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Environmental Laboratory

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Final report

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Preface

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Identifying degradation products responsible for increased toxicity of UV-Degraded insensitive munitions

A B S T R A C T

Degradation of insensitive munitions (IMs) by ultraviolet (UV) light has become a topic of concern following observations that some UV-degradation products have increased toxicity relative to parent compounds in aquatic organisms. The present investigation focused on the Army's IM formulation, IMX-101, which is composed of three IM constituents: 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and nitroguanidine (NQ). The IM constituents and IMX-101 were irradiated in a UV photo-reactor and then administered to *Daphnia pulex* in acute (48 h) exposures comparing toxicities relative to the parent materials. UV-degradation of DNAN had little effect on mortality whereas mortality for UV-degraded NTO and NQ (and associated degradation products) increased by factors of 40.3 and 1240, respectively, making UV-degraded NQ the principle driver of toxicity when IMX-101 is UV-degraded. Toxicity investigations for specific products formed during UV-degradation of NQ, confirmed greater toxicity than the parent NQ for degradation products including guanidine, nitrite, ammonia, nitrosoguanidine, and cyanide. Summation of the individual toxic units for the complete set of individually measured UV-degradation products identified for NQ only accounted for 25% of the overall toxicity measured in the exposures to the UV-degraded NQ product mixture. From these toxic unit calculations, nitrite followed by CN^- were the principal degradation products contributing to toxicity. Given the underestimation of toxicity using the sum toxic units for the individually measured UV-degradation products of NQ, we conclude that: (1) other unidentified NQ degradation products contributed principally to toxicity and/or (2) synergistic toxicological interactions occurred among the NQ degradation product mixture that exacerbated toxicity.

1. Introduction

As production of insensitive munitions (IMs) continues to expand, there is a need to understand how environmental processes affect the fate and effects of IMs during production and use. Given that IMs provide greater safety to soldiers due to reduction in unintended detonations, IMs are being pursued as a replacement for legacy munition compounds, such as trinitrotoluene (TNT). One of the Army's insensitive munitions, IMX-101, consists of a mixture of 2–4 dinitroanisole (DNAN), nitrotriazolone (NTO), and nitroguanidine (NQ) and is being increasingly deployed as a

replacement for conventional munitions across weapons platforms (Lee et al. 2010; Williams et al., 2014). While the NQ parent compound is less toxic than TNT, RDX, DNAN and NTO in aquatic exposures (Gong et al., 2018; Gust et al., 2018; Gust et al., 2014; Gust et al., 2011; Lotufo et al., 2018; Stanley et al., 2015; Warner et al., 2012), the UV-degradation of NQ can generate products with higher toxic potency than the UV-degradation products of the other munitions constituents and nearly equal to those of TNT (Kennedy et al., 2015; Kennedy et al., 2017; Gust et al., 2017).

Nitroguanidine and NTO are much more water soluble than the munitions they are expected to replace; coupled with their low adsorption affinity to soils, these compounds are likely to be more mobile in waters than TNT (Taylor et al., 2015a). Whether the introduction of IMs to aquatic environments is due to permitted discharges from manufacturing facilities (Ribeiro et al., 2012; Li et al., 2013) or live fire use on training ranges (Taylor et al., 2015b), more information on IM fate and ecotoxicity is warranted. Aqueous NQ has been found to UV-degrade into a number of small nitrogenous compounds including nitrite, nitrate, ammonia, hydroxylamine, cyanamide, cyanoguanidine, urea, guanidine, hydroxyguanidine, and melamine (Burrows et al., 1988; Spanggord et al., 1987; Noss and Chyreck, 1984; Haag et al., 1990). This degradation was rapid, with a half-life of 0.6 days when irradiated by natural sunlight in the summer months. DNAN was found to generate methoxy nitrophenols and nitrite/nitrate, and dinitrophenol and presumably methanol during UV-degradation (Rao et al., 2013; Halasz et al., 2018; Taylor et al., 2017). To date, little information regarding the UV-degradation of NTO has been published (Le Campion et al., 1999), whereas a greater number of studies have focused on biodegradation pathways for this compound (Krzmarzick et al., 2015; Le Campion et al., 1998; Le Campion et al., 1998). Overall, each constituent of IMX-101 has been observed to be susceptible to UV-degradation, thus we sought to determine the impacts of these degradation products on toxicity.

Nitroguanidine was found to have a marked increase in toxicity after exposure to ultraviolet light, 2–3 orders of magnitude in certain circumstances (Kennedy et al., 2017; Gust et al., 2017; van der Schalie, 1985), and represents a principle concern regarding the environmental risk of IMX-101 effluents that are either treated by UV-light (Felt, et al., 2013; Perreault et al., 2013) or are exposed to natural sunlight. Uncertainties associated with UV-degraded NQ have been a challenge for IMX-101 release permitting under the Clean Water Act, National Pollution Discharge and Elimination System (40CFR 122.44d). With the growing body of literature describing this increased toxicity of the undefined UV-degradation products of NQ, we set forth to determine if the toxicity of the product mixture could be explained by previously reported NQ degradation compounds (e.g., ammonia, nitrite, guanidine, etc.) in addition to other degradation-product chemicals detected in the present study. Within the larger scope, the objectives of this investigation were to: (1) contrast parent material toxicity versus UV-degraded IM toxicity for all IMX-101 constituents including DNAN, NTO, and NQ as well as the IMX-101 formulation using standard acute toxicity assessments in *Daphnia pulex* (US EPA 2002); (2) determine and quantify the UV-degradation products, specifically for NQ; and (3) characterize the relative toxicity for all individual UV-degradation products identified for NQ to determine the primary source(s) of toxicity within the UV-degraded NQ product mixture.

