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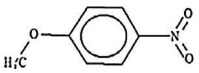
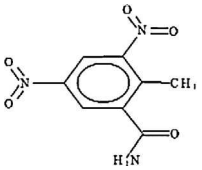
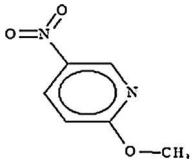
Table 1
Selected properties of test compounds 4-nitroanisole (4-NAN), 3,5-dinitro-*o*-toluamide (3,5-DNoTAME), and 2-methoxy-5 nitropyridine (2-M-5-NPYNE).

Name	4-NAN	3,5-DNoTAME	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-DNoTAME, 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-DNoTAME; 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/

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L) were 6/11, 29/65, 58/131, 116/223, and 232/289 for 4-NAN; 5/10, 48/100, 96/141, and 192/178 for 3,5-DNoTAME, and 6/11, 56/117, 113/178, 227/216 for 2-M-5-NPYNE. The experimental control treatment consisted of Römbke medium with no test chemicals added. All treatments were replicated ($n = 3$). The toxicity test with 2-M-5-NPYNE was repeated using nominal treatment concentrations of 2.3, 4.7, and 9.4 mg/L due to 100% mortality at all nominal concentrations ≥56 mg/L in the initial test. Corresponding analytically determined concentrations of 2-M-5-NPYNE were 14, 25, and 50 mg/L, respectively.

2.4. Bioconcentration studies

The design of the bioconcentration studies followed the protocol established in the international ring test for the assessment of bioaccumulation of chemicals in terrestrial oligochaetes (Bruns et al., 2001; Egeler et al., 2009). This method was modified for aqueous exposures of the earthworms. The BCFs were determined using a static aqueous exposure test system similar to the approach by Belfroid et al. (1993). The bioconcentration studies included exposures of the earthworms to individual test chemicals in Römbke medium in the presence of sand for up to 14 days. This experimental design was applied to studies with 4-NAN, 3,5-DNoTAME, and 2-M-5-NPYNE. The study design also included a control group (Römbke medium with no test chemicals added), and a lipid group (in both control and test chemical treatments) used to quantify earthworm lipid contents.

Glass jars (4.2 cm i.d. × 4.5 cm height) were used as test containers. Each test container was filled with 7.4 mL of exposure medium and 21 g of water-washed coarse silica sand. All treatments were appropriately replicated ($n = 5$ per harvest day). Two depurated earthworms were exposed in each replicate. All test containers were placed in an environmental chamber under a mean temperature of 21.6 ± 0.1 °C in the dark for the duration of the 14 day test. The exposure media in all test containers were renewed every 2-3 days. Replicates of each test chemical treatment were harvested after 0, 0.25, 1, 2, 3, 4, 7, and 14 days of exposure. The concentrations of test compound in the aqueous media were analytically determined for each harvest. Earthworms were rinsed with ASTM type I water and paper-dried to remove excess water. Wet weights of the earthworms were recorded, and the earthworms were then frozen at −80 °C. Frozen earthworms were lyophilized for 7 days prior to residue analysis and lipid content determinations. Uptake of test compounds by the earthworms from solutions was quantified under steady-state conditions to determine the empirical BCF_{ss} values (the ratio of concentrations in tissue compared to exposure media ($[C]_{tissue}/[C]_{media}$). A steady-state condition was accepted when test chemical concentrations in the earthworm tissues stabilized. Stability was reached when there were no significant ($p > 0.05$) differences in tissue concentrations for at least three harvest dates at the end of the study.

2.5. Chemical analyses and analytical determinations

A modified U.S. Environmental Protection Agency (USEPA) Method 8330B (USEPA, 2006) was used to extract and quantify the concentrations of test chemicals in exposure media at predetermined time points. Tissue extracts of the earthworms respectively exposed to 4-NAN, 3,5-DNoTAME, and 2-M-5-NPYNE were prepared according to Sarrazin et al. (2009). Earthworms in the control groups were exposed to the sand-liquid media without chemicals. For each replicate, all earthworms were lyophilized at the end of each exposure interval, then crushed using a mortar and pestle to obtain from 0.1 to 0.2 g of dry material for analyses. The crushed dry tissue was then extracted using a mixture of ASTM type I water and acetonitrile at 4 °C. Each tissue sample was vortexed in ASTM type I water for 10 s before addition of acetonitrile. All samples were then vortexed for an additional 60 s and sonicated (Branson Model 3200, Danbury, CT, USA) for 18 ± 2 h at 20 °C, then centrifuged (12,000 × g) for 10 min at 4 °C using a Sorval Super T21 centrifuge (Global Medical Instrumentation, Ramsey, MN, USA).

