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Meeting: **Water Directors meeting (Sofia, 12 June 2018)**

Agenda item: **9. COMMON IMPLEMENTATION STRATEGY (CIS) OF WFD - Documents for endorsement/acknowledgement**

Title: **Technical Guidance Document for Deriving Environmental Quality Standards (TGD-EQS)**

Version no.: 2 (some references added in chap.8)   Date: 05/06/2018

Author(s):   See List in Appendix 7 of the document

Proposal:

Water Directors are invited to endorse this revised guidance document.

Context :

The current version of this technical guidance document was endorsed by the SCG on behalf of the Water Directors in March 2011 as Guidance Document n°27. The revision of the TGD-EQS started in 2015. The aims of the revision were the following :

- Ensure that the TGD-EQS takes into account up-to-date scientific knowledge,
- Promote good practices to increase consistency,
- Where possible, take into account the recommendations made by the SCHER on the previous version of the document (if not already included).

After discussions in the dedicated subgroup, led by the UK and the JRC, and in the WG Chemicals, **the following main changes were introduced in the revised guidance document** :

- Revision of the section on metals to give more explicit guidance for deriving bioavailable quality standards,
- Introduction of a new methodology to protect top predators,
- Introduction of a new methodology as a possible option to assess the quality of the data (CRED),
- Changes in two default values (in the toxicologically based formula to protect human health against contamination via drinking water, and in the formula to protect thuman health against contamination via seafood consumption),
- Inclusion of marine experts' comments.

Before the SCG meeting of 17 May 2018, some Member States questioned whether there could be further harmonization with the Drinking Water Directive, when deriving the quality standard to protect human health against contamination via drinking water. **The reasons for keeping the**

current approach unchanged were presented during the SCG meeting, and no further question was raised on this point at the meeting.

During the meeting, the SCG agreed with the current version of the revised TGD and decided to forward it to the Water Directors for endorsement.

The SCG also concluded that the issue related to drinking water should be tackled in a next revision of the TGD together with the following items :

- The current approach favours the use of the standard from the food legislation over the standard derived using a toxicologically based formula to protect human health against contamination via consumption of seafood. The subgroup in charge of the revision proposed to change the approach, to use preferentially to tox-based quality standard. No consensus could be found on this proposal. Because this change would impact the EQSs for the priority substances, and no immediate revision of the EQS Directive is planned, it is proposed to postpone this discussion to a next revision of the guidance document.
- At the moment no overarching written rules seem to exist to describe what constitutes a sufficient scientific basis for inclusion of a new methodology in the TGD-EQS. Consequently it is proposed that experts working on a future revision of the guidance document look further into this question, with the aim of documenting such rules, in coordination with what may exist under other legislation.

Common Implementation Strategy

for the Water Framework Directive (2000/60/EC)

Final draft revised Guidance Document No. 27

Technical Guidance for Deriving

Environmental Quality Standards

FOREWORD

The EU Member States, Norway, and the European Commission in 2000 have jointly developed a common strategy for implementing Directive 2000/60/EC establishing a framework for Community action in the field of water policy (the Water Framework Directive). The main aim of this strategy is to allow a coherent and harmonious implementation of the Directive. The focus of this guidance is on methodological questions related to a common understanding of the technical and scientific implications of the Water Framework Directive (WFD). In particular, one of the objectives of the Common Implementation Strategy (CIS) is the development of non-legally binding and practical Guidance Documents on various technical issues of the Directive. These Guidance Documents are targeted to those experts who are directly or indirectly implementing the Water Framework Directive in river basins. The structure, presentation and terminology are therefore adapted to the needs of these experts and formal, legalistic language is avoided wherever possible.

Under the WFD Common Implementation Strategy, an Expert-Group (EG) on Environmental Quality Standards (EQS) was initiated in 2007 to produce guidance on the establishment of EQSs in the field of water policy. This activity was led by the UK and the Joint Research Centre (JRC) and supported by the Working Group E (WG-E), later renamed the Working Group Chemicals. This Working Group Chemicals is chaired by the Commission and consists of experts from Member States, EFTA countries, candidate countries and more than 25 European umbrella organisations representing a wide range of interests (industry, agriculture, water, environment, etc.). In 2014, a programme of work commenced to update this guidance. The updated guidance reflects feedback from users, marine experts, and also recent scientific developments, particularly with respect to metals and biomagnifying substances. This revised guidance also seeks to achieve greater distinction between the steps needed to derive an EQS, and guidance for implementing an EQS e.g. for classification. The update was led by the JRC and the UK and supported by Working Group-Chemicals, with major contributions from specialists in Member States. This revised Technical Guidance has been developed to support the derivation of EQSs for priority substances and for river-basin-specific pollutants that need to be regulated by Member States according to the provisions of the WFD. The Commission intends also to use this Technical Guidance to derive the EQSs for newly identified priority substances and to review the EQSs for existing substances.

Article 16 of the Water Framework Directive (WFD, 2000/60/EC) requires the Commission to identify priority substances among those presenting significant risk to or via the aquatic environment, and to set EU Environmental Quality Standards (EQSs) for those substances in water, sediment and/or biota. In 2001 a first list of 33 priority substances was adopted (Decision 2455/2001) and in 2008 the EQSs for those substances were established (Directive 2008/105/EC or EQS Directive, EQSD). In 2013, 12 new priority substances and groups of priority substances were added to the list, and the EQSs for some of the existing priority substances were revised (Directive 2013/39/EU, amending Directive 2008/105/EC). The WFD Article 16 requires the Commission to review periodically the list of priority substances. Article 8 of the EQSD requires the Commission to finalise its next review by 2018, accompanying its conclusion, where appropriate, with proposals to identify new priority substances, and to set EQSs for them in water, sediment and/or biota.

CONTENTS

TABLES

FIGURES

1. INTRODUCTION

1.1 Environmental Quality Standards (EQSs) under the Water Framework Directive

Article 16 of the Water Framework Directive (WFD) (EC, 2000) sets out the strategy against chemical pollution of surface waterbodies. The chemical status assessment is used alongside the ecological status assessment to determine the overall quality of a waterbody. Environmental Quality Standards (EQSs) are tools used for assessing the chemical status of waterbodies. The EQS Directive (EC 2008a) established the maximum acceptable concentration and/or annual average concentration for 33 priority substances and 8 other pollutants which, if met, allow(s) the chemical status of the waterbody to be described as 'good'. This Directive was updated in 2013 (EC, 2013), extending the number of Priority substances to 45. Some of the developments in the 2013 Directive have prompted a revision to the supporting technical guidance e.g. the establishment of biota EQSs for very hydrophobic substances.

