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Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX) Numerical Water Quality Criteria for the Protection of Freshwater Aquatic Organisms

Dennis T Burton* and Steven D Turley

University of Maryland, College of Agriculture and Natural Resources, Wye Research and Education Center Queenstown, Maryland, United States

***Corresponding author:** Dennis T Burton, University of Maryland, Wye Research and Education Center, P.O. Box 169, Queenstown, Maryland 21658, Tel: 443-446-4264, E-mail: dburton@umd.edu

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Abstract

Hexahydro-1,3,5-trinitro-1,3,5-triazine (CAS 121-82-4) is a high energetic explosive used extensively in a number of applications by the military. The compound can enter the aquatic environment during manufacturing activities, detonation, and other military activities. It has been shown to be toxic to aquatic organisms. The present study established the United States Environmental Protection Agency numerical freshwater ambient water quality criteria for the compound. The Criterion Maximum Concentration (CMC), which indicates that aquatic life should not be affected if the one-hour average concentration of RDX does not exceed the value more than once every three years, is 2.24 mg/L. The Criterion Continuous Concentration (CCC), which indicates that aquatic life should not be affected if the four-day average concentration of RDX does not exceed the value more than once every three years, is 1.76 mg/L.

Keywords: Hexahydro-1,3,5-trinitro-1,3,5-triazine; RDX; Aquatic Toxicity; Water Quality Criteria; Criterion Maximum Criterion (CMC); Criterion Continuous Criterion (CCC); Invertebrates; Fish

Introduction

Hexahydro-1,3,5-trinitro-1,3,5-triazine (CAS 121-82-4) is a high energetic explosive used extensively in a number of applications by the military. The compound may enter the aquatic environment during manufacturing activities and blending operations. It can also be released during firing and detonation of munitions, disposal of ordnance, and other military actives. In 2017 the United States Environmental Protection Agency (EPA) identified RDX at 32 sites on the EPA National Priorities List [1]. RDX has been shown to be toxic to a number of freshwater invertebrates and fish [2-4].

Definitive EPA numerical national water quality criteria for the protection of freshwater organisms and their uses have not been established for RDX [5]. EPA ambient water quality criteria (AWQC) consist of acute and chronic values. The acute AWQC or the Criterion Maximum Criterion (CMC) is the highest 1-hour average concentration over a 3-year period that should protect aquatic organisms and their uses [6]. The chronic AWQC or Criterion Continuous Criterion (CCC) is the highest 4-day average concentration over a 3-year period that should protect aquatic organism and their uses.

Etnier [2] and Hovatter, *et al.* [7] reviewed the toxicological literature and concluded that sufficient data were not available to establish AWQC for freshwater organisms. ENSR [3] established a provisional acute ambient water quality criterion for the protection of freshwater aquatic life; sufficient data to estimate a chronic freshwater AWQC were not available. ENSR [3] calculated toxicity reference values for saltwater organisms and used these values to estimate acute and chronic AWQC for saltwater organisms. Numerical national AWQC for saltwater organisms were not considered in this evaluation because sufficient RDX toxicity data still do not exist for saltwater numerical national water quality criteria. The objective of this study was to develop numerical water quality for freshwater organisms because of the continued manufacture, use, and number of sites contaminated by RDX. Both the Criterion Maximum Criterion and Criterion Continuous Criterion were calculated for freshwater organisms.

Materials and Methods

The data used in the criteria analyses were taken from bioassays conducted with published toxicity tests methods. RDX concentrations were measured in all toxicity tests with the exception of one acute study [8]. The EPA guidelines state that toxicity results of static and renewal acute tests based on initial nominal concentrations are acceptable if measured concentrations are not available. Toxicity values reported as "greater than" values and values that were at or near the solubility of RDX in the test systems were used [6].

Criterion Maximum Criterion

Toxicity data for at least one species in eight different families is required to develop a Criterion Maximum Criterion [6]. Sufficient acute toxicity data were available for invertebrates and fish (Table 1) to calculate a Final Acute Value (FAV) which was used to establish the CMC. The FAV is established using the Genus Mean Acute Value (GMAV) for one of more toxicity values for the genus. In the cases where more than one toxicity value is available for the genus, the geometric mean of the values is used for the GMAV.

