Environmental fate of 2,4-dinitroanisole (DNAN) and its reduced products

ARTICLE *in* CHEMOSPHERE · JANUARY 2015

Impact Factor: 3.34 · DOI: 10.1016/j.chemosphere.2014.05.047

Chemosphere 119 (2015) 16–23

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Environmental fate of 2,4-dinitroanisole (DNAN) and its reduced products

J. Hawari [a,*], F. Monteil-Rivera [a], N.N. Perreault [a], A. Halasz [a], L. Paquet [a], Z. Radovic-Hrapovic [a], S. Deschamps [a], S. Thiboutot [b], G. Ampleman [b]

[a] National Research Council Canada, 6100 Royalmount Ave, Montreal, Quebec H4P 2R2, Canada
[b] Defence Research Development Canada, Department of National Defence, Valcartier, QC, Canada

HIGHLIGHTS

- DNAN undergoes regioselective reduction at the *o*-NO_2 under abiotic and biotic conditions.
- Physicochemical parameters (S_w, pK_a, $\log K_{ow}$, K_d) are provided for DNAN and its amine products.
- Physicochemical behavior depends on type and position of substituent(s) on the aromatic ring.
- Amine products of DNAN irreversibly bind to soil under oxic conditions.

ARTICLE INFO

Article history:
Received 14 February 2014
Received in revised form 17 April 2014
Accepted 19 May 2014
Available online 14 June 2014

Handling Editor: I. Cousins

Keywords:
2,4-Dinitroanisole
2,4-Diaminoanisole
Regioselectivity
Insensitive munition

ABSTRACT

Several defense departments intend to replace 2,4,6-trinitrotoluene (TNT) in munitions formulations by the less sensitive 2,4-dinitroanisole (DNAN). To help understand environmental behavior and ecological risk associated with DNAN we investigated its key initial abiotic and biotic reaction routes and determined relevant physicochemical parameters (pK_a, $\log K_{ow}$, aqueous solubility (S_w), partition coefficient (K_d)) for the chemical and its products. Reduction of DNAN with either zero valent iron or bacteria regioselectively produced 2-amino-4-nitroanisole (2-ANAN) which, under strict anaerobic conditions, gave 2,4-diaminoanisole (DAAN). Hydrolysis under environmental conditions was insignificant whereas photolysis gave photodegradable intermediates 2-hydroxy-4-nitroanisole and 2,4-dinitrophenol. Physicochemical properties of DNAN and its amino products drastically depended on the type and position of substituent(s) on the aromatic ring. S_w followed the order (TNT < DNAN < 2-ANAN < 4-ANAN < DAAN) whereas $\log K_{ow}$ followed the order (DAAN < 4-ANAN < 2-ANAN < DNAN < TNT). In soil, successive replacement of $-NO_2$ by $-NH_2$ in DNAN enhanced irreversible sorption and reduced bioavailability under oxic conditions. Although DNAN is more soluble than TNT, its lower hydrophobicity and its tendency to form aminoderivatives that sorb irreversibly to soil contribute to make it less toxic than the traditional explosive TNT.

1. Introduction

The insensitive munition compound 2,4-dinitroanisole (DNAN) is currently under evaluation by the defense industry as a replacement for more sensitive explosives such as 2,4,6-trinitrotoluene (TNT). It has been reported that DNAN requires a higher temperature than TNT for detonation thus making it safer to manufacture, transport and store (Davies and Provatas, 2006). Before large scale production and deployment in the field, the fate and ecological risk associated with DNAN and its potential transformation products, especially the amine-derivatives, require investigation. Few studies have been reported on the biotic or abiotic transformation of DNAN (Platten et al., 2010; Ahn et al., 2011; Perreault et al., 2012; Olivares et al., 2013; Rao et al., 2013; Salter-Blanc et al., 2013). Several of these studies identify the reduced products, 2-amino-4-nitroanisole (2-ANAN) and 2,4-diaminoanisole (DAAN), as key initial or final products (Platten et al., 2010; Ahn et al., 2011; Perreault et al., 2012; Olivares et al., 2013). Currently, there is only limited knowledge available on the transport, transformation, and toxicity of DNAN and its amine derivatives (Fig. 1).

Other nitroaromatic compounds (NACs) such as TNT and dinitrotoluenes (DNTs) are known to undergo sequential reduction to their corresponding amino derivatives with selectivities depending

* Corresponding author. Tel.: +1 (514) 496 6267; fax: +1 (514) 496 6265.
E-mail address: jalal.hawari@cnrc-nrc.gc.ca (J. Hawari).

http://dx.doi.org/10.1016/j.chemosphere.2014.05.047
0045-6535/

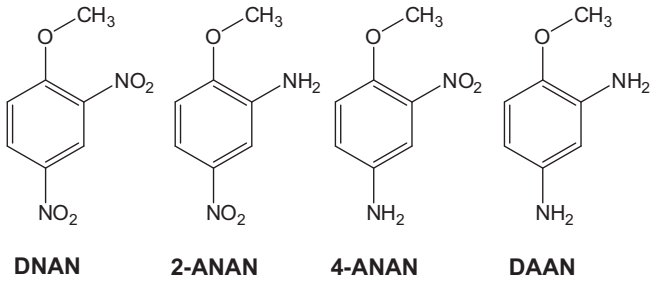

Fig. 1. Structures of DNAN and its amino products 2-amino-4-nitroanisole (2-ANAN), 4-amino-2-nitroanisole (4-ANAN), and 2,4-diaminoanisole (DAAN).

on redox conditions used and on the position of the —NO_2 group on the aromatic ring (Barrows et al., 1996). A number of papers reveal that aromatic amines, if formed in a soil environment, tend to irreversibly bind to soil organic matter through covalent bonding, e.g. —CO—NH— linkages (Haderlein and Schwarzenbach, 1995; Rieger and Knackmuss, 1995; Elovitz and Weber, 1999; Thorn and Kennedy, 2002). Once bound to soil, the amines are considered to represent a lower ecotoxicological risk due to their lack of bioavailability. One might exploit past observations on TNT environmental behavior, i.e. transformation, transport and ecotoxicity, to gain some insights into the environmental behavior of DNAN but the immediate challenge that one may face is how to deal with the dramatic differences in the reaction products of the two nitroaromatic compounds. In most cases TNT is regioselectively reduced at the *para*-position (Funk et al., 1993; Rugge et al., 1998; Elovitz and Weber, 1999; Wang et al., 2000), whereas DNAN seemingly favors reduction at the *ortho*-position (Perreault et al., 2012; Olivares et al., 2013). For example, Barrows et al. (1996) reported that abiotic reduction of TNT with bisulfite is 100% regioselective towards forming the *para* isomer 4-amino-2,6-dinitrotoluene as opposed to DNAN that under the same conditions produces only the *ortho* isomer 2-ANAN. Different products mean different physicochemical properties (aqueous solubility (S_w), pK_a, K_{ow}) which will strongly affect the partitioning of chemicals between water, soil, and environmental receptors, and consequently the ecological impact of these chemicals.

