Environmental aspects of RIGHTRAC TDP green munitions

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ENVIRONMENTAL ASPECTS OF RIGHTRAC TDP- GREEN MUNITIONS

Annual Report
NRC # ????

Submitted to

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Canada

Environmental Aspects of RIGHTTRAC
TDP- Green munitions

Annual Report

Submitted to: **Dr. Sylvie Brochu**
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2459 Blvd Pie-XI Nord, Quebec, Quebec G3J 1X5

October 2013

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ABSTRACT

The Defense Research and Development Canada (DRDC, Valcartier, QC) is developing new green explosive and propellant formulations, as part of a sustainable training strategy for the Canadian Army. The present research responds to the needs of DRDC by providing necessary physicochemical, chemical, and ecotoxicological data to help understand the environmental transport, fate and impact of new formulations developed within the RIGHTTRAC (Revolutionary Insensitive, Green and Healthier Training Technology with Reduced Adverse Contamination) project. The present study summarizes the dissolution, transport, transformation, and ecotoxicity of three propellant formulations, SP 7993, SP Unique, and CMR170, and their soluble components, NG, DPA, ATEC, MC, and EC. In addition, it gathers ecotoxicity data for an explosive formulation, GIM, which has been aged for periods varying from 6 to 24 months.

Amongst the three propellant formulations tested, the single base formulation SP 7993 was found to be the most stable in terms of dissolution, even more stable than the formulation New Green M1 identified as the most stable of previously studied formulations. If scattered on soil surface and subjected to precipitations SP 7993 will give rise to low leakage of ATEC and the latter will not persist in soil.

When comparing the two double base formulations, CRM170 appeared to be more stable than SP Unique. Although the MC/graphite coating present in CMR170 might have been responsible for the higher stability this could not be ascertained due to the concomitant higher NC content of CMR170, which also decreased its ability to dissolve. If released in the environment CMR170 will induce small leakage of NG and MC, whereas SP Unique will induce a substantial leakage of NG along with a small leakage of DPA. NG which was found to be relatively stable and highly mobile in Valcartier soil will likely occur in soil in a bioavailable form.

Based on the present ecotoxicological assessment of the SP 7993, SP Unique, and CMR170 formulations, the SP 7993 formulation was the least toxic formulation, and the SP Unique formulation was toxic to the aquatic and terrestrial organisms tested,

whereas the CMR170 formulation was toxic to the terrestrial organisms only. The toxicity of the SP Unique and CMR170 formulations can be attributed to the presence of nitroglycerin in both formulations.

The ecotoxicity of GIM (Green Insensitive Munitions) after weathering and aging (W-A) was compared with data previously obtained using fresh GIM. GIM-amended soil weathered and aged for 6 months showed a time-dependent decrease in TNT content. However, toxicity tests conducted using earthworms and plants did not show any significant toxicity decrease in the aged samples compared to freshly amended soil, suggesting that longer weathering times would be necessary to detect an effect. Aquatic toxicity tests (microtox assay and algae) of three GIM explosive samples that were weathered and aged outdoor for two years showed a clear decrease of toxicity compared to fresh GIM. The latter was directly related to the lower content of TNT remaining in the aged samples.

RESUME

Afin de développer des armes moins nocives pour l'environnement, l'armée canadienne étudie présentement la possible utilisation de nouvelles formulations d'explosifs ou de charge propulsive. La présente étude répond ainsi aux besoins de RDDC, Valcartier, QC, et vise à produire des données physiques, chimiques et écotoxicologiques nécessaires pour prédire le sort et l'impact de nouvelles formulations développées dans le cadre du projet RIGHTTRAC (Revolutionary Insensitive, Green and Healthier Training Technology with Reduced Adverse Contamination). La présente étude résume la dissolution, le transport, la transformation et l'écotoxicité de trois formulations de charge propulsive, SP 7993, SP Unique, et CMR170, ainsi que de leurs constituants, NG, DPA, ATEC, MC et EC. De plus, elle résume aussi l'écotoxicité d'une formulation d'explosifs, GIM, vieillie durant des périodes variant de 6 à 24 mois.

Parmi les trois formulations testées, SP 7993 s'est avérée être la plus stable en terme de dissolution, plus stable même que la formulation New Green M1 précédemment identifiée comme très stable. Si des particules de SP 7993 dispersées à la surface du sol sont sujettes à des précipitations elles devraient donner lieu à une dispersion minime d'ATEC. Ce dernier devrait rapidement disparaître étant donnée sa faible stabilité dans les sols.

Parmi les deux formulations à double-base, CMR170 est apparue moins soluble que SP Unique. Bien que l'enveloppe MC/graphite protégeant la formulation CMR170 puisse être à l'origine de sa stabilité, ceci ne peut être affirmé car CMR170 contient plus de NC que SP Unique ce qui limite aussi la dissolution de ses composés solubles. Si présentes dans l'environnement, CMR170 devrait donner lieu à une dispersion modérée de NG et MC tandis que SP Unique devrait donner lieu à une dispersion significative de NG et modérée de DPA. Etant données la stabilité et la grande mobilité de NG mesurées dans le sol Valcartier, la NG devrait demeurer très biodisponible dans de nombreux sols.

La présente évaluation écotoxicologique des formulations SP 7993, SP Unique, et CMR170 a permis de déterminer que la formulation SP 7993 était la moins toxique, que la formulation SP Unique était toxique pour les organismes aquatiques et terrestres testés, et que la formulation CMR170 était toxique pour les organismes terrestres seulement. La toxicité des formulations SP Unique et CMR170 peut être attribuée à la présence de nitroglycérine dans ces deux formulations.
La toxicité du GIM (Green Insensitive Munitions) vieilli climatiquement a été comparée à celle du GIM non vieilli. Les présents travaux montrent que la concentration de TNT mesurée dans un sol fortifié en GIM diminue au fur et à mesure du vieillissement. Cependant, après 6 mois de vieillissement, des tests de toxicité conduits sur des vers de terre et des plantes n'ont pas montré d'effet significatif, impliquant qu'un vieillissement plus long aurait été nécessaire pour discerner un effet. Des tests aquatiques (test Microtox et algues) réalisés avec des échantillons de GIM vieillis à l'extérieur durant 2 ans ont, eux, montré une nette diminution de toxicité expliquée par la concentration en TNT très inferieure dans les échantillons vieillis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

As part of a sustainable training of the Canadian Army, Defence Research and Development Canada (DRDC, Valcartier, QC) is developing new green explosive and propellant formulations. The newly developed formulations have to be tested for their safety, ballistic performance, as well as their environmental fate and impact. For most of the parameters tested, the new formulations must compare favourably to traditionally used munitions. The present research thus responds to the needs of DRDC by providing necessary physicochemical, chemical, and ecotoxicological data to help predict the environmental transport, fate and impact of new formulations.

During the years 2008-2012, NRC-Montreal has studied the fate and impact of numerous explosive and propellant formulations, the compositions of which are provided in Table 1 (top part for explosives; bottom part for propellants) (Hawari et al., annual reports 2009, 2010, 2011 & 2012). It was concluded from these studies that the presence of large content of nitrocellulose (NC) in propellant formulations decreases considerably the leakage of soluble components out of solid formulations, therefore decreasing the risk of contamination at firing sites. Based on this result, DRDC decided to investigate the fate of three new small arm propellant formulations containing large amounts of NC. One of the formulations, SP Unique, contains NC and triethyl-2-acetylcitrate (ATEC); the two other formulations, SP 7993 and CMR170, contain NC and nitroglycerin (NG), processed in a different way. While formulation SP Unique results from a bulk preparation with nitrocellulose and rosin serving as binders, CRM170 was prepared in two steps where a bulk formulation of NC, NG, and EC, was covered with a MC/graphite coating.

In a previous study aimed at investigating the environmental fate and impact of the new explosive formulation, GIM, we concluded that the latter was toxic to most tested aquatic and terrestrial receptors likely due to the leakage of TNT out of GIM solid particles (Hawari et al., 2009 & 2011). However, TNT, like other nitroaromatics, is known to transform in soil into amino-derivatives that can bind covalently and irreversibly to soil organic matter or polymerize oxidatively into insoluble azo and

hydrazo-oligomers (Thorn et al., 2002a, 2002b, 2008; Yang et al., 2008). The resulting products normally exhibit less toxicity than the original nitro compounds due to their unavailability in soil. In a separate request, DRDC therefore asked the NRC to investigate the toxicity of GIM after aging the latter in soil for several months.

The present report is thus constituted of three separate parts. Part I summarizes the results on the transport and fate of the three new small arm propellant formulations (SP unique, SP 7993, CMR170), by providing data on dissolution, sorption, and degradation of the whole formulations as well as the individual components, if not previously studied. Results are compared to those obtained with the most similar formulation we previously studied, *i.e.* New Green M1. Part II summarizes the environmental impact of the same three propellant formulations by providing data on the toxicity of the formulations to aquatic and terrestrial receptors. Finally, part III summarizes the toxicity of aged GIM to various aqueous and terrestrial receptors.

Table 1. Composition of RIGHTTRAC TDP explosive and propellant formulations studied at NRC-Montreal over the years 2008-2012

Formulation	Ingredient	Provided composition (% w/w)	Composition measured at BRI (% w/w) (*n* = 3)
Composition B (Explosive Reference)	RDX	60	48.3 ± 0.7
	TNT	40	40.4 ± 1.0
	HMX	0	12.0 ± 0.3
GIM (XRT)	ETPE	9.5	7.8 (by difference)
	HMX	51.3	51.5
	TNT	39.2	40.7
CX-85 (PBX)	HTPB-TDI	10.37	Non measured
	DOA	5.58	5.58 ± 0.07
	Lecithin	0.08	Non measured
	HDHA	0.08	Non measured
	Dantocol	0.15	Non measured
	HMX	83.74	86.7 ± 0.9

M1 (Propellant Reference)	NC	83.3	Non measured
	2,4-DNT	10.7	10.1 ± 0.2
	DBP	5	5.70 ± 0.08
	DPA	1	0.94 ± 0.03
Helova	ETPE	8.9	Non measured
	NC, Type 1, Gr A	14.8	Non measured
	TEGDN	6.9	8.8 ± 0.2
	HMX	67.8	70.9 ± 0.9
	TPA	0.4	0.37 ± 0.04
	CAB	0	Non measured
Green M1	NC, Type 1, Gr C	68.9	Non measured
	TEGDN	29.9	34.3 ± 0.9
	AK	1.0	0.93 ± 0.03
	Carbon black	0.2	Non measured
Triple base	NC	50.9	Non measured
	TEGDN	23.9	28.2 ± 0.3
	NQ	24.0	25.5 ± 0.3
	TPA	1.2	1.46 ± 0.12
	Carbon black	0.1	Non measured
New Green M1	NC, grade C	91.85	Non measured
	ATEC	7.15	7.16 ± 0.06
	AK	1.00	0.86 ± 0.04
	MC	(Added:4.30)	3.95 ± 0.08

Abbreviations: AK: Akardite II; ATEC: Acetyltriethylacetate; DBP: Dibutylphthalate; 2,4-DNT: 2,4-Dinitrotoluene; DOA: Dioctyladipate; DPA: Diphenylamine; ETPE: Energetic thermoplastic elastomer; HDHA: Hydroxy-6 dimethyl-N,N hexamide; HMX: Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; HTPB-TDI: Hydroxy-terminated polybutadiene and toluene di-isocyanate (= polyurethane); MC: Methyl centralite; NC: Nitrocellulose; TNT: 2,4,6-Trinitrotoluene.

PART I. ENVIRONMENTAL FATE OF PROPELLANT FORMULATIONS, SP 7993, SP UNIQUE, AND CMR170

I. SOLUBILITY AND DISSOLUTION OF PROPELLANT FORMULATIONS

Dissolution of formulations by precipitation is the departure point and one of the controlling factors for the transport, fate, and impact of their components (Morley et al., 2006; Furey et al., 2008). Several studies suggested that the dissolution rate of individual explosives was decreased when confined in formulations compared to the pure explosives (Lynch et al., 2002; Phelan et al., 2002; Lever et al., 2005; Taylor et al., 2009; Monteil-Rivera et al., 2010). More recently, we and others demonstrated that dissolution rates of components of propellant formulations were strongly affected by the presence of NC (Dontsova, et al., 2009; Taylor et al., 2011; Hawari et al., 2012; Taylor et al., 2012). In the present section, we will therefore determine the dissolution ability of the three new formulations of interest (SP 7993, SP unique, CMR170). The aqueous solubility of components that have not been studied earlier will be first determined and the propensity of all soluble components to migrate out of the solid formulations upon contact with water will be then assessed in batch or dripping experiments.

I.1. Materials and methods

I.1.1. Materials

The three formulations (SP 7993, SP unique, CMR170) were provided by DRDC in their original form as well as in powder if the size of particles was too large to allow homogeneous spiking in soil. Original SP 7993 particles appeared as greenish cylinders (2.5 mm $\times$ 5 mm), SP unique particles appeared as thin anthracite disks (1.6 mm $\times$ 0.5 mm) and CRM170 appeared as very tiny anthracite cylinders (0.81 mm $\times$ 0.85 mm) (Fig. 1). SP 7993 was grinded into powder to allow a better homogeneity in the ecotoxicology and dissolution tests; the two other formulations were used as received. The chemical composition of each formulation was

measured at NRC-Montreal by dissolving ~40 mg (precisely weighted) in acetonitrile (100 mL), sonicating for 15 min, stirring for 1 h at room temperature (250 rpm), and analyzing the supernatant by high performance liquid chromatography (HPLC) as described below. Results are summarized in Table 2 together with the theoretical compositions provided by DRDC. Concentrations measured at NRC-Montreal agreed well with the provided values; the more precise values measured in-house were the ones used in the environmental and ecotoxicological studies described later in this report.

In these formulations, nitrocellulose (NC) serves as an energetic binder, nitroglycerin (NG) as an energetic plasticizer, acetyltriethylcitrate (ATEC) as a plasticizer, carbon as an opacifier, K_2SO_4 as a visible flame suppressant, and diphenyl amine (DPA), methyl centralite (MC) and ethyl centralite (EC), as stabilizers.

Some of the organic hydrosoluble components were obtained to develop analytical tools and/or to determine their environmental fate. ATEC (colorless oily liquid, 99 %), EC (greyish solid, 99 %), and DPA (white solid, 99%) were purchased from Sigma-Aldrich (Oakville, ON). MC (white solid) was provided by Defence Research and Development Canada (Valcartier, QC). NG was purchased from Cerilliant Corporation (Round Rock, TX) as a solution in acetonitrile (1000 ppm).

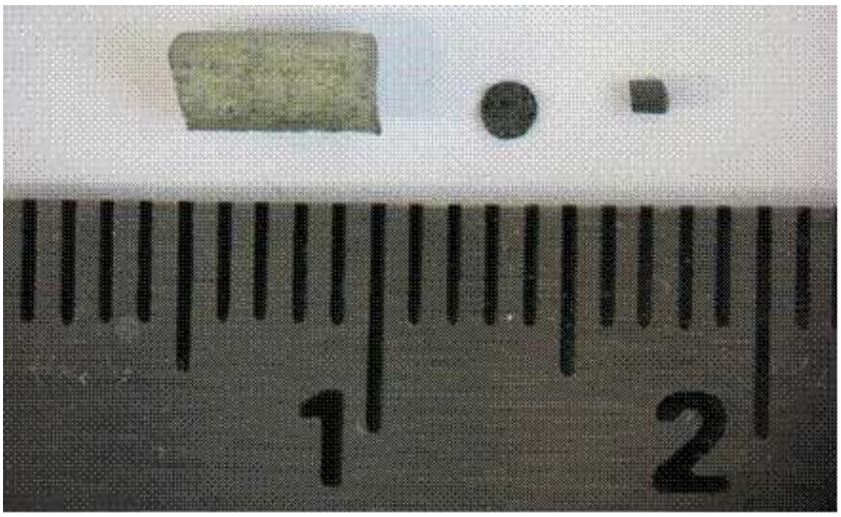


Figure 1. Micrograph of the three propellant formulations. From left to right: SP 7993, SP Unique, and CRM170

Table 2. Composition of the three propellant formulations as provided by DRDC and measured at NRC-Montreal

Formulation	Ingredient	Provided composition (% w/w)	Composition measured at BRI (% w/w) (*n* = 3)
SP 7993	NC	89.5	Non measured (89.6)
	ATEC	8.00	8.0 ± 0.6
	EC	1.50	1.39 ± 0.03
	K_2SO_4	1.00	Non measured
	Graphite	< 0.15	Non measured
SP Unique	NC & Rosin	58-96	Non measured (78.6)
	NG	4-40	20.6 ± 0.2
	EC	0-1	0.20 ± 0.01
	DPA	0-1	0.63 ± 0.01
CRM170	NC	86.3	Non measured (~89.4)
	NG	8.62	7.5 ± 0.2
	EC	0.97	0.93 ± 0.02
	K_2SO_4	1.55	Non measured
	Carbon black	0.14	Non measured
	MC (coating)	1.95	2.09 ± 0.05
	Graphite (coating)	0.49	Non measured

Abbreviations: ATEC: Acetyltriethylacetate; DPA: Diphenyl amine; EC: Ethyl centralite; MC: Methyl centralite; NC: Nitrocellulose; NG: Nitroglycerin.

I.1.2. Analytical methods

NG, ATEC, EC, and MC were analyzed in acetontrile/H_2O (50/50; v/v) solutions by reverse phase high performance liquid chromatography (HPLC)-UV. The system consisted of a W600 pump (Waters, Milford, MA, USA), a 717 plus autosampler, and a 2996 Photodiode-Array Detector. Samples (50 µL) were separated with a Discovery C18 column (25 cm × 4.6 mm × 56 µm) (Supelco, Oakville, ON), at 35°C. A water methanol gradient was run at 1 mL min^{-1}. The initial solvent composition was 50 % methanol/ water, which was held for 18 min. A linear gradient was run from 50% to 90% methanol over 2 min. This solvent ratio was held for 8 min and then changed to the initial conditions over 2 min. The initial conditions were held for an extra 15 min for a total run of 45 min. The detector was set to scan from 192 to 450 nm. Detection limits were estimated at 0.005 mg L^{-1} for DPA at 284 nm, 2.5 mg L^{-1}

for ATEC at 213 nm, 0.005 mg L^{-1} for MC at 242 nm, and 0.005 mg L^{-1} for EC at 246 nm.

Given the low sensitivity of the HPLC-UV method for ATEC, the latter was quantified at low concentration by LC-MS using a mass spectrometer (MS, Bruker MicroTOFQ mass analyzer) attached to an HPLC system (Hewlett Packard 1200 Series) equipped with a DAD detector. Aliquots (10 μL) were injected into a 3.5 micron-pore size Zorbax SB-C18 column (2.1 mm ID × 150 mm; Agilent, Mississauga, Canada) at 25°C. The solvent system was composed of 70% of CH_3OH in H_2O at a flow rate of 0.2 mL min^{-1}. For mass analysis, positive electrospray ionization mode (ES+) was used. Mass range was set from 40 to 1000 Da. ATEC was detected as sodium adduct ions $(M+Na)^+$ at *m/z* 341.1 ± 0.05. Quantification was done using external standard. Detection limit was estimated at 1 μg L^{-1}. Transformation products of ATEC were also identified using this method but with a mobile phase consisting of 60% MeOH in water to ensure a better separation.

DPA transformation products were analyzed using a Bruker Esquire3000Plus ion trap mass analyzer attached to a HPLC system (Hewlett Packard 1100 Series) equipped with a diode array detector. The samples were injected into a 5 micron-pore size Zorbax SB-C18 capillary column (0.5 mm ID × 150 mm; Agilent, Mississauga, Canada) at 30 °C. The solvent system was composed of a CH_3CN/H_2O gradient (50 to 90% v/v) at a flow rate of 12 μL min^{-1}. For mass analysis, positive electrospray ionization (ES+) was used to produce protonated molecules $[M+H]^+$. The mass range was scanned from m/z 40 to 1000. Auto MS/MS mode was set to fragment an unknown compound detected at *m/z* 337.

I.1.3. Aqueous solubility of individual components

EC was the only chemical for which we had not measured previously the aqueous solubility. Aqueous solubility of EC was therefore measured at 25ºC by suspending 0.1 g of the chemical in 10 mL of deionized water. The samples were stirred at 25ºC. At days 8, 15 and 50, aliquots (~ 1 mL) of the mixture was filtered, diluted 1:1 in acetonitrile, and analyzed by HPLC/UV. Solubility was considered reached when

value was stable between two successive measurements. Measurements were done in triplicate.

I.1.4. Leaching from formulations in water batch

Batch experiments were conducted to determine the aqueous concentrations of each component if the propellant formulations are equilibrated with an aqueous or wet medium like in ecotoxicity tests. The formulation powder (SP7973) or small particles (SP Unique and CRM170) were stirred for long times in water batches and water was changed after reaching sequential equilibriae. For all formulations, batch experiments were conducted in water at temperatures of (10.0 ± 1), (25.0 ± 1), and (30.0 ± 1) °C. A mass (10 or 500 mg) of formulation was added to 100 or 50 mL of pre-thermostated deionized water (pH 5.5) in a glass bottle. The capped samples were shaken (at 150 rpm) in a thermostated incubator protected from light. At various time intervals, aliquots (1.5 mL) of suspension were withdrawn, filtered through a Millex-HV 0.45 µm syringe filter, diluted (1:1 v/v) in acetonitrile, and analyzed for DPA, MC, EC, or NG by HPLC and for ATEC by LC-MS. Once the dissolved amounts of components reached equilibrium, the solid was isolated from supernatant and stirred again with a fresh batch of deionized water (100 or 50 mL) in order to determine the maximal potential of each component to dissolve. All experiments were done in duplicate.

I.1.5. Leaching from formulations under constant water dripping

In order to evaluate the ability of the three propellant formulations to leach under a rain event, experiments were set up where a particle of SP7993 (cylinder of 2.6 mm diameter and 4.9 mm height; 36.5 mg), SP unique (disk of 1.6 mm diameter and 0.5 mm thickness; 1.35 mg) or CRM170 (cylinder of 0.81 mm diameter and 0.85 mm height; 0.90 mg) was deposited on a glass funnel fitted with a nylon mesh and exposed to a continuous flow of water maintained with an HPLC pump at a rate of 0.5 mL min^{-1} corresponding to ~ 19 drops per min. Outflow samples were collected in glass flasks covered with aluminum foil and flasks were changed after increasing

intervals (from 1 h to 1 week). Each water fraction was analyzed for ATEC and EC for SP7993, NG, EC, and DPA for SP Unique, and NG, EC, and MC for CRM170.

I.2. Results and discussion

I.2.1. Aqueous solubility of components

Since ATEC, DPA, and MC were present in some of the formulations that we studied between 2010 and 2012 (see Table 1), their aqueous solubility had been previously measured (Hawari et al., 2012). As for NG several fairly coherent values of aqueous solubility were found in the literature (Mirecki et al., 2006). Thus, we only measured the aqueous solubility of EC in this study. Previous and present results of aqueous solubility are all gathered in Table 3 along with literature data, when available.

Table 3. Aqueous solubility of soluble components of formulations measured at 25 °C

Compound	Meas. Solubility (mg L^{-1}) / 25°C	Reported solubility (mg L^{-1}) (ref.)
NG	Non measured	1,950 (Mirecki et al., 2006)
ATEC	3,296 ± 41	688.2 (Chemspider, 2013)
MC	89.0 ± 0.4	Insoluble (Mirecki et al., 2006)
DPA	38.7 ± 0.4	39; 35-45 (Schetter, 1993, (Drzyzga, 2003)
EC	44.6 ± 0.7	80.0 (Mirecki et al., 2006)

NG is known to be reasonably soluble in water, with an aqueous solubility reported to range between 1,380 and 1,950 mg L^{-1} between 20 and 25°C mg L^{-1}. No data could be found in the literature on the solubility of ATEC but MSDS data available on the internet from several suppliers indicate a value of 7.2 g L^{-1} at 25°C and theoretical value predicted from the log K_{ow} using software WSKOW v1.41 of the EPIsuite (Chemspider 2013) led to 0.7 g L^{-1}. The value we measured (3,296 mg L^{-1}) was between these two values. MC aqueous solubility was reported in an encyclopedia of explosives (USA-ARDEC, 1983) to be insoluble in water although

the quality of the reported data was claimed to be equivocal by Mericki et al. (2006). We measured the aqueous solubility of MC at 25°C and found a value of 89.0 ± 0.4 mg L^{-1}. DPA aqueous solubility was measured at 25ºC and found to be 38.7 ± 0.4 mg L^{-1}, in perfect agreement with the value of 39 mg L^{-1} published by the Food and Agricultural Organization of United Nations (Schetter, 1993) or the value range of 35-45 mg L^{-1} reported by Drzyzga for various temperatures (2003). Finally, measurement of EC aqueous solubility led to a value (44.6 ± 0.7 mg L^{-1}) inferior to the value published in the Encyclopedia of Explosives and Related Items (USA-ARDEC, 1983) but logical compared to the solubility of MC.

According to these measurements, the aqueous solubility of each of the soluble components of the three formulations follows the order:

ATEC > NG > MC > DPA > EC.

I.2.2. Batch experiments for formulation dissolution

Leaching experiments were conducted to determine the amount of individual components that would leak from the propellant formulations if contacted with water. Powders were stirred for long times in water batches and water was changed after reaching sequential equilibriae.

SP 7993. Leaching of ATEC and EC from SP 7993 powder was first monitored under the conditions used at NRC for other formulations, *i.e.* 10 mg of formulation in 100 mL H_2O. Very low levels of EC (< 6 ppb) and ATEC (< 70 ppb) were detected in the first aqueous phase after two months of experiment while no chemical was detected after replacing the water with fresh water (2^{nd} run).

A second experiment was then set up with a larger concentration of formulation (500 mg / 100 or 50 mL). The resulting leaching of ATEC and EC is shown in Figures 1 and 2 for two successive runs.

