TOXICITY OF VANADIUM TO FRESHWATER ORGANISMS

REPRESENTATIVE OF THE ATHABASCA OIL SANDS REGION

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By

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ABSTRACT

Vanadium (V) is one of the most abundant trace elements in crude oils, making it an element of potential concern in aquatic ecosystems surrounding various industries, such as the Athabasca Oil Sands (AOS) industry in northeastern Alberta. When bitumen from the AOS region is upgraded to synthetic crude oil, V is removed and enriched at elevated concentrations (> 1000 mg V/kg) into the by-product petroleum coke. This coke is stored on-site of some major oil sands companies, until more practical ways of utilizing or storing it becomes available. Recently, coke has been evaluated as a treatment option for the removal of organic acids in oil sands process-affected water (OSPW) through adsorption processes. However, past studies have found coke to leach toxicologically relevant concentrations of V (> 1 mg/L) into solution upon contact with water, including OSPW. Thus, coke-leachates or coke-treated OSPW containing V could pose risks to aquatic organisms in nearby freshwater systems. Currently, a knowledge gap exists pertaining to the toxicity of V to aquatic organisms, which makes it difficult to assess how increased V in coke-treated OSPW could impact aquatic systems, if it were to be discharged into the surrounding freshwater environment. Thus, the overall goal of this research was to evaluate the toxicity of V (as vanadate oxyanions) to a diverse suite of aquatic organisms regionally-relevant to the AOS region to generate the data needed to derive sound V water quality benchmarks for this region. To achieve this goal, acute and chronic toxicity tests were conducted using comparable laboratory standard test-organisms and organisms more regionally-relevant to northern Alberta. Selected test-species included: four zooplankton species (*Daphnia pulex, D. dentifera, Simocephalus serrulatus,* and *Ceriodaphnia quadrangula*; 2-d and 8- to 21-d studies), two unicellular green algae species (*Pseudokirchneriella subcapitata* and *Scenedesmus quadricauda*; 72-h cell growth studies), two benthic invertebrates (*Chironomus dilutus* and *C. riparius*; 4-d and 30 to 40-d studies), and two freshwater fish species (*Oncorhynchus mykiss* and *Pimephales promelas*; 4-d and 28-d studies).

In short-term acute studies (≤ 4-d), the median lethal concentration (LC50) was the major endpoint reported and ranged from a low of 0.60 mg/L for *C. quadrangula* to a high of 63.2 mg/L for *C. riparius*.

During longer-term chronic toxicity tests ($\geq$ 4-d), effects (lethality and growth, reproductive, and adult emergence inhibition) occurred at concentrations greater than 0.1 mg V/L, and ranged upwards to concentrations of 37.3 mg V/L. When chronically exposed, V appeared to elicit toxicity directly through reduction of lifespan, with little to no effects on sublethal endpoints, such as cladoceran reproduction or fish growth. Sublethal toxicity was more evident in toxicity tests with benthic invertebrates. Here, V significantly impaired adult emergence of *C. dilutus* and *C. riparius* at concentrations $\geq$ 16.7 mg/L (31.6% reduction) and 8.3 mg/L (18.0% reduction), respectively. Overall, significant differences in V sensitivity among regionally-relevant green algal species and benthic invertebrate species and more commonly-used laboratory species were not observed in this research. One exception was the field-collected zooplankton species, *D. dentifera*, which was approximately 2- to 3-times more sensitive to acute and chronic V exposures than the comparable standard test species *D. pulex*. However, there were no significant differences in sensitivity of the field-collected cladocerans, *S. serrulatus* and *C. quadrangula* when compared to the standard test species, *D. pulex* and *C. dubia*, respectively. Similarly, *P. promelas* was 4 to 9 times more sensitive than the standard salmonid species, *O. mykiss*, in acute and chronic V tests, respectively.

The toxicity data generated in this research for V were combined with data from the peer-reviewed literature to construct acute (23 species) and chronic (21 species) species sensitivity distributions (SSDs). From these SSDs, the acute and chronic hazardous concentrations to 5% of species tested (HC5) were estimated as 0.64 and 0.09 mg V/L, respectively. Based on these SSDs and associated HC5 estimates, the most sensitive species to V were from the Cladocera (*Daphnia* and *Ceriodaphnia* species) and Cyprinidae (*P. promelas*). Toxicity threshold estimates for these species are < 1 mg V/L, and thus they could be adversely affected if V was elevated in future site-water or process-water discharges associated with Athabasca oil-sands operations. Many fish and unicellular green algae species were intermediate in their sensitivity to V, whereas benthic invertebrates were the least sensitive to acute and chronic exposures of V. These new toxicity data will not only supplement the current V toxicity database to ensure enough data are

available to develop sound national water quality guidelines, but also to develop regional guidelines for the protection of aquatic communities near oil sands industries, and other industrial sites with V contamination. In conclusion, interim V acute and chronic effects benchmarks of 0.64 and 0.09 mg V/L, respectively, are proposed for freshwater environments to protect aquatic life from exposure to hazardous levels of V in local AOS environments and elsewhere in Canada.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

A^2	Anderson-Darling statistic
ACR	Acute to chronic ratio
AER	Alberta Energy Regulator
ANOVA	Analysis of variance
AOS	Athabasca Oil Sands region
ARW	Athabasca River Water
ATRF	Aquatic Toxicology Research Facility
CCME	Canadian Council of Ministers of the Environment
CEPA	Canadian Environmental Protection Act
CI	Confidence interval
cm	Centimetre
CPCC	Canadian Phycological Culture Centre
CV	Coefficient of variation
CWQG	Canadian Water Quality Guideline
d	Day
dph	Days post hatch
DO	Dissolved oxygen
DOC	Dissolved organic carbon
ECx	Effective concentration estimated to cause an effect on x% of exposed organisms
g	Grams
$x\,g$	Times gravity
h	Hour
H:C	Hydrogen to carbon ratio
HC_5	Hazardous concentration to 5% of species tested
IC	Ion chromatography
ICP-MS	Inductively coupled plasma mass spectrometer
kg	Kilogram
L	Litre
LC50	Median lethal concentration
LOEC	Lowest observable effect concentration
mg	Milligram
mg/L	Milligrams per litre

MLSB	Mildred Lake Settling Basin
NA	Naphthenic acid
NaVO$_3$	Sodium metavanadate
n	Number of replicate
OSPW	Oil Sands Process-Affected Water
p	Probability
PAH	Polycyclic aromatic hydrocarbons
RAMP	Regional Aquatics Monitoring Program
ROS	Reactive oxygen species
SCL	Syncrude Canada Ltd.
SCO	Synthetic crude oil
SD	Standard deviation
SE	Standard error
SLRS	Standard reference material
SSD	Species Sensitivity Distributions
V	Vanadium
V(V)	Vanadate
V(IV)	Vanadyl
WQG	Water quality guideline
wt.	Weight
YCT	Yeast, cerophyll and trout-chow
°C	Degrees Celsius
µg	Microgram
µm	Micrometre
1640a	Standard reference material

NOTE TO READERS

This thesis is organized and formatted to follow the University of Saskatchewan College of Graduate Studies and Research guidelines for a manuscript-style thesis. Chapter 1 is a general introduction and literature review, including the study goal and objectives, and Chapter 4 contains a general discussion with overall conclusions. Chapters 2 and 3 of this thesis are organized as manuscripts for publication in scientific journals. Chapter 2 has been submitted to *Ecotoxicology and Environmental Safety* whereas Chapter 3 is being prepared for submission to *Environmental Toxicology and Chemistry*. As a result of the manuscript-style format, there may be some repetition of Introductions and Materials and Methods sections across each data chapter. The tables, figures, and references of submitted or in-preparation to submit have been reformatted to adhere to the thesis style. References cited in each chapter are combined and listed in the References section of the thesis.

The author contributions for each research chapter include:

Stephanie R. Schiffer (University of Saskatchewan) managed and conducted all experiments, collected and analyzed lab and field data, performed all chemical, statistical and speciation modeling analysis, and drafted each of the manuscripts and the thesis.

Dr. Karsten Liber (University of Saskatchewan) developed the project, supervised the research, and provided overall scientific guidance and input, review of manuscripts, and comments and editorial corrections on the thesis, and secured the funding.

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CHAPTER 1:

GENERAL INTRODUCTION

1.1.   An increased risk of V exposure in Alberta, Canada: The Athabasca Oil Sands region

The oil sands region in northeastern Alberta, Canada, contains the third largest deposits of crude bitumen in the world, following behind deposits in Venezuela and Saudi Arabia (Allen, 2008). Three major deposits make up this region, the Athabasca, Cold Lake and Peace River deposits that cover a combined area of 142 000 km^2 and contain an estimated 170 billion barrels of bitumen (Figure 1.1) (AEUB, 2002; Allen, 2008). Major oil sands mining companies (Syncrude Canada Ltd. and Suncor Energy Inc.) have been operating surface mining, extraction, and upgrading facilities on the Athabasca site located north of Fort McMurray, in close vicinity to the Athabasca River, since the 1970s. Currently, an estimated area of 602 km^2 has been mined and a remaining area of 4200 km^2 is still exploited via surface mining techniques. Despite the current economic downturn, the Alberta Energy Regulator (AER) still predicts strong growth in bitumen production over the next two decades, resulting in continued industrial activity in northern Alberta (AER, 2015). As an unconventional fuel source, bitumen is more costly to recover and process than conventional fuels. To produce a useable synthetic crude oil (SCO), the mined bitumen, which is a heavy, viscous hydrocarbon, undergoes extraction and upgrading processes. These processes generate large volumes of by-products, predominately oil sands process-affected waters (OSPW) and petroleum coke, containing high concentrations of vanadium (V), which are currently stored onsite (Allen, 2008; AER, 2015; Scott and Fedorak, 2004). Without proper storage and reclamation strategies in place, these by-products will continue to accumulate with ongoing oil sands operations, posing potential risks to the

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surrounding environment (Allen, 2008; Kannel and Gan, 2012; Pourrezaei et al., 2014a, b). For the purpose of this research, focus was primarily on Syncrude Canada Ltd. (SCL) operations.

Figure 1.1. Location of the primary oil sands deposits (orange-shaded areas) in Alberta, Canada. (*Source:* Alberta Energy Regulator, 2015)

1.1.1. Oil sands operations

1.1.1.1. Surface mining

Currently, surface mining is a feasible option for some oil sands recovery in the Athabasca bitumen deposit. An estimated 4200 km^2 of land remaining in the Athabasca region has a soil overburden layer of less than 75 m, therefore making it suitable for surface mining technology. The vegetation covering a deposit is removed and the overburden soil layer excavated to expose the oil sands contained within 75 m of the surface. Syncrude's surface mine is estimated to provide more than 500 kilotons of oil sands per day to the on-site extraction plant (Zubot et al., 2012). An estimated 80% of bitumen from the Athabasca oil sands is expected to be recovered in the future using in situ technology, in place of surface mining (AER, 2015).

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1.1.1.2. Extraction

Oil sands are naturally occurring deposits composed of bitumen, mineral solids (i.e., sand, silt and clay), organic compounds and water. Typically mined oil sands consist of about 10% bitumen, 85% mineral solids, and 5% water by weight, prior to extraction (Zubot et al., 2012). Bitumen must be extracted from its associated solids and organic compounds prior to upgrading using the Clark Hot Water process. This process separates the bitumen from residual sand, silt and clay using a combination of water, caustic soda (NaOH) and steam. Water and caustic soda are used to promote leaching of naturally occurring surfactants of bitumen into the water phase. The slurry is then piped into primary separation vessels and heated through the addition of steam. Bitumen is separated by flotation, forming a froth at the surface, which is then skimmed off and pumped to the treatment plant for upgrading (Allen, 2008; Zubot et al., 2012). The extraction process generates OSPW tailings, a complex aqueous mixture containing inorganic salts and organic compounds, which is more saline, alkaline and softer than the freshwater taken from the Athabasca River (Allen, 2008; Clemente and Fedorak, 2005). All OSPW is presently stored onsite in settling ponds (e.g., Mildred Lake Settling Basin) of major operators for an indefinite period of time.

1.1.1.3. Bitumen upgrading

The composition of bitumen is much different than the conventional oils normally used in refineries. Bitumen is highly viscous due to the high presence of asphaltene fractions, has a complex chemical composition, and contains many impurities, including metals, and is therefore unsuitable for use in conventional refineries. Asphaltenes are broadly defined as a complex mixture of high molecular weight compounds that are enriched in vanadyl porphyrins. To yield SCO as a final product of oil sands extraction, the viscosity and metal impurities (i.e., asphaltene fractions) need to be removed from the bitumen.

Syncrude upgrades heavy bitumen to the lighter, more valuable synthetic crude oil through the operation of three fluid cokers. Fluid coking technology heats bitumen to high temperatures (550 to 625 °C) to thermally break heavy hydrocarbon fractions into lighter, more volatile fractions. Bitumen is

converted to a lower viscosity through the removal of carbon (C), to increase the hydrogen: carbon (H:C) ratio (Scott and Fedorak, 2004; Zubot et al., 2012). However, during thermal upgrading, asphaltene fractions only undergo partial degradation. Therefore, the high molecular weight compounds associated with the asphaltene fraction polymerize and condense to form the solid, carbonaceous and heterogeneous by-product petroleum coke (Fedorak and Coy, 2006). Additionally, metal impurities, especially V and nickel (Ni) that are complexed with petroleum porphyrins in the bitumen, are also removed and concentrated in the coke.

1.1.2. Production and storage of coke

As a highly carbonaceous substance, coke could potentially be used as an energy source in the future, and is therefore required to be securely stored on site of all major oil sands producers. The Alberta Energy and Utilities Board (AEUB) dictates that coke should be stored on site for its potential recovery in the future as an energy source, similar to medium grade coal (~29.5 MJ/kg) (Baker, 2007; Fedorak and Coy, 2006; Scott and Fedorak, 2004). However, due to the high sulfur content (~6-8wt. %), low combustibility, high transport costs, elevated heavy metals content, and current availability of cheaper energy sources, such as natural gas and coal, petroleum coke is currently not a viable energy source (Chung et al., 1996; Furimsky, 1998; Scott and Fedorak, 2004; Baker, 2007; Small et al., 2012a, b).

It is reported that approximately 15% of extracted bitumen is converted to coke and that Syncrude generates approximately 2 million tons of coke annually. In 2014, the overall volume of coke produced by the entire oil sands industry had reached 90 million metric tons (Figure 1.2) (AER, 2015; Fedorak and Coy, 2006). Currently, coke is stockpiled on-site as required by the AEUB in mined-out pits, known as coke cells, or as "beaches" along active settling basins, such as the Mildred Lake Settling Basin (MLSB). These ever-accumulating volumes of coke, could eventually exceed safe storage capacity (Baker, 2007; Scott and Fedorak, 2004; Small et al., 2012a, b). Syncrude has begun hydraulically transporting coke via pipelines with OSPW and discharging it into the MLSB for alternative long-term storage (Puttaswamy and Liber, 2010). Coke will continue to be transported and stored in the MLSB, where it is exposed to natural

weathering, leading to its eventual incorporation into reclaimed landscapes, yet it is still available for

potential future recovery, if needed. Currently, coke is also being investigated as a potential option for the

removal of organic compounds causing the acute toxicity of OSPW.
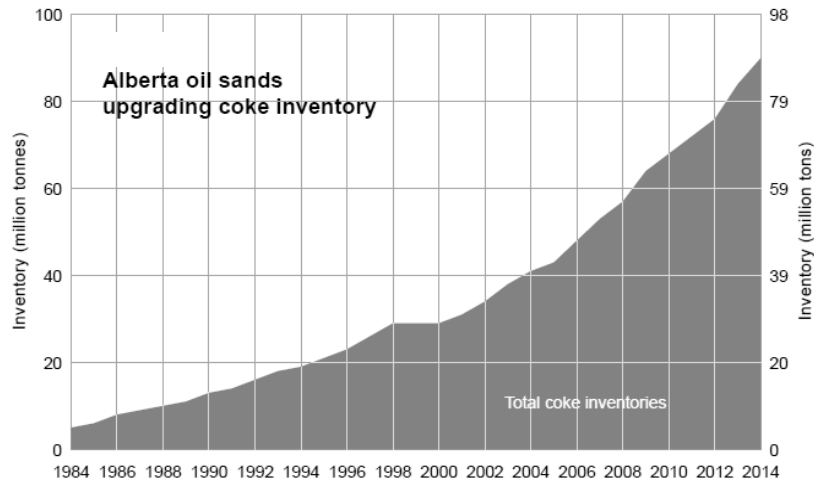
Figure 1.2. Alberta oil sands coke inventory from 1984-2014.
(*Source*: Alberta Energy Regulator, 2015)

1.1.3. Contaminants associated with petroleum coke

Syncrude has experimented with integrating oil sands coke into aquatic reclamation landscapes as

a potential solution to long-term storage. The high carbon content of coke could potentially provide a more

suitable substrate in reclaimed aquatic landscapes for colonization by benthic invertebrates, than fine oil-

sands tailings (Baker et al., 2012). Initially, coke was assumed to be inert, however, recent studies have

found coke to affect microbial processes and leach certain metals (i.e., V, Ni, Cu, Mn and Mo) when it is

exposed to water and weathering processes (Fedorak and Coy, 2006; Puttaswamy et al., 2010; Squires,

2005). Therefore, more investigations regarding coke impurities, such as the metals leached and the risks

they could present to the environment are needed before coke should be used as a substrate in reclaimed

wet landscapes (Baker et al., 2012; Puttaswamy et al., 2012; Squires, 2005).

Recently, studies have investigated the toxicity of coke leachates to the freshwater invertebrate, *Ceriodaphnia dubia* (Puttaswamy et al., 2010; Puttaswamy and Liber, 2011; Puttaswamy and Liber, 2012). Coke was collected from SCL coke stockpiles and placed in either a shallow or deep lysimeter, which differed only in the amount of overlying soil cover, constructed near the MLSB shore (on the coke beach). Undiluted (100%) leachates collected from the bottom of both lysimeters significantly reduced the survival and reproduction of *C. dubia* in 7-d exposures. When chronic toxicity identification and evaluation (TIE) tests were used to identify toxicants, V and Ni were confirmed as the two primary metal toxicants at two different pHs (5.5 and 9.5). At pH 5.5, both Ni and V were responsible for toxicity in coke leachates, whereas V was the stole toxicant of concern at pH 9.5 (Puttaswamy and Liber, 2012). Concentrations of V in the shallow and deep lysimeters were measured at concentrations as high as 2.9 and 14.7 mg/L, respectively, which greatly exceeded the *C. dubia* 7-d LC50 of 0.55 mg/L (Puttaswamy et al., 2010). Furthermore, V concentrations were significantly greater in the deep lysimeter relative to the shallow lysimeter because of the greater solubility of V in the more alkaline pH conditions present in the deep lysimeter. Although V concentrations gradually decreased in the deep lysimeters over time (February 2005 to September 2006) from 14.7 to 5.6 mg/L, respectively, V still continued to exceed the 7-d LC50 (0.55 mg/L). The rapid leaching of V from coke is supported by Squires (2005) who also reported high V in laboratory-generated coke leachates, under artificial weathering conditions. However, Squires (2005) reported no toxicity to *Chironomus tentans* suggesting this species is less sensitive to V than *C. dubia.* Furthermore, Baker et al. (2012) reported increased V concentrations in the tissues of benthic invertebrates (Aeshnid dragonfly larvae and chironomids) when exposed to coke used as a sediment substrate. Therefore, additional long-term studies of coke toxicity are necessary to better evaluate the risks coke and its leachates may pose to aquatic ecosystems (Baker et al., 2012; Squires, 2005; Puttaswamy and Liber, 2012). Additional studies of how toxic constituents in coke leachates could affect the growth, survival, and reproduction of higher trophic level organisms, would provide oil sands companies with valuable information to help formulate proper storage, remediation, and/or utilization options for the vast quantities of coke stored on-site.

1.1.4. Oil sands process-affected waters (OSPW)

Another issue of concern at the Alberta oil sands is the volume of oil sands process-affected waters (OSPW) generated during the extraction of bitumen from the sand, silt, and clay comprising mined oil sands. During the extraction process, the bitumen ore is mixed with hot water, caustic soda and naphtha, in order to recover the bitumen (Allen, 2008; Rogers et al., 2002). This process requires up to three barrels of freshwater, obtained from the Athabasca River, for every barrel of SCO produced (Allen, 2008). To reduce this freshwater consumption, OSPW can be recycled to some extent and reused in the extraction process, which acts to further concentrate organic toxicants. The OSPW is therefore stored on-site and not returned to the river. As result, the storage and management of OSPW presents a major operational and environmental challenge for oil sands producers.

Unlike the Athabasca River water, OSPW is much more alkaline and saline and has a complex mixture of inorganic salts and organic compounds (Allen, 2008; Pourrezaei et al., 2014b). The concentration of total dissolved solids (TDS) is in the range of 2000 to 2500 mg/L and is dominated by sodium, bicarbonate, chloride and sulphate (Allen, 2008). Dissolved organic compounds in OSPW contribute to the poor water quality. Acute toxicity of OSPW to a variety of aquatic organisms has been well characterized in the published literature, with the organic fraction being primarily responsible (i.e., naphthenic acids) (Anderson et al., 2012; Allen, 2008; He et al., 2010; Headley et al., 2009; Rogers et al., 2002; Scarlett et al., 2012; Scott et al., 2008). Naphthenic acids are a complex mixture of cycloalkanes containing a single carboxylic acid group, with over 200,000 structures present (Anderson et al., 2012; Small et al., 2012a; Zubot et al., 2012). In addition to NAs, other organic compounds such as pyrroles, thiopenes and phenols have also been detected in OSPW. Therefore the term acid-extractable organics is a more appropriate term to describe the range of organic compounds measured in OSPW (Small et al., 2012a; Verbeek et al., 1993; Zubot et al., 2012). Natural biodegradation of NAs has been reported to occur in tailings ponds found on-site of major operators; however, the overall toxicity reductions is a very slow process (Anderson et al., 2012). While simple NAs dissipate relatively quickly, more complex NAs with a

higher number of ring structures tend to be much more persistent in the environment (Holowenko et al., 2002). Therefore, a more rapid, cost-effective technique that specifically targets NAs and other organic acids, especially structures of greater molecular weights, is needed to aid in the remediation of this water and its eventual release to the natural environment.

1.1.5. Coke as a viable adsorbent in the treatment of OSPW

Allowing OSPW to age naturally in tailings ponds is not an effective treatment method for eliminating OSPW toxicity within a reasonable time-frame (Toor et al., 2013). Recent treatment approaches to mitigate OSPW toxicity have included the use of ozone to chemically oxidize NAs (Anderson et al., 2012; Scott et al., 2008). Studies have found ozone to effectively remove up to 95% of NAs and attenuate OSPW toxicity to *V. fischeri*, reduce steriodogenic effects in human cell lines and significantly improve survival, growth, pupation and emergence in *C. dilutus* (Anderson et al., 2012; He et al., 2010; Gamal El-Din et al., 2011). However, the cost associated with generating ozone for the treatment of large OSPW volumes would be very high for oil sands companies (Scott et al., 2008). Therefore, research has recently focused on the adsorption of organic acids onto activated carbon (AC), which is a proven and effective material in treatment of wastewaters and drinking water (Small et al., 2012a, b). Similar to ozone, however, AC is also expensive to obtain and use on an industrial scale of this magnitude, so coke has been suggested as a potential substitute. Coke would be very advantageous to oil sands companies as an absorbent, given that is high in C (> 80% by weight), and is available in large supply on site (Small et al., 2012a, b; Zubot et al., 2012).

Numerous studies have confirmed that coke, whether activated or not, can effectively remove complex organic compounds, with greater molecular weight and lower solubility, from OSPW (Gamal-El Din et al., 2011; Pourrezaei et al., 2014a, b; Small et al., 2012a, b; Zubot et al., 2012). Furthermore, removal of the more recalcitrant organic acids (i.e., high molecular weights, low solubility) also increases the overall biodegradation of organic fractions in OSPW and therefore results in greater toxicity reduction in a shorter time frame in settling ponds (Pourrezaei et al., 2014b; Zubot et al., 2012). These studies have

proven that coke can remove up to 90% of NAs from OSPW and can be used alone or in combination with ozone (Gamal-El Din et al., 2011; Zubot et al., 2012). In the latter case, coke serves as a pre-treatment step prior to ozonation, thus greatly reducing the ozone doses required. However, although coke can remove organic acids from OSPW, the alkaline pH of the OSPW causes the leaching of V from coke into coke-treated OSPW (Puttaswamy et al., 2010; Puttaswamy and Liber, 2012; Zubot et al., 2012). Therefore, given the absence of WQGs for V for the protection of freshwater organisms, it is unknown how these elevated concentrations of V in coke-treated OSPW could impact aquatic ecosystems downstream of future OSPW discharge sites.

1.2.   Sources of V in the environment

Vanadium (V) is a non-volatile transition element with an atomic number of 23 and an atomic weight of 50.9. It is the 22nd most abundant metal in the earth's crust averaging a concentration of 136 mg/kg (Crans et al. 1998; Langmuir et al. 2004). This concentration is similar to other metals, such as zinc, and makes V more common than copper and nickel. However, although widely distributed throughout the environment, naturally present in soils, sediments, and waters, V is rarely found in its pure state. Thus, economically significant ore deposits of V are seldom encountered. Instead it is more often found in combination with over 50 different minerals, such as vanadinite oxides ($Pb_3(VO_4)_3Cl$) and patronite (VS_4). Additionally, V also commonly occurs in the ores of other metals, such as copper, zinc, lead, iron, manganese, calcium, and uranium, as well as in phosphate ores (Aide, 2005; ASTDR, 1992; CCME, 1997; Moskalyk and Alfantazi, 2003).

The distribution of V in soils and sediments usually depends on the bedrock geology of the region. A large portion of aqueous V originates from the erosion of land surfaces by water. Humic substances are generally found to be naturally associated with V and often contain elevated concentrations of V (Crans et al. 1998). Carbonaceous sediments, such as shales, black shales, phosphate rocks, crude oil, asphalts, peat, bitumen and coal deposits, contain significant amounts of V in the form of porphyrin organometallic complexes (Crans et al. 1998; Duyck et al. 2007; Hamada, 1998; Hodgson et al. 1954; Jacob and Filby,

1983; Rehder, 1991; Rehder, 2015). Commonly, V has been used as an indicator in crude oil spills and oil pollution to identify the source of origin and maturation of the petroleum in question (Khalaf et al. 1982; Jacob and Filby, 1983). Crude oil from Venezuela and from the Athabasca Oil Sands (northern Alberta, Canada) region are known to contain highly elevated levels of V porphyrins at concentrations as high as 1180 and 640 mg/kg, respectively (Crans et al. 1998).

1.2.1. Sources of V contamination

Generally, natural background concentrations of V in aquatic ecosystems are quite low. However, V concentrations in freshwater systems are usually dependent on the geographical differences in leachates and effluents from both natural and anthropogenic sources, and can therefore be highly variable. Median background levels of aqueous V in freshwaters are usually reported within the range of 0.5 to 2.4 µg/L (Hamada, 1998; Hirayama and Kageyama et al., 1992; Rehder, 1999). Given that concentrations of V in aquatic environments tend to be low, this element has rarely been reported as a potential problem in terms of aquatic toxicity. However, near areas associated with heavy mining activity, dissolved V can be higher, with concentrations greater than 49.2 µg/L measured (Imtiaz et al., 2015; Linstedt and Kruger, 1969). Although contamination of V is unlikely to occur on a global scale, local environments near some industrial activities could be at increased risk (Hope, 1997).

Mobilization of V within environmental compartments occurs through both natural and anthropogenic processes. Natural processes include the deposition of atmospheric V particulates, marine aerosols, volcanic emissions, and erosion and weathering of soils and rocks, all of which act to influence environmental V concentrations (WHO, 2001). Combustion of fossil fuels, especially coal and oil, are the primary source of anthropogenic V released to the environment (de Beer and Coetzee, 1994; Duyck et al., 2007; Pacyna and Pacyna, 2001; Pyrzynska and Wierbicki, 2004; WHO, 2001). In crude oils, the asphaltene fraction is highly elevated in V, which is removed during bitumen upgrading and enriched into produced by-products. Therefore, the production and upgrading processes from the AOS region, and weathering of associated by-products, could potentially expose surrounding aquatic ecosystems to elevated

concentrations of biologically available forms of V. The concentrations of V in coke, ash and soot from petroleum products are also of concern, especially with ongoing and expected future expansion in mining and upgrading of heavy crude oils in the AOS region.

1.3. Speciation and environmental fate of V in aquatic systems

Vanadium can exist in multiple oxidation states, ranging from -1 to +5, however only the +3, +4 and +5 states are known to occur in the environment (Crans et al. 1998; Minelli et al. 2000; Wright and Belitz, 2010). The pentavalent anion, V(V), is the most stable, dominant and mobile form of V in aquatic systems (Crans et al. 1998; Wehrli and Stumm, 1989). The environmental fate of V in aqueous systems is greatly influenced by its redox behavior and aquatic geochemical factors, such as pH, oxygenation, the precipitation and dissolution of V-containing minerals, sorption processes, and its solution concentration (Lu et al. 1998; Zhou et al. 2011). These factors can alter the valence state of V, thus changing its speciation in aquatic systems, and ultimately influence its mobility, bioavailability and toxicity. Other abiotic factors, including water quality characteristics such as alkalinity, hardness, ion content, and temperature, could also influence V speciation in the aqueous phase, but these relationships are currently not well characterized.

1.3.1. Speciation of V in surface waters

In aquatic environments, V has complex surface water chemistry and behaves similarly to the metalloids arsenic (As) and selenium (Se), and the metals chromium (Cr) and molybdenum (Mo). In aerobic waters and soils, V covalently binds to oxygen to form oxyanion species (Fairbrother et al., 2007). Therefore, dominant forms of V most commonly as complexes rather than free ions. Vanadate (V) and vanadyl (IV) both comprise the dominant V species in overlying waters (Jen et al., 1997; Harita et al., 2005; Hirayama et al., 1992; Wang and Sanudo-Wilhelmy, 2009). The oxyanion species of V (V) (e.g., $H_2VO_4^-$ and HVO_4^{2-}) form the majority of dissolved V present in oxygenated surface waters because of their strong tendency to associate with oxygen atoms (Crans et al., 1998). As a redox sensitive element, V is most soluble under oxidizing conditions, thus its solubility increases with its valence state, as well as

with the pH and oxygenation of aquatic systems (Crans et al., 1998; Pourret et al., 2012; Wehrli and Stumm, 1989). However, under slightly more reducing conditions, the vanadyl (IV) oxocation (VO^{2+} and $VO(OH)^+$) dominates, with an increased tendency to precipitate or adsorb to sediment (Morford, 2005). Vanadium readily converts between these two oxidation states, via redox chemistry, under environmental conditions frequently encountered in aquatic systems (e.g., alkaline pH, oxygenated and positive redox conditions) (Crans et al., 1998). Elements, such as iron (Fe^{3+}), that are present as oxy-hydroxides and organic substances aid in the reduction of V(V).

