National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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A proposal for water quality standards in accordance with the Water Framework Directive

RIVM Letter Report 601714021/2012
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This investigation has been performed by order and for the account of Ministry of Infrastructure and Environment, within the framework of Chemical aspects of WFD".

Abstract

Environmental risk limits for vanadium in water
A proposal for water quality standards in accordance with the Water Framework Directive

RIVM has derived environmental risk limits (ERLs) for vanadium in freshwater. Vanadium is a natural element that is used for steel production. The compound is included in the Dutch decree on water quality objectives in the context of the Water Framework Directive (WFD). The current standard for vanadium has to be updated according to the new WFD-methodology. The ERLs in this report are advisory values that serve as a scientific background for the Dutch Steering Committee for Substances, which is responsible for setting those standards.

New information from REACH
Until now, updating the old standards was not possible because essential data were missing concerning the ecotoxicity of vanadium for algae, and potential food chain transfer. Both aspects are investigated in this report, partly based on information that has become available via REACH. Based on the available data, water quality standards are proposed for long-term exposure (1.2 microgram per litre) and for short-term concentration peaks (3.0 microgram per litre). Both values are expressed on the basis of dissolved concentrations, including the background concentration for Dutch surface waters.

Risk limits for effects on aquatic organisms
The proposed values are based on direct ecotoxicity for aquatic organisms. Exposure of humans and/or predatory birds and mammals due to consumption of fish is usually taken into account, but could not be included in the present calculations due to a lack of reliable information.

Keywords:
vanadium; environmental risk limits; WFD, water quality standards

Rapport in het kort

Milieurisicogrenzen voor vanadium
Een voorstel voor waterkwaliteitsnormen volgens de Kaderrichtlijn water

Het RIVM heeft in opdracht van het ministerie van Infrastructuur en Milieu (I&M), milieurisicogrenzen van vanadium voor zoet oppervlaktewater bepaald. Vanadium is een natuurlijke stof en wordt onder andere gebruikt voor de productie van staal. De stof is opgenomen in de Regeling monitoring kaderrichtlijn water, waarin staat aan welke eisen oppervlaktewater in Nederland moet voldoen. De nieuwe waterkwaliteitsnormen voor vanadium zijn nodig omdat de huidige norm niet is afgeleid volgens de meest recente methodiek. De Stuurgroep Stoffen stelt deze nieuwe normen vast op basis van de wetenschappelijke advieswaarden uit dit onderzoek.

Nieuwe informatie beschikbaar via REACH
In de afgelopen jaren konden geen nieuwe milieurisicogrenzen voor vanadium worden afgeleid. Essentiële gegevens die daarvoor nodig zijn ontbraken, zoals informatie over de effecten op algen en de mogelijke stapeling in de voedselketen. In dit onderzoek zijn beide aspecten opnieuw onderzocht, onder meer door gebruik te maken van de gegevens die voor de Europese verordening voor chemische stoffen REACH beschikbaar zijn gekomen. Op basis van de nieuwe informatie worden waterkwaliteitsnormen voorgesteld voor langdurige blootstelling (1,2 microgram per liter) en voor kortdurende piekbelasting (3,0 microgram per liter). Beide waarden zijn uitgedrukt als ‘opgelost vanadium’, inclusief de natuurlijke achtergrondconcentratie voor Nederlands oppervlaktewater.

Risicogrenzen gebaseerd op gevolgen voor waterorganismen
Deze risicogrenzen zijn gebaseerd op de mate waarin de stof direct giftig is voor waterorganismen. Doorgaans wordt in de berekeningen ook meegenomen in welke mate mensen en/of vogels en zoogdieren aan een stof staan blootgesteld via het eten van vis. Voor de stof vanadium was hierover echter onvoldoende betrouwbare informatie beschikbaar.

Trefwoorden:
vanadium; milieurisicogrenzen; KRW, waterkwaliteitsnormen

Contents

Summary

RIVM has derived environmental risk limits (ERLs) for vanadium in water. Vanadium is applied in many steel products, among which tools. The compound is included in the Dutch decree on water quality objectives in the context of the Water Framework Directive (WFD). The current standard for vanadium has to be updated according to the new WFD-methodology. Until now, updating the old standards was not possible because essential data were missing concerning the ecotoxicity of vanadium for algae, and the potential risks for humans and predatory birds and mammals resulting from accumulation of vanadium in fish and prey. Both aspects are investigated in this report, partly based on information that has become available via REACH. Although relevant information has been retrieved, derivation of ERLs is still hampered by considerable uncertainty concerning these issues.

With respect to direct ecotoxicity for freshwater organisms, a pragmatic approach was followed. In order to include as much information as possible, the available relevant and reliable data were used in a species sensitivity distribution (SSD), although strictly speaking the dataset does not meet the criteria for doing so. Based on the SSD, it is proposed to set the long-term ERL for direct toxicity ($MPC_{fw, eco}$) to 1.2 µg V/L and the ERL for short-term concentration peaks ($MAC_{fw, eco}$) to 3.0 µg V/L. These values refer to dissolved concentrations, including the background concentration of 0.82 µg V/L. Risk limits for saltwater based on direct ecotoxicity could not be derived.

Based on a re-evaluation of bioaccumulation data, the ERL for human exposure via food ($MPC_{water, hh\ food}$) is calculated as 0.89 µg V/L, the ERL for secondary poisoning ($MPC_{fw, secpois}$) as 0.34 µg V/L. Both values are at or below the natural background concentration and are therefore not suitable as a basis for the final MPC for fresh- or saltwater. In addition, the human toxicological risk limit is considered as "provisional" because of the large uncertainty in the underlying data. This also holds for the risk limit for predators. Furthermore, the information on bioaccumulation is not sufficient to derive a reliable estimate that is representative for Dutch surface waters.

As a result, it is proposed to use the $MPC_{fw, eco}$ of 1.2 µg V/L and the $MAC_{fw, eco}$ of 3.0 µg V/L for direct ecotoxicity for standard setting. These values are advisory values that serve as a scientific background for the Dutch Steering Committee for Substances, which is responsible for setting the final standards. Monitoring data indicate that these values will likely be exceeded at some locations.

1 Introduction

1.1 Current status of vanadium water quality standards

Vanadium is included in the Dutch *Regeling Monitoring Kaderrichtlijn Water*, the decree which sets the water quality standards for substances that are relevant for the Netherlands within the context of the Water Framework Directive (WFD). Updated standards according to the new methodology of the WFD have to be available by the end of 2012. The current environmental quality standard is 5.1 µg/L, expressed on the basis of total vanadium. This value is calculated from the Maximum Permissible Concentration (MPC) of 4.3 µg/L as derived by Crommentuijn et al. (1997), who used the data collected by Van de Plassche et al. (1992). The MPC of 4.3 µg/L is composed of a Maximum Permissible Addition (MPA) of 3.5 µg/L and a background concentration of 0.82 µg/L (both expressed as dissolved concentrations). The MPA was derived by putting an assessment factor of 1000 on the 48-h EC_{50} of 3.5 mg/L for *Daphnia magna*. Expressing this MPC as a total water concentration (including the fraction adsorbed onto suspended matter) results in the MPC of 5.1 µg/L cited above.

1.2 Recent reports on risk limits for vanadium

In 2005, RIVM published revised risk limits for vanadium, based on an update of the ecotoxicological literature (Van Vlaardingen et al., 2005). In 2007, the Dutch methodology of environmental risk limit (ERL) derivation was updated according to the requirements of the WFD. One of the main changes was the inclusion of secondary poisoning of predators and human exposure via consumption of fish or fishery products as routes for risk limit derivation. The 2005-report presents risk limits for direct ecotoxicity only, whereas human exposure and secondary poisoning of predators are potentially relevant due to the characteristics of the compound.
Another change in the guidance on ERL derivation is the treatment of freshwater and marine ecotoxicity data. In 2005, risk limits were derived using a dataset of combined endpoints for freshwater and marine species. According to the WFD-guidance, datasets for metals and metal-like compounds should be kept separated, unless it can be demonstrated "with high probability" that there is no difference in sensitivity between freshwater and marine species.
Van Vlaardingen and Verbruggen (2009) published an update of risk limits for vanadium in which the vanadium data collected in 2005 were split into a fresh- and saltwater dataset, and human exposure and secondary poisoning were included in the derivation of risk limits. The $MPC_{water,\ hh\ food}$ was derived as 0.71 µg V/L, the $MPC_{fw,\ secpois}$ was 0.27 µg V/L. It was not possible, however, to derive an MPC for direct ecotoxicity ($MPC_{fw,\ eco}$, $MPC_{sw,\ eco}$). This was due to the following reasons:

- After splitting the datasets for freshwater and marine species, the acute base set for deriving risk limits for freshwater (algae, daphnids, fish) was not complete because data on algae were missing; environmental risk limits for freshwater based on ecotoxicity could not be derived[1];

[1] The scientific literature was screened for additional toxicity data (preferably on algae) that could help overcome this data gap, but no valid data were found at that time.

- Acute data on marine algae were also missing, leading to an incomplete base set. Chronic data on algae were available, but in the absence of chronic data for other taxonomic groups, it was not possible to identify the potentially most sensitive species group.

The WFD-guidance states that final water quality standard should be based on the lowest of the three routes/protection aims, i.e. direct ecotoxicity, human exposure and secondary poisoning. In the absence of risk limits based on direct ecotoxicity, it was not possible to decide on the final risk limit.
As stated above, updated water quality standards have to be available by 2012. The current standard of 5.1 µg V/L is much higher than the values for human exposure and secondary poisoning as derived by Van Vlaardingen and Verbruggen (2009). This leads to the conclusion that those latter two risk limits should also be critically examined in order to advise on the final risk limit.

1.3 Risk limits and natural background

Another problem arises when using the values for the $MPC_{water,\ hh\ food}$ of 0.71 and $MPC_{fw,\ secpois}$ of 0.27 µg V/L for selection of a final risk limit for water. As indicated by Van Vlaardingen and Verbruggen (2009), the background concentration of vanadium is 0.82 µg V/L (dissolved fraction). The two above mentioned risk limits for vanadium of 0.71 and 0.27 µg V/L are both lower than this value. The so-called "added risk approach", in which a risk limit is expressed as a concentration that may be added to the natural background concentration, does not apply in this case, because the risk limits for human exposure and secondary poisoning are based on field-derived bioaccumulation factors (BAF) that do not distinguish between background and added concentrations.
An obvious question is also whether the background concentration that was derived previously is adequate. Several options for deriving background concentrations are presented in the new WFD-guidance (EC, 2011):

- to measure concentrations in deep groundwater. In some cases, however, the concentration of the metal may be higher in the groundwater than in the surface water, e.g. because of the groundwater's contact with deep lying mineral rocks or soils and subsequent dilution by rain.
- to gather information from national or international databases, such as the FOREGS Geological Baseline Programme (http://www.gsf.fi/foregs/geochem).
- geological modelling, to estimate the contribution from erosion.
- to estimate the concentration in the water from natural background concentrations found in the sediment by means of equilibrium partitioning models.

According to the FOREGS Geological Baseline Programme (Salminen, 2005), vanadium concentrations in filtered water in Europe range from <0.05 to 19.5 µg V/L, median 0.46, mean 0.829 and 90^{th} percentile 1.66 µg V/L. In view of these data, the reported background concentration of 0.82 µg V/L in the Netherlands may be considered as an adequate estimate. It should be realised, however, that detailed data from a national survey are not available. At the moment, a research project is performed in which the use of groundwater and/or sediment data is explored (Osté et al., in prep.). Preliminary results of this project indicate that the currently used background concentration may still be adequate.
In discussions on other naturally occurring elements, it was pointed out that for metals in general and essential elements in particular, attention should be paid to the selection of the BAF (see also the comments on this topic in the new EQS-guidance; EC, 2011). For many elements, organisms are able to regulate the

uptake and elimination to a certain extent. As a result, there is no linear increase of internal levels at increasing external concentrations, and in some cases more or less constant internal concentration may even be observed. Since the BAF is expressed as the ratio of the concentration in the organisms and the concentration in water, BAFs tend to increase with decreasing external concentrations and vice versa. For risk limit derivation, it is thus important to use a BAF that is relevant for the external concentration that is being considered. If a relationship can be established between the BAF and external water concentrations, this relationship can be used to establish the water concentration at which the critical concentration in food is reached.

1.4 Role of speciation

Vanadium can be present in various forms. The following is cited from Environment Canada (2010):

> "(…) The aqueous chemistry of the metal is complex and involves a wide range of oxygenated species for which stabilities depend mainly on the acidity and oxygen level of receiving waters. Under conditions commonly found in oxic fresh waters (i.e., pH between 5 and 9; redox potential [E_h] between 0.5 and 1 V), the pentavalent oxyanions $H_2VO_4^-$ and HVO_4^{2-} (also called vanadate ions) will be the dominant species in solution (Brookins 1988; Takeno 2005). Studying the speciation of vanadium in a lakewater sample of pH 7.5, Fan et al. (2005) did not detect vanadium(IV) oxidation states, supporting the idea that pentavalent forms dominate vanadium speciation in neutral surface fresh waters. Finally, it can be noted that polymerization of oxygenated species of vanadium will increase with increases in their concentrations (>10^{-4} M or 18.2 mg/L: Jennette 1981) and will be more prevalent in seawater (Petterson 1993).
>
> Vanadium is expected to be more mobile under oxidizing conditions than under reducing conditions (Garrett 2005), likely in part reflecting the difference in mobilities of the oxidized anionic and reduced cationic forms. Oxidized forms are generally less mobile under acidic conditions than under neutral to alkaline conditions (Reimann and de Caritat 1998). For example, the species $H_2VO_4^-$ and HVO_4^{2-} are among the most mobile forms of vanadium found in natural oxic waters (Crans et al. 1998)."

Environment Canada (2010) has performed speciation modelling using the WHAM VI-program. Modelling was done for some Canadian waters that are representative of the regions for which the Canadian risk assessment was performed. Modelled estimates indicate that the species $H_2VO_4^-$ and HVO_4^{2-} dominate chemical speciation in all types of water considered, with a minor contribution, less than 1%, from complexes with humic substances. The results are copied below in Table 1.

Table 1 Modelled results for chemical speciation of vanadium in relevant oxic surface waters in Canada. Table copied from Environment Canada (2010).

Water type	**General physical and chemical characteristics**			**Proportion of total aqueous vanadium (%)**		
	Degree of mineralization	**Acidity**	**DOC content**	**HVO_4^{2-}**	**$H_2VO_4^-$**	**HS–V_1**
Prairie						
Wabamun Lake (Alberta)	High; conductance ~500 µS/cm	Alkaline; pH ~8	High; >10 mg/L	38.6	61.4	<<1
Canadian Shield						
Allard River	Low; conductance ~60 µS/cm	neutral; pH ~7	High; >10 mg/L	2.9	97.1	<<1
Colombière River (Quebec)	Low; conductance ~30 µS/cm	Slightly acid; pH ~6.5	High; >10 mg/L	<1	99.3	<<1
Seawater						
St. Lawrence Gulf (eastern Canada)	Very high; salinity ~32 ppt	Alkaline; pH ~8	Very low; <1 mg/L	47.7	52.3	<<1

Abbreviations: DOC, dissolved organic carbon; HS–V_1, vanadate complex with humic substances; ppt, parts per thousand.

The characteristics of the water bodies modelled by Environment Canada (2010) are not representative for Dutch surface waters, in particular with respect to the high dissolved organic carbon content. A reference is cited in which on the basis of manipulations with dissolved organic matter content suggests that complexation is not important for vanadium. Further, reference is made to an analysis of data covering 71 rivers in the United States by Shiller and Mao (2000), who determined that DOC could play a "secondary" but nevertheless significant role in fluvial dissolved vanadium concentrations. However, as can be seen from the table above, speciation is similar in prairie freshwater with high DOC and seawater with low DOC. Conductivity seems to be more important than DOC content, as judged from the difference between prairie water and Canadian shield waters. Reported values for conductivity in Dutch freshwater are generally high (≈ 50-75 mS/m = 500-750 µS/cm; www.waterbase.nl), which might indicate that both $H_2VO_4^-$ and HVO_4^{2-} will be present.

Although the importance of speciation it recognised, it should be noted that speciation modelling can only be included in risk limit derivation if enough information is available with respect to the characteristic of the medium use in the ecotoxicity tests. For vanadium, this is not the case and it cannot be judged if the speciation in the ecotoxicity tests is similar to what is expected for the aquatic environment. The ecotoxicity tests have been performed with sodium orthovanadate (Na_3VO_4), sodium metavanadate ($NaVO_3$), ammonium metavanadate (NH_4VO_3), vanadium pentoxide (V_2O_5), and ammonium trivanadate ($NH_4V_3O_8$). Considering the expected environmental relevance of $H_2VO_4^-$ and HVO_4^{2-}, it might be most appropriate to conduct ecotoxicity tests with VO_4^{3-}, making sodium orthovanadate the most appropriate test compound.

1.5 Aim of the present report

The present report focuses on the two problems described above: the absence of ecotoxicity data for algae and the selection of input data that determine the $MPC_{water, hh\ food}$ and $MPC_{fw, secpois}$.

- Algae: The available studies were re-examined and the REACH dossiers were consulted in order to find additional information or references that may shed light on the relative sensitivity of algae as compared to other species groups.
- $MPC_{water, hh\ food}$ and $MPC_{fw, secpois}$: The derivation of the human threshold value and the MPC for birds and mammals was revisited. Furthermore, the input for selection of the BAF was evaluated and additional data were used to determine whether or not they are relevant for the Dutch situation.

Based on the available information, options are presented for derivation of the risk limits and a final proposal is made based on the discussions in the Scientific Advisory Group INS.

2 Risk limits for direct ecotoxicity

2.1 Introduction

As indicated in the introduction (see 1.2), valid chronic and acute data on freshwater algae and acute data on marine algae were not available in previous reports. For the present report, information was retrieved from a recent evaluation by Environment Canada (2010), and from the REACH-dossier. Furthermore, a paper by Meisch et al. (1980), which was in the list of non-used studies from the 2005 report, was re-examined and references that were retrieved from it were evaluated. The results from these studies are presented in the next sections. The studies are summarised in more detail in Appendix 1 and 2. The results are used to explore different options for risk limit derivation.

2.2 Additional laboratory data on freshwater algae

2.2.1 Information from Environment Canada

In the risk assessment of Environment Canada (2010), the following data on freshwater algae are included (Table 2).

Table 2 Endpoints for freshwater algae reported in Environment Canada (2010).

Species	Compound	pH	Endpoint/ duration	Value [mg V/L]	Reference
Anabaena flos-aquae	Na_3VO_4	6.8	IC_{100} (growth inhibition) / 7 d	0.1	Lee et al. (1979)
Chlorella pyrenoidosa	Na_3VO_4 [a]	6.8	MATC[b] (growth inhibition) / 7 d	0.32[b]	Lee et al. (1979)
Navicula pelliculosa	Na_3VO_4	6.8	NOEC (growth inhibition) / 7 d	1	Lee et al. (1979)
Scenedesmus obliquus	Na_3VO_4	6.8	NOEC (growth inhibition) / 7 d	0.32[b]	Lee et al. (1979)
Scenedesmus quadricauda	V_2O_5	n/a	EC_{50} (growth inhibition) / 12 d	2.23	Fargašová et al. (1999)

a: mistakenly reported as NH_4VO_4 in the evaluation
b: geometric mean of NOEC and LOEC, NOEC is thus considered to be 0.1 mg/L

The study of Fargašová et al. (1999) was considered not reliable by Van Vlaardingen et al. (2005), since endpoints were based on chlorophyll-a measurements after 12 days only, and no information was present as to whether exponential growth was maintained in the control.