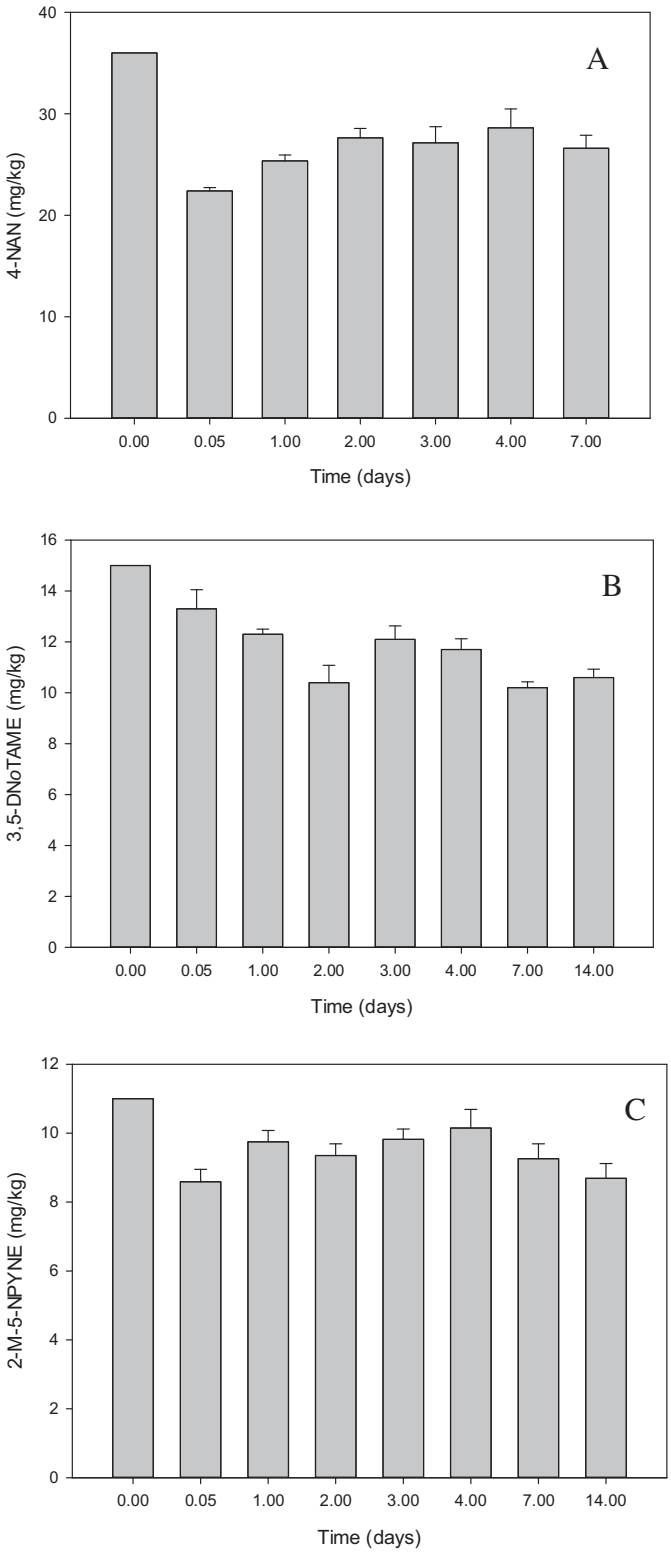

Fig. 1. Concentrations of 4-NAN (A), 3,5-DNoTAME (B), and 2-M-5-NPYNE (C) in aqueous exposure media during the bioconcentration studies.

