Investigating QSAR models for Chemical Warfare Agents: Biological, Biochemical, and Environmental Perspectives

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Abstract: - Quantitative Structure-Activity Relationship (QSAR) models are essential in predicting the characteristics of chemical warfare agents (CWAs), offering crucial insights into their biological, biochemical, and environmental activities. This paper examines how QSAR models elucidate the complex relationships between molecular structures and CWA actions. By leveraging principles from biology, biochemistry, and environmental research, QSAR models accurately predict key features such as CWA toxicity, reactivity, and environmental persistence. This study explores the fundamental mechanisms behind CWA interactions with biological systems, molecular targets, and environmental compartments, highlighting the potential of QSAR models to guide the development of novel antidotes, decontamination strategies, and environmental monitoring protocols. Integrating insights from various disciplines, this work underscores the significance of QSAR modeling in enhancing our understanding of CWA properties and supporting informed decision-making in defense, public health, and environmental management.

Key-Words: - Chemical warfare agents, A-Series, nerve agents, Novichok, Biology, Biochemistry, Environmental Systems, QSAR models, toxicity.

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1 Introduction

According to the Organization for the Prohibition of Chemical Weapons (OPCW), chemical weapons are chemical substances used to intentionally cause death or harm through various toxic properties. Examples of chemical weapons are ammunition and various devices and equipment specially designed and equipped with toxic chemicals. Chemical weapons are divided into different categories of agents such as choking agents, blistering agents, hematological agents, neurological agents and riot control agents.

The category of chemical weapons that will be extensively studied is the neurotoxic agents that block the enzyme acetylcholinesterase (AChE) in the nervous system. This causes neurotransmitter buildup between nerve cells or at synapses, resulting in overstimulation of muscles, glands, and other neurons. Neurotoxic substances are highly toxic and have immediate effects, primarily through absorption through the skin and lungs.

Agents of the G series and agents of the V series, named for military purposes, are the two primary classes of neurotoxic agents. Some G agents, particularly tabun and sarin, are only present in the environment for a short time. Other agents, such as soman and cyclosarin, last longer and are more dangerous to the skin. V agents are incredibly potent, requiring only milligrams to kill, and they can survive in the environment for extended periods. Tabun (GA), Sarin (GB), Soman (GD), Cyclosarin (GF), and the VX agent are examples of such chemicals. These agents cause overstimulation of the sympathetic nervous system, causing symptoms in the peripheral and central nervous systems such as tears, salivation, perspiration, impaired vision, headache, difficulty breathing, and vomiting. Neurotoxic chemicals cause seizures, loss of bodily control, muscle paralysis (including the heart and diaphragm), and loss of consciousness at larger doses.

The V- and G-series agents were initially utilized to create A- A-series compounds, with claims of synthesizing and testing over a hundred analogs. In the case of A-agents, the typical nerve agent alkoxy substituent (- OR) on the central phosphorus atom is replaced with a nitrogen substituent. Substance-84, also known as A-230, is a sarin derivative with an acetamidine moiety in place of the O- isopropyl group. [1], it's noteworthy that the majority of A-series agents use the A-230 design. A-232 and A-234 structures are

acetamidine-containing methoxy and ethoxy analogs of A-230. Other analogs are guanidine analogs, such as A-242 and A-262. A- 230 and A-242 are phosphonates, whereas A-232, A-234, and A-262 are phosphates. A-234 can be synthesized from direct binary precursors in the same way that V- and G-series agents are. The A-series constructions are presented in Table 1 (Appendix), [1], [2], [3], [4].

1.1 The Importance of Understanding the Toxicity behind A- Series

The toxicity of chemical warfare agents is important due to several reasons such as the immediate impact on everyone who is exposed and further implications for public health and safety. These substances possess a high degree of toxicity and can induce rapid and severe health repercussions. Chemical warfare agents, crafted for military applications, primarily serve strategic purposes. Public health and safety precautions are of paramount importance, given the substantial threats posed by accidental releases or intentional deployment in warfare or acts of terrorism.

These chemicals specifically, attack the enzyme acetylcholinesterase (AChe) which is used in the transmission of ongoing neuro signals, including acetylcholine, which is excitatory neurotransmitter. As a result, a nerve impulse will be slower than the chatochin decay rate, and ultimately the nervous system will be impaired. Correspondingly, the patient exhibits dyspnea, fitting, debility, or even death in severe instances. As a result, we observe the impairment of the respiratory system as well as the musculoskeletal system, and other organ systems are affected. It is essential to have an in-depth knowledge of the impact of certain agents on the environment, including the substances that are long-term pollutants that endanger both water and soil as resources. On the other hand, the modern medicine has pinpointed pills of atropine and pralidoxime that can reduce the degree of the effect. This field of research calls for further investigation to validate it.

1.2 The Importance of QSAR Modeling in Estimating A- Series Properties

When assessing the environmental or human health effects of these compounds, QSAR modeling can to a certain extent remedy the apparent lack of data. Thus, in the present study physico– chemical properties of nerve agents have been estimated using QSAR models. It should be emphasized that the environmental processes of these substances are no different from other substances. However, for environmental studies, the extreme toxicity of the nerve agents obviously must be considered.

QSAR modeling is beneficial when it comes to predicting the properties of these substances as there is no need for laboratory work or experiments, thus, there is no human exposure. Moreover, QSAR models provide the advantage of high speed in predictions, allowing the research to identify the substance, view the structure, and predict the biological activity, the toxicity, and lethality of the substance.

2 Model Assessment and QSAR Fundamentals

Toxicity, biological activity, and physicochemical properties are some of the parameters that are most often predicted using QSARs (Quantitative Structure-Activity Relationships), which are particularly relevant when gathering experimental data is difficult or very expensive. Actual measures like REACH, which requires the registration of all chemicals with production of more than one tonne and assesses their environmental influences and health, show the need for QSAR modeling. Other than that, it is realized that QSAR models are a better alternative for high-cost experimental data, [5], [6].

2.1 Statistical Methods

The development of quantitative structure-activity relationship (QSAR) models can be achieved using statistical approaches. These methods span numerous models, starting with simple and linear models, and advance to complex non-linear models which are designed to increase confidence in the data. These methods will be reviewed about model creation and use.

2.2 Modern Nonlinear Methods

Various machine learning technologies have been employed for pharmaceutical data analysis, covering a diverse range of approaches. These methods, often integrating the simpler techniques mentioned earlier, can generate nonlinear models with increased prediction accuracy. In the following section, we will present a concise overview of some frequently utilized techniques in the pharmaceutical industry.

The Random Forest approach [7] is a modern machine learning algorithm that has become the industry standard for developing comprehensive QSAR models. [8], a Random Forest model is made up of a large number (usually 100-500) of individual

decision or regression trees created using a process known as bagging.

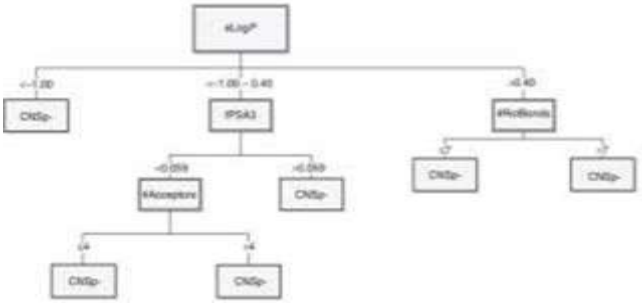

Fig. 1: A decision tree for predicting blood brain barrier permeation CNSp+ represents log PS measurement of>−2 and CNSp – represents a log PS measurement of < -3; alog P is the Ghose-Crippen partition coefficient; fpSA3 is the fraction polar surface area; #RotBonds is the number of rotatable bonds; and #Acceptors is the number of hydrogen bond acceptors

As shown in Figure 1, each tree in the forest is constructed using a different bootstrap sample of the training data, where a bootstrap sample consists of N compounds chosen with replacement for the original dataset. By examining only a portion of the descriptors for each tree node split, a second element of randomization is introduced. These two sources of randomness ensure that each tree represents the input data uniquely, and thus averaging predictions across the forest provides consistently accurate results. Furthermore, Random Forests give solid methods for assessing the relative relevance of input descriptors, and the variance of predictions across trees provides accurate estimates of expected prediction errors. The random Forest package in R can be used to create random forest models. The cForest machine learning approach, a variant of the Random Forest machine learning method, allows for the conditional relevance of input descriptors to be assessed.