2.2.2 Lee et al. (1979)

The study of Lee et al. (1979) could be retrieved and summarised in more detail in Appendix 1, study 1. They exposed cultures of *Anabaena flos-aquae*, *Chlorella pyrenoidosa*, *Navicula pelliculosa* and *Scenedesmus obliquus* in exponential growth phase in triplicate to concentrations of 0 to 1000 µg V/L (as Na_3VO_4) in standard growth medium (background concentration 2.7 µg V/L, pH 6.8, temperature 23 or 25 °C). Cell numbers, dry weight and chlorophyll-a content were determined after seven days. Cell numbers of *A. flos-aquae* were decreased at 0.1 µg V/L and higher, complete suppression was observed at 100 µg V/L.
The other species, however, were only inhibited at 1000 µg V/L (*C. pyrenoidosa*, *S. obliquus*) or did not show a clear response (*N. pelliculosa*). The endpoints that

were derived from the figures in the paper are presented in Table 3 (for details, see Appendix 1).

Table 3 Effect of vanadium on cell numbers, dry weight and chlorophyll-a content of algae and diatoms after 7 days exposure.

Species	Parameter	LOEC [µg/L]	NOEC [µg V/L]	EC_{10} [µg V/L]	EC_{50} [µg V/L]
Anabaena flos-aquae	cell numbers	0.1	0.01	0.013	1.43
	dry weight	1	0.1	0.36	6.7
	chlorophyll-a	1	0.1	1.5	12.5
Chlorella pyrenoidosa	cell numbers	1000	100	*curve fitting not possible*[a]	
	dry weight	1000	100	*or not reliable (r^2 < 0.8)*	
	chlorophyll-a	1000	100		
Scenedesmus obliquus	cell numbers	1000	100	873	977
	dry weight	1000	100	69	536
	chlorophyll-a	1000	100	*curve fitting not possible*[a]	
Navicula pelliculosa	cell numbers	> 1000	≥ 1000	> 1000	> 1000
	dry weight	> 1000	≥ 1000	> 1000	> 1000
	chlorophyll-a	> 1000	≥ 1000	> 1000	> 1000

a: no clear concentration response at concentrations ≤ 100 µg/L

The LOEC for *A. flos-aquae* of 0.1 µg V/L is extraordinary low taking into account the background concentration in the medium of 2.7 µg V/L. It is considered hardly possible that such a small addition would induce effects. It was tried to contact the authors for advice on this matter, but we did not succeed. On the other hand, Nalewajko et al. (1995a, see 2.2.4) also observed effects at very low added levels of vanadium using the same medium.
According to the current criteria, the results of this study would be considered not reliable, since endpoints were only determined after seven days and no information is given as to whether exponential growth was maintained in the control. For the present purpose, however, they are accepted in a "weight of evidence" approach for the derivation of risk limits for vanadium.

2.2.3 Studies by Meisch et al.

From the studies by Meisch et al. (see Appendix 1), it was concluded that vanadium (added as NH_4VO_3) has a stimulating effect on chlorophyll-a synthesis and increases dry weight of the unicellular algae *S. obliquus* and *C. pyrenoidosa*. An optimum was found at approximately 500 µg V/L (see Appendix 1, study 2 and 3), which is consistent with the above reported LOECs from Lee et al. (1979). In algal growth tests, cell numbers, chlorophyll-a content and biomass are usually all related and the three criteria give more or less similar endpoints. In the case of vanadium, however, dry weight and increased chlorophyll-a content of *C. pyrenoidosa* appeared to be associated with an increase in cell volume rather than with an increase in cell numbers (see Appendix 1, study 4; Meisch and Benzschawel, 1978). The exact way in which vanadium interacts with algal metabolism is not fully understood. Cell volume is not normally used as an endpoint for risk limit derivation. In the case of vanadium, the enlarged cells are considered to be a negative effect of vanadium exposure, since enlarged cells represent cells in which division is disturbed. After exposure of cells to 20 µg V/L under synchronous conditions (16:8 h L:D) over six light/dark periods, cell division in vanadium cultures stopped at the start of the fourth cycle, while the cells continued growing. When those cultures were transferred to fresh medium containing the same vanadium concentration, cell division started again for three cycles and then stopped. Information on effects on cell

division at lower concentrations is not available. Dr. Meisch was contacted by e-mail for advice on this matter and he responded as follows (mail dd. 01-03-2011):
"I can say that there are two different effects of trace amounts of vanadium on green algae. The first should be a positive influence on chlorophyll synthesis at a lower stage of the biosynthetic chain (most probably on the biosynthesis of 5-aminolevulinic acid[2]), while a toxic influence can be observed on cell division which leads to bigger cells. The latter phenomenon must not be a disadvantage (in a certain range of V-concentration, of course), because the biomass itself was not decreased. So it is hard to define a toxic level of V in water. I would say that trace amounts of V (up to about 0.1 ppm) are not very critical, while higher concentrations should be regarded as toxic. Don't forget that other water organisms could be more sensitive against vanadium than green algae."
The acceptable level of 100 µg V/L indicated by Dr. Meisch seems to be rather high, since complete inhibition of cell division was observed at 20 µg V/L. That level is highly comparable to the EC_{50} for cell volume increase of 24 µg V/L. It was suggested by the Scientific Advisory Group INS that the observed effect on cell volume and inhibition of cell division may be caused by excessive uptake of vanadium after deprivation during culturing in a vanadium-free medium. However, from the description of the experiment (see Appendix 1, study 4), it appears that cell cultures were maintained in the presence of peptone, which contains vanadium, and that other trace metals (a.o. iron) were present as well. This leads to the conclusion that the increase in cell volume is a toxic effect of vanadium, rather than an artefact. It is not known, however, if the effect on cell volume is also related to inhibition of cell division at vanadium concentrations lower than 20 µg V/L, although the data of Nalewajko et al. (1995a) on *S. obliquus* (see below, 2.2.4) suggest a strong relationship between the two parameters. It is also not clear if the conditions under which the effect has been observed are fully relevant for the field situation. Assuming that this is indeed the case, the EC_{10} for cell volume increase of *C. pyrenoidosa* (1.8 µg V/L) may be used.

2.2.4 *Nalewajko et al. (1995a)*

Nalewajko et al. (1995a) studied the effect of vanadium (as sodium orthovanadate[3], Na_3VO_4) on a range of freshwater algae and cyanobacteria, focussing on the interactions with phosphate (see Appendix 1, study 7). They showed that the phosphorus state of the cells (P-deficient vs. P-sufficient) is a major controlling factor of the inhibitory effect of vanadium. In P-sufficient cultures, vanadium was inhibitory when the vanadium concentration exceeded the phosphate concentration. In P-deficient cultures, inhibition of photosynthesis by vanadium increased with increasing phosphorus deficiency. Based on observations on P-kinetics, it is concluded that orthovanadate competes with phosphate for uptake. In a short-term experiment under P-sufficient conditions, a significant effect of vanadium on photosynthetic capacity was demonstrated for 16 algal species. The sensitivity towards vanadium was highly different between species, which according to the authors can be attributed to species-specific differences in phosphate-status among algae. As a follow-up, the effect of vanadium on growth rate was determined for eight species in 7-10 days growth tests with Na_3VO_4 at nominal concentrations of 10.2-50942 µg V/L in synthetic medium containing 57.5 µM PO_4^{3-} (5.5 mg/L). Growth rates were

[2] see Meisch and Bauer, 1978.
[3] reference states that study is conducted with "sodium orthovanadate ($NaVO_3$)", but $NaVO_3$ is denoted as sodium metavanadate, while sodium orthovanadate is Na_3VO_4. Latter is most likely meant.

calculated based on daily cell counts during the exponential growth phase and expressed as mean number of divisions per day of four replicates. Endpoints are presented in Table 4, based on the figures in the paper (for details, see Appendix 1, study 7). All values are based on nominal concentrations, actual concentrations were not measured. Since the same medium was used as in the above discussed study of Lee et al. (1979), it is assumed that a similar background level of vanadium of ≈ 2.5 µg/L was present.

Table 4 Effect on growth rate (divisions/day) of several species of algae, cyanobacteria and diatoms after 7 – 10 days exposure to vanadium.

Species	**EC_{10} [µg V/L]**	**EC_{50} [µg V/L]**	**r^2**
cyanobacteria			
Anabaena flos-aquae	4276	> 50942	0.93
Synechococcus leopoliensis	5649	> 50942	0.93
algae/diatomea			
Ankistrodesmus falcatus	0.29	188	0.93
Chlorella pyrenoidosa	6.90	24491	0.88
Diatoma elongatum	4.71	148	0.98
Dictyosphaerium planctonicum	31.2	> 50942	0.82
Kirchneriella lunaris	5.68	855	0.96
Scenedesmus acutus	863	3412	0.96

In this study, *A. flos-aquae* appeared to be much less sensitive than in the experiment of Lee et al. (1979; see Table 3). Since both studies were performed in the same medium, this cannot be caused by a difference in phosphate. A possible explanation is that different strains were used, which may differ in phosphate requirements.
The average total phosphate concentration reported for selected sampling points in the Netherlands is around 0.5 mg/L (RIWA, 2010a), which is about 10 times lower than in the test. If vanadium becomes more toxic at low phosphate levels, this probably means that that the values shown in Table 4 do not represent a worst case condition. It may be possible, however, that the interaction between vanadium and phosphate only plays a major role if algae that are adapted to high phosphate concentrations, are exposed to vanadium under phosphate limiting conditions.
In another experiment, Nalewajko et al. (1995a) showed that after exposure to 800 and 9017 µg V/L, cell volumes of *S. obliquus* increased by a factor of 1.9 and 8.5, respectively, as compared to the control, while cell numbers decreased to 78 and 9.5% of the control value. Colony dissociation and ultrastructural changes were also observed. From the relationship between cell volume increase and cell numbers, it appears that a 10%-effect on the latter is associated with a 1.5 fold increase in cell volume.
The effect of vanadium on cell volume of *S. obliquus* is consistent with the results of Meisch and Benzschawel (1978) described above for *C. pyrenoidosa*. Furthermore, the EC_{10} for growth rate of *C. pyrenoidosa* of 3.81 µg V/L from the study of Nalewajko et al. (1995a) is roughly similar to the EC_{10} of 1.8 µg V/L for cell volume increase from the study of Meisch and Benzschawel (1978). This indicates that cell volume increase is relevant in terms of effects on growth rate. It also underpins the conclusion that the cell volume increase reported by Meisch and Benzschawel (1978) was not an over-reaction towards previous vanadium-deficiency.

2.2.5 *Studies in the REACH dossiers*

Several studies with algae are included in the REACH dossiers on vanadium and related compounds, study summaries are available via the ECHA-website (ECHA, 2011). Among these, there are three GLP-studies with *Scenedesmus subspicatus* that are performed according to OECD 201 (OECD, 2006), for details see Appendix 2. The original studies are not available for review, but the summaries from the registrant contain detailed information on methodology and results. Tests were performed in supernatants after stirring the test substance for 24 hours, concentrations were measured. The endpoints for specific growth rate (based on cell counts) were recalculated by the registrant according to OECD 201. Although a final check on the results is not possible, the assignment Ri 2 as given by the registrant is agreed upon on the basis of the summary. Results are presented below in Table 5, all based on measured concentrations in supernatants. Background concentrations in the control were not reported, but it is assumed that these were negligible since vanadium is not a constituent of test media according to OECD 201.

Table 5 Accepted data on freshwater algae, based on the REACH dossier.

species	**test compound**	**EC_{10} [µg V/L]**	**EC_{50} [µg V/L]**
Scenedesmus subspicatus	V_2O_5-flakes	716	2907
Scenedesmus subspicatus	$NH_4V_3O_8$	1796	3865
Scenedesmus subspicatus	$NaVO_3$	4342	7619

Another study was submitted, in which bismuth vanadate was tested at 10 and 100 mg $BiVO_4$/L nominal. In this study, test solutions were prepared individually by weighing the required amount of test substance into 800 mL test medium. These solutions were covered and stirred for about 7 days. The pH-value of the test solutions were checked daily and adjusted to 8.5 with NaOH or HCl if necessary. After seven days the solutions were filtered through a 0.2 µm membrane filter. All test solutions were visibly clear and colourless over the exposure period. No effect on algal growth rate was observed at the highest test concentration. This study is considered reliable by the registrant (Ri 2), but this is not agreed upon because the actual concentration of vanadium will have been influenced by adjustment of pH and filtration. More important, it is not known to what extent the presence of bismuth influenced the potential effects of vanadium. An indicative MPC for bismuth of 0.7 µg/L was derived by RIVM in 1993, based on an acute EC_{50} for *Tubifex tubifex* of 0.66 mg/L (Booij et al., 1993).

2.2.6 *Summary of laboratory data on freshwater algae*

The available data on algae are summarised in Table 6. Preference is given to EC_{10}-values over NOECs, because the former represent a better estimate especially when spacing between concentrations is relatively large.

Table 6 Summary of endpoints for algae obtained from the above cited literature.

Species (in alphabetical order)	**Test compound**	**EC_{10} [µg V/L]**	**EC_{50} [µg V/L]**	**Parameter**	**Reference**
cyanophyta					
Anabaena flos-aquae	Na_3VO_4	0.013	1.43	cell number	Lee et al. (1979)
Anabaena flos-aquae	Na_3VO_4	4276	> 50942	growth rate	Nalewajko et al. (1995a)
Synechococcus leopoliensis	Na_3VO_4	5649	> 50942	growth rate	Nalewajko et al. (1995a)
algae/diatomea					
Ankistrodesmus falcatus	Na_3VO_4	0.29	188	growth rate	Nalewajko et al. (1995a)
Chlorella pyrenoidosa	Na_3VO_4	6.90	24491	growth rate	Nalewajko et al. (1995a)
Chlorella pyrenoidosa	NH_4VO_3	1.8	24	cell volume	Meisch and Benzschawel (1978)
Chlorella pyrenoidosa	Na_3VO_4	100[b]	- [c]	cell number	Lee et al. (1979)
Diatoma elongatum	Na_3VO_4	4.71	148	growth rate	Nalewajko et al. (1995a)
Dictyosphaerium planctonicum	Na_3VO_4	31.2	> 50942	growth rate	Nalewajko et al. (1995a)
Kirchneriella lunaris	Na_3VO_4	5.68	855	growth rate	Nalewajko et al. (1995a)
Navicula pelliculosa	Na_3VO_4	> 1000	> 1000	cell number	Lee et al. (1979)
Scenedesmus acutus	Na_3VO_4	863	3412	growth rate	Nalewajko et al. (1995a)
Scenedesmus obliquus	Na_3VO_4	873	977	cell number	Lee et al. (1979)
Scenedesmus subspicatus	V_2O_5-flakes	716	2907	growth rate	REACH
Scenedesmus subspicatus	$NH_4V_3O_8$	1796	3865	growth rate	REACH
Scenedesmus subspicatus	$NaVO_3$	4342	7619	growth rate	REACH

a: Reference states that study is conducted with "sodium orthovanadate ($NaVO_3$)", but $NaVO_3$ is denoted as sodium metavanadate, while sodium orthovanadate is Na_3VO_4. The latter is most likely used.
b: NOEC-value
c: Curve fitting not possible

From the information gathered, it appears that there is a large difference between the results for algae. Very low effect values are obtained for some species in some studies (*A. flos-aquae*, *A. falcatus*, *C. pyrenoidosa*), whereas other species are relatively insensitive (*N. pelliculosa*, *S. leopoliensis*). It is also apparent that there is a difference of several orders of magnitude between the endpoints for *A. flos-aquae* obtained in different tests (e.g. EC_{10} of 0.013 and 4276 µg/L, EC_{50} 1.43 and > 50942). To a lesser extent, this is also the case for *C. pyrenoidosa* (EC_{10} 1.8 and 6.9 µg/L, NOEC 100 µg/L; EC_{50} 24 and 24491 µg/L). Although part of the difference may be explained by different parameters observed (i.e. cell numbers, cell volume or growth rate), this cannot be the sole explanation. The difference is not consistent among studies, both the lowest value for *A. flos-aquae* and the highest value for *C. pyrenoidosa* originate from the same study. It is likely that differences in laboratory strain, vanadium compound, medium composition and parameters studied, all contribute to differences in sensitivity, alone or in combination.
Regarding *C. pyrenoidosa*, it can be argued that the EC_{10} of 6.9 µg/L for growth rate from Nalewajko et al. (1995a) is most appropriate, since this is considered the most relevant and reliable value and is in the same order of magnitude as the EC_{10} for cell volume increase of 1.8 µg/L obtained from the study by Meisch and Benzschawel (1978). For *A. flos-aquae,* there is no additional information that supports either the low or the high EC_{10} of 0.013 and 4276 µg/L, respectively. In general, EC_{10} values that are negligible as compared to the background concentration in the medium should be considered as not reliable. Because of the uncertainty with respect to the data for *A. flos-aquae*, the Scientific Advisory Group INS agreed to leave both values for this species out of consideration.

2.3 Field data

Lee et al. (1979) and Nalewajko et al. (1995b) report on the effect of vanadium on photosynthesis in enclosures in Canadian lakes (see Appendix 1, study 1 and 7). Additions were made as sodium orthovanadate (Na_3VO_4) at concentrations between 10 and 5000 or 6113 µg V/L. From the data of Lee et al. (1979), field EC_{10}-values of 20 and 60 µg added V/L were obtained for Lake St. George and Lake Erie, respectively. An EC_{10} of 38.6 µg added V/L was calculated for the average effect on photosynthesis in seven lakes reported by Nalewajko et al. (1995b). These EC_{10}-values are higher than the EC_{10}-values for growth rate of individual algae and cybanobacteria reported by Nalewajko et al. (1995a). In view of the reported background concentrations of Canadian lakes of 4 to 7 µg/L, adaptation to higher vanadium concentrations may have taken place. In addition, functional redundancy may take place in the field. Furthermore, the data of Meisch et al. indicate that biomass and chlorophyll-a content of algae may be stimulated by vanadium while at the same time cell division is inhibited. The data of Lee et al. (1979) also indicate that biomass and chlorophyll-a content are less sensitive than cell numbers. Therefore, these field values based on photosynthesis are not considered for use in ERL-derivation.

2.4 Additional information on marine algae

The evaluation of Environment Canada does not contain additional references, only the endpoints for the marine algae *Asterionella japonica*, *Dunaliella marina* and *Prorocentrum micans* from the study by Miramand and Unsal (1978) are mentioned. This study was already included in previous RIVM-reports. Van de Plassche et al. (1992) estimated a NOEC from this study by dividing the EC_{50} by a factor of 10, Van Vlaardingen et al. (2005) estimated the EC_{10}-values from a digitised scan of the concentration-response curve. The REACH dossiers do not contain additional information on marine algae either. Moreover, it should be noted that the study of Miramand and Unsal (1978) is not considered reliable by the registrant. Apparently, the registrant used information from Van Vlaardingen et al. (2005), because exactly the same EC_{10}-values are included in the REACH summary. According to the registrant, the study should be disregarded, because (arguments cited from dossier): the test method is poorly described, no standard guideline is followed, no statistics are presented, monitoring before/during test is not reported (physico-chemical parameters, V), the exposure period is not relevant (15 days), results are obtained from a digitised scan and the EC_{10} is extrapolated below the lowest test concentration, and refer to an irrelevant endpoint (mortality) for algae.
Not all arguments of the registrant are considered equally important. For instance the duration of the test might be relevant if the doubling time of these particular species is low, and when interpreting "mortality" as absence of cells, this is a relevant parameter. However, according to current insights the endpoints would not be considered reliable, mainly because it is not reported how cells were counted and apparently, counts were made after 15 days only. It is not known whether control algae were in the exponential growth phase.
One other study was retrieved, in which Fries (1982) observed a positive response of marine algae to vanadium. The study is summarised in Appendix 1, study 8. An increase in fresh weight of *Fucus spiralis* was observed after exposure for 53 days to 1, 10 and 100 µg V/L. The increase seems to be related to "true" growth, *i.e.* biomass increase, since broader leaves and more branches were present as compared to algae grown without additional vanadium. From this study, it appeared that negative effects on *F. spiralis* were not present at ≥ 100 µg V/L. The additional data on marine algae from Fries (1982) indicate that marine algae are probably not very sensitive to vanadium.

