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Position paper on a refined SSD approach to derive EQS values for cyanides

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Summary

This paper evaluates the recently proposed freshwater and marine water environmental quality standards (QS) for cyanides INERIS (2011) and suggests refined QS considering also data from studies considered for updating water quality criteria in the USA Gensemer R.W. et al. (2007) and new available GLP toxicity tests for the macrophyte *Lemna gibba* and the midge *Chironomus riparius.*

Because it was shown in previous reviews that the sensitivity of freshwater and saltwater species to cyanides is not significantly different, combined data sets were used to calculate Species Sensitivity Distributions (SSDs) and to derive HC5 Hazardous Concentration for 5% of the species = 5. centile of the SSD). Quality standards for short-term and long-term exposure (MAC-QS = Maximum Acceptable Concentration Quality Standard, AA-QS = Annual Average Quality Standard) applicable to fresh- and marine waters were derived following the recent Technical Guidance for Deriving Quality Standards under the Water Framework Directive (verison 8, 2011).

The MAC-QS proposed in this position paper are derived from an SSD with acute LC50 or EC50 for 43 freshwater and marine species covering 8 major taxonomic groups and including 15 marine species. The HC5 for this SSD is 15.9 µg CN^-/l with a 90 % confidence interval from 9.0 to 24.8 CN-/l.

Due to the large amount of acute toxicity data with broad taxonomic coverage, the low statistical uncertainty of the HC5, and the indication for several species that significant effects are unlikely at concentrations up to half the LC50 or EC50, it is suggested to reduce the assessment factor down to 5 to derive the MAC-QS.

Marine taxa were considered sufficiently represented in the SSD and so, no additional safety factor is considered necessary.

Thus, the $MAC\text{-}QS_{freshwater\ \&\ marine\ water}$ is proposed to be 15.8 µg/l / 5 = 3.2 µg/l.

For long-term exposure, 13 chronic NOECs or EC10 covering fish, crustaceans, molluscs, macrophytes and algae for 8 freshwater and 5 saltwater species were considered reliable for SSD calculation. Additional data available for insects, Echinoidea and Cnidaria did not indicate higher sensitivity of these taxa. Also the acute data set indicated that crustaceans, insects and molluscs likely include the

most sensitive invertebrates. Thus, the taxonomic coverage of the chronic data set was considered sufficient for QS derivation via the SSD approach.

The HC5 of the resulting chronic SSD was 2.0 µg CN^-/l with a 90% confidence interval of 0.9 to 3.3 µg CN^-/l.

In total, the quality of the data used is considered high, the data represent a sufficient diverse species assemblage with respect to taxonomy, trophic level and live form, the unspecific mode of action does not suggest that taxa not tested are more sensitive; and the statistical uncertainty on the HC5 is small. However, no model ecosystem or field data are available yet to compare field NOECs with the HC5. Therefore, an assessment factor of 2 is suggested for calculating the $AA\text{-}QS_{freshwater}$ from the HC5:

$$\mathbf{AA\text{-}QS_{freshwater} = 2.0\ \mu g\ CN/l\ /\ 2 = 1.00\ \mu g\ CN/l}$$

The data set used to calculate the chronic SSD includes marine algae, crustaceans, fish and molluscs. In addition, a short-term test on sublethal endpoints not included in the SSD indicated low sensitivity of Echinoidea larvae and the acute data set revealed very low sensitivity of a marine annelid species. Thus, it was considered appropriate to lower the standard additional assessment factor at least down to 5 to derive the $AA\text{-}QS_{saltwater}$:

$$\mathbf{AA\text{-}QS_{marine\ water} = 2.0\ \mu g\ CN/l\ /\ 2\ /\ 5 = 0.2\ \mu g\ CN/l}$$

Remaining uncertainty on the derived HC5 and QS could be reduced by monitoring data on cyanide concentrations and e.g. related macroinvertebrate community structure.

Natural occurrence of cyanides could result in natural background concentrations close to or above the proposed QS. In such cases the use of the added risk approach should be considered.

Author's signature

Schmallenberg, 31.10.2011

Dr. Udo Hommen

1 Introduction

In 2007 ECETOC published a report on the environmental risk of cyanides ECETOC (2007). Related to the aquatic environment it is concluded: ‘Cyanide is acutely very toxic to aquatic organisms. Probabilistic analysis of the large amount of available aquatic toxicity data led to the derivation of an acute tolerable concentration of 5 µg/l free cyanide and a long-term predicted no effect concentration (PNEC) of 1 µg/l free cyanide for the aquatic environment. This effect assessment was based on a review conducted by the Fraunhofer IME Hommen & Wellman P. (2003) of 261 literature references.

More recently, INERIS INERIS (2011) has prepared a dossier on Environmental Quality Standards (EQS) for free cyanides to be applied under the European Water Framework Directive (WFD, EC (2000)) proposed $MAC\text{-}QS_{freshwater\ \&\ marine\ water,\ eco}$ of 0.8 µg CN/l, $AA\text{-}QS_{freshwater\ eco}$ = 0.1 µg/l, and $AA\text{-}QS_{marine,\ eco}$ = 0.01 µg/l.[1] It is mentioned in the INERIS document that these EQS are preliminary because three tests on aquatic organisms have been commissioned by stakeholder associations of chemical and related industries which were not available for dossier preparation.

The aim of this paper is therefore to compare which data were used in the different reports, and to include the now available new GLP test data for *Lemna gibba* and *Chironomus riparius* for derivation of MAC-QS and AA-QS according to the now finalized guidance document to derive EQS under the WFD EC (2011)[2] .

MAC-QS = Maximum Acceptable Concentration Quality Standard, AA-QS = Annual Average Quality Standard

[2] For simplicity, this document is called WFD GD in this paper.

2 Material and methods

Available documents for this paper were the full data set used for the Fraunhofer review in 2003 as an Microsoft Excel file, the ECETOC report (ECETOC (2007), the reports of the new tests (Wenzel 2011, Simon 2011a & b), and the INERIS document INERIS (2011). In addition, the reviews of Sorokin & Atkinson (2007) and Gensemer et al. (2007) were used. The sponsor also provided the reports of three acute tests with *Cancer irroratus* which have been used to refine the US quality criteria Gensemer R.W. et al. (2007).

The re-evaluation started with the data listed in the ECETOC report by creating separate tables for short- and long-term data. From this data, reliable EC50 / LC50 and NOECs were selected to calculate SSD and HC5 values for short-term and long-term exposure.

In the next step, the data used in the INERIS report were added to the tables in order to highlight differences in the data selection in both reports and to check the SSDs calculated in the INERIS report.

In the third step, discrepancies between the ECETOC and the INERIS data sets were analysed and based on this it was decided which data should be used for the new SSD and HC5 calculation. The US report (Gensemer et al 2007) was screened for additional data. Finally, the new data for *Lemna gibba*, *Chironomus riparius* and *C. irroratus* were added to the data sets.

It has been assumed before, that there is no significant difference in sensitivity of freshwater and marine species ECETOC (2007), Sorokin & Atkinson (2007), INERIS (2011). For the acute and chronic datasets used here to derive the QS, the saltwater and freshwater data were tested on significant difference of variance and means. Because no significant differences were found, SSDs were only calculated for the combined data sets following the WFD GD (see below).

SSDs and HC5 were calculated using the ETX program van Vlaardingen P et al. (2004) which is based on the paper of Aldenberg & Jaworska (2000) where a log-normal distribution of the toxicity values is assumed. The program calculates the median HC5 (the 5% centile of the SSD) and the 90 % confidence interval of the HC5. Three tests are used to check the log-normal distribution of the data in ETX (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises test).

According to the number and taxonomy of the species in a SSD the WFD GD requires:

'*The output from an SSD-based QS is considered reliable if the database contains preferably more than 15, but at least 10 NOECs/EC10s, from different species covering at least 8 taxonomic groups. For estimating a* $QS_{fw,\ eco}$, *the following taxa would normally need to be represented:*

- ***Fish** (species frequently tested include salmonids, minnows, bluegill sunfish, channel catfish, etc.)*
- *A second family in the phylum Chordata (e.g. fish, amphibian, etc.)*
- *A **crustacean** (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.)*
- *An **insect** (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)*
- *A family in a phylum other than Arthropoda or Chordata (e.g**. Rotifera, Annelida, Mollusca**, etc.)*
- *A family in any order of **insect or any phylum not already represented***
- ***Algae***
- ***Higher plants**.'*[3]

For derivation of the QS an additional **assessment factor** should be applied to the HC5. For chronic data (thus, AA-QS derivation) the WFD GD suggests:

'An AF of 5 is used by default but may be reduced where evidence removes residual uncertainty. The exact value of the AF depends on an evaluation of the uncertainties around the derivation of the HC5. As a minimum, the following points have to be considered when determining the size of the assessment factor (ECHA, 2008):

[3] All highlighting by bold letters in citations of the WFD GD is done for this paper here and not in the original WFD GG.

- *the **overall quality of the database and the endpoints covered**, e.g., if all the data are generated from "true" chronic studies (e.g., covering all sensitive life stages);*
- *the **diversity and representativity of the taxonomic groups** covered by the database, and the extent to which differences in the life forms, feeding strategies and trophic levels of the organisms are represented;*
- ***knowledge on presumed mode of action** of the chemical (covering also long-term exposure). Details on justification could be referenced from structurally similar substances with established mode of action;*
- ***statistical uncertainties around the HC5 estimate**, e.g., reflected in the goodness of fit or the size of confidence interval around the 5th percentile, and consideration of different levels of confidence (e.g. by a comparison between the median estimate of the HC5 with the lower estimate (90% confidence interval) of the HC5);*
- ***comparisons between field and mesocosm studies**, where available, and the HC5 and mesocosm/field studies to evaluate the level of agreement between laboratory and field evidence.'*

With respect to the QS for marine waters the WFD guidance accounts for the higher diversity of marine ecosystems by applying an additional safety factor of 10:

*'If a combined dataset is used, the AF of 1-5 applied to the HC5 estimated from the SSD should only be applied for coastal and territorial waters if the data set used to establish the SSD comprises long-term NOECs or EC10s for at least 2 additional typically marine taxonomic groups, other than fish, crustaceans and algae. **When there are no additional marine taxonomic groups in the dataset, an AF of 10 is applied in addition to the AF of 1-5** to deal with residual uncertainty.'*

With respect to the MAC-QS derivation using the SSD approach, the WFD guidance refers to the recommendations for AA-QS derivation. However, the HC5 from an acute SSD is based on LC50 or EC50 values and thus 50 % effect concentrations (instead of NOEC or EC10 used in a chronic SSD). This means that at the HC5 of an acute SSD for 5% of species at least 50 % effect (mortality) is predicted. Because the WFD intends to avoid any effects as far as possible an additional extrapolation to acceptable acute effects is conducted. Therefore the default assessment factor for an acute HC5 is 10. This factor can be reduced for example based on the ratio of acute LC/EC50 to acute NOEC or LC/EC10 (‘*This AF should normally be 10, unless other lines of evidence (e.g. acute EC50:acute EC10 (or NOEC) ratios are narrow, or criteria presented in Section 2.9) suggest that a higher or lower one is appropriate*’ WFD GD).

