Review of Solution Chemistry of Uranium and Its Toxicity to Aquatic Organisms

Final Report

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Svensk sammanfattning

Lösligt uran (U) förekommer naturlig i vatten som kommer i kontakt med mineraliserade geologiska formationer men koncentrationen är mer påtaglig vid exempelvis gruvdrift. De flesta svenska gruvorna släpper ut en koncentration av U mellan 20µg/L och 800 µg/L. Detta kan jämföras med World Health Organisations dricksvattenstandard på 30µg/L av U för dricksvatten och det svenska EQS värdet för ytvatten ligger på årsmedelkoncentration på 0,17µg/L. Vad som anses skadligt för vattenlevande organismer varierar dock och är beroende på en mängd olika variabler. Art och vattenkemin är två av dessa. Tidigare toxicitets studier har visat på att det två giftigaste typerna av uranjoner är uranyl (UO_2^{2+}) och uranyl hydroxidjoner (UO_2OH^+) men EQS-värdet baseras på totalhalten av uran.

Man kan dra en mäng slutsatser från den forskning som har gjorts kring urans påverkan på vattenlevande organismer. De flesta studierna under det sista 20 åren pekar på att uran samt dess lösningskemi har stor betydelse för dess påverkan på miljön. För organismer som lever i dessa miljöer kan toxiciteten främst hänförs till mikroorganismer, vattenväxter, vattenlevande ryggradslösa djur och vattenlevande ryggradsdjur. Av dessa är tropiska mikroalger Chlorella sp. och vattenlevande ryggradslösa djur som Ceriodaphnia och Daphnia arter som är det organismer som löper störst risk vid höga halter av uran. Toxiska U-koncentrationer för dessa organismer verkar variera uppåt från 10 µg/L, även om det finns stor variation beroende på lösningskemi. Koncentrationer vid vilket hämmande effekt kan påvisas varierar uppåt från ca 1,0 µg/L.

Baserat på många av det studier som har gjorts kring toxiciteten av uran för vattenlevande organismer är den svenska EQS för U på 0,17 µg/L är mycket konservativ. Det är inte uppenbart att den strikta svenska standarden på 0.17 µg/L ger ytterligare skydd för vattenlevande organismer över en mildare standard.

Introduction

Low concentrations of soluble uranium (U) are often present in water that has come into contact with mineralized geologic formations, especially those which have been mined for ferrous and non-ferrous metals. It has been reported that most mines in Sweden have U concentrations in their discharge water of between 20 and 800 ug/L. The toxicity of high U concentrations to humans is well established and standards for drinking water have been developed accordingly. The World Health Organization (WHO) recommends a standard for U in drinking water of 30 µg/L. In contrast, and though it has been the subject of numerous investigations over many decades, the effect of elevated U concentrations on aquatic organisms varies widely and depends on a number of variables, especially the aquatic species of interest and the chemistry of the water.

The environmental quality standard (EQS) for average U concentration in northern Swedish streams is very stringent at 0.17 µg/L with a single sample maximum concentration of 8.6 µg/L. Uranium has a complicated chemistry in aqueous solution. In the natural environment U generally exists in one of two oxidation states; the +4 and +6 oxidation states which are written as U(IV) and U(VI) respectively. U(IV) minerals are very insoluble hence measurable U concentrations in water are nearly always assumed to represent dissolved U(VI) species. U(VI) may exist in aqueous solution as many different compounds or species which exhibit varying interactions with aquatic organisms. Aquatic toxicology studies suggest that the most toxic species are uranyl ions (UO_2^{2+}) and uranyl hydroxide ions (UO_2OH^+), thus the EQS for U has been interpreted as applying only to these species (Hogland et al., 2019).

The purpose of this report is to first provide a brief review of the solution chemistry of U(VI) and the species that predominate in natural waters similar to those that occur in northern Sweden. This is followed by a review of the aquatic toxicology of U and in particular, how it is affected by U speciation.

Brief Summary of Uranium Solution Chemistry Relevant to Its Toxicity

Free Ion Activity Model

The Free Ion Activity Model (FIAM) is the most commonly used model to describe the uptake of soluble constituents by cells in living organisms. It is based on a fundamental concept that the interaction of a soluble metal compound with the cells involves the three following steps (Campbell, 1995, Figure 4): 1) speciation of the metal in the external aqueous environment, 2) interaction between the metal species and the cell surface, and 3) metal partitioning within the organism and resulting biological effects. The FIAM is a mechanistic model which suggests that the toxicology of a metal in solution depends on its speciation rather than simply its total concentration, a process sometimes referred to as facilitated cation transport. In particular, the model recognizes the critical importance of the interaction between the metal and the characteristics of the cell's external membrane. Thus, if the solution chemistry and chemistry at the cell surface prevents the metal from binding to the surface, the metal cannot be transported into the cell to disrupt internal cell processes such as metabolism or replication.

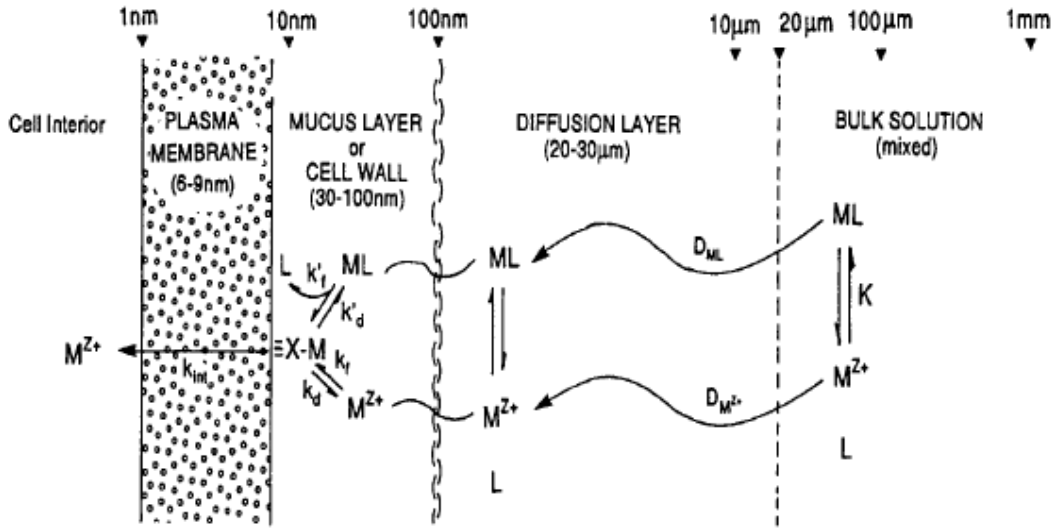

Figure 1. Conceptual model of the interactions between metals in solution and a biological cell. M = free metal ion, ML = metal complex, L = ligand (Campbell, 1995).

The FIAM concept was developed in the late 20th century and was in part the result of development of increasingly sophisticated aqueous equilibrium models that allowed calculation of the species present in aqueous solution under a wide range of solution chemistries. The FIAM has been applied to understanding the toxicity of a wide range of metals including U. Most of the studies of the past 20 years have included consideration of the U species predicted to be occur under the experimental and field conditions which are summarized below.

Although there is not universal agreement among these studies, there is a considerable body of knowledge which supports the concept that free uranyl ion (UO_2^{2+}) is the species that is primarily responsible for U toxicity in aqueous solutions. Accordingly, this review first summarizes the chemistry of U in solution then discusses its toxicity on a variety of microbial organisms, aquatic plants, invertebrates, and vertebrates (Fortin et al., 2004; Fortin et al., 2007).

