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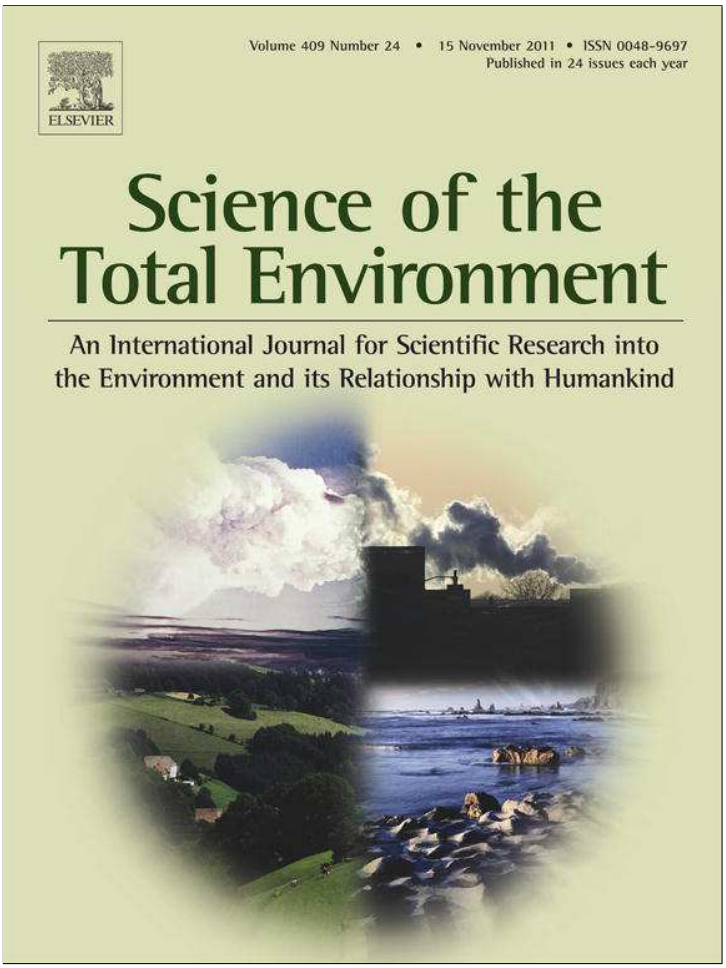

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ABSTRACT

Nitroglycerin (NG) is widely used for the production of explosives and solid propellants, and is a soil contaminant of concern at some military training ranges. NG phytotoxicity data reported in the literature cannot be applied directly to development of ecotoxicological benchmarks for plant exposures in soil because they were determined in studies using hydroponic media, cell cultures, and transgenic plants. Toxicities of NG in the present studies were evaluated for alfalfa (*Medicago sativa*), barnyard grass (*Echinochloa crusgalli*), and ryegrass (*Lolium perenne*) exposed to NG in Sassafras sandy loam soil. Uptake and degradation of NG were also evaluated in ryegrass. The median effective concentration values for shoot growth ranged from 40 to 231 mg kg^{-1} in studies with NG freshly amended in soil, and from 23 to 185 mg kg^{-1} in studies with NG weathered-and-aged in soil. Weathering-and-aging NG in soil did not significantly affect the toxicity based on 95% confidence intervals for either seedling emergence or plant growth endpoints. Uptake studies revealed that NG was not accumulated in ryegrass but was transformed into dinitroglycerin in the soil and roots, and was subsequently translocated into the ryegrass shoots. The highest bioconcentration factors for dinitroglycerin of 685 and 40 were determined for roots and shoots, respectively. Results of these studies will improve our understanding of toxicity and bioconcentration of NG in terrestrial plants and will contribute to ecological risk assessment of NG-contaminated sites.

1. Introduction

Nitroglycerin (NG), also called glyceryl trinitrate, trinitroglycerin or 1,2,3-propanetriol trinitrate, is a nitrate ester. Nitroglycerin is widely used for the production of dynamite, gunpowder, and rocket propellants, and in the pharmaceutical industry as a vasodilator for the treatment of angina pectoris (Podlipná et al., 2008; Husserl et al., 2010; Saad et al., 2010). Chronic exposure to NG causes severe headaches and methaemoglobinaemia to workers of the explosive manufacturing industry (Stucki, 2004).

Nitroglycerin is a component of several solid propellants used in rockets and artillery ammunitions. It is released into the environment at firing positions, and in the target areas due to low-order (partial) detonation of propellant-containing ordnance. Solid double- and triple-base propellants consist of nitrocellulose infused with either NG (double-base propellants) or with NG and nitroguanidine (NQ) (triple-base propellants). When released from the nitrocellulose matrix, NG is mobile in soil due to its moderate aqueous solubility of 1.8 g L^{-1} at 20 °C (Verscheuren, 1983; Pal and Ryon, 1986), and low partition coefficient values such as log K_{ow} of 1.62 (Sunahara et al., 2009) and log K_{oc} of 2.77 (Spanggord et al., 1980). However, simple leaching experiments showed that aqueous solubility alone does not control the release of NG from the nitrocellulose matrix (Mirecki et al., 2006), thus it cannot fully explain the fate and persistence of NG in soil. Therefore, it is not surprising that environmental assessments conducted at 23 military firing ranges in the United States and Canada identified NG as a soil contaminant at antitank rocket ranges with concentrations in soil as high as 4700 mg kg^{-1} (Jenkins et al., 2006). Such contamination can jeopardize the sustainable use of testing and training ranges at defense installations and requires assessment of potential ecological risks associated with NG contamination of soil.

One of the common methods for disposal of propellants at military sites is burning, which can leave unburned energetic residues on the soil surfaces (Walsh et al., 2010). Other physico-chemical remediation technologies for the removal of NG from the environment include oxidation, composting, phytoremediation, biodegradation (Pandey et al., 2007), iron reduction (Oh et al., 2004), and sorption using nano-structured silica based materials (Saad et al., 2010). However, none of these technologies are efficacious in removing energetic residues from soil, which can pose exposure risks for human and ecological receptors, such as terrestrial plants.

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Earlier studies developed ecotoxicological data for NG for various plant species using hydroponic solutions, liquid seed germination media, or cell culture media. Germination of white mustard seeds (*Sinapis alba*) in a liquid seed germination medium supplemented with NG was almost completely inhibited at NG concentration of 400 mg L^{-1}, while primary root growth was inhibited by 80% at NG concentration of 200 mg L^{-1} relative to growth in the negative control soil (Podlipná et al., 2008). Studies with wild-type and transgenic tobacco (*Nicotiana tabaccum* cv. *xanthi*) plants expressing pentaerythritol tetranitrate reductase (an enzyme derived from an explosives-degrading bacterium that enables degradation of nitrate ester and nitroaromatic explosives) showed that wild-type seeds failed to germinate at ≥0.5 mM (113.5 mg L^{-1}) of NG in liquid plant growth media. Conversely, transgenic tobacco seeds germinated and developed normally in medium containing 1 mM of NG but failed to germinate in medium containing 4 mM of NG (French et al., 1999; Hannink et al., 2003).