Despite the non-negligible aqueous solubilities of ATEC and EC at 25°C (3,296 and 45 mg L^{-1}, respectively) the maximum concentration of both chemicals remained largely inferior to these values (Fig. 2 & 3). During the first run, ATEC and EC were

released at a faster rate than in the second run suggesting that a continued contact with water would induce a decreasing release of chemicals in water.

The corresponding cumulative dissolved fractions of ATEC and EC are reported in Table 4. Although the releases were increased by increasing the temperature, less than 1.8% of ATEC and 0.2% of EC were released after 6 months at 30°C. Data thus show that very small proportions of initial amounts of ATEC or EC could be extracted from SP 7993 after several months of stirring in water. The losses measured correspond to low absolute masses of ATEC (0.75 mg at 30°C) or EC (0.07 mg at 30°C) thus suggesting that exposure of chunks of SP 7993 formulation to water in the environment would probably lead to extremely low levels of contamination. The nitrocellulose was probably responsible for the limited release of ATEC and EC. It might have swollen in water and blocked the inner pores of the matrix thus reducing the diffusion of the chemicals or acted as a potential adsorbent for the soluble components.

For comparison, when similar experiments were conducted with particles of New Green M1 (see Table 1 for composition) (Hawari et al., 2012), the release of components from these formulations was also found to be limited by processes other than pure dissolution and the extent of dissolution for ATEC (1.9% at 30°C) and MC (7% at 30°C) was slightly higher than the findings obtained with SP 7993.

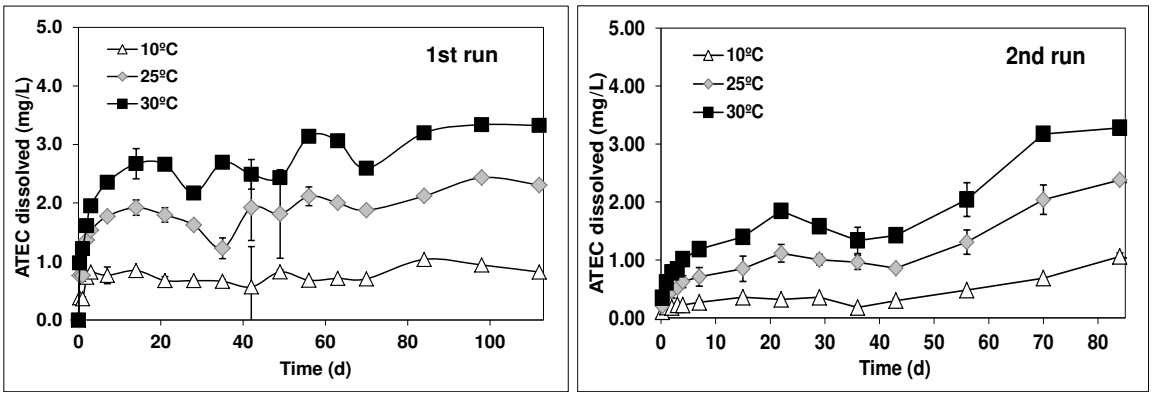


Figure 2. Leaching of ATEC from SP 7993 as a function of temperature (SP 7993: 5,000 ppm in 1st run; 10,000 ppm in 2nd run)

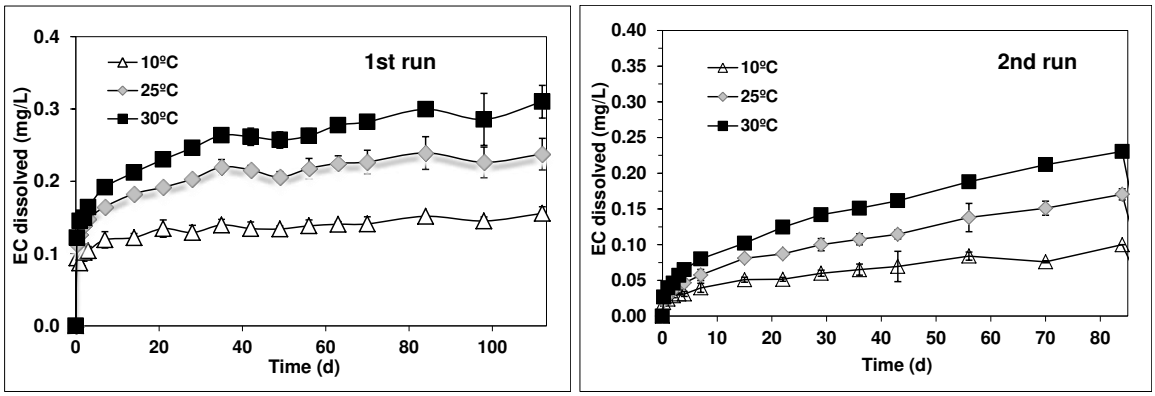


Figure 3. Leaching of EC from SP 7993 as a function of temperature (SP 7993: 5,000 ppm in 1st run; 10,000 ppm in 2nd run)

Table 4. Cumulative amounts of ATEC and EC released from SP 7993 (~500 mg) into water (1 × 100 mL; 1 × 50 mL) after the second run

	ATEC from SP 7993			EC from SP 7993		
	10°C	25°C	30°C	10°C	25°C	30°C
µg	401	514	714	33	51	67
% of initial chemical	1.00	1.29	1.79	0.08	0.13	0.17

SP Unique. SP Unique small particles were stirred in water at the same three temperatures (10, 25, and 30°C), and the release of NG, DPA, and EC was monitored over time (Fig. 4-6). Dissolution of the three chemicals was much faster than with SP 7993, and plateaus were reached after only one week of stirring. Although inferior to NG aqueous solubility, the concentration of NG in water reached high values, *i.e.* 450 and 500 mg L^{-1} at 25 and 30°C, respectively. After one month, the supernatant was replaced by fresh deionized water, and the dissolution continued to occur at a fast pace, with new plateaus being reached after only 2 to 3 weeks.

The dissolution curves are consistent with a very fast release of all chemicals followed by quasi-nil release. Changing the water induced a similar fast dissolution followed again by an absence of dissolution in the second phase of the run. The existence of plateaus could be explained by: 1/ reached solubility maxima; 2/ a physical change of the NC matrix which would hamper further diffusion of chemicals

from the core of the solid to the outer layers of the solid; 3/ reached sorption equilibriae. The aqueous concentrations of the three chemicals always remained markedly lower than the aqueous solubility thus ruling out the aqueous solubility as an explanation for the plateaus. SP Unique particles have not been dried before changing the water. Therefore, if physical changes were responsible for the absence of diffusion, they would still be effective at the beginning of the second run and would not allow additional release of chemicals in fresh water. Sorption/desorption processes thus seem to play an important role in the release of chemicals from SP Unique. K_d values calculated for NG at the three temperatures were found to increase from the 1st to the 2nd runs (Fig.4), which is not consistent with a pure reversible sorption process. The increasing K_d values could be due to some irreversible sorption or some hampered dissolution from the core of the particle. It thus seems that the release of chemicals from SP Unique is governed by sorption processes on the formulation itself, but it is not clear whether sorption is partially irreversible or combined with hampered dissolution/diffusion. The presence of rosin with NC might be the cause of the faster diffusion observed compared to other propellant formulations made of pure NC. It might also be responsible for the presence of sorption sites.

At the end of the second run, the experiment was stopped. The total amount of NG, DPA and EC dissolved at 30, 25 and 10°C are reported in Table 5. The present findings show that large amounts of NG were released from formulation SP Unique. It can thus be concluded that significant amounts of NG will leach out of SP Unique particles if the latter are scattered onto the soil surface and contacted with water in the field. In addition, NG leaking will occur within few days. DPA and EC which represent only 0.63 and 0.20% of the initial formulation mass will be present in water at very low or non-detectable levels.

SP Unique is therefore less stable than SP 7993. Given the observed important leaching of NG, the environmental impact of SP Unique formulation will be largely governed by the environmental impact of NG.

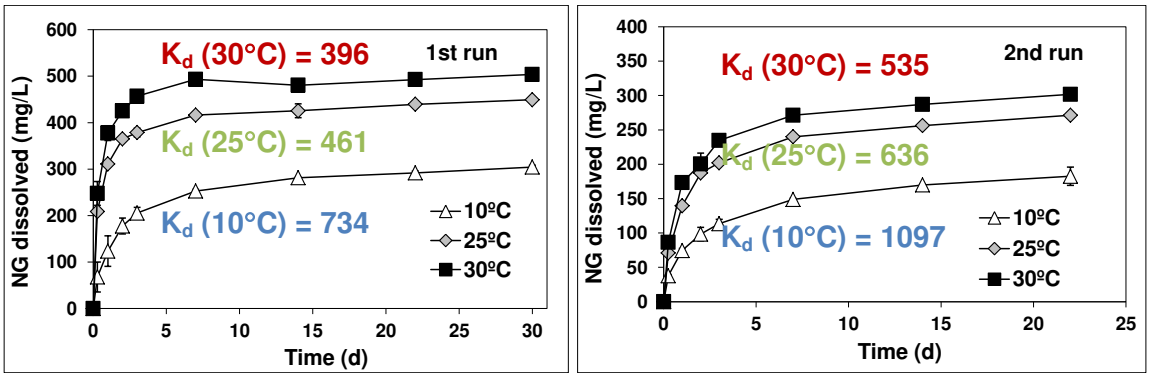


Figure 4. Leaching of NG from SP Unique as a function of temperature (SP Unique: 10,000 ppm in each run)

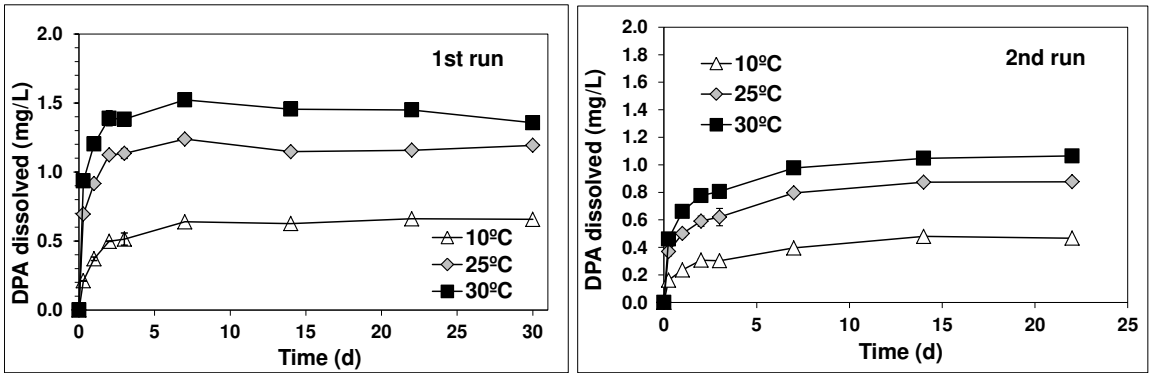


Figure 5. Leaching of DPA from SP Unique as a function of temperature (SP Unique: 10,000 ppm in each run)

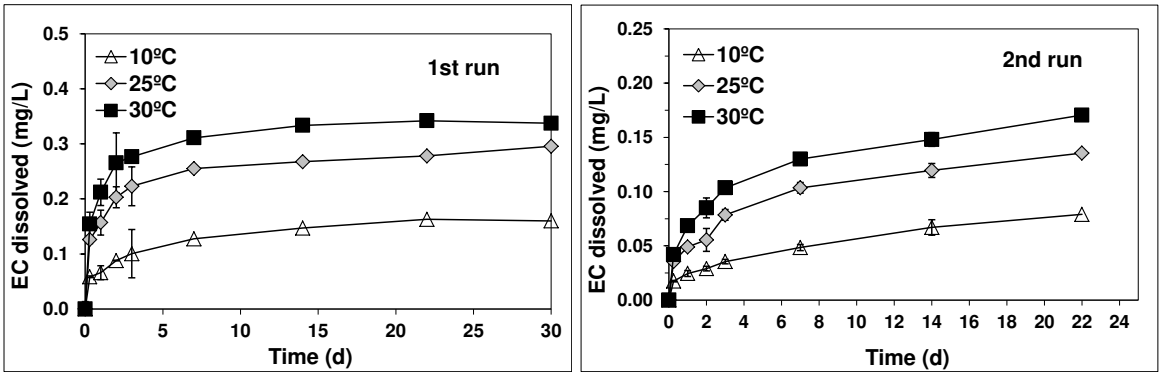


Figure 6. Leaching of EC from SP Unique as a function of temperature (SP Unique: 10,000 ppm in each run)

Table 5. Cumulative amounts of NG, DPA and EC released from SP Unique (~500 mg) into water (2 × 50 mL) after the second run

	NG from SP Unique			DPA from SP Unique			EC from SP Unique		
	10°C	25°C	30°C	10°C	25°C	30°C	10°C	25°C	30°C
mg	23.84	35.07	38.46	0.099	0.182	0.215	0.021	0.037	0.044
%	23.02	33.87	37.03	3.13	5.77	6.79	2.08	3.70	4.38

CMR 170. CMR170 small particles were stirred in water at the same three temperatures (10, 25, and 30°C), and the release of NG, MC, and EC was monitored over time (Fig. 7-9). Although CMR170, like SP Unique, contains NC, NG and EC, dissolution of NG and EC in water occurred at a much lower rate than it did from SP Unique and plateaus were not reached even after 4 months of stirring. The shape of the curves were indicative of an initial fast (though slower than for SP Unique) release of chemicals followed by a much slower release where diffusion from the core to the outside of the particles seemed to be a limiting factor. Recently, Taylor et al. (2012) reported in a study on the evaluation of the dissolution rate of propellant energetics from NC-based propellant formulations that NG had a fast initial dissolution followed by slower mass loss, and that the amount of NG dissolved was a function of the NG/NC ratio in the propellant. Unfortunately, in addition to having been prepared differently, formulations SP Unique and CMR170 had different NG/NC ratios (0.26 and 0.10, respectively), which made more complex the direct comparison of the manufacturing processes. According to the observation made by Taylor et al. (2012), it is expected that SP Unique, with its lower content of NC, releases higher amounts of NG in water.

After four months, the supernatant was replaced by fresh deionized water, and the dissolution continued to occur at a slower rate than in the 1st run (1st run rate/2), as expected with a diffusion process.

The experiment is currently ongoing but mass balances were calculated based on the amounts dissolved so far. The total amount of NG, MC and EC dissolved at 10,

25, and 30°C after a total of almost 5 months are reported in Table 6. NG was again the chemical that was released in larger amounts from CMR170 but the masses of released NG were around 10 times less than the masses released from SP unique. Small amounts of NG will thus leach out of CMR 170 particles if the latter are contacted with water in the field. MC and EC which represent only 2.09 and 0.93% of the initial formulation mass will be present in water at low or non-detectable levels.

The present findings indicate that CMR 170 is less stable than SP 7993 but more stable than SP Unique. The higher stability of CMR170 compared to that of SP Unique might be related to the manufacturing process which consisted in surrounding the NC-NG-EC matrix with a MC-Graphite coating. However, this cannot be ascertained since CMR170 also contains 10% more NC than SP Unique, which is less favorable to the diffusion and leaching of chemicals out of the matrix.

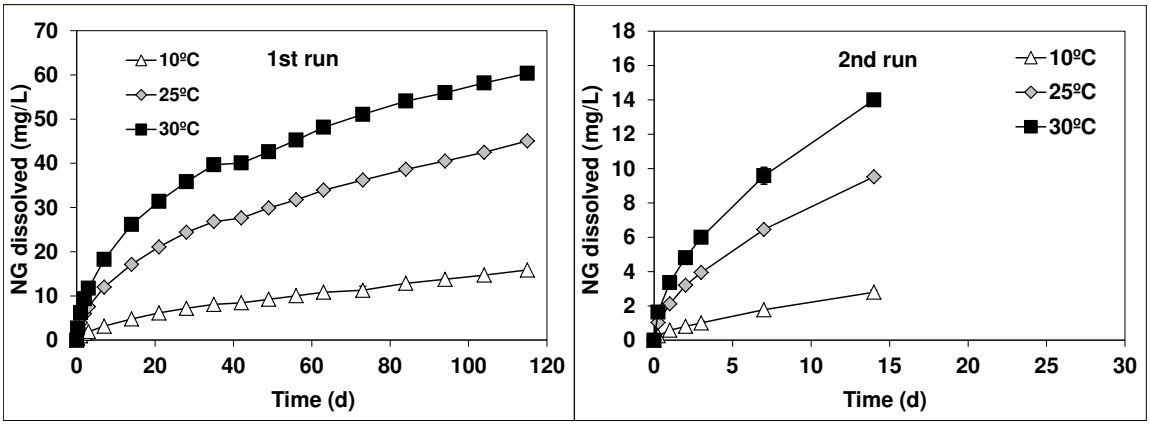


Figure 7. Leaching of NG from CMR 170 as a function of temperature (CMR170: 10,000 ppm in each run)

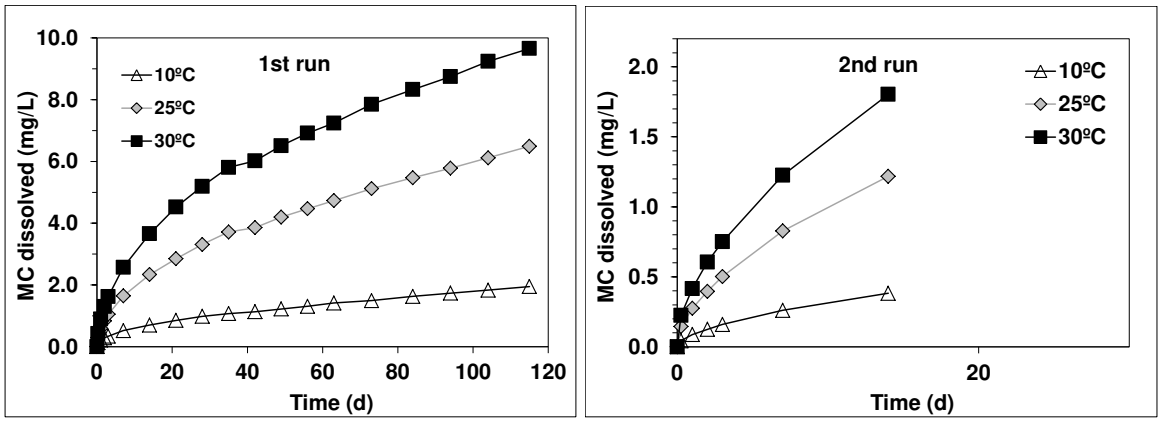


Figure 8. Leaching of MC from CMR 170 as a function of temperature (CMR170: 10,000 ppm in each run)

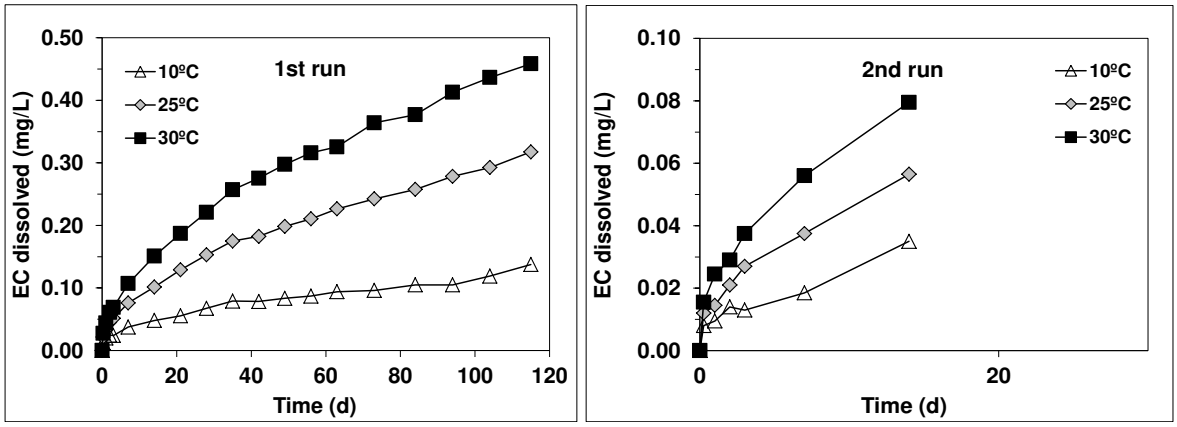


Figure 9. Leaching of EC from CMR 170 as a function of temperature (CMR170: 10,000 ppm in each run)

Table 6. Cumulative amounts of NG, MC and EC released from CMR170 (~500 mg) into water (2 × 50 mL) after the second run

	NG from CMR170			MC from CMR170			EC from CMR170		
	10°C	25°C	30°C	10°C	25°C	30°C	10°C	25°C	30°C
mg	0.72	2.09	2.92	0.09	0.30	0.44	0.007	0.014	0.021
%	1.95	5.65	7.86	0.90	2.90	4.24	0.14	0.31	0.45

I.2.3. Dripping experiments

To have an idea of what would happen over long periods of time and under more dynamic conditions, long term dissolution experiments were conducted using either SP 7993, SP Unique or CMR170 particles and a continuous flow of water. The free flowing of water on the particles mimicked a rainflow falling on a particle lying on a porous soil where the water would disappear quickly into the ground. Flow rate was chosen deliberately high to accelerate the dissolution processes.

SP 7993. A particle of SP 7993 (cylinder of 5 mm length × 2.6 mm diameter, 36.5 mg) was subjected to water dripping and the concentrations of ATEC and EC were monitored over time (Fig. 10). After the low concentrations of ATEC and EC detected in the above batch experiment conducted with SP 7993 powder (Table 4), short collecting times of 0.5 h were applied at the beginning of the dripping experiment to give rise to more concentrated samples. Despite the short times applied, EC remained at levels below the LOD (< 1 ppb) in all eluates, and ATEC was only detected in the first eluate at a concentration of 6.6 ppb (Fig. 10). Although no chemicals could be detected in the eluates after 0.5 h of dripping, the experiment was continued for 3 months.

After 3 months, the experiment was stopped. The remaining particle was let to dry under air and suspended in acetonitrile for quantification of residual ATEC and EC. After fast dissolution in acetonitrile, analysis of the acetonitrile solution by HPLC allowed recovering 2.67 mg of ATEC and 0.47 mg of EC corresponding to 92% of the initial ATEC and 93% of the initial EC, respectively. After a 3-month dripping, the SP 7993 particle had thus lost 0.278 mg in total corresponding to 0.8% of its total original mass. This finding shows the very high stability of formulation SP 7993 towards dissolution in water.

In line with these results, micrographs (Fig. 11) and size measurements of the SP 7993 particle before and after the dripping experiment did not reveal any detectable changes. The dripping test thus confirmed the observations made in batch experiment. SP 7993 formulation appeared to be very stable in water; it led to

very slow leaching of ATEC and EC into water with the former being detected at ppb levels in the most loaded eluates and the second remaining below LOD.

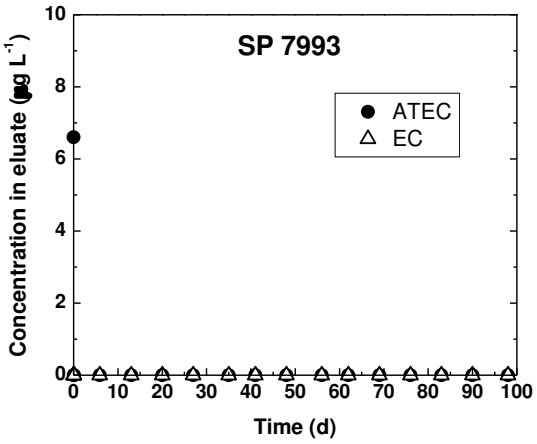


Figure 10. .Concentrations of ATEC and EC in the eluates of SP 7993 (35.6 mg) (T = 22°C; water flow: 0.5 mL min^{-1})

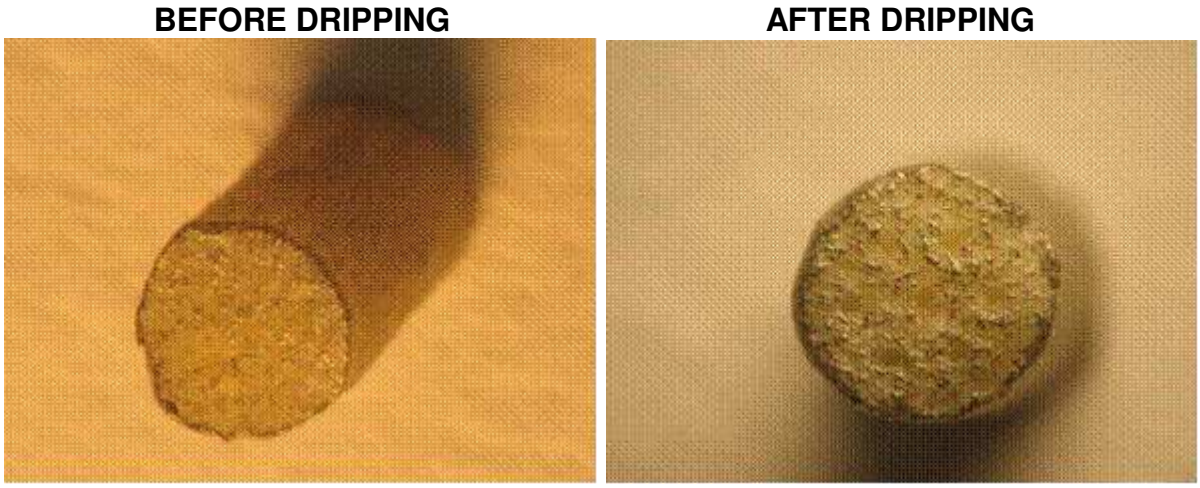


Figure 11. Microscopic photographs of a piece of SP 7993 non-exposed (left) and exposed (right) to a water dripping flow

SP Unique. SP unique particles that were provided to NRC were much smaller than SP 7993 particles. The largest particle available to conduct a dripping test was a disk (0.5 mm thickness ×1.6 mm diameter , 1.46 mg), which contained approx. 25 times less material than with SP 7993. When sweeping this small particle with the same water flow (0.5 mL min^{-1}), NG was detected in the two first eluates but not in the next ones, and neither DPA nor EC was detected in any of the eluates (Fig. 12). At first, this result seemed contradictory to the high NG concentrations measured in batch experiments. But it was explained by the low mass of solid and the ensuing high dilution of dissolved components which did not allow reaching LODs.