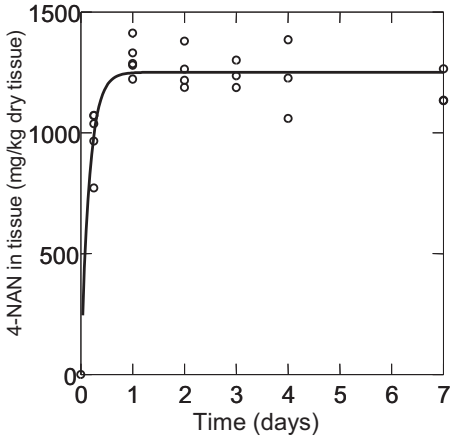

Fig. 2. Uptake of 4-NAN in earthworm tissue during 7 day exposure in structured aqueous medium.

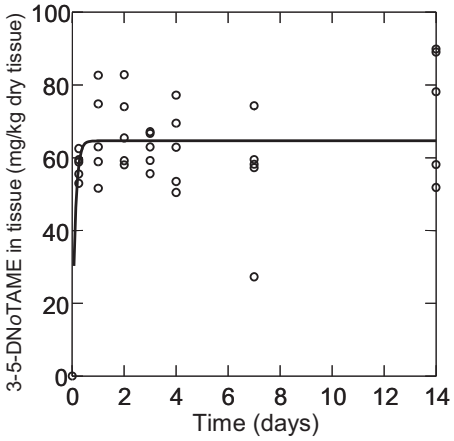

Fig. 3. Uptake of 3,5-DN*o*TAME in earthworm tissue during 14 day exposure in structured aqueous medium.

The HPLC system used for analyses consisted of a Waters 600 pump (Waters Corp, Milford, MA), a 717 plus autosampler, and a 2996 photodiode-Array Detector. Samples (50 μL) were separated with a Discovery C18 column (25 cm, 4.6 mm, 5 mm) (Supelco, Oakville, Canada) at 35 °C. The detector was set to scan from 192 to 450 nm. The analytical detection limit using HPLC was 0.005 mg/L for each compound. The limits of quantification were 0.6 mg/kg tissue for 4-NAN, 0.3 mg/kg tissue for 3,5-DN*o*TAME, and 0.3 mg/kg tissue for 2-M-5-NPYNE.

2.6. Determination of lipid content in earthworms

Depurated earthworms from the laboratory culture, and samples from the uptake studies were frozen at −80 °C (≥7 days), and later thawed on ice for lipid extraction. One or two earthworms were weighed directly in a Falcon™ 50 mL polypropylene conical centrifuge tube. Eight mL of isopropanol were added to the tube and vortexed for 5 s then 10 mL of cyclohexane was added and the content in the tube was vortexed for an additional 5 s. Earthworms in cyclohexane/propanol mixture were homogenized using the high speed homogenizer Ultra Turrax T25 for 2 min at 10,000 × g. A volume of 10.5 mL of ASTM type I water was added to the extraction mixture and homogenized for another 1 min. Five mL of concentrated HCl was added to dissolve the milky suspension prior to centrifugation at 460 × *g* for 5 min. Two phases were formed; the top organic layer (transparent and yellowish) was transferred into a pre-weighed vial. The bottom layer was cleaned by addition of 10 mL of a cyclohexane/isopropanol (87/13; v/v) mixture, vortexed for 5 s, then centrifuged at 460 × *g* for 5 min. The resulting top layer (transparent and yellowish) was transferred into the same pre-weighed vial containing the previous top layer. This cleaning step was repeated twice (normally) to remove traces of lipid, as evidenced by the top layer becoming more transparent after each cleaning step. The extract was then evaporated to dryness with the nitrogen stream. All vials were placed into an oven at 105 °C for 1 h. The vials were cooled in a desiccator and weights were recorded, then they were placed into an oven to dry again for another 2 h. The contents of each vial were transferred into a pre-weighed aluminum cup, using <5 mL of cyclohexane. The sample in the aluminum cup was concentrated to dryness under nitrogen stream, and placed into an oven at 105 °C, overnight. After a maximum of 16 h, the dried aluminum cups and their contents were cooled in a desiccator, and the weights were recorded. Lipid weights were calculated by difference.

2.7. Data analyses

Analysis of variance procedures were applied to the adult earthworm survival (except when 0 or 100% survival occurred), weight, lipid content, and test chemical concentration data. Mean separations were done using Fisher's least-significant difference (FLSD) pairwise comparison tests. A significance level of $p \leq 0.05$ (95% confidence level) was accepted for all statistical tests. Statistical analyses were performed using SYSTAT 11.0 (Systat Software, Inc., Chicago, IL) or SigmaPlot 10.0 (Systat Software, Inc., San Jose, CA).

