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S. I. Bendas[1], PhD student; https://orcid.org/0009-0003-6654-1061
B. M. Galkin[2], PhD in Biological Sciences, Professor;
https://orcid.org/0000-0002-9503-1277
N. A. Kyrylenko[1], PhD in Biological Sciences, Associate Professor;
https://orcid.org/0000-0002-2612-4656
Odesa I. I. Mechnikov National University, 2 Vsevoloda Zmiienka St,
Odesa, 65082, Ukraine
[1]Department of Physiology, Human Health and Safety and Natural Science Education;
[2]Biotechnology Science-Educational Centre, e-mail: bgalkin@ukr.net

IN SILICO STUDY OF THE ANTI-INFLAMMATORY PROPERTIES OF PFOA, PFOS, $KClO_4$, AND $AlCl_3$

In this study, we used a machine learning-based toxicity prediction tool to assess the potential toxic effects of military and civil chemical pollutants (perfluorooctanoic acid, perfluorooctane sulfonic acid, $AlCl_3$, and $KClO_4$). Additionally, molecular docking and molecular dynamic simulations were employed to explore the interaction of perfluorooctanoic acid and perfluorooctane sulfonic acid with key protein targets. The results indicate that these ligands exhibit high binding affinities for human serum albumin, monoamine oxidase A and COX-1, suggesting potential impacts on protein function.

Keywords: inflammation, molecular docking analysis, molecular dynamic, aluminium chloride, potassium perchlorate, perfluorooctanoic acid, perfluorooctane sulfonic acid, binding.

Military operations cause extensive environmental damage. This includes industrial and chemical contamination, wildfires ignited by shelling, pollution and waste from military vehicles, toxic substances from weapons and missiles, emissions from the displacement of people, nuclear hazards and destruction of water resources [14]. War-induced environmental destruction is likely to lead to higher rates of illness and death among civilians [12]. Chemical pollution poses the most significant environmental threat in this situation [18]. Detonation of rockets and artillery results in the emission of a complex mixture of chemical compounds. These include carbon oxides, water vapor, nitrogen oxides, formaldehyde, hydrogen cyanide, and a variety of toxic organic substances. Additionally, heavy metals such as lead, arsenic, and mercury are released into the environment. Aluminum, while a versatile and widely used metal, can become a pollutant in the context of war. Its presence as a pollutant in war-torn environments arises from several factors, including its use in military equipment, weaponry, and infrastructure. Aluminum (Al^{3+}) has a strong tendency to bind with and cross-link proteins. Unlike other common metals like iron, manganese,

and zinc, aluminum is not known to have any physiological role in the human body. Besides causing oxidative stress and attaching to negatively charged membrane structures in neurons, aluminum can also alter calcium signaling pathways in the hippocampus, which are essential for neuronal plasticity and, consequently, memory. [21, 13, 16]. Perchlorate is another chemical compound commonly employed in military ordnance and equipment, which poses a significant environmental and health concern. Its presence has been detected in various environmental matrices, including drinking water, air, soil, and even human breast milk. Human exposure to perchlorate primarily occurs in regions affected by armed conflict and areas adjacent to military training facilities [24, 8]. These pollutants are subsequently dispersed into soil, air, and water

Furthermore, munitions may contain per- and polyfluoroalkyl substances (PFAS), which are characterized by their exceptional environmental persistence [17]. PFAS constitute a group of synthetic chemicals renowned for their oil-, water-, and heat-resistant properties. These compounds, commonly used in producing a range of consumer and industrial products, including non-stick cookware, food packaging, firefighting foams, and munitions, have become ubiquitous environmental contaminants due to their persistent nature [4]. Humans are primarily exposed to these contaminants through inhalation of contaminated air and dust, as well as ingestion. Such exposure has been associated with endocrine disruption, compromised immune function, and an increased risk of developing certain cancers [27, 17, 5]. Among the most prominent PFAS are perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), which exhibit exceptional stability and resistance to degradation [20]. Bioaccumulation of PFAS in critical organs, such as the blood and liver, can induce adverse health effects even after cessation of exposure, underscoring the long-term implications of these persistent contaminants [1, 15]. Human serum albumin (HSA) is the main protein responsible for transporting substances in the blood and has been found to interact with PFAS at multiple binding sites. Consequently, it plays a significant role in the distribution and accumulation of PFAS in the body [9]. From the literature it is also known that PFAS activate nuclear receptor protein, specifically Peroxisome Proliferator-Activated Receptor alpha (PPARα) and Peroxisome Proliferator-Activated receptor gamma (PPARγ) [7].

HAS and PPAR-gamma have important roles in the inflammation process, either directly or indirectly. PPAR-gamma has strong anti-inflammatory effects and human serum albumin can modulate inflammatory responses, although its role is more indirect. These proteins are potential therapeutic targets for controlling inflammation in various diseases [9, 7]. The aim of this study is to investigate the potential toxicity of perfluorooctanoic acid, perfluorooctane sulfonic acid, $AlCl_3$, and $KClO_4$ using *in silico* approaches.

Materials and methods

Toxicity calculation for aluminium chloride, potassium perchlorate, perfluorooctanoic acid and perfluoro-octanesulfonic acid was performed with virtual lab ProTox 3.0 based on machine learning algorithms [2].

Molecular docking was provided using AutoDock Vina 1.2.5. (http://vina.scripps.edu), Schrödinger Maestro –2023–2 Glide (trial license). The molecular docking process was performed utilizing crystal structure of human serum albumin (7Z57), crystal structures of the ligand binding domain of human PPAR-gamma (2F4B), Human Monoamine Oxidase A in complex with Clorgyline (2BXR), Human COX-1 Crystal Structure (6Y3C) from the database of biological macromolecules PDB (http://www.rcsb.org/). During the research, perfluorooctanoic acid and perfluorooctane sulfonic acid were docked. The binding site was automatically identified based on the position of the reference ligand.