NG was readily taken up by yellow nutsedge (*Cyperus escalantus*), common rush (*Juncus effuses*), and yellow foxtail (*Setaria glacula*) from hydroponic solution containing initial NG concentration of 10 mg L^{-1}, although NG did not accumulate in yellow foxtail tissues, possibly due to effective enzymatic transformation of NG in the tissues of this species (Riefler and Medina, 2006). Flax (*Linum usitatissimum* L. cv. Viola) cell cultures accumulated NG and transformed it to the dinitroglycerin (DNG) isomers 1,2-dinitroglycerin (1,2-DNG) and 1,3-dinitroglycerin (1,3-DNG) in a 24-d study by (Podlipná et al., 2008). Sweet beet cell extracts metabolized NG to DNG and mononitrate glycerin (MNG) (Goel et al., 1997).

The present literature review revealed that no published studies were designed to determine the effects of plant exposure to NG in soil, and that the data resulting from hydroponic exposures, cell cultures, and transgenic plants cannot be applied directly to development of toxicity benchmarks acceptable for use in Ecological Risk Assessment (ERA) at sites contaminated with NG. In order to fill the existing knowledge gap in the discernment of potential ecological impacts of NG release into terrestrial ecosystems, studies were designed to specifically meet the United States Environmental Protection Agency (USEPA, 2005) criteria for the development of Ecological Soil Screening Level (Eco-SSL), the development of toxicity benchmarks for use in Screening Level ERA (SLERA). Additional experiments evaluated the potential for the uptake of NG from contaminated soil, and NG degradation, by perennial ryegrass (*Lolium perenne*).

2. Materials and methods

2.1. Chemicals and reagents

Nitroglycerin (Chemical Abstracts Service [CAS] No.: 55-63-0; purity: 99%) was obtained from General Dynamics Canada Inc. (Valleyfield, Quebec, Canada). Boric acid (CAS No.: 52869-79-1; 99.5% pure) was used as a positive control for the plant toxicity tests, and was obtained from EMD Chemicals Inc. (Gibbstown, NJ). High-performance liquid chromatography (HPLC)-grade acetone (CAS No.: 67-64-1) used to prepare individual EM solutions prior to soil amendments was obtained from Caledon Laboratories (Georgetown, ON, Canada). Acetonitrile (CAS No.: 75-05-8; HPLC-Grade) and methanol (CAS No.: 67-56-1, Chromatography Grade, 99.9% pure), obtained from Malinckrodt Baker Inc. (Philipsburg, NJ), and calcium chloride ($CaCl_2$; CAS No.: 10043-52-4; Reagent Grade) obtained from Fisher Chemical (Fairlawn, NJ), were used for the soil and plant extractions, and in analytical determinations by HPLC. Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX; CAS No.: 2691-41-0; purity: 99%) was used as the internal standard solution for soil extraction in toxicity tests, whereas hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX; CAS No.: 121-82-4; purity 99%) was used for plant tissue extraction. Both HMX and RDX were obtained from Defense Research and Development Canada-Valcartier (Quebec City, Quebec, Canada). Analytical standards of NG, 1,2-DNG, and 1,3-DNG were supplied by Cerilliant (Round Rock, TX). American Society for Testing and Materials (ASTM) type I water (American Society for Testing and Materials, 2004) was obtained using the Super Q water purification system (Millipore®, Nepean, Ontario, Canada) and was used throughout the studies. Glassware was washed with phosphate-free detergent, followed by rinses with tap water, ASTM type II water, analytical reagent grade nitric acid 1% (v/v), and then with ASTM type I water.

2.2. Soil collection and preparation

Studies were conducted using Sassafras sandy loam (SSL), [fine-loamy, siliceous, semiactive, mesic Typic Hapludult]; 55% sand, 28% silt, 17% clay, 2.3% organic matter (OM), 9.3 cmol kg^{-1} cation-exchange capacity (CEC), and pH 4.9. This soil was selected for developing ecotoxicological values protective of terrestrial plants, because it has physicochemical characteristics that support high bioavailability of organic chemicals according to the criteria for deriving the Ecological Soil Screening Level (USEPA, 2005). Soil was obtained from Aberdeen Proving Ground (Maryland, USA) by removing the vegetation and organic layers and collecting from the top 15 cm of the A horizon. Standard methods were used to measure soil pH, OM content, and particle size distribution (ISO (International Organization for Standardization), 1994). Soil analyses, using methods described below, showed that neither NG nor any of its degradation or transformation products were present at or above analytical detection level of 0.1 mg kg^{-1}. Soil batches were separately amended, with NG dissolved in an acetone carrier, by General Dynamics Inc. The acetone was allowed to volatilize in darkness in a chemical hood to prevent photolysis of NG for a minimum of 18 h. The carrier control was treated with acetone only. Each amended soil batch was mixed overnight (18 ± 2 h) using a three-dimensional rotary soil mixer. After mixing, the soil was hydrated with ASTM type I water to 60% of the SSL soil water holding capacity (WHC; 18% of SSL soil dry mass), to initiate the weathering-and-aging of NG in soil, or to a test-specific percentage of the WHC for studies with NG freshly amended in soil. These soil hydration levels (expressed as percent of the SSL soil WHC) were 75% for tests with alfalfa or ryegrass, and 60% for those with barnyard grass.

Weathering-and-aging of NG in soil was simulated in the assessments of the NG effects on plants to produce soil microenvironments that more closely resemble the bioavailability of NG and its transformation/degradation products under field conditions. The procedure for weathering-and-aging organic energetic chemicals in soil has been described elsewhere (Kuperman et al., 2005; Rocheleau et al., 2006). Briefly, weathering-and-aging of NG in soil included exposing individual hydrated soil batches in open glass containers at ambient temperatures in the greenhouse to alternating moistening-and-drying cycles for 1 month. All soil batches were weighed and readjusted to their initial mass by adding ASTM type I water to the soil each week. Soil surface crust formed during the week was broken with a spatula before addition of water. After the weathering-and-aging procedure concluded, each soil batch was hydrated with ASTM type I water to a test-required percent of the WHC, and was then allowed to equilibrate for a minimum of 24 h prior to the commencement of toxicity tests.

