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Maximum acceptable concentration levels of the chemical agent sarin in Canadian and Russian soil types

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Abstract: The lowest-observed-adverse-effect and no-observed-adverse-effect (NOAEC) concentrations of sarin were determined in soils typical to Canada and Russia. Maximum acceptable concentrations (NOAEC) were established to be $3.0 \times 10^{-4} \text{ mg kg}^{-1}$ for the standard reference soil, $2.0 \times 10^{-3} \text{ mg kg}^{-1}$ for prairie soil and $1.0 \times 10^{-2} \text{ mg kg}^{-1}$ for forest soil.

Keywords: sarin, chemical warfare agent, soil contamination, no observed effect concentration (NOEC), soil toxicology

Résumé: Les concentrations (lowest-observed-adverse-effect and no-observed-adverse-effect) maximales de sarin sont déterminées pour des sols typiques au Canada et à la Russie. Les concentrations maximales de sarin acceptables ont été établies comme $3.0 \times 10^{-4} \text{ mg kg}^{-1}$ pour le sol de référence, $2.0 \times 10^{-3} \text{ mg kg}^{-1}$ pour le sol de prairie et $1.0 \times 10^{-2} \text{ mg kg}^{-1}$ pour le sol de forêt.

Mots-clés: sarin, agent de guerre chimique, contamination de sol, concentration avec aucun effet observé, toxicologie de sol

Introduction

In 20 March 1995, at about 7 AM local time, the chemical warfare agent (CWA) sarin (O-ethyl-S-(2-diisopropylaminoethyl) methyl phosphonothiolate), was used in a terrorist attack at a Tokyo subway station. This agent was also reportedly used in Syria in and around Damascus (Ghouta) on 21 August 2013. Sarin (also known as GB) is a non-persistent nerve agent that was developed in the late 1930s in Germany and was weaponized by both sides of the cold war, among other nations. The Chemical Weapons Convention (CWC), with Syria becoming the 190th member state to sign on, monitors and controls the production, storage and use of CWAs for research and training purposes. This, however, as noted above, has not prevented their unauthorized use (OPCW 2015, UN 2013). This paper examines the acceptable levels of sarin in three different types of soils, with a view to establishing acceptable clearance levels after an accidental or deliberate release of the agent.

Soil was chosen as the medium of study, as it is a living system in which biological, biochemical and physical-chemical processes occur. As such, it is an essential habitat that supports life on the surface of the Earth. Due to its extremely complex structure, this biological environment not only accumulates exogenous contaminants, but can contribute to their migration into the air and water reservoirs (Sadovnikova 2006, Pavlov 2005, Mineev and Rempe 1990, Shilov 2006, Isidorov 2001, Strelnikov 2004 and Korolev 2001). Moreover, toxic substances in the ground can interact with communities of microorganisms, which can lead to microbiome, or the suppression and appearance of new forms of pathogenic bacteria (Sadovnikova 2006, Egorenkov and Kochurov 2005, Revazova 2000, Serikoff 1992, 1994, Egorova and Belolipetskaya 2000). Plants growing on the contaminated substrate absorb and accumulate exogenous chemicals, and, when eaten, may cause negative effects on humans and animals (Sadovnikova 2006, Mineev and Rempe 1990, Protasov 2005, Hotuntsev 2004, Slodkopevtsev 2005, Green et al 1979, Melnikov et al., 1977).

Highly toxic substances represent a significant threat to the normal functioning of the ecosystem. As a result, the latter's self-purification ability and biological activity become violated

(Kruglov 1991, Mishustin et al. 1979, and Yudanov 1989). This problem is particularly relevant in relation to the implementation of the destruction of chemical weapons, performed in accordance with international conventions (Chemical weapons 1996, Agadzhanov 1993, and Guidelines 1982). In addition, in recent years the cases of threats of terrorist attacks using poisonous substances have become more frequent all over the world. These hazardous substances can be used not only against humans, but also for environmental deterioration and degradation. Negative environmental effects are also possible in the case of industrial accidents.

The purpose of this paper is to report on an experimental study to determine lowest-observed-adverse-effect (LOAEC) and no-observed-adverse-effect (NOAEC) concentrations of sarin in three different types of soil.