All concentrations in this report are related to the free cyanide ion.

3 Derivation of MAC-EQS for cyanides

3.1 ECETOC data set

In the ECETOC report EC50 or LC50 data for 68 species are listed for the assessment of short-term effects. EC50 data for one algae species and the macrophyte *Myriophyllum* were only available from long-term tests over 10 and 32 days, respectively. However, these can be considered to be conservative for shorter-exposure periods. Therefore, the requirements for taxonomic coverage are fulfilled (because fish, Crustacea, insects, Turbellaria, Oligochaeta, Rotatoria, algae and macrophyte data are available).

Using all the 68 species data, the HC5 is 20 µg/l but the resulting distribution is not normal according to the goodness of fit tests included in the ETX program. As shown in Figure 1, this is mainly based on data in the tails of the SSD, i.e. the one exceptional low EC50 of 5 µg/l for *Cancer irroratus* Brix KV et al. (2011); Brix KV et al. (2000) and the taxa with EC50 > 3000 µg/l (*Myriophyllum* and invertebrates other than Arthropoda).

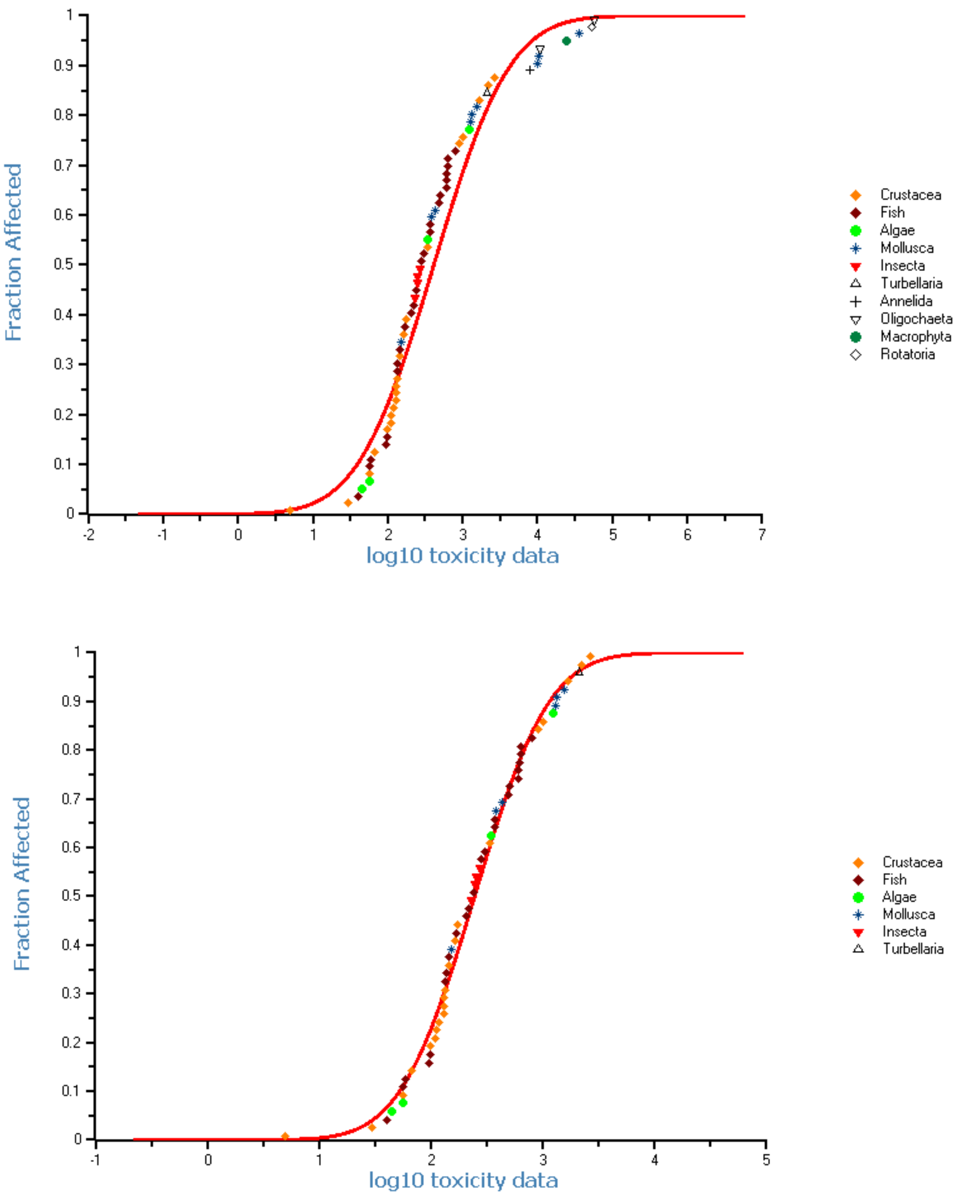

Figure 1: SSD for all the 68 (top) and only the 60 lowest (bottom) acute species data (EC50 or LC50) listed in the ECETOC report.

Ignoring the 8 least sensitive taxa (but keeping the *C. irroratus* value), the fit is much better and the hypothesis of normal distribution is fulfilled, which demonstrates that inclusion of very insensitive taxa (or unreliable high values) lead to lower HC5 values.

The value for *C. irroratus* is discussed and refined in section 3.3.

The resulting **HC5 is 34 µg/l** with a 90 % confidence interval of 21 to 48 µg/l (n=60).

3.2 INERIS data set

A large amount of the data from the ECETOC acute data set was not considered acceptable by INERIS (44 from 68). For the 21 species listed in the INERIS table, in most cases the ECETOC values were used (see table 2 in the appendix). For a few species the values listed by INERIS were slightly lower than in the ECETOC data set (for the two algae 43 instead of 45 and 55 instead of 57 µg/l. In 5 cases INERIS used the minimum value listed for a species where ECETOC used the mean. However, for *D. pulex* INERIS as ECETOC used the mean from 11 values. In two cases, neither the mean nor the minimum of the values per species was used (*Cancer productus* and *Lepomis macrochirus*). Two species (*Gasterosteus aculeatus* and *Chlamys asperrimus*) were considered by INERIS but not in the ECETOC dataset. Reasons for relevant discrepancies will be analysed in the next section.

However, the SSD shown in the INERIS dossier for acute effects includes 25 data points. The stickleback (*Gasterosteus aculeatus*) is listed twice, but *Perca fluviatilis, Pimephalis promelas* and *Pseudopleuronectes americanus* are in the SSD but not listed in the data table. Therefore, for the calculation here, the minimum values from the corresponding ECETOC values for these species were used as conservative estimations of the values likely used by INERIS. On the 5 % level, all tests for normality reject the Null-hypothesis.

In total, the INERIS data set lacks insects and macrophytes but was considered acceptable for SSD calculation because the crustaceans were considered to be the most sensitive taxon and to be present with several species in the data set. For *Cancer irroratus* only the data from Brix KV et al. (2000) but not the data reported in Gensemer R.W. et al. (2007) were used yet.

INERIS reports a HC5 of 8.14 µg/l while the recalculation revealed a slightly lower **HC5 of 7.62 µg/l**.

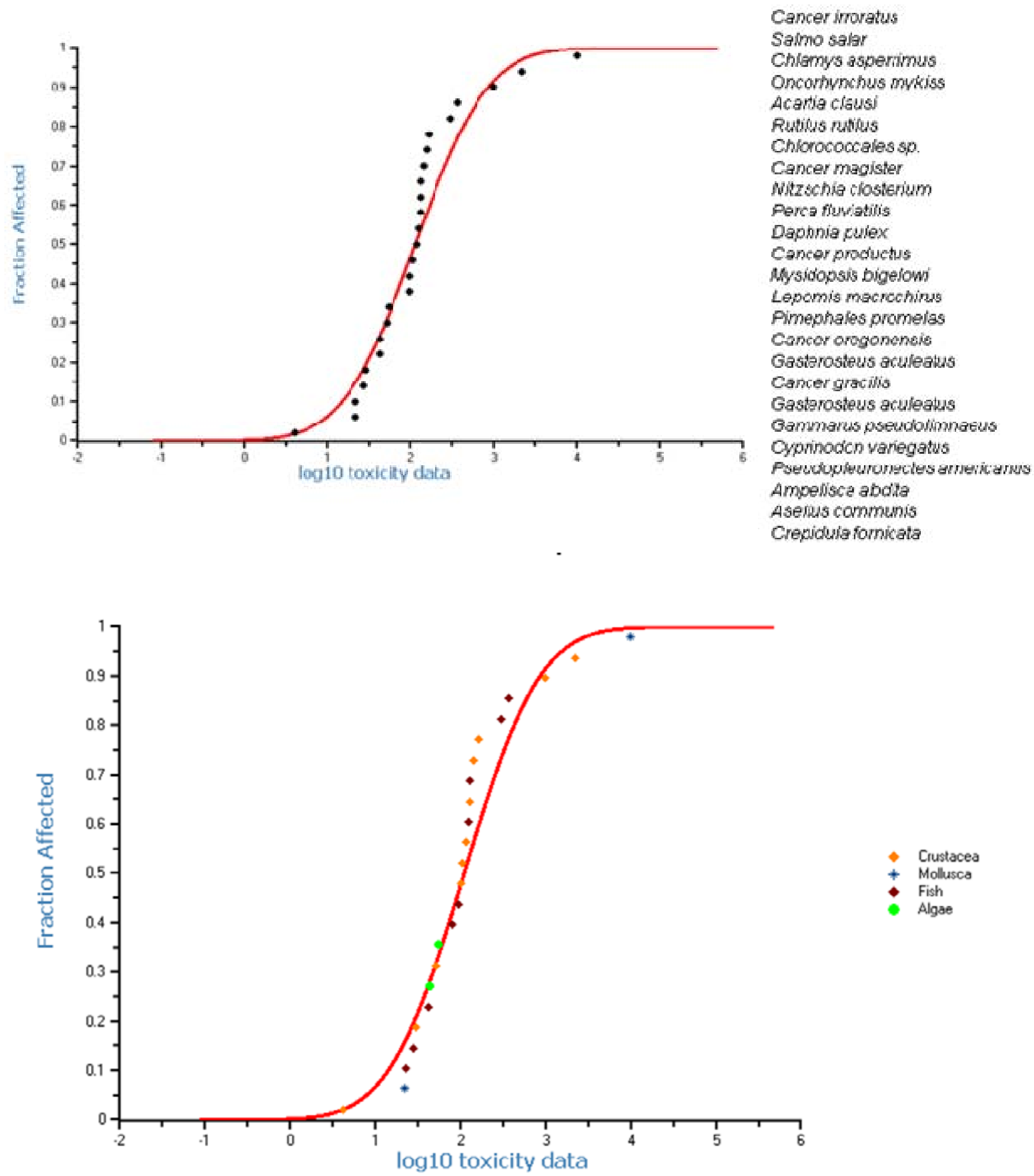

Figure 2: SSD for the acute data set in the INERIS report. Top: SSD as shown in INERIS report. Note that the species are listed with the most sensitive at the top.
Bottom: SSD recalculated from the data listed in the INERIS report supplemented by the minimum EC50 or LC50 values from the ECETOC data set for the 3 fish species without values listed by INERIS.

