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Do environmental quality standards for selected nerve agents ensure safety of the ecosystems?

Czy istniejące standardy jakości środowiska dla wybranych fosforoorganicznych bojowych środków trujących zapewniają również ochronę ekosystemów?

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Abstract
Possible hazard and risk posed by organophosphate nerve agents (OP-NA) towards higher organisms and humans is well recognized, but scientific data about environmental effects of these compounds are still limited. The main aim of this study was to inspect if established interim military drinking water and soil standards for selected nerve agents are also safe for ecosystems. The results of this research may indicate whether taking additional decontamination actions are needed after incidental or intended release of OP-NA in the environment. Three OP-NA were selected as model compounds: soman, sarin and VX. Results from aquatic and soil ecotoxicity test were used to estimate the predicted no effect concentrations according to the EU methodology. Risk quotients were calculated both for water and soil compartments. The results of this study indicate that existing OP-NA standards are underprotective for organisms living in soil and water. There is a necessity of preparing much more extensive testing strategy for chemical warfare agents' assessment in the environment leading to reduced high uncertainty in current risk estimation.

Streszczenie
Zagrożenie i ryzyko powodowane fosforoorganicznymi bojowymi środkami trującymi w stosunku do ludzi i organizmów wyższych jest stosunkowo dobrze rozpoznane i zbadane, jednakże dane naukowe dotyczące środowiskowego oddziaływania tego typu substancji są ciągle niedostateczne. Głównym celem pracy było zbadanie czy ustanowione w armii, tymczasowe kryteria jakości dla wody pitnej i gleby będą również zapewniały bezpieczeństwo dla ekosystemów. Wyniki analiz mogą wskazać czy istnieje konieczność podjęcia dodatkowych działań dekontaminacyjnych w przypadku zamierzonego lub przypadkowego uwolnienia fosforoorganicznych bojowych środków trujących do środowiska. Jako substancje modelowe wybrano trzy związki chemiczne: soman, sarin oraz VX. Wyniki testów ekotoksyczności przeprowadzonych z użyciem tych związków w stosunku do organizmów wodnych i glebowych posłużyły do wyznaczenia wartości PNEC zgodnie z metodologią UE. Ilorazy ryzyka obliczono zarówno dla środowiska wodnego jak i glebowego. Wyniki wstępnych analiz wskazują, że ustanowione standardy jakości są niewystarczające do zapewnienia bezpieczeństwa organizmom w ekosystemach. W związku z tym koniecznym jest przeprowadzenie znacznie bardziej rozszerzonych badań ekotoksyczności bojowych środków trujących w celu zmniejszenia poziomu niepewności w przeprowadzonych procedurach oceny ryzyka w środowisku.

1. INTRODUCTION

Chemical warfare agent (CWA) is defined as a chemical which is intended for use in military operations to kill, seriously injure, or incapacitate humans (or animals) through its toxicological effects. One of the most important groups of CWA consists of organophosphate esters, so-called nerve agents (OP-NA). OP-NA are colorless liquids, highly soluble in organic solvents, soluble in fats, and moderately soluble in water.

The reactivity of OP-NA is related to the electrophilic nature of the phosphorus ester bond. The main mechanism of action of these substances relies on blocking the enzyme acetylcholinesterase (AChE) and the accumulation of acetylcholine in the synaptic cleft. Organophosphates are generally considered to be readily biodegradable substances and not cumulative in living organisms [Manahan 2010].

The main aim of this study was to inspect if established field drinking water standards and health-based environmental soil screening levels for selected nerve agents are also safe for aquatic organisms.

2. MATERIALS AND METHODS

Selected OP-NA were used in this study, these are:
– soman;
– sarin;
– VX.

Substances were synthesized by Military Institute of Chemistry and Radiometry (Warsaw, Poland). Structures of tested OP-NA and some basic, environmentally relevant physical and chemical parameters are presented in Table 1.

The bioassays were preformed using crustacean *Daphnia magna* neonates (according to OECD 202 guideline [OECD 2004]) and seeds of higher plants: *Sorghum saccharatum*, *Lepidium sativum* and *Sinapis alba* (according to Phytotoxkit Standard Operational Procedure [Phytotoxkit 2010]). The detailed experimental setup and organisms' culturing regime are presented elsewhere [Kalinowski, 2011a; Kalinowski, 2011b]. Briefly, *Daphnia magna* neonates were exposed to series of dilution of OP-NA. Immobile organisms were inspected after 24 and 48 hrs. Plant seeds were

put in OP-NA spiked OECD soil, cultivated for 72 hrs in the dark, and after exposed germinated seeds were counted, the root and stem length were measured. All experiments were conducted in triplicate, and the EC50-t values were calculated using the probit method.

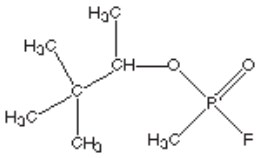
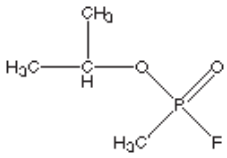
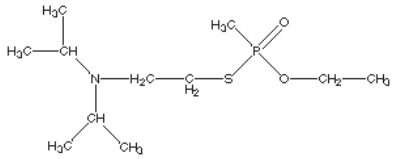
Predicted no effect concentrations (PNEC) were calculated using assessment factors according to EU methodology described in Technical Guidance Document on Risk Assessment [Technical Guidance Document 2003]. The risk assessment was made by risk quotients' (RQ) calculation defined as the proportion of military standards and PNEC as well, for the water and soil. Values of RQ above 1 indicate high risk for the ecosystem.