2.3. Plant toxicity tests

Plant toxicity tests were performed with alfalfa (*Medicago sativa*, variety Canada no. 1), barnyard grass (*Echinochloa crusgalli*, variety Common no. 1), and perennial ryegrass (*L. perenne*, variety Express) using methods adapted from ASTM (2002) and USEPA (1996) protocols. Alfalfa and barnyard grass were purchased from Williams Dam Seeds (Dundas, Ontario, Canada) and Labon Inc. (Boucherville, Quebec, Canada), respectively. Perennial ryegrass was obtained from Pickseed Canada Inc. (St-Hyacinthe, Quebec, Canada). Alfalfa seeds were inoculated with nitrogen-fixing bacteria *Rhizobium sp.* prior to sowing, as is typically done under field conditions. Twenty seeds were sown in 10-cm wide pots

containing 200 g dry soil, and incubated in sealed plastic bags to maintain soil moisture (USEPA, 1996) for the duration of the test. Plant toxicity tests were performed in a temperature and light controlled growth chamber (Conviron Inc., Winnipeg, Manitoba, Canada). Planted seeds were incubated in darkness for the first 2 d, then treatments were exposed to a diurnal photoperiod cycle thereafter. The growth chamber conditions were set as follows: light intensity at 5000 ± 500 lux, light for 16 h at 25 °C, dark for 8 h at 20 °C. Luminosity level was measured weekly using a photometer, and the light intensity was readjusted by moving the light canopy when needed. The measurement endpoints included seedling emergence, shoot wet mass, and shoot dry mass. Seedling emergence was measured after 5 d for alfalfa or barnyard grass, and after 7 d for ryegrass (ASTM, 2002). Shoot growth was measured after 16 d for alfalfa or barnyard grass, and after 19 d for ryegrass. Shoots were cut just above the soil line, and fresh mass was determined immediately to minimize moisture loss. Dry mass was determined after drying the tissue at 70 °C for 24 h.

All chemical concentrations in soil are expressed on dry mass basis. Nominal concentrations of NG in studies with freshly amended soil included 0, 1, 10, 100, 1000, and 5000 mg kg^{-1} Nominal concentrations in studies with NG weathered-and-aged in soil included 0, 5, 10, 20, 50, 100, 200, 400, and 650 mg kg^{-1}. Control treatments (0-added; non-detectable NG) included negative (ASTM type I water), carrier (acetone), and positive (boric acid at concentrations of 0, 175, 200, 230, 260, and 290 mg kg^{-1} for tests with alfalfa; 0, 65, 110, 175, 260, 350, and 450 mg kg^{-1} for tests with barnyard grass; and 0, 50, 80, 110, 150, and 200 mg kg^{-1} for tests with ryegrass). All definitive terrestrial plant tests were performed using three replicates per treatment. Results from control treatments complied with quality control requirements (USEPA, 1996; ASTM, 2002). Phytotoxicity tests were repeated if seedling emergence was less than 75% in the negative and carrier controls, in compliance with quality control procedures established in our laboratory.

2.4. Uptake and transformation of NG in perennial ryegrass

Twenty seeds of ryegrass were sown in pots containing 200 g dry SSL soil freshly amended with nominal NG concentrations of 0, 10, 30, 50, and 75 mg kg^{-1}. Soil was then hydrated with ASTM type I water to 75% of the SSL soil WHC. An additional set of pots containing similarly treated soil but without ryegrass seeds was prepared to evaluate contribution of plants to the fate of NG in soil. An airtight dessicator was used as a bioaccumulation microcosm to allow maximum growth of ryegrass during the study (Sarrazin et al., 2009). Each microcosm was opened once a week to allow air-exchange. Each microcosm was placed in a temperature and light controlled growth chamber at 24 °C in darkness for 2 d, then the growth chamber conditions were set as follows: light intensity at 5000 ± 500 lux, light for 16 h at 25 °C, dark for 8 h at 20 °C. Ryegrass shoots and roots were harvested after 14, 21, 28, and 35 d of exposure. Soil was washed away from roots with ASTM type I water, and excess water was absorbed with a paper towel. Shoots and roots were kept at −20 °C until wet extractions were performed in preparation for chemical analysis.

2.5. Extraction and analytical determinations of NG and its degradation products

Triplicate soil samples were collected from each soil NG treatment at the beginning of definitive toxicity tests, and throughout the uptake studies. These samples were extracted and analyzed using USEPA Method 8330B (USEPA, 2006) with some modifications. Briefly, the soil sample (2 g, dry mass basis) was placed in a glass tube, and 10 mL of acetonitrile and 100 µL of internal standard solution (HMX; 50, 250, 500 or 1250 mg L^{-1}, depending on NG nominal concentration) were then added to the soil sample. Glass tubes were vortexed for 1 min, then sonicated in darkness for 18 ± 2 h at 20 °C. Five mL of the sonicated sample was then transferred to another tube, to which 5 mL of 5 g L^{-1} $CaCl_2$ solution was added. Supernatant was filtered through a 0.45-µm Millex-HV Teflon cartridge to remove soil particles.

NG was extracted from fresh plant biomass (shoots or roots), in order to avoid volatilization of potential NG metabolites during a biomass drying process. Wet tissue was homogenized using a Dounce tissue grinder and 1 mL of internal standard consisting of equal volumes of RDX (1 mg kg^{-1}) and $CaCl_2$ (5 g L^{-1}) solutions. The tissue grinder was rinsed twice with the internal standard solution and the homogenate was transferred to a centrifugation tube. Samples were then vortexed for 1 min, sonicated for 18 ± 2 h at 20 °C and centrifuged (Allegra X-12R, Beckman Coulter) at 1500 rpm for 1 h. A one-mL aliquot of supernatant was then transferred into a glass vial. Samples were settled at 4 °C for 24 h, and supernatants were filtered through 0.45-µm Millex-HV Teflon cartridges to remove fine particles.

Filtered soil and plant extracts were analyzed using a Waters HPLC system composed of a Model 600 pump, a Model 717 Plus injector, a Model 2996 Photodiode-Array Detector and a Temperature Control Module. The column used was a Supelco Discovery C18 (25 cm × 4.6 mm; 5 µm particle size). Column temperature was maintained at 35 °C. The solvent system consisted of a 50% methanol/water isocratic mobile phase at a flow rate of 1.0 mL min^{-1}. The sample injection volume was 50 µL, and the total run time of a sample was 15 min. The detector scanned from 192 to 450 nm, and chromatograms were generated at a wavelength of 205 nm. The HPLC detection limits were 0.01 mg L^{-1} (soil extracts) and 0.1 mg L^{-1} (plant tissue extracts) for NG and its transformation products (1,2-DNG and 1,3-DNG), and the resulting limits of quantification of NG and its metabolites were 0.1 mg kg^{-1} in soil and 10 mg kg^{-1} in plant tissue. Extraction was repeated if the internal standard recovery was less than 90%.

