokoyama et al., 1998; Duffy et al., 1979; Duffy and Burchfield, 1980; Burchfield et al., 1976).

5. Biological markers of exposure to NAs

The diagnosis of acute exposure to high doses of NAs is confirmed by the aforementioned clinical picture, measuring of cholinesterase (ChE) activity, determination of OP NAs, their metabolites and reaction products in biological samples (Balali-Mood and Balali-Mood, 2008; Lionetto et al., 2013; Moshiri et al., 2012). Determination of ChE activity inhibition is generally sufficient for initial treatment in clinical settings and occupational health monitoring. Established ChE baseline levels in occupational settings, when compared to ChE levels after exposure, might be indicative of significant exposure. Sensitivity can be clearly improved, when baseline levels of AChE are known prior a potential exposure towards inhibitors of cholinesterases. However, the specificity of these parameteres for NAs is low as ChE inhibition might be a result of OP and carbamate pesticides exposure. For diagnostic purposes, in case of poisoning, no baseline levels exist, so inhibition levels less than 20% *per se* do not provide sufficient evidence of exposure (US EPA, 1995).

The parent NA has a relatively short self-life (G-type agents: up to several hours, V-type agents up to few days) due to its rapid hydrolysis and affinity for binding to the proteins in plasma and tissues, although its determination has the highest specificity. After the processes of absorption and distribution in tissues, the NAs undergo spontaneous, subsequent enzymatic hydrolysis by endogenous hydrolases – e.g. paraoxonases. Through irreversible binding to serine esterases other than AChE, such as Butyrylcholinesterase (BuChE) and carboxylesterases (CaE), they become inactive. Additionally, they may form covalent bonds with albumin and other proteins in the blood and tissues (VX has low affinity for CaE, whereas sarin and soman's affinity for CaE is high). In order to detect the non-metabolised agent, samples of blood from the casualties within hours of exposure should be collected; it is possible to detect V-agents for longer periods, possibly even days after exposure due to slow rate of absorption after dermal exposure and longer half-lifes (Balali-Mood and Balali-Mood, 2008; van der Schans et al., 2003). Despite the highest specificity and a relatively simple analytical technique, non-metabolised agent is rarely useful due to its short lifetime in circulation (Balali-Mood and Balali-Mood, 2008).

NA metabolites are not expected to be present for more than a few days after exposure, e.g. Isopropyl methyl phosphonic acid (IMPA) was detected in the urine of one of the sarin attack victims in Japan on the seventh day after the incident (Nakajima et al., 1999). Free metabolites have been used as biomarkers for confirming the NA exposure in terrorist attacks or other alleged uses as following: IMPA (identified in Matsumoto and Tokyo victims) and methylphosphonic acid – MPA (a secondary hydrolysis product also found after exposure to other NA) in urine and blood for sarin; pinacolyl MPA in urine and blood for soman; cyclohexyl MPA in urine and blood for cyclosarin, ethyl dimethylphosphoramidic acid (EDMPA) and ethyl phosphorocyanidic acid in urine for tabun; ethyl MPA in urine and blood for VX. Although detection methods for urinary metabolites have moderate to high specificity due to their occurrence in other sources, metabolites of tabun are not an unequivocal indicator of poisoning. The time frame for detection varies based on the severity of poisoning and the limits of detection of the method, but generally its durance is no more than a several days. Other analytical methods are also available: fluoride induced reactivation of BuChE and AChE/BuChE serine adducts in blood identified as phosphylated peptide; analysis of ChE adducts (albumin tyrosine adducts in plasma, keratin tyrosine adducts in the skin that is still being researched). The time window for adducts is no longer than for the free metabolites (up to several weeks or months), with limitations due to aging (albumin tyrosine adducts in plasma do not have this issue) (Nakajima et al., 1999; Okudera, 2002; Matsuda et al., 1998; van der Schans et al., 2004; Verstappen et al., 2012).

