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Accumulation of HMX (Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) in Indigenous and Agricultural Plants Grown in HMX-Contaminated Anti-Tank Firing-Range Soil

CARL A. GROOM,[†] ANNAMARIA HALASZ,[†] LOUISE PAQUET,[†] NEIL MORRIS,[‡] LUCIE OLIVIER,[§] CHARLES DUBOIS,[∥] AND JALAL HAWARI*,[†]

Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montreal, Quebec, Canada H4P 2R2; Beak International Incorporated, 14 Abacus Road, Brampton, Ontario, Canada L6T 5B7; Environment Canada, Quebec Region, Protection Directorate, 105 rue McGill, Montreal, Quebec, Canada H2Y 2E7; and Defense Research Establishment Valcartier, 2459 Pie-XI Boulevard North, Val Bélair, Quebec, Canada G3J 1X5

To investigate their potential for phytoremediation, selected agricultural and indigenous terrestrial plants were examined for their capacity to accumulate and degrade the explosive octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). Plant tissue and soil extracts were analyzed for the presence of HMX and possible degradative metabolites using high-performance liquid chromatography with diode-array UV detection (HPLC−UV), micellar electrokinetic chromatography with diode-array UV detection (MEKC−UV), and HPLC with electrospray ionization mass spectrometry (LC−MS). The pattern of HMX accumulation for alfalfa (*Medicago sativa*), bush bean (*Phaseolus vulgaris*), canola (*Brassica rapa*), wheat (*Triticum aestivum*), and perennial ryegrass (*Lolium perenne*) grown in a controlled environment on contaminated soil from an anti-tank firing range was similar to that observed for plants (wild bergamot (*Monarda fistulosa*), western wheat grass (*Agropyron smithii*), brome grass (*Bromus sitchensis*), koeleria (*Koeleria gracilis*), goldenrod (*Solidago* sp.), blueberry (*Vaccinium* sp.), anemone (*Anemone* sp.), common thistle (*Circium vulgare*), wax-berry (*Symphoricarpos albus*), western sage (*Artemisia gnaphalodes*), and Drummond's milk vetch (*Astragalus drummondii*)) collected from the range. No direct evidence of plant-mediated HMX (bio)chemical transformation was provided by the available analytical methods. Traces of mononitroso-HMX were found in contaminated soil extracts and were also observed in leaf extracts. The dominant mechanism for HMX translocation and accumulation in foliar tissue was concluded to be aqueous transpirational flux and evaporation. The accumulation of HMX in the leaves of most of the selected species to levels significantly above soil concentration is relevant to the assessment of both phytoremediation potential and environmental risks.

* Corresponding author phone: (514) 496-6267; fax: (514) 496-6265; e-mail: jalal.hawari@nrc.ca.
[†] Biotechnology Research Institute.
[‡] Beak International Incorporated.
[§] Environment Canada.
[∥] Defense Research Establishment Valcartier.

Introduction

The application of plant species to remediate contaminated ecosystems (phytoremediation) is a developing technology with potential for the treatment of contaminated soil environments and shallow aquifers (*1*−*3*). The estimated low expense of phytoremediation in comparison to that of conventional soil treatments is its most attractive feature (*4*, *5*), but the method is also less disruptive to soil organic compositions and associated fauna. Terrestrial plants are natural accumulators of limited nutrients (carbon dioxide, nitrate, and trace metals) from dilute environments, and this predisposes them for the uptake and concentration of anthropogenic chemical pollutants in a process referred to as phytoextraction (*2*). The contaminant may be irreversibly held in the plant root tissue (rhizofiltration) (*6*) or transported from subsurface soil and released through leaf tissue to the atmosphere (phytovolatilization) (*7*). Although plants have long been recognized for the synthesis of a wide variety of complex organic compounds, there is little evidence that terrestrial plants are capable of directly mineralizing contaminants to CO_2 and H_2O. In an indirect process (enhanced rhizosphere biodegradation) (*8*), plants are known to secrete nutrients and exudates that modify the surrounding microbial population, which then biodegrades the targeted contaminant.

The widespread acceptance of phytoremediation remains elusive, and further development and experimentation are desirable. Methods are described for the removal of radioactive waste (*9*), heavy metals (*10*), excess agricultural runoff (*11*), petroleum hydrocarbons (PHCs) (*12*), polynuclear aromatic hydrocarbons (PAHs) (*13*), trichloroethylene (TCE) (*14*), 2,4,6-trinitrotoluene (TNT) (*15*−*18*), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (*19*, *20*), with an ongoing development of protocols for the application and assessment of this relatively new technology (*21*, *22*). The method is currently being tested for the removal of residual explosives at a Canadian anti-tank firing range in central Alberta (WATC CFB Wainwright). The site has been identified as possessing elevated soil concentrations of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX; Figure 1) (*23*), an EPA-listed contaminant of concern (*24*) that poses credible ecotoxicological risks to soil invertebrate, bird, and mammal populations on the range. For example, the reported lowest observable adverse effects level (LOAEL) for mice (oral dosage) is 75 mg/kg/day (*25*). The weapon most frequently employed on the range (66-mm M72 LAW) is charged with the melt-cast explosive Octol, a nominal 70:30 wt % mix of HMX and TNT with small quantities of RDX present as a production contaminant (*23*). The range is still used extensively by Canadian and European NATO personnel, and phytoremediation is an attractive option for cleanup as it is inexpensive and it interferes little with firing schedules, or the need to clear unexploded ordnance. The dry prairie climate (plant hardiness zone 2a) may, however, have an adverse effect on plant survival and phytoremediation performance. As the dominant mechanism for contaminant uptake appears to be aqueous solubilization and transpirational flux, phytoremediation experiments involving the explosives 2,4,6-trinitrotoluene (TNT) and RDX often make