The speciation of V in aqueous solution is shown in Figure 1.1. The species V(II) (V^{2+}) and V(III) (V^{3+}, VOH^{2+}, $V(OH)_2^+$ and $V_2(OH)_2^{4+}$) are unstable in the aqueous phase, due to their high sensitivity to oxygen, and therefore exist only under strong reducing conditions or when complexed to organic ligands (Crans et al., 1998). Monomeric V(V) oxyanions ($H_2VO_4^-$ and HVO_4^{2-}) prevail in most oxygenated aquatic systems within a pH range of 5.8 to 9 (Crans et al., 1998; Wang and Sanudo-Wilhelmy, 2009; Wanty and Goldhaber, 1992; Wehrli and Stumm, 1989). These vanadate species are the most stable and mobile in oxidized waters, allowing for transport in surface waters, and can be bioavailable to aquatic biota. However, as V concentrations increase in solution (> 20 mg/L), the monomeric V(V) oxyanions begin to polymerize to less bioavailable species (e.g. $V_3O_9^{3-}$ and $V_4O_{12}^{4-}$) (Miramand and Unsal, 1978; Peacock and Sherman, 2004; Ringelband and Hehl, 2000). In contrast, the vanadyl (IV) cation is predominant under more reducing, acidic conditions. The vanadyl cation has a high tendency to adsorb to organic ligands and particulates in water and sediment, thus being easily removed from the water column. Vanadyl also has a strong affinity for hydrous oxide surfaces (i.e., iron and magnesium oxides, aluminum and titanium-based oxides and organic chelating ligands). Formation of complexes with humic acids and porphyrins allows V(IV) to extend its stability into more oxic waters with a pH range similar to V(V) (Wehrli and Stumm, 1989). Both V(V) and V(IV) species can co-exist in natural surface waters and can therefore cause adverse effects to aquatic organisms at elevated concentrations.
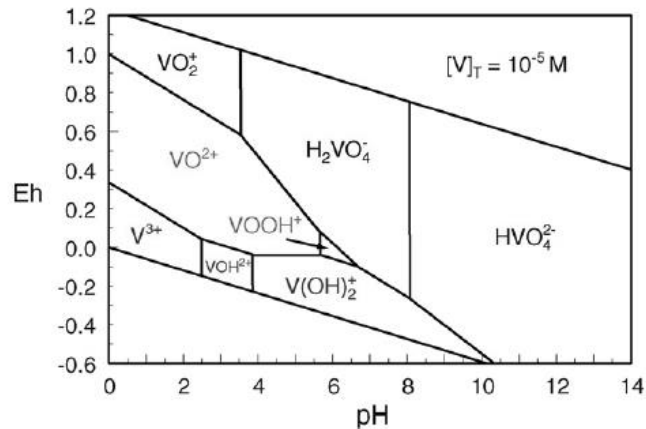
Figure 1.3. The speciation of V at low concentrations (0.5 mg/L) based on pH and Eh (V) conditions. (*Reproduced from:* Huang et al. 2015)

1.3.2. Accumulation of V in sediment

Most trace metals will readily partition from the water column and accumulate in sediment. Although bulk metals in sediments are largely unavailable to aquatic biota, aquatic systems are dynamic and metals can be continuously cycled between the aqueous and solid phases. Bound trace metals can also partition into the pore water of sediments, thus becoming biologically available and account for the majority of metal toxicity in sediments (Eggleton and Thomas, 2004). Other important components influencing metal speciation and bioavailability in sediment include clay minerals, iron (Fe) and manganese (Mn) oxyhydroxides, and carbonates (Eggleton and Thomas, 2004; Zhong and Wang, 2008).

The partitioning of V between overlying water and sediment is related to its redox behavior and its associated sorption onto sediment particles, especially oxides (Peacock and Sherman, 2004; Wehrli and Stumm, 1989). Whether or not V will associate with particulates in the water column depends on pH and redox conditions of the aquatic system in question (Huang et al., 2015). Anionic V(V) species, which are stable in oxic waters, do not readily complex to dissolved organic matter (DOM), dissolved organic carbon (DOC), or particle surfaces (Pourret et al., 2012; Wehrli and Stumm, 1989). However, V(V) species can be removed from the water column in the presence of some colloids (Fe and Mn oxyhydroxides) below pH 7

(Langmuir et al., 2004; Wright and Belitz, 2010). Iron and Mn oxyhydroxides scavenge V(V) from the water column, and reduce V(V) to V(IV) (Harita et al., 2005). Vanadate adsorption is favored through ligand exchange mechanisms, similar to that of phosphates, and is more likely to occur under lower pH and lower Eh conditions (Wehrli and Stumm, 1989). Increases in pH ($\geq$ 8) favor the desorption of V(IV) anions from mineral surfaces, allowing them to re-enter the water column as dissolved anions (Wright and Berlitz, 2010).

In general, accumulation of V in sediment is favored under reducing conditions or in the presence of reducing agents (i.e. humic acid, fulvic acid, and ascorbic acid), where the V(V) anion is reduced to the V(IV) cation (Huang et al. 2015). Although both species are surface reactive, the V(IV) cation has a stronger tendency to adsorb onto particles associated with the sediment phase. Vanadyl strongly adsorbs to organic chelating ligands, clay minerals, and oxide surfaces, such as Fe and Mn oxyhydroxides, Al_2O_3, and TiOH, through surface coordination reactions (Peacock and Sherman, 2004; Wang and Sanudo-Wilhelmy, 2009; Wehrli and Stumm, 1989). Because of a high affinity for organic ligands, V(IV) becomes highly enriched in organic-rich, carbonaceous sediments. Therefore, aqueous V can be precipitated from the water column as an insoluble oxyhydroxide, or reduced to the cationic form under anoxic conditions.

1.4. Toxicity of V

1.4.1. Bioavailability to aquatic biota

Bioavailability describes the amount of 'free' metal capable of interacting with aquatic biota in the environment. In aquatic systems, there is continuous cycling between aqueous and solid phases that control the transport, fate, and exposure of toxicants to aquatic biota (Ahlf et al., 2009). The solubility, mobility, and bioavailability of V in aquatic environments are affected by its oxidation state, with toxicity decreasing with lower oxidation states. The bioavailable vanadate oxyanions ($H_2VO_4^-$ and HVO_4^{2-}) are structurally similar to dihydrogen phosphate ($H_2PO_4^-$), therefore providing a probable mechanism of uptake in aquatic biota, through anionic phosphate and sulfate transport proteins, also known as ionic mimicry (Bridges and

Zalups, 2005; Rainbow, 1997). Abiotic and biotic sediment characteristics can further influence V oxidation states, and alter its environmental fate (Calace et al., 2006).

1.4.2. Proposed mechanisms of toxicity

Vanadium is an essential element in lower life forms (i.e., seaweed, bacteria, lichen and fungi) but an established role in vertebrates has not yet been determined. However, some studies have reported deficiency symptoms in conjunction with reproductive abnormalities and bone growth of goats and chickens, suggesting an essential role (Mukherjee et al., 2004; Nielsen and Uthus, 1990; Rehder, 2003). In lower trophic organisms, V has been detected in certain enzymes such as in the nitrogenases in bacteria and vanadate-dependent haloperoxidases in algae and fungi (Rehder, 2003). Certain nitrogen-fixing bacteria are found to use V in place of Mo in the enzyme nitrogenase when Mo is deficient in the environment (Crans et al., 1998; Rehder, 1991). Recent research into the uptake and speciation of V in the invertebrate, *Hyalella azteca,* found that 7-d exposures resulted in increased V (IV and V) tissue content with increasing exposure concentrations (Jensen-Fontaine et al., 2014). Although it is not well-studied, significant bioconcentration of V in aquatic organisms seems unlikely, given high V excretion rates and other studies reporting low V tissue concentrations from water exposures (Barceloux, 1999; Holdway et al., 1983). However, bioaccumulation has been observed to be greater from dietary sources in one study (Hilton and Bettger, 1987). Marine invertebrates belonging to the family Ascidaceace are also known to bioaccumulate and regulate elevated concentrations of V in specialized cells. The function of V in these cells still remains to be established (Crans et al., 1998; Crans, 2004; Rehder, 1991; Rehder, 2003).

Currently, there is uncertainty regarding the essentiality and physiological role of V in higher organisms. However, the structural and electronic similarities of vanadate (HVO_4^{2-}) to hydrogen phosphate (HPO_4^{2-}) suggest V could play an important role in both the inhibition and stimulation of phosphorylation processes (Rehder, 1999; Rehder, 2005). As an analogue to phosphate at physiological pH, V(V) can easily substitute for phosphate at the sites of membrane carrier proteins in cell membranes, through ion mimicry, thus allowing for uptake (Bridges and Zalups, 2005). This similarity allows V(V) to interact with various

physiological substrates that phosphate would otherwise utilize (Rehder, 2015). Therefore, V(V) species could interfere with several metabolic processes, such as ATP phosphahydrolase, ribonuclease, adenylate kinases, phosphofructokinase, and glyceraldehyde-3-phosphate dehydrogenase, by replacing their phosphate groups (Barceloux, 1999; Nechay, 1984; Rehder, 2003). When vanadate replaces phosphate in enzymes, it ultimately results in the inhibition of the enzyme, which has led to V(V) being identified as a potent inhibitor of the sodium (Na), potassium (K) ATPase pump in *in vitro* studies (Cantley and Aisen, 1979; Chasteen, 1983; Rehder, 2003). Thus, if V(V) is present at sufficiently high concentrations in certain tissues, it could potentially act as a specific regulator of Na, K-ATPase activity (Cantley, 1977; Capella et al. 2002). However, *in vitro* effects may not necessarily translate into *in vivo* effects as V speciation is not fully taken into account at this biological level. Furthermore, the potency of V(IV) as a Na, K-ATPase inhibitor is much less than V(V) (Cantley and Aisen, 1979). Overall, current research has suggested that a probable role of V in biological systems may be in the regulation of enzymatic phosphorylation and inhibition of the Na, K-ATPase pump. Further *in vivo* studies are needed to confirm these hypotheses.

Vanadium toxicity is primarily governed by its oxidation state, with the V(V) species generally being more toxic and bioavailable than the V(IV) species (Barceloux, 1999; Rehder, 2015). Vanadate oxyanions can enter cells via anionic transport pathways, whereas organically bound vanadyl complexes are taken up by passive diffusion processes (Yang et al. 2003). Once inside cells, V(V) species are gradually reduced to the less active V(IV) by intracellular reducing agents (e.g., NADPH and glutathione). If V(V) species are not reduced, they can interfere and inhibit the electron transport chain in the mitochondria and generate reactive oxygen species (ROS) through redox reactions. Spontaneous oxidation of vanadyl ions with oxygen or hydrogen peroxide *in vitro* can also create ROS within cells. These species can lead to oxidative damage, including DNA damage and lipid peroxidation in tissues, such as the liver and kidney, and eventual cell death (Soares et al., 2007; Soares et al., 2008; Valko et al., 2005). Therefore, another probable mechanism of V toxicity in aquatic biota may be cellular oxidative stress due to the generation of ROS species within the mitochondria.

1.4.3. Freshwater toxicity

Studies in the published literature have investigated the toxicity of V to freshwater organisms and the data that are available can be highly variable. These data make the establishment of water quality guidelines for the protection of aquatic organisms difficult. For example, a number of toxicity tests have determined the effects of V on freshwater algal species (Table 1.1). As an established essential element in certain algal species, V has been found to a play a role in growth stimulation, metabolism and photosynthesis at concentrations < 0.50 mg/L. However, at higher concentrations, V can inhibit growth and chlorophyll production, and significantly reduce photosynthesis (Lee et al., 1979; Meisch et al., 1975; Meisch and Bieling, 1975; Nalewajko et al., 1995a, b). In related studies, species from three taxonomic groups (Cyanobacteria, Chlorophyta, and Bacillariophyta) were exposed to sodium metavanadate to investigate the inhibitory effects of V on photosynthesis and growth (Nalewajko et al., 1995a, b). Results revealed large species differences with respect to V toxicity. The green algae, *Ankistrodesmus falcatus*, and the diatom, *Diatoma elongatum*, were the most sensitive species to V and exhibited threshold effects at 0.10 mg V/L. The least sensitive species was the blue-green alga (Cyanobacteria), *Anabaena flosaque*. Relative to these short-term tests evaluating V's effect on photosynthesis inhibition, chronic growth was a more sensitive endpoint for V in all three algal species. However, another study (Lee et al., 1979) found cell growth of *A. flosaque* to be 10 times more sensitive than the diatom, *Navicula pelliculosa*. Both studies reported species of green algae to be quite sensitive to V, with threshold effects in the 0.10 to 0.30 mg V/L range. Regardless, exposure durations in both growth bioassays ranged from 7 to 12 days; however, an exposure time of 72-h is generally sufficient to assess the same chronic endpoints (Environment Canada, 1992a; Nyholm and Kalliqvist, 1989). Prolonged toxicity tests result in a large biomass increase, which can be problematic in obtaining reliable endpoints; thus, additional tests with shorter exposure durations may be necessary.

Aqueous V toxicity has been investigated in several freshwater invertebrates, including crustaceans, oligochaetes, midges and amphipods (Table 1.1). The most commonly measured endpoint in

these studies is the median lethal concentration (LC50). However, there has been less investigation into sublethal effects of V. Acute effect concentrations were similar for all test species, except for the one oligochaete species, and found to range from 0.24 to 30.4 mg V/L. Fargasova (1997) reported the lowest 96-h LC50 of 0.24 mg V/L for the benthic invertebrate *Chironomus plumosus*, whereas the oligochaete, *Pristina leidyi*, was the most tolerant species to acute effects of V (Smith et al., 1991). Many of these acute toxicity endpoints for V were collected in the early 1980s and 1990s; therefore, more modern testing protocols and analytical techniques may be needed to verify these results.

Table 1.1. Toxicity of vanadium to freshwater algae and invertebrates (planktonic, epi-benthic and benthic)

Species	Endpoint	Effect concentration (mg/L)	Reference
Freshwater Algae			
Scenedesmus quadricauda	12-d EC50[1]	2.23	Fargasova et al. (1999)
Scenedesmus obliquus	7-10-d LOEC[2]	5.09	
Ankistrodesmus falcatus	7-10-d LOEC	0.10	Nalewajko et al. (1995b)
Diatoma elongatum	7-10-d LOEC	0.10	
Anabaena flos-aqueae	7-10-d LOEC	50.94	
Planktonic Invertebrates			
Ceriodaphnia dubia	7-d LC50[3]	0.55	Puttaswamy et al. (2010)
Daphnia magna	48-h LC50	4.1	Beusen and Neven (1987)
	23-d LC50	2.0	
Daphnia magna	21-d LC50	1.7	Van Leeuwan (1987)
Epi-benthic Invertebrates			
Hyalella azteca	96-h LC50	1.5	Lee et al. (2013)
	14-d LC50	1.2	
	7-d LC50	0.4	Borgmann et al. (2005)
Benthic Invertebrates			
Chironomus plumosus	96-h LC50	0.24	Fragasova (1997)
Pristina leidyi	96-h LC50	30.8	Smith et al. (1991)

[1]EC50 – median effect concentration
[2]LOEC – lowest observed effect concentration
[3]LC50 – median lethal concentration

Chronic toxicity in freshwater invertebrates has only been assessed in two zooplankton species: *Daphnia magna* and *Ceriodaphnia dubia* (Table 1.1). Beusen and Neven (1987) conducted a 23-day test with *D. magna* studying survival and reproductive endpoints. From this study, they concluded V caused toxicity by directly shortening the adult lifespan through mortality, rather than through the impairment of reproduction. Therefore, the induction of adult mortality could suggest that toxicity occurs through a direct action rather than chronic accumulation of V within the adult daphnid. Surviving daphnids reproduced similarly to the control daphnids (Beusen and Neven, 1987; Van Leeuwan et al., 1987). More recently, Puttaswamy and Liber (2012) generated 7-d IC50s ranging from 0.4 to 0.6 mg V/L for *C. dubia*. A 7-d *Hyalella azteca* exposure study conducted by Borgmann et al. (2005) in soft water reported an LC50 of 0.4 mg V/L, and a 14-d study by Lee et al. (2013) reported a LC50 of 1.2 mg V/L.

Vanadium toxicity to freshwater fish has been more thoroughly investigated than toxicity to invertebrates and algae, with the majority of V toxicity testing occurring in the 1980s (Table 1.2). These investigations differ in the V compounds tested, water quality parameters of test solutions, test procedures, exposure durations, and developmental stages of test organisms used. Furthermore, nominal concentrations were frequently reported rather than measured concentrations. A few studies have established V to be more toxic than some other metals, such as selenium, lithium, uranium and boron, in comparative 96-h exposures with five different species of fish (Hamilton, 1995; Hamilton and Buhl, 1997; Hamilton and Buhl, 1990). Relative to other metals, many studies have suggested that V is of moderate toxicity to freshwater fish.

Stendahl and Sprague (1982) researched the effects of water hardness and pH on V toxicity to *Oncorhynchus mykiss* and observed V to be most toxic at pH 7.7 compared to pH 5.5 or 6.6, regardless of water hardness. The observed toxicity differences at different pHs are now known to be attributed to the relative abundance of the toxic vanadate oxyanions ($H_2VO_4^-$). At pH 7.7, the $H_2VO_4^-$ anions are predicted to be the dominant V species, whereas HVO_4^{2-} species are expected to be dominant near pH 9 (Stendahl and Sprague, 1982; Giles et al., 1979). Stendahl and Sprague (1982) speculated that $H_2VO_4^-$ species are

slightly more toxic than HVO_4^{2-} due to the greater toxicity invoked at pH 7.7 rather than at pH 8.8; however, Holdway and Sprague (1979) reported a similar toxicity for both of these V(V) species. The estimated LC50s were highest at pH 6, which is likely due to the presence of decavanadates ($V_{10}O_{28}^{6-}$) at a more acidic pH. Decavanadates were found to exert less toxicity than the monomeric V(V) anion species (Crans et al., 1998; Stendahl and Sprague, 1982; Giles et al., 1979). Unlike pH, changes in water hardness did not significantly alter V toxicity to fish (Stendahl and Sprague, 1982). Often, increases in water hardness significantly reduce metal toxicity because of an increase in calcium (Ca^{2+}) cation competition for binding and uptake sites on gills. However, in oxidized waters V is present primarily as anion species, which are not expected to compete with Ca^{2+} cations.

Table 1.2. Toxicity of vanadium to freshwater fish.

Species	Endpoint, life stage	Effect concentration (mg/L)	Reference
Gasterosteus aculeatus	96-h LC50[1], adult	3.17	Gravenmier et al. (2004)
Catostomus latipinnis	96-h LC50, fry	11.5	Hamilton and Buhl (1997)
Ptychocheilus lucius	96-h LC50, fry	7.8	
	96-h LC50, juvenile	4.04	
Xyrauchen texanus	96-h LC50, fry	5.3	Hamilton (1995)
	96-h LC50, juvenile	3.35	
Gila elegans	96-h LC50, fry	8.8	
	96-h LC50, juvenile	3.46	
Oncorhynchus tshawytscha	96-h LC50, fry	16.5	Hamilton and Buhl 1990
Poecilia reticulata	96-h LC50	8	Beusen and Neven (1987)
Danio rerio	96-h LC50	4.0	
Salvelinus fontinalis	96-h LC50, yearling	7.0	
	96-h LC50, alevin	24.0	Ernst and Garside (1987)
	30-d LOEC[2], alevin survival	1.7	
Oncorhynchus mykiss	96-h LC50, juvenile	6.71	Stendahl and Sprague (1982)
	7-d LC50, fingerling	1.95	
	7-d LC50, juvenile	3.6	
Oncorhynchus mykiss	96-h LC50, eyed egg	118	Giles et al. (1982)
	96-h LC50, juvenile	11.4	
Oncorhynchus mykiss	96-h LC50, fingerling	6.4	Giles and Klaverkamp (1979)
	14-d LC50, fingerling	1.95	
Coregonus clupeaformis	96-h LC50, adult	17.4	
Jordanella floridae	96-h LC50, adult	11.2	Holdway and Sprague (1979)
	28-d LC50, fry	1.13	
Poecilia reticulate	6-d LC50	0.49	Knudston (1979)
Carassius auratus	6-d LC50	2.45	
Carassius auratus	7-d LC50, ELS[3] survival	0.46	Birge (1978)
Oncorhynchus mykiss	7-d LC50, ELS survival	0.17	Birge et al. (1980)

[1]LC50 – median lethal concentration

[2]LOEC – lowest observable effect concentration

[3]ELS – early life stage

1.5. Guidelines for the protection of aquatic life

In Canada, federal water quality guidelines (WQGs) are developed by the Canadian Council of the Ministers of the Environment (CCME) for the management and use of water resources. These guideline values are meant to apply to water quality outside of allowable effluent mixing zones, and establish contaminant concentrations below which adverse effects to aquatic biota are not expected. Their goal is to protect ecosystem function and structure within freshwater and marine surface waters. Recently, The Canadian Environmental Protection Agency (CEPA) put forth chronic guideline values for V in freshwaters and marine waters at 0.12 mg/L and 0.005 mg/L, respectively (Canada, 2016). However, this value only takes 8 chronic toxicity estimates into account and by-passes any kind of peer-review process. An interim provincial guideline set previously in Ontario was at 0.006 mg/L (MoEE, 1994). This value is based on the lowest-observable effect concentration of the most sensitive toxicity study using a 16.6 fold safety factor. Regardless, appropriately-derived, well-supported, site-specific guidelines for V would be advantageous to site managers in the AOS region in order to make informed decisions regarding the discharge and long-term management of accumulating by-products, as well as for the management of leachates and future effluent discharges that may contain elevated V concentrations.

In the past, the CCME employed what is now known as the Type B approach to derive WQGs for the protection of aquatic life (CCME, 1991). An important drawback to this approach is the use of only the most sensitive endpoint from a single toxicity study, usually the most sensitive study in a dataset, for a certain contaminant. An arbitrary safety factor of 10 was then applied to derive the guideline value. The safety factor is used to account for untested species sensitivity differences, different exposure conditions, and alternate test endpoints. Currently, this guideline derivation approach is only used when toxicological information is limited and thus inadequate for derivation using the preferred Type A (species sensitivity distribution) approach. If adequate toxicity data are not available for developing Type A guidelines, then interim guidelines are derived and implemented until more data become available.

The species sensitivity distribution (SSDs) approach (Type A guideline) is increasingly being incorporated into generation of WQGs and is now preferred by the CCME. This method is used when sufficient, high quality toxicity data are available (CCME, 2007b). Unlike the Type B approach, which relies on a single study with the most sensitive species tested, the SSD method includes all toxicity data from all species of all trophic levels representative of an aquatic ecosystem. Separate SSDs are generated for short (i.e., LC50s from acute tests) and long-term (i.e., EC10s from chronic tests) exposures. As a formal, statistical method, the SSD combines all of the available toxicity data into a cumulative distribution and interpolates the guideline value based on the protection of at least 95% of the species present. The contaminant concentration at this level is termed the hazardous concentration to 5% of species tested (HC5), which is estimated based on the best fit model to the data distribution, especially in the lower-left end of the SSD tail where the most sensitive species lie. The HC5 value is generally then established as the WQG. The statistical power of SSDs increases with increasing data points, especially from toxicity studies with different species (Duboudin et al., 2004; Newman et al., 2000; Wheeler et al., 2002). Therefore, prior to using this approach it is important to have a large number of toxicity studies covering a range of species. In order for SSDs to be used in WQG derivations, toxicity data from at least eight taxonomically different species are required (CCME, 2007b; Posthuma et al., 2002). Furthermore, the CCME requires high quality data from a minimum of three fish species, three aquatic invertebrate species, and one plant or algal species.

1.7. Study overview

Syncrude is currently facing the challenge of remediating OSPW toxicity prior to its eventual discharge into various receiving environments of the Athabasca Oil Sands region in northern Alberta. Currently, following a no-release practice, SCL stockpiles all OSPW on-site in large settling basins. Recent investigations of the percolation of OSPW through oil sands coke has shown that coke can significantly reduce acute OSPW toxicity through the removal of toxic organic acids. However, because of the elemental composition of coke, large increases in V have also been observed in coke-treated OSPW and

the toxicological data available on V toxicity were, until recently, inadequate for the derivation of sound WQGs for the protection of aquatic life (Puttaswamy et al., 2010; Zubot et al., 2012). Therefore, although the treatment of OSPW using coke could prove beneficial in the remediation of OSPW, the V released into coke-treated OSPW could be toxic to aquatic organisms downstream of future OSPW discharge sites. It is unclear at present which presents the greater ecotoxicological risk.

1.7.1 Study objectives

Elevated concentrations of aqueous V can affect important population endpoints, such as the survival, growth and reproduction of aquatic organisms, including fish, invertebrates and algae. Furthermore, a few species in the published V toxicity dataset would be considered representative of the aquatic ecosystems of northern Alberta. Therefore, the goal of this research was to evaluate the acute and chronic toxicity of V to a range of taxonomically diverse freshwater organisms that are representative of northern Alberta, where the Athabasca Oil Sands region is located. The data obtained from this research will be used to assess the ecological risks that V in SCL coke leachates could pose to various receiving environments of the Athabasca Oil Sands region and to aid in the eventual derivation of WQGs for V.

To achieve this research goal a select number of freshwater organisms, including both commonly-used laboratory test species and comparable, ideally field-collected species that are relevant, or representative, of northern Alberta were exposed to aqueous V in acute and chronic laboratory toxicity tests. The data collected from these tests was then combined with toxicity data present in the peer-reviewed literature to develop acute and chronic SSDs. Standard laboratory species were also compared to their comparable, regionally-relevant test species to determine if they differ in terms of sensitivity to V. From the acute and chronic SSDs, the hazardous concentration of V to 5% (HC5) of the species tested were derived. Toxicity data used to derive these HC5s will be useful to regulators to aid in the development of site-specific water quality guidelines for the protection of aquatic life for V in the Athabasca Oil Sands region.

Overall, this research included an assessment of the following:

i.   The effects of V on the growth of *Pseudokirchneriella subcapitata* and a comparable green algal species that is representative of northern Alberta (Chapter 2)

  H_O: There are no differences in growth between a standard laboratory green algae test-species (Pseudokirchneriella subcapitata) and an algae species representative of the AOS region exposed to V.

ii. The effects of V on the survival and reproduction of *Daphnia pulex* and a comparable field-collected zooplankton species obtained from a reference site in northern Alberta (Chapter 2)

  H_O: There are no differences in the survival and reproduction of a standard laboratory zooplankton test-species (Daphnia pulex) and field-collected zooplankton from the AOS region exposed acutely and chronically to V.

iii. The effects of V on the survival, growth and emergence of *Chironomus dilutus* and a comparable field-collected, or representative, macroinvertebrate species of northern Alberta (Chapter 3)

  H_O: There are no differences in the survival, growth and emergence of a standard laboratory benthic invertebrate (Chironomus dilutus) and a field-collected benthic invertebrate from the AOS region exposed acutely and chronically to V.

iv. The effects of V on the survival and growth of larval rainbow trout (*Oncorhynchus mykiss*) and larval fathead minnow (*Pimephales promelas*), the latter of which is native to the Athabasca River (Chapter 3)

  H_O: There are no differences in the survival and growth of a standard laboratory freshwater fish test species and a freshwater fish species representative of the Athabasca River exposed acutely and chronically to V.

v.   Species sensitivity distributions developed with toxicity data generated here and data published in the peer-reviewed literature and derive short-term and long-term hazardous concentrations to 5% of the test species (HC_5) (Chapter 3)

  H_O: Inclusion of regionally-relevant aquatic species (i.e. AOS region) in the larger V toxicity database will not significantly change the estimated short-term and long-term HC_5 values.

CHAPTER 2

TOXICTY OF AQUEOUS VANADIUM TO ZOOPLANKTON AND PHYTOPLANKTON SPECIES OF RELEVANCE TO THE ATHABASCA OIL SANDS REGION

2.1. Preface

This chapter describes the results of a laboratory investigation comparing the acute and chronic toxicity of dissolved vanadium (as vanadate oxyanions) to planktonic organisms that are either commonly-used laboratory species, or species more regionally-relevant to northern Alberta. Vanadium (V) is an abundant trace metal present in bitumen from the Athabasca Oil Sands (AOS) region of Alberta, Canada, and in the upgrading by-product, petroleum coke. Past studies have shown coke to leach ecotoxicologically relevant concentrations of V upon contact with water, including oil sands process-affected water. Therefore, a suite of acute and chronic toxicity tests using four cladoceran and two algal species were conducted to address toxicity data gaps for this metal and for use in the development of future water quality benchmarks specific to the AOS region. In addition to using standard test-species, the focus extended to include species of relevance to the AOS region. Overall, only small differences in V sensitivity between standard and regionally-relevant planktonic test species were observed. Regardless, inclusion of more regionally-relevant species will strengthen the toxicity database for V and allow for appropriate water quality benchmarks for use in the AOS region.

This chapter has been accepted to *Ecotoxicology and Environmental Safety* under joint authorship with Karsten Liber (University of Saskatchewan).

2.2. Abstract

Vanadium (V) is an abundant trace metal present in bitumen from the Athabasca Oil Sands (AOS) region in Alberta, Canada. The upgrading of bitumen can result in the production of large volumes of a carbonaceous material referred to as petroleum coke that contains V at elevated levels compared to the native bitumen. Previous studies have shown that coke has the capacity to leach ecotoxicologically relevant levels of V into water it contacts, yet limited data are available on the toxicity of aqueous V to planktonic organisms. Therefore, this study set out to evaluate the acute and chronic toxicity of V (as vanadate oxyanions) to freshwater zooplankton and phytoplankton species that are either commonly-used laboratory species, or species more regionally-representative of northern Alberta. Four cladoceran (2-d and 21-d tests) and two algal (3-d tests) species were exposed to V to obtain both acute and chronic toxicity estimates. Acute V toxicity (LC50s) ranged from 0.60 mg V/L for *Ceriodaphnia quadrangula* to 2.17 mg V/L for *Daphnia pulex*. Chronic toxicity estimates (EC50s) for cladoceran survival and reproduction were nearly identical within species and ranged from a low of 0.13 to a high of 0.46 mg V/L for *Daphnia dentifera* and *D. pulex*, respectively. The lack of sublethal toxicity in daphnia suggests a direct mechanism of toxicity through ion imbalance. Growth inhibition (EC50) of green algae occurred at concentrations of 3.24 and 4.12 mg V/L for *Pseudokirchneriella subcapitata* and *Scenedesmus quadricauda*, respectively. Overall, cladocerans were more sensitive to V than green algae, with survival of the field-collected *D. dentifera* being approximately 2.5 to 3 times more sensitive to acute and chronic V exposure than the standard test species *D. pulex*. However, there were no significant differences in V toxicity between the field-collected cladocerans *Simocephalus serrulatus* and *C. quadrangula*, compared with the respective standard species *D. pulex* and *Ceriodaphnia dubia*. Similarly, there were no significant differences in sensitivity to V in the two algal species evaluated. Based on V concentrations reported in laboratory-generated coke leachates, zooplankton survival could be adversely impacted under conditions of chronic leachate exposure if V concentrations in the environment exceed 0.1 mg/L. Furthermore, toxicity thresholds from commonly-used planktonic test species would likely have sufficed for derivation of a V water quality guideline (WQG) for

protection of local aquatic communities near oil sands operations, but the new data on V toxicity to more regionally-representative species will strengthen the database for WQG derivation.

2.3. Introduction

The Canadian oil sands, located in northern Alberta, rank third globally in terms of proven oil reserves, following only behind Saudi Arabia and Venezuela (Allen, 2008; National Energy Board, 2013). Within this area, the Athabasca Oil Sands (AOS) region is the largest of three bitumen deposits in Alberta and has been estimated to contain 170 billion barrels of bitumen, a viscous hydrocarbon (Allen, 2008).The Alberta Energy Regulator (AER) predicts continued growth in bitumen production over the next decade (AER, 2015). The bitumen extraction and upgrading processes used by some oil sands companies produce large volumes of liquid and solid by-products, predominately in the form of oil sands process-affected water (OSPW) and petroleum coke (Allen, 2008; AER, 2015; Scott and Fedorak, 2004). Following a no-release practice, substantial amounts of these products have been accumulating on-site of some oil sands companies and their eventual reclamation and/or utilization are of importance to the industry.

Syncrude Canada Ltd. (SCL) upgrades bitumen to a lighter more valuable synthetic crude oil through the operation of three fluid cokers. Fluid coking uses high temperatures to increase the hydrogen to carbon (H:C) ratio of bitumen through the removal of C (Zubot et al., 2012). Annually, SCL can generate up to 2 million tons of coke, with an overall coke production across the entire oil sands industry that reached 90 million tons at the end of 2014 (AER, 2015; Fedorak and Coy, 2006; Scott and Fedorak, 2004). Currently, operators are required to store petroleum coke on-site because it is viewed as a potential future energy resource, similar to medium grade coal (Baker, 2007; Fedorak and Coy, 2006). However, coke impurities such as high sulfur content (6-7% wt.), low combustibility and availability of cheaper energy sources, prevent its current use (Furimsky, 1998; Scott and Fedorak, 2004; Small et al., 2012a). With the vast volumes of coke being produced, some companies may eventually face challenges with available storage capacity. Initially, oil sands coke was thought to be biologically inert, however, recent studies have found coke to affect microbial processes and to leach certain metals (e.g., V, Ni, Cu, Mn, and Mo) when it

is exposed to water and weathering processes (Fedorak and Coy, 2006; Puttaswamy et al., 2010; Squires, 2005).