Three years ago, the OPCW faced the situation of alleged use of CWAs in Syria. As a historical precedent, the inspection teams of the UN and OPCW were to collect the samples within days of the incident, which, depending on the situation in the field, was not always an easy task. Nearly 90% of urinary metabolites of NAs is excreted within 72 h, so the protein adducts were more significant because of their longer half-lives of up to several weeks. The chemical attacks happened in August 2013 in Ghouta/Syria but reaching the scene in time was impossible. Aside from taking environmental samples, which entailed the likelihood of having NAs and/or their degradation products, and performing analyses of the aforementioned, only the analysis of biological samples could prove NA poisoning (including body tissues for the deceased). In contrast to armed conflicts, in terrorist attacks it may be assumed that samples could already be gathered within a few hours, as it was performed in Tokyo. When speaking of metabolites, it is desirable for them to have a good stability and not be subjects of hydrolysis or oxidation (primarily the issue with tabun, which hydrolyzes rapidly to the ubiquitary product of excretion, ethyl phosphoric acid). These substances can be detected in urine, blood and tissue samples by using liquid or gas chromatography. As they present a very high toxicity, very low concentrations in biological samples have to be assessed. Therefore, sofisticated GC–MS/MS or LC–MS/MS methods using single ion monitoring (SIM) or multiple reaction monitoring (MRM) modes are used. There is a specific demand for targeted analysis, for example, compounds such as IMPA. During the past decade there has been a significant development in off-site analysis as high-resolution mass spectrometry is now available in several designated laboratories for retrospective trace analysis (Pita and Domingo 2014).

In spite of these diagnostic techniques available, there is still a possibility for unidentified exposure especially in the case of very low levels of NAs without ChE inhibition, acute exposure followed by ChE inhibition but without symptoms, reduced clinical response if the patient had already been exposed to ChE inhibitors, or simple misidentification of symptoms of poisoning as a different diagnosis.

6. Risk assessment

The parameters for risk assessment are no-observed-effect level (NOEL), lowest-observed-effect level (LOEL) and effective dose (ED50). The level at which no noteworthy depression in red blood cells cholinesterase activity (RBC-AchE) occurs, is defined as the maximum acceptable level of exposure for the NAs. If RBC-AChE inhibition is the same or less than 20% and when it is not correlated to clinical signs and symptoms, only evidence of organophosphorus agent exposure exists.

Table 3
Summary of Multi-Media Chemical Agent Toxicity and Exposure Values: Existing Information as of 8/03/04 POC.V. Hauschild, USACHPPM, 410–436–5213. https://www.osha.gov./SLTC/emergencypreparedness/guides/nerve_cwa_othermedia_table_08032004.pdf, accessed 10.10.2016.

Media	Standard/Guideline Name	Population	Exposure Scenario	H/HD/HT (Mustard)	GA (Tabun)	GB (Sarin)	GD/GF	VX	Lewisite
WATER	**FDWS** (Field Drinking Water Standards) μg/L	designed for military[a] but can have civilian applications	safe for up to 7 days	*200*	*20*	*20*	*20*	*20*	*200*
			normal/humid climate – 5 L/day ingestion rate	**(140)**	**(12[a])**	**(12[a])**	**(12[a])**	**(12[a])**	**(80)**
			dry/arid climate – 15 L/day ingestion rate	**(47)**	**(4a)**	**(4[a])**	**(4[a])**	**(4[a])**	**(27)**
SOIL	**HBESL – Residential** (Health-Based Environmental Screening Levels) mg/kg	civilian general population: adults and children	daily exposure via ingestion, inhalation, and dermal contact for a lifetime	0.01	2.8	1.3	0.22	0.042	**0.3**
	HBESL – Industrial mg/kg	civilian general adult population	frequent exposures via ingestion, inhalation, and dermal contact: 250 days/year for 30 years	**0.3**	**68**	**32**	**5.2**	**1.1**	**3.7**
WASTE	**HWCLsol** (solid hazardous waste control limit) mg/kg	civilian/DoD worker	possible occasional exposure at HW treatment facility	**6.7**	**680**	**320**	**52**	10	**37**
	HWCL (liquid hazardous waste control limit) mg/L	worker civilian/DoD	possible occasional exposure at HW treatment facility	**0.7**	**20**	**8.3**	**0.3**	**0.08**	**3.3**
	NHWCL (non-hazardous waste control limit (haz waste exemption level) mg/kg	worker civilian/DoD	possible occasional exposures at a non-HW land disposal facility	**0.3**	**68**	**32**	**5.2**	**1.1**	**3.7**
Chronic Toxicity Reference Criteria (for use in risk assessment calculations)	**RfDo** (Oral Reference Dose) mg/kg-day	General population: adults and children	chronic (lifetime) ingested dose at or below which no adverse health effects are expected	**0.000007**	**0.00004**	**0.00002**	**0.000004**	**0.0000006**	**0.0001**
	CSFo Oral Cancer Slope Factor (mg/kg/day)$^{-1}$	General population: adults and children	represents the potency of the agent by ingestion to cause increased cancer risk	**7.7**			Not determined to be a carcinogen		
	Inhalation Unit Risk (μg/m^3)$^{-1}$	General population: adults and children	represents the potency of the agent by inhalation to cause increased cancer risk	**0.0041**					