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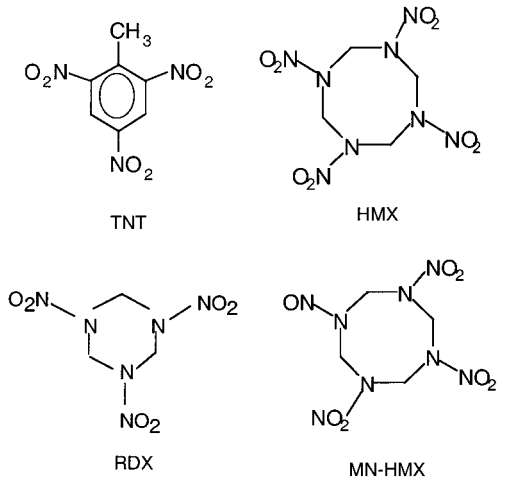

FIGURE 1. Molecular structures of 2,4,6-trinitrotoluene (TNT), 1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 1,3,5-trinitro-1,3,5-triazine (RDX), and 1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazocine (mononitroso-HMX, MN-HMX).

use of constructed wetlands populated by aquatic plants (*26*, *27*, *16*), or arboreal species with high water demand (*28*, *20*). Furthermore, whereas TNT and other aromatic explosives are enzymatically modified and sequestered in root tissues (*15*, *29*), RDX appears to be less readily transformed and is deposited in leaf tissues following the evaporative loss of water (*16*). Cyclic nitramine explosives such as RDX and HMX are reported to degrade in anaerobic bacterial liquid cultures, in aerobic fungal cultures, and in soil slurry environments (*30*, *31*), but almost no literature is available describing the transformation of HMX in terrestrial plants. This work reports on the uptake of HMX by commercial agricultural plant species cultivated in a controlled environment using HMX-contaminated firing-range soil, and also on the accumulation of HMX in indigenous plants collected from the firing range. The site history, and the physicochemical and biochemical data for the identified contaminants, were used to account for the persistence and fate of HMX in the natural setting.

Experimental Section

Materials. Commercial-grade RDX and HMX (with a purity > 99%) were provided by Defense Research Establishment Valcartier, Quebec, Canada. Methanol and acetonitrile were obtained from Fisher Scientific (Montreal, PQ) as HPLC grade. All other chemicals were obtained from Fisher as reagent grade.

Preparation of Soil Samples. Contaminated soil samples were obtained from the Wainwright firing range in Alberta, Canada. The firing-range soil is a sandy loam approximately 60% sand and 20% silt by weight. It has a low percentage of organic carbon (2%), limited ion exchange capacity (18 meq/100 g), and contains elevated levels of copper (790−1000 ppm), lead (85−96 ppm), zinc (100−120 ppm), and barium (100−120 ppm) that originate from the metal castings of exploded shells at the site. The soil pH is slightly basic (7.8) due to the high metal and potash content (*23*). Soil stocks for controlled environment studies (250 L) were extensively mixed in a cement mixer prior to shipment to provide a more homogeneous distribution of HMX. All samples were shipped frozen in polyethylene bags and stored at −20 °C. Soil samples were prepared and extracted using a modified EPA 8330 method as specified for the ppb-analysis of certain explosives in soil and sediment matrixes (*32*). Samples (400 g) were spread in a Pyrex dish, mixed with acetone, and then air-dried for 24 to 48 h and sieved (32 mesh) before extraction. Samples (16 g, 4 times the quantity specified in ref *32*) of soil were sonicated at 10 °C (Neptune jacketed sonication bath, Blackstone Ultrasonics, Jamestown, NY) with 40 mL of acetonitrile in 60-mL bottles for at least 6 h. For all samples a 5-mL extract volume was combined with 5 mL of aqueous $CaCl_2$ (5 g/L) and filtered (0.45-μm Millex) prior to analysis. Surface soil adjacent to indigenous plants selected on the firing range was collected and field tested colorimetrically for the presence of HMX and RDX according to the field protocol previously described by Jenkins et al (*33*).

Preparation of Plant Samples. *Plant Culture Conditions.* Agricultural plants were grown in HMX-contaminated Wainwright firing-range soil for 77 days as described in Morris et al (*34*). Three weeks prior to start-up, seeds of alfalfa (*Medicago sativa*), bush bean (*Phaseolus vulgaris*), canola (*Brassica rapa*), wheat (*Triticum aestivum*), and perennial ryegrass (*Lolium perenne*) were sown in soil-less potting medium seedling trays. Seeds were planted 3 per cell with the exception of bush beans which were planted 2 per cell owing to their relatively large size. All cells were monitored daily and watered on an as-needed basis. The majority of seeds had germinated after 3 weeks.

