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Ecotoxicological assessment of a high energetic and insensitive munitions compound: 2,4-Dinitroanisole (DNAN)

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HIGHLIGHTS

- DNAN was toxic to bacteria *V. fischeri* and freshwater green algae *P. subcapitata* in the mg/L range.
- DNAN was toxic to the earthworm *E. andrei* and perennial ryegrass *L. perenne* in the mg/kg range.
- DNAN toxicity was compared with TNT under the same experimental conditions.
- Chemical analyses of soil and tissue residues aid in the interpretation of toxicity data.

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ABSTRACT

The high explosive nitroaromatic 2,4-dinitroanisole (DNAN) is less shock sensitive than 2,4,6-trinitrotoluene (TNT), and is proposed as a TNT replacement for melt-cast formulations. Before using DNAN in munitions and potentially leading to environmental impact, the present study examines the ecotoxicity of DNAN using selected organisms. In water, DNAN decreased green algae *Pseudokirchneriella subcapitata* growth (EC_{50} = 4.0 mg/L), and bacteria *Vibrio fischeri* bioluminescence (Microtox, EC_{50} = 60.3 mg/L). In soil, DNAN decreased perennial ryegrass *Lolium perenne* growth (EC_{50} = 7 mg/kg), and is lethal to earthworms *Eisenia andrei* (LC_{50} = 47 mg/kg). At sub-lethal concentrations, DNAN caused an avoidance response (EC_{50} = 31 mg/kg) by earthworms. The presence of DNAN and 2-amino-4-nitroanisole in earthworms and plants suggested a role of these compounds in DNAN toxicity. Toxicity of DNAN was compared to TNT, tested under the same experimental conditions. These analyses showed that DNAN was equally, or even less deleterious to organism health than TNT, depending on the species and toxicity test. The present studies provide baseline toxicity data to increase the understanding of the environmental impact of DNAN, and assist science-based decision makers for improved management of potential DNAN contaminated sites.

1. Introduction

Highly energetic chemicals used for explosives and propellants can be found in soil at military training sites as well as at munitions production and disposal facilities. Contamination of soil, and ground and surface water by these chemicals is a serious health and environmental problem, and could result in high costs for managing and mitigation of contaminated sites [1,2]. There is a recent interest in the introduction of new shock-insensitive munitions compounds such as 2,4-dinitroanisole (DNAN) (Fig. 1) for use in various munitions compositions and applications (e.g., PAX-21, IMX-101, and IMX-104) [3–6]. Fig. 1 shows the structures of DNAN and its transformation products including 2-amino-4-nitroanisole (2A-4NAN) and 4-amino-2-nitroanisole (4A-2NAN) together with 2,4,6-trinitrotoluene (TNT). As Fig. 1 shows, these compounds share strong electron $-NO_2$ withdrawing groups, which are important in determining the environmental fate, transport, transformation, and ecological impact of the explosive [7]. Because DNAN is a nitroaromatic compound like TNT, it might then share similar acute and chronic toxic effects in humans and ecological receptors.

Like TNT, DNAN has been reported to transform easily under abiotic and biotic conditions to initially produce amino-reduced products (Fig. 1). For example, DNAN in water can undergo photo-transformation and can biologically reduce under anaerobic and aerobic conditions to form 2A-4NAN and 2,4-diaminoanisole [8–11]. These DNAN degradation studies suggest that DNAN can undergo abiotic and biotic transformation in the environment. Some of these pathways could be used for DNAN

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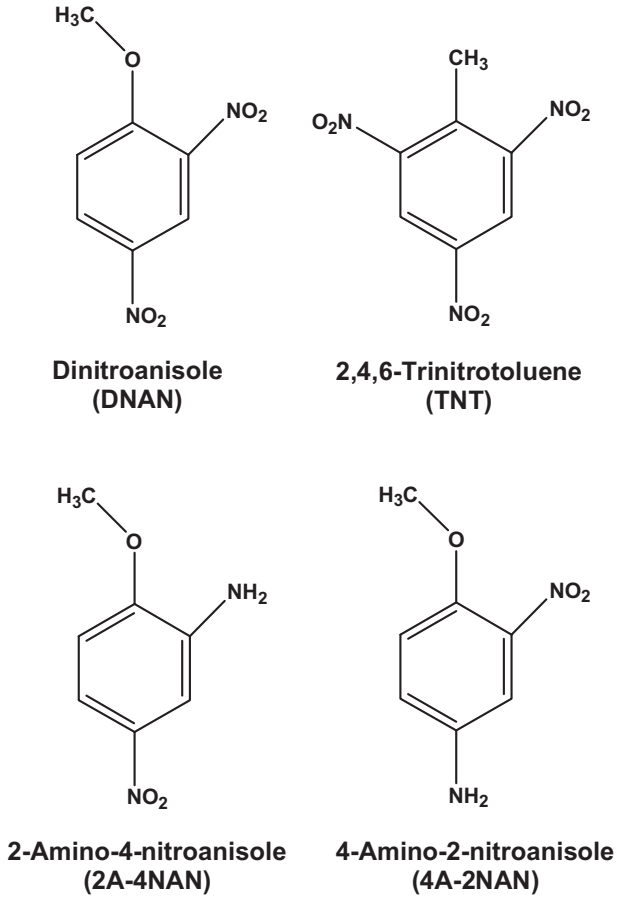

Fig. 1. Structures of DNAN, TNT, and some transformation products related to DNAN.

environmental remediation. For example, a reductive technology based on Fe/Cu bimetallic particles was proposed for the treatment of aqueous effluents contaminated with DNAN [12]. However, DNAN may also decrease the performance of microbial degradation processes of other munitions. Ahn et al. [13] reported that DNAN inhibited perchlorate respiring bacterial activity in batch studies of PAX-21 biodegradation.

