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Environmental risk limits for free cyanide in fresh- and marine surface water

Proposal for water quality standards according to the methodology of the Water Framework Directive

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Synopsis

Environmental risk limits for free cyanide in fresh- and marine surface water

Proposal for water quality standards according to the methodology of the Water Framework Directive

Cyanide exists as both a naturally-occurring substance and a man-made version. It sees frequent use in the processing of iron, steel and oil. Cyanide is also commonly used in the mining and energy industries. As a result, it sometimes ends up in the environment. Different forms of cyanide exist. The most toxic form is ‘free cyanide’. A high concentration of free cyanide in water can be toxic for the reproduction of aquatic plants and animals.

Although standards for free cyanide in surface water already exist in the Netherlands, they are outdated. This is because the method used to determine the standards has improved in recent years. In addition, a great deal of new knowledge about the toxicity of cyanide has been published in scientific literature.

Using these insights, RIVM has derived new standards for free cyanide in fresh and marine water. While the results are similar to the 2001 standards, they are now better supported. Water managers measure the concentrations of cyanide in surface water. This new standard will make it easier for them to judge whether the concentrations they find are harmful to aquatic life.

For freshwater, RIVM has determined that a concentration of 0.22 micrograms of free cyanide per litre of water is safe for aquatic plants and animals. For marine water, a slightly lower concentration of 0.044 micrograms per litre has been determined as safe. The concentration of free cyanide can fluctuate. The exposure to free cyanide for short amounts of time at a level of up to 1.7 micrograms per litre are not expected to affect aquatic life in either fresh or marine water.

Other countries and researchers have determined their own standards for free cyanide, using a variety of different methods. RIVM has examined these methodological differences and the ensuing results. The other parties’ results are very similar to those reached in the Netherlands.

Free cyanide is difficult to measure. For this reason, water managers in the Netherlands measure the total concentration of cyanide. Free cyanide accounts for part of this total. RIVM recommends exploring the possibilities for measuring free cyanide on its own.

Keywords: cyanide, surface water, environmental risk limit, standard, water quality

Publiekssamenvatting

Milieukwaliteitsnormen voor vrij cyanide in zoet en zout oppervlaktewater

Voorstel voor milieukwaliteitsnormen volgens de beoordelingsmethodiek van de Kaderrichtlijn Water

Cyanide is een natuurlijke stof die ook door de mens veel wordt gemaakt en gebruikt. Zo wordt de stof gebruikt bij de verwerking van ijzer, staal en olie. Ook wordt de stof veel in de mijnbouw en de energiesector gebruikt. De stof kan daarbij in het milieu terechtkomen. Cyanide komt in verschillende vormen voor. De giftigste vorm is 'vrij cyanide'. Een hoge concentratie vrij cyanide in water kan bijvoorbeeld schadelijk zijn voor de voortplanting van planten en dieren die daarin leven.

In Nederland bestaat er al een norm voor vrij cyanide in oppervlaktewater, maar deze is verouderd. In de afgelopen jaren is de methode om de norm te bepalen verbeterd. Ook is er veel nieuwe kennis over de giftigheid van cyanide in de wetenschappelijke literatuur gepubliceerd.

Met deze inzichten heeft het RIVM opnieuw de normen voor vrij cyanide in zoet en zout oppervlaktewater bepaald. De resultaten zijn vergelijkbaar met de normen uit 2001 maar zijn nu beter onderbouwd. Zo kunnen waterbeheerders beter inschatten of de concentraties cyanide die ze meten in het oppervlaktewater schadelijk zijn voor het waterleven.

Voor planten en dieren die in zoet water leven, heeft het RIVM een veilige concentratie van vrij cyanide berekend van 0,22 microgram per liter water. Voor zeewater is er een iets lagere concentratie van 0,044 microgram per liter berekend. Kortdurend mag de concentratie vrij cyanide op lopen tot 1,7 microgram per liter voor zoet en zout water. Ook dan worden er geen effecten op het waterleven verwacht.

Meer landen en onderzoekers hebben een norm voor vrij cyanide berekend. Ze hebben dat niet overal op precies dezelfde manier gedaan. Het RIVM heeft naar deze verschillen en de uitkomsten van hun berekeningen gekeken. Hun resultaten blijken sterk overeen te komen met die van Nederland.

Vrij cyanide is moeilijk te meten. In Nederland wordt daarom 'totaal' cyanide gemeten, waar vrij cyanide een onderdeel van is. Het RIVM adviseert te kijken naar de mogelijkheden om wel vrij cyanide te meten.

Kernwoorden: cyanide, oppervlaktewater, milieukwaliteitsnorm, waterkwaliteit

Contents

Summary

Environmental quality standards (EQSs) are used to safeguard the ecological status of water bodies, but they are also an important corner stone in discharge permits. In the latter, EQSs for surface water are needed for the risk assessment of a waste discharge using the 'immissietoets'. Rijkswaterstaat has commissioned RIVM to revise the EQS values for free cyanide in fresh- want marine surface water. This document describes the derivation of a maximum allowable concentration EQS (MAC-EQS) and an annual average EQS (AA-EQS)alues for free cyanide in freshwater and marine water, following the 2018 version of the Technical Guidance for Deriving Environmental Quality Standards guidance of the Water Framework Directive (WFD).

Cyanide inactivates cytochrome oxidase, an important enzyme in mitochondrial respiration and common in all eukaryotic organisms as well as in bacteria. The toxicity of cyanide is therefore considered to be non-species-specific. Ecotoxicity data were collected from previous national and international evaluations. In addition, a literature search was carried out. All ecotoxicity studies gathered from previous evaluations and the literature search were evaluated for reliability of and compliance with today's standards. The reliable data were used for the derivation of the EQS values. The acute ecotoxicity dataset consists of reliable study results for 35 species, distributed over 8 taxonomic groups. The chronic ecotoxicity dataset consists of study results for 13 species, distributed over 7 taxonomic groups. As both the acute and chronic dataset meet the WFD criteria for deriving an SSD, a probabilistic approach has been taken. The SSDs were fitted using the *E_TX* 3.0 R-package, as this model is also able to fit censored (unbound) data. The acute and chronic HC_5 values were 17 and 0.66 µg/L, respectively. With a default assessment factor of 10 on the acute HC_5 and an assessment factor of 3 on the chronic HC_5, the following EQS values were derived:

$MAC\text{-}EQS_{fw}$:	1.7 µg/L
$MAC\text{-}EQS_{sw}$:	1.7 µg/L
$AA\text{-}EQS_{fw}$:	0.22 µg/L
$AA\text{-}EQS_{sw}$:	0.044 µg/L

The MAC-EQS and AA-EQS values are generally in line with derivations by others.

Keywords: free cyanide, MAC-EQS, AA-EQS, WFD, SSD

1 Introduction

Cyanides are a group of chemicals characterised by one carbon atom connected with a triple bond to a nitrogen atom (C≡N). Cyanide occurs in many different chemical forms, ranging from free cyanide molecules in neutral and ionised state (HCN and CN^-, respectively), to simple cyanides (such as NaCN or KCN), cyanates, thiocyanates and metallocyanide complexes (such as $K_2Zn(CN)_4$ and $K_4Fe(CN)_6$) [1]. The free cyanide is considered to be the primary toxic form for aquatic life [2].

Cyanides originate from both natural and man-made sources. Natural sources of cyanides are cyanogenic glycosides which are found in, among others, almonds, apricots, bamboo, bean sprouts, cassava, cashews, cherries, lentils, olives, potatoes, sorghum, and soybeans [3, 4]. Cyanides are thereby produced, excreted, and degraded naturally by many animals, plants, insects, fungi, and bacteria.
Man-made sources include industrial sectors such as steel production, the (petro)chemical industry, the mining sector and the energy sector. There are additional sources of cyanide that may impact surface water quality, such as the use of the potassium ferrocyanide (potash) in road salt. Energy transition innovations may also lead to cyanide emissions [5]. Examples are gasification installations using wood type materials, where cyanide ends up in the waste water stream during gas production.

In the National surface water monitoring program, Rijkswaterstaat only measures total cyanides in surface water. The total cyanides content is photometrically determined in accordance with the NEN-14403-1 guideline. Analytical methodologies for the determination of free cyanide have been developed and published by industries. In fact, the NEN-14403-1 guideline also describes how to measure free cyanide in water samples. However, such analytical methods have not been adopted by Rijkswaterstaat as the practical applicability remains challenging.

