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Toxicity and uptake of cyclic nitramine explosives in ryegrass *Lolium perenne*

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Cyclic nitramine explosives accumulate in perennial ryegrass and exhibit distinct uptake patterns.

Abstract

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (CL-20) are cyclic nitramines used as explosives. Their ecotoxicities have been characterized incompletely and little is known about their accumulation potential in soil organisms. We assessed the toxicity and uptake of these explosives in perennial ryegrass *Lolium perenne* L. exposed in a Sassafras sandy loam (SSL) or in a sandy soil (DRDC, CL-20 only) containing contrasting clay contents (11% and 0.3%, respectively). A 21-d exposure to RDX, HMX or CL-20 in either soil had no adverse effects on ryegrass growth. RDX and HMX were translocated to ryegrass shoots, with bioconcentration factors (BCF) of up to 15 and 11, respectively. In contrast, CL-20 was taken up by the roots (BCF up to 19) with no translocation to the shoots. These studies showed that RDX, HMX, and CL-20 can accumulate in plants and may potentially pose a risk of biomagnification across the food chain.
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Keywords: RDX; HMX; CL-20; Plant uptake; Translocation pattern

1. Introduction

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) are monocyclic nitramines widely used as explosives. The more recently synthesized polycyclic nitramine 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (China Lake 20; CL-20) (Nielsen et al., 1998) is being considered as potential replacement for RDX and HMX because of its superior properties as an explosive and propellant material. While the ecotoxicities of RDX or HMX in soil have been studied in the past at concentrations up to 4000 mg/kg (Robidoux et al., 2003; Vila et al., 2007; Winfield et al., 2004), the potential ecological impacts of an accidental release of CL-20 in the environment remain uncertain.

Most studies of RDX or HMX uptake by plants used hydroponic systems (Best et al., 2001; Bhadra et al., 1999; Cataldo et al., 1990; Checkai and Simini, 1996; Thompson et al., 1999; Yoon et al., 2006) or irrigation water (Price et al., 2002). Few studies investigated the uptake of HMX or RDX from soil (Best et al., 2006; Cataldo et al., 1990; Groom et al., 2002; Harvey et al., 1991; Price et al., 2002; Winfield et al., 2004). Best et al. (2006) exposed ryegrass and alfalfa *Medicago sativa* at 1540 mg/kg, which was the greatest soil concentration studied to date. These authors reported that up to 3886 mg RDX/kg and 50 mg HMX/kg were measured in ryegrass tissue and calculated bioconcentration factors (BCF) ranging from 2 to 58 for RDX, and from 1 to 3 for HMX. Investigations of the effects of plant exposures to greater concentrations of

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explosives are necessary to better assess the ecological risks at explosives-contaminated sites, which were reported to contain RDX concentrations up to 74 000 mg/kg or HMX concentrations up to 5700 mg/kg (Best et al., 2006; Talmage et al., 1999). Information on the potential environmental impacts of CL-20 on plants is more limited. Studies of CL-20 bioaccumulation in plants by Gong et al. (2004) showed that up to 200 mg/kg was measured in ryegrass shoots exposed to 9832 mg/kg in Sassafras sandy loam (SSL) soil. Strigul et al. (2006) measured 12 mg/kg in ryegrass leaves and 16 mg/kg in ryegrass roots exposed to a soil CL-20 concentration of 100 mg/kg. The contrasting results of these studies show that the available data are insufficient to definitively quantify the uptake of CL-20 from soil to plants.

Both the toxicity and bioaccumulation of chemicals in soil can be affected by the bioavailability-modifying soil properties. Clay minerals and soil organic matter (SOM) are generally considered as the two most active soil components in the sorption of aqueous phase organic contaminants (Charles et al., 2006). However, many organic contaminants containing polar functional groups are substantially adsorbed from bulk water by clays.

In the present study, the effects and uptake potential of RDX, HMX, and CL-20 for ryegrass were assessed in SSL to establish benchmark data that are: (1) methodologically consistent with previous studies of ecotoxicity of these explosives in the same soil type (Rocheleau et al., 2006); and (2) better represent plant exposure conditions at contaminated sites. Additional studies were conducted with SSL and Defense Research and Development of Canada (DRDC) soils having similar SOM content (1.2%) but contrasting clay content (11 and 0.3%, respectively) to test the hypothesis that the phytotoxicity and uptake of CL-20 can be affected by the soil clay content.