Results from the earthworm uptake studies were fitted using the equation for a one-compartment model:

$$[C]_{\text{worm}} \text{at } t_{(i)} = [C]_{\text{ss}} \times \{1 - e^{(k \times t)}\},$$

where $[C]_{\text{worm}}$ is the test compound concentration measured in earthworm tissues (mg chemical/kg dry tissue); *t*is uptake time in d; $[C]_{ss}$ is the concentration of the compound in earthworm (mg chemical/kg dry tissue) at steady state; *k* is the uptake coefficient (1/day).

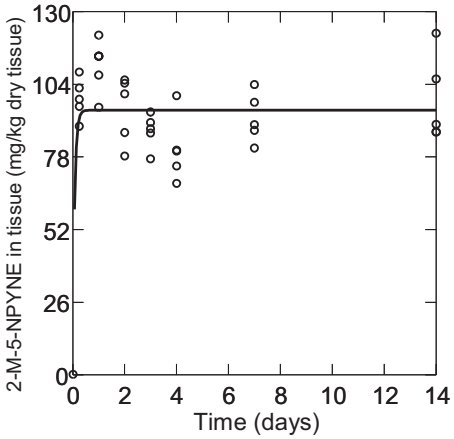

Fig. 4. Uptake of 2-M-5-NPYNE in earthworm tissue during 14 day exposure in structured aqueous medium.

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Table 2
Summary of 4-nitroanisole (4-NAN), 3,5-dinitro-*o*-toluamide (3,5-DNoTAME), and 2-methoxy-5 nitropyridine (2-M-5-NPYNE) uptake kinetics independently determined using the exposure of *Eisenia andrei* in the sand-liquid structured aqueous medium.

Compound	Uptake $[C]_{ss}$ mg/kg dry tissue	Media $[C]_{aq\ ss}$ mg/L	BCF_{ss}[a] mL/g dry tissue	% Lipid g lipid/g dry tissue	Lipid based BCF_{ss} mL/g lipid
4-NAN	1251	26.6	47	14.2	332
3,5-DNoTAME	64.6	10.6	6	10.5	58
2-M-5-NPYNE	94.7	8.7	11	12.1	90

[a] BCF_{ss} is based on the ratio of compound uptake concentration at steady-state $[C]_{ss}$ in tissue compared to aqueous exposure media concentration ($[C]_{ss}/[C]_{aqss}$).

3. Results and discussion

3.1. Aqueous toxicity studies

Mean earthworm survival (±SE) in the control and 29 mg/L nominal treatments for 4-NAN was 1.3 ± 0.67 ($n = 3$). Earthworm mass loss was 0.1 ± 0.05 g for the control treatment and 0.2 ± 0.03 for the 29 mg/L treatment. No earthworms survived exposures to 4-NAN concentration ≥58mg/L. In a study with 3,5-DNoTAME, the mean survival rates were 1.3 ± 0.67 in both the control and the 5 mg/L treatments. Earthworms survived in one of the three replicates used in the 50 mg/L treatment concentration. The mean earthworm mass loss was 0.1 ± 0.05 g for the control treatment and 0.2 ± 0.03 g for the 5 mg/L treatment. No earthworms survived in either 96 mg/L or 192 mg/L treatment concentrations of 3,5-DNoTAME. All earthworms survived in the toxicity study with 2-M-5-NPYNE. Based on the results of these toxicity tests, the following nominal exposure concentrations were selected for the bioconcentration studies: 30 mg/L for 4-NAN, and 10 mg/L for either 3,5-DNoTAME or 2-M-5-NPYNE. Corresponding analytically determined exposure concentrations at the start of the independent bioconcentration studies were 36 mg/L for 4-NAN, 15 mg/L for 3,5-DNoTAME, and 11 mg/L for 2-M-5-NPYNE, respectively.