EQSs for the 45 substances identified by the EU as Priority Substances (PSs) and Priority Hazardous Substances (PHSs) are derived at a European level and apply to all Member States. The list of these substances is also referred to as Annex X substances of the WFD.
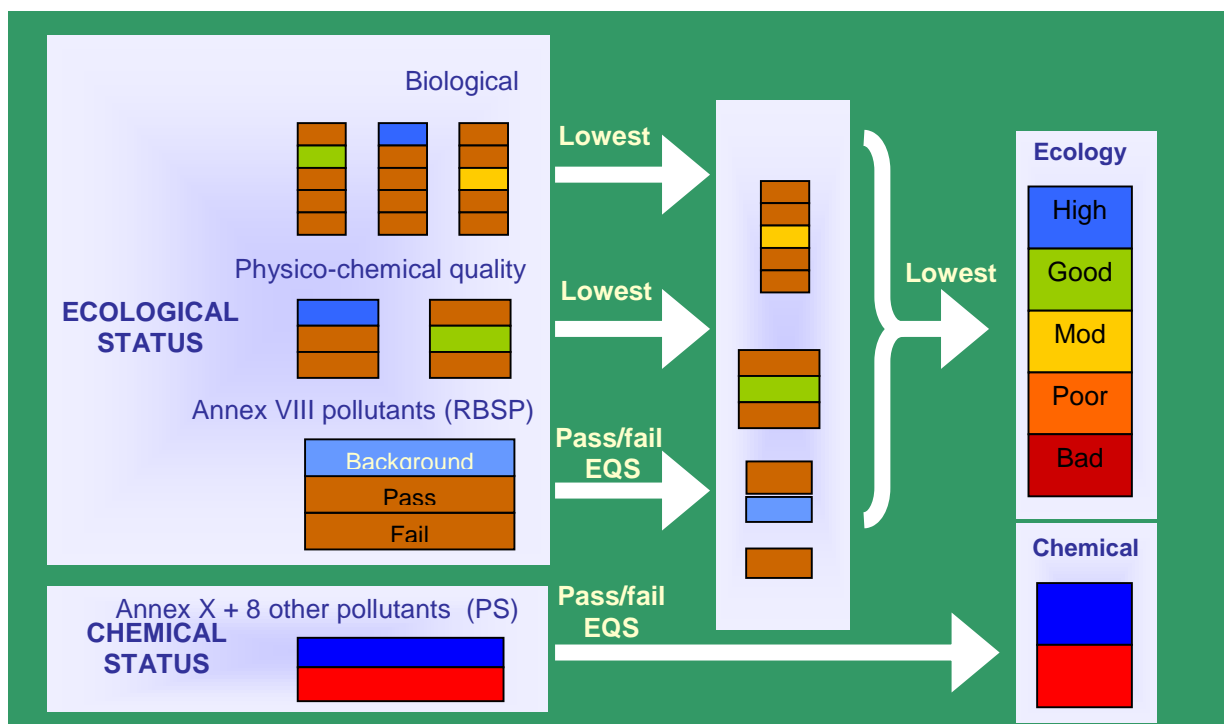
Figure 1: Role of EQSs in waterbody classification

In addition, the WFD (Annex V, Section 1.2.6) establishes the principles to be applied by the Member States to develop EQSs for Specific Pollutants that are 'discharged in significant quantities'. These are also known as Annex VIII substances of the WFD. Compliance with EQSs for Specific Pollutants forms part of the assessment of the ecological status (Figure 1). EQSs are therefore key tools in assessing and classifying the ecological status and can

therefore affect the overall classification of a waterbody under the WFD (Figure 1). In addition, EQSs will be used to set discharge permits to waterbodies, so that chemical emissions do not lead to EQS exceedance within the receiving waterbody.

EQSs protect several receptors (see Sections 2.3 and 2.4). Quality standards, also called QSs, are derived for each of the relevant routes of exposure. The lowest QS is proposed as the EQS to policy makers (except when this lowest QS is the drinking water QS, see section 2.5).

Whilst establishing the principles of EQS derivation, Annex V, Section 1.2.6 of the Water Framework Directive does not provide the necessary detail for practitioners to develop EQSs in a consistent manner or cover all the scientific issues that may be encountered.

In 2005, a technical guidance document was prepared (Lepper, 2005) for the purpose of EQS derivation. This covered many of the key technical issues involved in deriving EQSs. In recognition of technical advances, that guidance was revised in 2011, but the science has since moved on, requiring the need for a further update.

The risk assessment paradigm on which the technical guidance for EQS derivation is based (ECHA, 2008) relies on worst-case assumptions. Whilst this is entirely legitimate within a tiered assessment framework, to ensure environmental protection; however, when this paradigm is applied to EQS derivation it can lead to unworkable and/or unrealistically low EQS values (CSTEE[1], 2004; Lepper 2005). One of the factors leading to unmanageable water column standards is the very low concentrations that arise for some substances with low water solubility, or with a tendency to bioaccumulate through the food web. If these substances pose a significant risk through indirect toxicity (i.e. secondary poisoning resulting from food chain transfer), and their analysis is more feasible in other environmental matrices, such as biota and/or sediments, then a biota standard or a sediment standard may be required alongside, or instead of, the water column EQS, as referred to in the EQS Directive 2008/105/EC amended by Directive 2013/39/EC. For this reason, guidance on the derivation of biota and sediment EQSs is required. There is also a need for further guidance on setting EQSs for metals in ways that allow speciation and bioavailability to be accounted for, and which reflects the rapid development of regulatory tools in this field, such as Biotic Ligand Models. Furthermore, we are now in a position to refine the guidance for the derivation of water column standards in the light of technical advances and experience of EQS setting gained in recent years. These issues are amongst those covered in this new guidance.

The main areas of revision compared to the 2011 guidance are related to the derivation of biota standards for human health and secondary poisoning of wildlife, and the derivation of standards for bioavailable metals. No revisions were made in sections 6 and 7, and a minor change was made to figure 10 in section 5. This does not imply that no further scientific achievements have taken place in these areas, nor that these should not be considered in EQS derivation or EQS compliance checking; but rather that they are not yet at a stage where clear changes can be made or are demanded by practitioners. The main changes are summarised in the table below.

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[1] Scientific Committee on Toxicity, Ecotoxicity and the Environment

Revision	Content of the changes
Quality assessment of data	Advice on methods for the quality assessment of ecotoxicological data.
Revisions to biota quality standards for protecting human health	Changes to the allocation of diet from fish.
Revisions to biota quality standards for protecting predators (secondary poisoning)	Toxicity data normalised to the energy content of the diet.
Metals guidance	Improved clarity, more explicit guidance for deriving bioavailable QS.
Marine quality standards	Inclusion of marine's experts' comments.
Technical revisions	Various technical corrections.

1.2   Scope of the guidance

This guidance document addresses the derivation of environmental quality standards for water, sediment and biota. It builds on comments made by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 2004) and by the Scientific Committee on Health and Environmental Risks (SCHER) in 2010. It also reflects more recent feedback from marine experts, end-users of the guidance, and scientists involved in the development of chemical risk assessment methodologies.

The European Commission is currently putting emphasis on ensuring consistency and coherence between different pieces of legislation. In particular, it is beginning an examination of the approaches to risk assessment and risk management under the legislations related to chemicals, e.g. REACH, the plant protection products and biocidal products regulations, and probably other legislation such as the WFD. As far as possible, the guidance described here tries to ensure consistency with other legislation. **This guidance applies to the derivation of EQSs for priority substances (PSs), priority hazardous substances (PHSs) and River Basin Specific Pollutants (RBSPs)**. The guidance focuses on the steps required to derive EQSs that comply with the requirements of Annex V of the WFD. It assumes that the chemicals for which EQSs are required have been identified, i.e. the guidance does not cover chemical prioritisation. Whilst it does not cover the implementation of an EQS (e.g. design of monitoring programmes, sampling, chemical analysis) the guidance does highlight where the methods used to implement an EQS have a direct bearing on the way an EQS is derived and expressed.

