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Toxicity of the explosive metabolites hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) and hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) to the earthworm *Eisenia fetida*

Baohong Zhang, Ronald J. Kendall, Todd A. Anderson *

The Institute of Environmental and Human Health (TIEHH), Department of Environmental Toxicology, Texas Tech University, Lubbock, TX 79409-1163, USA

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Abstract

Toxicity of hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) to earthworm was evaluated. Both MNX and TNX had lethal and sublethal effects on earthworms. Exposure to MNX- or TNX-contaminated soil caused a significant concentration-dependent decrease in earthworm survival and growth. The lowest observed lethal concentration (LOLC) for both MNX and TNX was 100 and 200 mg kg^{-1} soil dry weight in the sandy loam soil and in the silt loam soil, respectively. No earthworms survived for 14 days in MNX- or TNX-spiked soil at 500 mg kg^{-1} soil dry weight. After 7 days exposure, the lowest observed effect concentration (LOEC) for earthworm growth was 50 mg kg^{-1} soil dry weight for TNX and 100 mg kg^{-1} soil dry weight for MNX in both soil types. The LC20 and LC50 for MNX in sandy loam soil were 114 and 262 mg kg^{-1} and for TNX, they were 114 and 254 mg kg^{-1} soil dry weight, respectively. The corresponding values for MNX and TNX in silt loam soil were 234 and 390 mg kg^{-1} soil dry weight, respectively, and 200 and 362 mg kg^{-1} soil dry weight, respectively. After 35 days exposure, earthworm growth was reduced 8–39% by TNX in sandy loam soil, whereas TNX only inhibited earthworm growth 5–18% at the same concentration range in silt loam soil. LC20 and LC50 for TNX were slightly lower than for MNX; this indicates that TNX was more toxic than MNX. No significant morphological or developmental abnormalities were observed in earthworms surviving exposure.

Keywords: MNX; TNX; Toxicity; Soil; Earthworm; Energetic material

1. Introduction

A variety of energetic compounds, such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX), have been produced and widely used in military and civil activities around the world (Talmage et al., 1999; Halasz et al., 2002). The production and use of energetic compounds has resulted in some water and soil contamination (Steinfield and Wormhoudt, 1998; Halasz et al., 2002). According to the US Department of Defense, an estimated 12 000 sites across the US have been contaminated by energetic compounds due to production activities, field usage, and disposal of munitions materials or their combustion products. Concentrations of energetic materials in soils were reported to exceed 3000 mg kg^{-1} for RDX, 87 000 mg kg^{-1} for TNT and 3000 mg kg^{-1} for HMX in some instances (Simini et al., 1995). The presence of energetic compounds in soil at these sites is of concern because of their potential effects on indigenous organisms. In some instances, these organisms include threatened or endangered species. An evaluation of the potential hazards

* Corresponding author. Tel.: +1 806 885 4567; fax: +1 806 885 4577.
E-mail addresses: baohong.zhang@tiehh.ttu.edu (B. Zhang), todd.anderson@tiehh.ttu.edu (T.A. Anderson).

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to terrestrial organisms/wildlife from energetic compounds commonly found in soil at these sites is warranted.

Recently, several laboratory and field studies have indicated that energetic compounds are toxic at relatively low concentrations to a number of organisms including microorganisms (Klausmeier et al., 1973; Spanggord et al., 1982; Sunahara et al., 1998; Juck et al., 2003), plants (Palazzo and Leggett, 1986; Winfield et al., 2004), invertebrates (Robidoux et al., 2000, 2002, 2004a,b; Kuperman et al., 2003; Simini et al., 2003), vertebrates (Homma-Takeda et al., 2002; Gogal et al., 2003), and humans (Bruns-Nagel et al., 1999; Lachance et al., 1999). Energetic compounds have several types of adverse effects, such as growth inhibition and development (Steevens et al., 2002), neurological effects in vertebrates, and possible genotoxicity or cancer (Honeycutt et al., 1996; Lachance et al., 1999). Most of these studies were focused on the parent compound. The products of the biotic and abiotic degradation of these compounds may also pose toxicological risk to terrestrial and aquatic organisms. For example, research has shown that the reduction metabolites of TNT adversely affected marine bacteria, freshwater green algae, amphipods, and earthworms (Lotufo et al., 2001; Steevens et al., 2002; Lachance et al., 2004).