3.3 WERF report

The report by the Water Environment Research Foundation (WERF, Gensemer et al. 2007) has included several new data meeting US EPA guidance for Ambient Water Quality Criteria (compared to the former derivation of US quality criteria form 1984).

An analysis of the data uses in the WERF report revealed that it contains some data not used in the ECETOC or INERIS report yet, i.e. tests with amphibian larvae (3 species), 3 GLP tests with the rock crab *Cancer irroratus* and a test with the marine copepod *Acartia tonsa*.

Especially the three tests with *C. irroratus* are of importance because in the former analyses by ECETOC and INERIS this species showed clearly the lowest EC50 based on data provided by Brix KV et al. (2000). However, this EC50 of approximately 5 µg/l was by one order of magnitude lower than the EC50 for four other *Cancer* species and it fits also not well to the species sensitivity distribution (see above). The three GLP studies conducted by North Western Aquatic Sciences, Newport, OR, USA, used in the WERF report revealed EC50 of 70.9, 44.2 and 70.4 µg/ which result together with the data by Brix in a geometric mean of 22 µg/l for this species used for the refinement of the US water quality criteria (Gensemer et al. 2007).

The species mean acute values (SMAV) of Table 1a) and 1b) in the WERF report are listed in Tab. 2 in the appendix. Note that these means were calculated after evaluating the individual studies and thus, have undergone a quality check following US EPA guidance. Plant and algae data were not used for SMAV calculation in the WERF Report.

The SSD resulting for the combined freshwater and marine SMAV listed in the WERF report are shown in the following diagram. The HC5 for this animal data set is 25.44 µg/l with a 90 % confidence interval from 14.34 to 39.87 µg/l. However, the fit to the normal distribution is bad – all tests reject the Null-hypothesis of normal distribution of the data set. The two lowest EC50 are from the marine copepod *Arcatia clausi* and the rock crab *Cancer irroratus*.

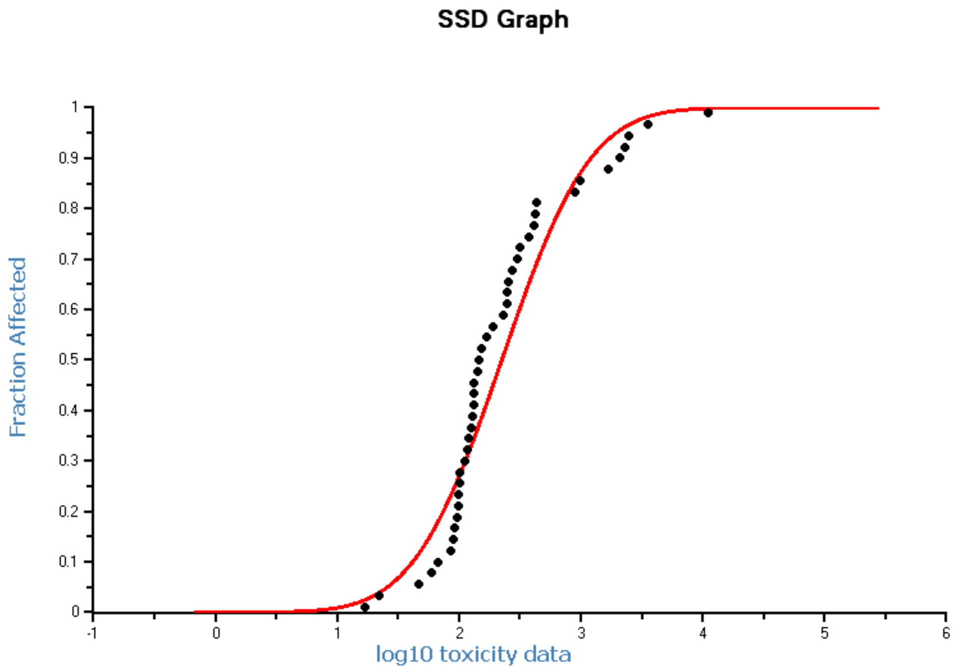

Figure 3: SSD for the Species Mean Acute Values listed in the WERF-Report (Gensemer et al. 2007). 'Lager- than- values' were not used.

3.4 Refined acute SSD

Based on the study evaluations and endpoint selections in the three reports (ECETOC, INERIS, WERF) and considering the two new GLP tests following OECD test guidelines (inhibition growth test with *Lemna gibba* (Wenzel 2011) and for the acute test with the midge *Chironomus riparius,* (Simon 2011a) a refined SSD was calculated.

If more than one reliable test was available per species, the (geometric) mean was considered to be most adequate. Thus, if for the INERIS report minima of separate tests were used, the geometric mean used in the ECETOC or the WERF report was used. If data evaluation has resulted in different means in the reports, the lower mean was used. Data only considered reliable (or evaluated) in one of the three reports were not used for the SSD here, except there were specific reasons (e.g. tests were not available during preparation of a report). Explanations of the final selection of data are given for each species in Table 2 of the appendix.

Thus, no detailed review on single studies has been conducted for this position paper but the data selection was based on the expert evaluations done independently before and discrepancies were handled in a conservative way if possible.

Finally data were selected for 43 species covering 8 major taxonomic groups (algae, macrophytes, annelids, molluscs, crustaceans, insects, amphibians and fish).

For the log-transformed LC50 or EC50 no statistical difference was found between the freshwater and the saltwater data set[4]. The normality test and the test on equal variance were passed and the t-test revealed no significant difference of the means (P=0.849, see annex 2), Thus, the combined data sets were used to create the SSD.

The hypothesis of normality was accepted by all tests on the 5 % level and the SSD revealed a **HC5 of 15.9 µg/l** (90 % CI = 9.0 - 24.8 µg/l, n=43).

There is no clear relationship of sensitivity to taxonomic group. If several species of a group have been tested the show large differences in their EC50 (i.e. Crustacea, insects, Mollusca, fish). The two new Fraunhofer tests provided the lowest EC50 values (11.6, and 12.4 µg/l for *Lemna gibba* and *Chironomus riparius*) followed by the marine crustaceans *Acartia clausi* and *Cancer irroratus* and the marine mollusc *Chlamys asperrrimus*.

[4] The data for the stickleback were excluded from this analysis because the species can be found in both habitats.

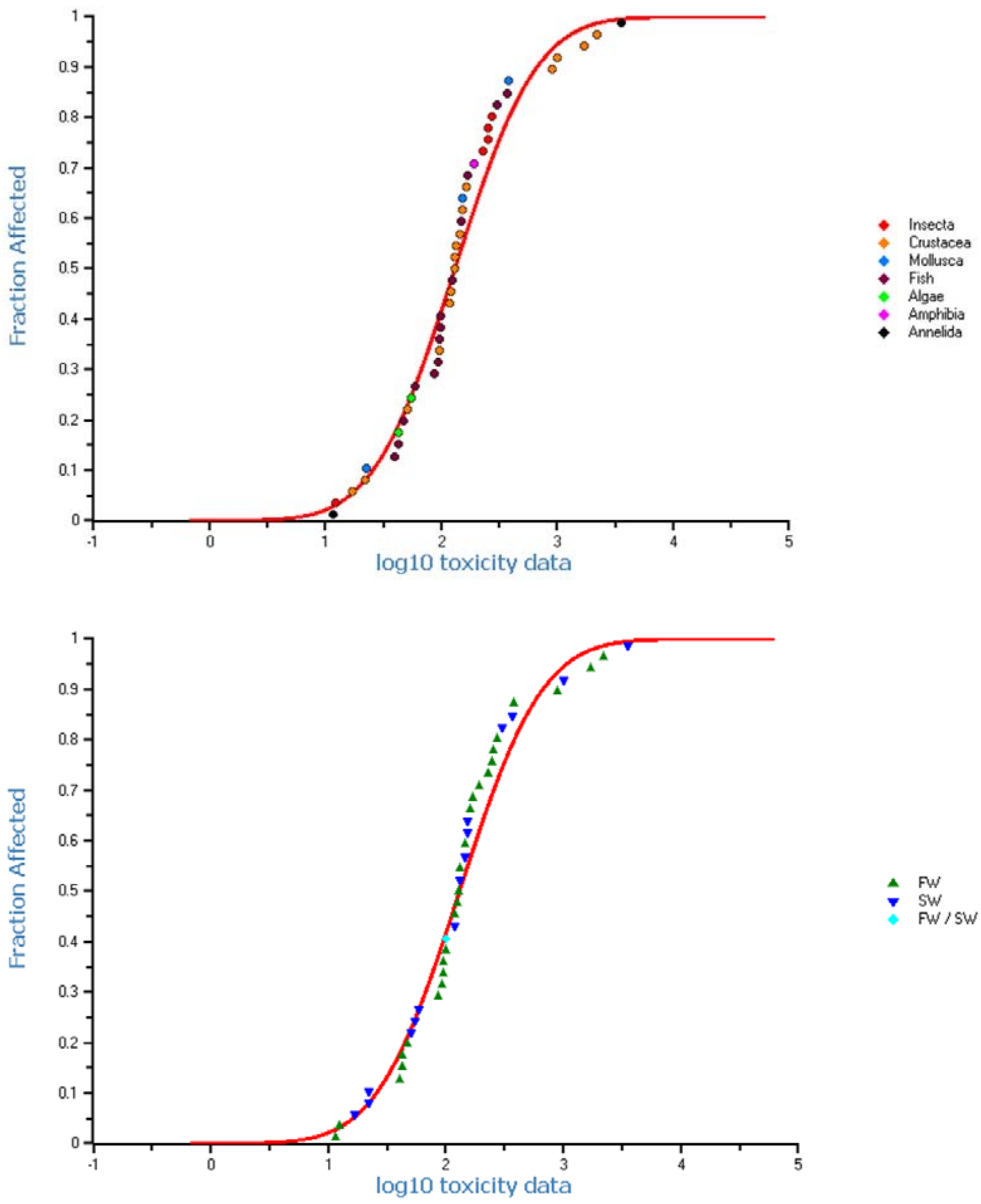

Figure 4: SSD for the refined acute toxicity data set based on the ECETOC, INERIS and WERFT reports and the new *Lemna* and *Chironomus* tests (HC5 =15.9 µg CN/l (90 % CI = 9.0 .- 24.8 µg CN/l, n=43). Top: taxonomic groups indicated, bottom: indication of water type FW = freshwater, SW = saltwater (the three-spiked stickleback was assumed to be able to live in salt- and freshwater).

