Response to the DG's request to the SAB to provide further advice on assistance and protection

The Science of Medical Countermeasures

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Member of the SAB

The Hague, 8 July 2015

Overview

1. Executive summary
2. Nerve agents
3. Adjunct agents and new trends in the treatment of NA poisoning
4. Acknowledgement

Executive summary

The Director-General requests the SAB to:

- identify best practices for preventing and treating the health effects that arise from acute, prolonged, and repeated organophosphorus (OP) nerve agent (NA) exposure; and

- identify any emerging medical countermeasures, intended for use at the point of exposure, that can reduce or eliminate longer term health effects arising from acute, prolonged, and repeated OP NA exposure.

This report addresses these questions and reviews current and promising developments in NA medical countermeasures.

Nerve agents

- Organophosphorus (OP) nerve agents (NAs) are stable OP compounds. They are easily dispersed and highly toxic when inhaled or absorbed through skin. They are classified into G and V agents, but some are hybrid in structure, and are called GV agents.

Nerve agents

G agents		
GA	Tabun	*O-ethyl N,N-dimethylphosphoramidocyanidate*
GB	Sarin	*O-isopropyl methylphosphonofluoridate*
GD	Soman	*O-pinacolyl methylphosphonofluoridate*
GF	Cyclosarin	*O-cyclohexyl methylphosphonofluoridate*
V agents		
VE		*O-ethyl S-2-(diethylamino)ethyl ethylphosphonothiolate*
VM		*O-ethyl S-2-(diethylamino)ethyl methylphosphonothiolate*
VG	Amiton	*O-O-diethyl S-2-(diethylamino)ethylphosphorothiolate*
VR		*O-isobutyl S-2-(diethylamino)ethyl methylphosphonothiolate*
VX		*O-ethyl S-(diisopropylamino)ethyl methylphosphonothiolate*
GV agents		
GV		*2-(dimethylamino)ethyl N,N-dimethylphosphoramidofluoridate*

Psychological reactions always have an organic background.

senses

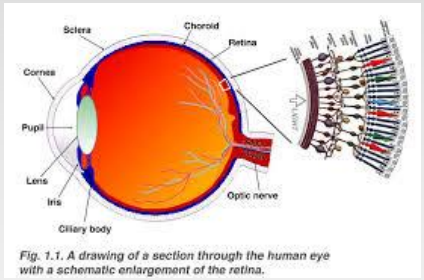
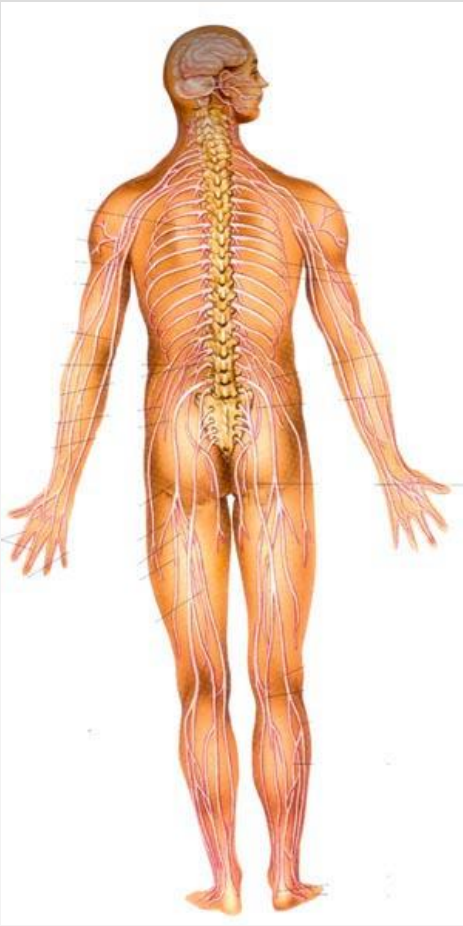


Fig. 1.1. A drawing of a section through the human eye with a schematic enlargement of the retina.

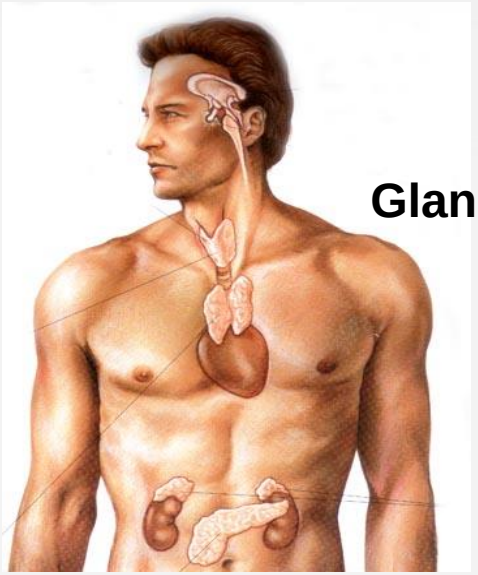
Nervous system is the organic base for psychological life. Every man has a couple of billions of neurons.

Senses, muscles and glands are important.