3.2. Bioconcentration studies

Chemical analysis revealed that 4-NAN concentration in the exposure medium rapidly decreased (by 38%) from the initial exposure concentration of 36 mg/L following introduction of the earthworms, then stabilized after 24 h, and remained relatively constant for the remainder of the 7-day study (Fig. 1A). Concentrations of 3,5-DNoTAME in the exposure medium also decreased after 6 h from the initial exposure concentration of 15 mg/L, following introduction of the earthworms, but the decrease continued for 2 d (31% from the initial 15 mg/L). Concentrations of 3,5-DNoTAME stabilized after 3 d and remained relatively constant for the remainder of the 14-day study (Fig. 1B). A rapid decrease (22% from the initial 11 mg/L), during the 6 h period after introduction of the earthworms was found in the study with 2-M-5-NPYNE. Similar to the pattern of results for 4-NAN, the concentrations of 2-M-5-NPYNE stabilized after 24 h and remained relatively constant for the rest of the 14-day study (Fig. 1C).

Tissue analyses revealed rapid uptake by the earthworms of each test chemical following earthworm introduction into the exposure medium (Figs. 2–4). These concentrations corresponded to rapid decreases in each test chemical concentration in the exposure medium. Steady-state conditions were attained in concentrations in respective earthworm tissues for the final three or more harvest days in these independent studies with 4-NAN ($p \geq 0.292$), 3,5-DNoTAME($p \geq 0.160$), and 2-M-5-NPYNE ($p \geq 0.110$). Steady-state concentrations of each test chemical in the earthworm tissues were used to calculate BCF_{ss} values. The estimates of the BCF_{ss} values (mL/g earthworm dry mass) for 4-NAN, 3,5-DNoTAME, and 2-M-5-NPYNE were 47, 6, and 11, respectively. The corresponding estimates of the lipid based L-BCF_{ss} values were 332 mL/g, 58 mL/g, and 90 mL/g earthworm lipid, respectively (Table 2).

The present bioconcentration studies have allowed us to separate the confounding factor of soil-water partitioning from that of organism exposure. This was necessary because experimental evidence suggests that the dissolved form of a chemical in interstitial or porewater may control its bioaccumulation from soils or sediments. Several studies demonstrated that the bioavailability of many organic compounds, including chlorophenols (Van Gestel and Ma, 1988) polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and linear alkylbenzenes (LABs) (Lamoureux and Brownawell 1999; Ma et al., 1995) was dependent on its concentration in the interstitial aqueous phase. Furthermore, studies by Savard et al. (2010) have shown that chemicals may be applied at loading rates that exceed aqueous solubility, which resulted in distorted BSAF values. This potential complication was avoided in the present studies by using aqueous exposures of the earthworms to test compounds below their aqueous solubility under the test conditions. Other potential complications can also arise from failure to maintain a constant exposure or dosage, as the source concentration typically decreases significantly over the course of the uptake experiment (Lord et al., 1980). We addressed this issue by using a static aqueous exposure test system with periodic (every 2-3 days) renewal of the test solution, which ensured consistent chemical exposure conditions during each study. Additionally, the potential importance of biota lipid content in determining BCF values has been noted in previous studies via various log K_{OW}–log BCF correlations (Lord et al., 1980; Connell and Markwell 1990; Belfroid et al., 1993). Reviewing the bioconcentration measurements for organic compounds persistent in the environment, Connell commented that the coefficient for log K_{OW}, which was considered as equivalent to log K_{LipidW}, should be the lipid content of the organism rather than a conveniently fitted parameter (Connell 1988; Connell and Markwell, 1990). Consequently, we included earthworm lipid content measurement in our experimental design and used these data for determining lipid-based BCF values. Overall, the features of experimental design in our present studies have allowed us to resolve complex technical challenges, described above, and facilitated the determination of the BCF values for 4-NAN, 3,5-DNoTAME, and 2-M-5-NPYNE that will contribute to the BCF database being developed for use in models aimed at predicting environmentally significant parameters for new munition compounds in soil.

