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Chemical aspects of cytotoxicity of nitroaromatic explosives: a review

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Nitroaromatic explosives and their degradation products are toxic environmental pollutants. In this paper, we summarize the available data on the chemical aspects of toxicity of the classical and the new generation nitroaromatic explosives, i.e. their flavoenzyme-catalyzed single- and two-electron reduction and the impact of these reactions on the mammalian cell cytotoxicity of explosives. The leading role of the oxidative stress-type cytotoxicity of nitroaromatic explosives has been demonstrated in bovine leukemia virus-transformed lamb kidney fibroblasts (line FLK) and, possibly, in mouse splenocytes.

Key words: nitroaromatic compounds, nitroaromatic explosives, toxicity, oxidative stress

INTRODUCTION

Nitroaromatic explosives and their degradation products are toxic and mutagenic to humans and other mammalians. The toxic effects of 2,4,6-trinitrotoluene (TNT) were observed as early as 1920s [1]. TNT, picric acid, and 2,4,6-trinitrophenyl-*N*-methylnitramine (tetryl) causes hemolytic anemia, methemoglobinemia, liver damage, splenomegaly, hypercholesterolemia, and testicular atrophy in rats and mice [2–6]. Data on the mutagenic activity of TNT and its metabolites in mammalian cells were equivocal, depending on the cell line and conditions [7–10]. The exposure of humans to nitroaromatic explosives is accompanied by multiple toxic effects which are influenced by the genetic or individual susceptibility and by the workplace standards [1, 11, 12]. Increased exposure to TNT causes methemoglobinemia [11], cataracts [12–14], reproductive toxicity [15], skin lessions and dermatitis [16], urinary tract, kidney, and liver tumours [17, 18]. An increased incidence of acute and chronic leukemia have also been reported following chronic exposure [19].

In spite of numerous clinical and ecotoxicological reports, the chemical mechanisms underlying the toxicity of nitroaromatic explosives are not well understood. One may expect that the cytotoxicity mechanisms of nitroaromatic explosives in mammalian cells will be related to their single- or two-electron enzymatic reduction, i.e. will be similar to other groups of nitroaromatic compounds used in pharmacy, or recognized as important environmental pollutants [20]. In this paper, we summarize the available data on the enzymatic reduction of the classical and the new generation nitroaromatic explosives [21–24] (Fig. 1) and the impact of these reactions on their mammalian cell cytotoxicity.

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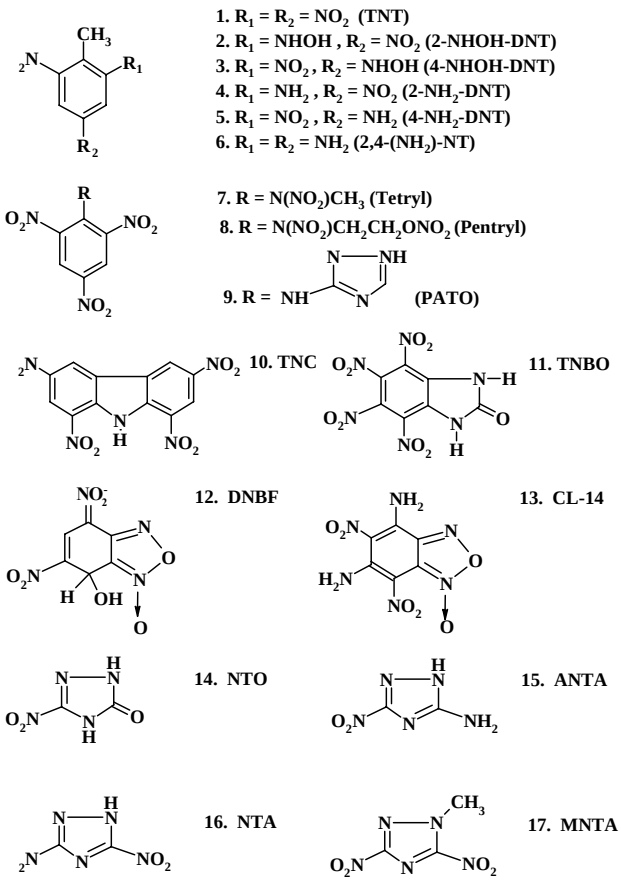


