Applied Soil Ecology 83 (2014) 210–218

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

An emerging energetic soil contaminant, CL-20, can affect the soil invertebrate community in a sandy loam soil

R.G. Kuperman*, R.T. Checkai, C.T. Phillips, M. Simini, J.S. Anthony

Environmental Toxicology Branch, Edgewood Chemical Biological Center, 5183 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5424, USA

ARTICLE INFO

Article history:
Received 31 October 2012
Received in revised form 4 September 2013
Accepted 9 September 2013
Available online 2 December 2013

Keywords:
Nitramine explosive
CL-20
Toxicity
Soil
Invertebrate community
Microcosm

ABSTRACT

We investigated the effects of nitramine explosive CL-20 (China Lake compound 20) on the indigenous soil invertebrate community in Sassafras sandy loam (SSL) soil using a 12-week soil microcosm assay. Freshly collected SSL soil was amended with CL-20 to prepare multiple treatment concentrations ranging from 0 (acetone control) to 10,300 mg kg^{-1}. The selected concentration range of CL-20 adequately assessed the concentration–response relationships for total microarthropods, and for individual microarthropod groups. The overall composition of microarthropod community in SSL soil was not affected by exposure to CL-20, based on the number of taxonomic groups present in the individual treatments after 12 weeks. However, community structure analysis revealed greater sensitivity to CL-20 by predatory mesostigmatid mites. Microarthropod and nematode communities showed contrasting sensitivities to CL-20 in SSL soil. Total numbers of nematodes were either unaffected or significantly ($p < 0.05$) increased in CL-20 treatments compared with control. Only predator group among nematodes was consistently adversely affected by exposure to CL-20. The abundance of predatory nematodes decreased in a concentration-dependent manner throughout the 12-week exposure. Microcosm assay with corresponding community structure analysis can provide the means for validating the ecotoxicity data from standardized laboratory tests, both complimenting and expanding upon the ecotoxicological significance of data from standardized single-species toxicity tests.

Published by Elsevier B.V.

1. Introduction

Preservation of soil fertility and structure is essential to protecting and sustaining ecological integrity of terrestrial ecosystems at military installations. Understanding the potential impacts on the soil ecosystems of an accidental release of explosives and their byproducts during manufacturing, use in training, storage or disposal operations is important to achieving this goal. Soil contaminated with energetic materials can affect soil biota directly or indirectly, by altering specific interactions among populations of soil organisms and by disrupting soil food webs. Populations of soil organisms are intimately linked and the effects of chemicals on any one species or group can impact the whole community. Ultimately, these effects can interfere with key soil processes that are important to the regulation, flow, and internal cycling of carbon and nutrients in ecosystems (Edwards and Bohlen, 1995; Kuperman and Carreiro, 1997; Kuperman et al., 1998; Parmelee et al., 1993). The use of multi-species tests in assessing soil contamination offers holistic tools for risk assessment and can provide a much broader understanding of the mechanisms by which soil contamination can affect the structure and function of soil ecosystems (Kuperman et al., 2002).

In spite of advances in soil ecotoxicological methodologies (Kuperman et al., 2002, 2009), only few studies investigated the community-level effects of soil contamination under controlled laboratory conditions (Bogomolov et al., 1996; Kuperman et al., 2007; Parmelee et al., 1993, 1997; Scott-Fordsmand et al., 2008). Soil microcosms can be used as tools for assessing the community level effects of chemicals while providing a large set of measurement endpoints from which an appropriate group can be selected for specific ecosystem structures and functions (Kuperman et al., 2002; Wentsel et al., 2003). Nevertheless, the current approach for assessing ecological risk in terrestrial ecosystems is to use limited data derived from standardized single-species laboratory tests and extrapolate these findings to contaminated sites without sufficient comprehensive regard to complexity of soil ecosystems in the field. Toxicity data established in standardized single-species tests can underestimate or overestimate the potential exposure effects on soil invertebrates in the field. For example, in a 7-d microcosm assay, total microarthropod numbers were reduced by 50% in the 30 mg kg^{-1} 2,4,6-trinitrotoluene (TNT) treatment compared with numbers in control oak-beech forest silt loam soil (Parmelee

* Corresponding author at: Environmental Toxicology Branch, Edgewood Chemical Biological Center, RDCB-DRT-E E5641, 5183 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5424, USA. Tel.: +1 410 436 4697.
E-mail address: roman.g.kuperman.civ@mail.mil (R.G. Kuperman).