2. Materials and methods

2.1. Chemicals and reagents

The RDX (99% purity) and HMX (99% purity) were obtained from the Defense Research and Development of Canada – Valcartier (Val Bélair, QC, Canada). Crystalline CL-20 (ε-isomer, 99.3% purity) was obtained from ATK Thiokol Propulsion (Ogden, UT, USA). The CL-20 impurity, 2-acetyl-4,6,8,10,12-pentanitro-2,4,8,10,12-hexaazaisowurtzitane (MAPNIW), was identified at concentrations up to 0.5%. The molecular structures of RDX, HMX, and CL-20 are presented in Fig. 1. All other chemicals were either analytical or certified grade. American Society for Testing and Materials (ASTM) type I water (ASTM, 2004) was obtained using the Super Q water purification system (Millipore®, Nepean, ON, Canada) and was used throughout the studies.

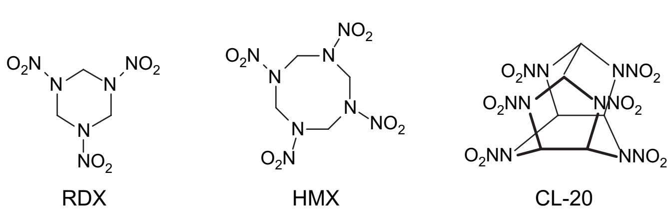

Fig. 1. Chemical structure of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and hexanitrohexaazaisowurtzitane (CL-20).

2.2. Test soils

Sassafras Sandy Loam (fine-loamy, siliceous, mesic Typic Hapludult) (USDA, 1999) was collected from a grassland field on the property of the U.S. Army Aberdeen Proving Ground (Maryland, USA). A sandy soil (DRDC) representative of soil found at some Canadian army sites was collected at DRDC-Valcartier. Vegetation and the upper layer were removed to just below the grass root zone, and the top 15 cm of the A horizon were then collected. These soils were selected because they had similarly low SOM content and low but contrasting clay contents (Table 1), thus were expected to support relatively high bioavailability of explosives (USEPA, 2005). Soil analyses showed that none of the tested explosives were present above analytical detection limits. Total concentrations of metals and nutrients in these soils were within respective regional background ranges (Robidoux et al., 2004). Soil batches were separately amended with RDX, HMX or CL-20 in acetone carrier, as described in previous studies (Rocheleau et al., 2006).

2.3. Plant toxicity and uptake tests

Perennial ryegrass "Express" was purchased from Pickseed Canada Inc. (St-Hyacinthe, QC Canada). Plant toxicity tests were performed according to the range-finding portion of USEPA Method EG-13 (USEPA, 1982) for 21 d with the following modifications. Fifty seeds were sowed in 10-cm wide pots containing 200 g dry soil, and incubated in sealed plastic bags to maintain soil moisture (USEPA, 1989). All seedlings were kept until the end of the tests. Luminosity was maintained at 5000 ± 500 lux during the 16-h daily light exposure. Nominal concentrations for each explosive included 10, 100, 1000, and 10 000 mg/kg. Control treatments included a negative (ASTM type I water), a carrier (acetone), and a positive control (boric acid at concentrations of 0, 50, 80, 110, 150, and 200 mg/kg). Results from control treatments complied with quality control requirements. All treatments were carried out in triplicate. Plant uptake studies were extended to 42 d to obtain greater biomass. Roots were separated from soil using a 2-mm sieve, and the soil was washed away from roots with ASTM type I water. Excess water was absorbed with a paper towel. Shoots and roots were kept at −80 °C until explosive extraction. Dry mass was determined after lyophilizing the plant tissue for 24 h.

2.4. Chemical extractions and analyses

For each soil treatment, aliquots of soil were extracted in triplicate using acetonitrile containing the appropriate recovery standard, according to the

Table 1
Selected physico-chemical characteristics of the test soils

Parameters	SSL[a]	DRDC[b]
PH	5.5	4.9
Water holding capacity (%, v/w)	18	23
Organic matter (%, w/w)	1.2	1.2
Texture	Sandy loam	Sandy
Sand, 50–2000 μm[c]	71	98
Silt, 2–50 μm[c]	18	1.7
Clay, <2 μm[c]	11	0.3
K_d of CL-20 for sorption[d]	2.43	0.84
K_d of CL-20 for desorption[d]	4.43	0.09