The degradation of RDX in soil and in plants has been previously characterized (for example, McCormick et al., 1981; Harvey et al., 1991; Sheremata et al., 2001). Under anaerobic conditions (i.e. flooded or saturated soils), some bacteria can biotransform RDX into several reduced metabolites, including hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) and hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) (Hawari et al., 2000a,b; Adrian and Arnett, 2004) (Fig. 1). Recently, MNX and TNX were detected in groundwater at the Iowa Army Ammunition Plant (Beller and Tiemeier, 2002) up to 430 $\mu g\,l^{-1}$ in some samples.

To our knowledge, very few data exist on the potential environmental impact of the degradation metabolites of energetic compounds such as HMX and RDX. To better understand the environmental and biological effects of RDX and the risk associated with RDX and its metabolites, the potential toxicity of reduced RDX metabolites should be investigated. Earthworms have been successfully employed to investigate the toxicity and bioavailability of contaminants in soil (Lanno et al., 2004; Spurgeon et al., 2004). In this study, we determined the toxicity of two RDX degradation metabolites (TNX and MNX) to earthworms (*Eisenia fetida*) in two soil types.

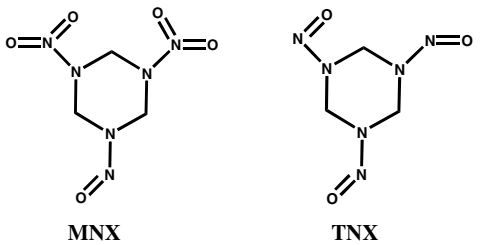

Fig. 1. Chemical structures of hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) and hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX).

2. Materials and methods

2.1. Chemicals and reagents

TNX and MNX were supplied by SRI International (Menlo Park, CA) at ⩾99% purity. Acetone and acetonitrile were purchased from Fisher Scientific (Pittsburg, PA). Ultra-pure water (>18 MΩ) was prepared by ultrafiltration with a Mili-Q water purification system from Millipore (Bedford, MA). All solvents were HPLC grade or analytical grade. Glassware was prepared by washing with phosphate-free detergent followed by rinses with acetone and deionized water.

2.2. Soil preparation and soil characteristics

Two types of natural soils, a sandy loam soil (Terry County, TX) and a silt loam soil (Harlan County, NE), were selected for this experiment in order to study the potential effects of different soil conditions. The physiochemical properties of the test soils were determined by A&L Midwest Laboratories (Omaha, NE) using standard techniques. The silt loam soil had the following characteristics: 34% sand, 54% silt, and 12% clay; 2.5% organic matter, and the soil pH was 7.0. The sandy loam soil had the following characteristics: 74% sand, 10% silt, and 16% clay; 1.3% organic matter, and the soil pH was 8.3. Each soil was mixed, air dried, and sieved (2 mm) prior to use in experiments.

Both soil types were spiked using TNX or MNX in acetone to obtain final soil concentrations of 0.1, 1, 10, 50, 100, 200, 400, and 500 mg kg^{-1} soil dry weight. Each toxicant was tested separately. Spiked soil samples were thoroughly mixed in order to distribute the contaminant evenly in soil and allow the solvent to evaporate. Spiked soil samples were stored for 24 h in the dark under a chemical hood to permit the complete evaporation of acetone. Soils were hydrated to 15% of soil weight prior to the start of the experiments; during the addition of water, soil samples were further mixed. Finally, spiked soils (250 g) were put into 500 ml glass jars. All experiments were conducted with three replicate test units per exposure concentration.

A solvent control (soil amended with acetone only) and negative control (soil without test compound or solvent) were included in all trials. The solvent controls were prepared in the same manner as toxicant-spiked soils only without the toxicant. The nominal exposure concentrations of MNX and TNX were verified using GC analysis as described below.

2.3. Earthworms and toxicity test

The earthworms (*Eisenia fetida*) were obtained from Ray's Worms and More (Raytown, MO, USA), and were