Species	Exposure Duration (Hours)	LC50/EC50[a] (mg/L)	GMAV[b] Values (mg/L)	Reference(s)
Invertebrates				
Asellus militaris (Sourbug)	48	>100	100	[9]
Ceriodaphnia dubia (Cladoceran)	48	>17.0	17	[10]
Chironomous tentans (Midge)	48	>100		[9]
Chironomous tentans	48	>15	39[c]	[9]
Daphnia magna (Water flea)	48	>100		[9]
Daphnia magna	48	>15		[9]
Daphnia magna	48	>55	44[c]	[8]
Gammarus fasciatus (Scud)	48	>100	100	[9]
Hydra littoralis (Hydra)	48	>32.3	32.3	[10]
Paratanytarsus parthenogeneticus (Midge)	48	>29.2	29.2	[10]
Fish				
Danio rerio (Zebrafish)	96	25.64		[11]
		22.98		[11]
	96	20.84		[11]
	96	27.71		[12]
	96	13.8	21.6[c]	[12]
Ictalurus punctatus (Channel catfish)	96	13		[9]
	96	4.1	7.3[c]	[9]
Lepomis macrochirus (Bluegill)	96	4.8[d]	4.8	[9]
Pimephales promelas (Fathead minnow)	96	28.9		[13]
	96	12.7		[14]
	96	6.6		[9]
	96	5.8		[9]
	96	4.5		[15]
	96	3.5		[12]
	96	1.8	6.3[c]	[12]
Oncorhynchus mykiss (Rainbow trout)	96	6.4	6.4	[9]

[a]LC50- concentration that kills 50% of the test organisms; EC50- concentration that causes a sublethal effect (e.g., loss of equilibrium) to 50% of the test organisms.

[b]Genus mean acute value.

[c]Geometric mean.

[d]Geometric mean of 14 static tests & 1 flow-through test

Table 1: Acute Toxicity of RDX to Freshwater Organisms

To determine the FAV the GMAV values in Table 1 were ranked from high to low. The GMAVs were assigned ranks, R, from 1 for the lowest to N for the highest. The cumulative probability, P, for each of the GMAVs was calculated as R/ (N+1). The four lowest GMAC values (Table 2) were used to calculate the FAV which is an estimate of the concentration of RDX corresponding to a cumulative probability of 0.05 (6). The cumulative probability of 0.05 is considered to protect 95% of the ecosystem. As discussed by Stephan, *et al.* [6], aquatic ecosystems can tolerate some stress and occasional adverse effects. Thus, protection of all species at all times and places are not deemed necessary.

Rank (R)	GMAV[a]	(ln GMAV)[b]	$(\ln GMAV)^2$	P = R/(N + 1)[c]	√P
4	7.3	1.9879	3.9517	0.33	0.5745
3	6.4	1.8563	3.4458	0.25	0.5
2	6.3	1.8405	3.3874	0.17	0.4123
1	4.8	1.5686	2.4605	0.08	0.2828
Sum		7.2533	13.2454	0.83	1.7696

[a]Four lowest genus mean acute values from Table 1.

[b]Natural log of GMAV.

[c]P is comparative probability for each GMAC; N = 12 GMAVs

$$S^2 = \frac{\sum\left[(\ln GMAV)^2\right] - \left[\left(\sum \ln GMAV\right)^2\right]/4}{\sum(P) - \left[\left(\sum\sqrt{P}\right)^2\right]/4} = 1.4307$$

S = 1.0949

L = [Σ(ln GMAV) – S(Σ√P)] / 4 = 1.1804

A = S(√0.05) + L = 1.5003

Final Acute Value (FAV) = $e^{1.5003}$ = 4.4830 mg/L

Criterion Maximum Concentration (CMC) = 4.4830 / 2 = 2.24 mg/L

Table 2: Ranking of RDX Acute Toxicity Studies

Criterion Continuous Criterion

The Criterion Continuous Criterion is estimated by considering the lowest of a Final Chronic Value, Final Plant Value, Final Residue Value, or Lowest Biological Important Value. Invertebrate and fish chronic toxicity data (Table 3) were available to calculate a Final Chronic Value (FCV). The FCV may be calculated is the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio (Table 3). The Final Acute-Chronic Ratio was used in the current analysis to determine the FCV. The Final Acute-Chronic Ratio was used because sufficient species were available to indicate that the acute-chronic ratio was probably the same for all species and was probably independent of water quality characteristics (e.g., pH, water hardness, etc.). The Final Acute-Chronic ratio was calculated as the geometric mean of the available species mean acute-chronic ratios. As discussed below, a Final Plant Value, Final Residue Value, and Lowest Biological Important Value were considered but were not used to establish the Criterion Maximum Criterion.