2.5 Pooling of ecotoxicity data

The new data on algae have been presented above in Table 6. The previously accepted data on freshwater and marine organisms are presented in Tables 7 and 8 below, data are copied from Van Vlaardingen et al. (2009). It should be noted that according to current criteria, the previously used chronic data for marine algae would probably not considered reliable as argued above. The Environment Canada evaluation and the REACH dossier also contain information for organisms other than algae, but the endpoints from the key-studies were already included in the previous dataset.

Table 7 Selected ecotoxicity data for freshwater species, copied from Van Vlaardingen et al. (2009).

Chronic			Acute		
taxon	**species**	**EC_{10}/NOEC [µg V/L]**	**taxon**	**species**	**L/EC_{50} [µg V/L]**
Crust.	*D. magna*	240	Protozoa	*T. pyriformis*	14000
Pisces	*J. floridae*	41	Annelida	*P. leidyi*	310
			Crust.	*C. pseudogracilis*	12000
				D. magna	1800
			Pisces	*D. rerio*	4000
				C. auratus	2500
				C. fasciatus	5000
				C. latipinnis	12000
				J. floridae	11000
				N. danrica	2600
				O. mykiss	3400
				O. tshawytscha	17000
				P. reticulata	370
				S. fontinalis	7000

Table 8 Ecotoxicity data for marine species, copied from Van Vlaardingen et al. (2009).

Chronic			Acute		
taxon	**species**	**EC_{10}/NOEC [µg V/L]**	**taxon**	**species**	**L/EC_{50} [µg V/L]**
Algae	*A. japonica*	50	Coelen.	*C. caspia*	4500
	D. marina	340	Moll.	*C. gigas*	910
	P. micans	54		*M. galloprovincialis*	64000
			Annel.	*N. diversicolor*	1100
			Crust.	*A. salina*	370
				C. maenas	35000
			Echin.	*P. lividus*	1100
			Pisces	*L. limanda*	28000
				T. jarbua	620

According to the WFD-guidance (EC, 2011), data for freshwater and marine species should not be combined in case of metals unless it can be demonstrated that there is no difference in sensitivity. Van Vlaardingen and Verbruggen (2009) concluded that, although the dataset comprises a range of different species, pooling is not allowed because for the individual taxa too few data are available to make a statistically sound comparison. Addition of the new data on algae (Table 6 and section 2.4) does not change that conclusion: valid acute data for marine algae are still absent and only few endpoints are available for crustacea and marine fish.

2.6 Derivation of the MPC and MAC for direct ecotoxicity

2.6.1 Derivation of the $MPC_{fw, eco}$

Based on the present data, it is clear that freshwater cyanobacteria and algae include the most sensitive as well as the least sensitive species in the dataset. In view of this, it is not considered justified to simply use the lowest available endpoint with an assessment factor. Several options are explored, i.e. using species sensitivity distributions (SSD), or applying assessment factors to the endpoint for fish.

Option 1: SSD

As a first option, it could be considered to use the HC_5 of the chronic data as starting point for the $MPA_{fw, eco}$. Figure 1 shows the SSD based on the endpoints for algae, cyanophyta and diatoms in Table 6. As argued above, the values for *A. flos-aquae* are omitted from the dataset, as is the case for the >-value for *N. pelliculosa*. For *C. pyrenoidosa*, the most relevant and reliable endpoint is chosen (EC_{10} 6.9 µg/L for growth rate), while for *S. subspicatus* the most sensitive endpoint (EC_{10} 716 µg/L) is used.
The goodness of fit is accepted at all levels. The median estimate of the HC_5 is 0.20 µg V/L (range 0.003-2.1), the HC_{50} is 56 µg V/L (range 72-427).

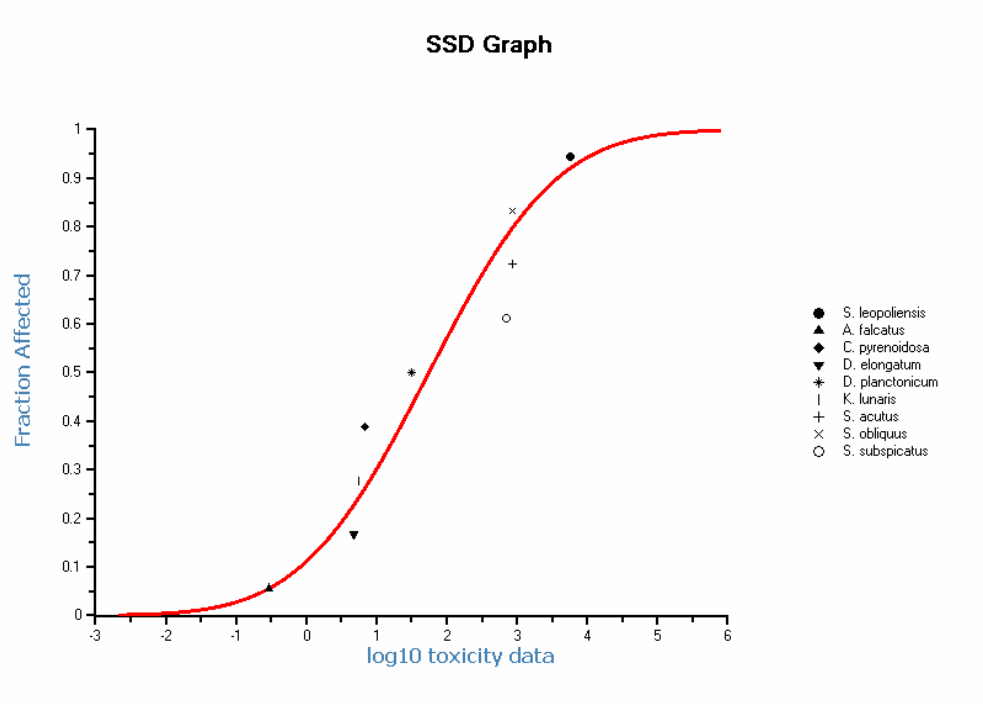

Figure 1 Species sensitivity distribution of cyanophyta, algae and diatoms. Most relevant and reliable chronic endpoint per species according to Table 6, data for A. flos-aquae and N. pelliculosa omitted. X-axis represents log EC_{10}, in µg/L.

When the data for fish and daphnids from Table 7 are added (see Figure 2), the median estimate of the HC_5 is increased by a factor of two, to 0.40 µg V/L (range 0.01-2.8), the HC_{50} is 62 µg V/L (range 12-315).

An assessment factor of 1-5 should be applied to the HC_5. Applying a factor higher than 1 would lead to a concentration that is again very close to the background concentration. Furthermore, the HC_5 of 0.40 µg V/L is protective for crustacea, fish and all algae, except for *A. falcatus*. Considering the specific characteristics of vanadium being an naturally occurring element which is probably essential, it is proposed from a pragmatic point of view to use the HC_5 of 0.40 µg V/L as the $MPA_{fw, eco}$ without a further assessment factor. Taking the background concentration of 0.82 µg V/L in the Netherlands into account, the $MPC_{fw, eco}$ is then 1.2 µg V/L.

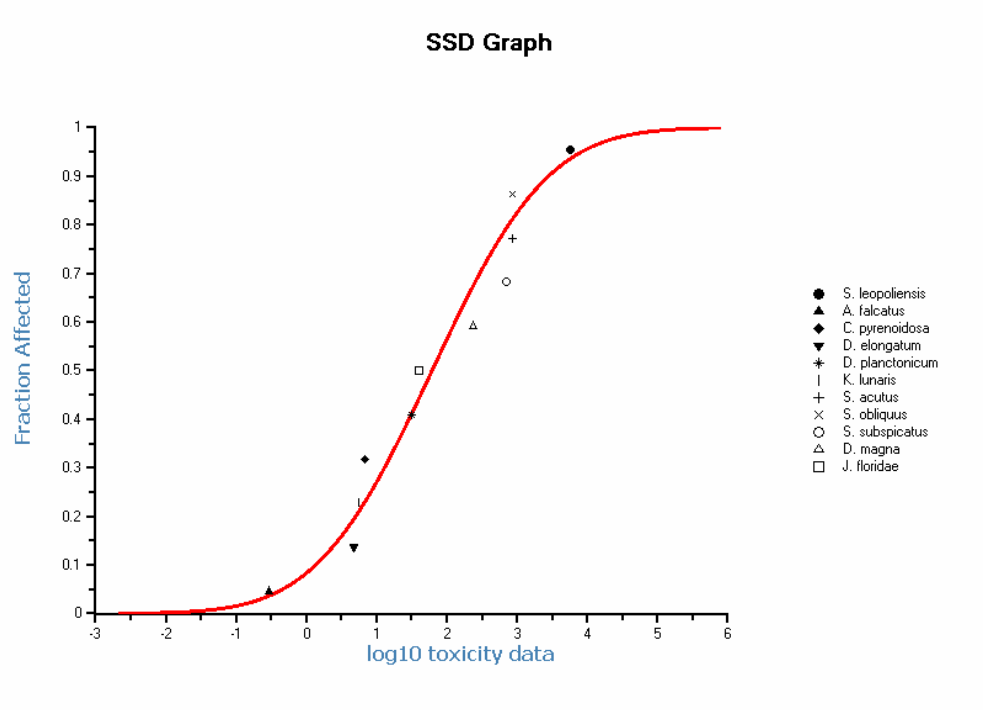

Figure 2. Species sensitivity distribution of cyanophyta, algae, diatoms, crustacea and fish. Most relevant and reliable chronic endpoint per species according to Table 6 and 7, data for A. flos-aquae and N. pelliculosa omitted. X-axis represents log NOEC or EC_{10}, in µg/L.

Option 2: Assessment factor approach

As a second option, it may be considered to use the NOEC of 41 µg V/L for the fish *Jordinella floridae* as the starting point for risk limit derivation, since the data on algae show such a high variation. An assessment factor of 50 could then be used to account for the uncertainty with respect to algae, which would lead to an $MPA_{fw, eco}$ of 0.82 µg V/L. Considering the SSD-curve in Figure 2, this would potentially affect about 5-10% of the species, while from the species listed in Table 6 only *A. falcatus* would be exposed above its EC_{10}. With a background concentration of 0.82 µg V/L, the $MPC_{fw, eco}$ would be 1.6 µg V/L. This is only slightly higher than the value according to option 1, and has the disadvantage that the available information on algae is not fully used.

The Scientific Advisory Group INS supported the use of the SSD for all aquatic species without an additional assessment factor, and advised to set the $MPA_{fw, eco}$ to 0.40 µg V/L, and the $MPC_{fw, eco}$ to 1.2 µg V/L.

2.6.2 *Derivation of the $MAC_{fw, eco}$*

Option 1: SSD

In line with the approach for derivation of the $MPC_{fw, eco}$, an SSD was constructed using the acute toxicity data. The data for *A. flos-aquae* and >-values were not used. For *C. pyrenoidosa*, the most relevant and reliable endpoint is chosen (EC_{50} 24491 µg/L for growth rate), while for *S. subspicatus* the most sensitive endpoint (EC_{50} 2907 µg/L) is used. The goodness of fit is accepted at all levels. The median estimate of the HC_5 is 219 µg V/L (range 76-455), the HC_{50} is 2722 µg V/L (range 1542-4806).

A default assessment factor of 10 is normally applied to account for residual uncertainty, and the fact that 50%-effect concentrations are used while the $MAC_{fw, eco}$ should protect from any effects. The resulting $MAA_{fw, eco}$ is then 22 µg V/L. Taking the background concentration of 0.82 µg V/L in the Netherlands into account, the $MAC_{fw. eco}$ is 23 µg V/L.

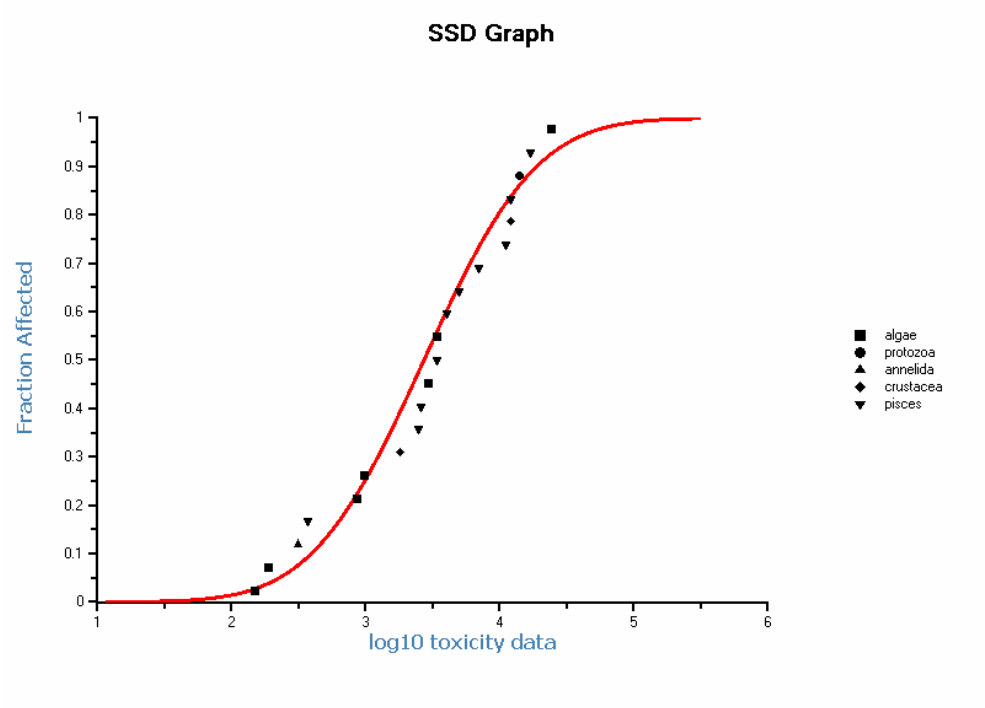

Figure 3. Species sensitivity distribution of cyanophyta, algae, diatoms, crustacea and fish. Most relevant and reliable chronic endpoint per species according to Table 6 and 7, data for A. flos-aquae and N. pelliculosa omitted. X-axis represents log NOEC or EC_{10}, in µg/L.

As can be seen from the data in Table 6, setting the $MAA_{fw, eco}$ to 22 µg V/L would mean that most algae species are exposed above the EC_{10}/NOEC. For algae short-term concentration peaks can still be considered as chronic exposure in view of the duration of their life-cycle. It is thus not sure that this $MAA_{fw, eco}$ is protective from long-term effects due to short-term concentration peaks, as it should be. A higher assessment factor is therefore considered necessary, which is consistent with the guidance for the assessment factor approach (see below).

Option 2: Assessment factor approach

The lowest acute L/EC_{50} is 148 µg V/L for *Diatoma elongatum*. Vanadium does not have a specific mode of action, and the variation between species is high as demonstrated by the standard deviation of the log-transformed L/CE_{50}-values of 0.67. In this case, an assessment factor of 100 should be applied according to the guidance (EC, 2011), leading to an $MAA_{eco, water}$ of 1.5 µg V/L. With a background concentration of 0.82 µg V/L, the $MAC_{fw, eco}$ is 2.3 µg V/L.

In view of the above, the Scientific Advisory Group INS advised to use an assessment factor of 100 to the acute HC_5, and set the $MAA_{eco, water}$ to 2.2 µg V/L This has the advantage of using all available data, while at the same time only a limited number of algae will be exposed above the EC_{10}/NOEC. With a background concentration of 0.82 µg V/L, the $MAC_{fw, eco}$ is 3.0 µg V/L.

2.6.3 Derivation of risk limits for saltwater

Although some additional information on marine algae was found, it is still not possible to derive an $MPA_{sw, eco}$. In case the only available chronic data are those for algae, the MPA should be based on the acute dataset. Acute data on marine algae are missing, whereas algae probably represent the potentially most sensitive taxon. In addition, the chronic endpoints for the marine algae *A. japonica*, *D. marina* and *P. micans* from the study by Miramand and Ünsal (1978) are not considered reliable. This also holds for the EC_{50}-values from this study which were used by Van de Plassche et al. (1992). On the basis of the present dataset, derivation of the $MPA_{sw, eco}$ is not possible. Since the acute base set is not complete, derivation of the $MAA_{sw, eco}$ is not possible either. Moreover, there is no established background concentration for saltwater.

2.7 Conclusions on the MPC and MAC for direct ecotoxicity

Based on the available information, it is proposed to set the $MPC_{fw, eco}$ to 1.2 µg V/L (0.40 µg V/L excluding the background concentration), and the $MAC_{fw, eco}$ to 3.0 µg/L (2.2 µg V/L excluding background). Risk limits for saltwater cannot be derived.

3 Human fish consumption and secondary poisoning

3.1 Human threshold limit and risk limit for predators

3.1.1 Background of the human threshold limit

At the time the report of Van Vlaardingen and Verbruggen (2009) was published, the human toxicological threshold limit for vanadium was under review. The re-evaluation was published by Tiesjema and Baars (2009). For their update, they included the previous evaluation of Janssen et al. (1998), and the additional literature published since then. This included reviews by the World Health Organization (WHO), National Toxicology Program (NTP), European Food Safety Authority (EFSA) and International Agencv for Research on Cancer (IARC) over the years 2000-2006.
With respect to carcinogenicity, Tiesjema and Baars (2009) concluded that it is unclear whether carcinogenic effects in inhalation studies with vanadium pentoxide are relevant for other vanadium compounds. In addition, the relevance of these studies for oral ingestion was questioned. The derivation of the human toxicological threshold limit is based on reproductive and developmental toxicity. In rats, repeated intragastric doses of 5 mg sodium metavanadate/kg_{bw}.d before mating (14 days in females, 60 days in males) resulted in effects on body weight, tale length, and relative organ weight of liver, spleen and kidneys in the pups (Domingo et al., 1986). The LOAEL of 5 mg sodium metavanadate/kg_{bw}.d is equivalent to 2 mg V/kg_{bw}.d. In mice, vanadyl sulphate pentahydrate induced embryotoxic effects at a dose equivalent to 7.5 mg V/kg_{bw}.d when administered on gestational days 6-15. A similar study in mice with sodium orthovanadate resulted in a NOAEL equivalent to 2 mg V/kg_{bw}.d.
Based on the LOAEL of 2 mg V/kg_{bw}.d for rats from Domingo et al. (1986), the TDI was derived using an uncertainty factor of 1000 (10 for LOAEL to NOAEL, 10 for inter- and 10 for intraspecies variation), leading to a value of 2 µg V/kg_{bw}.d. Because of the large uncertainty, this value is indicated as "provisional". Given the results from the other studies, the provisional TDI obtained for sodium metavanadate, is considered to be applicable to other vanadium compounds as well. Tiesjema and Baars (2009) report an estimated background exposure of 0.3 µg/ kg_{bw}.d, which is about seven times lower than the TDI. They note that much higher intakes (ca. 250 µg/ kg_{bw}.d) are reported for body builders using vanadium supplements.
Derivation of the $MPC_{water,\ hh\ food}$ is triggered by the reprotoxic effects of vanadium. The human toxicological threshold of 2 µg V/kg_{bw}.d is used to calculate the $MPC_{biota,\ hh\ food}$. For this, a body weight of 70 kg and a daily fish intake of 115 g are assumed. The contribution of consumption of fishery products to the threshold level is at most 10%.
The $MPC_{biota,\ hh\ food}$ is 2 x 70 x 0.1 / 0.115 = 122 µg V/kg_{fd}. This value is used to calculate the corresponding concentration in water ($MPC_{water,\ hh\ food}$).

