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Toxicity of Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) to Soil Microbes

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Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine, commonly known as HMX (High Melting Explosive), is a non-aromatic heterocyclic nitramine and one of the most powerful and commonly used conventional explosive compounds. Several studies have shown that HMX exerts neither acute nor chronic adverse effects on freshwater invertebrates, fish, and algae (Bentley et al. 1977, 1984). Up to its limit of aqueous solubility HMX showed no toxicity to *Vibrio fischeri* (Microtox®) and green alga *Selenastrum capricornutum* (Sunahara et al. 1998), V79 Chinese hamster lung cells and TK6 human lymphoblastic cells (Lachance et al. 1999). No mutagenicity to *Salmonella typhimurium* (Ames test) was observed with or without a rat liver metabolic activation system S9 (Lachance et al. 1999, Tan et al. 1992, Whong et al. 1980). However, Sullivan et al. (1979) questioned the statistical analysis of Bentley et al. (1977) and concluded after reanalysis that as low as 10 mg/l of HMX significantly increased cell growth in three algae species and the chlorophyll A content in one species. Its subchronic toxicity to mammals (rats and mice administered with HMX in diet) was reported with the lowest observable adverse effect level being 75-270 mg/kg/d (Everett et al. 1985, Everett and Maddock 1985).

A large data gap exists in the terrestrial ecotoxicology for HMX. Although HMX toxicity to soil invertebrates such as earthworms (*Eisenia foetida* or *E. andrei*), enchytraeid and collembola is relatively well-documented (Philips et al. 1993, Robidoux et al. 2001, Schäfer and Achazi 1999), no studies have been found so far that tested its toxicity to avian species, terrestrial plants and soil microorganisms (Talmage et al. 1999). In the present study, we examined the ecotoxicological effects of HMX on indigenous soil microbial processes or activities.

MATERIALS AND METHODS

Two uncontaminated garden soils (GS1 and GS3) were used. The GS1 soil was a sandy loam with a pH of 6.9 and total organic carbon of 11.2%, whereas the GS3 soil was a silty clay loam with a pH of 6.5 and total

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organic carbon of 3.5%. Immediately after collection in June 1999, both soils were sieved (2 mm) and frozen at –20°C. Before use, the soils were thawed overnight at 4°C and pre-incubated for 5-8 d at 25 ± 2°C.

HMX of technical grade (purity ≥ 99%) was obtained from the Defence Research Establishment Valcartier, Val-Bélair, PQ, Canada. HMX (powder) was added directly into the GS1 and GS3 soils without using any delivery solvent to achieve nominal concentrations of 0, 10, 50, 250, 1250, 6250 and 12500 mg/kg soil, followed by 5-min blending with an electric household mixer. Approximately one kg of soil (moist weight) was used for each treatment. Soils were sampled for chemical and microbiological analyses 4- and 12-wk after spiking. In a preliminary experiment, the dosage was set as 0, 10, 100, 1000 and 10000 mg/kg soil and the exposure time was one week. Each treatment was replicated four times. All soils were incubated at 25 ± 2°C in the dark. Soil moistures were adjusted every 2 weeks to maintain their original levels (24.3% for GS1 and 28.6% for GS3).

Soil dehydrogenase activity, potential nitrification activity, heterotrophic nitrogen fixation activity, substrate-induced respiration and basal respiration, all of which are microbiologically-mediated activities or processes frequently used for assessing the health of soil microbial communities, were analyzed for each soil sample. The methods for these microbial assays were described earlier (Gong et al. 1999, 2001). An acetonitrile sonication-HPLC analysis method was used to extract and determine HMX and its metabolites (if any) in soil (U.S. EPA 1997). All data were expressed on an oven-dried weight basis of soil and subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

As shown in Fig. 1, the recovery of spiked HMX varied tremendously at the three lowest levels, i.e., 10, 50, and 250 mg/kg. At higher levels, however, the variability in recovered HMX was low. We believe that this was largely due to the direct amendment of powder HMX, which was done purposely to avoid the introduction of any organic solvent that might kill some soil microbial species and meanwhile serve as a substrate to some others. Presumably, it is extremely difficult to achieve homogeneity when spiking a tiny amount of chemical (a few mg) into a relatively large quantity of soil (a few kg). During the 12-wk incubation, no metabolites were detected. Data from the preliminary experiment were similar to those shown in Fig. 1.