The present study was thus undertaken to help understand the behavior of DNAN under natural environmental conditions and to compare its behavior to that of TNT, which it may replace in the near future. We first investigated relevant transformation processes likely to occur under environmental conditions. Then we determined key relevant physicochemical and transport parameters (K_{ow}, pK_a, S_w, K_d) of DNAN and its observed products to predict their respective ecotoxicological risk and therefore provide an answer to the key question of whether DNAN represents an environmental risk higher or lower than that induced by TNT.

2. Materials and methods

2.1. Chemicals

DNAN (98.4%) was provided by Defense Research and Development Canada (Valcartier, QC). DAAN (99%), 2,4-dinitrophenol (DNP) (85%) and 1-octanol were purchased from Sigma–Aldrich (Oakville, ON). 2-ANAN (99%) was purchased from MD Biomedicals (Santa Ana, CA) and 4-ANAN (98%) was purchased from Apollo Scientific, Bradbury, UK. Zero valent iron (ZVI) was from Fisher Scientific (Nepean, ON). All solvents and reagents were used as received.

2.2. Soils

Two soils were used in this study. Table 1 lists their relevant properties. PETAWAWA soil was a silty soil sampled (5–15 cm depth) at Petawawa training range (ON, Canada), and TOPSOIL soil was a gardening top soil purchased from a local gardening company (Fafard, QC, Canada). Each soil was stored in a cold room (4 °C) until use, passed through a 2-mm sieve, air dried, and sterilized by gamma irradiation from a ^{60}Co source at the Canadian Irradiation Center (Laval, Quebec) with a dose of 50 kGy over 2 h.

2.3. Biological transformation of DNAN

Anaerobic biotransformation of DNAN was performed with resting cells of *Enterobacter* strain DM7 (our lab), *Shewanella oneidensis* strain MR-1 (ATCC 700550), *Pseudomonas fluorescens* I–C (NRRL B-59269) and *Burkholderia cepacia* strain JS872 (ATCC 700450). Cells were grown anaerobically, in LB (MR-1, I–C and JS872) or M9 minimal medium (Maniatis et al., 1982) plus 10 mM $NaNO_3$ (DM7), in the presence of 50 μM DNAN. The cells were harvested at late exponential phase, washed twice in sterile double-distilled water (ddH_2O), and resuspended at an optical density (at 600 nm) of ~3.3 in ddH_2O containing 200 μM DNAN. The reactions were performed in triplicate in serum bottles that were sealed and made anaerobic by briefly degassing (1 min) and then purging with argon for 10 min at 5 psi. The bottles were incubated at 25 °C in the dark. Samples were collected at selected times (1–22 h) for analysis of DNAN and its products as described below.

2.4. Reaction of DNAN with zero valent iron, ZVI

Experiments were carried out in 60-mL serum bottles each containing granular iron (*ca* 40 mesh) (0.5 g) and 50 mL aqueous solution of DNAN (50 mg L^{-1}) at room temperature. The bottles were sealed with Teflon coated septa under argon. Aliquots (2 mL) of the reaction mixture were withdrawn at selected times ranging from 15 to 360 min and analyzed by LC–MS as described below. Experiments were conducted in duplicate.

2.5. Photolysis of DNAN

Artificial sunlight (total irradiance of 590 000 mW m^{-2}) generated from a SolSim photoreactor (Luzchem Research Inc., Canada) was used to photolyze DNAN under solar-simulated conditions. Irradiation assays were conducted in duplicate at 25 °C in 20 mL quartz crucibles (25 mm ID) containing 5 mL of aqueous solutions of DNAN (50 mg L^{-1}). Samples were withdrawn at selected times ranging from 0.7 to 21 d and analyzed by HPLC and LC–MS as described below. DNP (70 mg L^{-1}) was also photolyzed using the same conditions. A control containing DNAN covered with aluminum foil was also prepared.

2.6. Solubility measurements

Aqueous solubility of DNAN, 2-ANAN, 4-ANAN, and DAAN was measured at 25 °C, in triplicate, as described previously (Monteil-Rivera et al., 2004). Briefly, suspensions of DNAN, 2-ANAN, or 4-ANAN were agitated at 150 rpm and analyte concentration was measured in the supernatant until constant values were measured. Solubility was reached within 2 weeks for DNAN and 2-ANAN, and 3 months for 4-ANAN. For DAAN, an anoxic suspension (0.4 g/10 mL) was prepared under argon to minimize decomposition and polymerization, then sonicated for 5 min, capped and sealed with an aluminum crimp and stirred at 150 rpm. At each sampling event, the suspension was deaerated with argon and sonicated for

Table 1
Physicochemical properties of soils investigated.

	Particle size distribution		Total Org. C (%)	pH	CEC[a] (meq/100 g)
	% Clay/silt (<80 μm)	% Sand (>80 μm)			
PETAWAWA	44.1	55.9	2.5	4.9	<10
TOPSOIL	0.6	99.4	34.0	6.1	35.0

[a] CEC = Cationic Exchange Capacity.

5 min. Aliquots of the aqueous phase were analyzed by HPLC as described below.

2.7. Octanol–water coefficient (K_{ow}) measurements

LogK_{ow} for DNAN, 2-ANAN, 4-ANAN, and DAAN was measured in triplicate at 23 ± 2 °C using the traditional flask shaking method as described previously (Monteil-Rivera et al., 2004). The aqueous phase was analyzed directly by HPLC whereas the octanol phase was diluted five times with a solution containing 70% methanol in water prior to HPLC analysis.

2.8. Measurement of dissociation constants, pK_a's

pK_a of 2-ANAN, 4-ANAN, and DAAN was measured spectrophotometrically as described by Albert and Serjeant (1971) using 10^{-2} M chloroacetic, formic, and chloroacetic and acetic buffers, respectively.