Contaminated soil (2,500 g of soil per pot) was added to 3.5-L pots 15 cm in diameter and 20 cm in depth. The pots were sealed (i.e., no drainage holes) to avoid the leaching of analyte during experiments. Pots with control and contaminated soils were then planted at a density of 3 plants per pot for bush bean and 9 plants per pot for the 4 other species. All of the 65 pots (including 25 controls) were arranged according to a randomized block design to reduce environmental bias. Containers with clean and contaminated soil without plants were also analyzed. The greenhouse was thermally regulated between 25 °C and 15 °C. On average, the maximum daily temperature was 25 °C and average daily minimum was 17.8 °C. Relative humidity ranged between 48 and 71%, averaging 59.4%. A photoperiod of 16 h (alternating with a dark period of 8 h) was artificially maintained with fluorescent lighting. Watering was completed as required in 125-mL increments to maintain a suitable level of soil moisture (less than saturation but greater than the wilting point). Soil moisture adequacy was indicated by the observation of plant vigor and using randomly placed moisture probes. The presence of disease and insect pests was monitored every 3 days, and, in general, these phenomena had no effect on plant performance.

Sample Collection. Plant tissue samples were collected to represent aerial and root tissues by cutting all plant stems at the soil surface. Roots were collected by fragmenting the soil into a sieve and extracting all visible material. Upon collection, all tissue samples were rinsed with deionized water, air-dried, weighed, bagged, and stored at −20 °C. Subsamples of plant tissue were collected for the determination of dry weight fractions. These samples were weighed fresh, and then oven dried at 65 ° C for 72 h.

Indigenous Plants Collected from the Firing Range. Samples of wild bergamot (*Monarda fistulosa*), western wheat grass (*Agropyron smithii*), brome grass (*Bromus sitchensis*), koeleria (*Koeleria gracilis*), goldenrod (*Solidago* sp.), blueberry (*Vaccinium* sp.), anemone (*Anemone* sp.), common thistle (*Circium vulgare*), wax-berry (*Symphoricarpus albus*), western sage (*Artemisia gnaphalodes*), and Drummond's milk vetch (*Astragalus drummondii*), identified as dominant species on the firing range in midsummer, were collected by cutting the plants at the stem crown, with their immediate storage in polypropylene bags on dry ice. The presence of unexploded ordnance on the range prevented the acquisition of root samples. Samples were subsequently stored at −20 °C. The plants were separated into stem, leaf, and flower tissues and rinsed with distilled water prior to extraction.

Extraction of HMX from Plant Tissues. The tissue extracts from all plant species were prepared as outlined by Larson et al (*35*). Briefly, finely cut samples of the plant (ap-

proximately 4 g) were suspended in 10−20 mL of ice-cold deionized water (18 MΩcm resistivity) and homogenized using a Kinematica (Kriens Switzerland) homogenizer fitted with a Brinkman Polytron PTA 20 S saw tooth generator (Brinkman Instruments, Mississauga, ON) suitable for fibrous plant or animal tissues. The homogenized samples were then lyophilized using a Virtis Freezmobile 24 (Virtis, Gardner, NY) lyophilizer until no further change in flask weight was observed (average time 20 h). Approximately 0.2 g of freeze-dried material was then sonicated for 18 h at 10 °C (Blackstone Ultrasonics, Jamestown, NY) in 15-mL Kimax screw-cap culture tubes containing 10 mL of acetonitrile. After sonication, the Kimax tubes were centrifuged at 5,000 rpm for 15 min (Fisher Centrific centrifuge, Fisher Scientific, Montreal, PQ). The supernatant was then decanted and allowed to settle for 20 min. A 2-mL aliquot of the supernatant was mixed with an equal volume of deionized water and filtered using Millex HV 0.45-μm filter cartridges for further analysis as described below.

HPLC−UV Analysis. A Waters chromatographic system comprising a model 600 pump, a model 717 plus injector, a model 996 photodiode-array detector, and a temperature control module was used for HPLC−UV analysis. The column was a Supelcosil LC-CN (25 cm, 4.6 mm; 5 μm particle size) with the column temperature held at 35 °C. The solvent system consisted of a methanol/water gradient at a flow rate of 1.5 mL/min. The initial solvent composition was 30% methanol and 70% water, which was held for 8 min. A linear gradient was then run from 30% to 65% methanol over 12 min. The solvent ratio was then returned to initial conditions over 5 min, and then maintained for 5 min, for a total run time of 30 min. The detector was set to scan from 200 to 325 nm with extraction of chromatograms at 254 and 230 nm. The injection volume was 50 μL. In general, this method has proven to be superior to that of EPA 8330 (C18 column with an isocratic 50% water/methanol mobile phase) for the analysis of HMX. The limits of detection and quantification for this method were 0.05 and 0.1 ppm, respectively.