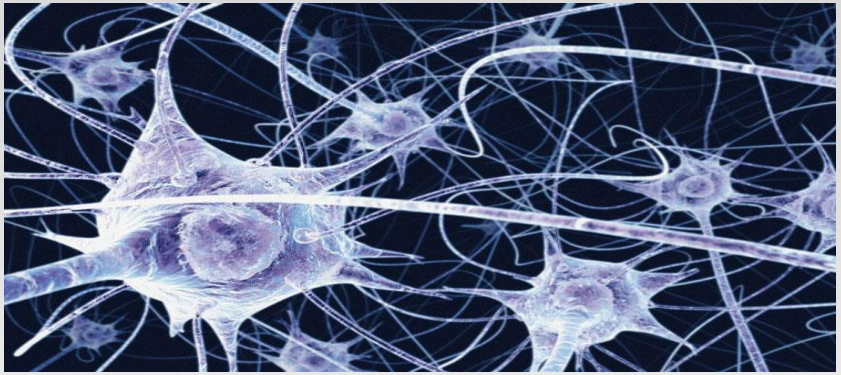
Nervous system

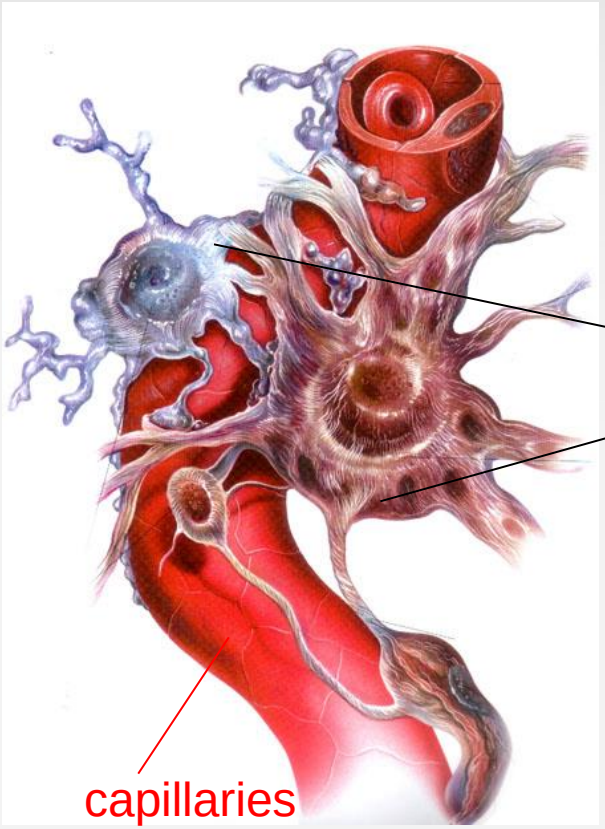
muscles

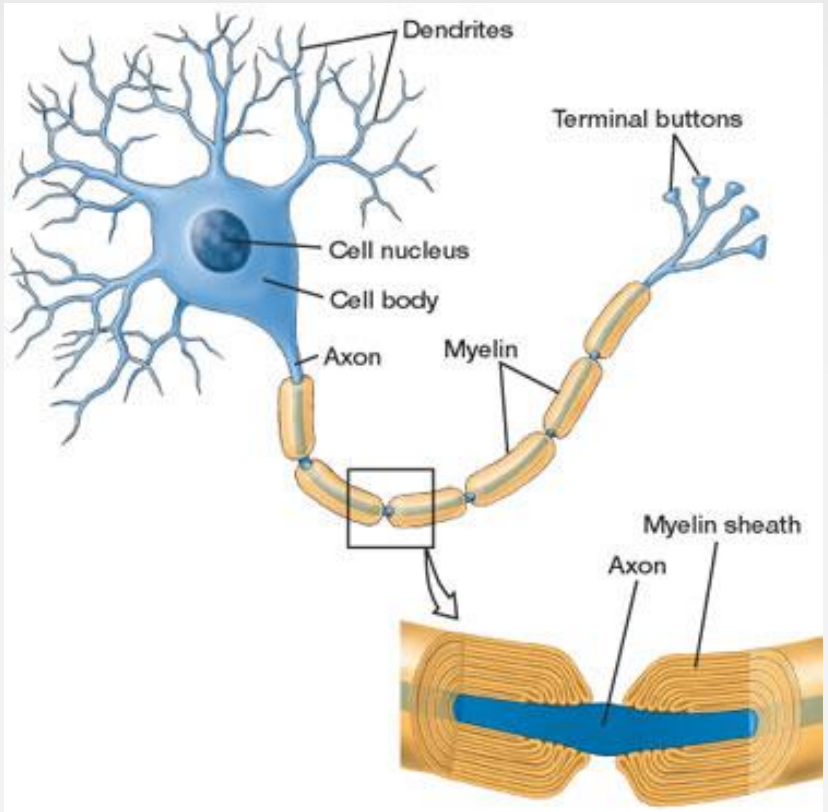


Glands

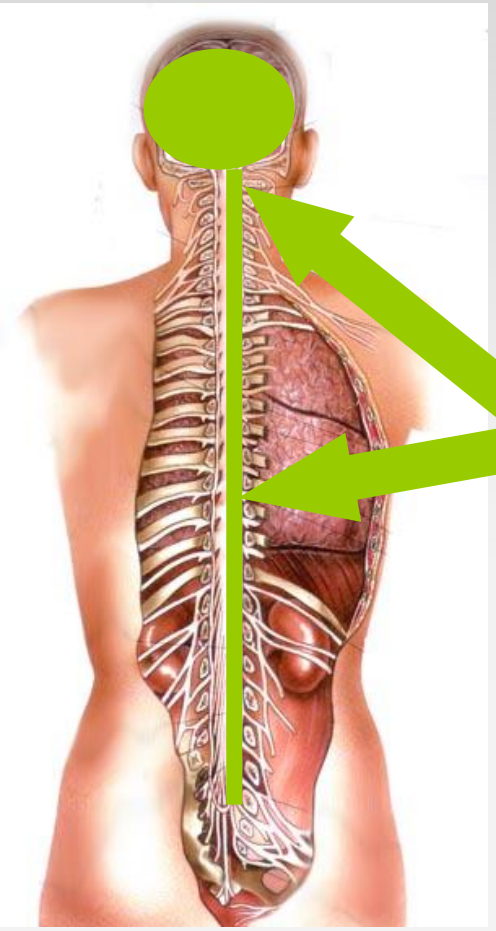
Neuron possesses a cell body (soma), dendrites and an axon. Generates and transmits the impulses between neurons and other types of cells (muscles, glands).