Figure. Structural formulae of nitroaromatic explosives and their metabolities

ENZYMATIC REACTIONS OF NITROAROMATIC EXPLOSIVES

Free radical reactions of nitroaromatic explosives

The single-electron enzymatic reduction of nitroaromatics ($ArNO_2$) to their anion-radicals ($ArNO_2^{\cdot-}$)

$$ArNO_2 + e^- \rightarrow ArNO_2^{\cdot-} \quad (1)$$

is followed by their reoxidation by O_2 with the formation of superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2)

$$ArNO_2^{\cdot-} + O_2 \rightarrow ArNO_2 + O_2^{\cdot-}, \quad (2)$$

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2, \quad (3)$$

and, subsequently, of the cytotoxic hydroxyl radical ($OH^{\cdot}$) in the transition metal-catalyzed Fenton reaction:

$$O_2^{\cdot-} + Fe^{3+} \rightarrow O_2 + Fe^{2+}, \quad (4)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH. \quad (5)$$

The single-electron reduction of nitroaromatics is catalyzed mainly by flavinmononucleotide (FMN) or flavinadeninedinucleotide (FAD)-containing dehydrogenases-electrontransferases. These enzymes transfer electrons from NAD(P)H or other two-electron donors to single-electron accepting oxidants, e.g., heme- or FeS-proteins. The nitroreductase reactions of microsomal NADPH : cytochrome P-450 reductase (P-450R, EC 1.6.2.4) and plant or algal ferredoxin $NADP^+$ reductase (FNR, EC 1.18.1.2) are most thoroughly studied [25–30]. P-450R is universally recognized as one of the most important generators of nitroradicals in mammalian cells [31]. P-450R contains both FAD and FMN in the active center, and transfers electrons in the sequence NADPH → FAD → FMN → cytochrome P-450, or nonphysiological oxidants (cytochrome *c*, quinones, nitroaromatics) [25, 26]. The bimolecular rate constants (k_{cat}/K_m) of explosives reduction by P-450R vary from ≥ 10^7 $M^{-1}s^{-1}$ (pentryl, tetryl, TNC) to 10^6–10^5 $M^{-1}s^{-1}$ (TNT, TNBO, DNBF) and to ~10^3 $M^{-1}s^{-1}$ (NTO, ANTA) [28–30]. FNR is responsible for the bioreductive activation of nitroaromatic pesticides in plants and is a useful model system for studying their reactivity. The rate-limiting step in the nitroreductase reaction of *Anabaena* FNR is the oxidation of the FAD semiquinone ($FADH^{\cdot}$), whereas the oxidation of two-electron reduced $FADH^-$ to semiquinone is 30 times faster. The k_{cat}/K_m in FNR-catalyzed reactions vary from ≥ 10^5 $M^{-1}s^{-1}$ (pentryl, tetryl, TNBO), and ≥10^4 $M^{-1}s^{-1}$ (DNBF, TNT), and ~ 10^2 $M^{-1}s^{-1}$ (NTO, ANTA) [27–30]. In general, the reactivity of nitroaromatics in P-450R- and FNR-catalyzed reactions is relatively insensitive to their molecular structure, but increases with an increase in their single-electron reduction potential (E_7^1, or standard potential of the $ArNO_2/ArNO_2^{\cdot-}$ redox couple at pH 7.0). The observed linear log k_{cat}/K_m vs. E^1 dependences with the slope $\Delta\log k_{cat}/K_m/\Delta E_7^1$ ~ 10 V^{-1} [25–28] are consistent with an "outer-sphere electron transfer" model with a weak electronic coupling between the reactants [32].

Due to the instability of free radicals, the E_7^1 values of nitroaromatics (Table) are usually obtained from the anaerobic pulse-radiolysis experiments [33, 34]. The E_7^1 values differ from half-wave potentials of electrochemical reduction, which reflect the net four-electron transfer [35]. The use of Hammett constants or other thermodynamic parameters obtained by means of quantum mechanical calculations to get the unavailable E_7^1 values for nitroaromatic explosives does not provide reliable results due to the large data scattering. Alternatively, the use of the linear log k_{cat}/K_m vs. E_7^1 relationships in the nitroreductase reactions of flavoenzymes [28–30] gives much better results [36]. The calculated reduction potentials ($E_{7\,(calc)}^1$, Table) deviate from the experimental ones no more than by 35 mV (standard deviation, ±18 mV) and thus should be considered as realistic.