AutoDockVina-1.2.5. The structures of the ligands (perfluorooctanoic acid and perfluorooctane sulfonic acid and reference ligands) were provided in *.pdb format and optimized by the internal energy value in the Avogadro program (v 1.2.0) and the Merck molecular force field - algorithm (MMFF94), protonation at a physiological pH value of 7.4. Protein structures were optimized using AutoDock tools 1.5.7. For protein optimization polar hydrogens, Kollman charges were added and pdbqt format structure was obtained. The area of docking process was determined using grid box. We determined the strong binding affinity by the most negative Autodock Vina score [21].

Schrödinger Maestro Glide. The structures of the ligands (perfluorooctanoic acid and perfluorooctane sulfonic acid and reference ligands) were optimized using LigPrep, OPLS2005 force field algorithm, the pH range for the generation of tautomers and protonated states was 7,4. The protein was modelled using the Protein Preparation of Schrödinger Suite; to prepare the protein structure, hydrogen atoms were added and hydrogen bonds were optimized, pH 7.4. The following formula was used to rank the ligands based on their G-scores: G-score = (0.05*vdW) + (0.15*Coul) + Lipo + Hbond + Metal + Rewards + RotB + Site (1); where vdW is the van der Waals energy, Coul represents the Coulomb energy, the term Lipo explains the lipophilicity Rewards describes the favorable hydrophobic interactions, Hbond is an indicator that reflects the hydrogen bond, Metal provides information about metal binding, RotB informs about the negative effect related to the freezing of spin bonds, and Site determines the polar interactions in the active site. The binding free energies (MM-GBSA) of the complexes were determined using the Prime module within the Schrödinger suite. The binding energy is determined using the formula: ΔG_bind = E_complex(minimized) - E_ligand(minimized) - E_receptor(minimized) [28, 29].

Visualization of docking results was performed using Schrödinger Maestro, DiscoveryStudio2021 (BIOVIA, Dassault Systèmes. Discovery Study), PyMOL (The PyMOL Molecular Graphics System, Version 3.0 Schrödinger, LLC).

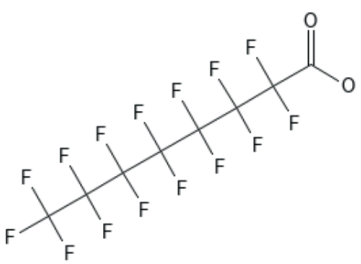
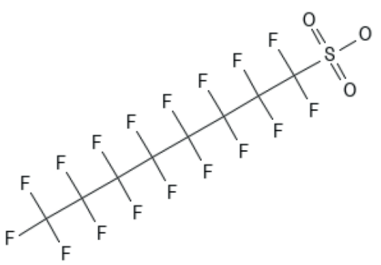
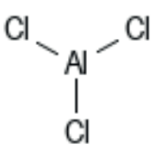
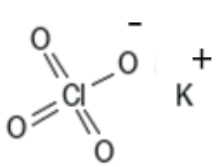
Molecular dynamic simulation was performed for complex (perfluorooctanoic acid-human serum albumin, $AlCl_3$ - human serum albumin) using software PlayMolecule. Ligands were prepared with GAFF2 force field and the parametrization function. We used ProteinPrepare tools for protein preparation [26] and SystemBuilder for building system for simulation (pH=7,4, forcefield Amber) [6, 26]. SimpleRun was used for the production molecular dynamic run, with a run time of 6 ns, globular simulation type [26].

Results and Discussion

We estimated the toxicity of a number of compounds that may have a military origin, using machine learning algorithm lab ProTox 3.0. ProTox 3.0 is particularly useful for predicting the toxicity of newly synthesized or experimentally unstudied compounds but can also be applied to already known chemicals to confirm or refine existing data. While these compounds may be part of the database on which the training algorithms were based, the new versions of ProTox 3.0 use improved machine learning models and more complete and updated datasets, potentially allowing for the consideration of new aspects of toxicity that may have been missed in previous studies [2]. Also, we provided docking analysis of perfluorooctanoic acid and perfluorooctane sulfonic acid, determining docking scores, free binding energy and mechanisms of binding. For a broader understanding of the stability of the complexes (human serum albumin with perfluorooctanoic acid and human serum albumin with $AlCl_3$), molecular modeling was carried out. All studied ligands in our research were presented in table 1.

Table 1

Structures of the studied compounds

Perfluorooctanoic acid (PFOA)	Perfluorooctane sulfonic acid (PFOS)
	
Aluminum chloride	**Potassium perchlorate**
	

Based on *in silico* toxicity study all studied substances ($KClO_4$, $AlCl_3$, PFOA, PFOS) may have the potential to cross the blood-brain barrier (Table 2). According to the prediction results, potassium perchlorate and aluminium chloride have influence on estrogenic receptors and have activity on transthyretin (TTR). Gorgogietas and colleagues indicate that aluminum salts cause a significant rise in estrogen receptor protein levels, potentially through alterations in estrogen receptor gene expression or the stability of the estrogen receptor protein. The authors assume that this effect could have impact on breast physiology by influencing estrogen receptor-mediated gene expression, either directly or indirectly, through interactions with estrogen receptor DNA binding [10]. Also, potassium perchlorate is primarily known for its role in disrupting thyroid function by inhibiting iodide uptake, which can lead to thyroid hormone imbalances [30]. The direct influence of $KClO_4$ on estrogenic receptors is less documented. The search results in scientific literature do not provide any information about the activity of potassium perchlorate or aluminum chloride on transthyretin (TTR). Furthermore, based on the results of ProTox 3.0 $KClO_4$ can have impact on pregnane X receptor (PXR). PXR is a nuclear receptor recognized as a key regulator of xenobiotics. It plays a crucial role in the regulation of the expression of genes responsible for drug-metabolizing enzymes and drug transporters, playing a vital role in detoxifying and removing xenobiotics and endotoxins from the body [23]. But there is no evidence in the scientific literature that KClO4 has this influence. $AlCl_3$ exceeds the threshold for cardiotoxicity and ecotoxicity. $AlCl_3$ is predicted to be active in CYP2C9 inhibition according to ProTox 3.0, which means that the compound is likely to inhibit the enzyme cytochrome P450 2C9 (CYP2C9). It may cause adverse interactions with drugs that are processed by this enzyme, affecting their efficacy and safety. PFOA reaches levels associated with nephrotoxicity, carcinogenicity, and Tumor Supressor p53. PFOS acid surpasses the threshold for respiratory toxicity.