2.9. Sorption assays

Sorption and fate of DNAN and its amino-derivatives were studied in long term batch experiments using the soils described in Table 1. Sorption experiments were conducted in borosilicate tubes (50-mL) containing either DNAN, 2-ANAN, 4-ANAN, or DAAN (50 mg L^{-1} each) and sterile soil (1.5 g) in 10 mL water. Samples were incubated aerobically, statically and away from light at room temperature (23 ± 2 °C). Samples were not tightly closed and were shaken twice a week to ensure a good aeration level. At time intervals varying from 1 d to 2 months, three replicates were sacrificed. The supernatant was withdrawn, filtered through a 0.45 m PVDF Millipore filter (Millipore Corp., Bedford, MA), diluted 1/1 in acetonitrile (CH_3CN) and analyzed by HPLC as described below. Sorbed analyte was extracted from soil by sonication in acetonitrile as described in the EPA SW-846 Method 8330 (USEPA, 1997). The soil water distribution coefficient (K_d in L kg^{-1}) was calculated as the ratio $[DNAN]_s/[DNAN]_{eq}$, where $[DNAN]_s$ is the concentration of DNAN adsorbed on soil (mg kg^{-1}) and $[DNAN]_{eq}$ is the concentration of DNAN in the aqueous phase at equilibrium (mg L^{-1}). The normalized distribution coefficient (K_{oc}) was calculated as the ratio K_d/f_{OC} where f_{OC} represents the fraction of organic carbon in soil. A percent recovery was calculated as $((DNAN_s + DNAN_l)/DNAN_{ini}) \times 100$, where $DNAN_s$, $DNAN_l$ and $DNAN_{ini}$ represent the amount (μg) of DNAN sorbed, soluble, and initially introduced, respectively.

2.10. Stability of DAAN in water

An aqueous solution of DAAN (100 mg L^{-1}) was prepared using water deaerated with 20-min argon bubbling and split into two bottles. One bottle (oxic) was vigorously aerated under air, screw capped and kept at room temperature in the dark. The second one (anoxic) was capped with a rubber stopper, crimped with aluminum seal, bubbled with argon for 10 min and incubated statically at room temperature in the dark. Aliquots of the oxic and anoxic solutions (3 mL) were periodically sampled and analyzed by HPLC–UV, UV–Vis, and LC–MS, as described below. The oxic sample was stirred for 10 min under air at each sampling event.

2.11. Analytical methods

UV–Vis spectra of aqueous solutions of DNAN or its products were collected using a UV1 Thermo Spectronic spectrophotometer.

DNAN and its reduced products were quantified by HPLC as described previously, using 50% methanol as mobile phase and a C18 column for separation (Perreault et al., 2012). Detection limits were estimated to be 0.010 mg L^{-1} at 298 nm for DNAN, 0.005 mg L^{-1} at 245 nm for 2-ANAN and 4-ANAN, and 0.010 mg L^{-1} at 245 nm for DAAN.

Unknown products were analyzed using a Bruker MicroTOFQ mass analyzer attached to an HPLC system (Hewlett Packard 1200 Series) equipped with a DAD detector. Samples (10 μL) were injected into a 2.5 μm-pore size Synergi-Polar column (2 mm ID × 100 mm; Phenomenex) at 25 °C. The solvent system was composed of CH_3OH and HCOOH (0.05%) at a flow rate of 0.15 mL min^{-1} using a gradient. For mass analysis, positive and negative electrospray ionization modes were used to produce protonated $[M + H]^+$ and deprotonated $[M–H]^-$ molecular mass ions. Mass range was scanned from 40 to 500 Da.

Nitrite, nitrate, formate and ammonium were quantified by ion chromatography as described previously (Balakrishnan et al., 2004). Formaldehyde derivatized with 2,4-pentadione was quantified by HPLC (Bhatt et al., 2006).

3. Results and discussion

3.1. Transformation of DNAN

When we investigated the aerobic biotransformation of DNAN by *Bacillus* sp., DNAN appeared to give regioselectively the *ortho* isomer, 2-ANAN, as a terminal reduced amine product (Perreault et al., 2012). No DNAN ring cleavage products were observed. Recently, Olivares et al. (2013) also reported regioselective reduction of DNAN to 2-ANAN in sludge that subsequently was reduced to DAAN under both aerobic and anaerobic conditions. In the present study, transformation of DNAN by resting cells of four strains of DNAN-reducing bacteria was investigated anaerobically. Resting cells of *Enterobacter* strain DM7 incubated anaerobically transformed DNAN at the rate of 9.4 ± 0.2 μmol min^{-1} g^{-1} protein with the formation of 2-HA-NAN (2-hydroxylamino-4-nitroanisole) that subsequently reduced to 2-ANAN (0.42 mol for each mole of DNAN degraded). Similarly, *B. cepacia* JS872 reduced the —NO_2 of DNAN at the *ortho*-position only. Resting cells of *S. oneidensis* MR-1 transformed DNAN into 2-ANAN at a rate of 2.1 ± 0.2 μmol min^{-1} g^{-1} protein. However in this case, 2-ANAN further transformed to DAAN. Anaerobic cells of *P. fluorescens* I–C followed the same pathway as MR-1, transforming DNAN to DAAN *via* 2-ANAN. In no case was 4-ANAN detected. This differs significantly from TNT, which was shown to be biologically reduced to its *para* amine derivative, 4-amino-2,6-dinitrotoluene (Funk et al., 1993).

Abiotically, Ahn et al. (2011) reported reduction of DNAN with ZVI to DAAN with the formation of intermediates such as 2-ANAN

