Current Topics in Toxicology
Vol. 19, 2023

Original Article

# Analyzing the impact of chemical warfare agents on environment and human health through toxicology-based QSAR models

**Pantelis Alexandros Roupas, Georgios Nikolaou and Michail Chalaris***
Department of Chemistry, International Hellenic University, St. Loukas, Kavala GR-65404, Greece.

## ABSTRACT

The spread of Novichok nerve agents and their various chemical forms has presented the international community with considerable challenges in terms of threat assessment and mitigation techniques. This article gives an in-depth look at the use of quantitative structure-activity relationship (QSAR) models to deduce the structure-activity relationships of several Novichok derivatives. QSAR models have emerged as invaluable tools for predicting the toxicological characteristics and reactivity profiles of these strong chemical warfare agents by using a wealth of experimental data combined with improved computational methodologies. The paper also discusses the crucial importance of QSAR techniques in unraveling the complex interplay between chemical structure and biological activity, throwing light on critical molecular properties influencing the cholinergic system, neurotoxicity, toxicity, respiratory failure and skin permeation. QSAR models have shown exceptional prediction accuracy through rigorous validation and refinement, allowing the prioritizing of chemicals for targeted research and the invention of more effective countermeasures. Ultimately, this analysis emphasizes the critical importance of QSAR modeling in furthering our understanding of Novichok nerve agents and their many structural variants. The combination of computational methodologies and QSAR techniques has greatly improved our ability to predict toxicological features, unravel binding mechanisms, and prioritize chemicals for further study. The findings of these studies offer significant promise for strengthening global security measures against this growing class of chemical warfare agents.

**KEYWORDS:** nerve agents, Novichock, QSAR models, toxicity, skin permeation.

## 1. INTRODUCTION

Neurotoxic agents are a subcategory of chemical warfare agents that primarily affect the nervous system of living organisms, mainly humans. The agents being analyzed first appeared in 1936, in Germany, when Gerhard Schroeder and his research team were working on the production of more effective pesticides to enhance the production and protection of agricultural foodstuffs against weeds and insects. The result was that the team created an organophosphate compound that proved deadly when applied to insects. However, it was also found to have a significant effect on various mammals and most importantly humans. Schrader, after presenting his results to the Nazi government in Germany, was invited to continue his research on this new poison, which later became widely known as Tabun. In 1938, Schrader and colleagues followed up their research by creating sarin, an even more deadly organophosphate toxin. These warfare agents were later given the name 'G-factors' [1].

History seems to be repeating itself once again, in 1950s Britain, as in the effort to produce effective insecticides, Ranajit Ghosh discovered a new neurotoxic agent. After extensive research carried

*Corresponding author: mchalaris@chem.ihu.gr

out not only in the UK but also in America and Canada, it was found that this toxin was less volatile, did not deteriorate or wash off easily and was more resistant than the 'G factors'. The chemical in question was named VX and now belongs to the 'V agents' along with other compounds discovered in later years such as VR, VM and many more [1].

Novichoks, also known as Class A neurotoxic agents, are a class of chemicals used as weapons of war that cause convulsions and paralysis [2]. This particular class was created during the Cold War by Russia. However, the end of the war prevented the application of these chemicals on the battlefield. Thus, there is quite limited information about these compounds, and so the question of how to deal with them directly. Still, with global compliance with the 1997 Chemical Weapons Convention, which prohibits the large-scale use, development, production, stockpiling and transfer of chemical weapons and their precursors, any hope that existed for the study of Novichoks was lost [3].

The first public application of a Novichok formula was made on 4 March 2018, to Sergei Skripas, a former Russian spy, and Yulia Skripas, his daughter. The passing incident happened in the UK and after being found unconscious they were taken to hospital for treatment. A month later scientists from the Organisation for the Prohibition of Chemical Weapons announced the existence of Novichok in the victims' bodies and the place where they were found [3].

And then again on August 20, 2020, prominent Russian political dissident Alexei Navalny was on a flight bound to Moscow when he was suddenly wracked with severe pain and was unable to breathe. The pilots conducted an emergency landing in the nearby Siberian city of Omsk where the previously healthy 44-year-old was quickly hospitalized. After extensive analysis, no toxic substances were traceable in his system, and it was thought he suffered from a severe bout of hypoglycemia. Only when Navalny was brought back to Germany and his blood and urine samples were submitted to the Bundeswehr Institute of Pharmacology and Toxicology, that trace amounts of the Novichok nerve agent were found in his system [4].

## 2. MATERIALS AND METHODS

Due to the abundance of the discovered nerve agents, it is impractical to study all of them within a single paper. Therefore, nine specific chemical warfare agents were selected. The chosen nerve agents are shown in Table 1:

As mentioned, Novichoks have not been widely used, which makes the information available on these compounds quite limited. Thus, in the event of a new chemical war, there will be no immediate ways to address the application of the compounds under consideration. Still, the laboratory-level reproduction of Novichok is now an illegal act under the 1997 Convention, making further study of them significantly more difficult.

