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

Guidance for the derivation of environmental risk limits

Additional guidance for some aspects of aquatic ERLs

version 2.0

Colophon

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This investigation has been performed by order and for the account of Ministry of Infrastructure and the Environment, within the framework of the project 'Nationaal stoffenbeleid ZZS'.

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Version history

VERSION	DATE	CHANGE
1.0	2015	
2.0	2025	version history added complete revision, deleting overlap with European WFD guidance

Contents

1 Introduction

1.1 Notes to this version

The previous version of this guidance was published in 2015, building on an RIVM report from 2007 [1]. In 2018, the European technical guidance for derivation of environmental quality standards (EQS) under the Water Framework Directive [2] has been revised, including a larger part of the national methodology. As a result, most of the 2015-guidance has become obsolete and the present document reflects this change in scope.

1.2 Scope of this guidance

The present ERL-report only deals with those aspects that are not (fully) covered in the WFD-guidance and/or for which specific (national) guidance has been developed. This document provides additional guidance for the following subjects:

- collection and tabulation of aquatic ecotoxicity data
- selection and aggregation of data
- data for micro-organism
- trophic levels and taxonomic groups in relation to the choice of assessment factors
- Species Sensitivity Distributions (SSDs) in case of a specific mode of action
- evaluation and use of mesocosm studies
- collection and selection of aquatic bioaccumulation data
- derivation of the Serious Risk Concentration

1.3 Terminology: EQS instead of MPC

Since the WFD methodology differs from the former national MPC-derivation, and compliance check was also changed, it was decided for the current guidance document to adopt the terminology as used in the European priority substances directive and WFD-guidance. This means that the term EQS will be used throughout this document instead of the Maximum Permissible Concentration (MPC). However, the values that are derived based on this guidance have a status of scientific advisory values and will be effective as official standards only after approval by the responsible ministry. This status of the results should be made clear when publishing reports based on this guidance.

2 Additional guidance on derivation of ERLs for direct ecotoxicity

2.1 Collection and tabulation of aquatic laboratory toxicity data

For general guidance on collection and evaluation of ecotoxicity data, first consult the relevant ERL guidance. International guidelines exist for performing aquatic toxicity studies for many species. The most frequently used guidelines are summarised in Appendix A.1.3 of the WFD-guidance.

The ecotoxicity data are summarised in data tables, an example of which is presented in Table 1. Separate tables are prepared for acute and chronic studies for freshwater and marine species. The aim is to fill the table as complete as possible. Guidance on the parameters that are reported in the aquatic toxicity data tables is given in Appendix A.1.3 of the WFD-guidance.

2.2 Selection and aggregation of aquatic laboratory data

Where multiple data are available for the same species/endpoint that are obtained under comparable test conditions, individual toxicity data may be aggregated using the same principles as those in Chapter R.10 of the REACH Guidance [3]. This process is performed separately with toxicity data for freshwater species and marine species.

For non-standard test species, preference is given to endpoints that are applicable to related standard test species, such as emergence, growth and survival or biomass. If for a species only alternative endpoints are available, these may be used, although this has to be judged on a case-by-case basis.

If endpoints are available for different durations, preference is given to the endpoints from tests that followed the minimum test duration as specified in the guideline, e.g. at least 72 hours for algae, 48 hours for daphnids, 96 hours for fish. If for *D. magna* endpoints are available from 24- and 48-hours test, the latter is preferred for risk assessment even when it is higher than the 24-hours value, since a test duration of 48 hours is prescribed in the guideline. In principle, the test duration for daphnids is considered applicable to other invertebrates as well.

If there is a clear relationship between test results and abiotic conditions, results are selected that refer to conditions relevant for Dutch surface waters. Any deselection of data should be motivated. The aggregated data should be presented in a new table. The selected acute and chronic values are presented separately for each species, and a footnote is added to explain how the value is derived from the summary data tables.

Table 1 Example of a freshwater ecotoxicity data-table.

Legend to column headings	
Species properties	relevant characteristics of the test species, such as age, size, origin
A	test water analysed Y(es)/N(o)
Test type	S = static; R = renewal; F = flow through; c = closed
Purity	refers to purity of active substance or content of active substance in formulation; ag = analytical grade; tg = technical grade
Test water	am = artificial medium; dtw = dechlorinated tap water; dw = deionised/dechlorinated/distilled water; nw = natural water; rw = reconstituted water; rtw = reconstituted tap water; tw = tap water
T	temperature
Ri	Reliability index according to [4]. Valid studies (Ri 2 or higher) are considered for EQS-derivation, depending on relevance and considering notes on data treatment (section 1.3.4)

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	T [°C]	Hardness $CaCO_3$ [mg/L]	pH	Exp. time	Criterion	Endpoint	Value [µg/L]	Ri	Note	Ref.
Bacteria																
Vibrio fischeri	strain NRRL-B-11,177	Y	S	active	ag		15			30 min	EC50	bioluminescence	61900	2	1	[a]
Cyanobacteria																
Anabaena flos-aquae		Y	S	product A	200 g/L					96 h	EC50		32800	4	2	[b]
Algae																
Desmodesmus subspicatus		Y	S	active	ag		21			72 h	EC50	growth rate	389000	2	3	[c]
Scenedesmus subspicatus	10000 cells/mL	N	S	active	tg		23		8.2-9.1	72 h	EC50	biomass	> 10000	3	4	[d]
Crustacea																
Asellus aquaticus	field collected	N		product A	200 g/L	am	10			1 h	NOEC	respiration	100	3	5	
Ceriodaphnia dubia	< 24 h	Y	S	product B	42.8%	dw	25	80-100		48 h	LC50	mortality	2.07	2	6	
Insecta																
Aedes aegypti	4th instar	N	S	active	97.4	dw	25			72 h	LC50	mortality	84	3	7	
Baetis rhodani	larvae, field collected	N	S	active	ag	am	15	180	7.4	48 h	LC50	mortality	8.49	3	8	
Amphibia																
Rana limnocharis	1 month old	N	R	active	> 95%	dw	20			96 h	LC50	mortality	82000	3	9	
Rana N. Hallowell	1.5 months old	N	R	active	> 95%	dw	20			96 h	LC50	mortality	129000	3	10	
Pisces																
Danio rerio		Y	S	active	ag	nw	21	140	8.4	96 h	LC50	mortality	241000	2	11	
Danio rerio		Y	S	product C	200 g/L	nw	21	140	8.4	96 h	LC50	mortality	214000	2	12	

Notes
1 Marine species, but tested in distilled water. Stability of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively.
2 Solvent 1% DMSO, solvent control included; no analysis of test concentrations, but short exposure time
3 etc.