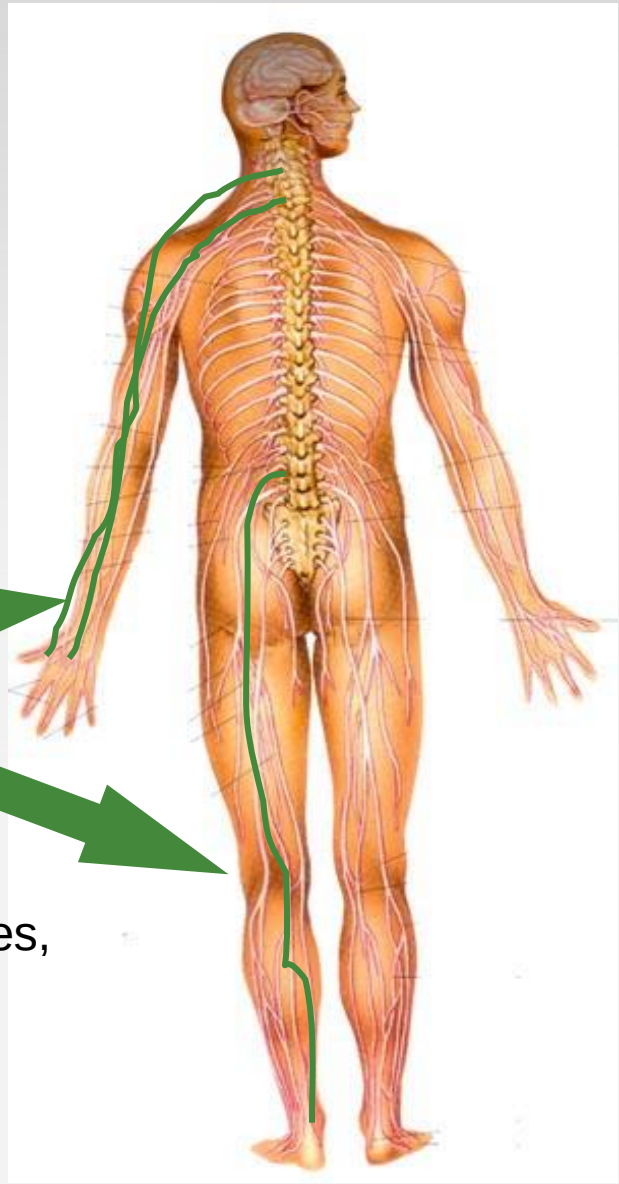


1. Central nervous system
Brain and spinal cord



CNS

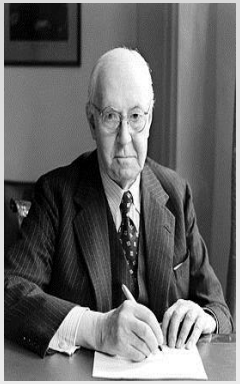
PNS



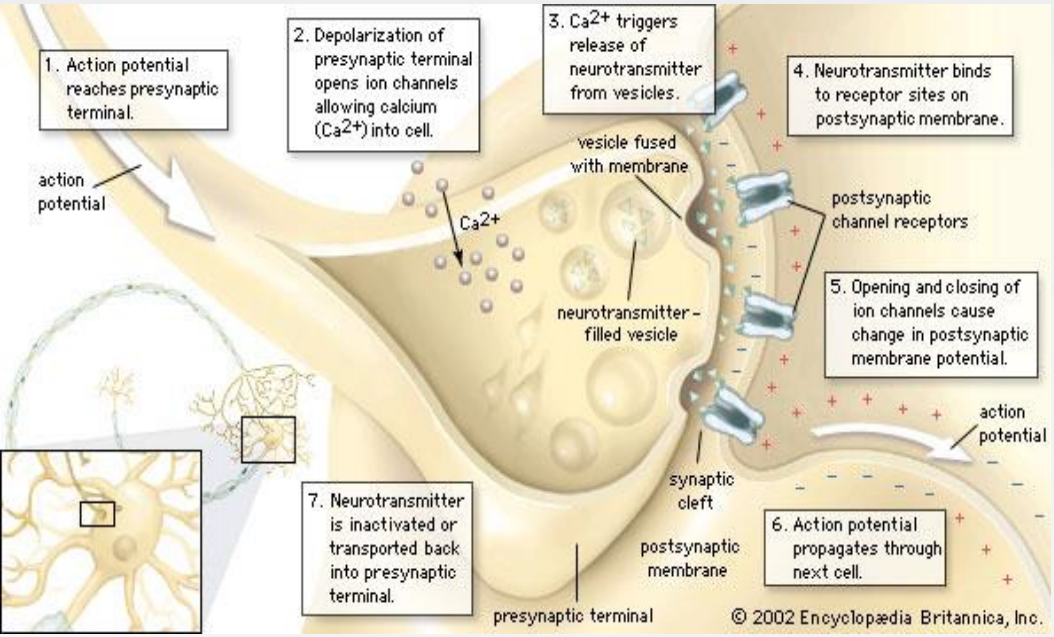
2. Perif. nervous system
Neurons outside the CNS, muscles, senses, organs