and 4-ANAN without any reference to regioselectivity. Koutsospyros et al. (2012) used a Fe/Cu system to degrade DNAN but no products were shown. In the present study, treatment of DNAN with ZVI gave the *ortho*-hydroxylamine (2-HA-NAN) in few min; the latter was further reduced to the *ortho*-amine derivative, 2-ANAN, which was subsequently reduced to the diamine DAAN product (Fig. 2). The nitroso-nitroanisole detected in samples at 15 and 80 min by LC–MS was presumably the *ortho*-nitroso derivative (2-NO-NAN) of the DNAN (Fig. 2). After 6 h of reaction DAAN was detected as the only product. The *para*-isomer, 4-ANAN, was not detected. Regioselectivity of Ar—NO_2 reduction in substituted NACs usually depends on the reducing agent used and the type and position of the substituent relative to the nitro group. In DNAN, regioselectivity might be controlled by two competing factors, one driven by steric effects favoring reduction at the *para* —NO_2 group and another driven by electronic effects favoring reduction at the more electronegative —NO_2 group, i.e., the *ortho* position (Davey et al., 1994; Barrows et al., 1996). This analysis is best exemplified by the products distribution observed during reduction of DNAN and its sulfur analogue 2,4-dinitrothioanisole using Baker's yeast. In the first case, the reduction was 80% regioselective at the *ortho* position (electronically favored) while in the second case the more sterically hindered S atom directs reduction mostly at the *para* position (Davey et al., 1994). Terpko and Heck (1980) found that triethyl ammonium formate reduces 2,4-DNT at the least hindered *para* —NO_2 group but reduces DNAN and other dinitroaromatics including 2,4-dinitroaniline and 2,4-dinitrophenol (DNP) at the more sterically hindered *ortho*- position. In addition to the electronic rationale given above to explain regioselective *ortho* reduction of DNAN, we attribute the *ortho* regioselective reduction to the stability gained through intramolecular H-bonding between the –OMe group and the *ortho* —NH_2 which are absent in the case of the *para*-isomer 4-ANAN. In summary, experimental evidences gathered from the present work and from literature reports indicate that reduction of DNAN occurs regioselectively at the *ortho* position.

When a DNAN aqueous solution was photolyzed under solar simulated conditions, DNAN disappeared following a first order kinetics at a rate of 0.22 d^{-1}. After complete disappearance of DNAN (t = 21 d), we detected nitrate anion (0.7 mol), ammonium (1.0 mol), and formaldehyde and formic acid (total of 0.9 mol) for each mole of DNAN degraded. 2-hydroxy-4-nitroanisole (2-HONAN) together with smaller amounts of DNP were detected (Fig. 3) but neither the *ortho*- nor the *para*- ANAN isomer was observed. Production of DNP started quickly and reached a maximum at 7 d of irradiation. In a separate experiment, we found DNP to be unstable under the same photolytic conditions giving nitrocatechol as a major degradation product. This finding is in line with an earlier study by Rao et al. (2013) who found the same products and neither 2-ANAN nor 4-ANAN during photolysis of DNAN. Interestingly, we detected several formamide derivatives of both aminonitroanisole and aminonitrophenol, which most likely resulted from the reaction of formaldehyde (or formic acid) originally generated from the demethylation of the ArO—Me group in DNAN with the amine groups of the amino derivatives.

As for hydrolysis, Hill et al. (2012), Salter-Blanc et al. (2013) indicated the much slower hydrolysis of DNAN compared to TNT. Although DNAN has been shown to hydrolyze to give mainly DNP (Murto and Tommila, 1962; Rochester, 1963; Davies and Provatas, 2006), the reaction occurred only under severe alkaline conditions ($\geqslant$pH 12). Under natural environmental conditions, DNAN does not hydrolyze.

3.2. Physicochemical properties of DNAN and its products

Table 2 summarizes the physicochemical properties (S_w, pK_a, and K_{ow}) measured herein for DNAN and its amino derivatives 2-ANAN, 4-ANAN, and DAAN. Relevant parameters of the more traditional explosive TNT are also gathered in Table 2 for comparison.

The aqueous solubility of DNAN and its major products follows the order: DNAN < 2-ANAN < 4-ANAN < DAAN (Table 2). Sequential reduction of the nitro groups into amino groups increased the water solubility of the aromatic chemical. However, while the solubility of 4-ANAN (4.43 ± 0.06 g L^{-1}) was twenty times higher than that of DNAN (0.213 ± 0.012 g L^{-1}), that of 2-ANAN (0.252 ± 0.008 g L^{-1}) was only slightly higher than DNAN. Two chemical phenomenons might explain these different solubilities: 1/the *para* isomer might have an ability to form solute–solvent intermolecular H-bonding that the *ortho* isomer cannot form due to the intramolecular H-bonding mentioned above, or 2/the *para* isomer is more easily protonable than the *ortho* isomer. In water, substituted aminoaromatics ($ArNH_2$) equilibrate with their acidic protonated forms ($ArNH_3^+$) and the dissociation constant (pK_a) of the latter depends on the relative position of the NH_2 group and other substituents, —MeO and —NO_2 in the present case, on the aromatic ring. The pK_a values measured herein show that 4-ANAN (pK_a 3.50) will be more protonated and hence more soluble than 2-ANAN (pK_a 2.55) in distilled water (pH 5.5). As for DAAN (pK_{a1} = 2.61; pK_{a2} = 5.46) half of the chemical is expected to be monoprotonated

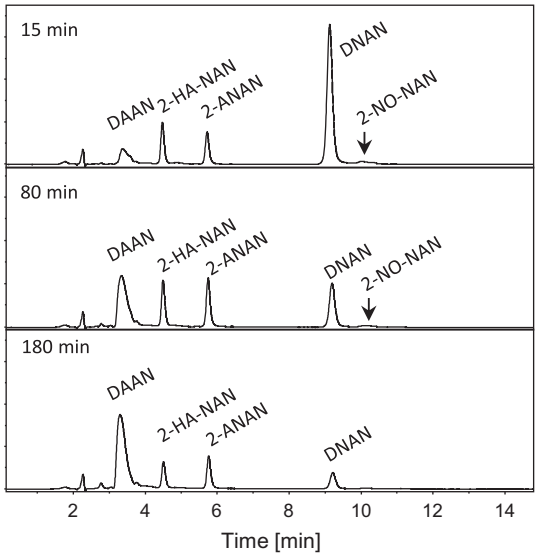

Fig. 2. UV chromatograms (Diode Array signals between 200 and 400 nm) of DNAN and its products after various times of ZVI reduction.

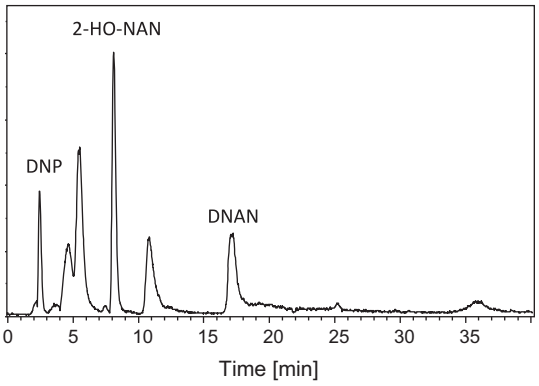

Fig. 3. LC–MS (ES-) extracted ion chromatogram (m/z 153) of DNAN and its products photolyzed after 1 d of simulated solar irradiation.