[a] SSL: Sassafras sandy loam soil from Aberdeen Proving Ground, MD, USA.
[b] DRDC: Sandy soil from Defense Research and Development Canada, Val Bélair, QC, Canada.
[c] Values are expressed as percent (w/w) and include organic matter contents.
[d] K_d: Dissociation coefficients from Balakrishnan et al. (2004a).

modified USEPA Method 8330A (USEPA, 1998) and as described in Rocheleau et al. (2006). Lyophilized plant tissues were ground using mortars and pestles, and at least 0.02 g was extracted using acetonitrile containing the appropriate recovery standard. Plant extracts were sonicated in the dark at 20 °C for 18 ± 2 h and then centrifuged (Centrific model 225, Fisher Scientific, Pittsburg, PA, USA) at 1500 rpm for one hour. Supernatants were filtered on 0.45 μm cartridges and analyzed using the modified USEPA Method 8330A. Soil and plant extracts were analyzed to quantify concentrations of explosives and their degradation products using an HPLC (Thermo Separation Products, San Jose, CA). The limits of detection were 3, 32, and 34 μg/l for CL-20, RDX, and HMX, respectively. The respective limits of quantification were 0.03, 0.32, and 0.34 mg/kg (dry weight) for soil, and 0.15, 1.6, and 1.7 mg/kg (dry weight) for plant tissue. Degradation products of CL-20 were identified using LC-MS, as described in Balakrishnan et al. (2004b). Bioconcentration factors (BCF) for RDX, HMX or CL-20 were calculated by dividing the ryegrass tissue concentration by the concentration measured in soil after 42 days of exposure. Translocation factors (TF) were calculated as the ratio of chemical concentrations in shoots to those in roots.

2.5. Data analyses

Shapiro–Wilk's tests were used to test for normal distribution and Bartlett's tests were used to test for equality of variance (ToxCalc v.5.0.18. Analysis of Variance (ANOVA) was applied to the ryegrass growth and chemical uptake data. Fisher's least significant difference tests were performed to determine the No Observed Effect Concentration (NOEC) or the No Observed Adverse Effect Concentration (NOAEC) values for shoot and root dry mass when ANOVA indicated a significant treatment effect (SYSTAT 7.01). Student's *t*-tests were used for comparisons of energetic material uptake in shoot and root tissues (Microsoft Excel 2002). A significance level of $p \leq 0.05$ was accepted for all statistical analyses.

3. Results and discussion

3.1. Effects of RDX, HMX or CL-20 on ryegrass growth

Exposures to RDX, HMX or CL-20 had no significant ($p > 0.05$) inhibitory effects on ryegrass growth in the standard 21-d exposures. The NOEC values for shoot or root growth in SSL were 9586 mg/kg for RDX and 9282 mg/kg for HMX (Figs. 2 and 3). These results are similar to those obtained in studies with lettuce and barley exposed to HMX in OECD (Organization for Economic Co-operation and Development) artificial soil or forest soil at concentrations up to and including 3320 mg/kg (Robidoux et al., 2003). Exposure to CL-20 significantly ($p = 0.001$) stimulated shoot growth at 9604 mg/kg SSL (a 38% increase compared with carrier control). Exposure to CL-20 had no effect on the root growth in either SSL or DRDC soil and no effect on the shoot growth in DRDC soil, establishing NOAEC of 9604 mg/kg for SSL and NOEC of 9810 mg/kg for DRDC soil. In a previous study, CL-20 stimulated shoot growth at concentrations up to 9832 mg/kg SSL, but this effect was not concentration-dependent after 19 d of exposure (Gong et al., 2004). Stimulation of ryegrass growth was also observed by Strigul et al. (2006) following the exposures to CL-20 in different types of sandy or sandy loam soil; however, these authors did not report statistical significance of the effects. The present study confirmed that RDX, HMX, and CL-20 have no adverse effects on the ryegrass growth at concentrations up to