2.6. Data analyses

Phytotoxicity data were analyzed using the appropriate regression models selected from among those described in Environment Canada Guidance Document (Environment Canada, 2005). During the model selection process, compliance with the normality assumptions and homoscedasticity of the residuals were determined by examining the stem-and-leaf graphs and histograms of the residuals. The best fit was evident when the regression lines generated by the models were the closest to the data points, the regression coefficients for point estimates were the highest, the residuals were homoscedastic (i.e., had most random scattering), and the means, standard errors, and variances of the residuals were the smallest. The selected models were:

$$\text{Logistic Gompertz model}: Y = a \times e\left([\log(1-p)] \times \left[C/EC_p\right]^b\right)$$

$$\text{Exponential model}: Y = a \times e\left(\left([\log(1-p)]/EC_p\right) \times C\right) + b$$

where Y is the number of emerged seedlings or the shoot mass, a is the y-intercept (i.e., the control response), e is the exponent of the base of the natural logarithm, p is the desired value for 'p' effect (e.g., 0.5 for EC_{50}), C is the analytically determined exposure concentration in test soil, EC_p is the estimate of effect concentration for a specified percent effect, and b is the scale parameter that defines the shape of the equation.

The EC_p estimates and 95% confidence intervals (CI) associated with the point estimates included the NG concentration producing 20% (EC_{20}) or 50% (EC_{50}) reduction in the measurement endpoint compared with the carrier control. Analysis of variance (ANOVA) was used to determine the chemical concentration associated with a statistically significant change compared with the results in carrier control, and to establish the No-Observable-Effect-Concentration (NOEC), the Lowest-Observable-Effect-Concentration (LOEC), and the Lowest-Observable-

Table 1
Concentrations of nitroglycerin (NG) in Sassafras sandy loam soil treatments used in phytotoxicity tests[a].

Nominal concentrations ($mg\ kg^{-1}$)	Measured concentrations in freshly amended soil ($mg\ kg^{-1}$)	Measured concentrations before weathering-and-aging NG in soil ($mg\ kg^{-1}$)	Measured concentrations after weathering-and-aging NG in soil ($mg\ kg^{-1}$)	Recovery of NG after 1 month of weathering-and-aging in soil (%)
1	0.8 ± 0.02	NA	NA	NA
5	NA	4 ± 0.4	0 ± 0	0
10	5 ± 0.2	7 ± 0.2	0 ± 0	0
20	NA	17 ± 2	0.2 ± 0.3	1
50	NA	48 ± 0.4	0.6 ± 0.3	1
100	85 ± 3	96 ± 2	2 ± 0.1	2
200	NA	204 ± 6	21 ± 1	10
400	NA	404 ± 8	121 ± 3	30
650	NA	673 ± 14	268 ± 4	40
1000	898 ± 97	NA	NA	NA
5000	4558 ± 119	NA	NA	NA

NA: not applicable; treatment was not included in the test.
[a] Separate soil treatment batches were prepared for studies with NG freshly amended and with NG weathered-and-aged in soil; NG concentrations in control treatments (0-added NG) were below analytical quantification limit of 0.1 $mg\ kg^{-1}$. Measured concentrations are means $\pm$ standard deviations ($n = 3$).

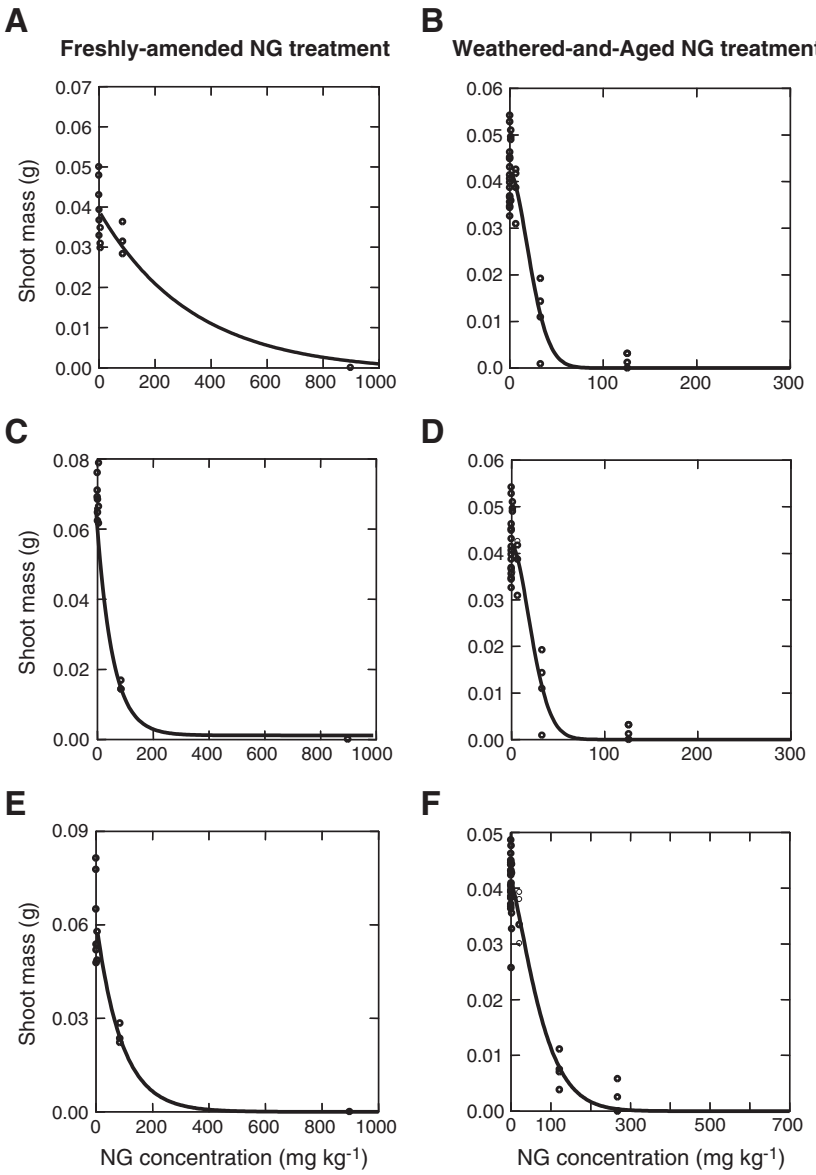

Fig. 1. Effects of nitroglycerin (NG) freshly amended (left) or weathered-and-aged (right) in Sassafras sandy loam soil on growth (shoot dry mass) of alfalfa (A and B), barnyard grass (C and D), and ryegrass (E and F) using measured NG concentrations ($n = 3$).