Chemistry of Uranium in Solution

Langmuir (1997) provided an extensive discussion of the chemistry of U in aqueous solutions. Uranium(IV) is generally considered to be very insoluble whereas U(VI) is quite soluble. A simplified phase diagram for dissolved U species is presented in Figure 1 and summarizes the oxidation-reduction (i.e. redox) and acid-base chemistry of a solution of 10^{-6} M uranium. These diagrams are commonly referred to as pe-pH of Eh-pH diagrams where pe is the negative logarithm of the electron activity and is analogous to pH ($pe = -\log\{e^-\}$) where Eh is the redox potential measured in volts.

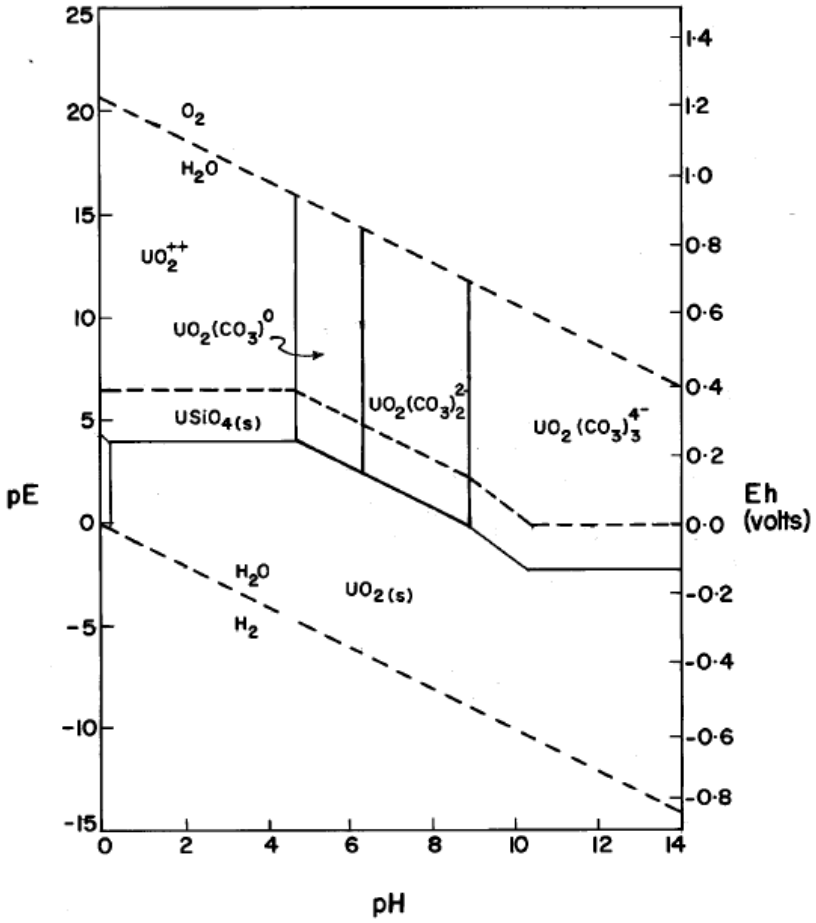

Figure 2. Simplified phase diagram showing major uranium species in solution as a function of pH and oxidation-reduction conditions represented as either pe or Eh. The dissolved inorganic carbon concentration is 10^{-3} M and the dissolved U concentration is 10^{-6} M.

It is difficult to analytically determine the oxidation state of dissolved U at concentrations below about 10^{-3} M (238 mg/L) hence it is commonly assumed that soluble U at concentrations less than this value are due to oxidized U(VI) species. As shown in Figure 1, U(VI) forms strong complexes with carbonate ions (CO_3^{2-}) at pH 5and higher. The degree of complexation and nature of the complexes affect the solution chemistry, environmental fate and transport of U, and the toxicity of U.

Figure 1 was prepared using thermodynamic data from Langmuir (1997) and is similar to the diagram in his book and many other references. In the last 10 years there has been recognition that U(VI)-carbonates also forms very strong ternary (three constituent) metal complexes with Ca and Mg. The U-Ca-CO_3 complexes $UO_2Ca(CO_3)_3^{2-}$ and $UO_2Ca_2(CO_3)_3$ are the strongest and equilibrium constants for them have been recently published in the data set by Mühr-Ebert et al. (2019). A speciation plot that considers complexation of UO_2^{2+} by OH^-, CO_3^{2-}, and mixed U-Ca-CO_3 is presented in Figure 2 for a moderately soft water (hardness = 50 mg/L as $CaCO_3$). There are two points that are especially important to consideration of the toxicity of U(VI) to aquatic species. The first is that over the pH range from 6 to 11 U-Ca-CO_3 complexes are the dominant form of dissolved U and exceed the concentration of all other species by orders of magnitude. This is likely to have significant effect on the biological uptake and bioavailability of dissolved U by aquatic organisms as it is proposed that uncomplexed UO_2^{2+} is the principal toxic constituent. This is the free ion activity model described by Campbell (1995).

The second and possibly more important point regarding U speciation in the presence of Ca and CO_3^{2-} is that the concentration of UO_2^{2+} and UO_2OH^+ drops to very low values above pH ~6. The Swedish stream standard of 0.17 µg/L corresponds to a U concentration of $7x10^{-10}$ M and is plotted on Figure 2 as a horizontal red line. The figure shows that the concentrations of UO_2^{2+} and UO_2OH^+ drop below the stream standard slightly below pH 7 and that the pH at which this occurs is relatively insensitive to whether the total U concentration in solution is 1 mg/L or 10 µg/L.

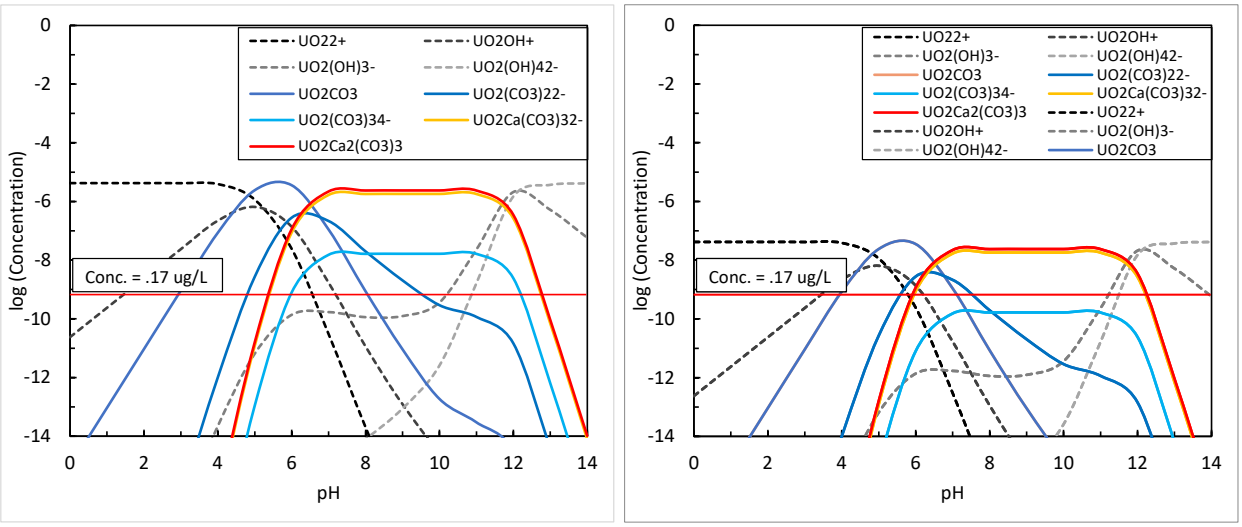

Figure 3. Diagram of major U(VI) species present in a solution with total concentration of Ca = 20 mg/L, and total dissolved inorganic carbon (DIC) concentration of 1 mM. A total U concentration 1 mg/L is plotted in the left diagram and 10 µg/L is plotted on the right diagram.