The most recent (December 2020) total cyanide concentration measurements in Dutch surface waters ranged between 1-2 µg/L for 5 out of the 14 measuring sites. For the other 9 sites, the concentrations were below the limit of detection (<1 or <2 µg/L)[6]. Even though the free cyanide content within the total cyanides is unknown, it is believed to be low as most free cyanide is considered to rapidly complex with iron particles, especially when the waste water treatment includes a Fenton process.

Environmental quality standards (EQSs) are used to safeguard the ecological status of water bodies, but they are also an important corner stone in discharge permits. In the latter, EQSs for surface water are needed for the risk assessment of a waste discharge using the 'immissietoets[1]'. The currently available Dutch quality standards for free cyanide and cyanide complexes are 0.23 and 0.13 µg/L, respectively [7, 8]. These values were taken from a report prepared in the context of soil remediation and are regularly debated by experts. Therefore, the Dutch Directorate-General for Public Works and Water Management (Rijkswaterstaat[2]) commissioned the National Institute for Public Health and the Environment (RIVM) to propose maximum acceptable concentrations (MAC) and annual average (AA) EQSs for cyanide.

The current EQS derivation focuses on the MAC-EQS and AA-EQS of free cyanide, which encompasses HCN and CN^-, the ratio of which depends on the pH. However, the free cyanide concentration is usually measured and expressed as CN^-. Also in the previous EQS derivation for free cyanide [9], values are expressed as CN^-, which is why the current derivation follows the same approach.

For the ease of reading, the term EQS is used throughout this report. However, the values derived in this report do not have an official status as a legal standard. They serve as scientifically based advisory values for the Dutch Ministry of Infrastructure and Water Management, that is responsible for environmental quality standard setting.

[1] The 'immissietoets' is an instrument used in the issue of discharge permits in the Netherlands. It assesses the permissibility of the discharge from a specific source -resulting after application of best available techniques- to a receiving surface water body.

[2] Rijkswaterstaat is part of the Dutch Ministry of Infrastructure and Water Management and responsible for the design, construction, management and maintenance of the main infrastructure facilities in the Netherlands.

2 Substance information

2.1 Identity and physico-chemical properties

Table 1 shows the chemical identifiers of hydrogen cyanide.

Table 1 Chemical identity of hydrogen cyanide.

chemical name (IUPAC)	Hydrogen cyanide
chemical name	Hydrogen cyanide
other names	hydrocyanic acid, formonitrile
CAS registry nr.	74-90-8
EC nr.	200-821-6
molecular formula	CHN
SMILES code	C#N

The cyanide ion, CN^-, has CAS registry nr. 57-12-5.

Table 2 shows relevant physico-chemical properties of hydrogen cyanide.

Table 2 Selected physico-chemical properties of hydrogen cyanide.

Parameter	Value	Unit	Remarks	Source
molecular weight	27.03	g/mol		[10, p. 43]
structural formula	HC≡N			
melting point	-13.24	°C	at 1013 hPa	[10]
vapour pressure	ca. 830	hPa	at 20°C	[10]
log K_{ow}	-0.25		at 20°C, pH ca. 7, value from Hansch et al.	[10]
log K_{ow}	-0.25		MlogP	[11]
water solubility	>100	vol%	at 20°C, pH ca. 7, value from handbook. 'Miscible in all ratios'	[10]
Henry's law constant (*H*)	13.48	$Pa/m^3/mol$	pH 4, 25°C; measured value	[10]
	13[a]	$Pa/m^3/mol$	20°C, measured value	[12]
pK_a	9.36±0.012		20°C	[10]
	9.11		30°C	[10]

Notes
a. Sander (2015) gives a range of values for *H*, from different origin (e.g. QSPR calculated, measured or review). We cite here the only measured value reported with a temperature of determination.

2.2 Classification and labelling

The harmonised classification of hydrogen cyanide according to Regulation (EC) No 1272/2008 (CLP) was retrieved from the CL inventory at the website of ECHA [13] (Table 3).

Table 3 CLP classification of hydrogen cyanide (CAS 74-90-8).

Hazard Class and Category Code	Hazard Statement Code
Acute Tox. 2 (oral)	H300
Acute Tox. 1 (dermal)	H310
Acute Tox. 2 (inhalation)	H330
Aquatic Acute 1	H400
Aquatic Chronic 1	H410

2.3 Environmental behaviour

Hydrogen cyanide is a gas which is highly soluble in water. In ecotoxicity tests, the test compound is usually a salt (usually NaCN or KCN), which, upon introduction, dissolves in the test medium into free cation species and free cyanide. For free cyanide, an equilibrium will be established according to the following reaction $HCN \rightleftharpoons H^+ + CN^-$. Hydrogen cyanide is a weak acid (pK_a ~9.2-9.3, Table 2) and is present for 99% in undissociated form (HCN) at pH 7. This means that CN^- is present for <1% at pH 7. At pH 8, this is 6%, at pH 8.5 this is 20% [2]. Both HCN and CN^- contribute to the overall ecotoxicity of free cyanide. But the free cyanide concentration is usually measured and expressed as CN^-.

From the Henry's law constants of 13 and 13.48 Pa/m^3/mol, a dimensionless Henry's law constant or $K_{air\text{-}water}$ of 0.0052 to 0.0054 is calculated for HCN. The latter value was determined at pH 4 and 25 °C. This roughly corresponds to a $K_{air\text{-}water}$ of 0.0024 at 10 °C. HCN can therefore be considered moderately volatile. Volatilisation is also expected to be the main removal route from the water compartment.

2.4 Mode of action

A brief description of the mode of action (MoA) is given here, which is derived from Zuhra and Szabo, 2022 [14], Eisler, 1991 [15] and ECETOC [16].

HCN is a small, soluble molecule, which rapidly crosses mucous membranes, resulting in a rapid uptake and tissue distribution. After uptake, cyanide is able to cause tissue anoxia through inactivation of cytochrome oxidase, an important enzyme in mitochondrial respiration. Specifically, cyanide inhibits cytochrome c oxidase (cytochrome a_3), the last enzyme (Complex IV) in the mitochondrial respiratory chain, from which electrons are transferred to molecular oxygen, producing water. Cyanide binds with ferric iron contained in the heme *a* part of the cytochrome c oxidase, resulting in a cytochrome c oxidase-CN complex, which subsequently blocks the electron transport from cytochrome to oxygen. A sequence of events follows: cessation of cellular respiration, hypoxia at cellular and tissue level, a shift to anaerobic metabolism and depletion of energy rich compounds (e.g. glycogen, ATP), lactate accumulation and internal pH decrease. The hypoxic situation affects the nervous system and results in respiratory arrest and death.

As mitochondrial respiration takes place in all eukaryotic cells, cyanide toxicity is considered to be non-species-specific. However, many plant species are able to endogenously produce cyanide as part of their molecular defense system and are therefore less sensitive than (other) eukaryotic organisms [14]. Cytochrome c oxidase is also an important enzyme in aerobic metabolism in bacteria and archaea, which suggests that also bacteria can be susceptible to cyanide [17]. In fact, bacteria are able to produce cyanide as an offense mechanism against other bacteria, which may explain why some bacteria are more sensitive to cyanide than others [18].

Some protection from cyanide poisoning can be provided by thiosulfate, which reacts with cyanide via thiosulphate sulfurtransferase in liver mitochondria, to form thiocyanate, which is excreted in urine. Eisler (1991) notes that species differ in the extent to which thiocyanate is formed and the rate at which it is excreted [15]. In addition, thiocyanate metabolites are less toxic than cyanide, but may accumulate and have been associated with adverse effects, such as developmental abnormalities. Some minor detoxification pathways exist, e.g. exhalation of HCN via breathing, conjugation with cystine and binding to vitamin B_{12}a (forming vitamin B_{12}) and methemoglobin in blood.

3 Methods

3.1 Determination of receptors at risk

In view of the low hydrophobicity of HCN, reflected in a log K_{ow} of -0.25, BCF or BMF values for HCN have not been collected. Environmental risk limit (ERL) derivation for biota (secondary poisoning) is not triggered.

None of the risk phrases that trigger risk limit derivation to protect humans via fish consumption is applicable to HCN. The substance does not have the potential to bioaccumulate and is not classified as carcinogenic, mutagenic or reprotoxic. In addition, HCN is not classified as having a risk of possible irreversible effects.

Conclusion: only ERLs to protect freshwater and marine surface water ecosystems will be derived.