Adverse-Effect-Concentration (LOAEC) values. Means separations were performed using Fisher's-Least-Significant-Difference (FLSD) tests (Systat Software Inc., Chicago IL USA).

Bioconcentration factors (BCF) were calculated by dividing the analytically determined (measured) concentration of the energetic material in plant tissue (shoot or root) by the measured concentration of the energetic material in soil at a specific exposure time and were expressed as g wet tissue mass per g dry soil. Translocation Factors (TF) were calculated by dividing the respective BCF for shoots by the BCF for roots.

3. Results and discussion

3.1. Phytotoxicity of nitroglycerin

The toxicities of NG to terrestrial plants were independently evaluated for NG freshly amended and for NG weathered-and-aged in SSL soil. Concentrations of NG in amended soil were determined at the beginning of each definitive toxicity test (Table 1). Samples prepared for weathering-and-aging of NG in test soils were analyzed to determine the initial NG concentrations. These concentrations were contrasted with NG concentrations measured at the end of weathering-and-aging procedure to assess the net effect of weathering-and-aging of NG in soil on the plant exposure conditions during toxicity testing (Table 1). Final concentrations of NG at the end of the one-month weathering-and-aging process ranged from 0% to 40% of initial concentrations in freshly amended soil treatments. These final concentrations represent exposure treatments used in definitive phytotoxicity tests of NG weathered-and-aged in SSL soil.

Seedling emergence of alfalfa, barnyard grass, and ryegrass in the carrier control was 92%, 82%, and 88% in studies where soils were freshly amended with NG; in studies with the NG weathered-and-aged in the soils, seedling emergence in carrier control was 83%, 76%, and 89%, respectively. These seedling emergence results complied with quality control criteria established in our laboratory for phytotoxicity studies. The shoot growth (dry mass) EC_{50} values ranged from 10 to 55 mg kg^{-1} in positive control and were consistent with the laboratory baseline established for the three plant species in studies with SSL soil. Compliance with the test quality control criteria, which is a seedling emergence percentage greater than 75%, confirmed that the toxicological effects determined in the definitive tests were attributable to the NG treatments.

The ranges of NG concentrations selected for the definitive tests were sufficient to establish the concentration–response relationships based on plant growth endpoints for the three test species (Fig. 1; shoot dry mass data shown as representative examples). The exponential model had the best fit for all phytotoxicity data obtained in tests with NG freshly amended in SSL soil (Table 2), while the logistic Gompertz model had the best fit for data obtained in tests with NG weathered-and-aged soil. Values for regression coefficients determined for all ECp estimates were ≥0.96, indicating a good fit of the models used for phytotoxicity data. Nonlinear regression analyses of toxicity data for NG freshly amended in soil yielded the EC_{20} values

Table 2
Summary of toxicological benchmarks for nitroglycerin (NG) freshly amended or weathered-and-aged in Sassafras sandy loam soil, determined in definitive tests with alfalfa, barnyard grass, and ryegrass.

Plant species	Alfalfa		Barnyard grass		Ryegrass	
Soil NG treatment	Freshly amended (mg kg^{-1})	Weathered-and-aged (mg kg^{-1})	Freshly amended (mg kg^{-1})	Weathered-and-aged (mg kg^{-1})	Freshly amended (mg kg^{-1})	Weathered-and-aged (mg kg^{-1})
Seedling emergence						
NOEC	85	122	0.8	33	85[1]	21
p	0.454	0.530	0.143	0.140	0.205	0.953
LOEC	898	268	5	126	898[2]	122
p	<0.001	0.011	0.023	<0.001	<0.001	<0.001
EC_{20}	95	286	97	56	105	97
CI (95%)	50–140	175–398	50–144	29–83	43–167	50–144
EC_{50}	296	485	301	126	325	250
CI (95%)	157–435	0–1132	156–447	96–157	135–515	195–306
Model used	Exponential	Gompertz	Exponential	Gompertz	Exponential	Gompertz
R^2	0.989	0.988	0.988	0.983	0.981	0.989
Growth — fresh mass						
NOEC	<0.8[3]	1.8	5	7	<0.8[3]	21
p	<0.001	0.875	0.088	0.894	<0.001	0.383
LOEC	0.8	21	85	33	0.8	122
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
EC_{20}	23	5	9	16	16	42
CI (95%)	14–32	0–13	6–13	0–38	7–25	9–75
EC_{50}	71	77	29	24	50	73
CI (95%)	43–100	29–125	19–40	9–39	23–78	43–103
Model used	Exponential	Gompertz	Exponential	Gompertz	Exponential	Gompertz
R^2	0.982	0.977	0.992	0.967	0.967	0.982
Growth — dry mass						
NOEC	<0.8[3]	21	5	7	<0.8[3]	1.8
p	0.001	0.347	0.378	0.468	<0.001	0.299
LOEC	0.8	122	85	33	0.8	21
p	<0.001	0.005	<0.001	<0.001	<0.001	0.020
EC_{20}	74	83	13	12	20	26
CI (95%)	13–136	25–141	9–16	1–23	9–31	12–41
EC_{50}	231	185	40	23	62	63
CI (95%)	40–421	131–238	29–50	14–32	29–95	44–81
Model used	Exponential	Gompertz	Exponential	Gompertz	Exponential	Gompertz
R^2	0.973	0.969	0.993	0.967	0.968	0.986

[1] Unbounded NOEC: Unbounded No-Observable-Effect-Concentration.
[2] NOAEC: No-Observable-Adverse-Effect-Concentration.
[3] LOAEC: Lowest-Observable-Adverse-Effect-Concentration.