As suggested above, sarin is an extremely toxic substance (for respiratory exposure, the lethal concentration for 50% of the population, or LC_{50} , is $100 \text{ mg-min m}^{-3}$, and for dermal exposure, the lethal dose or LD_{50} is 1700 mg) (Medscape 2015). Sarin is tightly controlled by the CWC, which extends to both storage and, when needed, synthesis. For both these reasons, only a very limited number of tests were possible, so that the usual statistical rigour was not able to be exercised, e.g., a statistically significant number of replicates were not examined. That said, facilities handling CWAs must be licensed by national authorities and registered with the Organization for the Prohibition of Chemical Weapons, or OPCW, who administer the CWC and conduct on-site inspections (OPCW 2015). For all these reasons, the experimental work reported upon here has been challenging to get approved to conduct and then to proceed with the extremely limited amount of agent available. The results below, nonetheless, fill an important gap in knowledge that could prove invaluable in addressing cleanup following the inadvertent or illegally-planned release of this substance.

Work was performed under a contract with the International Science and Technology Center (ISTC, Moscow) in collaboration with the Royal Military College of Canada, with financial support from the Government of Canada (Department of Foreign Affairs, Trade and Development).

Materials and Methods

O-ethyl-S-(2-diisopropylaminoethyl) methyl phosphonothiolate (sarin) of 95% purity obtained from the Federal State Unitary Enterprise “State Research Institute of Organic Chemistry and Technology” (GosNIIOKhT) was used in the study. Pit sand collected from a depth of at least 3 m from the ground surface was used as the standard reference soil (SRS), due to its maximum filtration and minimal sorption abilities, neutral pH and low organic carbon content (Guidelines 1982 and Goncharuk and Sidorenko 1986).

In addition to this, types of soils were chosen in the Russian Federation which were also good models for soils of the prairies and mixed forests in Canada. Based on the physical-chemical characteristics, soil from the steppe zone of the Lower Volga (PSM) was chosen as the analogue for Canadian prairie soil, while forest soil from the Republic of Udmurtia (FSM) was chosen as the analogue Canadian forest soil. Soil characteristics are presented in Table 1 (Lobova and Khabarov 1983 and Kaurichev 1982).

Experimental justification of the safe levels of sarin in these soils was carried out in accordance with Russian Federation Guidelines (1982). To conduct experimental studies on the toxic effects of an exogenous substance on soil microflora, the processes of nitrification and phosphorus exchange, it is necessary to create a soil mixture (SM), with certain characteristics. Our SMs were created from the forest or steppe soils and sand in such a way that the total organic carbon measured by Turin’s method did not exceed 0.5%, and microbiome was as close as possible to the one observed in the rendzina of the deciduous and coniferous forests and prairie soils of Canada.

The necessary physical and chemical properties of soil models (Tables A1-A3) were measured before the experiments by the following methods. The diameter of the soil particles was measured by the Kaczynski-Fedulov technique with Knopp’s sieves (Goncharuk and Sidorenko 1986 and

Kaurichev 1990). Full moisture capacity was estimated by drying to a constant weight (Guidelines 1982, Goncharuk and Sidorenko 1986 and Kaurichev 1990). The organic carbon content was determined by Turin's method and was done spectrophotometrically with the oxidation of organic compounds in soil by potassium dichromate (Mineev 2001). The pH of the aqueous extracts of soil samples, redox potential, exchange acidity, hydrolytic acidity and hydrolytic alkalinity were measured potentiometrically (Guidelines 1982, Goncharuk and Sidorenko 1986, Kaurichev 1990 and Mineev 2001). Spectrophotometric analysis was used to measure soluble phosphates. Nitrogen in ammonium was determined spectrophotometrically by reaction with Nessler's reagent, and in nitrites and nitrates by reaction with α -naphthylamine/sulfanilic acid and sodium salicylate, respectively (Mineev 2001). Finally, the density of the solid phase of the soil was determined by Kaurichev's method (Kaurichev 1990).

Soil mixtures were also analyzed for the presence of the main background microflora such as saprophytic bacteria, E.Coli, fungi and actinomycetes. Concentrations of sarin used in different experiments are summarized in Table A4. Mixtures of the model soils and standard reference soil in fixed ratios were used to study the soils' self-cleaning ability and microbiome (Guidelines, 1982 and Goncharuk and Sidorenko 1986).

