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Evaluating the bioavailability of explosive metabolites, hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), in soils using passive sampling devices

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Abstract

The uptake kinetics of two major RDX (hexahydro-1,3,5-trinitro-1,3,5-triazacyclohexane) metabolites, hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), into passive sampling devices (PSDs), and the ability of PSDs to serve as surrogates for evaluating bioavailability of MNX and TNX were investigated in laboratory sand and two soil types. The results indicate that MNX and TNX absorption into PSDs was best fitted with a polynomial curve model: $y = ax^2 + bx + c$ (y: amount of MNX or TNX absorbed into PSD; x: incubation time of PSDs in soil), with an excellent correlation coefficient (>0.95) for each type of soil amended with 10 mg/kg MNX or TNX. TNX was more readily absorbed by PSDs than MNX. Soil conditions, especially organic matter content, affected MNX and TNX uptake into PSDs. A relatively good correlation between MNX and TNX uptake into PSDs and uptake into earthworms was obtained in two types of natural soils (a silt loam soil from Nebraska and a sandy loam soil from Texas) and laboratory sand. A linear relationship between PSD uptake and earthworm uptake was observed. The correlation coefficients (r^2) were $\geq$0.82 for all test soils spiked with MNX or TNX. Organic matter content is one soil factor that affected the ratio of MNX or TNX uptake into earthworms versus uptake into PSDs. These data indicate that C_{18} PSDs may be used as a surrogate for soil organisms such as earthworms and provide a simple and easy chemical test for assessing the bioavailability of contaminants in soils.

Keywords: Semi-permeable membrane devices; PSD; Bioavailability; MNX; TNX; Earthworm; Soil organic matter

1. Introduction

Sampling is the most important step in any analytical procedure [1]. Studies have estimated that sampling and sample preparation usually account for 70–90% of total analytical time [1], and most sample preparation consumes resources (high purity solvents, for example). Thus, researchers have been trying to develop reliable sampling and sample preparation procedures that simplify the process and reduce consumption of resources. An example of progress in this field is the development of passive sampling techniques. Passive sampling devices (PSDs) offer several advantages over direct analysis of water, soil, or other solid wastes. The obvious advantages include simplifying and speeding up analysis procedures, saving labor and costs, eliminating the need to destroy or dispose of highly contaminated samples, eliminating secondary pollution, and using small volumes of solvent [2] or solventless procedures ("green analytical chemistry") [1].

Passive sampling is any sampling or monitoring technique based on passive diffusion of analyte molecules from the sampled medium to a collecting medium. Usually, the PSD is a container (such as permeable or semi-permeable membrane bag) containing sorbent that can absorb contaminant molecules. Although it was used for the first time to determine CO concentrations in air in the 1920s [3], successful examples of passive sampling also include the determination of SO_2 and NO_2 in air

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[4,5]. Currently, several PSDs have been developed to evaluate and monitor different types of environmental pollution [1,5]. However, a majority of these studies have monitored indoor air pollution [6] or have been used outdoors to monitor NO_2 [5,7], SO_2 [4,5], O_3 [5], CO_2 [8], H_2S [9], polyaromatic hydrocarbons (PAHs) [10], volatile organic compounds [11], or chlorinated semivolatile organic pollutants [12,13]. Water pollution by metals and organic chemicals also has been studied using passive sampling techniques [14–16].

Although passive sampling techniques have been used as monitoring devices for air and water quality for many years, their use in terrestrial environments (soil) is relatively new; only a few of the passive sampling systems reported have been used to monitor and evaluate soil contaminants [2,17–22]. For example, Johnson et al. [17] investigated passive sampling devices as a technique to monitor polychlorinated biphenyl (PCB) distribution at a contaminated site in South Carolina. In their experiments, they found that there was a strong correlation between PCB concentration in the soil and PCB concentration in PSDs deployed at the site. This correlation allowed them to use PSDs to monitor PCB concentration levels in soil.