The quantity of data available for deriving an EQS can vary. Where an EQS can be derived on the basis of a large dataset, there may be only small uncertainties in the final outcome. If, however, only a very small dataset is available, the residual uncertainties can be large. Uncertainty is accounted for by the use of assessment factors (AFs) but, clearly, there is a considerable difference in the robustness and reliability of such EQSs compared to those based on extensive data sets, and it may even be inadvisable to implement such EQSs. This technical guidance does not identify cases when uncertainties are so large that an EQS should not be implemented or used only an advisory capacity. That decision is for

policymakers, but this could come under review as we gain more experience in setting and using environmental standards for the WFD. However, **the scientist has an important role in advising the policymakers about the major uncertainties and key assumptions involved in deriving an EQS. This is particularly important for EQSs which are to be applied across Europe (e.g. for Priority Substances or Priority Hazardous Substances)**. It is also important to highlight to the policymakers the practical steps which might be taken to reduce uncertainty (e.g. generation of additional ecotoxicity data) and the benefits these would have e.g. reducing the size of the AFs. The scientist should also advise policymakers when uncertainties are small and the resulting EQS is correspondingly robust. With this in mind, a proforma technical report is appended (Appendix 2) to prompt the assessor for the information that should be reported, including advice to policymakers.

A further point to add is that confidence about regulatory decisions involving EQSs can also be affected by the way an EQS is implemented, e.g. how compliance is assessed. Although detailed monitoring guidance lies outside the scope of this guidance, it is useful to consider implementation issues during EQS setting. Although the final decision about EQS values should reflect the scientific risk, those responsible for EQS derivation are encouraged to discuss implications for water management practices with policy makers and those responsible for implementing an EQS. These might include, for instance, implications for permitting and emission controls, sampling (e.g. whole water vs filtered samples), consideration of backgrounds, statistical aspects of the compliance assessment, and availability of suitable analytical methods.

This guidance is intended for use by environmental scientists with an understanding of the principles of risk assessment. A detailed appreciation of the principles and practice of environmental chemistry and ecotoxicology is also recommended. Much of this guidance will be familiar to those used to dealing with effects assessments under REACH (Registration, Evaluation and Authorisation of Chemicals) (Regulation (EC) 1907/2006).

1.3   Links to chemical risk assessment

In Europe, various chemical regulatory regimes (covering industrial chemicals, pesticides, biocides and pharmaceuticals) are in place, and they have developed slightly different risk assessment procedures depending on the objectives of the relevant legislation. Nevertheless, the principles and process for deriving environmental standards have much in common with the effects (i.e. hazard) assessment required for risk assessment under these various regimes. Nevertheless, it is important to highlight some conceptual differences between an EQS and a PNEC (Predicted No Effect Concentration) from chemical risk assessment or TER (Toxicity Exposure Ratio), and a RAC (Regulatory Acceptable Concentration) for a pesticide. For example:

- Whereas there are opportunities to refine a risk assessment in the light of new data, this is often not the case in EQS derivation; although additional data may sometimes be voluntarily provided, we cannot usually demand the commissioning of new studies so have to utilise what is available to us.

- An exceedance of the EQS will not normally trigger a refinement of the standard.

- An underlying requirement of the WFD is to protect the most sensitive waters in Europe. For metal EQSs, where bioavailability is to be accounted for (Section 2.10) there may be a requirement to protect a higher proportion of waterbodies than for PNECs estimated as part of a risk assessment.

- In EQS derivation, field and mesocosm data have an important role as lines of evidence in helping define the standard (through helping reduce uncertainty) but would not be regarded as 'higher tier' data that would replace laboratory-based ecotoxicity data as is done in the assessment of the impacts of pesticides.

- For the purposes of the WFD, short- and long-term effects are of concern, the focus depending on the emission characteristics of the substance, and its persistence in the environment.

- EQSs represent a target to be reached in the context of risk management measures, in particular in relation to the reduction of emissions.

- The concept of an overall threshold (Sections 2.3 and 2.4) that protects all receptors and routes of exposure is a feature of WFD EQSs that does not normally apply in thresholds developed for other regulatory schemes.

- Unlike other chemical regimes that are focussed on authorisation of chemicals before they are used ('prospective' risk assessment), the WFD follows a retrospective approach. The chemicals evaluated have already been placed on the market, or they arise through unregulated activities.

- The guidance for undertaking risk assessment of pesticides (EFSA, 2013) allows for short term impacts from which recovery is possible. This is not a feature of the WFD or other regimes.

- When deriving an EQS under the WFD, the extrapolation method and choice of assessment factor is dictated by the quantity and relevance of the available toxicity data. In contrast, most of the other chemical risk assessment regimes have specific data requirements and consequently there is less flexibility in the choice of assessment factors.

A PNEC or RAC derived as part of a risk assessment will provide an important step in the derivation of an EQS and, in some cases, the PNEC from a risk assessment may be identical to the EQS. However, it will not be sufficient to simply adopt the PNEC as the EQS as a matter of course for the technical and policy reasons outlined above.

Authoritative guidance on effects assessment for chemicals has been developed, notably the technical guidance documents developed for industrial chemicals (now under REACH (ECHA, 2008-; developed since 2008 and regularly updated), the guidance documents on the biocidal products regulation (ECHA, 2015), as well as the guidance documents developed for pesticides' authorisation under Regulation 1107/2009 (EFSA, 2013)[2]. Annex V of the WFD refers directly to the methodology described for the Existing Substances Regulation (ESR) (now under REACH). As far as possible, the technical guidance for EQSs described here is consistent with the guidance for effects assessments performed for chemical risk assessment under other regimes such as REACH.

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[2] The EFSA Aquatic Guidance Document (EFSA, 2013) includes a section on differences in risk assessment procedures between Regulation No. 1107/2009 and the Water Framework Directive.

1.4   Structure of guidance

Generic issues and principles that apply to the derivation of EQSs across all media and receptors are outlined in Section 2. The guidance is separated into sections dealing with different environmental media, i.e. derivation of EQSs for the water column are considered in Section 3, derivation of EQSs in biota in Section 4 and those for sediment in Section 5. Risks from metals pose particular challenges and the guidance reflects the latest scientific developments for taking account of speciation and bioavailability in deriving thresholds. Detailed guidance for deriving EQSs for metals in water, biota and sediment is given in the respective Sections. Recognising the growing importance of computational and non-testing methods in the estimation of environmental hazard, guidance on the use of such methods when deriving EQSs is given in Section 6. Finally, Section 7 outlines how to estimate EQSs for mixtures.

At various points in the guidance, we refer to Appendices and scientific background documents to accompany the guidance. These are intended to provide more detailed explanations to complete the technical advice given in these sections.