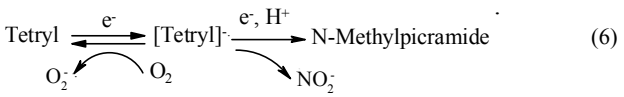
The reoxidation of nitroanion radicals by oxygen and their dismutation are most relevant to their cytotoxicity. The rate constants of nitroanion radical oxidation by O_2 (Eq. (2)) decrease with an increase in E_7^1 values, e.g. 7.7 × 10^6 $M^{-1}s^{-1}$ (nitrobenzene, E_7^1 = –0.485 V), 1.4 × 10^6 $M^{-1}s^{-1}$ (*p*-nitroacetophenone, E_7^1 = –0.355 V), 1.5 × 10^6 $M^{-1}s^{-1}$ (*p*-nitrobenzaldehyde, E_7^1 = –0.325 V), 2.5 ×

Table. **Single-electron reduction potentials of nitroaromatic explosives, their metabolites and model compounds (E_7^1 and $E_{7\,(calc.)}^1$), and their concentrations for the 50% survival (cL_{50}) of mammalian cells: FLK cells (24 h exposure)[28–30, 56, 63, 64], Chinese hamster ovary KI cells (24 h exposure) and rat hepatoma H4IIE cells (24 h exposure) [65], and Chinese hamster lung V79 cells (24 h exposure) and human lymphoblast TK-6 cells (48 h exposure) [9].**

No.	Compound	E_7^1 (V) [33,34]	$E_{7(calc.)}^1$ (V) [36]	cL_{50} (μM)					
				FLK	Splenocytes[a]	KI	V79	H4IIE	TK-6
Explosives and their metabolites:									
1	2,4,6-Trinitrotoluene (TNT)	–0.254	–0.253	25 ± 5.0	10 ± 2.0	106	197 ± 36	17.6	22 ± 5.0
2	2-Hydroxylamino-4,6-dinitrotoluene (2-HNOH-DNT)		–0.351	40 ± 7.0					
3	4-Hydroxylamino-2,6-dinitrotoluene (4-NHOH-DNT)		–0.429	112 ± 10	100 ± 20	18.8		28.2	
4	2-Amino-4,6-dinitrotoluene (2-NH_2-DNT)	–0.417	–0.423	440 ± 35		>1270	222 ± 76	91.3	168 ± 14
5	4-Amino-2,6-dinitrotoluene (4-NH_2-DNT)	–0.449	–0.453	316 ± 20	500 ± 80	>1270	>328	335	248 ± 51
6	2,4-Diamino-6-nitrotoluene (2,4-$(NH_2)_2$-NT)	–0.502	–0.467	350 ± 40		>1500	>600	>1500	>600
7	2,4,6-Trinitrophenyl-*N*-methylnitramine (tetryl)		–0.156	2.2 ± 0.3	6.0 ± 1.5				
8	2,4,6-Trinitrophenyl-*N*-nitraminoethylnitrate			–0.136	5.0 ± 1.0				
9	*N*-(1,2,4-Triazol-3-yl)-2,4,6-trinitro-phenyl-amine (PATO)			≥50	25 ± 5.0				
10	1,3,5,8-Tetranitrocarbazole (TNC)		–0.116	8.0 ± 2.0					
11	4,5,6,7-Tetranitrobenzimidazolone (TNBO)		–0.199	30 ± 5.0	9.0 ± 1.5				
12	4,6-Dinitrobenzofuroxan (DNBF)		–0.258	70 ± 8.0	150 ± 30				
13	5,7-Diamino-4,6-dinitrobenzofuroxane (CL-14)			–0.257	250 ± 40				
14	5-Nitro-1,2,4-triazol-3-one (NTO)		–0.509	≥3500	≥3000				
15	5-Nitro-1,2,4-triazol-3-amine (ANTA)		–0.466	3000 ± 400					
16	3,5-Dinitro-1,2,4-triazole (NTA)			2700 ± 380	2000 ± 400				
17	1-Methyl-3,5-dinitro-1,2,4-triazole (MNTA)			2300 ± 300	190 ± 20				
Model compounds:									
18	*p*-Dinitrobenzene	–0.257	–0.228	8.0 ± 2.0	2.5 ± 0.4				
19	*o*-Dinitrobenzene	–0.287	–0.309	30 ± 5.0	60 ± 15				
20	*m*-Dinitrobenzene	–0.348	–0.316	90 ± 20	100 ± 20				
21	Nitrobenzene	–0.485	–0.499	4370 ± 1470	1000 ± 200				