Therefore, in order to study Novichok, computational models (quantitative structure-activity relationship (QSAR)) are used. QSAR models can be defined as mathematical models capable of predicting physicochemical, biological, and environmental properties of compounds, using their chemical structures as a basis [5].

From the most accredited databases with QSAR models the following databases were used for this research:

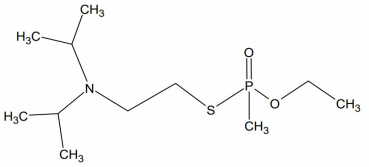
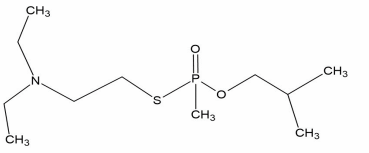
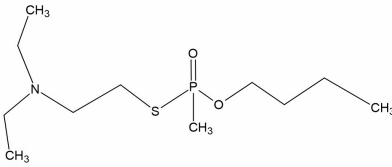
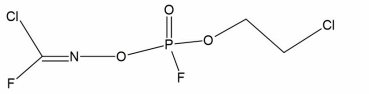
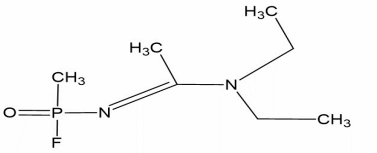
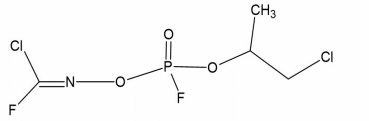
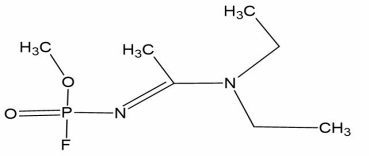
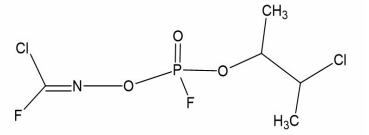
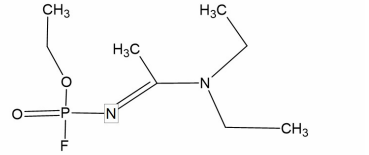
- PASS (prediction of activity spectra for biologically active substances)
- FDSP calculator (Finite dose skin permeation calculator)
- T.E.S.T (Toxicity estimation software tool)

### 2.1. Prediction of activity spectra for biologically active substances (PASS)

The PASS program is capable of calculating the properties of biologically active substances. In particular, it allows the calculation of more than 300 pharmacological properties and biological mechanisms based on the structure of the substance. The results of the calculators are given in the form of a table in which the activities of the properties studied (Activity) are assigned, as well as certain values indicating whether a compound is active (Pa) with respect to the property in question or inactive (Pi) [6].

- If Pa>0.7 the compound is likely to show the same activity at the experimental level, but there is a possibility that the compound is analogous to a known pharmaceutical substance.

**Table 1.** The substances to be examined and their chemical structures.

| Name | Chemical Structure |
|---|---|
| **VX (American)** |  |
| **VX (Russian)** |  |
| **VX (Chinese)** |  |
| **A-230 (American)** |  |
| **A-230 (Russian)** |  |
| **A-232 (American)** |  |
| **A-232 (Russian)** |  |
| **A-234 (American)** |  |
| **A-234 (Russian)** |  |

- If 0.5<Pa<0.7 the compound is likely to show the same activity at the experimental level, but with a lower probability, and there is no possibility that the compound is analogous to a known drug substance.
- If Pa<0.5 the compound is not likely to show the same activity at the experimental level. However, if this activity is observed at the experimental stage, then the substance in question may be a new chemical compound.

In the PASS platform a total of 4 activities for Novichok compounds was analyzed:

- Cholinergic
- Toxicity
- Neurotoxicity
- Respiratory failure

**2.1.1. Cholinergic**

In the parasympathetic system there appear to be certain sensory nerves known as cholinergic receptors. The term cholinergic is due to the existence of the neurotransmitter acetylcholine, which is the main neurotransmitter of the parasympathetic system. There are two classes of cholinergic neurotransmitters, categorized according to the compound that stimulates them in each case. These compounds may be nicotine and muscarinic [7, 8].

In the neurotransmitters that make up the cholinergic system, it is possible to influence their activity by various substances that stimulate, increase, or mimic the neurotransmitter acetylcholine. The phenomenon under consideration is termed cholinergic toxicity. The substances which have been found to be primarily responsible for cholinergic toxicity belong to the class of organophosphate and carbamate compounds.

