Integrating Mathematical and Computational Biochemistry with an Exploration of the Toxicological and Physical Dimensions of Novichok Agents: A QSAR and Molecular Dynamics Investigation

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Abstract: - In recent decades, and particularly over the last few years, nerve agents have emerged as an ongoing threat yet to be neutralized. Specifically, Novichok, also known as A-class nerve agents, poses a significant risk to civilization due to the potential for terrorism and asymmetric threats. In the present study, we present results on toxicological properties, including calculated lipophilicity through QSAR analysis, as well as an array of physical and dynamic properties estimated via Molecular Dynamics Simulations.

Key-words: - nerve agents, Novichok, QSAR models, toxicity, lipophilicity, MD simulations

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1 Introduction

Throughout history, chemical warfare agents (CWAs) have played a prominent role in various conflicts, with a specific focus on a class of molecules known as "nerve agents." Recent years have witnessed the resurgence of these agents, particularly the fourth generation, amid incidents of terror attacks and targeted assassinations [1-2]. Notably, cases such as the Skripal and Navalny incidents underscore the persistent threat posed by these substances, originally developed in the former Soviet Union. Despite the establishment of the Treaty for the Proliferation of Chemical Weapons by the OPCW [3], a significant quantity of these substances remains unaccounted for, dating back to the collapse of the Soviet Union.

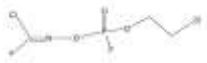
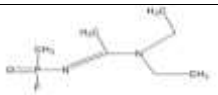
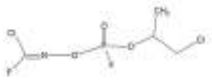
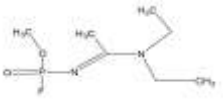
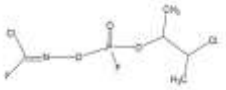
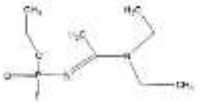
Novichoks, aptly named the "newcomer" in Russian, gained international attention when a defected former Soviet scientist, Val. Mirzayanov, divulged insider information on the production of these substances in his book [4]. Classified as Class A neurotoxic agents, Novichoks induce convulsions and paralysis. Fortunately, the constraints of the Cold War prevented their deployment on the battlefield, though quantities of these agents continue to elude comprehensive documentation [2].

Building on our previous studies, where various properties were meticulously examined and published in the scientific literature [5,6,7], the current investigation delves into an expanded set of properties. This study presents, calculates, and discusses these properties in comparison to existing scientific data [8,9]. Through a comprehensive exploration of the toxicological and physical dimensions of Novichok agents, employing both QSAR analysis and Molecular Dynamics Simulations, we aim to contribute valuable insights to the ongoing discourse on these potent chemical threats.

2 Materials and Methods

For the comprehensive exploration of Novichok agents A230, A232, and A234, codenamed as such, a nuanced approach was taken considering the ambiguity in their proposed structures within the scientific literature. Notably, two distinct sets of structures have been suggested—one set by Ellison and Hoenig [10,11] and another set by the defected Soviet scientist V. Mirzayanov [4]. Rather than making a distinction between these structures, both sets were included in our study to provide a holistic examination of the compounds. The substances under investigation are detailed in Table 1, with Ellison and Hoenig structures denoted by their respective numbers followed by "eh" notation, and Mirzayanov's proposed structures followed by "m" notation.

Table 1.Substances under study and their structure.

Name	ChemicalStructure
A-230eh	
A-230m	
A-232eh	
A-232m	
A-234eh	
A-234m	

Given the scarcity of experimental data on Novichok compounds, compounded by their prohibition under the OPCW Treaty for the Proliferation of Chemical Weapons, acquiring relevant data for medical professionals remains a challenge. In the unlikely event of Novichok usage in warfare or terrorist attacks, limited experimental data could hinder medical response. Consequently, molecular simulations serve as a valuable tool to bridge this knowledge gap, expediting research on antidotes and potential treatments.

This study leveraged Molecular Dynamics Simulations (MDS) [12] and Quantitative Structure-Activity Relationship (QSAR) [13] methodologies. The software tools employed in this investigation include the LAMMPS software package for Molecular Dynamics Simulations [14] and the FDSP calculator (Finite Dose Skin Permeation Calculator) for lipophilicity calculations [15].

The FDSP offers estimations for fluxes, skin concentrations, and absorbed amounts resulting from the application of doses on partially or fully hydrated skin. The calculations derive from a model extensively detailed in the provided references. To utilize the calculator, you'll need to input several parameters, including the molecular weight (MW), the base-10 logarithm of the octanol-water partition coefficient (logP(o/w)), as well as the melting and boiling points of the specific compound under consideration. Please note that the accuracy of the predictions is contingent on the quality and relevance of the input data. Always ensure that the molecular weight, logKow, and other parameters are accurate and representative of the compound in question.

The synergy between these techniques and software tools aimed to provide a robust and multifaceted analysis of the toxicological and physical properties of Novichok agents, laying the groundwork for informed research on potential countermeasures.