Extraction of sarin with water

The aim of the research was to determine the permissible level of sarin in the soils by measuring its concentration in the water passed through 1 meter of contaminated soil. Sarin concentration in such waters should not exceed $5.0 \times 10^{-5} \text{ mg L}^{-1}$ (Guidelines 1982 and Goncharuk and Sidorenko 1986). One-meter-high fiberglass columns with a cross sectional area of 100 cm^2 were used for the experiments. In accordance with the customary practice in Russia, each column was charged with an SRS of 60% humidity. Sarin solutions (with concentrations given in Table A4) were spread evenly in the upper dry layer (1 kg) of soil (SRS, FSM, PSM) and then the soil was washed with water. Three times a day, 32 mL of dechlorinated settled water was added to the

column. This amount was calculated based on the duration of the experiment (one month) and the mean annual precipitation in the geographic area of the corresponding type of soil (Guidelines 1982 and Goncharuk and Sidorenko 1986). Sampling of the seepage water was also carried out fractionally, three times a day, 30 min after column irrigation. Samples were combined at the end of the day, and the toxicant was extracted and its concentration was measured by gas-chromatography (Methodological guidelines 2004). Each experiment was performed in triplicate and average results were reported.

Migration of sarin into air

The ability of sarin to migrate into the air from SRS, FSM and PSM was studied by measuring its concentration above the soil surface in a 100 dm³ sealed chamber equipped with both a sampling device and an air ventilation rate adjuster. Following Russian Federation standards, the concentration of sarin in workspace air should not exceed 2.0×10^{-5} mg m⁻³. (The corresponding value from the Center for Disease Control/National Institute for Occupational Safety and Health (NIOSH) in the United States is 3.0×10^{-5} mg m⁻³ for workers over an 8-hour work shift. CDC 2015). Sarin was evenly distributed in the model soil by dissolving a given amount in an experimentally-established volume of distilled water (128 mL) needed to wet 1 kg of soil up to 60% of its maximum water-absorption capacity. The resulting mixture was stirred thoroughly and placed in a crystallization bowl (200×150 mm) in a sealed chamber with a constant temperature of 28 °C and humidity of 60-70% (which simulates summer conditions when sarin evaporates most readily from the soil surface). Air sampling was performed by passing air from the chamber at the rate 1 L/min for 15 min through an absorber filled with 5 mL of distilled water having a pH of 7.2 and porous plate #1. Sarin presence in the resulting solution was detected by reaction with propionylcholinesterase (State Research Institute 2005).

Sarin has the highest volatility among chemical warfare agents - 12.0 mg L⁻¹ (Methodological guidelines 2004). Therefore, air sampling was performed, on the first day of the

experiment, 1 h after soil contamination and then twice more every hour, and on the second day at 10 AM.

Each experiment was performed in triplicate and average results were reported.

Microbiological experiments

The ability of soils contaminated with sarin to self-purify and restore microbial activity was evaluated by counting the number of colonies of viable microorganisms involved in the recovery and purification of the soil and changes in the intensity of the biochemical processes (nitrification).

To study general health hazards, soil mixtures (SM) of FSM (33.3%) and PSM (20%) with SRS were used. The low content of organic matter in soil, e.g., the presence of soil carbon less than 0.5%, led to small amounts of soil microorganisms, minerals and enzymes, creating low sensitivity conditions for exposure to sarin.

To find microorganisms in the soil that have selective sensitivity to sarin, different groups of soil microbiome were studied. Saprophyte bacteria - the most numerous and active group of microflora, were studied on soil agar. Mushrooms (micromycetes), filamentous bacteria (actinomycetales), and *Escherichia coli*, characterizing the degree of contamination of the soil, were studied on the Chapek medium, starch-ammonia agar and Endo agar, respectively (Guidelines 1982, Goncharuk and Sidorenko 1986, Franke et al. 1967, Guidance 1976, Zvyagintsev 1980 and Babeva and Zenova 1989).

Microorganisms (bacteria, micromycetes and actinomycetes) from the soil mixtures, diluted from 10 to 10 000 times in sterile tap water, were seeded on Petri dishes by a dropwise method for bacteria and actinomycetes and by a spread plating technique for fungi (Guidance 1976, Zvyagintsev 1980 and Babeva and Zenova 1989). Microbiological sampling and seeding from soil mixtures were performed in triplicate on the 0-th, 1st, 3rd, 7th, 10th and 14th day from the moment of contamination (Guidelines 1982, Goncharuk and Sidorenko 1986 and Guidance 1976). Fungi,

actinomycetes and saprophytic bacteria were incubated in the dry-air oven at 24 – 26 °C; *E. coli* - at 37 °C (Guidance 1976, Zvyagintsev 1980 and Babeva and Zenova 1989).