3.1.2 Derivation of the risk limit for predators

Van Vlaardingen and Verbruggen (2009) used the unrounded LOAEL of 2.1 mg V/kg_{bw}.d for rats for derivation of the risk limit for predators. Expressed as a concentration in biota, this value is denoted as $MPC_{biota,\ secpois,\ fw}$ and $MPC_{biota,\ secpois,\ sw}$ for the freshwater and saltwater compartment, respectively. A factor of 20 was applied for conversion of the daily dose to a concentration in feed, an assessment factor of 10 was used to extrapolate from LOAEL to NOAEL

and the default assessment factor of 90 was applied to extrapolate the subchronic laboratory NOAEL to field based MPC-level. As a result, the $MPC_{biota,\ secpois}$ was set to 2.1 x 20 / (90 x 10) = 0.0467 mg V/kg_{fd} = 46.7 µg V/kg_{fd}. Using this value, a corresponding concentration in freshwater ($MPC_{fw,\ secpois}$) was calculated that is much lower than the background concentration in water.
For the present report, it was investigated whether the factor of 10 for the extrapolation from LOAEL to NOAEL could be lowered. However, inspection of the original publication of Domingo et al. (1986) reveals that at the level of the LOAEL, 12 to 20% effect was observed at body weight of litters, male and female pups. Analysis of the data using the standard benchmark dose approach indicates that the NOAEL might be as low as 0.04 mg V/kg bw.d, indicating that the factor of 10 is probably not worst case (Wout Slob, RIVM, pers. comm.)

3.1.3 Conclusion on the human toxicological threshold

Based on the information presented above, there is no reason to change the input for the calculation of the $MPC_{water,\ hh\ food}$ and $MPC_{fw,\ secpois}$ with respect to human toxicology (2 µg V/kg_{bw}.d, 122 µg V/kg_{fd}) and risk limits for predators (46.7 µg V/kg_{fd}). It is noted, however, that the human toxicological threshold limit is indicated as "provisional" because of the large uncertainty. To a lesser extent, this also holds for the risk limit for predators, taking into account that a true chronic study with oral exposure via feed is not available.

3.2 Re-evaluation of BCF- and BAF-values

3.2.1 Data used previously

Two studies are available from Van Vlaardingen and Verbruggen (2009) that report bioaccumulation of vanadium in aquatic organisms. From these studies by Ikemoto et al. (2008) and Ravera et al. (2007), a geometric mean BAF of 171 L/kg_{wwt} was derived for mussels and fish. This BAF was used for calculation of the $MPC_{water,\ hh\ food}$ and $MPC_{fw,\ secpois}$.

3.2.2 Additional BCF- and BAF-data

An additional paper by Ravera et al. (2003) is available from which field BAFs could be obtained. In addition, the REACH dossiers were consulted to retrieve additional data, since a systematic search for additional literature on bioconcentration/bioaccumulation had not been performed in the past. Resulting data are summarised in Appendix 3.

3.2.2.1 Laboratory studies

Bioconcentration as observed in laboratory studies is generally low. In most of the evaluated studies equilibrium was not reached, which is a reason to consider the resulting BCF as not reliable. The valid data are presented in Table 9.

Table 9 Bioconcentration of vanadium by aquatic organisms in laboratory studies.

Taxon	Species	Marine/ Fresh	Water conc. [µg V/L]	BCF [L/kg_{wwt}]
Crustacea	*Lysmata seticaudata*	M	2	11
	Lysmata seticaudata	M	2	7
	Lysmata seticaudata	M	2	8
	Lysmata seticaudata	M	2	20
	Lysmata seticaudata	M	2	9
	Lysmata seticaudata	M	2	11
	Lysmata seticaudata	M	25	7.2
	Lysmata seticaudata	M	50	6
	Lysmata seticaudata	M	100	5.5
Pisces	*Jordanella floridae*	F	41.4	25.5
	Jordanella floridae	F	41.4	27.9

Additional valid data are available for the crab *Carcinas maenas* (BCF 12 L/kg) and mollusc *Mytilus galloprovincialis* (BCF 22-38 L/kg). However, these BCF-values were based on whole animals including exoskeleton or shells. Since the exoskeleton and shells are generally discarded and the availability of vanadium from those parts is most likely negligible, the data are not included in Table 9. The majority of the data is obtained for marine species. There are indications that the uptake of vanadium is influenced by salinity. The BCF of the shrimp *Lysmata seticaudata* at salinity 38 ‰ was 9 L/kg, which is more than a factor of 2 lower than the BCF of 20 L/kg obtained at 28 ‰ (Miramand et al., 1981). A similar finding is reported by Ringelband and Hehl (2000) for the brackish water hydroid *Cordylophora caspia*. It is not known, however, whether this is a result of differences in bioavailability or speciation in the exposure medium, changes in the metabolism of the organisms, or a combination of both. Another observation is that although the BCF based on whole body residues was more or less stable in these studies, the internal distribution over the various organs was still changing with time.

3.2.2.2 Field bioaccumulation data

The available relevant field bioaccumulation data are presented in Table 10. Again, the majority of studies relate to marine organisms. All data for molluscs refer to soft tissue, thus excluding the shell. In the Environmental Canada report, a BAF of 333 L/kg_{wwt} is reported for the amphipod *Hyalella azteca*, based on data from Couillard et al. (2008). This value is recalculated from dry weight based concentrations reported in the original paper, assuming a dry weight content of 0.2. Because the actual moisture content is not known, these data are not included here.

Table 10 Bioaccumulation of vanadium by aquatic organisms: field data.

Taxon	Species	Marine/ Fresh	Water conc. [µg V/L]	BAF [L/kg_{wwt}]
Crustacea	*Macrobrachium rosenbergii*	M	1.05	105
	M. equidens	M	1.05	179
	Macrobrachium 3	M	1.05	65
	Macrobrachium 4	M	1.05	110
	Metapeneaus tenuis	M	1.05	26
Mollusca[a]	*Anodonta cygnea*	F	0.43	614
	Dreissena polymorpha	F	0.43	1163
	Unio pictorum	F	0.12	202
	U. pictorum	F	0.28	240
	U. pictorum	F	0.43	259
	V. decussata	M	4.9	130
	V. decussata	M	4.9	81
Pisces	*Clupeoides sp.*	M	1.05	66
	Cyclocheilichthys armatus	M	1.05	140
	Cynoglossus sp.2	M	1.05	166
	Eleotris melanosoma	M	1.05	220
	Glossogobius aureus	M	1.05	218
	Parambassis wolffii	M	1.05	44
	Pisodonaphis boro	M	1.05	57
	Polynemus paradiseus	M	1.05	77
	Puntioplites proctozysron	M	1.05	87

a: all data for molluscs refer to soft tissue

Because of the relatively large variation in BAFs, it was investigated whether there is a relationship between BAFs and weight. Wet weight BAFs were plotted against organism weight, as shown in Figure 4. Note that only marine species are included, since information on organism weight is not available for the freshwater mussels *Anodonta cygnea*, *Unio pictorum* and *Dreissena polymorfa*. There appears not to be a relationship between the two parameters.

In Figure 5, the BAFs are plotted as a function of the water concentrations at which they are determined. *U. pictorum* is plotted separately since this is the only species for which BAFs are available at different external concentrations. In general, there does not seem to be a relationship between BAFs and concentrations in water. The high BAFs observed for *A. cygnea* and *D. polymorpha* might indicate that these species show increased uptake to compensate for deficiency. However, a lower BAF is observed for the mollusc *U. pictorum* at even lower external concentrations. For this species there is no indication of decreasing BAFs at increasing concentrations, instead there seems to be a slight increase. Data from Couillard et al. (2008) for the amphipod *H. azteca* also point at higher BAFs at higher external concentrations.

In Figure 6, the internal concentration of vanadium in crustaceans, molluscs and fish is plotted against the concentrations in water. The dashed line represents the $MPC_{biota,\ hh\ food}$ of 122 µg V/kg_{fd}, the solid line the $MPC_{biota,\ secpois}$ of 46.7 µg V/kg_{fd}. The $MPC_{biota,\ secpois}$ is exceeded in 18 out of 21 cases, the $MPC_{biota,\ hh\ food}$ in nine cases.

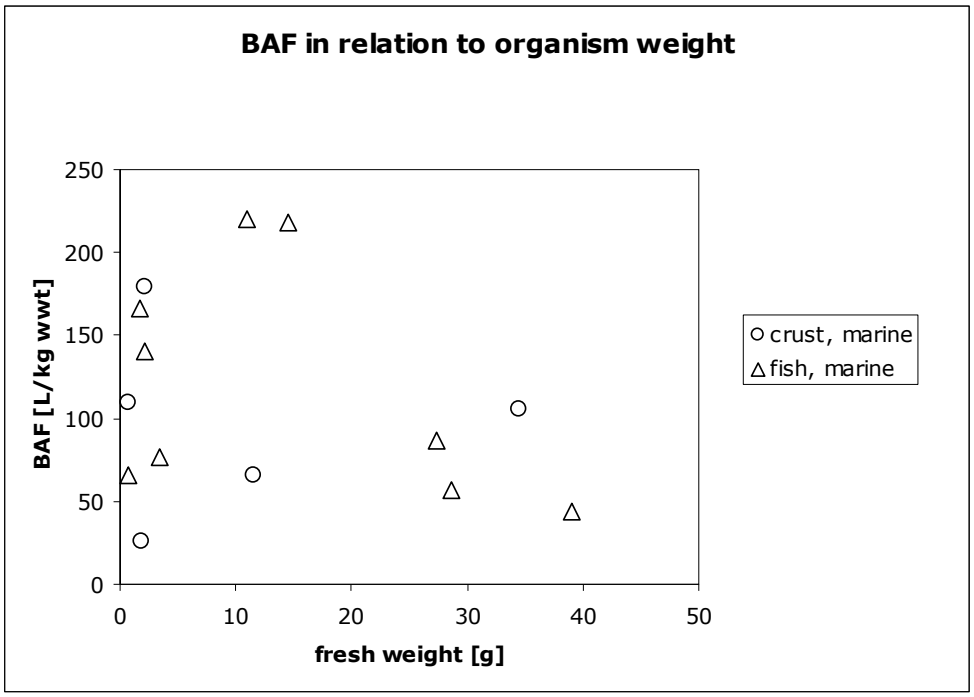

Figure 4 Bioaccumulation factors as a function of weight in water. Data from Ikemoto et al. (2008).

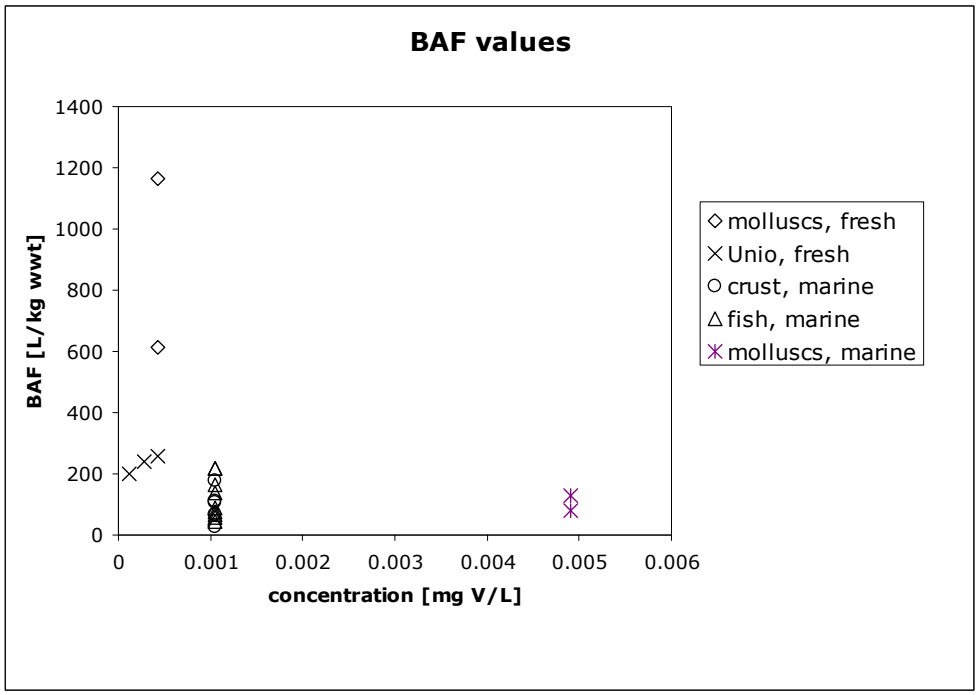

Figure 5 Bioaccumulation factors as a function of external concentrations in water. Data from Appendix 3.

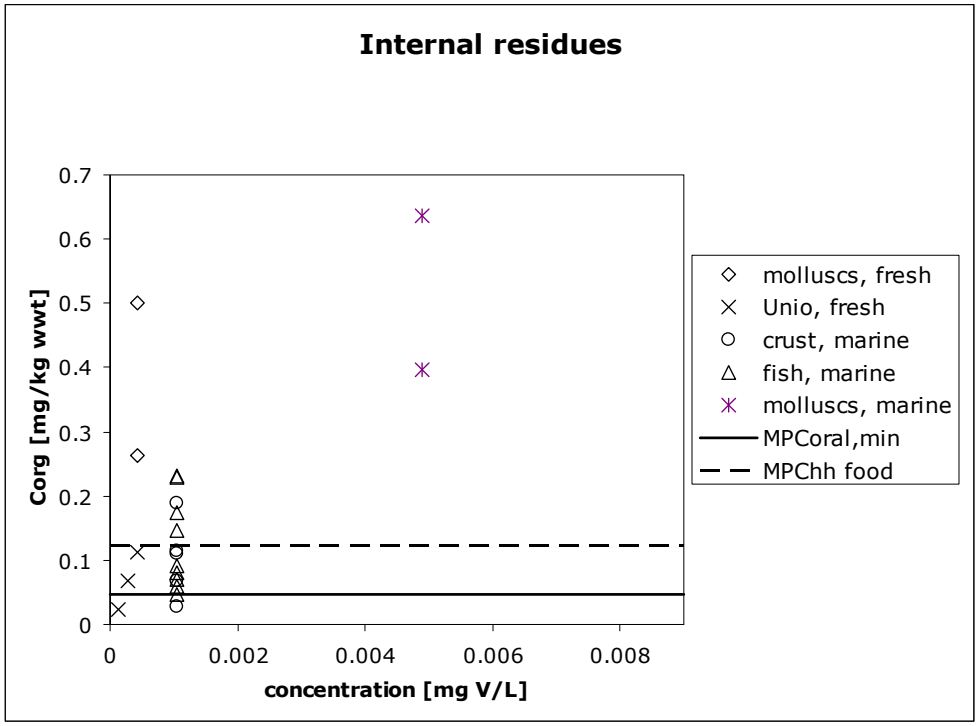

Figure 6 Concentration of vanadium in crustaceans, molluscs and fish as a function of external concentrations in water. Data from Appendix 3. The dashed horizontal line indicates the level of the $MPC_{biota,\ hh\ food}$ (122 µg V/kg_{fd}), the solid line the $MPC_{oral,min}$ (46.7 µg V/kg_{fd}).

3.2.2.3 Difference between freshwater and marine species

Similar to the treatment of ecotoxicity data, the BAFs for freshwater and marine species should be kept separated, unless it can be demonstrated that there is no difference between the two groups. The BAFs for the freshwater species (202-1163 L/kg_{wwt}) seem to be higher than those for marine organisms (26-220 L/kg_{wwt}). This is consistent with the above indicated trend towards lower vanadium uptake at higher salinities. However, a definitive conclusion on differences between freshwater and marine species cannot be drawn because for freshwater, wet weight based BAF-values are available for mussels only, while the marine data refer to crustaceans, mussels and fish. For crustaceans, a comparison of dry weight based BAFs can be made. From the data of Couillard et al. (2008), dry weight based BAFs for *H. azteca* can be calculated of 922 to 2254 L/kg_{dwt}. This is higher than the dry weight based BAFs for marine crustaceans of 105-686 L/kg_{dwt} calculated from the data of Ikemoto et al. (2008).

3.2.2.4 Conclusion on BCF- and BAF-values

Based on the information presented above, it is not possible to present a reliable estimate of bioconcentration or bioaccumulation values for vanadium that is representative for the conditions in Dutch surface waters. The laboratory BCF-values underestimate residues measured in field organisms. Equilibration time between water and organism is long, and even if the BCF based on whole body residues was more or less stable at the end of the experimental period, the internal distribution over the various organs was still changing with time.
With respect to the BAF-values, preference should be given to separate datasets for freshwater and marine species, unless it can be demonstrated that there is no difference between the two datasets. A sound statistical comparison cannot

be made, but there are indications of higher BAFs for freshwater species. This would support the separate treatment of BAFs for freshwater and marine species. However, using separate datasets would mean that derivation of the $MPC_{fw,\ secpois}$ and $MPC_{water,\ hh\ food}$ for freshwater would be based on BAFs for mussels only, ignoring the data for other taxa.
There is no consistent dataset for separate freshwater or marine taxa exposed to different external concentrations. It is therefore not possible to draw conclusions on regulation. If only those values are selected that are obtained at concentrations similar to the background concentration in the Netherlands, no freshwater species would be included. In view of this, further calculations are performed with the overall geometric mean BAF, which is 137 L/kg_{wwt}. Note that this is slightly lower than the BAF of 171 L/kg_{wwt} used by Van Vlaardingen and Verbruggen (2009).

3.3 Derivation of the $MPC_{water,\ hh\ food}$ and $MPC_{fw,\ secpois}$

The $MPC_{biota,\ hh\ food}$ is 122 µg V/kg_{fd}. Using the BAF of 137 L/kg_{wwt}, the corresponding $MPC_{water,\ hh\ food}$ is 122 / 137 = 0.89 µg V/L. As indicated in section 1.3, this value refers to a risk limit that includes the natural background concentration; the resulting value is similar to the natural background concentration and is therefore not useful as a basis for the final MPC for fresh- or saltwater. The $MPC_{fw,\ secpois}$ is calculated from the $MPC_{biota,\ secpois}$ of 46.7 µg V/kg_{fd} and the BAF of 137 L/kg_{wwt} as 46.7 / 137 = 0.34 µg V/L. This value is still below the natural background level of 0.82 µg V/L, and can thus not be used as the final MPC.
As discussed above, there is considerable uncertainty associated with the human toxicological threshold level and risk limit for predators, and the BAF. Regarding the $MPC_{water,\ hh\ food}$, an option could be to investigate whether the default 10% cut-off value can be reconsidered, based on information on intake of vanadium via other sources than fish. It should be noted, however, that vanadium is present in a large number of food items, including vegetable oil, soy and buckwheat. This probably means that the 10% value is a realistic estimate. For predators, considerations on daily consumption and maximum contribution to the daily intake do not apply, and such a refinement option is not possible for secondary poisoning. In addition, it would not reduce the uncertainty associated with the toxicological data and the information on bioaccumulation.
On the basis of the information presented above, the members of the Scientific Advisory Group INS advised against using the $MPC_{water,\ hh\ food}$ or $MPC_{fw,\ secpois}$ as a basis for the final MPC.

4 Discussion and conclusions

4.1 Choice of the MPC and MAC

Based on the available data on bioaccumulation of vanadium, human toxicology, toxicity to predators and effects on algae, an attempt is made to derive environmental risk limits for vanadium in water. There is considerable uncertainty concerning the input data for all routes under investigation.