Numerous studies have shown that transport of metals across cell membranes depends on the speciation of the metal. This is particularly true for U which, as shown, has a very complicated aqueous chemistry and forms numerous complexes. Transmembrane transport is usually the first step leading to toxicity and also affects bioaccumulation. A list of the U(VI) complexes in the WATEQ4F database that have been considered in modeling studies in support of the Viscaria

mine project is presented in Table 1. A recent compilation of equilibrium formation constants for these complexes has been published by Mühl-Ebert et al. (2019) and this information has been incorporated in the thermodynamic database used in this and related reports.

Consideration of the species of a metal in solution has been increasingly recognized in the past two decades based on the free ion activity model (FIAM) (Brown and Markich, 2000). This theory suggests that the biological response of a toxicant is proportional to the activity of the free metal ion. It is based on the conceptual model that in order to exhibit a biological effect the metal must diffuse to the surface of the organism, adsorb to that surface, then be transported across the cell membrane into the organism via facilitated cation transport. A consequence of this model is that for cationic metals such as UO_2^{2+}, high concentrations of other divalent cations such as Ca^{2+} or Mg^{2+} may affect both adsorption to the cell surface and transport across the membrane. The free ion activity model is the underlying justification for understanding U speciation in solution and, as will be seen in the following review of studies of aquatic toxicology of U, is frequently used to understand the results of these studies.

One of the early and frequently cited studies that evaluated the applicability of the FIAM was that by Markich et al. (2000) which considered the short term (48 hrs) sub-lethal toxic effects of both manganese (Mn) and U on valve movement in a freshwater bivalve mollusk. The study found that while the concentration of uncomplexed Mn^{2+} was directly correlated with toxicity to the organism, the toxic effects due to U depended on the concentrations of both UO_2^{2+} and UO_2OH^+. Furthermore, complexation of U(VI) by organic ligands similar to fulvic acid greatly reduced the toxic effects. The results of these and other studies demonstrated the importance of a thorough characterization of solution chemistry for a particular toxicant to understand its toxicity to aquatic organisms.

Table 1. U(VI) species included in the WATEQ4F thermodynamic database.

UO_2^+ & OH^- Complexes	**UO_2-Ca-CO_3 Complexes**	**Other Complexes**
UO_2^{2+}	$UO_2(CO_3)°$	$UO_2SO_4°$
UO_2OH^+	$UO_2(CO_3)_2^{2-}$	$UO_2(NO_3)^+$
$UO_2(OH)_3^-$	$UO_2(CO_3)_3^{4-}$	$UO_2(SO_4)_2^{2-}$
$(UO_2)OH^{3+}$	$(UO_2)_3(CO_3)_6^{6-}$	UO_2Cl^+
$(UO_2)_2OH^{3+}$	$UO_2Ca_2(CO_3)_3°$	$UO_2Cl_2°$
$(UO_2)_3(OH)^{3+}$	$UO_2Ca(CO_3)_3^{2-}$	$UO_2Mg(CO_3)$
$(UO_2)_3(OH)_4^{2+}$		
$(UO_2)_4(OH)_7^+$		

Review Literature on Toxicity of Uranium to Aquatic Organisms

There have been a large number of studies on the toxicity of U to aquatic organisms. Review papers by Gao et al. (2019) and Sheppard et al. (2005) cite roughly 100 papers each. A search of the literature for this review identified about 40 papers published in the last 20 years that may have relevance to possible water quality issues in Swedish streams. These are identified in Table 2 which also includes a very abbreviated summary of the findings.

Because there is so much information in these papers it is challenging to summarize each paper in a single table. Therefore, information summarized in each of the columns in Table 2needs explanation.

- Column 1 – Organism: The principal organism which was evaluated. Several of the studies considered multiple organisms and these are listed at the bottom of the table

- Column 2 – pH: The pH at which the study was conducted. Many of the studies were conducted over a range of pH values

- Column 3 – Speciation Considered?: Some of the studies did not consider whether U might be complexed and simply reported the total U concentration. Other studies recognized the importance of complexation, though few of them actually report the predominate U species that affected the results of their study.

- Column 4 – U Conc. (mg/L): All of the studies were conducted over a range of U concentrations. Some of them reported concentrations at which toxic effects were noted while others did not. Toxic effects were reported as EC_{50} – the concentration at which 50% of the organisms exhibited some manifestation of inhibition such as reduced reproduction, and LC_{50}, the concentration which was lethal to 50% of the organisms. LC_{50} values were reported for a duration of 48 or 96 hours by different investigators.

- Column 5 – Location: Many of the studies were done in the field or used water samples representative of field conditions. Others were lab studies using standardized solutions.

- Column 6 – Comments: Unique aspects of the studies are noted in this column.

- Column 7 – References: Citation of the study.

Regarding speciation, a common theme in much of the literature was the importance of uncomplexed uranyl ions (UO_2^{2+}), inorganic U complexes, the presence and possible complexation by dissolved organic carbon (DOC), and the effect of pH and alkalinity.

The discussion of these studies is broken into three sections; effects on single cell organisms (microalgae, bacteria and fungi), effects on plants and macrophytes, and effects on animals.

Table 2. Summary of references reviewed on aquatic toxicity of uranium.

Organism	pH	Speciation Considered?[1]	U Conc. (mg/L)	Location	Comment	Reference
Microorganisms/Algae						
Cyanobactreia	>7	Y		Marine	Summary of mechanisms of sequestration	Acharya & Apte, 2013
Fungi		N	12.5-45[1]	Portugal	Sporulation inhibited at U > 1 mg/L	Bergmann and Graça, 2020
Chlamydomonas sp.	5, 7	Y	0.0 – 0.5	Lab Study	Complexation by PO_4 reduces U uptake. Uptake by UO_2OH^+ occurs	Fortin et al., 2002
Chlorella sp.	5.7 – 6.2	Y	.044 - .078[2]	Australia	Competition between H^+ & metals on surface affected toxicity	Franklin et al., 2000
Chlorella sp.	7	Y	1.0 – 500	Lab study	Toxicity decreased when Ca > 2 meq/L	Charles et al., 2002
Chlorella sp.	6.0 – 6.2	Y	.120 - .187[4]	Australia	Toxicity reduced by complexation with DOC	Hogan et al., 2005
Chlamydomonas sp.	5 & 7	Y	.03 & .0004[2]	Lab study	Toxicity of UO_2^{2+} not total U	Lavoie et al., 2014
Pseudomonas sp.	7	Y		Lab study	Toxicity of UO_2^{2+} decreased at high HCO_3^- concs.	van Engelen et al., 2010
Macrophytes/Plants						
Soil-plant interactions		Y			Review paper, U uptake phytoremediation strategies	Chen et al., 2021
Nymphaea sp. (pygmy water lily		N		Lab study	Bioaccumulation for potential wetland phytoremediation	Chen et al., 2019
25 floating & emergent plant species	~7	N		Portugal	Bioaccumulation for natural attenuation	Cordeiro et al., 2016
28 floating & emergent plant species	~6.3	N		Portugal	Bioaccumulation for phytoremediation	Favas et al., 2014
Ceratophyllum sp.	6.2	Y	.13 - .55[2]	Lab study	Hardness of 20 & 550 mg $CaCO_3$/L	Markich, 2013
Lemna sp.	7	Y	0.9 – 7.4[2]	Lab study	Increase in EC_{50} at P concentration 8.0 mg/L	Mkandawire et al., 2006

Table 2 continued.

