Abstract

This doctoral dissertation was prepared based on four thematically related full-text publications devoted to predicting selected toxicological parameters for organophosphorus compounds from the Novichok group using *in silico* toxicology methods.

Novichoks, A-series compounds, are a group of nerve agents secretly created during the Cold War by the Soviet Union. They consist of the fourth generation of chemical warfare agents; like other nerve agents, they are organophosphate compounds designed to be incurable and undetectable. The chemical structure of organophosphorus compounds of the Novichok group still remains uncertain; two versions are postulated. The first variant, according to Vil S. Mirzayanov, is phosphoramides. A second alternative structure proposed by Hoenig and Ellison describes Novichoks as phosphorylated oximes. The mechanism of toxic action of A-series compounds consists of irreversible binding to acetylcholinesterase (AChE) and inhibition of the hydrolysis of the neurotransmitter acetylcholine (ACh) to acetate and choline. Excessive stimulation of cholinergic receptors as a result of the accumulation of ACh in the synaptic cleft leads, depending on the route, dose and time of exposure, to the manifestation of several toxic symptoms through three types of reactions: muscarinic, nicotinic and central nervous system. So far, we have witnessed the "show" of the Novichoks' enormous toxic potential three times. The first two use cases of these nerve agents occurred in 2018 in Salisbury and Amesbury (UK). The third example of using the Novichok compound was the poisoning of Alexei Navalny on a domestic flight in Russia. This case is the only published clinical study on the treatment of Novichok poisoning, which has proven the effectiveness of butyrylcholinesterase therapy and demonstrated the lack of reactivation effectiveness after the administration of obidoxime.

Due to its toxicity, working with Novichoks requires extreme precautions. Moreover, experimental studies should be preceded by the estimation of toxicological parameters. Moreover, after updating the CWA (Chemical Warfare Agent) list, more than 10,000 structures can be candidates for the Novichok agent. Therefore, synthesising and estimating target parameters for each structure is impossible. Given the global threat A-series compounds poses, many unknowns and data gaps must be addressed. Hence the inspiration for research using *in silico* toxicological methods in the form of prediction of selected Novichok parameters.

Novichok properties such as toxicity and environmental fate were determined using an *in silico*, quantitative structure-activity relationship (QSAR) approach. The QSAR Toolbox application package, Estimation Program Interface Suite and Toxicity Estimation Software Tool were used to estimate the median lethal dose (LD_{50}) parameters, biodegradation, hydrolysis half-life and hydrolysis rate constant.

So far, the literature has claimed that organophosphorus compounds from the Novichok group exceed their toxicity in the known conventional compounds of the -V or -G series. Compound A-230 was purported to be 5-8 times more toxic than VX, while A-232 was supposed to be 10 times more toxic than Soman (GD). The predictions contradicted these reports, the most dangerous of the tested Novichoks A-232 (LD_{50} = 0.41 mg/kg bw) was estimated as four times less toxic than the compound VX (LD_{50} = 0.1 mg/kg bw) and seven times weaker than GD (LD_{50} = 0.06 mg/kg bw). The estimated half-life and the constant rate of hydrolysis indicate that the Novichoks used can persist in the external environment, depending on the organophosphorus compound used, from several hours (A-230, A-242 and Iranian "Novichok"), for more than a month (A- 232, A-234, A-262 and C01A043-A045), even up to several years (C01A035-A042). In addition, using in silico models to predict rapid biodegradation showed that Novichoks are difficult to degrade aerobically and anaerobically. No resistant compounds were obtained, and the time frames for this process ranged from weeks (A-230, A-242, C01-A037 and C01-A041) to months.

To summarise, the prediction of parameters of organophosphorus compounds is a complex process and requires the use of various *in silico* approaches. With gratitude to *in silico* toxicological studies, a comprehensive insight into the nature of the Novichoks has been obtained in a health-safe manner. Although much information about the A-series compounds is still shrouded in mystery, the successful prediction of the toxic and environmental properties of Novichoks (n = 17) supplemented some of the essential data missing in the scientific literature.

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