With respect to direct ecotoxicity for freshwater organisms, it is proposed to set the $MPC_{fw, eco}$ to 1.2 µg V/L and the $MAC_{fw, eco}$ to 3.0 µg V/L. These values refer to dissolved concentrations, including the background concentration of 0.82 µg V/L. It is not possible to derive risk limits for saltwater based on direct ecotoxicity.

There is no reason to revise the previously used risk limits for human toxicology and predators. Based on a re-evaluation of bioaccumulation data, the $MPC_{water, hh\ food}$ is calculated as 0.89 µg V/L, the $MPC_{fw, secpois}$ as 0.34 µg V/L. Both values are at or below the natural background concentration and are therefore not suitable as a basis for the final MPC for fresh- or saltwater. In addition, the human toxicological risk limit is considered as "provisional" because of the large uncertainty in the underlying data. This also holds for the risk limit for predators. Furthermore, the information on bioaccumulation is not sufficient to derive a reliable estimate that is representative for Dutch surface waters.

In view of this, the Scientific Advisory Group INS advised to use the risk limits for direct ecotoxicity, and set the MPC_{fw} to 1.2 µg/L and the MAC_{fw} to 3.0 µg/L (dissolved concentrations, background included). Risk limits for the saltwater compartment could not be derived. If there is a policy need for standard setting, it may be considered to use the MPA_{fw} of 0.40 µg/L with an additional assessment factor of 10. However, a background concentration for saltwater is not established and derivation of an MPC_{sw} would still not be possible.

From this report and previous assessments of other natural elements, the lack of reliable information on bioconcentration and/or bioaccumulation appears to be a major problem for the derivation of risk limits for (essential) elements in general. For this purpose, studies should be performed in which organisms are exposed to, or sampled at, a range of external concentrations, including those relevant for the Dutch background. In addition, more research and monitoring data are needed to develop a method for derivation of background concentrations on a national scale.

4.2 Consequences for humans and predators

Using the BAF of 137 L/kg, the MPC_{fw} would potentially lead to residues in fish of 164 $\mu g/kg_{fd}$. This is only 1.3 times higher than the $MPC_{biota, hh\ food}$ of 122 µg/kg. The $MPC_{biota, hh\ food}$ is derived with an assessment factor of 1000, a daily fish consumption of 115 g and allowing that fish consumption contributes for at most 10% to the daily vanadium intake. Together, these factors lead to an $MPC_{biota, hh\ food}$ that is protective for worst case exposure conditions. The fact that at the level of the MPC_{fw} residues are potentially less than 1.3 times higher than the human risk limit, is therefore considered acceptable. At the level of the proposed MPC_{fw}, residues in fish are potentially 3.5 times higher than the risk limit for predators. It is not clear to what extent the MPC_{fw} may pose a risk to

birds and mammals, since considerations on daily consumption and maximum contribution to the daily intake do not apply.

4.3 Comparison with monitoring data

A full comparison with monitoring data is beyond the scope of this report. Data over 2010 from RIWA (2010ab) are used as an indication of environmental concentrations. Dissolved concentrations at RIWA monitoring stations are presented in Table 11. These data indicate that yearly average concentrations may exceed the MPC_{fw}. The MAC_{fw} was not exceeded at RIWA sampling points in 2010, but at other locations maximum concentrations reported for 2010 in Waterbase (www.waterbase.nl) are at or above the newly proposed value (e.g. 3.0 µg/L at Almelo, 2.9-5.2 µg/L at Sas van Gent).

Table 11 Monitoring data for vanadium over 2010, taken from RIWA (2010ab). All values refer to dissolved concentrations (filtration over 0.45 µm).

Location	Average [µg/L]	Maximum [µg/L]
Lobith	1.1	1.4
Nieuwegein	1.3	2.0
Nieuwersluis	1.1	1.5
Andijk	0.71	1
Brakel	0.50	0.76
Heel	1.3	2
Keizersveer	1.1	1.6

4.4 Conclusions

It is proposed to set the MPC_{fw} to 1.2 µg/L and the MAC_{fw} to 3.0 µg/L. Both values refer to dissolved concentrations including the background for Dutch surface waters. From a preliminary comparison with monitoring data it is concluded that the newly derived risk limits are likely to be exceeded.

Acknowledgement

The members of the Scientific Advisory Group INS are gratefully acknowledged for their comments and advise on this report. Thanks are due to Peter van Vlaardingen (RIVM) for comments on earlier drafts. Eric Verbruggen (RIVM) is acknowledged for reviewing the study evaluations and specific advise on the bioaccumulation part.

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List of abbreviations

BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
EC_x	Concentration at which x% effect is observed
ERL	Environmental Risk Limit
GLP	Good Laboratory Practice
I&M	Dutch Ministry of Infrastructure and Environment
INS	International and National Environmental Quality Standards for Substances in the Netherlands
LC_{50}	Concentration at which 50% mortality is observed
MAC_{eco}	Maximum Acceptable Concentration for ecosystems
$MAC_{fw, ecor}$	Maximum Acceptable Concentration for ecosystems in freshwater
$MAC_{sw, eco}$	Maximum Acceptable Concentration for ecosystems in the saltwater compartment
Marine species	Species that are representative for marine and brackish water environments and that are tested in water with salinity > 0.5 ‰.
MATC	Maximum Acceptable Toxicant Concentration, geometric mean of LOEC and NOEC (see below)
MPA	Maximum Permissible Addition = MPC (see below) for metals expressed as added concentration excluding the background; subscripts are used accordingly
MPC	Maximum Permissible Concentration
MPC_{fw}	Maximum Permissible Concentration in freshwater
MPC_{sw}	Maximum Permissible Concentration in the saltwater compartment
$MPC_{fw, eco}$	Maximum Permissible Concentration in freshwater based on ecotoxicological data
$MPC_{sw, eco}$	Maximum Permissible Concentration in the saltwater compartment based on ecotoxicological data
$MPC_{fw, secpois}$	Maximum Permissible Concentration in freshwater based on secondary poisoning
$MPC_{sw, secpois}$	Maximum Permissible Concentration in the saltwater compartment based on secondary poisoning
$MPC_{water, hh food}$	Maximum Permissible Concentration in freshwater and saltwater based on consumption of fish and shellfish by humans
$MPC_{dw, hh}$	Maximum Permissible Concentration in water used for abstraction of drinking water
LOEC	Lowest Observed Effect Concentration
NOEC	No Observed Effect Concentration
LOAEC/L	Lowest Observed Adverse Effect Concentration/Level
NOAEC/L	No Observed Adverse Effect Concentration/Level
OECD	Organisation for Economic Cooperation and Development
REACH	Registration, Evaluation and Authorisation of Chemicals according to Directive 1907/2006)
SSD	Species Sensitivity Distribution
TDI	Tolerable Daily Intake
TGD	Technical Guidance Document
WFD	Water Framework Directive (2000/60/EC)

Appendix 1 Summary of studies with algae from the open literature

Study 1. Lee et al. (1979)
Lee et al. (1979) studied the effect of vanadium addition on photosynthetic capacity and extracellular release of organic carbon substances in samples from Lake Erie, Lake St. George and Jack's Lake. In addition, the response of *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Navicula pelliculosa* and *Anabaena flos-aquae* were studied in 7-days growth tests.

Field studies
Field samples were collected and vanadium was added as a solution of sodium orthovanadate (Na_3VO_4) at concentrations between 10 and 5000 µg V/L. Concentrations of vanadium in Ontario lakes are generally between 4 and 7 µg/L. ^{14}C-$NaHCO_3$ was added and bottles were incubated in the lake or in an incubator. At intervals, triplicate aliquots were filtered and radioactivity retained on the filters was counted and extracellular organic ^{14}C-substances were determined. Concentrations < 50 µg V/L had no effect on, or were stimulating to photosynthesis, while above 50 µg V/L photosynthesis was consistently and significantly depressed (see copied figure below).

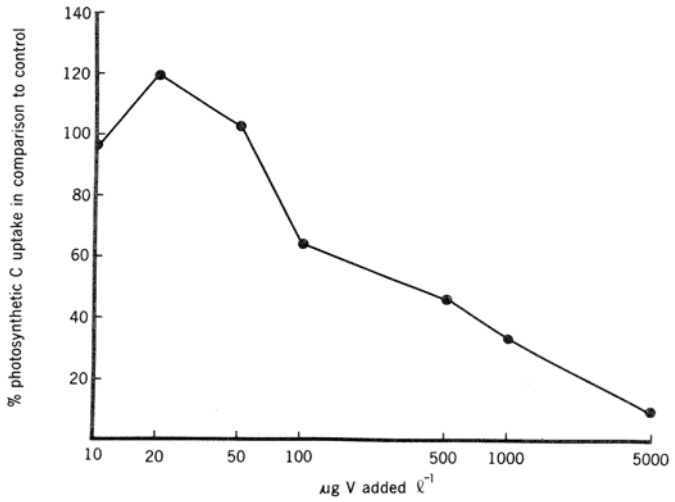
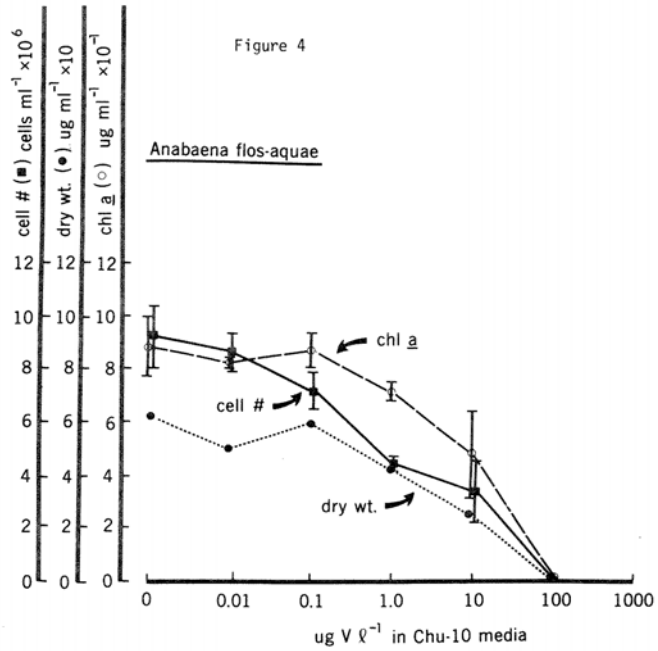


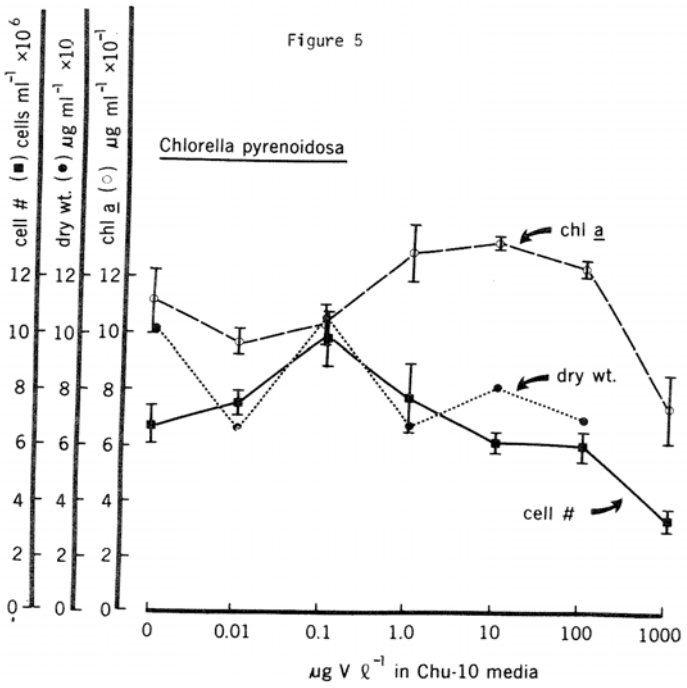
Figure 1. Effect of vanadium concentrations (0-5000 µg V ℓ^{-1}), on photosynthetic carbon uptake in Lake Erie; June 1, 1978. (Sample collected from the central basin of Lake Erie, 1 meter depth, incubated for 6 hours at ambient lakewater temperature and light conditions).

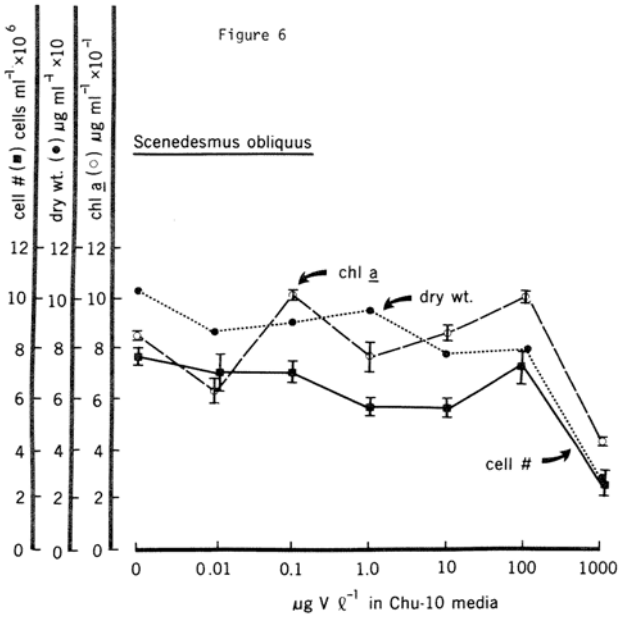
The photosynthesis data were read from the digitised graphs using the TechDig Program, and EC_{10} and EC_{50}-values were estimated by non-linear regression of a sigmoid concentration-response curve using the Graphpad Prism software, setting the top to 100% (because of the observed stimulation at lower concentrations) and the bottom to 0. The EC_{10} and EC_{50} for photosynthesis in Lake Erie was estimated as 58 and 475 µg added V/L, respectively, corresponding values for Lake St. George were 20 and 68 µg added V/L. In Lake St. George, vanadium stimulated extracellular release of organic compounds.

Laboratory experiments
For the laboratory experiments, algae cultures in exponential growth phase were exposed in triplicate to concentrations of 0 to 1000 µg V/L (as Na_3VO_4) in standard growth medium (Chu-10). Background concentration of the medium was 2.7 µg V/L. pH was adjusted to 6.8, temperature was 25 °C for *A. flos-aquae*, and 23 °C for the other species. Cell numbers, dry weight and chlorophyll-a content were determined after seven days. Cell numbers of *A. flos-aquae* were decreased at 0.1 µg V/L and higher, complete suppression was observed at 100 µg V/L. The other species, however, were only inhibited at 1000 µg V/L (*C. pyrenoidosa*, *S. obliquus*) or did not show a clear response (*N. pelliculosa*). The results are presented by concentration-response curves (see copied figures below).

Figure 4, 5, 6, and 7. Effects of vanadium concentration on the growth of the blue-green alga Anabaena flor-aquae; the green algae Chlorella pyrenoidosa, Scenedesmus obliquus and the diatom Navicula pelliculosa.
(Data represents biomass measurements after 7 days autotrophic growth).







The response of the respective parameters was read from the digitised graphs using the TechDig Program, and EC_{10} and EC_{50}-values were estimated by non-linear regression of a sigmoid concentration-response curve using the Graphpad Prism software. The control concentration was set to 10^{-5} µg/L, the bottom of the curve was set to 0. The LOEC was estimated from the graph as the lowest concentration with a clear difference with the control, taking into account the difference of error bars, the NOEC as the next lower concentration. The results are presented below. Figures in italics are considered not reliable due to bad fit.

Table A1.1 Effect of vanadium on cell numbers, dry weight and chlorophyll-a content of algae and diatoms after 7 days exposure.

Species	Parameter	LOEC [µg/L]	NOEC [µg V/L]	EC_{10} [µg V/L]	EC_{50} [µg V/L]	r^2
Anabaena flos-aquae (cyanobacteria)	cell numbers	0.1	0.01	0.013	1.43	0.97
	dry weight	1	0.1 [a]	0.36	6.7	0.96
	chlorophyll-a	1	0.1	1.5	12.5	0.98
Chlorella pyrenoidosa (green algae)	cell numbers	1000	100	*112.7*	*794*	*0.67*
	dry weight	1000	100 [a]	fit not possible [b]		
	chlorophyll-a	1000	100	*875*	*1066*	*0.53*
Scenedesmus obliquus (green algae)	cell numbers	1000	100	873	977	0.80
	dry weight	1000	100 [a]	69	536	0.91
	chlorophyll-a	1000	100	fit not possible [b]		
Navicula pelliculosa (diatomea)	cell numbers	> 1000	≥ 1000	> 1000	> 1000	-
	dry weight	> 1000	≥ 1000	> 1000	> 1000	-
	chlorophyll-a	> 1000	≥ 1000	> 1000	> 1000	-

a: error bars not given
b: no clear concentration response at lower concentrations

Study 2. Meisch and Bielig (1975)
Meisch and Bielig (1975) studied the effect of vanadium on growth, chlorophyll formation and iron metabolism in two green algae species. Addition of 20 µg V/L (as ammonium vanadate, NH_4VO_3) to synthetic nutrient medium containing 1 mg Fe/L (as $FeCl_3.6H_2O$), increased growth of *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. Dry weight after 7 days was increased by 500 and 380%, respectively, as compared to vanadium-free controls. *S. obliquus* takes up 90% of the amount offered and chlorophyll formation was stimulated in the presence of vanadium. Vanadium does not alter iron uptake, but the effect of vanadium decreases when iron was added in the form of stable complexes.

Study 3. Meisch et al. (1977)
Meisch et al. (1977) studied the effect of vanadium-additions on *Chlorella pyrenoidosa* that was cultured in vanadium-free medium. Starting cell material for assays in liquid cultures in presence of vanadium (> 1 µg V/L) was collected from solid medium (medium A containing 10 g/L agar, 1.0 g/L proteosepeptone, 0.2 g/L KNO_3, 0.02 g/L $MgSO_4.7H_2O$, 0.02 g/L $K_2HPO_4.3H_2O$). AAS–analysis revealed that peptone contained more than 2 mg V/kg, vanadium content of agar and the salts was below the detection limit (1 µg/kg). Liquid cultures with additions of less than 1 µg V/L therefore required an agar medium free of peptone (medium B, containing 10 g/L agar, 0.1 g/L urea, 0.2 g/L KNO_3, 0.12 g/L Ca(NO3)2.4H_2O, 0.1 g/L $MgSO_4.7H_2O$, 0.03 g/L KH_2PO_4, 0.04 g/L $K_2HPO_4.3H_2O$; trace elements according to Arnon, 1953 and 1 mg Fe/L iron as Fe(III)citrate).
After 7 days in autotrophic liquid culture, additions between 0.01 and 1 µg V/L (added as NH_4VO_3) caused a continuous increase in dry weight. Dry weight at 1 µg V/L was 4.89 g/L as compared to 2.92 g/L in the vanadium-free control. Chlorophyll content was increased as well, but the effect was less pronounced. In a similar experiment, a series of concentrations between 5 µg V/L and 100 mg V/L was tested. Dry weight increased continuously, with an optimum at 500 µg V/L, and decreased thereafter. Chlorophyll content showed a similar response, but the optimum was found at a broader range of 50 to 1000 µg V/L. At 25 mg V/L, the response was similar to that of the vanadium-free cultures, complete inhibition was observed at 100 mg V/L.

Study 4. Meisch and Benzschawel (1978)

Meisch and Benzschawel (1978) showed that the previously observed increase in algal biomass by vanadium is caused by a shift towards cells with a higher volume. NH_4VO_3 was added to cultures of *Chlorella pyrenoidosa* (cultured in medium A as described above, with vanadium-containing peptone) at concentrations of 1 µg V/L to 100 mg V/L and distribution of cell volumes was determined after 3 days. Cell volumes increased with increasing vanadium concentrations. A significant increase to larger volumes was observed as from 20 µg V/L, the maximum was found at 500 µg V/L after which cell volumes decreased.