Table 2
Properties of TNT, DNAN, and DNAN major products.

Compound	S_w at 25 °C (g L^{-1})		Log K_{ow} at 25 °C		pK_a at 25 °C	
	Value[a]	Reference	Value[a]	Reference	Value[a]	Reference
TNT	0.150	Rosenblatt et al., 1991	1.86–2.00	Rosenblatt et al., 1991	NA[b]	–
DNAN	0.213 ± 0.012	This work	1.58 ± 0.01	This work	NA	–
2-ANAN	0.252 ± 0.008	This work	1.47 ± 0.01	This work	2.55 ± 0.03	This work
4-ANAN	4.43 ± 0.06	This work	0.80 ± 0.01	This work	3.50 ± 0.02	This work
DAAN	>40	This work	<−1	This work	2.61 ± 0.02 (*ortho*) 5.46 ± 0.04 (*para*)	This work

[a] Values are given as mean ± standard deviation.
[b] NA = nonapplicable.

at the water pH of 5.5, thus explaining its markedly higher water solubility. TNT was the least soluble of all chemicals (0.15 g L^{-1}).

The octanol/water partition coefficient, logK_{ow}, for DNAN and its reduced products followed the order: DNAN > 2-ANAN > 4-ANAN > DAAN (Table 2), which is the exact opposite of the observed solubility trend. Successive replacement of the —NO_2 groups by —NH_2 groups in DNAN therefore reduces the hydrophobicity (Table 2). The precursor, DNAN, which is less soluble than any of its amine derivatives, exhibited a higher logK_{ow} value (1.58). The fully reduced DAAN, which partially ionizes in water, is very polar and has a marked preference for water over organic solvents (logK_{ow} < −1). TNT, with its three —NO_2 groups, was the most hydrophobic chemical of all (1.8 < logK_{ow} < 2.0).

With significantly higher solubility in water and equal or lower hydrophobicity than DNAN, DNAN products including 2-ANAN, 4-ANAN, and DAAN have a high potential to migrate through subsurface soil unless their migration is slowed down by immobilization mechanisms different from simple hydrophobic partitioning. The next section is aimed at elucidating soil–water interactions for DNAN and its products.

3.3. Sorption and fate of DNAN and its products in soil

Sorption and fate of the four chemicals were measured individually in the two sterile soils under aerobic conditions (Fig. 4). The amounts of chemicals sorbed on soil were obtained by extraction in CH_3CN, which should only desorb chemicals sorbed by weak (electrostatic, electron donor–acceptor) and stronger (hydrophobic partitioning) reversible interactions but not the ones irreversibly chemisorbed by covalent binding (Elovitz and Weber, 1999). While DNAN sorbed reversibly on the two soils, the monoamines sorbed both reversibly and irreversibly on both soils, and DAAN sorbed or reacted (see stability of DAAN below) irreversibly in the two soils (Fig. 4). K_d values corresponding to reversible non-covalent binding are provided in Table 3 along with normalized K_{oc} values. The concurrent reversible and irreversible processes observed with 2-ANAN and 4-ANAN led to non-equilibrium situations that forced us to measure K_d values for both monoamines at day 2, when irreversible binding was at its minimum.

Nitroaromatics (NACs) can sorb reversibly to organic matter, as dictated by their K_{ow}, or to clay minerals through electron donor–acceptor complexes with the oxygen of the siloxane surface(s) of the clays or through complexation with exchangeable cations (Haderlein et al., 1996; Zhang et al., 2005; Qu et al., 2011). The large K_d values measured in TOPSOIL soil despite its low clay content and the overall correlation observed between K_d's and K_{ow}'s confirm the binding of DNAN, 2-ANAN and 4-ANAN with organic matter. On the other hand, the markedly larger K_{oc} values measured for all three amino compounds in PETAWAWA soil (Table 3) suggest the occurrence of interactions between the three amines and clay. The concurrent sorption of nitroaromatics to both organic matter and clay in natural soil was also recently demonstrated by Qu et al. (2011) using 2,4-DNT. As previously reported for other NACs (Haderlein et al., 1996), in the present study, reversible sorption decreased with the number of nitro groups and followed the order (DNAN > 2-ANAN > 4-ANAN > DAAN ≈0).

Opposite to reversible sorption, irreversible sorption increased with the number of amino groups and was more pronounced for 4-ANAN than for 2-ANAN. While DNAN was fully recovered after two months in sterile PETAWAWA and TOPSOIL soils, less than 40% of 2-ANAN and 20% of 4-ANAN was recovered after 2 months in either soil, and DAAN did not persist after 3 d in either soil. The increasing irreversible binding observed for DNAN amine products with increasing number of amino groups has previously been observed when contacting other NACs, e.g. TNT, and their reduced products with organic rich soils under oxic conditions (Haderlein and Schwarzenbach, 1995; Rieger and Knackmuss, 1995; Elovitz and Weber, 1999). In the absence of catalyst, the —NH_2 group of aromatic amines undergo nucleophilic addition with quinones and other C═O groups of the natural organic matter (Thorn and Kennedy, 2002). Using ^{15}N NMR and labeled chemicals, Thorn and Kennedy, 2002; Thorn et al., 2008) showed that amino-derivatives of TNT or DNTs bind covalently to soil organic matter through aminohydroquinone, aminoquinone, imine, and amide linkages. The increasing pK_a values measured for 2-ANAN, 4-ANAN, and DAAN are indicative of an increase of nucleophilicity of the —NH_2 in the order 2-ANAN < 4-ANAN < DAAN, which supports a similar order for the increase of binding capacity of the chemical with electrophilic sites (C═O, COOH). The most common products of DNAN, 2-ANAN and DAAN, will be less mobile than DNAN or completely immobilized, respectively, in soil under oxic conditions. If nontransformed, DNAN will be slightly more mobile than TNT in soil (Table 2).