[a] Data of this work. The culture of mouse splenocytes (10^6 cells/ml) was obtained as described [66], their viability was examined after a 24 h exposure to nitroaromatic compounds.

10^5 $M^{-1}s^{-1}$ (nitrofurantoin, E_7^1 = –0.255 V), 1.5×10^5 $M^{-1}s^{-1}$ (nifuroxime, E_7^1 = –0.255 V) [37]. The reduction of TNT and other nitroaromatics by P-450R and FNR is accompanied by redox cycling, i.e. oxidation of a significant excess of NADPH over nitroaromatic compound and a stoichiometric to NADPH consumption of oxygen. The single-electron reduction of tetryl and pentryl by FNR and P-450R is accompanied by its redox cycling, *N*-denitration and nitrite formation [28, 38]:



(6)

The pentryl radical undergoes *N*-denitration at a greater rate than tetryl, since pentryl forms a higher amount of nitrite during its redox cycling [28].

The dismutation of nitroanion radicals yields the nitroso compounds (ArNO):

$$2ArNO_2^{\cdot -} + 2H^+ \rightarrow ArNO_2 + ArNO + H_2O. \quad (7)$$

The dismutation rate constant ($2k_d$) for $TNT^{\cdot -}$ and other radicals of explosives is not reported; however, for the radicals of *o-*,*m-*,*p*-dinitrobenzenes, $2k_d$ are equal to 2.4×10^6 $M^{-1}s^{-1}$, 8.0×10^6 $M^{-1}s^{-1}$, and 3.3×10^8 $M^{-1}s^{-1}$, respectively, at pH 7.0 [39]. It is important to note that the fraction of stable reduction products of nitroaromatics may be formed under aerobic conditions due to a competition between the nitroradical oxidation by oxygen and its dismutation. The rate of dismutation of free radicals (V_{dism}) may be expressed as

$$V_{dism} = 2k_d\ ((k_{ox} \times [O_2]/4\ k_d)^2 + V/2k_d)^{1/2} - k_{ox} \times$$

$$\times\ [O_2]/4k_d)^2, \qquad (8)$$

where V is the rate of free radical formation, k_{ox} is the rate constant of its oxidation by oxygen, and $[O_2]$ is the oxygen concentration. Using $V = 10^{-6}$ M/s, $k_{ox} = 10^5$ $M^{-1}s^{-1}$, $[O_2] = 2.5 \times 10^{-4}$ M, and $2k_d = 10^7$ $M^{-1}s^{-1}$, we obtain $V_{dism} = 1.6 \times 10^{-8}$ M/s, i.e., 1.6% of the total nitroreduction rate. Using $V = 10^{-5}$ M/s, we obtain $V_{dism} = 12.3 \times 10^{-7}$ M/s, i.e. 12.3% of the total rate. This shows that the yield of the nitroreduction metabolites should exhibit a square dependence on a single-electron transferring enzyme concentration, which has been confirmed experimentally [40].

