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Developing earthworm bioconcentration factors of nitrogen-based compounds for predicting environmentally significant parameters for new munition compounds in soil

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ABSTRACT

We investigated the bioconcentration potential of nitrogen-based compounds 4-nitroanisole (4-NAN), 3,5-dinitro-*o*-toluamide (3,5-DN*o*TAME), and 2-methoxy-5 nitropyridine (2-M-5-NPYNE) using earthworm *Eisenia andrei* exposures in aqueous exposure media in sand. Separate toxicity studies were conducted prior to bioconcentration studies using a range of chemical concentrations to establish the sublethal exposure conditions for the earthworms. The objectives of the present studies were to: (1) develop an experimental test system for estimating bioconcentration potentials of new and emerging munition compounds that partition into earthworms, using aqueous exposure media; and (2) apply this experimental model to establish original bioconcentration data for 4-NAN, 3,5-DN*o*TAME, and 2-M-5-NPYNE. Experimental design includes earthworm exposures to chemicals for up to 14 days in aqueous media (Römbke medium; 0.08 mM KCl, 2 mM $CaCl_2$, 0.5 mM $MgSO_4$, and 0.8 mM $NaHCO_3$) in the presence of water-washed coarse sand (0.5–1.0 mm) substrate. Concentrations of test chemicals in respective exposure media remained relatively stable during these independent studies. Tissue analyses revealed a rapid uptake of each chemical by the earthworms; a steady state was attained within 24 h from commencement of these exposures. Estimated steady-state bioconcentration factors (BCF_{ss}; mL/g dry tissue) were 47, 6, and 11 for 4-NAN, 3,5-DN*o*TAME, and 2-M-5-NPYNE, respectively. These results will contribute to the BCF database being developed for use in models aimed at predicting environmentally significant parameters for new munition compounds in soil.

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1. Introduction

A new generation of highly energetic chemicals is expected to replace traditional explosives and propellants. Examples include new shock-insensitive munitions (IM) compounds that minimize the probability of inadvertent detonation as a result of accident, combat, or terrorist actions. To address a pressing need for the United States Department of Defense (DoD) to reduce the environmental risks associated with military operations, investigations of environmental fate and ecotoxicological effects of some of these compounds, such as 2,4-dinitroanisole (DNAN) and nitro-triazolone (NTO), have been initiated (Ampleman, 2010; Ampleman et al., 2012; Davies and Provatas, 2006; Dodard et al., 2013; Haley et al., 2009). However, ecotoxicological data developed in these studies are pertinent only to the existing compounds. A more critical need in reducing the environmental risks of energetics is the development of new approaches to predict environmental fate, such as uptake by soil organisms, of future compounds while they are still in the initial design phases.

The use of quantitative structure activity relationships (QSARs) to estimate the environmental and toxicological properties of potential contaminants has been an active area of research for

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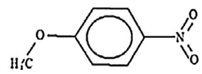
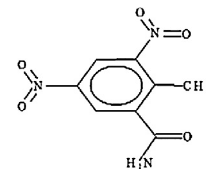
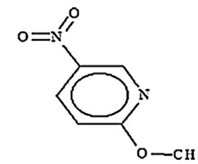
Table 1
Selected properties of test compounds 4-nitroanisole (4-NAN), 3,5-dinitro-*o*-toluamide (3,5-DN*o*TAME), and 2-methoxy-5 nitropyridine (2-M-5-NPYNE).

Name	4-NAN	3,5-DN*o*TAME	2-M-5-NPYNE
CAS- Reg. Number	100-17-4	148-01-6	5446-92-4
Molecular mass (g/mol)	153.1	225.2	154.1
Structural formula			
Log Kow (L/kg)	2.03	0.19	1.55
Water solubility[a] (mg/L)	552.9	447.5	1406

[a] Values based on the U.S. EPA Estimation Programs Interface Suite (EPI Suite); estimated at 25 °C.