The performance of a model is often evaluated using both graphical representation and various statistical measurements. In this context, QSAR models can be divided into two types:
(1) regression models, which provide quantitative predictions of the modeled property, and (2) classification models, which provide predictions in the form of class labels, such as active or inactive. Charts displaying the correlation between predictions and measured values for either the external test set or cross-validated predictions are used to analyze regression models. The strength of the correlation, the range of input and predicted data, the existence of outliers, and the even distribution of predictions across the value range should all be considered while analyzing correlation charts. Detecting clusters around specific values may indicate that the model is detecting simple patterns, such as distinguishing separate subseries within the data with varying activity levels.

3 Clinical Implications for Human Health

The exposure to organophosphate compounds leads to distinct clinical effects, typically manifesting in three stages in humans. [9], initially, there is an acute cholinergic phase, occurring shortly after exposure and lasting around 12 to 24 hours. This phase encompasses various symptoms, such as muscarinic, nicotinic, and central nervous system symptoms. Muscarinic symptoms involve chest tightness, increased secretions, coughing, nausea, vomiting, abdominal cramps, diarrhea, sweating, salivation, tearing, blurred vision, and incontinence. Nicotinic symptoms may include muscle spasms and weakness. Furthermore, the central nervous system is affected, resulting in emotional instability, dizziness, sleep disturbances, nightmares, headache, confusion, stupor, speech difficulties, seizures, coma, and potentially fatal outcomes in severe cases.

The second stage involves the intermediate syndrome (IS), [10]. IS appears 1-4 days after the acute cholinergic phase and before the onset of delayed polyneuropathy. It is characterized by severe weakness of the proximal muscles of the limbs and cranial nerve disorders. Difficulty in breathing can progress to respiratory failure following paralysis of the diaphragm and other respiratory muscles. Complete recovery occurs within 4-

21 days with appropriate care. Although the exact pathogenesis of the intermediate syndrome is currently unknown, there may be alterations in the function and activity of nicotinic receptors at the neuromuscular junction, [11]. IS has not been described in cases of poisoning by neurologic agents; however, there is a connection with muscle changes observed after poisoning by tabun, soman, and sarin in experimental animals, [12]. Following organophosphate insecticide poisoning in humans, it has been reported that 10- 30% of patients may develop IS, [13], [14].

The third and final stage involves delayed polyneuropathy and typically manifests 7-14 days after exposure to the organophosphate compound. It usually results in symmetric weakness of the

peripheral muscles in the hands and feet with varying degrees of sensory impairment. Currently, phosphorylation of another enzyme, esterase, a target of organophosphate-induced neuropathy (OPIDN), is believed to be responsible for the dysfunction. [15], [16], unlike the intermediate syndrome, it is highly unlikely for neurotoxic agents to have the capability to induce polyneuropathy, possibly due to the low concentrations of neurotransmitters required to affect acetylcholinesterase compared to the high concentrations needed to inhibit NTE. Studies have shown that only a small percentage of NTE inhibition from the brain and spinal cord of hens undergoes aging.

3.1 Other Effects of Organophosphate Compounds

Studies involving both humans and animals have documented a range of disorders affecting various functional systems following exposure to organophosphate compounds, [17].

3.1.1 Effects on the Central Nervous System

Exposure to organophosphate compounds can lead to intricate changes in mental functioning, [18] including diminished memory and alertness, reduced information processing speed, and psychomotor abilities. Additionally, symptoms like depression, anxiety, and irritability may manifest. These effects are particularly concerning in wartime situations, as they could significantly impair soldiers' combat effectiveness. Studies suggest that the long-term consequences of cognitive impairment may persist for up to a year, whether from a single exposure or chronic exposure to low-level nerve agents or pesticides. [19], furthermore, individuals may experience subsequent behavioral and psychological changes, [20], [21].

In an industrial accident, 40 civilians were accidentally exposed to GB vapors, [22]. This did not result in significant changes in the cholinesterase of red blood cells (RBC) in those exposed. However, there was a temporary incapacity among the exposed. Individuals reported symptoms such as weakness, headaches, sensations of heat-cold, dizziness, nightmares, feeling warmth or increased sweating, drowsiness, insomnia, irritability, tremors, sweating, facial spasms, anorexia, diarrhea, and pseudo-sensory edema. Other studies on individuals with neurological factors have revealed symptoms and signs such as frontal headaches, occipital pain, eye vessel dilation, rhinorrhea, nausea, vomiting, chest tightness, and blurred vision. These are similar to those observed after exposure to insecticides. Fatigue, lethargy, and poor or no sleep were observed in some who were exposed, while skin exposure caused sweating for periods of up to 34 days. Cognitive dysfunctions, which may be particularly relevant for military effectiveness, include disturbances in three-dimensional spatial coordination and judgment.

In animals, after exposure to the agents, initial changes observed include edema, astrocytic, and peri-vascular hemorrhages. Neuronal degeneration and occasionally diffuse necrosis may be observed along with more discrete infarctions. These changes may be particularly detected in the hippocampus and the cerebellar cortex, [23].

3.1.2 Genotoxic and Carcinogenic Effects

The genetic toxicity and carcinogenic effects are worrisome because of the irreversible nature of the disease mechanisms and the extended latent period before they become apparent. Certain scholars [24], [25] propose that these agents possess the capability to undergo alkylation. Nonetheless, in comparison to chemical substances, the reaction rate of these agents with enzymes like AChE and phosphorylation is significantly higher.

3.1.3 Mutation

The hazardous effects of mutagenesis will rely on the capability of these agents to cause observable genetic damage to DNA, encompassing mutations, deletions, displacements, and chromosomal abnormalities. Both the pesticides malathion and dimethoate have induced sister chromatid exchanges in human cells and chromosomal aberrations in human lymphocytes. Likewise, Dichlorvos, an insecticide containing organophosphate compounds, has also been associated with mutagenic abnormalities, [26], [27].

3.1.4 Teratogenesis

Teratogenesis is defined as the induction of dysplasia in living offspring without a decrease in the number of births. Embryonic defects did not arise after continuous administration of agents in animals, except at doses significantly affecting the health of the mother. Many agents are teratogenic in bird and fish embryos. The mechanism of teratogenesis in birds may involve the inhibition of an enzyme, cyanoformamidase [28], which does not appear to be significant for humans.

3.1.5 Pregnancy

In experimental animals, exposure to organophosphate compounds during pregnancy induces prenatal and postnatal death and congenital

abnormalities, including spinal deformities, limb defects, polydactyly, intestinal hernia, cleft palate, and hydronephrosis. [29], damage to organs related to male fertility, such as the testes, has been reported following exposure to these agents. [30], [31], in humans, intoxication during the third month of pregnancy results in miscarriage as continuing the pregnancy is deemed hazardous, [32].

3.1.6 Immune System

Epidemiological studies have indicated that these agents may impact the human immune system. [33], [34], compounds like parathion can suppress lymphocytes and chemical immunity, following doses that produce cholinergic effects. Significant impairment of protective white blood cell (neutrophil) function and increased upper respiratory tract infection frequency have been observed in workers occupationally exposed to pesticides. Reduction in both serum cholinesterase and RBC activity has also been noted, [35].

3.1.7 Metabolic Function

Several metabolic (e.g., glucose metabolism) [36] and endocrine activity (adrenal and thyroid hormones) disorders have been reported in animals and humans following exposure to organophosphate substances. [37], in evaluating the health impacts of nerve agent exposure, it is essential to investigate the presence of such disorders.

4 Environmental Impacts of Neurological Agents

4.1 Effects on Soil Environment

The environmental repercussions of neurological agents are significant, particularly concerning the soil ecosystem. Soil chemical properties frequently suffer degradation due to military actions aimed at harming individuals and infrastructure, or disrupting agricultural activities, along with the cessation of military training exercises. This often results in large-scale population movements to safer regions amidst armed conflicts. However, the influx of people into these areas often exacerbates environmental challenges due to the potential overuse of natural resources. This can lead to severe ecological disruptions, including extensive deforestation, desertification, unsustainable exploitation of groundwater, and contamination of both soil and groundwater. These adverse effects are commonly observed near densely populated refugee camps and migration routes across international borders.