3.2 EQS derivation

For the derivation of the EQS values, we followed the 2018 version of the Technical Guidance for Deriving Environmental Quality Standards of the Water Framework Directive (WFD) [19]. This guidance is also the basis for EQS derivation at the Dutch National level.

For reasons of efficiency, we deviated from this guidance on some aspects of data collection and evaluation. We did not perform a full literature search to retrieve all existing ecotoxicological data. Instead, we have used four reports in which EQS for cyanide were derived as primary data source (see Section 3.1.1). *(When considered necessary)* The ecotoxicity data retrieved from these reports were evaluated on reliability based on current WFD standards. Cases where the original source was checked and the resulting test outcomes were adapted are described in Sections 3.4, 3.5 or noted in the explanatory notes to the individual ecotoxicity data. These can be found in the ecotoxicity data tables in Appendix 1-4. In addition, a literature search was performed to gather additional ecotoxicity data generated since the publication of these four reports (described below).

3.3 Data search

3.3.1 Existing datasets

We retrieved ecotoxicity data collected for earlier environmental risk limit derivations and tabulated these. Sources used are:

- Verbruggen et al. 2001 [9, 20]. Environmental risk limit derivation for cyanide, performed at the Dutch national level in 2001.
- Hommen (2011) [21]. This report by Fraunhofer considers previous EQS derivations and derives refined values based on additional ecotoxicological data.
- Peters et al. (2012) [22]. This is a draft report concerning the EQS derivation for cyanide under the WFD framework. A draft 2014 update of the report was obtained and used.

- Sorokin et al. (2008) [23]. EQS proposal by Water Framework Directive – United Kingdom Technical Advisory Group (WFD UKTAG).

3.3.2 *Additional literature search*

The US EPA ECOTOX database [24] was scanned for the substances HCN, KCN, and NaCN. All potentially relevant publications were downloaded, evaluated and added to the tables with ecotoxicity data. Any relevant secondary literature was also considered.

In addition, a literature search was performed using Scopus for the period 2011-2022 (the period since the publication of the EQS derivation by Peters et al.; 2012), with the search strings provided below:
*cyanide AND aquatic AND ecotox**
TITLE-ABS (ec50 OR ec20* OR ec10* OR lc50* OR lc20* OR lc10* OR noec* OR loec* OR matc OR tlm OR chv OR ecx OR bioassay*) AND TITLE-ABS (cyanide) AND TITLE-ABS (aquatic* OR water OR freshwater OR surface AND water).*

Since the number of relevant hits was limited an additional search was done using the following search string:
TITLE-ABS-KEY (cyanide)) AND ((toxicity)) AND (aqua)*
over the period 2011-2022.

The reference lists from all evaluated publications were screened for potentially relevant studies (retrospective search) in the period 2011-2022.

3.3.3 *Data compilation*

All relevant ecotoxicity data retrieved from the studies (freshwater and marine water; acute and chronic) were collated in data tables, presenting study information as outlined in the WFD guidance [19]. These tables are included in Appendix 1-4.

3.4 Data evaluation

Generally, data from Verbruggen et al. (2001) and Peters et al. (2012) were included in the data tables without re-evaluation. However, in some cases, re-evaluation of the underlying literature was deemed necessary. It is known that the authors of both reports only presented data that were considered acceptable and reliable for use. Peters et al. explicitly stated that the 36 studies selected for their draft EQS derivation had a Klimisch reliability score of 1 (reliable) or 2 (reliable with restrictions). The main drawback of the data from Peters et al. is that they lack any tabulated information on test species characteristics (such as life stage), test substance (identity, purity) and test set up (analytical verification, static, renewal, etc.) or test endpoints. Therefore, the reliability score could not be confirmed without revisiting the original study. The tables in Verbruggen et al. (2001) are much more detailed, but do not report temperature.

For some species, a range is reported from tests at different temperatures without specifying the individual results. In such cases it is not possible to decide on combining data into a geometric mean per species as required in the guidance. Therefore, if the lowest value for a species originated from Verbruggen et al. (2001) or Peters et al. (2012), the underlying reference was checked and Ri was adapted where necessary. In addition, revisiting the underlying study was sometimes necessary to verify a study result, e.g. when results cited in two sources were different or in case the result was aberrant compared to values published by other authors.

Based on the non-specific mode of action of free cyanide (see 2.4), limited variation in sensitivity between and within species is expected. Therefore special attention was paid to effect concentrations that were much lower (e.g., factor of 10) than other values for the same or related species. In case the re-evaluation led to a different effect concentration, this was noted in the comments to the specific ecotoxicity study in the data table. For example, the LC_{50} values from Calleja et al. [25], that were recalculated using the original publication, as well as for the results for *Gammarus pseudolimnaeus* from Smith et al. [26] and Oseid and Smith [27]. Otherwise, values were added to the data tables in the appendices without further evaluation.

Studies that were retrieved from the additional literature searches (see Section 3.2) were evaluated and scored on reliability according to Klimisch et al. (1997) and the current WFD standards [28]. Study details and reliability scores were tabulated in the data tables, accordingly (see Appendix 1-4).

3.5 Data selection

In order to derive quality standards, the ecotoxicity dataset has been reduced to a single ecotoxicity value per species, as explained below, which results in aggregated data tables, see Table 4, Table 5 and Section 3.6.

Following the WFD guidance, results of multiple studies with the same species can be averaged to arrive at one value per species in case test endpoint, test conditions, life stage, etc. are equal [19]. For this report, such multiple results per species were not averaged when there was a difference in test temperature (see 5^{th} bullet below), pH or exposure set up (static versus renewal and flow through). In such situations, the lowest test result for the relevant exposure duration was selected. Effectively, this means that in the majority of cases, multiple results for the same species and the same endpoint and test duration were not averaged. Specific choices on data selection are detailed below.

- Upon evaluating literature, we have noticed that analytical measurements (when performed) usually retrieve relatively low concentrations of cyanide (CN^-) compared to nominal concentrations, even when exposure containers were covered or sealed. This can be explained by the high volatility of the substance (see Section 2.3.4). We therefore decided to only consider results based on measured data to be reliable. All studies in which no analytical verification was conducted were not taken into account for the ERL derivation.

- The literature search retrieved some studies in which the test material was zinc cyanide (ZnCN) or copper cyanide (CuCN). As zinc and copper may also be toxic, these studies were left out.
- Study results were considered not reliable when dissolved oxygen in the exposure media were reported to be<60%.
- Studies with activated sludge bacteria were excluded. These studies are not performed with a species or consortium that resembles natural water bodies.
- Studies reporting responses of biochemical parameters, e.g. catalase, glutathione peroxidase, glutathione S-transferase activity or lipid peroxidation, glycogen levels, etc., were not included as these are considered non population-relevant endpoints. The same holds for histopathological findings on various organs when the findings could not be related to a population-relevant parameter. Such studies usually investigated endpoints only at one or two concentrations, insufficient for deriving EC_x values [19].
- In case acute ecotoxicity studies were conducted with a species for which a test guideline is available, but the test was performed with different exposure durations, we selected the result for exposure duration prescribed in the guideline or closest to that duration, also mentioned in the WFD guidance [19]. These relatively short exposures (typically 24 – 96 h) match with the concept of the MAC-EQS which is a standard that protects against short-term peak exposures. We deviated from this principle when a clear increase in ecotoxicity is observed at longer test durations. This was done for the study by Jaafarzadeh et al. (2013), in which *D. magna* showed a clear decrease in mobility over longer test periods. In this case, the lowest EC_{50} was used for the EQS-derivation.
- Several authors investigated the effect of temperature on the ecotoxicity of cyanide. When studies with the same species were conducted at different temperatures, we selected tests conducted within the range 0 to 30°C, based on measured surface water temperatures (2015-2019) for two large rivers (Rhine, Meuse) in The Netherlands [29]. In some studies an effect of temperature was observed, although the pattern was not consistent. In some studies with fish tested at three temperatures, ecotoxicity was markedly higher at the higher temperature [30], while other fish studies [31, 32] showed the highest ecotoxicity at lower temperatures. However, in the latter studies, ecotoxicity seemed to increase with pH as well. To account for a potential effect of temperature on ecotoxicity and to arrive at one value per species, we therefore selected the lowest test result (EC_{50}, NOEC, etc.) if multiple values were available for the same species, obtained at different test temperatures.
- When replication of a study is not performed or not reported, it cannot be considered reliable. For example, Verbruggen et al. (2001) [9] tabulated a NOEC <0.01 mg/L for *Salmo salar* by Leduc [33]. Since this was a relatively low and unbound result on a relevant endpoint (hatching success) in a chronic study, we evaluated the original paper. It is a study in which eggs were tested at five test concentrations and a control. The initial number of eggs varied between 855 and 1041 eggs per treatment. Controls showed 94% hatching. However, only the control

treatment was replicated, the other treatments were performed without replication. Not knowing the variation between independent treatments is a major deficiency in set up, because statistical evaluation to derive e.g., LOEC and NOEC is therefore not possible. Due to the lack of replication of treatments, the study is given a Klimisch score of 3 and is therefore not used in the ERL derivation.