Sarin toxic effects on the soil microbial community have been evaluated as an average total number of colonies of microorganisms. Significant results were defined as those differing more than 50 % from controls. The dynamics of biochemical processes were assessed by the content of ammonia, nitrite and nitrate ions (Guidelines 1982, Goncharuk and Sidorenko 1986, Lobova and Khabarov 1983, Kaurichev 1982, 1990 and Mineev 2001 and Babeva and Zenova 1989). The experiments were performed at room temperature (20 – 25 °C) and 60% humidity of PSM and FSM samples. Containers with samples studied were kept in the dark.

The following timeframe was used to perform soil sample analysis. On the day of contamination, control background samples were analyzed. On the 1, 3, 7, 10 and 14th days, contaminated and control soil samples were analyzed. Main indicators of the nitrification process were as described above. Significant results were defined as those that differ more than 25% from the control experiments within 14 days of observation.

For sarin's phytotoxicity study in the SRS, PSM and FSM soils, seeds from crops widely represented in the human diet, such as cereals (wheat), root vegetables (beets), legumes (beans) and vegetables (zucchini), were used (Guidelines 1982 and Goncharuk and Sidorenko 1986). All seeds of the test plants had certificates of quality.

Seed germination under the influence of the toxicant was studied by placing an equal number of seeds (10 pieces) on moist contaminated and uncontaminated soils (SRS, PSM, FSM) in Petri dishes for 3 days. Seeds were covered with filter paper on both sides. Germination was determined based on the appearance of sprouts in comparison to the controls.

The effect of the toxicant on plants' root growth was evaluated in the soils of interest (SRS, PSM, FSM) by placing 50 g of soil into a Petri dish with the addition of 0.1 mL of a Pryanishnikov's nutrition mixture (to improve the plants' living conditions) and brought to full moisture capacity by adding a water solution of sarin of a given concentration (Guidelines 1982 and

Goncharuk and Sidorenko 1986). The soil was then covered with a filter paper and 10 plant seeds were placed in each cup. Seeds were then covered with another piece of dry filter paper. After that, closed Petri dishes were placed in the incubator for 14 days at a temperature of 23 °C and a relative humidity of 50 - 70%. During the experiment, constant soil moisture was maintained in the Petri dishes.

Phytotoxic effects were determined by changes in the root growth of seeds germinated on the substrate. Root lengths were measured 3, 7, 10 and 14 days after toxicant addition to the soil. The overall appearance of the roots (color, shape, branching) was recorded as well.

The following criteria were considered while choosing plants for the vegetation experiments: low sensitivity to the toxicant's effects on seed germination; belonging to the cereal family, which allow determination of growth and development of the stem; possible depigmentation; and the green mass of the plants (haulm cut directly before measurement). Based on the results of preliminary experiments of the sarin toxic effects assessment on the germination of seeds, wheat met the above mentioned requirements and was chosen for these tests.

To determine threshold and no observed effect concentrations (NOECs) of sarin on the growth of the plants, three plant seeds were planted into $4.0 \times 4.0 \times 7.0$ cm containers containing 80.0 g of PSM or FSM soil. Then, the substance, dissolved in dechlorinated settled water, was introduced into the system. Controls were performed using settled dechlorinated water without sarin. Trays with containers were kept in a climatic chamber MLR-351H (Sanyo) at temperatures ranging from 17-28 °C and relative humidities of 65-80%, depending on the time of the day.

The toxic effects of the substance were determined by changes in the growth of the stems, possible depigmentation, and sprout development (deformation, branching). These parameters were registered on the 3rd, 7th, 10th and 14th days after soil contamination. The mass of the green part of the plant was determined after exposure. Results were considered meaningful if values were more than 20% different from the control value (Guidelines 1982 and Goncharuk and Sidorenko 1986).

Results and Discussion

Standard Reference Soil

During the study of sarin migration in soil with water it was shown that sarin's residence time in soil is proportional to its concentration. It was found that at the initial concentration of sarin, $4.0 \times 10^{-4} \text{ mg kg}^{-1}$, agent was leached from the soil in the period from the 5th to the 15th day of the experiment, with the maximum on the 10th day ($5.6 \times 10^{-5} \text{ mg L}^{-1}$). On the other hand, a lower initial sarin concentration ($3.0 \times 10^{-4} \text{ mg kg}^{-1}$) led to longer agent residence time in the soil. It was washed out from the 9th to the 11th day, with concentrations below the occupational exposure limit for drinking water. Based on the results above, the lowest significant dose for migration in water was $4 \times 10^{-4} \text{ mg kg}^{-1}$, while the permissible level not exceeding the occupational exposure limit was $3.0 \times 10^{-4} \text{ mg kg}^{-1}$.