0929-1393/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.apsoil.2013.09.011

et al., 1993), which suggested an order of magnitude greater toxicity when contrasted with LC_{50} value of 360 mg kg^{-1} reported for acute effect (14-d) of TNT on adult enchytraeid worm (potworm) *Enchytraeus crypticus* in freshly amended Sassafras sandy loam soil (Kuperman et al., 2005).

Ecotoxicological data established for an emerging polynitramine energetic material hexanitrohexaazaisowurtzitane (CL-20; China Lake compound 20) in standardized single-species tests indicated very high toxicity to soil invertebrates (Dodard et al., 2005; Kuperman et al., 2006a,b; Robidoux et al., 2004). However, such ecotoxicological data did not account for the community-level effects of exposure to test chemical or possible interactions among soil invertebrate populations. In order to address the knowledge gap regarding the soil invertebrate community-level effects of CL-20, we designed our studies to test the hypotheses that the effects of CL-20 on the indigenous soil invertebrate community will be specific for individual taxonomic or trophic groups, and will be affected by the duration of exposure of these organisms in a natural soil. This was accomplished by conducting definitive microcosm toxicity assays with soil containing the indigenous invertebrate community to address the objectives of this investigation which included: (i) assessing the respective toxicity of CL-20 to the populations within soil invertebrate community groups; (ii) determining whether the toxicity of CL-20 to individual groups within the soil invertebrate community can be affected by the duration of exposure; and (iii) assessing the utility of the microcosm assay as a tool for developing ecotoxicological parameters for use in ecological risk assessment of contaminated soils.

2. Materials and methods

2.1. Chemicals and reagents

Crystalline CL-20 (Chemical Abstracts Service [CAS] No.: 135285-90-4; ε-isomer, purity 99.3%) was obtained from ATK Thiokol Propulsion (Ogden, UT, USA). High-performance liquid chromatography (HPLC)-grade acetone (CAS No.: 67-64-1; Fisher Scientific, Pittsburgh, PA, USA) was used to prepare individual CL-20 solutions prior to soil amendments. Acetonitrile (CAS No.: 75-05-8; HPLC-Grade; Pharmco, Brookfield, CT, USA), methanol (CAS No.: 67-56-1, Chromatography Grade, Purity: 99.9%; Pharmco, Brookfield, CT, USA), and calcium chloride ($CaCl_2$; CAS No.: 10043-52-4; Reagent Grade 100%, J.T. Baker, Phillipsburg, NJ, USA), were used for the soil extractions, and in analytical determinations by HPLC. Ethanol (CAS No.: 64-17-5, purity 99.98%; Pharmco, Brookfield, CT, USA) was used as preservative for extracted microarthropods. Sodium bisulfate monohydrate ($NaHSO_4 \cdot H_2O$, CAS No.: 10034-88-5, purity 99%; Sigma–Aldrich, St. Louis, MO, USA) was used to acidify stock solutions in preparation of chemical extracts from soil for determinations by HPLC. American Society for Testing and Materials (ASTM) type I water (18 MΩ cm @ 25 °C; ASTM D1193-99, 2004) was the grade of purified water used throughout the toxicity studies. It was obtained using Milli-RO® 10 Plus followed by Milli-Q® PF Plus systems (Millipore®, Bedford, MA). The same grade of purified water was used throughout the analytical determinations. Glassware was washed with phosphate-free detergent, followed by rinses with tap water, ASTM type II water (>5 MΩ cm @ 25 °C), analytical reagent grade nitric acid 1% (v/v), then with ASTM type I water.