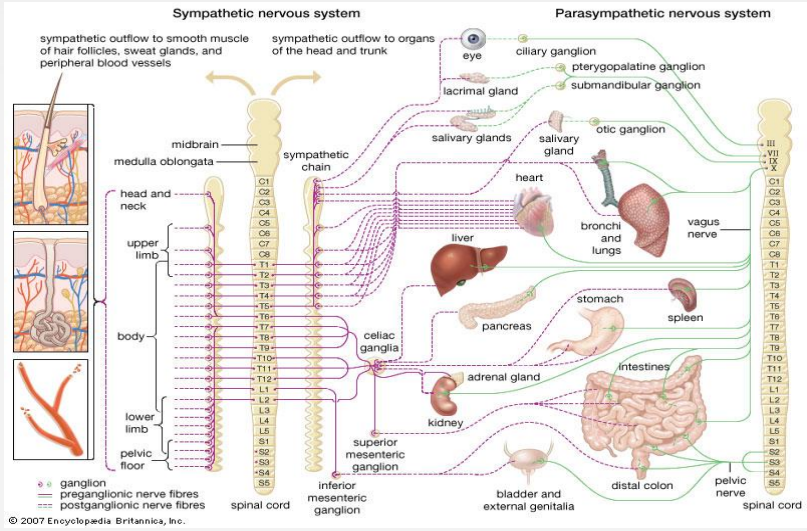
ACh, AChE, Mechanism of OP NA

ACh functions in the PNS (activates muscles) and
CNS (forms a cholinergic system with other neurons- inhibitory actions).
Major NT in ANS.

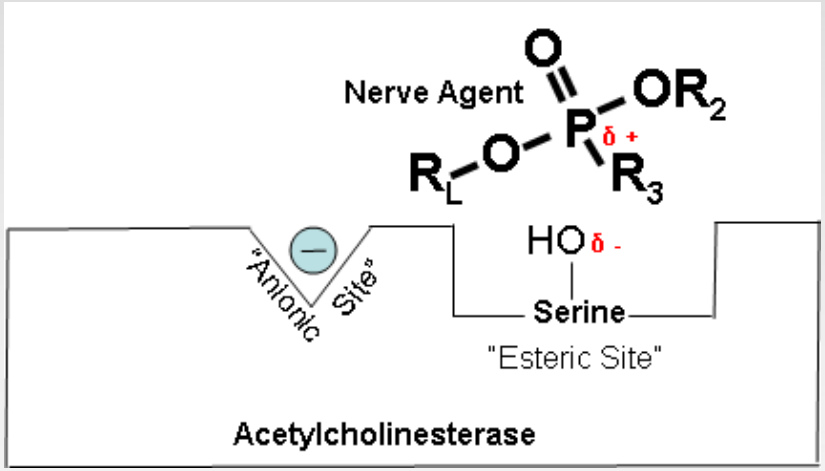


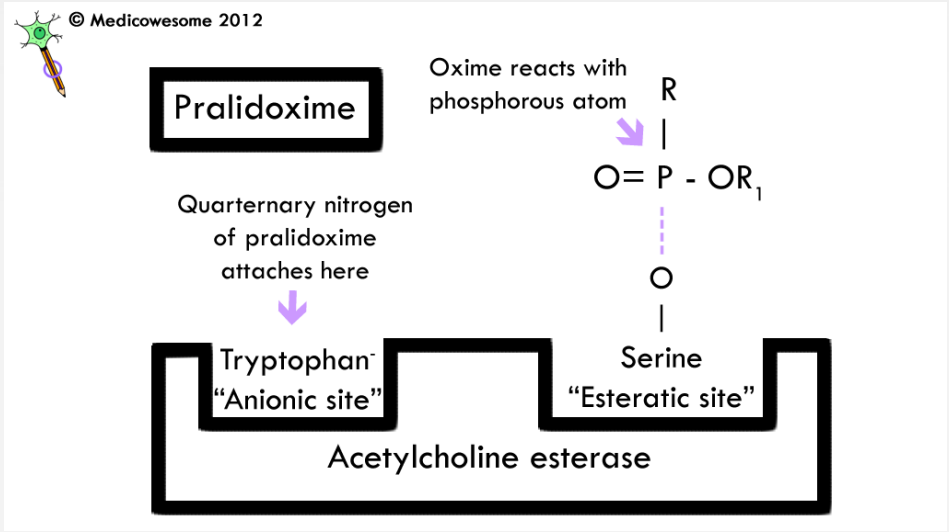
Henry Hallett Dale,1914, Otto Loewi 1936





Nerve agent, acetylcholinesterase and oxime





SIGNS AND SYMPTOMS AFTER ACUTE INHALATION EXPOSURE TO NA	SIGNS AND SYMPTOMS AFTER ACUTE DERMAL EXPOSURE TO NA
Low-dose with mild effects	***Low-dose with mild effects***
Runny nose	Localized sweating at exposure site
Miosis (blurred vision)	Fine muscle fasciculations at exposure site
Conjuctival inflammation	Miosis not an early sign and may be absent
Bronchoconstriction (chest tightness)	
Mild bronchosecretion	
Medium-dose with moderate effects	***Medium-dose with moderate effects***
Shortness of breath	Nausea and vomiting
Coughing	Severe headache
Wheezing	Generalized fasciculation
Nausea and vomiting	Feelings of weakness
Fasciculation	BEWARE: No respiratory signs present yet
Generalized feelings of weakness	
High-dose with severe effects	***High-dose with severe effects***
Loss of consciousness	Sudden loss of consciousness
Seizures	Seizures
Flaccid paralysis	Flaccid paralysis
Apnea	Apnea
Death usually within minutes	Death

From Crit. Care Med. 30 (2002)

Medical treatment of NA exposure

- Pretreatment and prophylaxis
- Post-exposure therapy

Pretreatment

- Pretreatment - before poisoning, to increase the efficacy of treatment post-exposure. Carbamates, e.g. pyridostigmine, have the ability to carbamoylate AChE, preventing the OP inhibitor from binding.

- Pyridostigmine (30 mg/8 h) provides good protection against lethality within 2 h of the 1st dose, but is not optimal until the 3rd dose. To be stopped upon observation of NA poisoning symptoms and post exposure therapy started.

- Pretreatment for poisoning (tablets, sublingual or transcutaneous patch).