ProTox 3.0 predictions indicate that PFOA and PFOS may have higher toxicological risks, particularly related to carcinogenicity, nephrotoxicity, and potential endocrine disruption. In November 2023, the International Agency for Research on Cancer (IARC) designated PFOA as a “carcinogen to humans” (Group 1) and PFOS as “possibly carcinogenic to humans” (Group 2B) [30]. Ducolomb and his colleagues have shown that PFOS and PFOA act on steroidogenic ovarian cells, acting as endocrine disruptors, which could influence the functions dependent on sexual steroids [3]. $KClO_4$ and $AlCl_3$ also demonstrate significant potential for toxicity through multiple pathways, with $KClO_4$ potentially posing risks to the endocrine systems, and $AlCl_3$ demonstrating cardiotoxicity. In the scientific literature, there is evidence that potassium perchlorate induces the development of dystrophic processes in the bone tissue of the jaws of rats with hypothyroidism. $KClO_4$ blocks the absorption of iodine by the thyroid gland and the synthesis of iodinase, which confirms the results of prediction [30]. $AlCl_3$ is recognized for its propensity to accumulate in several body tissues, including the liver, pancreas, kidneys, brain, and heart. It has been linked to liver damage, kidney toxicity, nervous system impairment, and heart toxicity [11].

Table 2

Prediction toxicity of potassium perchlorate, aluminum chloride, PFOA and PFOS

Target	$KClO_4$	$AlCl_3$	PFOA	PFOS
	Probability/ Prediction	Probability/ Prediction	Probability/ Prediction	Probability/ Prediction
1	2	3	4	5
Hepatotoxicity	0.98/In	0.94/In	0.81/In	0.89/In
Neurotoxicity	0.91/In	0.68/In	0.88/In	0.94/In
Nephrotoxicity	0.62/In	0.70/In	**0.51/Ac**	0.52/In
Respiratory toxicity	0.61/In	0.67/In	0.59/In	0.54/Ac
Cardiotoxicity	0.97/In	**0.55/Ac**	0.89/In	0.88/In
Carcinogenicity	0.80/In	0.55/In	**0.63/Ac**	0.68/In
Immunotoxicity	0.99/In	0.99/In	0.99/In	0.99/In
Mutagenicity	0.75/In	0.84/In	0.92/In	0.64/In
Cytotoxicity	0.73/In	0.68/In	0.68/In	0.75/In
BBB-barrier	0.91/Ac	0.99/Ac	0.97/Ac	0.91/Ac
Ecotoxicity	0.52/In	**0.68/Ac**	0.85/In	0.61/In
Clinical toxicity	0.85/In	0.83/In	0.79/In	0.76/In
Nutritional toxicity	0.62/In	0.74/In	0.92/In	0.86/In
AhR	1/In	0.99/In	1/In	1/In
AR	1/In	1/In	1/In	1/In
AR-LBD	0.99/In	0.99/In	1/In	1/In
Aromatase	0.99/In	0.99/In	1/In	0.99/In
ER	**1/Ac**	0.99/In	1/In	0.99/In
ER-LBD	0.98/In	0.96/In	1/In	0.98/In
PPAR-Gamma	0.99/In	1/In	1/In	1/In
nrf2/ARE	1/In	0.90/In	1/In	0.96/In
HSE	1/In	0.90/In	1/In	0.96/In
MMP	0.99/In	0.93/In	1/In	0.93/In
Tumor Supressor p53	0.98/In	0.89/In	**0.77/Ac**	0.97/In
ATAD5	0.99/In	0.99/In	1/In	1/In
THRα	0.85/In	0.94/In	0.90/In	0.90/In
THRβ	0.81/In	0.93/In	0.78/In	0.78/In

Table 2

1	2	3	4	5
TTR	**0.58/Ac**	**0.56/Ac**	0.97/In	0.97/In
RYR	0.97/In	0.95/In	0.98/In	0.98/In
GABAR	0.71/In	0.71/In	0.96/In	0.96/In
NMDAR	0.90/In	0.93/In	0.92/In	0.92/In
AMPAR	0.99/In	0.99/In	0.97/In	0.97/In
KAR	1/In	1/In	0.99/In	0.99/In
AChE	0.58/In	0.64/In	0.81/In	0.89/In
CAR	0.99/In	1/In	0.98/In	0.98/In
PXR	**0.59/Ac**	0.55/In	0.92/In	0.92/In
NADHOX	0.69/In	0.75/In	0.97/In	0.97/In
VGSC	0.8/In	0.75/In	0.95/In	0.95/In
NIS	0.94/In	0.94/In	0.98/In	0.98/In
CYP1A2	0.92/In	0.87/In	0.9/In	0.93/In
CYP2C19	0.88/In	0.84/In	0.86/In	0.89/In
CYP2C9	0.54/In	**0.59/Ac**	0.55/In	0.60/In
CYP2D6	0.88/In	0.79/In	0.87/In	0.87/In
CYP3A4	0.98/In	0.98/In	0.99/In	0.99/In
CYP2E1	0.96/In	0.92/In	0.99/In	0.90/In

Notes: In-inactive, Ac-active, Aryl hydrocarbon Receptor (AhR), Androgen Receptor (AR), Androgen Receptor Ligand Binding Domain (AR-LBD), Estrogen Receptor Alpha (ER), Estrogen Receptor Ligand Binding Domain (ER-LBD), Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma), Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE), Heat shock factor response element (HSE), Mitochondrial Membrane Potential (MMP), Phosphoprotein (Tumor Supressor) p53, ATPase family AAA domain-containing protein 5 (ATAD5), Thyroid hormone receptor alpha (THRα), Thyroid hormone receptor beta (THRβ), Transtyretrin (TTR), Ryanodine receptor (RYR), GABA receptor (GABAR), Glutamate N-methyl-D-aspartate receptor (NMDAR), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR), Kainate receptor (KAR), Achetylcholinesterase (AChE), Constitutive androstane receptor (CAR), Pregnane X receptor (PXR), NADH-quinone oxidoreductase (NADHOX), Voltage gated sodium channel (VGSC), Na+/I- symporter (NIS)