Organism	pH	Speciation Considered?[1]	U Conc. (mg/L)	Location	Comment	Reference
Callitriche sp., *Potamogeton* sp.,	6.5	N		Portugal	Phytofiltration reduced U conc. From >0.2 mg/L to < 0.1 mg/L in 14 d	Pratas et al., 2014
Hydrilla sp.	2 – 6	N	20 – 100	India	U uptake for possible phytoremediation	Srivastava, 2010
Animals						
Aquatic invertebrates	~7	Y	24-262[3]	Lab study	U is less toxic than other metals	Bergmann et al., 2018
Lymnaea sp.	~7	Y		Lab study	Uptake by snails reduced by U-Ca-CO_3 complexation	Croteau et al., 2015
Ceriodaphnia & *Daphnia* sp.	8.2	N	0.04	Brazil	High hardness (> 500 mg/L) reduced toxicity	Ferrari et al., 2017
Fish		Y			Review. Toxicity reduced by complexation with Ca, Mg, etc.	Goulet et al., 2011
Ceriodaphnia & *Hyalella*	7.9 – 8.5	N	10.5 & 1.5[3]	New Mexico		Kuhne et al., 2002
Velesunio angasi (freshwater bivalve)	5.0 – 6	Y		Australia	Toxicity due to UO_2^{2+} & UO_2OH^+	Markich et al., 1996
Daphnia sp.	7	N		Lab study	Multi-generation effects observed at U ~ 0.010 mg/L	Massarin et al., 2010
Daphnia sp.	~7.5	N	0.524[4] & >0.799[3]	Lab study, Canadian water	DOC reduced toxicity, no effect of hardness	Muscatello et al., 2020
Chironomus tentans	7.8	N	0.157[4]	Lab study	U accumulated in larvae but was lost during metmorphosis	Muscatello et al., 2009

Table 2 continued

Organism	pH	Speciation Considered?[1]	U Conc. (mg/L)	Location	Comment	Reference
Chironomus tentans	8.3	N	No mortality at U = 0.3	Lab study	U accumulated in larvae but was lost to depuration in 3 d	Muscatello et al., 2010
Brook trout			59.[5]	Colorado	No impact at U = 4 mg/L Hardness reduced toxicity	Parkhurst et al., 1984
Daphnia sp.			6.[5]	Columbia River	Hardness reduced toxicity	Poston et al., 1984
Hydra viridissima	6	Y	0.144[2] at hardness = 6.6	Australia	Doubling hardness reduced EC_{50} by 24%. Alkalinity had little effect	Riethmuller et al., 2001
Daphnia sp.	6.7	N	0.045 – 0.194[6]	Norway	High U uptake & depuration rates, gene expression	Scheibener et al., 2021
Daphnia sp.	7 & 8	Y	0.39 & 7.8[3]	Lab study	Effects attributed to uncomplexed UO_2^{2+}	Zeman et al., 2008 Zeman et al., 2010
Multiple Organisms						
Algae, crustaceans, insects	<6	N		Portugal	Assays of water & sediment. Toxicity highest at low pH	Antunes et al. 2007
Microorganisms, plants, invertebrates					Extensive review of U toxicity to aquatic organisms >100 references	Gao et al., 2019
Fish, crustaceans, algae, floating plants	6.5-7.3	Y		Lab study	Toxicity depends on alkalinity more than hardness	Goulet et al., 2014
Terrestrial & aquatic plants, aquatic invertebrates, etc.					Lit. review of predicted no effect concentrations (PNECs), ~100 references	Sheppard et al., 2005
Fish, hydra, green algae	6.2	Y		Australia	DOC reduced bioavailability & LC_{50} for all 3 species	Trenfield et al. 2011a Trenfield et al., 2011b

Table 2 continued

Organism	pH	Speciation Considered?[1]	U Conc. (mg/L)	Location	Comment	Reference
Fish, hydra, green algae, macrophyte, gastropod	3 – 6	Y		Australia	Synergistic effects of constituents & DOC reduced toxicity of mine water	Trenfield et al., 2021
Chlorella sp., *Hydra* sp., *Moinodaphnia* sp.		Y			Re-analysis of 46 datasets. DOC was best predictor followed by hardness	van Dam et al., 2012

Notes

1 – Was U speciation considered in report?

2 – Toxicity reported as EC50 = U concentration causing 50% inhibition

3 – 96 hour LC50 – U concentration causing 50% mortality

4 – 72 hr lowest observed effect concentration (LOEC)

5 – 48 hour LC50 – U concentration causing 50% mortality

6 – U concentrations affecting growth

Toxicity to Microorganism

Acharya and Ante (2013) studied the toxicity of U to a marine cyanobacteria which is not relevant to conditions in Swedish streams. Several studies considered the toxicity of U to micro-algae (Franklin et al., 2000; Charles et al., 2002; Hogan et al., 2005; and Lavoie et al., 2014). These studies are especially relevant because they report that the organism studied, microalgae of the *Chlorella* sp., was among the most sensitive to low concentrations of U. The U concentrations exhibiting inhibition or toxic effects were much higher than the EQS. Franklin et al. (2000) considered U complexation and pH and suggested that H^+ interactions at the algal surface may affect the membrane's permeability to the metal. Complexation, particularly formation of metal-organic complexes, may affect trans-membrane transport.

Reduced toxicity associated with increased DOC found by Hogan et al. (2005) lends support to this suggestion. Lavoie et al. (2014) found that the EC_{50} for free UO_2^{2+} was pH dependent and was 40 μg/L and 0.4 μg/L at pH 5 and 7 respectively. van Engelen et al. (2010) correlated bioaccumulation and toxicity on a bacteria (*Pseudomonas* sp.) directly to uncomplexed uranyl ions and found that increasing alkalinity, and therefore U-CO_3 complexation, decreased U toxicity. The suggested explanation is that the increased toxicity of uncomplexed uranyl ions is that these molecules are more readily transported across the cell wall than much larger U-CO_3 molecules. This hypothesis would suggest that the toxicity of U-Ca-CO_3 molecules would be even less due to their larger size.

Toxicity to Aquatic Macrophytes

Macrophytes are aquatic plants living in or near water. A large number of studies have been done on bioaccumulation of U, much of it related to possible use of plants for phytoremediation processes. One application of this technology might be to use plants in constructed wetlands to treat U contaminated water (Chen et al., 2021; Chen et al. 2019; Cordeiro et al., 2016; Favas et al., 2014; Pratas et al., 2014; Srivastava, 2010). Chen et al. (2021) reviewed over 200 recent studies, most since 2010, on U uptake by plants from both soil and water, though the emphasis was on contaminated soil.

Toxic effects for aquatic plants became evident at U concentrations of 1 mg/L or greater. U primarily accumulates on root material but can be translocated to other parts of the plant depending on the plant species, U species, and other factors. All of the studies that considered U bioaccumulation by aquatic plants focused on plants grown in temperate or tropical climates, not organisms adapted to northern Sweden.

There is an active research group in Australia led by Markich, Trenfield and van Dam that has published numerous studies on the aquatic toxicity of U to a variety of species that includes both plant and animal life. Much of this work has been conducted to determine U toxicity to understand potential impact of U mining in Australia. The paper by Markich et al. (1996) was one of the first to discuss the importance of U speciation on freshwater biota. They reported that UO_2^{2+} and UO_2OH^+ were the U species responsible for toxic effects to a freshwater mollusk, not the total U concentration. All subsequent studies by this group has included consideration of U species.