The experiment was extended for 1 month regardless of the absence of chemical detection in the eluates. After one month of dripping, microscopic photographs of the particle showed a rough surface with some cavities in the surface of the particle (Fig. 13). Moreover, dissolution of the particle in acetonitrile followed by HPLC analysis allowed recovering 16.1% of NG, 23.5% of DPA, and 99.1 % of EC. Most of NG and DPA have thus leached out of the small particle during the 1-month dripping experiment, whereas the content of EC remained largely unaffected. These findings show that a formulation that contains close to 80% NC can still loose most of its constituents if the latter, like NG, are highly hydrosoluble and poorly hydrophobic.

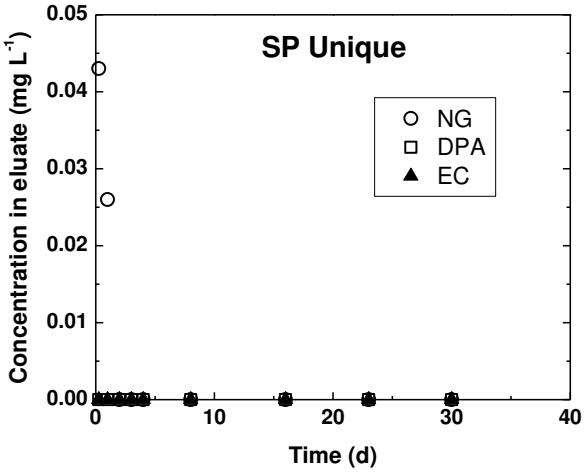


Figure 12. Concentrations of NG, DPA, and EC in the eluates of SP Unique (1.46 mg) (T = 22°C; water flow: 0.5 mL min^{-1})

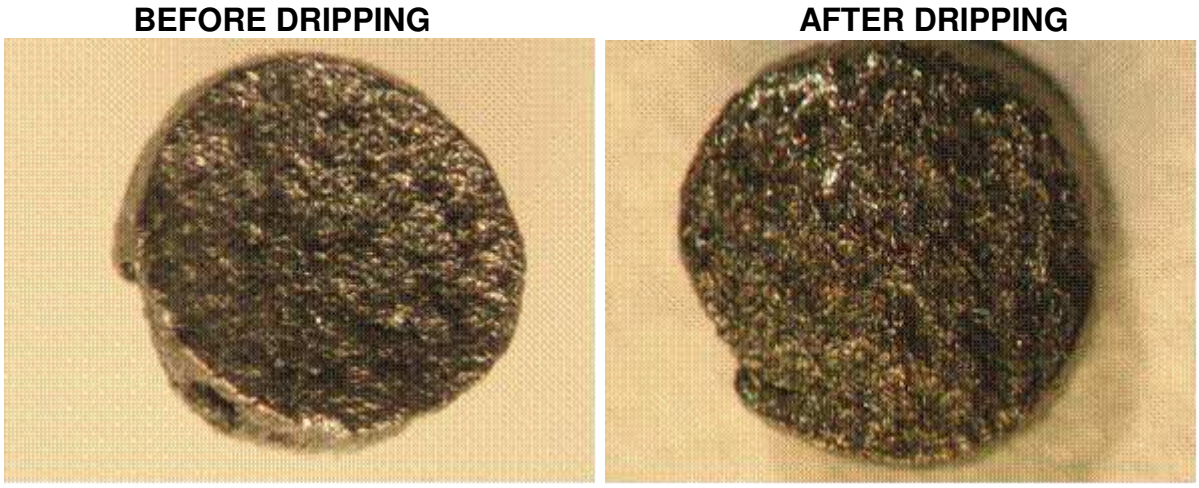


Figure 13. Microscopic photographs of a piece of SP Unique non-exposed (left) and exposed (right) to a water dripping flow

CMR 170. CMR170 particles that were provided to NRC were even smaller than SP Unique particles. The largest particle available to conduct a dripping test was a small cylinder (0.85 mm thickness ×0.81 mm diameter , 0.90 mg), which contained approx. 40 times less material than with SP 7993. When sweeping the small particle with the same water flow (0.5 mL min^{-1}), neither NG, nor MC, nor EC were detected in any of the eluates. Again, like for SP Unique particle, this result was explained by the low mass of initial solid and the ensuing high dilution of dissolved components which did not allow attaining concentrations higher than LODs.

The experiment was also continued for 1 month regardless of the absence of chemical detection in the eluates. After one month of dripping, microscopic photographs of the particle showed a rough surface with some cavities in the surface of the particle (Fig. 14). Dissolution of the particle in acetonitrile followed by HPLC analysis allowed recovering 79.2% of NG, 79.2% of MC, and 85.3% of EC. The higher recovery of NG in CMR170 compared to that measured in SP Unique (16%) after a similar period of water dripping is indicative of a slower dissolution rate of NG in CMR170 than in SP Unique. The lower NG dissolution rate observed with CMR170 despite the smaller particle used in the dripping test for this formulation is in line with the results observed in the batch experiments (Figs. 4 & 7) and commented in section I.2.2. MC dissolved more than EC which is consistent with the outer location of MC in CMR 170 as well as the higher aqueous solubility of MC (89

mg L^{-1}) compared to EC (45 mg L^{-1}). The lower recovery of EC in CMR170 than in SP Unique was most likely attributable to analytical error due to the very low amount of EC in the small particles used. The present results confirm the possible leaching of NG from a formulation that contains NC. They also show that the rate and extent of NG leaching are inversely proportional to the proportion of NC in the formulation.

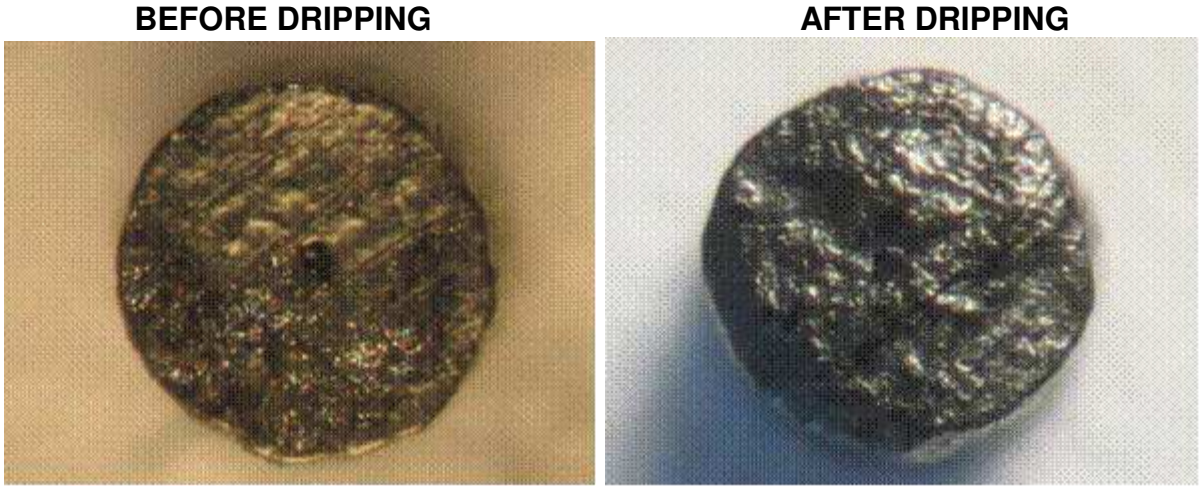


Figure 14. Microscopic photographs of a piece of CMR170 non-exposed (left) and exposed (right) to a water dripping flow

I.2.4. Conclusion

The aqueous solubility and dissolution rates of three formulations which all contain more than 75% NC were estimated using batch and dripping experiments. The single base formulation, SP 7993, (NC (90%), ATEC (8%), EC (1.4%), K_2SO_4, graphite), appeared to be the most stable in water, followed by CMR170 (NC (90%), NG (7.5%), EC (0.9%), K_2SO_4, C, MC (2.1%), graphite), and finally by SP Unique (NC & rosin (79%), NG (20.6%), EC (0.2%) and DPA (0.6%) . If exposed to a water flow, the latter will rapidly release significant amounts of NG in the environment.

II. TRANSPORT OF FORMULATIONS SP 7993, SP UNIQUE, AND CMR170 AND THEIR INDIVIDUAL COMPONENTS IN SOIL

Several individual components of the three formulations (NG, ATEC, DPA, MC) were present in other formulations previously studied at NRC-Montreal (Hawari et al., 2010a; report for NG Hawari et al., 2012). The transport studies of these components were not replicated but the data previously obtained are included in this report for comparison purposes. EC was the only organic component that had not been investigated earlier. The octanol/water partition coefficient of EC was therefore determined to evaluate its relative hydrophobicity. In addition, its transport behavior was assessed in a soil from DRDC-Valcartier.

II.1. Experimental part

II.1.1. Materials

A soil sampled in 2009 at a DRDC training range in Valcartier, QC, was used in this study. Selected specific properties of the soil - named “DRDC-09” throughout this report - are given in Table 7 along with properties of DRDC-08 soil also sampled at a DRDC training range but in 2008. DRDC-09 was markedly richer in silt/clay and organic matter than the soil sampled in 2008 at Valcartier.

Table 7. Physico-chemical properties of DRDC soils

	Particle size distribution		Total Org. C (%)	pH	CEC[a] (mequiv./100g)
	% Clay/Silt (< 80μm)	% Sand (>53μm)			
DRDC-08	6	94	0.36	4.2	6.69
DRDC-09	27.5	72.5	2.08	6.7	13.2

[a] CEC = Cationic Exchange Capacity

II.1.2. K_{ow} measurements

The octanol-water partition coefficient (K_{ow}) of ATEC, DPA, MC, and EC was measured at 22 ± 2 °C as described in the OECD Guideline 107 (OECD, 1981). The measurements were performed with non-saturated solvents. A volume (5 mL) of an aqueous solution of the analyte at approx. 10 mg L^{-1} was added to octan-1-ol (2, 3 or 4 mL) in a 16-mL PTFE-lined capped glass tube. The mixtures were equilibrated for four 10-min shaking periods spaced 10 min apart. The tubes were centrifuged at 3190 × *g* for 10 min. The octanol fraction was diluted (1:5) with a solution containing 70% methanol in water and the concentration of the substrate in the two fractions was determined by HPLC (or LC/MS for ATEC). Experiments were run in triplicate.

II.1.3. Batch sorption experiments

Batch equilibration was used to quantify the sorption of individual soluble compounds of the three propellant formulations in soils DRDC-08 (for NG) or DRDC-09 (for the four other components) under aerobic conditions. A volume (10 mL) of filtered aqueous solution of analyte was added under sterile conditions to DRDC-09 soil (1.5 g) in autoclaved 125-mL headspace glass bottles. The latter were closed, but not sealed, with butyl rubber stoppers. The bottles were kept in the dark at 22 ± 1°C and opened and shaken twice a week to ensure aerobic conditions. At various time intervals varying from 1 day to 3 months, three replicates were sacrificed. After 2 h of deposition, the supernatant was withdrawn, filtered through a 0.45 μm Millipore filter (Millipore Corp., Bedford, MA), diluted 1/1 in acetonitrile (CH_3CN) and analyzed by HPLC or LC/MS as described in section I.1.2. Sorbed analyte was extracted from DRDC-09 soil as described in the EPA SW-846 Method 8330 (USEPA, 1997). Briefly, soil was sonicated with CH_3CN (10 mL) at 20°C for 20 h, diluted 1/1 in an aqueous $CaCl_2$ solution and filtered through a Millex-HV 0.45-μm filter prior to HPLC analysis. A percent recovery was calculated by adding the soluble and sorbed fractions of analytes. The soil water distribution coefficient (K_d) was calculated as the soil to water analyte concentrations ratio.

II.2. Results and discussion

The previous section demonstrated that five of the organic components of the three formulations might leach into water at the long term. Chemical formulae of the five chemicals are provided in Figure 15.

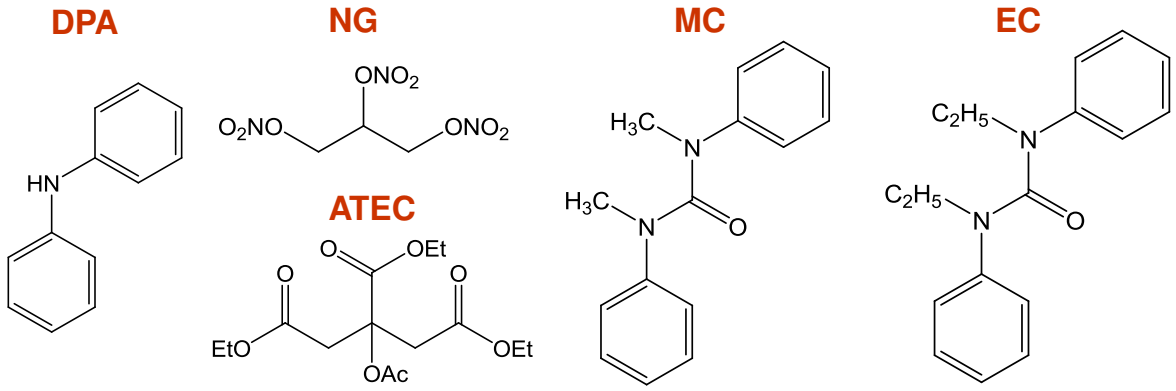


Figure 15. Chemical formulae of hydrosoluble components of formulations SP 7993, SP Unique, and CMR170

II.2.1. Octanol-water partition coefficients (K_{ow}'s)

The measured octanol-water partition coefficients (K_{ow}'s) of the soluble components of SP 7993, SP Unique, and CMR170 are reported in Table 8. Measured data agreed well with reported experimental values but somewhat less with predicted data generated using the program KOWWIN v1.67 of the US Environmental Protection Agency's EPISuite™ and available in the Chemspider database (Chemspider, 2013). The discrepancies observed between the experimental measurements and predicted values *confirm the caution that researchers should apply when using theoretical values for physico-chemical properties. Based on the present experimental measurements,* DPA and EC are the most hydrophobic chemicals amongst the five and they exhibit a moderate potential for bioaccumulation ($3.3 < \log K_{ow} < 4.2$); If released in the environment, they will thus adsorb significantly on soil organic matter. MC also exhibits a relatively high hydrophobicity. In contrast, NG and ATEC are less hydrophobic. They will thus not be strongly bound to the NC matrix and if released in soil, they will sorb partially but in lesser extent than DPA, EC and MC.

Table 8. Measured K_{ow}'s of soluble components of SP 7993, SP Unique and CMR170

Component	Measured Log K_{ow}[a]	Reported values for log K_{ow}
NG	N.D.[b]	1.51 (Chemspider); 1.62 (Hansch et al. (1995))
DPA	3.46 ± 0.07	3.50 (Hansch et al. (1995); McDougal and Jepson (1998)) ; 3.62 (Dave et al. (2000)) ; 3.42 (Drzyzga (2003)) 3.29 (chemspider, 2013)
ATEC	1.99 ± 0.06	1.34 (chemspider, 2013)
MC	2.90 ± 0.05	3.22 (Chemspider, 2013)
EC	3.61 ± 0.07	4.20 (Chemspider, 2013) (McDougal and Jepson (1998))

[a] K_{OW} = octanol water coefficient (dimensionless) measured at 22 ± 1°C.
[b] N.D. = No data.

II.2.2. Soil water partition coefficients (K_d's)

Sorption experiments were conducted with DRDC-08 soil (NG) or DRDC-09 soil (2,4-DNT, AK, DPA, ATEC, MC, and EC), and the measured K_d values were converted into log K_{oc} values to allow a comparison with reported experimental or predicted values (Table 9). Interestingly, K_d values measured with DRDC-09 soil were not well correlated with the K_{ow} values of each component (r^2 = 0.4042) (Tables 8 and 9, Fig. 16). This suggests that sorption processes of all the investigated components did not only consist of hydrophobic partitioning. In particular sorption of DPA appeared to be markedly high given its K_{ow} value. Aromatic amines are known to sorb on soil by covalent binding between the amino groups and quinone units of the soil organic matter. For instance, using ^{15}N NMR and labeled chemicals, Thorn et al. (2002a, 2002b & 2008) showed that amino-derivatives of TNT or DNTs can bind covalently to soil organic matter through both heterocyclic and non-heterocyclic aminohydroquinone, aminoquinone, and imine linkages. When we studied the fate of 2,4-DNT in marine sediment, we showed that its diamino derivative was immobilized

through both irreversible binding to sediment and oxidative coupling to azo and hydrazo-oligomers (Yang et al., 2008). It is thus likely that processes including covalent binding to organic matter and oxidative polymerization were involved in DPA sorption to soil.

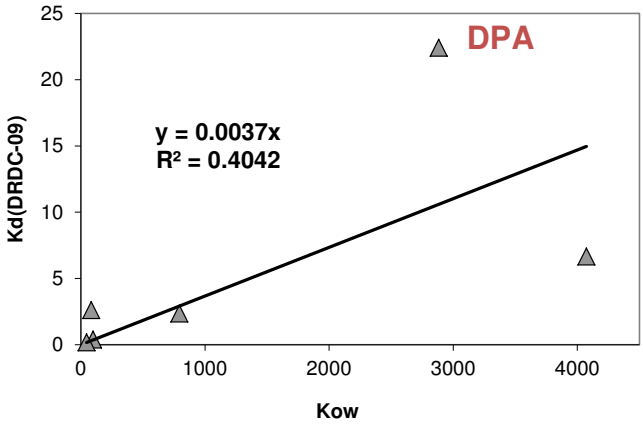


Figure 16. Correlation between K_{ow}'s and $K_{d(DRDC-09)}$'s

No experimental data could be found in the literature for ATEC, MC, and EC K_{oc}'s, but predictions generated using the program PCKOCWIN v1.66 of the US Environmental Protection Agency's EPISuite™ were available in the Chemspider database. As seen in Table 9, close similarities were obtained between experimental measurements conducted by various laboratories whereas discrepancies were noted again between the present measurements and predictions obtained from the Chemspider database. For instance, measured ATEC K_{oc} was 2 orders of magnitude lower than the available predicted value. From the modest value of predicted ATEC log K_{ow} (1.34), and from its high water solubility (> 3,000 mg L^{-1}), it is probable that the high value predicted for ATEC log K_{oc} (3.45) was erroneous. Similarly, even if the same difference was observed between the theoretical K_{oc} values of MC and EC as between the experimental K_{oc} values of MC and EC, the absolute measured K_{oc}'s for MC and EC were more than one order of magnitude lower than the predicted values. An explanation for this gap might come from the theoretical log K_{ow} values which were already overestimated. These findings show that one should be critical when using theoretical physico-chemical parameters and one should not hesitate to conduct real measurements when doubt hangs over predictions.

Table 9. Measured K_d's and log K_{oc} for soluble components of SP 7993, SP Unique, and CMR170

Component	K_d(DRDC-09)	Log K_{oc}	Reported values for log K_{oc}
DPA	22.41	3.03	2.78 (Meylan et al. (1992) 3.28 (chemspider, 2013)
ATEC	0.38	1.26	3.49 (chemspider, 2013)
MC	2.34	2.05	3.30 (Chemspider, 2013)
EC	6.65	2.50	3.87 (Chemspider, 2013)
Component	**K_d(DRDC-08)**	**Log K_{oc}**	**Reported values for log K_{oc}**
NG	0.28	1.89	2.12 (Chemspider, 2013)

[a] K_d = soil water distribution coefficient (L kg^{-1}) measured at 22 ± 1°C.
[b] K_{oc} = Soil water distribution coefficient normalized to the C content.

II.2.3. Conclusion

In the previous section, leaching studies carried out with the three formulations, SP 7993, SP Unique, and CMR170, showed that high concentrations of NG and traces of ATEC, MC, EC, and DPA might be released into the environment if these formulations are contacted with water. Based on the above sorption data, a major fraction of DPA released from SP Unique will be immobilized on soils containing a minimum amount of carbon such as DRDC-09 soil, MC, EC, and NG will sorb partially onto soil, whereas ATEC might be mobile in soil.

III. FATE OF FORMULATIONS SP 7993, SP UNIQUE, CMR170 AND THEIR CONSTITUENTS

The fate studies carried out in this section were limited to the individual constituents of the three formulations that are capable of leaching in water, *i.e.* **NG, ATEC, DPA, MC and EC**. Biotic and abiotic degradations of DPA, ATEC, and MC were measured in DRDC-09 soil in order to predict the stability (or degradability) of these chemicals once they are released in soils typical of DRDC training ranges (Hawari et al., 2012). Before this, biotic and abiotic degradation of NG had been studied in DRDC-08 soil (Hawari et al., 2010). In the present work, results previously obtained with the other four components will be summarized to allow the reader having a general view of the three new formulations under investigation. Then, we will report the fate of EC assessed under various environmental conditions.

III.1. Fate of NG in soil and water

Stability of NG in soil and water was previously investigated at NRC (Hawari et al., 2010) and is thus not detailed in the present report. If thorough data are of interest to the reader, the latter is invited to consult the original report (Hawari et al., 2010) and published work (Saad et al., 2010; Halasz et al., 2010).

In brief, when stirring for more than 3 months NG in suspensions of biologically active DRDC-08 soil, NG was found to be highly persistent thus suggesting a low susceptibility to both microbial and hydrolytic degradation in DRDC-08 soil. In addition, NG was not well retained by DRDC-08 soil.

NG is an oxidized chemical, and as such is sensitive to reduction. Under highly reducing conditions induced by zerovalent nanoparticles, NG was found to fully (100%) degrade to the two benign products, glycerol and ammonium (Saad et al., 2010). When heated under alkaline conditions, NG was found to degrade into nitrite,

nitrate and various oxidized products of glycerol or ethylene glycol (Halasz et al., 2010).

Table 10. Measured degradation rates and products for NG

Matrix / Stress	Transformation rate (d^{-1})	Products
Water / neutral pH	~ 0	None
DRDC-08 Soil suspension / Non-sterile	~ 0	None
Water / pH 9 / 50°C		NO_2^-, NO_3^-, $HCOO^-$, $CH(O)CH(OH)CH(O)$, $CH_2(OH)COOH$
Water/ Fe^0	331	Glycerol, NH_4^+

III.2. Fate of DPA in soil and water

Although DPA has been used in America as plant growth regulator on harvested apples (USEPA, 1998), it belongs to the third European Union (EU) list of priority pollutants, and as such should be carefully investigated for its environmental fate and impact. Abiotic and biotic degradation of DPA has only been investigated to a small extent (Drzyzga, 2003). DPA was found to be stable towards hydrolysis at pH values of 5, 7, and 9 (USEPA, 1998). On the contrary, it degraded fast under irradiation at 313 nm, pH 7.0, and 25°C with carbazole and tetrahydrocarbazole as main products (Mallon et al., 1988). Under aerobic conditions, DPA biologically transformed into aniline, indole, 4-hydroxydipheylamine, and other unidentified products, as deduced from a study conducted with ^{14}C-labelled DPA (Gardner et al., 1982). Aniline was also identified as a major breakdown product in studies on the microbial degradation of DPA under anoxic conditions (Drzyzga and Blotevogel, 1997). Finally, hydroxylated products of DPA (2-OH-, 3-OH-, 4-OH- and 2,4'-diOH-DPA) were identified in apples treated with ^{14}C-labelled DPA (Kim-Kang et al., 1998).

DPA serves as a stabilizer in M1 formulation, the reference propellant formulation used in the United States. We thus investigated the fate of DPA in soil and water during our previous research work on propellant formulations which included M1 formulation (Hawari et al., 2012). When stirring DPA in an aerobic suspension of biologically active DRDC-09 soil, the amine disappeared at a rate of 0.08 d^{-1}. Degradation of DPA also occurred in sterilized DRDC-09 soil, but at a slower rate (0.01 d^{-1}) thus indicating the prevalence of biological processes over abiotic ones in the transformation of DPA in soil. Disappearance of DPA was accompanied by the formation of at least three products, one more polar and two less polar than the initial DPA. The less polar chemicals were identified by LC/MS as dimers of DPA ($C_{24}H_{20}N_2$, MW 336). Various oxidative dimerization processes have been reported for DPA, e.g. *para*- C-C coupling of two phenyl rings of DPA cation radical ($Ph_2NH^{\cdot+}$) (Orlov et al. 2006, Yang and Bard, 1991), N-N coupling during MS analysis (Ho et al. (1990)), or C-N coupling with tricyclic products formation during photolysis of DPA (Baur and Robinson (1993)). The structures of the dimers biologically formed in DRDC-09 soils were not clearly established but they could correspond to any of the formulae provided in Table 11.