MEKC−UV Analysis. Separations to detect HMX, RDX, or their metabolites in plant extracts were performed using a Hewlett-Packard (HP) 3D CE instrument interfaced with an HP Vectra personal computer running HP Chemstation software. The HP 3D CE was fitted with a HP G-1600-31232 fused silica bubble capillary with a total length of 64.5 cm, and an effective length (inlet to detection window) of 56 cm. The voltage was set at 30 kV, and the temperature was set at 25 °C. Samples were injected by applying 50 mbar pressure to the capillary inlet for 5 s. The separation buffer was composed of 2.5 mM sodium tetraborate and 12.5 mM boric acid (pH 8.5) containing 50 mM sodium dodecyl sulfate. Absorbances were monitored at wavelengths of 214, 230, and 280 nm. Unless otherwise indicated, the separation time was 8 min with post-conditioning flushes of the capillary after each run in the following sequence: methanol (0.5 min), 0.1 M NaOH (0.5 min), and running buffer (3 min). The total analysis time was therefore 12 min. The limits of detection and quantification for this method were 0.2 and 1.0 ppm, respectively.

LC/MS Analysis. The concentration of HMX and other analytes was analyzed by LC/MS with a Micromass Platform benchtop single quadropole mass detector fronted by a Hewlett-Packard 1100 series HPLC system equipped with a photodiode array detector. Samples (50 μL) were injected into a Supelcosil LC-CN column (25 cm × 4.6 mm; 5 μm particle size) thermostated at 35 °C. The solvent system consisted of a methanol/water gradient at a flow rate of 1 mL/min. A first linear gradient was run from 10% to 20% methanol over 15 min, followed by a second linear gradient from 20% to 60% over 5 min which was held for 3 min. This solvent ratio was returned to the initial conditions over 2 min and held for an extra 10 min. Analyte ionization was done in a negative electrospray ionization (ES (−)) mode producing mainly $[M - H]^-$ mass ions. The electrospray probe tip potential was set at 3.5 kV with a skimmer voltage of 30 V and an ion source temperature of 150 °C. The mass range was scanned from 40 to 400 Da with a cycle time of 1.6 s, and the resolution was set to 1 Da (width at half-height). The detection limit for HMX was determined to be 50 ppb.

TABLE 1. HMX Concentrations (mg/kg dry weight) in Wainwright Firing-Range Soil Indicating a Heterogenous Distribution of Soil HMX

sample identification	HMX (mg/kg)	RSD[c] (%)
DND 1[a]	28.8	29
DND 2	50.7	31
DND 3	32.3	24
AT 1[b]	25.3	1.7
AT 2	31.8	3.3

[a] DND, samples mixed and analyzed using method EPA 8330. Aliquots of 4 g of soil sonicated for 16 h in 10 mL of acetonitrile. [b] AT, samples with four-fold increases in extraction amount to reduce HMX variation. Aliquots of 16 g of soil sonicated for 16 h in 60 mL of acetonitrile. [c] RSD, relative standard deviation = standard deviation/average (n = 3).

Results and Discussion.

HMX Persistence in Firing-Range Soil. The average HMX concentrations found in the soil samples are listed in Table 1, indicating that the spatial distribution of HMX varied greatly because of soil heterogeneity and the granular nature of the explosive formulation used on the site. Jenkins et al (*33*) observed that the variation in HMX measurements caused by spatial soil heterogeneity exceeded analytical error by a factor of 10 or more. The HMX concentrations in soil samples using 16 g of soil for extraction in place of 4 g (see Experimental Section) are also included in Table 1, and the diminution of the heterogeneic variation is evident in the reported relative standard deviations. At the time of collection, RDX and TNT were not detected in the contaminated firing-range soil used for the cultivation of agricultural plants, but small amounts of mononitroso-HMX were detected in addition to HMX. The nitroso derivative is a reduced form of HMX that can form as a result of the high metal content in soil, microbial degradation, or even photodegradation (*30*).

Previous measurements of firing-range soil (*23*) indicated that the concentration of HMX exceeded that of RDX and TNT at all times. In a related ecotoxicological study (*25*), low concentrations of RDX were identified in the range surface-soil samples located directly in front of the targets, whereas no RDX was detected in subsurface soils. A survey conducted 3 years before (*23*) indicated contamination of RDX and TNT in the low ppm range at the same sampling locations with elevated concentrations (30−80 ppm) of HMX. At no time did the concentration of TNT approach the percentage found in the original Octol formulation (i.e., 30% TNT, 1% RDX by weight) and significant disappearances of TNT and RDX were indicated along with the persistence of HMX.