Prophylaxis

- Administration of drugs before poisoning, designed to prevent poisoning.
- In the last decades, several topical skin protectants (TSP) have been produced (SERPACWA, AG7, IB1) but they have not always been fielded. They will increase the protection afforded by other protective equipment.
- Its purpose is to reduce or delay the absorption of CWA through the skin. However, effectiveness can only be expected when the TSP is applied prior to exposure.

Post - exposure treatment

A therapeutic scheme for NA poisoning includes early decontamination, supportive measures and specific pharmacological treatment to achieve:

- muscarinic cholinergic blockade (atropine),
- enzyme reactivation (oximes),
- and anticonvulsant effect (benzodiazepines associated with other drugs in case of refractory seizures).

Emergency field therapy

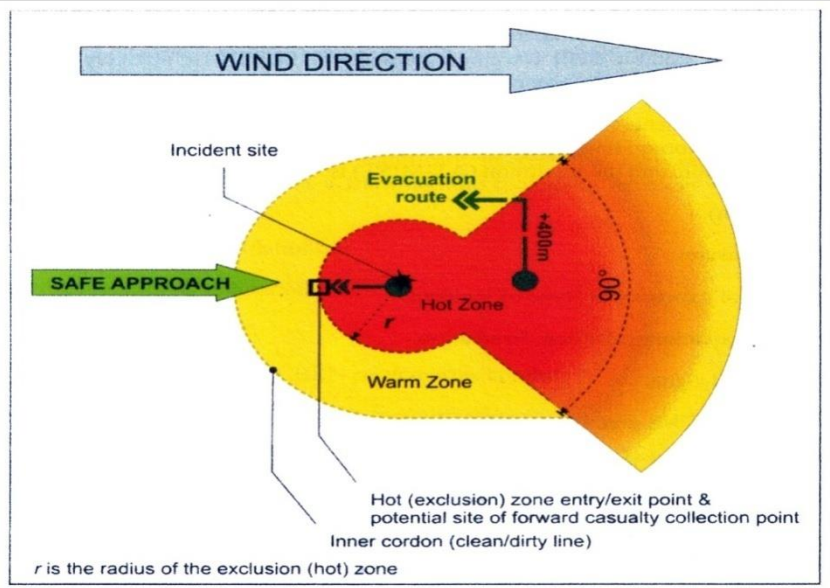
- Pralidoxime chloride (600 mg) and atropine (2 mg)
- Pralidoxime methylsulfate (350 mg), atropine (2 mg) and avizafone chlorhydrate (20 mg)
- Obidoxime chloride (220 mg) and atropine (2 mg)
- TMB-4 (80 mg) and atropine (2 mg)
- HI-6 dimethanesulfonate (750 mg), atropine (2 mg) and diazepam (10 mg)

Strategies using these autoinjectors depend on the country in which they are used.

Hot Zone

- Responders should have received training and wear protective clothing before entering a Hot Zone.

- If PPE is unavailable, or rescuers have not been trained, a call for assistance should be made according to local Emergency Operational Guides (EOG).



First aid recommendations under field conditions

Treatment should be commenced immediately and the casualty given an antidote by self or buddy aid via autoinjectors:

- **MARK I kit:** Atropine (2 mg, 0.7 ml) and 2-PAM (600 mg)
- **AIBC Ineurope®:** Pralidoxime methylsulfate (350 mg), atropine (2 mg) and avizafone chlorhydrate (20 mg)
- **ATOX II:** Atropine (2 mg, 0.7 ml) and obidoxime (220 mg)
- **ATNAA:** Atropine (2 mg/0.7 ml) and 2-PAM (600 mg/2 ml); 1 needle injects both drugs
- **ATROPEN:** Atropine (2 mg, 0.7 ml). Each soldier must have 3 kits and 1 auto-injector with diazepam (10 mg) (if warned of NA attacks).
- Based on the severity of poisoning, I-III autoinjectors are applied.

Emergency medical treatment of NA poisoning

Symptoms and signs	Mark-1 Kit	Repeat dosing
Severe difficulties with breathing, apnea, cyanosis, muscle fasciculation or twitching, seizure, loss of consciousness	ABC (Maintain patent airway; assist breathing as needed, give oxygen, provide suction, restore normal cardiac rhythm) Administer Mark I Kit (3 times at 10-15 min intervals)	Diazepam autoinjector may be repeated 3 times every 10-15 min
Severe respiratory distress	Administer Mark I Kit (2 doses)	
Sweating, miosis, rhinorrhea, nausea, vomiting, anxiety	Administer Mark I Kit (1 dose)	
NOTE:	Monitor for symptoms every 10 min. Repeat atropine if needed	

Civilian population

- Important differences between relatively well protected armed forces and civilians.
- Do not have PPE, and are not pretreated by PB. At least 30 min delay for administration of specific therapy.

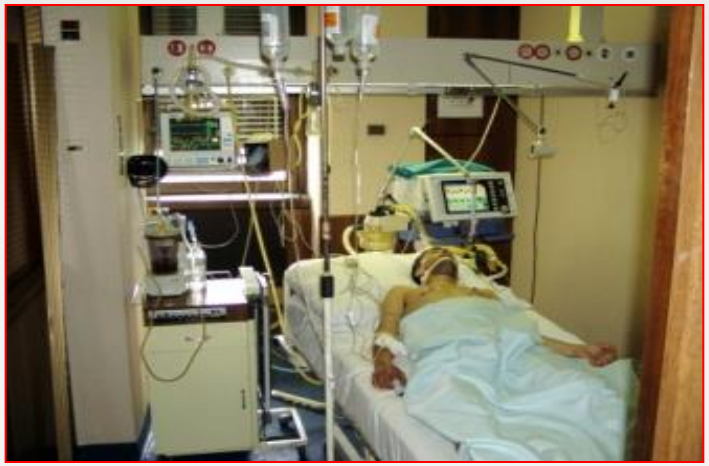
Atropine dosage after transfer to hospital

- Lower atropine doses needed.
- No established atropine dosage protocol.
- Individual titration of atropine dose.
- High concentration (100 mg/ml) or large volume ampules (10 or 20 mL) of 2 mg/mL atropine solutions are recommended for stockpiling.
- After initial hyper-atropinisation, 10-20% of a loading dose of atropine should be used in 5% glucose solution as a continuous infusion.