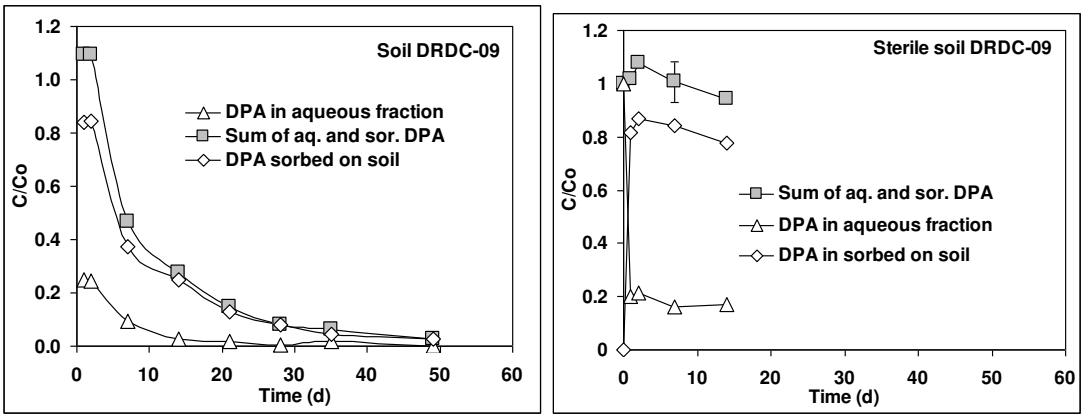
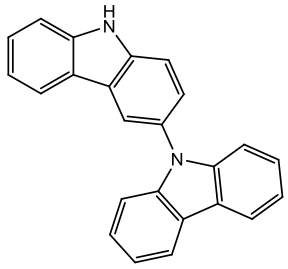


Figure 17. Fate and distribution of DPA in (left) non-sterile and (right) sterile DRDC-09 soil suspensions (Initial concentration of DPA: 20 mg L^{-1})

Table 11. Degradation rates and products for DPA

Matrix / Stress	Transformation rate (d^{-1})	Products
Water / neutral pH	Not measured	Not measured
DRDC-09 Soil suspension / Sterile	0.01	Not analyzed
DRDC-09 Soil suspension / Non-sterile	0.08	**Dimers:** Ph-N(H)-Ph-Ph-N(H)-Ph $(Ph)_2N$-$N(Ph)_2$  (Residues covalently bound to soil OM)

III.3. Fate of ATEC in soil and water

ATEC reaction in water. ATEC was found to react in water upon solubilization to form two main products, **I** and **II**, identified by LC/MS (MW = 258; $C_{12}H_{18}O_6$) as the products of acetic acid elimination, *i.e.* the *cis* and *trans* aconitic acid triethyl esters. Given the highest stability of trans aconitic acid, it is probable that the major product (**II**) corresponds to the trans configuration of the aconitate, as summarized in Table 12. Similar solvolytic elimination was reported to occur when tertiary substrates like ATEC are dissolved in polar solvents such as water (Toteva and Richard, 1996).

ATEC reaction in alkaline water. ATEC remained stable (results not shown), when stirred in slightly acid aqueous solutions (pH 4.7), however, under neutral (pH 6.75) or slightly alkaline (pH 8.3) conditions, ATEC was transformed into the two above aconitate. The reaction was faster and more selective under slightly alkaline

conditions (pH 8.3), with the isomer **II** being the major product formed after 4 days (Fig. 18). At pH 6.75 like at pH 8.3, a third product was also detected in low levels, which was analyzed by LC/MS and found to have a MW of 278 Da matching a molecular formula of $C_{12}H_{20}O_7$. The latter was tentatively identified as the hydrolysis product originating from the cleavage of the acetyl group in ATEC, *i.e.* the triethylcitrate **III** (Table 12).

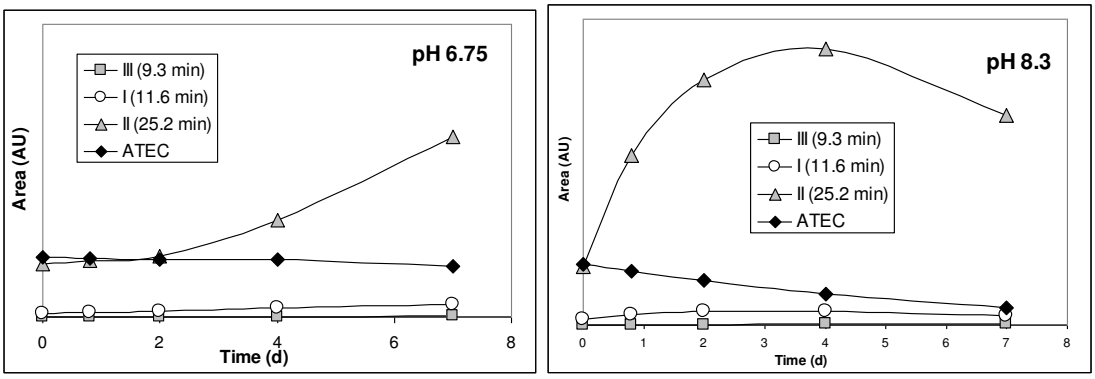


Figure 18. HPLC-UV$_{\lambda=213nm}$ peak area of ATEC and products in aqueous solutions (<u>left</u>: pH 6.75; <u>right</u>: pH 8.3)

ATEC reaction in soil. ATEC was transformed at a rate of 0.88 d^{-1} in non-sterile DRDC-09 soil (Fig. 19). This transformation was mainly biological as suggested by the much slower disappearance measured under sterile conditions (results not shown). In non-sterile DRDC-09 soil, ATEC as well as products **I** and **II** initially present in the aqueous phase degraded in few days (Fig. 19). The disappearance of ATEC was accompanied by the formation of triethylcitrate (**III**), indicative of a microbially induced hydrolysis of the acetyl group. However, triethylcitrate appeared to be a transient product that had also completely disappeared after 1 month in this soil. The triethylcitrate most likely hydrolyzed to citric acid via the intermediate formation of partially acid forms of the citrate but the polar character of the carboxylated products did not allow retaining them on the HPLC column and distinguishing them from the elution peak always present in soil extracts. Citric acid is a common nutriment easily assimilated by microorganisms that, if formed, should not persist in biologically active soils. ATEC products are all summarized in Table 12.

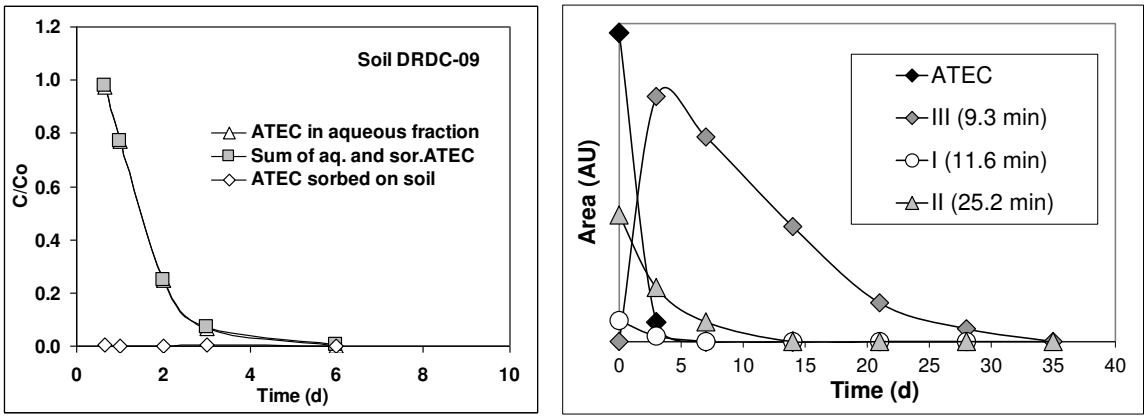
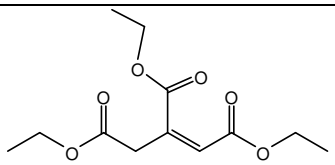
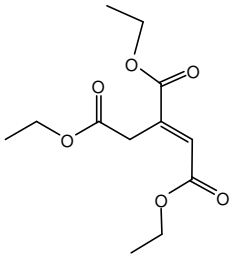
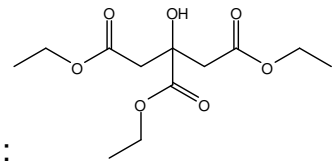
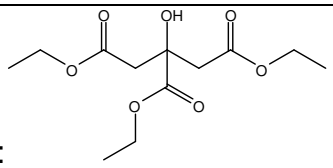


Figure 19. left: Fate and distribution of ATEC in non-sterile DRDC-09 soil suspensions (Initial concentration of ATEC: 5 mg L^{-1}); right: HPLC-$UV_{\lambda=213nm}$ peak area of ATEC and products in soil supernatant

Table 12. Degradation rates and products of ATEC

Matrix / Stress	Transformation rate (d^{-1})	Products
Water / acid	~ 0	None
Water / neutral or alkaline	Varies with pH	**I**:  II:  III: 
DRDC-09 Soil suspension	0.88	*III: 

*Further transformation of III would give citric acid.

III.4. Fate of MC and EC in soil and water

As a constituent of New Green M1 formulation, MC fate was studied in non-sterile DRDC-09 soil for over 3 months (Fig. 20). MC appeared to be stable over this long period of time thus signifying a high stability of the chemical if released in the environment. Approximately 80% of MC remained in the soluble form and 20% was reversibly sorbed to DRDC-09 soil, indicative of a fairly high capacity to migrate in poorly organic soils. In organic soils, however, MC might be well immobilized due to its non-negligible hydrophobicity.

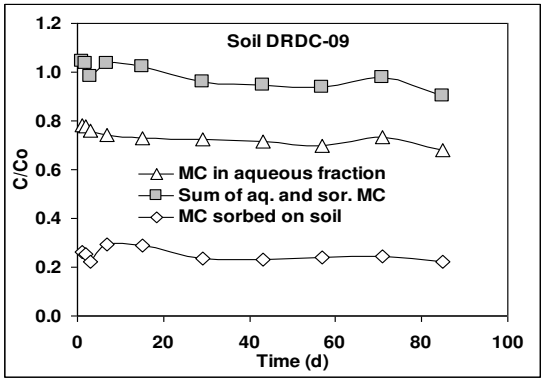


Figure 20. Fate and distribution of MC in non-sterile DRDC-09 soil suspensions (Initial concentration of MC: 56 mg L^{-1})

The fate of EC was also investigated in non-sterile DRDC-09 soil. Replacing methyl groups by ethyl ones in centralite did not affect considerably the stability of the chemical and EC appeared to be almost as stable as MC in non-sterile DRDC-09 soil (Fig. 21). However, the hydrophobicity of EC (log K_{ow} = 3.6) is higher than that of MC (log K_{ow} = 2.9), and this had a direct effect on the bioavailability of the chemical. Approximately 50% of EC was reversibly sorbed on DRDC-09 soil against 20% for MC. In addition, when EC was stirred in an organic rich soil that containing 34% of organic carbon, the chemical was sorbed at more than 95%, thus indicating a low propensity to migrate.

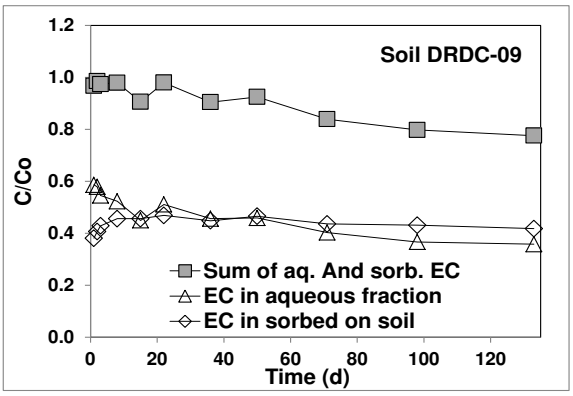


Figure 21. Fate and distribution of EC in non-sterile (left) DRDC-09 soil or (right) suspensions (Initial concentration of MC: 56 mg L^{-1}; EC: 39 mg L^{-1})

III. 5. Conclusion

Five chemicals expected to be released from the three propellant formulations, *i.e.* NG, DPA, ATEC, MC, and EC, were studied in terms of degradability and availability in a soil collected from Valcartier base. The stability of the chemicals in water and soil suspensions was found to increase according to the following order: ATEC < DPA < NG ≈ MC ≈ EC, while their ability to be immobilized on soil was found to follow the order: ATEC < NG < MC < EC < DPA.

By combining the two trends, the following order could be deduced for the availability - and hence migrability or potential risk - of the five chemicals in soil: ATEC < DPA < EC < MC < NG

IV. SUMMARY ON FATE AND TRANSPORT

Dissolution, transport, and transformation of three propellant formulations, SP 7993, SP Unique, and CMR170, and their soluble components, NG, DPA, ATEC, MC, and EC, were investigated in this study and compared to properties obtained with a similar formulation (New Green M1) previously studied at NRC.

The single base formulation SP 7993 was found to be the most stable amongst the three formulations in terms of dissolution, even more stable than formulation New Green M1 which had been identified as the most stable of previously studied formulations. If contacted with a water flow in the environment SP 7993 will only give rise to low aqueous concentrations of ATEC, which will not persist due to the demonstrated high instability of ATEC in soil.

When comparing the two double base formulations, CRM170 appeared to be more stable than SP Unique. Although the MC/graphite coating present in CMR170 might have been responsible for the higher stability this could not be ascertained due to the high NC content of CMR170, which would also decrease dissolution. If released in the environment CMR170 will induce small leakage of NG and MC, whereas SP Unique will induce a 10 times more intense NG leakage along with a small leakage of DPA. NG being relatively stable and highly mobile in Valcartier soil, the ecotoxicity of formulation SP Unique should be carefully investigated.

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PART II. ECOTOXICOLOGICAL ASSESSMENT OF PROPELLANT FORMULATIONS, SP 7993, SP UNIQUE, AND CMR170

I. OBJECTIVES OF THE ECOTOXICOLOGICAL ASSESSMENT

The objective of the present ecotoxicological assessment was to evaluate the adverse effects of three propellant formulations designated as SP 7993, SP Unique, and CMR170, respectively. The detailed composition of the formulations was previously presented in Table 2. Direct contact soil toxicity tests included ryegrass seedling emergence and growth inhibition, as well as earthworm lethality and avoidance tests. Aquatic toxicity was assessed using soil leachates samples and by measuring bioluminescence inhibition in the bacteria *Vibrio fischeri (*Microtox assay) and growth inhibition of freshwater algae.

II. MATERIAL AND METHODS

II.1. Preparation of amended soil

The following soil preparation procedure was used in the soil-water equilibrium and bioavailability studies, as well as in the terrestrial plant and earthworm toxicity tests. Formulation was weighed directly in pre-weighed glass vials and then added to the DRDC2010 reference soil (Table 13). Emptied glass vials were weighed to measure the exact amount of formulation added to the soil. No water or organic solvents was added to dissolve the formulation at this point. Each amended soil treatment was mixed for 24 $\pm$ 2 h using a three-dimensional soil mixer.

Table 13.Selected physico-chemical properties of DRDC2010 control soil

	Particle size distribution			
Soil	% Clay/Silt (< 80μm)	% Sand (>53μm)	Total Organic Carbon (%)	pH
DRDC2010	2.3	97.6	2.00	5.96

Prior to the initiation of the toxicity evaluation, soil-water equilibrium studies were conducted using two concentrations (1000 and 10,000 mg/kg) in order to determine the homogeneity of the energetic materials in soil, and to determine the time to equilibrium (steady-state) period of each energetic material between soil and interstitial water. The soil-water equilibrium preliminary study was conducted over a period of 21 to 42 days. Prior to addition of water to the amended soil, acetonitrile extractions (USEPA Method 8330, 2007) were performed. Each soil treatment was then transferred to a large glass dish and hydrated to 75% water holding capacity with deionized water. Following the addition of water, acetonitrile and interstitial water extractions were performed on the wet soil samples. Soil aliquots were sampled in triplicate at each time point using at least six sampling scoops for each replicate.

Following the soil-water equilibrium study, DRDC2010 soil was amended independently with each formulation using a wide range of concentrations, and deionized water was added according to each terrestrial toxicity test requirement. Acetonitrile extractions and interstitial water extractions were performed on the wet soil samples at the beginning of each toxicity test.

II.2. Preparation of soil leachates

Prior to the preparation of the soil leachates, DRDC2010 soil was treated with acetone to inactivate any residual microorganisms that may interfere with the activity

of the bacteria *Vibrio fischeri* used in the Microtox assay, the freshwater algae *Pseudokirchneriella subcapitata.* An equivalent of 15% (v/w) of acetone was added to the DRDC2010 soil, which was evaporated for 48 h in a chemical hood. Each formulation was then amended directly, as described for the amendment of soil samples (Section II.1). Two concentrations (1,000, and 10,000 mg/kg) of SP 7993, SP unique and CMR170, and two negative controls (no chemical added) were tested. Each amended soil treatment was mixed for 24 ± 2 h using a three-dimensional soil mixer. Following hydration, amended soil samples were left to equilibrate for 7 d (SP 7993), 21 d (SP Unique), and 28 d (CMR170). The two control soils were prepared, and were left to equilibrate for 21 and 28 d. Leachates of the amended soil samples were prepared using the USEPA Method 1312 (USEPA, 1997). Each amended soil was leached with acidified deionized water (pH 4.5 ± 0.1). The acid solution used was composed of 60% H_2SO_4 and 40% HNO_3. The extraction was performed by mixing 7.5 ± 0.5 g of dry soil with 140 mL of extraction liquid for 18 ± 2 h, at 22°C ± 3°C with mixing at 30 ± 2 rpm. The soil suspension was then filtered through a 0.5 μm Millex-HV cartridge using a 20-mL syringe. At the end of the process, the pH was measured, and when needed, adjusted between pH 6.5 and 8.5 in order to comply with test conditions of Microtox and freshwater algae assays.

II.3. Analytical measurement of energetic materials in soil samples

II.3.1. Extraction of soil samples

Energetic materials from soil samples individually amended with each propellant formulation were extracted using the USEPA Method 8330A (USEPA, 2007). Briefly, soil aliquot, acetonitrile solvent, and internal standard (1,3-dinitrobenzene; 1,3-DNB) were vortexed for 1 min, and then sonicated in the dark for 18 ± 2 h at 20°C. Five mL of the sonicated sample was transferred to a new tube, to which 5 mL of 5 g/L $CaCl_2$ solution was added. Supernatant was filtered through a 0.45 μm Millex-HV cartridge. Soil extracts were analyzed and quantified using the

appropriate HPLC method (See Part I - section I.1.2). The HPLC detection limits, provided in Part I - section I.1.2., correspond to quantification limits in soil of 25 mg/kg for ATEC, of 1 mg/kg for NG, and 0.05 mg/kg for diphenylamine (DPA), ethyl centralite (EC), and methyl centralite (MC). Extraction was repeated if the internal standard recovery was less than 90%.

II.3.2. Extraction of soil interstitial water

Soil interstitial water was extracted from soil using a centrifugation method (Lock and Janssen, 2003). Aliquots of 8 to 12 g soil were placed in a Sera-Separa® (Evergreen Scientific, Los Angeles, CA, USA) polypropylene tube fitted with an HPDE filter in its base. The Sera-Separa® was inserted in a conical polypropylene tube and centrifuged at 3000 rpm for 45 min at 20°C. The collected interstitial water was filtered with a 0.45 µm Millex-HV cartridge and was mixed with acetonitrile (1:1, v:v). Concentrations of the different energetic materials measured in the soil interstitial water samples were determined by HPLC.

II.4. Toxicity tests

II.4.1. Microtox assay

The Microtox standardized toxicity test (Environment Canada, 1992a), which uses the luminescent marine bacterium *Vibrio fischeri* was performed using the soil leachates. Endpoint of this acute toxicity test used for aqueous solutions is the measurement of bioluminescence reduction. The bioluminescence emitted by *V. fischeri* was first measured after 10 min of incubation after which, the aqueous contaminant was added to the bacterial suspension. The bioluminescence reduction was determined after a 15-min exposure to the contaminant, and was expressed as the average percentage of light emission inhibition compared to the negative control.

The test was done in triplicate and each soil leachates were salted (2% NaCl) prior to testing. The negative control was 2% NaCl and the reference toxicant was phenol.

II.4.2. Green algae inhibition assay

The chronic toxicity test measuring the growth inhibition of freshwater algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum)* performed using a 96-h exposure, as recommended by the Canadian Ministry of Environment (Environment Canada, 1992b). *P. subcapitata* was exposed for 96 h to different concentrations of soil leachates in a 96-well microplate and under continuous lighting at 24 ± 2°C. Following exposure to the water samples, the number of algae cells was measured using a cell counter, and the percentage of growth inhibition was calculated in comparison to the negative control. The test was done in triplicate, the negative control was deionized water, and the reference toxicant was zinc sulfate.

II.4.3. Terrestrial plant seedling emergence and growth inhibition test

Terrestrial plant toxicity tests were performed according to ASTM (2002) and USEPA (1989b) methods. Perennial ryegrass *Lolium perenne* Express was obtained from Pickseed Canada Inc. (St-Hyacinthe, Quebec, Canada). Twenty seeds were sown in 10-cm wide pots containing 200 g dry soil, and incubated in sealed plastic bags to maintain soil moisture for the duration of the test (USEPA, 1989b). Plant toxicity tests were performed in a temperature and light controlled growth chamber. Seeds were incubated in the dark for the first two days, and then exposed to a diurnal photoperiod cycle afterwards. 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. The luminosity level measured weekly using a photometer was readjusted when needed. Nominal concentrations of the propellant formulations were 0, 30, 100, 300, 1000, 3000, and 10,000 mg/kg. Control treatments included negative (water) and

positive controls (boric acid at concentrations of 0, 50, 80, 110, 150, and 200 mg/kg). DRDC2010 soil samples were hydrated to 75% of their water holding capacity and all treatments were carried out using three replicates. Seedling emergence was determined after 7 d, whereas shoot growth (fresh mass and dry mass) was determined after 19 d. 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 shoot tissue at 70°C for 24 h. The EC_{50} and EC_{20} (concentrations which inhibit 50% and 20% of the seedling emergence or plant growth), the lowest observable effect concentration (LOEC), and no observable effect concentration (NOEC) were measured using the linear interpolation model (TOXCALC v5.0, 1997).

II.4.4. Earthworm lethality test

The earthworm lethality test is an acute toxicity test that measures the effect of contaminants in soil on the survival of earthworms. Adult *Eisenia andrei* earthworms were obtained from Carolina Biological Supply (Burlington, NC, USA) and were used to establish the initial laboratory cultures. Animals were maintained at room temperature in earthworm bedding (Magic Products, Amherst Jct., WI, USA) supplemented with dry cereal (Magic Worm Food, Magic Products). Earthworms (with a clitellum and weighing from 325 to 552 mg) were used for the toxicity studies. Earthworms were acclimated in an environment-controlled incubator under a 16 h-light and 8 h-dark photoperiod cycle with a mean light intensity of 800 ± 400 lux, mean temperature of 20 ± 1°C, and relative humidity of 70% for 24 h in a non-contaminated soil (DRDC2010) prior to the experiment. Nominal concentrations of the propellant formulations were 0, 30, 100, 300, 1000, 3000, and 10,000 mg/kg. Ten earthworms were placed into 1-L glass jar (*i.e.*, test unit), previously filled with 200 g of test soil (dry weight). Test units were prepared in triplicate for each concentration of formulation. Each test unit was then covered by a perforated cap and a chemically inert porous geotextile (Landscape Fabric, Select). After 14-d exposure, surviving earthworms were counted, rinsed, and weighed. Missing earthworms, due to rapid tissue decomposition, are considered dead. To be a valid

test, the 14-d earthworm survival rate in the negative control soil must be at least 90%. This average survival rate is based on the mean survival across all replicates for the negative control treatment. Sensitivity of the assay was measured using potassium chloride amended in OECD artificial soil as a reference toxicant. Concentrations used were 2000, 4000, 6000, 8000, and 10,000 mg/kg. Soils were hydrated to 75% of their water holding capacity. Potassium chloride solutions were prepared in water in different concentrations to obtain the targeted concentrations in soil. The average of calculated EC_{50} value for potassium chloride in OECD soil (USEPA, 1989a) was in between 5500 and 7000 mg/kg. Each calculated EC_{50} value was in the warning and confidence limits of the laboratory quality control chart.

II.4.5. Earthworm avoidance test

The acute avoidance test is a sensitive sub-lethal screening test that measures the avoidance behavior of earthworms during 48 h (Environment Canada, 2007). Tests were done in stainless steel circular containers with a central chamber and six pie-shaped interconnecting compartments. Three amended soil samples were distributed alternately with three negative control soil samples into the stainless steel circular containers. This test is based on the earthworm's ability to detect and avoid a toxic concentration in soil. The avoidance behavior of earthworms is considered with a criterion of > 60% avoidance response.

Earthworms were acclimated to laboratory conditions in the DRDC2010 soil for 24 h before testing. Selected *E. andrei* used in tests were between 303 and 649 mg. The culturing method was the same as that in the earthworm acute lethality test (section 2.5.5). Earthworms were neither fed nor disturbed during the 48-h acute avoidance test. Each compartment was filled with 300-350 mL of test soil or control soil (approximately 450-470 g at 75% of the soil WHC) and was separated by a stainless plate to avoid transfer of the worms during counting. Soil treatments and negative control soils were prepared, homogenized, and hydrated 7 d prior the initiation of the test. Nominal concentrations used were 30, 300, 1000, 3000, and

10,000 mg/kg for SP 7993 formulation, and 10, 100, 300, 1000, and 10,000 mg/kg for SP Unique and CMR170 formulations. Three units were used per concentration, for a total of 15 avoidance units. The units were then covered with a fitted lid. Each test unit contained ten *E. andrei* adults. Test units were maintained with no disturbance at 20 ± 2°C in an environmentally-controlled growth chamber, under continuous darkness for 48 ± 2 h. Soil moisture content and pH were measured in each control and contaminated soil samples. After the 48-h exposure period, each compartment was carefully emptied and worms were counted. To be valid, earthworm survival percentage in each test unit containing negative control and test soils must be at least 90% at the end of the test.

Reference toxicant for the acute avoidance test is boric acid amended in OECD artificial soil (USEPA, 1989a). Concentrations used in these soils were 400, 800, 1600, 2400, and 4000 mg/kg. Soils were hydrated to 75% of their water holding capacity. A volume of 350 mL of soil corresponds to about 225 g of OECD soil. Boric acid solutions were prepared in water in different concentrations to obtain the targeted concentrations in soil. Contaminant was added 24 h before the initiation of the test and was well homogenized. The negative control soil sample was also hydrated and homogenized 24 h prior to the initiation of the test. The average of calculated EC_{50} value for boric acid in OECD soil was between 1519 and 2285 mg/kg. Each calculated EC_{50} value complied with this quality control criterion.

III. ANALYTICAL MEASUREMENTS OF THE ENERGETIC MATERIALS CONTAINED IN THE THREE SELECTED PROPELLANT FORMULATIONS

III.1. Soil-water equilibrium and bioavailability of SP 7993 formulation

As determined by the NRC Analytical and Environmental Chemistry laboratory, the SP 7993 formulation is composed of 8.0% ATEC, and 1.39% ethyl centralite (EC) (See Table 2). The remaining 89.6% is composed of non-extractable Grade C nitrocellulose.