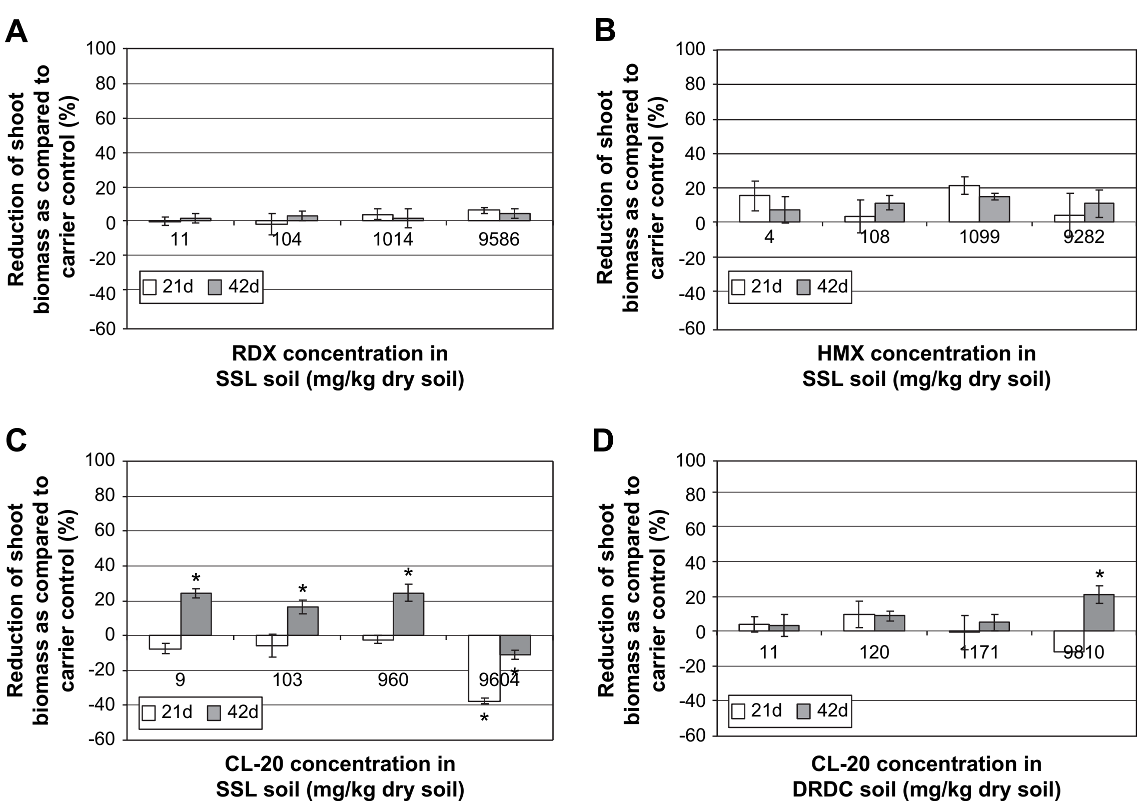

Fig. 2. Effects of RDX (A), HMX (B), and CL-20 in SSL soil (C), and of CL-20 in DRDC soil (D) on ryegrass shoot growth as compared to carrier (acetone) control. Concentrations determined at the initiation (To) of the toxicity tests are based on acetonitrile extraction and HPLC using USEPA Method 8330A. Values are means and standard errors ($n = 3$). Significant ($p \leq 0.05$, Fisher's LSD) change from carrier control is indicated by [*]. Negative values indicate stimulation of shoot growth.