2.   GENERIC ISSUES

2.1   Use of EQSs in waterbody classification

The WFD establishes a framework for protection of all surface waters and groundwaters, with an obligation to prevent any deterioration of status, and to achieve good status. The overall good status is reached for a certain waterbody if both, ecological and chemical status, are classified as good.

EQSs established at EU level by the EQS Directive (2013/39/EC) for the 45 Priority Substances are used within the WFD to assess the chemical status of a waterbody. Good chemical status is achieved where a surface waterbody complies with all the environmental quality standards listed in Part A of Annex I of the EQS Directive, applied according to the requirements set in Part B of Annex I of the same directive. If not, the waterbody shall be recorded as failing to achieve good chemical status.

For Annex VIII substances (Specific Pollutants), each Member State shall establish their EQSs according to Annex V, Section 1.2.6 of the WFD. Specific Pollutants are supporting parameters for biological quality elements; thus they contribute among other parameters to the ecological status classification. If the EQSs for these substances are not met, the waterbody cannot be classified as either 'Good' or 'High' status, even if the biological quality is 'Good' or 'High' (Figure 1).

2.2   Overview of the steps involved in deriving an EQS

Figure 2 illustrates the key steps that are involved in deriving an EQS, irrespective of the compartment or receptor at risk. The key steps are broadly consistent across all media/receptors. However, the detail within each step can differ markedly between compartments and receptors.
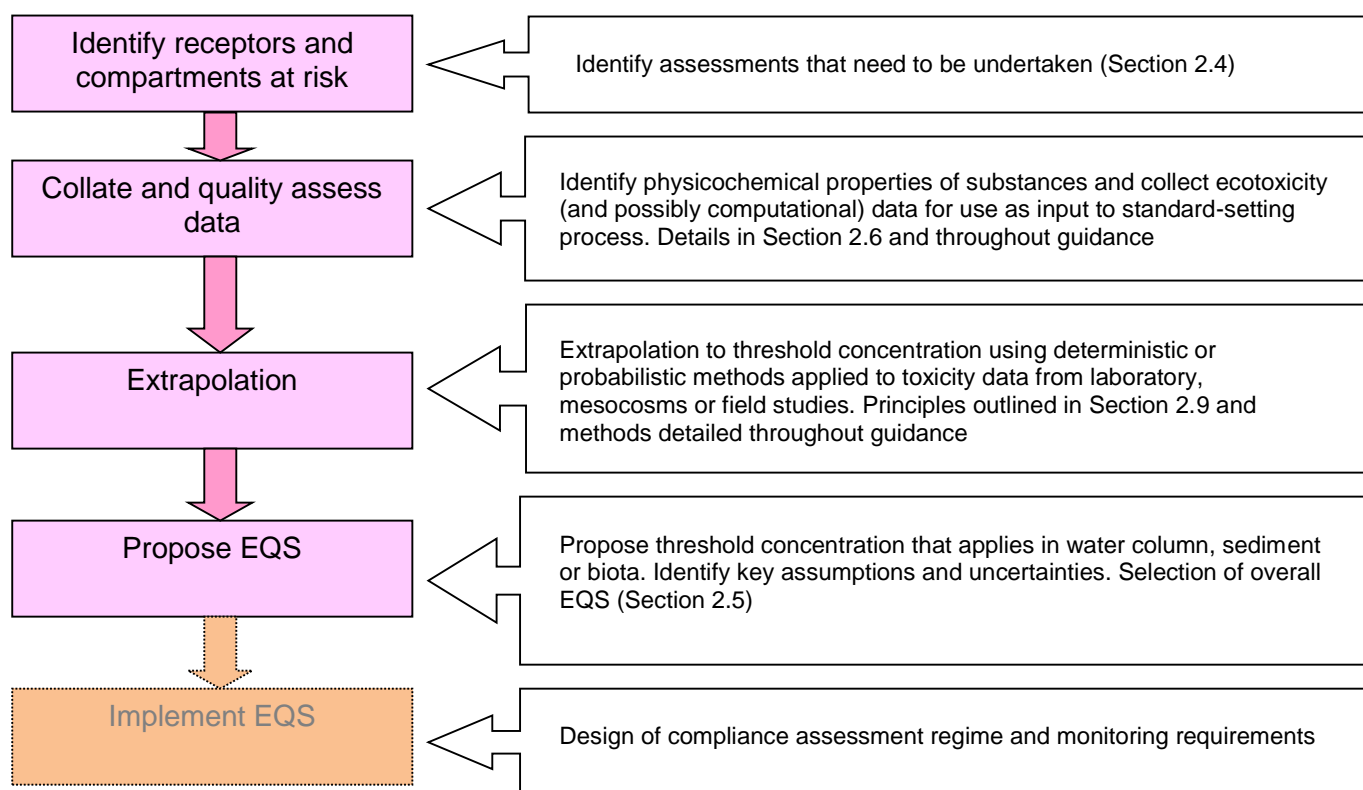
Figure 2: Key steps involved in deriving an EQS

2.3 Receptors and compartments at risk

EQSs should protect freshwater and marine ecosystems from possible adverse effects of chemicals as well as human health via drinking water or ingestion of food originating from aquatic environments. Several different types of receptor therefore need to be considered, i.e. the pelagic and benthic communities in freshwater, brackish or saltwater ecosystems, the top predators of these ecosystems and human health.

The receptors and media of concern to EQS setting covered in this guidance are illustrated in Figure 3.

		Environmental compartment		
		Water	**Sediment**	**Biota**
Receptor(s) at risk	**Humans**	**Yes[3]**	**No**	**Yes (consumption of fish products)**
	Sediment dwelling biota	**No**	**Yes**	**No**
	Pelagic biota	**Yes**	**No**	**Yes (secondary poisoning)**
	Top predators (birds, mammals)	**Yes**	**No**	**Yes (secondary poisoning)**

Figure 3: Receptors for which an assessment may be required

Yes = potential risks to receptor need to be considered in EQS derivation
No = risks do not need to be addressed in EQS derivation

Not all receptors need to be considered for every substance. This depends on the environmental fate and behaviour of the substance. For example, if a substance does not bioaccumulate (or does not have high intrinsic toxicity), there is no risk of secondary poisoning and so a biota standard is not required. However, where a possible risk is identified, quality standards should be derived for that receptor (Figure 4). Criteria to help identify which of the assessments are needed for a particular substance are given in Section 2.4. Where several assessments are performed, the lowest (most stringent) of the thresholds will be selected as an 'overall' EQS as illustrated in Figure 4 and detailed in Section 2.5. By ensuring that the most sensitive receptor is protected, risks from other routes of exposure should automatically be addressed. Moreover, all direct and indirect exposure routes in aquatic systems, i.e. exposure in the waterbody via water and sediment or via bioaccumulation, as well as possible exposure via drinking water uptake, are accounted for. Figure 4 presents the routes taken into account for the freshwater compartment, similar routes are considered for the saltwater compartment, but indicated with different subscripts ("fw" is replaced by "sw" in the figure below) See appendix 6 for clarification of the 'temporary' standards used during EQS derivation.