2.3 Data for micro-organisms

According to the WFD-guidance [2], data for bacteria representing a further taxonomic group may only be used if non-adapted pure cultures were tested. Furthermore, studies with bacteria (e.g., growth tests) are regarded as short-term tests. Consequently, the WFD-guidance states that unlike for algae, NOECs or EC10-values derived from bacterial studies may not be used in the derivation of the AA-EQS using assessment factors. EC50 values from bacterial tests may be used as additional acute data. The same principle applies to toxicity data using protozoans. Nevertheless, NOECs or EC10 values from bacterial studies are valuable and should be tabulated amongst the toxicity data because they are relevant as inputs in an SSD.

It is noted that there is no scientific reason to exclude such endpoints from the dataset, if reliable tests are comparable to an algae test in terms of duration and endpoint (i.e., 72 hours and specific growth rate). Depending on the mode of action, NOECs and EC10-values for bacteria and protozoans may be considered as additional evidence when deciding on the assessment factors for EQS-derivation, if obtained in a comparable way as those for algae.

The WFD-guidance does not make reference to fungi as a specific taxonomic group. Data on fungi are considered relevant for fungicide risk assessments and may become available in the (near) future. If growth tests with fungi are present, it is advised for the time being to treat the data similarly to algae, i.e., include the EC50 for the acute dataset and the NOEC in the chronic dataset.

2.4 Trophic levels or taxonomic groups

The quantity and type of data available determines the assessment factors used. The assessment schemes for derivation of the QS_{eco} and MAC_{eco} are presented in detail in the WFD-guidance [2]. The schemes have been developed for all types of chemicals, including those for which ecotoxicity data are scarce, and offer the possibility to derive a QS and MAC in case only acute data for algae, *Daphnia* and fish are available. The use of an AF of 10 on the lowest NOEC or EC10 is allowed if additional chronic NOECs (EC10) are present for three species from three trophic levels, provided that the species tested represent one of the more sensitive taxonomic groups. This is made clear in one of the footnotes to the table with assessment factors for fresh water in the WFD-guidance, which states (footnote d):

'…When examining the results of long-term toxicity studies, the $QS_{fw, eco}$ should be calculated from the lowest available long term result. Extrapolation to the ecosystem can be made with much greater confidence, and thus a reduction of the assessment factor to 10 is possible. This is only sufficient, however, *if the species tested can be considered to represent one of the more sensitive groups*. This would normally only be possible to determine if data were available on at least three species across three trophic levels.'

The link with trophic level that is made in the assessment schemes is complicating. Crustaceans and insects may belong to the same trophic

level, while depending on the mode-of-action large differences in sensitivity may exist between these taxonomic groups. Similarly, the primary producers algae and macrophytes may also show large differences in sensitivity. For the choice of the assessment factor, the availability of data for the potentially most sensitive taxonomic group is most important, rather than having three trophic levels [5].

Determining whether or not the potentially sensitive species group is included in the dataset may be difficult [6]. Given the fact that test results for the same species may easily differ by a factor of 10, the question is which difference between test results should be considered as indicative for a taxon-related difference in sensitivity. As a pragmatic approach, if the lowest test results per taxon differ by more than a factor of 3, this is considered as an indication that one taxon is more sensitive than another. If this sensitive taxon is not represented in the chronic dataset, a higher assessment factor should be applied. All additional relevant information that substantiates the choice of the assessment factor should be considered, including information from additional (non-standard) studies, read-across and QSAR-data [5]. Information from e.g. mesocosm studies may also point at sensitive taxa that are not adequately represented in the laboratory dataset. This may lead to a higher assessment factor than originally selected on the basis of the schemes alone. Guidance on the differentiation between taxonomic groups is given in the relevant ERL report.

2.5 Species Sensitivity Distributions

2.5.1 Data requirements

The WFD-guidance gives criteria for construction of a Species Sensitivity Distribution (SSD), which are in accordance with REACH guidance [3]. 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 data points, from different species covering at least the following eight taxonomic groups:

- Fish
- A second family in the phylum Chordata
- A crustacean
- An insect
- A family in a phylum other than Arthropoda or Chordata
- A family in any order of insect or any phylum not already represented
- Algae
- Higher plants

If freshwater and marine datasets cannot be pooled, the requirements should be met for each of the two datasets. However, some of the taxa mentioned above (e.g. insects, higher plants) are not (well) represented in marine environments and may be replaced by other taxa. Also in that case, the minimum number of taxa and data points should be met [2].

In some cases, where a large dataset is available, but one of the listed taxa is missing, it may be considered to use SSDs. In this case, using only the lowest endpoint with an assessment factor would mean that a

lot of valuable information is neglected. For plant protection products with a specific mode of action, additional data will most often focus on the potentially sensitive species groups. For insecticides, authorisation dossiers will most often not contain data on aquatic macrophytes, since only data for algae are required. Similarly, data on insects may not be included in the dataset for herbicides, since only crustaceans are required for authorisation. Moreover, because reduction of vertebrate testing is an important issue, authorisation dossiers may no longer include multiple fish studies in the future. Examples of justification of the use of SSDs for datasets that did not fully meet the requirements can be found in several RIVM-reports [7-11].