Toxicity to Aquatic Animals

Two of the animals that are most widely used for characterizing aquatic toxicity are the invertebrate freshwater planktonic crustaceans of the order Cladocera (often referred to as water fleas), especially *Daphnia* sp. and the related genus *Ceriodaphnia* sp. They are used primarily because they are easy to culture, grow rapidly, are transparent which facilitates observation of their internal organs, and are sensitive to a wide range of contaminants. Nine of the U toxicity studies identified in Table 2 used one or both of these organisms.

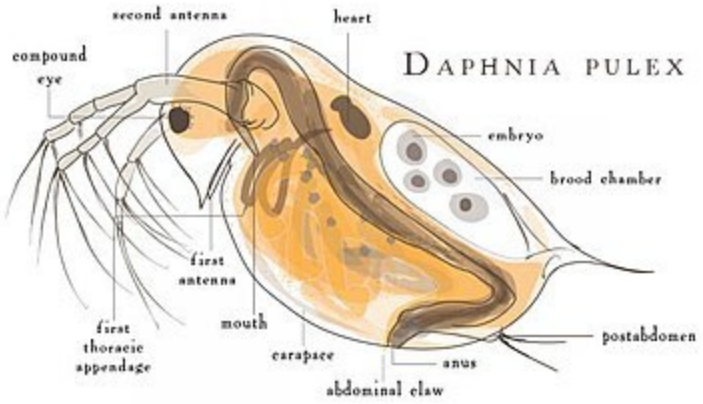

Figure 4. Anatomy of *Daphnia pulex* (source: Wikipedia).

While there was a large difference in the study design, specific organism tested, the water chemistry, and findings of these studies, two of the common findings were that toxicity was reduced at high pH and high hardness (Ferrari et al., 2017; Poston et al., 1984). One of the most widely cited studies was that by Zeman et al. (2008, 2010) which found that the 48 hr LC_{50} toxic concentration (concentration which kills 50% of the organisms) was 0.39 mg/L and 3.2 mg/L near pH 7 and 8 respectively. Speciation calculations suggested that the uncomplexed UO_2^{2+} concentration was 0.17 µg/L and 0.00017 µg/L at pH 7 and 8. Chronic effects evidenced by reduced feed consumption and increased oxygen respiration occurred at lower U concentrations. Consideration of U species present in the solutions studied by Zeman et al. (2008) led them to suggest that the explanation for U toxicity is more complicated than simply relating it to the uncomplexed UO_2^{2+} concentration and might be due to inhibition of H+ for uranium transport and/or the involvement of other bioavailable chemical species of uranium.

A recent study by Muscatello et al. (2020) is one of the investigations most relevant to conditions in streams of northern Sweden. This study examined the effects of elevated U concentrations on *Ceriodaphnia dubia* in contaminated stream water in the Yukon territory of northern Canada. This study found a no observed effect concentration (NOEC) of 0.999 mg/L during the summer and 0.381 mg/L during the winter. The reduced toxicity during the summer was attributed to higher alkalinity (80 mg/L as $CaCO_3$) than in the winter (56 mg/L as $CaCO_3$ which caused more of the U to be complexed and thus less bioavailable to the organism. They also reported that DOC concentrations as low as 4 mg/L reduced the U toxicity, a finding consistent with that reported by Australian researchers van Dam et al., (2012) and Trenfield et al. (2011a, 2011b, 2021).

One study that found higher U toxicity for aquatic animals was that by Kuhne et al. (2002). They found that the 96 hour LC_{50} was less at pH 8.5 (1.5 mg/L) than at pH 7.9 (10.5 mg/L). This study was conducted in NM stream water and did not consider U speciation. Also, it should be noted that the U concentrations that were found to be toxic are much higher than those reported in most other studies.

Several studies reported on the non-lethal effects that elevated U concentrations might have on Cladocera organisms including reproduction, growth, respiration, feeding, and U accumulation. Massarin et al. (2010) reported measurable effects on growth and reproduction at total U concentrations as low as 10 μg/L. However, they also found that cultures exposed to U concentrations of 25 μg/L quickly recovered when placed in a non-contaminated media (referred to as depuration). More recently Scheibener et al. (2021) found rapid uptake by *Daphnia* in solutions with U concentrations ranging up to 200 μg/L, but also depuration (loss of U from the organism) in clean solutions was almost as rapid. Greater than 50% of U was associated with the organism's exoskeleton and was lost during shedding, a phenomenon found for other aquatic crustaceans (Muscatello et al., 2009).

U toxicity to *Chironomus* species, a midge whose larvae live in water, is generally similar to that of the Cladocera. U concentrations which are toxic are relatively high (> 0.10 mg/L) and are reduced by complexation with CO_3^{2-} (Muscatello et al, 2009). Uranium uptake was rapid but most of it accumulated in the exoskeleton and was lost when it shed during metmorphosis. Uranium was also rapidly lost by depuration (Muscatello et al., 2010). Studies of U effects on fish found that toxic concentrations were considerably higher than for planktonic crustaceans as discussed in a literature review by Goulet et al (2011). High hardness also reduced toxicity as occurred for the crustaceans (Parkhurst et al., 1984).

Synthesis of Uranium Toxicity for Multiple Organisms

Antunes et al. (2007) reported that U toxicity from an abandoned U mine in Portugal was highest for algae, crustaceans and dipterans (aquatic flies) and principally depended on the pH of mine water. The pH of the mine water exhibited a seasonal variation that ranged from 3.44 in autumn to 5.67 in the fall.

In a remarkable study Goulet et al. (2015) challenged the findings of other studies that increased hardness reduced U toxicity and reported that the reduction was instead due to increased alkalinity. Their study investigated the toxicity to six different organisms including fish (fathead minnows and rainbow trout), crustaceans (*Ceriodaphnia dubia* and *Hyalella azteca)*, aquatic macrophytes (*Lemna minor)*, and micro algae (*Pseudokirchneriella subcapitata*). Their results showed species sensitivity to U in the following order: *H. azteca, C. dubia, P. subcapitata*, *O. mykiss* alevin/egg stage, *P. promelas*, *O. mykiss* fry stage, and *L. minor.* The study reported both U concentrations which exhibited toxic effects as well as non-toxic impairments. A compilation of their results in presented in Appendix I. They proposed that the lack of an effect of hardness on toxicity for four of the six species studied was because U did not compete with Ca and Mg for uptake sites on the cell membranes. They argue that studies suggesting an effect of hardness did not properly consider an associated increase in alkalinity.

The numerous studies by the Australian researchers (Trenfield, Markich, van Dam) are among those that suggest that toxicity depends on hardness and the resulting formation of ternary U-Ca-CO_3 complexes. Their work appears to have been carefully done, thus the apparent contradiction between the conclusion by Goulet et al. (2015) and the findings of this group and other researchers has not been resolved.

Two widely cited literature reviews on the aquatic toxicology of U were those by Gao et al. (2019) and Sheppard et al. (2005). In addition to a comprehensive review of the literature on U toxicity to both terrestrial and aquatic plants and animals, Gao et al. (2019) provides a brief review of the mechanisms which cause U toxicity. These include damage to the respiratory chain through generation of reactive oxygen species, DNA damage, and apoptosis (U induced eukaryotic cell death). Sheppard et al. (2005) provides a more detailed interpretation of the results of the studies cited. It also includes a brief summary of toxicity to birds and mammals. In part because the studies reviewed by Sheppard et al. (2005) are older, there is little consideration of the effects of U speciation on its toxicity. As a result of their analysis, Sheppard et al. developed a table summarizing the proposed no effect concentration (PNEC) for all classes of organisms considered. The PNEC values for aquatic organisms is presented in Table 3. In addition to these classes of organisms, PNEC values were also reported for terrestrial plants, soils, birds, and mammals.