Treatment approaches to mitigate OSPW toxicity have been directed at removing the bitumen-derived organic fractions, including naphthenic acids (NAs) and other acid-extractable organic compounds that are mostly responsible for toxicity. Chemical oxidation of NAs in OSPW by ozone effectively removes up to 95% of the NAs and attenuates OSPW toxicity to *Vibrio fischeri* and *Chironomus dilutus*, as well as reduces steroidogenic effects in human cell lines (Anderson et al., 2012; Gamal El-Din et al., 2011; He et al., 2010; Scott et al., 2008). However, full-scale treatment of the approximately 1 billion m^3 of OSPW stored on-site at the AOS may be impractical (Scott et al., 2008). Consequently, activated carbon (AC) has also been investigated as a treatment option for OSPW, but it too would be expensive to obtain and use on an industrial scale of this magnitude (Small et al., 2012a, b). Therefore, petroleum coke, which is high in carbon (> 80% wt.) and available in large supply on-site, has been proposed as an option to treat OSPW in an engineered facility (Small et al., 2012a, b; Zubot et al., 2012). In appropriate applications, petroleum coke can remove complex organic compounds with higher molecular weight and low solubility from OSPW resulting in a reduction in acute toxicity over a shorter time frame than untreated OSPW (Gamal El-Din et al., 2011; Pourrezaei et al., 2014a, b; Small et al., 2012a, b; Zubot et al., 2012).

While most metals do not appreciably leach into water that is in contact with coke, increased concentrations of V have been observed in coke leachates and coke-treated OSPW (Small et al., 2012a, b; Puttaswamy et al., 2010; Squires, 2005). However, Zubot (2011) reported that leached V will decline with prolonged coke-water residence times due to predicted adsorption of V onto metal hydroxides that will be present within saturated coke deposits. The V leached from coke is primarily V(V), which is favored under the more alkaline pH conditions of OSPW (pH 8-9). The presence of V in petroleum coke is not surprising given it is a highly abundant trace metal in crude oil where it forms organic porphyrin complexes. Bitumen from the AOS contains one of the greatest concentrations of V in the world, an estimated 220 mg/kg (Moskalyk and Alfantazi, 2003; Ventura et al., 2015; Zubot et al., 2012). When bitumen is upgraded, V is

removed and enriched into coke at concentrations of up to 1226 mg/kg, therefore making it the most elevated metal in coke (Zubot et al., 2012). Several studies have documented the leaching of coke upon contact with water, potentially generating ecotoxicologically relevant V concentrations (Puttaswamy et al., 2010; Puttaswamy and Liber, 2011; Small et al., 2012b; Squires, 2005; Zubot et al., 2012). For example, V can be released into leachates at concentrations greater than the 7-d median lethal concentration (LC50) for the aquatic invertebrate, *Ceriodaphnia dubia* (Puttaswamy et al., 2010). In addition, the amount of V that leached from coke in an 18-month lysimeter study resulted in V concentrations > 1000 µg/L (Puttaswamy et al., 2010). Typically, median background levels of aqueous V in freshwater ecosystems are reported within the range of 0.5 to 2.4 µg/L (Hirayama and Kageyama, 1992; Rehder, 1991).

The release of V from petroleum coke suggests that coke leachates could expose aquatic ecosystems of northern Alberta to relatively high concentrations of V, depending on how the coke leachate is generated, stored and handled prior to discharge. However, the limited V toxicity data available in the peer-reviewed literature presently prevents the development of appropriate water quality guidelines (WQGs) for the protection of aquatic life. Furthermore, future development of site-specific guidelines or objectives for V will become a priority if oil sands companies want to discharge process water containing V into the natural aquatic ecosystems of northern Alberta. To address this data gap, this study set out to characterize the acute and chronic toxicity of V to freshwater planktonic organisms for which limited data were available. In addition, the study aimed to determine whether species more regionally representative of northern Alberta were more or less sensitive to V than more conventional (standard) test species. These data will be useful to assess the ecological risks that V in Syncrude coke leachates would pose to the biota in receiving environments of the AOS region.

2.4. Materials and methods

2.4.1. Source and culturing of test organisms

Standard test organisms, *Daphnia pulex* and *Pseudokirchneriella subcapitata*, were obtained from in-house cultures maintained at the Toxicology Centre, University of Saskatchewan (U of S), Saskatoon,

SK, Canada. Culturing procedures followed general guidelines described elsewhere (Environment Canada, 1990a; Environment Canada, 1992a). Daphnids were maintained in groups of 25 to 30 adults in 2-L glass jars in environmental chambers at $23 \pm 1°C$ under a 16:8 h light:dark photoperiod. Water renewals occurred three times weekly and daphnids were fed a daily diet of green algae, *P. subcapitata*. Regionally-relevant test species were either collected from a reference pond on Syncrude's mine site or were purchased based on species most representative of the region. Field-collected zooplankton from Syncrude's Test Pond 1 included *Daphnia dentifera, Simocephalus serrulatus* and *Ceriodaphnia quadrangula*. The green algae, *Scenedesmus quadricauda*, was identified as a regionally-representative green algal species based on its reported presence and abundance in a local reference lake, Mildred Lake (Hayes, 2006). This alga was purchased from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo, ON, Canada. Stock cultures were incubated on a rotary shaker at 100 rpm in an environmental chamber at $24 \pm 1°C$ under continuous lighting (3000-4000 lux) and maintained in log-phase growth in 1-L Erlenmeyer flasks containing 250 ml of axenic nutrient media (Environment Canada, 1992a).

2.4.2. Test material and dilution water

The test chemical, sodium metavanadate ($NaVO_3$, anhydrous, min. 96% pure), was obtained from Strem Chemicals Inc., Newburyport, MA, USA. Concentrated stock solutions were prepared for toxicity tests by dissolving sodium metavanadate in ultrapure water (Barnstead NANOpure, 18.2 MΩ-cm) in 1-L, acid-washed, glass volumetric flasks. Stock solutions were then diluted with appropriate volumes of dilution water to achieve the target V concentrations (modeled as $H_2VO_4^-$) for the different treatments.

The dilution water used for all toxicity tests was reconstituted water prepared to mimic the general characteristics of the Athabasca River (ARW) in terms of conductivity, hardness, and alkalinity, based on field data from various local sampling sites generated by the Regional Aquatics Monitoring Program (RAMP, 2011). To achieve target values, carbon-filtered, bio-filtered municipal water from the City of Saskatoon, was diluted with approximately 30% reverse osmosis (RO) water. This water was then aerated for a minimum of 24 h prior to initiating toxicity tests. All test organisms were acclimated and cultured in

reconstituted ARW prior to testing. General water quality characteristics of ARW, reconstituted ARW and solutions for each test are provided in Table 2.1.

2.4.3. Collection and culturing of field test organisms

Zooplankton species were collected in June 2013 from Test Pond 1, a reference pond on Syncrude's Mildred Lake mine site, located north of Fort McMurray, AB, Canada. Species were collected from both the littoral and open water zone. Prior to zooplankton collection, site water was collected and filtered through a 53-μm mesh into a 50-L carboy for subsequent use in initial culturing. Zooplankton collection was performed with a zooplankton net and captured organisms transferred to a sorting tray containing filtered site water. Individual organisms were then pipetted from the sorting tray into 2-L plastic containers, filled with filtered site water. Approximately 200 individuals were placed in each of five 2-L containers. Zooplankton in each container was fed with approximately 10 ml of *P. subcapitata* and a few drops of Nutrafin® (Rolf C. Hagen Inc., Montreal, QC, Canada) prior to transport back to the U of S Toxicology Centre in coolers.

Separate water samples were collected in triplicate from Test Pond 1 for water quality characterization (Table 2.1), and for analysis of metals, major ions and polycyclic aromatic hydrocarbons (PAHs) to verify that this was in fact a reference site. Seventeen priority PAHs (CCME, 1999) were measured by ALS Laboratories, Saskatoon, SK; all were found to be below the limit of detection (< 0.05 μg/L). Major ion concentrations were as follows (mean $\pm$ SD in mg/L; n=3): chloride 4.9 $\pm$ 0.1; sulphate 204.5 $\pm$ 0.6; sodium 62.6 $\pm$ 0.1; potassium 3.4 $\pm$ 0.02; magnesium 29.8 $\pm$ 0.1; and calcium 58.7 $\pm$ 0.1. The concentration of V in the pond water was measured at 0.42 $\pm$ 0.03 μg/L (n=3). All other measured metals were present at concentrations below Canadian water quality guidelines for the protection of aquatic life (mean $\pm$ SD in μg/L, n=3: B 154.8 $\pm$ 4.9; Fe 14.0 $\pm$ 5.9; Ni 1.9 $\pm$ 0.0; Cu 1.1 $\pm$ 0.3; Zn 1.9 $\pm$ 1.0; As 2.5 $\pm$ 0.2; Mo 0.4 $\pm$ 0.1; Cd 0.03 $\pm$ 0.0; Pb 0.1 $\pm$ 0.0; and U 0.5 $\pm$ 0.1) (CCME, 2006).

At the Toxicology Centre, different zooplankton species were isolated and cultured in 2-L glass jars containing surface water from where they were collected. Culturing conditions were similar to those required for *D. pulex* (Environment Canada, 1990a). The field-collected species were gradually acclimated from Test Pond 1 water (Table 1) to reconstituted ARW conditions over a four week period. All field-collected species were cultured under static-renewal conditions at 23 ± 1°C and an 18:6 h light:dark photoperiod. In order to ensure healthy individuals for toxicity tests, 40 individuals of each species were maintained individually in 30-ml beakers until they had produced a minimum of three healthy broods. Neonates from healthy individuals were then used to start new cultures that were maintained for a minimum of two generations in reconstituted ARW prior to use of neonates in toxicity tests. *Daphnia dentifera* and *S. serrulatus* were fed daily with a mixture of *P. subcapitata* and *Chlamydomonas reinhardtii* at a 1:1 ratio. *Ceriodaphnia quadrangula* was fed daily with a mixture of *P. subcapitata* and *C. reinhardtii* at a 2:1 ratio, and a yeast, cerophyll, and trout chow (YCT) concoction. Their culture media was renewed every other day, rather than three times weekly, as done for the other daphnids. Independent confirmation of cladoceran taxonomy was performed by John Bailey and Lynne Witty from Laurentian University, ON, Canada.

2.4.4. Toxicity testing

All acute and chronic toxicity tests with standard zooplankton species were conducted in controlled environmental chambers at 23 ± 1°C under a 16:8 h light:dark photoperiod. Field-collected zooplankton were also maintained at 23 ± 1°C, but under an 18:6 h light:dark photoperiod. Tests with green algae were conducted at the same temperature, but under continuous lighting. Treatment groups for zooplankton consisted of an untreated control and five geometrically increasing concentrations of V. Algal tests consisted of an untreated control and six geometrically increasing V concentrations chosen to provide a concentration-response effect.

2.4.4.1. Acute lethality tests with zooplankton

Acute lethality tests were conducted with *D. pulex* and the three field-collected zooplankton species, *Daphnia dentifera*, *Simocephalus serrulatus* and *Ceriodaphnia quadrangula*. All tests followed general guidelines established by Environment Canada (1990a). Tests were static, performed in 250-ml acid washed, glass beakers and were of 48-h duration without feeding. Trace amounts of vitamin B12 and selenium were added to all test solutions at the start of every test. Each test was conducted with five replicates of 10 neonates per beaker ($\leq$ 24 h old) placed in either 200 ml of control or test solution. Mortality, defined as lack of movement following gentle prodding, was recorded at 48 h. A test was considered acceptable if greater than 90% of control organisms survived.

Water samples (20 ml) were collected at the start and end of each test period from three randomly chosen beakers per treatment for analysis of routine water quality: dissolved oxygen (DO), temperature, pH, conductivity, total hardness and alkalinity. Samples for vanadium analysis were also collected at the start and end of each test to quantify actual exposure concentrations. All water samples (5 ml) were collected from three randomly chosen beakers in each treatment group, filtered through a 0.45-µm polyethersulfone membrane (VWR International, Mississauga, ON, Canada), placed into pre-cleaned 8-ml Nalgene bottles, and acidified with 145 µl of high purity nitric acid (HNO_3) to a pH of $\leq$ 2.

2.4.4.2. Chronic survival and reproduction tests with zooplankton

Two chronic 21-d tests were performed using *D. pulex* and *D. dentifera*, and one 8-d test was performed with *C. quadrangula*, following guidelines described in ASTM (1997), OECD (2008), and Environment Canada (2007). Tests were static-renewal with 10 replicates of individual neonates. Daphnid neonates ($\leq$ 24 h old) from the third brood were placed in 80 ml of control or test solution in 100-ml glass beakers, whereas *C. quadrangula* ($\leq$ 24 h old) from the third brood were placed in 25 ml of solution in 30-ml glass beakers. Full water renewals were performed every 48 h for the *Daphnia* species and every 24 h for *Ceriodaphnia*. At each water renewal, trace amounts of vitamin B12 and selenium were added to all test solutions. The daphnia were fed green algae daily (*P. subcapitata* and *C. reinhardtii*) at a 1:1 ratio, and

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C. quadrangula was also fed YCT, in addition to the algal mixture. Each day, daphnids were checked for mortality and reproductive output (presence of offspring). When neonates were present, they were counted and discarded. The *Ceriodaphnia* test was considered acceptable if 80% of control organisms survived and 60% of surviving adults produced at least three broods with a total of at least 15 neonates per adult. The *Daphnia* tests were considered acceptable if survival in the controls was $\geq$ 80%.

Temperature and DO were monitored in control, low, medium and high treatment groups before and after each water renewal. Triplicate water samples (20 ml) were collected at the start and end of each test period from three randomly chosen beakers per treatment, as well as weekly, to measure pH, conductivity, total hardness and alkalinity throughout the chronic exposures in both old and new test solutions. Samples for V (5 ml) were taken at the start and end of each experiment, and periodically throughout all exposure durations to document actual exposure concentrations.

2.4.4.3. Growth inhibition tests with green algae

The *P. subcapitata* and *S. quadricauda* growth inhibition tests were conducted for 72 h following the guidelines of Environment Canada (1992a), with slight deviations. The nutrient spike used in toxicity tests with both species was prepared with 13.75 times the concentration of the nutrient stock solutions used to make the culture media described in Environment Canada (1992a), but reducing the amount of Na$_2$EDTA by 75% to minimize complexation of metal. Prior to testing, all glassware and reconstituted ARW used to prepare control and V test concentrations were autoclaved. Tests were performed in 10-ml glass beakers with 5 replicates per treatment to provide adequate water volume for water quality characterization and V analysis. Algal inoculum, obtained from exponentially growing stock cultures, was prepared by centrifuging the liquid stock culture for 10 minutes at 270 x *g*. The supernatant was then discarded and the algae re-suspended in 15 mg/L of sodium bicarbonate solution, centrifuged once more, discarded and re-suspended in reconstituted ARW to a final cell concentration of approximately 220 000 cells/ml. From this, 0.25 ml of algae inoculum was added to each 10-ml glass beaker containing 5 ml of either control or test solution and 0.25 ml of nutrient spike. Beakers were covered with parafilm [TM] (VWR

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International, Mississauga, ON, Canada) and placed on a rotary shaker (Orbital Shaker, Standard 3500, VWR International, Mississauga, ON, Canada) in an environmental chamber for the duration of each test. After 72 h, test solutions were homogenized via swirling and a 10 µl sample removed for cell enumeration using a light microscope and haemocytometer. Unlike *P. subcapitata, S. quadricauda* cells tend to form 2 to 8 celled colonies, however, each cell was counted individually. Tests were considered acceptable since cell concentrations of the controls increased at least 16 times during 3 days. The measurement endpoint was number of algal cells per ml and was used to calculate cell population growth rates and percent inhibition at each V concentration based on the following equation (Eq. 1):

$$\%I = \frac{\mu_c - \mu_t}{\mu_c} \times 100 \qquad (1)$$

where µ is the average specific growth rate based on cell growth at time (T) = 0 and T = 72 of treatments (t) relative to the control (c). Triplicate sub-samples of 1 ml were collected from the control and each treatment for V analysis at the start and end of the 72-h exposure period. To standardize test volumes at the start the test, 1 ml was removed from the remaining replicates. Three replicates of four mock test solutions (control, low, medium and high) were also prepared at test initiation for routine water chemistry: DO, temperature, conductivity, pH, total hardness and alkalinity. Samples for V (1 ml) analysis were taken prior to cell enumeration from three replicates. After algal cell enumeration, replicates of the control and each treatment were pooled for water chemistry analysis at the end of the test.

2.4.5. Chemical analyses

Dissolved concentrations of V in test waters from all toxicity tests were quantified in-house using an inductively coupled plasma mass-spectrometer (ICP-MS) equipped with collision cell technology (X-Series II ICP-MS, Thermo Electron Ltd, Mississauga, ON, Canada). To evaluate the accuracy of ICP-MS measurements, a river water standard reference material (SLRS-5) and a natural water standard reference material (1640) were purchased from the National Research Council (Ottawa, ON, Canada) and the National Institute of Standards & Technology (Gaithersburg, MD, USA), respectively. The instrumental

limit of quantification for V was ≤ 0.05 µg/L. Procedural blanks and sample duplicates were included with each analytical run to further ensure accuracy and precision. Reproducibility of reference values (SLRS-5 and 1640) and precision of duplicates, exceeded 85% in all cases. Measurements of major cations (Ca, K, Mg, Na) and major anions (Cl and SO_4) were performed in-house using ion chromotagraphy (Dionex ICS-3000, Sunnyvale, CA, USA) on select samples collected at the beginning and end of the chronic *D. dentifera* test and at the beginning and end of the *P. subcapitata* test. In addition, 20 ml water samples were collected from the control and low, medium and high test concentrations in the chronic *D. dentifera* test, filtered through 0.45-µm polyesthersulfone membranes and analyzed for dissolved organic carbon (DOC) content using a TOC analyser (TOC-V CPN model 5000, Shimanzu Co., Kyoto, Japan) for descriptive purposes.

2.4.6. Statistical analysis and V speciation modeling

All LC50 estimates and associated 95% confidence intervals were calculated using either the trimmed Spearman-Karber method, version 1.5 (US EPA, 1990), or the probit method based on mean measured V concentrations (Hamilton et al., 1977). Differences in toxicity response between standard and regionally-representative species were considered significant if there was no overlap of the 95% confidence intervals. Reproduction (21 d, 7-8 d) and growth (72 h) data were used to estimate IC50 values using the Inhibition Concentration (ICp) approach, version 2.0 (US EPA, 1993; Norberg-King, 1993). Again, significant differences between endpoints were assessed based on non-overlapping 95% confidence limits. Dissolved chemical species of V were modelled using the program Visual MINTEQ, version 3.1 (KTH, Department of Land and Water Resources, Stockholm, Sweden) based on the mean measured concentrations of dissolved V at effect concentrations and major ions, and general water quality data (Table 2.1). All other statistical analyses were performed using the computer program SigmaPlot[®], version 11.0 (San Jose, CA, USA) with a 95% ($p \leq 0.05$) level of confidence. Significant differences between treatment groups and the control were determined using one-way analysis of variance (ANOVA) followed

by a Dunnett's multiple comparison test. In the case of non-normally distributed data, a Kruskal-Wallis ANOVA followed by the Dunn's post-hoc test was used.

2.5. Results

2.5.1. Exposure conditions

Similar water quality (i.e., conductivity, pH, hardness, and alkalinity; Table 1) of Test Pond 1 and synthetic ARW facilitated successful acclimation of field-collected zooplankton to appropriate test conditions. Furthermore, dissolved V concentrations measured in Test Pond 1 were low (0.42 ± 0.03 µg/L; n=3), did not differ from background concentrations in the Athabasca River (0.4 µg/L), and were within ranges typically reported for aquatic systems in the region (Hamada, 1998; RAMP, 2011). Experimentally, water quality parameters were similar across treatments in all acute and chronic zooplankton and algal tests, and remained within recommended ranges (Environment Canada, 1990a; Environment Canada, 1992a; Environment Canada, 2007) and within reasonable approximation of ARW characteristics (Table 1). Most major ions varied little among the different tests and averaged (mean $\pm$ SD in mg/L; n=12): (i) chloride 7.8 ± 0.2; sulphate 33.1 ± 0.7; sodium 19.3 ± 0.4; potassium 2.0 ± 0.0; magnesium 12.4 ± 0.2 and calcium 31.4 ± 0.6 in the zooplankton tests; and (ii) chloride 12.1 ± 0.3; sulphate 36.4 ± 0.7; sodium 28.4 ± 1.5; potassium 2.3 ± 0.1; magnesium 13.3 ± 0.3; and calcium 27.0 ± 0.5 in the algal tests. The only parameter that changed significantly from the controls was sodium, which increased slightly due to the use of $NaVO_3$ as the source of V. Based on the general water quality recorded for each test (Table 2.1) and major ion concentrations, the pH-dependent monomeric V(V) oxyanions (HVO_4^{2-} and $H_2VO_4^-$) were the dominant species accounting for 98.9-99.9% of dissolved V. When pH ranged from 8.2 to 8.4, $H_2VO_4^-$ represented 64-72% of species, whereas HVO_4^{2-} represented approximately 26-30%. However, an increase in pH to 8.6, as observed in the *P. subcapitata* test, resulted in a modeled increase in the HVO_4^{2-} anion to 45% and a decrease in $H_2VO_4^-$ to 54%.

2.5.2. Acute toxicity of vanadium to zooplankton

Vanadium displayed acute toxicity to *D. pulex, D. dentifera, S. serrulatus* and *C. quadrangula* in the low mg/L range (Figure 2.1), with 48-h LC50s listed in Table 2.2. The concentrations of V that resulted in 100% mortality for all species ranged from 1.3 to 8.2 mg V/L. The 48-h LC50s ranged from 0.60 to 2.17 mg/L for *C. quadrangula* and *D. pulex*, respectively. All four species displayed similar concentration-response slopes.

Small differences in V sensitivity were observed for the four zooplankton species evaluated (Figure 2.1). The commonly-used laboratory test species, *D. pulex*, was the most tolerant species although its 48-h LC50 value did not differ significantly from that of the field-collected species, *S. serrulatus*. The 48-h LC50 values for *D. pulex* and *S. serrulatus* were 2.17 and 1.72 mg V/L, respectively. However, the field-collected *D. dentifera* was significantly more sensitive to V than both *D. pulex* and *S. serrulatus*, with a 48-h LC50 value of 0.88 mg V/L. Based on this LC50, *D. dentifera* is approximately 2.5 times more sensitive to V than *D. pulex*. The most sensitive zooplankton species tested was the field-collected *C. quadrangula*. Its 48-h LC50 of 0.60 mg V/L was lower than that estimated for *C. dubia* (0.97 mg V/L) based on raw data from Puttaswamy et al. (2010).

Table 2.1. General water quality characteristics of Test Pond 1 water, Athabasca River water, reconstituted Athabasca River water, and test solutions from all V toxicity tests. Data are mean ± SD calculated over all relevant test periods except for Athabasca River water which are ranges reported by RAMP (2011).

Water source or water from a specific test	DO[1] (mg/L)	Temperature (°C)	Conductivity (µS/cm)	pH	Alkalinity (mg/L as CaCO$_3$)	Total hardness (mg/L as CaCO$_3$)	DOC[2] (mg/L)
Syncrude Test Pond 1	8.4 ± 0.1	22.9 ± 0.2	656 ± 1	8.1 ± 0.0	176 ± 2	247 ± 1	
Athabasca River Water			250-290		85-110	90-120	
Reconstituted ARW[3]			287 ± 3		101 ± 1	110 ± 2	
Algae							
Pseudokirchneriella subcapitata[4]	8.4 ± 0.1	23.7 ± 0.7	317 ± 22	8.6 ± 0.1	87 ± 6	110 ± 6	
Scenedesmus quadricauda[4]	8.3 ± 0.1	23.4 ± 1.0	337 ± 12	8.4 ± 0.1	91 ± 3	111 ± 3	
Zooplankton							
Daphnia pulex[4]	8.4 ± 0.0	22.7 ± 0.0	311 ± 20	8.3 ± 0.1	98 ± 2	108 ± 2	1.8 ± 0.1
Daphnia pulex[5]	8.50 ± 0.2	23.1 ± 0.3	287 ± 7	8.3 ± 0.1	96 ± 5	114 ± 5	1.6 ± 0.2
Daphnia dentifera[4]	8.4 ± 0.0	23.5 ± 0.4	282 ± 2	8.2 ± 0.1	92 ± 1	109 ± 0	
Daphnia dentifera[5]	8.6 ± 0.2	23.2 ± 0.3	287 ± 7	8.3 ± 0.1	94 ± 10	113 ± 6	
Simocephalus serrulatus[4]	8.2 ± 0.0	23.7 ± 0.3	275 ± 3	8.2 ± 0.0	87 ± 2	100 ± 5	
Ceriodaphnia quadrangula[4]	8.4 ± 0.0	23.7 ± 0.5	280 ± 2	8.2 ± 0.1	93 ± 1	109 ± 1	
Ceriodaphnia quadrangula[6]	8.8 ± 0.3	23.3 ± 0.3	285 ± 14	8.2 ± 0.0	81 ± 5	108 ± 5	

[1] DO = dissolved oxygen
[2] DOC = dissolved organic carbon (mean ± SD; $n = 12$)
[3] Reconstituted ARW (mean ± SD; $n = 3$)
[4] Acute 2-d zooplankton toxicity tests ($n = 36$) and chronic 3-d algal tests ($n = 8$); measured in triplicates at test start and end of test.
[5] Chronic 21-d zooplankton toxicity tests ($n = 60$-132); measured in triplicate at test start, weekly throughout the test period, and at test completion.
[6] Chronic 8-d zooplankton toxicity test ($n = 66$); measured in triplicate at test initiation, once throughout test, and at test completion
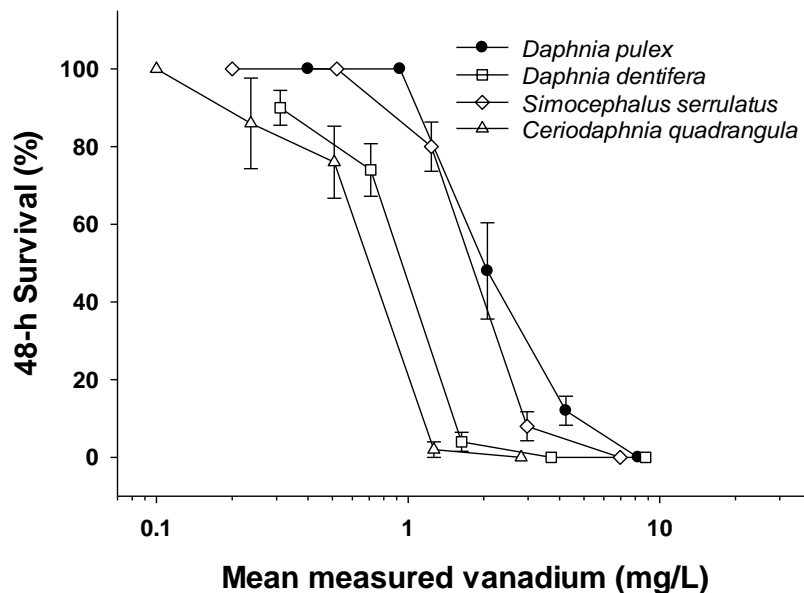
Figure 2.1. Acute toxicity of vanadium to the standard laboratory test species, *D. pulex*, and three field-collected zooplankton species, *S. serrulatus*, *D. dentifera* and *C. quadrangula*. Each data point represents the percent survival (mean ± SE; *n* = 5) at each V concentration.

2.5.3. Chronic toxicity of vanadium to zooplankton

Mean survival in the control groups during the 21-d chronic toxicity tests with *D. pulex* and *D. dentifera* was 100% and 90%, respectively. Mean reproduction per surviving adult over the 21 days was 49 and 53 neonates for *D. pulex* and *D. dentifera*, respectively. The 21-d LC50 for *D. pulex* was 0.46 mg/L (Table 2.2). Interestingly, the 21-d LC50 and EC50 values for *D. pulex* were identical, suggesting that V caused no sublethal effects in terms of neonate production. However, the number of neonates per surviving adult was significantly reduced at V concentrations greater than the 21-d LC50 (Table 2.3). The two highest V treatments (1.35 and 2.72 mg/L) did not yield any neonates and 100% mortality occurred following four days of exposure. There were no significant differences in average adult survival and number of neonates of the two lowest V treatments (0.16 and 0.32 mg/L) relative to the control.

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As observed for the acute studies, the field-collected *D. dentifera* was slightly more sensitive to V during the chronic exposure than *D. pulex*, with a 21-d LC50 of 0.13 mg/L and an EC20 and EC50 of 0.08 and 0.13 mg/L, respectively (Table 2.2). Furthermore, 21-d LC50 and EC50 estimates for *D. dentifera* were identical (Figure 2.2a), an observation similar to that made for *D. pulex*. A 100% reduction in both survival and reproduction occurred at ≥ 0.22 mg V/L. There were no significant effects observed for any endpoint assessed at 0.05 and 0.10 mg V/L (Table 2.3).

Although *C. quadrangula* was the most sensitive species to V in the acute tests that was not found to be the case under chronic conditions (Table 2.2). However, the 8-d LC50 of 0.61 mg/L was similar to the 7-d LC50 of 0.50 mg/L reported in a previous study for *C. dubia* (Puttaswamy et al., 2010) (Figure 2.2b; Table 2.2). The EC20 and EC50 (reproductive impairment) for *C. quadrangula* were 0.44 and 0.58 mg V/L, respectively (Table 2.3). Reproduction was found to be significantly impaired at 0.4 mg V/L (reduced by 25.7%, Table 2.3).

Acute-to-chronic rations (ACRs) for cladocerans were calculated based on the acute LC50 and the chronic EC50 for the most sensitive endpoint (Table 2.2). The ACRs for the five cladoceran species ranged from 1.03 for *C. quadrangula* to 6.77 for *D. dentifera*.

Table 2.2. Acute and chronic toxicity data (mg/L) generated in the present study.[1]

Test Species	Acute 48-h LC50 (lethality)	Chronic test duration (days)	Chronic Endpoint	Chronic toxicity threshold			
				EC10	EC20	EC50	LC50
Daphnia pulex	2.17 (1.91, 2.45)	21	Reproduction	0.12 (0.06, 0.33)	0.35 (0.13, 0.36)	0.46 (0.43, 0.47)	0.46 (0.40, 0.47)
Daphnia dentifera	0.88 (0.77, 1.01)	21	Reproduction	0.05 (0.01, 0.11)	0.08 (0.07, 0.12)	0.13 (0.09, 0.16)	0.13 (0.12, 0.16)
Ceriodaphnia dubia[2]	0.97 (-)	7	Reproduction	0.28 (0.04, 0.34)	0.32 (0.10, 0.45)	0.45 (0.19, 0.64)	0.50 (0.38, 0.62)
Ceriodaphnia quadrangula	0.60 (0.52, 0.68)	8	Reproduction	0.28 (0.01, 0.47)	0.44 (0.03, 0.51)	0.58 (0.32, 0.65)	0.61 (-)
Simocephalus serrulatus	1.72 (1.53, 1.94)	NA	NA	NA	NA	NA	NA
Pseudokirchneriella subcapitata	NA	3	Growth	1.38 (0.55,1.78)	1.72 (0.99, 2.30)	3.69 (2.90, 4.29)	NA
Scenedesmus quadricauda	NA	3	Growth	0.27 (0.19, 1.75)	1.69 (0.35, 2.63)	4.12 (3.06, 4.85)	NA

[1]Numbers between parentheses represent the 95% confidence intervals (CI). If a CI is not reported, no reliable confidence intervals could be calculated due to a steep concentration-response curve.

[2] Raw data from Naveen Puttaswamy (unpublished)

NA = Not applicable

Table 2.3. Chronic effects (mean neonates per females, time to first brood, broods per female with standard deviations) in three zooplankton species exposed to different measured concentrations of V for 21 days (10 daphnids per concentration).