Full recoveries of ATEC and EC in the acetonitrile extracts were obtained at the beginning of the soil equilibrium study, with a slight decrease of recovery to 73% in the 1000 mg/kg SP 7993 soil treatment after 14 d, and to 87-89% in the 10,000 mg/kg SP 7993 soil treatment after 7 d respectively (Figures 22 and 23). Full recoveries of ATEC and EC were then obtained after 21 d and 14 d of equilibrium, respectively.

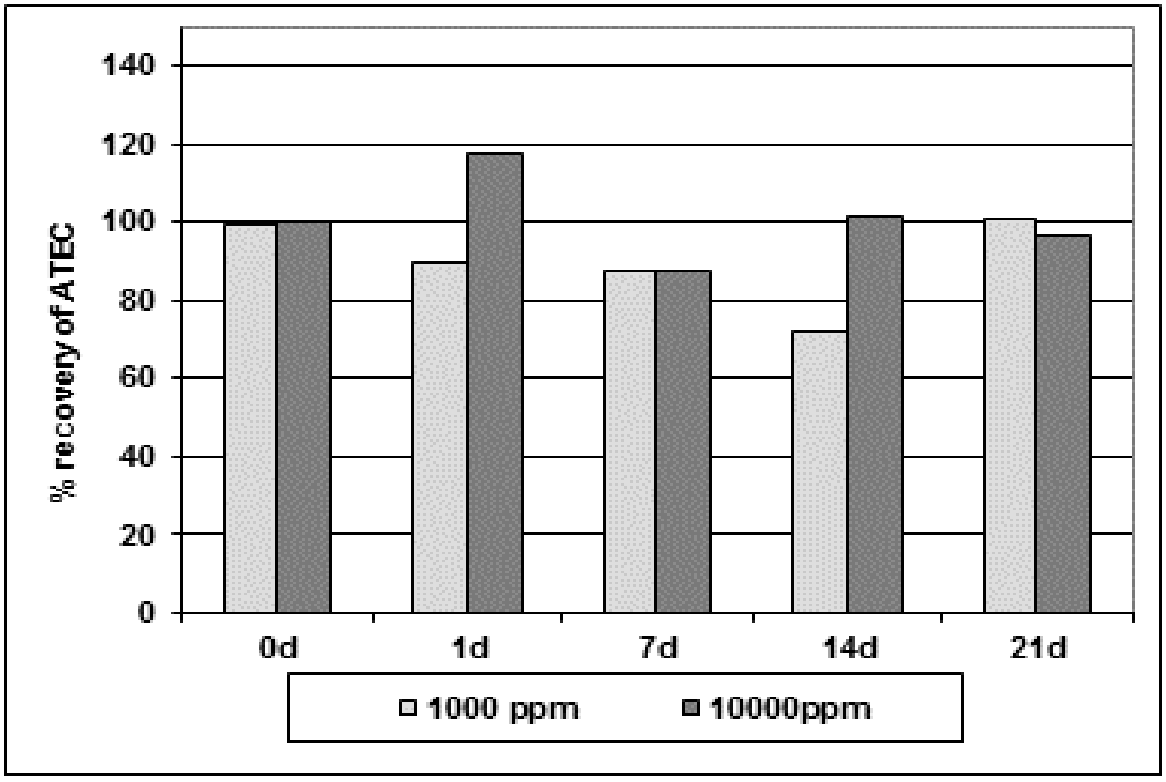

Figure 22. Recovery of ATEC during the SP 7993 equilibrium study using acetonitrile extraction (n = 3).

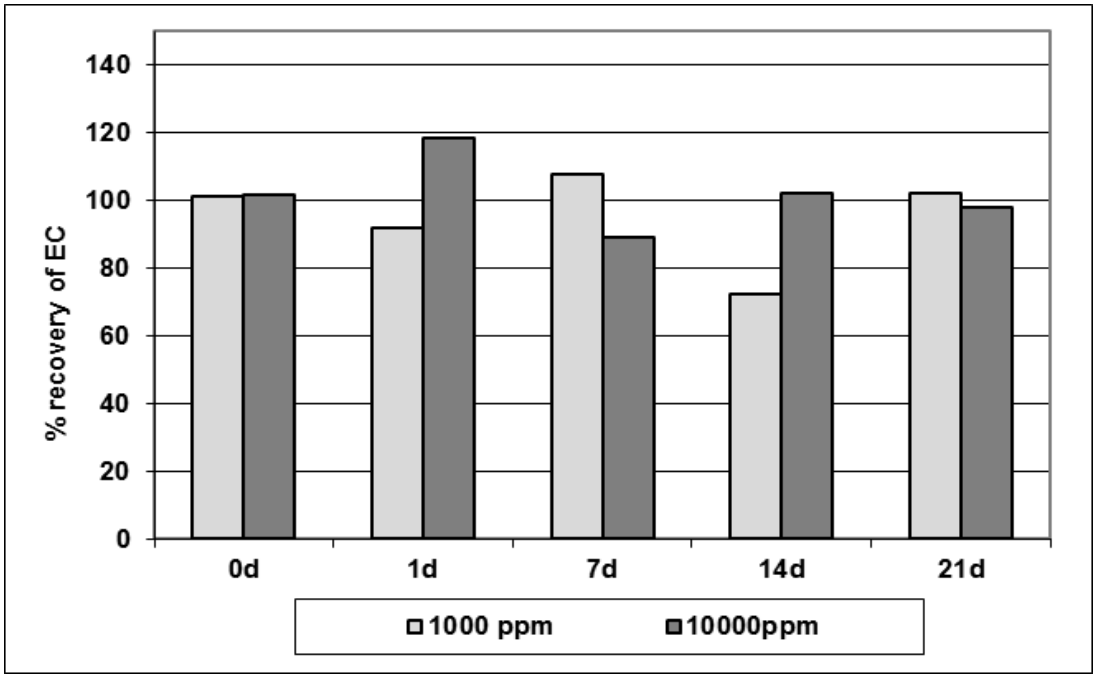

Figure 23. Recovery of ethyl centralite (EC) during the SP 7993 equilibrium study using acetonitrile extraction (n = 3).

The water-soluble fractions of ATEC and EC were measured using the soil interstitial water extraction method (Figures 24 and 25). Because of its low concentration and its relatively low leachability from the SP 7993 formulation (Table 4), no ATEC was measured in the 1000 mg/kg SP 7993 soil treatment, whereas 7 to 12 mg/L of ATEC was measured in the 10,000 mg/kg SP 7993 soil treatment during the first week of equilibrium, and then, none was detected at and after 14 d (Figure 24). Leachable concentration of ATEC from the SP 7993 formulation was 0.514 mg / 50 mL (Table 4), which corresponds to 10.28 mg/L, and which is comparable to the values of 7 to 12 mg/L measured in water-soluble fractions at the 10,000 mg/kg SP 7993 soil treatment.

Some ethyl centralite (EC) was leached out from the SP 7993 formulation, with the highest concentrations of 0.23 and 0.50 mg/L measured after one day of equilibrium in the 1000 and 10,000 mg/kg SP 7993 soil treatments, respectively. Leachable concentration of EC from the SP 7993 formulation was 0.051 mg / 50 mL (Table 4), which corresponds to 1.02 mg/L. Afterwards, EC concentrations stabilized

at 0.04 and 0.2 mg/L after 7 d of equilibrium in the 1000 and 10,000 mg/kg SP 7993 soil treatments, respectively, which is below the measured leachable concentration of 1.02 mg/L.

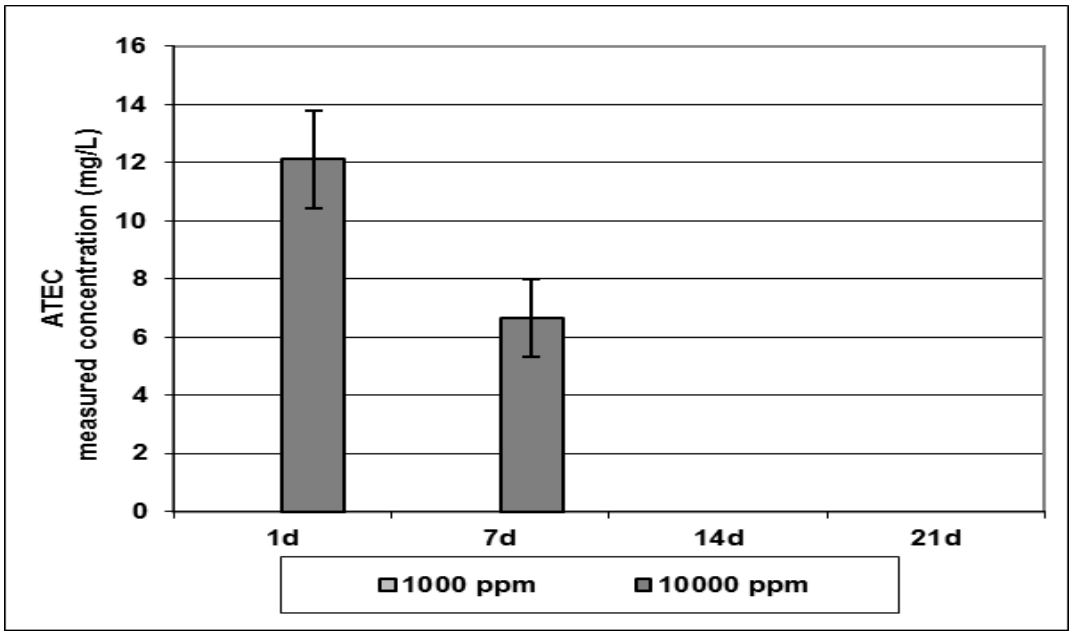

Figure 24. ATEC concentrations measured in the interstitial water during the equilibrium study of the SP 7993 formulation in soil (n = 3).

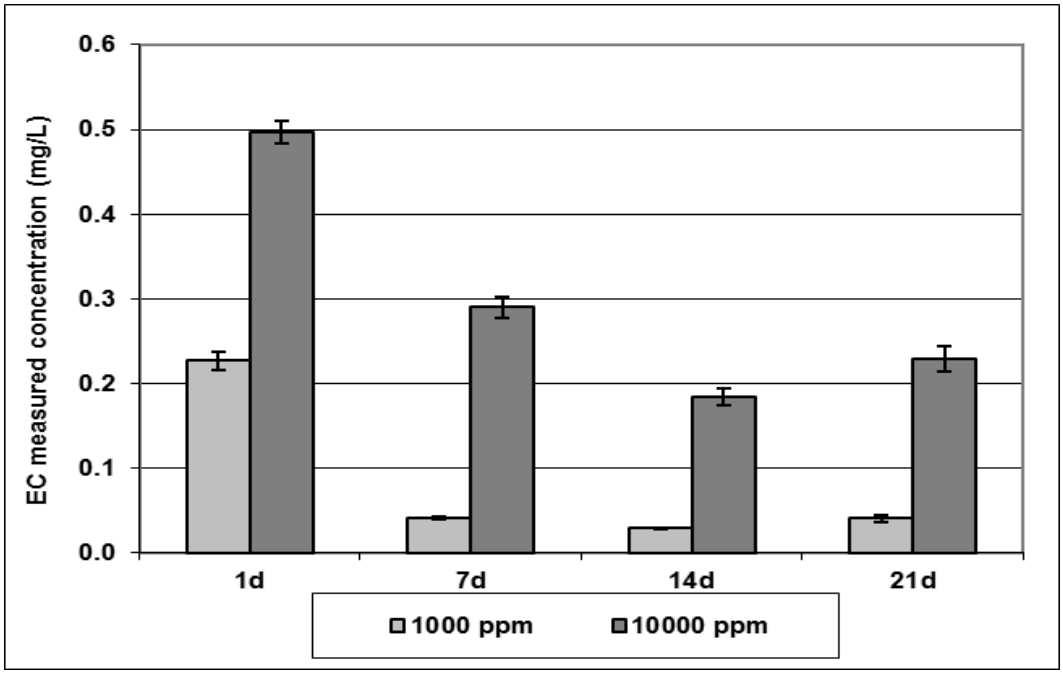

Figure 25. EC concentrations measured in the interstitial water during the equilibrium study of the SP 7993 formulation in soil (n = 3).

Considering that ATEC and EC were fully extractable in acetonitrile at the beginning of the soil equilibrium study, that no water-soluble fraction of ATEC was measured after 7 d of equilibrium, and that the concentrations of water-soluble EC stabilized after 7 d of equilibrium, it was decided to hydrate and equilibrate the SP 7993 soil samples at room temperature for 7 d prior to the initiation of the toxicity tests.

III.2. Soil-water equilibrium and bioavailability of SP Unique formulation

As determined by the NRC Analytical and Environmental Chemistry laboratory, the SP Unique formulation is composed of 20.6% NG, 0.20% EC, and 0.63% DPA (Table 2). The remaining 78.6% is composed of non-extractable nitrocellulose and rosin.

Full recoveries of NG, EC, and DPA in the acetonitrile extracts were immediately obtained after one day of equilibrium and remained stable throughout the 21-d equilibrium study (Figures 26-28).

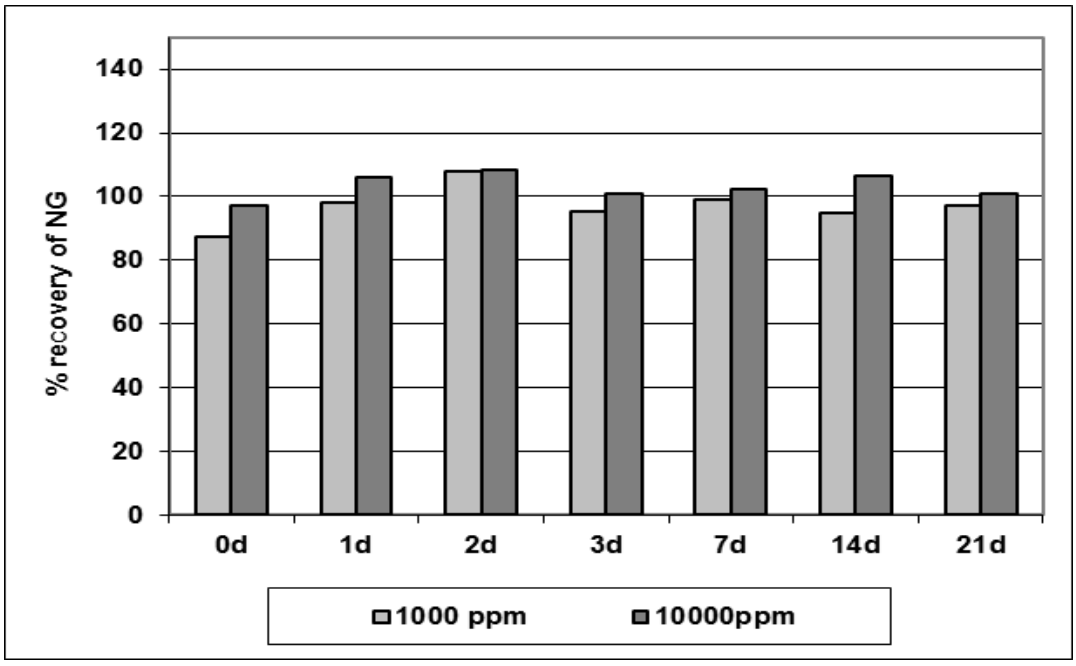

Figure 26. Recovery of nitroglycerin (NG) during the SP Unique equilibrium study using acetonitrile extraction (n =3).

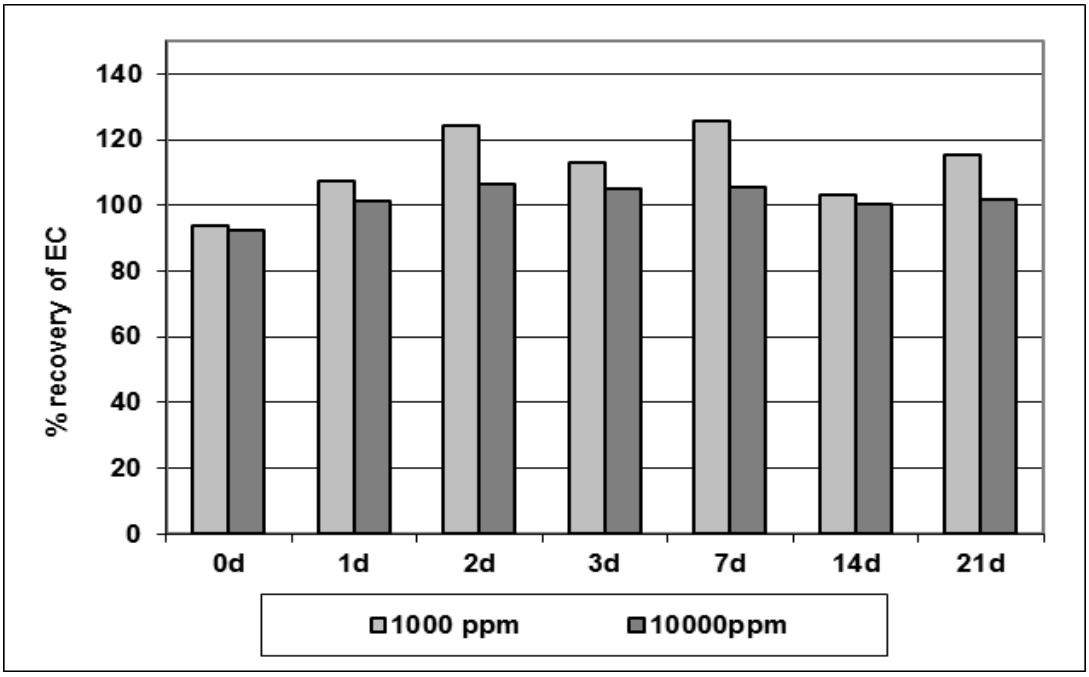

Figure 27. Recovery of ethyl centralite (EC) during the SP Unique equilibrium study using acetonitrile extraction (n=3).

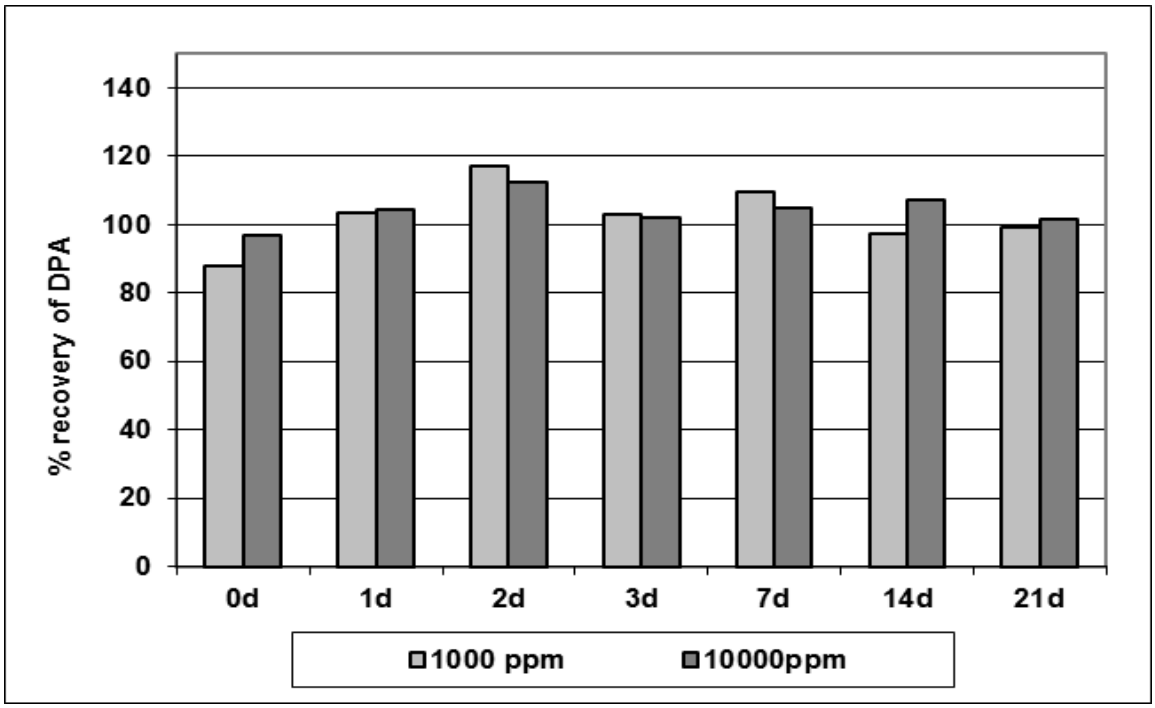

Figure 28. Recovery of diphenylamine (DPA) during the SP Unique equilibrium study using acetonitrile extraction (n=3).

The water-soluble fractions of NG, EC, and DPA were measured using the soil interstitial water extraction method. Concentrations of soluble NG gradually increased throughout the 21-d equilibrium study from 147 to 286 mg/L, and from 540 to 700 mg/L in the 1000 and 10,000 mg/kg SP Unique soil treatments, respectively (Figure 29). The same pattern of gradual concentration increase over time was measured for DPA, with concentrations increasing from 0.01 to 0.1 mg/L, and from 0.1 to 0.3 mg/L in the 1000 and 10,000 mg/kg SP Unique soil treatments, respectively (Figure 30). Ethyl centralite (EC) had a different dispersion pattern, with low but stable measurable concentrations of 0.04 mg/L in the 1000 mg/kg SP Unique soil treatment, and variable but relatively low concentrations of 0.2 mg/L to 0.3 mg/L in the 10,000 mg/kg SP Unique soil treatment (Figure 31).

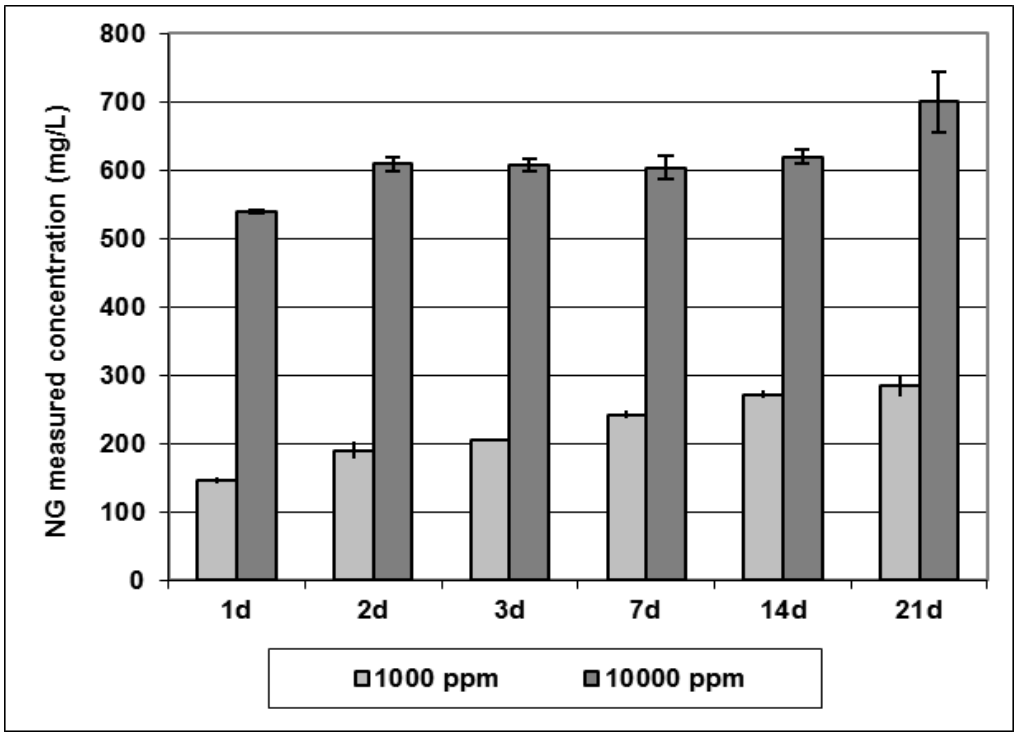

Figure 29. NG concentrations measured in the interstitial water during the equilibrium study of the SP Unique formulation in soil (n =3).

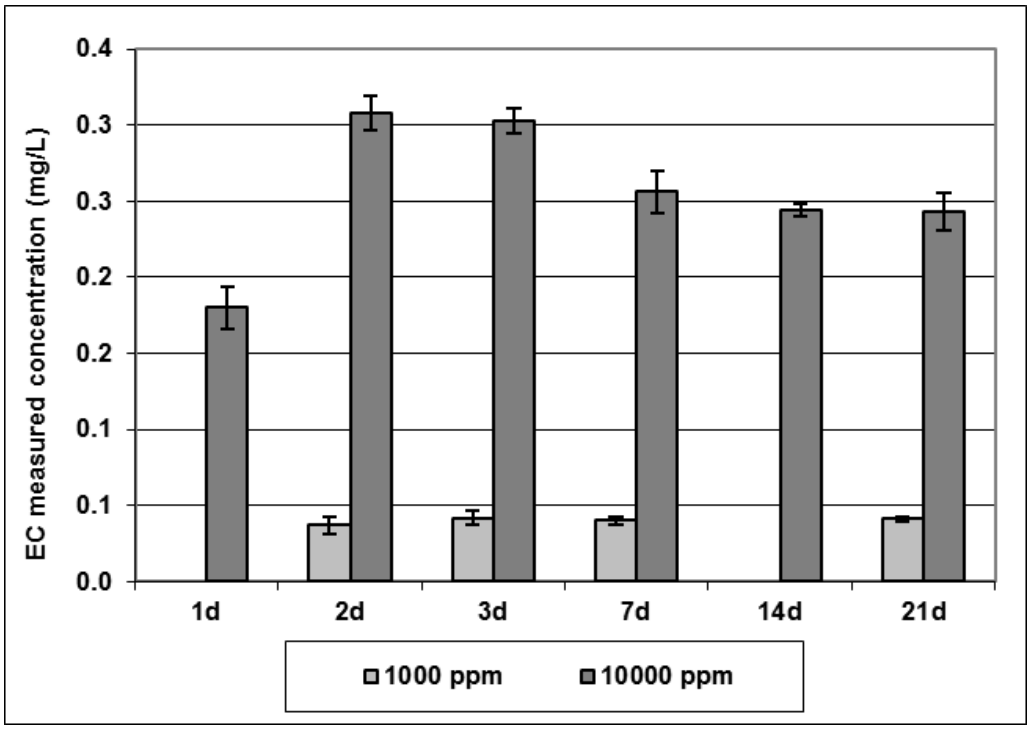

Figure 30. EC concentrations measured in the interstitial water during the equilibrium study of the SP Unique formulation in soil (n=3).