Reactions of nitroaromatic explosives with a number of potentially important single-electron transferring flavo-enzymes have not been studied. However, their reactivities may be predicted by the linear log k_{cat}/K_m vs E^1_7 relationships that exist in nitroreductase reactions. The main source of nitroradicals in mammalian mitochondria are the outer membrane NAD(P)H-oxidizing nitroreductase(s) whose properties remain uncharacterized so far [41]. NADH:ubiquinone reductase (complex I of the inner mitochondrial membrane, EC 1.6.5.3) contains FMN, two Fe_2S_2 and minimum three Fe_4S_4 clusters, and transfers electrons to ubiquinone-10. The nitroreductase reaction of complex I is not inhibited by rotenone, a competitive inhibitor to ubiquinone, which binds at the N2 Fe_4S_4 cluster, but is inhibited by NAD^+ and ADP-ribose [42], demonstrating that nitroaromatics are reduced via FMN. It seems likely that complex I may reduce nitroaromatics both by single- and two-electron transfer. FAD-dependent NADPH : adrenodoxin reductase (ADR, EC 1.18.1.2) transfers electrons to Fe_2S_2 protein adrenodoxin (ADX) which in turn reduces cytochromes P-450 in adrenal cortex mitochondria. The low nitroreductase activity of ADR is markedly stimulated by ADX acting as a redox mediator [43]. The nitroreductase reactions of complex I and ADX are characterized by the linear log k_{cat}/K_m vs. E^1_7 dependences. In terms of k_{cat}/K_m, the activity of complex I is close to that of FNR, and the activity of ADX is 3–5 times higher [42, 43]. FAD-dependent NADH: cytochrome b_5 reductase (EC 1.6.2.2), which performs the cytochrome b_5-mediated reduction of cytochromes P-450 in microsomes, participates in microsomal nitroreduction as well [44]. Xanthine oxidase (EC 1.1.3.22) contains FAD, two Fe_2S_2 clusters and molybdopterin cofactors, and catalyzes mixed single- and two-electron reduction of nitroaromatics [45]. This enzyme is supposed to be the main source for nitroreduction in the cytosol [46]. However, the nitroreductase reactions of cytochrome b_5 reductase and xanthine oxidase have not been investigated in detail.

FORMATION OF STABLE METABOLITES OF NITROAROMATIC EXPLOSIVES

In mammalian systems, nitroaromatic compounds are further reduced to amines and / or hydroxylamines which may subsequently form DNA and protein adducts. These stable metabolites may be formed by: a) the reduced oxygen tension and a high local single-electron transferring enzyme concentration, e.g., P-450R in microsomes, may favour the free radical dismutation over their reoxidation by oxygen (Eq. (8)). Subsequently, the nitroso compounds formed (Eq. (7, 8)) will be reduced to hydroxylamines (ArNHOH) and / or amines ($ArNH_2$); and b) the two-electron reduction of nitroaromatics by certain flavoenzymes. In fact, the enzymatic two-electron reduction may be considered as the four-electron reduction, since after the first two-electron (hydride) transfer, the reduction of an intermediate nitroso compound to hydroxylamine (ArNHOH) proceeds 10^4 times faster (Eq. (9)) [47]:

$$ArNO_2 \xrightarrow[-H_2O]{+2e^- + 2H^+} ArNO \xrightarrow{+2e^- + 2H^+} ArNHOH. \qquad (9)$$

Due to the high redox potential (the polarographic reduction potentials of nitroso compounds are by 0.2–0.8 V higher than those of parent nitroaromatics [48]), nitrosobenzenes may be also nonenzymatically reduced by NAD(P)H, reduced glutathione (GSH) and other reductants [47–49]. Alternatively, aromatic hydroxylamines may be formed during the *N*-hydroxylation of amines by cytochrome P-450 [50].

The administration of TNT to laboratory animals leads to the excretion of 4-NHOH-DNT, 2-NH_2-DNT, 4-NH_2-DNT in the urine [51] and to the formation of covalent adducts with microsomal liver and kidney proteins, hemoglobin, and other blood proteins [52]. The acid hydrolysis of adducts yielded mainly 2-NH_2-DNT and 4-NH_2-DNT. Incubation of rat liver microsomes with TNT and NADPH under aerobic conditions resulted in the formation of NH_2-DNTs and the transient metabolite 4-NHOH-DNT, as well as of covalent protein adducts with TNT metabolites [49]. Presumably, the adducts were formed in the reaction of the nitroso metabolite (NO-DNT) reaction with protein or nonprotein thiols.