3.4. Stability of DNAN products in water

We found both monoaminated products of DNAN to be stable over six months in water, under air, at ambient temperature (23 ± 2 °C), and away from light. Under the same conditions, DAAN remained stable under argon but disappeared under air at a rate of approximately 0.08 d^{-1}. Disappearance of DAAN under air was accompanied by the formation of several products as indicated by the broad shoulder appearing between 250 and 500 nm in UV–Vis spectra (Fig. 5). LC–MS (ES+) analysis of the oxic solution after 18 d showed two peaks with protonated molecular mass ions $[M+H]^+$ at m/z 273.116 and m/z 259.101 matching the empirical formulae $C_{14}H_{16}N_4O_2$ and $C_{13}H_{14}N_4O_2$, respectively, which were tentatively identified as the azo dimer of DAAN and its demethylated azo derivative. Previously, Platten et al. (2010) observed a product with an m/z 259 after exposing DAAN to air in the effluent of an anaerobic digester fed with DNAN and attributed the mass ion to a mass fragment of the azo dimer of DAAN with $[CH_2]^+$ cleaved off. Our study suggests that both the azo dimer and the demethylated

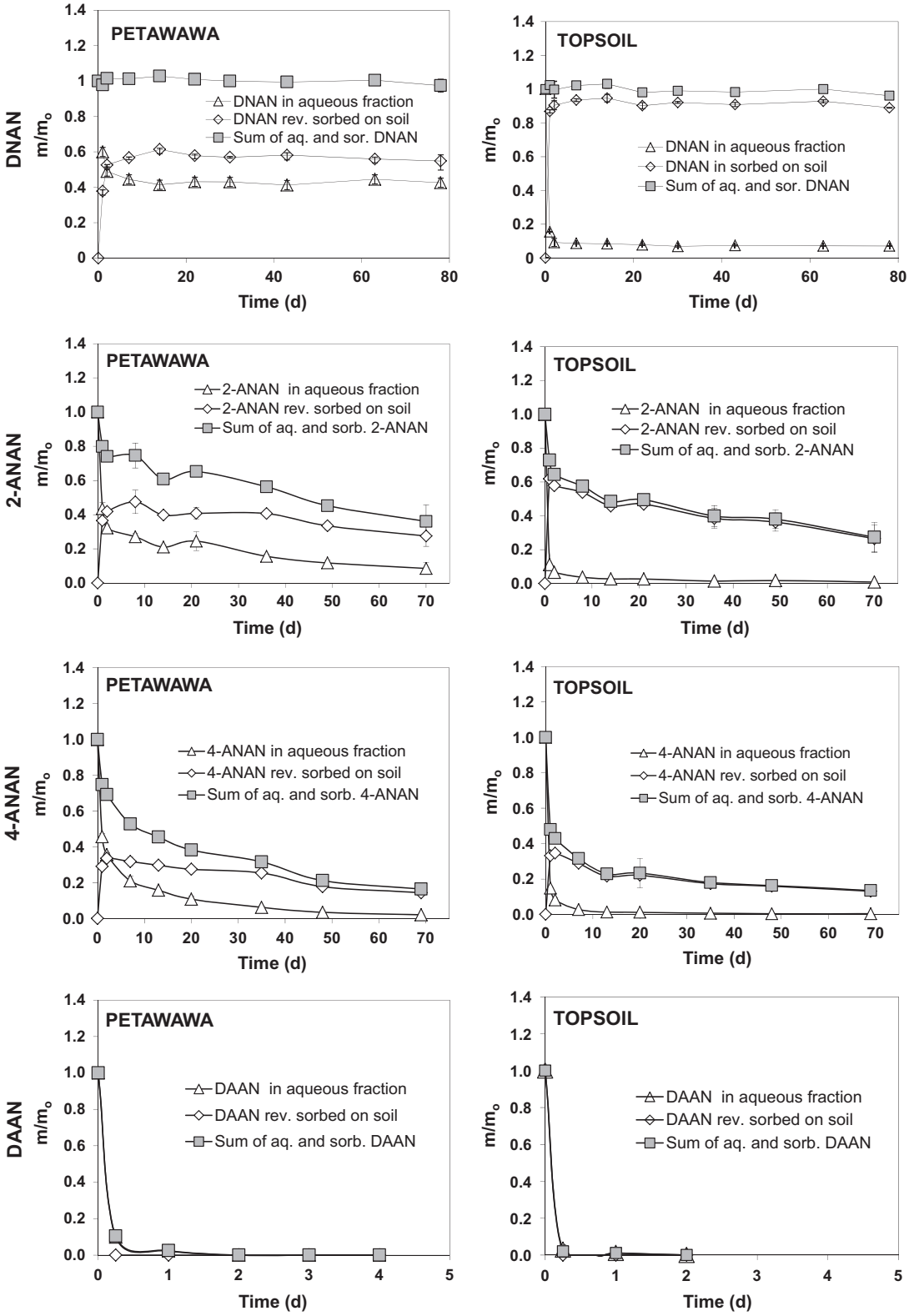

Fig. 4. Overall mass balance for the reaction of DNAN and its major products in sterile aerobic soils (error bars represent standard deviation).

azo dimer of DAAN were formed when exposing an aqueous solution of DAAN to air.

Instability of DAAN in water under oxic conditions is similar to what we observed earlier with the autooxidation of diamino derivatives of 2,4-DNT (Yang et al., 2008) and triamino derivatives of TNT (Hawari et al., 1998).

3.5. Implication on toxicity of DNAN and its products

In a recent study, Dodard et al. (2013) compared the toxicity of DNAN and TNT and found that TNT was more toxic than DNAN when either aqueous (*Vibrio fischeri*, green algae) or terrestrial (earthworms) receptors were investigated. Donlon et al. (1995)

Table 3
Soil–water partition coefficients, K_d and K_{oc}, for DNAN and its amino derivatives.

Component	PETAWAWA		TOPSOIL	
	K_d[a] (L kg^{-1})	K_{oc}[a] (L kg^{-1})	K_d[a] (L kg^{-1})	K_{oc}[a] (L kg^{-1})
DNAN	9.1 ± 0.7	364 ± 28	73 ± 3	215 ± 9
2-ANAN	7.9 ± 0.8	316 ± 32	46 ± 3	134 ± 9
4-ANAN	6.0 ± 0.3	240 ± 12	29 ± 5	84 ± 15
DAAN	<0.01	<0.5	<0.01	<0.03

[a] Values are given as mean ± standard deviation.