2. Materials and methods

2.1. Materials

Military grade NTO (>95% pure), and the IM formulation IMX-101 were obtained from the Holston Army Ammunition Plant (BAE Systems, Holston Army Ammunition Plant, TN) and used without further purification. Nitroguanidine, (NQ; 25% hydrated to stabilize), 2,4-dinitroanisole (DNAN), cyanamide, cyanoguanidine, nitrite, nitrate, guanidine hydrochloride, ethyl acetate, pyridine, FerroZine™ (97%) and zinc dust were purchased from Sigma Aldrich (St. Louis, MO). Potassium cyanide was purchased from Hach (Loveland, CO), and ammonium chloride, methanesulfonic acid was purchased from Fisher (Waltham, MA). Chloramine T (98%) and barbituric acid were purchased from Spectrum (New Brunswick, NJ). All chemicals were >99% purity unless otherwise stated and used as received. Nitrosoguanidine (GuNO, 11% pure) was synthesized from literature procedures (Davis and Rosenquist, 1937) where specific methods are provided in the Supplemental Materials. All chemical solutions were made using high-purity water (>18.2 MΩ) produced using a Gemini High Purity Water Filtration system (Aries Filter Works, Berlin, NJ).

2.2. UV-degradation

UV-Degradation was conducted using solutions of the parent compounds for both toxicity exposures and degradation product analysis. For product identification studies, solutions of nominally 0.96 mM (NQ, 100 mg/L), 0.58 mM (DNAN, 115 mg/L), and 0.08 mM (NTO, 10.4 mg/L) were prepared in high purity water and 5 mL added to each of 6 quartz test tubes (Technical Glass, Painesville Twp., OH, 1.18 cm ID) and 100 mL of DNAN solution was added to a quartz beaker (Technical Glass, 3.2 cm ID) capped with a rubber stopper. The tubes were arranged in the outer ring of the carousel inside a Rayonet photoreactor (Model RPR-100, Southern New England Ultraviolet Co. Branford, CT) equipped with sixteen, 300 nm lightbulbs (RPR-3000A, Southern New England Ultraviolet Co.). Irradiation times were carefully controlled with a timer controller (Control Co. Webster, TX), and photon flux measured to be $1.38 \pm 0.16 \times 10^{-8}$ E per second by ferrioxalate actinometry (Goldstein and Rabani, 2008) where the formation of ferrous iron was monitored by UV–vis spectroscopy (Cary 8454, Agilent, Santa Clara, CA) after chelation with FerroZine™.

The UV-degradation of IMs for toxicity assessments were performed as we previously described in Kennedy et al. (2017). Briefly, solutions of nominally 100 mg/L NQ, 121 mg/L NTO, 100 mg/L DNAN and 100 mg/L IMX-101 were prepared in 220 μS cm^{-1} and 100 mL was added to each of 10 quartz beakers (Technical Glass, 3.2 cm ID) inside the previously described photo-reactor for 4 h. The beakers were arranged in a custom made carousel holder (to accommodate larger volumes required for bioassays) along the outer ring. UV-degradation solutions were mixed well prior to determination of degradation by high performance liquid chromatography (HPLC, Agilent 1200 series, Agilent Technologies Santa Clara, CA), and transported in 1L amber bottles to the toxicity lab to avoid further UV-degradation.

2.3. Analytical

Degradation for all IMs were measured using HPLC (Agilent 1200 series, Santa Clara, CA Bruker Hystar software Billerica, MA) using a C_{18} column (Phenomenex, Synergi™ 4 μm Hydro-RP 80 Å liquid chromatography column (LC), 250 × 4.6 mm, Torrance, CA) at a flow rate of 1.1 mL/min, a column temperature of 25 °C, and diode array detection (Russell et al., 2014). NQ was analyzed using a 100% water mobile phase, NTO was analyzed using a 0.005% aqueous trifluoroacetic acid mobile phase, and DNAN was analyzed using an isocratic mobile phase of 40% methanol in water. Ammonia and guanidine were analyzed by ion chromatography (Dionex ICS-5000, Sunnyvale, CA), with a CS16 analytical column and a

conductivity detector, using an isocratic mobile phase of 30 mM methanesulfonic acid in high-purity water flowing at a rate of 0.6 mL/min.