2.5.2 *Constructing specific SSDs*

The WFD-guidance offers the option to derive quality standards on the basis of specific SSDs for sensitive taxonomic groups. In this case, the minimum number of data points (10, preferably 15) should be maintained. However, this is only possible if from a generic SSD with all required taxa there is clear evidence of a 'break' in the distribution between the sensitive and other species (bimodal distribution), or if there is poor model fit [2]. In principle, this guidance is followed, meaning that the mode of action alone is not enough reason to justify an SSDs for a potentially sensitive group, without having data on the above listed required taxa. In other words, the minimum requirements to perform an SSD should also be met for a compound with a specific mode of action, in order to be able to demonstrate deviations from the expected distribution [5].

However, it is recognised that for herbicides and insecticides often large datasets are present for sensitive species groups, while data for other taxa are missing. Sometimes, it may be possible to demonstrate a break in the distribution for the acute dataset, but too many taxa are missing to construct a generic chronic SSD. In this case, it may be considered to apply specific SSDs for both datasets. However, the implicit assumption that the sensitivity distribution is similar on the acute and long term time scale has not been proven yet. Comparing the position of specific taxa in species sensitivity distributions between acute and chronic SSDs was identified as an important topic for future research [5].

In principle, a specific SSD is made for the most sensitive taxon, but multiple sensitive taxa may be combined in one specific SSD based on the distribution of data. This specifically applies to insecticides for which insects and crustaceans may be combined in one SSD for arthropods if the data show that sensitivities of the respective species groups overlap [5]. If a specific SSD is constructed, it should always be checked if the result is sufficiently protective for taxa that were not included in the SSD.

2.5.3 *Assessment factors for a specific SSD*

For derivation of the $QS_{fw,\ eco}$, a default assessment factor of 5 is applied to the Hazardous Concentration for 5% of the species (HC5) that is derived from an SSD based on chronic ecotoxicity data. The WFD-guidance lists five topics that are relevant when considering a lower factor:

- 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 representativeness 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.

Based on case studies, some examples for justifying a lower factor are given in [5]. A default factor of 10 is used for the SSD-based MAC-$QS_{fw, eco}$. This factor may be adapted if other lines of evidence suggest that a higher or lower one is appropriate. Such evidence may consist of information on the ratio between acute L(E)50 and EC10/NOEC-values and the topics that are listed in the section on the $QS_{fw, eco}$. When specific SSDs are constructed for sensitive species groups, some of the uncertainty described in the WFD-guidance still remains and should be addressed, however, lowering the assessment factors is reasonable because uncertainty about the representativeness of the tested species is reduced.

The scheme in Table 2 may be used as a starting point for derivation of standards for fresh water [5]. An important note is that when deriving an SSD-based MAC-$QS_{fw, eco}$ using L(E)C50-values, an assessment factor >1 is needed because the SSD-result relates to a 50% effect level, whereas the MAC-QS_{eco} refers to no effects.
If a pooled dataset is used, the corresponding saltwater standards are derived using an additional assessment factor of 10, which can be decreased to 5 if one typically marine species is represented in the dataset. If at least two typically marine species are present, no additional assessment factor is needed for the saltwater assessment.

Table 2 Assessment factors to be applied to a HC5 to derive freshwater standards based on different types of datasets [5]. Shaded cells represent the values given in the WFD-guidance [2]. Saltwater standards are derived using an additional AF of 10 or 5, depending on the presence of typically marine species.

	$QS_{fw, eco}$	$MAC\text{-}QS_{fw, eco}$	
	input: chronic NOEC/EC_{10}	input: acute L(E)C_{50}	input: acute NOEC/L(E)C_{10}
generic SSD	**default 5** **range 5-1**	**default 10**	default 5 range 5-1
specific SSD	default 3 range 3-1	default 6 range 6-2	default 3 range 3-1

2.6 Use of mesocosm data

➔ Location in WFD guidance: section 3.3.1.3.

2.6.1 Assessment of reliability

Aquatic micro- and mesocosm studies are frequently submitted in the context of registration of agricultural pesticides. According to the WFD-guidance, they are a valuable tool to assess the impact of a chemical on populations or communities of aquatic ecosystems under more realistic environmental conditions than is achievable with standard single species laboratory studies. If reliable mesocosm data are available, they may be used either as the basis of aquatic ERLs, or used as additional information for the selection of the assessment factor applied to an SSD [2,12]. General guidance on the design of mesocosm studies is given in several documents [13-16]. A guidance document on the evaluation and interpretation of study results was published in 2008 [17]. The following questions should be answered to assess the reliability of mesocosm studies:

- Is the test system adequate and does the test system represent a realistic freshwater community?
- Is the description of the experimental set-up adequate and unambiguous?
- Is the exposure regime adequately described?
- Are the investigated endpoints sensitive and in accordance with the working mechanisms of the compound, and with the results of the first-tier studies?
- Is it possible to evaluate the observed effects statistically and ecologically?

To facilitate answering these questions, De Jong et al. provide more information on the aspects to be considered and contains a detailed checklist to assess the scientific reliability of the study [17]. A critical part of the evaluation of mesocosm studies is the statistical analysis of measurement endpoints related to effects. Various univariate and multivariate techniques are available for evaluation of effects at the population and at the community level. Detailed information on methodology and statistical evaluation can be found in [17] and references therein.

In 2013, the European Food Safety Authority (EFSA) published guidance on the risk assessment of plant protection products (PPP) [18]. This guidance elaborates on the aforementioned guidance documents and specifically addresses the set-up, evaluation and use of mesocosm studies for risk assessment of PPP in edge-of-field surface waters. For example, EFSA specifies that, besides representatives of different trophic levels, at least 8 different populations of the sensitive taxonomic group need to be present in the micro-/mesocosm test systems and for which a concentration–response relationship can be derived.

Regarding statistical evaluation, detailed information on EFSA introduces the Minimum Detectable Difference (MDD) as an additional criterion. The MDD expresses the difference between control and treatment that can be detected as significant, given a specific test design and control performance. The MDD is particularly important if no effect is observed, since when a LOEC can be calculated the statistical power apparently is high enough to detect an effect. However, if the MDD is >100%, due to e.g. low abundance or variability in the control, it is not possible to derive a meaningful NOEC, since in this case it is not possible to underpin statistically that there is no difference between treatment and control [18]. EFSA advises that the MDD is reported for each measurement endpoint and states that the MDD should preferably be lower than 70-90%. It is noted that for field studies with earthworms and non-target arthropods, a lower level of 50% effect should be detectable [19,20]. However, EFSA [18] also requires that for at least 8 sensitive taxa a statistical evaluation of the dose-response relationship should be possible, meaning that the MDD should be sufficiently low. The case study with an insecticide that is included in the EFSA guidance shows that low MDDs for sensitive endpoints are indeed possible.