Study of sarin migration in air has shown that agent evaporates from the surface within the first hour after contamination, independent of the initial concentration. The LOAEC of the agent was $5.0 \times 10^{-3} \text{ mg kg}^{-1}$. On the other hand, the concentration of sarin in air did not exceed the occupational exposure limit for air ($3 \times 10^{-5} \text{ mg m}^{-3}$ for the short term exposure limit) (OSHA 2015) when the initial contaminant concentration was $2.0 \times 10^{-3} \text{ mg kg}^{-1}$. Therefore this concentration was used as the NOAEC for standard reference soil.

It was found that sarin had a negative effect on the germination of all seeds tested at the concentration of 10 mg/kg. At the lower sarin concentration of 1 mg/kg, negative germination effect was observed for all seeds but zucchini. And, finally, at the lowest concentration of 0.1 mg/kg, only the germination of beet seeds was affected, which represents its sensitivity towards the studied organophosphorus compound.

Based on sensitivity toward sarin toxicity, plant seeds can be placed in the following order: beets > beans > wheat > zucchini. As zucchini seeds were not very sensitive to the sarin concentration in soil they were excluded from further study.

A study of sarin phytotoxic properties has shown its negative impact on higher plants. The minimal effective concentration of sarin on seed germination was 0.1 mg kg^{-1} . The impact of chemical agent on plant root systems was assessed and the LOAEC and NOAEC concentrations were found to be 2.5×10^{-2} and $1.0 \times 10^{-2} \text{ mg kg}^{-1}$ respectively (determined from triplicates). Based on all the above, the limiting hazard index (or lowest concentration for which effects were detected) for sarin was its migration in water, which was found to be $3.0 \times 10^{-4} \text{ mg kg}^{-1}$ (Table 2).

Prairie Soil Model

Our studies showed that sarin does not migrate with water in the prairie soil model, due to its poor water permeability. Therefore, the hazard index for migration in water was not defined and a subthreshold level was set as 0.1 mg kg^{-1} - the highest concentration of the agent used. The concentrations used in this determination are contained in Table A4, with all tests conducted in triplicate.

The study of sarin migration in air has shown that agent evaporates from the soil surface only within the first hour after contamination. The LOAEC of the agent was 0.1 mg kg^{-1} and maximum NOAEC was found to be $5.0 \times 10^{-2} \text{ mg kg}^{-1}$.

Analysis of the results of the general health hazard of contaminated soil showed that nitrifying bacteria were the most sensitive to negative sarin effects. LOAEC and NOAEC were 0.1 and $5.0 \times 10^{-2} \text{ mg kg}^{-1}$, respectively, for microbiome and, correspondingly, 5.0×10^{-3} and $2.0 \times 10^{-3} \text{ mg kg}^{-1}$ for nitrification. Therefore, the maximum permissible concentration of sarin in PSM, based on the general health hazard, was $2.0 \times 10^{-3} \text{ mg kg}^{-1}$.

It was shown that sarin introduced into PSM in the concentrations 2.0×10^{-3} , 1.0×10^{-2} and $5.0 \times 10^{-2} \text{ mg kg}^{-1}$ did not affect seed germination, using triplicates in each case. Therefore $5.0 \times 10^{-2} \text{ mg kg}^{-1}$ was chosen as the NOAEC.

At the same time, the toxicant showed a negative effect on the root systems of higher plants, significantly slowing growth and development. The 21% decrease of wheat roots growth was observed on the 3rd day of growth at the sarin concentration 0.05 mg/kg. Zucchini root growth was decreased by 27% on the 7th day of growth at 0.01 mg of sarin per kg of PSM soil. At concentrations below 0.002 mg/kg, no effects of sarin were observed on root system development of all plant seeds tested.

Plants can be distributed in the following order based on its root system development sensitivity towards sarin in PSM soil: wheat > zucchini > beets > beans. The LOAEC and NOAEC of the agent were found to be 1.0×10^{-2} mg kg⁻¹ and 2.0×10^{-3} mg kg⁻¹, respectively.

Vegetation experiments have shown that sarin had a negative influence on the growth of wheat stalks, but did not affect the green part mass (haulm cut directly before measurement). LOAEC and NOAEC values were 0.45 and 0.15 mg kg⁻¹, respectively.