Currently, there is a lack of published information on the toxicity of DNAN and its related amine products on ecological species. For toxicity, DNAN has been used by the armed forces as a lice egg pesticide in MYL formula [14] and has been found to be mutagenic in the *Salmonella*/mammalian microsome test [15]. Recent studies conducted by the U.S. Army described a 90-d toxicity study of DNAN using rats [16]. Toxicological effects included organ-specific effects, including neurotoxicity. Another study reported the acute and chronic toxicity of DNAN to aquatic vertebrate and invertebrate species including the larval fish *Pimephales promelas* and the water flea *Ceriodaphnia dubia* [17]. The 48-h median lethal concentration (LC_{50}) ranged from 37 to 42 mg/L DNAN, whereas the sublethal and chronic effects (EC_{50}, median effect concentration) ranged from 11 to 15 mg/L DNAN, using these two species. Effects on unicellular primary producers (bacteria and algae) were not reported, and detailed information on the terrestrial toxicity of DNAN is scant. Preliminary unpublished studies [18,19] reported that DNAN was toxic to earthworms *Eisenia fetida* exposed for 28 d in amended field soil (100% mortality at 300 mg/kg). DNAN is toxic to wheat exposed to a 0.01 M DNAN solution on filter paper for 7 d [20]. These studies indicate that more information on the ecotoxicological effects of DNAN is needed for environmental hazard and risk assessment.

The objectives of the present study were to determine the toxicity of DNAN to various test species representing different trophic levels including bacteria (*Vibrio fischeri*), freshwater green algae (*Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*), earthworms (*Eisenia andrei*), and plants (*Lolium perenne*). Results will be compared with TNT, and finally, we will examine the behavioral effects of DNAN amended soil on earthworms because in earlier studies, earthworms avoided TNT-contaminated soils [21]. Chemical analyses of soil fractions as well as test organism tissue residues, were used to confirm DNAN exposure concentrations and the presence of DNAN transformation products such as 2A-4NAN and 4A-2NAN in the test systems. Bioavailability of DNAN in soil was monitored using chemical analyses of the soil interstitial water (IW) fractions, based on the soil (sediment) water equilibrium partitioning [22,23].

2. Materials and methods

2.1. Chemicals and reagents

2,4-Dinitroanisole (purity 98.4%) was obtained from Defense Research Development Canada (DRDC) – Valcartier, whereas TNT (purity > 99.9%) was obtained from ICI explosives Canada (McMasterville, QC, Canada). Reference standards including DNAN, TNT, 2A-4NAN, 4A-2NAN, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), were purchased from AccuStandard (New Haven, CT, USA) or Sigma–Aldrich Chemical (Canada). Acetone and acetonitrile (HPLC grade) were obtained from Caledon Laboratories (Georgetown, ON, Canada). ASTM type I water [24] was obtained using a Milli-Q system (Millipore, Canada).

2.2. Preparation of exposure media for toxicity testing

For the aquatic toxicity tests, a stock solution of DNAN (30 mg in 100 mL water) was prepared and kept in the dark at room temperature. Aliquots were taken every other day for up to two weeks to verify the DNAN concentration using HPLC analysis (described below). For terrestrial toxicity testing, a natural sandy soil (DRDC2010; 0.7% clay, 2.0% organic matter, 97.6% sand, 1.6% silt, and pH 5.5–6.0) provided by DRDC Valcartier in 2010, was used. This soil was amended with DNAN using acetone as the carrier solvent as described previously [25], and was used throughout the study. Following solvent evaporation, three replicates from each dry soil batch were hydrated individually to 75% of the soil water holding capacity. Soil samples amended with different DNAN concentrations (0 (control), 10–1000 mg/kg) were taken at days 1, 3, 7, and 14, following soil hydration.

Soil equilibration studies were then performed in the dark to estimate the minimum time required for DNAN to equilibrate in the hydrated soil and to maximize its bioavailability prior to terrestrial toxicity testing. Concentrations of DNAN and transformation products in soil acetonitrile extracts (total extractable fraction) and in soil IW (bioavailable fraction) were determined separately at each sampling time. The total DNAN added in soil was confirmed using the acetonitrile extraction procedure, whereas the amount of dissolved DNAN in soil IW was determined using the coupled filtration-centrifugation method [26,27]. The collected IW (about 1 mL) was analyzed using HPLC.

2.3. Toxicity to bacteria, algae, and terrestrial plants

Toxicity assays conducted in the current study complied with our in-house laboratory control charts, and results for known reference toxicants (as suggested in the cited standard toxicity protocols) are reported in Tables 1 and 2. The standard 30-min Microtox toxicity test was performed on the aqueous samples as previously described [28] using the following nominal test concentrations of