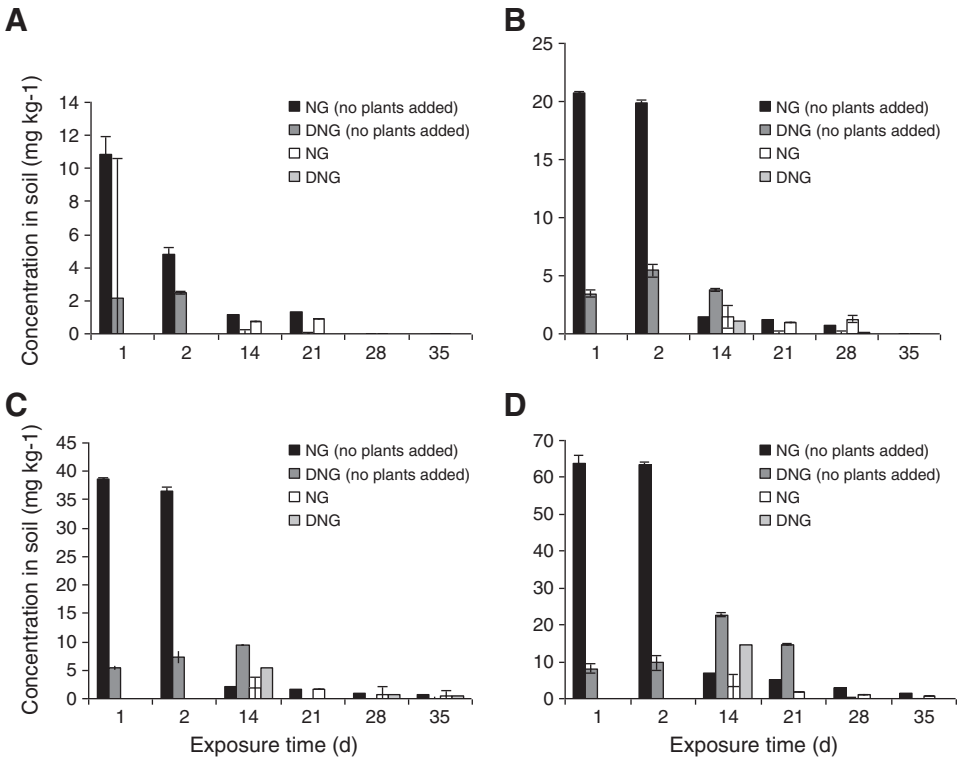

Fig. 2. Measured concentrations of nitroglycerin (NG) and dinitroglycerin (DNG) in Sassafras sandy loam soil treatments with or without ryegrass during the 35-d exposure to nominal NG concentrations (A) 10 mg kg^{-1}, (B) 30 mg kg^{-1} , (C) 50 mg kg^{-1}, and (D) 75 mg kg^{-1}. Data are means and standard deviations (n = 3).

(mg kg^{-1}) for shoot growth (dry mass) ranging from 13 to 74, and from 40 to 231 for corresponding EC_{50} values (Table 2). The EC_{20} and EC_{50} values (mg kg^{-1}) for shoot growth (dry mass) determined for NG weathered-and-aged in soil ranged from 12 to 83, and from 23 to 185, respectively (Table 2). Corresponding toxicity benchmarks for plant growth determined on the basis of fresh mass data are shown in Table 2.

To our knowledge, ecotoxicological benchmarks established in the present studies are the first reported for NG effects on plants grown in soil. Results of the present studies indicate that barnyard grass (monocotyledon) was the most sensitive, and alfalfa (dicotyledon) was the least sensitive to NG and related compounds in soil, among the three plant species tested. The phytotoxicity of NG amended SSL soil was not affected by the weathering-and-aging of soil treatments based on both the EC_{20} and EC_{50} values, and their respective 95% CI for either seedling emergence or growth endpoints (Table 2). This result for NG contrasted with findings of our previous studies which demonstrated that the weathering-and-aging of other nitroaromatic energetic compounds in soil significantly increased their respective toxicities, including that of 2,4,6-trinitroluene (TNT), 1,3,5-trinitrobenzene (TNB), and dinitrotoluenes (Rocheleau et al., 2006; 2008). This difference may be explained, in part, by a relatively high biodegradability of NG, which is a nitrate ester, compared to the less easily degraded nitroaromatic compounds, such as TNT (Podlipná et al., 2008). Furthermore, the lower toxicity of NG transformation products, DNG and mononitroglycerin isomers was determined and compared with the effects of the parent material in a study with mustard seedlings exposed in a liquid growth medium (Podlipná et al., 2008). Relevance of the latter studies for plant exposures to NG transformation products in soil will require confirmation in future studies.

3.2. Uptake of nitroglycerin by ryegrass

Uptake studies were performed with ryegrass using soil freshly amended with NG at nominal concentrations of 0, 10, 30, 50, and 75 mg kg^{-1}. These NG concentrations were selected to encompass the shoot growth (dry mass) EC_{20} and EC_{50} values determined for ryegrass in the phytotoxicity studies described here (Table 2). Concentrations of NG in soil decreased as the exposure time increased (Fig. 2). The decrease was consistently greater in treatments having both soil and plants, compared to soil without plant treatments (Fig. 2), while no NG was detected in either shoots or roots (data not shown). This suggests that plants can affect the fate of NG in soil through rhizosphere processes that promote microbial transformation of NG. Using hydroponic exposures, Riefler and Medina (2006) showed that NG accumulated in the leaves and roots of yellow nutsedge and common rush but did not accumulate in yellow foxtail tissues. Total NG accumulated in yellow nutsedge and common rush tissues only accounted for 12% and 5% of the initial NG added in the hydroponic solution, respectively, indicating that substantial transformation of NG occurred either by bacteria in solution or in the plant tissue. An improved understanding of mechanisms controlling the fate of NG in soil, aqueous solution or plant tissue will require additional studies.

Trace amounts of NG (0.6 mg kg^{-1}) were detected in soil amended with nominal NG concentrations 50 and 75 mg kg^{-1} at the end of the 35-d uptake studies (Table 3). NG transformation products, 1,2-DNG and 1,3-DNG, were present in soil and tissue samples in the nominal NG treatments ≥30 mg kg^{-1} (Table 3). Because both dinitroglycerin isomers eluted at similar retention times, and could not be separately quantified by HPLC analysis, they are reported together as DNG. The highest concentration of DNG in soil (14.8 mg kg^{-1}) was found after 14 d in the nominal NG treatment of 75 mg kg^{-1} (Table 3). DNG was detected in soil and shoots of ryegrass exposed to nominal NG concentrations of 50 or 75 mg kg^{-1} for 14 d, establishing the BCF values for DNG of 35 and 40 g wet tissue g^{-1} dry soil for the respective nominal NG treatments (Table 3). DNG was also detected in roots of ryegrass exposed to nominal NG treatments 30, 50, and 75 mg kg^{-1}. The highest amount of DNG (1964 μg g^{-1} tissue) was found in ryegrass roots in nominal NG treatment 75 mg kg^{-1} after 21 d of exposure. The BCF values of DNG in roots ranged from 107 to 261 g wet tissue g^{-1} dry

Table 3
Concentrations of nitroglycerin (NG) or dinitroglycerin (DNG) in soil and ryegrass tissue during the 35-d exposure in Sassafras sandy loam soil.