The expected outcome of neurological agents on soil involves the eradication of specific organisms residing in the area. However, there is a lack of studies aiming to confirm this impact, as noted in the available literature. One prominent neurological agent widely utilized, called VX, presents as a clear, amber-colored, odorless, oily liquid that can dissolve in water and poses severe toxicity even at minimal exposure levels. Unlike other neurological agents such as tabun (GA), sarin (GB), and soman (GD), VX exhibits notably lower atmospheric pressure and persists in the soil environment due to its strong absorption properties. [38], once absorbed into soil colloids, its toxicity diminishes significantly. [39], research on VX-induced soil contamination has observed near-complete degradation of the pollutant within three weeks, regardless of soil type. However, intact VX was still detectable in soil samples stored at 4°C for over a year post-contamination. [40], evaporation is succeeded by hydrolysis, serving as the primary mechanism for the loss of neurological agents from soil. Sarin can permeate through the soil in gaseous form under hot, arid conditions, weighing about five times that of air. Its hydrolysis occurs through the loss of fluorine, followed by a slower loss of the alkoxy group. Hydrolysis rates depend on soil temperature and pH, with resultant products typically being non-toxic. Soman, while having lower atmospheric pressure and volatility compared to sarin, undergoes a similar hydrolysis process. However, soman's decomposition products, generally non-toxic, are more water- soluble and have less affinity for organic matter than sarin decomposition products, [41].

Many microbial species can break down organophosphorus compounds in soil, demonstrating their effectiveness in the colonization and restoration of contaminated soils. The phosphotriesterase (PTE) from soil bacteria, such as Pseudomonas diminuta, is involved in deactivating a broad range of neurological organophosphorus agents. [42], recent studies have led to significant advancements in the catalytic activities of recognized PTE variants, with improvements up to 15,000 times compared to the wild-type enzyme [43], supporting a combined strategy of rational design and directed evolution as a potent tool for discovering increasingly effective enzymes for the detoxification of organophosphorus neurological agents.

Neurological agents persist in poorly drained soils, where anaerobic conditions prevail. [44], soil

recovery in such conditions can be achieved through injections of evaporated hydrogen peroxide. [45], [46], most degradation products are less toxic than the parent compounds, but VX, HN-2, L, and ED form toxic intermediates that are more resilient than the parent compounds. [47], organophosphorus agents are essentially converted into low-solubility phosphoric salts, resulting in a net gain for the mineralogical aggregation of the soil. According to UN Resolution 687, the production and storage of neurological agents were outlawed by the Chemical Weapons Convention of 1993.

4.2 Impact on the Marine Ecosystem

4.2.1 Regulatory Aspects of Chemical Weapon Disposal at Sea

Certain international organizations have addressed the issue of chemical munitions being discarded at sea. The quantity of weapons disposed, the locations (both actual and documented), and the potential impacts on humans and marine ecosystems have been evaluated, both through conducting new primary research and assimilating existing available data. [48], [49], the chemical agents remaining in disposed munitions pose two main threats: environmental risk associated with the chronic or acute release of agents from their casings into the subsea environment, and the risk of human health associated with human exposure to agents leaching onto land or otherwise surfacing. [50], traditional rates of dissolution and hydrolysis of an agent serves as useful indicators of its ecological toxicity and indirect threat to humans. Agents highly soluble in water dissolve rapidly and are expected to disperse quickly in seawater due to the vast volume of the ocean. Agents undergoing rapid dissolution and hydrolysis should thus lead to lower long-term toxicity than environmentally resistant agents. Phosgene, chloroacetophenone, and blood agents have dissolution and hydrolysis rates that make them unlikely to chronically threaten underwater ecosystems. LD_{50} or LCt_{50} values may be unknown or may only come from animal models.

4.2.2 Neurological Agents

Tabun dissolves in seawater and breaks down within a few days, with cyanide being the only toxic byproduct of its degradation. Sarin, being colorless and odorless, remains a liquid at 10°C. It does not mix with water and rapidly breaks down (within a few days) into hydrofluoric acid and isopropyl methylphosphonic acid. Both resulting compounds from the hydrolysis process degrade quickly in water, hence they are considered relatively safe within the oceanic environment. Soman, more lipophilic than tabun and sarin, decomposes slowly into pinacolyl methylphosphonic acid and methylphosphonic acid. These breakdown products also exhibit minimal toxicity. [51], soman is the only G-agent expected to possess some environmental persistence. [52], VX demonstrates an uncommon characteristic of increased water solubility at lower temperatures. [53], with its slow hydrolysis rate, VX is anticipated to have a subsea half-life of 5.4 years. The initial hydrolysis products retain toxicity and acetylcholinesterase activity, thereby prolonging potential adverse effects on marine ecosystems. Predicting post-release concentrations is challenging as they depend on local oceanic disturbances and currents. Neurotoxic agents typically undergo eventual hydrolysis in seawater, forming non-toxic degradation products.

4.2.3 Impact on Fish from Chemical Weapons

Triphenylarsine, adamsite, and sulfur mustard are expected to exhibit chronic toxicity in fish. This may be related to the hydrophobic nature of sulfur mustard and its arsenic oil components, as they are expected to persist in the environment much longer than other factors. [54], the relative resilience of the sea may also contribute to the persistence of sulfur mustard lumps and continuous exposure of fishermen. Of these compounds, fish and tissue modeling predicted that only adamsite would be present in fish muscles: adamsite concentrations in cod and haddock were predicted to be 0.485 mg/kg and 0.167 mg/kg, respectively. These levels were considered too low to cause health impacts from fish consumption, although a separate model predicted an oral RfD for adamsite to be 0.00003 mg/kg body weight/day. Several mass marine "deaths," including starfish, benthic fish, and dolphins, were initially attributed to chemical weapons, but the causation has either not been proven or definitively attributed to another cause. To date, mass marine deaths have not been confirmed to be caused by chemical weapons disposed of at sea. Predicting the risk to humans from consuming fish exposed to underwater chemical agents is challenging. Scenario modeling for water concentration and bioaccumulation data in combination with oral RfD has suggested a risk. Triphenylarsine appears to be the chemical agent posing the greatest threat to humans consuming seafood, followed by sulfur mustard and adamsite. [55], the potential toxicity of triphenylarsine arises from its higher bioaccumulation rate. In MEDEA's assessment of disposal in the Arctic seas, arsenic in seafood from discarded chemical weapons was estimated to pose moderate risk to indigenous

populations repeatedly consuming seafood from the same collection areas throughout their lives.

4.2.4 Impact on Marine Microorganisms from Chemical Weapons

It has been demonstrated that chemical agents in landfills affect local marine organisms. Of particular interest are bacterial species resistant to arsenic, sulfur mustard, and the breakdown products of sulfur mustard, thus participating in the degradation of arsenic, sulfur mustard, and thiodiglycol. Basidiomycota fungal species are found in marine environments and are capable of breaking down sulfur mustard, but they are not regularly assessed during landfill research cited in the literature. Arthrobacter, Achromobacter, and Pseudomonas species resistant to thiodiglycol were found to be functional in the breakdown of thiodiglycol at relevant seawater temperatures (280 K). In two landfills, one in the Bornholm Basin and one off the Norwegian coast, it was found that 20-90% of heterotrophic organisms were resistant to sulfur mustard. Up to 58% of bacteria were resistant to sulfur mustard breakdown products, with dominant species being Pseudomonas and Bacillus. Sampling studies in Baltic and Skagerrak disposal areas showed that 20% to 98% of bottom- dwelling heterotrophs are resistant to sulfur mustard breakdown products. The increase in the microbiome resistant to sulfur mustard breakdown products may also have mitigating effects on pollutants, as toxic agents would degrade and be removed from the environment more rapidly. However, in cases where contamination of harvested seafood has occurred, such seafood has been preemptively destroyed, [56], [57].

5 Results of QSAR Model Development for the A SERIES

5.1 Finite Dose Skin Permeation (FDSP) Calculator

The finite dose skin permeation calculator facilitates the computation of the skin permeation coefficient (Kp). [58], numerous models have been devised to ascertain steady-state permeation from an aqueous solution of limitless volume, yet these models do not align with typical workplace exposure scenarios. However, when a dose, regardless of its size, is administered to partially or fully hydrated skin, this program determines fluxes, concentrations in the skin, and absorbed amounts. Specifically, the software computes the skin penetration coefficient and absorption of chemicals concerning evaporation following the application of a test substance to the skin. The skin permeability coefficient (Kp) serves as a predictor for chemical skin penetration. Although many mathematical models rely on Kp data, conflicting Kp values have been observed, prompting concerns about the overall reliability of these measurements. Kp is measured in units of (cm h^{-1}). To ensure the effectiveness of the finite dose skin permeation calculation and obtain the desired outcome, it is essential to input specific parameters. These include the chemical name and type of the substance under testing, LogKow, melting and boiling points, molecular weight, vapor pressure, permeability, and information regarding double or triple bonds and the presence of a ring. The hydrophilic/lipophilic property of a compound is determined by the octanol-water partition coefficient (Kow). Initially applied in drug and pesticide discovery and design, Kow has become a critical factor for any chemical, significantly influencing its behavior within a living organism and in the environment. The results of the program in relation to the VP, Kp, Jmax and Tmax of the A-series are presented in Table 2 and percentages of systemic absorption, evaporation, stratu corneum, Molacular Weight and log Kow are presented in Table 3, respectively.