4. Conclusions

Bioconcentration potentials of new and emerging munition compounds that partition into earthworms were determined in independent aqueous exposure media studies. These studies showed that the earthworm *Eisenia andrei* can survive in a sand-Römbke exposure medium for 28 d, and allow determination of BCF values. The present bioconcentration studies were designed to separate the factors that arise from soil-water partitioning from

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that of organism-exposure, thus avoiding the potential confounding influence of soil characteristics on the uptake data. This was especially important for data development for a BCF model that is applicable to a wide range of munition compounds which have nitro groups that bind strongly to the clay fraction of the soil. The BCF values determined in the present studies will contribute substantially to the BCF database being developed for use in models aimed at predicting environmentally significant parameters for new munition compounds in soil. The corresponding estimates of the lipid based L-BCF_{ss} will advance comprehension of the organism factors that directly affect bioconcentration. Together these factors are important for the assessment of new and emerging munition compounds as they are evaluated for their potential manufacture and use, and corresponding release into the environment.

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References

Ampleman, G., 2010. Development of a new generation of insensitive explosives and gun propellants. Int. J. Energetic Mater. Chem. Propul. 9, 107–132.
Ampleman, G., Brousseau, P., Thiboutot, S., Rocheleau, S., Monteil-Rivera, F., Radovic-Hrapovic, Z., Hawari, J., Sunahara, G., Martel, R., Coté, S., Brochu, S., Trudel, S., Beland, P., Marois, A., 2012. Evaluation of GIM as greener insensitive melt-cast explosive. Int. J. Energetic Mater. Chem. Propul. 11, 59–87.
ASTM International, 2004. Standard Specification for Reagent Water; ASTM D1193-99. ASTM International, West Conshohocken, PA.
Belfroid, A., van Wezel, A., Sikkenk, M., van Gestel, K., Seinen, W., Hermens, J., 1993. The toxicokinetic behavior of chlorobenzenes in earthworms (*Eisenia andrei*): experiments in water. Ecotoxicol. Environ. Saf. 25, 154–165.
Bruns, E., Egeler, P., Römbke, J., Scheffczyk, A., Spoerlein, P., 2001. Bioaccumulation of lindane and hexachlorobenzene by the oligochaetes *Enchytraeus luxuriosus* and *Enchytraeus albidus* (Enchytraeidae, Oligochaeta, Annelida). Hydrobiologia 463, 185–196.
Connell, D.W., 1988. Bioaccumulation behaviour of persistent organic chemicals with aquatic organisms. Rev. Environ. Contam. Toxicol. 101, 117–154.
Connell, D.W., Markwell, R.D., 1990. Bioaccumulation in the soil to earthworm system. Chemosphere 20, 91–100.
Davies, P.J., Provatas, A., 2006. Characterization of 2,4-Dinitroanisole: An Ingredient for Use in Low Sensitivity Melt Cast Formulations, DSTO-TR-1904. Defense Science and Technology Organization, Edinburgh, Australia.
Dodard, S., Sarrazin, M., Hawari, J., Paquet, L., Ampleman, G., Thiboutot, S., Sunahara, G.I., 2013. Ecotoxicological assessment of a high energetic and insensitive munitions compound: 2,4-dinitroanisole (DNAN). J. Hazard. Mater. 262, 143–150.
Dodard, S., Sunahara, G.I., Sarrazin, M., Gong, P., Kuperman, R.G., Ampleman, G., Thiboutot, S., Hawari, J., 2005. Survival and reproduction of enchytraeid worms (Oligochaeta) in different soil types amended with cyclic nitramine explosives. Environ. Toxicol. Chem. 24, 2579–2587.
Egeler, P., Gilber, D., Scheffczyk, A., Moser, T., Römbke, J., 2009. Validation of a soil bioaccumulation test with terrestrial oligochaetes by an international ring test. Technical Report UBA-FB 204 67 458 for the Research and Development Project of the German Federal Environmental Agency. ECT Oekotoxikologie, Flörsheim/Main, Germany.