3.5 Proposal of MAC-QS

The large data set fulfils the requirements regarding taxonomic coverage of the tested species as listed in the WFD GD. The standard assessment factor of 10 would reveal a $MAC\text{-}QS_{freshwater}$ of 15.9 µg/l / 10 = 1.6 µg/l. However, according to the WFD GD a lower assessment factor can be used '*where evidence removes residual uncertainty*'. In the following the criteria listed in the WFD guidance are considered:

- Overall quality of the database and the endpoints covered:
  The quality of the data set is considered to be high because the data (except the new GLP tests for *Chironomus* and *Lemna*) have checked in independent reviews.
- Diversity and representativity of the taxonomic groups covered by the database: The SSD covers 8 major groups and in most of these groups, e.g. fish, insects, crustaceans and molluscs also different families and orders have been tested. fish, insects, crustaceans, molluscs, algae and fish. Available data for Rotatoria, and Turbellaria indicated low sensitivity of these groups not considered in the SSD. The data set includes also 15 of marine (or brackish water) species covering algae, Annelida, crustaceans, molluscs and fish. However, the data set lacks representatives from specific saltwater taxonomic groups (e.g. Echinodermata).
- Knowledge on presumed mode of action of the chemical:
  The general mode of action of cyanides as well as the ranking of the major taxa in SSD do not indicate that a single taxonomic group is significantly more sensitive than others. The eight species with the lowest EC50 or LC50 cover 6 major taxonomic groups (algae, macrophytes, molluscs crustaceans, insects and fish). Thus, there is no indication that the SSD lacks any sensitive taxa due to a specific mode of action.
- Statistical uncertainties around the HC5
  The fit of the SSD to the data is good and the hypothesis of normal distribution is accepted by all three tests conducted. The 95^{th} centile of the HC5 (9.0 µg/l) is close to the median HC5 of 15.8 µg/l (by a factor < 2). Thus, uncertainty around the HC5 is considered low due to the large sample size and the goodness of fit.

- Comparisons between field and mesocosm studies:
  Model ecosystem or field data on short-tern effects of cyanides seem not to be available yet.
- Ratio of LC50 or EC50 to acute NOECs or EC10
  According to data for *Lepomis, Onkorhynchus, Daphnia* and *Mysidopsis* listed in the ECETOC report, the acute dose response curve is steep with LC50/IC10 ratios between 1.1 to 5.4. For the new *Lemna* test the EC50 / EC10 ratio was 11.6 / 3.7 = 3.1. However, for the *Chironomus* test the ratio is 12.4 / 0.9 = 13.7. It seems that in most cases a factor of 5 is sufficient to extrapolate form an LC50 to a concentration with up to 10 % effect. For the *Chironomus*-test it should be considered that the NOEC was significantly larger than the EC10, i.e. 7.0 µg/l. Thus, a statistically significant increase in mortality was not observed in this test at a concentration of half the EC50.

In total, due to the large amount of acute toxicity data with broad taxonomic coverage, the low statistical uncertainty of the HC5, and the indication for several species that at concentrations up to half the LC50 or EC50 significant effects are unlikely, it is suggested to reduce the standard assessment factor down to 5 to derive the MAC-QS.

Thus, the MAC-QS$_{freshwater}$ is proposed to be 15.8 µg/l / 5 = 3.2 µg/l.

For marine water, the WFD GD suggests an additional assessment factor of 10 applied to the HC5 of a combined fresh and saltwater data set if not at least two marine taxonomic groups, other than fish, crustaceans and algae, have been tested. In this case, data from marine fish, crustaceans, and algae, but also marine molluscs and Annelida have been used to derive the SSD. Thus, an additional safety factor is not required. INERIS (2011) also applied no additional assessment factor in their analysis of acute data '*considering the lack of significant differences between freshwater and marine species and the fact that marine species are fairly represented in the dataset*'.

Thus, the MAC-QS$_{marine\ water}$ is proposed to be 3.2 µg/l.

4 Derivation of AA-QS

4.1 ECETOC data set

In the ECETOC report chronic data for 16 species (11 freshwater, 5 marine) were considered reliable to calculated a SSD and resulted in a **HC5 of 1.1 µg/l** (90% confidence interval from 0.4 to 2.3 µg/l). Using only the freshwater data the HC5 was slightly higher (1.4 µg/l with 90% confidence interval 0.4 - 2.8 µg/l). For both SSDs the 'goodness of fit' tests revealed no significant deviations from the normal distribution. However, the slope of the SSD seems to be affected by the lowest and the highest NOEC. The lowest NOEC of 1 µg/l was estimated from the LOEC of 5 µg/l reported for a long-term test with the bluegill *Lepomis macrochirus.*

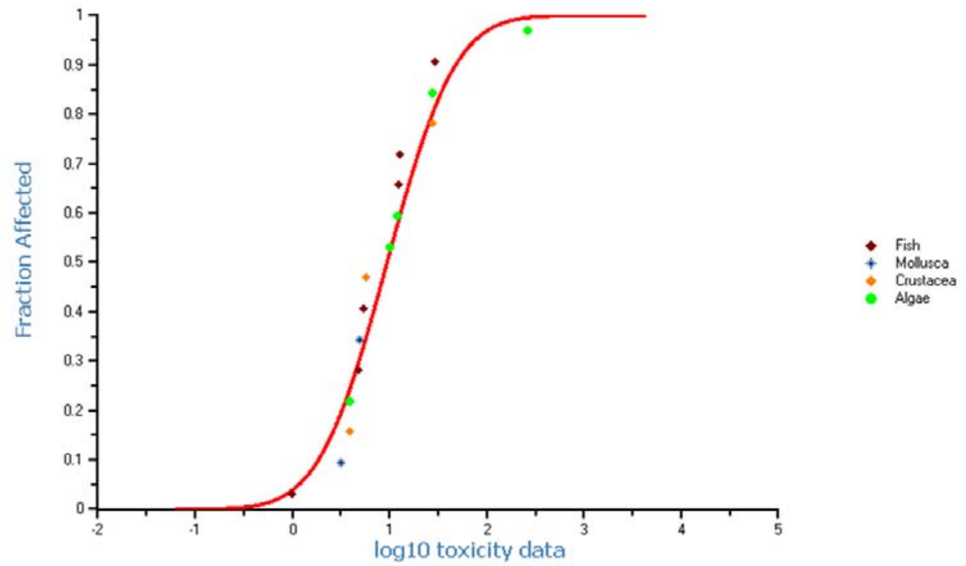

Figure 5: Chronic SSD from the ECETOC report (HC5 = 1.1 µg CN/l (90% c.il = 0.4 to 2.3 µg/l, n= 16))

4.2 INERIS data set

INERIS considered 10 chronic data as reliable and reports an HC5 of 2.8 µg/l. However, recalculation of the SSD resulted in a **HC5 of 1.3** (90 % c. i. 0.4 -2.5 µg/l) which is very close to the results obtained in the ECETOC report. The INERIS SSD diagram indicates that for *Lepomis* a LOEC of 5 µg/l was used instead of the LOEC / 5 as reported. It is also unclear why not two data points at log(NOEC) of 0.6 are shown because for two species a NOEC of 3.9 µg/l is reported.

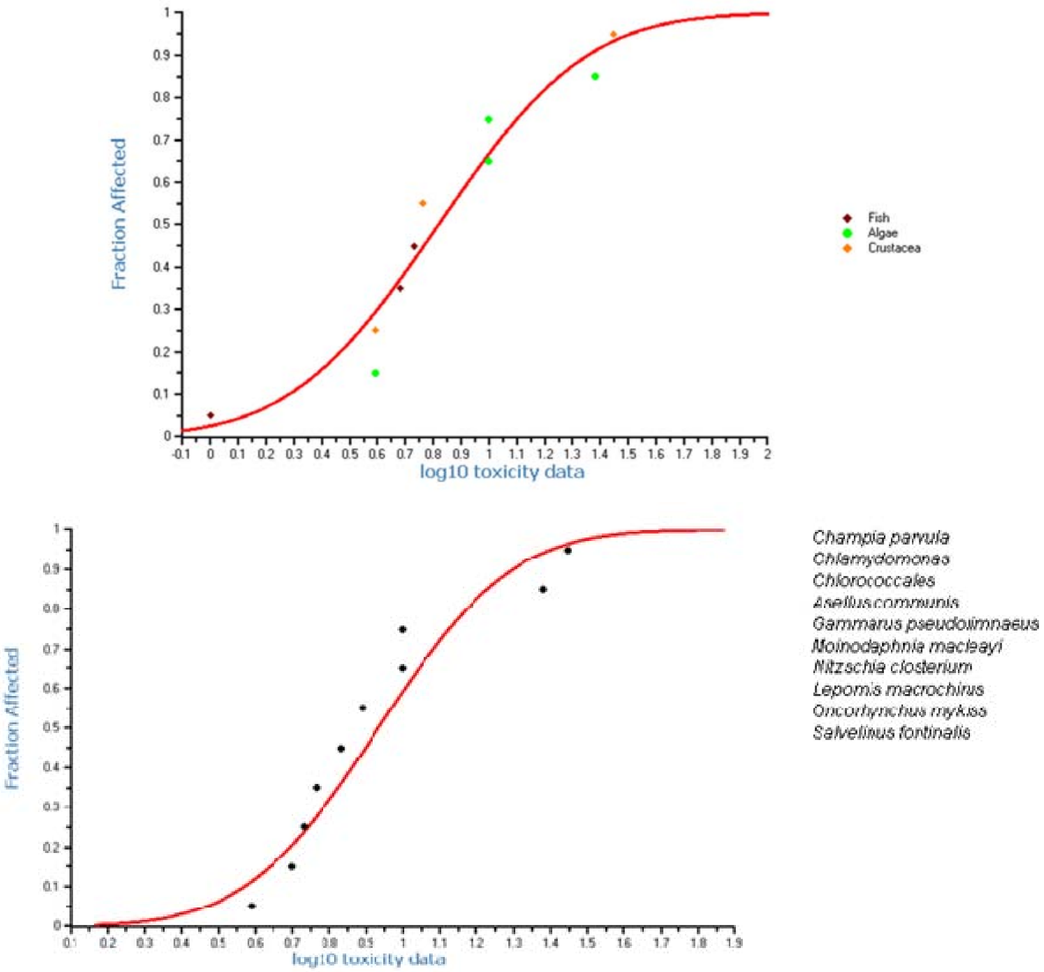

Figure 6: Chronic SSD based on the INERIS data set. INERIS SSD (top) and recalculated SSD based on the data listed in the INERIS report (HC5 of 1.3 µg CN/l (90 % c. i.) 0.4 -2.5 µg/l)

4.3 WERF data set

Chronic values and EC20 values are only listed for seven (animal) species in Table 2a and 2b of the WERF report (Gensemer et al. (2007): *Asellus communis,* *Gammarus pseudolimenaeus, Lepomis macrochirus, Pimephales promelas, Salvelinus fontinalis, Americamysis bahia, Cyprinodon variegatus*). The EC20 are listed in Table 3 of the appendix here.

The use of these data for chronic assessment in the WERF report was considered as additional indication of the reliability of the tests and thus, a support to use the data in the chronic SSD. However, because in Europe the EC10 and not the EC20 is usually preferred to be used in chronic SSDs, EC10 were extrapolated (see below).