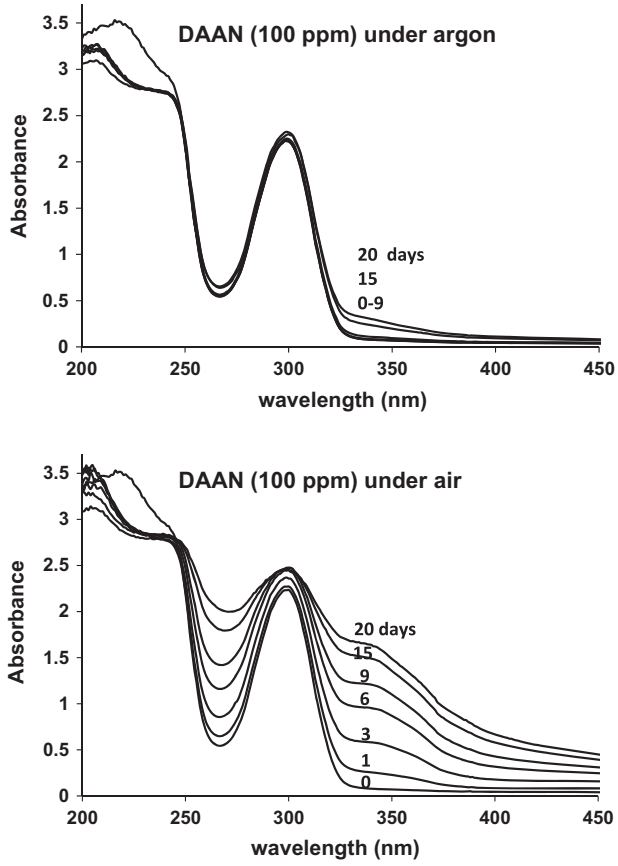

Fig. 5. UV–Vis monitoring of DAAN transformation in water, away from light.

reported that NACs were approximately 500-fold more toxic towards methanogens than their corresponding amines. Recently, Liang et al. (2013) confirmed this trend for DNAN but showed that only complete reduction to DAAN decreased the toxicity of DNAN to *Aliivibrio fischeri*, used in the Microtox assay. From literature data it appears that the toxicity of DNAN and its products in aqueous media follows the order TNT > DNAN > (or =) 2-ANAN > DAAN. This trend is perfectly in line with the decreasing hydrophobicity ($\log K_{ow}$) measured herein (Table 2) and is likely related to the higher ability of hydrophobic compounds to bioaccumulate in receptors. Based on the K_{ow} measured in the present study (Table 2), 4-ANAN should exhibit a toxicity markedly lower than that of DNAN, between that of 2-ANAN and DAAN. In soil, the amino-derivatives will be less bioavailable than their nitro analogous due to possible irreversible binding, so that the lower toxicity observed in water should be confirmed in soil.

4. Conclusion

To help understand the environmental behavior and ecological risk associated with DNAN we investigated its key initial abiotic and biotic reaction routes and determined relevant physicochemical parameters (S_w, pK_a, $\log K_{ow}$, K_d) for the chemical and its products. 2-ANAN and DAAN were identified as DNAN major products, abiotically and biotically. DNP was found to form under photolysis conditions and photodecompose. S_w followed the order (TNT < DNAN < 2-ANAN < 4-ANAN < DAAN) whereas $\log K_{ow}$ followed the order (DAAN < 4-ANAN < 2-ANAN < DNAN < TNT). In soil successive replacement of —NO_2 by —NH_2 in DNAN enhanced irreversible sorption and reduced bioavailability under oxic conditions. Toxicity of DNAN was found to be lower than that of TNT and toxicity of DNAN amino products was found comparable to or lower than that of DNAN (Dodard et al., 2013; Liang et al., 2013). The present findings therefore favor the use of DNAN against that of TNT in munitions manufacturing.

Acknowledgement

We acknowledge Defence Research Development Canada, Department of National Defence, for funding of this project.

References

Ahn, S.C., Cha, D.K., Kim, B.J., Oh, S.-Y., 2011. Detoxification of PAX-21 ammunitions wastewater by zero-valent iron for microbial reduction of perchlorate. J. Hazard. Mater. 192, 909–914.
Albert, A., Serjeant, E.P., 1971. The Determination of Ionization Constants. Chapman and Hall Ltd., London, UK.
Balakrishnan, V.K., Monteil-Rivera, F., Halasz, A., Corbeanu, A., Hawari, J., 2004. Decomposition of the polycyclic nitramine explosive, CL-20 by Fe0. Environ. Sci. Technol. 38, 6861–6866.
Barrows, S.E., Cramer, C.J., Truhlar, D.G., Elovitz, M.S., Weber, E.J., 1996. Factors controlling regioselectivity in the reduction of polynitroaromatics in aqueous solution. Environ. Sci. Technol. 30, 3028–3038.
Bhatt, M., Zhao, J.-S., Halasz, A., Hawari, J., 2006. Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine by novel fungi isolated from unexploded ordnance contaminated marine sediment. J. Ind. Microbiol. Biotechnol. 33, 850–858.
Davey, C.L., Powell, L.W., Turner, N.J., Wells, A., 1994. Regioselective reduction of substituted dinitroarenes using Baker's yeast. Tetrahedron Lett. 35, 7867–7870.
Davies, P.J., Provatas, A., 2006. Characterization of 2,4-dinitroanisole: an ingredient for use in low sensitivity melt cast formulations. Weapons Systems Division, Defense Science and Technology Organization, DSTO-TR-1904 Department of Defense, Commonwealth Australia.
Dodard, S.G., Sarrazin, M., Hawari, J., Paquet, L., Ampleman, G., Thiboutot, S., Sunahara, G.I., 2013. Ecotoxicological assessment of high energetic and insensitive munitions compound: 2,4-dinitroanisole (DNAN). J. Hazard. Mat. 262, 143–150.
Donlon, B.A., Razo-Flores, E., Field, J.A., Lettinga, G., 1995. Toxicity of N-substitued aromatics to acetoclastic methanogenic activity in granular sludge. Appl. Environ. Microbiol. 61, 3889–3893.
Elovitz, M.S., Weber, E.J., 1999. Sediment-mediated reduction of 2,4,6-trinitrotoluene and fate of the resulting aromatic (poly)amines. Environ. Sci. Technol. 33, 2617–2625.
Funk, S.B., Roberts, D.J., Crawford, D.L., Crawford, R.L., 1993. Initial-phase optimization for bioremediation of munition compound-contaminated soil. Appl. Environ. Microbiol. 59 (7), 2171–2177.
Haderlein, S.B., Schwarzenbach, R.P., 1995. Environmental processes influencing the rate of abiotic reduction of nitroaromatic compounds in the subsurface. In: Spain, J.C. (Ed.), Biodegradation of Nitroaromatic Compounds. Plenum Press, New York, pp. 199–225.
Haderlein, S.B., Weissmahr, K., Schwarzenbach, R.P., 1996. Specific adsorption of nitroaromatic explosives and pesticides to clay minerals. Environ. Sci. Technol. 30, 612–622.
Hawari, J., Halasz, A., Paquet, L., Zhou, E., Spencer, B., Ampleman, G., Thiboutot, S., 1998. Characterization of metabolites in the biotransformation of 2,4,6-trinitrotoluene with anaerobic sludge: the role of triaminotoluene. Appl. Environ. Microbiol. 64 (6), 2200–2206.
Hill, F.C., Sviatenko, L.K., Gorb, L., Okovytyy, S.I., Blaustein, G.S., Leszczynski, J., 2012. DFT M06-2X investigation of alkaline hydrolysis of nitroaromatic compounds. Chemosphere 88, 635–643.
Koutsospyros, A., Pavlov, J., Fawcett, J., Strickland, D., Smolinski, B., Braida, W., 2012. Degradation of high energetic and insensitive munitions compounds by Fe/Cu bimetal reduction. J. Hazard. Mat. 219–220, 75–81.
Liang, J., Olivares, C., Field, J.A., Sierra-Alvarez, R., 2013. Microbial toxicity of the insensitive munitions compound, 2,4-dinitroanisole (DNAN), and its aromatic amine metabolites. J. Hazard. Mater. 262, 281–287.
Maniatis, T., Fritsch, E.F., Sambrook, J., 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Monteil-Rivera, F., Paquet, L., Deschamps, S., Balakrishnan, V.K., Beaulieu, C., Hawari, J., 2004. Physico-chemical measurements of CL-20 for environmental applications – comparison with RDX and HMX. J. Chromatogr. A 1025, 125–132.