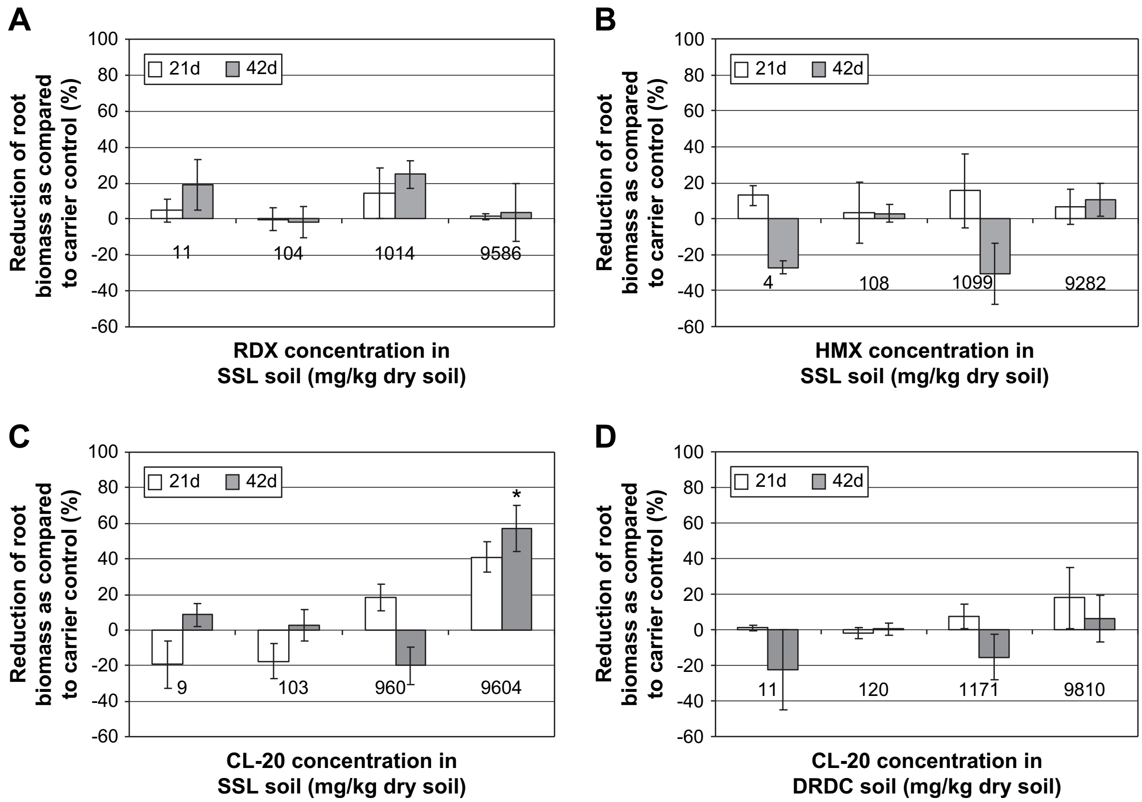

Fig. 3. Effects of RDX (A), HMX (B), and CL-20 in SSL soil (C), and of CL-20 in DRDC soil (D) on ryegrass root growth as compared to carrier control. Concentrations determined at the initiation (To) of the toxicity tests are based on acetonitrile extraction and HPLC using USEPA Method 8330A. Values are means and standard errors ($n = 3$). Significant ($p \leq 0.05$, Fisher's LSD) change from carrier control is indicated by [*]. Negative values indicate stimulation of shoot growth.

approximately 10 000 mg/kg, and that CL-20 can have a stimulatory effect on ryegrass shoot growth at the greatest concentration tested (9604 mg/kg SSL) after the 21-d exposure.

Extending ryegrass exposure to the explosives for up to 42 d revealed statistically significant effects of CL-20 on growth endpoints (Figs. 2 and 3). Inhibition ($p \leq 0.05$) of shoot growth up to 24% was determined at CL-20 concentrations ranging from 9 to 960 mg/kg and an 11% stimulation ($p \leq 0.0001$) of shoot growth was determined at CL-20 concentration of 9604 mg/kg. No concentration-dependent relationship could be determined between the ryegrass shoot growth and the CL-20 concentrations in SSL but this bimodal response may require confirmation in future studies. The ryegrass root growth was inhibited by 58% ($p \leq 0.0001$) at CL-20 concentration of 9604 mg/kg. The only effect of CL-20 in DRDC soil was a 17% inhibition ($p = 0.003$) of shoot growth at 9810 mg/kg after the 42-d exposure. No statistically significant effects of RDX or HMX in SSL were determined for ryegrass growth after the 42-d exposure.

3.2. Uptake of RDX, HMX or CL-20 in ryegrass

Traces of hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), a degradation product of RDX, were found in SSL, and in ryegrass shoots and roots. No degradation products of HMX were detected, indicating that this compound is more resistant to degradation. Traces of mono-nitroso-pentanitro-2,4,8,10,12-hexaazaisowurtzitane (mononitroso-CL-20), a degradation product of CL-20, were found in both SSL and DRDC soils at all tested concentrations and in the roots of ryegrass exposed to 9457 mg/kg SSL (data not shown). The detection of the mononitroso derivatives of RDX and CL-20 indicated that the two nitramines were metabolized in soil and plant tissue. Formation of trace quantities of these transformation products was not sufficient to affect the calculation of the BCF values for either RDX or CL-20.