ProTox 3.0 has predicted that PFOA and PFOS could have pharmacophore fit to Amine Oxidase A (MAO-A) 42.24%, 33.43%, and 38.99%, 50.55% to Prostaglandin G/H Synthase 1 (PGHS-1), respectively. Docking of PFOA and PFOS with MAO-A and PGHS-1 might be considered reasonable if the predicted pharmacophore fits suggest potential binding interactions. ProTox 3.0 predictions, although showing low prediction percentages, still indicated potential pharmacophore matches, which provides grounds for molecular docking (Table 3).

Table 3

Possible toxicity targets

Toxicity Target	PFOA	PFOS
Amine Oxidase A	42.24%	33.43%
Prostaglandin G/H Synthase 1	38.99%	50.55%

We performed docking of PFOA and PFOS with human serum albumin (7Z57), PPAR-gamma (2F4B), monoamine oxidase A (2BXR) and COX-1 (6Y3C) (Table 4). According to AutoDock Vina and MMGBSA binding free energy calculations, the investigated ligands exhibit a strong binding affinity for human serum albumin, -9.2 kJ/mol for PFOA and -8.9 kJ/mol for PFOS (AutoDock Vina). We obtained moderate binding affinity results for the studied PFAS with PPARγ, particularly from the analyses conducted using Schrödinger Maestro. The MM-GBSA binding energy for 2F4B with PFOA was −10.2 kJ/mol, while for 2F4B with PFOS, it was -4.8 kJ/mol. Scientific literature suggests that PFAS activate nuclear PPARγ [7], leading us to hypothesize that PPARγ undergoes conformational changes *in vivo* that enhance binding, which may not be accurately reflected in static crystal structures. On the other hand, ligands have moderate affinity of interaction with monoamine oxidase A. PFOS has docking score with MAO-A AutoDock Vina -9.5 kJ/mol and MMGBSA value -25.4 kJ/mol. PFOS also demonstrates reliable free binding energy of interaction with human COX-1 (-26,1 kJ/mol).

Table 4

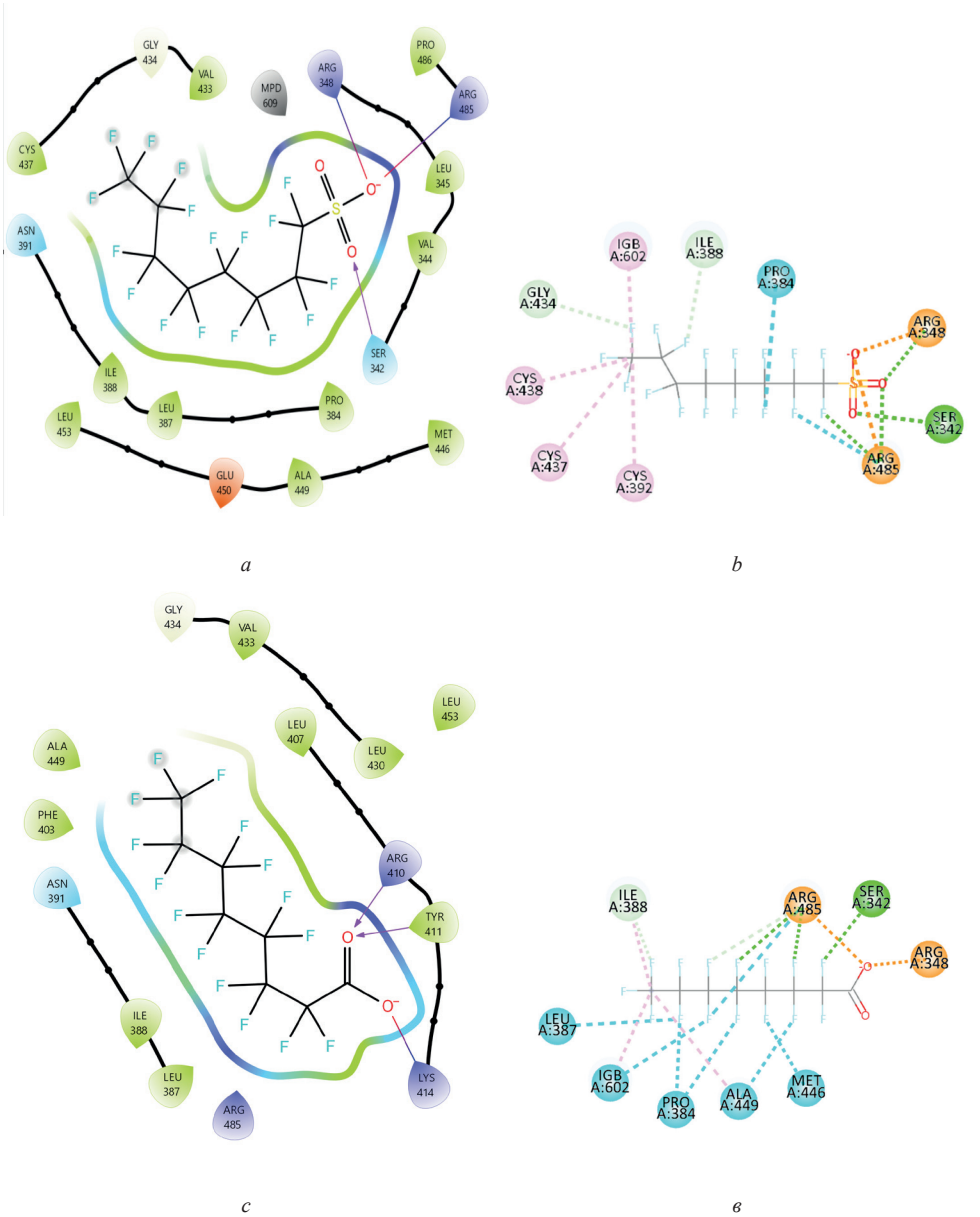
Docking scores and free binding energy of PFAS with human serum albumin and PPAR-gamma