Cyanide was detected on a Lachat QuikChem Flow Injection Analysis System (Hach, Model QC8500 Series II, Loveland, CO) with high purity water as the carrier using the following aqueous solutions: 1 M sodium dihydrogen phosphate with pH adjusted to 8 with 1 M NaOH, 4 g/L chloramine T, and a color reagent consisting of 15.0 g barbituric acid, 75 mL pyriadine, and 15 mL concentrated hydrochloric acid (12 M HCl) diluted to a volume of 1 L. Samples were distilled by using either a Lachat microdist or minidist system (Hach, Loveland, CO) prior to analysis to remove interfering compounds. Samples generated via NQ UV-degradation had 0.024 g of sulfamic acid added to each 6 mL sample prior to the distillation to avoid nitrite and nitrate interferences.

Products of DNAN UV-degradation were identified by gas chromatography mass spectrometry (GC-MS) using an Hewlett Packard 6890 Series (Agilent, Santa Clara, CA), GC and Hewlett Packard 5973 MS following modifications of USEPA method 8270D (1998) and matching to the NIST spectral library (NIST v17, Software v2.3 Gaithersburg, MD). The UV-degraded mixture (30 mL) was acidified to facilitate liquid-liquid extraction into ethyl acetate (3×25 mL), and the combined organic layers concentrated by rotary evaporation.

2.4. Aquatic hazard testing

Acute (48-h) toxicity bioassays were conducted for each test chemical using to a standard method (U.S. EPA 2002) relevant to the National Pollutant Discharge Elimination System, enforced by the Clean Water Act. *Daphnia pulex* was selected because it is a standard freshwater ecotoxicological model, has shown greater sensitivity to some chemicals relative to a conspecific (*D. magna*), and was more sensitive to DNAN than *Ceriodaphnia dubia* (Kennedy et al., 2015), yet has no literature reported for UV-degraded IM toxicity. The source of the *D. pulex* was A. Beckerman (University of Sheffield, UK), a previously sequenced genotype (Kennedy et al., 2015) that is particularly well studied in genomics investigations of ecotoxicological effects (Eads et al., 2008) and can be considered a human health model due to a large number of shared genes (Colbourne et al., 2011).

Briefly, acute toxicity bioassays were conducted at $25 \pm 1\,^{\circ}C$ using a 16L:8D photoperiod (wide spectrum florescent light, no UV) in environmentally controlled chambers (Darwin Chambers Company, St. Louis, MO, USA). The parent (undegraded) compounds: IMX-101 (348 mg/L, measured), NQ (1,619 mg/L, measured), NTO (1,507 mg/L, measured) and DNAN (120 mg/L, measured) were used in bioassay exposures at relatively high concentrations that were selected specifically to induce a toxicological effect for comparison to the UV-degraded IMs toxicity. For each assessment, the parent IMs, UV-degraded IMs, and five individual UV-degradation products identified in the UV-degraded NQ product mixture included 5 to 8 exposure concentrations generated from a 50% dilution series (Supplemental Table S1), using a standard reconstituted water formulated according to US EPA (2002) and diluted to 220 μS/cm, which was found optimal for *D. pulex* (Laird et al., 2015). This water was used as the diluent and the control. Each exposure concentration consisted of eight replicate 40 mL glass scintillation vials, each containing 5 organisms. The individual nominal exposure concentrations tested are provided for each compound in the supporting information (Supplemental Table S1). Water quality parameters (temperature, pH, dissolved oxygen, conductivity) were measured at test termination and water samples were collected at test initiation and termination for analytical determination of the chemical exposures. Toxicological endpoints were generated from measured concentrations summarized by arithmetic means (if the concentration changed < 20% from test initiation to termination) or geometric means (if concentration changed >20% from test initiation to termination), as described in OECD methods (OECD, 2012). The test endpoint assessed was survival following 48-h exposure. Median Lethal Concentrations (LC50) and partial lethal concentrations (e.g., LC10) were calculated using ToxCalc (Tidepool Scientific, McKinleyville, CA) using the trimmed Spearman Karber Method. No effect concentrations were calculated using U.S. EPA BenchMark Dose Software (BMDS) (v2.7; US Environmental Protection Agency, 2018).

To infer information on individual IM constituent contribution to IMX-101 mixture toxicity, as well as contribution of NQ degradation products to the UV-degraded NQ toxicity, toxic units (TUs) were determined as the quotient of the measured parent compound concentration and the LC50 of each constituent, as previously described (Han et al., 2006). All concentrations were based on the original parent compound concentration so that a relative UV-degraded-to-parent compound comparisons in toxicity could be determined as well as the relative contribution to the UV-degraded product to the IMX-101 mixture formulation.

3. Results and discussion

3.1. Comparative toxicity of parent versus UV-Degraded IMX-101 and constituents

UV-degradation of IMX-101 caused an approximately 1 order of magnitude increase in toxic potency, decreasing the 48 h LC50 from 62.5 mg/L to 4.65 mg/L (Table 1, Supplemental Fig. S1). Comparative evaluation of parent versus UV-degraded IM constituents that make up the IMX-101 mixture indicated significant increases in toxicity for NQ and NTO, but not DNAN (Table 1, Supplemental Fig. S1). The most dramatic increase in toxicity was a >3 order of magnitude increase in toxicity for NQ when 90% of the parent compound had been degraded, leading to a decrease in the LC50 from 1,485 mg/L to 1.2 mg/L. Even relatively minor degradation of NQ (7% of parent NQ degraded) lead to a nearly 2 order of magnitude increase in toxicity (Table 1). UV-degradation of NTO caused a 3.7-fold increase in toxicity, decreasing the LC50 of NTO from 113.6 mg/L to 30.5 mg/L in non-pH adjusted exposures (Table 1). The increases in NQ and NTO toxicity when UV-degraded are consistent with previous results with another cladoceran, *C. dubia* (Kennedy et al., 2017).