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[3] In addition to exposure via drinking water, consider hazards from dermal exposure during swimming and seek specialist advice if these are likely to be significant.
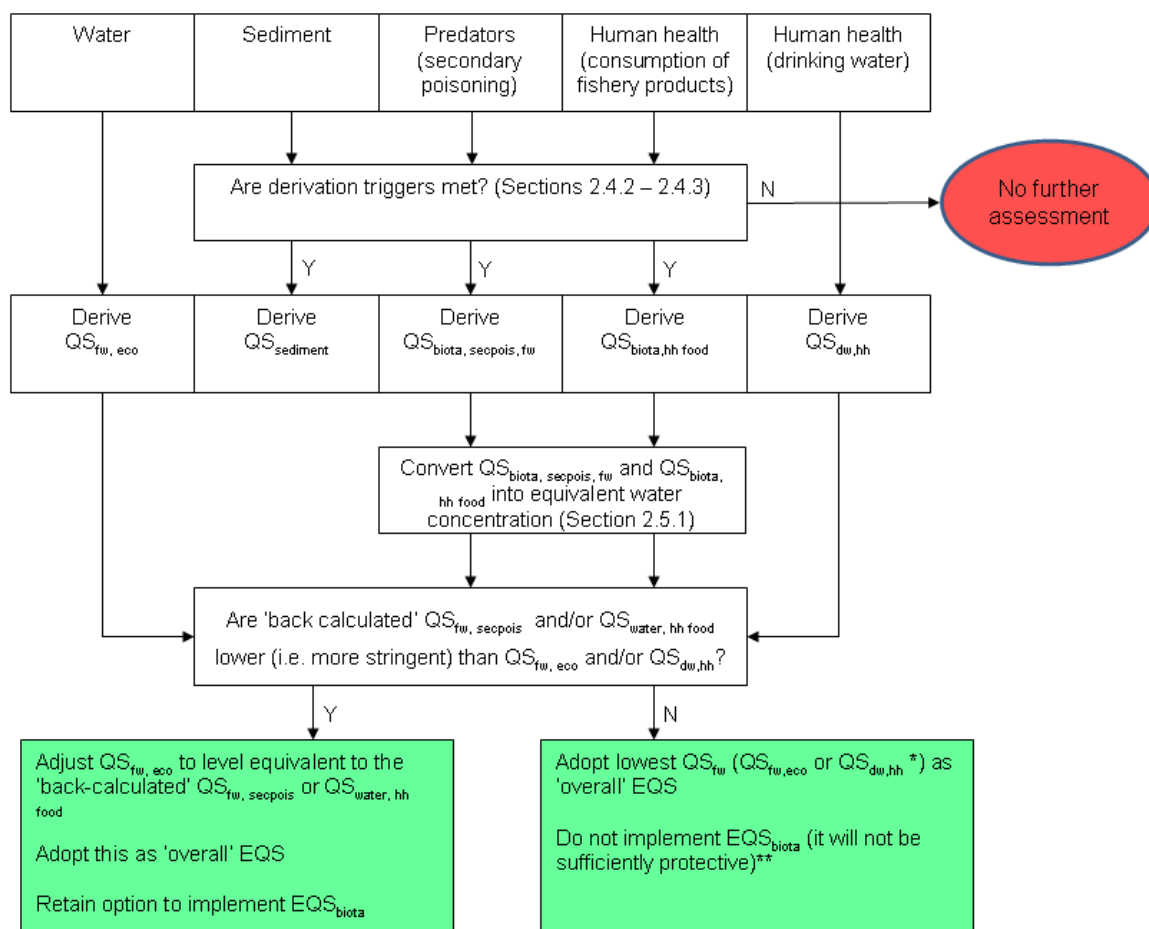
* $QS_{dw,hh}$ can only be adopted as the lowest QS_{water} for waters intended for drinking water use

** Unless monitoring in biota is strongly preferred. Under these circumstances, calculate QS_{biota} that is equivalent to lowest (i.e. most protective) QS_{water} and select this value as EQS_{biota}

Figure 4 Overview of assessments needed and selection of an 'overall' EQS

The mode of toxic action for a chemical is not always known but, when carrying out an assessment, all relevant modes of toxicity need to be considered. No plausible toxicological hazard should be excluded from consideration. The stressors for which an EQS could be derived, but do not act by chemical toxicity (e.g. temperature, pH), may require a different approach than that described here. Such physical stressors lie outside the scope of this guidance.

2.4   Identifying the assessments to be performed (receptors at risk)

According to the 2008 and 2013 EQS Directives, quality standards shall apply to contaminant concentrations in water, sediments and/or biota. As illustrated in Figure 4, **an assessment for several compartments is needed when a substance could pose a risk through direct toxicity in the water column, to predators through the food chain, or to benthic (sediment-dwelling) biota.** On the other hand, **a QS is not required if a substance will not pose a risk to a particular compartment**. For instance, a quality standard for sediment is not necessary if the substance is unlikely to partition to, or accumulate in, sediment. Similarly, quality standards for biota are not required if a substance does not bioaccumulate (or doesn't have high intrinsic

toxicity), in which case it is reasonable to conclude that there is no risk of secondary poisoning of top predators, or to human health from consumption of fishery products.

The criteria for identifying which assessments are required are outlined below.

2.4.1 Water column

An assessment to protect pelagic (i.e. water column) organisms from direct toxicity of chemicals is always undertaken. A drinking water threshold is also required for waters used for drinking water abstraction. For these waters, existing health-based standards from either the Drinking Water Directive 98/83/EC or the World Health Organization (WHO) could be used, if available, as the basis for the QS derivation, as described in Section 3.7. If no existing standards are available, an assessment of risks to human health from drinking water will be required. However, a QS to protect waterbodies designated for drinking water abstraction is required only when it is lower (i.e. more stringent) than the water column QS to protect aquatic life. A derivation is not required if existing drinking water standards are less stringent (i.e. higher) than the water column QS to protect aquatic life.

In the derivation of QSs to protect human health two major exposure routes are considered (consumption of fishery products and consumption of drinking water). There may be other routes of exposure, such as exposure during recreation (dermal exposure, ingestion of water). These routes are of minor importance compared to the other routes considered (see for example Albering *et al*, 1999) and are therefore not considered in this guidance.

2.4.1.1 EQSs for transitional waters

Separate EQSs are recommended for freshwaters and saltwaters. However, transitional (e.g. estuarine) waters are intermediate in salinity, which can vary on a diurnal cycle. For waters with low salinity and supporting communities that are closely related to freshwater ecosystems, the freshwater scheme is more appropriate. At salinity levels between 3 and 5‰ there is a minimum number of species present and this can be considered as a switch from communities that are dominated by freshwater species to communities that are dominated by saltwater species. Therefore, EQSs in this document are not reported for 'transitional and marine waters', but either for freshwaters or saltwaters. As a default, a salinity of 5‰ is recommended as the cutoff unless other evidence suggests that a different one is appropriate for a particular location. For instance, Bothnian Sea (inner Baltic Sea) is a brackish water body that has a salinity of around 5‰, and has, so far, been treated as a saltwater system.