Bioconcentration of RDX in the shoots was determined for ryegrass exposed to 8.1 and 91 mg/kg SSL establishing the BCF values of 14.6 and 8.9, respectively (Table 2). Similarly, bioconcentration of HMX in the shoots was determined for ryegrass exposed to 3.6 and 100 mg/kg SSL establishing the BCF values of 10.7 and 2.0, respectively. In contrast, the accumulation of CL-20 occurred mainly in the roots, with BCF of 11.9 and 3.1 determined in the 8.6 and 104 mg/kg SSL treatments, respectively, and with BCF of 18.8 and 4.6 determined in the 9.7 and 99 mg/kg DRDC treatments, respectively. The lower BCF values calculated for greater soil treatment concentrations may be an artifact of the traditional BCF calculation procedure. The greatest BCF values of 14.6, 10.7, and 11.9 established for RDX, HMX, and CL-20, respectively, represent the more conservative estimates for use in ecological risk assessment. Results show that RDX and HMX were

Table 2
Soil and tissue concentrations of RDX, HMX, and CL-20 determined in studies with perennial ryegrass after the 42-d exposure in Sassafras sandy loam (SSL) soil or Defense Research and Development Canada (DRDC) sandy soil used for establishing the bioconcentration factors (BCF) for respective energetic compounds

Energetic compound	Soil	Concentration in soil at T_0[a] (mg/kg dry soil)	Concentration in soil at T_f[b] (mg/kg dry soil)	Concentration in shoots (mg/kg dry plant)	Concentration in roots (mg/kg dry plant)	Shoot BCF[c]	Root BCF[c]	Translocation factor (TF)[d]
RDX	SSL	11.1 ± 0.4[e]	8.1 ± 0.1	119 ± 15	44 ± 6	14.6 ± 1.9	5.4 ± 0.8	2.7
RDX	SSL	104 ± 2	91 ± 2	804 ± 199	178 ± 32	8.9 ± 2.2	2.0 ± 0.4	4.5
RDX	SSL	1014 ± 49	1023 ± 37	764 ± 207	382 ± 24	0.7 ± 0.2	0.37 ± 0.02	2.0
RDX	SSL	8867 ± 118	9780 ± 346	1690 ± 327	1383 ± 603	0.17 ± 0.03	0.14 ± 0.06	1.2
HMX	SSL	3.9 ± 0.1	3.6 ± 0.1	39 ± 2	9 ± 2	10.7 ± 0.4	2.4 ± 0.5	4.3
HMX	SSL	107 ± 1	100 ± 2	201 ± 5	159 ± 117	2.0 ± 0.1	1.6 ± 1.2	1.3
HMX	SSL	1099 ± 89	1001 ± 75	206 ± 62	430 ± 274	0.21 ± 0.06	0.4 ± 0.3	0.5
HMX	SSL	9282 ± 513	9976 ± 443	325 ± 75	7878 ± 5864	0.03 ± 0.01	0.8 ± 0.6	0.04
CL-20	SSL	8.9 ± 0.2	8.6 ± 0.2	2 ± 2	102 ± 10	0.2 ± 0.2	11.9 ± 1.1	0.02
CL-20	SSL	103 ± 13	104 ± 3	20 ± 4	327 ± 9	0.19 ± 0.03	3.1 ± 0.1	0.06
CL-20	SSL	960 ± 145	1024 ± 57	24 ± 4	332 ± 65	0.020 ± 0.004	0.32 ± 0.06	0.07
CL-20	SSL	9604 ± 1187	9457 ± 129	90 ± 28	1649 ± 647	0.010 ± 0.003	0.17 ± 0.07	0.06
CL-20	DRDC	11.2 ± 0.7	9.7 ± 0.3	5 ± 3	186 ± 20	0.54 ± 0.45	18.8 ± 2.2	0.03
CL-20	DRDC	120 ± 6	99 ± 3	24.0 ± 0.3	464 ± 9	0.24 ± 0.01	4.6 ± 0.1	0.05
CL-20	DRDC	1171 ± 117	1085 ± 90	38 ± 4	1558 ± 407	0.04 ± 0.01	1.4 ± 0.4	0.02
CL-20	DRDC	9810 ± 1052	10 312 ± 423	95 ± 8	5521 ± 1566	0.009 ± 0.001	0.5 ± 0.1	0.02

All concentrations are based on acetonitrile extraction and HPLC using USEPA Method 8330A (1998).
[a] T_0: Initial concentration.
[b] T_f: Concentration after 42 days.
[c] BCF: Bioconcentration factors were calculated using the T_f (42 d) concentrations.
[d] TF: Translocation factor is the ratio of chemical concentrations in shoots to those in roots.
[e] Average ± standard deviation ($n = 3$).