Data sets for *D. pulex* (generated in the present study) and *C. dubia* (Kennedy et al., 2017) were investigated utilizing a toxic units approach to provide insight into the relative contribution of each constituent of IMX-101 to overall toxicity before and after UV-degradation (Fig. 1). Prior to UV-degradation, DNAN was the most toxic constituent within the IMX-101 mixture formulation, whereas after UV-degradation, the toxicity of IMX-101 increased in both *D. pulex* and *C. dubia* where the toxic units indicated UV-degraded NQ was the principle driver of toxicity (Fig. 1). The similarity between the toxic responses between the two species support the observation that toxicological responses are conserved and consistent between cladoceran species. Although increased toxicity of UV-degraded NQ is pervasive across species (Kennedy et al., 2017; Gust et al., 2017; van der Schalie, 1985), the >3 order of magnitude increase observed in the *D. pulex* exposed to the 90% UV-degraded NQ (Supplemental Fig. S1) is the greatest increase in toxicity associated with UV-degraded IMs reported in the published literature to date.

Table 1
Toxicity values for munitions and UV-transformed munitions. All test data were for *Daphnia pulex*, unless otherwise specified. All units are in mg/L. LC50 curves for each of the tested munitions and the concentrations used for each exposure can be found in Supplemental Fig. S1 and Supplemental Table S1).

munition	LC50	LC10	reference
TNT	5.1 (1.5–17.9)[A]	–	Johnson et al. (1994)
	4.0 (3.7–4.5)[A]		Burton et al., 1993
IMX-101	62.5 (52.3–72.4)	35.6 (24.0–44.2)	this study
DNAN	25.2 (20.1–30.0)	12.28 (7.5–16.2)	this study
NTO (neutralized pH)	1234.8 (1040.7–1463.6)	718.6 (445.8–888.3)	this study
NTO (pH ≈ 2.7)	113.6 (111.4–115.9)	–	this study
NQ	1485.7 (999.1–3220.2)	318.6 (197.3–433.9)	this study
TNT UV-degradation products	1.0 (0.6–1.7)	–	Johnson et al. (1994)
IMX-101 UV-degradation products	4.7 (4.1–5.3)	2.8 (2.2–3.3)	this study
DNAN UV-degradation products	27.6 (25.0–30.6)	NA	this study
NTO UV-degradation products	30.5 (27.5–34.2)	22.1 (18.2–24.9)	this study
NQ UV-degradation products (90% degraded)	1.2 (0.4–2.3)	0.4 (0.0–0.8)	this study
NQ UV-degradation products (7% degraded)	17.4 (15.2–19.9)	4.4 (0.1–10.1)	this study

[A] for *Daphnia magna*.

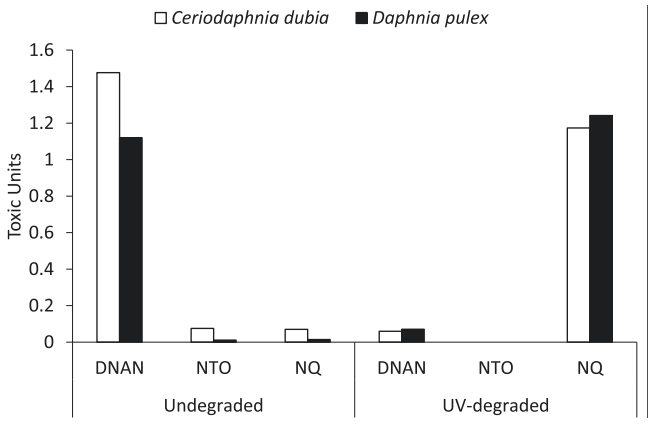

Fig. 1. Toxic units of individual IM constituents for two cladoceran species, *D. pulex* and *C. dubia*, before and after the IMs were subjected to UV-irradiation. Data for *C. dubia* is derived from Kennedy et al. (2017).

3.2. Kinetics of UV-Degradation for each IMX-101 constituent

In parallel to the acute toxicity assays described above, the UV-degradation rates were determined for each of the IM-101 constituents (Supplemental Fig. S2). For DNAN and NQ, the degradation kinetics were best described by 0^{th} order reactions with rate constants of 0.0032 M h^{-1} and 0.0397 M min^{-1}, respectively. For NTO, the concentration was sufficiently low for the UV-degradation to be modeled as a pseudo-first order reaction with a rate constant of 0.015 min^{-1}. The different starting concentrations (i.e., optical density) hindered the ability to make direct comparisons of the rate constants, but relative comparisons of the concentrations tested indicated the trend of $k_{NQ} > k_{DNAN}$, which corresponds with previous observations (Moores et al., 2019; Halasz et al., 2018).