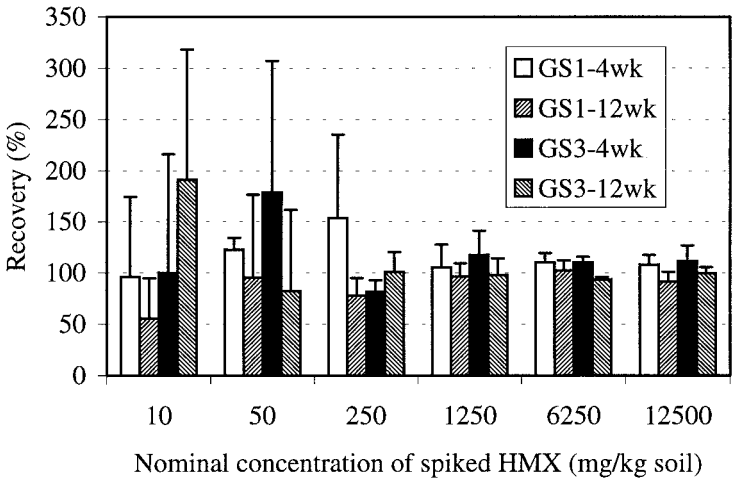

Figure 1. Recovery of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) 4 and 12 weeks after being spiked into the GS1 and GS3 soils. Data are expressed as mean (column) + standard deviation (error bar) (n = 4).

TNT (2,4,6-trinitrotoluene) was used as a positive control and was spiked into the GS3 soil at 0, 30, 60, 125, 500, 1000 and 2000 mg/kg soil. The soil was sampled only once, i.e., one week after spiking. Results show that TNT inhibited all the microbial indicators except for basal respiration in a dose-response manner, which has been reported elsewhere (Gong et al. 2001). Our earlier studies also indicated that TNT adversely affected DHA, NFA and PNA in both laboratory spiked and field contaminated soils (Gong et al. 1999). On the contrary, HMX did not show significant effects (ANOVA, $p > 0.05$) on any of the five microbial indicators in both GS1 and GS3 soils at all three sampling times (i.e., 1-, 4- and 12-wk). Up to 12500 mg/kg soil HMX caused less than 10% inhibition or 5% stimulation of microbial activities (Fig. 2a&b), which is both statistically and biologically insignificant.

HMX is structurally similar to RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and they are expected to exhibit similar biochemical reactivities. However, HMX is less water soluble (6.6 vs. 38.4 mg/l at 20°C) (Talmage et al. 1999) and more chemically stable than RDX (Akhavan 1998). Therefore, under the aerobic conditions in our studies, HMX hardly influenced soil microorganisms whereas RDX only showed slightly significant effects (Gong et al. 2001).

Soil contamination with HMX is widespread (Hawari 2000), especially in military-related sites. However, the environmental fate and impact of HMX

are not well understood. Information obtained from the present study can be important and useful for ecological risk assessment of explosives (HMX)-contaminated sites. Our results indicate that HMX is recalcitrant to biodegradation by indigenous soil microorganisms, most likely due to the absence of co-substrates. It is through a co-metabolic process that HMX can be removed from soil by native soil bacteria and fungi under aerobic conditions (Axtell et al. 2000, Boopathy 2001, Hawari 2000). On the other hand, our results also suggest that HMX at the levels in this study, does not constitute a threat to soil microflora.