For plants and algae (listed in Table 4a and 4b of the WERF report no comparable endpoints are listed (e.g. LOEC, EC50, LC50).

4.4 Refined chronic SSD

Considering the data used by ECETOC (2007), INERIS (2011) and WERF (2007) as well as the new available data (for *Lemna gibba* and *Chironomus riparius,* Wenzel 2011, Simon 2011b) a refined chronic SSD was calculated.

The *Lemna* test revealed a NOEC of 3.7 µg/l based on mean measured concentrations.

No effects on emergence and development rates were found in the chronic *Chironomus* test up to the highest test concentration. Thus, following the test guideline the NOEC was reported to be >= 5 µg CN/l based on nominal concentrations. However, due to fast disappearance of cyanide in the water NaCN solution was applied daily. Initial measured concentrations were within 80 – 120 % of the nominal concentrations but a dissipation studies indicates the over 24 h concentrations likely decreased down to approximately 10% of initial concentrations. Thus, for the risk assessment here, the geometric mean of 100 and 10 % of the nominal concentration is considered a better description of the exposure over the 28 d study duration and a NOEC of >= 1.6 µg CN/l for *Chironomus riparius* was considered.

For the other species, tests were considered to be valid if they have been considered reliable in the ECETOC and the INERIS or the WERF report.

Regarding the type of data which can be used to derive the EQS, the recommendations of the WFD GD were followed (see annex 3 for details), e.g. not to use NOEC >= highest test concentration for EQS derivation and not to use NOEC < lowest test concentration, except that a EC10 can be derived; otherwise the NOEC can be estimated as ECx/2 for (10 < x <= 20) or MATC / sqrt(2),).

The data set and the finally used values are listed in Annex 1 including the explanation why data were included or excluded from SSD derivation. The most relevant decisions are listed below:

- Results of Bringmann & Kuhn (1978) for the algae *Microcystis* and *Scendesmus* were not used for the SSD because they were related to a 3 % deviation from control and no further information on the dose-response curve was available. However, the data provide evidence that cyanophyta (represented by *Microcystis*) are likely not more sensitive than other algae. Finally, for algae only the NOECs for Chlorococcales and *Nitzschia* were used which were also considered as reliable by INERIS (2007).
- Based on the evaluation in the WERF report, data for the saltwater shrimp *Americamysis bahia* were considered for the chronic SSD. This species is a common test species in the US with guidelines available. Thus, it was assumed that, if the test was assessed to meet US EPA quality criteria, it could also be considered valid here. The NOEC for reproduction was equal to or larger than the highest test concentration of 43 µg/l. However, at this concentration the effect was already 24 % while there was no effect up to 20 µg/l. Thus, the EC10 would between 20 and 43 µg/l and estimated as the geometric mean of these two concentrations.
- Also *Cyprinodon variegatus* test is a common saltwater test species used in the US and the test was accepted by WERF (2007). The NOEC was reported to be 29 µg/l which was the lowest concentration tested.

- *Pimephale promelas* test results were used in the ECETOC report but not by INERIS. *P. promelas* (fathead minnow) is a frequently uses freshwater test fish, especially in the USA. In the WERF report, an EC20 of 6 µg/l for inhibition of egg production is calculated which is below the NOEC (which was related to 47 % effect). To be conservative and also consistent with the use of other EC20, a refined NOEC for the SSD was calculated by dividing the EC20 by 2.
- *For Lepomis macrochirus the* NOEC < 5 µg/l is based on no reproduction in all treatments except an early spawning in the highest treatment. Because this 100 % effect was given at the lowest test concentration and thus, no dose response relation is given, an EC10 cannot be extrapolated. The WERF evaluation was based on survival until day 57 only. According to the WFD GD, the test was not used for EQS derivation.
- In the *Chironomus* test no effects were found up to the highest test concentration. Thus, the test was not included in the SSD calculation.
- For the ECETOC report two marine mollusc data were considered (5 µg/l for *Chlamys asperrima* and 3.2 µg/l for *Mytilus galloprovincialis*). They were not considered as chronic studies in the INERIS report and were also not considered in the WERF report (because for US water quality criteria only native species are considered). Both studies are short-term studies because they only lasted 48 h. However, they assessed sublethal endpoints (growth and development) of a potentially sensitive life stage (larvae). Both tests gave very similar results and indicate potential high sensitivity of marine mollusc larvae. Thus, it was decided to include these data in the chronic SSD.

This procedure resulted in data for 13 species data for calculation a chronic SSD for cyanide which is considered to be a sufficient number according to the WFD GD. With respect to the required coverage of 8 different taxonomic groups the following should be considered:

1. Fish (here 4 fish species are included in the SSD)

2. A second family in the phylum Chordata (here three families of fish are represented)
3. A crustacean (four species)
4. An insect (one species, Chironomus riparius, was tested, but not included in the SSD because the highest test concentration showed no significant effect.)
5. A family in a phylum other than Arthropoda or Chordata (two marine mollusc species are included in the SSD)
6. A family in any order of insect or any phylum not already represented (see comment below)
7. Algae (two species)
8. Higher plants (one species)

Critical is requirement 6 because not chronic data for e.g. Annelida, rotifers or Echinoidea could be included in the SSD. However, in the ECETOC review a NOEC > 200 µg/l is reported for *Hydra viridissima* from the phylum Cnidaria. However >=x values were not included in the SSD. In the ECETOC data set also a short-term test on development of *Anthocidaris crassispina* larvae (Echinoidea) is listed with an NOEC of 125 µg/l. Despite this is an sublethal endpoint the test duration (12h) was considered too short to represent chronic exposure of the life stage. From the acute data set, indication of very low sensitivity of Annelida, Rotatoria, or Turbellaria compared to other was found was found. Thus, there is some evidence that other invertebrate taxa are likely not significantly more sensitive than e.g. insects, crustaceans or molluscs. In addition, it should also be considered that algae are represented in this data set by Cyanobacteria, green algae and diatoms which are taxonomically very different.

Thus, in total it is concluded that the data base is sufficiently broad and that the SSD likely covers the sensitivity of different taxa, i.e. invertebrates.

Comparison of the freshwater (n=8) and saltwater (n=5) values used in the SSD revealed no statistical difference (see annex 2). Thus, construction of the SSD based on the pooled data set is justified.

The hypothesis of normal distribution of the data was accepted on the 5% error level by all three tests conducted. The **HC5 of the SSD is 2.0 µg CN/l** with a 90 % confidence interval from 0.9 – 3.3 µg CN/l.

The SSD plots (Figure 7) do not indicate any clear pattern related to taxonomy or type of water.

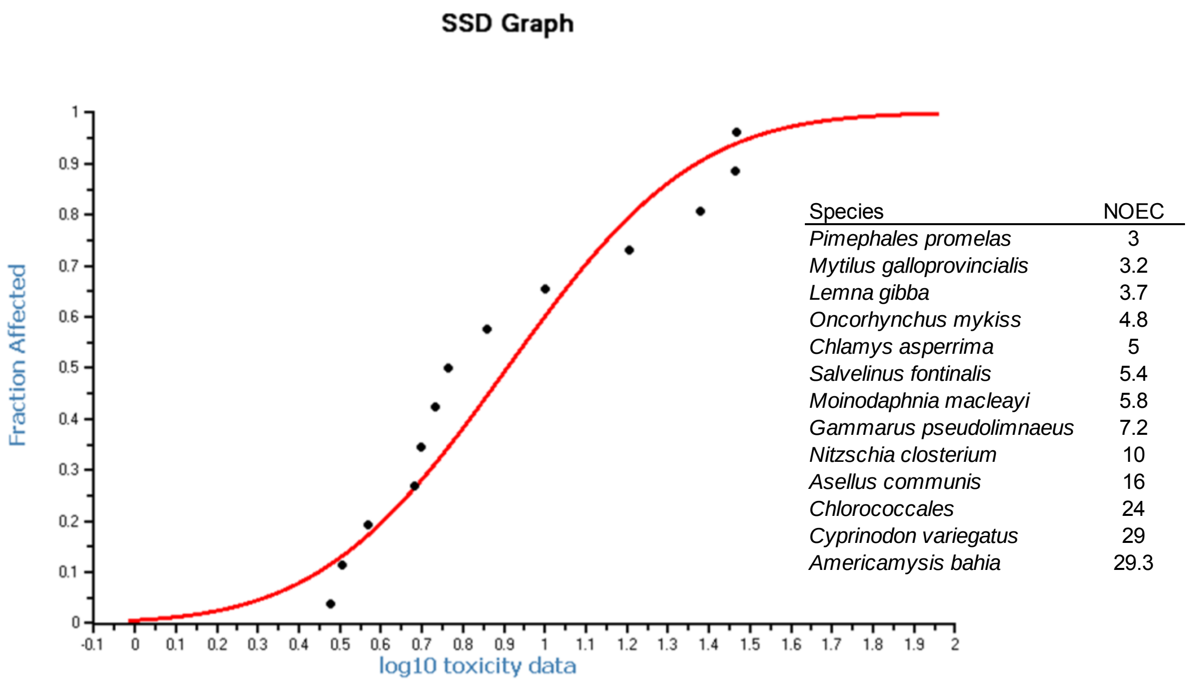

Figure 7: Refined SSD based on chronic NOEC or EC10 values (HC5 = 2.0 µg CN/l, 90 % c.i, = 0.9 – 3.3 µg CN/l, n = 13).

4.5 Proposed AA-QS

The usually recommended assessment factor to be applied to a chronic SSD fulfilling the requirements of representing different taxa is 5. The criteria of the WFD GD to reduce this assessment factor are considered as follows:

- Overall quality of the database and the endpoints covered: The data have been reviewed by independent institutes (except the two new GLP studies of the Fraunhofer IME following OECD test guidelines) and thus, are considered to be reliable. Note, that several additional chronic test data are available which might be less reliable but do not indicate any higher sensitivity of other species.
- Diversity and representativeness of the taxonomic groups covered by the database:
  As outlined above the SSD data set covers six of the taxonomic groups listed in the WFD GD while total data set covers also additional groups (Insecta, Bacteria, Cnidaria and Echinoidea) with no indication of higher sensitivity than the taxa considered in the SSD. A GLP test was conducted to fill the data gap for insects, but because no effects were found on emergence and survival of Chironomus larvae at the highest test concentrations (5 µg/l nominal, 1.6 µg/l mean measured) the test was not included in the SSD.
  Also the much broader acute data set does not indicate that e.g. the sensitivity of invertebrate is not covered by insects, crustaceans and molluscs. The species included in the chronic SSD cover several different life forms and four trophic levels. Thus, the representativity of the available chronic test data for the diversity of taxa is assumed to be good.
- Knowledge on presumed mode of action of the chemical (covering also long-term exposure): Details on justification could be referenced from structurally similar substances with established mode of action: See also the section on acute toxicity. The distribution of taxa in the SSD does not indicate that a specific group is more sensitive than others which indicates no specific mode of action.
- Statistical uncertainties around the HC5 estimate:
  The statistical tests did not detect significant deviation from normal distribution. However, the fitted curve is conservative compared to the data given in the lower left tail of the distribution. The empirical HC5 is expected to be around 3 µg/l. The fitted HC5 of 2 µg/l is below the lowest NOEC used in the SSD and close to the minimum NOEC obtained for Chironomus riparius (>= 1.6 µg/l). Considering

sampling uncertainty, the 90 % confidence interval of the HC5 indicates that with 95 % probability the true HC5 is above 0.9 µg/l. Thus, the statistical uncertainty on the median HC5 of 2.0 µg/l is relatively low.