Cholinergic toxicity can show certain symptoms in the human body depending on the type of acetylcholine neurotransmitter affected in each case. An excess of acetylcholine in a muscarinic neurotransmitter leads to increased secretions (sweat, tears, saliva, stomach fluids), constriction in the bronchi of the lungs, a decrease in heart rate and cramps in the abdominal region. In nicotine neurotransmitters similarly, excess acetylcholine can cause muscle spasms or even paralysis due to excessive stimulation of the nerves that carry messages to the muscles [9, 10].

**2.1.2. Toxicity**

Toxicity is the ability of a substance to cause harmful health effects to a single cell, a group of cells, an organ system, or even the entire body. All chemicals are able to cause harm to a certain degree, but only when a small amount can be harmful to a living organism, the chemical is considered toxic. On the contrary if only a large amount of the chemical can cause damage, the chemical is considered to be relatively non-toxic.

The toxicity of a substance can be narrowed down to three key factors:

- The chemical structure.
- The extent to which the substance is absorbed by the body.
- The ability of the organism's body to detoxify the substance and eliminate it from the body.

However, for comparing the toxicity between two compounds, the only necessary factor is their structure. The reason being that the amount of a substance that an organism can absorb or detoxify can differ not only from different species but among organisms of the same species [11].

**2.1.3. Neurotoxicity**

Neurotoxicity refers to the capability of inducing adverse effects in the central nervous system, peripheral nerves or sensory organs. A chemical is considered to be neurotoxic if it is capable of inducing a consistent pattern of neural dysfunction or change in the chemistry or structure of the nervous system [12].

Neurotoxicity can be observed at any time in the life cycle, from gestation through senescence, and its manifestations and its symptoms can vary with age. The nervous system seems to be particularly vulnerable to damage while it is still developing, but the results of some early injuries may become evident only as the nervous system matures and ages [13]. The grading of the severity of neurotoxic effects is depicted in Table 2.

**2.1.4. Respiratory failure**

The respiratory system is responsible for providing oxygen to and removing carbon dioxide from the body; however, the inability to perform either or both of these task's results in respiratory failure [14].

**Table 2.** Table depicting the grading of the severity of the neurotoxic effects (modified from [12]).

| Level | Grouping | Explanation/Examples |
|---|---|---|
| 6 | Morphological changes | Cell death and axonopathy as well as subcellular morphological changes. |
| 5 | Neurological changes | Abnormal findings in neurological examinations of single individuals. |
| 4 | Physiological/behavioural changes | Changes in evoked potentials and EEG, or changes in psychological and behavioural tests. |
| 3 | Biochemical changes | Changes in relevant biochemical parameters (e.g., transmitter level, GFA-protein content or enzyme activities). |
| 2 | Irreversible, subjective symptoms | There is no evidence of abnormality on neurological, psychological or other medical examination. |
| 1 | Reversible, subjective symptoms | There is no evidence of abnormality on neurological, psychological, or other medical examination. |

Novichoks, akin to other organophosphate compounds, upon ingress into the human organism induce the phosphorylation of serine hydroxyl residues on the acetylcholine esterase enzyme. This enzymatic modification leads to the accumulation of acetylcholine, a neurotransmitter pivotal in cholinergic signaling pathways. The elevated levels of acetylcholine lead to dysregulation within the cholinergic system, resulting in both central and peripheral clinical presentations. The perturbation of the cholinergic system can give rise to deleterious physiological effects, necessitating a nuanced understanding of the intricate mechanisms underlying Novichok-induced biochemical disruptions.

One if not the most devastating cholinergic feature of organophosphate poisoning is respiratory failure, which upon further investigation has been attributed to central mechanisms. To be more specific, the respiratory center known as the pre-Botzinger complex situated in the ventrolateral medulla is composed of glutaminergic and muscarinic fibers. Thus, the existence of excess acetylcholine can depress respiratory activity in these areas, leading to respiratory failure [14].

### 2.2. Toxicity estimation software tool (T.E.S.T)

To measure the toxicity of the test substances, the T.E.S.T. software was used, expressing the $LD_{50}$ values. Specifically, the $LD_{50}$ was calculated based on the oral administration of each substance to mice and then, based on the ratio of body surface area between the mouse and the human, the $LD_{50}$ was converted to allow the toxicity to be expressed in terms of human toxicity [15].

The T.E.S.T. program was created to facilitate the approach of quantitative structure-activity relationships (QSARs) for the evaluation of substance toxicity. The calculation of toxicity with this QSAR model is done in three ways, either by plotting it in a chemical planner window, by typing it into a structure text file or by importing it from a structure database [16].

The $LD_{50}$ is the amount of the substance administered to a set of test animals to kill half of the population. The most common animal used to measure the $LD_{50}$ is the mouse. The unit of measurement used varies depending on the size of the animal in which each toxic substance is studied. For the most part, for the rats, it is expressed as the amount of the chemical (in milligrams) per kilogram of the test animal's body weight (mg/kg) [17].