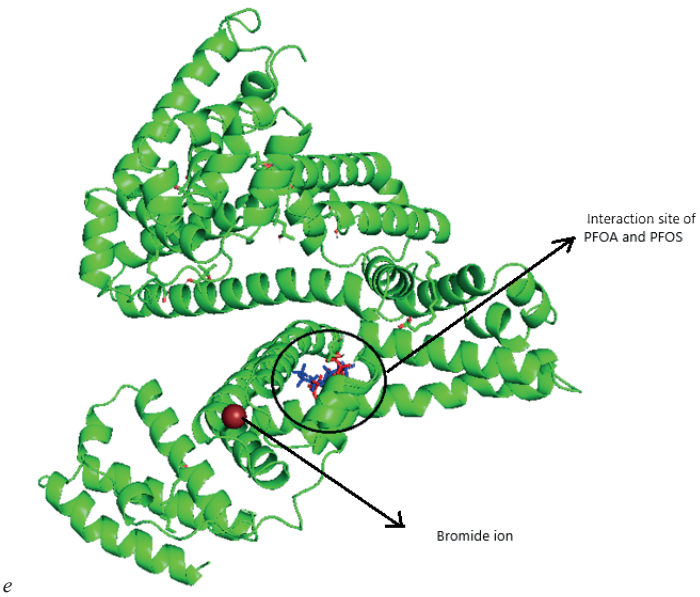
Protein	Docking score	PFOA	PFOS	Reference ligand
7Z57	AutoDock Vina	-9.2	-8.9	-8.6
	Maestro Schrodinger	-6.2	-7.3	-7.3
	MMGBSA	-22.3	-27.9	-24.7
2F4B	AutoDock Vina	-7.4	-7.7	-10.6
	Maestro Schrodinger	-5.3	-4.5	-9.4
	MMGBSA	-10.2	-4.8	-41.1
2BXR	AutoDock Vina	-8.9	-9.5	-6.3
	Maestro Schrodinger	-5.8	-6.1	-2.7
	MMGBSA	-5.9	-25.4	-7.9
6Y3C	AutoDock Vina	-7.7	-7.0	-4.9
	Maestro Schrodinger	-5.5	-5.2	-6.3
	MMGBSA	-21.2	-26.1	-5.9

Notes: 7z57 -Crystal structure of Human Serum Albumin in complex with surfactant GenX (2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoate);
2f4b - Crystal structure of the ligand binding domain of human PPAR-gamma in complex with an agonist;
2bxr - Human Monoamine Oxidase A in complex with Clorgy line, Crystal Form A;
6y3c - Human COX-1 Crystal Structure.

This interaction may influence the enzyme's function, potentially leading to toxicological or inflammation effects. Although there is no mention in the scientific literature of the effects of PFAS on MAO-A and human COX-1.

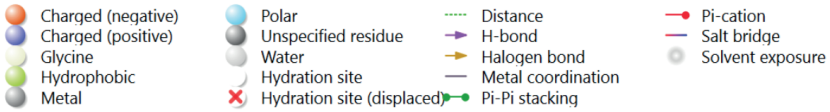
The investigated PFAS create the hydrogen bonds with SER 342, ARG 410 and TYR 411 of the HSA. PFOS creates hydrogen interaction by the sulfonic acid functional group and PFOA by the carboxyl group (Fig.1).





e

Legend for Schrödinger Maestro Glide results (a, c):



Legend for AutoDock Vina results (b, d):

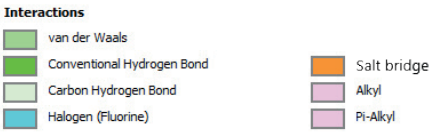


Fig. 1 *a) Visualized position of PFOS in the specific binding sites of human serum albumin (Schrödinger Maestro Glide); b) Visualized position of PFOS in the specific binding sites of human serum albumin (AutoDock Vina); c)Visualized position of PFOA in the specific binding sites of human serum albumin (Schrödinger Maestro Glide); d) Visualized position of PFOA in the specific binding sites of human serum albumin (AutoDock Vina); e)Whole structure of complex human serum albumin with PFOA and PFOS;*

Based on the molecular docking results ARG 348 and ARG 485 participated in salt bridges interactions with the studied ligands. Sepúlveda and colleagues observed that ligands with strong binding affinity to human serum albumin (HSA) predominantly form hydrogen bonds and salt bridges with the Arg485 and Arg348 residues [9].

Guo and colleagues reported in their studies that PFOA forms hydrogen bonds with the Ser289, His449, and Tyr473 residues of PPARγ [19]. The predicted results

of our research indicated that PFOA creates a hydrogen bond with ARG 288 and PFOS with TYR 327. In our case Ser289, His449 form polar contacts with ligands. We performed molecular docking of the studied ligands at the clorgyline-binding site of monoamine oxidase A, to analyze their interactions. PFOS creates a hydrogen bond interaction with ALA 68 and arginine TYR 69 of the binding site of monoamine oxidase A. Given that amine oxidase A regulates amines such as dopamine, serotonin, and norepinephrine, the possibility of PFOA and PFOS interacting suggests that they may affect neurotransmitter levels, potentially leading to mood-related or neurological problems.

Both PFOA and PFOS form of the hydrogen bonds interaction with glutamate 524 and arginine 120 of the cyclooxygenase 1 (COX -1). Interaction with COX-1 raises concerns about potential effects on inflammation, gastric health, and blood clotting. PFOS, with a higher probability of interaction with COX-1, may pose a slightly higher risk for these pathways than PFOA. Based on the positive molecular docking results of PFOA with HSA, we performed molecular dynamics (MD) simulations of these complexes to further explore the mechanisms of interaction. We also provided MD simulation of the HSA with $AlCl_3$ to modulate presence of cations Al^{3+} (fig. 2, 3).