The recalcitrance of HMX in surface soil and apparent losses of RDX and TNT can be attributed to their adsorption/desorption properties and (bio)chemical reactivity in the relatively dry soil condition. Table 2 provides physical and chemical data for TNT, RDX, and HMX. Losses through volatilization from soil or groundwater to the atmosphere are negligible, as HMX, RDX, and TNT all possess low vapor pressures and moderately low Henry's law constants (Table 2). The low water solubility of HMX (6.6 mg/L) would appear to limit leaching to groundwater, however the low K_p ($K_p = [HMX_{sediment}]/[HMX_{water}]$) and K_{oc} ($K_{oc} = K_p/$[fraction organic carbon]) values observed in sediments (Table 2) indicate that

TABLE 2. Physical and Chemical Constants for TNT, RDX, and HMX (References Provided in Parentheses)

compound	TNT	RDX	HMX
vapor pressure (mmHg)	1.99×10^{-4} (*36*)	4.0×10^{-9} (*37*)	3.3×10^{-14} (*37*)
Henry's law constants (atm-m^3/mol)	4.57×10^{-7} (*36*)	1.2×10^{-5} (*37*)	2.6×10^{-15} (*37*)
solubility in H_2O (mg/L, 20 °C)	130 (*36*)	38 (*38*)	6.6 (*39*)
partition coefficients			
Log K_{ow}[a]	1.84 (*36*)	0.86 (*38*)	0.13, 0.06 (*38*)
Log K_p[b]	4−53 (*40*, *41*, *42*)	0.83−4.13 (*43*, *44*)	< 8.7 (*38*)
Log K_{oc}[c]	3.2 (*40*)	0.8−4.2 (*38*)	2.8 (*38*)

[a] Octanol water partition coefficient. K_{ow} = concentration in octanol (mol/L)/concentration in water (mol/L). [b] Sediment partition coefficient. K_p = concentration in sediment (mg/kg)/concentration in water (mg/L). [c] Organic carbon partition coefficient. $K_{oc} = K_p$/fraction organic carbon in sediment.

solubilized HMX can be readily transported through subsurface soil. Soil columns packed with contaminated Wainwright surface soil yield distilled water leachate that is mildly basic (pH 7.8) and contains 1.14 ± 0.05 ppm HMX. Similarly, the solubility in water (38 mg/L), and low Log K_{oc} (0.8) reported for RDX indicate little adsorption to soil particles and a high degree of mobility. The moderate water solubility of TNT (130 mg/L) and low Log K_p (4−53) and Log K_{oc} (3.2) values also favor the movement of dissolved TNT in groundwater with little sorption to the soil matrix. However, TNT readily (bio)transforms under both aerobic and anaerobic conditions (*45*) to give products such as hydroxylamine and amine derivatives that can irreversibly bind to organic soil fractions.

During periods of drought at Wainwright, water flows toward the soil surface where the explosive contaminants may be subject to photodegradation. TNT was observed to photolyze in pure water with a half-life of 0.5 days, and at the soil surface photolysis may be the primary abiotic transformation process for this compound (*24*). Spanggord (*38*) reported photolytic half-lives of 0.85 and 1.4 days for RDX and HMX respectively in pure water, while the attenuation of light in lagoon water by UV-absorbing species increased the half-life for HMX to 70 days (*40*). The direct photolysis of HMX in soil was not observed ($t_{1/2} = 39$ yr, (*46*)) and this result would agree with the high concentrations of HMX observed in firing-range surface soils.

HMX Accumulation in Agricultural Plant Trials. Five agricultural plant species (alfalfa (*Medicago sativa*), bush bean (*Phaseolus vulgaris*), canola (*Brassica rapa*), wheat (*Triticum aestivum*), and perennial ryegrass (*Lolium perenne*)) were cultivated in firing-range soil (32 ppm HMX, Table 1) in a greenhouse under controlled meteorological conditions, and were subsequently analyzed for explosives content in their various tissues. The species were selected on the basis of commercial availability and sufficient hardiness for their cultivation at the site. Other attributes considered were rapid growth, root penetration, cover potential, and known capacity for contaminant uptake if available (*16*, *19*, *28*). Figure 2 compares the biomass obtained for all species grown on HMX-contaminated and control firing-range soils. All plant species appeared to be in good health, and no significant differences in biomass production were associated with the presence of HMX in soil. Figure 3a shows the concentration of HMX (mg/kg dry weight) in the roots and in the viable and nonviable aerial (leaf and stem) tissue for each species. Very low levels of extractable HMX were detected in root samples; but the concentration of HMX in scenescent leaf samples was greatly elevated, as high as 677 mg/kg in the case of canola (*Brassica rapa*, Figure 3a). No explosive contaminants or their derivatives were detected in control plant or soil samples.

Comparisons on a dry weight basis are somewhat misleading in this case as the senescent tissue represents a small proportion of the total plant mass. Figure 3b represents

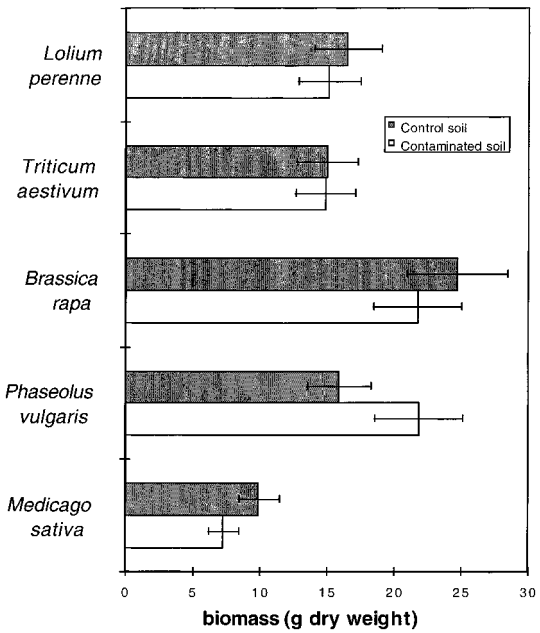

FIGURE 2. Biomasses obtained for agricultural species grown on HMX-contaminated (white bars) and noncontaminated control (black bars) firing-range soils.

the amount of HMX present in fresh plant samples adjusted for the proportional masses of each tissue as collected. The values, therefore, indicate the total mass of HMX available in each tissue in harvesting one kg of each species. The majority of accumulated HMX is found in the viable leaf tissue, and this result is consistent with that reported for RDX in bush bean (*19*) and other plant species (*20*).