### 2.3. Finite dose skin permeation (FDSP) calculator

The finite dose skin permeation calculator enables the calculation of the skin permeation coefficient (Kp). To determine the steady-state permeation

from an aqueous solution of unlimited volume, several models have been developed. These, however, do not fit the model of a normal workplace exposure situation. However, with a dose of any size administered to skin that is partially or fully hydrated, this program determines fluxes, concentrations in the skin, and amounts absorbed [18]. More specifically, this software calculates the skin penetration coefficient and absorption of chemicals in relation to evaporation after a dose of the test substance is applied to the skin [19].

The skin permeability coefficient (Kp) predicts the chemical skin penetration. Most mathematical models have been developed using Kp data, but of course, conflicting values of Kp have been observed, which raises concerns about the overall reliability of these measurements. The units of measurement of Kp are (cm $h^{-1}$) [20].

In order for the finite dose skin permeation to work and give the desired result, it is necessary to enter the following parameters: the chemical name and chemical type of the substance to be tested, $LogK_{ow}$, melting and boiling point, molecular weight, vapor pressure, permeability and whether the compound contains double or triple bonds and whether it has a ring.

The hydrophilic/lipophilic property of a compound is determined by the octanol-water partition coefficient ($K_{OW}$). It was first considered in the discovery and design of drugs and pesticides, but today it is a critical aspect of any chemical, as it greatly influences the way a chemical behaves both within a living body and in the environment. The decimal logarithm of $K_{OW}$ ($logK_{OW}$, also known as logP) is often referred to instead of $K_{OW}$ itself with the range of $logK_{OW}$ values ranging from 3 (very hydrophilic) to +10 (very hydrophobic) [21].

## 3. RESULTS

The analysis of the chemical warfare agents' impact is multifaceted, encompassing their cholinergic, toxic, neurotoxic, and respiratory failure activities, as assessed using the PASS platform. This robust program offers insights into the pharmacological properties and biological mechanisms of these agents by evaluating over 300 properties based on their structural attributes. The results from PASS are presented in a tabular format, classifying the activity levels of the properties as active (Pa) or inactive (Pi).

For the cholinergic system, which is integral to the parasympathetic nervous system and involves acetylcholine neurotransmitters, we investigated how different compounds affect its activity. Cholinergic toxicity can lead to diverse symptoms, depending on which type of acetylcholine neurotransmitter is influenced. In this context, organophosphate and carbamate compounds play a pivotal role. The following information (Table 3) provides the PASS results for cholinergic activity.

Neurotoxicity, encompassing adverse effects on the nervous system, can manifest across the lifespan. Different manifestations may occur as the nervous system matures, and these effects can be graded based on their severity (see Table 4 for PASS results for neurotoxic activity).

**Table 3.** PASS results for the cholinergic activity.

| Compound | Pa | Pi | Activity |
|---|---|---|---|
| **VX American** | 0.108 | 0.097 | Cholinergic |
| **VX Russian** | 0.866 | 0.003 | Cholinergic |
| **VX Chinese** | 0.816 | 0.003 | Cholinergic |
| **A-230 American** | 0.113 | 0.088 | Cholinergic |
| **A-230 Russian** | 0.205 | 0.023 | Cholinergic |
| **A-232 American** | 0.147 | 0.052 | Cholinergic |
| **A-232 Russian** | 0.198 | 0.025 | Cholinergic |
| **A-234 American** | 0.129 | 0.07 | Cholinergic |
| **A-234 Russian** | 0.2 | 0.025 | Cholinergic |

**Table 4.** PASS results for the neurotoxic activity.

| Compound | Pa | Pi | Activity |
|---|---|---|---|
| **VX American** | 0.975 | 0.002 | Neurotoxic |
| **VX Russian** | 0.978 | 0.002 | Neurotoxic |
| **VX Chinese** | 0.98 | 0.002 | Neurotoxic |
| **A-230 American** | 0.847 | 0.012 | Neurotoxic |
| **A-230 Russian** | 0.662 | 0.034 | Neurotoxic |
| **A-232 American** | 0.898 | 0.005 | Neurotoxic |
| **A-232 Russian** | 0.64 | 0.37 | Neurotoxic |
| **A-234 American** | 0.932 | 0.004 | Neurotoxic |
| **A-234 Russian** | 0.695 | 0.03 | Neurotoxic |

**Table 5.** PASS results for the toxic activity.

| Compound | Pa | Pi | Activity |
|---|---|---|---|
| **VX American** | 0.898 | 0.012 | Toxic |
| **VX Russian** | 0.925 | 0.008 | Toxic |
| **VX Chinese** | 0.927 | 0.008 | Toxic |
| **A-230 American** | 0.872 | 0.015 | Toxic |
| **A-230 Russian** | 0.566 | 0.067 | Toxic |
| **A-232 American** | 0.895 | 0.012 | Toxic |
| **A-232 Russian** | 0.558 | 0.069 | Toxic |
| **A-234 American** | 0.899 | 0.012 | Toxic |
| **A-234 Russian** | 0.619 | 0.058 | Toxic |

The toxicity evaluation (Table 5) explores the potential harmful health effects of these agents, underscoring their capacity to cause harm at various levels of biological organization. The assessment considers factors like chemical structure, absorption, and the organism's ability to detoxify the substance.