3.3. Identification of UV-Degradation products for DNAN and NTO

UV-degradation of DNAN resulted in the formation of nitrite (Supplemental Fig. S3), nitrate (Supplemental Figs. S4 and S5), dinitrophenol (Supplemental Fig. S6) and both isomeric methoxynitrophenols (Supplemental Fig. S7), in agreement with previous observations (Rao et al., 2013; Halasz et al., 2018; Taylor et al., 2017). A number of other products were observed as products of UV-degradation for DNAN which could not be unequivocally identified, and their spectra and discussion can be found in the Supplemental Materials (Supplemental Figs. S8–S11). Formation of nitrite (Supplemental Fig. S12) and nitrate (Supplemental Fig. S13) was observed during the UV-degradation of NTO, and presumably the corresponding hydroxylated triazolone or its tautomer, urazole (Le Campion et al., 1998). During these initial UV-degradation product identification experiments, a greater focus was made on identifying the products of NQ, since there is now abundant evidence that these UV-degradation products are the most important for determining the increased toxicity of the UV-degraded IMX-101 formulation.

3.4. Identification of UV-Degradation products for NQ

We first identified UV-degradation products for NQ from literature observations (Noss and Chyreck, 1984; Spanggord et al., 1987; Burrows et al., 1988) and empirically measured product formation in NQ UV-degradation kinetics experiments (Fig. 2). The results indicated that nitrate + nitrite formed the most rapidly followed by guanidine. Only trace amounts of ammonium and cyanoguanidine were formed after >40% degradation of the parent NQ. Previously, formation of the monomer of cyanoguanidine, cyanamide, and its trimer, melamine has been demonstrated (Noss and Chyreck, 1984; Spanggord et al., 1987; Burrows et al., 1988). The HPLC chromatograms in Fig. 3 show the overlay of cyanoguanidine and a sample of NQ before and after UV-degradation demonstrating the growth of poorly resolved peaks with retention times of 1.5–2.5 min, which are likely ionic constituents and guanidine eluting with the injection peak, a peak with a retention time of 2.9 min that could not be identified in the initial screening, and a peak with a retention time of 3.6 min which corresponds to cyanoguanidine formation. The ratio of the integrations for cyanoguanidine using wavelengths of 216 nm and 230 nm is 3.82 compared to the integration ratio of 3.81 for the NQ UV-degradation product, each at the same retention time. The similarities in the cyanoguanidine and NQ UV-degradation product retention times and integration ratios gives good evidence that the identification of the degradation product observed in the present study is cyanoguanidine. Retention times found for cyanamide was very similar to cyanoguanidine, but had 216/230 ratios of 0.26. Melamine eluted later than cyanamide and cyanoguanidine, but did not have a corresponding peak in the NQ UV-degradation chromatograms. Summing the products in Fig. 2 accounted for 65.6% and 76.7% of carbon and nitrogen, respectively, on a molar basis.

3.5. Toxicity screening of previously identified NQ UV-Degradation compounds

Toxicity results for the five NQ UV-degradation products

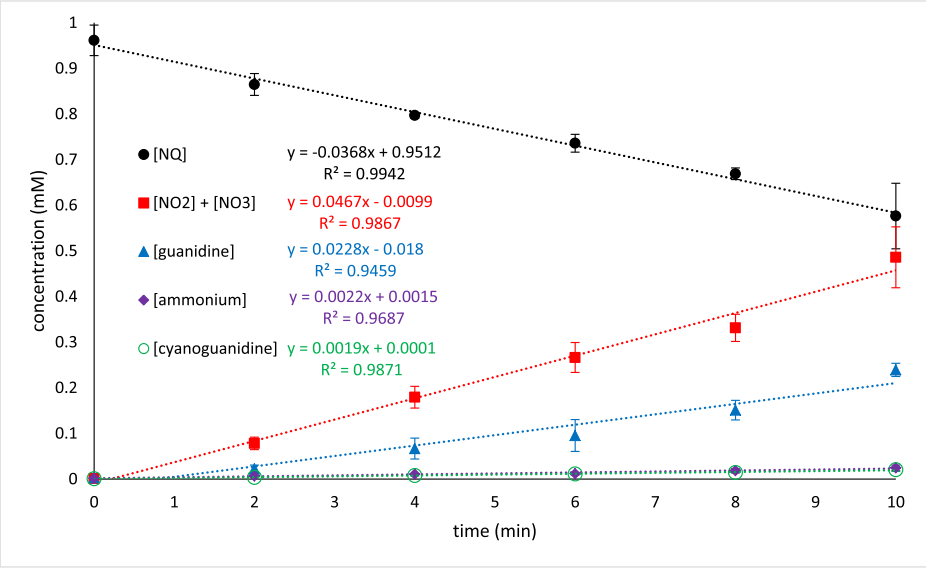

Fig. 2. UV-degradation of NQ (black) and formation of identified products, nitrite and nitrate (red), guanidine (blue), ammonia (purple), and cyanoguanidine (green). Error bars represent the standard deviation of the measured concentrations of triplicate experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