2.6.2 Effect classes

If a study based on the abovementioned criteria is considered reliable, Effect classes are used to summarise the observed effects in a transparent and comparable way. The original classes were developed by [21,22] and adapted later on [17,18]. The Effect classes are summarised as follows (after EFSA):

Table 3 Description of Effect classes used to classify effects in mesocosms.

Effect class	Description
0	*Treatment related effects cannot be evaluated*. Due e.g. low abundance and variability the MDD was always larger than 100 % so even very strong effects could not be determined for the endpoint evaluated. If this class is consistently assigned to endpoints that are deemed most relevant for the interpretation of the study, the regulatory reliability of the micro-/mesocosm tests is questionable.
1	*No treatment-related effects demonstrated for the most sensitive endpoints*. No (statistically and/or ecologically significant) effects observed as a result of the treatment. Observed differences between treatment and controls show no clear causal relationship.

Effect class	Description
2	*Slight effects* Effects concern short-term and quantitatively restricted responses usually observed at individual samplings only.
3A	*Pronounced short-term effects (< 8 weeks, followed by recovery)* Clear response of endpoint, but full recovery of affected endpoint within 8 weeks after the first application or, in the case of delayed responses and repeated applications, the duration of the effect period is less than 8 weeks and followed by full recovery[1]. Treatment-related effects demonstrated on consecutive samplings.
3B	*Pronounced effects and recovery within 8 weeks post last application* Clear response of the endpoint in micro-/mesocosm experiment repeatedly treated with the test substance and that lasts longer than eight weeks (responses already start in treatment period), but full recovery[1] of affected endpoint within eight weeks post last application.
4	*Pronounced effect in short-term study* Clear effects (e.g. large reductions in densities of the population) observed, but the study is too short to demonstrate complete recovery within eight weeks after the (last) application.
5A	*Pronounced long-term effect followed by recovery* Clear response of sensitive endpoint, effect period longer than 8 weeks and recovery did not yet occur within 8 weeks after the last application but full recovery[1] is demonstrated to occur in the year of application.
5B	*Pronounced long-term effects without recovery* Clear response of sensitive endpoints (> 8 weeks post last application) and full recovery cannot be demonstrated before termination of the experiment or before the start of the winter period.

The Effect classes are assigned to all different endpoints measured in the study, e.g. abundance of specific taxa based on univariate statistics, diversity indices or community endpoints based on multivariate analyses. A summary of the Effect classes is made to enable the overall assignment of Effect classes to the respective treatments (Figure 1).

[1] An endpoint is considered as recovered if the MDD allows statistical evaluation during the relevant recovery period (so excluding MDD class 0) and the conclusion of no statically significant effect between treated systems and controls is not caused by a decline of that endpoint in controls (e.g. at the end of the growing season). If these criteria are violated a higher effect class has to be selected.

Box 2 Example of the summary of the Effect classes observed for several endpoints in the outdoor microcosm study with xxxx; ↓ indicates a downward trend; ↑ indicates an upward trend. *TWA*, Time weighted average, *PRC*, principal response curve

	Water concentration (µg a.s./L)				
Nominal concentration:	3	15	30	150	300
Measured peak concentration:	2.8	14.5	28	146	292
7-day TWA concentration:	2.5	12.9	24.9	130	260
21-day TWA concentration:	2.0	11.5	22.2	116	231
Species/group					
Chlorophyll a – periphyton	1	1	1	1	1↑
Chlorophyll a – phytoplankton	1	1	1	1	1↑
Periphyton (PRC)	1	1	1	1	1↑
Periphyton (populations)	1	1	1	1	2↑
Phytoplankton (PRC)	1	1	3A↓	3A↓	3A↓
Phytoplankton (populations)	1	1	3A↓	3A↑↓	3A↑↓
Zooplankton (PRC)	1	1	3A↓	5A↓	5B↓
Zooplankton (populations)	1	2↓	3A↓	5A↓	5B↓
Macroinvertebrate, sweep net (PRC)	1	1	1	1	4↓
Macroinvertebrate populations	1	1	1	1	5B↓

Figure 1 Illustration of a summary of Effect classes for various endpoints measured in five mesocosm treatments. Based on this overview, Effect class 1 is assigned to the nominal concentration of 3 µg/L, Effect class 2 to 15 µg/L. Box copied from [17].

2.6.3 *Use of Effect classes for EQS-derivation*

The WFD-guidance only refers to the NOEC or EC10 of a mesocosm, but does not make reference to the Effect classes. It is stated, though, that ecological recovery is not considered when deriving aquatic EQSs within the context of the WFD (see WFD-guidance [2], section 2.8.2, 3.3.1.3). In a Dutch proposal for aquatic effects assessment of pesticides [5] additional guidance is given on the use of mesocosm data for EQS-derivation. According to this guidance at least Effect Class 3 concentrations and higher are not relevant for EQS-derivation, because an initial treatment-related effect on a relevant ecological endpoint is demonstrated. Strictly speaking, Effect Class 1 concentrations are equal to the NOEC, since at that concentration no consistent and statistically significant treatment-related effects are found. According to [5], Effect class 2 concentrations may be used as well, since they relate to situations in which 'treatment-related effects are reported as 'slight', 'transient', or other similar descriptions. It concerns a short-term and/or quantitatively restricted response of one or a few sensitive endpoints, usually observed at individual samplings only. Application of a larger assessment factor to Effect Class 2 concentrations may ensure appropriate protection and a cost-effective use of micro-/mesocosm experiments [5].