2.4.2 Sediments

Not all substances require an assessment for a sediment standard. The criteria for triggering an assessment are consistent with those under REACH Regulation (EC) No 1907/2006 (ECHA, 2008, Chapter R.7b). In general, substances with an organic carbon adsorption coefficient (K_{oc}) of <500–1000 l·kg^{-1} are not likely to be sorbed to sediment. Consequently, a log K_{oc} or log K_{ow} of ≥3 is used as a trigger value for sediment effects assessment. Some substances can occur in sediments even though they do not meet these criteria so, in addition, evidence of high toxicity to aquatic organisms or sediment-dwelling organisms or evidence of accumulation in sediments from monitoring, would also trigger derivation of a sediment EQS.
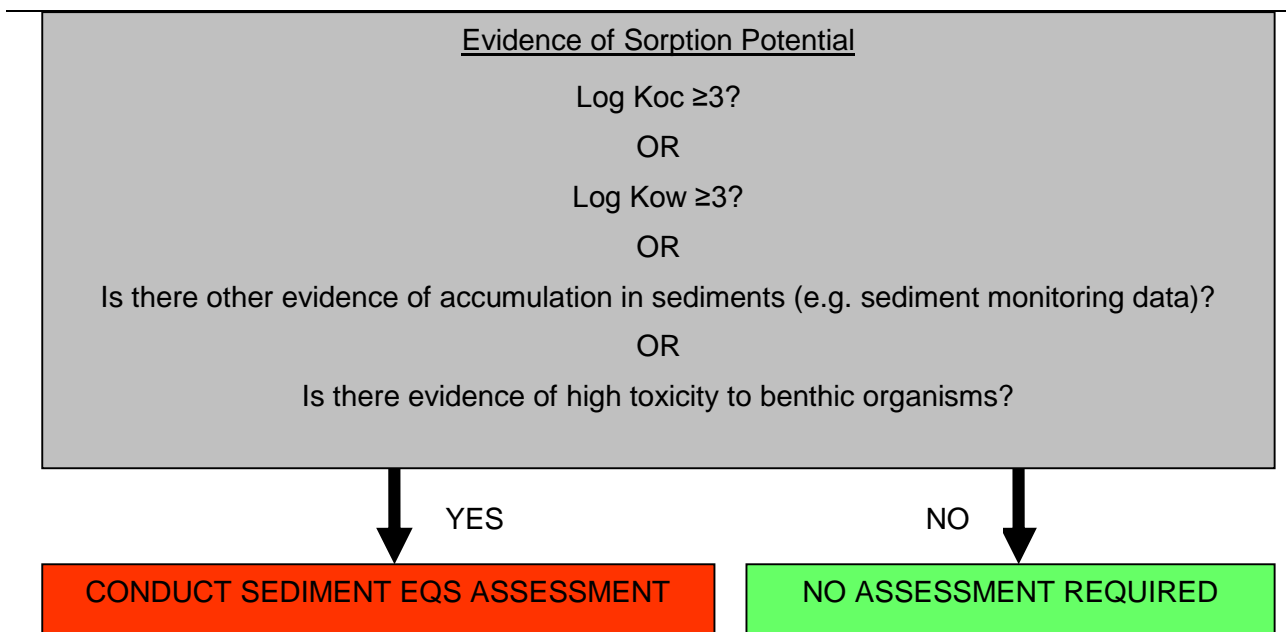
2.4.3 Biota

The criteria determining whether or not a biota standard is needed are more complex. A standard would be required if there was a risk of secondary poisoning of predators (e.g. mammals or birds) from eating contaminated prey ($QS_{biota,secpois}$), or a risk to humans from eating fishery products ($QS_{biota, hh food}$).

The triggers are based on those used to determine whether a secondary poisoning assessment is necessary for a substance under REACH Regulation (EC) No 1907/2006 (ECHA, 2008)[4]. The triggers for deriving a $QS_{biota, hh food}$ are dominated by hazard properties, whereas a $QS_{biota sec pois}$ is triggered by the possibility of accumulation in the food chain in conjunction with hazard properties. There are differences between how metals and organic substances are dealt with, and these are highlighted below.

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[4] The criteria used to determine whether a substance is Persistent, Bioaccumulative and Toxic (PBT) or very Persistent and very Bioaccumulative (vPvB) under Annex XIII of REACH are more stringent and not suitable for use as a screening decision tree since a substance meeting the PBT/vPvB criteria would require stricter management control than standard settings.

2.4.3.1 *Protection of predators from secondary poisoning*

(1) Organic substances

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Step 1: Evidence of Bioaccumulation Potential

Is measured BMF>1 or BCF (BAF) ≥100?

OR

If no valid measured BMF or BCF (BAF) is available, is Log Kow ≥ 3?

OR

Is there other evidence of bioaccumulation potential (e.g. biota monitoring data, structural alerts)?

PROVIDED THAT there is no mitigating property such as rapid degradation (ready biodegradability or hydrolysis half-life <12h at pH 5-9, 20°C) or obvious molecular size exclusion

OR

Does the substance have high intrinsic toxicity to mammals and birds (except carcinogenicity)?

</div>

YES → **UNDERTAKE BIOTA ASSESSMENT**

NO → **BIOTA ASSESSMENT NOT REQUIRED**

The assessor should determine whether the substance has the potential to accumulate through food chains and thus expose top predators via their diet. The biomagnification factor (BMF) is the ratio of the concentration of a substance in an organism compared to the concentration in food (prey) items. The bioconcentration factor (BCF) is the ratio of the concentration of a substance in an organism to the concentration in water. A BMF greater than 1 or, in the absence of this information, a BCF greater than or equal to 100 is used as an indication of the potential for bioaccumulation. **When both BMF and BCF data are available, the most <u>reliable</u> should be used, not necessarily the worst case (highest) value**. Usually this will be the BCF data, except for metals, where BCF data can be influenced by the water concentration used in the study (See Section 2.4.3.1 (2)).

If neither BMF nor BCF data are available, the octanol–water partition coefficient (K_{ow}), can be used as a surrogate for bioaccumulation potential. A log K_{ow} ≥3 would be expected to capture substances with a BCF ≥100. Other evidence of bioaccumulation potential should also be taken into account where available, such as structural features of the molecule or monitoring data from top predators. In addition, factors mitigating bioaccumulation potential should be considered. These include rapid degradation and molecular size. Rapid degradation may lead to relatively low concentrations of a substance in the aquatic environment and thus low concentrations in aquatic organisms. Information on the molecular size can be an indicator of the limited bioaccumulation potential of a substance, as very bulky molecules will pass less easily through cell membranes. Further guidance on molecular size and its impact on bioaccumulation potential are available in the REACH guidance R.10 (ECHA, 2008).

(2) Metals

Biomagnification of metals in aquatic organisms is rarely observed and, if it does occur, it usually involves the organo-metallic forms of metals (e.g. methyl mercury) (Brix *et al.*, 2000). However, the assessor should examine their potential to biomagnify or cause secondary poisoning in food

chains, even for inorganic metal forms. It is especially important to look for evidence of organo-metallic species being formed in some compartments, or if the range over which homeostasis occurs is relatively small (e.g. selenium). Therefore, a useful first step is to review the information available for the metal in question in order to assess whether an in-depth secondary poisoning assessment is needed.