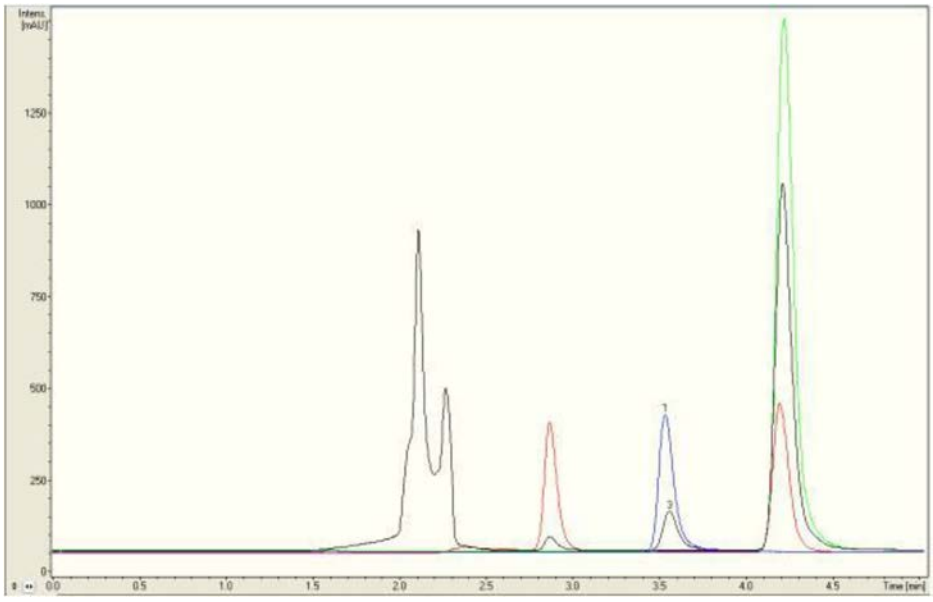

Fig. 3. UV chromatograms of NQ UV-degradation (t = 0, green; t = 10 min, black), cyanoguanidine (blue), and synthesized nitrosoguanidine (red). Note the synthesized nitrosoguanidine contains the starting material, NQ. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

identified in the literature (Gersich and Hopkins, 1986; Xiang et al., 2010; Scott and Crunkilton, 2000) and further confirmed using analytical chemistry in the present study were determined. We used a combination of literature values for *D. pulex* as well as generation of new toxicity data for previously untested chemicals to provide context for the sum toxicity of UV-degraded NQ (Table 2). Cyanamide and its oligomers, cyanoguanidine (detected here) and melamine, did not elicit a toxic response by *D. pulex*, thus no analytical confirmations were conducted for these tests. Of these products found in the initial screening, guanidine was the most toxic with an LC50 of 14.2 mg/L. Although relatively toxic, the presence of guanidine in the UV-degraded NQ product mixture was relatively low (~1.0 mg/L) after the 1:100 sample dilution (Table 2, Column 7) that was required to eliminate 100% mortality of *D. pulex* in the highly-potent 90% UV-degradation product of the 100 mg/L NQ solution (Supplemental Fig. S1). In fact, after accounting for all of the initially screened UV-degradation products, the toxicity of the NQ UV-degradation product mixture could not be explained. Therefore, we sought to determine if additional, presently unidentified UV-degradation products were present, and if present, the contribution of each to toxicity in *D. pulex*.

3.6. Further NQ UV-Degradation product identification and toxicity assessments

A more rigorous investigation of the UV-degradation chemistry

Table 2
Toxicity values for individual NQ UV-degradation products. All test data were for *Daphnia pulex*, unless otherwise specified. All concentrations have the units mg/L.

chemical	LC50	reference	7% degraded		90% degraded	
			conc. in product mixture[e]	conc. at LC50[f]	conc. in product mixture[e]	conc. at LC50[f]
previously identified products						
ammonia-N[a]	26.25 (24.11–28.75) [b]	ich and Hopkins, 1986	0.256	0.045	2.66	0.0313
nitrite-N	18.72 (14.7–30.51)[c]	Xiang et al., 2010				
	28.78 (24.58–35.13)[c]	Xiang et al., 2010				
nitrate-N	323 (198–469)[d]					
	453 (299–659)[d]					
	611 (455–820)[d]	t and Crunkilton,	4.69[g]	0.814[g]	135[g]	1.59[g]
guanidine	14.2 (12.4–16.2)	this study	3.23	0.562	84.8	1.00
cyanoguanidine	>100 (nominal)	this study	1.15	1.990	10.8	0.128
cyanamide	>100 (nominal)	this study	N/A		N/A	
melamine	>100 (nominal)	this study	N/A		N/A	
Newly Identified Products						
Nitrosoguanidine	>11 (nominal)[h]	this study	N/A		N/A	
cyanide	0.09 (0.07–0.10)					
	0.13 (0.11–0.16)	this study	0.07	0.012	0.35	0.0041
	0.17 (0.15–0.20)					