translocated in the ryegrass from roots to shoots, whereas CL-20 accumulated primarily in the roots with only limited translocation to the shoots. Translocation factors (TF) have been used to characterize chemical uptake with soil water, passage through the plant roots, and subsequent translocation to the above-ground portion of the plant (Lunney et al., 2004; Tu and Ma, 2002). In the present study, the greatest TF values for RDX (4.5) and for HMX (4.3) were established in ryegrass exposed to 91 and 3.6 mg/kg SSL, respectively (Table 2). Groom et al. (2002) reported that most of accumulated HMX was found in the viable leaf tissue and a very small quantity of extractable HMX was detected in the roots. Thompson et al. (1999) found that poplar trees accumulated up to 60% of RDX in leaves. Vila et al. (2007) determined that 89% of RDX was translocated to rice leaves, and more than 80% of RDX was translocated in the leaves of maize, wheat, and soybean. In contrast to RDX or HMX, translocation factors of CL-20 were all below 0.07 (Table 2), indicating that CL-20 was taken up by the roots but barely translocated into the shoots. We hypothesize that the differential uptake of these cyclic nitramines by ryegrass can be related to the differences in their molecular structures, bioavailability, and hydrophobicity. The uptake of RDX or HMX (monocyclic nitramines) in shoots appears to become saturated with respect to concentrations in the roots based on the non-linear relationships shown in Figs. 4A,B, whereas the uptake of CL-20 (polycyclic nitramine) is linear (Figs. 4C,D). Vila et al. (2007) hypothesized that the RDX saturation of translocation in shoots could result from its limited bioavailability in soil resulting from low aqueous solubility of RDX (43 mg/l at 20 °C; Monteil-Rivera et al., 2004). The differences in uptake of explosives could also be related to the differences in octanol/water partitioning coefficients (log K_{ow}), which are 0.9 for RDX, 0.2 for HMX, and 1.9 for CL-20 (Burken and Schnoor, 1998; Monteil-Rivera et al., 2004). Briggs et al. (1982) determined an optimum lipophilicity for maximum translocation to shoots at log $K_{ow} = 1.8$. The differential accumulation patterns determined in our studies for RDX and HMX (log $K_{ow} < 1.8$), and for CL-20 (log $K_{ow} > 1.8$) are consistent with this optimum lipophilicity value. The transpiration stream concentration factor, which accounts for the mass of the chemical accumulated in the shoots and for a volume of water transpired (Briggs et al., 1982; Yoon et al., 2005), could also contribute to the differential uptake of monocyclic and polycyclic nitramines. Additional studies would be required to resolve the mechanisms responsible for different uptake patterns of cyclic nitramines in plants.

Concentration of CL-20 was significantly greater in the roots of ryegrass exposed in DRDC soil compared with concentration in the roots from SSL (Table 2). These results suggest that CL-20 bioavailability is greater in DRDC soil compared with that in SSL. The greater CL-20 bioavailability in DRDC soil is consistent with the lower sorption of CL-20 in this soil ($K_d = 0.84$) compared with SSL ($K_d = 2.43$), and can be related to the lower clay content (0.3% in DRDC vs. 11% in SSL soil, Table 1). Although Balakrishnan et al. (2004a) determined low sorption of CL-20 to clay minerals and a direct non-linear relationship between sorption of