Aromatic hydroxylamines can modify DNA either directly or via formation of an O-acetylated intermediate. The acetylated intermediate can be transformed to a strongly electrophilic nitrenium ion ($ArNH^+$) capable of modifying guanine bases with the formation of N-(deoxyguanosin-8-yl)-NHAr adducts. However, the formation of TNT adducts with DNA in mammalian cells has not been reported. On the other hand, NHOH-DNT may undergo the transition metal-catalyzed redox cycling which may cause the oxidative damage of DNA [53]:

$$NHOH\text{-}DNT + Cu^{2+} \rightarrow NHO^{\cdot}\text{-}DNT + Cu^+ + H^+, \qquad (10)$$

$$Cu^+ + O_2 \rightarrow Cu^{2+} + O_2^{\cdot-}, \qquad (11)$$

$$NHO^{\cdot}\text{-}DNT + NADH \rightarrow NHOH\text{-}DNT + NAD^{\cdot}, \qquad (12)$$

$$NAD^{\cdot} + O_2 \rightarrow NAD^+ + O_2^{\cdot-}. \qquad (13)$$

In liver microsomes, the P-450R-catalyzed reactions according to Eq. (1, 7, 8) may be the main source of TNT

metabolites [54]. Other formation pathways may be predicted by the analogous reactions of other polynitroaromatics. Rat liver cytosol catalyzed the NAD(P)H-dependent formation of the monoamino- and mononitroso metabolites of 1,3- and 1,6-dinitropyrenes under both aerobic and anaerobic conditions [55]. The amine formation was inhibited by oxygen by 10–60%, whereas the nitroso metabolite formation was either not affected or even enhanced by oxygen. In contrast, the microsomal formation of both amino and nitroso metabolites was inhibited by oxygen by 80–90% [55]. Xanthine oxidase and NAD(P)H : quinone oxidoreductase (DT-diaphorase, NQO1, EC 1.6.99.3) were responsible for the formation of 3-nitroaniline from 1,3-dinitrobenzene in the cytosolic fraction of rat small intestinal mucosa under aerobiosis [46], thus being the potential sources for the formation of the stable metabolites of TNT and other nitroaromatic explosives in the cytosol.

The nitroreductase reactions of NQO1 have been studied more thoroughly [28, 37, 56, 57]. Rat NQO1 contains FAD with the standard (two-electron reduction) potential at pH 7.0, E_7^0, of -0.159 V [58]. The ability of NQO1 to perform two-electron transfer is most probably determined by the instability of its anionic FAD semiquinone, because the E_7^1 of FAD/FAD$^{\cdot-}$ and FAD$^{\cdot-}$/FADH$^-$ couples are equal to -0.200 V and -0.118 V, respectively [58]. The majority of nitroaromatic compounds, including TNT, are very slow NQO1 substrates (k_{cat}/K_m = 10^2–10^4 $M^{-1}s^{-1}$, k_{cat} = 0.1-1.0 s^{-1}), with the exception of TNBO, tetryl and pentryl whose reactivities are intermediate ($k_{cat}/K_m \geq 10^5$ $M^{-1}s^{-1}$, k_{cat} > 10 s^{-1}). Possibly, NQO1 reduces TNT to NHOH-DNTs which is further reduced to dihydroxylamino-NT at a similar rate [30]. NQO1 performs reductive *N*-denitration of tetryl and pentryl (Eq. (6)) with the formation of picramides, other unidentified products, and $O_2^{\cdot-}$, which points to the involvement of single-electron transfer steps [28, 58]. In general, the reactivity of nitroaromatics towards NQO1 increases with an increase in their E_7^1, however, the dependence is very scattered ($r^2 \leq 0.7$). Their reactivity is strongly influenced by the structural parameters which are currently poorly identified, except the positive dependence of the reactivity on the torsion ankle of nitrogroups with respect to the aromatic ring.