[a] presented as total ammonia-N for units consistency, albeit unionized ammonia is more predictive of toxicity.
[b] Back calculated from unionized ammonia LC50 of 2.94 (2.7–3.22) using pH 8.5 and temperature of 20 °C (11.2% unionized).
[c] For *Daphnia similoids*.
[d] For *Daphinia magna*.
[e] Concentrations calculated from rates of formation.
[f] Concentrations calculated by accounting for dilution of 100 ppm starting concentration to the determined LC50 for UV-degraded NQ (17.36 ppm and 1.18 ppm for the 7% and >90% degraded experiments, respectively) to estimate the maximum exposure concentration.
[g] Expressed as the sum of nitrite and nitrate concentrations due to interconversion during UV-degradation experiments.
[h] *D. pulex* exposed to an impure mixture of 11 ppm GuNO and 89 ppm NQ.

of NQ was conducted and toxic responses to the degradation products assessed. First, nitrosoguanidine (GuNO) has been proposed to be a UV-degradation intermediate of NQ (Noss and Chyreck, 1984; Burrows et al., 1988). However, this had been refuted initially due to the finding that the N–NO_2 scission was homolytic (Pace and Holmes, 1983), and presumed to be the result of microbial reduction (Kaplan, et al., 1982). Investigation of the chromatogram's unidentified peak (Fig. 3, RT = 2.9 min) corresponded well with the elution of GuNO in chromatograms from literature accounts (Burrows et al., 1984). Given no commercially available analytical standard for GuNO, we conducted synthesis of the chemical for analytical and toxicity bioassay use. HPLC analysis of the synthesized product showed poor purity of the synthetized GuNO (11%, 89% NQ), but sufficient overlap with the unidentified peak and nearly identical 254nm/300 nm integration ratios of 21.1 and 21.2 for the synthesized GuNO and UV-degraded NQ, respectively to provide reasonable certainty of its presence. The GuNO/NQ product mixture was utilized in an acute *D. pulex* toxicity test indicated that approximately 11 mg/L GuNO +89 mg/L NQ was not acutely toxic. It should be noted that more or less of the GuNO may have been present in the product mixture, as no analytical determinations against chemical standards were possible with the low purity synthesized GuNO. Overall, Nitroso compounds are typically regarded as hazardous materials due to their carcinogenic properties (Swann, 1975), which may be of lesser importance in an acute test in *D. pulex*; however, chronic exposure assessments and further fate studies of this product are warranted. Regarding the environmental relevance of GuNO formation, the greater photolytic rate compared to NQ (Burrows et al., 1988) suggests this product can only form when penetration depths are kept short (i.e., high optical density, large concentrations of NQ). It is not anticipated that this product would form in significant quantities under sunlight-driven UV-degradation of NQ in natural waters, but in might be a product of concern as a potential byproduct of wastewater treatment employing UV-degradation.

With the results from GuNO showing no acute toxicity at the concentration tested, further experimentation was undertaken to identify products that might explain the toxicity of the UV-degraded NQ product mixture. Preliminary colorimetric testing gave a positive result for cyanide; however, cyanamide, cyanoguanidine, melamine, nitrite and nitrate were all found to give false positive results. Upon distillation of solutions containing each individual product, only UV-degraded NQ product mixture and potassium cyanide were capable of being distilled. The formation of cyanide was determined analytically during the UV-degradation of NQ and its formation is shown in Fig. 4. To the authors' knowledge, this is the first time, cyanide was analytically confirmed to be a product of NQ UV-degradation. Although CN is highly toxic, the concentration in the UV-degraded product mixture was very low

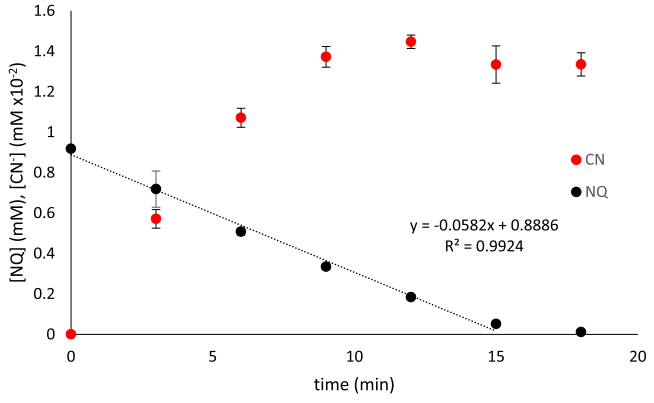

Fig. 4. Formation of cyanide (red) and NQ UV-degradation (black). Note the different units for cyanide and NQ concentrations. Error bars represent the standard deviation of the analytically determined concentrations of NQ and CN^- at each sampling interval in triplicate experiments. %RSD for each of the triplicate samples was <1.0% for CN^-. The trend line representing pseudo-0^{th} order kinetics was observed through the sample at 12 min, but deviated from linearity after this point as the solution became optically dilute where pseudo-1st order kinetics would best represent the UV-degradation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