Oxime treatment after transfer from the first line to hospitals

- Pralidoxime (30 mg/kg in 5% glucose solution i.v, followed by 8 mg/kg/h continuously, until clinical recovery, or 12 h after the last dose of atropine was given.

- Obidoxime is a more potent reactivator in the case of VX, sarin. Dosage: 8 mg/kg i.v initially, followed by 3 mg/kg/h (500 mg loading dose, followed by 750-1000 mg in a continuous infusion).



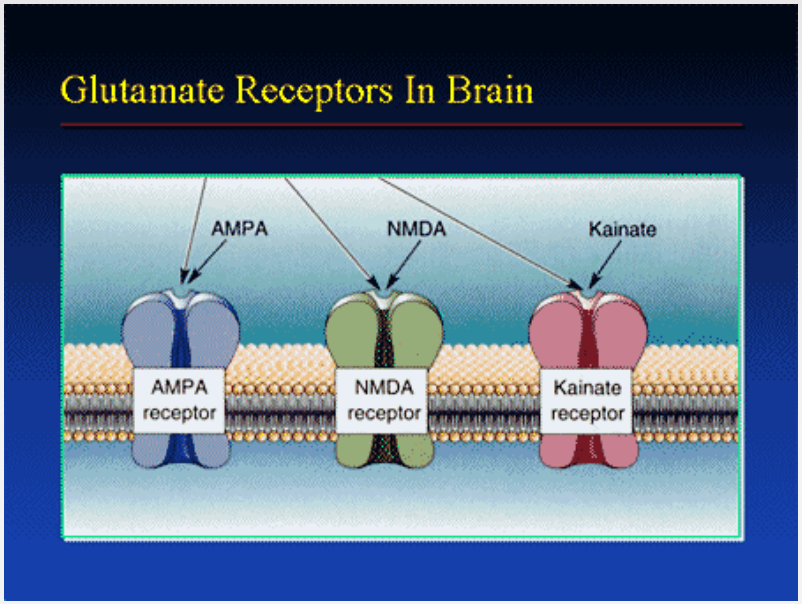
Anticonvulsants

- Anticonvulsants (e.g. diazepam, lorazepam or midazolam) having a neuroprotective effect, should be administered as necessary.
- Diazepam should be injected i.m starting with a 10 mg dose (adjusting the frequency of later injections).
- Midazolam should replace diazepam in cases requiring urgent treatment.

Adjunct agents and new trends

Gacyclidine

- A non-competitive NMDA receptor antagonist with neuroprotective properties.
- Prevents glutamate - induced neuronal death, enhances the neuroprotective activity of antidotes in soman poisoning (inhibits neuropathologic changes occuring 3 weeks after a soman challenge), and prevents convulsions.
- Unfortunately not anymore available – could be replaced by ketamine.



Adjunct agents and new trends

Tezampanel

- A competitive antagonist of the AMPA and kainate sub-type receptor. It reduces the length of status epilepticus and neuropathy induced by soman (exp). The best results - 1 h after exposure.

Ketamine

- Anaesthetic, a non-competitive antagonist of NMDA receptors. Experimentally confirmed to stop seizures and reduced related brain damage (1 h after exposure).

- Large clinical use world wide, should be considered for the treatment of NA - induced **refractory status epilepticus**.

Adjunct agents and new trends

Huperzine A

- NMDA receptor antagonist (prevents status epilepticus, reduces the severity of seizures), inhibits AChE reversibly, similar to donepizil, rivastigmine or galantamine. It is used to treat Alzheimer's disease and myasthenia gravis.

Caramiphen

- Antimuscarinic drug with antiglutamergic and gaba-ergic properties. Therapeutic efficacy against OP-poisoning as a prophylactic and post-exposure (confirmed experimentally).

Adjunct agents and new trends

Galantamine

- Galantamine (GAL) inhibits AChE and potentiates ACh-induced currents in brain neurons, potentiates the activity of NMDA receptor.
- In contrast to pyridostigmine that inhibits BuChE also, it should help preserve the scavenger capacity of plasma BuChE for OPs.
- Experimentally confirmed (VX challenge) to reduce lethality, impairment of muscle tension, EEG changes.

Adjunct agents and new trends

Scopolamine

- Anticholinergic. No randomized controlled studies.

Penehyclidine hydrochloride

- The anticholinergic agent used clinically for treating poisoning by OPs. Crosses the blood-brain barrier and antagonizes muscarinic and nicotinic receptors in the brain.

- Pauses ongoing seizures and has a better neuroprotective effect if administered soon after seizure onset in soman poisoning (experimentally). However compared to other drugs the body of evidence is smaller.