Murto, J., Tommila, E., 1962. The influence of the solvent on reaction velocity XXI. The reaction of 2,4-dinitrophenyl alkyl ethers with hydroxyl ion in water and in methanol–water and ethanol–water mixtures. Acta Chem. Scand. 16, 63–70.

Olivares, C., Liang, J., Abrell, L., Sierra-Alvarez, R., Field, J.A., 2013. Pathways of reductive 2,4-dinitroanisole (DNAN) biotransformation in sludge. Biotechnol. Bioeng. 110, 1595–1604.

Perreault, N., Manno, D., Halasz, A., Thiboutot, S., Ampleman, G., Hawari, J., 2012. Aerobic biotransformation of 2,4-dinitroanisole in soil and soil *Bacillus* sp. Biodegradation 23, 287–295.

Platten III, W.E., Bailey, D., Suidan, M.T., Maloney, S.T., 2010. Biological transformation pathways of 2,4-dinitroanisole and *N*-methyl paranitro aniline in anaerobic fluidized-bed bioreactors. Chemosphere 81, 1131–1136.

Qu, X., Zhang, Y., Li, H., Zheng, S., Zhu, D., 2011. Probing the specific sorption sites on montmorillonite using nitroaromatic compounds and hexafluorobenzene. Environ. Sci. Technol. 45, 2209–2216.

Rao, B., Wang, W., Cai, Q., Anderson, T., Gu, B., 2013. Photochemical transformation of the insensitive munitions compound 2,4-dinitroanisole. Sci. Tot. Environ. 443, 692–699.

Rieger, P.-G., Knackmuss, H.J., 1995. Basic knowledge and perspectives on biodegradation of 2,4,6-trinitrotoluene and related nitroaromatic compounds in contaminated soil. In: Spain, J.C. (Ed.), Biodegradation of Nitroaromatic Compounds. Plenum Press, New York, pp. 1–18.

Rochester, C.H., 1963. Correlation of reaction rates with acidity functions in strongly basic media. Trans. Faraday Soc. 59, 2826–2828.

Rosenblatt, D.H., Burrows, E.P., Mitchell, W.R., Parmer, D.L., 1991. Organic explosives and related compounds. In: Part, G., Hutzinger, O. (Eds.), The Handbook of Environmental Chemistry, vol. 3. Springer-Verlag, Berlin Heidelberg, pp. 195–234.

Rugge, K., Hofstetter, T.B., Haderlein, S.B., Bjerg, P.L., Knudsen, S., Zraunig, C., Mosbaek, H., Christensen, T.H., 1998. Characterization of predominant reductants in an anaerobic leachate-contaminated aquifer by nitroaromatic probe compounds. Environ. Sci. Technol. 32, 23–31.

Salter-Blanc, A.J., Bylaska, E.J., Ritchie, J.J., Tratnyek, P.G., 2013. Mechanisms and kinetics of alkaline hydrolysis of the energetic nitroaromatic compounds 2,4,6-trinitrotoluene (TNT) and 2,4-dinitroanisole (DNAN). Environ. Sci. Technol. 47, 6790–6798.

Terpko, M., Heck, R.F., 1980. Palladium catalyzed triethylammonium formate reductions. 3. Selective reduction of dinitroaromatic compounds. J. Org. Chem. 45, 4992–4993.

Thorn, K.A., Kennedy, K.R., 2002. ^{15}N NMR investigation of the covalent binding of reduced TNT amines to soil humic acid, model compounds, and lignocellulose. Environ. Sci. Technol. 36, 3787–3796.

Thorn, K.A., Pennington, J.C., Kennedy, K.R., Cox, L.G., Hayes, C.A., Porter, B.E., 2008. N-15 NMR study of the immobilization of 2,4- and 2,6-dinitrotoluene in aerobic compost. Environ. Sci. Technol. 42, 2542–2550.

USEPA (U.S. Environmental Protection Agency), 1997. Method 8330 SW-846 update III Part 4: 1 (B). Nitroaromatics and nitramines by high performance liquid chromatography (HPLC). Office of Solid Waste, Washington, DC.

Wang, C.Y., Zheng, D., Hughes, J.B., 2000. Stability of hydroxylamino and amino-intermediates from reduction of 2,4,6-trinitrotoluene, 2,4-dinitrotoluene, and 2,6-dinitrotoluene. Biotechnol. Lett. 22, 15–19.

Yang, H., Halasz, A., Zhao, J.-S., Monteil-Rivera, F., Hawari, J., 2008. Experimental evidence for *in situ* natural attenuation of 2,4- and 2,6-dinitrotoluene in marine sediment. Chemosphere 70, 791–799.

Zhang, D., Zhu, D., Chen, W., 2005. Sorption of nitroaromatics to soils: comparison of the importance of soil organic matter versus clay. Environ. Toxicol. Chem. 28, 1447–1453.