A lack of biomagnification should not be interpreted as lack of exposure or no concern for trophic transfer. Even in the absence of biomagnification, aquatic organisms can bioaccumulate relatively large amounts of metals and this can become a significant source of dietary metal to their predators (U.S. EPA 2007; Reinfelder *et al.* 1998).

For metals, a BCF should not be used. This is because the model of hydrophobic partitioning, giving a more or less constant ratio C_{biota}/C_{water} with varying external concentration, does not apply to metals. For a number of metals an inverse relationship between BCF and external (water-) concentration is observed (McGeer *et al.*, 2003). Consequently, BCFs and BAFs are not constant with water concentration. Furthermore, some metals are essential for life and many organisms possess mechanisms for regulating internal concentrations, especially essential metals such as copper and zinc.

Instead, a case-by-case evaluation of the possibility of dietary toxicity is required:

- Information on metal mode of action and homeostatic (internal regulation) controls;

- Information on essentiality;

- Information on biomagnification (BMF). An example of a study relevant in addressing this question is Ikemoto et al (2008a);

- Information on major toxicities, i.e. whether main risks are through direct toxicity to pelagic organisms or secondary poisoning. With regards to the potential for secondary poisoning the assessment of the mode of toxic action in both prey and predator is a key consideration. If there is no evidence of biomagnification (i.e. BMF<1) and no specific toxicity in birds and mammals compared to fish (on a dose-based approach), the $QS_{water,\,eco}$ should be protective for birds and mammals as well as pelagic organisms.

If the balance of evidence points to a risk of secondary poisoning, then an assessment is required.

2.4.3.2   Protection of humans from consuming fishery products

For humans, the derivation of a biota standard is triggered solely on the basis of the hazardous properties of the chemical of interest. The available mammalian and bird toxicity data is used to give an indication of possible risks to top wildlife predators as well as humans since there is usually standard mammalian toxicity data available for well-studied chemicals. Effects on reproduction, fertility and development are of particular concern since these are long-term effects which could impact on populations of organisms.

Specific triggers[5] are as follows:

- a known or suspected carcinogen (Cat. I-II, R-phrases R45 or R40) or

- a known or suspected mutagen (Cat. I-II, R-phrases R46 or R40) or

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[5] In accordance with Directive 67/548/EEC.

- a substance known or suspected to affect reproduction (Cat. I-III, R-phrases R60, R61, R62, R63 or R64) or

- possible risk of irreversible effects (R68) or

- the potential to bioaccumulate (see protection of top predators) plus danger of serious damage to health by prolonged exposure (R48) or harmful/toxic/fatal when swallowed (R22/R25/R28).

Note that applicability of these toxicological triggers should follow from R or H phrases, but information obtained from the evaluation of toxicological data and not necessarily reflected in the classification and labelling phrases should not be neglected. It may warrant derivation of a risk limit for human health based on the consumption of fishery products.

The H-statements have replaced the R-phrases in EU chemicals legislation via the Classification, Labelling and Packaging Regulation (2008) (EC, 2008). For example, H302 ('harmful if swallowed') was formerly known as R22. However, in some older assessments R-phrases can still be found. The conversion between H and R phrases is provided below. For those substances where R or H phrases have not been harmonised at the EU-level, consultation with (a) human toxicological expert(s) is needed.

R22   H302: Harmful if swallowed

R25   H301: Toxic if swallowed

R28   H300: Fatal if swallowed

R40   H351: Suspected of causing cancer

R45   H350: May cause cancer

R46   H340: May cause genetic effects

R48   H373: May cause damage to organs through prolonged or repeated exposure

R60   H360: May damage fertility or the unborn child

R61   H360: May damage fertility or the unborn child

R62   H361: Suspected of damaging fertility or the unborn child

R63   H361: Suspected of damaging fertility or the unborn child

R64   H362: May cause harm to breast-fed children

R68   H341: Suspected of causing genetic effects

2.5   Selecting an overall standard

Standards for water, sediment and biota are derived independently and they should all be made available for possible implementation. Where several assessments are performed for the same compartment (e.g. water: protection of pelagic species, protection of human health from drinking water; biota: protection of biota from secondary poisoning, protection of human health from consuming fisheries products), **the lowest standard calculated for the different objectives of protection will normally be adopted as the overall quality standard for that compartment**. An exception will be when the drinking water route results in the lowest (most stringent) QS but a

waterbody is not designated as a source of drinking water. It is not sufficient to simply report the 'overall' EQS; the assessor must make available all the relevant QSs and their derivations. Standards for freshwater and saltwaters will be derived independently so the overall $EQS_{saltwater}$ may be different to the overall $EQS_{freshwater}$.

To select an overall EQS, quality standards will need to be expressed in the same units (i.e. mass/volume). This means that biota standards must be 'back-calculated' to the corresponding water concentration. This is referred to in Figure 4 and further guidance is given in Section 2.5.1. However, sediment QSs are dealt with independently from water column and biota standards, because they cannot be inter-converted with confidence. This leads to the selection of a separate, overall $EQS_{sediment}$.

2.5.1 Converting biota standards into an equivalent water concentration

Procedures for converting biota standards into water column concentrations are given in Section 4.6.2. It should be noted that the conversion from a biota standard into an equivalent water concentration can introduce uncertainty, especially for (a) highly lipophilic substances and (b) metals.

(a) Where it is necessary to convert a biota QS into an equivalent water column concentration for a highly lipophilic substance, the uncertainties may be taken into account by performing the conversion for extreme BAF values as well as the typical BAF value. If the QS for water lies within the range of possible extrapolated values of the QS for biota, when considering the uncertainties of the extrapolation, it is not possible to determine with high confidence which is the 'critical' QS. These should be reported as key uncertainties, outlining the implications for implementing an EQS.

As explained in Section 2.4.3.1, BCF data for metals may be unreliable. Instead, BAF or BMF data are preferable. To compare a biota standard with water column standards, refer to Section 4.6.2.

(b) For an organic substance, if the log K_{OW} ≥3 criterion is met, but no experimental evidence is available on BCF or BMF then the assessor should estimate BCF or BMF from log K_{OW} and translate the biota standard to a water concentration for comparison with water column standards (Section 4.6.2). If the estimated QS for biota is the most stringent (i.e. lowest) value, then further investigation to improve BCF and BMF values would be necessary; otherwise, there is a risk of developing an unrealistically low QS value for water.

2.6 Data – acquiring, evaluating and selecting data

Comprehensive and quality assessed data are key inputs to QS derivation. Indeed, most of the resources required for QS derivation are expended on collecting and assessing data. Appendix 1 provides detailed guidance on how to locate relevant data, evaluate the data to assess their suitability for QS derivation, and select data that will be used to determine a QS.

A brief summary of the main types of data required for deriving QSs is provided below (Section 2.6.1), along with details of the quality assessment of data (Section 2.6.2), and the identification of 'critical' and 'supporting' data (Section 2.6.3).