2.6.4 *Treatment of freshwater and saltwater data*

Little information is present on the representativeness of freshwater studies for marine risk assessments. Differences in physico-chemical characteristics, water exchange rate and sensitive taxa may contribute to differences in ecological response. According to the WFD-guidance

(section 3.3.2.3), freshwater mesocosm studies may be used as a basis for a marine risk assessment, but an additional assessment factor of 10 should be applied in line with the AF-approach (see section 2.4). Supplementary to the WFD-guidance, it may be considered to lower the additional assessment factor if the laboratory dataset indicates that the sensitivity of the typically marine species is covered by the freshwater species (i.e., effect levels for the typically marine species are in between those for freshwater species).

Regarding the use of marine mesocosms for the derivation of a freshwater EQS, it should be noted that according to the WFD-guidance marine mesocosm data often apply solely to small pelagic organisms. It should be considered that such studies may therefore seriously under-represent many taxa, e.g., benthic epifauna and macrophytes. On the other hand, marine mesocosms may point at sensitive taxa that are not represented in the freshwater dataset (e.g., molluscs). If for the laboratory dataset it is decided that freshwater and marine data can be pooled, there is no scientific objection to use a valid marine mesocosm also in the freshwater assessment. However, if the critical endpoint in the marine mesocosm is for a typically marine taxon which has no freshwater representatives (e.g., Echinoderms), the representativeness of the result for a freshwater assessment should be carefully considered, e.g., considering the size and diversity of the freshwater dataset in relation to the diversity in the mesocosm. Brock et al. [5] advice that a single marine mesocosm should not be used as the sole basis for a freshwater standard.

The following sections discuss the use of mesocosm results for derivation of the QS or MAC-QS, which is considered applicable to freshwater and saltwater mesocosms. However, for the ease of reading, only the subscript for freshwater is used.

2.6.5 Assessment of exposure

The evaluation and selection of mesocosm data as discussed in the previous sections results in identification of Effect Class 1 and/or 2 treatments that may be used for derivation of the $QS_{fw, eco}$ or MAC-$QS_{fw, eco}$ and respective Whether or not a particular mesocosm study is indeed relevant depends on the exposure regime that was applied in the study. Basically, the same considerations have to be made as for laboratory tests: the exposure conditions should match the purpose of QS-derivation, e.g., the $QS_{fw, eco}$ should preferably be based on studies with long-term continuous exposure, whereas studies with peak exposure may be used for derivation of the MAC-$QS_{fw, eco}$. However, since existing mesocosms for pesticides have often been submitted for authorisation of PPP, they are designed to reflect the agricultural use and may not (fully) meet the requirements for QS-derivation. Studies may simulate single or replicated applications and depending on the dissipation rate, the following exposure patterns may be found in the water phase:

- single pulse with decline of concentrations to 0 within a few days
- single pulse with decline to 0 within days to weeks
- multiple pulses with decline to 0 in between applications
- multiple pulses with accumulation of concentrations between applications

Guidance on how these patterns may be used for EQS-derivation is based on [5,18,23]. There are two issues:

- the duration of exposure should reflect the relevant duration in the field, i.e. a short-term peak for the MAC-$QS_{fw, eco}$ and long-term exposure for the $QS_{fw, eco}$
- the concentration in the mesocosm that is associated with the no-effect level should be adequately expressed, i.e. a choice should be made between nominal, measured peak of time weighted average (TWA) concentrations

Regarding the relevant duration, it is advised that studies involving single or multiple pulses with a relatively fast decline can only be used for derivation of the MAC-$QS_{fw, eco}$. For the $QS_{fw, eco}$, the substance should have been present in the water phase for a longer period of time. For tests with multiple applications of fast dissipating substances, it is stated in [5] that concentrations should not drop below 10% of the peak concentration in between applications, while tests with single pulses can only be used when dissipation rate is relatively slow, but no further guidance on dissipation rate is given. Following EFSA [18], a single pulse study can only be used for chronic QS-derivation when the concentration has not declined to levels lower than 20% of nominal during the time-window that is used for calculating the TWA concentration that is associated with the NOEC-treatment (Effect class 1 or 2, see 2.6.3). This relates to the second issue, the expression of the results.

For QS-derivation it is advised to express the no-effect treatment in a mesocosm on the basis of a TWA concentration. The length of the TWA time window should be guided by the length of the relevant critical test from the laboratory dataset, i.e. the test that delivered the lowest L(E)C50 or NOEC/EC10. Additional information on the time to onset of maximum effects, the length of the most sensitive life stage, the acute to chronic ratio may be used to further underpin or adapt the choice of the time window. For example, for derivation of the $QS_{fw, eco}$, the mesocosm-NOEC is initially derived by expressing the Effect class 1 or 2-treatment on the basis of 21-days TWA if the 21-days NOEC for *Daphnia magna* was the lowest endpoint from the laboratory dataset. However, if in the treatment level above the level identified as Effect Class 1 the time to onset of maximum effects is 30 days, the NOEC of the Effect class 1 treatment should be calculated as the 30-days TWA concentration. For derivation of the MAC-$QS_{fw, eco}$, the Effect class 1 or 2-treatment is expressed on the basis of the 48- or 72-hours TWA after the highest peak, depending on whether arthropods or algae are most sensitive in the laboratory tests.

Taking this criterion as a starting point, this means that if the lowest laboratory NOEC is obtained from a 21-days *Daphnia* study, a single pulse mesocosm can only be used for a $QS_{fw, eco}$ if the concentration in the water phase during 21 days is at least 20% of the initial peak. In this case, the DT50 for dissipation from the water phase in the mesocosm should have been 9 days or higher. Figure 2 gives a graphical representation of an Effect class 1 treatment not meeting the criterion (left hand side) and one just meeting this criterion (right hand side).