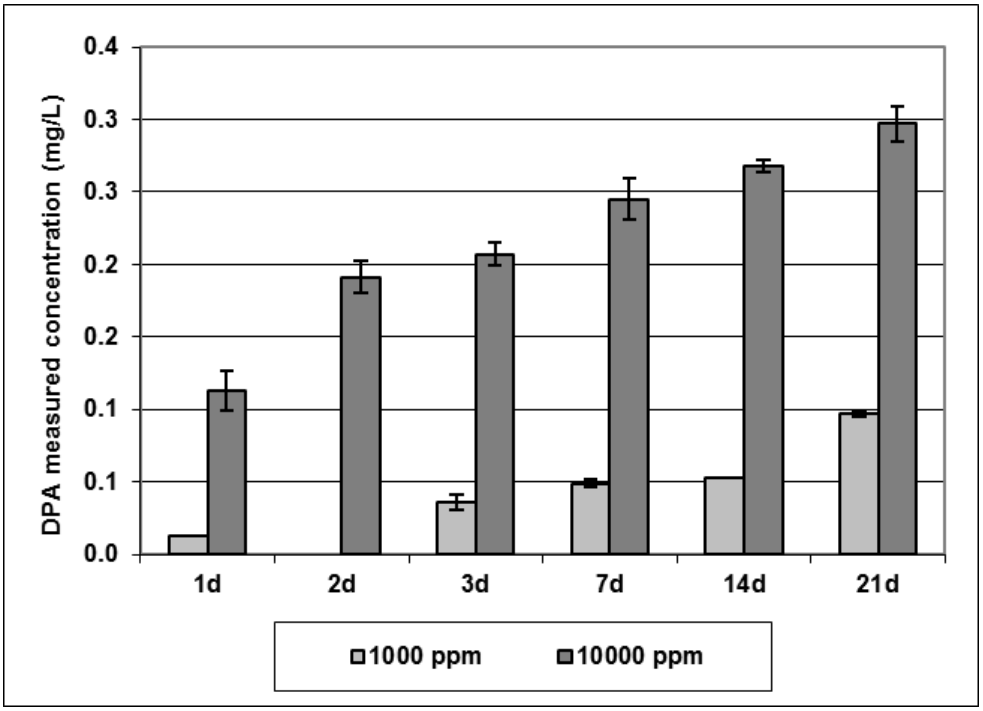

Figure 31. DPA concentrations measured in the interstitial water during the equilibrium study of the SP Unique formulation in soil (n=3).

Considering that NG, EC, and DPA were fully extracted in acetonitrile right from the beginning of the soil equilibrium study, and that the concentrations of water soluble NG and of DPA increased throughout the 21-d equilibrium study, it was decided to hydrate and equilibrate the SP Unique soil samples at room temperature for 21 d prior to the initiation of the toxicity tests.

III.3. Soil-water equilibrium and bioavailability of CMR170 formulation

As determined by the NRC Analytical and Environmental Chemistry laboratory, the CMR170 formulation is composed of 7.5% NG, 0.93% EC, and 2.09% MC (Table 2). The remaining 89.4% is composed of non-extractable Grade C nitrocellulose.

Full recovery of NG, EC, and MC in the acetonitrile extracts was obtained at the beginning of the CMR170 soil equilibrium study, with a slight decrease of recovery to 79%, 82%, and 80%, respectively, after 14 d in the 1000 mg/kg CMR170 soil treatment (Figures 32-34). Afterwards, full recovery (96 % and higher) was reached until the end of the 42-d equilibrium study. In the 10,000 mg/kg CMR170 soil treatment, full recovery of NG, EC, and MC in the acetonitrile extracts was also obtained at the beginning of the CMR170 soil equilibrium study, with a decrease to 90-92% after 28 d, and a decrease to 70-73% after 42 d.

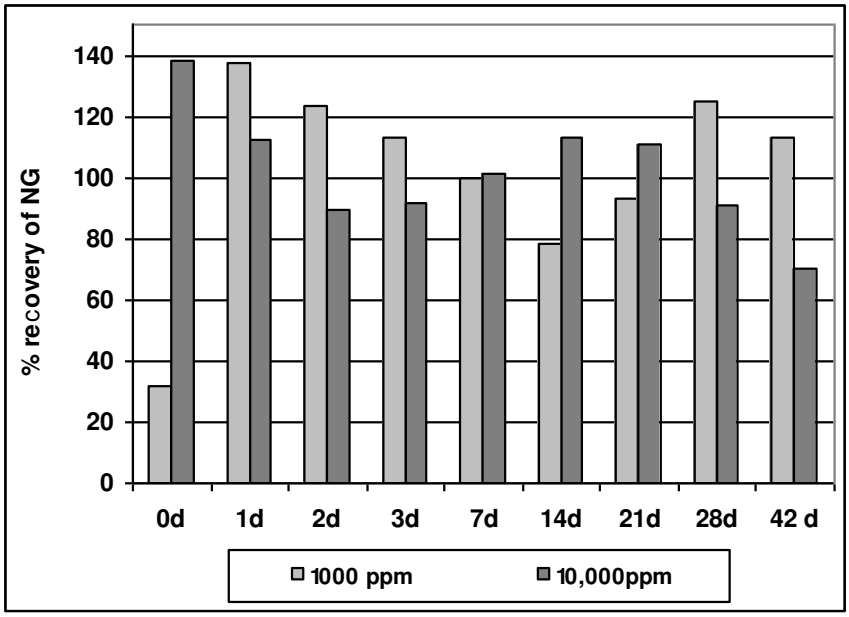

Figure 32. Recovery of nitroglycerin (NG) during the CMR170 equilibrium study using acetonitrile extraction (n = 3).

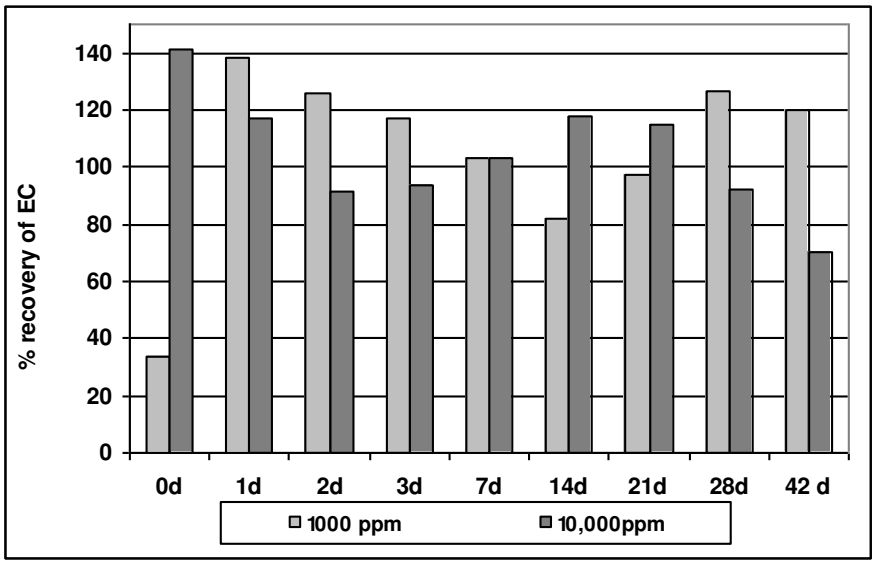

Figure 33. Recovery of ethyl centralite (EC) during the CMR170 equilibrium study using acetonitrile extraction (n = 3).

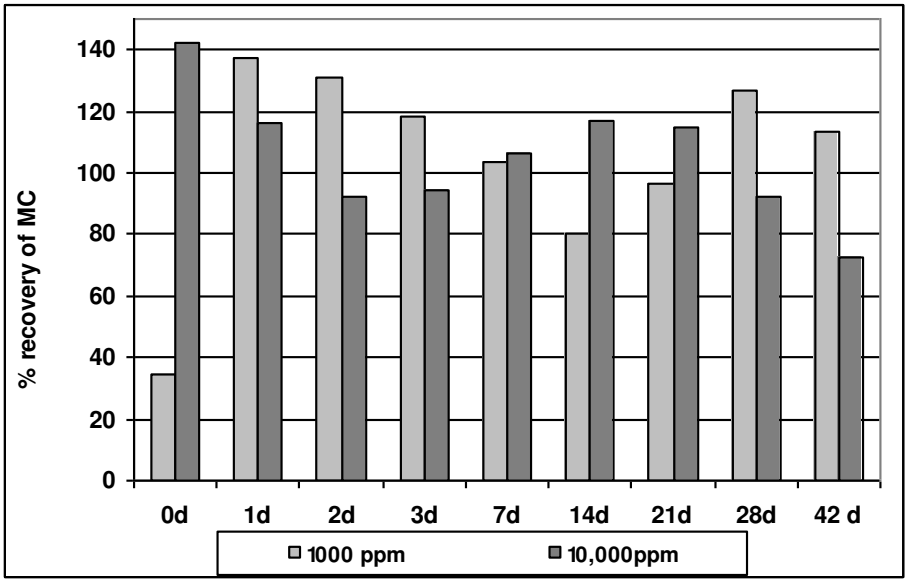

Figure 34. Recovery of methyl centralite (MC) during the CMR170 equilibrium study using acetonitrile extraction (n = 3).

The water-soluble fractions of NG, EC, and MC in the CMR170 formulation were measured using the soil interstitial water extraction method (Figures 36-38). Concentrations of soluble EC and MC were relatively stable and at low levels, reflecting their low leachability from the CMR170 formulation. Leachable concentrations of EC and MC were 0.014 and 0.30 mg/ 50 mL (Table 6), respectively, which corresponds to 0.28 and 6.0 mg/L, respectively. EC concentration was below the detection limit in the 1000 mg/kg CMR170 soil treatment, and ranged between 0.09 and 0.19 mg/L in the 10,000 mg/kg CMR170 soil treatment (Figure 36), which is just below the 0.28 mg/L concentration measured in the leachability study at 25°C (Table 6). MC concentrations ranged between 0.3 and 0.6 mg/L in the 1000 mg/kg CMR170 soil treatment, and between 2.2 and 5.1 mg/L in the10,000 mg/kg CMR170 soil treatment (Figure 37), which is just below the 6.0 mg/L concentration measured in the leachability study at 25°C (Table 6).

NG gradually increased throughout the 42-d equilibrium study, with a very steep increase at 28 d, reaching concentrations of 4.9 mg/L in the 1000 mg/kg CMR170 soil treatment, and of 52 mg/L in the 10,000 mg/kg CMR170 soil treatments after 42

d of equilibrium, respectively (Figure 35). The measured concentration of NG in the leachability study was 41.8 mg/L at 25°C (Table 6), which is slightly lower than what we measured in the 10,000 mg/kg CMR170 soil treatments after 42 d of equilibrium.

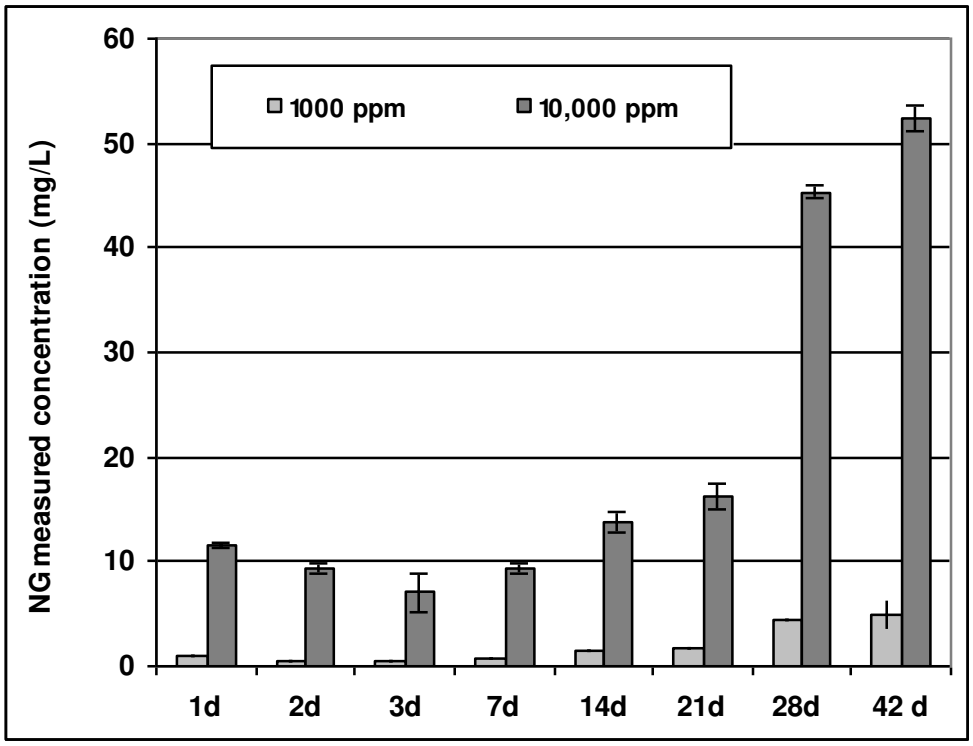

Figure 35. NG concentrations measured in the interstitial water during the equilibrium study of the CMR170 formulation in soil (n = 3).

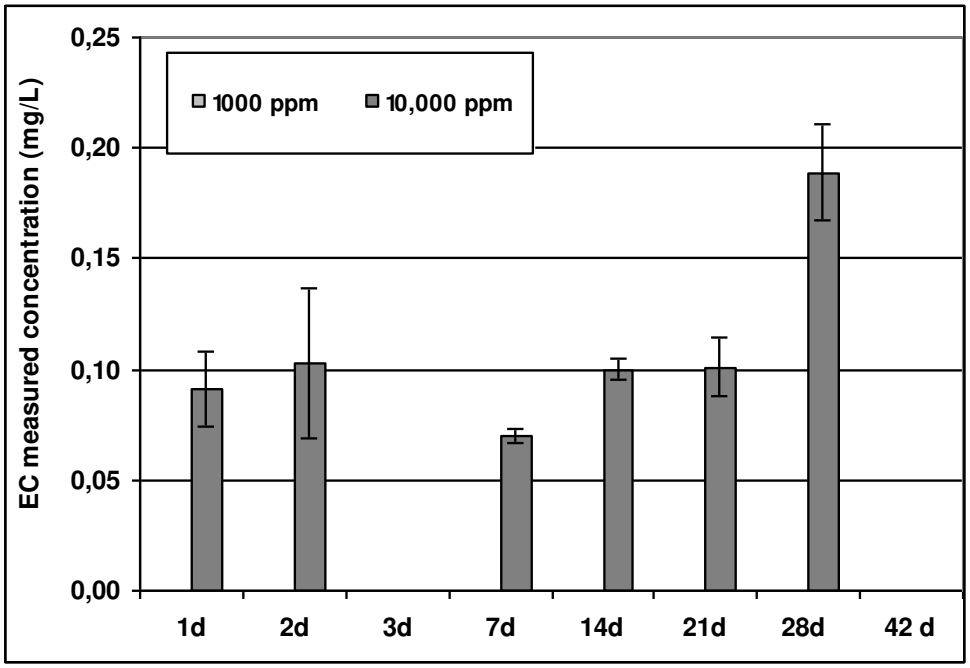

Figure 36. EC concentrations measured in the interstitial water during the equilibrium study of the CMR170 formulation in soil (n = 3).

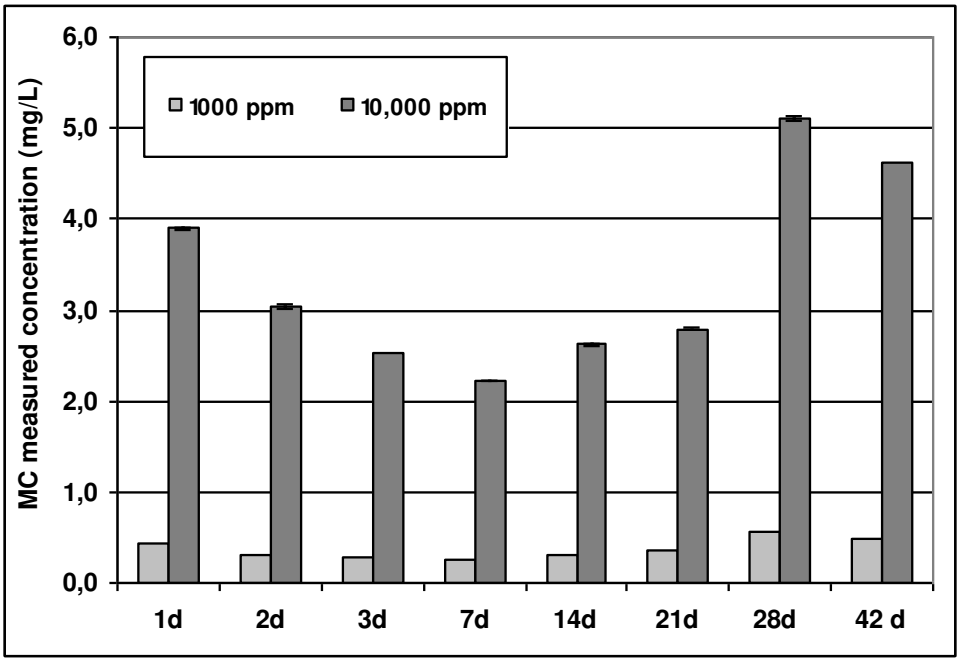

Figure 37. MC concentrations measured in the interstitial water during the equilibrium study of the CMR170 formulation in soil (n = 3).

Considering that NG, EC, and MC were fully extracted in acetonitrile right from the beginning of the soil equilibrium study, that the concentrations of water soluble EC and MC were relatively stable throughout the 42-d equilibrium study, and that water soluble concentrations of NG gradually increased over the first 21 d of the equilibrium study, with a very steep increase at 28 d, it was decided to hydrate and equilibrate the SP Unique soil samples at room temperature for 28 d prior to the initiation of the toxicity tests.

III.4. Summary of the soil-water equilibrium and bioavailability equilibrium studies

Table 14 summarizes the equilibrium period needed to obtain a relative homogeneous distribution of the energetic substances in soil amended with the different formulations. Therefore, prior to the initiation of the toxicity tests, soil samples will be amended with the different formulations, deionized water will added according to the toxicity tests requirements and soil samples will be kept at room temperature, in the dark, for the respective period determined in the preliminary equilibrium studies (Figures 22-37).

Table 14. Summary of the equilibrium study of the different explosive formulations in soil and sediment samples

Formulation	Equilibrium period (days)
SP 7993	7
SP Unique	21
CMR170	28

III.5. Quantification of energetic materials contained in the SP 7993-amended soil used for the terrestrial plant growth and earthworm lethality tests

Results of the chemical analyses of DRDC2010 soil amended with SP 7993 measured at the beginning of the terrestrial plant growth and earthworm lethality tests are presented in Tables 15 and 16. Total extractable concentrations of ATEC were between 3 and 716 mg/kg, and between 0.7 and 123 mg/kg for EC, in the 30, 100, 300, 1000, 3000, and 10,000 mg/kg SP 7993 soil treatments. Respective recovery percentages of ATEC and EC were between 38 and 90%, and between 40 to 89%, respectively. EC recovery of 168% in the 30 mg/kg SP 7993 soil treatment indicates the high variability of the measurement at such low amendment concentration. Recovery of the 1,3-DNB internal standard generally complied with the quality control criteria.

No ATEC was detected in the soil interstitial soil water samples, whereas water soluble EC was measured at concentrations ranging between 0.008 and 0.24 mg/L in the 300, 1000, 3000, and 10,000 SP 7993 soil treatments (Table 16).

Table 15. Chemical determinations of total extractable ATEC and EC from the SP 7993-amended soil in the soil samples measured at the beginning of the terrestrial plant growth and earthworm lethality tests (acetonitrile extraction).

Nominal Concentration (mg/kg)	ATEC (mg/kg)	Standard deviation (mg/kg)	% recovery	EC (mg/kg)	Standard deviation (mg/kg)	% recovery
0	BDL		0	BDL		
30	BDL		0	0.7	0.1	168
100	3	5	38	0.6	0.6	40
300	12	10	50	2.3	1.6	55
1000	63	22	79	11	4	78
3000	202	103	84	37	16	89
10,000	716	174	90	123	39	89

Recovery percentages were calculated using NRC relative quantification of ATEC and EC. BDL: Below detection limit. Detection limit of ATEC = 2.5 mg/L; Detection limit of EC = 0.005 mg/L, which correspond to quantification limits of 25 and 0.05 mg/kg, respectively.

Table 16. Chemical determinations of water soluble EC from the SP 7993-amended soil in the soil samples measured at the beginning of the terrestrial plant growth and earthworm lethality tests (extraction of interstitial water).

Nominal Concentration (mg/kg)	EC (mg/L)	Standard deviation (mg/L)
0	BDL	
30	BDL	
100	BDL	
300	0.008	0.002
1000	0.025	0.005
3000	0.085	0.003
10,000	0.243	0.011

BDL: Below detection limit. Detection limit of EC = 0.005 mg/L.

III.6. Quantification of energetic materials contained in the SP 7993-amended soil used for earthworm avoidance test

Table 17 presents the results of the chemical analyses of DRDC2010 soil amended with SP 7993 measured at the beginning of the earthworm avoidance tests. Measured concentrations of ATEC and EC ranged between 16 and 771 mg/kg, and between 0.2 and 142 mg/kg, respectively. These measured concentrations of ATEC and EC are similar to those measured at the beginning of the terrestrial plant growth

and earthworm lethality tests (See Table 16). Respective recovery percentages of ATEC and EC ranged between 68 and 97%, and between 49 to 102%, respectively. Recovery of the 1,3-DNB internal standard complied with the quality control criteria, except for the 30 mg/kg SP 7993 soil treatment.

Table 17. Chemical determinations of total extractable ATEC and EC from the SP 7993-amended soil in the soil samples measured at the beginning at the beginning of the earthworm avoidance test (acetonitrile extraction).

Nominal Concentration (mg/kg)	ATEC (mg/kg)	Standard deviation (mg/kg)	% recovery	EC (mg/kg)	Standard deviation (mg/kg)	% recovery
0	BDL			BDL		
30	BDL		0	0.20	0.19	49
300	16	16	68	3	2	74
1000	70	14	88	12	2	89
3000	185	94	77	34	16	82
10,000	771	60	97	142	10	102

Recovery percentages were calculated using NRC relative quantification of ATEC and EC. BDL: Below detection limit. Detection limit of ATEC = 2.5 mg/L; Detection limit of EC = 0.005 mg/L, which correspond to quantification limits of 25 and 0.05 mg/kg, respectively.

III.7. Quantification of energetic materials contained in the SP Unique-amended soil used for the terrestrial plant growth and earthworm lethality tests

Results of the chemical analyses of DRDC2010 soil amended with SP Unique measured at the beginning of the terrestrial plant growth and earthworm lethality tests are presented in Tables 18 and 19. Total extractable concentrations of NG, EC, and DPA were between 1 and 2148 mg/kg, 0.1 and 21 mg/kg, and 0.05 and 70 mg/kg, respectively. Recovery percentages ranged between 23 and 104%, 61 and 106%, and 26 and 111% for NG, EC, and DPA, respectively (Table 18). Low recoveries of NG (23%) and DPA (26%) were only measured in the 30 mg/kg SP

Unique soil treatment. Recovery of the 1,3-DNB internal standard complied with the quality control criteria.

Water soluble fractions of NG, EC, and DPA were measured at concentrations between 0.5 and 721 mg/L, 0.08 to 5 mg/L, and 0.4 to 3.3 mg/L, respectively (Table 19).

Table 18. Chemical determinations of total extractable NG, EC, and DPA from the SP Unique-amended soil in the soil samples measured at the beginning of the terrestrial plant growth and earthworm lethality tests (acetonitrile extraction).

Nominal Concentration (mg/kg)	NG (mg/kg)	Standard deviation (mg/kg)	% recovery	EC (mg/kg)	Standard deviation (mg/kg)	% recovery	DPA (mg/kg)	Standard deviation (mg/kg)	% recovery
0	BDL			BDL			BDL		
30	1.4	1.3	23	BDL			0.05	0.04	26
100	19	6	90	0.1	0.2	61	0.6	0.2	95
300	60	2	98	0.8	0.1	136	1.7	0.1	93
1000	208	18	101	2.3	0.1	117	6.6	0.6	104
3000	681	67	110	7	1	118	22	2	116
10,000	2148	130	104	21	2	106	70	4	111

Recovery percentages were calculated using NRC relative quantification of NG, EC, and DPA. BDL: Below detection limit. Detection limit of NG = 0.1 mg/L; Detection limits of EC and DPA = 0.005 mg/L, which correspond to quantification limits of 1, and 0.05 mg/kg, respectively.

Table 19. Chemical determinations of water soluble NG, EC, and DPA from the SP Unique-amended soil in the soil samples measured at the beginning of the terrestrial plant growth and earthworm lethality tests (extraction of interstitial water).

Nominal Concentration (mg/kg)	NG (mg/kg)	Standard deviation (mg/kg)	EC (mg/kg)	Standard deviation (mg/kg)	DPA (mg/kg)	Standard deviation (mg/kg)
0	BDL				BDL	
30	0.5	0.1	BDL		BDL	
100	29	3	BDL		BDL	
300	123	4	0.08	0.01	BDL	
1000	302	4	0.32	0.02	0.4	0.1
3000	538	37	2.7	0.3	1.8	0.6
10,000	721	16	5.0	0.4	3.3	0.8

BDL: Below detection limit. Detection limit of NG = 0.1 mg/L; Detection limit of EC and DPA = 0.005 mg/L.