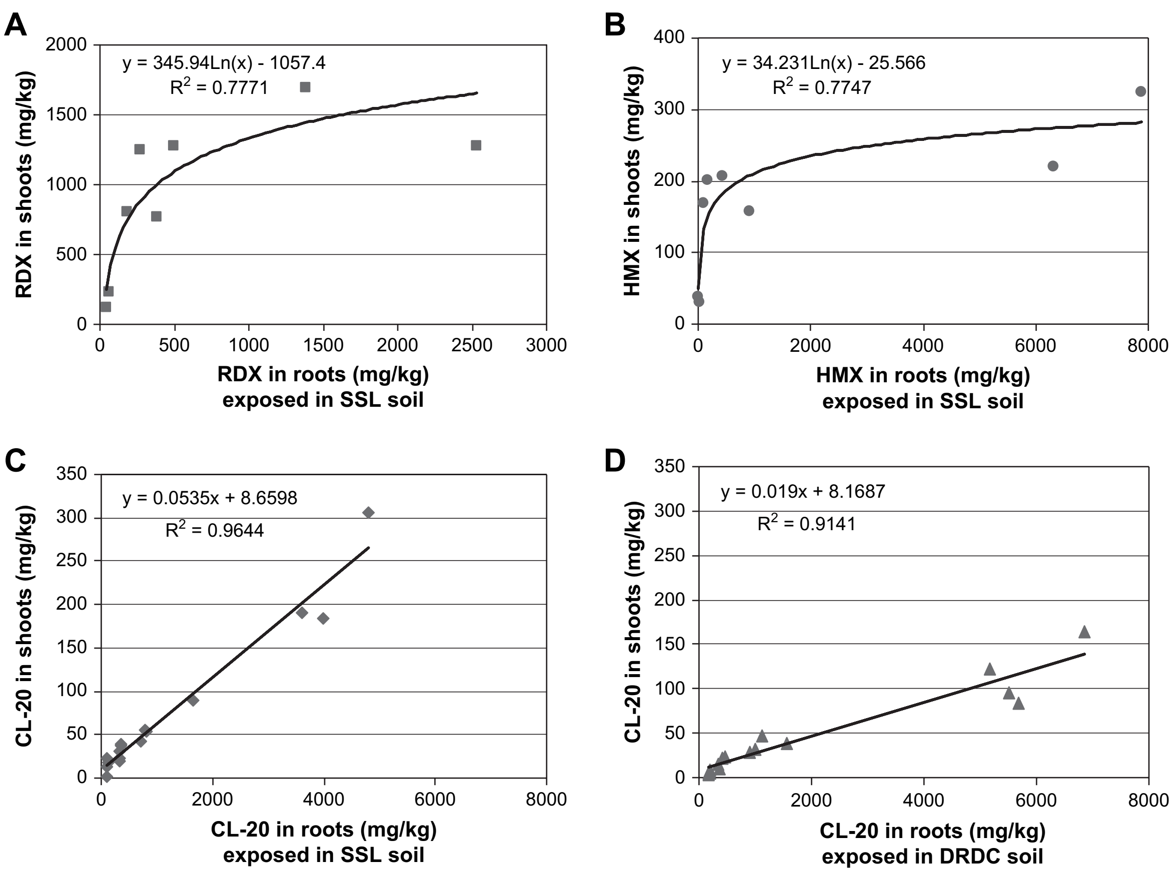

Fig. 4. Uptake in shoots versus uptake in roots of ryegrass exposed to RDX in SSL soil after 21 and 42 d (A), HMX in SSL soil after 21 and 42 d (B), CL-20 in SSL soil after 21, 28, 35, and 42 d (C) or CL-20 in DRDC soil after 21, 28, 35, and 42 d (D). Concentrations are based on acetonitrile extraction and HPLC using USEPA Method 8330A.

CL-20 and soil organic carbon (OC) content, application of these findings to the results of our studies is limited because those authors did not consider sorption in soils with similar OC content but contrasting clay contents. Monteil-Rivera et al. (2003) showed that clay plays a significant role in the sorption of HMX. Greater than 90% of the CL-20 sorption was controlled by mineral surfaces and not by OC in studies with sediments by Szecsody et al. (2004). Similar preferential adsorption by clays compared with that by OC was demonstrated for several organic nitro-compounds (Boyd et al., 2001; Charles et al., 2006; Johnston et al., 2002). Leggett (1985) suggested formation of hydrogen bonds between surface hydroxyl groups in soil and nitro groups of nitramines as a possible sorption mechanism for RDX and HMX. These nitro groups can contribute to the formation of electron donor (clay)-acceptor complexes and affect nitramine bioavailability in soil as was demonstrated for nitroaromatic compounds (Weissmahr et al., 1997).

4. Conclusions

The present study showed that although RDX, HMX, and CL-20 are generally not phytotoxic, they can accumulate in ryegrass and consequently pose a low risk of exposure to RDX and HMX for grazers of above-ground vegetation, and to CL-20 for grazers of below-ground vegetation. A study with prairie voles fed with leaves of corn and alfalfa containing ^{14}C-RDX mixed with commercial rabbit feed showed retention of nearly 20% of the radioactivity in the tissues of the voles, which suggested that such herbivorous animals can accumulate RDX or RDX-radio-labeled fragments and allow the movement of RDX through trophic levels (Fellows et al., 2006). Additional studies using several trophic levels (plants−invertebrates−mammals−birds) would be required to assess the potential for biomagnification of RDX, HMX, and CL-20 across the food chain.

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