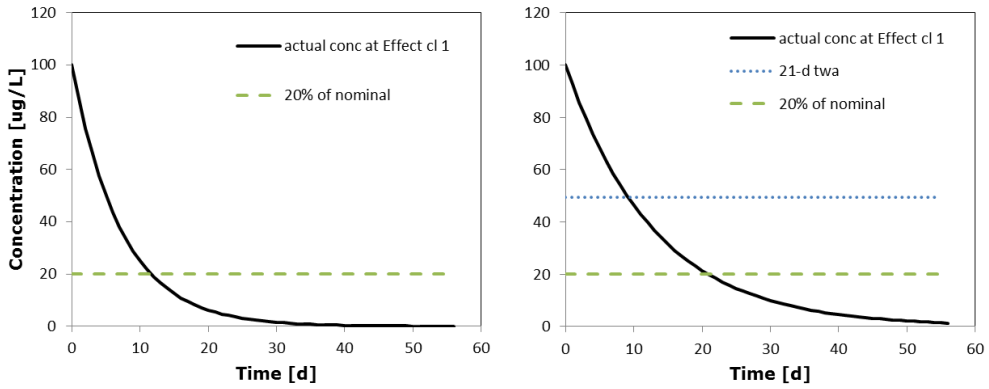

Figure 2 Development of concentrations in a single pulse Effect class 1 mesocosm-treatment. The critical laboratory test is a 21-d Daphnia test. The initial concentration is 100 µg/L. The green dashed line represents 20% of initial. The treatment at the left hand side does not meet the criterion, because after 21 days, the concentration has declined to 5% of initial.The treatment at the right hand side meets the criterion of 20% of initial left after the critical time window and the NOEC is expressed as the 21-days TWA (blue dotted line).

If decline is faster than required, studies may still be used for derivation of the $QS_{fw, eco}$, provided that repeated dosing is applied and the concentrations in between treatments does not fall below 20%. In addition, the application period should be long enough to cover the required time window (Figure 3). The appropriate effect class

concentration is then calculated as the time weighted average concentration over the test period.

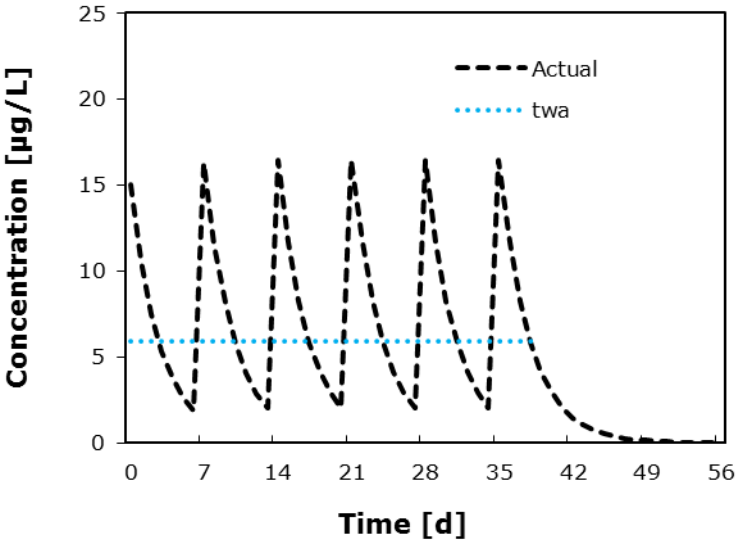

Figure 3 Development of concentrations after repeated applications, minimum concentration between dosing is >20% of initial and the application period is longer than the critical laboratory test of 21 days.

The requirement of at least 20% of initial left within the appropriate time window can also be applied to the MAC-$QS_{fw, eco}$: with a time-window of 48 to 72 hours, the minimum DT50 for dissipation from the water phase should be 0.9 to 1.3 days, respectively. Studies with multiple applications can be considered as a worst case exposure regime for derivation of the MAC-EQS, which may be reflected in the choice of the assessment factor [5].

If the concentration of a substance has fallen below 20% of initial within the appropriate time window, a case-by-case decision has to be made, by e.g. considering the time to effect in the laboratory or mesocosm studies. If a shorter time window is not appropriate, the test cannot be used for EQS-derivation, unless repeated dosing is applied.

2.6.6 Assessment factors to be used on mesocosm results

The WFD-guidance gives a default assessment factor of 5. A more differentiated assessment factor scheme is given in Table 6, based on [5]. The height of the assessment factor is always based on expert judgement considering all available information.

Table 4 Assessment factors for mesocosm studies [5].

TWA concentration associated with	**$QS_{fw, eco}$**	**MAC-QS**
NOEC = Effect class 1 for most sensitive structural endpoint	2-4*	1-2* (multiple applications) 2-3* (single application)
Effect class 2 for most sensitive structural endpoint	4-5*	2-3* (multiple applications) 3-4* (single application)

If a single adequate study is available, the higher assessment factor is used. If several adequate micro/mesocosm studies are available the assessment factor is applied to the highest test result or the lower assessment factor is applied to the most critical test result. Since mesocosms generally do not contain fish, it should always be checked if the resulting mesocosm-based QS is protective for fish.

If a mesocosm study cannot be used as such for derivation of the QS or MAC, it may still be useful to underpin the choice of the assessment factor for the AF- or SSD-method.

As indicated above (see section 2.6.4), an additional assessment factor may be needed when using a freshwater mesocosm for the derivation of the $QS_{sw, eco}$.

3 Aquatic bioaccumulation and biomagnification

3.1 Data collection and evaluation

In principle, the evaluation of bioaccumulation data follows the general guidance on evaluation for ecotoxicity to a large extent. All retrieved literature is read and evaluated with respect to its usefulness and reliability.

For the aquatic compartment, the most relevant BCF-, BMF, and BAF-studies are those performed with fish, but studies performed with other taxa are important for secondary poisoning as well. BAF and BCF data for non-standard species should be carefully checked because they are prone to experimental errors. The accumulation may not reflect uptake but adsorption to the outside of the organism. For this reason, BCF values for algae should be regarded as unreliable.

A reliable laboratory BCF study should be similar in experimental set-up to the updated OECD guideline 305 [24]. At least the concentration of the (parent) compound in the aqueous phase, and in the organisms, has to be measured at several time points.