Based on the most sensitive value above, i.e., the NOAEC for root systems, the exposure limit for sarin in the prairie soil model was found to be 2.0×10^{-3} mg kg⁻¹ (Table 3).

Forest Model Soil

Three different concentrations of sarin were used to study the migration of the agent with water in the forest model soil: 1.0×10^{-2} , 5.0×10^{-2} and 0.2 mg kg⁻¹, all examined in triplicate. Sarin in the leachate was found on the 4th day in a concentration below the exposure limit. That can be explained by the nature of the soil's relatively high pH (7.83) and high content of organic compounds (1.97% by Turin's method) and microflora. It is well known that these factors accelerate sarin degradation. Therefore a LOAEC was not determined, and 0.2 mg kg⁻¹, as the highest concentration of sarin, was used as the NOAEC value.

The study of sarin migration in air has shown that, as for other soil models, agent evaporates from the surface only within the first hour after contamination. The LOAEC and NOAEC of the agent were found to be 0.3 and 0.1 mg kg⁻¹, respectively.

It was found that sarin has a strong influence on the soil microbial community. For example, nitrification processes were more sensitive to sarin presence than was microbiome. The LOAEC and NOAEC were found to be 5.0×10^{-2} and 1.0×10^{-2} mg kg⁻¹, respectively for the microbiome, and 3.0×10^{-2} and 1.0×10^{-2} mg kg⁻¹, respectively, for nitrification processes. Therefore, 1.0×10^{-2} mg kg⁻¹ of sarin was chosen as the maximum allowable concentration for forest soil models.

Seed germination was studied at sarin concentrations of 2.0×10^{-2} , 5.0×10^{-2} , 0.1 and 0.3 mg kg⁻¹, in triplicates. It was found that the two highest concentrations of sarin influenced beet root germination. Sarin, at the three highest concentrations, also showed significant inhibition of beet and wheat roots at 0.3 mg kg⁻¹ on the 7th day of the experiment. LOAEC and NOAEC concentrations of sarin for this criterion were 5.0×10^{-2} and 2.0×10^{-2} mg kg⁻¹, respectively.

Vegetation experiments were performed with three different concentrations of sarin: 0.3, 0.9 and 2.7 mg kg⁻¹. It was found that the highest concentration inhibits wheat development in the period from the 3rd to 7th day. The subsequent measurement of the average mass of the green part of the wheat (haulm cut directly before measurement) did not show any significant difference from the controls. Based on that, the LOAEC and NOAEC were taken as 2.7 and 0.9 mg kg⁻¹, respectively.

Based on the lowest concentration above, the NOAEC of sarin on biome, the exposure limit for sarin in the forest soil model was found to be 1.0×10^{-2} mg kg⁻¹ (Table 4).

In summary, sarin has been found to be an ecotoxic hazard in different soil models. It is able to migrate from soil into the air and ground water and have a negative influence on the growth and development of higher plants, as well as the general microbial soil condition.

It should be noted that a research methodology was developed to determine the maximum allowable contamination of different types of soils by a highly toxic substance (sarin). For other types of soil, when a similar study cannot be conducted, the value 3×10^{-4} mg/kg (measured for the standard reference soil in the extreme laboratory conditions) may be used and corrected for the type of soil and climate conditions of interest.

Conclusions

It is concluded that: the introduction of sarin to different types of soils results in a significant adverse change in their ecosystem functions; the limiting indicators of sarin in soil are its migration in water for standard reference soil and the general health of soil constituents and plants for prairie and forest model soils, and that the maximum exposure limits of sarin in SRS, PSM and FSM are 3.0×10^{-4} , 2.0×10^{-3} and 1.0×10^{-2} mg kg⁻¹, respectively.

Future Work

Based on recent developments in Syria, it would be highly recommended that a similar study be performed on soil types typical to that part of the world.