Species	Mean measured exposure (mg V/L)	Survival after 21-d (%)	Total number of neonates produced	Average number of young/ Surviving female	Time to first brood (days)	Broods per female
D. pulex	0.00	100	492	49.2 ± 4.7	8.0 ± 0.0	5.6 ± 0.7
	0.16	90	429	47.7 ± 4.4	8.1 ± 0.3	5.2 ± 0.4
	0.32	100	430	43.0 ± 4.1*	8.4 ± 0.5	5.2 ± 0.6
	0.63	10*	99*	10*	10.9 ± 2.3*	1.4 ± 0.5*
	1.34	0*	0*	0*	0*	0*
	2.72	0*	0*	0*	0*	0*
D. dentifera	0.00	90	541	59.3 ± 15.2	7.5 ± 1.3	6.3 ± 2.1
	0.05	90	526	56.1 ± 9.5	7.2 ± 1.3	6.3 ± 1.3
	0.10	80	473	53.5 ± 9.4	6.8 ± 0.8	6.0 ± 1.6
	0.22	0*	133*	0*	6.7 ± 0.8	2.6 ± 1.4*
	0.47	0*	0*	0*	0*	0*
	1.06	0*	0*	0*	0*	0*
C. quadrangula	0.00	100	380	38 ± 2.9	4.4 ± 0.5	3.0 ± 0.0
	0.05	100	374	37.4 ± 6.0	4.3 ± 0.5	3.2 ± 0.4
	0.10	100	365	36.5 ± 6.6	4.1 ± 0.3	3.0 ± 0.0
	0.21	100	347	34.7 ± 6.4	4.5 ± 0.7	3.0 ± 0.5
	0.40	100	282*	28.2 ± 4.7*	4.2 ± 0.4	3.0 ± 0.0
	0.83	0*	0*	0*	0*	0*

[*] Significantly different from the control ($p \leq 0.05$).
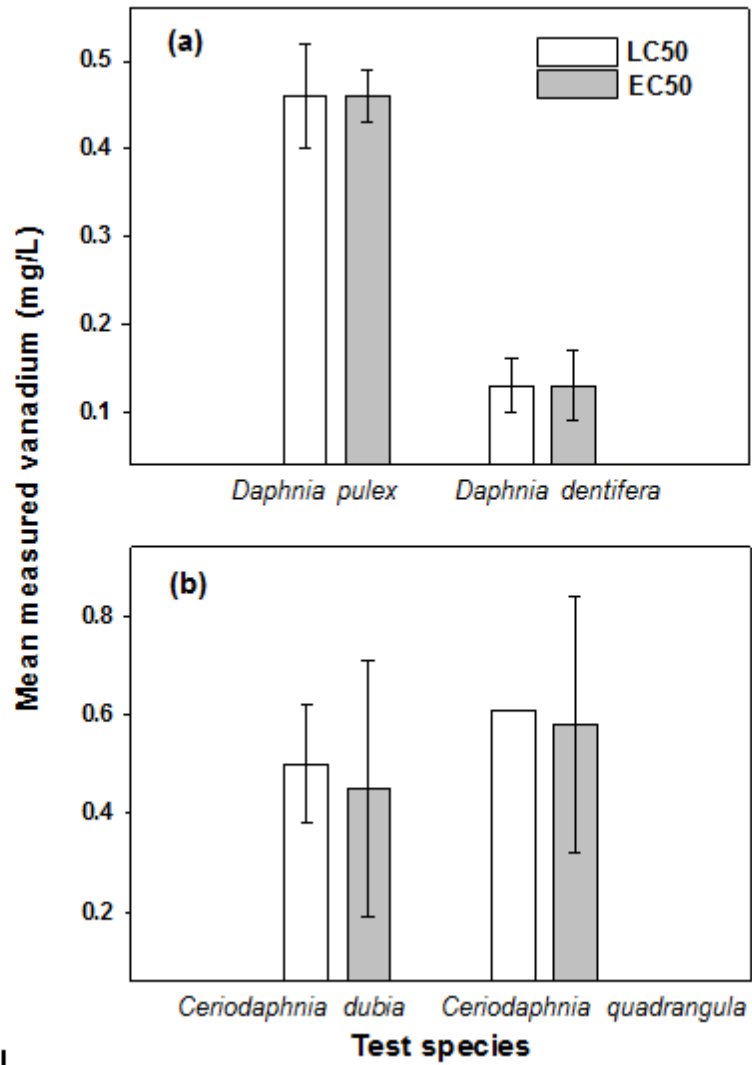
Figure 2.2. Comparison of LC50 and EC50 estimates for two standard laboratory test species, *D. pulex* and *C. dubia* and two comparable field-collected zooplankton species, *D. dentifera* and *C. quadrangula*. Each bar represents the (a) 21-d LC/EC50 and (b) 7-d/8-d LC/EC50 for each species and its associated 95% confidence interval.

2.5.4. Chronic toxicity of vanadium to green algae

The mean cell densities in both the *P. subcapitata* and *S. quadricauda* tests were greater than 16 times the initial cell counts. The coefficient of variance (CV) was 17.2% and 18.3% for *P. subcapitata* and *S. quadricauda*, respectively. Growth inhibition of *P. subcapitata* appeared to begin at 3.3 mg V/L (40% inhibition), but was not statically significant until 6.1 mg V/L. Similarly, growth inhibition of *S. quadricauda* appeared to start at 5.1 mg V/L (48% inhibition), but was not statically significant until 8.9 mg V/L. Although the power of the ANOVA tests was 0.87 and 0.92, significant differences at the lower V concentrations were not detected, likely due to high variability of cell counts within replicate treatments. The EC20 and EC50 estimates for growth inhibition of *P. subcapitata* were 1.72 and 3.69 mg/L, respectively (Table 2). Growth of *P. subcapitata* cells was significantly inhibited at concentrations greater than 6.1 mg V/L, relative to control growth (74.6% inhibition; Figure 3). Growth reductions of *P. subcapitata* growth at V concentrations > 1 mg/L over 72-h exposures was described best using a logistic relationship ($r^2 = 0.98$).

The field-representative species, *S. quadricauda*, was only marginally more tolerant to V than *P. subcapitata*. However, unlike *P. subcapitata*, a significant increase in cell density was not observed at lower concentrations of V (Figure 3). The EC20 and EC50 estimate for growth inhibition of *S. quadricauda* were 1.69 and 4.12 mg/L, respectively (Table 2). Cell growth was significantly inhibited at 8.9 mg V/L, relative to the control growth (71.2% inhibition; Figure 3). A logistic relationship best described the growth inhibition of *S. quadricauda* at V concentrations > 2.9 mg/L throughout the 72-h exposure ($r^2 = 0.99$). Overall, the toxicity of V to *S. quadricauda* was not significantly different from that of *P. subcapitata*.
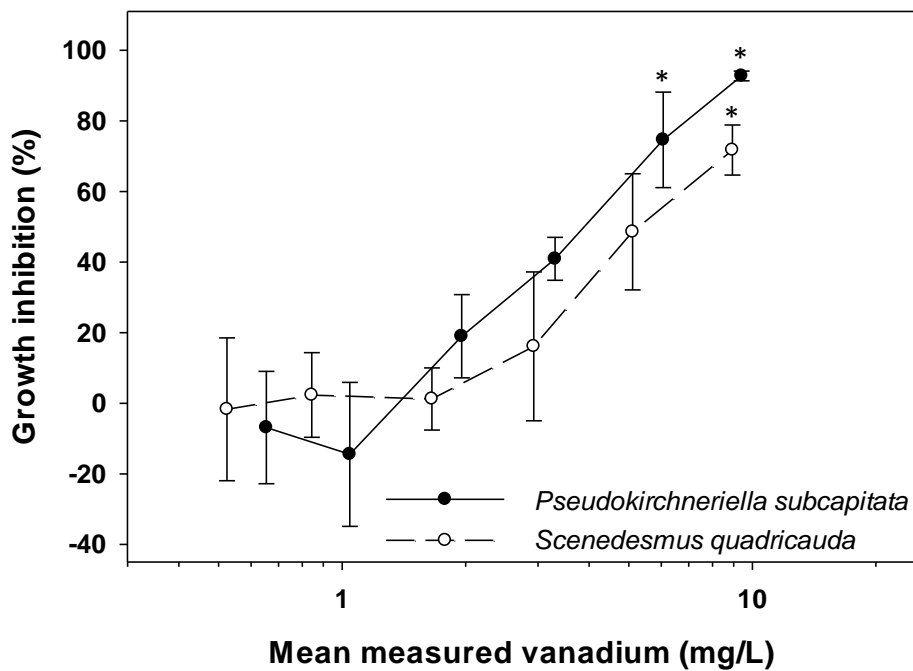
Figure 2.3. Relationships between measured vanadium concentration and percent growth inhibition relative to the control of the green algae species, *Pseudokirchneriella subcapitata* and *Scenedesmus quadricauda*. Each point represents the mean ± SE of five replicate samples. *Significantly different from the respective control (Kruskal-Wallis ANOVA, Dunnett's test, $n = 5$, $p \leq 0.05$).

Table 2.4. Vanadium toxicity for planktonic organisms available in the peer-reviewed literature.

Species	Endpoint	Effect concentration (mg/L)	Reference
Freshwater Algae			
Scenedesmus quadricauda	12-d EC50[1]	2.23	Fargasova et al. (1999)
Ankistrodesmus falcatus	7-10-d LOEC[2]	0.102	
Diatoma elongatum	7-10-d LOEC	0.102	
Scenedesmus obliquus	7-10-d LOEC	5.09	Nalewajko et al. (1995b)
Anabaena flos-aqueae	7-10-d LOEC	50.94	
Planktonic Invertebrates			
Ceriodaphnia dubia	7-d LC50[3]	0.55	Puttaswamy et al. (2010)
Daphnia magna	48-h LC50	4.1	
Daphnia magna	23-d LC50	2.0	Beusen and Neven (1987)
Daphnia magna	21-d LC50	1.7	Van Leeuwan (1987)

[1] EC50 – median effect concentration.

[2] LOEC – lowest observed effect concentration.

[3] LC50 – median lethal concentration

2.6. Discussion

Vanadium is a widely distributed metal in the Earth's crust with an average abundance of 0.14 mg/kg, making it the 5th most abundant transition metal (Amorim et al., 2007; Moskalyk and Alfantazi, 2003). Currently, a lack of published V aquatic toxicity data in the peer-review literature is making it difficult for agencies to generate sound V water quality guidelines for the protection of aquatic life. Some of the most recent approaches used the most sensitive toxicity study and applied a safety factor, which may generate guidelines that are over or under protective of aquatic biota (CCME, 2007; MoEE, 1994). Bitumen from northern Alberta contains considerable amounts of V, up to 220 mg/kg (Jacob and Filby, 1983; Zubot et al., 2012). The majority of this V is bound up in porphyrins in the IV oxidation state and is unavailable to aquatic life (Ventura et al., 2015). In aqueous environments, V can exist in multiple oxidation states ranging from -1 to +5, depending on the pH and redox conditions present (Crans et al., 1998; Wehrli and Stumm, 1989; Ventura et al., 2015) but generally, vanadyl (IV) and vanadate (V) are the dominant species (Crans et al., 1998; Hirayama et al., 1992). In terms of aquatic toxicity, the vanadate oxyanions ($H_2VO_4^-$ and HVO_4^{2-}) are considered the most toxic chemical species. These species are soluble and dominate in oxic waters, allowing for easy transport and are readily bioavailable (Crans et al., 1998). These oxyanions are structurally and functionally similar to phosphate (PO_4^{2-}), and not only interfere with phosphate uptake, but also with many phosphate-metabolizing enzymes, and are strong inhibitors of the Na, K-ATPase pump, at least in vertebrates (Cantley et al., 1977). Inhibition of such activity affects the active transport mechanism of key ions in aquatic organisms. Currently, data regarding V's biological mechanisms of action in invertebrates remains sparse.

Speciation of V in the present planktonic tests showed predominance towards the mono- and divanadate oxyanions (HVO_4^{2-} and $H_2VO_4^-$) in solution (98.9-99.9%) at the measured effect (LC/EC50) concentrations. The DO and pH in these tests ranged from 8.2 to 8.8 mg/L and 8.2 to 8.6, respectively, with a higher pH observed in the algal toxicity tests. Under these conditions V(V) is known to be the dominant species in solution (Crans et al., 1998). Studies that have investigated V lethality to rainbow

trout fingerlings have attributed its toxicity to these two species because of their similarity to phosphate

ions (Crans et al., 1998; Rainbow, 1997; Stendahl and Sprague, 1982). While $H_2VO_4^-$ is known to be the

dominant ionic form at pH 7, as pH increases HVO_4^{2-} becomes the dominant ion, which was reflected by

the speciation modeling in the *P. subcapitata* test (pH 8.6). Stendahl and Sprague (1982) observed

slightly higher toxicity to rainbow trout at pH 7, suggesting that $H_2VO_4^-$ may be the more toxic of the two

forms. However, Holdway and Sprague (1979) observed similar toxicity between the two species. At the

lower V concentrations (< 2 mg/L), the monomeric species (HVO_4^{2-} and $H_2VO_4^-$) composed the majority

of V speciation. However, when the V concentrations increased (> 6 mg/L), polyvanadate species

($H_2V_2O_7^{2-}$ and $V_4O_{12}^{4-}$) increased in solution (1-4%), but remained at relatively low concentrations. This

trend is supported by Crans et al. (1998). Interestingly, under higher salinity conditions where

polyvanadate species tend to be greater in solution, the uptake of V into invertebrates was reduced. This

suggests that mono- and divanadate species are favored over polyvanadate species for uptake into cells

(Miramand and Unsal, 1978; Ringelband and Hehl, 2000).

Few V toxicity tests have been completed with freshwater invertebrates, and very little is known

of the mechanistic behavior of V in these species. The uptake of V(V) is understood to be through

competition of similar anions (i.e., phosphate and sulphate) via anionic transporters (Nalewajko et al.,

1995b; Puttaswamy et al., 2012; Rainbow, 1997). Additionally, studies with brackish water invertebrates

have suggested *in vitro* inhibition of the Na, K-ATPase as a potential mode of action in growth of colonial

hydroids, and in the gills of eels and shore crabs, at approximately 0.05-1.0 mg/L (Bell and Sargent,

1979; Holleland and Towle, 1990; Ringelband and Karbe, 1996). However, further *in vivo* studies are

needed to confirm this hypothesis. For freshwater zooplankton species, the acute toxicity of V has been

investigated by others and their results are very similar to those reported here. Beusen and Neven (1987)

reported 48-h LC50s for *Daphnia magna* of 3.4 to 4.8 mg V/L (Table 2.4), values only slightly higher

than our estimate of 2.17 mg/L for *D. pulex*. Similarly, the field-collected *C. quadrangula* was slightly

more sensitive than the standard test species, *C. dubia*, with 48-h LC50s of 0.60 and 0.97 mg V/L,

respectively (Table 2). These differences are likely inherent to the test species used since the water quality conditions were similar, thus ensuring that test concentrations and V speciation should have been similar.

The chronic toxicity of V to cladocerans has been reported by Beusen and Neven (1987), Van Leeuwan et al. (1987), and Puttaswamy et al. (2010) (Table 4). Their toxicity threshold estimates are in good agreement for *Ceriodaphnia*, but higher for *Daphnia*. Beusen and Neven (1987) and Van Leeuwan et al. (1987) reported 21-d or 23-d LC50 values for *D. magna* of 1.7 and 2.0 mg/L. Our LC50 estimates for *D. pulex* and *D. dentifera* were 0.46 and 0.13 mg V/L, respectively (Table 2.2). These observations support the general notion that *D. magna* is less sensitive to contaminants than most other *Daphnia* species (Koivisto et al., 1992; Mount and Norberg, 1984; Versteeg et al., 1997). Therefore, it is important to demonstrate that sensitivity is comparable over the broad zooplankton community often present in the natural environment, because selection of test species can greatly influence the outcome of laboratory based toxicity estimates (Koivisto, 1995). Conversely, the 8-d LC50 obtained for the field-collected *C. quadrangula* (0.58 mg V/L) was comparable to the 7-d LC50 reported by Puttaswamy et al. (2010) for *C. dubia* (0.45 mg V/L). Generally, *Ceriodaphnia* species tend to be more sensitive to toxicants than larger daphnids (Versteeg et al., 1997).

Both *D. pulex* and *D. dentifera* exhibited nearly identical 21-d LC50 and EC50 estimates (Figure 2.2a). The EC50 estimates were based on surviving females at the end of the 21-d exposure period. This similarity in LC50 and EC50 estimates could be due to the steep concentration-response curve for V, and the choice of test concentrations not being narrow enough to capture an effect of V on neonate output. At high V concentrations, the total numbers of neonates were significantly reduced relative to the controls. However, this was due to high mortality of adults, thereby shortening the reproductive output of females. While V appeared to impair reproduction at 0.63 and 0.22 mg/L for *D. pulex* and *D. dentifera*, respectively, those impairments were directly proportional to reductions in adult survival. Neonate production and smaller brood sizes were significantly reduced at these concentrations and high mortality in adults prior to test end contributed to such reductions. Similarities between survival and reproductive

endpoints for V suggest a direct toxic action of V on adult survival, rather than a more sublethal effect such as reproductive inhibition. Beusen and Neven (1987) also observed negligible differences between cumulative numbers of living neonates per adult and adult survival. Similar LC50 and EC50 values were also observed for the two *Ceriodaphnia* species (Figure 2.2b).

The toxicity of V to algae has also been investigated by others, but toxicity thresholds are more variable and generally lower than the EC50 estimates produced here. The best comparison found for *S. quadricauda* was Fargasova (1999) who reported a 12-d EC50 of 2.23 mg/L, compared to the 3-d EC50 of 4.12 mg/L reported here. This difference could very likely be due to the very different exposure duration used in the two studies. In addition, Lee et al. (1979) and Nalewajko et al. (1995b) have investigated V toxicity to a variety of different algal species, including cyanobacteria, Chlorophyta, and Bacillariophyceae. Lee et al. (1979) noted complete suppression of growth at 0.1 mg V/L in the cyanobacteria *Anabaena flos-aque*, whereas *Scenedesmus oliquus*, *Chlorella pyrenidasa*, and the diatom *Navicula pelliculosa* were slightly more tolerant (Table 4). Conversely, Nalewajko et al. (1995a, b) observed the opposite, where the diatom, *Diatoma elongatum*, and the green alga, *Ankistrodesmus falcatus*, were the more sensitive and *A. flos-aqueae* the least sensitive. These species differences were attributed to differences in phosphate requirements. In the latter study, Nalewajko et al. (1995b) confirmed that algae were unable to distinguish vanadate from phosphate, resulting in competitive uptake of the two ions at the cell surface. The addition of phosphate to medium ameliorated the toxic effects of V. Therefore, differences in toxicity may be attributed to differences in phosphate requirements among taxonomic groups that can determine responses to potential phosphate competitors, such as vanadate. Unlike cyanobacteria, diatoms are more likely to become phosphate deficient due to smaller cellular phosphate quota and lower phosphate uptake affinity, thus making them more susceptible to V toxicity. The phosphate requirement for green algal species is more varied than cyanobacteria and diatoms, resulting in more intermediate responses to increased concentrations of V. Consequently, V is more toxic to phytoplankton in freshwater systems where phosphate is limited (Nalewajko et al., 1995a).

Several studies have suggested that V is an essential micronutrient for algae and plants given its stimulatory effects on algal growth and metabolism at low concentrations (Meisch and Bieling, 1975). As an anion, toxicity of V (V) will be similar to that of arsenate, with the interference of phosphate uptake at the cell surface as the primary effect (Planas and Healey, 1978). Concentrations greater than 0.5 to 1.0 mg V/L have been found to inhibit green algal growth, chlorophyll synthesis, photosynthesis and cell division, prevent complete synchronization of cell division through the uncoupling of phosphorylation reactions, and disrupt ATPases (Nalewajko et al., 1995a, b). In contrast, Meisch et al. (1977) noted that the green alga, *Chlorella pyrenoidosa*, had maximal growth at 0.5 mg V/L, with cell growth and chlorophyll being reduced at concentrations greater than 25 mg V/L. The possibility that V could serve as a micronutrient in algae could explain the small increases in cell counts observed at lower V treatments in the *P. subcapitata* test. Here, cell counts were 4.5% and 10% higher than in the control at 0.7 and 1.0 mg V/L, respectively. However, such an increase was not observed in the *S. quadricauda* test.

One of the objectives of the present study was to compare the sensitivity of V to commonly used test species to species more representative of northern Alberta. The more commonly used test species included *D. magna*, *D. pulex*, *C. dubia*, and *P. subcapitata*. While *D. magna* is the most widely used cladoceran in toxicity testing (Koivisto, 1995), the use of *D. magna* has often been discouraged given its large size, limited geographical range, and general confinement to small, fishless water bodies (Bossuyt and Janssen, 2005). Furthermore, many studies have investigated differences in sensitivity between *D. magna* and other cladoceran species (Hickey, 1989; Mount and Norberg, 1984; Koivisto et al., 1992; Versteeg et al., 1997) and concluded that *D. magna* is generally the least sensitive. Versteeg et al. (1997) demonstrated that smaller *Ceriodaphnia* species were 2.4 times more sensitive than *D. magna* and *D. pulex*; a similar trend was observed here. Furthermore, smaller cladocerans exhibit higher sodium turnover rates, making them more sensitive to acute toxicity (Grosell et al., 2002), especially for compounds that may interfere with Na and K homeostasis. The data presented here suggest that zooplankton species more representative of aquatic environments in the vicinity of the Alberta oil sands

have comparable or slightly greater sensitivity to V than more conventional test species. Inclusion of such regionally-relevant species in the V toxicity database will help ensure that any future water quality guideline developed for V is protective to cladocerans in the region. The unicellular, non-motile green alga, *P. subcapitata*, is routinely used in establishing water quality criteria and has been shown to exhibit greater sensitivity to some metals than other algal species (Rojickova-Padrtova and Marsalek, 1999). However, the phytoplankton is an incredibly diverse group and very few species have been used in toxicity assessments. *Scenedesmus quadricauda* is one of the most common green algae in the Alberta oil sands area and is abundant in Mildred Lake on the Syncrude lease site (Hayes, 2006). Overall, differences in V sensitivity of these two species were relatively insignificant and both were approximately an order of magnitude less sensitive than cladocerans. However, it should be reiterated that other studies (see Table 2.4) have reported lower toxicity thresholds for algae. In short, both zooplankton and phytoplankton will likely feature prominently in the future derivation of a Canadian V water quality guideline for the protection of aquatic life.

Treatment of OSPW prior to discharge to the natural environment will be necessary for sustaining continued operations in the oil sands sector. Petroleum coke presents a unique approach to OSPW treatment, as it is a stockpiled by-product with minimal current use and in large supply, and has been shown to remove > 90% of the organic fraction associated with acute OSPW toxicity (Gamal El-Din et al., 2011; Small et al., 2012a, b; Zubot et al., 2012). However, the leaching of V from coke could negatively impact water quality of treated process-affected water (Small et al., 2012a, b). The toxicity data presented here suggest that V in coke leachate could negatively impact long-term survival of sensitive cladoceran species in leachate-receiving aquatic systems if the V concentration in those systems was to exceed 0.1 mg/L. Considering the solubility of V, which increases under the alkaline conditions of OSPW, and data suggesting that fresh coke leachate can contain up to 5 mg V/L (Pourrezaei et al., 2014b; Small, 2011), it is apparent that V will have to be removed from process-affected waters prior to

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discharge to the natural environment. If not, it may be necessary to rely on dilution in the receiving aquatic systems to reduce V to ecologically safe levels.

2.7. Conclusion

Differences in the sensitivity to V toxicity between commonly used laboratory test species (*D. pulex, C. dubia, P. subcapitata*) and species more regionally-representative of northern Alberta (*S. serrulatus, D. dentifera, C. quadrangula, S. quadricauda*) were relatively small (within a factor of 4), suggesting that toxicity data from commonly-used planktonic species would suffice for the derivation of a V water quality guideline for use in the AOS region. Overall, zooplankton exhibited greater sensitivity to V than phytoplankton. Acute and chronic toxicity thresholds for V to zooplankton were within and below the range of V that could potentially be released from petroleum coke when it comes in contact with water, and possibly within the range that could occur in subsequent receiving environments depending on the method of storage, use and treatment of such leachates. Consequently, development of water quality guidelines for V for use in Alberta or elsewhere will have important implications for the storage, use and/or remediation of coke at major oil sands companies, and for other industries that may have elevated concentrations of V in their process waters and environmental discharges, such as uranium and phosphate mining operations and steel industries.

CHAPTER 3:

ESTIMATION OF VANADIUM WATER QUALITY BENCHMARKS FOR THE PROTECTION OF AQUATIC LIFE WITH RELEVANCE TO THE ATHABASCA OIL SANDS REGION USING SPECIES SENSITIVITY DISTRIBUTIONS

3.1. Preface

This chapter further describes the acute and chronic toxicity of dissolved vanadium (as vanadate oxyanions) in addition to toxicity data generated in Chapter 2, in this case to macrobenthic invertebrates and freshwater fish species that are either commonly-used laboratory test-species, or species more regionally-representative of the Athabasca Oil Sands (AOS) region. Acute and chronic toxicity estimates for several apical endpoints (survival, growth and insect emergence) were generated for two *Chironomus* species and two species of fish. Research from this chapter and Chapter 2 were then combined with data from the peer-reviewed literature to generate acute and chronic species sensitivity distributions (SSDs) based on the sensitivity of multiple aquatic organisms to V. From these SSDs, an acute and chronic hazardous concentration to 5% of species present (HC5) were estimated. These values could be used as interim water quality benchmarks to protect aquatic life in local aquatic environments surrounding oil sands companies from exposure to hazardous levels of V.

This chapter is being prepared for submission to *Environmental Toxicology and Chemistry* under joint authorship with Karsten Liber (University of Saskatchewan).

3.2. Abstract

Elevated vanadium (V) concentrations in oil sands coke, which is currently produced and stored on-site of some major Athabasca Oil Sands (AOS) companies, could pose a risk to aquatic ecosystems in northern Alberta, depending on its future storage and utilization. Here, V toxicity was determined in reconstituted Athabasca River water to various freshwater organisms, including two midge species (*Chironomus dilutus* and *Chironomus riparius*; 4-d and 30 to 40-d exposures) and two freshwater fish species (*Oncorhynchus mykiss* and *Pimephales promelas*; 4-d and 28-d exposures) to facilitate calculation of appropriate water quality benchmarks. The acute toxicity of V was 52.0 and 63.2 mg/L for *C. dilutus* and *C. riparius*, respectively, and 4.0 and 14.8 mg V/L for *P. promelas* and *O. mykiss*, respectively. Vanadium significantly impaired adult emergence of *C. dilutus* and *C. riparius* at concentrations ≥ 16.7 (31.6% reduction) and 8.3 (18.0% reduction) mg/L, respectively. Chronic toxicity in fish presented primarily as lethality, with chronic 28-d estimates of 0.5 and 4.3 mg/L for *P. promelas* and *O. mykiss*, respectively. There were little to no effect on growth of surviving fish (i.e., weight and length). These data were combined with data from the peer-reviewed literature and separate acute and chronic species sensitivity distributions (SSDs) constructed. The acute and chronic hazardous concentrations endangering 5% of species (HC5) were estimated as 0.64 and 0.09 mg V/L, respectively. Based on these SSDs, the most sensitive species to V, species that could be adversely affected if V was elevated in future site-water or process-water discharges associated with Athabasca oil-sands companies, were from the Chlorophyta (*Diatoma elongatum*), Arthropoda (*Daphnia* and *Ceriodaphnia* species) and Cyprinidae (*P. promelas*). These new data for V toxicity to aquatic organisms ensure that there are now adequate data available for regulatory agencies to develop appropriate water quality guidelines for use in the AOS region. Until then, the HC5 values presented here could serve as interim benchmarks for the protection of aquatic life from exposure to hazardous levels of V in local aquatic environments.

3.3. Introduction

Vanadium (V), a ubiquitous transition metal in the environment, has surfaced as a potential aquatic contaminant because of its high abundance in bitumen from the Athabasca Oil Sands in Alberta, Canada (Crans et al., 1998; Hamada, 1998; Hodgson et al., 1954; Jacob and Filby, 1983). In most aquatic ecosystems, the background concentrations of V are generally low, ranging from 0.5 – 2.4 µg/L (Hamada, 1998; Hirayama et al., 1992; Rehder, 1991). Rather than occurring as a concentrated ore, V is more likely to occur as a constituent in many minerals and in ores of other metals (e.g., uranium) (Crans et al., 1998; Moskalyk and Alfantazi, 2003). However, it is also elevated in many types of carbonaceous sediments, especially oil sands, which is a substantial and growing industry in Western Canada (Alberta Energy Regulator, 2015; Crans et al., 1998; Duyck et al., 2007; Huang et al., 2015; Wehrli and Stumm, 1989). Other significant sources of V contamination to surface waters include the combustion of fossil fuels, the generation of liquid wastes from steel, chemical and polymer industries, and the increasing presence of V in rechargeable batteries (Huang et al., 2015).

In fresh waters, V displays interesting aquatic chemistry, which is highly influenced by ecosystem pH, redox conditions and its solution concentration. The dominant and most soluble forms of V in oxic ecosystems (pH 7-9) are the vanadate oxyanions (V) (Crans et al., 1998; Li et al., 2007; Wang and Sanudo-Wilhemy, 2009). Under reducing, slightly acidic (pH <6) conditions, the mobile V(V) is reduced to vanadyl oxocations (IV). Unlike V(V), V(IV) is less toxic and less bioavailable, and can be removed from solution via complexation with organic matter (Wehrli and Stumm, 1989). The V(V) oxyanions ($H_2VO_4^-$ and HVO_4^{2-}) are structurally and electronically similar to phosphate anions ($H_2PO_4^-$ and HPO_4^{2-}) and can therefore compete for uptake into biotic cells via ion mimicry (Bridges and Zalups, 2005). This allows V(V) to elicit toxicity through the interference of phosphate-metabolizing enzymes and substrates (Crans et al., 1998; Nechay et al., 1986; Rainbow, 1997; Rehder, 1992; Wanty and Goldhaber, 1992). *In vivo* studies have also shown V to inhibit Na-K-ATPase activity in the gills of fish and crabs (Bell and Sargent, 1979; Holleland and Towle, 1990). Additionally, V has been identified in several important

enzyme systems (e.g. haloperoxidases and nitrogenases) in bacteria, algae, marine invertebrates and fungi, suggesting a role as an essential element (Rehder, 1991). However, its essentiality in higher organisms remains uncertain. Except for a few older studies, little information is available regarding the toxicity of V to freshwater organisms, especially for long-term exposures.

As an abundant trace element in crude oils, V can range from 150-290 mg/kg in bitumen mined from the Athabasca Oil Sands (AOS) region in northern Alberta, Canada (Giles et al., 1979; Zubot et al., 2012). In crude oils, V is complexed to organic ligands, known as vanadyl porphyrins, in the heavier fraction of oil (i.e. asphaltenes) (Amorim et al., 2007; Breit and Wanty, 1991; Ventura et al., 2015). When bitumen is upgraded, V is removed and enriched in the by-product petroleum coke at concentrations upwards of 1000 mg/kg (Zubot et al., 2012). Currently, some oil sands companies, such as Syncrude Canada Ltd. (SCL) stockpile vast amounts of petroleum coke on-site because it is designated as a potential future energy source. As of 2014, approximately 90 million tons of coke had been produced across all oil sands companies in the AOS region (AER, 2015). The majority of coke will continue to be stockpiled until more practical ways of utilizing or storing it becomes available.

Previous studies have found coke to leach high concentrations of V into solution upon exposure to water, including process-affected waters (Small et al., 2012a, b; Puttaswamy et al., 2010; Puttaswamy and Liber, 2011). An 18-month lysimeter study found V to leach from coke into solution at concentrations >1000 µg/L, orders of magnitude greater than background concentrations (Puttaswamy et al., 2010). Such V concentrations could negatively impact cladoceran survival in chronically exposed aquatic ecosystems (Puttaswamy et al., 2010; Schiffer and Liber, submitted). Currently, coke is being evaluated as a treatment option for the removal of organic acids in oil sands process-affected waters. This is a unique approach where an oil sands by-product available in large supply on-site could potentially be used to treat another growing by-product, also stored on-site. However, V can leach from coke at concentrations up to 5000 µg/L into treated process-affected waters (Pourrezaei et al., 2014b; Small et al., 2012b; Zubot et al., 2012).