The toxicity data presented above are based on oral administration. The skin is the most likely route of absorption. A review of the International Journal of Molecular Science yielded data on vapor pressures, melting and boiling points, and octanol-water partitioning coefficients for the 12 compounds investigated. Table 3 contains the estimated vapor pressures for the chemicals examined. It should be observed that very low vapor pressures were detected for the A-230, A- 232, A-234, A-038, A-039, A-042, and especially for the A- 242, but slightly higher vapor pressures appear to prevail for the compounds A-036, A-040, A-041, and A-037, respectively. For probable absorption through the skin, the vapor pressure, as well as structural properties such as the presence of hydrophilic/hydrophobic groups, will be assessed. The skin permeation data were then computed using the Finite Dose Skin Permeation calculator, and the results are shown in Table 3. The skin permeation (Kp) of A-230 was determined to be 3.952E-5 cm/hr, while the maximal flux, Jmax, was determined to be 0.029 mg/ cm2hr.

Table 2. Calculated vapor pressure, skin permeation, and maximum absorptive flux. Tmax corresponds to the time to maximum systemic absorption

Compound	VP mm Hg	Kp cm/hr	Jmax µg/cm^{2}hr	Tmax hr
A-230	0,01598	$3,952*10^{-5}$	0,029	3,081
A-232	0,0111	$1,862*10^{-5}$	0,038	2,85
A-242	0,01275	$3,397*10^{-5}$	0,021	1,855
A-035	0,00434	$3,876*10^{-5}$	0,00348	25,022
A-036	0,13875	$2,282*10^{-4}$	0,003678	5,476
A-037	0,5535	$8,553*10^{-4}$	0,002008	6,785
A-038	2,28	$3,290*10^{-3}$	0,001575	7,964
A-039	0,01148	$1,821*10^{-5}$	0,004598	13,282
A-040	0,0528	$9,545*10^{-5}$	0,005173	3,378
A-041	0,2055	$3,572*10^{-4}$	0,002979	3,609
A-042	0,825	$1,356*10^{-3}$	0,001803	2,85

Table 3. The percentage distribution of systemic absorbed, evaporated, and sorbed to the stratum corneum. Further, the molecular weight (MW) and the octanol-water partitioning coefficient are given

Compound	Systemic Absorbed pct.	Evaporation pct.	Stratu Corneum pct.	MW g/mol
A-230	12,13	95,03	0,06	194,19
A-232	15,78	89,31	0,08	210,19
A-234	8,02	109,07	0,01	224,21
A-242	15,34	90,00	0,13	239,27
A-035	2,25	166,49	0,02	209,93
A-036	1,42	286,10	0,01	193,47
A-037	1,11	618,57	0,01	177,02
A-038	8,86	101,75	0,08	240,03
A-039	3,31	134,17	0,02	223,96
A-040	1,83	199,20	0,01	207,50
A-041	1,26	375,51	0,01	191,05
A-042	13,51	92,75	0,13	254,05

It should be emphasized that these statistics are subject to some uncertainty because skin permeation is highly dependent on where the skin is taken from the human body. Several intriguing features arise from the data reported in Table 5. Several intriguing features arise from the data reported in Table 5. To begin, the skin permeation value, Kp, of Novichoks A-042, A-038, and A-232 is much lower than that of A-037 and A-041. Second, significant changes in the percentage of the chemical that is absorbed systemically should be noticed. Thus, the absorbed percentage for compounds A-232 and A-242 is approximately 15 times that of compounds A-037 and A-041. Concurrently, a large increase in the proportion of compound evaporated is seen, which is perfectly consistent with the fluctuation in vapor pressure. Third, all substances have a relatively low percentage of the stratum corneum and, as a result, always reach the plasma. Fourth, the variance in time to maximal flux is highlighted. When exposed to one of these substances, this lag time may be significant. It should be noted that these figures are for healthy skin. Possible skin damage may reduce the lag-time dramatically. Because Novichoks have a low molecular volume in general, the proceding observations cannot be explained only by a molecular size.

5.2 Prediction of Activity Spectra for Biologically Active Substances (PASS)

The PASS program can compute the characteristics of biologically active compounds. Specifically, it enables the computation of over 300 pharmacological properties and biological mechanisms using the substance's structure as a basis. The outcomes from these calculations are presented in a tabular format, indicating the activities of the studied properties (Activity) and providing specific values that determine whether a compound is active (Pa) or inactive (Pi) concerning the relevant property, [59].

- 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.
- 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.

Yet, should this behavior be identified during the experimental phase, it suggests that the substance under consideration might constitute a novel chemical compound. The PASS platform scrutinized a total of eight activities related to Novichok compounds.

5.2.1 Cholinergic

In the parasympathetic system, specific sensory nerves known as cholinergic receptors are evident. The term "cholinergic" is derived from the presence of the neurotransmitter acetylcholine, which serves as the principal neurotransmitter in the parasympathetic system. Cholinergic neurotransmitters fall into two classes, distinguished by the compound that stimulates them in each case—nicotine and muscarinic. [60], [61], various substances can impact the activity of these neurotransmitters, either by stimulating, enhancing, or imitating the neurotransmitter acetylcholine. This phenomenon is referred to as cholinergic toxicity. Organophosphate and carbamate compounds primarily account for substances responsible for cholinergic toxicity. Depending on the specific acetylcholine neurotransmitter affected, cholinergic

toxicity can manifest distinct symptoms in the human body. An excess of acetylcholine in muscarinic neurotransmitters results in increased secretions (sweat, tears, saliva, stomach fluids), bronchi constriction, decreased heart rate, and abdominal cramps. Similarly, in nicotine neurotransmitters, an excess of acetylcholine can induce muscle spasms or even paralysis due to overstimulation of the nerves transmitting messages to the muscles. [62], [63], the results of the program for the cholinergic activity are presented in Table 4.

5.2.2 Toxicity

Toxicity refers to a substance's capacity to induce harmful side effects on a single cell, a group of cells, an organ system, or the entire body. While all chemicals can cause some degree of harm, a chemical is deemed toxic when even a small amount can be detrimental to a living organism. Conversely, if a large quantity of the chemical is necessary to cause harm, it is considered relatively non-toxic. The toxicity of a substance hinges on three crucial factors: its chemical structure, the extent of absorption by the body, and the organism's ability to detoxify and eliminate the substance. However, when comparing the toxicity of two compounds, their structure is the sole essential factor. This is because the absorption and detoxification capabilities can vary not only between different species but also among organisms of the same species. [64], the results of the program for the toxic activity are presented in Table 6.

5.2.3 Neurotoxicity

Neurotoxicity pertains to a substance's ability to induce adverse effects in the central nervous system, peripheral nerves, or sensory organs. A chemical is classified as neurotoxic if it can instigate a consistent pattern of neural dysfunction or bring about changes in the chemistry or structure of the nervous system. [65], neurotoxicity can manifest at any stage in the life cycle, from gestation through senescence, and its symptoms may vary with age. The nervous system appears particularly susceptible to damage during its developmental stages, and the consequences of early injuries may become apparent only as the nervous system matures and ages. [66], the results of the program for the neurotoxic activity are presented in Table 5.

5.2.4 Respiratory Failure

The respiratory system plays a crucial role in supplying the body with oxygen and expelling carbon dioxide. Any failure in performing these tasks can result in respiratory failure. [67], Novichoks, similar to other organophosphate compounds, induce the phosphorylation of serine hydroxyl residues on the acetylcholine esterase enzyme upon entering the human body. This modification to the enzyme leads to an accumulation of acetylcholine, a neurotransmitter essential in cholinergic signaling pathways. The heightened levels of acetylcholine cause dysregulation in the cholinergic system, manifesting in both central and peripheral clinical symptoms. Given the potential for detrimental physiological effects, it is imperative to comprehend the intricate mechanisms underlying the biochemical changes generated by Novichoks.