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Table 1. Characteristics of the soil types

	<i>Canadian soils</i>	<i>Analogue Russian soils</i>
Soil location	Prairie	Steppe zone of the Lower Volga
Soil description	Brown calcareous, brown semidesert low gypseous. The soil profile is greyish-brown, powdery humus horizon.	Brown calcareous, brown steppe low gypseous. The soil profile is greyish-brown, powdery humus horizon.
pH	7.0 – 12.0	7.5 – 10.0
Humus content (%)	1.2 – 3.0	1.2 – 4.0
Soil location	Sod-calcareous soils of deciduous and mixed forests	Forest zone of the Republic of Udmurtia
Soil description	Light gray and gray forest's illuvium-humus-iron podzols with residual carbonate. The soil is loose, brownish-gray in color, unstructured or with weak thin-layer structure. Consists mainly of sandy-loam. Sandy and loamy soils are less common.	Sod-carbonate and sod-podzolic with residual carbonate soils of brown color of different intensity. Individual ashed lighter spots are visible under the sod and topsoil layer. Consists mainly of sandy-loam. Sandy and loamy soils are less common
pH	4.0 – 6.5	5.5 – 6.5
Humus content (%)	2.5 – 9.0	2.5 – 7.5

Table 2. Experimentally determined lowest-observed-adverse-effect (LOAEC) and no-observed-adverse-effect (NOAEC) concentrations of sarin in the standard reference soil (SRS)

Hazard index	LOAEC of sarin in SRS, mg kg⁻¹	NOAEC of sarin in SRS, mg kg⁻¹
Translocation:	2.5×10^{-2}	1.0×10^{-2}
Germination	0.1	-
Root length	2.5×10^{-2}	1.0×10^{-2}
Migration in air	5.0×10^{-3}	2.0×10^{-3}
Migration in water	5.0×10^{-4}	3.0×10^{-4}

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Table 3. Experimentally determined lowest-observed-adverse-effect (LOAEC) and no-observed-adverse-effect (NOAEC) concentrations of sarin in the prairie model soil (PMS)

Hazard index	LOAEC of sarin in SRS, mg kg ⁻¹	NOAEC of sarin in SRS, mg kg ⁻¹
General soil health:	5.0×10^{-3}	2.0×10^{-3}
Microbiome	0.1	5.0×10^{-2}
Nitrification	5.0×10^{-3}	2.0×10^{-3}
Translocation:	1.0×10^{-2}	2.0×10^{-3}
Germination	-	5.0×10^{-2}
Root length	1.0×10^{-2}	2.0×10^{-3}
Vegetation	0.45	0.15
Migration in water	-	0.1
Migration in air	0.1	5.0×10^{-2}

Table 4. Experimentally determined lowest-observed-adverse-effect (LOAEC) and no-observed-adverse-effect (NOAEC) concentrations of sarin in the forest model soil (FMS)

Hazard index	LOAEC of sarin in SRS, mg kg ⁻¹	NOAEC of sarin in SRS, mg kg ⁻¹
General health:	3.0×10^{-2}	1.0×10^{-2}
Microbiome	5.0×10^{-2}	1.0×10^{-2}
Nitrification	3.0×10^{-2}	1.0×10^{-2}
Translocation:	5.0×10^{-2}	2.0×10^{-2}
Germination	0.1	5.0×10^{-2}
Root length	5.0×10^{-2}	2.0×10^{-2}
Vegetation	2.7	0.9
Migration in water	-	0.2
Migration in air	0.3	0.1

Table A1. Physico-chemical properties of the Standard Reference Soil (SRS)

Composition	Sand
- Particles with diameter > 0.1 mm, %	95.0
- Particles with diameter < 0.1 mm, %	5.0
- Maximum particle diameter, mm	0.5
Moisture capacity, %	21.2
pH of the aqueous extract of soil samples	7.22
Organic Carbon, %	0.02
Ammoniacal Nitrogen, mg/kg	Not observed
Nitrite-Nitrogen (NO ₂ ⁻), mg/kg	Not observed
Nitrate-Nitrogen (NO ₃ ⁻), mg/kg	Not observed
Mobile Phosphorus, mg/kg	Not observed
Actinomycetes, ×1000 cells per 1g of soil	Not observed
Micromycetes, ×1000 cells per 1g of soil	Not observed
Saprophytic bacteria, ×1000 cells per 1 g of soil	Not observed
Escherichia Coli, ×1000 cells per 1 g of soil	Not observed

Table A2. Physico-chemical properties of the forest soil model (FSM) from the Republic of Udmurtia and its mixture with Standard Reference Soil (SRS).