III.8. Quantification of energetic materials contained in the SP Unique-amended soil used for earthworm avoidance test

Results of the chemical analyses of DRDC2010 soil amended with SP Unique measured at the beginning of the earthworm avoidance tests are presented in Table 20. Measured concentrations of NG, EC, and DPA ranged between 1.5 and 2087 mg/kg, between 0.3 and 23 mg/kg, and between 0.1 and 65 mg/kg, respectively (Table 20). These measured concentrations are similar to those measured at the beginning of the terrestrial plant growth and earthworm lethality tests (See Table 18). Respective recovery percentages ranged between 72 and 123%, between 94 and 138%, and between 85 to 105%. Recovery of the 1,3-DNB internal standard complied with the quality control criteria.

Table 20. Chemical determinations of total extractable NG, EC and DPA from the SP Unique-amended soil in the soil samples measured at the beginning at the beginning of the earthworm avoidance test acetonitrile extraction).

Nominal Concentration (mg/kg)	NG (mg/kg)	Standard deviation (mg/kg)	% recovery	EC (mg/kg)	Standard deviation (mg/kg)	% recovery	DPA (mg/kg)	Standard deviation (mg/kg)	% recovery
0	BDL			BDL			BDL		
10	1.5	0.9	72	BDL			0.07	0.04	105
100	19	4	92	0.28	0.12	138	0.6	0.1	101
300	53	2	86	0.57	0.01	94	1.6	0.1	85
1000	230	34	111	2.3	0.5	116	6	1	99
10,000	2087	123	101	23	4	113	65	7	103

Recovery percentages were calculated using NRC relative quantification of NG, EC, and DPA. BDL: Below detection limit. Detection limit of NG = 0.1 mg/L; Detection limits of EC and DPA = 0.005 mg/L, which correspond to quantification limits of 1, and 0.05 mg/kg, respectively.

III.9. Quantification of energetic materials contained in the CMR170-amended soil used for the terrestrial plant growth and earthworm lethality tests

Results of the chemical analyses of DRDC2010 soil amended with CMR170 measured at the beginning of the terrestrial plant growth and earthworm lethality tests are presented in Tables 21 and 23. Total extractable concentrations of NG, EC, and MC were between 11 and 654 mg/kg, 1.4 and 82 mg/kg, and 0.9 and 186 mg/kg, respectively. Recovery percentages ranged between 77 and 146%, 78 and 152%, and 78 and 142% for NG, EC, and MC, respectively (Table 21). Recovery of the 1,3-DNB internal standard complied with the quality control criteria.

Water soluble fractions of NG, EC, and MC were measured at concentrations between 0.1 and 36 mg/L, 0.04 to 0.17 mg/L, and 0.03 to 4.2 mg/L, respectively (Table 23).

Considering that the concentration of NG increased drastically between 28 d and 42 d during the preliminary equilibrium test, chemical analyses of DRDC2010 soil

amended with CMR170 were also measured at the end of the terrestrial plant growth test (Tables 22 and 24). Total extractable concentrations of NG, EC, and MC were between 3 and 833 mg/kg, 0.4 and 106 mg/kg, and 0.9 and 238 mg/kg, respectively (Table 22).

Water soluble fractions of NG, EC, and MC were measured at concentrations between 0.1 and 12 mg/L, 0.06 to 0.27 mg/L, and 0.02 to 7 mg/L, respectively (Table 24). These results indicate that, in contrast with what was observed during the equilibrium study, concentrations of NG, EC, and MC were relatively stable throughout the plant toxicity test.

Table 21. Chemical determinations of total extractable NG, EC, and MC from the CMR170-amended soil in the soil samples measured at the beginning of the terrestrial plant growth and earthworm lethality tests (acetonitrile extraction).

Nominal Concentration (mg/kg)	NG (mg/kg)	Standard deviation (mg/kg)	% recovery	EC (mg/kg)	Standard deviation (mg/kg)	% recovery	MC (mg/kg)	Standard deviation (mg/kg)	% recovery
0	BDL			BDL			BDL		
30	BDL			BDL			0.9	1.6	141
100	11	12	146	1.4	1.6	152	3	3	142
300	21	1	95	2.8	0.1	99	6	1	92
1000	57	40	77	7	7	78	16	11	78
3000	223	24	99	28	28	102	63	7	101
10,000	654	42	88	82	82	88	186	11	89

Recovery percentages were calculated using NRC relative quantification of NG, EC, and MC. BDL: Below detection limit. Detection limit of NG = 0.1 mg/L; Detection limit of EC and MC = 0.05 mg/L, which correspond to quantification limits of 1, 0.05, and 0.5 mg/kg, respectively.

Table 22. Chemical determinations of total extractable NG, EC, and MC from the CMR170-amended soil in the soil samples measured at the end of the terrestrial plant growth test (acetonitrile extraction).

Nominal Concentration (mg/kg)	NG (mg/kg)	Standard deviation (mg/kg)	% recovery	EC (mg/kg)	Standard deviation (mg/kg)	% recovery	MC (mg/kg)	Standard deviation (mg/kg)	% recovery
0	BDL			BDL			BDL		
30	BDL			BDL			BDL		
100	3	6	46	0.4	0.7	46	0.9	1.6	44
300	25	34	113	3.4	4.6	123	7	10	112
1000	55	13	73	7	2	75	16	4	75
3000	200	43	89	25	6	91	57	12	92
10,000	833	36	112	106	6	114	238	12	114

Recovery percentages were calculated using NRC relative quantification of NG, EC, and MC. BDL: Below detection limit. Detection limit of NG = 0.1 mg/L; Detection limit of EC and MC = 0.05 mg/L, which correspond to quantification limits of 1, 0.05, and 0.5 mg/kg, respectively.

Table 23. Chemical determinations of water soluble NG, EC, and MC from the CMR170-amended soil in the soil samples measured at the beginning of the terrestrial plant growth test (extraction of interstitial water).

Nominal Concentration (mg/kg)	NG (mg/kg)	Standard deviation (mg/kg)	EC (mg/kg)	Standard deviation (mg/kg)	MC (mg/kg)	Standard deviation (mg/kg)
0	BDL		BDL		BDL	
30	BDL		BDL		BDL	
100	0.1	0.1	BDL		0.03	0.004
300	0.6	0.1	BDL		0.11	0.01
1000	3.3	0.4	BDL		0.45	0.04
3000	13	3	0.04	0.02	1.4	0.2
10,000	36	3	0.17	0.05	4.2	0.2

BDL: Below detection limit. Detection limit of NG = 0.1 mg/L; Detection limits of EC and MC = 0.05 mg/L.

Table 24. Chemical determinations of water soluble NG, EC, and MC from the CMR170-amended soil in the soil samples measured at the end of the terrestrial plant growth test (extraction of interstitial water).

Nominal Concentration (mg/kg)	NG (mg/kg)	Standard deviation (mg/kg)	EC (mg/kg)	Standard deviation (mg/kg)	MC (mg/kg)	Standard deviation (mg/kg)
0	BDL		BDL		BDL	
30	BDL		BDL		BDL	
100	BDL		BDL		0.024	0.003
300	0.1	0.2	BDL		0.12	0.01
1000	0.7	0.2	BDL		0.49	0.04
3000	3.0	0.4	0.06	0.02	2.1	0.3
10,000	12	2	0.27	0.13	7	2

BDL: Below detection limit. Detection limit of NG = 0.1 mg/L; Detection limits of EC and MC = 0.05 mg/L.

III.10. Quantification of energetic materials contained in the CMR170-amended soil used for earthworm avoidance test

Results of the chemical analyses of DRDC2010 soil amended with CMR170 measured at the beginning of the earthworm avoidance tests are presented in Table 25. Measured concentrations of NG, EC, and MC ranged between 7 and 653 mg/kg, between 1 and 83 mg/kg, and between 2 and 184 mg/kg, respectively (Table 25). These measured concentrations are similar to those measured at the beginning of the terrestrial plant growth and earthworm lethality tests (See Table 21). Respective recovery percentages ranged between 72 and 98%, between 74 and 99%, and between 78 to 96%. Recovery of the 1,3-DNB internal standard complied with the quality control criteria.

Table 25. Chemical determinations of total extractable NG, EC, and MC from the CMR170-amended soil in the soil samples measured at the beginning at the beginning of the earthworm avoidance test (acetonitrile extraction).

Nominal Concentration (mg/kg)	NG (mg/kg)	Standard deviation (mg/kg)	% recovery	EC (mg/kg)	Standard deviation (mg/kg)	% recovery	MC (mg/kg)	Standard deviation (mg/kg)	% recovery
0	BDL			BDL			BDL		
10	BDL			BDL			BDL		
100	7	11	98	1	1	99	2	3	96
300	16	8	72	2	1	74	5	2	78
1000	66	15	89	9	2	92	19	4	90
10,000	653	94	87	83	14	89	184	29	88

Recovery percentages were calculated using NRC relative quantification of NG, EC, and MC. BDL: Below detection limit. Detection limit of NG = 0.1 mg/L; Detection limits of EC and MC = 0.05 mg/L, which correspond to quantification limits of 1, 0.05, and 0.5 mg/kg, respectively.

III.11. Quantification of energetic materials and pH measurements of the SP 7993, SP Unique and CMR170-amended soil leachates used for the Microtox and freshwater algae toxicity tests

Chemical analyses were performed on soil leachates and showed absence of any chemical in the Control elutriate and the SP 7993 samples. NG was present in both SP unique and CMR170 leachates, with highest amount (19.78 mg/L) in the SP unique leachate amended with 10,000 mg/kg (Table 26).

The pH values of soil leachates amended with SP 7993, SP unique, and CMR170 formulations were slightly acidic, and were adjusted between 6.5 and 7.0 prior to both Microtox and 96-h algae growth inhibition assays (Table 26).

Table 26. Summary of the physico-chemical characteristics of the different formulation amended soil leachates.

		Compounds detected in soil leachates (mg/L)			
Soil leachates	**pH***	**DPA**	**NG**	**EC**	**MC**
Control-1	5.50 / 7.00	BDL	BDL	BDL	BDL
Control-2	5.63 / 6.52	BDL	BDL	BDL	BDL
SP 7993 1,000 mg/kg	5.50 / 6.56	NA	BDL	BDL	NA
SP 7993 10,000 mg/kg	6.03 / 6.50	NA	BDL	BDL	NA
SP unique 1,000 mg/kg	5.98 / 6.59	0.026	2.73	BDL	BDL
SP unique 10,000 mg/kg	5.68 / 6.59	0.20	19.78	0.028	BDL
CMR170 1,000 mg/kg	5.62 / 6.61	NA	BDL	BDL	BDL
CMR170 10,000 mg/kg	5.80 / 6.94	NA	0.356	0.012	0.08

*Soil leachates represented as: measured / adjusted.
DPA: diphenylamine; NG: nitroglycerine; EC: ethyl centralite; MC: methyl centralite.
NA: not analyzed; BDL: below detection limit. Detection limit of NG = 0.1 mg/L; Detection limits of EC and MC = 0.05 mg/L.

IV. TOXIC EFFECTS OF THE PROPELLANT FORMULATIONS

IV.1. Effects of formulation-amended soil leachates to Microtox

Leachates of SP 7993, SP unique, and CMR170 amended soil had no significant effect on the bioluminescence of *V. fischeri*. As indicated in Table 27, the leachates of soil amended with SP 7993 and CMR170 formulations were not toxic to the photo-bacteria *V. fischeri*. The leachate of SP Unique amended with 10,000 mg/kg was toxic (maximum inhibition = 17.7%). Therefore, preliminary data indicates that only leachate of SP Unique amended at a very high concentration had toxic effect on the bacteria, whereas neither SP 7993 nor CMR170 soil leachates had an adverse effect on the bioluminescence of marine bacteria *Vibrio fischeri*.

Table 27. Summary of toxicity responses of the different propellant amended soil leachates to *V. fischeri.*

Soil treatment concentration	Maximum inhibition (%)*		
	SP 7993	**SP unique**	**CMR170**
DRDC2010 soil (Negative control)	7.5 ± 4.8	7.5 ± 4.8	-6.5 ± 0.0
1,000 mg/kg	3.7 ± 1.3	0.1 ± 0.0	-0.06 ± 0.0
10,000 mg/kg	0.0 ± 0.0	17.7 ± 4.7	-0.01 ± 0.0
Reference toxicant	**EC_{50}****	**Maximum inhibition (%)***	
Phenol	20.5 (18 - 23)	71.9 ± 1.1	

* Maximum inhibition is expressed as % of test control ± standard deviation. Negative values (-) indicate a stimulation of bioluminescence.
** EC_{50} value expressed as mg/L with the 95% confidence intervals in brackets.

IV.2. Effects -amended soil leachates to freshwater algae

Leachate of the RDDC2010 control soil was not toxic and had a significant stimulatory effect on the growth of *P. subcapitata*, as indicated by a maximum inhibition value of -39% (Table 28). Therefore, any toxic effect of the amended-soil leachates measured on algae would be associated to chemicals in the formulations.

Results indicate that only the 10,000 mg/kg soil leachate of SP Unique was toxic to *P. subcapitata* (EC_{50} = 26% v/v of soil leachate). Both concentrations of SP 7993 soil leachates (1,000 and 10,000 mg/kg) as well as the 1000 mg/kg of SP Unique and CMR170 were not toxic to algae. The CMR170 soil leachate of the 10,000 mg/kg soil treatment stimulated the algae growth, as indicated by a maximum inhibition value of -243%.

Table 28. Summary of toxicity responses of the different propellant amended soil leachates to *P. subcapitata*

	SP 7993		SP Unique		CMR170	
Soil treatment concentration	**EC_{50}***	**Maximum inhibition (%)****	**EC_{50}***	**Maximum inhibition (%)****	**EC_{50}***	**Maximum inhibition (%)****
DRDC2010 soil (Negative control)	Nd	-39 ± 15	Nd	-39 ± 15	Nd	-59 ± 3
1,000 mg/kg	Nd	6 ± 7	Nd	-36 ± 1	Nd	-34 ± 6
10,000 mg/kg	Nd	7 ± 5	26 (25-27)	97 ± 0.3	Nd	-243 ± 26
Zinc sulfate (Reference toxicant)	38 (35 - 40)	93 ± 2				

* Values are expressed as % of leachates (v/v) for soil leachates, and as µg/L for the reference toxicant zinc sulfate with the 95% confidence intervals in brackets.

** Negative values (-) indicate a stimulation of growth.

Nd: Not determined.

IV.3. Effects of formulation-amended soil to terrestrial plants

The effects of SP 7993, SP Unique, and CMR170 amended soil on the seedling emergence and shoot growth of ryegrass are presented in Table 29. Results indicate that SP 7993 and CMR170 formulations had no toxic effect on ryegrass seedling emergence, and that S7993 formulation had no toxic effect on ryegrass shoot growth at concentrations up to 10,000 mg/kg. The CMR170 formulation had a slight toxic effect on shoot growth, with EC_{20} of 4975 mg/kg (fresh mass) and 5903 mg/kg (dry mass), respectively. The SP Unique formulation was the most toxic formulation, with seedling emergence EC_{50} value of 442 mg/kg, and a shoot growth (dry mass) EC_{50} value of 88 mg/kg. These results indicate that the toxicity of the SP Unique formulation can be attributed almost completely to the presence of NG. Seedling emergence of ryegrass in the negative (water) control was 83%, which complies with the quality control requirements.

Table 29. Effects of SP 7993, SP Unique, and CMR170 formulations amended soil on ryegrass seedling emergence and growth.

Toxicity endpoints	Seedling emergence (mg/kg)	Shoot growth (fresh mass) (mg/kg)	Shoot growth (dry mass) (mg/kg)
SP 7993			
EC_{20}	>10,000	>10,000	>10,000
EC_{50}	>10,000	>10,000	>10,000
SP Unique			
EC_{20}	192 (147-266)	148 (111-164)	88 (42-211)
EC_{50}	442 (140-722)	229 (196-251)	197 (103-289)
CMR170			
EC_{20}	>10,000	4975	5903
EC_{50}	>10,000	>10,000	>10,000
Boric acid in DRDC2010 Control Soil (Reference toxicant)			
Seedling emergence (%)	83.3		
EC_{50}	92 (70-112)	83 (72-94)	75 (70-82)

EC_{50} values are expressed as mg/kg dry soil.
The 95% confidence intervals are presented in brackets.

IV.4. Effects of amended soils to earthworm survival and avoidance behavior

Results of the earthworm lethality test using SP 7993, SP Unique, and CMR170 amended soil indicate that both SP 7793 and CMR170 formulations were not lethal to earthworm *Eisenia andrei* at concentrations up to 10,000 mg/kg (Table 30). An LC_{50} value of 6441 mg/kg was measured for the SP Unique formulation, indicating that this formulation was lethal to earthworms.

Results also indicate that the SP 7993 formulation had no detrimental effect on the avoidance behavior of *Eisenia andrei* at concentration up to 10,000 mg/kg (Table 30). In contrast, significant effects on the avoidance behavior were measured in the SP Unique and CMR170 formulations. An avoidance percentage of 60% or above is considered to be a significant response, as recommended by Environment Canada (2007). An 87% avoidance behavior was measured in the SP Unique soil treatment of 100 mg/kg, and 100% avoidance was measured at concentrations of 300 mg/kg and higher. In the 10,000 mg/kg of CMR170 soil treatment, an avoidance behavior of 100% was also measured.

Table 30. Effects of SP 7993, SP Unique, and CMR170 formulations amended soil on earthworm avoidance behavior and survival.

Toxicity endpoints	% avoidance behavior ± Standard deviation	Avoidance behavior EC_{50} (mg/kg)	Survival LC_{50} (mg/kg)
SP 7993 soil treatment (mg/kg)			
30	7 ± 42		
300	-7 ± 31	>10,000	>10,000
1000	-27 ± 12		
3000	-27 ± 76		
10,000	-40 ± 35		
SP Unique soil treatment (mg/kg)			
10	13 ± 23		
100	87 ± 23	36	6441 (6178-6565)
300	100 ± 0		
1000	100 ± 0		
10,000	100 ± 0		
CMR170 soil treatment (mg/kg)			
10	13 ± 50		
100	-13 ± 31	2028	>10,000
300	0 ± 60		
1000	20 ± 53		
10,000	100 ± 0		
KCl in OCDE soil (Reference toxicant)		Between 1519 and 2285	Between 5500 and 7000

The 95% confidence intervals are presented in brackets.

V. DISCUSSION OF THE ECOTOXICOLOGICAL ASSESSMENT RESULTS

The ecotoxicological effects of SP 7993, SP Unique, and CMR170 formulations are summarized in Table 31.

Leachates of SP 7993, and CMR170 amended soils were not toxic to the aquatic species used in the present study. The leachate of the DRDC2010 amended with SP unique formulation at 10,000 mg/kg had a significant effect on bacteria and algae growth. Considering the presence of NG (19.78 mg/L in the 100% leachate), the growth EC_{50} value for algae corresponds to 5.1 mg/L or 22.6 µM of NG. This EC_{50} value was higher than the reported EC_{50} values (from 1.8 to 5.1 µM) of pure NG (Nipper et al., 2009). Therefore, the NG in SP unique was less toxic than pure NG.

The SP 7993 and CMR170 formulations had no toxic effect on ryegrass seedling emergence and in addition, the SP 7993 formulation had no toxic effect on ryegrass shoot growth at concentrations up to 10,000 mg/kg, respectively. The CMR170 formulation had a slight toxic effect on ryegrass shoot growth, with an EC_{20} value of 4975 mg/kg for fresh mass endpoint, and of 5903 mg/kg for dry mass endpoint, respectively. The SP Unique formulation was the most toxic formulation, with a seedling emergence EC_{50} value of 442 mg/kg and a shoot growth (dry mass) EC_{50} value of 88 mg/kg, respectively. These toxicological endpoints measured in the SP Unique formulation are similar to those obtained previously with pure NG. As described in Rocheleau et al. (2011), a seedling emergence EC_{50} value of 325 mg/kg and a shoot growth (dry mass) EC_{50} value of 62 mg/kg were measured in Sassafras sandy loam soil. The toxicity of the SP Unique formulation can therefore be attributed to the presence of NG, with a total extractable concentration of up to 2148 mg/kg in soil, and a bioavailable concentration of up to 721 mg/L in the soil interstitial water at the soil treatment of 10,000 mg/kg. Mirecki et al. (2006) reported that the solubility of NG in water can be up to 1950 mg/L (See Table 3). The toxic

effect on ryegrass shoot growth exposed to the CMR170 formulation can also be attributed to the presence of NG with total extractable concentration of up to 654 mg/kg measured in soil, and a bioavalable concentration of up to 36 mg/L measured in the soil interstitial water at the soil treatment of 10,000 mg/kg.

The SP 7993 and CMR170 formulations were not lethal to earthworm *Eisenia andrei* at concentration up to 10,000 mg/kg. An LC_{50} value of 6441 mg/kg was measured for the SP Unique formulation, indicating that this formulation has a significant effect on the survival of earthworms but at relatively high formulation concentrations. In addition, the SP 7993 formulation had no detrimental effect on the avoidance behavior of *Eisenia andrei* at concentrations up to 10,000 mg/kg, whereas significant effects on the avoidance behavior were measured at concentrations of 100 mg/kg and higher of the SP Unique formulation, and at 10,000 mg/kg of the CMR170 formulation. The deleterious effect of SP Unique and CMR170 formulations on the avoidance behavior of *Eisenia andrei* can also be attributed to the presence of NG, with total extractable concentrations of up to 2087 mg/kg and 653 mg/kg in the soil treatment of 10,000 mg/kg.

Table 31. Summary of the toxic effects of the different formulations on different organisms

Test organisms	SP 7993	SP Unique	CMR170
Toxicity tests using soil elutriates samples (10,000 mg/kg)			
	Maximum inhibition (% of test control ± standard deviation)	**Maximum inhibition (% of test control ± standard deviation)**	**Maximum inhibition (% of test control ± standard deviation)**
Bacteria *Vibrio fischeri* (Microtox)	0.0 ± 0.0	17.7 ± 4.7	-0.01 ± 0.0
Freshwater algae *Pseudokirchneriella subcapitata*	7 ± 5	97 ± 0.3	-243 ± 26
	EC_{50} (% of elutriate)	**EC_{50} (% of elutriate)**	**EC_{50} (% of elutriate)**
Freshwater algae *Pseudokirchneriella subcapitata*		26 (25-27)*	
Toxicity tests using soil samples			
	EC_{50} (mg/kg)	**EC_{50} (mg/kg)**	**EC_{50} (mg/kg)**
Terrestrial plant ryegrass *Lolium perenne* growth inhibition (dry mass)	>10,000	197 (103-289)	>10,000
Earthworm *Eisenia andrei* (lethality)	No effect at ≤ 10,000 mg/kg	6441 (6178-6565)	No effect at ≤ 10,000 mg/kg
Earthworm *Eisenia andrei* (avoidance)	No effect at ≤ 10,000 mg/kg	36	2028

*95% confidence intervals are indicated in brackets.

VI. CONCLUSION OF THE ECOTOXICOLOGICAL ASSESSMENT

Based on the present ecotoxicological assessment of the SP 7993, SP Unique, and CMR170 formulations, we concluded that the SP 7993 formulation was the least toxic, the SP Unique formulation was toxic to the aquatic and terrestrial organisms tested, whereas the CMR170 formulation was toxic to the terrestrial organisms only. The toxicity of the SP Unique and CMR170 formulations can be attributed to the presence of nitroglycerin in both formulations.

In addition, data indicate that preliminary soil equilibrium studies are necessary because the equilibrium time can be formulation and soil specific. Future ecotoxicological studies could focus on direct contact soil toxicity assays, such as soil microbial activity tests, terrestrial plant seedling emergence and growth, and earthworm assays, such as lethality, reproduction, or avoidance tests. The earthworm lethality assay is not a very sensitive endpoint, is not labor-intensive, and is not really useful for risk assessment. The earthworm reproduction assay is a more sensitive endpoint and useful for risk assessment, but is more labor-intensive and more expensive. The earthworm avoidance assay is a very sensitive endpoint, can be used as a screening test, but has limited usefulness for risk assessment. The selection of the appropriate earthworm assay will depend on the objectives and the resources allocated for the future ecotoxicological studies.

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PART III. TOXICOLOGICAL EVALUATION OF WEATHERED AND AGED GIM

INTRODUCTION

In a previous study (Hawari et al., 2011), the NRC investigated the fate and ecotoxicity of a new explosive formulation, namely GIM (Green Insensitive Munition), constituted of HMX (53%), TNT (39%) and an energetic elastomer used to make the formulation less sensitive. It was concluded that GIM exhibited higher ecotoxicity than the reference explosive formulation, PBX (83% HMX, 10% polyurethane, 6% DOA,…), used in the study. A probable origin for the higher ecotoxicity of GIM was the rapid and abundant release of TNT from GIM in the environment. Since TNT is known to transform in soil into aminated products that tend to adsorb irreversibly to soils, it was decided to determine the ecotoxicity of GIM after several weeks of aging. Two aging protocols were used: (1) a soil from DRDC was amended with fresh GIM and aged in a greenhouse at the NRC for 6 months, and (2) water was amended with GIM that had been weathered and aged outdoors for 2 years. Ecotoxicity data collected using the two types of samples are presented in this report.

I. WEATHERING-AGING OF GIM IN SOIL

I.1. Materials and methods

Materials. Fresh GIM (HMX (53%), TNT (39.5%), Energetic ThermoPlastic Elastomer (ETPE) copolymer (7.5%)) was provided by DRDC-Valcartier. The soil used in this study (DRDC-09 soil, see Table 7 for physico-chemical properties) was provided by DRDC-Valcartier.