Haley, M.V., Kuperman, R.G., Checkai, R.T., 2009. Aquatic Toxicity Screening of 3-Nitro-1,2,4-triazol-5-one. Technical Report No. ECBC-TR-726, U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD. September 2009.
Kuperman, R.G., Checkai, R.T., Johnson, M., Robidoux, P.Y., Lachance, B., Thiboutot, S., Ampleman, G., 2009. Ecological risk assessment of soil contamination with munition constituents in North America. In: Sunahara, G.I., Lotufo, G., Kuperman, R.G., Hawari, J. (Eds.), Ecotoxicology of Explosives. CRC Press, Boca Raton, FL.
Kuperman, R.G., Checkai, R.T., Simini, M., Phillips, C.T., Kolakowski, J.E., Lanno, R., 2013. Soil properties affect the toxicities of TNT and RDX to the enchytraeid worm, *Enchytraeus crypticus*. Environ. Toxicol. Chem. 32, 2648–2659.
Lamoureux, E.M., Brownawell, B.J., 1999. Chemical and biological bioavailability of sediment-sorbed hydrophobic organic contaminants. Environ. Toxicol. Chem. 18, 1733–1741.
Lanno, R., Wells, J., Conder, J., Bradham, K., Basta, N., 2004. The bioavailability of chemicals in soil for earthworms. Ecotoxicol. Environ. Saf. 57, 39–47.
Lord, K.A., Briggs, G.G., Neale, M.C., Manlove, R., 1980. Uptake of pesticides from water and soil by earthworms. Pestic. Sci. 11 (40), l–408.
Ma, W.-C., Immerzeel, J., Bodt, J., 1995. Earthworm and food interactions on bioaccumulation and disappearance in soil of polycyclic aromatic hydrocarbons: studies on phenanthrene and fluoranthene. Ecotoxicol. Environ. Saf. 32, 226–232.
Monteil-Rivera, F., Halasz, A., Groom, C., Zhao, J.-S., Thiboutot, S., Ampleman, G., Hawari, J., 2009. Fate and transport of explosives in the environment: a chemist's view. In: Sunahara, G.I., Lotufo, G., Kuperman, R.G., Hawari, J. (Eds.), Ecotoxicology of Explosives. CRC Press, Boca Raton, FL.
Robidoux, P.-Y., Svendsen, C., Sarrazin, M., Hawari, J., Thiboutot, S., Ampleman, G., Weeks, J.M., Sunahara, G.I., 2002. Evaluation of tissue and cellular biomarkers to assess 2,4,6-trinitrotoluene (TNT) exposure in earthworms: effects-based assessment in laboratory studies using *Eisenia andrei*. Biomarkers 7, 306–321.
Rocheleau, S., Kuperman, R.G., Simini, M., Hawari, J., Checkai, R.T., Thiboutot, S., Ampleman, G., Sunahara, G.I., 2010. Toxicity of 2,4-dinitrotoluene to terrestrial plants in natural soils. Sci. Total Environ. 408, 3193–3199.
Römbke, J., Knacker, T., 1989. Aquatic toxicity test for enchytraeids. Hydrobiologia 180, 235–242.
Sarrazin, M., Dodard, S.G., Savard, K., Lachance, B., Robidoux, P.Y., Kuperman, R.G., Hawari, J., Ampleman, G., Thiboutot, S., Sunahara, G.I., 2009. Accumulation of hexahydrohexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by the earthworm *Eisenia andrei* in a sandy loam soil. Environ. Toxicol. Chem. 28, 2125–2133.
Savard, K., Sarrazin, M., Dodard, S.G., Monteil-Rivera, F., Kuperman, R.G., Hawari, J., Sunahara, G.I., 2010. Role of soil interstitial water in the accumulation of hexahydro-1,3,5-trinitro-1,3,5-triazine in the earthworm *Eisenia andrei*. Environ. Toxicol. Chem. 29, 998–1005.
Selassie, C.D., 2003. The history of quantitative structure activity relationships, 6th edition In: Abraham, D.J. (Ed.),), vol. 1. John Wiley and Sons Publishers, New York, NY.
Sunahara, G.I., Lotufo, G., Kuperman, R.G., Hawari, J., 2009. Ecotoxicology of Explosives. CRC Press, Boca Raton, FL.
United States Environmental Protection Agency (USEPA), 2006. Method 8330B. Nitroaromatics, nitrate esters, nitramines by high performance liquid chromatography (HPLC). Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. Office of Solid Waste. SW-846, Washington, DC.
Van Gestel, C.A.M., Ma, W.-C., 1988. Toxicity and bioaccumulation of chlorophenols in earthworms, in relation to bioavailability in soil. Ecotoxicol. Environ. Saf. 15, 289–297.
Van Gestel, C.A.M., Ma, W.-C., 1990. An approach to quantitative structure–activity relationships (QSARs) in earthworm toxicity studies. Chemosphere 21, 1023–1033.

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