Adjunct agents and new trends

Sodium hydrogencarbonate and blood alkalinization

- To increase the hydrolysis of OP molecules *in vivo*, the effects of higher doses of $NaHCO_3$ (5 mEq/kg in 1 h, followed by 5 mEq/kg/day) were assessed.
- Increasing one unit of pH (accompanied by a 10-fold increase in OP hydrolysis, and alkalinization products of NAs).
- **Better control of cardiotoxicity, increased bio-availability of oximes, increased atropine activity, and/or a direct effect of $NaHCO_3$ on neuromuscular function. Not a standardized procedure so far.**

Adjunct agents and new trends

Magnesium sulfate

- The mechanism - inhibition of ACh release through blocking Ca^{2+} channels in the CNS and at peripheral sympathetic and parasympathetic synapses.
- In acute OP poisoning - decreased mortality and reduced overstimulation of the CNS due to NMDA receptor activation.
- No side effects with doses of 4-16 g.
- **Insufficient evidence to recommend routine use in NA casualties.**

Adjunct agents and new trends

Antioxidants

- Possible additional mechanism for NA: induction of oxidative stress and generation of free oxygen radicals.
- Chronic toxicity studies have revealed an increased level of oxidative stress biomarkers as well as increased DNA damage.
- A beneficial effect of **vitamin E and *N*-acetyl-cysteine** has been shown (exp. studies).
- **Insufficient evidence to recommend routine use in NA casualties.**

Adjunct agents and new trends

Protective bioscavengers

- New medical treatment of NA exposure should provide reduced lethality, reverse toxicity following exposure, and help eliminate the need for further treatment.

- The need to start treatment within 1 min after exposure has prompted the development of pretreatment therapy, such as bioscavengers of different profile.

Adjunct agents and new trends

Bioscavengers - enzymes or antibodies that sequester and neutralize toxic OP compounds before they reach their biological targets.

Conditions:

- a) broad spectrum vs different NAs and a rapid activity;
- b) suitable retention time in circulation (ideally 11-15 days);
- c) be available in sufficient concentration to be effective;
- d) have no adverse immunogenic properties.
- e) be available at a reasonable cost

Adjunct agents and new trends

Bioscavengers:

- a) **Stoichiometric bioscavengers** - (ChEs), especially BChE, and carboxylesterases (CaEs) react stoichiometrically with OP compounds (1 mole of enzyme neutralizes 1 mole of OP, inactivating it).

- b) **"Pseudo catalytic bioscavengers"** that combine AChE (that has scavenging properties and binds NA) and oxime (that acts as a pseudocatalytic bioscavenger reactivating ChE) and thus restore AChE function;

- c) **Catalytic bioscavengers** (OP hydrolase, OP anhydrase, and paraoxonase (PON) that trap and degrade neurotoxic OP compounds rendering them non-toxic.

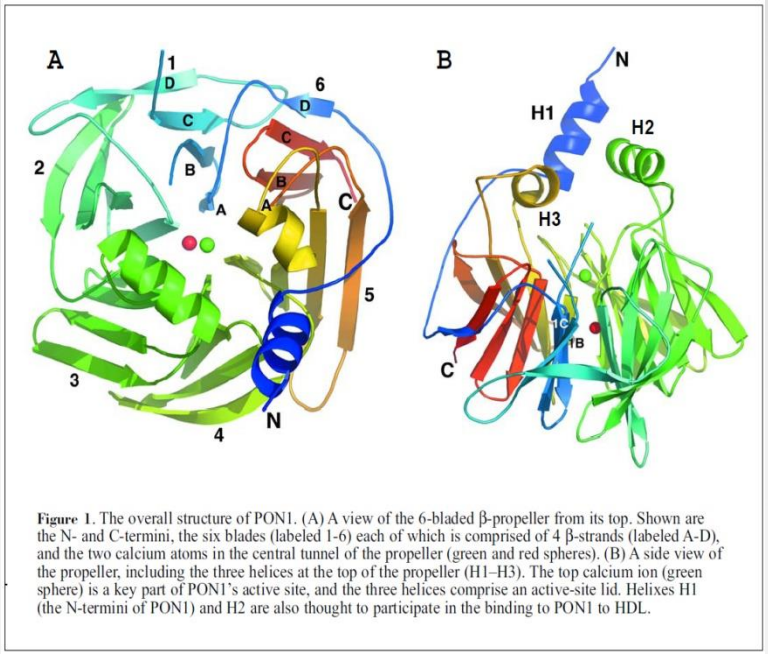


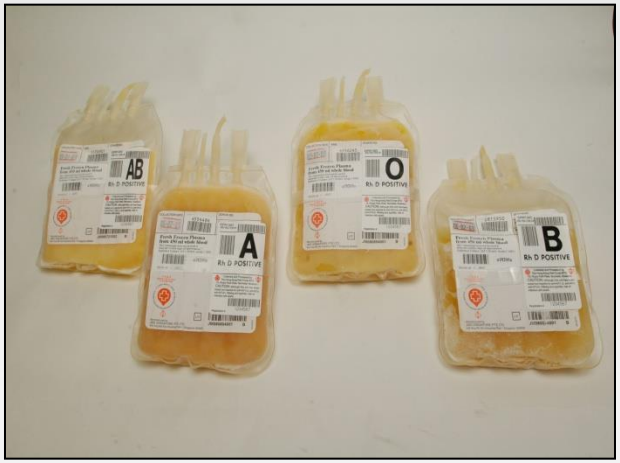
Figure 1. The overall structure of PON1. (A) A view of the 6-bladed β-propeller from its top. Shown are the N- and C-termini, the six blades (labeled 1-6) each of which is comprised of 4 β-strands (labeled A-D), and the two calcium atoms in the central tunnel of the propeller (green and red spheres). (B) A side view of the propeller, including the three helices at the top of the propeller (H1–H3). The top calcium ion (green sphere) is a key part of PON1's active site, and the three helices comprise an active-site lid. Helixes H1 (the N-termini of PON1) and H2 are also thought to participate in the binding to PON1 to HDL.

Adjunct agents and new trends

- **pHuBChE** has scavenging properties against different NAs (soman, sarin, VX).
- Advantages for human use: rapid reaction with a broad spectrum of OPs, a good retention time in circulation, and no immunogenic activity.
- Methods for mass production of pHuBChE: **purification of the enzyme from human plasma,** recombinant HuBChE (rHuBChE) **produced in the milk of transgenic goats** ('Protexia') developed by Nexia.
- Possible sources of **rHuBChE** are transgenic plants, transgenic animals, adenovirus or algae, and it can be derived in cell-lines.