Accumulations of HMX in Indigenous Plants. While locations were being identified for field experiments, a selection of dominant plant species was analyzed for explosive content to determine the extent to which indigenous phytoextraction may be occurring. At this time munition clearances were not available for the safe collection of root samples. Prairie grasses (western wheatgrass (*Agropyron smithii*), Brome grass (*Bromus sitchensis*), and koeleria (*Koeleria gracilis*)) dominated 70% of the surface area on the range, and their presence was assisted by periodic brush fires that tend to defoliate competing species during summer training exercises. Other species selected for analysis included wild bergamot (*Monarda fistulosa*), lowbush blueberry (*Vaccinium* sp.), anemone (*Anemone* sp.), common thistle (*Circium vulgare*), wax-berry (*Symphoricarpos albus*), western sage (*Artemisia gnaphalodes*), Drummond's milk vetch (*Astragalus drummondii*), and goldenrod (*Solidago* sp). With the exception of goldenrod, all species were observed to contain extractable HMX, and no TNT or RDX. The concentrations of HMX in individual tissues (i.e., leaves, stems,

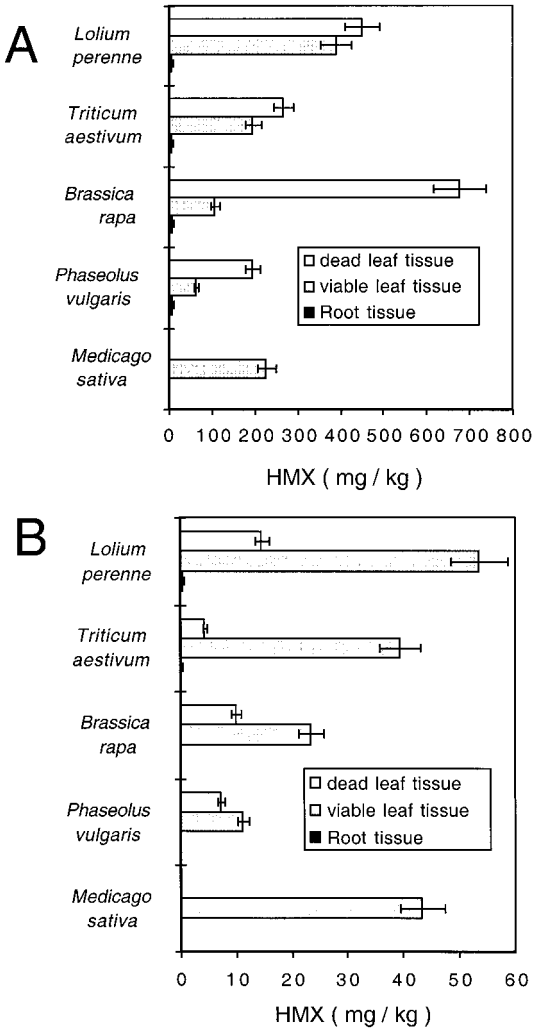

FIGURE 3. (A) HMX concentrations in senescent leaf (white bars), viable leaf (grey bars), and root (black bars) tissues (mg/kg desiccated sample mass) for agricultural plants grown on firing-range soil. (B) HMX amounts (mg/kg) adjusted for the proportions of tissue observed in fresh samples.