3. RESULTS

The test results indicate that VX is the most toxic compound among tested OP-NA towards *Daphnia magna*. EC50-48hrs was calculated at level 0,29 ng/l. Sarin seems to be over 500 times less toxic with EC50-48hrs value of 161,2 ng/l (Table 2).

All three tested OP-NA did not cause significant influence on germination of bioindicators' seeds. EC50-72hrs values were higher than 200 mg/kg d.w. soil. The highest toxicity toward early growth of plants (measured as root and stem elongation inhibition) was observed in VX and sarin treatment, the lowest in soman treatment. *Sorghum saccharatum* seems to be more sensitive to organophosphate influence than *Lepidium sativum* and *Sinapis alba* (Table 2).

Table 1. Structures and some physical and chemical properties of the tested OP-NA.

OP-NA	Soman	Sarin	VX
Structure			
CAS number	96-64-0	107-44-8	50782-69-9
Molecular weight [g/mol]	182,18	140,1	267,38
Density [g/mL]	1,0222 (at 25°C)	1,102 (at 20°C)	1,006 (at 20°C)
Water solubility [g/L]	21 (at 20°C)	miscible	30 (at 25°C)
Log K_{ow}	1,02	0,15	2,36
Log K_{oc}	1,17	0,45	1,18

Values taken from Gupta [Gupta 2009] and Rosenblatt [Rosenblatt 1996]

Table 2. Determination of PNEC values for the selected OP-NA for aquatic and terrestrial compartments.

Environmental compartment	OP-NA	Species and endpoint	Test duration [hours]	Lowest EC50-t(*),(**)	Assessment factor	PNEC(**)
Aquatic	Soman	*Daphnia magna* immobilization	48	59,7 (46,7-72,7)	1000	0,0597
	Sarin			161,2 (154,5-167,9)		0,1612
	VX			0,29 (0,1-0,39)		0,00029
Terrestrial	Soman	*Sorghum saccharatum* Stem elongation	72	67,5 (60,7-74,3)		0,0675
	Sarin	*Sorghum saccharatum* Stem elongation		30,7 (27,6-33,8)		0,0307
	VX	*Sorghum saccharatum* Root elongation		31,6 (29,0-34,2)		0,0316

(*) EC50-t values are taken from Kalinowski et al. 2011a and Kalinowski et al. 2011b

(**) EC50-t and PNEC values are expressed in ng/L and mg/kg dw for aquatic and terrestrial compartments respectively

The existing military standard for water and soils are presented in Table 3. Obviously standard values were derived based on toxicity to humans and risk assessment. These values may therefore be a guideline for decontamination troops as a limit value that clean up procedures must reach after OP-NA release. It must be pointed out that no environmental standards for OP-NA in surface water exists in the world, therefore drinking water MPC is used as PEC in risk quotient calculations. It should be noted that for numerous substances (e.g. pesticides) drinking water standards are orders of magnitude higher than the surface water guidelines. One of the reasons of such large differences is much more effective biotransformation mechanism in humans and limited intake of contamination only via ingestion route, not whole body surface like it takes place for water living organisms. All risk calculations that are based on such assumption may lead to large underestimate of RQ values.

The question asked in this study is whether these values are also protective for ecosystems. Simple risk quotient (RQ) approach is used for this purpose. Guideline values are compared to the $PNEC_{water/soil}$ values calculated based on previously conducted ecotoxicity studies with OP-NA (Table 2). If calculated RQ exceeds 1 (that means that the acceptable guideline value is higher than the predicted no-effect concentration) the substance may pose significant risk to the environment.

Table 3. OP-NA drinking water and soil standards.

OP-NA	Drinking water MCP* [µg/l]	HBESL** for residential soil [mg/kg]	HBESL** for industrial soil [mg/kg]
Soman	6,0	1.6	41
Sarin	13,8	0.31	8.1
VX	7,5	0.047	1.2

* Military field drinking water Maximum Permissible Concentration for selected OP-NA assumes a 70-kg person consuming field drinking water at 5 L/day for up to 7 days, and possible AChE depression not exceeding 25% [DANIELS 1990]
** Health-based environmental screening levels [RABER at al. 2004]

Table 4. Risk quotients' values for OP-NA.

OP-NA	Risk quotient [-]		
	Aquatic compartment	Soil compartment	
		residential soil	industrial soil
Soman	100503	23,7	607,4
Sarin	85608	10,1	263,8
VX	25862069	1,5	38

4. CONCLUSION

All RQ values (Table 4) exceed 1, which means that OP-NA present in environment in concentrations not exceeding existing threshold values may pose significant risk to aquatic and soil biota although they will not pose any risk to human health.
It must be pointed out that the presented risk assessment is done based on only single, short-term ecotoxicity test results from single trophic level both for soil and water. Usage of additional single species test (e.g. algae and fish for aquatic compartment or earthworms and microorganisms for terrestrial compartment), long term studies or even model ecosystems will highly reduce uncertainty and would provide much more precise risk estimation.
This study indicates a necessity of further much extensive testing of OP-NA towards aquatic and soil organisms, that will allow us to establish environmental guideline values for these highly dangerous compounds.

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