Lastly, we examined respiratory failure (Table 6), a critical aspect of organophosphate poisoning. Understanding the mechanisms behind respiratory failure and the impact on central and peripheral cholinergic features is vital.

### 3.1. Toxicity

As previously mentioned, the toxicity was first calculated when the test substance was administered to mice and then the value obtained

**Table 6.** PASS results for the respiratory failure activity.

| Compound | Pa | Pi | Activity |
|---|---|---|---|
| **VX American** | 0.961 | 0.004 | Respiratory Failure |
| **VX Russian** | 0.991 | 0.002 | Respiratory Failure |
| **VX Chinese** | 0.988 | 0.002 | Respiratory Failure |
| **A-230 American** | 0.824 | 0.014 | Respiratory Failure |
| **A-230 Russian** | 0.663 | 0.034 | Respiratory Failure |
| **A-232 American** | 0.921 | 0.006 | Respiratory Failure |
| **A-232 Russian** | 0.64 | 0.38 | Respiratory Failure |
| **A-234 American** | 0.762 | 0.021 | Respiratory Failure |
| **A-234 Russian** | 0.705 | 0.028 | Respiratory Failure |

**Table 7.** $LD_{50}$ values, calculated from T.E.S.T. administered to rats and their conversion for humans.

| Compound | Oral rat $LD_{50}$ mg/kg | $LD_{50}$ oral human mg/kg |
|---|---|---|
| **VX (American)** | 1.95 | 0.31 |
| **VX (Russian)** | 1.08 | 0.17 |
| **VX (Chinese)** | 1.23 | 0.2 |
| **A-230 (American)** | 99.17 | 15.99 |
| **A-230 (Russian)** | 7.65 | 1.23 |
| **A-232 (American)** | 26.37 | 4.25 |
| **A-232 (Russian)** | 7.57 | 1.22 |
| **A-234 (American)** | 578.01 | 93.23 |
| **A-234 (Russian)** | 3.2 | 0.51 |

was divided by 6.2 to express the $LD_{50}$ result for humans [22].

Table 7 shows the $LD_{50}$ values of each substance tested for both rats and humans. It is initially observed that the Russian and Chinese VX have almost similar $LD_{50}$ values, but they are slightly lower, by about 0.11-0.14 mg/kg compared to the result given for the American VX. Thus, it is observed that these two substances have approximately the same toxic effect, with American VX being slightly more toxic. As for the Novichoks whose structures have been given by the Russians, with its values being higher than those of VX, it was found that the $LD_{50}$ values of A-230 and A-232 are almost identical, while the $LD_{50}$ value of A-234 is lower by about half compared to the values of A-230 and A-232. However, the $LD_{50s}$ of the A agents of Americans can be found to have the highest values with A-234 holding the most extreme value.

### 3.2. Skin permeability

Skin permeability is important to study for these warfare agents because, like many other nerve agents, they have a low vapor pressure such that the delivery of the substance to living organisms is by absorption through the skin [21].

First, for all the compounds studied, the amount used to obtain the results was 1 mg/cm$^2$. From the Table 8 it can be observed that A-230, A-232 and A-234 of the Russians show, with a significant difference, the lowest values in Kp and as a result they have the lowest dermal absorbency compared to the other nerve agents. On the other hand, the corresponding compounds given by the Americans show the highest values with A-230 having a slightly higher value than the American VX while A-232 and A-234 hold double and triple values, respectively than A-230.

## DISCUSSION

### 4.1. Prediction of activity spectra for biologically active substances (PASS) (Cholinergic)

#### 4.1.1. VX

In the case of the VX nerve agents we can clearly see that the Russian VX has the strongest activity (0.866), followed by the Chinese VX with its activity being slightly lower than that of the Russian VX (0.816), meaning they are highly active compounds when they enter the cholinergic system. As for the inactivity of the said compounds, which is 0.003 for both, it is dramatically low, meaning those forms of VX will almost never be inactive when they react with the cholinergic system.

On the other hand, the American VX seems to have a completely different value for the cholinergic activity (0.108) and a value for inactivity that's quite close to it (0.097). With those numbers in mind, the American VX seems to have little to no influence on the cholinergic system relative to the other VX agents. The values of this VX seem a bit peculiar since it's an organophosphate chemical compound related to the other VX agents.