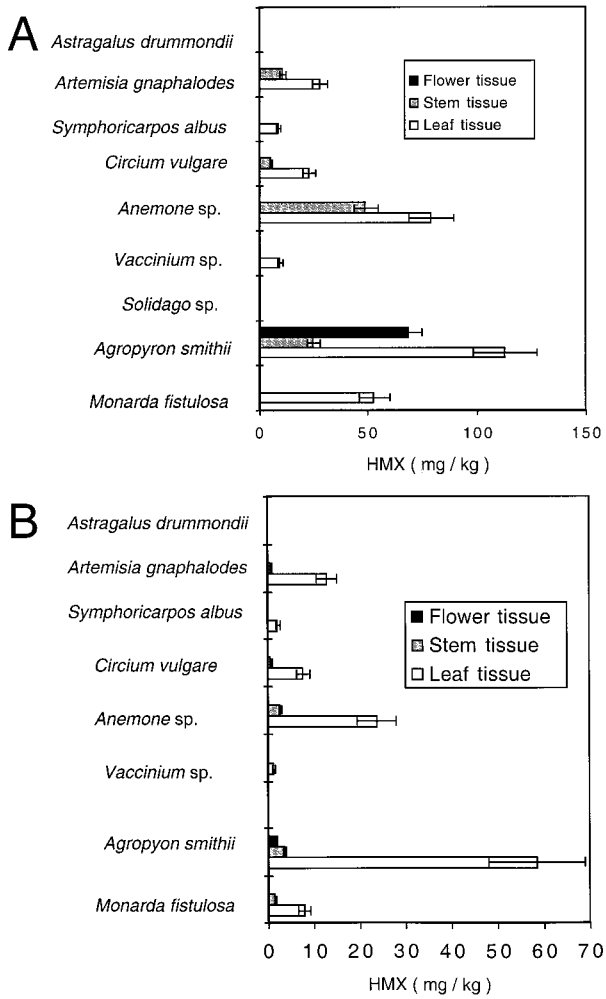

FIGURE 4. (A) HMX concentrations in leaf (white bars), stem (grey bars), and flower (black bars) tissues (mg/kg desiccated sample mass) for samples of indigenous plants collected from the firing range. (B) HMX amounts (mg/kg) adjusted for the proportions of tissue observed in fresh samples.

or flowers if present) are presented in Figure 4a. Figure 4b represents the amount of HMX present in fresh plant samples adjusted for the proportional masses of each tissue as collected. HMX is present in the leaves of all plant species with the exceptions of goldenrod and milk vetch where only trace quantities were detected in the leaves and no analyte was detected in other tissues. The results also indicate that significant quantities of HMX (20−50 mg/kg, Figure 4b) are accumulated in plants at the firing range and retained over the summer months, primarily in the grasses, as they are the most populous species at the range.

Fate of HMX in Plant Tissue. The biomass, moisture content, and composite HMX concentrations for all species are shown in Table 3. It cannot be concluded that the agricultural plant species are intrinsically more capable of accumulating HMX than the indigenous plant species, as the growth conditions in the greenhouse study (constant soil humidity and temperature, no eolian effect) were more conducive to plant growth than those encountered at the firing range. The moisture content of the controlled environment species (75%) was consistently greater than that of the field species (60%), and the availability of water appears to affect the accumulation of HMX. In a similar manner, RDX accumulation was observed to be dependent on moisture content and water demand for various plant species (*20*).

The analytical methods employed in this study failed to detect any HMX transformants in leaf tissue, with the exception of mononitroso-HMX that was also observed in soil samples. HMX in the extracts from plant tissues was identified by its LC/MS (ES-) and by comparison with a reference analytical standard. Both the extracted analyte and the standard showed a chromatographic peak at a retention time of 25 min with a characteristic deprotonated mass ion $[M - H]^-$ at 295 Da.

In preliminary work, lyophilized ryegrass samples extracted with supercritical CO_2 were found to contain only HMX, and no other transformation products were found as identified by the available analytical methods. It is interesting to note that while HMX can be degraded abiotically in basic aqueous solution (*48*), the acidic pH encountered in leaf tissue (pH 5) due to the phytosynthetic adsorption of carbon dioxide favors its stability. A recent review (*30*) of RDX and HMX degradation reported that chemical or biological reactions directly associated with the parent ring structure result in rapid ring cleavage to create low-molecular-weight products including formaldehyde (HCHO) and nitrous oxide (N_2O). Any hydrolytic or oxidative chemical or biochemical activity in leaf tissue would produce similar gaseous products which are better monitored in closed environments. In a study involving the uptake of ^{14}C-labeled RDX (*49*) into terrestrial plants no radioactive gaseous products (CO_2, HCHO) were detected. However, gel permeation chroma-

TABLE 3. Biomass (kg), Moisture Content (wt %), and HMX Concentration (mg/kg) for Composite Samples of Agricultural and Indigenous Plants Grown on HMX-Contaminated Soil

species	total individual plants	total biomass (kg)[a]	moisture content (%)	HMX in plant (mg/kg)[a]	RSD HMX (%)[b]
		agicultural (greenhouse) species			
Medicago sativa	72	0.052	78	289.3	9.8
Phaseolus vulgaris	24	0.126	77	123.3	8.6
Brasica rapa	72	0.175	78	223.5	11
Lolium perenne	72	0.125	75	459.7	8.9
Triticum aestivum	72	0.119	70	295.1	13
		indigenous species			
Monarda fistulosa	10	0.085	61	20	12
Agropyon smithii	>100	0.073	21	76	13
Bromus sitehensis					
Koeleria gracilis					
Solidago sp.	5	0.185	64	ND	-
Vaccinium sp.	5	0.088	61	9	10
Anemone sp.	5	0.076	35	80	20
Circium vulgare	5	0.100	72	50	12
Symphoricarpos albus	10	0.182	43	9	14.
Artemisia gnaphalodes	5	0.042	61	30	9.0
Astragalus drummondii	10	0.177	64	Trace	-

[a] All values are averages reported as mg/kg dry weight. [b] RSD = sample standard deviation/average ($n = 3$).

