THE SITUATION IN LITHUANIA: THE STUDIES ON THE EXPLOSIVE CONTAMINATION, THEIR TOXIC ACTION AND BIODEGRADATION

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The Lithuanian contribution to the European studies of explosives starts from 1650s, having been summarized in the book of Kazimieras Simonavičius (1600-1651) *Artis Magnae Artilleriae* (Latin, Amsterdam, 1650 (French - 1651, German - 1676, English and Dutch - 1729) (Fig. 1)). This textbook served as a manual for the European artillerymen for 150 years, including their contribution to the development of Congreave rockets in 1800s.

Fig. 1 The title page of K. Simonavičius book *Artis Magnae Artilleriae*, Pars I. (Amsterdam, 1650).

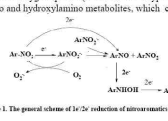
The German occupation during the WWI (1915-1918), the occupation of Lithuanian Republic by the Soviet Union (1940), the WW2 events (1941-1945, the fights between the Red Army and Wehrmacht), and the post war events (the fights between the Soviet Army and the Lithuanian resistance (1945-1951), and the training activities of the occupant Soviet Army (1945-1991)), resulted in the massive occurrence of the unexploded devices (mines, artillery and aviation bombs, rocket(s) in Lithuanian territory (terrain, groundwater, and the territory of the Baltic sea), and its general contamination by the unexploded ordnance residues (Pabradė, Kairiai, Rūdninkai, and Rukla firing fields). Totally, 222 territories (240 km^2) are heavily affected by those activities, which resulted in 19 casualities (13 killed, 6 wounded between 1990-2006).

After the reestablishment of the independence of Lithuania (1990), ca. 200000 explosive devices were destroyed till 2007 (Fig. 2A,B). According to the Lithuanian Government Decision of 2007.04.17, the final destruction of the explosive devices in the terrain may last untill 2018 (the main responsible Juozas Vitkus Engineering Battalion (Kaunas)). The implementation of these tasks seems feasible, however, it is complicated by the unexpected findings (e.g., the 500-3000 kg aviation bombs from the Soviet period (1945-1990), including the 500 kg bomb found in Kaunas (Aleksotas suburb) in 2006). Typically, the unexploded bombs are found both in rural (Pravieniškės, Žalioji forests), and in urban (Vilnius, Kaunas, Klaipėda, Šiauliai, Panevėžys, Alytus, Tauragė) regions.

In contrast, no activities on the decontamination/bioremediation of the terrain, including the territories of the firing fields in Lithuania, has been carried out, generally due to the lack of funds for the scientific research. However, since 1998 the efforts of several scientific teams of the Institute of Biochemistry and the Institute of Immunology of Vilnius University were directed to the analysis of the mechanisms of the mammalian cell cytotoxicity of nitroaromatic explosives.

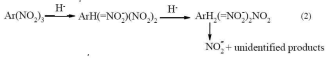
Fig.2

Briefly, nitroaromatic explosives ($ArNO_2$) are toxic and mutagenic to humans and other mammalian species. Their toxicity is partly attributed to the single-electron enzymatic reduction to radicals accompanied by the formation of the reactive oxygen species (oxidative stress type of cytotoxicity), and their two-(four)-electron reduction to nitroso and hydroxylamino metabolites, which covalently bind to proteins and DNA:



Scheme 1. The general scheme of 1e⁻/2e⁻ reduction of nitroaromatics by flavoenzymes

It is important to note that 2e⁻/4e⁻ reduction being catalyzed by the 'oxygen-insensitive' nitroreductases of numerous bacteria, microorganisms, and fungi, also make a first step in the biodegradation of nitroaromatic explosives in the environment. In parallel, certain nitroreductases reduce the aromatic ring with the formation of Meisenheimer-type hydride adducts, which further undergo denitration (**Scheme 2**):



These reactions also contribute to the biodegradation of explosives.

In our studies [**1-5, 7**] since 1998 we examined the following mechanisms of flavoenzyme-catalyzed reduction of nitroaromatic explosives:

a) single-electron reduction by flavoenzymes dehydrogenases-electrontransferases NADPH:cytochrome P-450 reductase, ferredoxin:NADP$^+$ reductase, and others. These reactions follow an outer-sphere electron-transfer model, i.e., the reactivity increases with an increase in their single-electron reduction potential of compounds (E^1_7, $E^1_{7(calc.)}$ (**www.bchi.lt/potential.pdf**)), and is relatively insensitive to their structural peculiarities (Fig.) . Among thje explosives, the typical reactivity order is tetryl ~ pentryl ~ TNC > TNBO ~ TNT ~ DNBF > NHOH-DNTs ~ NH2-DNTs > NTO ~ ANTA.