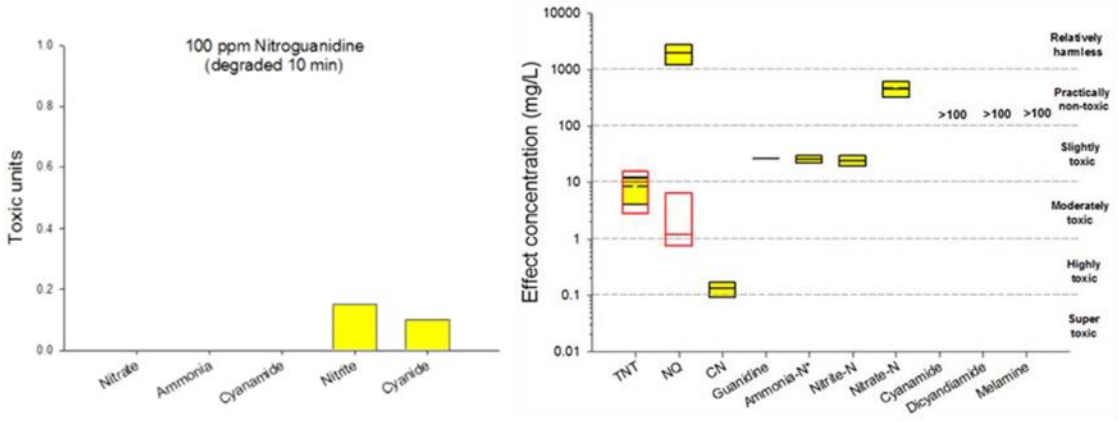

Fig. 5. Toxic units and relative toxicity of UV-degraded NQ solutions.

(0.004 mg/L) after the 1:100 dilution that was required to eliminate 100% mortality of *D. pulex* in the UV-degraded NQ product exposures (Table 1, Supplemental Fig. S1). Further investigation on the formation, stability and fate of the cyanide may be warranted, as there is an apparent decrease in concentration after NQ UV-degradation is complete. This could be due to the reaction of cyanide with hydroxyl radicals generated from the photolysis of nitrate/nitrite (Mack and Bolton, 1999), volatilization of HCN, or sorption by the septa used to deter HCN volatization during the UV-degradation experiments.

4. Conclusions

The toxicity of the UV-degraded IMX-101 mixture was confirmed to be in large part due to the degradation products of NQ. Many of the UV-degradation products found for NQ, NTO and DNAN from previous work were confirmed by this study. Additionally, for the first time, cyanide was detected during UV-degradation of NQ. Among the analytically determined NQ UV-degradation products, cyanide was the most toxic, followed by guanidine, nitrite, and ammonia. However, individual concentrations of each of these products cannot fully account for the observed toxicity of the UV-degradation product mixture. Specifically, nitrite and CN contributed TUs of approximately 0.15 and 0.10, respectively (Fig. 5), thus accounting for only ~25% of the observed toxic response. Evaluation of nitrosoguanidine requires further testing with a pure material to provide a robust toxicity assessment, however our initial evaluation suggests that it is not formed in sufficient quantity and does not have high enough potency to explain the sum-balance of the observed toxicity of UV-degraded NQ. It is, therefore, hypothesized that the acute toxicity of the UV-degradation product mixture may be attributable to as yet to be identified degradation products or a toxicologically synergistic effect among degradation products. Additional efforts are required to better characterize the mixture toxicology among NQ UV-degradation products and to elucidate the mode(s) of action utilizing a toxicogenomics approach to understand the degree to which these products and interactions contribute to the observed toxic response. Finally, efforts to characterize the real-world scenarios of IMX-101 constituent UV-degradation and the toxicity of NQ UV-degradation by natural sunlight and the rate by which these processes occur is needed to determine the environmental implications of UV-induced NQ degradation on IMX-101 toxicity in nature.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.124958.

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14. ABSTRACT

Degradation of insensitive munitions (IMs) by ultraviolet (UV) light has become a concern following observations that some UV-degradation products have increased toxicity relative to parent compounds in aquatic organisms. This investigation focused on the Army's IM formulation, IMX101, composed of three IM constituents: 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and nitroguanidine (NQ). The IM constituents and IMX101 were irradiated in a UV photo-reactor and then administered to Daphnia pulex in acute (48 h) exposures comparing toxicities relative to the parent materials. UV-degradation of DNAN had little effect on mortality whereas mortality for UV-degraded NTO and NQ increased by factors of 40.3 and 1240, making UV-degraded NQ the principle driver of toxicity when IMX101 is UV-degraded. Toxicity investigations for specific products formed during UV-degradation of NQ, confirmed greater toxicity than the parent NQ for degradation products. Summation of the individual toxic units for the complete set of individually measured UV-degradation products identified for NQ only accounted for 25% of the overall toxicity measured in the exposures to the UV-degraded NQ product mixture. Given the underestimation of toxicity using the sum toxic units for the individually measured UV-degradation products of NQ, we conclude that: (1) other unidentified NQ degradation products contributed principally to toxicity and/or (2) synergistic toxicological interactions occurred among the NQ degradation product mixture that exacerbated toxicity.

15. SUBJECT TERMS

Nitroguanidine, Cyanide, Nitrosoguanidine, Daphnia pulex, Toxicity, IMX-101

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