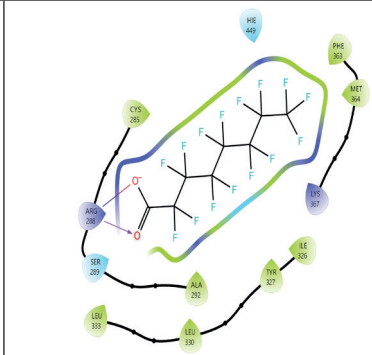
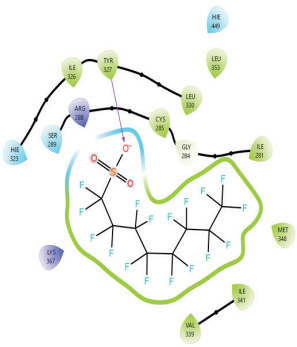
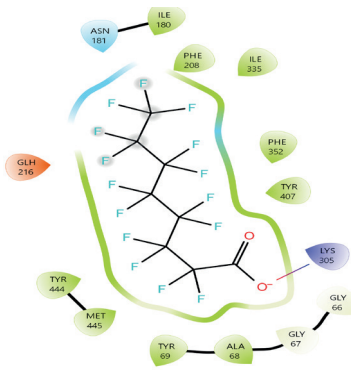
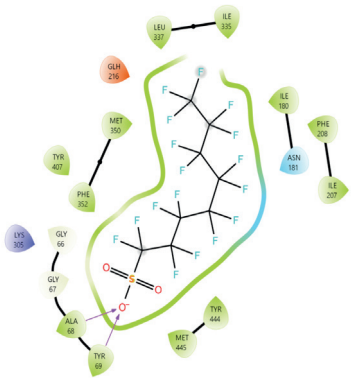
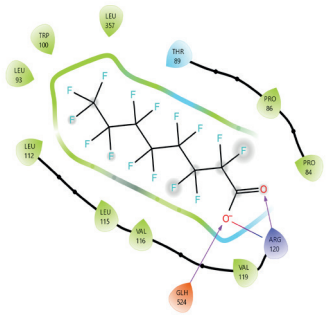
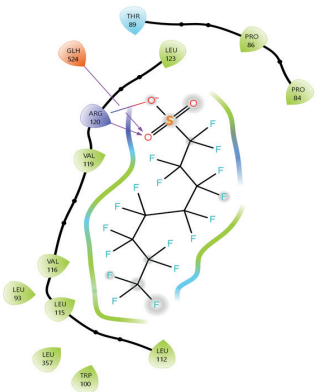
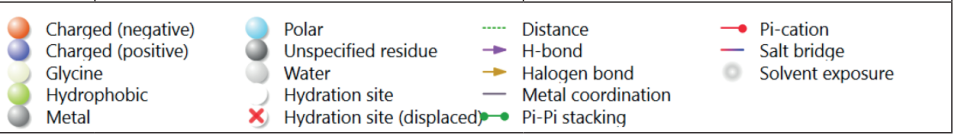
The difference between the RMSD (root mean square deviation) values at 6 ns and 3 ns for the protein without a ligand is 1.733. It shows a significant increase in RMSD over time. This suggests that the protein structure undergoes substantial conformational changes when unbound, indicating flexibility or instability in the absence of a ligand. The protein with the PFOA ligand has a smaller RMSD range (0.973), indicating that PFOA binding helps stabilize the protein structure to some extent. The overall RMSD values are also lower compared to the unbound protein, suggesting that PFOA may confer some structural stability. The protein with $AlCl_3$ shows moderate structural changes over time with an RMSD range of 1.047. The initial RMSD at 3 ns is slightly higher than for PFOA, which might suggest a more flexible starting structure with this ligand. However, its overall stability appears similar to PFOA, although it does not stabilize the protein as effectively as PFOA. The complex HAS with $AlCl_3$ shows the highest RMSD fluctuations, reaching peaks close to or above 2.5 Å, indicating that it undergoes larger structural deviations.

If PFOA binds tightly to HAS, it could compete with the molecules for binding sites on HSA, potentially displacing essential substances or reducing the transport efficiency of these molecules. It could lead to accumulation of PFOA in the bloodstream, since it may bind strongly and remain bound to albumin over time. HSA plays a crucial role in maintaining colloid osmotic pressure (oncotic pressure) in the blood, which helps to retain fluid within blood vessels. If PFOA binding alters the structure or functional sites of HSA, it could impact HSA's ability to maintain this pressure.

The RMSF (Root Mean Square Fluctuation) analysis was performed to assess the movement of residues following ligand binding (Fig.3). $AlCl_3$ often causes the highest fluctuation across several residues (51–101 and 501–551), indicating that

Table 5

Visualization of the location of PFOA and PFOS in the specific binding site of the studied targets Maestro Schrödinger Suite

Protein	PFOA	PFOS
2F4B		
2BXR		
6Y3C		
		

Notes: 2F4B - Crystal structure of the ligand binding domain of human PPAR-gamma in complex with an agonist; 2BXR - Human Monoamine Oxidase A in complex with Clorgy line, Crystal Form A; 6Y3C - Human COX-1 Crystal Structure

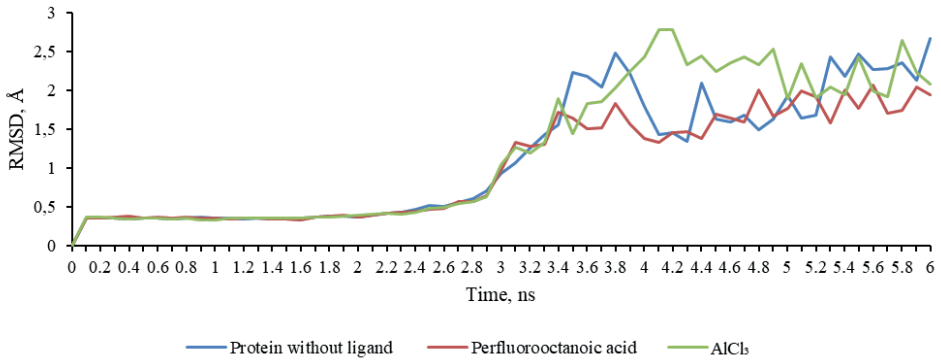


Fig. 2 *RMSD values of HSA without and in complex with ligands*

$AlCl_3$ presence increases the flexibility of certain parts of the protein more than PFOA or the unbound state. PFOA tends to show the lowest RMSF across most residues, indicating that it stabilizes the protein more, potentially reducing the flexibility in these regions.