Respiratory failure is one of the most serious cholinergic effects of organophosphate poisoning, and it is mostly caused by central processes. Specifically, the neural fibers that function with glutaminergic and muscarinic neurotransmission form the pre-Bötzinger afferent complex, a region in the posterior medulla. Excessive production of the substance acetylcholine in this area can suppress breathing, resulting in respiratory failure. The results of the program for the respiratory failure are presented in Table 7.

5.2.5 Multiple Organ Failure

The mechanisms underlying the multiorgan failure syndrome remain poorly understood, suggesting that multiple biological pathways may be involved in the early stages of the disease. In critically ill patients, functional abnormalities may be the main cause of organ failure, rather than anatomical defects. Likely, a mechanism of a defensive or reactive nature - rather than simple failure - is the main process at play in this context. This theory states that a decrease in oxidative phosphorylation and mitochondrial activity sets off the loss of organ function, which in turn causes a decrease in cellular metabolism. This effect on mitochondria may be related to acute phase changes in inflammatory mediators and hormones. The results of the program for the multiple organ failure activity are presented in Table 8.

5.2.6 Hematotoxic

The hematotoxic effects induced by chemical substances can be broadly categorized into two primary groups: (i) alterations in the number of circulating blood cells and cell types, and (ii) modifications to the oxygen-carrying capacity of hemoglobin. Anemia and leukopenia characterize conditions where the numbers of red and white blood cells per unit volume of blood decrease, while polycythemia and leukemia denote conditions

marked by an increase in the numbers of red and white blood cells. Disorders such as methemoglobinemia and carboxyhemoglobinemia are associated with a diminished capacity of red blood cells to transport oxygen. Methemoglobinemia stems from the oxidation of ferrous iron in hemoglobin to the ferric state, while carboxyhemoglobinemia arises from the complexation of carbon monoxide with hemoglobin. The results of the program for the hematotoxic activity are presented in Table 9.

5.2.7 Carcinogenic

Carcinogenic properties refer to a substance's capacity to either induce or exacerbate cancer. Carcinogens can cause genetic alterations or interfere with regular cell processes, which can result in unchecked cell division. If you are exposed to certain compounds at work, in the environment, or through your lifestyle, there is a possibility that cancer will develop. It is critical to recognize and understand the properties of substances that have the potential to cause cancer to safeguard public health. The results of the program for the carcinogenic activity are presented in Table 10.

5.2.8 Teratogen

Teratogens are chemicals that can cause congenital abnormalities in a developing embryo or baby. These are substances known for their ability to worsen fetal abnormalities when treated or taken during pregnancy. Among the teratogens are pharmaceuticals, drugs, chemicals, certain diseases, and poisonous substances. In addition, teratogen exposure may increase the chance of stillbirth, premature labor, and miscarriage. The results of the program for the teratogenic activity are presented in Table 11.

Warfare chemical agents are assessed for their effects by detailed examination, taking into account a variety of factors, such as their effects on cholinergic, toxic, neurotoxic, respiratory, multiple organ failure, haematotoxic, carcinogenic, and teratogenic activities. For this assessment, PASS software, an advanced tool providing a comprehensive understanding of the pharmacological properties and biological mechanisms of action of the different chemical agents, is used. The PASS platform evaluates more than 300 attributes according to the agents' structural features. The outcomes are then tabulated and the activity levels of these attributes are classified as either active (Pa) or inactive (Pi).

Table 4. PASS results for the cholinergic activity

Compound	Pa	Pi	Activity
A-230	0,205	0,023	Cholinergic
A-232	0,198	0,025	Cholinergic
A-234	0,2	0,025	Cholinergic
A-242	0,179	0,033	Cholinergic
A-262	0,173	0,036	Cholinergic
Novichok 5	0,123	0,077	Cholinergic
Novichok 7	0,15	0,049	Cholinergic
A-038	0,198	0,025	Cholinergic
A-039	0,206	0,023	Cholinergic
A-040	0,302	0,011	Cholinergic
A-041	0,319	0,01	Cholinergic
A-042	0,242	0,016	Cholinergic
A-043	0,113	0,088	Cholinergic
A-044	0,147	0,052	Cholinergic
A-045	0,129	0,07	Cholinergic
Unknown 1	n/a	n/a	Cholinergic
Unknown 2	n/a	n/a	Cholinergic
Unknown 3	0,2	0,025	Cholinergic
Iranian Novichok	0,137	0,062	Cholinergic
A-230	0,113	0,088	Cholinergic
A-232	0,147	0,052	Cholinergic
A-234	0,129	0,07	Cholinergic

Table 5. PASS results for the neurotoxic activity

Compound	Pa	Pi	Activity
A-230	0,662	0,034	Neurotoxic
A-232	0,64	0,037	Neurotoxic
A-234	0,695	0,03	Neurotoxic
A-242	0,745	0,024	Neurotoxic
A-262	0,726	0,026	Neurotoxic
Novichok 5	0,685	0,031	Neurotoxic
Novichok 7	0,746	0,023	Neurotoxic
A-038	0,727	0,025	Neurotoxic
A-039	0,842	0,013	Neurotoxic
A-040	0,881	0,008	Neurotoxic
A-041	0,877	0,008	Neurotoxic
A-042	0,842	0,013	Neurotoxic
A-043	0,847	0,012	Neurotoxic
A-044	0,898	0,005	Neurotoxic
A-045	0,932	0,004	Neurotoxic
Unknown 1	0,893	0,006	Neurotoxic
Unknown 2	0,56	0,052	Neurotoxic
Unknown 3	0,623	0,04	Neurotoxic
Iranian Novichok	0,748	0,023	Neurotoxic
A-230	0,847	0,012	Neurotoxic
A-232	0,898	0,005	Neurotoxic
A-234	0,932	0,004	Neurotoxic

Table 6. PASS results for the toxic activity.

Compound	Pa	Pi	Activity
A-230	0,566	0,067	Toxic
A-232	0,558	0,069	Toxic
A-234	0,548	0,068	Toxic
A-242	0,612	0,059	Toxic
A-262	0,604	0,061	Toxic
Novichok 5	0,526	0,075	Toxic
Novichok 7	0,585	0,064	Toxic
A-038	0,606	0,06	Toxic
A-039	0,744	0,037	Toxic
A-040	0,808	0,026	Toxic
A-041	0,798	0,028	Toxic
A-042	0,731	0,039	Toxic
A-043	0,872	0,015	Toxic
A-044	0,895	0,012	Toxic
A-045	0,899	0,012	Toxic
Unknown 1	0,882	0,014	Toxic
Unknown 2	0,538	0,073	Toxic
Unknown 3	0,522	0,075	Toxic
Iranian Novichok	0,64	0,055	Toxic
A-230	0,872	0,015	Toxic
A-232	0,895	0,012	Toxic
A-234	0,899	0,012	Toxic

Table 7. PASS results for the respiratory failure activity

Compound	Pa	Pi	Activity
A-230	0,663	0,034	Respiratory Failure
A-232	0,64	0,038	Respiratory Failure
A-234	0,705	0,028	Respiratory Failure
A-242	0,741	0,024	Respiratory Failure
A-262	0,723	0,026	Respiratory Failure
Novichok 5	0,708	0,028	Respiratory Failure
Novichok 7	0,759	0,021	Respiratory Failure
A-038	0,76	0,021	Respiratory Failure
A-039	0,849	0,012	Respiratory Failure
A-040	0,907	0,007	Respiratory Failure
A-041	0,903	0,008	Respiratory Failure
A-042	0,864	0,01	Respiratory Failure
A-043	0,824	0,014	Respiratory Failure
A-044	0,921	0,006	Respiratory Failure
A-045	0,762	0,021	Respiratory Failure
Unknown 1	0,684	0,031	Respiratory Failure
Unknown 2	0,461	0,079	Respiratory Failure
Unknown 3	0,646	0,037	Respiratory Failure
Iranian Novichok	0,759	0,021	Respiratory Failure
A-230	0,824	0,014	Respiratory Failure
A-232	0,921	0,006	Respiratory Failure
A-234	0,762	0,021	Respiratory Failure