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Until very recently, there were no established water quality guidelines (WQGs) for the protection of aquatic life for V in Alberta or Canada, despite the abundance of V in bitumen and associated by-products produced by oil sands companies in the AOS region. Guideline values are set to protect aquatic ecosystems from hazardous concentrations of contaminants in the water column and are ideally established using the species sensitivity distribution (SSDs) approach, when adequate data are available (CCME, 2007). Compared to traditional methods, SSDs introduce greater confidence into a risk assessment and consider all of the available toxicity data, rather than a single toxicity study presenting the most sensitive endpoints. Instead, SSDs are cumulative distributions of interspecies sensitivity response to a contaminant, which are used to estimate the hazardous concentration to the most sensitive 5% of species tested (HC5), thereby aiming to protect 95% of species (Posthuma et al., 2002). The HC5 can then be established as a benchmark value. However, an effective SSD requires a large dataset composed of multiple organisms from different trophic levels representative of diverse aquatic ecosystems. Such dataset diversity and completeness are often lacking for less-studied contaminants, such as V (CCME, 2007). While no federal guidelines had been generated until May 2016, an interim provincial guideline for V was established in Ontario in 1994 based on a lowest observable effect concentration (LOEC) from a single study with a safety factor (16.6) applied due to insufficient data (MOEE, 1994). Federal WQGs for V were recently established under CEPA 1999 using a process that requires no public consultation (Canada, 2016). These values (120 µg/L for freshwater, 5 µg/L for marine waters) were generated using a more limited dataset, but did follow the SSD approach. While these guidelines are helpful, well-supported V guidelines (both in number and type of species) will be required for the AOS region to help oil sands companies make informed decisions regarding the management and use of coke and the potential future discharge of leachates and effluents containing elevated concentrations of V.

To facilitate further water quality benchmark development for V, additional studies of V toxicity to a diverse suite of aquatic organisms were needed. The study described here aimed not only to increase toxicity data for V, but also to address the toxicity of V to organisms representative of the AOS region,

where increased V concentrations would be expected. Currently, organisms representative of northern Alberta have not been well represented in the existing V toxicity database. Therefore, this study also set out to determine if species more regionally-representative of northern Alberta were more or less sensitive to V than conventional laboratory test organisms. This will assist regulators and managers by making the toxicity database and future guidelines development more relevant to the AOS region. Here, acute and chronic toxicity data were generated for two benthic macro-invertebrate species (*Chironomus dilutus* and *C. riparius*) and two freshwater fish species (*Oncorhynchus mykiss* and *Pimephales promelas*). Subsequently, data from this research were used in combination with other published data to calculate acute and chronic HC5 values using SSDs that could serve as interim water quality benchmarks for the AOS region.

3.4. Materials and methods

3.4.1. Test chemical and Athabasca River water

Sodium metavanadate ($NaVO_3$, anhydrous, min. 96% pure, Strem Chemicals, Inc., Newburyport, MA, USA) was used to create V stock solutions for all experiments because V(V) oxyanions are the dominant species in oxic, aquatic ecosystems (Crans et al., 1998; Wehrli and Stumm, 1989). Stock solutions were prepared by dissolving $NaVO_3$ in ultra-pure water (Barnstead NANOpure®, 18 MΩ-cm, Thermo Scientific, Dubuque, IA, USA). To achieve the target V concentrations for each treatment, stock solutions were diluted using test waters.

Reconstituted Athabasca River Water (ARW) was used in all experiments and mimicked the general water characteristics (i.e., conductivity, alkalinity, total hardness, and pH) of the Athabasca River in northern Alberta (Table 3.1). These characteristics were obtained from field samples collected from various sampling sites by the Regional Aquatics Monitoring Program (RAMP, 2011). Carbon-filtered, bio-filtered municipal water from the City of Saskatoon (culture water) was diluted with approximately 30% reverse osmosis (RO) water to approximate ARW characteristics (Table 3.1). Prior to conducting toxicity tests, reconstituted ARW was aerated for a minimum of 24 h and test species cultured in ARW

for a minimum of 24 h leading up to testing. The general culture water, ARW, reconstituted ARW, and test-solution characteristics are reported for each test in Table 3.1.

3.4.2. Source and culturing of test organisms

Four freshwater organisms were used in separate toxicity tests: two chironomid species (*C. dilutus* and *C. riparius*) and two fish species (*O. mykiss* and *P. promelas*). The midge larvae, *C. dilutus*, were obtained from an in-house culture maintained at the Toxicology Centre, University of Saskatchewan (U of S), Saskatoon, SK. Culturing procedures followed general guidelines described elsewhere (Environment Canada, 1997). Multiple attempts to culture field-collected chironomid larvae collected from the Syncrude mine lease area north of Fort McMurray, AB, Canada, were unsuccessful due to poor hatching success of egg masses obtained from emerged adults. Therefore, *C. riparius* egg cases were obtained from the Canada Centre for Inland Waters in Burlington, ON, Canada, and used for comparison to our laboratory-cultured, *C. dilutus*. *Chironomus* species are representative of the Athabasca River and the AOS area. Egg masses from established, healthy cultures were raised to test age in 15-L glass aquaria containing reconstituted ARW in an environmental chamber maintained at 23 ± 1°C under a 16:8 h light:dark photoperiod.

Rainbow trout (*Oncorhynchus mykiss*) was selected as a standard laboratory test species and eyed eggs purchased from a commercial supplier (Troutlodge, Inc., Summer, WA, USA). Eggs were held in 9-L MacDonald jars under a flow-through regime using culture water in the Aquatic Toxicology Research Facility (ATRF) at the U of S Toxicology Centre until hatch (Table 3.1). A hatch day was established when an estimated 50% of the eggs had hatched. At this time, larvae were transferred to 20-L glass aquaria containing reconstituted ARW in an environmental chamber maintained at 12 ± 1°C under a 16:8 h light:dark photoperiod. Trout larvae were held in several aquaria with continuous aeration for 24 h prior to test initiation. Breeding stocks of fathead minnow (*P. promelas*), an abundant species in the Athabasca River, were also maintained in-house in the ATRF in culture water. Eggs were collected and subsequently

hatched in an environmental chamber under conditions similar to those used for *O. mykiss*, but at an average temperature of $24 \pm 1°C$.

3.4.3. Toxicity tests

All experiments were conducted under the same environmental conditions as described above in Section 3.2.2 in controlled environment chambers at the Toxicology Centre. They were performed as static-renewal assays, with experimental groups consisting of an untreated control and a minimum of five geometrically increasing V concentrations chosen to provide a concentration-response for V. Treatments in the chironomid toxicity tests consisted of four replicates, whereas treatments for fish contained five replicates. The experimental procedures performed using fish were approved by the University of Saskatchewan's Animal Research Ethics Board (protocol no. 20140036), and followed the Canadian Council on Animal Care guidelines for humane animal use.

3.4.3.1. Acute lethality tests with chironomids

Acute lethality tests with *C. dilutus* and *C. riparius* followed Environment Canada (1997) guidelines with slight modifications. Tests were static-renewal and 96 h in duration, using 300-ml glass beakers containing a thin layer of washed quartz sand (particle size: 250-425 µm) and gentle aeration. Ten second instar larvae of *C. dilutus* (4 to 5-d post-hatch) and *C. riparius* (3 to 4-d post-hatch) were randomly assigned to each of four replicate beakers per experimental group containing either 250 ml of control or test solution. An additional three replicates of ten larvae were collected and set aside for determination of initial dry weights for growth calculation. A partial water change was conducted following 48-h of exposure to maintain good water quality. Larvae were fed 3 mg of Nutrafin® fish flake (Rolf C. Hagen Inc., Montreal, QC, Canada) slurry per beaker at times 0 and 48 h. Following 96 h, the exposure was terminated and survival rates calculated. Tests were valid if chironomid survival was $\geq 80\%$ in the controls.

Dissolved oxygen (DO), temperature and ammonia were measured at times 0, 48, and 96 h from three randomly selected beakers of each treatment using an Orion 3-Star RDO Portable Meter (Thermo Fischer Scientific Inc., Nepean, ON, Canada) and an Orion Aquafast$^®$ II meter (Thermo Electron Corporation, Beverly, MA, USA), respectively. Water samples (5 ml) for quantification of dissolved V were collected at similar times. Water samples (20 ml) were also collected at the start and end of each test period from three beakers per treatment for analysis of routine water quality: conductivity, pH, alkalinity, and total hardness.

3.4.3.2. Acute lethality tests with freshwater fish

Two 96-h tests were performed with *P. promelas* (initiated 24-h post-hatch) and *O. mykiss* (initiated14-d post-hatch) following guidelines described in Environment Canada (1990b). Eyed eggs were obtained for both species and held in either culture water (*O. mykiss*) or reconstituted ARW (*P. promelas*) until hatch. Following hatch, *O. mykiss* larvae were transferred into several aquaria containing reconstituted ARW and held there until test initiation, which occurred after 50% of the larvae reached the swim-up stage. These tests were static-renewal and performed in 1-L glass beakers that were continuously aerated to maintain DO saturation. Each test was conducted with five replicates of 10 fish per beaker randomly placed in either 900 ml of control or test solutions. At 48 h, 80% of the solution was renewed to maintain good water quality. Mortality was monitored at least six times daily and a test was considered valid if survival was ≥ 90% in control treatments.

Ammonia, DO and temperature were measured daily in three randomly selected beakers from each treatment, including before and after the water replacement on day 2. Water samples for quantification of dissolved V were collected at 0, 48 (before and after water renewal) and 96 h. Other water quality variables (conductivity, pH, alkalinity, and total hardness) were measured at the start and end of the test period.

3.4.3.3. Partial lifecycle tests with chironomids

Partial lifecycle toxicity tests were performed with *C. dilutus* and *C. riparius* based on slight modifications of the *C. tentans* lifecycle static-renewal protocol (Benoit et al., 1997; Environment Canada, 1997). Tests were initiated with 10 early instar larvae (3- to 5 d post-hatch) per beaker with a total of eight replicate beakers per treatment. Replicate 300-ml tall-form glass beakers containing a thin layer of rinsed quartz sand (particle size: 250-425 μm) served as the experimental units. On day 0, 10 larvae were randomly introduced into each beaker, with gentle aeration provided. For determination of initial dry weights, an additional three replicates of 10 organisms were collected as described in Section 3.2.3.1. To ensure adequate water quality and constant V exposure concentrations throughout the experimental period, test solutions were renewed every 3 days. Test organisms were fed daily with 6 mg of Nutrafin® fish flake slurry per beaker.

Long-term effects on larval survival and growth (as dry weight) were evaluated prior to adult emergence by taking down four replicates from each treatment on day 10 (*C. riparius*) and day 14 (*C. dilutus*). The different days were chosen to account for differences in adult emergence times for the two species. Surviving larvae were counted, collected in weigh boats, and dried for a minimum of 24 h at 60°C. Exposures were continued in the remaining replicates until all larvae had either emerged or died at the life stage they were able to achieve. Once pupation began, daily records were kept of adult emergence, including how many larvae were able to pupate and successfully emerge, and the sex of each emerged adult.

In the test solutions, DO, temperature and ammonia were measured every other day from the control, low, medium and high V treatments before and after water renewals. Other water quality variables (conductivity, pH, alkalinity and total hardness) were monitored before and after renewals at the beginning, middle and end of each test. Similarly, samples for V analysis were collected from old and new test solutions every four days throughout the exposure period from all treatments.

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3.4.3.4. Chronic tests with freshwater fish

Chronic toxicity tests were conducted using *P. promelas* and *O. mykiss* to evaluate the effects of V on their longer term survival and growth, covering both the larval (i.e. alevin) and post yolk-sac adsorption (i.e. swim-up fry) stages. Tests were conducted following the general recommendations of Environment Canada (2011), but extended from 7 to 28 days to account for the longer yolk-sac stage in *O. mykiss*. Unlike *P. promelas*, which absorbs the yolk-sac within 48 h of hatch, *O. mykiss* requires approximately 14-d to absorb the yolk-sac. Prior to test initiation, *O. mykiss* larvae were held in holding tanks containing reconstituted ARW for 24 h following hatch, whereas *P. promelas* eggs were maintained and hatched in reconstituted ARW. Tests were performed in 1-L glass beakers, with five replicates per treatment. On day 0, ten larvae were randomly assigned to 900 ml of either control or test solutions. Beakers were continuously aerated to maintain the DO above 7 mg/L; specifically to means of 83.1% and 66.4% DO saturation at temperatures of approximately 24.0 and 13.0°C, respectively. As a static-renewal test, 80% water replacements were performed every second day for the first 14 d and then increased to daily for the remainder of the experiments. In addition to water renewals, waste siphoning was performed at least twice daily, and beakers were replaced half-way through the test to maintain low ammonia concentrations. When approximately 50% swim-up of fish occurred, *O. mykiss* (14 d post-hatch) were fed a fine trout chow (Aqua Balance Trout 54:17 Starter #0 crumble) *ad libitum* three times daily, whereas *P. promelas* (2 d post-hatch) were fed live brine shrimp *ad libitum* twice daily. At test termination, surviving fish were counted, euthanized with a lethal dose of buffered MS-222 (ethyl 3-aminobenzoate methanesulfonate), and collected into weigh boats. Wet weights were recorded and fish were then dried for a minimum of 24-h at 60°C to obtain dry weights. Tests were valid if ≥ 90% of control fish survived.

At each water renewal, DO, temperature and ammonia were measured in the old and new test solutions in the control, low, medium, and high treatment concentrations. Excrement and excess food were siphoned off during water renewals, as well as multiple times each day. Other water quality variables (conductivity, pH, alkalinity, and total hardness) were measured before and after water

replacements in all treatments at the start of the test, twice during the testing period, and at test termination. Samples to confirm actual dissolved V concentrations were collected every four days from the new and old test solutions from each treatment.

3.4.4. Chemical analyses

Dissolved V in tests waters was measured in-house using an inductively coupled plasma-mass spectrometer (ICP-MS) equipped with collision cell technology (X-Series II ICP-MS, Thermo Fisher, Mississauga, ON, Canada). Water samples for metal analysis were membrane filtered (0.45 μm polyethersulfone membrane, VWR International, Mississauga, ON, Canada) and acidified to $\leq$ pH 2 using a high purity nitric acid (HNO$_3$; Optima grade, Fisher Scientific Company, Ltd., Whitby, ON, Canada). Samples were kept at 4°C until analysis could be completed. The ICP-MS limit of quantification for V was ≤ 0.5 μg/L. To evaluate the accuracy of ICP-MS measurements, a river water standard (SLRS-5, National Research Council, Ottawa, ON, Canada) and a natural water standard reference material (1640a, National Institute of Standards & Technology, Gaithersburg, MD, USA) were analyzed with each analytical run. Procedural blanks and sample duplicates were also included. Reproducibility of reference values (SLRS-5 and 1640a) and precision of duplicates exceeded 90% in all cases. Measurements of major cations (calcium, potassium, magnesium, and sodium) and major anions (sulphate and chloride) were performed in-house using a dual ion chromatography system (3000i/SP, Dionex, Sunnydale, CA, USA) on select samples from the acute and chronic *C. dilutus* and *O. mykiss* tests. Dissolved organic carbon (DOC) was also measured in the same tests on samples collected from the control, low, medium, and high concentrations using a TOC analyser (TOC-500, Shimadzu, Duisburg, Germany).

3.4.5. Statistical analysis and V speciation modeling

Data are presented as the mean $\pm$ standard error (SE) unless otherwise stated. All LC50 estimates and associated 95% confidence intervals (CIs) were calculated using either the trimmed Spearman-Karber method, version 1.5 (US EPA, 1990), or the probit method using mean measured V concentrations (Hamilton et al., 1977). Sublethal data were analyzed using the Inhibition Concentration (ICp) approach,

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version 2.0 (US EPA, 1993) and reported as EC50 and EC20 values (Norberg-King, 1993). Differences in toxicity responses among standard and comparable regionally-relevant test species were considered significant if there was no overlap of the 95% CIs. All other statistical analysis were conducted using SigmaPlot®, version 11.0 (San Jose, CA, USA) with a 95% (p≤0.05) level of confidence. Significant differences between treatment groups and the control were determined using a one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test. In the case of non-normally distributed data, the Kruskal-Wallis test followed by the Dunn's post-hoc test was used. Visual MINTEQ, version 3.1 (Stockholm, Sweden) was used to model V speciation based on mean measured dissolved V, major ions, and routine water quality data (Table 3.1). To develop SSDs, toxicity data were gathered and compiled from the present study and from other studies published in the peer-reviewed literature (Table 3.2 and 3.3). If multiple toxicity data points were present for the same species, the data were combined and plotted as the geometric mean. The SSDs were plotted using the CCME SSD Master, version 3.0, which creates cumulative graphs based on species taxonomic information and concentration values. If both lethal and sublethal endpoints were available to support the chronic effects benchmark for V, then the most sensitive endpoint was selected to include in the SSD compilation. Four different analyses (log-normal, log-logistic, gumbel, and extreme values) were performed, the best-fit distribution chosen using the Anderson-Darling statistic (A^2), and the HC5 and 95% CIs calculated from that distribution.

3.5. Results

3.5.1. Exposure Conditions

The water quality characteristics of the Athabasca River and the reconstituted ARW are listed in Table 1. Overall, the measured water quality variables for reconstituted ARW from all experiments fell within recommended ranges for tests using chironomids and fish, as well as within reasonable approximation of ARW characteristics (Table 1) (Environment Canada, 1997; Environment Canada, 1990b; Environment Canada, 2011). The high variability in conductivity observed in the acute tests was related to the addition of $NaVO_3$ in a dose-dependent manner. Major ions composition varied little among

the chironomid and fish chronic tests; averages across tests were (mean $\pm$ SD in mg/L; $n = 80$): chlorine 8.9 $\pm$ 0.7; sulphate 58.8 $\pm$ 3.6; sodium 27.6 $\pm$ 11.8; potassium 2.6 $\pm$ 0.7; magnesium 13.1 $\pm$ 0.8; and calcium 29.8 $\pm$ 2.3. Only sodium differed significantly among the chironomid and fish species, measuring as high as 92.3 $\pm$ 0.2 mg/L in the *C. dilutus* acute test due to the high V concentrations required to establish an LC50. High variability in ammonia values, reported as an average over the chronic test periods (Table 3.1), was a result of averaging measurements prior to and following water renewals during the fry stages of the fish tests. Over the static periods of the fry stage, ammonia tended to increase to approximately 0.8 mg/L, however, following a water renewal, ammonia would decrease to < 0.1 mg/L. The mean measured V concentrations in test solutions were within 5% and 25% of nominal concentrations in the acute and chronic chironomid tests, respectively. In the fish tests, mean measured V concentrations were within 5 and 15% of nominal concentration in the acute and chronic tests, respectively. The mono- and dimeric V(V) oxyanion species (HVO_4^{2-} and $H_2VO_4^-$) were identified as the dominant species in solution using the MINTEQ model, ranging from > 90% in the fish tests and > 80% in the chronic chironomid tests, at the measured effect concentrations (LC/EC50). However, monomeric V species were reduced to 64-72% in the acute chironomid tests, followed by an increase in polymeric V(V) species ($H_2VO_7^{3-}$, $V_4O_{12}^{4-}$, and $V_5O_{15}^{5-}$) (28-36%) because of the high V concentrations required to establish effect concentrations. Overall, $H_2VO_4^-$ represented a greater portion of the monomeric V(V) oxyanions than HVO_4^{2-} in all experiments.

3.5.2. Acute effects of V on chironomid survival

. The acute effects of V on *C. dilutus* and *C. riparius* during 96-h exposures occurred in the high mg/L range, with estimated 96-h LC50s of 52.1 and 63.2 mg V/L for *C. dilutus* and *C. riparius*, respectively (Figure 3.1a; Table 3.2). Both *Chironomus* species displayed very similar dose-response curves. Larval growth (measured as dry weight) over the test period was also found to be significantly less at the highest test concentrations (56.4 and 72.9 mg V/L for *C. dilutus* and *C. riparius*, respectively).

3.5.3. Acute effects of V on fish survival

The acute toxicity of V to *O. mykiss* and *P. promelas* during 96-h exposure periods are illustrated in Figure 3.1b. There was no mortality in the control treatments during the exposure periods. Vanadium was found to exert acute lethality to fish in the low mg/L range, with 96-h LC50s of 14.8 and 4.0 mg V/L for *O. mykiss* and *P. promelas*, respectively (Table 3.2). *Pimephales promelas* (24-h post-hatch) were significantly more sensitive to V toxicity than *O. mykiss* swim-up fry (14-d post-hatch). Furthermore, additional exposures conducted with the alevin life-stage of *O. mykiss* (24-h post-hatch) yielded a 96-h LC50 of > 25 mg V/L, showing that swim-up *O. mykiss* fry were significantly more sensitive to V than the alevin life-stage (Figure 3.1b). The 96-h LC50s for *O. mykiss* fry was 14.8 mg V/L (Table 3.2). Compared to *O. mykiss*, *P. promelas* was approximately 3.8 times more sensitive to V, with a 96-h LC50 of 4.1 mg V/L (Table 3.2).
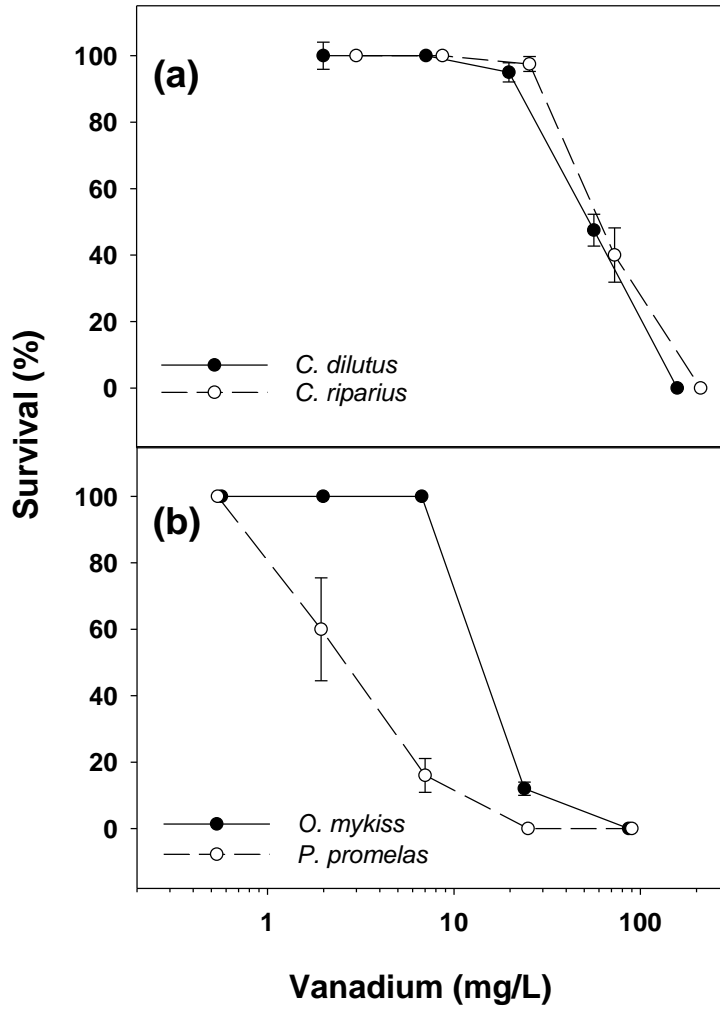
Figure 3.1. Acute lethality tests with standard laboratory species, *Chironomus dilutus* (96-h, Fig. 1a) and *Oncorhynchus mykiss* (96-h, Fig. 1b), and field representative species, *Chironomus riparius* (96-h, Fig. 1a) and *Pimephales promelas* (96-h, Fig. 1b). Each data point represents the percent survival (mean ± SE, *n*=4-5) at each V concentration.

Table 3.1. General water quality characteristics are for Athabasca River water (ARW), reconstituted Athabasca River water and test solutions from all V toxicity tests. Data are mean± SD calculated over all relevant test periods, except for Athabasca River water which are ranges reported by RAMP (2011).

Water source or water from specific tests	DO[1] (mg/L)	Temperature (°C)	Conductivity (µS/cm)	pH	Ammonia (mg/L)	Alkalinity (mg/L as CaCO₃)	Total hardness (mg/L as CaCO₃)	DOC[3] (mg/L)
Culture water[2]	8.0 ± 0.2	23.4 ± 0.7	420 ± 2	8.4 ± 0.1	0.1 ± 0.2	113 ± 20	150 ± 23	
Athabasca River Water			250-290	8.0-8.5		85-110	90-120	
Reconstituted ARW[4]			287 ± 3			101 ± 1	110 ± 2	
Benthic invertebrates								
Chironomus dilutus[5]	8.4 ± 0.1	23.1 ± 0.2	382 ± 89	8.1 ± 0.2	0.2 ± 0.2	96 ± 8	117 ± 8	4.6 ± 2.3
Chironomus dilutus[6]	8.1 ± 0.2	23.2 ± 0.3	332 ± 16	8.2 ± 0.1	0.2 ± 0.2	96 ± 10	120 ± 6	3.6 ± 1.3
Chironomus riparius[5]	7.9 ± 0.3	23.8 ± 0.3	406 ± 135	7.9 ± 0.1	0.4 ± 0.3	91 ± 6	99 ± 5	
Chironomus riparius[6]	8.1 ± 0.4	23.4 ± 0.3	350 ± 23	8.0 ± 0.1	1.1 ± 0.8	93 ± 6	104 ± 7	
Freshwater fish								
Oncorhynchus mykiss[5]	9.8 ± 0.2	13.1 ± 0.0	297 ± 2	8.0 ± 0.1	0.05 ± 0.04	89 ± 1	108 ± 2	
Oncorhynchus mykiss[6]	9.8 ± 0.1	13.2 ± 0.3	283 ± 5	8.0 ± 0.1	0.4 ± 0.6	95 ± 3	109 ± 3	2.5 ± 0.3
Pimephales promelas[5]	8.2 ± 0.2	24.0 ± 0.3	303 ± 33	8.0 ± 0.2	0.07 ± 0.16	86 ± 3	92 ± 10	
Pimephales promelas[6]	8.6 ± 3.2	24.0 ± 0.3	331 ± 12	8.1 ± 0.1	0.2 ± 0.2	92 ± 3	106 ± 5	

[1] DO = dissolved oxygen.

[2] Culture water = carbon-filtered, bio-filtered municipal water (mean ± SD; *n*=14).

[3] DOC = dissolved organic carbon (mean ± SD; *n*=16).

[4] Reconstituted ARW (mean ± SD; *n*=3).

[5] Acute 4-d chironomid (*n*=33-66) and fish (*n*=33-66) toxicity tests; measured in triplicates at days 0, 2, and 4.

[6] Chironomid partial lifecycle test (*n*=130-300) and 28-d fish toxicity tests (*n*=89-309); measured in triplicate at test start, weekly throughout test period, and at test end.

Table 3.2. Acute and chronic vanadium toxicity data (mg/L) for chironomids and juvenile fish generated in the present study.[1]

Test Species	Acute 96-h LC50 (lethality)	Chronic test duration (days)	Chronic endpoint	Chronic toxicity thresholds		
				EC20[2]	EC50	LC50[3]
Chironomus dilutus	52.0 (43.4, 62.2)	14	Growth	11.5 (3.2, 19.2)	36.3 (30.7, 42.7)	54.2 (-)
		Lifecycle[4]	Emergence	8.3 (4.8, 11.5)	32.4 (27.5, 40.5)	NA
Chironomus riparius	63.2 (53.4, 74.7)	10	Growth	12.4 (0.6, 26.1)	37.3 (14.6, 46.3)	43.5 (40.5, 46.8)
		Lifecycle[4]	Emergence	9.0 (6.4, 13.1)	17.4 (13.7, 20.6)	NA
Oncorhynchus mykiss	14.8 (13.1, 16.6)	28	Growth	3.7 (3.5, 4.2)	6.0 (5.5, 7.2)	4.3 (3.7, 4.9)
Pimephales promelas	4.0 (3.3, 4.8)	7	Survival	NA	NA	1.0 (0.8, 1.1)
		28	Growth	0.8 (0.8, 0.8)	1.2 (1.2, 1.2)	0.5 (0.4, 0.6)

[1] Numbers between brackets represent the 95% confidence intervals.

[2] Concentration estimated to cause an effect to 20% (EC20) or 50% (EC50) of the individuals of the species tested.

[3] Median lethal concentration.

[4] Exposure duration covered chironomid larvae (2nd instar) and adult life stages (a 40-d exposure period for *C. dilutus* and a 30-d exposure period for *C. riparius*).

NA = Not applicable.

3.5.4. Chronic effects of V on chironomids

Mean larval survival in control treatments for the initial 14-d old *C. dilutus* and 10-d *C. riparius* exposure periods were both 100%, exceeding the minimum acceptable criterion of ≥ 70% (Benoit et al., 1997; Environment Canada, 1997). The concentration-response relationship for larval survival prior to adult emergence showed survival to decrease significantly at the highest V concentrations of 51.1 and 74.9 mg V/L for *C. dilutus* and *C. riparius*, respectively. However, only 40% mortality was obtained for *C. dilutus* when exposed to 51.1 mg V/L. Therefore, the 14-d LC50 was > 48.6 mg V/L, however a value of 52.3 mg V/L was obtained based on the extrapolation of the 14-d concentration-response curve (logistic, r^2= 0.99). Based on the higher 96-h LC50 for *C. riparius*, higher V concentrations were selected for the chronic study yielding a 10-d LC50 of 43.5 mg V/L (Table 3.2). Survival in the remaining treatments for both *Chironomus* species remained ≥ 90%.

Larval growth, as dry weight, was also assessed for the chironomid larvae surviving the 10-14-d exposure periods (Figure 3.2b). Compared to larval survival, larval growth was a more sensitive indicator of V toxicity for both *Chironomus* species. The dry weights obtained in the control treatments for *C. dilutus* and *C. riparius*, were 2.7 ± 0.2 and 1.2 ± 0.3 mg per test organism, respectively, exceeding the recommended minimum dry weight at the end of a 10-d test of 0.6 mg per test organism (Environment Canada, 1997). Relative to the control treatment, *C. dilutus* growth was significantly inhibited at concentrations ≥ 16.7 mg V/L (by ≥25.9%) and *C. riparius* at V concentrations ≥ 30.8 mg/L (by ≥36.9%) (p<0.05) (Figure 3.2b). The 14-d EC50 for *C. dilutus* and *C. riparius* growth was 36.3 and 37.3 mg V/L, respectively (Table 3.2). Emergence of adult chironomids was also significantly inhibited in the presence of V in the high mg/L range. The percent emergence of adult *C. dilutus* and *C. riparius* in the control treatments were 95 ± 3% and 97.5 ± 3%, respectively, by the end of each test. Adult emergence was found to decrease with increasing concentrations of V and was a slightly more sensitive endpoint for V toxicity than either larval survival or growth (Table 3.2). Pupation was greatly reduced at the highest tested V concentrations (51.1 mg V/L) for *C. dilutus*; however, this reduction was likely a result of

reduced larval survival (Figure 3.2a). The remaining *C. dilutus* treatments had pupation rates of > 90%.

Many of the pupae present at 16.7 and 51.1 mg V/L did not emerge as adults. The estimated EC20 and

EC50 for *C. dilutus* were 8.3 and 32.4 mg V/L, respectively (Table 3.2). Unlike *C. dilutus*, *C. riparius*

only achieved a pupation rate of $30 \pm 9\%$ and $3 \pm 3\%$ at 30.8 and 74.9 mg V/L, respectively. Prior to

pupation, larvae in the 30.8 mg V/L treatment were found to leave their cases and die on the sediment

surface. The calculated EC20 and EC50s for *C. riparius* were 9.0 and 17.4 mg V/L, respectively (Table

3.2).