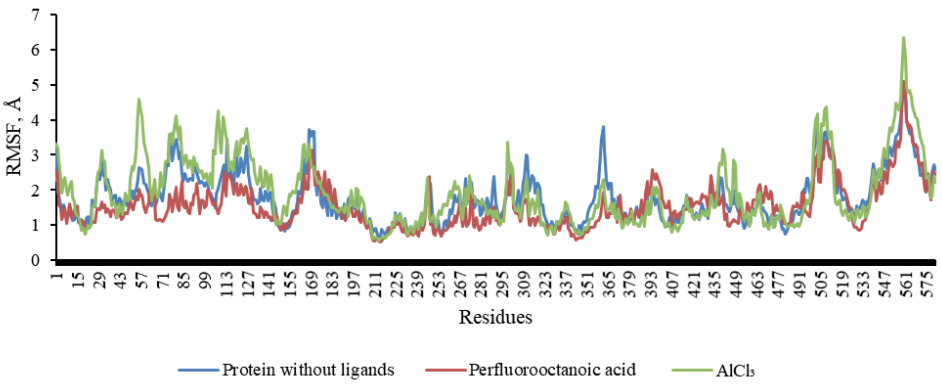


Fig.3 *RMSF values of human serum albumin without and in complex with ligands*

Conclusion

Based on the prediction toxicity machine learning based virtual lab ProTox 3.0 all compounds ($KClO_4$, $AlCl_3$, PFOA, PFOS) demonstrate significant potential for toxicity through multiple pathways, such as carcinogenicity, nephrotoxicity for PFOA, respiratory toxicity for PFOS, cardiotoxicity for $AlCl_3$ and estrogenic receptor alpha toxicity for $KClO_4$. According to ProTox 3.0 PFOA and PFOS can create interaction with Prostaglandin G/H Synthase 1 and Amine Oxidase A.

HSA showed the best docking score and binding free energy among the target proteins when docked with PFOA and PFOS. Binding of PFOA to human serum albumin, leading to its stabilization, could disrupt many of the essential functions that HSA performs in the blood. This binding could contribute to bioaccumulation, interfere with normal transport processes, impact drug efficacy, and potentially lead to chronic health issues by continuously exposing the body to PFAS toxicity. The persistence of PFOA-bound HSA in circulation thus represents a pathway for prolonged exposure and associated health risks.

The molecular docking results indicate that PFOS has moderate binding affinities with Monoamine Oxidase A and COX-1. These results are theoretical predictions and require further experimental studies.

These findings contribute valuable insights into the toxicity profiles and biological interactions of these pollutants, providing a foundation for further toxicological studies.

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С. І. Бендас[1], Б. М. Галкін[2], Н. А. Кириленко[1],
Одеський національний університет імені І. І. Мечникова;
[1]Кафедра фізіології, здоров'я і безпеки людини та природничої освіти;
[2]Біотехнологічний науково-навчальний центр, Одеса, вул. Всеволода Змієнка, 2, м. Одеса, 65082, Україна, e-mail: bgalkin@ukr.net

IN SILICO ДОСЛІДЖЕННЯ ПРОТИЗАПАЛЬНИХ ВЛАСТИВОСТЕЙ PFOA, PFOS, $KClO_4$ ТА $AlCl_3$

Резюме

Вступ. Військові дії завдають великої шкоди навколишньому середовищу. Найбільшу екологічну загрозу в цій ситуації становить хімічне забруднення (оксиди вуглецю, оксиди азоту, формальдегід, ціанід водню, калію перхлорат, алюміній та різноманітні токсичні органічні речовини). Крім того, боєприпаси можуть містити пер- та поліфторалкільні речовини (PFAS), які характеризуються винятковою екологічною стійкістю. Серед найвідоміших PFAS є перфтороктансульфонова кислота (PFOS) і перфтороктанова кислота (PFOA), які виявляють виняткову стабільність і стійкість до деградації. На нашу думку ці хімічні сполуки є високо токсичними та можуть підсилювати запальні процеси в організмі.

Мета: дослідження потенційної токсичності перфтороктанової кислоти, перфтороктанової сульфокислоти, $AlCl_3$ і $KClO_4$ за допомогою підходів *in silico*.

Методи. Розрахунок токсичності хлориду алюмінію, перхлорату калію, перфтороктанової кислоти та перфтороктансульфокислоти проводили за допомогою віртуальної лабораторії ProTox 3.0 на основі алгоритмів машинного навчання. Молекулярний докінг був забезпечений за допомогою AutoDock Vina 1.2.5. (http://vina.scripps.edu), Schrödinger Maestro–2023–2 Glide (пробна ліцензія). Процедуру молекулярного докінгу проводили з використанням кристалічної структури сироваткового альбуміну людини (7Z57), кристалічних структур лігандзв'язуючого домену PPAR-гамма людини (2F4B), моноамінокисидази А людини в комплексі з клоргіліном (2BXR), ЦОГ людини -1 Crystal Structure (6Y3C) з бази даних біологічних макромолекул PDB (http://www.rcsb.org/). Під час досліджень проводили докінг перфтороктанової кислоти та перфтороктанової сульфокислоти. Молекулярно-динамічне моделювання було виконано для комплексу (перфтороктанова кислота – сироватковий альбумін людини, $AlCl_3$ – сироватковий альбумін людини) за допомогою програмного забезпечення PlayMolecule.