3.6 Recalculation

In most studies, substances such as KCN and NaCN are used as test substances to determine cyanide ecotoxicity. In most of these studies, also the effect values are expressed as the concentration of KCN and NaCN, respectively. In the current EQS derivation, we converted these values to CN^- concentration, using the corresponding molecular weights:

$$EV_{CN^-} = EV_{substance} * (MW_{CN^-} / MW_{substance})$$

Where:
EV = Effect value
MW = molecular weight

4 Overview of ecotoxicity data

4.1 Aggregated data tables

The data selection was applied to the data shown in Appendix 1-4. This led to Table 4 and 5 with aggregated data for acute and chronic ecotoxicity data, respectively, for both freshwater and marine organisms. These data tables are the basis for the current EQS derivation.

Table 4 Aggregated data table with selected acute ecotoxicity data for freshwater- and marine organisms.

Taxon/species	L(E)C50 [mg CN^-/L]	Remark	Ref.
Cyanobacteria			
Anabaena flos-aquae	>0.7	96-h chlorophyll a measurements	Shehata et al., 1988
Algae			
Chlamydomonas sp.	>0.04	10-d growth rate	Cairns et al., 1975
Chlorella vulgaris	0.14	72-h chlorophyll a measurements	Liu et al. 2018
Raphidocelis subcapitata	0.04	72-h growth rate	Environment Agency 2008
Nitzschia closterium	0.06	72-h growth rate; marine species	Pablo et al., 1997 c
Macrophyta			
Lemna gibba	0.03	7-d growth rate	Wenzel 2011
Mollusca			
Chlamys asperrimus	0.03	48-h larval abnormality; marine species	Pablo et al., 1997 a
Villosa iris	0.58	96-h mobility, heart beat	Pandolfo et al 2012
Crustacea			
Acartia tonsa	0.19	48-h mortality; marine species	Parametrix, 2006
Americamysis bahia	0.11	96-h mortality; marine species	Lussier et al., 1985
Cancer gracilis	0.14	96-h mortality; marine species	Brix et al., 2000
Cancer irroratus	0.04	96-h mortality; marine species	Northwestern Aquatic Sciences, 2003
Cancer magister	0.05	96-h mortality; marine species	Brix et al., 2000
Cancer oregonensis	0.15	96-h mortality; marine species	Brix et al., 2000
Cancer productus	0.11	96-h mortality; marine species	Brix et al., 2000
Daphnia magna	0.01	96-h immobility	Jaafarzadeh et al., 2013
Gammarus pseudolimnaeus	0.08	96-h mortality	Smith et al., 1979
Penaeus monodon	0.11	96-h mortality; marine species	Pablo et al., 1997 b
Insecta			
Chironomus riparius	0.01	48-h immobility; 1st instar	Bertow 2011a
Tanytarsus dissimilis	2.49	48-h mortality; 3rd and 4th instar	Call et al., 1983

Taxon/species	L(E)C50 [mg CN^-/L]	Remark	Ref.
Pisces			
Ictalurus punctatus	0.16	26-h mortality	Cardwell et al., 1976
Carassius auratus	0.32	96-h mortality	Cardwell et al., 1976
Gasterosteus aculeatus	0.10	96-h mortality	Parametrix, 2005
Lepomis macrochirus	0.07	96-h mortality	Smith et al., 1978
Micropterus salmoides	0.10	96-h mortality	Smith et al., 1979
Oncorhynchus mykiss	0.03	96-h mortality	Kovacs 1979; Kovacs & Leduc, 1982
Perca flavescens	0.09	96-h mortality	Smith et al., 1978
Pimephales promelas	0.10	96-h mortality	Smith et al., 1978
Pomoxis nigromaculatus	0.10	96-h mortality	Smith et al., 1979
Rutilus rutilus	0.11	168-h mortality	Solbé et al.,1985
Salmo salar	0.08	24-h mortality; tested under marine conditions	Alabaster et al., 1983
Salmo salar	0.07	24-h mortality	Alabaster et al., 1983
Salvelinus fontinalis	0.07	96-h mortality	Smith et al., 1978
Amphibia			
Rana berlandieri	0.41	96-h mortality	ENSR, 2005c
Rana pipiens	0.19	96-h mortality	ENSR, 2005a
Xenopus laevis	0.25	96-h mortality	ENSR, 2005b

Table 5 Aggregated data table with selected chronic ecotoxicity data for freshwater- and marine organisms.

Taxon/species	NOEC/EC_{10} [mg CN^- /L]	Remark	Ref.
Cyanobacteria			
Anabaena flos-aquae	>0.7	96-h chlorophyll a measurements	Shehata et al., 1988
Algae			
Chlorella vulgaris	0.055	72-h chlorophyll a measurements	Liu et al. 2018
Nitzschia closterium	0.0060	72-h growth rate; marine species	Pablo et al., 1997a
Raphidocelis subcapitata	0.0096	72-h growth rate	Environment Agency 2008
Scenedesmus quadricauda	0.3	96-h chlorophyll a measurements	Shehata et al., 1988
Macrophyta			
Lemna gibba	0.0055	7-d growth rate	Wenzel, 2011
Mollusca			
Chlamys asperrimus	0.0050	48-h embryo development; marine species	Pablo et al., 1997c
Crustacea			
Americamysis bahia	0.043	29-d mortality; marine species	Lussier et al., 1985

Taxon/species	NOEC/EC_{10} [mg CN^- /L]	Remark	Ref.
Insecta			
Chironomus riparius	>0.0017	28-d emergence; development rate	Bertow, 2011
Pisces			
Lepomis macrochirus	<0.005	289-d spawning	Kimball et al., 1978; Smith et al., 1979
Oncorhynchus mykiss	0.005	20-d growth	Kovacs, 1979
Pimephales promelas	0.012	107-d egg production	Lind et al., 1977; Smith et al., 1979
Salvelinus fontinalis	0.0055	144-d egg production	Koenst et al., 1977

5 EQS derivation

5.1 Pooling of ecotoxicity data for freshwater and marine organisms

The acute freshwater and marine ecotoxicity datasets may be pooled, because a 2-sided t-test for unequal variances (F-test: $p = 0.022$) showed that the datasets are not significantly different ($p = 0.50$, $\alpha = 0.05$). For these calculations, the censored values ('higher than' or 'lower than' values) were not taken into account.

The chronic freshwater and marine ecotoxicity datasets may be pooled as well. A 2-sided t-test for equal variances (F-test: $p = 0.85$) showed that the datasets are not significantly different ($p = 0.71$, $\alpha = 0.05$). For these calculations, the censored values ('higher than' or 'lower than' values) were not taken into account.

Based on the results of the statistical comparisons above, both the acute and the chronic ecotoxicity data are pooled for freshwater and marine species for the ERL derivation. This is in line with all previous EQS derivations for free cyanide.

5.2 Deterministic or probabilistic approach

5.2.1 Acute ecotoxicity data

The combined dataset for acute ecotoxicity consists of reliable study results (EC_{50} or LC_{50} values) for 35 species, distributed over 8 taxonomic groups: cyanobacteria, algae, macrophyta, mollusca, crustacea, insecta, pisces and amphibia. The dataset meets the criteria for construction of a Species Sensitivity Distribution (SSD) as listed in the WFD-guidance. According to the guidance, the output from an SSD-based quality standard is considered reliable if the database contains preferably more than 15, but at least 10 datapoints, from different species covering at least eight taxonomic groups [19, p. 43-44]. Below, the criteria are listed, together with the representative species from the present acute dataset:

- Fish: *Oncorhynchus mykiss (*family Salmonidae*)*
- A second family in the phylum Chordata: *Lepomis macrochirus* (family Centrarchidae)
- A crustacean: *Daphnia magna*
- An insect: *Chironomus riparius* (order Diptera, family Chironomidae)
- A family in a phylum other than Arthropoda or Chordata: *Chlamys asperrimus* (phylum Mollusca)
- A family in any order of insect or any phylum not already represented: *Anabaena flos-aquae* (phylum cyanobacteria)
- Algae: *Chlorella vulgaris*
- Higher plants: *Lemna gibba*

In addition, the acute dataset contains several examples of species representing the same phylum, but having a very different life form and survival, reproduction and feeding strategy. Amphibia and fish, for example, are very different in that regard although both represent the phylum Chordata. The same argument can be made for crabs and cladocerans, both representing Crustacea.