In an additional experiment, the authors studied the effect of vanadium on cell division. For this, cultures were started under synchronous conditions (16:8 h L:D) over six light/dark periods with and without the addition of NH_4VO_3 (20 µg V/L). During the first three periods, there was no difference between the cultures without and with vanadium: cell division began after 2 h in the dark and terminated about 4 h later. At the start of the fourth cycle, however, cell division in vanadium cultures stopped, while the cells continued growing. When those cultures were transferred to fresh medium containing the same vanadium concentration, cell division started again for three cycles and then stopped. The inhibition of cell division was also shown under continuous light. Staining revealed that vanadium-free cells had nuclei with diameters < 1 µm. Vanadium-inhibited, undivided cells had larger nuclei, often more than one nucleus was present. The authors conclude that vanadium, although called an essential trace element, has a toxic effect. During growth, duplication of the genetic material goes on without disturbance, but normal nuclear division is inhibited in the presence of vanadium.

Table A1.2 shows the mean cell volume of *C. pyrenoidosa* at different concentrations of vanadium. The reported data were used to estimate a concentration-response relationship, using the concentrations of 0 to 500 µg V/L (maximum cell volume). The EC_{10} and EC_{50} were estimated by non-linear regression of a sigmoid concentration-response curve using the Graphpad Prism software. The EC_{10} is 1.8 µg V/L (95% CI 0.32-9.4 µg/L), the EC_{50} is 24 µg V/L (95% CI 8.0-70; r^2 0.9966). Top and bottom were not fixed because setting minimum or maximum cell volume is not considered adequate.

Table A1.2 Cell volume of Chlorella pyrenoidosa after 3 days exposure to vanadium.

Vanadium concentration [µg/L]	**Mean cell volume**	
	[µm³]	**[% of control][a]**
0	319	100.0
1	468	146.7
5	573	179.6
20	938	294.0
100	1316	412.5
500	1510	473.4
1000	1161	363.9
10000	1147	359.6
100000	920	288.4

a: calculated by evaluator

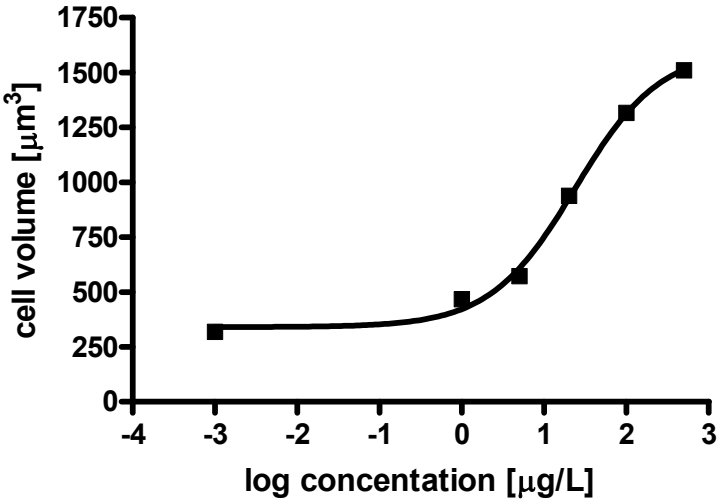


Figure A1.1 Cell volume of Chlorella pyrenoidosa after exposure to vanadium.

Study 5. Meisch et al. (1980)
In this study, Meisch et al. (1980) studied interactions between iron and vanadium in *Chlorella fusca*. They showed that iron and vanadium deficient *C. fusca* have increased rate of starch synthesis, accompanied by a limited ability of the algae to use these carbohydrates. High starch contents are observed in algae that are deficient for both iron and vanadium, intracellular starch is reduced by addition of trace metals. In the presence of Fe(III)citrate or $FeCl_3$, addition of NH_4VO_3 at 20 µg V/L increased dry weight by 22 and 158%, respectively. At the same time, chlorophyll content was increased by 43 and 90%, but cell numbers decreased by 27 and 32%.

Study 6. Nalewajko et al. (1995a)
Nalewajko et al. (1995) studied the effect of vanadium on a range of freshwater algae and cyanobacteria, focussing on the interactions with phosphate. They report that they used "sodium orthovanadate ($NaVO_3$)", but $NaVO_3$ is denoted as sodium metavanadate, while sodium orthovanadate is Na_3VO_4. It is thus not clear which form was used, most likely Na_3VO_4. They showed that the phosphorus state of the cells (P-deficient vs. P-sufficient) is a major controlling factor of the inhibitory effect of vanadium. In P-sufficient cultures, vanadium was inhibitory when the vanadium concentration exceeded the phosphate concentration. In P-deficient cultures, inhibition of photosynthesis by vanadium increased with increasing phosphorus deficiency. The authors concluded that vanadium competes with phosphate, based on the observations that the influx of $^{32}P\text{-}PO_4$ into P-deficient cells was decreased in the presence of vanadium; addition of phosphate reduced the effect of vanadium on photosynthesis; and vanadium accumulated in the cells. As part of their study, photosynthetic capacity was studied in 2-hours experiments in which 16 algal species, that were grown under P-sufficient conditions, were exposed to different concentrations of vanadium in phosphate free medium. A significant concentration-related response was shown for all species. Sensitivity was significantly different between species. As a follow-up, the effect of vanadium on growth rate was determined for eight species. This experiment is most relevant for the present purpose, and is discussed in more detail.
The cyanophyta *Anabaena flos-aquae and Synechococcus leopoliensis*, green algae *Ankistrodesmus falcatus*, *Chlorella pyrenoidosa*, *Dictyosphaerium*

planctonicum, *Kirschneriella lunaris*[4], *Scenedesmus acutus* and diatom *Diatoma elongatum*, from exponentially growing cultures were exposed for 7-10 days to six vanadium concentrations in synthetic medium to which 57.5 µM PO_4^{3-} (5.5 mg/L) was added. It is assumed that in this case the normal Chu-10 medium is meant, which according to information on the internet contains 0.01 g/L K_2HPO_4 = 5.5 mg PO_4^{3-}/L. Nominal concentrations were 0.2, 2, 20, 200, 1000 µM V, added as sodium orthovanadate, $NaVO_3$, equivalent to 10.2-50942 µg V/L. Concentrations were not measured. Initial cell density was 2000 cells/mL. During the experiment pH, which was adjusted to 6.5 at the start, increased but never exceeded pH 8. Cell counts were made using a hemacytometer, filamentous algae were briefly sonicated before counting. Growth rates were reported as mean number of divisions per day of four replicates. The results of this experiment are presented by concentration-response curves for the respective species (see below). For each species, growth rates were read from digitised graphs using the TechDig Program, and EC_{10} and EC_{50}-values were estimated by non-linear regression of a sigmoid concentration-response curve using the Graphpad Prism software. The control concentration was set to 10^{-5} µg/L, the bottom of the curve was set to 0. The results are presented in Table A1.3 below. All values are based on nominal concentrations. Since the same medium was used as in the study of Lee et al. (1979, study 1), it is assumed that a similar background level of vanadium of ≈ 2.5 µg/L was present. The exposure time of 7-10 days is longer than generally applied, but cell counts were made daily and it is stated that growth rates were calculated from the exponential growth phase.

Table A1.3 Effect on growth rate (divisions/day) of several species of algae, cyanobacteria and diatoms after 7 – 10 days exposure to vanadium.

Species	**EC_{10} [µg V/L]**	**EC_{50} [µg V/L]**	**r^2**
cyanobacteria			
Anabaena flos-aquae	4276	> 50942	0.93
Synechococcus leopoliensis	5649	> 50942	0.93
algae/diatomea			
Ankistrodesmus falcatus	0.29	188	0.93
Chlorella pyrenoidosa	6.90	24491	0.88
Diatoma elongatum	4.71	148	0.98
Dictyosphaerium planctonicum	31.2	> 50942	0.82
Kirchneriella lunaris	5.68	855	0.96
Scenedesmus acutus	863	3412	0.96

In another experiment, *S. obliquus* was exposed to 15.7 and 177 µM V (800 and 9017 µg V/L) for nine days. Cell volumes had increased to 137.4 and 607 μm^3, respectively, as compared to 71.6 μm^3 in the control (1.9 and 8.5 times increase), whereas cell numbers decreased to 78 and 9.5% of the control value. Based on these data, the EC_{10} for cell numbers would be around 400 µg/L, the EC_{50} would be around 1900 µg/L (see Figure A1.2). The EC_{10} for cell volume increase would be around 1000 µg V/L, the EC_{50} around 6000 (Figure A1.3).

[4] According to algaebase.org, the name should be spelled as Kirchneriella

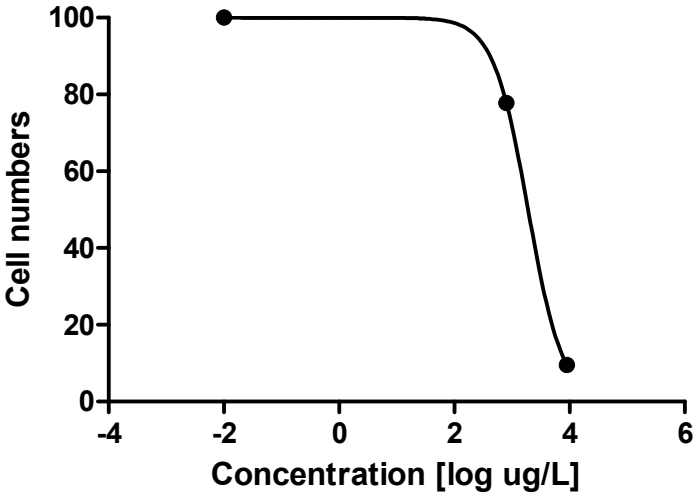


Figure A1.2 Cell numbers of Scenedesmus obliquus after 9 days as a function of vanadium concentration.

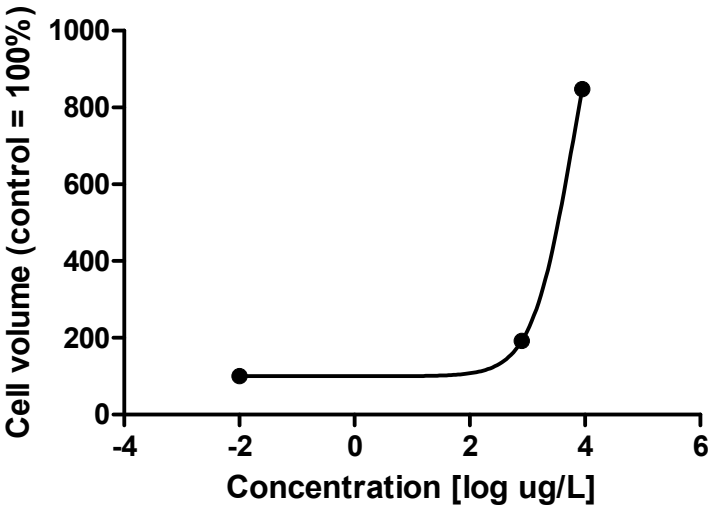


Figure A1.3 Cell volume increase of Scenedesmus obliquus after 9 days as a function of vanadium concentration.

From the correlation between cell volume increase and cell numbers (Figure A1.4), it can be seen that an EC_{10} for cell volume increase is relevant in terms of effects on growth rate.

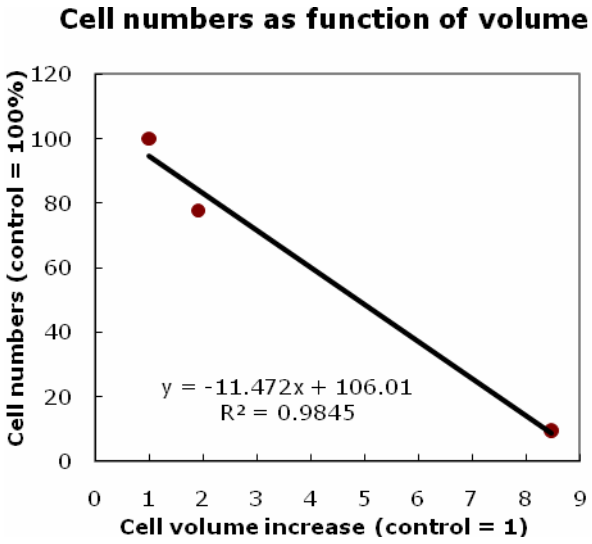


Figure A1.3 Relationship between cell volume increase and cell number decrease.

Colony dissociation was found: in the absence of vanadium, 90% of the cells was found in four-celled colonies, but the proportion of these decreased progressively with increasing concentrations. Ultrastructural changes included thicker cell walls, numerous vacuoles, and lipid and starch bodies. Normal growth rates and cell types were observed 4-6 days following resuspension of abnormal cells in vanadium-free medium. The observed increase in cell volume after exposure to vanadium is consistent with the observations of Meisch and Benzschawel (1978) described above (Study 4). The relationship between cell volume increase and decrease in cell numbers is plotted below. Since only three data points are available, the regression line should be considered as indicative only, and the relationship is probably not fully linear at low volume changes.

Study 7. Nalewajko et al. (1995b)
Nalewajko et al. (1995b) performed field experiments similar to those of Lee et al. (1979). Field samples were collected in seven Canadian lakes and vanadium was added as a solution of sodium orthovanadate (Na_3VO_4) at concentrations between 2 x 10^{-7} M and 1.2 x 10^{-4} M (10 - 6113 µg V/L). Photosynthetic rate was measured *in situ* after addition of ^{14}C-$NaHCO_3$. Average photosynthesis measured in all lakes was plotted against concentration as percentage of the control (100%), see copied figure below. The photosynthesis data were read from the digitised graphs using the TechDig Program, and EC_{10} and EC_{50}-values were estimated by non-linear regression of a sigmoid concentration-response curve using the Graphpad Prism software, setting the bottom to 0. The EC_{10} and EC_{50} for photosynthesis was estimated as 39 and 2260 µg added V/L, respectively.

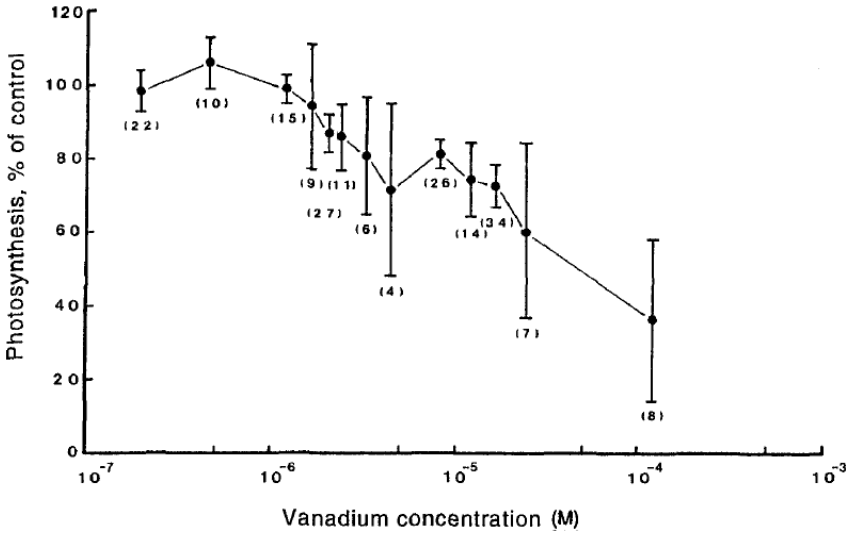


Fig. 2. Effects of vanadium on rates of phytoplankton photosynthesis in 7 lakes. In brackets: number of experiments at each vanadium concentration. Error bars represent 95% confidence limits.

For Lake Ontario, a separate regression plot is presented of photosynthesis as percentage of the control against concentration (see copied figure below). The regression line is described by y=-0.062x + 98.2 (r^2 0.90, n=4), with x being concentrations in 10^{-7} M. From this formula, an EC_{10} and EC_{50} of 673 and 3958 µg V/L can be calculated. It should be noted that a log-logistic regression would probably more appropriate.

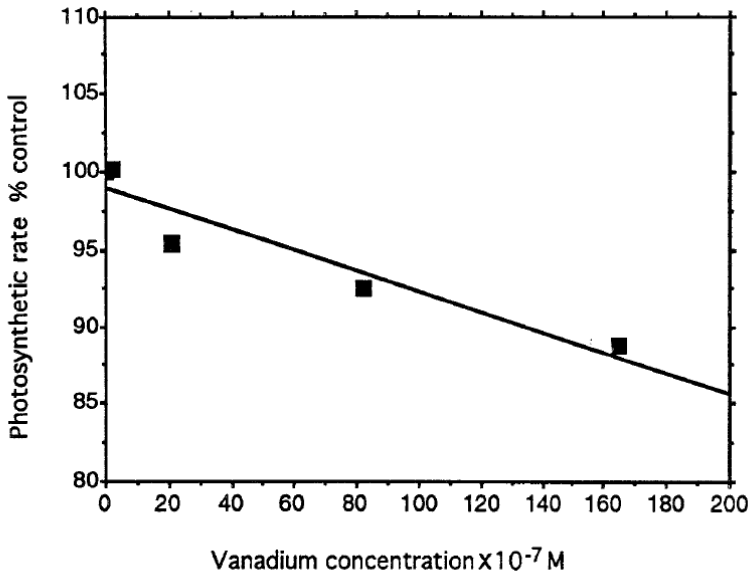


Fig. 4. Relationship between photosynthetic rate (as % control value) and added vanadium concentration in Lake Ontario, 19/07/79.

Study 8. Fries (1982)

Fries (1982) studied the effect of vanadium on marine algae. In 53-days growth experiments, additions of NH_4VO_3 at 1, 10 and 100 µg V/L increased fresh weight of the multicellular brown algae *Fucus spiralis* to 53, 79 and 65 mg as compared to 14 mg in the vanadium-free control (279, 464 and 364% increase, see Figure copied below). Morphology was strikingly changed with broader blades and more branches at higher concentrations. No differences in chlorophyll

content were observed after 18 days, but significant changes were seen after 45 days, with increases up to 50%. An increase in fresh weight of about 90% was observed for the green seaweed *Enteromorpha compressa* when grown at 10 µg V/L, but morphology was not changed strongly.

Fig. 1. Growth of *Fucus spiralis* with vanadium, 1–100 µg l^{-1}, added as NH_4VO_3. All inocula in a horizontal row originated from the same plant. Fresh weight given as total weight of the five plants from one series. Experimental time 53 d (November–December). 15° C, 15 h light. The bar corresponds to 2 cm

Appendix 2 Summary of studies with algae from the REACH dossiers

The ECHA website was searched for additional information submitted in the REACH dossiers. Information on registered substances is available via http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances
Vanadium was used as search string, which resulted in a number of dossier names. All dossiers were checked on information on algae. For the other taxa the key-studies as indicated by the registrant were already included in the dataset. Below, the ecotoxicity tables are presented that are based on the study summaries.

Legend to column headings	
A	test water analysed Y(es)/N(o)
Test type	S = static; Sc = static closed; R = renewal; F = flow through; CF = continuous flow; IF = intermittent flow system
Purity	refers to purity of active substance or content of active in formulation; ag = analytical grade
Test water	am = artificial medium; dtw = dechlorinated tap water; dw = deionised/dechlorinated/distilled water; nw= natural water; rw = reconstituted water; rtw = reconstituted tap water; tw = tap water
T	temperature
Ri	Reliability index according to Klimisch et al. (1997); asterisk indicates citation

Table A2.1. Toxicity of vanadium to freshwater algae.