	FSM	FSM : SRS (1:2)
Mechanical Composition:	Sand, Loam, Organics	Sand, Loam, Organics
Particles with diameter > 7 mm, %	19.70	Not observed
Particles with diameter > 4 mm, %	27.03	Not observed
Particles with diameter > 2 mm, %	32.10	12.50
Particles with diameter > 1 mm, %	9.73	7.40
Particles with diameter > 0.3 mm, %	7.53	27.80
Particles with diameter < 0.3 mm, %	2.30	15.80
Maximum particle diameter, mm	2.0	0.3
Moisture capacity, %	55.21	27.75
pH of the aqueous extract of soil samples	7.83	7.71
Organic Carbon, %	1.97	0.55
RedOx potential Eh, mV	334	295
Soil porosity, %	56	46
Dry soil density, g/cm ³	2.59	2.47
Hydrolytic acidity, Eq/100 g soil	Not observed	Not observed
Exchange acidity, Eq/100 g soil	0.056	0.024
Hydrolytic alkalinity, Eq/100 g soil	0.64	2.52
Ammoniacal Nitrogen, mg/kg	20.10	7.87
Nitrite-Nitrogen (NO ₂ ⁻), mg/kg	0.621	0.55
Nitrate-Nitrogen (NO ₃ ⁻), mg/kg	2.13	1.66
Mobile Phosphorus, mg/kg	33.82	17.14
Actinomycetes, ×1000 cells per 1g of soil	433.47	243.17

Micromycetes, ×1000 cells per 1g of soil	25.75	13.95
Saprophytic bacteria, ×1000 cells per 1 g of soil	1755.73	997.34
Escherichia Coli, ×1000 cells per 1 g of soil	57.15	23.41

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Table A3. Physico-chemical properties of the Canadian prairie soil model (PSM) from the Lower Volga region and its mixture with Standard Reference Soil (SRS).

	PSM	PSM : SRS (4:1)
Mechanical Composition:	Sand, Loam, Organics	Sand, Loam, Organics
Particles with diameter > 7 mm, %	9.3	Not observed
Particles with diameter > 4 mm, %	7.7	Not observed
Particles with diameter > 2 mm, %	15.9	7.2
Particles with diameter > 1 mm, %	10.1	19.8
Particles with diameter > 0.3 mm, %	32.1	52.3
Particles with diameter < 0.3 mm, %	24.0	20.7
Maximum particle diameter, mm	0.3	0.3
Moisture capacity, %	52.56	42.19
pH of the aqueous extract of soil samples	8.45	8.62
Organic Carbon, %	1.2	0.59
RedOx potential Eh, mV	288	261
Soil porosity, %	54	48
Dry soil density, g/cm ³	2.69	2.57
Hydrolytic acidity, Eq/100 g soil	Not observed	Not observed
Exchange acidity, Eq/100 g soil	0.06	0.059
Hydrolytic alkalinity, Eq/100 g soil	1.53	1.57
Ammoniacal Nitrogen, mg/kg	0.753	0.476
Nitrite-Nitrogen (NO ₂ ⁻), mg/kg	0.122	0.105
Nitrate-Nitrogen (NO ₃ ⁻), mg/kg	1.755	1.68
Mobile Phosphorus, mg/kg	28.3	24.24
Actinomycetes, ×1000 cells per 1g of soil	203.47	153.47

Micromycetes, ×1000 cells per 1g of soil	2.15	1.73
Saprophytic bacteria, ×1000 cells per 1 g of soil	975.73	875.73
Escherichia Coli, ×1000 cells per 1 g of soil	47.25	41.25

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Table A4. Concentrations of O-ethyl-S-(2-diisoproylaminoethyl) methyl phosphonothiolate (sarin) in mg/kg used for determination of LOAEC and NOAEC in soils.

Experiment	Soil Type		
	SRS	PSM	FSM
Extraction by water	4×10^{-4} ; 3×10^{-4} ; 2×10^{-4} 1×10^{-4} ; 0.5×10^{-4}	0.1; 0.05; 0.025	0.2; 0.05; 0.01
Migration to air	2×10^{-2} ; 5×10^{-3} ; 2×10^{-3}	0.1; 5×10^{-2} ; 5×10^{-3}	0.3; 0.1; 0.03
Microbiology		0.1; 5×10^{-2} ; 2.5×10^{-2}	0.3; 0.1; 0.05; 0.03; 0.01
Nitrification		0.1; 0.05; 0.01; 5×10^{-3} ; 2×10^{-3}	0.3; 0.1; 0.05; 0.03; 0.01
Germination	10; 1; 0.1	0.05; 0.01; 0.002	0.3; 0.1; 0.05
Root length	0.05; 0.025; 0.015	0.05; 0.01; 0.002	0.3; 0.1; 0.05; 0.02
Vegetation		0.45; 0.15; 0.05	2.7; 0.9; 0.3