As the WFD-criteria are met, the MAC-EQS are derived using an SSD and an assessment factor on the derived HC_5 value.

5.2.2 *Chronic ecotoxicity data*

The combined dataset for chronic ecotoxicity consists of study results (NOEC or EC_{10} values) for 13 species, distributed over 7 taxonomic groups: cyanobacteria, algae, mollusca, crustacea, insecta, pisces and macrophyta. Below, the WFD criteria for the construction of an SSD are listed, together with the representative species from the present chronic dataset:

- Fish: *Oncorhynchus mykiss (*family Salmonidae*)*
- A second family in the phylum Chordata: *Lepomis macrochirus* (family Centrarchidae)
- A crustacean: *Americamysis bahia*
- An insect: *Chironomus riparius* (order Diptera, family Chironomidae)
- A family in a phylum other than Arthropoda or Chordata: *Chlamys asperrimus* (phylum Mollusca)
- A family in any order of insect or any phylum not already represented: *Anabaena flos-aquae* (phylum cyanobacteria)
- Algae: *Chlorella vulgaris*
- Higher plants: *Lemna gibba*

As the WFD-criteria are met, the AA-EQS are derived using an SSD and an assessment factor on the derived HC_5 value.

5.2.3 *SSD modelling*

Both the acute and chronic datasets contain a few censored or 'unbound' effect values ('greater than' or 'lower than' values); for example, the chronic effect value for the insect *C. riparius* is an unbound NOEC, i.e. reported as > 0.00165 mg/L. Traditionally, such values cannot be used in a species sensitivity distribution. The E_TX 2.3 software tool mentioned in the WFD guidance, for example, does not allow integrating censored data in fitting an SSD. However some calculation models currently have integrated statistical methods that allow the use of censored values for fitting an SSD. The R-package E_TX 3.0, which is a follow-up of E_TX 2.3, provides functions for fitting univariate distributions to different types of data, including censored data. The E_TX 3.0 R-package is available on request[3] from the author (T. Aldenberg). Also available is the R-package 'fitdistrplus' which also provides SSD fitting with censored data [34]. For both the current acute and chronic dataset, E_TX 3.0 is used for fitting the SSDs and deriving the HC_5 values.

Results will be compared with those obtained with *fitdistrplus* (section 6.4). A relevant difference between both packages is that E_TX 3.0 handles censored data using Bayesian statistics via numerical Markov Chain Monte Carlo (MCMC) simulation (i.e. not via extrapolation constants as in E_TX 2.3), explicitly allowing to address small sample

[3] ETX_3.0-132.tar.gz is available upon request at tom.aldenberg@gmail.com.

sizes; while *fitdistrplus* makes use of the (non-Bayesian) likelihood-plus-bootstrap approach (classical approach).

To confirm the statistical output generated by *fitdistrplus*, the MOSAIC SSD application, published by the University of Lyon [35-37], was used on the same datasets. This model also runs the R-package *fitdistrplus* and is very user-friendly, as it can be used as a web-application, but is limited in its user-customisation [38]. See Section 6.4 for the comparison of the results between the two methods.

5.3 MAC-EQS for freshwater and marine water

The MAC-EQS was derived using an SSD and an assessment factor on the derived HC_5 value (see Section 5.2.1). A log-normal distribution was fitted through the aggregated acute toxicity data, combining both freshwater and marine water data, using the *E$_T$X* 3.0 R-package. The HC_5 estimated from the log-normal distribution is 17 µg/L, with a 90% confidence interval of 9.6 to 25 µg/L. Figure 1 presents the SSD plot of the acute ecotoxicity dataset, as derived from the *E$_T$X* 3.0 output file. The output file (including the R-script) is embedded in this document under Annex 1.

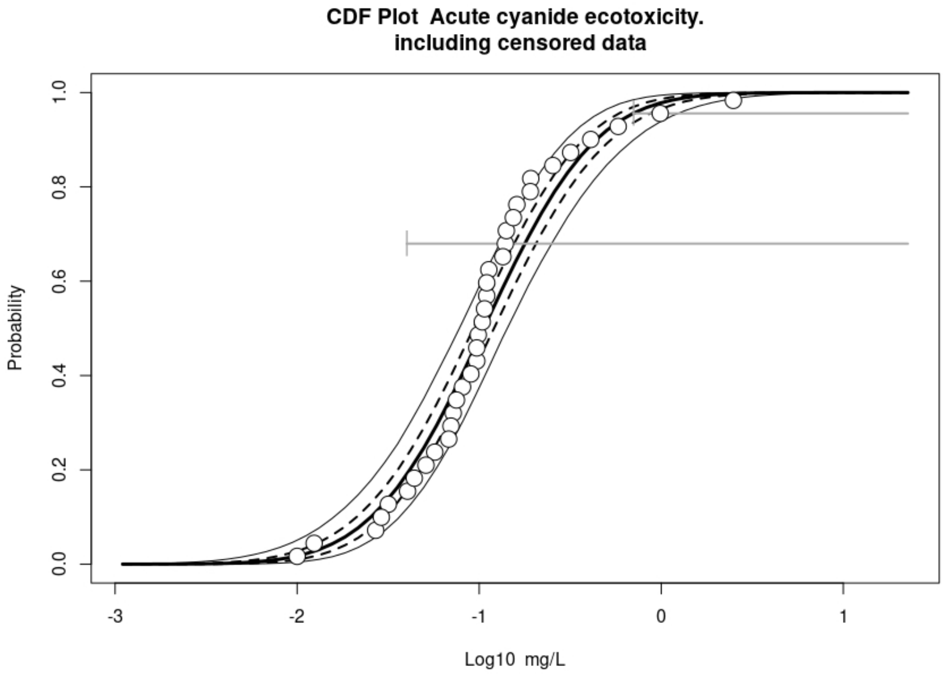

Figure 1 Cumulative distribution function of acute ecotoxicity data for cyanide; combining freshwater and marine water data (35 species). A log-normal distribution was fitted through the data using E$_T$X 3.0. Censored data are indicated by horizontal grey lines, the vertical part indicating the truncated value.

Figure 1 shows the fitted log normal distribution through the combined freshwater and marine acute toxicity data set. The position of the truncated value of the censored data points is indicated by a small vertical grey line with a horizontal grey line indicating the (uncertain) position of the real data point. E.g. the EC_{50} of >0.04 mg/L for

Chlamydomonas sp., is positioned at the x-axis at -1.40 on the left ($\log_{10}$ 0.04 = -1.40). As the location of the real data point on the x-axis is fully uncertain (except from being higher than -1.40), a probability distribution for it is fitted (Bayesian bootstrap using MCMC simulation) from the truncated value at -1.40 to infinity at the right side. The median of the bootstrapped distribution to the right of this unbound value is the estimate for this specific data point. The data point is plotted at the x-axis position of this median estimate; it has been sorted as such with the other data points of the SSD. The horizontal line through the data point ending at the truncated (">") value indicates the 'original' tabulated data point.

The default assessment factor on the HC_5 for derivation of the MAC-EQS is 10. This assessment factor can be lowered in case other lines of evidence suggest that a higher or lower assessment factor is appropriate. However, the current dataset and background information does not allow deviation from the default assessment factor of 10.

Figure 1 shows that the interspecies variation is high (i.e. the SSD is not steep): the spread of the toxicity data amounts to three orders of magnitude. Moreover, the difference between acute and chronic ecotoxicity is considerable. The difference between the acute HC_{50} and the chronic HC_{50} values is 5 and the difference in HC_5 values is 20. Based on the above, the assessment factor on the HC_5 for derivation of the MAC-EQS is kept at 10.

Even though cyanide toxicity follows by a non-specific mode of action (targeting the mitochondrial respiration chain), the SSD spread covers three orders of magnitude and the chronic HC_5 is 20-fold lower than the acute HC_5. This can be explained by the difference in natural tolerance of aquatic species to cyanide as well as the intra- and inter-laboratory variation of toxicity data.

The MAC-EQS for freshwater is 17/10 = 1.7 µg/L.