[a] Military application.

According to the 1995a EPA report a statistically significant restraint of AChE in many organs and tissues is hazardous, provided there are clinical results. If clinical effects are not exhibited, this inhibition might be not of biological importance (Augerson, 2000; US EPA, 1995; Dabish et al., 2001; Young et al., 1999).

Health-Based Environmental Screening Levels (HBESLs) were derived from using chronic toxicity criteria with risk assessment models. They are used for assessing potential long-term human exposure to soil, water and waste contaminated from a liquid agent as it is not likely that ambient vapour alone would result in deposition or soil contamination (Augerson, 2000; Agency for Toxic Substances & Disease Registry, 2014; Department of the Army, Memorandum Subject, 2004). Specific exposure scenarios are estimated and shown in Table 3. The field drinking water standards were designed for a military scenario in which a small water container might be deliberately contaminated with signifiant amount of NA. The values < 12 μg/L of GA, GB, GD and VX up to 7 days exposure at 5 L/day water consumption (higher rate of drinking in hot environments) are safe. Daily exposure for a lifetime to soil contaminated from liquid GB (via ingestion, inhalation and dermal contact) is safe up to the limit of 1.3 mg/kg. In hazardous waste treatment facilities, possible occasional exposure of workers to waste water that may contain 8.3 mg/L of GB will not endanger worker's health.

Also risk assessment approaches that are used to determine the safe limits for chronic exposure to NAs should include all the possible synergism or potentiation effects that could appear in real life exposure from different sources between them and other chemicals with the same mechanism of action as organophosphorous compounds used as pesticides (Tsatsakis et al., 2016; Tsatsakis et al., 2017).

The so called "Gulf War" syndrome has inspired numerous studies and extensive research related to NAs, and a few relevant questions have been raised:

a) Is it possible to have NA effects from low-level exposures without the typical clinical picture?
b) Are long-term effects possible after mild exposure?
c) If the exposure goes unrecognized are the delayed effects possible?

One cannot exclude the possibility seeing as how there are cases of occupationally exposed workers that were found to have very low ChE levels but without clinical picture. Also, impaired thinking, memory, calculating ability were documented in volunteers exposed to VX and were asymptomatic.

There are case reports of workers with mild exposures who had fatigue, poor memory and concentration difficulties 4–10 months after the exposure. Duffy and Burchfiel (1980) found nonspecific EEG changes in workers exposed to sarin, but explained that it had no clinical significance (Duffy et al., 1979; Duffy and Burchfiel, 1980; Burchfiel et al., 1976).

No human studies exist that accurately describe that effects of NAs on neurotoxic esterase (NTE) caused delayed neurotoxicity. Effects on NTE were confirmed in experimental model on animals. However, the relevance of these effects in humans exposed to a single dose of NAs is unclear. So far there is no evidence that NAs can cause delayed effects similar to those associated with NTE in a military, asymetric conflict or terrist scenario, but the possibility is not excluded for atypical forms (Augerson, 2000).

7. Conclusion

Suspected exposure to an OP NA can occur in an environment where the agent can be found as a liquid, and when perhaps not even the afflicted know they are exposed to a harmful chemical. The onset of signs and symptoms of NAs poisoning can also be delayed, up to 18 h after the exposure. In order to provide successful medical countermeasures it is necessary to understand the physical, chemical and toxicological properties of these agents. Depending on the type of exposure there are different requirements that need to be fulfilled for the exposure to be confirmed.

Conflict of interest

None.

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