Species	Exposure Duration (Days)	GMCV Values (mg/L)	Acute-Chronic Ratio[a]	Reference(s)
Invertebrates				
Ceriodaphnia dubia (Cladoceran)	7	4.7	3.62	[10]
Chironomous tentans (Midge)	23	21	2.00[b]	[9]
Hydra azteca (Hydra)	35	12.2	2.71	[16]
Paratanytarsus parthenogeneticus (Midge)	17	20.82	2.00[b]	[10]
Fish				
Pimephales promelas (Fathead minnow)	365	0.83	-	[17]
	240	4.3	-	[9]
	30	4.2	-	[9]
	28	1.8	-	[14]
		2.30[c]	2.74	
Geometric Mean			2.5486	

[a]Acute-Chronic ratios calculated from the genus mean acute values in Table 1 and genus mean chronic values in Table 3.

[b]The calculated C. tentans and P. parthenogeneticus acute-chronic ratios are 1.86 and 1.40, respectively; however, the EPA guidelines state that a value of 2 should be used when and acute-chronic ratios are <2.00 [6].

[c]Geometric mean.

Final Chronic Value (FAV/FACR) = 4.4830 mg/L / 2.5486 = 1.76 mg/L

Criterion Continuous Concentration (CCC) = 1.76 mg/L

Table 3: Chronic Toxicity of RDX to Freshwater Organisms

Results and Discussion

Acute toxicity data for RDX were found for 12 species which met the criteria of at least eight different families specified in the EPA guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms [6]. Two or more values were available for the invertebrates *Chironomous tentans* and *Daphnia magna* and the fish *Danio rerio, Ictalurus punctatus*, and *Pimephales promelas* (Table 1). The geometric mean of each of the two or more values was calculated to provide the Genus Mean Acute Value for each species. The genus mean acute values for the 12 species are given in Table 1.

In general RDX was acutely more toxic to fish than invertebrates (Table 1). All 48-hour toxicity values for the invertebrates occurred at the solubility limit of the compound or the highest concentration tested. The genus mean acute values for invertebrates ranged from a low of 17 mg/L to a high of 100 mg/L. The genus mean acute values for fish ranged from a low of 4.8 mg/L to a high of 21.6 mg/L. The Final Acute Value based on the four lowest Genus Mean Acute Values of the organisms listed in Table 1 is 4.48 mg/L as calculated by the equations shown in Table 2. The Criterion Maximum Concentration, which is equal to one-half of the FAV, is 2.24 mg/L.

Chronic RDX toxicity data were available for five species which met the EPA criteria of at least on fish, one invertebrate, and one organism which was an acutely sensitive species (Table 3). Four chronic tests were available for the invertebrates. The tests ranged from a 7-day cladoceran (*C. dubia*) neonate production test [9] to a 35-day survival, growth, and reproduction test with hydra (*H. azteca*) [10]. Data were available for four fathead minnow (*P. promelas*) chronic tests. The tests ranged from a 28-day early life stage test [14] to a 365-day multigenerational test [17]. The genus mean chronic values for the invertebrates ranged from 4.7 to 20.8 mg/L. The four genuses mean chronic values for the flathead minnow ranged from 0.83 to 4.3 mg/L. In all cases, RDX was more toxic to the fish than the four invertebrates.

The Genus Mean Acute Values in Table 1 and Genus Mean Chronic Values in Table 3 were used to calculate the acute-chronic ratios for each species given in Table 3. The Final Acute-Chronic Ratio (FACR), which is the geometric mean of the acute-chronic ratios, was used to determine the Final Chronic Value. The Final Chronic Value of 1.76 mg/L was established by dividing the Final Acute Value by the FACR. Thus, the Final Chronic Value is the Criterion Continuous Concentration. A Lowest Biological Important Value less than the FCV of 1.76 mg/L was not found in the literature.