Since there is one reliable acute value for an additional marine taxonomic group available (mollusc; *Chlamys asperrimus*), as well as an acute value for crabs (*Cancer sp.*), which are also invertebrates, but have a different life form and feeding strategy than the representatives of the freshwater invertebrates, the uncertainty of the marine acute toxicity is reduced. Therefore, no additional assessment factor is applied for the marine MAC-EQS.

Thus, the marine MAC-EQS is also 1.7 µg/L.

5.4 AA-EQS for freshwater and marine water

The AA-EQS was derived using an SSD and an assessment factor on the derived HC_5 value (see Section 5.2.2). A log-normal distribution was fitted through the aggregated chronic toxicity data, combining freshwater and marine water data, using the *E_TX* 3.0 R-package. The HC_5 estimated from the log-normal distribution is 0.66 µg/L, with a 90% confidence interval of 0.078 to 2.5 µg/L. Figure 2 presents the SSD plot of the chronic ecotoxicity dataset, as derived from the output file. The

E_TX 3.0 output file (including the R-script) is embedded in this document under Annex 1.

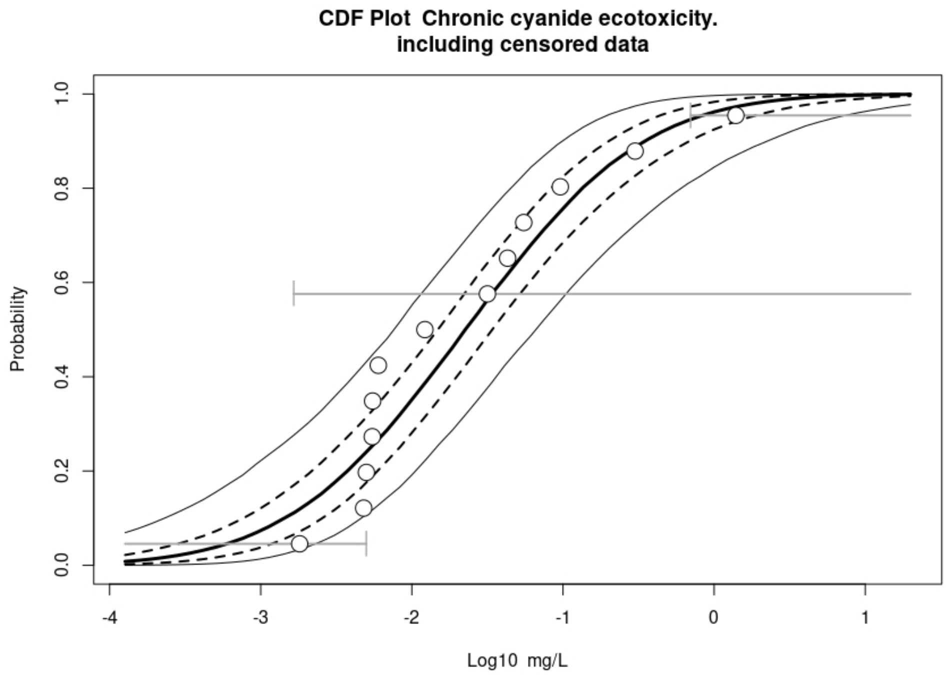

Figure 2 Cumulative distribution function through the aggregated chronic ecotoxicity data for cyanide (13 species); combining freshwater and marine water data, including three censored data points. A log-normal distribution was fitted through the data using ETX 3.0.

As a probabilistic approach is followed on the chronic dataset using an SSD, an additional assessment factor (1-5) should be applied on the extrapolated HC_5 value to account for residual uncertainties that are not accounted for by the SSD model. The height of this assessment factor depends on a variety factors, as outlined in the WFD Guidance (p.31-32), which are discussed below.

Mode of action:
the MoA of cyanide is known (see section 2.4) and is non-species-specific, since mitochondrial respiration occurs in all eukaryotic organisms. Even though the acute and chronic datasets cover a large variety of aquatic species, inclusion of the most sensitive aquatic species cannot be guaranteed. On the other hand, given the non-species-specific MoA, it is unlikely that other aquatic species, not represented in the current dataset will be significantly more sensitive.

Background concentrations
There is limited information on free cyanide concentrations in Dutch surface waters. Analytical methods for measuring free cyanide in water samples are available. However, the limits of detection of such methods are usually not low enough to be able to measure concentrations below levels of concern, given the high ecotoxicity of free cyanide. As analytical monitoring of free cyanide is not routinely performed in the

Netherlands, there is insufficient information available on the free cyanide concentrations in Dutch surface waters.

Rijkswaterstaat does monitor the background concentrations of total cyanides at several freshwater bodies in the Netherlands. The most recent (December 2020) total cyanide concentration measurements ranged between 1-2 μg/L for 5 out of the 14 measuring sites. For the other 9 sites, the concentrations were below the limit of detection (<1 or <2 μg/L) [6]. As previously described, even though the free cyanide content within the total cyanides is unknown, it is believed to be low as most free cyanide is considered to rapidly complex with iron particles; especially when the water treatment includes a Fenton process.

The size of the AF should normally not result in a QS that is below the natural background level. Knopf et al. (2021) developed and validated a continuous flow analysis (CFA) analytical technique to measure free cyanide [39]. The LOD of this is ranged around 0.05 μg/L. The analytical method was tested in the Lenne river (a tributary of the river Ruhr) upstream and downstream of an industrial area in Germany (Schmallenberg). Measured concentrations upstream were between 0.05 and 0.1 μg/L. Measured concentrations downstream were higher, ranging between 0.1 and 0.2 μg/L.

Field and mesocosm data
The retrieved dataset does not contain any field or mesocosm studies. Therefore, the assessment factor cannot be lowered based on these grounds.

Other lines of evidence
The overall quality of the database and the endpoints covered are considered to be good. For all data points, a reliability evaluation has been conducted either based on previous EQS derivations (Peters *et al*. (2012), Verbcruggen *et al*. (2001), Hommen (2011) and Sorokin *et al*. (2008)) or directly by evaluating the studies. Only studies that are considered reliable for assessment (Klimisch score 1 or 2) were taken into account. Both the acute and chronic dataset cover a wide variety of species and taxonomic groups. However, the interspecies variation is high, as can be observed in the SSD plots. The spread of the toxicity data is between two and three orders of magnitude.

Also, the difference between chronic and acute toxicity is considerable, which is shown, for example, by comparing the HC_5 and HC_{50} values between the acute and chronic SSD, of which the ratios are 20 and 5, respectively.

The datapoints in the chronic dataset are not well in line with the fitted SSD curve. This can be explained by the many datapoints around the 0.005 μg/L range and the low number of datapoints at higher concentrations. This could have resulted in a slightly conservative derivation of the HC_5 of 0.66 μg/L.

Assessment factor
Based on the present considerations, some uncertainty about the derivation of the HC_5 remains, but given the reliability of the dataset

which covers a large variety of species, an assessment factor of 3 is considered reasonable for the AA-EQS derivation.

The AA-EQS for freshwater is 0.66/3 = 0.22 µg/L.

As one additional marine taxonomic group (mollusca) is available in the chronic dataset, an additional assessment factor of 5 (instead of a default assessment factor of 10) is be applied for the marine AA-EQS.

Therefore, the marine AA-EQS is 0.22/5 = 0.044 µg/L.

6 Discussion

6.1 Comparison with environmental risk limits from other frameworks

The current freshwater and marine MAC-EQS are derived at 1.7 µg CN^-/L. The freshwater and marine AA-EQS values are derived at 0.22 and 0.044 µg CN^-/L, respectively. How these values compare with previously derived EQS (or similar) values is examined in Table 6, where the currently proposed EQS-values are presented in grey.

The freshwater MAC-EQS is in line with the other values. Most values are within a factor of 2 from the MAC-EQS of 1.7 µg/L. The current $AA\text{-}EQS_{fw}$ (0.22 µg CN^-/L) is in the same order of magnitude as the values from Peters et al. (2012) (0.50 µg/L), JRC (2015) (0.57 µg/L) and very close to the value from Verbruggen et al. (2001) (0.23 µg/L). The current $AA\text{-}EQS_{sw}$ is close to the value from JRC (2015) (0.057 µg/L).

Table 6 Environmental risk limits for cyanide from other frameworks and countries.