many years (Van Gestel and Ma, 1990; Selassie, 2003). However, predicting bioconcentration factors (BCFs) of new munition compounds is challenging because models currently available do not accurately account for either partitioning of a compound into an organism or degradation. Furthermore, predicting the BCF from soil exposures to soil biota is difficult because soil properties affect the bioavailability of the compound (Dodard et al., 2005; Kuperman et al., 2009, 2013; Lanno et al., 2004; Rocheleau et al., 2010; Sunahara et al., 2009). This particular challenge can be resolved, in part, by the use of aqueous exposures, thus separating the soil effects from the organism uptake mechanisms when developing empirical data for use in models. Therefore, we conducted the present experiments to develop the necessary uptake data for selected compounds for use in model development. The selected compounds that have nitro-group functionality can serve as proxies for new munitions. These compounds share strong electron —NO_2 withdrawing groups, which are important in determining the environmental fate, transport, transformation, and ecological effects of the explosives (Monteil-Rivera et al., 2009). Compound-specific properties are summarized in Table 1. To test the hypothesis relating the uptake of selected nitrogen-based organic compounds to their molecular structures, we designed the present studies to: (1) develop an experimental test system for estimating bioconcentration potentials of new and emerging munition compounds that partition into earthworms, using aqueous exposure media; and (2) apply this experimental model to establish original bioconcentration data for 4-NAN, 3,5-DN*o*TAME, and 2-M-5-NPYNE.

2. Material and methods

2.1. Test species

The earthworm (Oligochaeta, Annelida) *Eisenia andrei* Bouché (1972) was used in the present studies. Earthworms were bred in plastic containers filled with approximately 14 kg of a 1:1 mixture of PRO-GRO sphagnum peat moss (Gulf Island Peat Moss Co., PEI, Canada) and BACCTO® potting soil (Michigan Peat Co., Houston, TX, USA). The pH was adjusted to 6.2 ± 0.1 by adding calcium carbonate (pulverized lime). The culture was kept moist at 21 ± 2 °C, under continuous light. Earthworm colonies were fed biweekly with alfalfa food, consisting of dehydrated alfalfa pellets (27% fiber, 17% protein, 1.5% fat; Ohio Blenders of PA, York, PA) that were prepared by hydrating, fermenting for at least 14 days, air-dried, and then ground to a course powder. Earthworm cultures were synchronized so that all worms used in each test were approximately the same age. Individual earthworms with fully developed clitella, and weighing from 0.3 to 0.6 g (wet weight) were allowed to depurate their gut content on moist filter paper for at least 24 h, and then randomly assigned to each test container. Measurement endpoints in toxicity tests included adult earthworm survival and wet weight.

2.2. Exposure media

Preliminary studies were conducted to select the appropriate exposure medium for subsequent toxicity and bioconcentration studies. Formulations of aqueous exposure media were prepared according to Römbke and Knacker (1989; the Römbke medium) or Robidoux et al. (2002; the Lumbricus Balanced Salt Solution (LBSS) medium). The Römbke medium contained 0.08 mM KCl, 2 mM $CaCl_2$, 0.5 mM $MgSO_4$, and 0.8 mM $NaHCO_3$. The LBSS contained 71.5 mM NaCl, 4.8 mM KCl, 3.8 mM $CaCl_2$, 1.1 mM $MgSO_4$, 0.4 mM KH_2PO_4, 0.3 mM K_2HPO_4, and 4.2 mM $NaHCO_3$. Solutions of each medium were prepared using ASTM type I water (ASTM International, 2004). The Control medium consisted of ASTM type I water only. Each aqueous exposure medium was replaced regularly (1–3 day intervals) during the studies. Solid components were included in the studies to determine whether availability of solid substrate saturated with the aqueous exposure medium extends the survival of the earthworms. The solid components tested were either coarse sand (0.5–1.0 mm), or glass beads (10 mm). Sand and aqueous medium were changed daily, while the glass beads were rinsed with ASTM type I water or Römbke medium, respectively, every 1–3 days. These preliminary studies showed that earthworm survival was greatest in a sand-Römbke exposure medium for 28 d (data not shown). Consequently, this medium was selected for the earthworm toxicity and bioconcentration studies with test chemicals.

2.3. Toxicity tests

No ecotoxicological data were available for the test compounds; therefore, toxicity tests were conducted to determine the appropriate exposure concentration of each test compound for use in the bioconcentration studies. A concentration was deemed appropriate when it sustained survival of the earthworms for the duration of the test. Separate toxicity tests were conducted with 4-nitroanisole (4-NAN; an intermediate in the manufacture of synthetic organic dyes and pharmaceuticals), 3,5-dinitro-*o*-toluamide (3,5-DN*o*TAME; used as a veterinary coccidiostat (to control coccidiosis) and food additive), and 2-methoxy-5 nitropyridine (2-M-5-NPYNE; used as a precursor in organic chemistry syntheses). Toxicity tests included exposing earthworms to a range of chemical concentrations in sand-Römbke medium for up to 21 days. Nominal/analytically determined treatment concentrations (mg/