Four chronic plant values were found in the literature for RDX. *Anabeana flos-aquae* (blue-green alga), *Microcystis aeruginosa* (blue-green alga), *Naviula pelliculosa* (diatom), and *Raphidocelis subcapitata* formally known as *Selenastrum capricornutum* and P*seudokirchneirella subcaptita* (green alga) were evaluated in 96-hour assays by Bentley, *et al.* [9]. However, the algal tests did not meet the EPA numerical national water quality criteria for a Final Plant Value because nominal test concentrations were used instead of measured concentrations [6]. One 96-hour algal test with *Raphidocelis subcapitata* was available that met the EPA chronic guidelines of measured test concentrations [18]. The EPA guidelines state that if the algal species is among the most sensitive organisms to the test material the results of a test with a plant in another phylum should also be available [6]. Thus, a Final Plant Value was not used in the Criterion Continuous Concentration estimation.

No Final Residue Value was established for the Criterion Continuous Criterion estimate. Little if any bioconcentration or bioaccumulation of RDX by aquatic organisms should occur because the octanol-water partition coefficients (log k_{ow}) for RDX range from 0.81 to 0.90 [19-21]. Low bioconcentration factors (BCF) for fish have been shown to range from 0.6 to 1.7 for the sheepshead minnow, *Cyprinodon variegatus* [22,23], 0.91 to 2.23 for zebrafish [24], and 2 for channel catfish [25]. Bentley, *et al.* [9] also established low BCFs for bluegill, fathead minnow, and channel catfish. A BCF of 2.1 for the aquatic oligochaete, *Lumbriculus variegatus* has be reported by [25]. Dietary transfer of RDX from the invertebrate, L. *variegatus*, to both fathead minnows [26] and channel catfish [25] have been shown to have a negligible bioaccumulation potential in fish. Gust, *et al.* [17] concluded in a one-year exposure of fathead minnow to 0.83 mg/L RDX that the compound had limited impacts on genomic, individual, and population-level endpoints.

Several studies have shown that RDX is decomposed via photolysis in aqueous solutions at ultraviolet wavelengths shorter than approximately 290 nm and at longer wavelengths above 290 nm which can occur from irradiance in natural sunlight [27-29]. The photolysis of RDX in distilled water and natural water samples follows first order kinetics when RDX is photolyzed in sunlight or at 313 nm [29]. The reduction in toxicity to the cladoceran *Ceriodaphnia dubia* following photolysis in sunlight has been studied by Burton and Turley [30]. A 10 mg/L solution, which was toxic to the cladoceran in a 7-day exposure, was photolyzed for a total of 28 hours in direct sunlight in early October in Shady Side, Maryland (38.8418 latitude: -76.5122 longitude) until the parent compound reached non-detectable levels. Photolyzed RDX did not affect cladoceran neonate production. The lowest-observed-effect concentration and no-observed-effect concentration for neonate production exposed to the parent compound were 6.0 and 3.6 mg/L, respectively.

Conclusion

The current analysis of RDX toxicity to freshwater organisms established the United States Environmental Protection Agency numerical freshwater ambient water quality Criterion Maximum Criterion (CMC) of 2.24 mg/L. In contrast, a provisional acute ambient water quality criterion of 3.096 mg/L was derived by ENSR [3]. The CMC is the highest 1-hour average concentration over a 3-year period that should protect 95% of the aquatic organisms and their uses. A definitive Criteria Continuous Concentration (CCC) of 1.76 mg/L was determined in this evaluation. A provisional CCC of 1.032 mg/L was suggested by ENSR [3] because sufficient data were not available to derive a definitive CCC. A year-long exposure of the fathead minnow to RDX at a concentration of 0.83 mg/L by Guest, *et al.* [17] showed that the compound had limited effects on genomic, individual, and population-level endpoints. The CCC is the highest 4-day average concentration over a 3-year period that should protect aquatic organism and their uses.

The above criteria should provide adequate ecosystem protection without being over protective or under protective. The criteria can be useful for developing effluent limitations, mixing zone standards, water quality standards, etc. As discussed in the criteria guideline (6), the use of the criteria may have to consider additional factors such the hydrology of a body of water where the compound is released, relationships between species for which data are available and ecologically important species in the body of water of concern, etc.

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