Type	Cyanide species	Value [µg/L]	Institute/ framework	Year	Based on	Method	Source	Note
Acute freshwater								
MAC-EQS	CN^-	1.7	RIVM, WFD	2023	SSD, 35 species, 8 taxonomic groups	HC_5 / 10	this study	
MAC-EQS	free cyanide	3.2	WFD (draft)	2014 [f]	SSD, 28 species, nr. taxa not reported	HC_5 / 5	[22]	
MAC-EQS	free cyanide	1.0	WFD (draft)	2014 [f]	Lowest LC_{50} of 10 µg/L free cyanide for the fish *Lepomis macrochirus*	AF = 10	[22]	a
AWQC freshwater	free cyanide	23	US EPA	2006	28 species; *Oncorhynchus mykis*s most sensitive	LC_{50min}/2	[40]	
short-term PNEC	free cyanide	5	ECETOC	2007	Lowest LC_{50}/EC_{50} values are 'around' 50 µg/L. An AF of 100 would be too conservative	AF = 10	[16]	
MAC-QS freshwater and marine water	free cyanide	3.2	Fraunhofer	2011	SSD, 43 species covering 8 taxonomic groups, freshwater and marine water pooled, HC_5 = 15.9 µg CN-/L	HC_5 / 5	[21]	
MAC freshwater, eco	free cyanide	4.2	WFD, JRC (draft)	2015	Lowest LC_{50} of 42 µg/L free cyanide for the fish *Oncorhynchus mykiss*	AF = 10	[41]	g
Acute, marine water								
MAC-EQS	CN^-	1.7	RIVM, WFD	2023	SSD, 35 species, 8 taxonomic groups	$MAC\text{-}EQS_{sw}$ = $MAC\text{-}EQS_{fw}$	this study	
MAC marine water, eco	free cyanide	0.42	WFD, JRC (draft)	2015	Lowest LC_{50} of 42 µg/L free cyanide for the fish *Oncorhynchus mykiss*	AF = 100	[41]	g
AWQC marine water	free cyanide	20	US EPA	2006	SSD, 9 species, HC_5 = 39.4 µg/L	HC_5/2	[40]	
Chronic, freshwater								
AA-EQS	CN^-	0.22	RIVM, WFD	2023	SSD, 13 species, 7 taxonomic groups	HC_5 / 3	this study	
AA EQS	free cyanide	0.5	WFD (draft)	2014 [f]	NOEC embryo development; scallop, *Chlamys asperrimus*	AF = 1000	[22]	
AWQC freshwater	free cyanide (CN)	4.8	US EPA	2006	28 species; *Oncorhynchus mykis*s most sensitive	LC_{50min} / ACR of 9.659	[40]	e

Type	Cyanide species	Value [µg/L]	Institute/ framework	Year	Based on	Method	Source	Note
PNEC fresh and marine water	free cyanide	1	ECETOC	2007	SSD, 4 taxa, 16 NOECs, HC_5 = 1.1 µg CN-/L	HC_5 / 1	[16]	h
AA-QS freshwater	free cyanide	1	Fraunhofer	2011	SSD, 8 taxa, 13 NOECs, HC_5 = 2.0 µg CN-/L	HC_5 / 2	[21]	h
AA-EQS freshwater	free cyanide	0.57	WFD, JRC (draft)	2015	Lowest NOEC of 5.7 µg/L for the fish *Salvelinus fontinalis*	AF = 10	[41]	g
PNEC	HCN	0.04	ECHA, BPR	2012	Lowest EC_{50} of 40 µg/L based on *Scenedesmus subspicatus*	AF = 1000	[42] [c]	b
MPC	CN^-	0.23	RIVM	2001	SSD, 6 taxa, 8 NOECs	HC_5	[7, 8, 20]	d, g
PNEC freshwater	free cyanide	5	REACH dossier	2022	Lowest NOEC	AF = 10	[10]	i
Chronic, marine water								
AA-EQS	CN^-	0.044	RIVM, WFD	2023	SSD, 13 species, 7 taxonomic groups	$AA\text{-}EQS_{fw}$ / 5	this study	
AA-EQS marine water	free cyanide	0.057	WFD, JRC (draft)	2015	Lowest NOEC of 5.7 µg/L for the fish *Salvelinus fontinalis*	AF = 100	[41]	B
AWQC marine water	free cyanide (CN)	4.1	US EPA	2006	HC_5 (acute; 9 species)	HC_5/ ACR of 9.659	[40]	e
AA-QS marine water	free cyanide	0.2	Fraunhofer	2011	SSD, 8 taxa, 13 NOECs, HC_5= 2.0 µg CN-/L	AA-QS freshwater / 5	[21]	h
PNEC marine water	free cyanide	1	REACH dossier	2022	SSD	HC_5/ 1	[10]	i

Notes

For abbreviations, see page 49, section 7.

a. Spread in acute tox dataset is a factor 3.3.

b. cyanide species not reported (HCN or CN^-).

c. Biocides competent authority report (CAR) for product type 8. Identical information on the PNEC is found in the CARs for product type 14 and 18.

d. No assessment factor applied to HC_5.

e. See Gensemer [40, p. 7 and 8].

f. A draft version from 2014 was available, first draft: 2012.

g. Formally not approved

h. SSD based on assumptions of insensitivity to CN for some taxonomic groups. SSD criteria formally not met.

i. Data used unclear from the dossier, but most probably taken over from ECETOC.

6.2 The role of pH and temperature

The mode of action of cyanide can be considered non-specific or general as it targets the mitochondrial respiration which occurs in all eukaryotic and certain bacterial organisms. Still, the ecotoxicity observed across the different species covered in this study, varies to up to 3 orders of magnitude. This variation can be attributed to many factors. Firstly, it must be understood that difference in sensitivity to environmental stressors between species is a natural phenomenon. Secondly, ecotoxicity data has been gathered from many different sources describing different laboratory test set ups which generate intra- and inter-laboratory variation between ecotoxicity data. Particularly for cyanide are the influence of pH and temperate. The toxicity of cyanide is believed to be predominantly exerted through HCN, and CN^- toxicity is thought to be of little importance [15]. This is because HCN, in contrast to CN^-, is able to cross biological membranes [43, 44]. Thus, the pH may play a role in the ecotoxicity of free cyanide. As only analytically verified study results have been taken into account, the ecotoxicity of free cyanide can be directly related to the exposure to the substance. The influence of pH and temperature is implicitly included in this approach, hence no correction on the pH or temperature is deemed necessary.

6.3 Difference in sensitivity between insects

In the acute ecotoxicity dataset, two chironomid species are included (*Chironomus riparius* and *Tanytarsus dissimilis*). Despite their close taxonomic relatedness and ecological similarity, they have very different acute effect concentrations (0.01 mg/L vs 2.49 mg/L, respectively). This difference can be explained by the difference in larval development stage during exposure in the ecotoxicity tests. *C. riparius* was exposed as first instar, while the *T. dissimilis* were exposed as 3rd or 4th instar. Chironomid sensitivity to chemicals stressors significantly decreases at later larval stages [46-48], which can explain the difference in sensitivity observed in the current dataset.

6.4 Comparison between *fitdistrplus,* MOSAIC and ETX 3.0 calculations

The program E_TX 2.3 is commonly used by RIVM for the construction of an SSD. However, in the current EQS derivation, the acute and chronic dataset only meet the criteria for probabilistic EQS derivation (SSD) when censored values are taken into account. Since E_TX 3.0 is not yet publicly available and limited experience with fitting SSDs with censored data is available, we also constructed the SSDs and plots using the *fitdistrplus* R-package, as described Section 5.2.3. To confirm the manually constructed SSD using *fitdistrplus*, the automated MOSAIC SSD application was used on the same datasets. The HC_5 and HC_{50} values of the *fitdistrplus* and MOSAIC calculations are presented in Table 7 along the with estimates determined using ETX 3.0.

Table 6 Comparison of the results from SSD models fitdistrplus (R), MOSAIC (web based) and E_TX 3.0 (R) on the acute and chronic dataset. All values in µg/L.

		fitdistrplus	**MOSAIC**	**ETX_3.0-132**
Acute dataset	HC5 LL – UL	17 11 - 29	17 10 – 32	17 9.9 – 25
	HC50 LL – UL(	105 78 - 142	100 7380 - 150	106 78 – 143
Chronic dataset	HC5 LL – UL	0.88 0.32 – 2.6	0.88 0.22 – 3.1	0.66 0.078 – 2.5
	HC50 LL - UL	22 9.1 - 62	22 7.9 - 80	23 7.6 - 66

LL – lower limit, UL – upper limit of the 90% confidence interval.