Table 8. PASS results for the multiple organ failure activity

Compound	Pa	Pi	Activity
A-230	0,748	0,02	Multiple Organ Failure
A-232	0,683	0,035	Multiple Organ Failure
A-234	0,646	0,046	Multiple Organ Failure
A-242	0,779	0,014	Multiple Organ Failure
A-262	0,721	0,026	Multiple Organ Failure
Novichok 5	0,687	0,034	Multiple Organ Failure
Novichok 7	0,779	0,014	Multiple Organ Failure
A-038	0,329	0,205	Multiple Organ Failure
A-039	0,522	0,09	Multiple Organ Failure
A-040	0,565	0,073	Multiple Organ Failure
A-041	0,536	0,084	Multiple Organ Failure
A-042	0,302	0,229	Multiple Organ Failure
A-043	0,43	0,134	Multiple Organ Failure
A-044	0,398	0,153	Multiple Organ Failure
A-045	0,398	0,153	Multiple Organ Failure
Unknown 1	0,366	0,175	Multiple Organ Failure
Unknown 2	0,353	0,185	Multiple Organ Failure
Unknown 3	0,592	0,063	Multiple Organ Failure
Iranian Novichok	0,729	0,024	Multiple Organ Failure
A-230	0,43	0,134	Multiple Organ Failure
A-232	0,398	0,153	Multiple Organ Failure
A-234	0,398	0,153	Multiple Organ Failure

Table 9. PASS results for the carcinogenic activity

Compound	Pa	Pi	Activity
A-230	0,284	0,047	Carcinogenic
A-232	0,248	0,055	Carcinogenic
A-234	0,265	0,051	Carcinogenic
A-242	0,357	0,032	Carcinogenic
A-262	0,322	0,038	Carcinogenic
Novichok 5	0,411	0,026	Carcinogenic
Novichok 7	0,465	0,021	Carcinogenic
A-038	0,683	0,009	Carcinogenic
A-039	0,242	0,057	Carcinogenic
A-040	0,328	0,036	Carcinogenic
A-041	0,308	0,041	Carcinogenic
A-042	0,26	0,052	Carcinogenic
A-043	0,614	0,011	Carcinogenic
A-044	0,541	0,015	Carcinogenic
A-045	0,567	0,014	Carcinogenic
Unknown 1	0,664	0,009	Carcinogenic
Unknown 2	0,292	0,045	Carcinogenic
Unknown 3	0,14	0,125	Carcinogenic
Iranian Novichok	0,155	0,107	Carcinogenic
A-230	0,614	0,011	Carcinogenic
A-232	0,541	0,015	Carcinogenic
A-234	0,567	0,014	Carcinogenic

Table 11. PASS results for the Teratogen activity.

Compound	Pa	Pi	Activity
A-230	0,213	0,136	Teratogen
A-232	0,253	0,112	Teratogen
A-234	0,29	0,096	Teratogen
A-242	0,283	0,098	Teratogen
A-262	0,348	0,08	Teratogen
Novichok 5	0,36	0,076	Teratogen
Novichok 7	0,433	0,061	Teratogen
A-038	n/a	n/a	Teratogen
A-039	0,21	0,138	Teratogen
A-040	0,296	0,094	Teratogen
A-041	0,27	0,103	Teratogen
A-042	0,177	0,16	Teratogen
A-043	0,23	0,125	Teratogen
A-044	0,422	0,063	Teratogen
A-045	0,25	0,114	Teratogen
Unknown 1	0,879	0,006	Teratogen
Unknown 2	0,374	0,073	Teratogen
Unknown 3	n/a	n/a	Teratogen
Iranian Novichok	0,252	0,113	Teratogen
A-230	0,23	0,125	Teratogen
A-232	0,422	0,063	Teratogen
A-234	0,25	0,114	Teratogen

5.3 ProTox-II

ProTox-II is an online toxicity lab that may be accessed via a web server. It is intended to forecast different toxicological consequences linked to a given chemical structure. The platform forecasts the potential toxicity of both real and virtual chemicals using computer-based models trained on real data, whether gathered from in vitro or in vivo research. ProTox-II determines the acute toxicity class and multiple endpoints for an input compound by evaluating chemical similarities to known dangerous compounds and applying trained machine learning models.

The platform aims to position itself as a freely available and comprehensive computational tool for in silico toxicity prediction, catering to toxicologists, regulatory agencies, computational chemists, and medicinal chemists. The ProTox web server utilizes both chemical similarity and the identification of toxic fragments to accurately predict toxicity. It introduces a distinctive feature for predicting toxicity class through methods based on both similarity and fragments, accompanied by alerts indicating potential toxicity targets. An important advantage of ProTox-II lies in its adaptability for future enhancements. It incorporates an oral toxicity model that relies on a predictive method analyzing two-dimensional similarity to compounds with known LD50 values and identifying fragments overrepresented in toxic substances. The validation method employs leave-one-out cross-validation, calculating the three nearest neighbors from the training set for each compound using fingerprint similarity. Results for the input compound's oral toxicity prediction are presented as a predictive LD50 value (mg/kg), as presented in Table 12.

Table 12. ProTox-II results for acute toxicity (LD50) and the predicted toxicity class

Compound	Predicted LD50 (mg/kg)	Predicted Toxicity Class
A-230 Russian	280	3
A-232 Russian	1	1
A-234 Russian	100	3
A-242 Russian	1340	4
A-262 Russian	1	1
Novichok 5	1600	4
Novichok 7	3000	5
A-230 American	49	2
A-232 American	14	2
A-234 American	1017	4
A-035	14	2
A-036	14	2
A-037	2	1
A-038	1000	4
A-039	8	2
A-040	8	2
A-041	2	1
A-042	1000	4
A-043	49	2
A-044	14	2
A-045	1017	4
Unknown 1	150	3
Unknown 2	300	3
Unknown 3	14	2
Iranian Novichok	233	3

Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethal dose meaning the dose at which 50% of test subjects die upon exposure to a compound. Toxicity classes are defined according to the globally harmonized system of classification of labeling of chemicals (GHS). LD50 values are given in [mg/kg].

5.3.1 Class I

Fatal if swallowed (LD50 ≤ 5): High-risk classes in ProTox-II are the categories that can cause serious

health consequences to humans if they are ingested. The body can uptake of these compounds which lead rapid onset of symptoms and even this may be life-threatening. The LD50 value of ≤ 5 mg/kg is equivalent to extreme toxicity and exhibits high potency to induce poisoning, even in low doses.

5.3.2 Class II

Fatal if swallowed (5 < LD50 ≤ 50): Compounds in this class are predicted to have LD50 values that indicate a high level of acute toxicity. These chemicals represent a major threat to human well-being and the environment, with some adverse reactions being reported even at fairly low concentrations. Extremely poisonous substances may cause rapid occurrence of symptoms, damage to organs, and, ultimately, can lead to fatality. Extreme examples of very toxic substances include some pesticides, heavy metals, and a few industrial chemicals.

5.3.3 Class III

Toxic if swallowed (50 < LD50 ≤ 300): Compounds in this class have less acute toxicity than in class II. However, exposure to substances in this class may not have immediate effects, but prolonged exposure may lead to serious effects on human health.

5.3.4 Class IV

Harmful if swallowed (300 < LD50 ≤ 2000): Those substances calculated to produce mild acute toxicity are predicted to have LD50 values reduced as compared with class III, II and I. However, exposure to harmful agents can physically be harmful at higher doses or with constant administration over time. These types of substances could be industrial solvents, household chemical agents and even some pharmaceuticals.

5.3.5 Class V

May be harmful if swallowed (2000 < LD50≤ 5000): Compounds classified in class V are predicted to cause irritation or inflammation upon contact with skin, eyes, or mucous membranes, rather than systemic toxicity. While irritants may not pose significant acute health risks at typical exposure levels, they can still cause discomfort, allergic reactions, and skin sensitization in susceptible individuals. Common examples of irritants include certain cleaning agents, cosmetics, and environmental pollutants.

5.3.6 Class VI

Non-toxic (LD50 > 5000): Compounds assigned to this class are predicted to have LD50 values indicating minimal acute toxicity. These substances are unlikely to cause significant adverse effects even at high doses and are considered safe for general use. However, it's essential to note that even substances classified as practically nontoxic may still pose risks at very high concentrations or under certain exposure scenarios. Examples of practically nontoxic substances include many food additives, some cosmetic ingredients, and certain environmental contaminants at low concentrations.