NG nominal concentration in soil (mg kg^{-1})	Measured concentration of NG in soil (mg kg^{-1})	Measured concentration of DNG in soil (mg kg^{-1})	Measured concentration of DNG in shoots (μg g^{-1})	Measured concentration of DNG in roots (μg g^{-1})	DNG BCF in shoots (g w.w. tissue g d.w. $soil^{-1}$)	DNG BCF in roots (g w.w. tissue g d.w. $soil^{-1}$)
1-d exposure						
10	10.8 ± 8.5	2.2 ± 1.1	NA	NA	NA	NA
30	20.8 ± 0.4	3.5 ± 0.1	NA	NA	NA	NA
50	38.7 ± 0.4	5.4 ± 0.3	NA	NA	NA	NA
75	63.8 ± 1.4	8.2 ± 2.1	NA	NA	NA	NA
14-d exposure						
10	0.8 ± 0.2	ND	ND	ND	ND	ND
30	1.5 ± 0.1	1.1 ± 1.0	ND	295 ± 256	ND	261
50	1.9 ± 0.3	5.5 ± 1.9	194 ± 47	879 ± 73	35	160
75	3.3 ± 0.6	14.8 ± 3.1	596 ± 300	1586 ± 525	40	107
21-d exposure						
10	0.9 ± 0.1	ND	ND	ND	ND	ND
30	1.0 ± 0.3	ND	ND	469 ± 100	ND	ND
50	1.7 ± 0.2	ND	ND	1262 ± 444	ND	ND
75	2.0 ± 0.2	ND	486 ± 104	1964 ± 442	ND	ND
28-d exposure						
10	ND	ND	ND	ND	ND	ND
30	1.3 ± 0.2	0.2 ± 0.3	ND	111 ± 15	ND	685
50	0.8 ± 0.3	0.7 ± 1.3	ND	493 ± 255	ND	677
75	1.3 ± 0.2	ND	69 ± 76	744 ± 100	ND	ND
35-d exposure						
10	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	13 ± 23	ND	ND
50	0.6 ± 0.5	0.5 ± 0.9	ND	170 ± 32	ND	346
75	0.6 ± 0.1	ND	212 ± 368	208 ± 41	ND	ND

DNG: mixture of 1,2-dinitroglycerin and 1,3-dinitroglycerin isomers.
BCF: bioconcentration factor.
NA: not assessed.
ND: not detected; concentration below the limit of quantification of 0.1 mg kg^{-1} for soil and 10 μg g^{-1} for plant tissue.
Measured concentrations are means ± standard deviations (n = 3).

soil after the 14-d exposure, and were 685 and 677 in the 30 and 50 mg kg^{-1} nominal NG treatments, respectively, after the 28-d exposure. The root BCF value of 346 was determined for the 35-d exposure only in nominal NG treatment 50 mg kg^{-1}. DNG translocation factors of 0.22 and 0.38 were determined for ryegrass exposed to NG concentrations of 50 and 75 mg kg^{-1}, respectively, for the 14-d exposures in SSL soil. By the end of the 35-d study, DNG accumulated in ryegrass roots in a concentration-dependent manner with the highest concentration of 208 μg g^{-1} determined in nominal NG treatment 75 mg kg^{-1}, and NG was translocated to ryegrass shoots in the amount of 212 μg g^{-1} in that nominal NG treatment.

Uptake of organic contaminants by plants is affected by both, the properties of the soil (e.g., clay and OM content, pH, water content) and the properties of the compound (e.g., aqueous solubility, partition coefficients values), in addition to physiologically-determined species-specific uptake mechanisms. Sassafras sandy loam used in our studies supports high bioavailability of energetic organic contaminants based on qualitative ranking used in Eco-SSL guidance document (USEPA, 2005). Following uptake into roots, organic contaminants may be translocated to other plant tissues, subsequently transpired, undergo partial or complete degradation, or be transformed to compounds that are more or less toxic than the parent compound. These degradation and transformation products can bind to plant tissues in available or non-available forms (Salt et al., 1998). For example, Podlipná et al. (2008) showed that NG accumulated in flax cell cultures and was transformed to 1,2-DNG and 1,3-DNG. Goel et al. (1997) reported that sweet beet cell extracts metabolized NG to DNG and glycerol mononitrate (GMN). French et al. (1999) and Hannink et al. (2003) also showed that both wild-type and transgenic tobacco seedlings were able to denitrate NG into DNG and GMN. Contrasting with bacteria, plants can fully mineralize very few organic chemicals. Denitrification pathways for biodegradation of NG to glycerol by different bacteria species have been proposed in several studies (Meng et al., 1995; Salt et al., 1998; Marshall and White, 2001). More recently, Husserl et al. (2010) isolated *Arthrobacter sp.* strain JBH1 that can denitrate and mineralize NG to glycerol and CO_2. Studies presented here showed that NG was not accumulated in ryegrass but was transformed in soil and in the root tissue to DNG, which was subsequently translocated into the shoots.

4. Conclusions

Toxicological benchmarks for NG were determined in definitive studies on the basis of concentration–response relationships for growth endpoints of three terrestrial plant species (monocotyledonous barnyard grass, ryegrass, and dicotyledonous alfalfa) exposed in a natural sandy loam soil. The EC_{20} values ranged from 13 to 74 mg kg^{-1} for NG in freshly amended soil and from 12 to 83 mg kg^{-1} for NG weathered-and-aged in soil. Weathering-and-aging of NG in soil did not significantly affect phytotoxicity based on either seedling emergence or growth endpoints. These new data filled the knowledge gap in the understanding of phytotoxicity of NG in compliance with USEPA (2005) requirements for the development of toxicity benchmarks that are acceptable for derivation of an Ecological Soil Screening Level for terrestrial plants, and can be applied in ecological risk assessment at NG-contaminated sites.

Studies of NG uptake by perennial ryegrass revealed that NG was not accumulated in ryegrass tissues but was transformed into DNG in the soil or the roots, or both. The highest BCF of 685 was determined for

DNG in roots. A portion of DNG was subsequently translocated into the shoots, resulting in the highest shoot BCF of 40. DNG translocation factors of 0.22 and 0.38 were determined for ryegrass after 14 d of exposure to NG concentrations of 50 and 75 mg kg^{-1}, respectively. Our results suggest that NG transformation products can accumulate in plants, and consequently pose a potential risk of exposure to DNG for grazers of above-ground and below-ground vegetation. Additional studies using several trophic levels will be required to assess the potential for biomagnification of DNG within the food chain.