- Comparisons between field and mesocosm studies:
  No mesocosm studies seem to be available for cyanides. However, if monitoring data on cyanide concentrations and e.g. related macroinvertebrate communities are available these could be used to test the uncertainty of the HC5 and the reliability of the proposed EQS.

In summary, the quality of the data used is considered high; the data represent a sufficient diverse species assemblage with respect to taxonomy, trophic level and live form; the unspecific mode of action does not suggest that taxa not tested are more sensitive; and the statistical uncertainty on the HC5 is small. However, no model ecosystem or field data are available to compare field NOECs with the HC5. Therefore, an assessment factor of 2 is suggested for calculating the $AA\text{-}QS_{freshwater}$ from the HC5:

$$\mathbf{AA\text{-}QS_{freshwater} = 2.0\ \mu g\ CN/l\ /\ 2 = 1.0\ \mu g\ CN/l}$$

For the marine AA-QS the WFD GD recommends to us an additional safety factor of 10 when there are not at least 2 additional typically marine taxonomic groups, other than fish, crustaceans and algae. When only one additional marine taxonomic group is presented, the additional safety factor can be reduced to 5. In the data set used to calculate the chronic SSD here, marine algae, crustaceans, fish and, in addition, molluscs are represented. A short-term test on sublethal endpoints not included in the SSD indicated low sensitivity of Echinoidea larvae and the acute data set revealed very low sensitivity of a marine annelid species. Thus, an additional safety factor of 5 seems to be sufficient to derive the $AA\text{-}QS_{saltwater}$:

$$\mathbf{AA\text{-}QS_{saltwater} = 2.0\ \mu g\ CN/l\ /\ 2\ /\ 5 = 0.2\ \mu g\ CN/l}$$

5 Discussion

In the following table, the criteria derived in the different reports are summarized.

Table 1: Comparison of proposed water quality standards for cyanides [µg CN^-/l] in different reports

	short-term			**long-term**		
Data set	**acute HC5**	**Freshwater MAC-QS**	**Marine MAC-QS**	**chronic HC5**	**Freshwater AA-QS**	**Marine AA-QS**
ECETOC 2007	34 (n=60)	5	5	1.1 (n=16)	1	1
US EPA 2007 (based on GMAV	Not used	23	20	Not used	4.8	4.1
INERIS 2011	8 (n=24)	0.8	0.8	NOEC= 1	0.1	0.01
This paper	**15.8 (n=43)**	**3.2**	**3.2**	**2.0 (n=13)**	**1.0**	**0.2**

Thus, inclusion of the new data and the refinement of the single older LC50 for *C. irrogatus* resulted in a significant refinement of the proposed MAC-QS compared to the INERIS proposal. The proposed MAC-QS here is close to the one derived in the ECETOX report and around a factor of 4 lower than the proposed water quality criteria for the US (based on a different data set and using a different approach).

Considering the new GLP tests conducted recently by Fraunhofer and following the recommendations of the WFD GD the AA-QS proposed here confirms the freshwater AA-QS of the ECETOC report. It is by a factor of 5 lower than the US criterion which might be a result of the use of different data (e.g. the US use only data from species living in the US, algae and plants seem not to be included in the calculation) and approaches (e.g. use of geometric means per genus). The proposed AA-QS for freshwater on 1 µg/l is by a factor of 10 above the one proposed by INERIS, which was based on the assessment factor method.

In contrast to the ECETOC report, based on an evaluation in 2003, additional uncertainty for extrapolation to marine environments was considered by an additional safety factor of 5. This was done in accordance to the recent WFD GD under the assumption that the available NOEC for two marine molluscs can be considered as chronic tests because they are based on sublethal endpoints of potentially sensitive life stages. Therefore the default additional safety factor was reduced from 10 to 5. Following the WFD GD, chronic data for an additional typically marine taxon should be available to reduce this factor further. Despite that such data are not available yet further reduction could be considered based on the acute data set for cyanides and the non-specific mode of action of cyanides.

For both, freshwater and marine AA-QS remaining uncertainty in the QS derivation could be reduced further based on chemical and biological monitoring data, e.g. a analysis of the relation between cyanide concentrations and invertebrate community structure.

For the use of the proposed QS it should be considered that cyanide can also be a product of natural processes, e.g. algae metabolism and destruction of plant material. The WFD GD states that '*the size of the AF should not normally result in a QS that is below the natural background level unless an 'added risk' approach to compliance assessment is to be adopted (Section 3.5). However, if uncertainties in the extrapolation are largely responsible for the QS being below the background level (e.g. an AF > 50 is required), this must be highlighted in the datasheet as a key uncertainty for the policymaker*'. In the case of cyanides, the assessment factors used in this paper are not very high (<=10). Thus, for comparison with measured concentrations it could be necessary to use the added risk approach to take natural background concentrations into account.

It should also be noted that LOQ for determination of cyanide in monitoring programs might be above proposed QS-values.

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Annex 1 – Data set tables

Table 2: Acute data sets, LC50 or EC50 [µg/l]

Group	Species	Water type	ECETOC EC or LC50	INERIS EC or LC50	WERF SMAV	New SSD EC or LC50	Selection for the new SSD based on values considered in at least 2 of the 3 reports (except new studies)
Algae	*Ankistrodesmus falcatus*	FW	1250.0				only used by ECETOC, not very sensitive
Algae	Chlorococcales sp.	FW	45.0	43.0		43.0	use of the lowest of ECETOC - INERIS value
Algae	*Navicula seminulum*	FW	344.0				only used by ECETOC, not very sensitive
Algae	*Nitzschia closterium*	SW	57.0	55.0		55.0	use of the lowest of ECETOC - INERIS value
Amphibia	*Rana berlandieri*	FW			410.0		only used by WERF, not very sensitive
Amphibia	*Rana pipiens*	FW			192.0	192.0	most sensitive frog to represent amphibians but not overemphasize them
Amphibia	*Xenopus laevies*	FW			254.3		only used by WERF, not very sensitive
Annelida	*Aeolosoma headleyi*	FW	56470.0				not used, very insensitve
Annelida	*Lumbriculus variegatus*	FW	11000.0		11000.0		not used, very insensitve
Annelida	*Dinophilus gyrociliatus*	SW	8074.0		3560.0	3560.0	use of WERF value as conservative EC50 for Annelida
Crustacea	*Asellus aquaticus*	FW	2680.0				only used by ECETOC, other Asellus values available, not very sensitive
Crustacea	*Asellus communis*	FW	2210.0	2210.0	2326.0	2210.0	lowest values used
Crustacea	*Asellus intermedius*	FW	1700.0		1700.0	1700.0	only used by ECETOC, other Asellus values available, not very sensitive
Crustacea	*Cyclops viridis*	FW	130.0		130.0	130.0	representative for freshwater copepoda
Crustacea	*Daphnia magna*	FW	174.0		120.0	120.0	use of the lowest of ECETOC - WERF mean
Crustacea	*Daphnia pulex*	FW	100.0	100.0	96.0	96.0	lowest value used
Crustacea	*Daphnia spp.*	FW	132.0		132.0		excluded because potential overlap with D. magna and D. pulex
Crustacea	*Diaptomus sp.*	FW	133.0		133.0	133.0	used by ECETOC and WERF
Crustacea	*Gammarus fasciatus*	FW	900.0		900.0	900.0	used by ECETOC and WERF
Crustacea	*Gammarus pseudolimnaeus*	FW	163.0	163.0	167.0	163.0	lowest mean value used
Crustacea	*Acartia clausi*	SW	30.0	30.0	17.0	17.0	lowest mean value used
Crustacea	*Americamysis bahia*	SW	113.0		113.0		used by ECETOC and WERF
Crustacea	*Ampelisca abdita*	SW	996.0	996.0	996.0	996.0	used in all three reports
Crustacea	*Cancer gracilis*	SW	144.0	144.0	144.0	144.0	used in all three reports
Crustacea	*Cancer irroratus*	SW	5.0	4.2	22.0	22.0	considering 3 GLP tests not included by ECETOC & INERIS
Crustacea	*Cancer magister*	SW	68.0	51.0	68.0	51.0	mean used (INERIS used species min)
Crustacea	*Cancer oregonensis*	SW	131.0	131.0	131.0	131.0	used in all three reports
Crustacea	*Cancer productus*	SW	338.0	107.0	153.0	153.0	mean used (INERIS used species min according WERF data)
Crustacea	*Leptomysis mediterranea*	SW	57.0				only used by ECETOC
Crustacea	*Mysidopsis bigelowi*	SW	118.0	118.0	118.4	118.0	used in all three reports
Crustacea	*Penaeus monodon*	SW	110.0				only used by ECETOC

Table 2 continued

Group	Species	Water type	ECETOC EC or LC50	INERIS EC or LC50	WERF SMAV	New SSD EC or LC50	Selection for the new SSD based on values considered in at least 2 of the 3 reports (except new studies)
Fish	*Carassius auratus*	FW	169.0		318.0	169.0	lowest value used
Fish	*Catla catla*	FW	633.0				only used by ECETOC
Fish	*Cirrhinus mrigala*	FW	511.0				only used by ECETOC
Fish	*Danio rerio*	FW	490.0				only used by ECETOC
Fish	*Ictalurus punctatus*	FW	242.0				only used by ECETOC
Fish	*Labeo bata*	FW	639.0				only used by ECETOC
Fish	*Labeo calbasu*	FW	615.0				only used by ECETOC
Fish	*Labeo rohita*	FW	600.0				only used by ECETOC
Fish	*Lepomis macrochirus*	FW	144.0	126.0	99.0	99.0	lowest mean value used
Fish	*Leuciscus idus melanotus*	FW	209.0				only used by ECETOC
Fish	*Microperus salmoides*	FW			102.0		only used by WERF
Fish	*Notemigonus crysoleucas*	FW	369.0				only used by WERF
Fish	*Oncorhynchus mykiss*	FW	57.0	28.0	47.0	47.0	used of lowest mean, INERIS used min value
Fish	*Perca flavescens*	FW	134.0		93.0	93.0	lowest mean used
Fish	*Perca fluviatilis*	FW	96.0	96.0		96.0	not listed in INERIS table but in the SSD plot
Fish	*Pimephales promelas*	FW	137.0	79.0	125.0	125.0	used of lower mean, INERIS used min value
Fish	*Poecilia reticulata*	FW	800.0		147.0	147.0	use of lower value
Fish	*Rhinichthys atratulatus*	FW	220.0				only used by ECETOC
Fish	*Rutilus rutilus*	FW		42.4		42.4	Only used by INERIS, but WERF would use only american species
Fish	*Salmo salar*	FW	40.0	23.0	90.0	40.0	Lowest mean used, INERIS used single
Fish	*Salvelinus fontinalis*	FW	99.0		86.0	86.0	lowest mean used
Fish	*Tilapia mossambica*	FW	600.0				only used by ECETOC, relatively insenstive
Fish	*Gasterosteus aculeatus*	FW / SW		131.0	99.0	99.0	new data in WERF report used
Fish	*Boleophthalmus boddarti*	SW	279.0				only used by ECETOC
Fish	*Cyprinodon variegatus*	SW	300.0	300.0	300.0	300.0	used by all
Fish	*Menidia menidia*	SW	59.0		59.0	59.0	used by ECETOC and WERF
Fish	*Pomoxis nigromaculatus*	SW			102.0		old test, used only by WERF
Fish	*Pseudopleuronectes america*	SW	372.0	372.0	372.0	372.0	used by all