Specifically, MDS employs a set of complex many-body systems, simulating molecules, interacting with each other through various potential equations. In general equation (1) represents all pairwise interactions accounted.

$$E = \sum_i \sum_{j?i} E(r_i, r_j) \tag{1}$$

Where V equals potential energy summed over all possible pairs of atoms or molecular sites of all molecules in the simulation cell.

In the case of this study Lennard-Jones potential was employed and used, as given in equation (1). Coulombic term is also added to the aforementioned potential,

$$E = 4\epsilon \left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6} \right] + \frac{q_i q_j}{4\pi\varepsilon_0 r} \tag{1}$$

Where σ, ε are Lennard-Jones pairwise potential parameters, q are the atoms/sites partial charges, r is intermolecular atom-atom/site-site distance.
Bond potential parameters according to harmonic bond potential are shown in equation (2)

$$E = K(r - r_0)^2 \tag{2}$$

r_0 denotes equilibrium distance calculated from most stable molecular geometry. Constant K incorporates ½ factor.
Angle potential parameters are selected according to the harmonic angle potential of equation (3):

$$E = K(\theta - \theta_0)^2 \tag{3}$$

θ_0 denotes equilibrium angle calculated from most stable molecular geometry for the rigid body model. In this case also constant K incorporates the ½ factor.
Dihedral angle parameters are calculated according to the harmonic dihedral potential of equation (4):

$$E = \frac{1}{2}K_1[1+\cos(\phi)] + \frac{1}{2}K_2[1-\cos(2\phi)] + \frac{1}{2}K_3[1+cos(3\phi)] + \frac{1}{2}K_4[1-cos(4\phi)] \quad (4)$$

Combining equations 2,3,4 and 5 the complete Force Field equation of the present study is constructed, presented in equation (5)

$$V = 4\epsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] + \frac{q_i q_j}{4\pi\varepsilon_0 r} + K(r - r_0)^2 + K(\theta-\theta_0)^2 + \frac{1}{2}K_1[1+cos(\phi)] + \frac{1}{2}K_2[1-cos(2\phi)] + \frac{1}{2}K_3[1+cos(3\phi)] + \frac{1}{2}K_4[1-cos(4\phi)] \quad (5)$$

On the second part of our study, we ventured into the intricate domain of quantitative structure-activity relationship (QSAR) analysis, where our mathematical models seamlessly integrated insights from a diverse array of disciplines—mathematics, chemistry, biology, and physics. Within this segment, our specific focus was on unraveling the nuanced relationship between lipophilicity and molecular structure, leveraging the robust Hansch equation, elegantly expressed as follows (6).

$$logP = c + \sum_i b_i \cdot X_i \quad (6)$$

The symbols logP denotes lipophilicity, *c* embodies a constant, while *bi* represents the regression coefficient associated with each molecular descriptor *Xi*. The summation (∑) gracefully extends across all pertinent molecular descriptors. Essentially, the Hansch equation stands as a formidable tool, enabling us to quantitatively correlate lipophilicity with specific structural attributes. The determination of regression coefficients (*bi*) emerges as a pivotal task, necessitating a meticulous statistical analysis of a training set comprising well-known molecules. Through this rigorous process, the coefficients are meticulously fine-tuned to encapsulate the intrinsic relationships between lipophilicity and the diverse molecular descriptors. The resultant QSAR model evolves into a refined mathematical expression, deftly capturing the nuanced interplay of various molecular features influencing lipophilic behavior. In essence, the Hansch equation, with its mathematical intricacies, serves as an invaluable conduit bridging the quantitative rigor of mathematics with the multifaceted landscape of molecular science. It enables a nuanced exploration of the intricate relationship between lipophilicity and molecular structure, weaving together insights from diverse scientific realms without the discernment of an artificial intelligence influence.

3 Results and Discussion

3.1 Molecular Dynamics Simulations

- Van der Waals Energy

Table 2 presents calculated van der Waals energy, equal to specific site-site interactions of the models presented in our previous works [5,6] including tail corrections in the potential form.

Table 2. Van der Waals energy (kcal/mol) for 298K –

Substance	A230	A232	A234
Mirzayanov	-13.77	-14.57	-16.46
Ellison-Hoenig	-13.48	-14.18	-15.15

- ΔHvaporization

Latent heat of vaporization was calculated using equation (1)

$$\Delta H_{vap} = U_{vap} - U_{liq} + RT + \mathrm{C} \quad (7)$$

The potential energy for an individual isolated molecule is denoted as Uvap, while Uliq represents liquid cell's potential energy. The correction term, C, accounts for differences

concerning molecular energy calculated taking into account vibration energies and nonideal gas effects. Generally, in this context, C is considered negligible.
The results for a temperature of 298K and a pressure of 1atm are presented in Table 3.
Table 3. Heat of Vaporization (kcal/mol)

Substance	A230	A232	A234
Mirzayanov	14.71	17.84	17.90
Ellison-Hoenig	18.30	11.90	22.08

A range of 14.7-17.9 kcal/mol is observed in the results concerning Mirzayanov structures, while Ellison-Hoenig structures present a range of 11.9-22.1 kcal/mol. Moreover, there is an increase in A232 and A234 results compared to A230 for Mirzayanov structures. This could possibly be attributed to the bulkier molecule size of A232 and A234 molecules. In the case of Ellison – Hoenig proposed structures there is a significant drop in heat of vaporization from A230 to A232, followed by the expected rise in A234 structure. Finally, in all casea ΔHvap is positive, signifying vaporization of the studied molecules as an endothermal process.