TABLE 4. Experimentally Determined Values and Transpiration Stream Concentration Factor (TSCF) Based Estimates for HMX Uptake in Agricultural Plants Grown in Controlled Environment[a]

species	final amount HMX in soil (mg/pot)[b]	final amount HMX in plant (mg/pot)	% partitioned in plant biomass	total aqueous volume (L/pot)	predicted HMX in plant (mg/pot)[c]
Medicago sativa	100.9	1.915	1.9	6.75	7.70
Phaseolus vulgaris	85.0	1.807	2.2	9.25	10.5
Brasica rapa	90.5	4.69	5.2	9.875	11.3
Lolium perenne	88.7	7.199	8.1	9.625	9.29
Triticum aestivum	88.9	4.383	4.9	9.75	11.1

[a] Reported values are averages ($n = 3$) as mg or kg dry weight. [b] Total soil HMX based on 2.6 kg soil per container and soil surface area per container of 0.0177 m^2. [c] Predicted total HMX = bulk H_2O concentration (mg/L) × TSCF × total aqueous volume (L). Bulk H_2O concentration = 1.14 mg/L. log TSCF = 0.784 exp $[-(\log K_{ow} - 1.78)^2/2.44] = 1.0$ (*50*).

tography fractions with molecular weights of 800−1000 Da were observed to contain the ^{14}C radioactive label. It was assumed that sequestered transformants of RDX in tissue were present with higher molecular weights than the parent molecule (222 Da) but no further analyses of the individual fractions were performed to identify the radioactive compounds. It is unclear whether the labeled products in the studies of Larson et al. (*49*) were direct conjugates of intact RDX species, or the high-molecular-weight products resulting from the incorporation of ring cleavage fragments or low-molecular-weight products into plant organic constituents.

Plant HMX Uptake Mechanisms. As no evidence of rapid HMX degradation was observed, the low retention of HMX in root or stem tissues and accumulation in the leaves for both greenhouse and field samples suggested that transpirational flux was the dominant mechanism for the transfer of HMX from soil to leaf tissue. Another possible mechanism for the transport of HMX is the deposition of HMX dust directly onto the exposed plant surfaces, but this possibility was ruled out by the washing of all samples prior to extraction. Also, the lack of wind and high humidity in the greenhouse reduced the availability of airborne particulate; as indicated by the absence of contamination in plant and soil analyses for control pots that were randomly placed among the contaminated soil pots. The large differences in HMX concentration observed for living and nonliving leaf tissue of equivalent surface area in contaminated soil pots also suggested that aerial deposition was not a significant path for HMX translocation in this case.

The octanol−water coefficient is often used to correlate the accumulation of toxin in animal and plant species (*24*). For plant species in general, compounds with log K_{ow} values less than 0.5 do not passively enter the root endodermis and require active uptake for adsorption. Compounds with high K_{ow} values are expected to absorb to extracellular wall components and remain in root tissue. Moderately hydrophobic compounds (log K_{ow} values between 0.5 and 3.0) move passively through the endodermis and are transported to other portions of the plant with little accumulation in root tissue (*50*). The low observed concentrations of HMX in the roots for all agronomic species, combined with the significant accumulation in leaf tissue indicated the passive transport of HMX through the roots. Correlations based on log K_{ow} relationships are used to estimate root concentration factors (RCF) and transpirational stream concentration factors (TSCF) which approximate the uptake of organic contaminants in root and vascular tissues. The reported log K_{ow} values for HMX (0.13 and 0.06) (*38*) result in RCF values (*50*) (0.86 and 0.85, respectively) which predict limited exclusion of HMX at the root surface and no accumulation in root tissue. The calculated TSCF values are slightly greater than 1.0 (1.19 and 1.30) and indicate the unrestricted movement of HMX within the transpiration stream. (By definition, the TSCF cannot exceed unity, as no aqueous solute is assumed to migrate faster than water in the transpiration stream.) A comparison of predicted and measured total HMX uptake (Table 4) revealed uptake estimates for ryegrass (9.29 mg) close to experimentally observed values (7.2 mg, RSD 8.9%). Using the midpoint (30 days) and final (77 days) experimentally observed average daily uptake rates of HMX for ryegrass (0.09 mg HMX/day) an initial estimate for the complete transfer of HMX from soil to ryegrass leaf tissue

under controlled environment is 1,022 consecutive days or approximately 3 years. Projections for time to clean up in the field must account for obtainable planting densities, transpiration rates, and the relatively short (130 day) growing season at the firing range. Field trials currently in progress will assist in these assessments.

In conclusion, HMX was observed to accumulate in the leaf tissue of agricultural plants cultivated on HMX-contaminated soil taken from the anti-tank firing range at CFB Wainwright Alberta. The pattern of its accumulation in plants collected from the range matched that of the agricultural plant species, with little evidence for the rapid degradation of HMX in leaf tissue. The persistence of HMX in the firing-range soil is most likely due to its low chemical reactivity and poor aqueous solubility which limits its aqueous transport in the dry soil environment. Wheat and ryegrass demonstrated rapid growth in the presence of HMX, and similar grasses are known to dominate much of the surface area of the firing range. The capacity to survive and accumulate significant quantities of HMX identified these agricultural plant species as leading candidates for future field trials and applications.

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