DEVELOPMENT OF A SHORT-TERM (<12 DAYS), PLANT-BASED SCREENING METHOD TO ASSESS THE BIOAVAILABILITY, BIOCONCENTRATION, AND PHYTOTOXICITY OF HEXAHYDRO-1,3,5- TRINITRO-1,3,5-TRIAZINE (RDX) TO TERRESTRIAL PLANTS

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EXECUTIVE SUMMARY

Limited amounts of information have been published on the environmental impacts of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to terrestrial plant communities. RDX is one of the two high-explosive compounds used by the U.S. military (Davis 1998) and classified as a priority pollutant by the U.S. Environmental Protection Agency (USEPA). Millions of acres of land on military installations, as well as manufacturing, storage, and disposal sites, have been contaminated with RDX (Jenkins 1989). Therefore, environmental risk assessments (ERAs) are conducted to determine the potential environmental impacts of RDX on receptors. Research on the environmental impacts of RDX on terrestrial plants is needed to facilitate filling data gaps and decrease the level of uncertainty and costs associated with ERAs on RDX.

Studies were designed and conducted to evaluate the responses of 15 terrestrial plans to short-term (< 12 days) and long-term (2, 4, and 6 weeks) exposures to military grade RDX. Emphasis was placed on cover plants (nontraditional crop plants) because they are more likely to occur in the remote areas near military installations with RDX-contaminated soils. The target plants included species recommended for use in assessing toxic compounds by the USEPA and the Organization for Economic and Cooperative Development (Fletcher and others 1988). Additionally, the target plants naturally occur in the regions around the military installations, are important economically, and are a source of food for wildlife or livestock. The objectives of the short-term (< 12 days) studies were to: (1) identify RDX sensitive plants for use in developing a plant-based, short-term, screening method for assessing RDX bioavailability; and (2) provide information useful for filling data gaps on the environmental impacts of RDX in terrestrial plants. The objectives of the long-term (2, 4, and 6 weeks) studies were to develop RDX uptake coefficients and evaluate the efficacy of experiments in the short-term study for estimating plant responses to long-term RDX exposures. A toxicological approach evaluating a spectrum of plant responses to RDX exposure was used during both studies.

During the short-term studies, 10 cover plants (sanfroin, sunflower, bush grass, Delar small Burnett, red clover, white clover, tall fescue grass, Kentucky blue grass, orchard grass, and rye grass) and 5 crop plants (cucumber, lettuce, rape, corn, and wheat) were exposed to Grenada, Memphis, and Bowdre soils amended with environmentally relevant concentrations of RDX (0 to 4000 ppm). The assessment parameters were percent emergence, root and shoot lengths, and adverse developmental effects. The experiments were concluded 2 days after 50 percent of the control seedlings had emerged, typically < 12 days.

Statistically significant differences were measured in root length, shoot length, or percent emergence in over 50 percent of the plants (sunflower, sanfroin, Delar small Burnett, rape, lettuce, corn, tall fescue, and orchard grass). However, there were no consistent patterns. The most consistent indicators of detrimental impacts following RDX exposures were the observed adverse developmental effects, regardless of soil type. The observed adverse developmental effects included: underdeveloped roots, fused or bifurcated leaves, atypical bilateral symmetry, atypical pigmentation, atypical seedling emergence, curled or irregular leaf margins, necrotic lesions, chlorosis, and yellow spots. Sunflower (a forb), sanfroin (a legume), and corn (a cereal grass) were identified as RDX-sensitive plants. Cover and dicot plants were generally more RDX sensitive than monocot and crop plants. Overall, sunflower was the most RDX sensitive of the test plants and was selected for use during the long-term studies.

Inherent plant characteristics, seed size, and root type were more important than taxonomic status in the development of adverse developmental effects during the short-term studies. The seeds of sunflower, sanfroin, and corn (47.8, 22.3, and 300.5 mg per seed, respectively) were larger than those of the other test plants (0.5 to 6.0 mg per seed for cover plants and 1.4 to 37.3 mg per seed for crop plants). Sunflower, sanfroin, and corn seeds had to imbibe larger volumes of the soil solution containing soluble (bioavailable) RDX in order to germinate as compared to the other test plants. The nitro groups of the RDX molecule are electron deficient (Freeman and others 1976) whereas the cellular biomolecules, i.e., nucleic acids and enzymes, are electron dense. Electron-deficient molecules can participate in electophillic substitution reactions with electron-dense

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Citation for proceedings: Holland, Marjorie M.; Warren, Melvin L.; Stanturf, John A., eds. 2002. Proceedings of a conference on sustainability of wetlands and water resources: how well can riverine wetlands continue to support society into the 21st century? Gen. Tech. Rep. SRS-50. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 191 p.

molecules (Gregus and Klaassen 1996). Sunflower, sanfroin, and corn developed branched roots within 8 days following initial RDX exposure, which increased the root surface available for absorption of bioavailable RDX. These factors enhanced the possibilities for interaction between RDX and cellular biomolecules.

The efficacy of the short-term screening experiment for estimating plant responses to long-term RDX exposure was evaluated during the long-term (2, 4, and 6 weeks) studies. The effects of life stage (embryos and 2-week-old seedlings) and exposure duration were also evaluated. The adverse developmental effects observed following short-term RDX exposures were generally less severe when the plants were exposed to RDX-amended Grenada soil. Therefore, Grenada soil was used during the long-term studies to develop RDX uptake coefficients that would be conservative. The RDX concentrations (0, 5.8, 50, and 100 ppm) used were low to enhance the chance for plant survival, yet predetermined to cause adverse developmental effects. The assessment parameters included: adverse developmental effects, growth effects (above- and belowground biomass, maximum shoot and root lengths, maximum stem diameter, number of leaves, root biovolume), and bioconcentrated RDX in leaves and flowers.

The efficacy of the short-term screening experiments for estimating plant responses to long-term RDX exposure was validated. The adverse developmental effects observed during the short-term and long-term exposures were similar, regardless of life stage or exposure duration, and included: underdeveloped roots, fused or bifurcated leaves, atypical bilateral symmetry, curled or irregular leaf margins, necrotic lesions, chlorosis, and yellow spots. Additional adverse developmental effects observed following long-term RDX exposure were wrinkled leaf surfaces and poor seedling development. Statistically significant differences were measured in several of the growth parameters (biomass, number of leaves, shoot length, root length, and root biovolume), but there were no consistent patterns.

Statistically significant differences were measured in the bioconcentrated RDX content of leaves using both life stages during all exposure durations. The amount of flowers produced was not sufficient to provide data for statistical analysis. The highest coefficient of determination (R-square) was calculated using data for embryos following the 6- week exposure (0.80) and following the 4-week exposure using the 2-week-old seedling (0.73).

The short-term screening experiments can be used for site characterizations, risk assessments, and to evaluate remediation activities. Additionally, they are cost effective, simple, and require significantly less time than more traditional approaches. The growth parameters commonly used to evaluate herbicides, inorganic, or organic contaminants did not adequately assess the potential for adverse environmental impacts from RDX exposure. The toxicological approach used during these studies was more appropriate.

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