Table 3. Proposed No Effect Concentration (PNEC) for uranium for different classes of aquatic organisms (Sheppard et al., 2005).

Class of Organisms	**Proposed No Effect Concentration (PNEC)**	**Explanation**
Freshwater Invertebrates	0.005 mg/L	Derived as the 5th percentile of the distribution of observed effect concentrations, with the implication that 95% of biota would be protected using this as a guideline concentration
Freshwater Benthos	100 mg U/kg dry sediment	Based on the LEL approach of observed benthic populations in U-impacted sediments.
Freshwater Fish	0.4 mg/L for hardness < 10 mg/L 2.8 mg/L for hardness 10 – 100 mg/L 23 mg/L for hardness > 100 mg/L	There was a good relationship between effect concentrations and water hardness from a number of studies, the functional expression (units of mg L−1) was: effect concentration = 0.26 (hardness).
Freshwater Plants	0.005 mg/L	Equivalent to the GM effect concentration for Chlorella, with a safety factor of about 10-fold. Because this resulted in a value very similar to that proposed for aquatic invertebrates, that number was used.

International Standards for Uranium in Water

This section presents a brief summary of federal regulations standards for U in water for the United States, Canada and the European Union.

The drinking water standards for U in the U.S. are 30 μg/L (USEPA, 2021) and are applicable to all public water systems. The Canadian government issued Drinking Water Guidelines for public water systems that established a maximum U concentration of 20 μg/LL (Health Canada, 2021). The World Health Organization issued a drinking water guideline of 30 μg/L for maximum allowable U concentration (WHO, 2017). The principal health threat to humans caused by ingestion of high U containing water is renal damage (i.e. damage to kidneys). These standards or guidelines refer to the total U concentration; U speciation is not considered. It is interesting to note that the drinking water standards/guidelines for U are almost 200 times greater than the Swedish EQS standard of 0.17 μg/L.

The PNEC U concentrations listed in Table 3 have an appealing simplicity to them and, as noted, are widely cited. Sheppard et al. (2005) argue that because they have considered a large number of species in each class of organisms they are sufficiently protective of the environment. However, they do not consider the complexity of aquatic systems that has been the subject of considerable investigation since this paper was published.

In the U.S., stream water quality standards are set by the individual states but must be approved by the USEPA. These standards are largely based on guidance documents published by the USEPA. There is no federally recommended standard for U in the U.S. and the guidance documents (USEPA, 1976 and 1986), though quite old, do not discuss the aquatic toxicology of U.

The Canadian Council of Ministers of the Environment published a rather comprehensive discussion of the water quality guidelines for the protection of aquatic life from exposure to U (CCME, 2011) that considered the sources of U, its speciation, its fate, behavior, and partitioning, and its aquatic toxicity. Uranium toxicity was considered for aquatic plants, both invertebrate and vertebrate aquatic animals. The guidelines considered both short term (48 hr and 96 hr) toxicity as well as long term effects (≥ 7 d for fish and invertebrates and ≥ 24 hr for plants and algae). The document determined the centralized species sensitivity distribution (SSD) for short term and long term U exposure. This allowed direct comparison the aquatic toxicity of U for different species for short duration exposure (Figure 5) and long duration exposure (Figure 6). Based on this analysis, Canadian water quality guidelines recommend a total U concentration of 33 μg/L for protection of short term toxic effects and 15 μg/L for protection from long term effects (CCME, 2011). The summary notes that short term guidelines do not protect aquatic life.

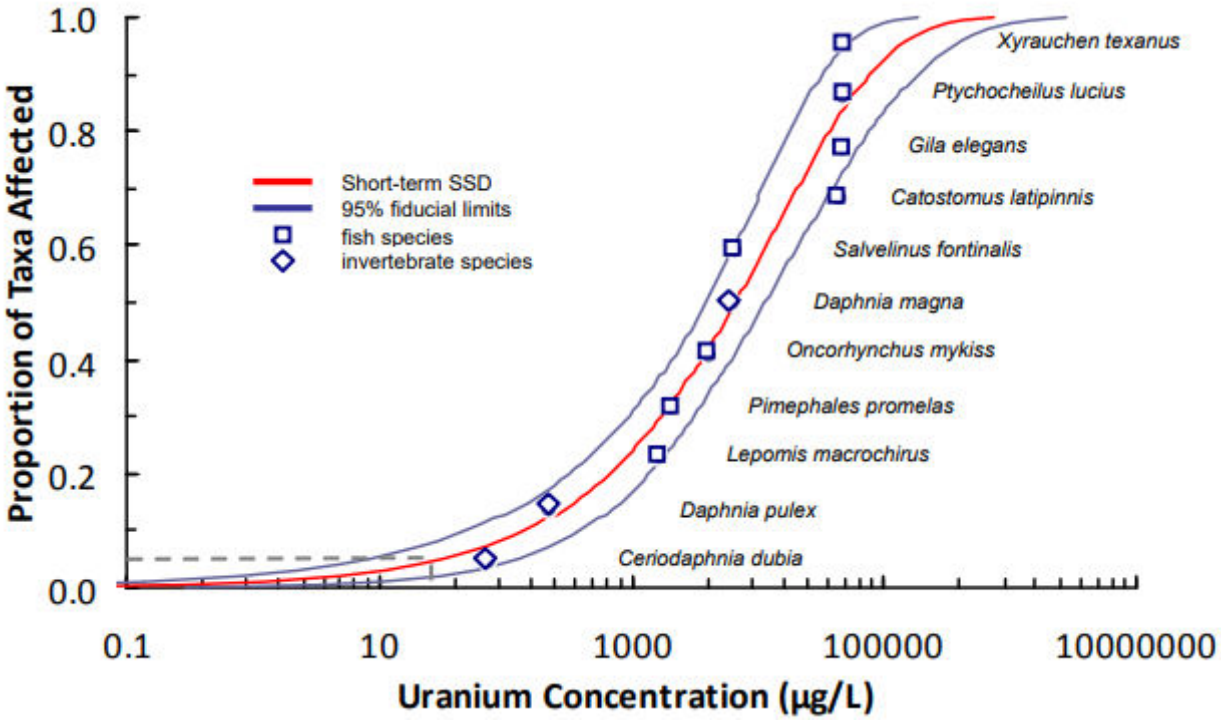

Figure 5. Short-term species sensitive distribution (SSD) representing the toxicity of uranium in fresh water consisting of acceptable short-term LC50s of eleven aquatic species versus proportion of species affected over 48 hr and 96 duration (CCME, 2011).

The European Commission recently launched an online public consultation process to obtain input on proposed water quality criteria and the list of priority pollutants established by Decision 2455/2001/EC (European Commission, 2021). The amended Water Framework Directive (WFD) 2013/39/EU amended previous lists of priority pollutants. Annex X of this directive identifies 45 compounds as priority substances in the field of water policy. Only four of these 45 compounds are metals, cadmium, lead, mercury, and nickel; U is not included. Directive 2013/39/EU also contains watch list of constituents that member countries are required to monitor at least once per year. Eighteen compounds are on the watch list, none of which are metals.