The *fitdistrplus* and MOSAIC results are in line with each other. Both the HC_5 and HC_{50} estimates are highly comparable, whereas the 90% confidence intervals slightly differ. The more narrow confidence intervals calculated by *fitdistrplus* can be explained by the higher bootstrap iterations used (10000 vs 5000). The HC_5 and HC_{50} estimates obtained by *E_TX* 3.0 are also highly comparable to the values determined using *fitdistrplus/MOSAIC*. We note that the distribution of the HC_5 is not symmetrical. This can be seen in the lower limit (LL, 5^{th} percentile) estimate of the HC_5 estimate of the chronic data set by *E_TX* 3.0, which is lower than the LL estimate of *fitdistrplus*/MOSAIC. The HC_5 calculated by *E_TX* 3.0 is a bit more conservative.

For comparison, we also show the plotted CDFs obtained with *fitdistrplus* for the acute and chronic data sets. *Fitdistrplus* visualises the plotting of the data points as 'steps' rather than symbols. Also, the position of the censored data points is unclear. This makes *fitdistrplus* plots more difficult to read.

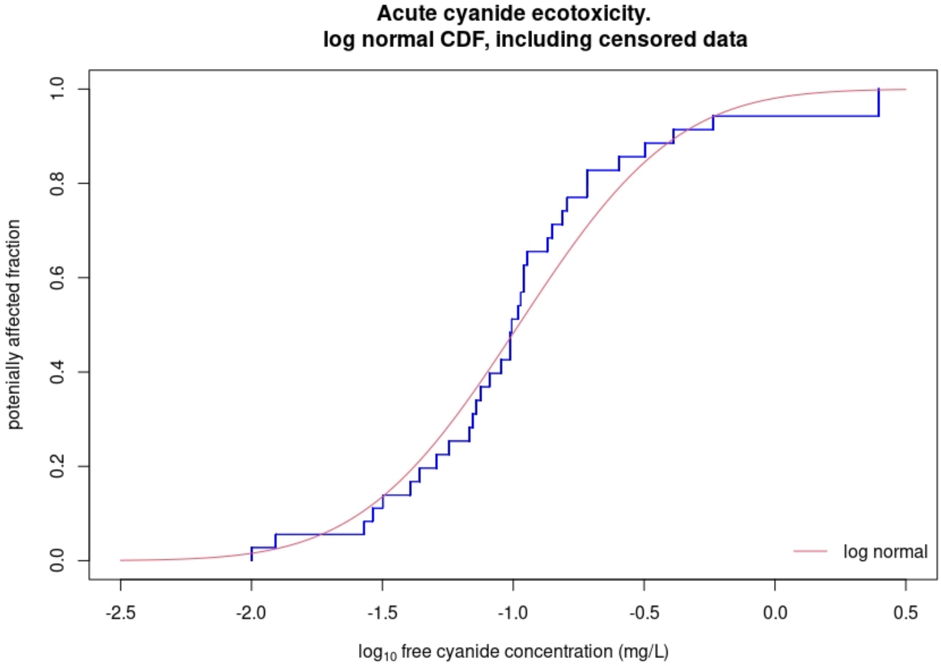

Figure 3 Cumulative distribution function of acute ecotoxicity data for cyanide; combining freshwater and marine water data (35 species). A log-normal distribution was fitted through the data using R-package fitdistrplus. Data set contains two censored data points.

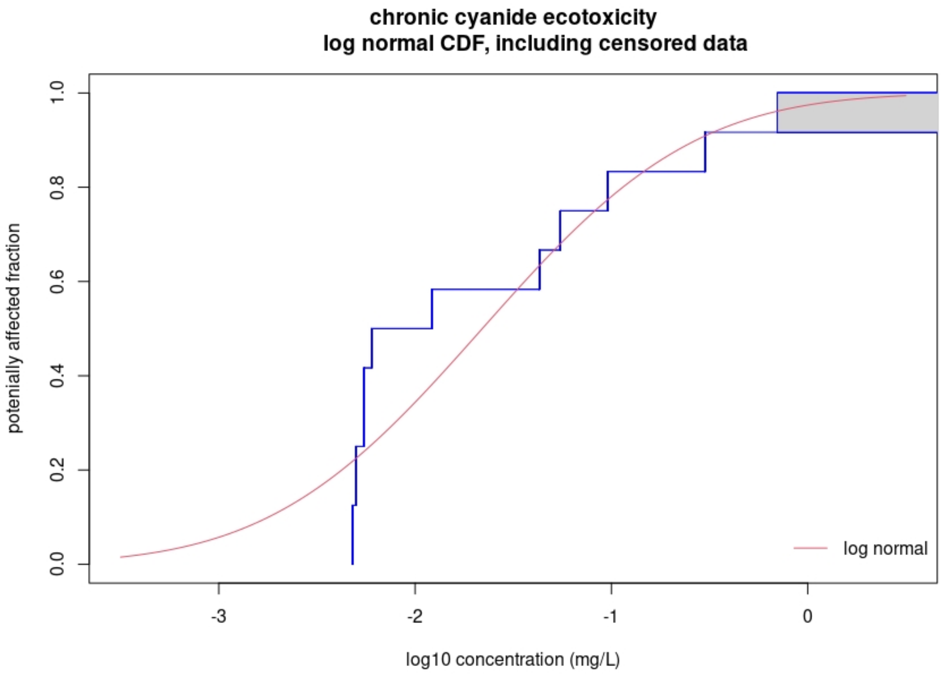

Figure 4 Cumulative distribution function of chronic ecotoxicity data for cyanide; combining freshwater and marine water data (13 species). A log-normal distribution was fitted through the data using R-package fitdistrplus. Data set contains three censored data points.

6.5 Conclusion on the MAC-EQS and AA-EQS values

In summary, the following EQS values for free cyanide (CN^-; CAS registration number: 57-12-5) were derived:

MAC-EQSfw:	1.7 µg/L
MAC-EQSsw:	1.7 µg/L
AA-EQSfw:	0.22 µg/L
AA-EQSsw:	0.044 µg/L

It should be emphasised that these standards are derived for free cyanide (expressed as CN^-). Rijkswaterstaat currently does not routinely monitor concentrations of free cyanide in Dutch waters. Instead, total cyanide is measured in accordance with the NEN-14403-1 guideline. Even though analytical methods for free cyanide have been published and methodology on measuring free cyanide is also described in the NEN-14403-1 guideline, practical applicability remains challenging for water managers.

Different forms of cyanide have different physico-chemical properties and ecotoxicity. As the most toxic form, free cyanide is a more relevant measure of toxicity to aquatic life than total cyanide, because total cyanide can include nitriles and other stable metallo-cyanide complexes that are relatively harmless to aquatic life. Therefore, monitoring of total cyanide has limited value for the aquatic risk assessment of cyanide.

Indeed, at the majority of monitoring sites total cyanides are not detected at concentrations above 1 µg/L and it is considered that most free cyanides will complex with iron once in the surface water. Still, when total cyanides above 1 µg/L are detected which was the case in 2020 for 5 out of the 14 measuring sites, it remains unclear how much of this concentration can be attributed to free cyanide.

Thus, to be able to assess the environmental risks of cyanides in surface waters, it is advised to investigate the possibilities to measure free cyanide (as the most toxic form). In doing so, it is advised to express the free cyanide concentrations as CN^- and compare these with the corresponding water quality standards.

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Abbreviations

AA	annual average
ACR	acute to chronic ratio
AF	assessment factor
AWQC	ambient water quality criterion
CAS	chemical abstract service
CL	classification and labelling
CDF	cumulative distribution function
EC	European Commission;
EC_x	Effect Concentration causing x% effect
ECHA	European Chemicals Agency
ERL	environmental risk limit
EQS	environmental quality standard
FAV	final acute value
fw	freshwater
HC_5	hazardous concentration; corresponding to the 5^{th} percentile in a species sensitivity distribution
IUPAC	International Union of Pure and Applied Chemistry
LOEC	lowest observed effect concentration
MAC	maximum acceptable concentration
MoA	mode of action
MCMC	Markov Chain Monte Carlo
NOEC	no observed effect concentration
PNEC	predicted no effect concentration
QSPR	quantitative structure property relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RIVM	National Institute for Public Health and the Environment
SSD	species sensitivity distribution
SMILES	simplified molecular input line entry system
sw	saltwater (marine)
US EPA	United States Environmental Protection Agency
WFD	Water Framework Directive

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