The acute-to-chronic ratios (ACRs) for the two midge species were calculated based on the acute

LC50 and chronic EC20 for the most sensitive endpoint, adult emergence. The ACRs were 6.3 and 7.0 for

C. dilutus and *C. riparius*, respectively.
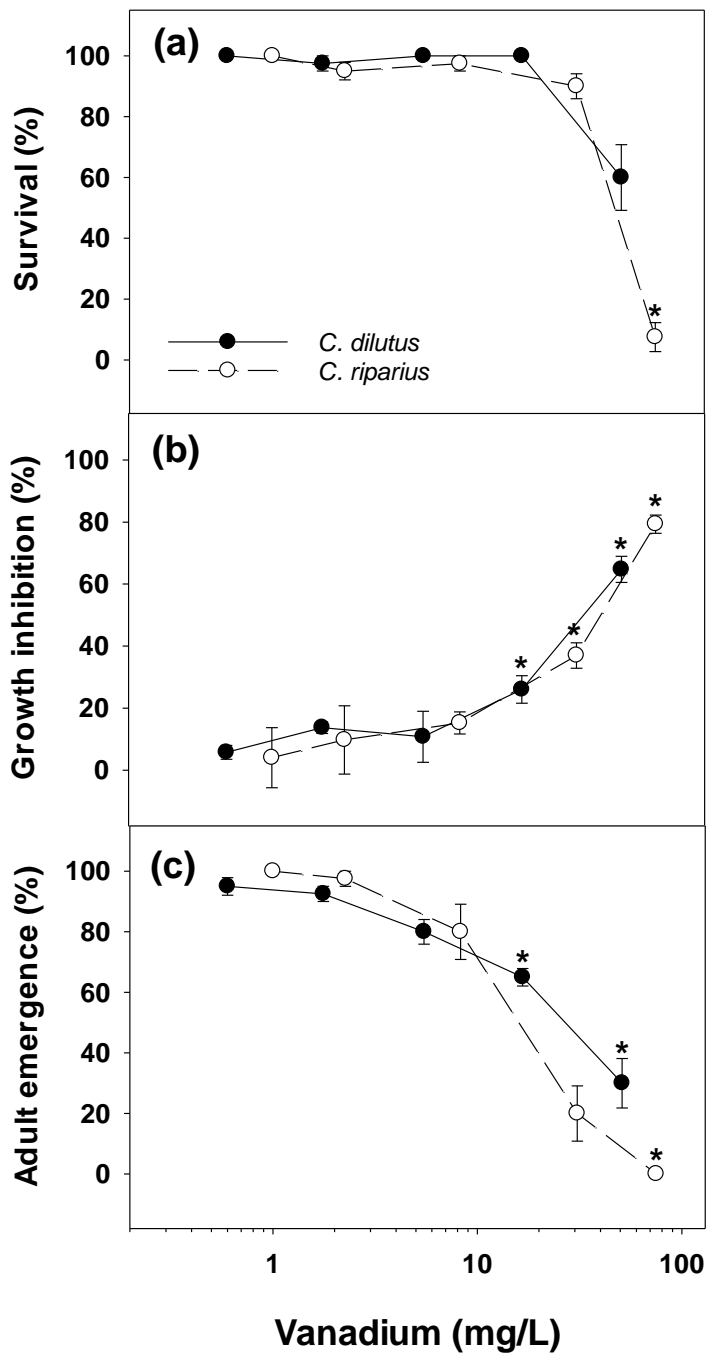
Figure 3.2. Survival (% Fig. 3.2a), growth inhibition (%, Fig. 3.2b) and emergence (%, Fig. 3.2c) for *Chironomus dilutus* and *Chironomus riparius* following 30-40-d exposure periods starting with second-instar larvae. Each data point represents the mean ± SE (*n*=4) at each V concentration. [*] Significantly different from the respective control (One way ANOVA, Tukey's test, *n*=4, p ≤ 0.05).

3.5.5. Chronic effects of V on fish

There were significant concentration-dependent decreases in survival during exposure of V to *O. mykiss* and *P. promelas* over a 28-d test period, beginning at 24-h post-hatch for both species (Figure 3.3).. Survival in the control treatments were 98 ± 2% for both *O. mykiss* and *P. promelas*, meeting acceptable test guidelines (Environment Canada, 2011). The survival of *O. mykiss* was substantially reduced at V concentrations ≥ 9.25 mg/L (98%; Figure 3.3a); the 28-d LC50 was 4.3 mg V/L (Table 3.2). Similar to the acute studies, *P. promelas* were more sensitive to chronic V exposure than *O. mykiss*. Survival was significantly reduced at V concentrations ≥ 1.91 mg V/L (100%; Figure 3.3b) and survival was only 40 ± 3% at 0.58 mg V/L by day 28 (Figure 3.3b). Overall, *P. promelas* was approximately 8-times more sensitive to V than *O. mykiss*, with a 28-d LC50 of 0.51 mg V/L (Table 2). At V concentrations < 1.91 mg/L and < 9.25 mg/L for *P. promelas* and *O. mykiss*, respectively, survival remained above 90% for the duration of each test. Fish growth (dry weight and length) were assessed for surviving fish following exposures to V. Overall, growth inhibitions for both dry weight and length were negligible at concentrations ≤ 0.58 and 2.46 mg V/L for *P. promelas* and *O. mykiss*, respectively. The 28-d EC50s for *O. mykiss* and *P. promelas* growth were 6.0 and 1.2 mg V/L, respectively (Table 3.2). The ACRs for fish, based on the acute LC50 and the EC50 for growth, were 2.3 and 4.3 in *O. mykiss* and *P. promelas*, respectively.
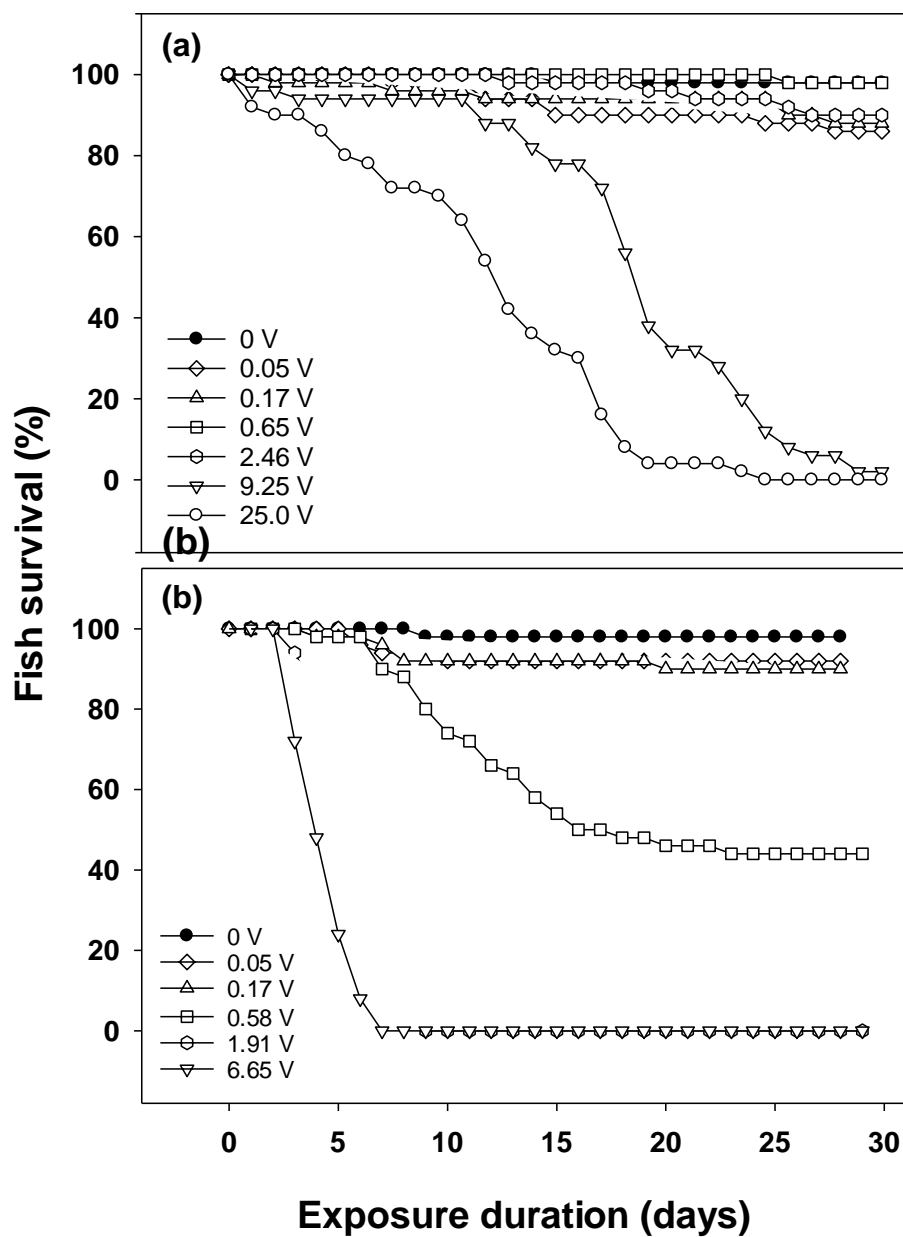
Figure 3.3. Survival of *Oncorhynchus mykiss* (Fig. 3.3a) and *Pimephales promelas* (Fig. 3.3b) over the course of a 28-d exposure period to increasing concentrations of V (mg/L).

Table 3.3. Acute vanadium toxicity data used to generate the acute species sensitivity distribution.

Test Species	Age	Endpoint	V (mg/L)	Geometric mean	Rank	Reference
Ceriodaphnia quadrangula	< 24 h	2-d LC50[2]	0.6		1	Schiffer and Liber (submitted)
Daphnia dentifera	< 24 h	2-d LC50	0.88		2	Schiffer and Liber (submitted)
Ceriodaphnia dubia	< 24 h	2-d LC50	0.97		3	Puttaswamy, unpublished
Hyalella azteca		4-d LC50	1.5		4	Lee et al. (2013)
Simocephalus serrulatus	< 24 h	2-d LC50	1.72		5	Schiffer and Liber (submitted)
Daphnia pulex	< 24 h	2-d LC50	2.17		6	Schiffer and Liber (submitted)
Gasterosteus aculeatus	Adult	4-d LC50	3.17		7	Gravenmier et al. (2005)
Danio rerio	Adult	4-d LC50	4		8	Beusen and Neven (1987)
Pimephales promelas	< 1 dph[1]	4-d LC50	4.02		9	This Study
Daphnia magna	< 24 h	2-d LC50	4.1		10	Beusen and Neven (1987)
Xyrauchen texanus	7-29 dph	4-d LC50	5.3	4.21	11	Hamilton (1995)
	102-116 dph		3.35			
Gila elegans	4-19 dph	4-d LC50	8.8	5.52	12	
	100-110 dph		3.46			
Ptychocheilus lucius	8-15 dph	4-d LC50	7.8	5.61	13	
	155-110 dph		4.04			
Salvelinus fontinalis	Alevin	4-d LC50	24		14	Ernst and Garside (1987)
	Yearling (18g)		7			
Poecilia reticulate	Adult	4-d LC50	8		15	Beusen and Neven (1987)
Oncorhynchus mykiss	2-2.5 g	4-d LC50	6.71	8.59	16	Stendahl and Sprague (1982)
	Fingerling		6.4			Stendahl and Sprague (1982)
	14 dph		14.8			This Study
Jordanella floridae	Adult (130 mg)	4-d LC50	11.2		17	Holdway and Sprague (1979)
Catotomus latipinnis	Fry	4-d LC50	11.5		18	Hamilton and Buhl (1997)
Oncorhynchus tschawtyscha	Fry	4-d LC50	16.5		19	Hamilton and Buhl (1990)
Coregonus clupeaformis	Adult	4-d LC50	17.4		20	Giles et al. (1979)
Pristina leidyi		2-d LC50	30.8		21	Smith et al. (1991)
Chironomus dilutus	2nd instar	4-d LC50	52.0		22	This Study
Chironomus riparius	2nd instar	4-d LC50	63.2		23	This Study

[1] dph = days post-hatch.

[2] LC50 = Lethal concentration to 50% of test population.

Table 3.4. Chronic vanadium toxicity data used to generate the chronic species sensitivity distribution.

Test Species	Age	Endpoint	V (mg/L)	Geometric mean	Rank	Reference
Diatoma elongatum		7-10-d LOEC[2]	0.10		1	Nalewajko et al. (1995b)
Ankistrodesmus falcatus		7-10-d LOEC	0.10		2	Nalewajko et al. (1995b)
Daphnia dentifera	< 24 h	21-d EC50[3]	0.13		3	Schiffer and Liber (submitted)
Ceriodaphnia quadrangula	< 24 h	8-d LC50[4]	0.45		4	Schiffer and Liber (submitted)
Daphnia pulex	< 24 h	21-d LC50	0.46		5	Schiffer and Liber (submitted)
Pimphales promelas	< 1dph[1]	28-d LC50	0.51		6	This Study
Ceriodaphnia dubia	< 24 h	7-d LC50	0.55		7	Puttaswamy et al. (2010)
Hyalella azteca	1-11 days old	7-d LC50	0.40	0.69	8	Borgmann et al. (2005)
		14-d LC50	1.2			Lee et al. (2013)
Jordanella floridae	7 dph	28-d LC50	1.13		9	Holdway and Sprague (1979)
Salvelinus fontinalis	Alevins	30-d LOEC	1.7		10	Ernst and Garside (1987)
Daphnia magna	< 24 h	21-d LC50	2.0	1.84	11	Beusen and Neven (1987)
	< 24 h	23-d LC50	1.70			Van Leeuwan (1987)
Danio rerio		7-d LC50	2.3		12	Beusen and Neven (1987)
Carassuis auratus		7-d LC50	2.45		13	Knudston (1979)
Scenedesmus quadricauda		12-d EC50	2.23	3.03	14	Fargasova et al. (1999)
		3-d EC50	4.12			Schiffer and Liber (submitted)
Oncorhynchus mykiss	Juvenile (2-2.5 g)	7-d LC50	3.7	3.14	15	Stendahl and Sprague (1982)
	Fingerlings	14-d LC50	1.95			Giles et al. (1979)
	< 1 dph	28-d LC50	4.3			This Study
Pseudokirchneriella subcapitata		3-d EC50	3.24		16	Schiffer and Liber (submitted)
Poecilia reticulate		7-d LC50	3.30		17	Beusen and Neven (1987)
Scenedesmus obliquus		7-10-d LOEC	5.09		18	Nalewajko et al. (1995b)
Chironomus riparius	2nd instar	30-d EC50	17.4		19	This Study
Chironomus dilutus	2nd instar	40-d EC50	32.4		20	This Study
Anabaena flos-aqueae		7-10 d LOEC	50.94		21	Nalewajko et al. (1995b)

[1] dph = Days post-hatch.

[2] LOEC = Lowest observed effect concentration.

[3] EC50 = Effect concentration to 50% of test population.

[4] LC50 = Lethal concentration to 50% of test population.

3.5.6. Species sensitivity distributions

The SSD plots and associated HC5 estimates were generated from source data collected from the peer-reviewed literature, as well as the present and previous studies conducted in our laboratory (Figure 3.4; Table 3.3 and 3.4). The data included for each distribution are based on the most sensitive endpoints reported for each species and include LC50 values (i.e., mortality) and EC50/20 values (e.g., growth, reproduction, and emergence inhibition). For SSD development, Canadian WQGs require studies from plants (i.e., algae), invertebrates and fish, and require a minimum of 10 data points for appropriate guideline formation (CCME, 2011). The acute SSD (Figure 3.4a; Table 3.3; 23 data points) represents the toxicity data for invertebrates and fish, whereas the chronic SSD (Figure 3.4b; Table 3.4; 21 data points) contains plant, invertebrate and fish data. The calculated HC5s for the acute and chronic distributions were 0.64 mg V/L (95% CIs: 0.54-0.76 mg/L) and 0.09 mg V/L (95% CIs: 0.06-0.12 mg V/L), respectively (Table 3.5). These values were obtained based on the best-fit curve, which was in both cases determined to be the log-logistic distribution. This distribution produced the lowest A^2 result for the four tested models using the Anderson-Darling goodness-of-fit test.

Table 3.5. Species sensitivity distribution (SSD) fitting parameters and test results for acute and chronic V toxicity data.

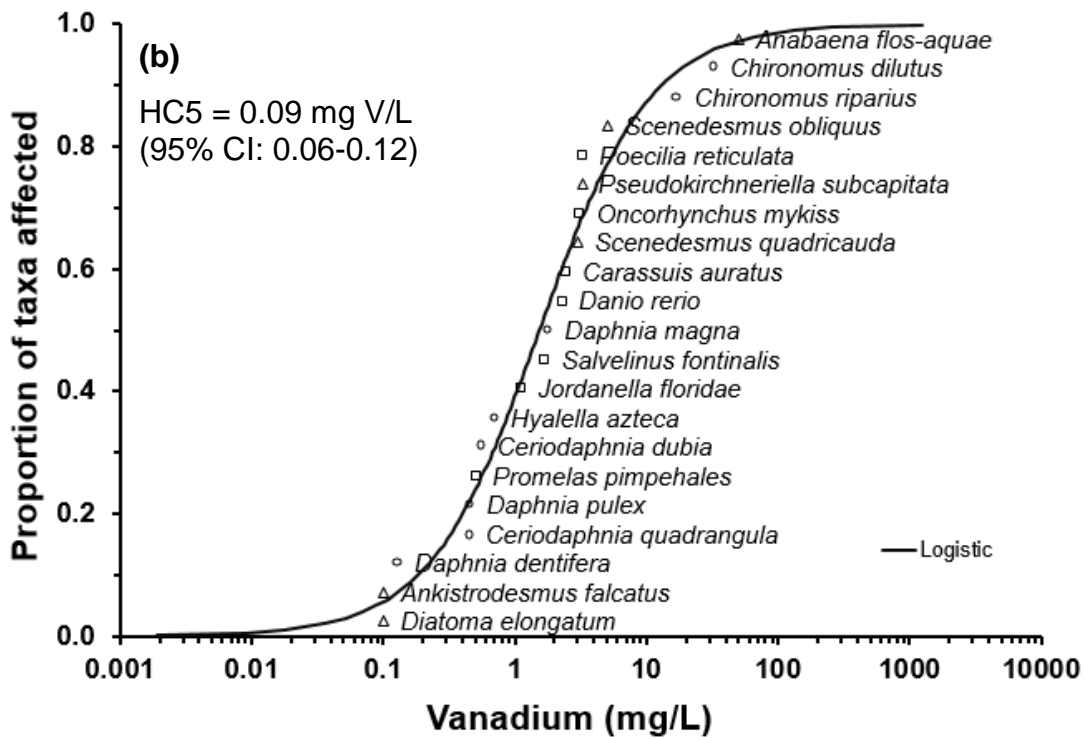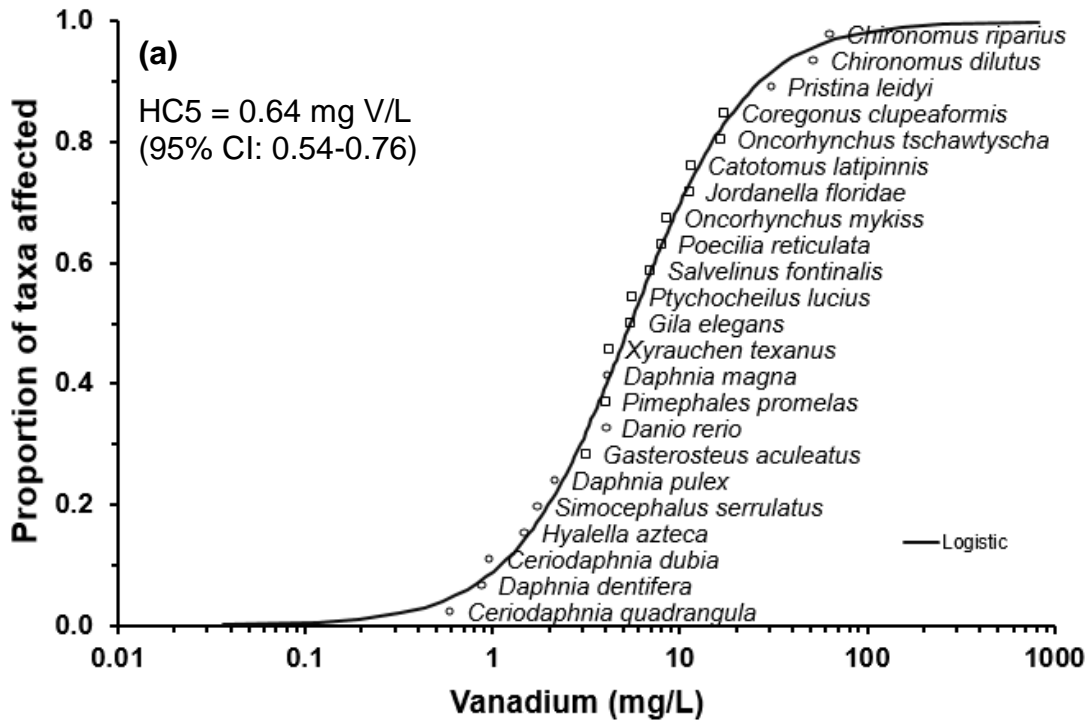| SSD	HC5 (mg/L)	Goodness of fit test (A^2)[a]	Model
Acute	0.73	0.16	Log-Normal
	0.64	0.15	Log-Logistic
	0.39	0.71	Extreme Value
	1.06	0.36	Gumbel
Chronic	0.11	0.38	Log-Normal
	0.09	0.32	Log-Logistic
	0.06	1.37	Extreme Value
	0.24	1.13	Gumbel

[a] Anderson statistic.

Figure 3.4. Species sensitivity distributions for V toxicity to aquatic species: (a) acute data, and (b) chronic data fitted using a log-logistic model. ○ = invertebrates, □ = fish, and Δ = algae species.

3.6. Discussion

This study investigated the toxicity of V to select benthic macroinvertebrates and fish and revealed significant impairment of long-term survival and insect emergence at V concentrations in the 0.5 to 37.3 mg/L range (Table 3.2). The toxicity threshold values obtained were higher than previous estimates obtained for zooplankton and phytoplankton species under similar experimental conditions (Schiffer and Liber, submitted). At reported effect concentrations and water quality conditions (DO 7.9-9.8 and pH 7.9-8.2), the dominant V species in solution were the soluble mono- and dimeric V(V) oxyanions (HVO_4^{2-} and $H_2VO_4^-$). These observations were expected based on another study conducted under similar water characteristics, which additionally reported oxidation of V(IV) to V(V) to occur fairly rapidly in exposure waters (Jenson-Fontaine et al., 2014). Furthermore, $H_2VO_4^-$ represented the majority of V(V) species (55-80%) across all exposure concentrations, as was the case in earlier studies (Holdway and Sprague, 1979; Schiffer and Liber, submitted; Stendahl and Sprague, 1982). Similarity in toxicity between the two ionic species (HVO_4^{2-} and $H_2VO_4^-$) has been documented in past studies (Stendahl and Sprague, 1982). A modeled increase in polymeric V species ($H_2VO_7^{3-}$, $V_4O_{12}^{4-}$, and $V_5O_{15}^{5-}$) was greatest in the acute chironomid studies (28-36%), where high V concentrations were present in solution (17-30 mg V/L); in addition to pH and redox conditions, the concentration of V is also important in its speciation (Crans et al., 1998). Little is known about the toxicity of polymeric V(V) species; however, mono- and divanadate species are favored more for uptake and are therefore likely more toxic (Miramand and Unsal, 1978; Ringelband and Hehl, 2000).

In addition to the work presented here, one other study has investigated the acute toxicity of V to a benthic oligochaete, *Pristina leidyi*, with results consistent with those reported here for *C. dilutus* and *C. riparius*. Smith et al. (1991) reported a 48-h LC50 of 30.8 mg/L for *P. leidyi*, exposed under water quality conditions similar to those used here (Table 3.3). Other evaluated invertebrate species included the epibenthic amphipod, *Hyalella azteca*, which was more sensitive to V than sediment-dwelling organisms. The 96-h LC50 for *H. azteca* was 1.5 mg V/L (Lee et al., 2013), whereas Borgmann et al. (2005) reported

a more sensitive 7-d LC50 estimate of 0.4 mg V/L (Table 3.3). In terms of acute toxicity, *P. leidyi* and the

two chironomid species were the least sensitive to V in water-only exposures (Figure 3.4a; Table 3.3).

However, it is important to note that exposures were primarily through the overlying water. In aquatic

environments, true sediment-dwelling organisms are also exposed to contaminants via the pore water and

sediment, important sinks for many contaminants. Baker et al. (2012) observed increased V

concentrations in chironomids exposed to oil sands coke used as a substrate in microcosm experiments.

Therefore, the potential exists for chironomids to be exposed to higher V concentrations through sediment

pore water than overlying water.

Other studies have investigated the chronic toxicity of V to benthic marine species, including the

polychaete, *Nereis diversicolor*, the littoral crab, *Carcinus maenas,* and the mussel, *Mytilus*

galloprovincialis (Miramand and Unsal, 1978). The 9-d LC50s for these species were 10, 35, and 65 mg

V/L, respectively. These results suggest that marine invertebrates display similar sensitivity to V as

freshwater species. Similar to previous studies with zooplankton where lethality was the most sensitive

endpoint (Schiffer and Liber, submitted), long-term survival was also more sensitive than growth

endpoints for *H. azteca* (Lee et al., 2013). However, our studies with chironomids found larval growth

and adult emergence to be more sensitive than long-term survival. Pupation and successful emergence

rates were inhibited at V concentrations ≥ 16.7 (31.6% reduction) and 8.3 mg/L (18.0% reduction) for *C.*

dilutus and *C. riparius*, respectively, but concentrations > 30 mg V/L were required for larval survival to

be affected (Figure 3.2c). This is in general agreement with results from studies with *C. dilutus* exposed

to other metals (e.g. uranium). Usually, under stress conditions, chironomids will allocate more resources

to detoxification processes rather than to growth and therefore prolong their time to adult emergence

(Liber et al., 1996). In this study, male and female emergence was delayed at the highest V treatments,

likely as a result of reduced growth during the larval stage.

Several studies have investigated the acute toxicity of V to freshwater fish of multiple life stages;

however, many species reported in the literature are not representative of northern Alberta (Table 3.3).

Regardless, many of the acute toxicity estimates found in the literature are similar to the values reported here. In this study, acute toxicity to *P. promelas* was approximately 3.5 times greater than for *O. mykiss* (Table 3.2). This difference could be due to inherent physiological differences between the two species, and also to differences in body size. The 96-h LC50 of 4.0 mg V/L for *P. promelas* was similar to LC50s for other small fish models, such as *Gasterosteus aculeatus*, *Danio rerio*, and the swim-up fry of *Xyrauchen texanus*, *Gila elegans*, and *Ptychocheilus lucius*, which ranged from 3.2 to 4.0 mg V/L (Table 3.3) (Beusen and Neven, 1987; Gravenmeir et al., 2005; Hamilton, 1995). However, *O. mykiss* fry (96-h LC50 of 14.8 mg V/L) displayed similar toxicity to other salmonids such as *Salvelinus fontinalis*, *O. tshawtyscha*, and *Coregonus clupeaformis*, which had 96-h LC50s ranging from 7 to 17.4 mg V/L (Table 3.3) (Ernst and Garside, 1987; Giles et al., 1979; Hamilton and Buhl, 1990). Such allelometric relationships are not uncommon. Generally, the acute toxicity of many water-borne metals occur at the gill-water interface through ion imbalance, and this general mode of action has also been proposed for V oxyanions (Cantley et al., 1979; Nechay, 1986; Rehder, 1992). As a potent inhibitor of the Na, K-ATPase pump, V could interfere with ion regulation at the gill level; however, this has not been researched thoroughly (Cantley et al., 1979; Rehder, 1992). Additional research on the mechanistic toxicity of V would be needed to determine if there are physiological differences in sensitivity to V among fish species.

The tolerance of the alevin (yolk-sac) life stage to dissolved V was quite apparent in this study, as well as from other studies reported in the literature. The alevin life stage was substantially more tolerant to V than the exogenously feeding swim-up fry, an observation seen in both the acute and chronic studies. In acute exposures, swim-up *O. mykiss* fry (14-d post-hatch) were approximately 2 times more sensitive than the alevin life stage. Ernst and Garside (1987) reported similar observations using *S. fontinalis*, with the alevin life stage (96-h LC50 of 24 mg V/L) being 3.4 times more tolerant than yearling fish (96-h LC50 of 7 mg V/L) (Table 3.3). Hamilton (1995) also reported larval stages (1-d post-hatch) to be more tolerant to acute V toxicity than juvenile life stages (100 to 150-d post-hatch) for three different fish species. Similarly, another study observed early juvenile white sturgeon to be more sensitive to copper

than the yolksac stage (Vardy et al., 2013). These differences could be due to differences in uptake mechanisms between the two life stages. Gills in the larval stage are largely undeveloped, so ion exchange takes place primarily through the skin, whereas in swim-up fry it occurs through the now fully-functional gills. Furthermore, when exogenous feeding begins, the swim-up stage experiences increased respiration because they are more physically active, which further increases the amount of water moving through the gills (Vardy et al., 2013). Therefore, similar to other ion exchange-disrupting metals, acute V toxicity could be predicted based on the relationship between the gill surface area and body size and/or volume (Zimmer et al., 2004).

The 28-d LC50 estimated for *O. mykiss* from the present study was in agreement with other toxicity estimates found in the published literature (Table 3.2; Table 3.4). For example, Giles et al., (1979) and Stendahl and Sprague (1982) reported 7-d and 14-d LC50s of 3.6 and 2.0 mg V/L, respectively, for juvenile *O. mykiss* under similar water quality conditions as reported for this study. Furthermore, Stendahl and Sprague (1982) evaluated V toxicity to *O. mykiss* at different pHs, concluding that similar toxicity existed between pH 7 and 9, and that toxicity was only reduced at pH 6. This reduction in toxicity was due to the polymerization of V(V) oxyanions to the relatively non-toxic decavanadates species at pH < 7. In the present study, *P. promelas* was 8.4-fold more sensitive to V than *O. mykiss* (Table 3.2). The chronic LC50s for *P. promelas* (7-d LC50 of 1 mg V/L) reported was also consistent with published data for other small fish species, such as the 7-d LC50s of 2.3, 2.5 and 3.3 mg/L for *D. rerio*, *Carassuis auratus*, and *Poecilia reticulata*, respectively, and the 28-d LC50 of 1.13 mg/L for 7-d post-hatch *Jordenella floridae* larvae (Table 3.4) (Beusen and Neven, 1987; Knudston, 1979; Holdway and Sprague, 1979). Similar to what was observed in chronic tests with zooplankton (Schiffer and Liber, submitted), sublethal effects of V on fish hatch, growth (i.e., weight and length), reproduction (i.e., daily egg production) and the transition from yolk-sac to swim-up fry were largely negligible in fish surviving higher V exposure concentrations (Ernst and Garside, 1987; Giles et al., 1979; Holdway and Sprague, 1979). In fact, *P. promelas* exposed to 0.58 mg V/L were larger than control fish at 28-d.

However, increased fish growth was likely due to the reduced fish volume per beaker at this treatment which resulted from increased mortality (66%) compared to the control group. Currently, little is known with regard to V accumulation in biological tissues. Some studies have found accumulation of V in invertebrates and fish to be low in water-borne exposures, reporting bioconcentration factors in the range of 13.5 to 27.5 L/kg wet weight (Holdway et al., 1982; Ringelband and Hehl, 2000). Jensen-Fontaine et al. (2014) observed a concentration-dependent increase of V in *H. azteca* tissue following 14-d water-only exposures, with V concentrations ranging from 0.2 to 1.0 mg/L. At V concentrations ranging from 3 to 22.5 mg/L, target organs included the gill and kidney where increased incidences of gill hyperplasia, and hydropic and hyaline degeneration in the kidney have been observed (Giles et al., 1979). Previous research with mice and rats has shown V to increase peroxidation *in vivo*, possibly through the formation of reactive oxygen species (Donaldson et al., 1984; Valko et al., 2005).

The V toxicity dataset prior to this project contained few species that would be considered relevant to aquatic ecosystems in northern Alberta. A comparison of responses to V between commonly used laboratory test species (*Daphnia magna* and *Ceriodaphnia dubia*) and zooplankton species collected within the vicinity of the Athabasca Oil Sands (AOS) region (*D. dentifera, Simocephalus serrulatus,* and *C. quadrangula*), found the more regionally-representative species to be comparable or slightly more sensitive to V (Schiffer and Liber, submitted). Prior to this study, macroinvertebrates were poorly represented in the acute and chronic V database. Unfortunately, field-collected chironomid species could not be brought into culture successfully (i.e. they could be kept alive and raised to adults, but viable egg masses could not be obtained). Regardless, the Chironomidae family dominates the macroinvertebrate community in many aquatic systems in the AOS region, with genera such as *Tanytarsus, Procladius, Polypedilum, Paralauterborniella*, and *Stempellinella* being the most common (RAMP, 2015). It is difficult to predict how these species would respond to V, but based on the relative tolerance of the two *Chironomus* species tested it is unlikely that data for these species would have substantially influenced the HC5 estimates.