6 Discussion

6.1 Finite Dose Skin Permeation (FDSP) Calculator

After successfully running the finite dose skin permeation calculator program we conclude that the primary route of absorption is likely through the skin. Data on vapor pressures, melting and boiling points, and the octanol-water were collected, revealing relatively low values for A-230, A- 232, A-234, A-038, A-039, A-042, and especially A-242, while somewhat higher vapor pressures are observed for A- 036, A-040, A-041, and A-037. Vapor pressure, coupled with structural features like the presence of hydrophilic/hydrophobic groups, plays a crucial role in potential skin absorption. Skin permeation data were then estimated using the Finite Dose Skin Permeation calculator, and the results are summarized in Table 3. For A-230, the skin permeation (Kp) value was determined to be 3.952E-5 cm/hr, with a maximum flux, Jmax, of 0.029 mg/cm^{2}hr. It's important to note the uncertainty in these data, given that skin permeation appears to be influenced by the specific location on the human body from which the skin is obtained.

The skin permeation value (Kp) for Novichoks A-042, A-038, and A-232 is significantly lower than those for A-037 and A-041. There are substantial differences in the percentage of the compound systemically absorbed, with A-232 and A-242 exhibiting absorption percentages approximately 15 times higher than A-037 and A-041. All compounds display a markedly low percentage in the stratum corneum, consistently reaching the plasma. Lastly, there is a noteworthy variation in the time it takes to reach maximum flux, emphasizing the potential significance of this lag time when exposed to these compounds. It must be emphasized that these data

correspond to undamaged skin, and any skin damage could significantly reduce the lag time.

Understanding the behavior of the chemical during skin contact is as important as understanding the mechanism of the skin absorption process. Central considerations comprising vapor pressures, melting and boiling points and octanol-water partition coefficients determine the speed and depth to which a chemical diffuses into the skin. Vapor pressure, being the balance between the vapor and the condensed phase, represents how much a chemical will try to turn into the air. The high vapor pressure enables the product to dissipate rapidly from the skin surface, which may result in a decrease in the absorption time. Additionally, melting and boiling points define the temperature ranges on which substances change from one phase into another. A lower melting point translates to the liquid state even at normal temperature making the substance more disposed to skin contact while lower boiling point may trigger rapid evaporation which will eventually reduce skin absorption. The octanol-water partition coefficient, indicating the solubility of a substance in lipophilic (octanol) versus hydrophilic (water) environments, exhibits the layer passage across skin layers. Lipid rich stratum corneum has a higher partition coefficient, and the substances with a higher partition coefficient penetrate through it and reach the deeper layers of the epidermis. Together, understanding these physicochemical properties brings new light about chemicals and the skin, in turn, provides a basis for the absorption kinetics and toxicological implications of those chemicals

There are different skin penetration levels of the A-series chemicals. These levels have profound effects on the organism along several domains. In this regard, products with higher penetration rates pose the risk of systemic dissemination and therefore, require detailed safety evaluations which may result in tighter regulatory norms.

In addition, the formulation of topical products depends much on an in-depth understanding of these variations so that, a specialized formulation might be needed to mitigate systemic absorption for potent drugs associated with high penetration property while at the same time assuring effective localized therapy. On the one hand, compounds with decreased permeation may need formulation adjustments for the purpose of achieving an adequate skin penetration potential for optimal therapeutic outcomes. This is accompanied by increased impact of the exposure risk management, where higher penetration rate compounds imply more exposure and hazards for both, handlers and users, which in turn, requires robust risk reduction measures for human health protection. Differences in the skin permeation level also provide a foundation for various regulatory decisions, requiring more efforts on the penetration and absorption if it is a novel or re-evaluated product. However, those differences can also promote more studies and the development of new delivery systems and improved treatment efficacy as the result of better probing of the intricacies of biology and the physicochemical properties.

FDSP (Finite Dose Skin Permeation) calculators provide important hints about the performance of compounds on the skin and their absorbability into the body. It is, however, mostly important their misinterpretation by an observer should be taken under consideration. Built-in assumptions and simplifications can oversimplify the nature and complexities of substance features and one's skin, thereby continuously preventing the model from giving expected or real data. Skin variability and suboptimal experimental data contribute to the difficulty in predicting the measure. The application area becomes limited by uncertainties concerning input parameters and calibration, thus preventing the generalization of the findings to all types of scenarios. A further concern is that such erratic dynamics might defeat the very aims of the FDSP calculators. Combining the experimental data, factually mentioning uncertainties, and verifying the hypotheses will be inevitable procedures for the FDSP simulator to produce accurate and trustworthy results.

Skin damage greatly affects the skin permeability and the dermal lag time, which play a critical element in the chemical absorption exams, needed for an effective and accurate toxicological evaluation. Through any type of skin damage such as breakdown of the barrier function, the chemicals penetrate deeper into the skin with higher absorption rates. This means, on the one hand, damaged skin would be much more permeable, allowing compounds to penetrate the bloodstream faster and deeper than in healthy skin. This thorough permeability may completely remove the slack time that can cause delays in risk assessments and formulation decisions. FDSP calculators might underestimate the breadth of consequences, triggering modified safety policies and adjusted experiment settings as a remedy. Skin damage assessment sheds light on the safety requirements of clinical and occupational environments and elevates the level of risk assessment for human purposes.

6.2 Prediction of Activity Spectra for Biologically Active Substances (PASS)

The Prediction of Activity Spectra for Biologically Active Substances (PASS) is a tool in the cheminformatics computational model that enables prediction of the biologically active substances. PASS, in this case, examines the structural similarities of a chemical to the database for various activities and outcomes, so crucial for detecting chemical weapons. Applying the structure-activity relationship (SAR) principles, the task of predicting activities at PASS will be based on the comparison of structural properties to well-known patterns. The very first step of this process is the input of compound structure, followed by the search for the important molecular components that can contribute to biological activity. The similarities allow the prediction of a formula which facilitates such demands as pharmaceutical business and ecological security programs, with chemical warfare detection being key.

The structure-activity relationships (SAR) principle that asserts that the chemical composition of a compound governs what action it will carry in a biological system is the key found in the model of Prediction of Activity Spectra for Biologically Active Substances (PASS). Research by analyzing the rigid structure of the compound and, thus, comparing it to database patterns, PASS ultimately determines the possible biological activities by probability, based on common motifs in the structures. Think of such structural considerations as functional groups and molecule properties promoting size and solubility for a better understanding of the chance establishments. PASS identifies and classifies physicochemical properties and binding modes/interactions with biological targets to predict how molecules will behave based on size, stereoisomerism, and solubility. Numbers assigned imply molecular building block resemblance with known active compounds of different contexts, and thus activation predictions become more accurate.

After successfully running the program Pass for all 8 activities, we can discuss about the possibility of the occurrence of each activity. For the cholinergic activity, there is a higher chance of occurring in substances A-040 and A-041 possibly due to the F and Cl atoms. While there's a lower chance of occurring in substances A-043 and A-044. For the neurotoxic activity, there is a 40% more chance of occurring in A-045 and A-234 than in any other substance. For the toxic activity, we can see that substances A-040, A-043, A-044, A-045, A-230, A-232, and A-234.

For the respiratory failure activity, it is observed that the substances A-040, A-041, A-044, and A-232 American have the highest probability of inducing this activity, while the substances A-230, A-232, and A-234 have the lowest. On the other hand for the multiple organ failure activity we can see that substances A-230, A-232, A-234, A-242, and A-262 have the highest probabibility while A-038, A-039, and A-040 have the lowest. For the hematotoxic, carcinogenic and teratogen activities all the substances have low probabilities of causing these activities except for the A-045 and the A-232 American which have high chances of causing the hematotoxic activity. Lastly, A-038 has a high chance of causing carcinogenic activity and the Unknown 1 substance has a high chance of causing teratogenic activity, respectively.

While the Prediction of Activity Spectra for Biologically Active Substances (PASS) tool offers valuable insights into chemical compound activities, it has limitations and uncertainties to consider. Prediction accuracy heavily depends on the quality and diversity of the reference database, potentially skewing results towards certain compounds or activities and limiting applicability, especially for novel drugs. Even though PASS predictions produce credible estimates for the activity likelihood, there are no guarantees that this may be directly observable. The biological processes are extremely complex and systematic and the interactions between compounds with the environment are non-static. On the other hand, PASS's overly-dependence on structural features enables it to overlook the nuanced molecular interactions or face the variables that may bother its accuracy such as target specificity and cellular milieu. To verify the PASS predictions one must use critical interpretation uniting the computational chemistry studies, biology, and experimental data in favor of correct interpretations.

