

## Project abstract

Novichoks are a group of chemical warfare agents with paralytic and convulsive effects. One probable mechanism of Novichok's toxic effects is irreversible binding to acetylcholinesterase (AChE) and inhibition of the hydrolysis of the neurotransmitter acetylcholine (ACh) to acetate and choline. Overstimulation of cholinergic receptors due to the accumulation of ACh in the synaptic cleft due to AChE inhibition leads to the manifestation of toxic symptoms (cholinergic toxidrome) through three types of reactions: muscarinic, nicotinic and central nervous system (CNS). The structures of these unique organophosphates are still unclear, with two possible structures postulated as phosphoramides or phosphorylated oximes. Novichoks were designed to be undetectable and bypass the Chemical Weapons Convention (CWC) list. So far, little is known about the Novichoks, and much data needs to be completed - for example, knowledge about the danger level of these compounds, i.e. their toxicity. We have already witnessed the "show" of the Novichoks' enormous toxic potential three times. The first two use cases of these nerve agents occurred in 2020 in Salisbury and Amesbury (UK) and sparked a public debate that made everyone aware of the dangerous nature of these compounds. The third example of the use of Novichok paralyzing agents was the case of the acute poisoning of Alexei Navalny during a domestic flight in Russia. Based on the results of clinical and laboratory studies, the use of a cholinesterase inhibitor has been identified.

The above examples indicate the presence of Novichoks in public spaces and confirm the threat and huge effects of poisoning. The toxicity of Novichok nerve agents should be a critical national issue. Therefore, from the point of view of social security, it is crucial to study their properties, especially toxicological aspects. There are many problematic issues, and the fundamental questions are: What are the risks of these substances in contact with humans? Exposure to what dose of these dangerous compounds is lethal? Do Novichoks surpass previous generations of nerve agents (-V and -G) in toxicity? What is the impact of these hazardous materials on the environment? Is there genuine concern about their presence and persistence in the environment? If so, how long will they likely be an environmental problem after HAZMAT/CBRN incidents? To answer these questions, it is essential to determine the basic physical and chemical parameters and their toxicity and environmental persistence.

Considering the cases of chemical attacks involving Novichoks, there is no doubt that determining the parameters of these dangerous substances is essential but also tricky due to their high reactivity and toxicity. The rationale for carrying out several studies within the project is the lack of primary data in the scientific literature on Novichoks. The few references in existing reports only refer to single examples of these hazardous substances. Recognising the vulnerability to the threat of terrorist activity, the most desirable and justified approach in this situation seems to be using *in silico* toxicological tools. In addition, due to the threat posed by Novichoks, the safest way to assess is to use *in silico* methods. Alternative methods are desirable and necessary to predict many Novichok parameters. An alternative that we plan to use will be computer methods (in particular, techniques for quantitative modelling of relationships between chemical structure and activity/toxicity, QSAR). In addition, experimental studies should be preceded by estimating toxicological parameters. To fulfil the modern requirements of 21<sup>st</sup>-century toxicology research and to consider next-generation risk assessment (NGRA) with the prediction of toxicological parameters, it is necessary to first apply *in silico* toxicological methods to eliminate unnecessary animal testing. The use of alternative methods allows, according to the 3R principle of W. Russell and R. Burch, to reduce the number of animals (Reduction), replace them (Replacement) and improve methods (Refinement). Moreover, after updating the CWA (Chemical Warfare Agent) list, more than 10,000 structures are candidates to be called Novichoks. It is, therefore, impossible to synthesise and estimate target parameters for each structure separately. Thus, developing a reliable theoretical method for estimating selected toxicological parameters is a priority in preparation for the future use of this type of organophosphorus compound. Such research will be of key importance not only in the context of toxicological research but also in the context of international security (examination with a global impact on people and the environment). In the future, they will enable efficient monitoring and management (toxicovigilance) of newly developed and existing cholinergic chemical warfare agents.