In this study, V SSDs were constructed from data obtained from the peer-review literature and data generated here (Table 3.3 and 3.4; Figure 3.4). There were 23 acute toxicity values covering 13 fish species, including three salmonid species, six zooplankton, one epi-benthic invertebrate, and three macroinvertebrate species (Figure 3.4; Table 3.3). For the chronic SSD, there were 21 toxicity values covering six fish species, including two salmonid species, five zooplankton, one epi-benthic invertebrate, two macroinvertebrates, and six unicellular algae species (Figure 3.4; Table 3.4). For the studies included, each had fairly similar water quality conditions with pH ranging from 7 to 9, a range known to have similar V speciation. Data collected on various metals by Birge et al. (1978, 1980) is not included in the United States Environmental Protection Agency (US EPA) water criteria derivation for a number of metals (i.e. aluminum, arsenic, cadmium, copper, and selenium) and is thus not included here either. Additionally, other investigations have not included these data either because of the low toxicity thresholds that have proven to be non-reproducible (De Schampelaere et al., 2010; McPherson et al., 2014). The 28-d LC50s reported for *O. mykiss* by Birge et al. (1978) and Birge et al. (1980) were 25-fold lower than our 28-d estimates beginning with 1-d post-hatch alevins. Another study not included in the acute SSD presented here was by Fargasova (1997) who calculated a 96-h LC50 of 0.24 mg V/L for *Chironomus plumosus*, a value more than 200-fold lower than estimates for other chironomids presented here, as well as for other benthic macroinvertebrates. Exclusion was based on a poor study design (only one concentration used), poor test conditions, unreported control responses, and unknown age and health of the field-collected larvae used. Overall, cladocerans and diatoms were among the most sensitive organisms to V (Figure 3.4). Based on the data collected, the acute and chronic HC5s for V were calculated to be 0.64 and 0.09 mg V/L, respectively (Figure 3.4; Table 3.5). The chronic HC5 is 15 times greater than the interim provincial WQG of 0.006 mg V/L currently used in Ontario, Canada, which is based on the most sensitive algae toxicity value (LOEC) with an applied safety factor of 16.6 (MoEE, 1994). If EC50s from this and a previous study (Schiffer and Liber, submitted) were substituted for EC20s in the chronic SSD, as recommended for developing water quality benchmarks, the HC5 would decrease from 0.09 to 0.08 mg V/L (95% CIs: 0.06, 0.10) based on a log-logistic distribution. A Canadian

federal environmental quality guideline for V was released in May 2016, after the present research had been completed. It presents a chronic freshwater guideline value of 0.12 mg/L and a marine water quality guideline value of 0.005 mg/L (Canada, 2016). It should, however, be noted that these values are based on data identified up to June 2010 and used a much more limited V toxicity database than the ones presented here. Despite only relying on 8 toxicity values, the WQG of 0.12 mg/L is not too dissimilar to the chronic HC5 of 0.09 mg/L presented here.

The acute SSD revealed cladoceran species (*Daphnia dentifera* and *Ceriodaphnia* species) to be the most sensitive of the species investigated to V toxicity. In the chronic SSD, the diatom, *Diatoma elongatum*, and the green algae, *Ankistrodesmus falcatus* were also very sensitive to V toxicity, in addition to cladoceran species (Figure 3.4). Surprisingly, these toxicity estimates were more than one order of magnitude lower than those presented here for green algae, but their duration of exposure was also approximately two times longer. Currently, V concentrations in coke leachates and coke-treated process-affected waters from the AOS have been reported as > 1 mg/L (Puttaswamy et al., 2010; Zubot et al., 2012). Furthermore, so far only acute toxicity has only been investigated with respect to coke-treated process-affected waters, yielding no toxicity to *O. mykiss* or *D. magna* in 96-h and 48-h exposures, respectively (Zubot et al., 2012). However, based on our toxicity data, it is possible that long-term survival of cladocerans would be negatively impacted in aquatic ecosystems chronically exposed to ≥ 1 mg V/L (Schiffer and Liber, submitted). Furthermore, growth of diatoms, which are prevalent in northern Alberta, and survival of regionally-relevant fish such as *P. promelas*, would likely also be impaired at V concentrations observed to leach from coke. Although relatively tolerant to V, benthic organisms, including native chironomid species and Aeshnid dragonflies, were found to accumulate high concentrations of V when exposed to coke substrates (Baker et al., 2012). Consequently, development of V water quality guidelines for the AOS region will have important implications for future utilization of coke and/or remediation of coke leachates and for protection of local aquatic life.

CHAPTER 4:

GENERAL DISCUSSION

4.1. Project summary and rationale

Increased concentrations of vanadium (V) in aquatic environments are most likely to occur through anthropogenic sources, including increased industrial activities (i.e. crude oils and coals), the combustion of fossil fuels, generated effluent from steel, chemical and polymer industries, use in phosphate fertilizers, and in landfills from an increasing presence in rechargeable batteries (Huang et al., 2015; Imtiaz et al., 2015). However, this research focused specifically on the abundance of V in bitumen mined from the Athabasca Oil Sands (AOS) region in northeastern Alberta, Canada. During bitumen upgrading, V is removed from bitumen and enriched into the by-product, petroleum coke. Some oil sands companies, such as Syncrude Canada Ltd. (SCL), produce enormous volumes of coke during this process. Currently, the coke produced must be stored on-site of SCL operations. Until recently, little was known of the potential environmental risks associated with coke. Recent studies have found laboratory-generated coke leachates, from petroleum coke collected from SCL stockpiles, to contain elevated concentrations of dissolved vanadium (V) (>14 mg/L in leachate from a lysimeter) (Puttaswamy et al., 2010). Additionally, when coke was exposed to weathering processes (i.e. pH, dissolved oxygen, and freeze-thaw cycles), V was consistently identified as a metal of concern because of initial high concentrations in the pore water and overlying water (Squires, 2005). These experiments were conducted based on the scenario that coke could be used as a sediment substrate in reclaimed wetlands and indicated the potential of coke to leach toxicologically-relevant levels of metals, especially V, into the environment if coke was used as a

sediment substrate. Surrounding aquatic environments could be at risk to increased V, depending on how this by-product is stored and/or utilized in the future.

Another by-product that needs to be reclaimed to ensure sustainable operation in the AOS region is oil sands process-affected water (OSPW). Because of its well-characterized aquatic toxicity, OSPW cannot be released back into the natural environment and is contained on-site in growing tailings ponds (Allen, 2008). Biodegradation of the chronically toxic organic fraction of OSPW is not feasible within a reasonable time-frame, and thus, alternative treatment methods have been proposed, such as adsorption onto petroleum coke (Gamal El-Din et al., 2011; Small et al., 2012a, b; Zubot et al., 2012). Using coke as a treatment method would be an ideal approach because it uses one by-product, available in large supply on-site, to treat another by-product, which will be important in sustaining future oil sands operations.

Investigations into the use of coke to treat OSPW showed >90% removal of complex organic acids within OSPW and a reduction in acute toxicity to standard test-species (Zubot et al., 2012). However, an inverse relationship is shown for V in coke-treated OSPW. As the organic contaminant fraction in coke-treated OSPW decreased, V increased to high concentrations, initially measuring as high as 1-5 mg/L (Pourrezaei et al., 2014b; Small et al., 2012a, b). The high V concentrations in coke (>1000 mg/kg) and the water chemistry of OSPW (pH $\geq$8) favors the leaching of V from coke into coke-treated OSPW (Lopez and Lo Monaco, 2004; Zubot et al., 2012). Very recently, a Canadian federal environmental water quality value for V was released. A chronic freshwater value of 0.12 mg V/L was presented (Canada, 2016). However, this value is based on a limited toxicity database for V (8 chronic toxicity data-points) and has not undergone a peer-review process. In the absence of water quality guidelines (WQGs) specific for V the AOS region, increased V in coke-treated OSPW could negatively impact water quality, if the purpose of treatment was to re-release the coke-treated OSPW back into the local aquatic environment.

This research compared the toxicity of V (as vanadate oxyanions) to freshwater organisms of various trophic levels that were either commonly-used laboratory species, or species more regionally-representative of northern Alberta, to determine if commonly-used laboratory species would suffice for V water quality guideline derivations in the AOS region. Moreover, because coke has been shown to leach toxicologically-relevant concentrations of V upon contact with water, having water quality benchmarks specific to the AOS region would be advantageous to site managers for future use and/or reclamation plans for on-site coke. Overall, inclusion of regionally-relevant species should strengthen the toxicity database for V and allow for development of appropriate V WQGs by regulators for use in the AOS region and elsewhere in Canada.

4.1.1. Species sensitivity distributions

In freshwaters, the acute toxicity (24-96-h LC50s) of V to freshwater organisms has been documented for 14 different freshwater organisms in the published literature. However, many of the species represented are largely surrogate fish species, with only two species (*Salvelinus fontinalis* and *Coregonus clupeaformis*) considered relevant to the northern Alberta environment (Table 1.2). Furthermore, the dataset only contained two invertebrate species (*Daphnia magna* and *Pristina leidyi*) (Table 1.1). Extrapolation of an acute HC5 value based on an SSD constructed from these species, would give an estimate of 1.53 mg V/L.

Incorporation of the data generated in this research increased the acute toxicity database from 14 to 23 freshwater organisms, with an improved representation of freshwater invertebrates. Overall, freshwater organisms from different taxonomic groups included crustaceans, amphipods, annelids, insects, and fish. Three cladoceran species (*Daphnia dentifera, Ceriodaphnia quadrangula*, and *Simocephalus serrulatus*) were field-collected from the AOS region and an insect and fish species, which were not locally-collected but known to occur in the region, were obtained from elsewhere. Acute toxicity values ranged from 0.6 to 63.2 mg/L for invertebrates and 3.2 to 17.4 mg/L for fish. Toxicity thresholds calculated for *Oncorhynchus mykiss* in this study were similar to values reported in the literature (Ernst

and Garside, 1987; Giles et al., 1979; Hamilton and Buhl, 1990). Endpoints for *C. dubia* and the epi-benthic amphipod *H. azteca*, not published in the literature, were also included in developing the acute SSD (Lee et al., 2013; Puttaswamy, unpublished data). When data (24-96-h LC50s) were combined into an SSD, the preferred model fit was a logistic distribution. In addition to having the lowest Anderson-Darling statistic (A^2), indicating good fit, the logistic distribution also had the best fit at the lower end of the tail of the distribution where the most sensitive species lie. The calculated HC5 from the logistic distribution was (HC5, 95% CIs): 0.64 mg V/L (0.54, 0.76). With the exception of *C. quadrangula*, which had a 48-h LC50 of 0.60 mg/L, the toxicity estimates for all species exceeded this potential benchmark value for V. One data point (96-h LC50 for *Chironomus plumosus*) was excluded from the dataset. Fargasova (1997) calculated a 96-h LC50 of 0.24 mg V/L for field-collected *C. plumosus* larvae. Use of larvae of various ages, stressful testing conditions (e.g., no substrate or food, and low dissolved oxygen) and testing at only one V concentration likely resulted in 96-h LC50s magnitudes lower than those for similar species, such as *C. dilutus* and *C. riparius*. Furthermore, Squires (2005) did not observe any toxicity to *C. tentans* when exposed to coke leachates containing > 1 mg/L in 10-d toxicity tests. According to the SSD, cladoceran species and *H. azteca* exhibited the greatest sensitivity to V; fish were moderately sensitive to V with smaller fish models slightly more sensitive than larger salmonid species, whereas benthic chironomids and oligochaetes were very tolerant to dissolved V (Figure 3.5a).

Chronic toxicity studies (≥24-h for algae and ≥ 7-d exposures for invertebrates and fish) were fairly abundant in the peer-review literature and covered 13 freshwater organisms. Endpoints from those studies and from those reported here were primarily LC/EC50s, with a few lowest observed effect concentrations (LOECs) reported as well. Algae appeared well represented in the literature, with toxicity estimates available for three taxonomic groups (Cyanobacteria, Chlorophyta and Bacillariophyta). The toxicity of V, reported as 7-10-d LOECs for cell division, greatly varied from 0.1 to 50.9 mg V/L for diatoms and cyanobacteria, respectively. This variation could be due to differences in phosphate regulation among these species, thus affecting V uptake (Nalewajko et al., 1995b). However, a similar

study observed the opposite, with toxicity estimates ranging from 0.1 to 1 mg/L for cyanobacteria and diatoms, respectively (Lee et al., 1979). Data for three invertebrate species were present in the literature, including the zooplankton *D. magna* and *C. dubia*, and the amphipod *H. azteca*; however, benthic macroinvertebrates species were absent (Table 1.1). Of the five fish species, only one (*S. fontinalis*) could be considered representative of northern Alberta fish species, whereas the others (*Carassuis auratus, Poecilia reticulata, Jordanella floridae*, and *O. mykiss*) may be considered surrogate species (Table 1.2).

The study presented here generated new toxicity thresholds for algae, invertebrates and fish, and increased the chronic V toxicity database from 13 to 21 species. Data for freshwater organisms now include species of algae, crustaceans, amphipods, insects and freshwater fish. Two cladoceran species (*D. dentifera* and *C. quadrangula*) were field-collected from the AOS region, and a benthic macro-invertebrate (*C. riparius*), a green algae (*Scenedesmus quadricauda*) and a fish (*Pimephales promelas*) were chosen to represent local species in the AOS region. Interestingly, *S. quadricauda* was less sensitive (72-h EC50 of 4.1 mg/L) than *Ankistrodesmus falcatus* (7-10-d LOEC of 0.1 mg/L), although both are green algae. Nalewajko et al. (1995b) found green algae to be intermediate in their response to V compared to blue-green algae and diatoms; however, their green algae was reported to be as sensitive as the diatom species, *Diatoma elongatum*. Lee et al. (1979) also reported low toxicity estimates for green algae (*Chlorella pyrenoidosa,* 7-d MATC of 0.32 mg V/L). The EC20s from the present research were combined with data (LC/EC50s and LOECs) published in the peer-review literature in order to develop the chronic SSD. A logistic distribution was the preferred fit for this SSD, both in terms of goodness-of-fit (lowest A^2) and visual assessment of the lower left-end of the distribution tail. The chronic HC5 was calculated to be (HC5, 95% CIs): 0.09 mg/L (0.06, 0.12). The toxicity of V for the species tested here all exceeded the generated HC5 value. Therefore, it appears that an HC5 of 0.09 mg/L would be an appropriate interim water quality benchmark for all aquatic life for an indefinite period of exposure. It should be noted that studies conducted by Birge (1978) and Birge et al. (1980) were excluded from the

toxicity database due to poor reproducibility of their study using other metals. One example is the metal molybdenum (Mo) (De Schamphelaere et al., 2010). These studies by Birge are also excluded from the US EPA database for the derivation of certain water quality criteria for different metals. Overall, similar to the acute SSD described above, cladocerans again exhibited the greatest sensitivity to V, along with *H. azteca* and smaller fish (i.e. *P. promelas, C. auratus, P. reticulata*, and *J. floridae*). Again, benthic macro-invertebrates and cyanobacteria were most tolerant taxa to high concentrations of dissolved V in freshwaters.

4.1.2. Standard and regionally-relevant test species

An important objective of this thesis research was to determine if regionally-relevant aquatic species representative of the AOS region would be more or less sensitive to dissolved V than more commonly-used, standard laboratory species. Usually when WQGs are established in Canada they are largely based on a recommended set of commonly-used laboratory cultured, standardized test organisms (CCME, 2007b). This results in derivation of federal guidelines that apply to a broad range of aquatic ecosystems across the country. Surrogate species chosen to represent trophic levels commonly present in aquatic ecosystems, such as *Pseudokirchneriella subcapitata, Daphnia magna, Chironomus dilutus,* and *Oncorhynchus mykiss* have standardized culturing and test protocols ensuring organism health and that standardized testing methods are reproducible across multiple laboratories (CCME, 2007b). However, these species are not necessarily present in or representative of organisms in a specific area. Species compositions and their sensitivities to contaminants vary greatly among different aquatic ecosystems. Therefore, it is important to understand if toxicity thresholds from standard species can provide adequate protection for species in local environments at risk, or if they are either over- or under-protective (Chen et al., 2016; CCME, 2003; Jin et al., 2015).

It is impossible to evaluate the sensitivity of all species that make up a biological community of a specific aquatic ecosystem; therefore a few species were selected, either through field-collection from a reference pond on the SCLs mine lease, or obtained from other laboratory cultures. Test species were

selected based on their occurrence in the AOS region. Unfortunately, the culturing of a field-collected benthic invertebrate species was unsuccessful, so a different *Chironomus* species was chosen in its place. A species from the *Tanytarsini* genus, reported to be abundant in the AOS region (RAMP, 2015), would have been an ideal species. However, the *Chironomus* genus is also present in the AOS region, but at lower abundances, which was the primary reasoning for selecting *C. riparius* as a regionally-representative species. With the exception of freshwater fish (*O. mykiss* and *P. promelas*) and cladoceran species (*D. dentifera* and *D. pulex*), differences in sensitivity among comparable species tested were not observed. Greater sensitivity was observed in the field-collected cladoceran, *D. dentifera*, than either of the standard test-species, *D. pulex* and *D. magna*. *Daphnia dentifera* was significantly more sensitive ($\geq$ 2.5 x) than *D. pulex* and *D. magna* in both acute and chronic tests. Although one of the most commonly-used organisms in aquatic toxicity testing, *D. magna* is often reported to be among the most tolerant zooplankton species to contaminants (Koivisto et al., 1992; Mount and Norberg, 1984; Versteeg et al., 1997). It is also unlikely to occur in most aquatic ecosystems at risk. The smaller *D. dentifera* was similar to *Ceriodaphnia dubia* in sensitivity to V with 48-h LC50s of 0.88 and 0.97 mg V/L, respectively. However, *S. serrulatus* (48-h LC50 of 1.72 mg/L), which is a larger zooplankton species, had similar sensitive to *D. pulex* (48-h LC50 of 2.17 mg/L), but was significantly more sensitive than *D. magna* (48-h LC50 of 4.1 mg/L) (Table 2.2). Differences were not observed in V sensitivity in acute and chronic exposures using either comparable green algal species (*Pseudokirchneriella subcapitata* and *S. quadricauda*) or benthic macroinvertebrates (*C. dilutus* and *C. riparius*).

Fathead minnow (*P. promelas*) were significantly more sensitive to V than the standard salmonid species, *O. mykiss*. Typically, the opposite has been observed. In this V SSD, smaller fish species, such as goldfish (*C. auratus*), three-spine stickleback (*G. aculeatus*), zebra fish (*D. rerio*), and guppies (*P. reticulata*) appeared at the lower left-end of the SSD tails compared to larger species, including brook trout (*S. fontinalis*), Chinook salmon (*O. tschawtyscha*), and whitefish (*C. clupeaformis*). In this research, fathead minnow was approximately 4 times more sensitive to V than rainbow trout. The toxicity estimates

reported for rainbow trout in this study (96-h LC50 of 15 mg/L and 28-d LC50 of 4 mg/L) were similar to values reported for Chinook salmon (96-h LC50 of 16.5 mg/L) and yearling brook trout (10-d LC50 of 4 mg/L). Therefore, the addition of data for species, such as fathead minnow, known to occur in the AOS region is valuable when developing V guidelines for protection of aquatic life in northern Alberta. Overall, the chronic HC5 of 0.09 mg V/L presented here was similar to the chronic federal environmental quality guideline of 0.12 mg V/L for freshwaters, thus suggesting that surrogate species would have likely sufficed in deriving V water quality guideline values.

4.1.3. Significance to water quality guidelines

Water quality guidelines are values recommended for the protection of aquatic ecosystems from harmful concentrations of contaminants. Below established WQG values, adverse environmental effects are not expected to occur (CCME, 2007b). Generally, generic WQGs are derived by regulators that can then be applied to a broad geographical scale. Despite the fact that establishing generic WQGs is a more effective management tool for regulators, many contaminants are confined to specific regional areas, where these generic WQGs often fail to take into account physiochemical, biological, and species sensitivity differences (CCME, 2003). Thus, WQGs can be either under- or over-protective to the local aquatic community present. Vanadium is unlikely to occur as a contaminant on a broad scale. Instead V is more likely to be elevated in areas of increased industrial activity, such as the AOS region, where it potentially could impact water quality in local aquatic ecosystems. Therefore, developing more region- or site-specific WQGs could improve protection in such areas.

Despite the importance and use of SSDs, there are some uncertainties associated with this approach (Duboudin et al., 2004; Forbes and Forbes, 1993; Newman et al., 2000; Wheeler et al., 2002). Many of those uncertainties are difficult to address, including overlooking species interactions at a community and/or ecosystem level, exclusion of keystone species, and a large dependence on single-species laboratory data. Furthermore, disagreement on the quality and quantity of data required for development of short- and long-term SSDs exists among various protocols. While some protocols suggest

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as little as five species are needed for development of a SSD, others recommend 10 to 15 species (Maltby et al., 2005; Wheeler et al., 2002). Newman et al. (2000) suggests using much higher numbers of toxicity data (i.e. a sample size anywhere from 15 to 55, averaging approximately 30), from a range of taxonomically-diverse species, than currently proposed by regulatory agencies. More toxicity data for a greater number of test-species provides greater confidence for fitting SSD models to derive HC5s. Furthermore, especially for long-term SSDs, HC5s are commonly calculated based on a mixture of toxicity endpoints. However, ideally EC10/20s are required for generating long-term HC5s, but often these values are under-reported in older toxicity studies. Many V toxicity studies were completed in the 1980-1990s. Duboudin et al. (2004) also recommends taking into account intra-species variation, in addition to inter-species variation.

To address some of these issues, this research attempted to include as many field-relevant species as possible in the generation of the SSDs. It can be difficult to culture field-collected organisms under controlled laboratory conditions for use in standardized, high quality tests. Commonly-used, standard test-species were often originally chosen because they were easy to culture and breed under laboratory conditions. However, an attempt to include regionally-relevant test-species into SSDs also expanded the diversity of species included into the model generation. Taxonomically diverse species, with a wide range of sensitivities to V were represented in the acute and chronic SSDs presented in this research. Compared to the limited toxicity database used to derive federal environmental quality guidelines, expanding the number and diversity of species could improve HC5 estimates for V (Canada, 2016). The quantity of data entered into the acute and chronic SSDs consisted of 23 and 21 data points, respectively, of either primary or secondary quality, which exceeds the numbers recommended by most protocols. Jin et al. (2015) recommend using a minimum of 20 species in SSDs. In such a case, each species represents 5% of the total, thus resulting in greater statistical power and reduced variability of the analysis. Rather than extrapolate separate acute and chronic HC5s for regionally-relevant and standard test-species, the regionally-relevant and standard toxicity data were pooled within SSDs to obtain effective acute and

chronic HC5 estimates. Also, this study did not take into account intra-species variation. However, multiple data points for a single species only came up twice: for *D. magna* and *O. mykiss*. The variation among toxicity thresholds for each of these two test-species was small and within a factor of three. Therefore, use of the geometric mean method should suffice in the SSD input data for those species.

Vanadium has been listed as an element of concern given its abundance at some contaminated sites, or in areas of increased industrial activities (i.e. AOS region), and because of its potential toxicity to aquatic biota (Langmuir et al., 2004). Moreover, toxicity estimates for V specific to the AOS region have been recommended by previous authors to better determine the proper storage, use and remediation options for petroleum coke (Baker et al., 2012; Puttaswamy and Liber, 2011; Squires, 2005). In order to properly evaluate the risk from elevated V concentrations in coke leachates and coke-treated OSPW, WQGs for V relevant to northern Alberta will need to be established. According to the acute and chronic HC5s calculated here, the initial concentrations of V released into coke leachates upon contact with water and into coke-treated OSPW (> 1 mg V/L) could adversely impact receiving aquatic systems downstream of discharge points with limited mixing zones. Therefore, pre-treatment steps should be taken, or increased storage time in engineered facilities allowed, in order to reduce V concentrations in treated waters prior to discharge. Overall, the toxicity data generated in this research will supplement the V toxicity database and should be included in the V toxicity database to strengthen WQG derivation for V for application in Alberta and elsewhere. An increased number of data points and species in acute and chronic SSDS will help reinforce HC5 estimates, which are ultimately established as guideline values.

4.2. Future work and recommendations

The research presented in this thesis helped to supplement the V toxicity database for dissolved V for both acute and chronic exposure scenarios. The inclusion of regionally-relevant aquatic organism to the northern Alberta environment will improve HC5 estimates to provide appropriate protection of local aquatic systems at increased risk of elevated V concentrations. However, further research is required to better understand potential risks of V to aquatic systems. A few areas are discussed below.

- The acute and chronic HC5s presented here of 0.64 and 0.09 mg V/L, respectively, may present an issue to oil sands industry depending on the storage and utilization of petroleum coke stockpiles in the future. It has been shown that if used as a treatment for oil-sands process-affected waters (OSPW), V can exceed concentrations of 1 mg/L. Pre-treatment steps would need to be taken prior to continuous re-release of newly coke-treated OSPW back into the natural environment, if this was the primary intention of treating OSPW. However, Zubot (2011) did find leached V to decline with increased coke-water residence times (6-8 months) due to the adsorption of V onto metal hydroxides present in the saturated coke deposits. More research would be needed to confirm this, as well as other pre-treatment steps that could be taken to reduce V concentrations in the coke-treated OSPW.

- This research only considered V toxicity under laboratory settings, which favored the speciation of vanadate oxyanions. However, once released into the environment, many environmental-modifying factors can further influence V speciation. More research into the environmental fate and speciation of V under water quality conditions present in northern Alberta, not considered here (i.e., organic matter, Fe and Mn oxyhydroxides, etc.), would be useful to compare exposure data to generated concentration-response data. Moreover, since sediments will likely be an important sink for V in aquatic systems, V toxicity via other exposure routes, such as sediment and/or dietary, will also be important areas for additional research.

- In Chapter 2, algal toxicity estimates were generated for two species of green algae (*Pseudokirchneriella subcapitata* and *Scenedesmus quadricauda*); however, given the variability of toxicity data for other taxonomic groups (i.e., Cyanobacteria and Bacillariophyta) it would be worthwhile to expand on this research. This could be especially important if coke were to be used as a sediment substrate in reclaimed wetlands, because algae are generally early colonizers. Furthermore, diatom species are very prevalent in

oligotrophic aquatic systems of northern Alberta and thus could be better represented in chronic SSDs.

- In Chapter 3, the acute and chronic SSDs were presented, constructed with a mixture of surrogate and regionally-relevant aquatic organisms. Overall, a large range of taxonomically diverse species are now represented in both SSDs. Given the similarities in our chronic HC5s and the newly derived chronic federal water quality guideline, it could be assumed that overall regionally-representative aquatic organisms are well represented in the SSD. However, algal species lie in the lower left end of the distribution. More in-depth investigation of dissolved V toxicity to algal species other than Chlorophyta could influence their position, which in turn could influence HC5 estimates.

- In Chapter 3, toxicity estimates for survival and growth were generated for larval and swim-up fry of *Oncorhynchus mykiss* and *Pimephales promelas*. Furthermore, literature toxicity data only exist for these life-stages as well. Since early life-stages are generally more sensitive, additional toxicity studies could be completed looking at V toxicity from fertilization and onwards. These earlier life-stages of fish could be more susceptible to dissolved V toxicity.

- This research presented the toxicity of V to important apical endpoints (i.e., survival, growth, reproduction, and emergence of insects); however, there is still uncertainty regarding the mechanistic behavior of V and the mechanisms through which it elicits toxicity to aquatic organisms. More investigation into a mechanistic mode of action may help explain the differences in sensitivity of aquatic organisms to dissolved V, especially for freshwater fish species.

4.3. Conclusions

Based on the findings of this research, the overall conclusions reached were:

i. Of all the aquatic organisms tested in this research, the zooplankton species were the most sensitive to aqueous V exposures. However, based on the generated chronic species sensitivity distribution, previous literature values indicate a few algae species (e.g., *Diatoma elongatum, Ankistrodesmus falcatus*) may be more sensitive to V than zooplankton.

ii. Based on the regionally-relevant species tested here, regionally-relevant and standard test species generally had similar sensitivities to V toxicity, especially for species of green algae, benthic invertebrates, and fish. However, one field-collected zooplankton species (*Daphnia dentifera*) was slightly more sensitive (within a factor of 4) to V than a related, more commonly used test-species (*Daphnia pulex*).

iii. Acute and chronic hazardous concentrations to 95% of the species tested, estimated from species sensitivity distributions, were 0.64 and 0.09 mg V/L, respectively.

iv. Addition of regionally-relevant test species to the V database will help improve future V guidelines for protection of aquatic communities in northern Alberta.

REFERENCES

Alberta Energy Regulator (AER). 2015. ST98-2015: Alberta's Energy Reserves 2014 and
  Supply/Outlook 2015-2024, Calgary, AB, Canada.

Alberta Energy Utilities Board (AEUB). 2002. Statistical series 2002-98: Alberta's reserves 2001 and
  supply/demand outlook 2002-2011, Calgary, AB, Canada.

Ahlf W., Drost, W., Heise, S. 2009. Incorporation of metal bioavailability into regulatory frameworks –
  metal exposure in water and sediment. *Journal of Soils and Sediments*, *9,* 411-419.

Aide, M. 2005. Geochemical assessment of iron and vanadium relationships in oxic soil environments.
  Soil & Sediment Contamination, 14, 403-416.

Allen, E. W. 2008. Process water treatment in Canada's oil sands industry: I. Target pollutants and
  treatment objectives. *Journal of Environmental Engineering and Science*, *7*(2), 123-138.

Amorim, F. A., Welz, B., Costa, A. C., Lepri, F. G., Vale, M. G. R., Ferreira, S. L. 2007. Determination
  of vanadium in petroleum and petroleum products using atomic spectrometric
  techniques.*Talanta*,*72*(2), 349-359.

Anderson, J., Wiseman, S. B., Moustafa, A., Gamal El-Din, M., Liber, K., Giesy, J. P. 2012. Effects of
  exposure to oil sands process-affected water from experimental reclamation ponds on *Chironomus
  dilutus*. *Water Research*, *46*(6), 1662-1672.

Agency for Toxic Substances and Disease Registry (ASTDR). 1992. Toxicological profile for vanadium
  and compounds. U.S. Government Printing Office: 1662-636-281.
  (http://www.atsdr.cdc.gov/toxprofiles/tp58.pdf)

American Society for Testing and Materials (ASTM). 1997. Standard guide for conducting *Daphnia
  magna* life –cycle toxicity tests. Report nr ASTM/E1193-97.

Baker, L.F., Ciborowski, J.J.H., MacKinnion, M.D. 2012. Petroleum coke and soft tailings sediment in
  constructed wetlands may contribute to the uptake of trace metals by algae and aquatic
  invertebrates. *Science of the Total Environment*, *414*, 177-186.

Baker, L. F. 2007. The effects of petroleum coke amendments on macrophytes and aquatic invertebrates
  in northern Alberta, Canada constructed wetlands. PhD Thesis. University of Windsor, Windsor,
  ON, Canada.

Barceloux, D. G. 1999. Vanadium. *Clinical Toxicology, 37,* 265-278.

Benoit, D.A., Sibley, P.K., Juenemann, J.L., Ankley, G.T. 1997. Chironomus tentans life-cycle test: design and evaluation for assessing toxicity of contaminated sediments. *Environmental Toxicology and Chemistry, 16*, 1165-1176.

Bell, M. V., Sargent, J. R. 1979. The partial purification of sodium-plus-potassium ion-dependent adenosine triphosphatase from the gills of *Anguilla anguilla* and its inhibition by orthovanadate. *Biochemical Journal*, *179*(2), 431-438.

Beusen, J. M., Neven, B. 1987. Toxicity of vanadium to different freshwater organisms. *Bulletin of environmental contamination and toxicology*, *39*(2), 194-201.

Birge, W.J. 1978. Aquatic toxicology of trace elements of coal and fly ash. In J.H. Thorp, J.W. Gibbons (eds) Energy and Environmental Stress in Aquatic Systems: Selected Papers From a Symposium Held at Augusta, Georgia, November 2-4, 1977. Dep Energy Symp Ser 48: 219-240.

Birge, W.J., Black, J.A., Westerman, A.G. 1980. Aquatic toxicity tests on inorganic elements occurring in oil shale. In Gale, C. (ed.)., Oil Shale Symposium: Sampling, Analysis and Quality Assurance. United States Environmental Protection Agency, Cincinnati, OH, USA. pp. 519-534.