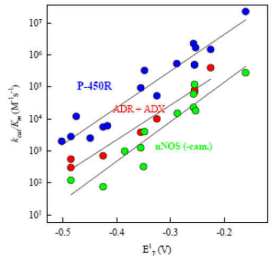
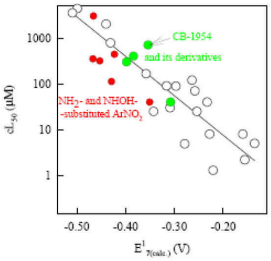


Fig. 3 QSAR in the reduction of $ArNO_2$ by electrontransferases cytochrome P-450 reductase (P-450R), NO-synthase (nNOS), adrenodoxin reductase/adrenodoxin complex (ADR-ADX).

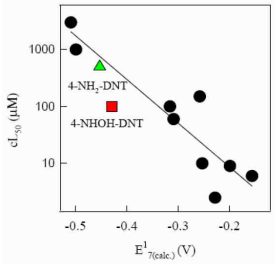
b) Two-electron reduction of explosives and other nitroaromatics (n = 39) by liver DT-diaphorase (NQO1), and the gastrointestinal tract *Enterobacter cloacae* nitroreductase (NR). The obtained results show that in general, the reactivity also increases with E^1_7 of compounds, however, it is more sensitive to their structure. In NQO1-catalyzed reactions, reactivity increases with an increase in the highest torsion angle between the nitrogroup(s) and the aromatic ring, and is relatively insensitive to their Van der Waals volume (VdWvol). In NR-catalyzed reactions, reactivity decreases after an increase in VdWvol above 0.3 nm^3. A similar trend is observed in the reactions of *E. cloacae* PB2 NADPH:PETN reductase (PETNR), which performs both two-electron reduction of nitrogroup (**Scheme 1**) or aromatic ring (**Scheme 2**).

We also examined the the mammalian cell cytotoxicity of nitroaromatic explosives. Using two cell lines, we found that the cytotoxicity of explosives increases with an increase in their E^1_7 or $E^1_{7(calc.)}$, except for the amino- and hydroxylamino- metabolites of TNT, which possess higher than expected cytotoxicity [**2, 4**]. Typically, the order of cytotoxicity is tetryl, pentryl > TNC > TNT, DNBF >> NTO, ANTA. The protective effects of antioxidants show that the main cytotoxicity mechanism id oxidative stress (free radical formation). Inhibitor of NQO1, dicoumarol, shows controversial effects, thus, the role of two-electron reduction by NQO1 in the mammalian cell cytotoxicity of nitroaromatic explosives remains further elucidation.



Toxicity of explosives and other nitroaromatics in FLK cells (fibroblasts)

Fig. 4



Toxicity of explosives and other nitroaromatics in mice splenocytes

Fig. 5

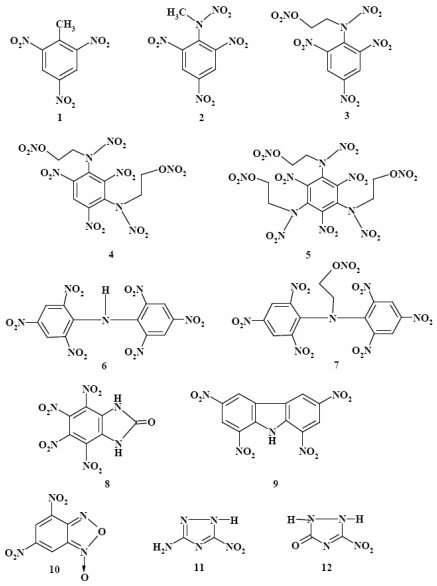


Fig. 6 Structural formulae of examined nitroaromatic explosives: 2,4,6-trinitrotoluene (**TNT**) (1), 2,4,6-trinitrophenyl-*N*-methylnitramine (**tetryl**) (2), 2,4,6-trinitrophenyl- *N*-nitroxyelthylnitramine (**pentryl**) (3), **dipentryl (4), tripentryl** (5), 2,2',4,4',6,6'-hexanitro-diphenylamine (**hexyl**) (6), **hexyl**-*N*-ethylnitrate (7), 4,5,6,7-tetranitrobenzimidazolone (**TNBO**) (8), 1,3,6,8-tetranitrocarbazole (**TNC**) (9), 4,6-dinitrobenzofuroxane (**DNBF**) (10), 3-amino-5-nitro-1,2,4-triazole (**ANTA**) (11), and 5-nitro-1,2,4-triazol-3-one (**NTO**) (12).

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