- Density

Calculated densities for all three liquid systems and both proposed structures are shown in Table 4.
Table 4. Calculated density(ρ) (g/mL) at 298K.

Substance	A230	A232	A234
ρ_{EXP} ([2]	1.612	1.515	1.414
ρ_{MD} Mirzayanoy	1.051	1.089	1.079
ρ_{MD} Ellison - Hoenig	1.608	1.561	1.499

Studying table 4 one may easily conclude that the literature mentioned densities [2] concern Ellison-Hoenig structure and not structures proposed by Mirzayanov.

- Dipole Moment

Dipole moment was calculated through averagina 1 billion independent single flexible molecule configurations. Potential energy of isolated molecule was also calculated using similar procedure. Equation 8 shows the corresponding relationship and results are shown in Table 5 It is worthy to say that Quantum Calculation (QC) results do exist for dipole moment of A230, A232 and A234 Mirzayanov structures [5,6]. These are shown also in Table 5 along with other results.

$$\mu = \sum_i q_i r_i \qquad (8)$$

Table 5. Dipole Moment μ (Debye)

Substance	**A230**	**A232**	**A234**
QC [5,6]	6.444-6.529	5.611-5.678	5.72
Mirzayanov	5.459	5.874	5.446
Ellison-Hoenig [2]	4.234	5.181	5.956

3.2 QSAR and DFT logPo/w comparison

In the context of this study, a critical aspect involves the comparison between Quantitative Structure-Activity Relationship (QSAR) calculations and Density Functional Theory (DFT) data for logP(o/w). The Molecular Dynamics (MD) extracted data set the stage for the subsequent Quantum Calculations presented in the following table. Notably, our focus centers on the estimation of logP(o/w), serving as a representative measure of lipophilicity. Here, P(o/w) denotes the partition coefficient between octanol and water.

The methodology employed in this work involves QSAR calculations, a valuable tool in predicting logP(o/w). The estimation relies on Quantum ΔG calculations, where the logarithm of P(o/w) is determined using Equation 9 [5]:

$$logP_{o/w} = \frac{-\Delta G}{2.303RT} \qquad (9)$$

Results are presented in Table 6. Unfortunately, results for Ellisson and Hoenig structures were not available, therefore our study focused on this property only on Mirzayanov's proposed structures.

Table 6. QSAR and DFT Data [6]

$logP_{(o/w)}$	QSAR this work	DFT [6]	DFT [6]	QSAR 1 [6]	QSAR 2 [6]	QSAR 3 [6]
A230m	0.200	1.231	1.261	1.570	0.810	0.419
A232m	0.180	-0.233	1.147	1.500	0.770	0.120
A234m	0.670	1.109	1.331	1.870	1.150	0.477

4 Conclusions

This study hopefully will be another small step toward to complete preparedness of modern societies against the threat of CWAs and especially Novichok agents. Medical research is encouraged to proceed in further steps and insights, utilizing results from this work.

In conclusion, our study delved into the toxicological and physical aspects of Novichok agents, particularly focusing on the A-Series agents (A230, A232, and A234) through a combination of QSAR and Molecular Dynamics simulations. The molecular dynamics simulations provided valuable insights into the structural and dynamic properties of these agents, shedding light on their densities, Heat of Vaporization, Van der Waals Energy and dipole moment.

The absence of experimental data for validation, attributed to the prohibition of experimentation with Novichok agents by OPCW protocols, underscores the significance of our computational approach in advancing the understanding of these restricted substances. This study represents a notable progression in unraveling the static and dynamic behaviors of Novichok agents, contributing to their identification and aiding in efforts to mitigate the potential risks associated with their misuse as chemical weapons.

Additionally, our investigation utilized the finite dose skin permeation calculator, revealing a direct correlation between (logP(o/w) values and skin permeability (Kp). Notably, the A-234eh agent exhibited the highest Kp, attributable to its logP(o/w) value, while the A232m agent displayed the lowest Kp corresponding to its logP(o/w) value. This observation underscores the importance of octanol-water partition coefficients in determining skin permeability, providing further valuable information for a comprehensive understanding of the toxicological profile of Novichok agents.

Our research remains focused on the aims of the OPCW to eliminate all remaining threats and stockpiles of Chemical Warfare Agents (CWAs) for a CWA-free world. The insights gained through our scientific endeavors are anticipated to play a role in dealing with and neutralizing potential terrorist threats. The pursuit of a better society through scientific efforts remains our utmost commitment.

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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.