Borgmann, U., Couillard, Y., Doyle, P., Dixon, D.G. 2005. Toxicity of sixty-three metals and metalloids to *Hyalella azteca* at two levels of water hardness. *Environmental Toxicological and Chemistry*, *24*, 641-652.

Bossuyt, B. T., Janssen, C. R. 2005. Copper toxicity to different field-collected cladoceran species: intra- and inter-species sensitivity. *Environmental Pollution*, *136*(1), 145-154.

Breit, G.N., Wanty, R.B. 1991. Vanadium accumulation in carbonaceous rocks: a review of geochemical controls during deposition and diagenesis. *Chemical Geology, 91,* 83-97.

Bridges, C.C., Zalups, R.K. 2005. Molecular and ionic mimicry and the transport of toxic metals. *Toxicology and Applied Pharmacology*, *204* (3), 274-308.

Calace N., Petronio, B.M., Pietroletti, M. 2006. Metal bioavailability: how does its significance change in the time? *Annali di Chimica*, *96*.

Canada. Environment and Climate Change Canada. 2016. *Canadian Environmental Protection Act,* 1999. Federal Environmental Quality Guidelines: Vanadium. Available from < http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=48D3A655-1>

Canadian Council of Ministers of the Environment (CCME). 1991. Appendix IX – A protocol for the derivation of water quality guidelines for the protection of aquatic life (April 1991). In: Canadian Water Quality Guidelines, Canadian Council of Resource and Environment Ministers, 1987. Prepared by the Task Force on Water Quality Guidelines.

Canadian Council of Ministers of the Environment (CCME). 1999. Canadian environmental quality guidelines for the protection of aquatic life: Polycyclic aromatic hydrocarbons (PAHs).In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Canadian Council of Ministers of the Environment (CCME). 2003. Canadian water quality guidelines for the protection of aquatic life: guidance on the site-specific application of water quality guidelines in Canada: Procedures for Deriving Numerical Water Quality Objectives. In: Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment, 1999, Winnipeg.

Canadian Council of Ministers of the Environment (CCME). 2007a. A protocol for the derivation of water quality guidelines for the protection of aquatic life 2007. In: Canadian Environmental Quality Guidelines, 1999, Canadian Council of Ministers of the Environment, 1999, Winnipeg.

Canadian Council of Ministers of the Environment (CCME). 2007b. Canadian water quality guidelines for the protection of aquatic life: Summary table. Updated December, 2007. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Cantley L.C., Aisen, P. 1979. The fate of cytoplasmic vanadium. Implications on (Na,K)-ATPase Inhibition. *The Journal of Biological Chemistry, 254,* 1781-1784.

Cantley, L. C., Josephson, L., Warner, R., Yanagisawa, M., Lechene, C., Guidotti, G. 1977. Vanadate is a potent (Na, K)-ATPase inhibitor found in ATP derived from muscle. *Journal of Biological Chemistry*, *252*(21), 7421-7423.

Capella, L.S., Gefe, M.R., Silva, E.F., Affonso-Mitidieri, O., Lopes, A.G., Rumjanek, V.M., Capella, M.A.M. 2002. Mechanisms of vanadate-induced cellular toxicity: role of cellular glutathione and NADPH. *Archives of Biochemistry and Biophysics, 406*, 65-72.

Chasteen, N.D. 1983. The biochemistry of vanadium. *Structure and Bonding*, 53.

Chen, Y., Yu, S., Tang, S., LI, Y., Li, Y., Liu, H., Zhang, X., Su, G., Li, Bing, Yu, H., Giesey, J.P. 2016. Site-specific water quality criteria for aquatic ecosystems: A case study of pentachlorophenol for Tai Lake, China. *Science of the Total Environment, 541,* 65-73.

Chung, K., Janke, L., Dureau, R., Furimsky, E. 1996. Leachability of cokes from Syncrude stockpiles. *Environmental Science and Engineering,* 50-53.

Clemente, J.S., Fedorak, P.M. 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere, 60,* 585-600.

Crans, D.C., Amin, S.S., Keramidas, A.D. 1998. Chemistry of relevance to vanadium in the environment. In: Niragu, J.O. (Ed.), Vanadium in the Environment Part 1: Chemistry and Biochemistry. John Wiley and Sons, Inc., Toronto, pp. 73-91.

Crans, D.C., Smee, J.J., Gaidamauskas, E., Yang, L. 2004. The chemistry and biochemistry of vanadium and the biological activities exerted by vanadium compounds. *Chemical Rev*iews, *104* (2), 849-902.

De Beer, H., Coetzee, P.P. 1994. Vanadium speciation by ion chromatography. *Fresenuis' Journal of Analytical Chemistry, 348,* 806-809.

De Schamphelaere, K.A.C., Stubblefield, W., Rodriguez, P., Vleminckx, K. Janssen, C.R. 2010. The chronic toxicity of molybdate to freshwater organisms. I. Generating reliable effects data. *Science of the Total Environment, 408*, 5362-5371.

Donaldson, J., Hemming, R., LaBella, F. 1984. Vanadium exposure enhances lipid peroxidation in the kidney of rats and mice. *Canadian Journal of Physiology and Pharmacology, 63*(3), 196-199.

Duboudin, C., Ciffroy, P., Magaud, H. 2004. Effects of data manipulation and statistical methods on species sensitivity distributions. *Environmental Toxicology and Chemistry, 23*(2), 489-499.

Duyck, C., Miekeley, N., Porto da Silveira, C., Aucelio, R.Q., Campos, R.C., Grinberg, P., Brandao, G.P. 2007. The determination of trace elements in crude oil and its heavy fractions by atomic spectrometry. *Spectrochimica Acta Part B, 62,* 939-951.

Eggleton, J., Thomas, K. V. 2004. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environment International, 30*,973-980.

Environment Canada. 1990b. Biological test method: acute lethality test using rainbow trout (EPS1/RM/09). Ottawa, ON, Canada.

Environment Canada. 1990a. Biological test method: acute lethality test using '*Daphnia*' spp*.* (EPS1/RM/11). Ottawa, ON, Canada.

Environment Canada. 1992a. Biological test method: growth inhibition test using a freshwater alga (EPS1/RM/25). Ottawa, ON, Canada.

Environment Canada. 1997. Biological test method: test survival and growth in sediment using the larvae of freshwater midges (EPS1/RM/32). Ottawa, ON, Canada.

Environment Canada. 2007. Biological test method: test of reproduction and survival using the Cladocera *Ceriodaphnia dubia* (EPS1/RM/21). Ottawa, ON, Canada.

Environment Canada. 2011. Biological test method: test of larval growth and survival using Fathead Minnows (EPS1/RM/22) Second edition. Ottawa, ON, Canada.

Ernst, W.R., Garside, E.T. 1987. Lethal effects of vanadium to two life stages of brook trout *Salvelinus fontinalis* (Mitchill). *Canadian Journal of Zoology*, *65*, 628-634.

Fairbrother, A., Wenstel, R., Sappington, K., Wood, W. 2007. Framework for metals risk assessment. *Ecotoxicology and Environmental Safety*, *68*, 145-227.

Fargašová, A. 1997. Sensitivity of *Chironomus plumosus* larvae to V^{5+}, Mo^{6+}, Mn^{2+}, Ni^{2+}, Cu^+ metal ions and their combinations. *Bulletin of Environmental Contamination and Toxicology*, *59*, 956-962.

Fargašová, A., Bumbálová, A., Havránek, E. 1999. Ecotoxicological effects and uptake of metals (Cu^+, Cu^{2+}, Mn^{2+}, Mo^{6+}, Ni^{2+}, V^{5+}) in freshwater alga *Scenedesmus quadricauda*. *Chemosphere*, *38*(5), 1165-1173.

Fedorak, P. M., Coy, D. L. 2006. Oil sands cokes affect microbial activities. *Fuel*, *85*(12), 1642-1651.

Forbes, T.L., Forbes, V.E. 1993. A critique of the use of distribution-based extrapolation models in ecotoxicology. *Functional Ecology, 7,* 249-254.

Furimsky, E. 1998. Gasification of oil sand coke: Review. *Fuel Processing Technology, 56*(3), 263-290.

Gamal El-Din, M. G., Fu, H., Wang, N., Chelme-Ayala, P., Pérez-Estrada, L., Drzewicz, P., Martin, J. W., Zubot, W., Smith, D. W. 2011. Naphthenic acids speciation and removal during petroleum-coke adsorption and ozonation of oil sands process-affected water. *Science of the Total Environment*, *409*(23), 5119-5125.

Giles, M.A., Klaverkamp, J.F., Lawrence, S.G. 1979. The acute toxicity of saline groundwater and of vanadium to fish and aquatic invertebrates. Alberta Oil Sands Environmental Research Program. Final Report Sub. Project AF 3.2.1.

Giles M.A., Klaverkamp J.F. 1982. The acute toxicity of vanadium and copper to eyed eggs of Rainbow Trout (*Salmo gairdneri*). *Water Research*, *16*, 885-889.

Gravenmier, J.J., Johnston, D.W., Arnold, W.R. 2005. Acute toxicity of vanadium to the Threespine Stickleback, *Gasterosteus aculeatus*. *Environmental Toxicology*, 20, 18-22.

Grosell, M., Nielsen, C., Bianchini, A. 2002. Sodium turnover rate determines sensitivity to acute copper and silver exposure in freshwater animals. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *133*(1), 287-303.

Hamada T. 1998. High vanadium content in Mount Fuji groundwater and its relevance to the ancient biosphere. In: Nriagu J, ed. Vanadium in the environment. Part 1: Chemistry and Biochemistry. New York, NY, John Wiley and Sons, pp. 97-123.

Hamilton S.J. 1995. Hazard assessment of inorganics to three endangered fish in the Green River, Utah. *Ecotoxicology and Environmental Safety*, *30*, 134-142.

Hamilton S.J., Buhl K.J. 1990. Safety assessment of selected inorganic elements to fry of Chinook salmon (*Oncorhynchus tshawytscha*). *Ecotoxicology and Environmental Safety*, *20*, 307-324.

Hamilton S.J., Buhl K.J. 1997. Hazard evaluation of inorganics, singly and in mixtures, to Flannelmouth Sucker *Catostomus latipinnis* in the San Juan River, New Mexico. *Ecotoxicology and Environmental Safety*, *38*, 296-308.

Hamilton, M. A., Russo, R. C., Thurston, R. V. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science & Technology*, *11*(7), 714-719.

Harita, Y., Hori, T., Sugiyama, M. 2005. Release of trace oxyanions from littoral sediments and suspended particles induced by pH increase in the epilimnion of lakes. *Limnology and Oceanography*, *50*(2), 636-645.

Hayes, T.M.E., 2006. Examining the ecological effects of naphthenic acids and major ions on phytoplankton in the Athabasca oil sands region. MSc Thesis. University of Waterloo, Waterloo, ON, Canada.

He, Y., Wiseman, S. B., Zhang, X., Hecker, M., Jones, P. D., Gamal El-Din, M. G., Martin, J. W., Giesy, J. P. 2010. Ozonation attenuates the steroidogenic disruptive effects of sediment free oil sands process water in the H295R cell line. *Chemosphere*, *80*(5), 578-584.

Headley, J.V., Peru, K.M., Barrow, M.P. 2009. Mass spectrometric characterization of naphthenic acids in environmental samples: a review. *Mass Spectrometry Reviews, 28*, 121-134.

Hickey, C. W. 1989. Sensitivity of four New Zealand cladoceran species and *Daphnia magna* to aquatic toxicants. *New Zealand journal of marine and freshwater research*, *23*(1), 131-137.

Hilton, J.W., Bettger, W.J. 1988. Dietary vanadium toxicity in juvenile rainbow trout: a preliminary study. *Aquatic Toxicology, 12,* 63-71.

Hirayama, K., Kageyama, S., Unohara, N. 1992. Mutual separation and preconcentration of vanadium (V) and vanadium (IV) in natural waters with chelating functional group immobilized silica gels followed by determination of vanadium by inductively coupled plasma atomic emission spectrometry. *Analyst*, *117*(1), 13-17.

Hodgson, G.W. 1954. Vanadium, nickel, and iron trace metals in crude oils of western Canada. *Bulletin of the American Association of Petroleum Geologists*, *38*(12), 2537-2554.

Holdway, D.A., Sprague, J.B. 1979. Chronic toxicity of vanadium to flagfish. *Water Research*, *13,* 905-910.

Holdway, D.A., Sprague, J.B., Dick, J.G. 1982. Bioconcentration of vanadium in American flagfish over one reproductive cycle. *Water Research, 17*(8), 937-941.

Holleland, T., Towle, D.W. 1990. Vanadate but not ouabain inhibits Na++ K+-ATPase and sodium transport in tight crab gill (*Carcinus maenas*). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 96*(1), 177-181.

Holowenko, F.M., MacKinnon, M.D., Fedorak, P.M. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. *Water Research, 36*, 2843-2855.

Hope, B.K. 1997. An assessment of the global impact of anthropogenic vanadium. *Biogeochemistry, 37,* 1-13.

Huang, J., Huang, F., Evans, L., Glasauer, S. 2015. Vanadium: global (bio) geochemistry. *Chemical Geology, 417*, 68-89.

Imtiaz, M., Rizwan, M.S., Xiong, S., Li, H., Ashraf, M., Shahzad, S.M., Shahzad, M., Rizwan, M., Tu, S. 2015. Vanadium, recent advancements and research prospects: a review. *Environmental Internation, 80,* 79-88.

Jacob, F.S., Filby, R.H. 1983. Solvent extraction of oil-sand components for determination of trace elements by neutron activation analysis. *Analytical Chemistry*, 55(1), 74-77.

Jen, J., Wu, M., Yang, T.C. 1997. Simultaneous determination of vanadium (IV) and vanadium (V) as EDTA complexes by capillary zone electrophoresis. *Analytica Chimica Acta. 339*, 251-257.

Jensen-Fontaine, M., Norwood, W.P., Brown, M., Dixon, D.G., Le, X.C. 2014. Uptake and speciation of vanadium in the benthic invertebrate *Hyalella azteca. Environmental Science and Toxicology, 48*, 731-738.

Jin, X., Wang, Z.,Wang, Y., Lv, Y., Rao, K., Jin, W., Giesy, J.P., Leung, K.M.Y. 2015. Do water quality criteria based on non-native species provide appropriate protection for native species? *Environmental Toxicology and Chemistry, 34*(8), 1793-1798.

Kannel, P.R., Gan, T.Y. 2012. Naphthenic acids degradation and toxicity mitigation in tailings wastewater systems and aquatic environments: A review. *Journal of Environmental Science and Health, Part A, 47*(1), 1-21.

Khalaf, F., Literathy, P., Anderlini, V. 1982.Vanadium as a tracer of oil pollution in the sediments of Kuwait. *Hydrobiologia*, *91*, 147-154.

Knudston, B.K. 1979. Acute toxicity to vanadium to two species of freshwater fish. *Bulletin of Environmental Contamination and Toxicology*, *23*, 95-99.

Koivisto, S. 1995. Is *Daphnia magna* an ecologically representative zooplankton species in toxicity tests?. *Environmental Pollution*, *90*(2), 263-267.

Koivisto, S., Ketola, M., Walls, M. 1992. Comparison of five cladoceran species in short-and long-term copper exposure. *Hydrobiologia*, *248*(2), 125-136.

Langmuir, D., Chrostowski, P., Vigneault, B., Chaney, R. 2004. Issue Paper on the Environmental Chemistry of Metals. US Environmental Protection Agency, Risk Assessment Forum, Washington, DC.

Lee K., Nalewajko, C., Jack, T.R. 1979. Effects of vanadium on freshwater algae. Proceedings of the 5th Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fisheries and Marine Service Technical Report. 862: 297-310.

Lee, K., Muttray, A., McPherson, C., Elphick, J. 2013. Senstivity of Hyalella azteca to strontium, vanadium and potassium (PO). Proceedings of the 39th Annual Aquatic Toxicity Workshop, Sun Peaks, British Columbia, Sept. 30 – Oct. 3, 2012. Canadian Technical Report of Fisheries and Aquatic Sciences, 3030: 55.

Li, X.S., Le, X.C. 2007. Speciation of vanadium in oilsand coke and bacterial culture by high performance liquid chromatography inductively coupled plasma mass spectrometry. *Analytica Chimica Acta, 602,* 17-22.

Liber, K., Call, D.J., Dawson, T.D., Whiteman, F.W., Dillon, T.M. 1996. Effects of *Chironomus tentans* larval growth retardation on adult emergence and oviposting success: implications for interpreting freshwater sediment bioassays. *Hydrobiologia*, *323*, 155-167.

Lindstedt, K.D., Kruger, P. 1969. Vanadium concentrations in Colorado River basin waters. *American Water Works Association, 61*(2), 85-88.

Lopez, L., Lo Monaco, S. 2004. Geochemical implications of trace elements and sulfur in the saturate, aromatic and resin fractions of crude oil from the Mara and Mara Oeste fields, Venezuela. *Fuel, 83*, 365-374.

Lue, X., Johnson, X.D., Hook, J. 1998. Reaction of vanadate with aquatic humic substances: an ESR and [51]V NMR study. *Environmental Science & Technology, 32*(15), 2257-2263.

Maltby, L., Blake, B., Brock, T.C.M., Van Den Brink, P.J. 2005. Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. *Environmental Toxicology and Chemistry, 24*(2), 379-388.

McPherson, C.A., Lawrence, G.S., Elphick, J.R., Chapman, P.M. 2014. Development of a strontium chronic effects benchmark for aquatic life in freshwater. *Environmental Toxicology and Chemistry, 33(11)*, 2472-2478.

Meisch, H. U., Benzschawel, H., Bielig, H. J. 1977. The role of vanadium in green plants II. Vanadium in green algae – two sites of action. *Archives of microbiology*, *114*(1), 67-70.

Meisch, H. U., Bielig, H. J. 1975. Effect of vanadium on growth, chlorophyll formation and iron metabolism in unicellular green algae. *Archives of microbiology*, *105*(1), 77-82.

Minelli, L., Veschetti, E., Giammanco, S., Mancini, G., Ottaviani, M. 2000. Vanadium in Italian waters: monitoring and speciation of V (IV) and V (V). *Microchemical Journal*, *67*, 83-90.

Miramand, P., Unsal, M. 1978. Acute toxicity of vanadium to some marine benthic and phyto-planktonic species. *Chemosphere, 7*(10), 827-832.

Ministry of Environment and Energy (MoEE). 1994. Water Management: Policies, Guidelines, Provincial Water Quality Objectives of the MoEE. Ontario, PIBS 3303E.

Moford, J.L., Emerson, S.R., Breckel, K.J., Kim, S.H. 2005. Diagenesis of oxyanions (V, U, Re, and Mo) in pore waters and sediments from a continental margin. *Geochimica et Cosmochimica Acta*, *69*(21), 5021-5032.

Moskalyk, R. R., Alfantazi, A. M. 2003. Processing of vanadium: a review. *Minerals Engineering*, *16* (9), 793-805.

Mount, D. I., Norberg, T. J. 1984. A seven-day life cycle cladoceran toxicity test. *Environmental toxicology and chemistry*, *3*(3), 425-434.

Mukherjee, B., Patra, B., Mahapatra, S., Banerjee, P., Tiwari, A., Chatterjee, M. 2004. Vanadium – an element of atypical biological significance. *Toxicology Letters, 150,* 135.143.

Nalewajko, C., Lee, K., Jack, T.R. 1995a. Effects of vanadium on freshwater phytoplankton photosynthesis. *Water, Air and Soil Pollution. 81,* 93-105.

Nalewajko, C., Lee, K., Olaveson, M. 1995b. Responses of freshwater algae to inhibitory vanadium concentrations: the role of phosphorus. *Journal of Phycology*, *31*(3), 332-343.

National Energy Board. 2013. Canada's Energy Future 2013: Energy Supply and Demand Projections to 2035 – An Energy Market Assessment. ISSN 2292-1710. <https://www.neb-one.gc.ca/nrg/ntgrtd/ftr/2013/index-eng.html> (accessed on 05.09.15.).

Nechay, B.R. 1984. Mechanisms of action of vanadium. *Annual Review of Pharmacology and Toxicology, 24,* 501-524.

Newman, M.C., Ownby, L.C., Mezin, L.C.A., Powel, D.C., Christensen, S.B.L., Andersen, B.A. 2000. Applying species-sensitivity distributions in ecological risk assessment: assumptions of distribution type and sufficient number of species. *Environmental Toxicology and Chemistry*, *19*, 508-515.

Nielsen, F.H., Uthus, E.O. 1990. The essentiality and metabolism of vanadium. In *Vanadium in biological systems*, pp. 51-62. Spring Netherlands.

Norberg-King, T. J. 1993. A linear interpolation method for sublethal toxicity: The inhibition concentration (ICp) approach (version 2.0). *National Effluent Toxicity Assessment Center Technical Report*, *39*, 3-93. Environment Protection Agency, Duluth, MN, USA.

Nyholm N., Kallqvist T. 1989. Methods for growth inhibition toxicity tests with freshwater algae. *Environmental Toxicology and Chemistry*, **8**: 689-703.

Organisation for Economic Co-operation and Development (OECD). 2008. OECD guidelines for the testing of chemicals – 211: Daphnia magna Reproductive test. Report nr211.

Pacyna, J.M., Pacyna, E.G. 2001. An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. *Environmental Reviews, 9*, 269-298

Peacock, C.L., Sherman, D.M. 2004. Vanadium (V) adsorption onto goethite α-FeOOH at pH 1.5 to 12: a surface complexation model based on ab initio molecular geometries and EXAFS spectroscopy. *Geochimica et Cosmochimica Acta*, *68*, 1723-1733.

Planas, D., Healey, F.P. 1978. Effects of arsenate on growth and phosphorus metabolism of phytoplankton 1, 2. *Journal of phycology*, *14*(3), 337-341.

Posthuma, L., Suter, G.W., Traas, T.P. 2002. Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers: Boca Baton.

Pourret, O., Dia, A., Gruau, G., Davranche, M., Coz, M.B. 2012. Assessment of vanadium distribution in shallow groundwaters. *Chemical Geology, 294-295*, 89-102.

Pourrezaei, P., Alpatova, A., Chelme-Ayala, P., Perez-Estrada, L.A., Jensen-Fontaine, M., Le, X.C., Gamal El-Din, M.G. 2014a. Impact of petroleum coke characteristics on the adsorption of the organic fractions from oil sands process-affected water. *International Journal of Environmental Science and Technology*, *11*(7), 2037-2050.

Pourrezaei, P., Alpatova, A., Khosravi, K., Drzewicz, P., Chen, Y., Chelme-Ayala, P., Gamal El-Din, M.G. 2014b. Removal of organic compounds and trace metals from oil sands process-affected water using zero valent iron enhanced by petroleum coke. *Journal of Environmental Management, 139*, 50-58.

Puttaswamy, N., Turcotte, D., Liber, K. 2010. Variation in toxicity response of *Ceriodaphnia dubia* to Athabasca oil sands coke leachates. *Chemosphere*, *80*(5), 489-497.

Puttaswamy, N., Liber, K. 2011. Identifying the causes of oil sands coke leachate toxicity to aquatic invertebrates. *Environmental Toxicology and Chemistry*, *30*(11), 2576-2585.

Puttaswamy, N., Liber, K. 2012. Influence of inorganic anions on metals release from oil sands coke and on toxicity of nickel and vanadium to *Ceriodaphnia dubia*. *Chemosphere, 86*(5), 521-529.

Pyrzynska, K., Wierzbicki, T. 2004. Determination of vanadium species in environmental samples. *Talanta*, *64*, 823-829.

Rainbow, P.S. 1997. Ecophysiology of trace metal uptake in crustaceans. *Estuarine, coastal and shelf science, 44*(2), 169-175.

Regional Aquatics Monitoring Program (RAMP) 2011. Oil Sands Regional Aquatics Monitoring Program (RAMP) 2011. Technical Report Final.

Regional Aquatics Monitoring Program (RAMP) 2015. Regional Aquatics Monitoring in support of the Joint Oil Sands Monitoring Plan 2014 Program Report. Annual Technical Report, JOMSP6784.2.

Rehder, D. 1991. The bioinorganic chemistry of vanadium. *Angewandte Chemie International Edition in English, 30*, 148-167.

Rehder, D. 1992. Structure and function of vanadium compounds in living organisms. *BioMetals*, *5*(1), 3-12.

Rehder, D. 1999. The coordination chemistry of vanadium as related to its biological functions. *Coordination Chemistry Reviews, 182*, 297-322.

Rehder, D. 2003. Biological and medicinal aspects of vanadium. *Inorganic Chemistry Communications*, *6,* 604-617.

Rehder, D. 2015. The role of vanadium in biology. *Metallomics, 7*, 730-742.

Ringelband, U., Hehl, O. 2000. Kinetics of vanadium bioaccumulation by the brackish water hydroid *Cordylophora caspia* (Pallas). *Bulletin of environmental contamination and toxicology, 65*(4), 486-493.

Ringelband, U., Karbe, L. 1996. Effects of vanadium on population growth and Na-K-ATPase activity of the brackish water hydroid *Cordylophora caspia*. *Bulletin of environmental contamination and toxicology, 57*(1), 118-124.

Rogers, V.V., Wickstrom, M., Liber, K., MacKinnon, M.D. 2002. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. *Toxicological Sciences, 66*, 347-355.

Rojickova-Padrtova, R., Marsalek, B., 1999. Selection and sensitivity comparisons of algal species for toxicity testing. *Chemosphere, 38*(14), 3329-3338.

Scarlett et al. 2012. Scarlett, A.G., West, C.E., Jones, D., Galloway, T.S., Rowland, S.J. 2012. Predicted toxicity of naphthenic acids present in oil sands process-affected waters to a range of environmental and human endpoints. *Science of the Total Environment, 425,* 119-127.

Scott, A.C., Fedorak, P.M. 2004. Petroleum coking: a review of coking processes and the characteristics, stability, and environmental aspects of coke produced by the oil sands companies. Report prepared for Suncor Energy Inc., Syncrude Canada Ltd., and Canadian Natural Resources Ltd. University of Alberta, Department of Biological Sciences, Edmonton, AB, Canada.

Scott, C.S., Zubot, W., Mackinnon, M.D., Smith, D.W., Fedorak, P.M. 2008. Ozonation of oil sands process water removes naphthenic acids and toxicity. *Chemosphere, 71*(1), 156-160.

Shiller, A.M., Boyle, E.A. 1987. Dissolved vanadium in rivers and estuaries. *Earth and Planetary Science Letters, 86,* 214-224.

Small, C.C. 2011. Activation of delayed and fluid petroleum coke for the adsorption and removal of naphthenic acids from oil sands tailings pond water. MSc. Thesis. University of Alberta, Edmonton, AB, Canada.

Small, C.C., Hashisho, Z., Ulrich, A.C. 2012a. Preparation and characterization of activated carbon from oil sands coke. *Fuel*, *92*(1), 69-76.

Small, C.C., Ulrich, A.C., Hashisho, Z. 2012b. Adsorption of acid extractable oil sands tailings organics onto raw and activated oil sands coke. *Journal of Environmental Engineering*, *138*(8), 833-840.

Smith, D.P., Kennedy, J.H., Dickson, K.L. 1991. An evaluation of a nadid oligochaete as a toxicity test organism. *Environmental Toxicology and Chemistry*, *10,* 1459-1465.

Soares, S.S., Martins, H., Gutierrez-Merino, C., Aureliano, M. 2008. Vanadium and cadmium in vivo effects in teleost cardiac muscle: metal accumulation and oxidative stress markers. *Comparative Biochemistry and Physiology, Part C*, *147*, 168-178.

Soares, S.S., Martins, H., Duarte, R.O., Moura, J.J.G., Coucelo, J., Gutierrez-Merino, C., Aureliano, M. 2007. Vanadium distribution, lipid peroxidation and oxidative stress markers upon decavanadate *in vivo* administration. *Journal of Inorganic Biochemistry, 101*(1), 80-88.

Squires, A.J. 2005. Ecotoxicological assessment of using coke in aquatic reclamation strategies at the Alberta oil sands. MSc Thesis. University of Saskatchewan, Saskatoon, SK, Canada.

Stendahl, D., Sprague, J.B. 1982. Effects of water hardness and pH on vanadium lethality to rainbow trout. *Water research, 16*(10), 1479-1488.

Toor, N.S., Franz, E.D., Fedorak, P.M., MacKinnon, M.D., Liber, K. 2013. Degradation and aquatic toxicity of naphthenic acids in oil sands process-affected waters using stimulated wetlands. *Chemosphere*, *90*, 440-458.

Valko, M., Morris, H., Cronin, M.T.D. 2005. Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, *12,* 1161-1208.

Van Leeuwen, C.J., Niebeek, G., Rijkeboer, M. 1987. Effects of chemical stress on the population dynamics of *Daphnia magna*: a comparison of two test procedures. *Ecotoxicology and Environmental Safety*, *14*(1), 1-11.

Van Staalen, N.M. 2002. Threshold models for species sensitivity distributions applied to aquatic risk assessment for zinc. *Environmental Toxicology and Pharmacology, 11,* 167-172.

Vardy, D.W., Oellers, J., Doering, J.A., Hollert, H., Giesy, J.P., Hecker, M. 2013. Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper. *Ecotoxicology, 22*, 139-147.

Ventura, G.T., Gall, L., Siebert, C., Prytulak, J., Szatmari, P., Hurlimann, M., Halliday, A.N. 2015. The stable isotope composition of vanadium, nickel, and molybdenum in crude oils. *Applied Geochemistry*, *59*, 104-117.

Verbeek, A., McKay, W., MacKinnon, M. 1993. Isolation and characterization of the acutely toxic compounds in oil sands process water from Syncrude and Suncor. In Proceedings of Oil Sands-Our Petroleum Future Conference, Fine Tails Fundamentals Consortium, Edmonton, Alta. Pp. F10-1-F10-11.

Versteeg, D.J., Stalmans, M., Dyer, S.D., Janessen, C. 1997. *Ceriodaphnia* and *daphnia*: a comparison of their sensitivity to xenobiotics and utility as a test species. *Chemosphere, 34*(4), 869-892.

Wang, D., Sanodu-Wilhelmy, S.A. 2008. Development of an analytical protocol for the determination of V (IV) and V (V) in seawater: application to coastal environments. *Marine Chemistry*, *112*, 72-80.

Wanty, R.B., Goldhaber, M.B. 1992. Thermodynamics and kinetics of reactions involving vanadium in natural systems: accumulation of vanadium in sedimentary rocks. *Geochmica et Cosmochimica Acta, 56,* 1471-1483.

Wehrli, B., Stumm, W. 1989. Vanadyl in natural waters: adsorption and hydrolysis promote oxygenation. *Geochimica et Cosmochimica Acta, 53*(1), 69-77.

Wheeler, J.R., Grist, E.P.M., Leung, K.M.Y., Morritt, D., Crane, M. 2002. Species sensitivity distributions: data and model choice. *Marine Pollution Bulletin, 45,* 192-202.

World Health Organization (WHO). 2001. Vanadium pentoxide and other inorganic vanadium compounds. Concise International Chemical Assessment Document 29.

Wright, M.T., Belitz, K. 2010. Factors controlling the regional distribution of vanadium in groundwater. *Ground Water*, *48,* 515-525.

Zhong, H., Wang, W.X. 2008. Effects of sediment composition on inorganic mercury partitioning speciation and bioavailability in oxic surficial sediments. *Environmental Pollution, 151,* 222-230.

Zhou, Z., Wei, C., Li, M., Qiu, S., Li, X. 2011. Thermodynamics of vanadium-sulfur-water systems at 298 K. *Hydrometallurgy*, *106,* 104-211.

Zimmer, A.M., Brauner, G.J., Wood, C.M. 2014. Exposure to waterborne Cu inhibits cutaneous Na+ uptake in post-hatch larval rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology, 150,* 151-158.

Zubot, W., MacKinnon, M.D., Chelme-Ayala, P., Smith, D.W., Gamal El-Din, M.G. 2012. Petroleum coke adsorption as a water management option for oil sands process-affected water. *Science of the Total Environment*, *427*, 364-372.

Zubot, W. 2011. Removal of naphthenic acids from oil sands process water using petroleum coke. MSc. Thesis. University of Alberta, Edmonton, AB, Canada.