**Table 8.** The skin absorption coefficient Kp and the octanol-water partition coefficient $logK_{OW}$ calculated by the finite dose skin permeation calculator.

| Compound | Effective kp [cm/h] | $LogK_{ow}$ |
|---|---|---|
| **VX (American)** | 0.0003871 | 2.12 |
| **VX (Russian)** | 0.0001036 | 1.2 |
| **VX (Chinese)** | 0.0002558 | 1.9 |
| **A-230 (American)** | 0.0005761 | 2.14 |
| **A-230 (Russian)** | 0.00003345 | 0.2 |
| **A-232 (American)** | 0.001009 | 2.55 |
| **A-232 (Russian)** | 0.00002449 | 0.18 |
| **A-234 (American)** | 0.001809 | 2.97 |
| **A-234 (Russian)** | 0.0000486 | 0.67 |

Realistically, the difference between the values of American VX and the others should have been similar to that of the Russian and the Chinese VX.

#### 4.1.2. A-230

Between the two A-230 agents the Russian undoubtedly is the most effective when it comes to the cholinergic system. The activity value of the Russian agent (0.205) is almost double that of the American one (0.113), resulting in it being twice as effective. At the same time the inactivity of the Russian (0.023) agent is almost four times lower that of the American one (0.088), evidently making the American A-230 four times more inactive than the Russian. In comparison though with the VX agents, the A-230 agents are less effective, except for the American VX, as seen by the activity and inactivity values.

#### 4.1.3. A-232

Likewise, from the A-232 agents, the Russian compound seems to have an activity (0.198) bigger than that of the American (0.147). Additionally, the inactivity of the Russian compound (0.025) is half that of the American (0.052), resulting again in the Russian agent being the most effective of the two.

#### 4.1.4. A-234

Finally, A-234 are like the A-230 and A-232 agents. The activity of the Russian agent (2.0) is again bigger than that of the American (1.29) and the inactivity of the Russian is almost three times smaller than the activity of the American agent.

### 4.2. Neurotoxicity

#### 4.2.1. VX

By studying the results for the neurotoxic activity, it is quite clear that all three agents have close activity values, with the Chinese having the largest (0.98), followed by the Russian (0.978) and then the American (0.975). The values being so close to each other, in combination with the same value of inactivity for all the agents (0.002), brings us to the conclusion that these compounds result in almost the same amount of neurotoxicity when they enter a living organism.

#### 4.2.2. A-230

In the case of the A-230 agents, we can see results that are opposite of those of cholinergic. The activity of the American agent (0.847) is stronger than that of the Russian agent (0.662). At the same time the inactivity of the American agent (0.012) is almost three times lower that of the Russian A-230 agent (0.034). That being set the American A-230 is a far more effective agent when it comes to neurotoxicity, than the Russian A-230.

#### 4.2.3. A-232

The same can be said for the A-232 agents. That is, the activity of the American agent (0.898) seems again to be stronger than that of the Russian agent (0.64). And looking at the inactivity, the American agent (0.005) seems to have a lower value than that of the Russian (0.37), instantly making it a superior agent when it comes to neurotoxicity.

#### 4.2.4. A-234

At last, we can observe that the A-234 agents do not differ from the previous ones since again the American agent has a stronger activity (0.932) when it comes to neurotoxicity and a lower inactivity (0.004) value than that of the Russian one (0.695-0.03).

### 4.3. Toxicity

#### 4.3.1. VX

By studying once again the results from the PASS program we can see that in terms of toxicity, the Chinese (0.927) and the Russian (0.925) VX agents have almost the same values of activity and exact same values of inactivity (0.008). These agents do seem to cause the same amount of toxicity (the Chinese being slightly stronger because of the value of its activity). Now when it comes to the American VX, it has an activity value (0.898) that's lower than the other VX agents and an inactivity value that's 50% higher than that of the other VX agents (0.012). Those values make it slightly worse version of the Chinese and Russian agents when it comes to their toxicity.

#### 4.3.2. A-230

The values of the American agent instantly make it a more toxic compound, since its activity (0.872) is higher than that of the Russian agent (0.566) and has an inactivity that's almost four times smaller than it (0.015-0.064).

#### 4.3.3. A-232

The same can be said for the A-232 agents. Being more specific, the activity values of the American agent (0.895) once again are higher of those of the Russian agent (0.558). Additionally, the inactivity values for the American compound (0.012) are lower that the those of the Russian agent (0.069). Having said that, the American form of A-230 evidently is more toxic than the Russian one.

#### 4.3.4. A-234

As for A-234 agents, they follow the same pattern as the A-230 and A-232 agents. To be more specific the Russian agent has greater activity (0.899) relative to the American one (0.619) and its inactivity (0.012) is lower to that of the American agent (0.058), making once more the Russian agent more toxic than the American.