Another advantage of PSDs is their potential as biological surrogates to determine bioavailability of chemicals to organisms [23] due to the similarities of PSDs to biological systems (hydrophobic depots covered with a semi-permeable membrane). This potential as biological surrogates has attracted interest among scientists who have investigated their practical use in aquatic environments and have indicated their potential as surrogates for aquatic organisms [24,25]. For example, Peven et al. [25] evaluated the possibility of a semi-permeable membrane device (SPMD) as a surrogate for mussels in assessing bioavailability of PCBs, PAHs, and DDT in an aquatic system. Their results indicated that SPMDs had similar uptake and response times as mussels. On the contrary, research to investigate PSD or SPMD use as biological surrogates in terrestrial environments is relatively new. Recently, passive sampling devices were used to assess the availability of aged organic compounds. Good correlations were observed between the uptake of DDT, lindane, heptachlor, aldrin, dieldrin, and endrin by earthworms and the quantity of chemical sorbed by C_{18} PSDs [18,19].

Hexahydro-1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) is a second generation explosive. It has been widely used in military and civil activities, which has resulted in some contamination of soil and water with RDX [26]. According to a recent report, 583 RDX-contaminated sites (and an additional 88 sites suspected of having RDX contamination) have been identified in the USA [27]. Under anaerobic conditions, RDX can be reduced to hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) by some bacteria [28–30]. Our experiments indicated that MNX and TNX have some toxicity to earthworms [31]. In this study, a passive soil-sampling device similar to that used by Awata et al. [18,19] and Johnson et al. [17] was evaluated for determining availability of TNX and MNX residues in soil. Residue concentrations obtained by this method were compared to results obtained in earthworm tests and to data from chemical extractions to assess the availability of explosive residues in soil. An additional goal of these tests was to develop relationships between PSD concentrations and earthworm concentrations.

2. Materials and methods

2.1. Chemicals and reagents

TNX and MNX were purchased from SRI International (Menlo Park, CA, USA). The purity of these reagents was ≥99%. Octadecyl sorbent (C_{18}) was obtained from Fisher Scientific (Houston, TX, USA). Granular sodium sulfate (purity ≥99%) was from EM Science. Extraction solvents (acetone and acetonitrile) were purchased from Fisher Scientific (Pittsburg, PA, USA). Ultra-pure water (>18 MΩ) was prepared by ultrafiltration with a Milli-Q water purification system from Millipore (Bedford, MA, USA). All solvents were HPLC grade, and the other chemicals were HPLC or analytical grade.

Glassware was washed with phosphate-free detergent followed by rinses with acetone and deionized water.

2.2. Passive sampling devices (PSDs)

Passive sampling devices (PSDs) were constructed as described by Awata et al. [18] and Johnson et al. [17]. Five hundred milligrams of C_{18} was weighed and placed into a 4-oz polyethylene Whirl-Pak sampling bag (Nasco, Fort Wilkinson, WI, USA). Nominal film thickness of the bag was 63–71 μm. Bags were cut into approximate final dimensions of $(5.0 \pm 0.2) \times (5.0 \pm 0.2)$ cm and then heat-sealed. The approximate surface area of each bag was $50 \pm 5\ cm^2$.

2.3. Soil preparation and soil characteristics

Laboratory sand (Fisher Scientific Inc.) and two types of natural soils, a sandy loam soil from Texas and a silt loam soil from Nebraska, were used in these experiments in order to study the effect of different soil environmental conditions on bioavailability of MNX and TNX. Silt loam soil was collected near Stamford, Harlan County, NE, USA. Sandy loam soil was collected near Ropesville, Terry County, TX, USA. The physiochemical properties of the study soils (Table 1) were determined by A&L Midwest Laboratories (Omaha, NE, USA) using standard techniques. Each soil was mixed, air dried, and prepared by sieving through a 2-mm sieve prior to use in experiments.

Silt loam soil was spiked using TNX and MNX in acetone to produce a series of concentrations (0.1, 1, 10, 50, 100, 200, 400, or 500 mg/kg). Since the goal of our tests was to evaluate soil

Table 1
Physiochemical properties of soils used in studies on the bioavailability of MNX and TNX

Soil	Soil type	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	pH
Nebraska	Silt loam	34	54	12	2.5	7.0
Texas	Sandy loam	74	10	16	1.3	8.3
Sand	Sand	100	0	0	0	7.0