Vankamp and Sanderson (2016) analyzed the variability between national environmental quality standards (EQS) for member states of the European Union to investigate possible reasons for the high degree of variability for some these standards. Six countries have EQS for U that range from 0.015 µg/L in Denmark to 24 µg/L in the Czech Republic. According to this document the Swedish EQS is based on the Canadian study discussed above (CCME, 2011). The standard is based on 72 hr. EC10 (concentration which affects 10% of the organisms) U toxicity to a freshwater algae, *Chlorella sp.* as reported in a 2002 study by Charles et al. (2002). This study reported an EC10 concentration for U in soft water (Ca concentration of 0.16 meq/L) of 0.7 µg/L

Charles et al. (2002) found that U toxicity decreased as water hardness increased as has been reported in many other studies (see Table 2). This was presumably due to formation of a ternary U-Ca-CO_3 complex, although these complexes were not widely recognized in 2002 and were not mentioned by Charles et al. (2002). An assessment factor (sometimes referred to as an

uncertainty factor) of 10 was used to account for uncertainty in the results (Vankamp and Sanderson, 2016). It is interesting to note that the Canadian study which was cited as the origin of the Swedish EQS did not report on the U toxicity to *Chlorella sp*. and is not plotted in either Figure 5 or Figure 6.

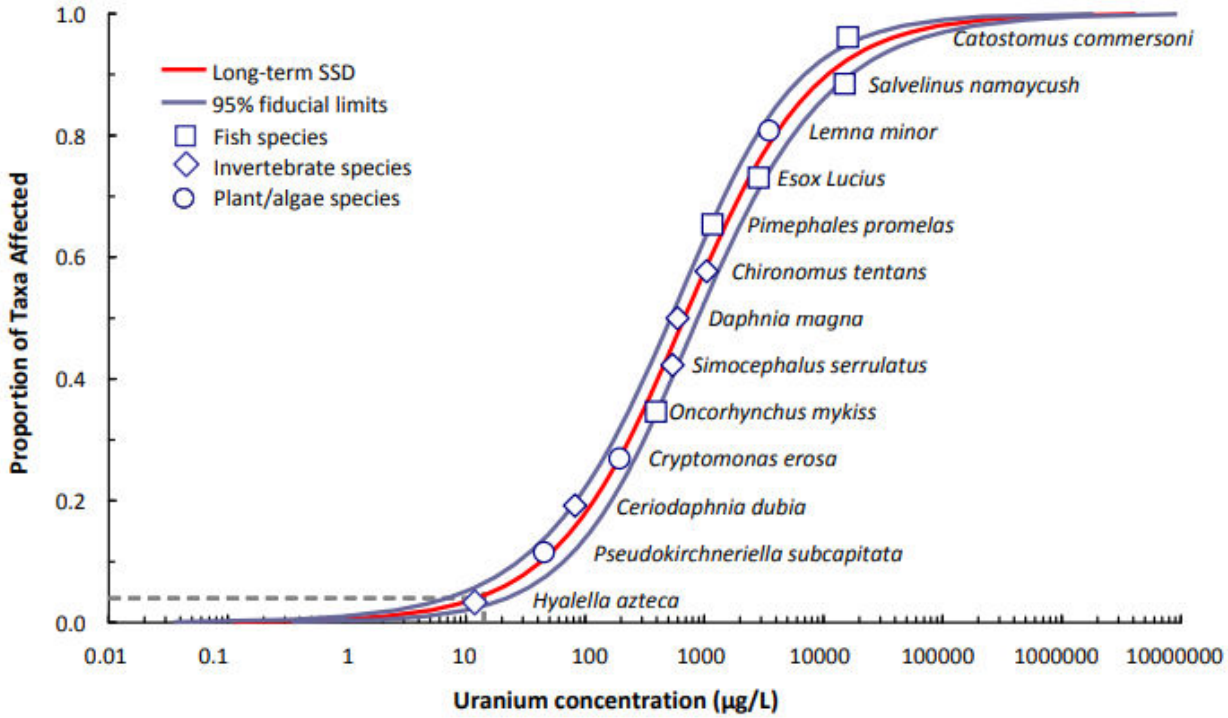

Figure 6. Long term species sensitive distribution (SSD) representing the toxicity of uranium in fresh water consisting of acceptable long term no effect endpoints of ten aquatic species versus proportion of species affected over long duration exposure (≥ 7 d for animals and ≥ plants and algae) (CCME, 2011).

More recently van Herwijnen and Verbruggen (2014) have conducted an analysis of the Dutch EQS for U in water under the European Union WFD. Two types of EQS were derived to cover both long term and short term effects for a large number of aquatic organisms including microorganisms and algae, plants, invertebrates and vertebrates. The most sensitive organisms were microalgae of *Chlorella sp.* This conclusion was primarily based on three studies thatwere done by the Markich group in Australia using tropical green algae. All are somewhat dated as they were published in 2005 or earlier (Franklin et al., 2000; Charles et al., 2002; Hogan et al. 2005). Based on the results of this review van Herwijnen and Verbruggen (2014) proposed a water quality standard for U consisting of an annual average concentration of 0.5 µg/L and a maximum concentration of 8.9 µg/L.

Conclusions

Most studies of the toxicity of U to aquatic organisms that have been conducted in the last 20 years have recognized that the nature of the U species in solution and the solution chemistry is important to its environmental impact. Many of the studies interpret their results in terms of the free ion activity model (FIAM) which proposes that toxicity of a metal ion in solution is primarily due to the uncomplexed cationic U species UO_2^{2+} in aqueous solution and to a lesser extent the hydroxyl complex of this ion (UO_2OH^+). The results of the large number of studies reviewed in this report support the hypothesis that the toxicity of U to aquatic organisms including microorganisms, aquatic plants, aquatic invertebrates and aquatic vertebrates can be attributed primarily to these species.

The aqueous chemistry of U is complicated because the uranyl ion (UO_2^{2+}) forms strong binary complexes with carbonate species (CO_3^{2-}) and ternary complexes with Ca^{2+} and Mg^{2+}. These give U-CO_3. U-Ca-CO_3 and U-Mg-CO_3 complexes respectively. Over the pH range of 6 to 11 these complexes dominate the solution U chemistry while above about pH 6 the concentration of the uncomplexed uranyl ion is extremely low. The presence of complexing agents such as phosphate (PO_4^{3-}) and organic molecules will further reduce the concentration of uncomplexed U species in solution. This emphasizes the importance of including solution chemistry when considering U toxicity to aquatic organisms.

Most of the studies of aquatic U toxicity have shown that the toxicity decreases as the pH goes up. Furthermore, toxicity generally decreases with increasing hardness. One study (Goulet et al., 2015) argues that the reduction in toxicity at high pH is due to increasing alkalinity rather than hardness; the inconsistency of this report and those attributing reduce toxicity to hardness is unresolved. Regardless, multiple reports of decreased toxicity with increasing pH and increasing hardness or alkalinity supports the general FIAM hypothesis that uncomplexed UO_2^{2+} is the principal cause of U toxicity to aquatic organisms. This lends support to the position that the Swedish EQS should only be applied to uncomplexed uranyl ions. Since a uranyl-hydroxyl complex (UO_2OH^+) is only present at environmentally relevant concentrations between about pH 5.0 and pH 6.0 it is not clear whether inclusion of this species in determining compliance with the standard is justified.

The results of the papers cited in this review suggest that the organisms which are most sensitive to aqueous solutions of U are the tropical microalgae *Chlorella* sp. and aquatic invertebrates such as *Ceriodaphnia* and *Daphnia* species. Toxic U concentrations for these organisms appear to range upward from 10 μg/L, though there is wide variability depending on solution chemistry. Concentrations at which an inhibitory effect is detectable range upward from about 1 μg/L.

Based on the findings of this literature review it is apparent that the Swedish EQS for U of 0.17 μg/L is very conservative. It is not clear that a standard this stringent provides additional protection of aquatic life over a more lenient standard.

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Appendix I

Summary of results of results of toxicity testing for six aquatic organisms (Goulet et al., 2015).