Результати. На основі віртуальної лабораторії ProTox 3.0, заснованої на машинному навчанні прогнозування токсичності, усі сполуки ($KClO_4$, $AlCl_3$, перфтороктанова кислота, перфтороктанова сульфонова кислота) демонструють значні потенціали токсичності, такі як канцерогенність, нефротоксичність

для перфтороктанової кислоти, респіраторна токсичність для перфтороктанової сульфокислоти, кардіотоксичність для $AlCl_3$ і альфа-токсичність естрогенного рецептора для $KClO_4$. Відповідно до ProTox 3.0 перфтороктанова кислота та перфтороктансульфонова кислота можуть взаємодіяти з простагландин G/H синтазою 1 та аміноксидазою А. Людський сироватковий альбумін показав найкращий показник докінгу та вільну енергію зв'язування серед цільових білків при докуванні з перфтороктановою кислотою та перфтороктановою сульфокислотою. Результати молекулярного докінгу демонструють, що перфтороктансульфонова кислота має помірну афінність зв'язування з моноаміноксидазою А та ЦОГ-1. Ці результати є теоретичними прогнозами і потребують подальших експериментальних досліджень.

Висновки. Дослідження токсичності за допомогою віртуальної лабораторії ProTox 3.0 та молекулярного докінгу виявило значний токсикологічний потенціал досліджуваних сполук ($KClO_4$, $AlCl_3$, перфтороктанової кислоти, перфтороктанової сульфонової кислоти). Зв'язування PFOA з сироватковим альбуміном людини, що призводить до його стабілізації, може порушити багато основних функцій, які HSA виконує в крові. Це зв'язування може сприяти біоакумуляції, перешкоджати нормальним транспортним процесам, впливати на ефективність ліків і потенційно призводити до хронічних проблем зі здоров'ям через постійний вплив на організм токсичності PFAS. Таким чином, стійкість HSA, пов'язаного з PFOA, у циркуляції, може бути шляхом для тривалого впливу та пов'язаних із цим ризиків для здоров'я.

Ключові слова: запалення, аналіз молекулярного докінгу, молекулярна динаміка, перфтороктанова кислота, перфтороктан сульфокислота, зв'язування.

S. I. Bendas[1], **B. M. Galkin**[2], **N. A. Kyrylenko**[1]
Odesa I. I. Mechnikov National University,
[1]Department of Physiology, Human Health and Safety and Natural Science Education
[2]Biotechnology Science-Educational Centre; 2 Vsevoloda Zmiienka St, Odesa, 65082, Ukraine, e-mail: bgalkin@ukr.net

IN SILICO STUDY OF THE ANTI-INFLAMMATORY PROPERTIES OF PFOA, PFOS, $KClO_4$, AND $AlCl_3$

Summary

Introduction. Military operations cause great damage to the environment. The great-est environmental threat in this situation is chemical pollution (carbon oxides, nitro-gen oxides, formaldehyde, hydrogen cyanide, potassium perchlorate, aluminum and various toxic organic substances). In addition, ammunition may contain per- and polyfluoroalkyl substances (PFAS), which are characterized by exceptional envi-ronmental stability. Among the most famous PFAS are perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), which exhibit exceptional stability and resistance to degradation. In our opinion, these chemical compounds are highly toxic and can enhance inflammatory processes in the body.

Aim. to study the potential toxicity of perfluorooctanoic acid, perfluorooctane sulfonic acid, $AlCl_3$ and $KClO_4$ using *in silico* approaches.

Methods. The toxicity of aluminum chloride, potassium perchlorate, perfluorooctanoic acid, and perfluorooctane sulfonic acid was calculated using the ProTox 3.0 virtual laboratory based on machine learning algorithms. Molecular docking was performed using AutoDock Vina 1.2.5. (http://vina.scripps.edu), Schrödinger Maestro–2023–2 Glide (trial license). The molecular docking procedure was performed using the crystal structure of human serum albumin (7Z57), crystal structures of the ligand-binding domain of human PPAR-gamma (2F4B), human monoamine oxidase A in complex with clorgyline (2BXR), human COX-1 Crystal Structure (6Y3C) from the PDB database of biological macromolecules (http://www.rcsb.org/). During the studies, docking of perfluorooctanoic acid and perfluorooctane sulfonic acid was performed. Molecular dynamics simulations were performed for the complex (perfluorooctanoic acid – human serum albumin, $AlCl_3$ – human serum albumin) using the PlayMolecule software.

Results. Based on the ProTox 3.0 virtual laboratory based on machine learning for toxicity prediction, all compounds ($KClO_4$, $AlCl_3$, perfluorooctanoic acid, perfluorooctanoic sulfonic acid) show significant toxicity potential through several pathways such as carcinogenicity, nephrotoxicity for perfluorooctanoic acid, respiratory toxicity for perfluorooctanoic sulfonic acid, cardiotoxicity for $AlCl_3$ and estrogen receptor alpha toxicity for $KClO_4$. According to ProTox 3.0, perfluorooctanoic acid and perfluorooctanoic sulfonic acid can interact with prostaglandin G/H synthase 1 and amine oxidase A. Human serum albumin showed the best docking score and binding free energy among the target proteins when docked with perfluorooctanoic acid and perfluorooctanoic sulfonic acid. The molecular docking results indicate that PFOS has moderate binding affinities with Monoamine Oxidase A and COX-1. These results are theoretical predictions and require further experimental studies.

Conclusions. Toxicity studies using the ProTox 3.0 virtual laboratory and molecular docking revealed significant toxicological potential of the compounds tested ($KClO_4$, $AlCl_3$, perfluorooctanoic acid, perfluorooctanoic acid). Binding of PFOA to human serum albumin, resulting in its stabilization, may disrupt many of the essential functions that HSA performs in the blood. This binding may promote bioaccumulation, interfere with normal transport processes, affect drug efficacy, and potentially lead to chronic health problems due to ongoing exposure to PFAS toxicity. Thus, the persistence of PFOA-bound HSA in the circulation may be a pathway for long-term exposure and associated health risks.

Keywords: inflammation, molecular docking analysis, molecular dynamics, perfluorooctanoic acid, perfluorooctane sulfonic acid, binding.

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