Species	**Species properties**	**A**	**Test type**	**Test compound**	**Purity [%]**	**Test water**	**pH**	**T [°C]**	**Hardness $CaCO_3$ [mg/L]**	**Exp. time**	**Criterion**	**Test endpoint**	**Value [µg V/L]**	**Ri**	**Notes**	**Ref.**
Algae																
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	V_2O_5 flakes	55.9%		7.9-8.1	21-25		72 h	EC10	growth rate	716	2	1	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	V_2O_5 flakes	55.9%		7.9-8.1	21-25		72 h	EC50	growth rate	2907	2	1	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	V_2O_5 flakes	55.9%		7.9-8.1	21-25		72 h	NOEC	biomass	16.8	2	1	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	V_2O_5 flakes	55.9%		7.9-8.1	21-25		72 h	EC50	biomass	989.4	2	1	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	$NH_4V_3O_8$; powder, 100% < 3 mm			7.85-8.80	21-25		72 h	EC10	growth rate	1796	2	2	ECHA, 2011

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg V/L]	Ri	Notes	Ref.
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	$NH_4V_3O_8$; powder, 100% < 3 mm			7.85-8.80	21-25		72 h	EC50	growth rate	3865	2	2	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	$NH_4V_3O_8$; powder, 100% < 3 mm			7.85-8.80	21-25		72 h	NOEC	biomass	30	2	2	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 0^4 cells/mL	Y	S	$NH_4V_3O_8$; powder, 100% < 3 mm			7.85-8.80	21-25		72 h	EC50	biomass	1162	2	2	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	$NaVO_3$	6.82%	rw	7.9±0.3	21-25		72 h	EC10	growth rate	4342	2	3	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	$NaVO_3$	6.82%	rw	7.9±0.3	21-25		72 h	EC50	growth rate	7619	2	3	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	$NaVO_3$	6.82%	rw	7.9±0.3	21-25		72 h	NOEC	biomass	75	2	3	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 10^4 cells/mL	Y	S	$NaVO_3$	6.82%	rw	7.9±0.3	21-25		72 h	EC50	biomass	2177	2	3	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 0.5×10^4 cells/mL	N	S	$BiVO_4$	100.80%	am	8.5	22.4-22.6		72 h	NOEC	growth rate	≥ 100000	3	4	ECHA, 2011
Scenedesmus subspicatus	86.81 SAG; 0.5×10^4 cells/mL	N	S	$BiVO_4$	100.80%	am	8.5	22.4-22.6		72 h	EC50	growth rate	>100000	3	4	ECHA, 2011

NOTES	
1	Remarks registrant: According to OECD (1984) guidelines, test medium not specified. Results for average specific growth rate calculated according to updated OECD 201 (2006) guidelines based on raw data reported and log-logistic dose-response model. To obtain a maximum concentration on the aqueous medium 48 hours before use, 1000 mg of the test substance was given into 1 l exposure medium and stirred at room temperature for 24 hours. After the 24h stirring period the mixture was allowed to stand for a further 24 hours without stirring. The supernatant was used for the test. Measured dissolved concentrations: control, 16.8, 100, 850, 7350 and 83000 µg V/L; EbC50 for biomass was calculated by means of linear regression analysis. The 95% confidence limits for this value were determined according to Litchfield and Wilcoxon. Notes reviewer: classification Ri 2 by registrant is agreed upon. Concentration of vanadium in control is not presented, but since vanadium is not a constituent of test media according to OECD 201, concentrations in the control are assumed to be negligible.
2	Remarks registrant: According to OECD (1984) guidelines, test medium not specified. Results for average specific growth rate calculated according to updated OECD 201 (2006) guidelines based on raw data reported and log-logistic dose-response model. To obtain a maximum concentration on the aqueous medium 48 hours before use, 1000 mg of the test substance was given into 1 l exposure medium and stirred at room temperature for 24 hours. After the 24h stirring period the mixture was allowed to stand for a further 24 hours without stirring. The supernatant was used for the test. Measured dissolved concentrations: control; 30, 150, 650, 2700 and 16000 µg V/L; EbC50 for biomass was calculated by means of linear regression analysis. The 95% confidence limits for this value were determined according to Litchfield and Wilcoxon. Notes reviewer: classification Ri 2 by registrant is agreed upon. . Concentration of vanadium in control is not presented, but since vanadium is not a constituent of test media according to OECD 201, concentrations in the control are assumed to be negligible.

3	Remarks registrant: According to OECD (1984) guidelines, test medium not specified. Results for average specific growth rate calculated according to updated OECD 201 (2006) guidelines based on raw data reported and log-logistic dose-response model. To obtain a maximum concentration on the aqueous medium 48 hours before use, 1000 mg of the test substance was given into 1 l exposure medium and stirred at room temperature for 24 hours. After the 24h stirring period the mixture was allowed to stand for a further 24 hours without stirring. The supernatant was used for the test. Measured dissolved concentrations: control, 75, 217, 700, 2050, 6600 and 21000 µg V/L; EbC50 for biomass was calculated by means of linear regression analysis. The 95% confidence limits for this value were determined according to Litchfield and Wilcoxon. Notes reviewer: classification Ri 2 by registrant is agreed upon. Concentration of vanadium in control is not presented, but vanadium compounds are not added to test media according to OECD 201
4	Remarks registrant: Based on the results of an OECD 29 dissolution study with the test substance (see Analytical Report 07L00001) it was concluded by the sponsor that the elution of the test substance as well as the aquatic studies should be conducted at a pH of 8.5. The test solutions were prepared individually by weighing the required amount of test substance into a 1L glass beaker and adding 800 mL test medium.These test solutions were covered with parafilm and stirred on a magnetic stir plate for about 7 days at approximately 20 + 2 °C. The pH-value of the test solutions were checked daily and adjusted to 8.5 with NaOH or HCl if necessary. The first check was made immediately after the test media had been introduced to the beakers. After seven days the solutions were filtered through a membrane filter (pore size 0.2 µm). All test solutions were visibly clear and colorless over the exposure period. Notes reviewer: Reference to OECD is not correct, not clear which guideline is meant; classification Ri1 by registrant not agreed upon, because endpoints are based on nominal concentrations and fluorescence used instead of cell counts; presence of bismuth may have influenced result (indicative MPC bismuth is 0.7 µg/L)

Appendix 3 Bioconcentration and bioaccumulation of vanadium

Legend to column headings	
A	analysis method
Test type	S = static; Sc = static closed; R = renewal; F = flow through; CF = continuous flow; IF = intermittent flow system
Purity	refers to purity of active substance or content of active in formulation; ag = analytical grade
Test water	am = artificial medium; dtw = dechlorinated tap water; dw = deionised/dechlorinated/distilled water; nw= natural water; rw = reconstituted water; rtw = reconstituted tap water; tw = tap water
T	temperature
Ri	Reliability index according to Klimisch et al. (1997); asterisk indicates citation

Table A3.1. Bioconcentration of vanadium in freshwater organisms.

Species	**Species properties**	**Compound**	**A**	**Test type**	**Test water**	**pH**	**Hardness CaCO3 [mg/L]**	**T [°C]**	**Exp. time**	**Exp. conc. [mg V/L]**	**BCF [L/kg ww]**	**BCF type**	**Method**	**Ri**	**Notes**	**Ref.**
Pisces																
Anguilla anguilla	0.56-3.55 g, mean 1.55	$^{48}VO_4^{3-}$	LSC	R	nw	7.68	57	15	56 d	1.15	0.45	whole body	Cfish/Cw	3	1	Bell et al., 1981
Carassius auratus	6.2-8.4 g	$^{48}VO_4^{3-}$	LSC	S	nw	7.68	57	22	96 h	0.026	1	whole body	Cfish/Cw	3	2	Edel and Sabbioni, 1993
Jordanella floridae	F0/F1	V_2O_5	AAS			8.15	347	24.5	28-30 d	0.041	17	whole body	Cfish/Cw	3	3	Holdway et al., 1983
Jordanella floridae	F0/F1	V_2O_5	AAS			8.15	347	24.5	28-30 d	0.17	16	whole body	Cfish/Cw	3	4	Holdway et al., 1983
Jordanella floridae	F0/F1	V_2O_5	AAS			8.15	347	24.5	28-30 d	0.48	6.5	whole body	Cfish/Cw	3	5	Holdway et al., 1983
Jordanella floridae	F0/F1	V_2O_5	AAS			8.15	347	24.5	28-30 d	1.5	2	whole body	Cfish/Cw	3	6	Holdway et al., 1983
Jordanella floridae	F0	V_2O_5	AAS			8.15	347	24.5	70 d	0.041	25.5	whole body	Cfish/Cw	2	7	Holdway et al., 1983
Jordanella floridae	F0	V_2O_5	AAS			8.15	347	24.5	70 d	0.17	22	whole body	Cfish/Cw	3	8	Holdway et al., 1983
Jordanella floridae	F0	V_2O_5	AAS			8.15	347	24.5	70 d	0.48	11.5	whole body	Cfish/Cw	3	9	Holdway et al., 1983
Jordanella floridae	F0	V_2O_5	AAS			8.15	347	24.5	96 d	0.041	27.9	whole body	Cfish/Cw	2	10	Holdway et al., 1983
Jordanella floridae	F0	V_2O_5	AAS			8.15	347	24.5	96 d	0.17	24.5	whole body	Cfish/Cw	3	11	Holdway et al., 1983
Jordanella floridae	F0	V_2O_5	AAS			8.15	347	24.5	96 d	0.48	11.6	whole body	Cfish/Cw	3	12	Holdway et al., 1983
Jordanella floridae	F0	V_2O_5	AAS			8.15	347	24.5	96 d	1.5	4.5	whole body	Cfish/Cw	3	13	Holdway et al., 1983

NOTES	
1	freshwater, 1.2 µm filtered; radiochemical concentration of [48V]orthovanadate 40 µCi/L; sodium orthovanadate added to give 10^{-5} M solution, assumed to relate to VO_4^{3-} => 1.15 mg V/L; pH adjusted with HCl; fish fed twice a week, excess food removed, radioactivity adsorbed to unconsumed food reported to be <1% of added RA; concentration in whole fish 1.01 x 10^{-5} g atom/kg wwt = 1.01 x 50.942 x 10^{-5} g/kg wwt = 0.515 mg/kg wwt; value for whole fish most likely estimated on the basis of individual organs; steady state in liver, kidney, bone and carcase not reached after 8 weeks (reason for Ri 3), during depuration concentrations increased in liver and kidney but decreased in other organs
2	water from Lake Maggiore, < 1 µg V/L, 0.45 µm filtered; exposure solution prepared by dilution of 10 µM stock NH4VO3 with lake water and addition of [^{48}V]-radiotracer; exposure concentration reported as 50 ng/mL [48V]vanadate, but in results section concentrations are referred to as "µg V/L"; BCF calculated assuming that water concentrations refer to vanadate, and concentrations expressed as vanadium are thus about twice as low; assumed that reported vanadium content in whole fish of 26.3 µg/kg wwt is indeed based on vanadium; accumulation in whole fish reported as 0.1% of the dose; exposure duration short, no information whether steady state is reached (reason for Ri 3)
3	not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is NOEC for growth/reproduction for this species; BCF refers to F0 and F1, F0 started with 1 week old larvae, exposure for 28 d; F1 started with eggs and exposed for 30 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state not reached, since longer duration did result in significantly higher residues (reason for Ri 3)
4	not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is more than two times higher than NOEC for growth/reproduction (reason for Ri 3); BCF refers to F0 and F1; F0 started with 1 week old larvae, exposure for 28 d; F1 started with eggs and exposed for 30 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state is not reached, since longer duration did result in significantly higher residues (reason for Ri 3)
5	Not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is more than 10 times higher than NOEC for growth/reproduction (reason for Ri 3); BCF refers to F0 and F1; F0 started with 1 week old larvae, exposure for 28 d; F1 started with eggs and exposed for 30 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state not reached, since longer duration did result in significantly higher residues (reason for Ri 3)
6	Not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is about 1/8 of LC50, and caused mortality during the experiment (reason for Ri 3); BCF refers to F0 and F1; F0 started with 1 week old larvae, exposure for 28 d; F1 started with eggs and exposed for 30 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state not reached, since longer exposure did result in higher residues (reason for Ri 3)
7	Not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is NOEC for growth/reproduction for this species; BCF refers to F1, started with eggs and exposed for 70 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state probably reached, since longer duration did not result in significantly higher residues
8	Not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is more than two times higher than NOEC for growth/reproduction (reason for Ri 3); BCF refers to F1, started with eggs and exposed for 70 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state probably reached, since longer duration did not result in significantly higher residues
9	Not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is more than 10 times higher than NOEC for growth/reproduction (reason for Ri 3); BCF refers to F1, started with eggs and exposed for 70 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state probably reached, since longer duration did not result in significantly higher residues
10	Not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is NOEC for growth/reproduction for this species; BCF refers to F1, started with eggs and exposed for 96 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state probably reached, residues did not significantly differ from 70-days exposure
11	Not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is more than two times higher than NOEC for growth/reproduction (reason for Ri 3); BCF refers to F1, started with eggs and exposed for 96 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state probably reached, residues did not significantly differ from 70-days exposure
12	Not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is more than 10 times higher than NOEC for growth/reproduction (reason for Ri 3); BCF refers to F1, started with eggs and exposed for 96 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; steady state probably reached, residues did not significantly differ from 70-days exposure
13	Not clear if based on analysed water concentrations; exposure described as "constant", but no information on system; exposure concentration is about 1/8 of LC50, and caused mortality during the experiment (reason for Ri 3); BCF refers to F1, started with eggs and exposed for 96 d; BCF on wwt basis calculated from dwt residues using average dwt/wwt ratio of 0.24; not clear if steady state has been reached, no fish available for 70-days exposure (reason for Ri 3)

Table A3.2. Bioconcentration of vanadium in marine organisms.

Species	Species properties	Compound	A	Test type	Test water	pH	Salinity [‰]	T [°C]	Exp. time	Exp. conc. [mg V/L]	BCF [L/kg_{ww}]	BCF type	Method	Ri	Notes	Ref.
Crustacea																
Carcinas maenas	males, 16 g	$48VOCl_2$	LSC	R	nw		38	13	21 d	0.002	12	whole organism	Corg/Cw	2	1	Miramand et al., 1992
Carcinas maenas	18 g	$NaVO_3$	AAS	R	nw		37.8	14	15 d	0.5	7.6	whole organism	Corg/Cw	3	2	Ünsal, 1983
Carcinas maenas	18 g	$NaVO_3$	AAS	R	nw		37.8	14	30 d	0.5	8.8	whole organism	Corg/Cw	3	3	Ünsal, 1983
Lysmata seticaudata	collected from field, 1.0 g	$48VOCl_2$	LSC	R	nw		38	13	21 d	0.002	11	whole body	Corg/Cw	2	4	Miramand et al., 1981
Lysmata seticaudata	collected from field, 1.0 g	$48VOCl_2$	LSC	R	nw		38	13	14 d	0.002	7	whole body	Corg/Cw	2	5	Miramand et al., 1981
Lysmata seticaudata	collected from field, 1.0 g	$48VOCl_2$	LSC	R	nw		38	18	14 d	0.002	8	whole body	Corg/Cw	2	5	Miramand et al., 1981
Lysmata seticaudata	collected from field, 1.0 g	$48VOCl_2$	LSC	R	nw		28	13	21 d	0.002	20	whole body	Corg/Cw	2	5	Miramand et al., 1981
Lysmata seticaudata	collected from field, 1.0 g	$48VOCl_2$	LSC	R	nw		38	13	21 d	0.002	9	whole body	Corg/Cw	2	5	Miramand et al., 1981
Lysmata seticaudata	collected from field, 1.0 g	$48VOCl_2$	LSC/AAS	R	nw		38	13	18 d	0.002	11	whole body	Corg/Cw	2	6	Miramand et al., 1981
Lysmata seticaudata	collected from field, 1.0 g	$48VOCl_2$	LSC/AAS	R	nw		38	13	18 d	0.025	7.2	whole body	Corg/Cw	2	6	Miramand et al., 1981
Lysmata seticaudata	collected from field, 1.0 g	$48VOCl_2$	LSC/AAS	R	nw		38	13	18 d	0.05	6	whole body	Corg/Cw	2	6	Miramand et al., 1981
Lysmata seticaudata	collected from field, 1.0 g	$48VOCl_2$	LSC/AAS	R	nw		38	13	18 d	0.1	5.5	whole body	Corg/Cw	2	6	Miramand et al., 1981
Mollusca																
Mytilus edulis	collected from field, 25-31 g	$^{48}VO_3^-$	LSC	S	am	7.68	20	15	96 h	0.00026	1.9	soft tissues	Corg/Cw	3	7	Edel and Sabbioni, 1993
Mytilus edulis	collected from field, 25-31 g	$^{48}VO_3^-$	LSC	S	am	7.68	20	15	96 h	0.0026	1.6	soft tissues	Corg/Cw	3	7	Edel and Sabbioni, 1993
Mytilus edulis	collected from field, 25-31 g	$^{48}VO_3^-$	LSC	S	am	7.68	20	15	96 h	0.026	1.7	soft tissues	Corg/Cw	3	7	Edel and Sabbioni, 1993
Mytilus edulis	collected from field, 25-31 g	$^{48}VO_3^-$	LSC	S	am	7.68	20	15	96 h	0.26	1.4	soft tissues	Corg/Cw	3	7	Edel and Sabbioni, 1993
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC	R	nw			13	19 d	0.002	34	whole body	Corg/Cw	3	8	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC	R	nw			18	19 d	0.002	34	whole body	Corg/Cw	3	8	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC	R	nw			24	19 d	0.002	22	whole body	Corg/Cw	3	8	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC	R	nw		19	13	21 d	0.002	11.6	soft tissues	Corg/Cw	3	9	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC	R	nw		28	13	21 d	0.002	8.4	soft tissues	Corg/Cw	3	9	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC	R	nw		38	13	21 d	0.002	12.1	soft tissues	Corg/Cw	3	9	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC/AAS	R	nw		38	13	21 d	0.002	38	whole body	Corg/Cw	3	10	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC/AAS	R	nw		38	13	21 d	0.025	32	whole body	Corg/Cw	3	11	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC/AAS	R	nw		38	13	21 d	0.05	27	whole body	Corg/Cw	3	12	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC/AAS	R	nw		38	13	21 d	0.1	19	whole body	Corg/Cw	3	13	Miramand et al., 1980
Mytilus galloprovincialis	collected from field, 5-6 g	$^{48}VOCl_2$	LSC/AAS	R	nw			13	21 d		7	soft tissues	Corg/Cw	3	14	Miramand et al., 1980
Pisces																

Species	Species properties	Compound	A	Test type	Test water	pH	Salinity [‰]	T [°C]	Exp. time	Exp. conc. [mg V/L]	BCF [L/kg$_{ww}$]	BCF type	Method	Ri	Notes	Ref.
Gobius minutus	collected from field, 3.6 g	$^{48}VOCl_2$	LSC	R	nw		38	13	5 d	0.002	0.3	whole fish	Corg/Cw	3	15	Miramand et al., 1992
Gobius minutus	collected from field, 3.6 g	$^{48}VOCl_2$	LSC	R	nw		38	13	10 d	0.002	0.2	whole fish	Corg/Cw	3	15	Miramand et al., 1992
Gobius minutus	collected from field, 3.6 g	$^{48}VOCl_2$	LSC	R	nw		38	13	16 d	0.002	0.4	whole fish	Corg/Cw	3	15	Miramand et al., 1992
Gobius minutus	collected from field, 3.6 g	$^{48}VOCl_2$	LSC	R	nw		38	13	21 d	0.002	1	whole fish	Corg/Cw	3	15	Miramand et al., 1992
Echinodermata																
Marthasterias glacialis L	collected from field, 35 g	$^{48}VOCl_2$	LSC	R	nw		38	13	22 d	0.002	18	whole body	Corg/Cw	3	16	Miramand et al., 1982
Paracentrotus lividus	collected from field, 35 g	$^{48}VOCl_2$	LSC	R	nw		38	13	22 d	0.002	6	whole body	Corg/Cw	3	16	Miramand et al., 1982
Holothuria forskali	collected from field, 35 g	$^{48}VOCl_2$	LSC	R	nw		38	13	22 d	0.002	5	whole body	Corg/Cw	3	16	Miramand et al., 1982