Experimental BMF values generally originate from field studies. Laboratory derived BMF values according to the OECD 305 test guideline cannot be used for this purpose, because these were derived in the absence of simultaneous aqueous exposure. Field-based BAFs are preferred over the use of separate BCF- and BMF-values. No guidance exists to derive field-based BAFs, BMFs or TMFs. For a valid BAF-study, insight into the corresponding concentrations in water at the time of organism sampling is needed. For a reliable BMF value it is necessary to know that the prey and predator species originate from the same area and from the same period in time.

➔ Location in WFD guidance: Appendix A1.4 and A3.

3.2 Trophic magnification studies

TMFs are derived from field studies investigating contaminant concentrations in species and surrounding medium in specific food webs. The trophic level (TL) of the various sampled species is estimated using tissue concentrations of stable isotopes, expressed as a ratio, e.g. $^{15}N/^{14}N$. These ratios are offset against a standard $^{15}N/^{14}N$ ratio (usually in air) which gives a $\delta^{15}N$ value (increase in the case of ^{15}N) that is a measure of trophic position of the sampled species. TLs as whole integers are derived from $\delta^{15}N$ using enrichment factors per trophic level, which is usually 3.4‰, based on literature reviews, but the value may differ for specific organism diets. The slope of a linearised regression of the (log) contaminant concentration against TL gives the TMF, which is the antilog of the regression slope. Detailed information on TMFs and guidance on how to derive these are given in e.g., Burkhard et al., 2013, Borgå et al., 2012 and Conder et al., 2012 [25-27].

A valid BCF/BAF ≥ 100 L/kg and/or BMF greater than 1 is used as an indication of the potential for bioaccumulation. Bioaccumulation data for metals should be treated with special care, since for some metals organisms are able to regulate internal concentrations. In this case, bioconcentration and bioaccumulation may depend on the external water concentrations.

3.3 Data tables for laboratory studies

After evaluating a study, the results are summarised by entering it into the appropriate data table, examples of which is given below for a freshwater BCF study. The aim is to fill the table as completely as possible. Guidance on the parameters to be filled in is given in the WFD-guidance, Appendix A1.4.3. Note that studies may yield different type of results. Report each of these type of endpoints in separate tables and adapt the tables where necessary. Data tables for field BAF- or TMF studies are organised in a similar way as those for laboratory studies, adapting the columns to the specific study type.

3.4 BCF and BAFs for metals

Many organisms can keep their body concentration of metal relatively constant within certain concentration range, while the water concentration varies. Variation in BCF or BAF is then not caused by accumulation but by regulation. Inverse relationships of BCF/BAF with external water concentration have been observed [28,29]. The BCF concept as applicable to many organic substances is not valid for metals and BCFs (BAFs) for metals should not be used in the same way, nor can they be simply averaged. If a relevant relationship between BAF and external water concentration is observed BAf values derived in this way should be preferred. An example of this is the use of BAF values for the derivation of a QS for uranium in water for the protection goal secondary poisoning [30].

➔ WFD guidance deals with the use of BCF values for metals in:
section 2.4.3.1
section 4.6.2

Table 5 Example of a bioconcentration data-table for freshwater organisms.

Legend to column headings	
Species properties	relevant characteristics of the test species, such as age, size, origin
Analysis method	GC = gas chromatography; MS = mass spectrometry; LSC = liquid scintillation counting; TLC = thin layer chromatography; HPLC = high performance liquid chromatography
Test type	S = static; R = renewal; F = flow through; c = closed
Purity	refers to purity of active substance or content of active substance in formulation; ag = analytical grade; tg = technical grade
Test water	am = artificial medium; dtw = dechlorinated tap water; dw = deionised/dechlorinated/distilled water; nw = natural water; rw = reconstituted water; rtw = reconstituted tap water; tw = tap water
T	Temperature
Exp. / Dep. time	exposure and depuration time
BCF-type	ethe basis of the BCF, e.g. wet weight, whole fish, edible parts
Method	method for calculation of the BCF, e.g. steady state concentrations or kinetic approach
Ri	Reliability index according to [4]. Valid studies (Ri 2 or higher) are considered for EQS-derivation, depending on relevance and considering notes on data treatment (section 1.3.4)

Species	Species properties	Analysis Method	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Dep. time	Exp. conc. [µg/L]	BCF [L/kg]	BCF-type	Method	Ri	Note	Ref.
Mollusca																		
Mytilus edulis	field collected, shell length 4 cm	GC-MS	F	active	99.5	am	30		10	96 h		0.5	2300	ww; edible	Corg/Cw	2	1	[a]
Pisces																		
Cyprinus carpio	8 cm	LSC	F	active, ^{14}C	99	rw		6.0-8.5	25	56 d		0.5	11000	whole fish	Corg/Cw	3	2	[b]
Oncorhynchus mykiss	1.2 g, 3% lipid	LSC, TLC, HPLC	F	active, ^{14}C	99	rw	50	7.5	21	63 d		0.005	9600	whole fish	k1/k2	2	3	[c]

Notes
1 steady state reached
2 significant mortality occurred; only information on total radioactivity
3 result based on total RA, parent confirmed by TLC and HPLC
4
5

4 Derivation of national risk limit SRC

The SRC_{eco} is the geometric mean of all available chronic toxicity data (that have been judged valid and have been compiled in the aggregated data table). If not enough chronic toxicity data are available, the SRC_{eco} is calculated as the geometric mean of all (aggregated) acute data, divided by an assessment factor of 10. The two values are compared and the lowest value is selected as SRC_{eco}.

The aggregated data tables with acute and chronic aquatic toxicity data are used for the derivation of the SRC_{eco} according to the assessment factor scheme in Table 6. In case a pooled data set for freshwater and marine toxicity data is used for QS derivation (see section 2.3), the pooled (aggregated) data set is also used for SRC derivation. In this case, one $SRC_{water, eco}$ is derived that is valid for both the freshwater and the marine compartment. No additional assessment factor is used for derivation of the $SRC_{sw, eco}$. When the freshwater and marine data have not been pooled for QS derivation, the assessment factor scheme in Table 6 is applied to the separate freshwater and marine aggregated data sets to derive an $SRC_{fw, eco}$ and $SRC_{sw, eco}$.