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References

American Society for Testing and Materials (ASTM). Standard Guide for Conducting Terrestrial Plant Toxicity Tests. E1963–02; ASTM International, West Conshohocken, PA; 2002.

American Society for Testing and Materials (ASTM). Standard specification for reagent water. ASTM D 1193-99e1, Book of ASTM Standards, Vol. 11.01. Philadelphia, PA, USA: ASTM International, West Conshohocken, PA; 2004. p. 116–8.

Environment Canada. Guidance document on statistical methods for environmental toxicity tests. Environment Canada Report EPS 1/RM/46; 2005.

French CE, Rosser SJ, Davies GJ, Nicklin S, Bruce NC. Biodegradation of explosives by transgenic plants expressing pentaerythritol tetranitrate reductase. Nat Biotechnol 1999;17:491–4.

Goel A, Kumar G, Payne GF, Dube SK. Plant cell biodegradation of a xenobiotic nitrate ester, nitroglycerin. Nat Biotechnol 1997;15:174–7.

Hannink NK, Rosser SJ, French CE, Bruce NC. Uptake and metabolism of TNT and GTN by plants expressing bacterial pentaerythritol tetranitrate reductase. Water Air Soil Pollut 2003;3:251–8.

Husserl J, Spain JC, Hughes JB. Growth of *Arthrobacter sp*. strain JBH1 on nitroglycerin as the sole source of carbon and nitrogen. Appl Environ Microbiol 2010;76:1689–91.

International Organization for Standardization (ISO). Soil Quality — Determination of pH. Geneva, Switzerland: ISO 10390; 1994.

Jenkins TF, Hewitt AD, Grant CL, Thiboutot S, Ampleman G, Walsh ME, et al. Identity and distribution of residues of energetic compounds at army live-fire training ranges. Chemosphere 2006;63:1280–90.

Kuperman RG, Checkai RT, Simini M, Phillips CT, Kolakowski JE, Kurnas CW. Weathering and aging of 2,4,6-trinitrotoluene in soil increases toxicity to potworm *Enchytraeus crypticus*. Environ Toxicol Chem 2005:2509–18.

Marshall SJ, White GF. Complete denitration of nitroglycerin by bacteria isolated from a washwater soakaway. Appl Environ Microbiol 2001;67:2622–6.

Meng M, Sun WQ, Geelhaar LA, Kumar G, Patel AR, Payne GF, et al. Denitration of glycerol trinitrate by resting cells and cell extracts of *Bacillus thuringiensis/cereus* and *Enterobacter agglomerans*. Appl Environ Microbiol 1995;61:2548–53.

Mirecki JE, Porter B, Weiss CA. Environmental transport and fate process descriptors for propellant compounds. U.S. Army Engineer Research and Development Center Report ERDC/EL TR-06-7; 2006. US Army Corps of Engineers, Vicksburg, MS. http://dodreports.com/pdf/ada450743.pdf.

Oh SY, Cha DK, Kim BJ, Chiu PC. Reduction of nitroglycerin with elemental iron: pathway, kinetics, and mechanisms. Environ Sci Technol 2004;38:3723–30.

Pal BC, Ryon MG. Database Assessment of Pollution Control in the Military Explosives and Propellants Production Industry. ORNL-6202, NTIS # DE86007934Oak Ridge, TN: Oak Ridge National Laboratory; 1986.

Pandey RK, Asthana SN, Bhattacharya B, Tiwari I, Ghole VS. Waste explosives and other hazardous materials — hazard potential and remedial measures: an overview. J Environ Sci Eng 2007;49:195–202.

Podlipná R, Fialová Z, Vaněk T. Toxic effect of nitroesters on plant tissue cultures. Plant Cell Tissue Organ Cult 2008;94:305–11.

Riefler RG, Medina VF. Phytotreatment of propellant contamination. Chemosphere 2006;63:1054–9.

Rocheleau S, Kuperman RG, Martel M, Paquet L, Bardai G, Wong S, et al. Phytotoxicity of nitroaromatic energetic compounds freshly amended or weathered and aged in sandy loam soil. Chemosphere 2006;62:545–58.

Rocheleau S, Lachance B, Kuperman RG, Hawari J, Thiboutot S, Ampleman G, et al. Toxicity and uptake of cyclic nitramine explosives in ryegrass *Lolium perenne*. Environ Pollut 2008;156:199–206.

Saad R, Thiboutot S, Ampleman G, Hawari J. Sorptive removal of trinitroglycerin (TNG) from water using nanostructured silica-based materials. J Environ Qual 2010;39:580–6.

Salt DE, Smith RD, Raskin I. Phytoremediation. Ann Rev Plant Biol 1998;49:643–68.

Sarrazin M, Dodard SG, Savard K, Lachance B, Robidoux PY, Kuperman RG, et al. Accumulation of hexahydro-1,3,5-trinitro-1,3,5-triazine by the earthworm *Eisenia andrei* in a sandy loam soil. Environ Toxicol Chem 2009;28:2125–33.

Spanggord RJ, Mill T, Chou TW, Smith JH, Lee S. Environmental fate studies on certain munitions wastewater constituents. Final Report, Phase II — Laboratory Studies. SRI Project Number LSU-7934, (ADA099256). Menlo Park, CA: SRI International; 1980. http://www.stormingmedia.us/65/6529/A652990.html.

Stucki H. Toxicity and degradation of explosives. Chimia 2004;58:409–13.

Sunahara GI, Lotufo GL, Kuperman RG, Hawari J. Ecotoxicology of Explosives. Boca Raton, FL: CRC Press; 2009.

United States Environmental Protection Agency (USEPA). Ecological Effects Test Guidelines, Early Seedling Growth Toxicity Test. EPA 712-C-96-347, OPPTS 850-4230, Washington, DC, USA, Prevention, Pesticides and Toxic Substances (7101). U.S. Environmental Protection Agency; 1996.

United States Environmental Protection Agency (USEPA). Ecological Soil Screening Level Guidance. OSWER 9285.7-55, Washington, DC, USA: U.S. Environmental Protection Agency; 2005.

United States Environmental Protection Agency (USEPA). Method 8330B — Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC) 2006. http://www.epa.gov/osw/hazard/testmethods/pdfs/8330b.pdf.

Verscheuren K. Handbook of Environmental Data on Organic Chemicals. 2nd ed. New York, NY: Van Nostrand Reinhold; 1983. p. 1310.

Walsh MR, Walsh ME, Hewitt AD. Energetic residues from field disposal of gun propellants. J Hazard Mater 2010;173:115–22.