2.2. Soil preparation

A natural soil, Sassafras sandy loam [SSL; fine-loamy, siliceous, semiactive, mesic Typic Hapludult] (USDA-NRCS//ARS 1999) was used in this investigation to assess CL-20 toxicity to the soil invertebrate community. This soil was selected for developing ecotoxicological values protective of soil biota because: (a) it has been previously used for establishing ecotoxicological benchmarks for CL-20 in standardized soil invertebrate toxicity tests (Dodard et al., 2005; Kuperman et al., 2006a,b), and (b) it has physical and chemical characteristics that support "very high" Qualitative Relative Bioavailability for organic chemicals in natural soils (USEPA, 2005), including low organic matter and clay contents (2.6% organic matter, 14% clay, 58% sand, 28% silt, 9.8 cmol kg^{-1} cation-exchange capacity, pH 5.1). Total concentrations of metals and nutrients were within regional background ranges, and were reported previously (Robidoux et al., 2004).

Fresh SSL soil containing the indigenous invertebrate community was collected from an open grassland field in the coastal plain on the property of the U.S. Army Aberdeen Proving Ground, Harford County, Maryland, in May 2003. Soil was gently sieved using a 5-mm sieve to remove large debris and regularize distribution of soil invertebrates. Samples ($n = 5$) of prepared soil were extracted immediately to establish the baseline data for abundance of microarthropods and nematodes in SSL soil. These baseline data were used to determine the effects on subsequent soil preparation procedures on the soil invertebrate community. Prepared soil was stored in covered plastic containers overnight to preserve the initial field moisture content.

Dry SSL soil collected earlier and sieved through a 2-mm sieve was used to prepare the CL-20 soil concentrates. Soil concentrates were required to uniformly amend fresh field-moist SSL soil during preparation of nominal target treatment concentrations without harming soil organisms by exposure to solvent. During the soil concentrate preparation procedure, appropriate amounts of CL-20 were amended into separate aliquots of soil using an organic solvent (acetone) as a carrier. This was necessary in order to more evenly and uniformly distribute the CL-20 to a large soil surface area, rather than by addition of solid chemical crystals to soil. Soil was spread to a thickness of 2.5 cm. CL-20 was dissolved in acetone in glass volumetric flasks then pipetted across the soil surface, ensuring that the volume of solution added at any one time did not exceed 15% (v w^{-1}) of the soil dry mass. After adding the designated CL-20 solution to a respective aliquot of SSL soil, the volumetric flask was rinsed twice with a known volume of acetone and rinses were also pipetted onto the soil. If the total volume of solution needed to amend the soil exceeded 15% (v w^{-1}), the solution was added in successive stages, allowing the acetone to evaporate between additions for a minimum of 2 h in darkness within a chemical hood.

The same total CL-20/acetone solution volume at different CL-20 concentrations was added to every treatment, equaling the volume required to dissolve CL-20 at the greatest dissolved concentration amended. This approach was used to prepare nominal CL-20 treatments of 100, 500, 1000, and 2500 mg kg^{-1}. The nominal CL-20 treatments of 5000, 7500, and 10,000 mg kg^{-1} substantially exceeded solubility levels of CL-20 in acetone carrier so these were prepared by directly mixing appropriate amounts of dry crystalline CL-20 with dry SSL soil. Acetone was added to these three treatments in the same amount as was used in preparation of other treatments to maintain the uniformity of treatments (i.e., solvent addition) throughout all exposure concentrations. Amended soil was subsequently air-dried overnight (minimum of 18 h) in a chemical hood in darkness, to prevent photolysis of CL-20. Each soil treatment sample was then transferred into a fluorocarbon-coated high-density polyethylene container and mixed for 18 h on a three-dimensional rotary soil mixer.

Nominal target concentrations for all freshly amended treatments were prepared one-day after collecting soil in the field, by individually combining and gently mixing CL-20-amended soil