### 4.4. Respiratory failure

#### 4.4.1. VX

By examining for one last time the results from the PASS program it is clear that the Chinese (0.988) and the Russian (0.991) VX agents have almost the same values of activity and at the same time the exact same values of inactivity (0.002). These agents do seem to cause the same amount of respiratory failure (the Russian being slightly stronger thanks to the value of its activity). Now when it comes to the American VX, it has an activity value (0.961) that's lower than the other VX agents and an inactivity value that's two times higher than that of the other VX agents (0.004). The values consequently make it slightly worse version of the Chinese and Russian agents when it comes to respiratory failure.

#### 4.4.2. A-230

The values of the American agent without a question make it more effective in causing respiratory failure, since its activity (0.824) is higher than that of the Russian agent (0.663). Additionally, the inactivity of the American (0.014) agent is almost three times smaller than that of the Russian (0.034).

#### 4.4.3. A-232

The same can be observed in the case of the A-232 agents. In detail, the activity values of the American agent (0.921) are higher of those of the Russian agent (0.64). In addition, the inactivity values for the American compound (0.006) a found to be lower than those of the Russian agent (0.038). With that being said, the American form of A-230 evidently is able to cause respiratory failure way more efficiently than the Russian one.

#### 4.4.4. A-234

Lastly the A-234 agents are a bit different than the A-230 and A-232 agents. That is the Russian agent has activity (0.762) that's a bit higher than the American one (0.705) and its inactivity (0.021) is slightly lower than that of the American agent (0.028), making once more the Russian agent more effective in causing respiratory failure than the American. But in the case of the A-234 agents the activity and inactivity values seem to be closer to each other, making the effectiveness of the two compounds quite similar.

Conclusively, after examining every value calculated from the PASS program, we can clearly see that from all the compounds the VX agents have the highest values for every type of activity, making them undeniably the most effective. In terms of which agent is the most effective we can clearly see that the American compound always had the smallest activity compared to the other two and the highest values of inactivity (except the times the values of all substances were equal). By comparing the Chinese and the Russian agents, we can see that their values most of the time are close to each other, with none having always the highest value. This makes it impossible to choose which is the strongest. The only value that doesn't seem relative to the values of the VX agents is that of the American VX as stated before.

When it comes to A-230, A-232, A-234, when comparing the compounds of Russia and America separately we can observe that their values of activity and inactivity are quite close to each other, making it difficult to choose which is the best. Although, it must be said that depending on the activity the values of the American compounds are higher than the Russian ones and vice versa. In detail, for the activities of cholinergic the American compound have higher values of inactivity and lower values of inactivity. The same can be said for the Russian compounds for the toxicity, neurotoxicity and respiratory failure activities, which is quite surprising since the cause

of respiratory failure in the first place is cholinergic toxicity. That being the case, we would expect the values of the respiratory failure and cholinergic to be comparable to each other.

Overall, the VX agents seem to have the required activity values that certify the effectiveness of their use, according to the PASS program. On the other hand, the A-230, A-232, A-234 agents exhibit smaller values than those of the VX agents and depending on activity the American and Russian compounds can have big differences in values, that unavoidably will affect the choice of a compound at a certain situation.

### 4.5. Toxicity estimation software tool (T.E.S.T)

As for the three VXs studied, it was observed that the American VX has the highest LD50 value, followed by the Chinese VX and last is the Russian VX. Thus the most toxic VX is the American one and the least toxic VX is the Russian, with its toxicity more closely converging with that of the Chinese VX.

Comparing the results for Novichok's, is evident that those of the Americans have considerably higher toxicity than the Russian ones while the values of Russian A-230, A-232 and A-234 are relatively higher than those of VX; the same is not true for the American ones.

### 4.6. Finite dose skin permeation calculator

Using the finite dose skin permeation calculator software program it was observed that the key factor in obtaining the Kp result was logKow. For this reason, the values of logKow were also placed in the table to have a more direct comparison. The The connection between Kp and logKow is shown by the lowest values of both Kp and logKow for A-232 (Russian), while the A-234 of the Americans has the highest value in both cases. It is further identified that for the three VX types and for the Americans' A-230 which have almost similar logKow, the Kp values obtained are also similar. The same conclusion can be drawn by comparing the Kp and logKow values for the A-230 and A-232 of Russians, where the difference in logKow of these neural factors is 0.02 and the difference they have in Kp appears in the fifth decimal place. Thus it is found that the main factor affecting the permeability in the skin is the octanol-water partition coefficients, i.e. as this coefficient increases, the Kp will also increase accordingly.

## 5. CONCLUSION

In conclusion, the analysis of various chemical warfare agents, including VX, A-230, A-232, and A-234, using toxicology-based QSAR models, provides crucial insights into their potential impacts on the cholinergic and respiratory systems and the possible toxicity and neurotoxicity of certain compounds.