TNT and other polynitrobenzenes may be reduced in erythrocytes with the formation of hydroxylamines and covalent protein adducts [51, 60], with a possible involvement of an unidentified NADH-oxidizing flavoenzyme. The formation of stable metabolites of TNT and other nitroaromatic explosives by intestinal microflora or their enzymes has not been investigated, except the study of isolated *Enterobacter cloacae* NAD(P)H:nitroreductase (NR, EC 1.6.99.7) [47, 61]. However, it is possible that other "oxygen-insensitive" nitroreductases of the intestinal microflora will reduce nitroaromatic explosives in a similar way, e.g., the *Escherichia coli* nitroreductase NfsB which shares a 80% sequence identity with *E. cloacae* NR. In the active center, *E. cloacae* NR contains FMN (E_7^0 = -0.19 V) whose semiquinone state is extremely unstable (E_7^1 of FMN$^{\cdot-}$/FMNH$^-$ couple, ~ -0.01 V, and E_7^1 of the FMN / FMN$^{\cdot-}$ couple, ~ -0.37 V) [62]. The reactivity of nitroaromatics towards *E. cloacae* NR increases as their reduction potential increases, showing a little specificity towards their molecular structure [61]. However, the reactivity of 4,6-dinitrobenzofuroxan (DNBF, Fig. 1) is much lower than expected [63]. This is most probably caused by a partial loss of compound aromaticity due to its existence in the form of Meisenheimer adduct with water (Fig. 1). The nitroaromatic explosives with high $E_{7(calc)}^1$ values (tetryl, pentryl, TNC, TNBO) are reduced more rapidly (k_{cat}/K_m = 10^6–10^7 $M^{-1}s^{-1}$, k_{cat} > 100 s^{-1}) than NTO and ANTA (($k_{cat}/K_m \geq 10^4$ $M^{-1}s^{-1}$, $k_{cat} \geq 5$ s^{-1}). NR reduces nitrobenzene to phenylhydroxylamine [47]; however, TNT, tetryl, and pentryl oxidize more than two equivalents of NADH [61]. The reduction of NHOH-DNT's by *E. cloacae* NR is accompanied by a close to stoichiometric O_2 consumption, as well as the oxidation of more than two-fold excess of NADH by TNT [30]. Most probably, *E. cloacae* NR reduces TNT to dihydroxylamino-NT, which undergoes a rapid autoxidation. The reduction mechanism of tetryl and pentryl by *E. cloacae* NR is different from that described by Eq. (6), since no picramides are formed, and nitrite is formed not during the explosive reduction by two NADH equivalents, but in subsequent steps [61].

MAMMALIAN CELL CYTOTOXICITY OF NITROAROMATIC EXPLOSIVES

Mammalian cell culture cytotoxicity data of TNT, its metabolites, and other nitroaromatic explosives, expressed as a concentration causing 50% cell death (cL_{50}), are summarized in Table [9, 28–30, 56, 63–65]. Other less comprehensive studies show that cL_{50} of TNT and 2-NH_2-DNT are above 450 μM for human neuroblastoma NG108 (7 h incubation) [67], and that cL_{50} of TNT for human hepatocarcinoma HepG2 is 460 ± 26 μM (48 h incubation) [10]. The explosive-contaminated soil extract containing 68% TNT and 12% NH_2-DNTs, was toxic to human fibroblasts GM05757 with cL_{50} close to 2.1 mg/l (1 h incubation, 48 h growth in extract-free medium) [68]. The extracts of TNT-contaminated soil show an immunotoxic activity *in vitro* [69], however, the data were not expressed in quantitative terms.

The most thorough cytotoxicity studies were performed with bovine leukemia virus-transformed lamb kidney fibroblasts (line FLK) [28–30, 56, 63, 64]. The cytotoxicity of TNT, tetryl, PATO, NTO, ANTA, MNTA and TNBO in FLK cells was reduced by the antioxidant *N,N'*-diphenyl-*p*-phenylene diamine and desferrioxamine, an iron ion chelator which prevents the Fenton reaction (Eq. (4-6)). In contrast, the alkylating agent 1,3-bis-(2-chloroethyl)-1-nitrosourea, which inactivates glutathione reductase and depletes intracellular GSH, potentiated the cellular toxicity. Cytotoxicity was accompanied by lipid peroxidation, suggesting the involvement of oxidative stress resulting from the enzymatic redox cycling of anion-radicals of