Table 2 continued

Group	Species	Water type	ECETOC EC or LC50	INERIS EC or LC50	WERF SMAV	New SSD EC or LC50	Selection for the new SSD based on values considered in at least 2 of the 3 reports (except new studies)
Insecta	*Chironomus riparius*	FW				12.4	New GLP test (Fraunhofer)
Insecta	*Corixa sp.*	FW	250.0		250.0	250.0	checked by ECETOC and WERF
Insecta	*Dytiscus sp.*	FW	252.0		251.0	251.0	checked by ECETOC and WERF
Insecta	*Nepa sp.*	FW	274.0		273.0	273.0	checked by ECETOC and WERF
Insecta	*Pteronarcys dorsota*	FW			426.0		only used by WERF
Insecta	*Ranatra sp.*	FW	230.0		230.0	230.0	checked by ECETOC and WERF
Insecta	*Tanytarsus dissimilis*	FW			2490.0		only used by WERF, not very sensitive
Macrophyta	*Lemna gibba*	FW				11.6	New GLP test (Fraunhofer)
Macrophyta	*Myriophyllum spicatum*	FW	24319.0				not used, insensitive
Mollusca	*Anculosa sp.*	FW	10402.0				not used, insensitive
Mollusca	*Helisoma trivolvis*	FW			>50 000		not used, insensitive
Mollusca	*Lymnaea luteola*	FW	1326.0				used only by ECETOC, not very sensitive
Mollusca	*Physa heterostropha*	FW	383.0		432.0	383.0	lowest mean used
Mollusca	*Pila globosa*	FW	1293.0				used only by ECETOC, not very sensitive
Mollusca	*Viviparus bengalensis*	FW	1550.0				used only by ECETOC, not very sensitive
Mollusca	*Chlamys asperrimus*	SW		22.4		22.4	not used by WERF because species not resident in North America
Mollusca	*Crepidula fornicata*	SW	10000.0	10000.0	>10 000		larger than value, very insensitive species
Mollusca	*Mytilus edulis*	SW	36000.0				very insensitve
Mollusca	*Mytilus galloprovincialis*	SW	154.0			154.0	used only by ECETOC, but sensitive mollusc
Rotatoria	*Philodina acuticornis*	FW	54000.0				used only by ECETOC, very insenstive, however represents tribe
Turbellaria	*Dugesia tigrina*	FW	2100.0		2100.0		used only by ECETOC and relativel insentive,however, represents tribe

Table 3: Chronic data set, NOECs or EC10 [µg/l]

Group	Scientific Name	Medium	ECETOC	INERIS	WERF EC20	End-point	test design	CN conc.	GLP report available	conc. [µg CN/L] (not used in SSD)	Comment	Author	Year
Algae	*Anabaena flosaquae*	FW	>= 700			NOEC	static	nom	no	(> 700)	not checked further here, very insenstive, increased growth observed	Shehata, S.A., S.I. Abo-Eela, and G.H. Ali	1988
Algae	*Ankistrodesmus falcatus*	SW	265.0			EC10	static	nom	no	(265)	Not checked further because insensitive algae, no informatio on actual exposure over 10 d.	Tscheu-Schluter, M.	1983
Algae	*Champia parvula*	SW	3.9	3.9		NOEC, EC10	static	measured	no	(< 11)	Significant effect on growth on female gametophytes at lowest test concentration (11 µg/L). MATC reported to be in the range of 0 to 11 µg/L. Effect at 11 µg/L > 20 %. No data available to fit dose response. Thus, extrapolation to NOEC or EC10 is not possible.	Steele, R.L., and G.B. Thursby	1983
Algae	*Chlamydomonas*	FW	>= 10	10.0		NOEC	static	?	no	(>= 10)	>= x value and no clear dose-response	Cairns, J., A.L.Jr Buikema, A.G. Heath, and B.C. Parker	1978
Algae	*Chlorococcales*	FW	24.0	24.0		EC10	static	?	no	**24.0**	EC10 for oxygen production is not a common endpoint, no species identified. However, used by INERIS Paper not available	Krebs, F.	1991
Algae	*Microcystis aeruginosa*	FW	28.0			EC3	static	nom	no	(28)	Graphical determination of EC3 = 70 µg/L KCN with no further information on dose-response curve. Should not be used according to GD.	Bringmann, G., and R. Kuhn	1978
Algae	*Nitzschia closterium*	SW	10.0	10.0		NOEC	static	measured	no	**10.0**	NOEC < 10, LOEC = 10 µg/L, according diagram the effect at 10 µg/L seems to be (slightly) < 10%. Values for dose response fitting are not available. ECx (x<10) should not be used according to GD. However, because the NOEC is < 10 µg/L while the EC10 is > 10 µg/L, the use of 10 µg/L for the SSD was considered reliable.	Pablo, F., J.L. Stauber, and R.T. Buckney	1997
Algae	*Phytoplankton*	FW	< 100						no	(<100)	taxon unclear, not checked further	Shehata, S.A., S.I. Abo-Eela, and G.H. Ali	1988
Algae	*Scenedesmus quadricauda*	FW	12.0			EC3	static	nom	no	(12)	Graphical determination of EC3 = 30 µg/L KCN with no further information on dose-response curve. Should not be used according to GD.	Bringmann, G., and R. Kuhn	1978
Bacteria	*Bacteria*	FW	9.2						no	(9.2)	Studies with bacteria (e.g. growth tests) are regarded as short-term tests.' (WFD TG 2008)	Shkodich PE	1966
Cnidaria	*Hydra viridissima*	FW	>= 200						no	(>=200)	not checked further and not used because >= x value	Rippon, G.D., C.A. Le Gras, R.V. Hyne, and P.J. Cusbert	1992
Crustacea	*Americamysis bahia*	SW	> 30.4		43.0	EC10	flow - through	measured	no	**29.3**	Test accepted also by WERF (Gestermer et al. 2007), NOEC >= highest test concentration (43 µg/)L with effect of 24 %. WERF lists 43 as EC20 and 67.7 as MATC. Up to 20 µg/L reproduction was not inhibited at all (higher than in controls). Thus, the EC10 would be between 20 and 43 µg/L and the geometric mean was used (29.3 µg/L).	Lussier, S.M., J.H. Gentile, and J. Walker	1985
Crustacea	*Asellus communis*	FW	27.9	27.9	32.0	EC10	?	measured	no	**16.0**	The tests are poorly described and variability between replicates was partly high. No statistically derived NOECs or EC10 are given, effect – no effect concentrations based on difference to mean of the two control replicats by more than 2 standard errors of mean. Gammarus value in competition exp. with Asellus not used because focus on intrinsic tox. However, the data were used in the WERF report where EC20 of 32 µg/L for Asellus and 14.5 µg/L for Gammarus were derived based on the data. Here EC10 were extrapolated from the EC20 by division by 2.	Oseid, D.M., and Jr.	1979
Crustacea	*Gammarus pseudolimnaeus*	FW	3.9	3.9	14.3	EC10	?	measured	no	**7.2**		Oseid, D.M., and Jr.	1979
Crustacea	*Moinodaphnia macleayi*	FW	5.8	5.8		?	?	measured	no	**5.8**	used by ECETOC and INERIS. Not checked further now (pdf not available).	Rippon, G.D., C.A. Le Gras, R.V. Hyne, and P.J. Cusbert	1992

Table 3 continued

Group	Scientific Name	Medium	ECETOC	INERIS	WERF EC20	End-point	test design	CN conc.	GLP report available	conc. [µg CN/L] (not used in SSD)	Comment	Author	Year
Echinoidea	*Anthocidaris crassispina*	SW	125.0			?	?	?	no	(125)	high value, short-term test (12 h) but sublethal endpoint (development), not checked further	Kobayashi, N.	1971
Fish	*Cyprinodon variegatus*	SW	29.0		29.0	NOEC	?	?	no	**29.0**	Test accepted also by WERF (Gensemer 2007), NOEC = lowest test concentration, related to 23 % effect, pdf not	Schimmel, S.C.	1981
Fish	*Gnathonemus petersi*	FW	< 5						no	(< 5)	irrelevant endpoint (electronic organ activity), not checked further	Lewis, J.W., A.N. Kay, and N.S. Hanna	1992
Fish	*Gnathonemus tamandua*	FW	< 5						no	(<5)	irrelevant endpoint (electronic organ activity), not checked further	Lewis, J.W., A.N. Kay, and N.S. Hanna	1992
Fish	*Jordanella floridae*	FW	12.6			NOEC			no	(< 63)	Effect on egg number at lowest test concentration > 20 %, reliable extrapolation to NOEC or EC10 not possible	Cheng, S.K., and S.M. Ruby	1981
Fish	*Lepomis macrochirus*	FW	1.0	1.0	5.6	NOEC	flow through	measured	no	(<5)	The NOEC < 5 in the ECETOC report was based on no reproduction in all treatments except an early spawning in the highest treatment. Because this 100 % effect was given at the lowest test concentration and thus, no dose response relation is given, an EC10 can not be extrapolated. The WERF evaluation was based on survival until day 57 only.	Kimball, G.L., L.L. SmithJr., and S.J. Broderius	1978
Fish	*Oncorhynchus mykiss*	FW	4.8	4.8		NOEC	flow through	measured	no	**4.8**	NOEC of 5 µg HCN/L based on growth rate at 6°C with appr. 10 % effect, lower sensitivity at higher temperature. However, effect on fat gain = 40 %.	Kovacs, T.G.	1979
Fish	*Pimephales promelas*	FW	12.3		6.0	EC10	flow trough	measured	no	**3.0**	Egg production per female significantly reduced at 19.6 µg HCN/L (NOEC = 12.85 µg HCN/L = 12.4 µg CN/L). However, the effect at the NOEC was 47 %. Gensemer et al. (2007) re-calculated an EC20 of 6 µg CN/L and used this for criteria derivation. Thus, an EC10 could be estimated to be 6 µg /L / 2.	Lind, D.T., L.L. SmithJr., and S.J. Broderius	1977
Fish	*Salvelinus fontinalis*	FW	5.4	5.4	7.7	NOEC	flow trough	measured	no	**5.4**	No statistical NOEC, however, egg production at 5.7 µg HCN/L above value of 1 of the 2 controls while at 11.2 42 % reduction compared to the lowest control value. Gensemer et al. (2007) calculated an EC20 of 7.7 µg/L.	Koenst, W.M., L.L. SmithJr., and S.J. Broderius	1977
Insects	*Chironomus riparius*	FW				NOEC	semi-static	measured	yes	(>= 1.6)	New GLP study, no significant effect up to highest test concentration; EC10 > 1.6 µg/L	Simon	2011
Macrophytes	*Lemna gibba*	FW				NOEC	semi-static	measured	yes	**3.7**	New GLP study, NOEC based on mean measured conc., close to lowest EC10	Wenzel	
Mollusca	*Chlamys asperrima*	SW	5.0			NOEC	static	measured	no	**5.0**	Short-term test (48 h) but focussing on sublethal endpoint - development of the embryo into a shelled, straight-hinged larva. At the NOEC, free CN decreased from 6.2 to 5.6 µg CN over the 48 h, thus, the nominal conc was used.At the NOEC sensitive life stage	Pablo, F., R.T. Buckney, and R.P. Lim	1997
Mollusca	*Mytilus edulis*	SW	< 18			NOEC	flow trough	?	no	(< 18)	14 day study, statistically significant inhibition of glycine uptake at 32 µg/L but effect on growth not tested. Inhibiton of growth at 18 µg/L > 50 %. because no data are tabled extrapolation to EC10 is not possible	Thompson, R. S.	1984
Mollusca	*Mytilus galloprovincialis*	SW	3.2			NOEC	flow through	measured	no	**3.2**	48 h test on sublethal endpoint - growth of primary shell of straight-hinge veliger larvae.	Pavicic J, Pihlar B.	1982