NOTES	
1	crabs purchased in local fishmarket; radiotracer solution added to cotton filtered natural seawater at 0.28 μCi/L; 48V assumed to be present in the +5 oxidation state; added amount of stable V together with radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater, which is assumed to be 2 μg/L based on previous studies; renewal every two days; BCF calculated as ratio of radioactivity in whole crab and water counted regularly during exposure; graph indicates that equilibrium is reached
2	crabs purchased in local fishmarket in Nice, 1978; exposure to contaminated water, uncontaminated food (Nereis diversicolor); residue 10.13 μg/g dwt, dwt 7.14 g, wwt 19.14 g => residue (10.13*7.14)/19.14 = 3.78 μg/g wwt; equilibrium probably not reached since BCF is higher after 30 d (reason for Ri 3)
3	crabs purchased in local fishmarket in Nice, 1978; exposure to contaminated water, uncontaminated food (Nereis diversicolor); residue 11.22 μg/g dwt, dwt 7.35 g, wwt 18.72 g => residue (11.22*7.35)/18.72 = 4.4 μg/g wwt; not clear if equilibirum is reached (reason for Ri 3)
4	shrimp collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 μCi/L; 48V assumed to be present in the +5 oxidation state; added amount of stable V together with radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater, which is assumed to be 2 μg/L based on previous studies; renewal every two days; BCF calculated as ratio of radioactivity in whole non-moulting shrimp and water counted regularly during exposure; graph of whole body BCFs indicates that equilibrium is reached, but this is not the case for individual tissues
5	shrimp collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 μCi/L; 48V assumed to be present in the +5 oxidation state; added amount of stable V together with radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater, which is assumed to be 2 μg/L based on previous studies; renewal every two days; BCF calculated as ratio of radioactivity in whole non-moulting shrimp and water counted regularly during exposure; BCF read from graph, graph indicates that equilibrium is reached
6	shrimp collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 μCi/L; ^{48}V assumed to be present in the +5 oxidation state; added amount of stable V plus radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater, which is assumed to be 2 μg/L; renewal every two days; BCF calculated as ratio of radioactivity in whole non-moulting shrimp and water counted after 18 d of exposure; BCF calculated from graph with residues against water concentration; authors report that uptake is not proportional to vanadium concentration in water (significantly different from 10:1 line); not clear if equilibrium is reached, in view of other experiments this is expected to be the case
7	mussels collected at Wexford, Ireland; exposure solutions prepared by dilution of aliquots of 10 μM stock NH4VO3 with artificial seawater and addition of [^{48}V]-radiotracer; exposure concentration reported as 0.5 ng/mL [^{48}V]vanadate, but in results section concentrations are referred to as "μg V/L"; BCF calculated assuming that water concentrations refer to vanadate, and concentrations expressed as vanadium are thus about twice as low; assumed that reported vanadium content in soft tissues is indeed based on vanadium; exposure duration short, no information whether steady state is reached (reason for Ri 3)

8	mussels collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 µCi/L; ^{48}V assumed to be present in the +5 oxidation state; added amount of stable V together with radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater, which is assumed to be 2 µg/L; renewal every two days; BCF calculated as ratio of radioactivity in whole mussels and water counted regularly during exposure; salinity not reported, most likely 38 ‰, since this is the salinity of "full strength seawater"; BCF read from graph; according to the authors there is no indication of equilibrium after 14 d
9	mussels collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 µCi/L; ^{48}V assumed to be present in the +5 oxidation state; added amount of stable V together with radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater; renewal every two days; BCF calculated as ratio of radioactivity in whole mussels and water counted regularly during exposure; BCF-values in whole animals (incl. shell, byssus, pallial fluid) show a more or less constant increase with time, equilibrium not reached (reason for Ri 3)
10	mussels collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 µCi/L; ^{48}V assumed to be present in the +5 oxidation state; added amount of stable V together with radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater, which is assumed to be 2 µg/L; renewal every two days; BCF calculated as ratio of radioactivity in whole mussels and water counted regularly during exposure; BCF read from graph; BCF-values seem to level off as from day 10, but increase is observed between day 18 and 21, equilibrium not reached (reason for Ri 3)
11	mussels collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 µCi/L; ^{48}V assumed to be present in the +5 oxidation state; 25 µg/L stable V added as sodium metavanadate; renewal every two days; BCF calculated as ratio of radioactivity in whole mussels and water counted regularly during exposure; salinity not reported, most likely 38 ‰, since this is the salinity of "full strength seawater"; BCF read from graph; BCF-values seem level off as from day 10, but equilibrium not reached (reason for Ri 3); uptake not significantly different from control (=background V)
12	mussels collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 µCi/L; ^{48}V assumed to be present in the +5 oxidation state; 50 µg/L stable V added as sodium metavanadate; renewal every two days; BCF calculated as ratio of radioactivity in whole mussels and water counted regularly during exposure; salinity not reported, most likely 38 ‰, since this is the salinity of "full strength seawater"; BCF read from graph; BCF-values level off as from day 10, but increase between 15 and 21 d, equilibrium not reached (reason for Ri 3); uptake not significantly different from control (=background V)
13	mussels collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 µCi/L; ^{48}V assumed to be present in the +5 oxidation state; 100 µg/L stable V added as sodium metavanadate; renewal every two days; BCF calculated as ratio of radioactivity in whole mussels and water counted regularly during exposure; salinity not reported, most likely 38 ‰, since this is the salinity of "full strength seawater"; BCF read from graph; BCF-values level off as from day 10, but increase between 17 and 21 d, equilibrium not reached (reason for Ri 3); uptake significantly different from control (= background V)
14	mussels collected near Port of Monaco; experimental conditions not clear, probably no stable V added, only radiotracer at 0.28 µCi/L and background concentration of 2 µg/L stable V present; BCF read from graph, most likely estimated from the respective tissue parts; equilibrium not reached.
15	fish collected from littoral zone near Monaco; radiotracer solution added to cotton filtered natural seawater at 10.4 kBq/L; 48V assumed to be present in the +5 oxidation state; added amount of stable V together with radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater, which is assumed to be 1.7 µg/L based on previous studies; renewal every two days; BCF calculated as ratio of radioactivity in whole fish and water counted regularly during exposure; low residues in organs, highest residues in digestive tract, probably adsorption of ingested metal; BCF shows continuous rise with time, equilibrium not reached after 21 d (reason for Ri 3)
16	organisms collected from littoral zone near Monaco; radiotracer solution added to cotton filtered natural seawater at 10.4 kBq/L (0.28 µCi/L); 48V assumed to be present in the +5 oxidation state; added amount of stable V together with radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater, which is assumed to be 2 µg/L based on previous studies; renewal every two days; salinity not reported, assumed to be 38 ‰ in line with other studies; radioactivity in tissues and water counted regularly during exposure, whole body BCF reconstructed from BCFs for individual tissues; highest residues in body wall or test; BCF shows continuous rise with time, equilibrium not reached after 21 d (reason for Ri 3)

Table A3.3. Bioaccumulation of vanadium in marine organisms, laboratory studies with food intake.

Species	Species properties	Compound	A	Test type	Test water	pH	Salinity [‰]	T [°C]	Exp. time	Exp. conc. [mg V/L]	BAF [L/kg$_{ww}$]	BAF type	Method	Ri	Notes	Ref.
Crustacea																
Carcinas maenas	18 g	NaVO3	AAS	R	nw		37.8	14	15 d	0.5	5.2	whole organism	Corg/Cw	3	1	Ünsal, 1983
Carcinas maenas	18 g	NaVO3	AAS	R	nw		37.8	14	30 d	0.5	13.0	whole organism	Corg/Cw	3	2	Ünsal, 1983
Mollusca																
Mytilus edulis		NaVO3	AAS	R	nw			18	7 d	0.5	11 (dwt)		Corg/Cw	3	3	Ünsal, 1982
Mytilus galloprovincialis	5-6 g	48VOCl2	LSC/AAS	R	nw		38	13	4 d	0.002	2	soft tissues	Corg/Cw	3	4	Miramand et al., 1980
Mytilus galloprovincialis	5-6 g	48VOCl2	LSC/AAS	R	nw		38	13	4 d	0.025	1.1	soft tissues	Corg/Cw	3	5	Miramand et al., 1980
Mytilus galloprovincialis	5-6 g	48VOCl2	LSC/AAS	R	nw		38	13	4 d	0.05	1.7	soft tissues	Corg/Cw	3	5	Miramand et al., 1980
Mytilus galloprovincialis	5-6 g	48VOCl2	LSC/AAS	R	nw		38	13	4 d	0.1	1.8	soft tissues	Corg/Cw	3	5	Miramand et al., 1980

NOTES	
1	crabs purchased in local fishmarket in Nice, 1978; exposure to contaminated water, fed on V-contaminated food (Nereis diversicolor); residue 6.57 µg/g dwt, dwt 6.14 g, wwt 15.57 g => residue (6.57*6.14)/15.57 = 2.6 µg/g wwt; equilibrium probably not reached since BCF is more than two times higher after 30 d (reason for Ri 3)
2	crabs purchased in local fishmarket in Nice, 1978; exposure to contaminated water, fed on V-contaminated food (Nereis diversicolor); residue 14.0 µg/g dwt, dwt 7.63 g, wwt 16.39 g => residue (14*7.63)/16.39 = 6.5 µg/g wwt; equilibrium probably not reached since BCF is more than two times higher after 30 d (reason for Ri 3)
3	mussels exposed to contaminated water and fed on algae that had been exposed to 0.5 mg V/L; exposed organisms contained 6.17 µg/g dwt, controls 0.67 µg/g dwt; using these figures, the dwt based BCF can be calculated as (6.17-0.67)/0.5 = 11 L/kg dwt, dry to wet weight ratio not known; not clear if reported residue includes shell; steady state is not reached (reason for Ri 3)
4	mussels collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 µCi/L; ^{48}V assumed to be present in the +5 oxidation state; added amount of stable V together with radiotracer ≈ 8 ng/L, negligible as compared to total background level of stable V in natural seawater, which is assumed to be 2 µg/L; renewal every two days; animals fed on phytoplankton previously exposed under same conditions; BAF calculated as ratio of radioactivity in whole mussels and water/food mixture ; exposure time too short, equilibrium most likely not reached (reason for Ri 3)
5	mussels collected near Port of Monaco; radiotracer solution added to cotton filtered natural seawater at 0.28 µCi/L; ^{48}V assumed to be present in the +5 oxidation state; stable V added as sodium metavanadate; renewal every two days; animals fed on phytoplankton previously exposed under same conditions; BAF calculated as ratio of radioactivity in whole mussels and water/food mixture ; exposure time too short, equilibrium most likely not reached (reason for Ri 3)

Table A3.4. Bioaccumulation of vanadium in freshwater organisms, field observations.

Species	**Species properties**	**A**	**Test water**	**pH**	**Exp. conc. [mg V/L]**	**BAF [L/kg$_{ww}$]**	**BAF type**	**Ri**	**Notes**	**Ref.**
Crustacea										
Hyalella azteca	4-10 weeks old	ICP-MS	river	6.7	0.00065	2205 (dwt)	whole body	2	1	Couillard et al., 2008
Hyalella azteca	4-10 weeks old	ICP-MS	river	7.1	0.00082	1944 (dwt)	whole body	2	1	Couillard et al., 2008
Hyalella azteca	4-10 weeks old	ICP-MS	river	7.0	0.00092	2122 (dwt)	whole body	2	1	Couillard et al., 2008
Hyalella azteca	4-10 weeks old	ICP-MS	river	6.4	0.00033	922 (dwt)	whole body	2	1	Couillard et al., 2008
Hyalella azteca	4-10 weeks old	ICP-MS	river	7.5	0.00034	2254 (dwt)	whole body	2	1	Couillard et al., 2008
Hyalella azteca	4-10 weeks old	ICP-MS	river	6.2	0.00036	1078 (dwt)	whole body	2	1	Couillard et al., 2008
Mollusca										
Anodonta cygnea		ICP-MS	lake		0.00043	614	soft tissues	2	2	Ravera et al., 2003
Dreissena polymorpha		ICP-MS	lake		0.00043	1163	soft tissues	2	3	Ravera et al., 2003
Unio pictorum mancus	shell length 64-71 mm	ICP-MS	lake		0.00012	202	soft tissues	2	4	Ravera et al., 2007
Unio pictorum mancus	shell length 64-71 mm	ICP-MS	lake		0.00028	240	soft tissues	2	5	Ravera et al., 2007
Unio pictorum mancus	shell length 64-71 mm	ICP-MS	lake		0.00043	259	soft tissues	2	6	Ravera et al., 2003

NOTES	
1	laboratory organisms exposed in-situ; BAF calculated from reported on measured concentrations in animals and 0.45 µm-filtered water; dry weight content not given, recalculation to wwt BAF not possible, therefore not used
2	mussels sampled from Lake Maggiore; residue 2 µg/g dwt, BAF calculated using reported wwt/dwt ratio of 7.58
3	mussels sampled from Lake Maggiore; residue 2 µg/g dwt, BAF calculated using reported wwt/dwt ratio of 4.0
4	mussels sampled from Lake Candia; residue 0.21 µg/g dwt, BAF calculated using reported wwt/dwt ratio of 9.66
5	mussels sampled from Lake Maggiore; residue 0.65 µg/g dwt, BAF calculated using reported wwt/dwt ratio of 8.65
6	mussels sampled from Lake Maggiore; residue 0.6 µg/g dwt, BAF calculated using reported wwt/dwt ratio of 5.38

Table A3.5. Bioaccumulation of vanadium in marine organisms, field observations.

Species	**Species properties**	**A**	**Test water**	**Exp. conc. [mg V/L]**	**BAF [L/kg$_{ww}$]**	**BAF type**	**Ri**	**Notes**	**Ref.**
Crustacea									
Lysmata seticaudata	1.1 g	AAS	sea	0.002	410	whole body	3	1	Miramand et al., 1980
Carcinas maenas	males/females, 25 g	AAS	sea	0.002	450	whole body	3	2	Miramand et al., 1980
Macrobrachium rosenbergii	98.8 mm; 34.5 g	ICP-MS	sea	0.00105	105	whole body	2	3	Ikemoto et al., 2008
Macrobrachium equidens	41.5 mm; 2.2 g	ICP-MS	sea	0.00105	179	whole body	2	4	Ikemoto et al., 2008
Macrobrachium sp. 3	79.7 mm; 11.5 g	ICP-MS	sea	0.00105	65	whole body	2	5	Ikemoto et al., 2008
Macrobrachium sp. 4	33.0 mm; 0.7 g	ICP-MS	sea	0.00105	110	whole body	2	6	Ikemoto et al., 2008
Metapeneaus tenuis	52.7 mm; 1.9 g	ICP-MS	sea	0.00105	26	whole body	2	7	Ikemoto et al., 2008
Mytilus galloprovincialis	collected near Monaco, 36 g	AAS	sea	0.002	35	soft tissues	3	8	Miramand et al., 1980
Pisces									
Clupeoides sp.	35.8 mm; 0.7 g	ICP-MS	sea	0.00105	66	whole fish	2	9	Ikemoto et al., 2008
Cyclocheilichthys armatus	153.4 mm; 82.1 g	ICP-MS	sea	0.00105	140	whole fish	2	10	Ikemoto et al., 2008
Cynoglossus sp.2	56.4 mm; 1.7 g	ICP-MS	sea	0.00105	166	whole fish	2	11	Ikemoto et al., 2008
Eleotris melanosoma	71.9 mm; 11 g	ICP-MS	sea	0.00105	220	whole fish	2	12	Ikemoto et al., 2008
Glossogobius aureus	81.5 mm; 14.6 g	ICP-MS	sea	0.00105	218	whole fish	2	13	Ikemoto et al., 2008
Gobius minutus	3 g	AAS	sea	0.0017	412	whole fish	3	14	Miramand et al., 1980
Parambassis wolffii	102.3 mm; 39.0 g	ICP-MS	sea	0.00105	44	whole fish	2	15	Ikemoto et al., 2008
Pisodonaphis boro	492.9 mm; 28.6 g	ICP-MS	sea	0.00105	57	whole fish	2	16	Ikemoto et al., 2008
Polynemus paradiseus	53.6 mm; 3.4 g	ICP-MS	sea	0.00105	77	whole fish	2	17	Ikemoto et al., 2008
Puntioplites proctozysron	89.1 mm; 27.4 g	ICP-MS	sea	0.00105	87	whole fish	2	18	Ikemoto et al., 2008
Echinodermata									
Marthasterias glacialis L	100 g	AAS	sea	0.002	150	whole body	2	19	Miramand et al., 1982
Paracentrotus lividus	65 g	AAS	sea	0.002	600	whole body	2	19	Miramand et al., 1982
Holothuria forskali	240 g	AAS	sea	0.002	215	whole body	2	19	Miramand et al., 1982

NOTES	
1	not clear if crabs were purchased locally or captured in the field; BAF estimated on the basis of assumed concentration in seawater of 2 μg V/L; actual concentration not measured (reason for Ri 3)
2	BAF estimated on the basis of assumed concentration in seawater of 2 μg V/L; actual concentration not measured (reason for Ri 3)

3	river prawn purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.37 µg/g dwt, BAF calculated using reported moisture content (70.2%)
4	river prawn purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; exposure concentration is geomean of two locations; residue 0.72 µg/g dwt, BAF calculated using reported moisture content (73.9%)
5	river prawn purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.24 µg/g dwt, BAF calculated using reported moisture content (71.4%)
6	river prawn purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.54 µg/g dwt, BAF calculated using reported moisture content (78.7%)
7	river prawn purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.11 µg/g dwt, BAF calculated using reported moisture content (75.2%)
8	large mussels collected from rocks near Monaco in 1979; BAF determined by analysis of soft tissues, not reconstructed; BAF estimated on the basis of assumed concentration in seawater of 2 µg V/L; actual concentration not measured (reason for Ri 3)
9	fish purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.38 µg/g dwt, BAF calculated using reported moisture content (81.9%)
10	fish purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.57 µg/g dwt, BAF calculated using reported moisture content (74.3%)
11	fish purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.85 µg/g dwt, BAF calculated using reported moisture content (79.5%)
12	fish purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.95 µg/g dwt, BAF calculated using reported moisture content (75.7%)
13	fish purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.98 µg/g dwt, BAF calculated using reported moisture content (76.7%)
14	fish collected from littoral zone near Monaco in 1988; BCF determined by analysing whole fish; highest residues in gills and digestive tract (BAF 15.1); BAF estimated on the basis of assumed concentration in seawater of 1.7 µg V/L; actual concentration not measured (reason for Ri 3)
15	fish purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.20 µg/g dwt, BAF calculated using reported moisture content (76.9%)
16	fish purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.23 µg/g dwt, BAF calculated using reported moisture content (74.2%)
17	fish purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.39 µg/g dwt, BAF calculated using reported moisture content (79.2%)
18	fish purchased from local fishermen at Can Tho, Mekong Delta, South Vietnam, April 2004; water samples filtered 0.45 µm; salinity not reported, but assumed that water is at least brackish; exposure concentration is geomean of two locations; residue 0.36 µg/g dwt, BAF calculated using reported moisture content (74.7%)
19	BAF estimated on the basis of assumed concentration in seawater of 2 µg V/L; actual concentration not measured (reason for Ri 3); BAF determined by analysing whole organism; highest residues in body walls or "shell"

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