Soil amendment. DRDC-09 soil was amended with GIM in the powdered form, as described in a previous report (Hawari et al., 2011). Briefly, the dry explosive

formulation was weighed and added to the desired amount of DRDC-09 soil into a high-density polyethylene container coated with Teflon. Four soil concentrations of GIM (100, 500, 1000, and 10,000 mg/kg) were prepared. The contaminated soil samples were mixed overnight using a three-dimensional soil rotary mixer. Aliquot samples of freshly amended soil (< 1 d) were collected in triplicate from each soil treatment batch and were extracted using acetonitrile for subsequent HPLC analysis (USEPA, 2007). The remaining soil in each batch was transferred to glass containers, and hydrated with ASTM type I water to 75% of the soil water holding capacity (WHC) for weathering and aging. GIM-amended or un-amended hydrated DRDC-09 soil was weathered and aged (W-A) by alternating wetting and drying cycles in open glass containers in a greenhouse, as described by Kuperman et al. (2006). For these cycles, ASTM type I water was added once a week to readjust the soil weight to its initial hydrated weight. Each treated soil (100-10,000 mg/kg) was analyzed after 4, 8, 12, 18, and 24 weeks. Both the acetonitrile extracts and the interstitial water (IW) collected using the centrifugation-filtration method (Savard et al. 2010) were analyzed by HPLC for HMX and TNT. Only the 24-week aged samples were used for the toxicity tests.

Analytical methods. TNT and HMX were analyzed in acetonitrile/H_2O (50/50; v/v) solutions by reverse phase high performance liquid chromatography (HPLC)-UV. The system consisted of a W600 pump (Waters, Milford, MA, USA), a 717 plus autosampler, and a 2996 Photodiode-Array Detector. Samples (50 µL) were separated with a Discovery C18 column (25 cm × 4.6 mm × 56 µm) (Supelco, Oakville, ON), at 35°C. A water methanol gradient was run at 1 mL min^{-1}. The initial solvent composition was 50 % methanol/ water, which was held for 18 min. A linear gradient was run from 50% to 90% methanol over 2 min. This solvent ratio was held for 8 min and then changed to the initial conditions over 2 min. The initial conditions were held for an extra 15 min for a total run of 45 min. The detector was set to scan from 192 to 450 nm. Detection limits were estimated at 0.005 and 0.004 mg L^{-1} for TNT and HMX, respectively at 254 nm,

II.2. Results and discussion

Chemical analyses of DRDC-09 soil freshly amended with GIM showed a significant level of variation for the recoveries of HMX and TNT (standard deviations were from 19 to 35%, Table 32). The perfect match between the recoveries of TNT and HMX in each sample indicates that this heterogeneity was due to the irregular spatial distribution of GIM particles in soil.

Table 32. Initial HMX and TNT measured in GIM-amended DRDC-09 soil before starting the W-A procedure

GIM in soil (mg/kg)	Measured in soil (mg/kg)		Recovery (%)*	
	HMX	TNT	HMX	TNT
100	37	29	70 ± 19	73 ± 35
500	201	147	75 ± 34	75 ± 20
1,000	551	418	103 ± 34	106 ± 29
10,000	4793	3479	90 ± 21	88 ± 25
		Average	84.5	85.5
		SD	15.0	15.2

Recovery data expressed as mean ± standard deviation (n=3).

During the W-A procedure, the concentration of HMX in GIM-amended soil was stable over 24 weeks (Fig. 38), with recoveries varying from 73 to 101% of the original concentrations in soil. As for TNT, its concentration decreased constantly up to the week 18. At week 24, a slight increase in TNT concentration compared to the week 18 was observed but the latter was attributed to variability in the spatial distribution of GIM. After 24 weeks of weathering and aging, from 19 to 54% of the TNT and from 86 to 98% of the HMX originally measured in GIM amended soils were recovered.

The decrease in TNT was accompanied by the detection of TNT amino derivatives 2-aminodinitrotoluene (2-ADNT) and 4-aminodinitrotoluene (4-ADNT) (Table 33). No diamino, and no azoxy compounds were measured in any of the aged samples.

The analyses of soil IW after the 24 weeks of W-A of the GIM-contaminated soil show the presence of TNT (from 0.2 to 118.8 mg/L) and HMX (from 2.8 to 3.4 mg/L) (Table 34). While the concentration of HMX in IW was close to its water solubility limit (3.34 at 20°C; Monteil-Rivera et al., 2004) at all levels of GIM spiking, that of TNT increased with the level of GIM, likely due to the prevailing transformation and sorption of TNT at lower concentrations. TNT solubility limit was only reached in the IW of highly concentrated samples (10,000 mg/kg).

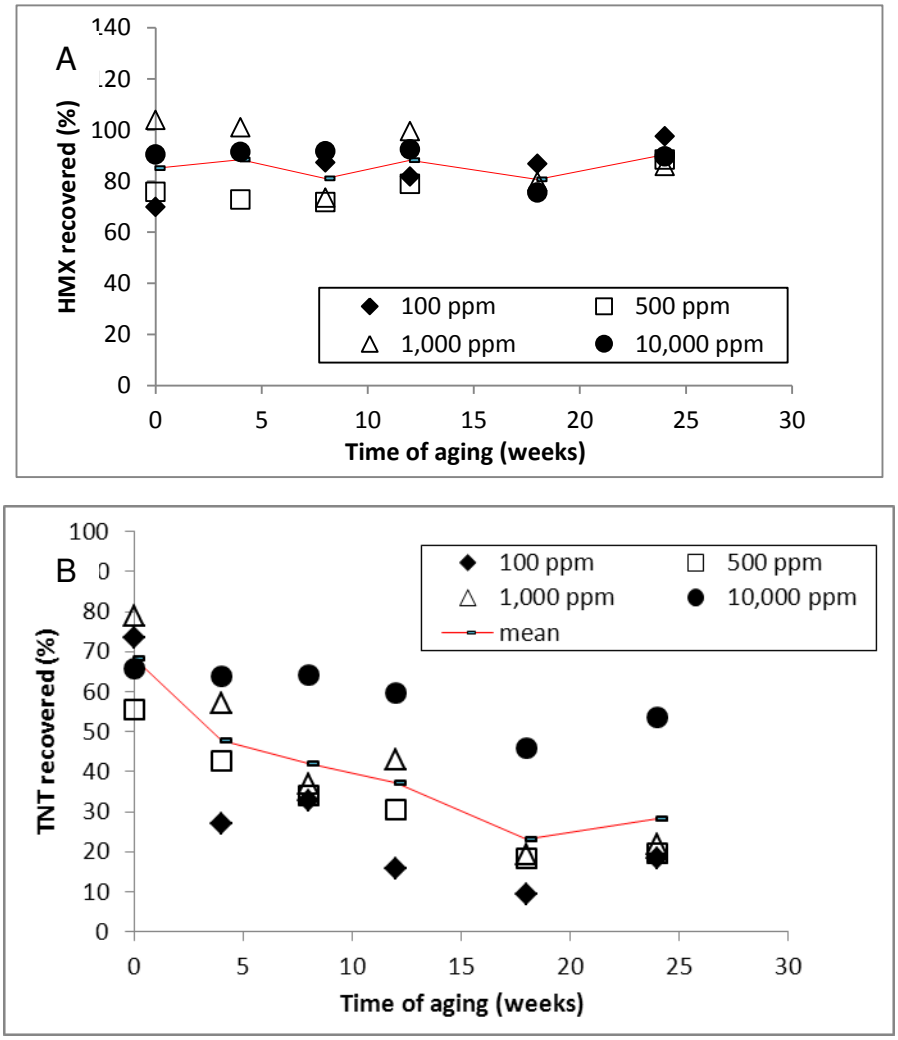

Figure 38. HMX (A) and TNT (B) found in GIM-amended DRDC-09 soil during the 24 weeks of W-A (Nominal concentrations are indicated in Fig. legends)

Table 33. TNT transformation products recovered in DRDC-09 soil over the 24 weeks of W-A

GIM in soil	2-ADNT (mg/kg)				4-ADNT (mg/kg)			
(mg/kg)	8-w	12-w	18-w	24-w	8-w	12-w	18-w	24-w
100	< 0.5	1.7	2.1	1.6	< 0.5	4.6	6.1	5.6
500	5.6	6.9	9.9	1.3	7.8	9.0	11.5	1.4
1,000	8.3	12.5	16.5	2.0	13.5	17.7	22.5	2.5
10,000	nd	nd	nd	10.3	nd	nd	nd	15.1

The minimum quantification limit was 0.5 mg/kg in soil. nd: not determined.

Table 34. TNT, HMX, and TNT transformation products recovered from DRDC-09 soil prior to the toxicity assays (24-week aging)

GIM in soil (mg/kg*)	Total TNT in soil (mg/kg)	TNT in IW of soil (mg/L)	2-ADNT in IW of soil (mg/L)	4-ADNT in IW of soil (mg/L)	Total HMX in soil (mg/kg)	HMX in IW of soil (mg/L)
100	7.3	0.2 ± 0.0	0.2 ± 0.0	0.9 ± 0.0	51.7	3.2 ± 0.1
500	52.0	9.9 ± 0.5	1.9 ± 0.1	1.9 ± 0.1	234.0	3.4 ± 0.0
1,000	116.0	32.1 ± 0.0	3.3 ± 0.1	4.1 ± 0.1	455.0	3.1 ± 0.0
10,000	2842	118.8 ± 0.7	1.7 ± 0.1	2.2 ± 0.2	4751	2.8 ± 0.0

* Concentration expressed on a dry basis ± standard deviation (n = 3).

II. EFFECTS OF WEATHERED-AND-AGED GIM-AMENDED SOIL ON EARTHWORMS

II.1. Materials and methods

The 24-week W-A GIM-amended soils were used for the earthworm survival assay. Water was added to reach 75% of the WHC capacity. Hydrated soils were left for 1 d prior to the beginning of the experiment. *Eisenia andrei* originally obtained from Carolina Biological Supply (Burlington, NC, USA) were cultured in our laboratory for at least one year prior to their use as a test species. Twenty four hours prior to the initiation of the assay, the earthworms were acclimated in a moist 'clean' soil using DRDC-09 (without amendment). An environment-controlled incubator (20 ± 1°C, humidity set at 70%, photoperiod cycle of 16 h-light (800 ± 400 lux) and 8 h-dark) was used. Ten *Eisenia andrei* earthworms (with well-developed clitellum, weighing from 0.39 to 0.55 g) were then exposed to the GIM-amended soil. The surviving earthworms were counted after 7 and 14 d, rinsed and weighed after 14 d. The criterion for test validity was set at > 90% survival in negative control as stated in the OECD guideline (1984). This average survival value is based on the number of surviving earthworms in all replicates of the negative control groups. The test exposure period ends at 14 d or at 7 d if survivors are not detected in the treatment groups.

II.2. Results and discussion

In a previous study, the lethality of earthworms after exposure to soil samples contaminated with fresh GIM was attributed to the presence of TNT. After weathering and ageing GIM-contaminated soil (1,000 mg/kg) for 24 weeks, a

decrease in total TNT concentration (116 mg/kg (aged) compared to 260 mg/kg (fresh)) was observed. However, the earthworm survival test showed that the aged GIM soil sample was as toxic as the GIM freshly-amended soil samples (Table 35). TNT measured in IW of GIM-amended soil at 1,000 mg/kg was 32 mg/L (Table 34). Earlier study indicated that the TNT concentration in IW of a soil freshly amended with 1,000 mg/kg GIM was 40 mg/L (Hawari et al., 2011), supporting our belief that observed lethality was caused by TNT and indicating that only the soluble fraction of TNT seems to negatively affect earthworms.

Table 35. Summary of lethality endpoints for earthworms exposed to freshly-amended or W-A GIM-amended soil samples

GIM in soil (mg/kg)	Total TNT in freshly-amended soil (mg/kg)	Total TNT in W-A GIM soil (mg/kg)	Lethality freshly-amended soil (%)	Lethality in W-A soil (%)
100	29.0	7.3	0	0
500	Nd	52.0	Nd	0
1,000	260.0	116.0	100	93
10,000	3120	2842	100	100

Data for fresh soil were taken from Hawari et al., 2011. Nd: not determined.

III. EFFECTS OF WEATHERED-AND-AGED GIM-AMENDED SOIL ON RYEGRASS (*LOLIUM PERENNE)*

III.1. Material and methods

The toxicity of 24-week W-A GIM-amended soil to ryegrass was determined using ASTM and USEPA methods, as described by Rocheleau et al. (2006). The test species, perennial ryegrass *Lolium perenne* Express, was obtained from Pickseed Canada Inc. (St-Hyacinthe, Quebec, Canada). Preliminary tests were done using nominal concentrations ranging from 100 to 10,000 mg/kg in soil. All treatments including 0 (control) were carried out in triplicate, and were incubated in sealed plastic bags to maintain soil moisture for the duration of the test. Plant toxicity tests were performed in a temperature and light-controlled growth chamber (Conviron, Winnipeg, Manitoba, Canada). The shoot growth (dry mass) was determined after 19 d of growth. Shoots were cut just above the soil line, and dry mass per treatment groups (mg tissue) was determined after drying the shoot tissue in an oven set at 70°C for 18 h. The EC_{20} and EC_{50} values, determined using linear interpolation (using the ToxCalc software), denoted the concentrations causing a 20% and 50% decrease in ryegrass shoot biomass, respectively, compared to their respective negative controls (no toxicant added).

III.2. Results

The first signs of toxicity were obtained when using soil spiked at 1,000 mg/kg for both fresh and W-A soil samples, in which the W-A sample is less toxic. In the study with W-A soil a target concentration of 500 mg/kg (between the EC_{20} and EC_{50}, based on fresh GIM) was added, and was comparable to the control soil (0.04 mg tissue) (Fig. 39). The present test was aimed at finding ranges that affect the EC_{20}

and EC_{50} rather than determining accurately the two values. Wide concentration ranges were thus used, which resulted in an overlap between the concentration ranges causing a toxic effect with fresh and aged GIM-amended soil (Table 36). Despite the overlap, the current preliminary data seem to show a lower toxicity of aged GIM-contaminated soil compared to the freshly amended one (Table 36). Fig. 39 shows a growth stimulation in the 100 mg/kg sample compared to the control (having no GIM added). This finding might be attributed to the aging process as in earlier study this growth stimulated effect was not observed.

Table 36. Effect of GIM-amended soil (fresh or W-A) on ryegrass

19-d Shoot growth (based on dry mass)	Soil freshly amended with GIM* (mg/kg)	W-A soil amended with GIM (mg/kg)
EC_{20} (95% C.I.)	308 (0-464)	520 (270-716)
EC_{50} (95% C.I.)	736 (540-910)	903 (718-1086)

* Data for freshly amended soil taken from Hawari et al., 2011.

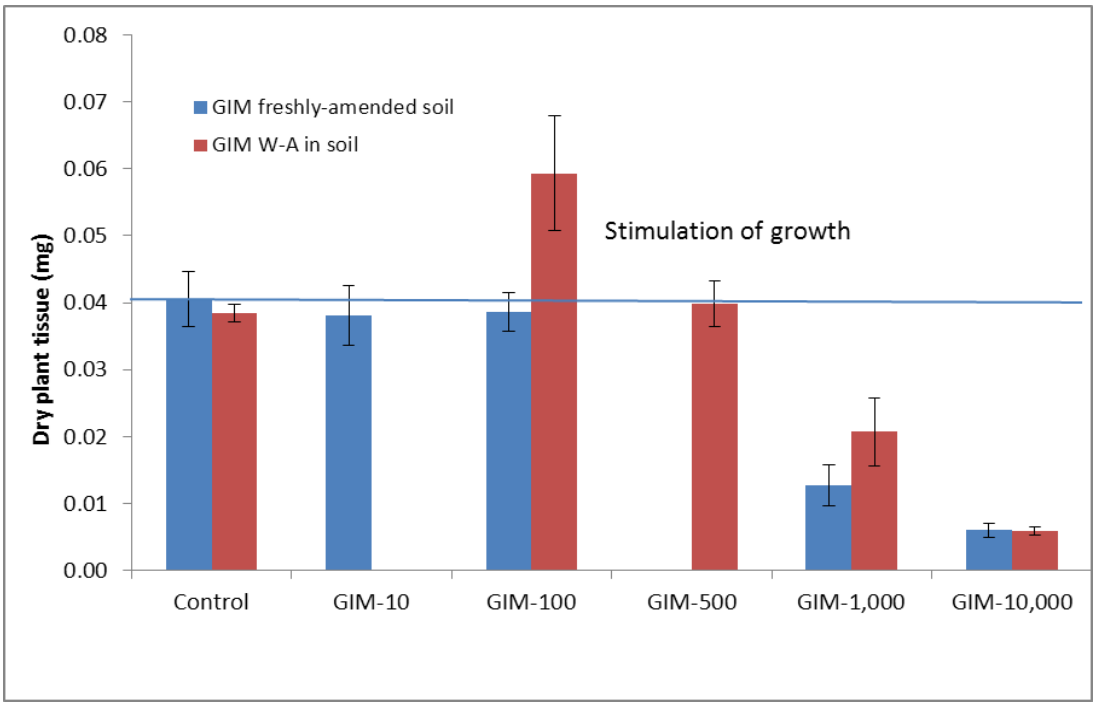

Figure 39. Ryegrass plant biomass after 19-d growth in GIM-amended soil W-A for 24 weeks

IV. TOXICITY OF 2-YEAR WEATHERED GIM FORMULATION

IV.1. Materials and methods

Three vials of GIM weathered outdoors for 2 years were provided by DRDC. The three samples identified as GIM-EX-1B, GIM-EX-2B and GIM-EX-3B were ground individually using a mortar and a pestle and fine particles (powder) were separated from coarse particles (plastic chunks) (Fig. 40). Only the fine particles of each sample were used for chemical analysis and ecotoxicological testing. To evaluate the dissolution of different constituents of the GIM samples, the powder (0.02 g) was added to either acetonitrile (100 mL) or water (100 mL), and stirred for 7 d at room temperature, then sonicated in an ultra-sonic bath for 30 min. Samples were filtered using syringes fitted with 0.45-µm Millex HV filters. The filtrate was diluted with either acetonitrile or water to have an acetonitrile:water solution of ratio 1:1 for the HPLC analysis. Water-dissolved samples (= water extracts) were used to evaluate the toxicity with selected receptors (Microtox or algae), whereas the acetonitrile-dissolved samples (acetonitrile extracts) were used for chemical analyses to quantify the remaining TNT and HMX in the aged GIM samples.

Figure 40. Photograph of the 2-year aged GIM sample after hand mixing

IV.1.1. Microtox assay

The Microtox standardized toxicity test was performed using the water extracts of the three aged GIM samples. Each sample was salted to 2% w/v NaCl prior to testing and diluted using the test diluent (ASTM type I water with 2% w/v NaCl). The negative control was the test diluent and the reference toxicant was phenol. Endpoint of this acute toxicity test is the decrease in bioluminescence of *Vibrio fischeri* (Environment Canada, 1992). The Microtox response was expressed as the average percentage of light emission inhibition compared to the negative control. The test was done in triplicate.

IV.1.2. 96h-Algal growth inhibition assay

The chronic toxicity test measuring the growth inhibition of freshwater algae *Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*, was performed as recommended by the Canadian Ministry of Environment (Environment Canada, 2007). *P. subcapitata* was exposed to various dilutions of the aqueous extract of aged GIM in a 96-well microplate and under continuous lighting at 24 °C. Following 96-h exposure to the water samples, the number of algae cells was measured using a Coulter Z2 cell counter, and the percentage of growth inhibition was calculated in comparison to the negative control. The test was done in triplicate, the negative control was deionized water and the reference toxicant was zinc sulfate.

IV.1.3. Statistical analyses

Analysis of variance (ANOVA) or the Student's t–test was performed to compare control versus different treatment groups. The EC_{20} and EC_{50} values, determined using linear interpolation or maximum likelihood-probit (using the ToxCalc software), denoted the concentrations respectively causing a 20% and 50% decrease in Microtox bioluminescence or algal growth, compared to their respective negative controls (no toxicant added).

IV.2. Results

The GIM samples weathered outdoors for 2 years did not dissolve completely in either acetonitrile or water, as expected from the insolubility of ETPE in either solvent. Table 37 provides the amount of HMX or TNT dissolved in either acetonitrile or water based on the total weight of aged GIM used in the test. No TNT transformation products were detected in either the aqueous or acetonitrile solutions. The content of ETPE was calculated as the difference between the total mass of aged GIM and the HMX and TNT mass recovered from the acetonitrile solutions (Table 37).

Dissolution of the three aged samples in acetonitrile clearly showed some differences between them (Table 37). While sample GIM-EX-1B exhibited a composition close to that of fresh GIM, GIM-EX-2B and GIM-EX-3B had clearly a lower content of TNT and higher content of HMX indicative of more extensive aging. Indeed, as previously reported (Hawari et al., 2011), the weathering and aging of GIM decreases primarily its content of TNT, leaving behind an aged GIM more concentrated in HMX and ETPE. According to the analyses of acetonitrile extracts, it thus seems that the severity of aging applied to the three samples followed the order: GIM-EX-1B < GIM-EX-2B < GIM-EX-3B.

Samples prepared in water accounted for 20.6 to 26.5% of the total GIM mass (Table 37), due to the water solubility limit of both TNT and HMX.

Table 37. HMX and TNT recovered from GIM samples weathered for 2 years and dissolved in acetonitrile or water over 7 d

	In acetonitrile		In water		Insoluble in acetonitrile
	HMX (wt.%)	TNT (wt.%)	HMX (wt.%)	TNT (wt. %)	ETPE (wt.%) deduced by difference
GIM-EX-1B	55.7± 1.6	34.5 ± 0.5	1.8 ± 1.6	24.8 ± 1.6	10.4 ± 1.7
GIM-EX-2B	65.6 ± 1.7	26.5 ± 0.5	3.8 ± 1.6	18.5 ± 1.6	8.0 ± 1.2
GIM-EX-3B	65.8 ± 1.7	19.1 ± 0.1	1.9 ± 1.6	18.8 ± 1.6	15.2 ± 1.7

Data expressed as mean ± standard deviation, (n=3).

The aqueous extracts of the three aged GIM samples were toxic to both Microtox bacteria and algae (Tables 38, 39). Based on these results, the toxicity responses overlapped for the selected endpoints, but data seem to show a lower toxicity of aged GIM (11.4 to 11.9 mg/L (bacteria); 2.3 to 4.0 mg/L (algae)) compared to the fresh GIM (6.8 mg/L (bacteria); 1.5 mg/L (algae)). When expressing the toxicity as concentration of TNT, it appeared that the aged GIM samples were slightly less toxic to Microtox bacteria (EC_{50} = 2-3 mg/L) than neat TNT (EC_{50}= 0.6 to 1.1 mg/L) (Table 38). The lower toxic effects observed in the Microtox assay may be due to the presence of other compounds such as HMX in the water extract of aged GIM sample. As for the algae assay (Table 39), the toxicity expressed as concentration of TNT was in the same range for GIM (EC_{50} of 0.5 to 0.7 mg/L) and neat TNT (EC_{50} of 0.5 to 0.7 mg/L). These two findings confirm the major role played by TNT in the toxicity of GIM, and are in agreement with earlier finding using fresh GIM (Hawari et al., 2011). With less TNT in its composition, aged GIM appeared to be less toxic than fresh GIM.

Table 38. Aqueous toxicity of a 2-year aged GIM samples using bacteria

	Concentration of GIM (mg/L)		**Concentration of TNT in GIM (mg/L)**	
	EC_{20}	**EC_{50}**	**EC_{20}**	**EC_{50}**
Fresh GIM	0.5 (0.4–0.6)	6.8 (5.1-9.0)	0.2 (0.1–0.2)	2.4 (1.8-3.2)
GIM-EX-1B	1.1 (0.4–2.2)	11.6 (7.4-18.8)	0.3 (0.1-0.6)	3.1 (2.0-5.0)
GIM-EX-2B	1.4 (0.2-3.1)	11.9 (5.1-120.7)	0.3 (0.0-0.6)	2.2 (0.9-4.9)
GIM-EX-3B	1.1 (0.2-2.3)	11.4 (4.0-13.1)	0.2 (0.0-0.5)	2.0 (0.7-4.7)
TNT				0.6 -1.1**

* Data expressed as mean with 95% Confidence Interval in brackets.
** From Dodard et al. (1999).

Table 39. Aqueous toxicity of a 2-year W-A GIM sample using freshwater green algae

	Concentration of GIM (mg/L)		Concentration of TNT in GIM (mg/L)	
	EC_{20}	EC_{50}	EC_{20}	EC_{50}
Fresh GIM	1.1 (1.0–1.3)*	1.5 (1.5-1.6)	0.4 (0.1–0.4)	0.5 (0.5-0.6)
GIM-EX-1B	1.1 (0.6–1.3)	2.3 (2.1-2.4)	0.3 (0.2-0.4)	0.60 (0.6-0.6)
GIM-EX-2B	2.0 (1.9-2.2)	3.4 (2.9-3.8)	0.4 (0.3-0.4)	0.63 (0.5-0.7)
GIM-EX-3B	2.2 (1.9-2.5)	4.0 (3.4-4.8)	0.4 (0.3-0.5)	0.7 (0.6-0.9)
TNT				0.5 – 0.7**

* Data expressed as mean with 95% confidence interval in brackets.
** From Dodard et al. (1999).

V. CONCLUSIONS

The 6-month weathering-aging procedure conducted in the present study showed a significant decrease in TNT content of GIM-amended soil at concentrations up to 1,000 mg/kg. Toxicity tests conducted using earthworms and plants did not show any significant toxicity decrease in the aged soils, likely due to the high concentrations of TNT remaining after 6 months in the samples spiked with 1,000 mg/kg of GIM and up. Based on the decrease in TNT concentrations observed using this procedure, soils amended with GIM at 1,000 and 10,000 mg/kg would need more than a year of W-A to reach non-toxic concentrations. Aqueous toxicity tests conducted with a GIM weathered and aged outdoors for 2 years did show a clear decrease of toxicity for the aged samples compared to fresh GIM. This lower toxicity was explained by the lower content of TNT remaining in the aged samples.

VI. ACKNOWLEDGEMENTS

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