For the VX agents, it is evident that Russian VX exhibits the highest cholinergic activity, closely followed by Chinese VX. In contrast, American VX demonstrates significantly lower cholinergic activity, indicating a reduced influence on the cholinergic system. These results diverge from what might be expected, given their common organophosphate nature.

In the case of neurotoxicity, all three VX agents exhibit similar activity, signifying nearly identical neurotoxic effects when introduced into living organisms.

For the A-230, A-232, and A-234 agents, the Russian counterparts consistently demonstrate higher activity and lower inactivity than their American counterparts across all examined toxicity types, making them more effective in terms of toxicity.

Analyzing the T.E.S.T results, the American VX is found to be the most toxic, followed by the Chinese VX, with the Russian VX being the least toxic. Novichok agents of American origin also show significantly higher toxicity compared to their Russian counterparts.

Finally, using the finite dose skin permeation calculator, we observe that logKow values have a direct impact on skin permeability (Kp). The A-234 American agent exhibits the highest Kp due to its logKow value, while the A-232 Russian agent has the lowest Kp, corresponding to its logKow value. Furthermore, similar logKow values lead to similar Kp values, emphasizing the role of octanol-water partition coefficients in skin permeability.

This comprehensive analysis underscores the varying levels of activity and toxicity among different chemical warfare agents and highlights

the potential impact on both the cholinergic and neurotoxic systems. Understanding these differences is crucial in determining the most effective compounds for specific situations. Additionally, skin permeability assessments emphasize the importance of logKow values in predicting permeation behavior.

## ACKNOWLEDGEMENT

CPU time of GRID Computing Center, which is located at the International Hellenic University (IHU), Kavala Campus (Greece), is gratefully acknowledged. This research has received funding from the European Union's Erasmus+ Programme: ERASMUS-EDU-2022-CB-VET call under grant agreement No 101092458 with the acronym GROWTH.

## CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare regarding the publication of this paper.

## REFERENCES

1. Aroniadou-Anderjaska, V., Apland, J. P., Figueiredo, T. H., Furtado, M. D. A. and Braga, M. F. 2020, Neuropharmacology., 181, 108298.
2. Noga, M., Michalska, A. and Jurowski, K. 2023, Arch Toxicol., 97, 1692.
3. Chai, P. R., Hayes, B. D., Erickson, T. B. and Boyer, E. W. 2018, Toxicol Commun., 2(1), 45-46.
4. Platteborze, P. L. 2021, therapeutic drug management and toxicology division news 1-4.
5. QSAR models, European Chemicals Agency. https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/qsar-models
6. Lagunin, A., Stepanchikova, A., Filimonov, D. and Poroikov, V. 2000, Bioinformatics, Moscow, Pogodinskaya, Russia.
7. Tiwari, P., Dwivedi, S., Singh, M. P., Mishra, R. And Chandy, A. 2013, Asian Pacific Journal of Tropical Disease, 3(5), 413–420.
8. The editors of Encyclopedia Britannica, 2023, Acetylcholine chemical compound, Britannica.
9. Lott, E. L. and Jones, E. B. 2022, Cholinergic Toxicity, StatPearls publishing.
10. Cole, B. 2012, Neurology, 79(2), 200.
11. Rosenberg, J., Nicas, M. and Yomashiro, Y. 1986, California Hazard Evaluation and Information Service (HESIS), California, America, 2-3.
12. Chemical Neurotoxic Agents, 2011, Encyclopedia of Occupational Health and Safety.
13. Giyanwani, P. R., Zubair, U., Salam, O., Zubair, O. and Zarafshan. 2017, Cureus, 9(9), e1651.
14. National Research Council. 1992, Environmental Neurotoxicology, National Academies Press (US).
15. Martin, T. 2016, User's Guide for T.E.S.T. (version 5.1.2) (Toxicity Estimation Software Tool), EPA/600/R-16/058, U.S. EPA/National Risk Management Research Laboratory/ Sustainable Technology Division.
16. Martin, T. M. 2020, User's Guide for T.E.S.T. (Toxicity Estimation Software Tool), U.S. Environmental Protection Agency., 8-20.
17. Chemicals and Materials, 2018, Canadian Center for Occupational Health and Safety.
18. Dancik, Y., Miller, M. A., Jaworska, J. and Kasting, G. B. 2013, Adv. Drug Delivery Rev., 65, 221-223.
19. Carlsen, L. 2018, Molecular Informatics., 37, 4-5.
20. Kladt, C., Dennerlein, K., Göen, T., Drexler, H. and Korinth, G. 2018, International Archives of Occupational and Environmental Health.
21. Cumming, H. and Rücker, C. 2017, ACS Omega., 2(9), 6244.
22. Nair, A. B. and Jacob, S. 2016, J. Basic Clin. Pharm., 7, 27-31.