In addition, take account of the following:

- In principle, an acute-to-chronic ratio (ACR) of 10 is applied to the acute toxicity data to compare acute L(E)C50s with chronic NOECs (or EC10s). One may deviate from this factor of 10 if more information on the ACR for the specific compound or endpoint is available [31].
- For the aquatic compartment, comparison between chronic data and acute data is not performed when chronic data are available for *at least* three species, which should represent the three specified trophic levels from the base set of REACH guidance: algae, *Daphnia* and fish (see Table 6).
- When the SRC_{eco} is to be reported with confidence limits, the computer program *ETX* 2.3.1 [32] is used to calculate the median HC_{50} and its 90% confidence interval. The HC_{50} is equal to the geometric mean of log-normally distributed toxicity data.
- The SRC_{eco} is always taken as the geometric mean of (either acute or chronic) toxicity data, irrespective of whether these data are log-normally distributed or not. If the data from which the SRC_{eco} is calculated do not fit a normal distribution, it suffices to note this briefly in the report section where the SRC_{eco} derivation is presented.

Table 6. Assessment factors used to derive the SRC_{eco} for the aquatic compartment.

Available test results	**Additional criteria**	**SRC_{eco} based on**	**Assessment factor**
only L(E)C50s and no NOECs		geometric mean of L(E)C50s	10
1 NOEC[1]	**none** of three specified taxa[2] is represented	geometric mean of L(E)C50s	10
1 NOEC[1]	one of three specified taxa[2] is represented AND geometric mean of L(E)C50s / 10 < NOEC value	geometric mean of L(E)C50s	10
1 NOEC[1]	one of three specified taxa[2] is represented AND geometric mean of L(E)C50s / 10 ≥ NOEC value	NOEC value	1
≥ 2 NOECs[1]	**none** of three specified taxa[2] is represented	geometric mean of L(E)C50s	10
≥ 2 NOECs[1]	one or two of three specified taxa[2] is represented AND geometric mean of L(E)C50s / 10 < geometric mean[3] of NOECs	geometric mean of L(E)C50s	10
≥ 2 NOECs[1]	one or two of three specified taxa[2] is represented AND geometric mean of L(E)C50s / 10 ≥ geometric mean[3] of NOECs	geometric mean[3] of NOECs	1
≥ 3 NOECs[1]	≥ 3 of three specified taxa[2] are represented	geometric mean[3] of NOECs	1

1: this may also be an EC10 value.
2: the 3 taxa for which NOEC data (and/or EC10 values) should be available are **algae, *Daphnia* and fish**.
3: the geometric mean of all available NOECs (and/or EC10 values) is calculated; including the values that do not belong to the specified taxa.

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List of abbreviations

AA-EQS	annual average environmental quality standard
ACR	acute to chronic ratio
ADI	acceptable daily intake
AF	assessment factor
ag	analytical grade
am	artificial medium
AMA	amphibian metamorphosis assay
BAF	bioaccumulation factor
BCF	bioconcentration factor
BMF	biomagnification factor
bw	body weight
c	closed (exposure) system
CAS	chemical abstract service
CD	commission directive
CF	continuous flow system
c.i.	confidence interval
CMR	carcinogenic, mutagenic, reprotoxic
d	days
DT50	half life time for dissipation of a substance from an environmental compartment
dtw	dechlorinated tap water
dw	de-ionised water, dechlorinated water or distilled water dry weight
DW	drinking water
DWQG	drinking-water quality guidelines
DWS	drinking-water standard
EC	European commission; effect concentration
ECx	effect concentration at which an effect of x% is observed, generally EC10 and EC50 are calculated
ECHA	European Chemicals Agency
EEC	European economic community (replaced by EU)
EFSA	European Food Safety Authority
ELS	early life stage
EqP	equilibrium partitioning
EQS	environmental quality standard
ERL	environmental risk limit
EU	European union
F	flow through system
FHI	Fraunhofer Institute
FID	flame ionisation detection
FSDT	fish sexual development test
FSTRA	fish short term reproduction assay
GC	gas chromatography
h	hours
HCx	hazardous concentration at which x percent of species is potentially affected
HPLC	high pressure liquid chromatography
IenM	Dutch Ministry of Infrastructure and Environment
IF	intermittent flow system

INS	International and National Environmental Quality Standards for Substances in the Netherlands (In Dutch: (Inter)nationale Normen Stoffen)
ISO	international organisation for standardisation
LCx	effect concentration at which x% lethality is observed, generally LC50 and LC10 are calculated
lg	laboratory grade
LSC	liquid scintillation counting
LOEC	lowest observed effect concentration
MAC	maximum acceptable concentration
MAC-EQS	maximum acceptable concentration-environmental quality standard
MDD	Minimum Detectable Difference
min	minutes
mo	months
MPC	maximum permissible concentration
MS	mass spectrometry, Microsoft™
NA	negligible addition
NC	negligible concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
nw	natural water, such as lake water, river water, sea water, well water
OECD	organisation for economic co-operation and development
pa	pro analyse
PRC	principal response curve
PPP	plant protection product
ppt	parts per thousand or parts per trillion
psu	practical salinity unit
QS	quality standard
QSAR	quantitative structure activity relationship
R	renewal system
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical substances
rg	reagent grade
rtw	reconstituted tap water: tap water with additional salts
rw	reconstituted water: (natural) water with additional salts
RIVM	national institute for public health and the environment
S	static
Sc	static, closed system
sp.	species
SRA_{eco}	ecotoxicological serious risk addition
SRC	serious risk concentration
SRC_{eco}	ecotoxicological serious risk concentration
susp	suspended particulate matter
SSD	species sensitivity distribution
TDI	tolerable daily intake
tg	technical grade
TGD	Technical Guidance Document
TL	trophic level in secondary poisoning assessment and biomagnification studies
TLC	thin layer chromatography
TTL_{hh}	toxicological threshold level for human health

TLm	median tolerance limit; also encountered as: median threshold limit
TMF	trophic magnification factor
tw	tap water
TWA	time weighted average
UV	ultraviolet
w	weeks
WFD	water framework directive
WHO	world health organisation
ww	wet weight
y	years