Table 5. Summary of toxicity test results in total U ($mg\,L^{-1}$) with 95% confidence limits (CI) compared with other studies found in the literature

Organism[a]	Test	Endpoint	Duration (d)	pH	HCO_3^- ($mg\,L^{-1}$)	Nominal water hardness ($mg\,L^{-1}$ as $CaCO_3$)					Ref.
						5 $mg\,L^{-1}$	15 $mg\,L^{-1}$[b]	60 $mg\,L^{-1}$	120 $mg\,L^{-1}$	240 $mg\,L^{-1}$	
L. minor	Frond no.	IC25 (95% CI)	7	5.8–7.4	3.1–37	—	4.7 (3.6–5.9)[c]	—	12.3 (10.6–12.9)	—	Present study
		IC50 (95% CI)				—	7.4 (6.4–9.2)[c]	—	16.4 (14.8–18.2)	—	
L. minor	Dry wt	IC25 (95% CI)	7	5.8–7.4	3.1–37	—	6.4 (4.9–8.5)[c]	—	13.3 (11.9–30.2)	—	Present study
		IC50 (95% CI)				—	13.1 (9.1–45.6)[c]	—	35.5 (5.6–332)	—	
P. subcapitata	Growth	NOEC	3	6.8–8.2	43	0.01	0.06	0.06	0.22	0.11	Present study
		LOEC				0.03	0.11	0.11	0.43	0.21	
		IC25 (95% CI)				0.05 (0.02–0.05)	0.09 (0.08–0.12)	0.06 (0.02–0.08)	0.10 (0.05–0.15)	0.15 (0.10–0.16)	
		IC50 (95% CI)				0.16 (0.11–0.19)	0.17 (0.08–0.26)	0.10 (0.08–0.11)	0.20 (0.11–0.36)	0.20 (0.16–0.21)	
P. subcapitata	Growth	NOEC	3	7.8–8.4	53–72	—	—	0.4–0.8	—	—	[28]
		LOEC				—	—	0.78–1.59	—	—	
		IC25				—	—	0.11–0.33	—	—	
		IC50				—	—	0.52–2.19	—	—	
C. Dubia	Survival	NOEC	7 d 1	6.5–7.3	2.4–4.3	0.07	0.06	—	0.17	0.06	Present study
		LOEC				0.18	0.16	0.44	0.44	0.18	
		LC25 (95% CI)				0.15 (0.12–0.16)	0.12 (0.09–0.16)	—	0.06 (0–0.11)	0.05 (0.02–0.09)	
		LC50 (95% CI)				0.16 (0.12–0.17)	0.14 (0.12–0.18)	—	0.10 (0.01–0.30)	0.11 (0.07–0.21)	
C. Dubia	Survival	NOEC	7	5.9–6.2	2	0.068	—	—	—	—	[29]
C. Dubia	Reproduction	LOEC	7	5.9–6.2	2	0.092–0.097	—	—	—	—	[29]
C. dubia	Reproduction	NOEC	7 d 1	6.5–7.3	2.4–4.3	0.05	0.06	—	0.02	0.06	Present study
		LOEC				0.07	0.16	—	0.06	0.18	
		IC25 (95% CI)				0.05 (0.05–0.06)	0.05 (0.07–0.06)	—	0.05 (0.0003–0.05)	0.05 (0.009–0.05)	
		IC50 (95% CI)				0.09 (0.08–0.10)	0.11 (0.11–0.11)	—	0.05 (0.04–0.10)	0.1 (0.06–0.11)	
C. Dubia	Reproduction	NOEC	7	8.1	69	—	—	1.54	—	—	[26]
		LOEC				—	—	6.4	—	—	
		IC25				—	—	2.7	—	—	
		IC50				—	—	3.97	—	—	
H. azteca	Survival	NOEC	14	6.4–7.1	4.3–5.5	0.006	0.006	0.07	0.06	0.09	Present study
		LOEC				0.011	0.011	0.17	0.13	0.19	
		LC25 (95% CI)				0.005 (0.001–0.01)	0.005 (0.001–0.01)	0.10 (0.08–0.12)	0.13 (0.09–0.16)	0.13 (0.06–0.26)	
		LC50 (95% CI)				0.02 (0.009–0.04)	0.02 (0.009–0.04)	0.14 (0.12–0.16)	0.20 (0.17–0.26)	0.34 (0.19–1.8)	
H. azteca	Survival	NOEC	14	8.1	69	—	—	0.02	—	—	[26]
		LOEC				—	—	0.06	—	—	
		LC25				—	—	—	—	—	
		LC50				—	—	0.03	—	—	
H. azteca	Survival	LC50	14	7.91	157	—	—	—	1.52	-	[30]
C. riparius	Early life stage	NOEC	20–31	6.4–7.0	6.1–7.9	<0.28	—	0.31	—	—	Present study
		LOEC				0.28	—	0.61	—	—	
		IC25 (95% CI)				0.34 (0.28–0.39)	—	0.55 (0.49–0.59)	—	—	
		IC50 (95% CI)				0.46 (0.40–0.51)	—	0.66 (0.63–0.65)	—	—	
G. roseus	Survival	NOEC	4	6.4–7.0	6.1–7.9	—	2.6	2.4	2.5	2.4	Present study
		LOEC				—	6.7	6.3	6.3	5.9	
		LC50 (95% CI)				—	4.2 (2.6–6.7)	3.9 (2.4–6.5)	4.0 (2.5–6.3)	3.8 (2.4–5.9)	
G. roseus	Survival	LC50	4	6.8–7.0	26	—	8	-	-	-	[32]
P. promelas	Survival	NOEC	4	6.4–7.0	6.1–7.9	—	1.3	1.2	0.81	1.2	Present study
		LOEC				—	2.1	1.9	1.2	2	
		LC25				—	1.7 (1.6–2.0)	1.9 (1.5–2.6)	1.8 (1.3–2.2)	1.6 (1.1–1.6)	
		LC50				—	2.0 (1.8–2.2)	2.0 (2.0–2.1)	2.1 (2.0–2.3)	1.8 (1.5–2.0)	
P. promelas	Survival	LC50	4	7.4–8.2	18	—	3.1	—	—	—	[33]
P. promelas	Survival	NOEC	7	6.4–7.0	6.1–7.9	—	0.84	1.2	0.81	1.2	Present study
		LOEC				—	1.3	1.9	1.2	2.0	
		LC25 (95% CI)				—	1.4 (1.1–1.5)	1.6 (1.2–1.9)	1.2 (1.1–1.6)	1.2 (0.98–1.4)	
		LC50 (95% CI)				—	1.6 (1.5–1.8)	2.1 (2.0–2.1)	2.0 (2.0–2.1)	1.5 (1.3–1.7)	
P. promelas	Growth	NOEC	7	6.4–7.0	6.1–7.9	—	0.84	1.9	2.0	2.0	Present study
		LOEC				—	1.3	>1.9	>2.0	>2.0	
		IC25 (95% CI)				—	1.3	1.5	>2.0	>2.0	
		IC50 (95% CI)				—	>1.3	>1.9	>2.0	>2.0	

[a] Organisms were *Lemna minor*, *Pseudokirchneriella subcapitata*, *Ceriodaphnia dubia*, *Hyalella azteca*, *Oncorhynchus mykiss*, and *Pimephales promelas*.
[b] *Lemna minor* was tested at hardnesses of 30 $mg\,L^{-1}$ and 120 $mg\,L^{-1}$ as $CaCO_3$.
NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect concentration; LC25 and LC50 = lethal concentration at 25% and 50%; IC25 and IC 50 = inhibitory concentration at 25% and 50%.