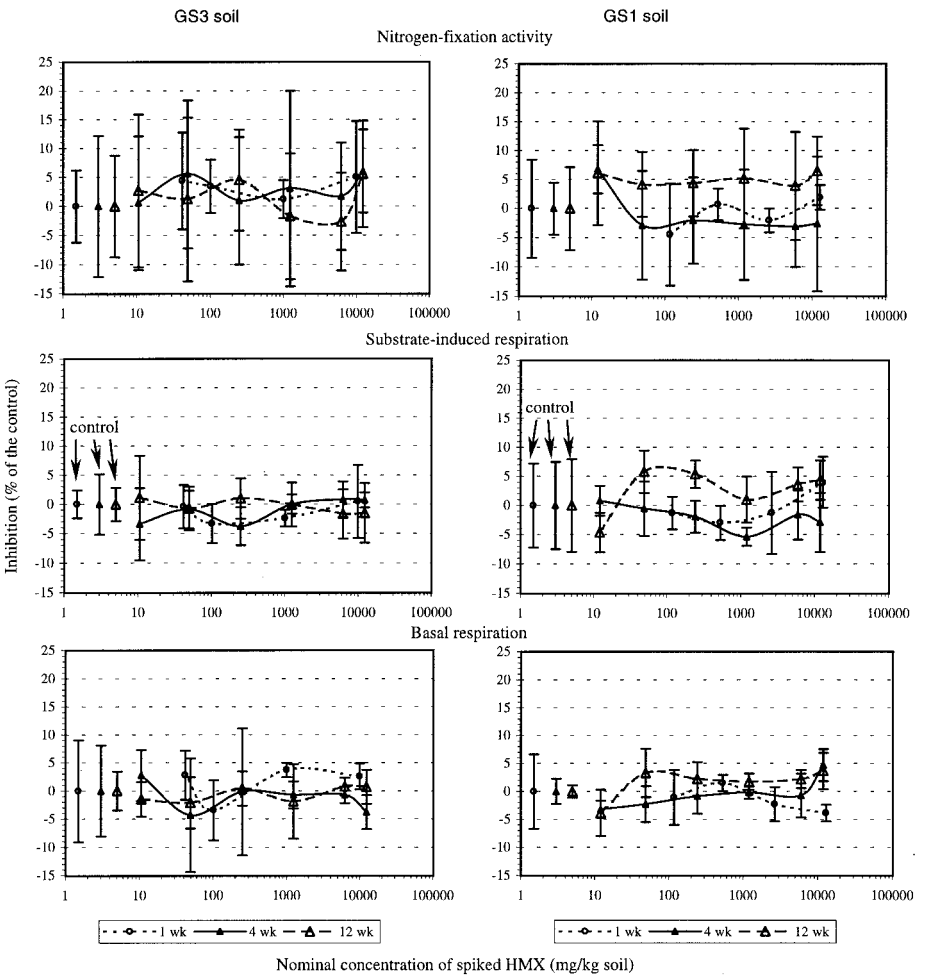

Figure 2a. Effects of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) on soil microbial activities in two garden soils. Data are given in mean (points) ± standard deviation (error bars) (n = 4).

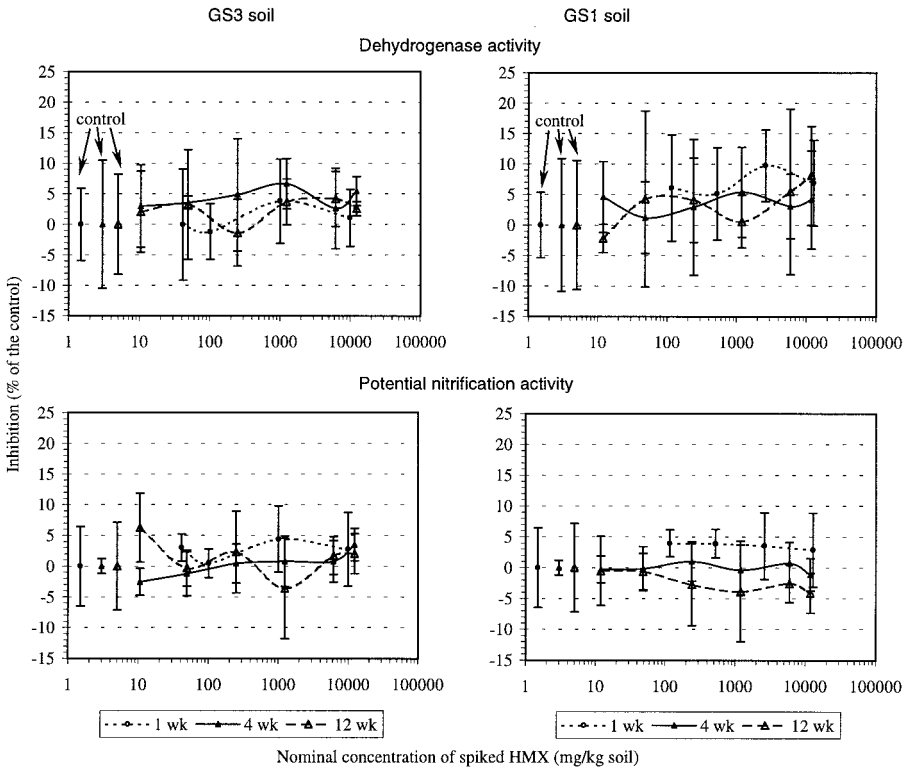

Figure 2b. Effects of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) on soil microbial activities in two garden soils. Data are given in mean (points) ± standard deviation (error bars) (n = 4).

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