Adjunct agents and new trends

- **Fresh frozen plasma (FFP)** is a blood fraction prepared by removing the cellular components by apheresis.
- It contains clotting factors, proteins, and enzymes.
- It is hypothesised that in OP poisoning BuChE from FFP will sequester the poison present in blood and remove it from circulation.
- **No general agreement about the routine use for the treatment of exposure to OP compounds.**



Methods for decontamination

Methods of decontamination of CW agents: (i) mechanical, (ii) physical, and (iii) chemical. They were presented last year by SAB members (*OPCW Today*, Vol. 3, No. 1, Aug 2014).

Oxidiser gels

- To detoxify NA, a formulation with a adsorbent (e.g. silica, alumina or alumino-silicate clays) and oxidising agent (aqueous sodium hypochlorite) can be prepared and applied at the site of decontamination. **Suitable for field implementation**.

Bacterial phosphotriesterase

- Phosphotriesterase (PTE) is an enzyme isolated from the bacterium *Pseudomonas diminuta.* Modified by biotechnological processes, engineered PTE enzymes are useful for detoxification of NA ***in vivo***.

Methods for decontamination

Nanosized metal oxides as CWA decontaminants

- Nanosized metal oxide aerogels (MgO, Al_2O_3 and CaO) (with a high surface area, potent adsorbent properties and reactivity towards NAs) are promising sorbent materials for removing NAs from **contaminated surfaces** and degrading them *in situ*, leading to non-toxic products.

Future trends

- Formulations have to be user and eco-friendly,
- Without corrosive properties,
- Stable active ingredients.
- Nanoporous materials,
- Nanosized metal oxide aerogels

Methods for decontamination

- TSP (RSDL)
- Intended to remove or neutralize CWAs (GA; GB; GD; GF; VX; HD), T-2 toxin and many pesticides.
- Originally developed by Canadian Department of National Defense, adopted by several military services around the world.
- FDA has approved use thereof in 21 and 42 mL packets.
- EXTERNAL USE ONLY, CONTACT WITH EYES AND MUCOUS MEMBRANES SHOULD BE AVOIDED!

Identify best practices for preventing and treating the health effects that arise from acute, prolonged and repeated NA exposure

- **In the case of NA exposure**, it is necessary to administer the adequate antidotal treatment:

  - **the reactivator of NA** - inhibited AChE,

  - **the anticholinergic drug** to counteract the overstimulation of peripheral and central cholinergic muscarinic receptors, and

  - **the anticonvulsive drug** to prevent centrally mediated seizures and subsequent tonic - clonic convulsions.

- Treatment must continue as long as NA - induced clinical and laboratory signs and symptoms are visible.

Identify best practices for preventing and treating the health effects that arise from acute, prolonged and repeated NA exposure

- **In the case of repeated NA exposure**, each exposure must be treated **in the same way as the first exposure** using adequate antidotes and supportive symptomatic drugs.
- Humans can be more sensitive to acute toxicity of NAs in the case of repeated exposure (lower activity of AChE due to previous NA exposures).
- The prognosis of repeated exposure to NA is more severe and the antidotal and supportive treatment must be as intensive as possible.

Identify any emerging medical countermeasures, intended for use at the point of exposure that can reduce or eliminate longer term health effects arising from acute, prolonged and repeated NA exposure

- To reduce or eliminate longer term health effects - treat correctly acute cholinergic crisis.
- Delayed and prolonged effects of NA are mostly caused by damage to the CNS through centrally - mediated seizures.
- To prevent seizures, it is necessary to **prevent prolonged stimulation of muscarinic receptors by a centrally - acting anticholinergic drug** and **an anticonvulsive drug.**
- If longer term health effects (especially neurological, including symptoms such as increased excitability and a deficit of cognitive function) emerge **symptomatic and supportive treatment should be recommended in this situation**.

The role of prophylactic antidotes against NAs

- Prophylactic antidotes should increase the:
- **Resistance of the organism against acute toxicity of NAs**
- **Efficacy of post-exposure antidotal treatment of NA poisoning**
- Prophylactic antidotes to NAs should be administered in response to the threat of exposure to NAs. Generally, the combination of pre-treatment and post - exposure adequate antidote treatment increases the probability of avoiding the delayed and prolonged effects of NAs.

The role of prophylactic antidotes against NA

- **Pyridostigmine** drawbacks: limited dosage (due to adverse effects), it cannot penetrate the blood-brain barrier.
- A combined oral prophylaxis called **PANPAL** was developed in the Czech Republic. It consists of **pyridostigmine** and two centrally-acting anticholinergic drugs (**benactyzine and trihexyphenidyl**).
- Higher efficacy than pyridostigmine alone to avoid or diminish the acute toxicity and to prevent delayed and long-lasting health effects from acute, prolonged and repeated exposure to NA.
- **Clinical approval from the FDA and EMA would be necessary prior a general recommendation.**

The role of prophylactic antidotes against NA

- Another approach is to administer reactivators of NA - inhibited AChE in advance.
- A special prophylactic antidote called TRANSANT (involving the **oxime HI-6**) was developed and introduced into the Czech Army.
- The combination of both prophylactic antidotal means (PANPAL and TRANSANT) represents an effective prevention, increases the resistance of humans and prevents centrally - mediated seizures as well as subsequent delayed and prolonged health effects from acute, prolonged and repeated exposure to NA.
- **Clinical approval of the FDA and EMA would be necessary prior a general recommendation.**

The role of prophylactic antidotes against NA

- Recent alternative approach to the development of prophylaxis - **bioscavengers** able to bind or hydrolyse NA before they reach the biological target.
- Valuable, but until now has not been prepared for clinical use.
- However, it represents a promising approach to preventing the longer term health effects arising from acute, prolonged and repeated NA exposure.

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