explosives (Eq. (3–6)). Enzymes responsible for free radical formation in FLK cells have not yet been identified. Dicumarol, an inhibitor of NQO1, partly reduced the cytotoxicity of TNT and TNBO but, surprisingly potentiated the cytotoxicity of tetryl and TNC. Both the protective and potentiating effects of dicumarol may not be considered as significant, since it affected the cL_{50} values of the above compounds approximately 1.5 times, showing that the cytotoxic consequences of reaction of NQO1 with all explosives in FLK cells are equivocal, evidently depending on the further metabolic fate of reaction products. For a number of explosives and model nitroaromatics, log cL_{50} in FLK cells decreased with an increase in their $E^1_{7(calc)}$ (Table) with a coefficient ~ -6.7 V^{-1} (r^2 = 0.80). This type of dependence shows that the main mechanism of the cytotoxicity of explosives in this cell line is the oxidatives stress initiated by the flavoenzyme-catalyzed single-electron reduction (Eq. (1–5)) [70]. Our preliminary studies of an immunocompetent cell line, mouse splenocytes (Table), show that this cytotoxicity mechanism may be common for a number of cell types, because there exists a linear relationship (r^2 = 0.83) between the log cL_{50} of nitroaromatic explosives in FLK cells and splenocytes. This finding may be important for the quantitative description of immunotoxic action of explosives. On the other hand, it should be noted that FLK cell cytotoxicity of NHOH-DNTs, NH_2-DNTs and 2,4-diamino-6-nitrotoluene ($(NH_2)_2$-NT) (Table) is somewhat higher than could be expected from their $E^1_{7(calc.)}$ [30]. Analyzing the FLK cell cytotoxicity, we found that the omission of compounds 2–6 (Table) from the correlation improves it (r^2 = 0.88) and gives the coefficient $\Delta \log cL_{50} / \Delta E^1_7 = -7.9$ V^{-1}. Besides, in other cell lines except splenocytes, NHOH-DNTs are as toxic as TNT, or even more (Table). These data may be indicative of a role of the parallel cytotoxicity mechanisms, e.g., redox cycling of hydroxylamines (Eq. (10–13)), their binding to macromolecules, and the possibility of formation of toxic hydroxylamines from amines [50] whose importance may depend on the cell type.

CONCLUSIONS

This paper summarizes the present state of knowledge on the chemical aspects of cytotoxicity of nitroaromatic explosives So far, the leading role of the oxidative stress-type cytotoxicity of explosives has been demonstrated in FLK cells and, possibly, in mouse splenocytes. However, because of the incomprehensive studies, it is impossible to establish the main cytotoxicity mechanisms of explosives in other cell lines (Table). In this aspect, comparison of the cytotoxicity of explosives and model compounds with a wide range of E^1_7 values could be a promising approach. Nevertheless, data on the enzymatic reactivity and mammalian cell cytotoxicity of explosives provide some background for the understanding of their overall toxicity. For example, NTO is almost nontoxic in rats and mice (cL_{50} > 5g/kg when given perorally [71]), whereas cL_{50} for TNT and tetryl are equal to 660–1320 mg/kg, and above 300 mg/kg, respectively [1, 5]. This is consistent with a much lower $E^1_{7(calc.)}$ of NTO and cell culture cytotoxicity (Table). This paper also outlines some future directions for research: i) identification and characterization of both the enzyme and metabolites of nitroaromatic explosives in mammalian cell mitochondria, cytosol, erythrocytes and gastrointestinal microflora; ii) enzymatic and cytotoxicity studies of the nitroaromatic explosives of a new generation, and iii) identification of protein targets of the explosive toxic action in the organism, including the proteomic approach, as well as characterization of the mode of their binding to DNA.

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CHEMINIAI NITROAROMATINIŲ SPROGMENŲ CITOTOKSIŠKUMO ASPEKTAI (APŽVALGA)

Santrauka

Nitroaromatiniai sprogmenys ir jų degradacijos produktai yra toksiški aplinkos teršalai. Šiame straipsnyje apibendrinami duomenys apie klasikinių ir naujos kartos nitroaromatinių sprogmenų toksiškumo cheminius aspektus, t. y. apie jų vien- ir dvielektroninę redukciją, katalizuojamą flavininiais fermentais, ir šių reakcijų reikšmę sprogmenų citotoksiškumui žinduolių ląstelėse. Šiuo metu nustatyta, kad pagrindinis nitroaromatinių junginių citotoksiškumo mechanizmas galvijų leukemijos virusu transformuotuose ėriuko inkstų fibroblastuose (linija FLK) ir, greičiausiai, pelės blužnies ląstelėse yra oksidacinis stresas.