6.3 ProTox-II

ProTox-II is a computational tool that calculates the toxicological effects of a certain chemical compound, with the ability to detect if it is hazardous to life or not. Median lethal dosage (LD50) the laboratory test aiming at evaluating the probability of being killed by 50% of the test population is a very important parameter of toxicology.

The numerical representation of LD50 values is indispensable as it allows for a scientific basis for acute toxicity. Toxicologists can evaluate the relative toxicity of various compounds and develop dose-response connections by calculating the LD50

of a compound through experimental testing on animal models. Greater toxicity is indicated by a lower LD50 value, which suggests that less of the substance is needed to cause harm. Predicted LD50 values are used in ProTox-II to group substances into toxicity groups according to how likely they are to cause acute toxicity. Generally speaking, these toxicity groups span from extremely toxic to almost harmless, with several intermediary categories denoting different toxicity levels.

All substances were studied under the ProTox-II program and the results were significant. Substances A-232, A-262, A-037 and A-041 were found to have the lowest median lethal dose (LD50) and thus, categorized as the number 1 predicted toxicity class. Substances A-230 American, A-232 American, A-035, A-036, A-039, A-040, A-043, A-044, and the Unknown 3 substance have a median lethal dose from 5 to 50 mg/kg and thus categorized as class 2 of predicted toxicity. Substances A- 230, A-234, Unknown 1, Unknown 2, and Iranian Novichok have a median lethal dose from 50 to 300 mg/kg and are categorized as class 3 of predicted toxicity. Substances A-242, Novichok 5, A-234, A-038 American, A-042 American, and A- 045 American have a median lethal dose from 300 to 2000 mg/kg and are categorized as class 4 of predicted toxicity. Lastly, the substance Novichok 7 has the lowest lethal dose of 3000 mg/kg and is categorized as class 5 of predicted toxicity.

While ProTox-II offers valuable insights into chemical compound toxicological characteristics, users must recognize its limitations and uncertainties. Unlike computational models based on structure-activity relationships (SAR), ProTox-II predictions might not have a full representation of the real biological systems and special compound structures, which may result in inaccuracies, especially for the compounds which is not in the training set. Model accuracy depends on the quality and diversity of the training set with new drugs resulting in increased bias. The lack of training sets data that is open to the public makes it even more difficult to evaluate. Besides that, the toxicological reactions disparity among species and individuals claim a careful interpretation. Exploring both the principles of Toxicology and Computational Chemistry is therefore of great importance as it plays a role in robustly evaluating ProTox-II predictions, avoiding false conclusions without experiment confirmation.

7 Conclusion

In conclusion, QSAR models like FDSP, PASS, and ProTox-II are very useful for the toxicological studies of chemical warfare agents, including those from the A-series. Skin Permeation analysis by Finite Dose Spread Spectrometer (FDSP) will also reveal the significance of studying through the skin as the main source of input to the test substances. Vapor pressures, molecular structure parameters, and skin permeability values, particularly those of A-230, revealed certain absorption routes, which highlights their significance of in the assessment of skin porosity. Nevertheless, uncertainties do exist as a result of unforeseeable displacements of skin locations, which can influence the precision of the predictions. The PASS software is found to help provide critical information on how compounds may act. Differentiation in the cholinergic, neurotoxic, pulmonary, hematotoxic, carcinogenic, and teratogenic actions of the drugs caused the occurrence of various types of toxicological profiles. The observation of how some drugs bring out some behaviors shows how sophisticated their toxicological effects are and promotes the understanding of their biological interactions. Moreover, the ProTox-II tool provides an in-depth evaluation of the toxicological problems related to the compounds by putting into different toxicity categories the compounds according to the expected toxicity. Certain substances like A-232 and A-262 are being classified in the highest predicted toxicity level category, meaning a severe risk coming from each one's exposure. In contrast, some compounds demonstrate varying toxicity within the A-series, as the compounds are classified on the toxicity scale according to their average LD values. The findings strengthen the knowledge we have on the possible threats that are associated with each substance, and hence, they serve as a guide for future studies and inform risk mitigation efforts employed in defense and environmental applications.

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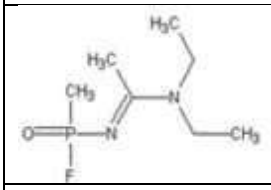
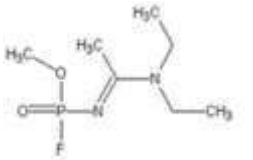
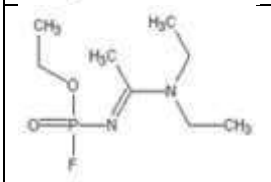
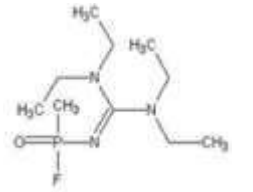
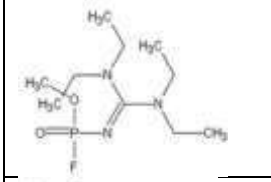
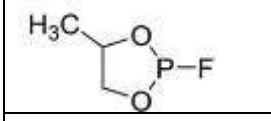
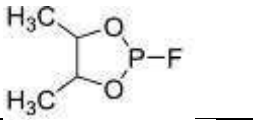
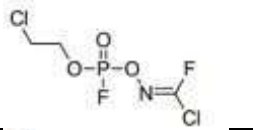
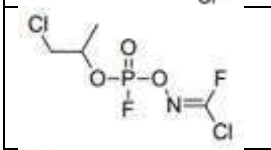
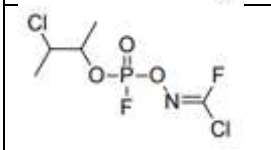
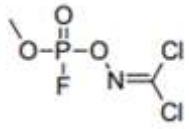
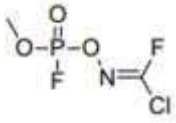
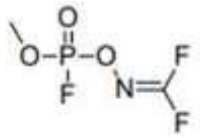
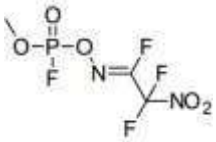
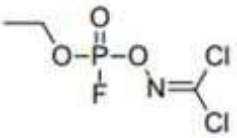
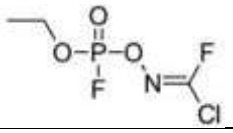
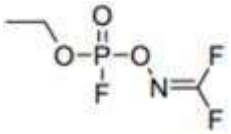
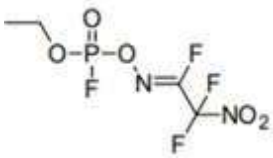
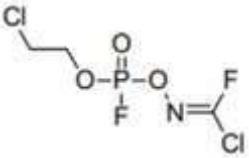
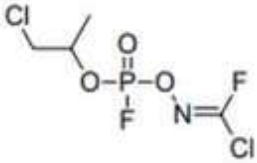
APPENDIX

Table 1. A-Series structure

Structure	Name	Citation
	A-230	[2]
	A-232	[2]
	A-234	[2]
	A-242	[2]
	A-262	[2]
	Novichok 5	[2]
	Novichok 7	[2]
	A-230	[3]
	A-232	[3]
	*A-234*	[3]

Structure	Name	Citation
	*A-035*	[4]
	*A-036*	[4]
	*A-037*	[4]
	*A-038*	[4]
	*A-039*	[4]
	*A-040*	[4]
	*A-041*	[4]
	*A-042*	[4]
	A-043	[4]
	A-044	[4]

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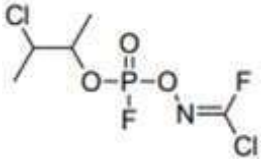
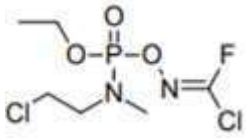
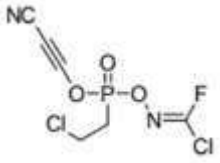
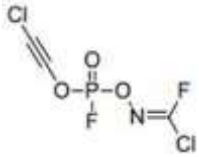
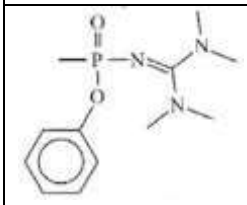
	A-045	[4]
	Unknown 1	[4]
	Unknown 2	[4]
	Unknown 3	[4]
	Iranian Novichok	[4]

Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Michail Chalaris was responsible for the supervision. The authors equally contributed to the present research, at all stages from the formulation of the problem to the final findings and solution.

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Conflict of Interest

The authors have no conflicts of interest to declare.