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

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HIGHLIGHTS

- DNAN undergoes regioselective reduction at the *o*-NO_2 under abiotic and biotic conditions.
- Physicochemical parameters (S_w, pK_a, logK_{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.

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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, logK_{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 logK_{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

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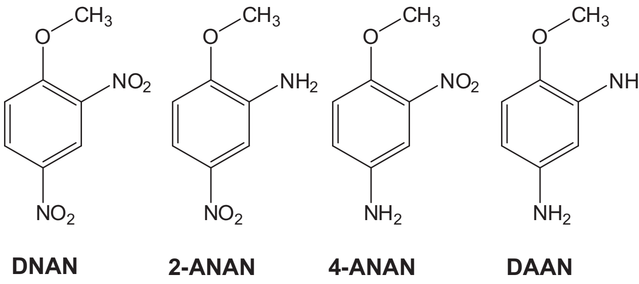

Fig. 1. Structures of DNAN and its amino products 2-amino-4-nitroanisole (2-ANAN), 4-amino-2-nitroanisole (4-ANAN), and 2,4-diaminoanisole (DAAN).

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 590000 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