Annex 2 – Testing differences between freshwater and saltwater data

Table 4: Acute data base [µg/l] used to test on significant differences between freshwater and saltwater species

Freshwater LC50	Saltwater LC50	Log (Freshwaer LC50)	Log (Saltwater LC50)
11.6000	17.0000	1.0645	1.2304
12.4000	22.0000	1.0934	1.3424
40.0000	22.4000	1.6021	1.3502
42.4000	51.0000	1.6274	1.7076
43.0000	55.0000	1.6335	1.7404
47.0000	59.0000	1.6721	1.7709
86.0000	118.0000	1.9345	2.0719
93.0000	131.0000	1.9685	2.1173
96.0000	144.0000	1.9823	2.1584
96.0000	153.0000	1.9823	2.1847
99.0000	154.0000	1.9956	2.1875
120.0000	300.0000	2.0792	2.4771
125.0000	372.0000	2.0969	2.5705
130.0000	996.0000	2.1139	2.9983
133.0000	3560.0000	2.1239	3.5514
147.0000		2.1673	
163.0000		2.2122	
169.0000		2.2279	
192.0000		2.2833	
230.0000		2.3617	
250.0000		2.3979	
251.0000		2.3997	
273.0000		2.4362	
383.0000		2.5832	
900.0000		2.9542	
1700.0000		3.2304	
2210.0000		3.3444	

Report on statistical tests conducted with SigmaStat for Windows Version 3.5 (Systat Software 2006)

Normality Test: Passed (P = 0.107)
Equal Variance Test: Passed (P = 0.332)

Group Name	N	Missing	Mean	Std Dev	SEM
log10(col(1))	27	0	2.132	0.527	0.101
log10(col(2))	15	0	2.097	0.631	0.163

Difference 0.0349
t = 0.191 with 40 degrees of freedom. (P = 0.849)
95 percent confidence interval for difference of means: -0.333 to 0.403

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.849).
Power of performed test with alpha = 0.050: 0.050
The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Table 5: Chronic data base (NOECs or EC10 [µg/l]) used to test on significant differences between freshwater and saltwater species

Freshwater NOEC	Saltwater NOEC	Log (Freshwater NOEC)	Log (Saltwater NOEC)
3.0000	3.2000	0.4771	0.5051
3.7000	5.0000	0.5682	0.6990
4.8000	10.0000	0.6812	1.0000
5.4000	29.0000	0.7324	1.4624
5.8000	29.3000	0.7634	1.4669
7.2000		0.8573	
16.0000		1.2041	
24.0000		1.3802	

Report on statistical tests conducted with SigmaStat for Windows Version 3.5 (Systat Software 2006)

Normality Test: Passed (P = 0.398)
Equal Variance Test: Passed (P = 0.102)

Group Name	N	Missing	Mean	Std Dev	SEM
log10(col(6))	8	0	0.833	0.310	0.110
log10(col(7))	5	0	1.027	0.437	0.195

Difference -0.194
t = -0.940 with 11 degrees of freedom. (P = 0.367)
95 percent confidence interval for difference of means: -0.647 to 0.260

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.367).
Power of performed test with alpha = 0.050: 0.050
The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Annex 3 – Suggested use of summary statistics for EQS derivation following WFD GD

The following table and additional information is copied from the TECHNICAL GUIDANCE FOR DERIVING ENVIRONMENTAL QUALITY STANDARDS (Table 5, Final Version 8 after formatting, July 2011, p. 150, 151)

Table 6: Summary statistics derived from toxicity studies and their use in EQS derivation.

Test type	Criterion	Use in EQS derivation?	Action
acute test	EC10 or LC10	No a	▪ Tabulate value; may be valuable as additional information
acute test	EC50 or LC50	Yes	▪ Tabulate value
acute test	ECx or LCx	No	▪ Tabulate value; may be valuable as additional information
acute test	LOEC	No	▪ Omit if NOEC is also available from same experiment ▪ Else: tabulate value; may be valuable as additional information
acute test	MATC5	No	▪ Omit if NOEC is also available from same experiment ▪ Else: tabulate value; may be valuable as additional information
acute test	NOEC	No a	▪ Tabulate value; may be valuable as additional information
acute test	TLm	Yes	▪ Tabulate as LC50 b
Chronic test	EC10 or LC10	Yes	▪ Tabulate value
Chronic test	EC50 or LC50	No [a]	▪ Tabulate value; may be valuable as additional information
Chronic test	ECx (x < 10)	No	▪ Omit if NOEC is also available from same experiment ▪ If more than one ECx value is available, try to establish an EC10 from a reliable dose-response relationship ▪ Else: tabulate value; may be valuable as additional information
Chronic test	ECx (10 < x < 20)	Yes	▪ Omit if NOEC is also available from same experiment ▪ If more than one ECx value is available, try to establish an EC10 from a reliable dose-response relationship ▪ Tabulate value if the ECx is the lowest effect concentration measured. Calculate NOEC = ECx/2 (TGD guidance) and tabulate this NOEC [c]
Chronic test	ECx (x ≥ 20)	No	▪ Tabulate value; may be valuable as additional information ▪ If more than one ECx value is available, try to establish an EC10 from a reliable dose-response relationship

[5] The MATC is the geometric mean of NOEC and LOEC.

Test type	Criterion	Use in EQS derivation?	Action
Chronic test	LOEC	No	▪ Omit if NOEC is also available from same experiment ▪ Else: (i) if percentage effect is known, see ECx in this table for further guidance ▪ Else: (ii) if percentage effect is unknown: tabulate value; may be valuable as additional information
Chronic test	MATC - single value, no further information	Yes	▪ Omit if NOEC is also available from same experiment ▪ Else, if no further information is available, calculate NOEC = MATC/√2 (TGD guidance) and tabulate this NOEC [d]
Chronic test	MATC - reported as a range	Yes	▪ Omit if NOEC is also available from same experiment ▪ Else, if no further information is available, tabulate the lowest value of the range as NOEC [e]
Chronic test	MATC – spacing factor is given [f]	Yes	▪ Omit if NOEC is also available from same experiment ▪ Else, if no further information is available, calculate NOEC = MATC/√(spacing factor)[f] and tabulate this NOEC [g]
Chronic test	NOEC	Yes	▪ Omit LOEC if it is also available from same experiment

a) For toxicity tests with algae and *Lemna* sp., both the EC50 and the EC10 or NOEC are used in the EQS derivation, if available.
b) A footnote should be added to the toxicity data table stating that the TLm is used as LC50.
c) A footnote should be added to the toxicity data table stating that the NOEC is calculated as ECx/2.
d) A footnote should be added to the toxicity data table stating that the NOEC is calculated as MATC/√2.
e) A footnote should be added to the toxicity data table stating that the lowest value of the MATC range is taken as NOEC.
f) The spacing factor is the factor of difference between two subsequent testing concentrations employed in the toxicity experiment.
g) A footnote should be added to the toxicity data table stating that the NOEC is calculated as MATC/√(spacing factor).

Additional information to Table 5 (in the WFD GD, here table 6)

The most common summary statistics are either EC50 or LC50 in the case of acute toxicity tests and EC10 or NOEC in the case of a chronic test. Other examples of summary statistics that are regularly found in the literature are LOEC, MATC (the geometric mean of NOEC and LOEC) and TLm, which is equivalent to the LC50. If a NOEC is reported, the LOEC can be omitted. If the endpoint presented is an ECx or LOEC value with an effect between 10 and 20% (i.e., x = 10-20), then a NOEC can be derived according to the TGD, by dividing the ECx by a factor of 2. In such a case, the NOEC can be presented in the toxicity data table, with a note that this value is estimated from an ECx value.

In a strict sense, calculating NOEC as ECx/2, according to the TGD, is only allowed for ECx values with an effect smaller than 20%. However, EC20 values are often presented in the literature. If there is no other information on the dose-response relationship (e.g. a companion EC50, which enables the calculation of an EC10), the EC20 divided by 2 can be considered as NOEC as well, accompanied by a footnote in the table with selected toxicity data (see Section 0).

The information on dose–response relationship should be used as much as possible. If it is possible to derive EC50 and EC10 values from a range of tabulated or graphically presented ECx values, these derived endpoints can be included in the toxicity data table as well, accompanied by a footnote stating the method of derivation.

p. 143 of the WFD GD (version 8 after formatting):

“If the highest concentration in a toxicity test is not high enough to determine the NOEC or L(E)C50, the result of that study should be tabulated as NOEC ≥ or L(E)C50 >, followed by the value of the highest test concentration. The test result should be reported in the toxicity data tables. The result itself cannot be used in calculations of EQSs. However, it is valuable information that a species from this taxon (or trophic level) has been tested and that it was not sensitive to the toxicant at a known concentration. It may therefore have a useful supporting role. For example: when NOEC values for algae, Daphnia and fish are found, of which one is a ‘NOEC ≥ ’ value, and this value is not the lowest effect concentration, an assessment factor (AF) of 10 may be applied, whereas the AF would have been 50 if the study had been rejected.

For similar reasons, the data from tests resulting in an effect at the lowest test concentration should be tabulated as NOEC < or L(E)C50 <, followed by the value of the lowest test concentration. Although these values cannot be used directly for the derivation of EQSs, useful information can be obtained from comparing the sensitivity of that species with the EQS. This comparison may permit an adjustment to the AF.”