2.6.1 Types of data required for deriving QSs

2.6.1.1 Data on physical and chemical properties

Properties that can be very important when interpreting laboratory and field ecotoxicity are: water solubility, vapour pressure, photolytic and hydrolytic stabilities, and molecular weight (when

assessing risks of bioaccumulation). Such data will make it clear when steps to control exposure concentrations in ecotoxicity experiments are particularly important. This, in turn, helps assess how reliable a toxicity study is (Section 2.6.2). In addition, partition coefficients are needed when deriving a sediment QS using a EqP method, as also to conduct transformation calculations (e.g. from mass/volume [mg/L] to mass/mass [mg/kg]). These coefficients (K) include, for example: Koctanol-water (K_{ow}), K suspended particulate matter – water ($K_{susp-water}$), K sediment – water ($K_{sed-water}$), K organic carbon (K_{oc}).

2.6.1.2   Ecotoxicological data

According to Annex V of the WFD, the base set of taxa that should be used in setting quality standards for water are algae and/or macrophytes, *Daphnia* (or representative invertebrate organisms for saline waters), and fish, both in relation to water column standards. For sediment QSs, the range of species should be expanded to include benthic species (Section 5). However, for the purpose of quality standard setting, the data should not be restricted to this base set. **<u>All</u> available data for <u>any</u> taxonomic group or species should be considered, provided these data meet quality requirements for relevance and reliability** (Section 2.6.2). This may include data for alien species and even exotic species[6], although care should be taken with data generated from experiments using species from extreme environments (e.g. thermophiles, halophytes).

If there are indications of endocrine activity (e.g. bioassays), but no studies are available that allow assessment of adverse effects through this mechanism, this should be highlighted as an uncertainty in the technical report.

Often, multiple data are available for the same species and endpoint (e.g. several studies assessing acute toxicity to *Daphnia*). Unless there is a clear reason for differences between toxicity (e.g. different test conditions, different exposure periods, different life stages or forms of the substance tested, like different metal species), any variation in toxicity may simply reflect random error and the valid data may be aggregated into a single value for each species and endpoint. Detailed guidance on data aggregation is given in Appendix 1

Finally, using ecotoxicological data to derive QSs for metals requires additional considerations. These are dealt with in detail in the relevant sections.

2.6.1.3   Mammalian toxicity data

QSs to protect human health utilise information about effects on mammals from oral exposure, repeated dose toxicity, carcinogenicity, mutagenicity and effects on reproduction, typically No Observable Adverse Effect Level (NOAEL), Acceptable Daily Intake (ADI) and Tolerable Daily Intake (TDI) values identified in the human health section of risk assessments performed under the REACH regime. Oral Reference Doses (RfD), ADI or TDI values adopted by national or international bodies such as the World Health Organization may also be used. For some substances, a threshold level cannot be established (e.g. some genotoxic carcinogens). For these, risk values corresponding to an additional risk of, e.g. cancer over the whole life of 10^{-6} (one additional cancer incident in 10^6 persons taking up the substance concerned for 70 years), may be used, if available.

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[6] This is because test species not only represent species that occur in European waterbodies but taking them into consideration will ensure that a range of sensitivities is represented in the dataset, so that that any resulting QS is more likely to protect the range of species sensitivities found in nature.

To assess the risk of secondary poisoning of predators, bird and mammal toxicity data are also used. Further details are to be found in Appendix 1.

2.6.1.4   Data on bioaccumulation

Data on bioaccumulation (bioconcentration, biomagnification and/or the octanol-water partition coefficient (K_{ow})) are required if a substance has a potential to bioaccumulate (i.e. it exceeds the trigger-values given in Section 2.4.2). Where data are available that give different indications of bioaccumulation potential, preference should be given to field observations on bioaccumulation and biomagnification factors (BAFs, BMFs), or to experimentally derived BCFs and BMFs (and TMFs – Trophic Magnification Factor), if available.

Further details on how to obtain and evaluate data on bioaccumulation can be found in Appendix 1.

2.6.2   Quality assessment of data

A rigorous assessment of the data is needed to ensure that data are **reliable** and **relevant**. This will normally entail a review of the original study report(s), especially for critical data that are likely to have a major impact on the QS (Section 2.6.3).

Reliability refers to the inherent quality of the method used to conduct the test. A reliable study requires all relevant details about the test to be described. **Relevance** means the extent to which a test provides useful information about the hazardous properties of a chemical. **Only data that are both reliable and relevant should be considered valid for use in setting a quality standard.**

Appendix 1 goes into more detail about the quality assessment of data used to derive an EQS. It explains that assessment may be performed according to the scheme developed by Klimisch et al. (1997) or CRED (Moermond et al., 2016). The Klimisch system is a long-established one that is also used in other chemical assessment regimes, but CRED offers the ability to further assess relevance of aquatic ecotoxicity data in addition to the reliability criteria. Appendix 4 offers more detailed guidance about the CRED approach to assess data quality, and it summarises the results of the comparison between the Klimisch and CRED evaluation methods, which were compiled in a ring test by experienced risk assessors (Kase et al. 2016).

2.6.2.1   Reliability

Guidance on the principles of data validation and the aspects to be considered is given in Appendix 1. A score is assigned to the data according to the reliability of the study.

Further assessment of data generated or assessed under Community legislation such as Regulations (EEC) 793/93 and (EC) 1488/94 (existing chemicals, now replaced by REACH) or Directives 91/414/EC (plant protection products, now replaced by Regulation (EC) No 1107/2009) or 98/8/EC (biocide products, now replaced by Regulation (EU) No 528/2012) is required unless the data published in the risk assessment reports under these legal frameworks have already been subjected to data quality assurance controls and peer-review. The same applies to peer-reviewed data or guidance values (e.g. Tolerable Daily Intakes or Drinking Water values) published by (inter)national organisations such as the World Health Organization (WHO), the United Nations Food and Agriculture Organization (FAO), the Organisation for Economic Development (OECD) or the OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic.

Studies on pesticides may be performed on technical material or formulated product. Preference is given to data using technical material because toxicity of the active ingredient is less prone to modification by other formulation ingredients, but specific guidance on treatment of ecotoxicological data for pesticides when formulations have been tested is given in Appendix 1. Not all studies on plant protection properties are suitable for EQS derivation because the exposure

regimes are sometimes very short to simulate specific exposure scenarios (mesocosm studies for example).

Studies that have been performed according to 'Good Laboratory Practice' (GLP) are also subject to review but, if they have already been reviewed by a competent authority they may be accepted without further assessment. An exception to this would be if ecotoxicity studies submitted as part of a regulatory dossier have been performed in such a way that they might not be relevant to QS derivation e.g. unusual exposure regimes, or if the test duration is very short.

Detailed guidance for the selection of data to be used for standard setting is provided in Appendix 1, but the principles are highlighted here:

1. Only data that can be considered as reliable may be used, irrespective of the source of the data. **Admissible data are <u>not</u> confined to GLP studies**.

2. Data should be collated into a database with quality scores clearly assigned to each datum. **Only those considered as reliable (see Appendix 1, section "Toxicity data") should be used as 'critical' data (Section 2.6.3) in deriving an EQS**.

3. If a QS for a particular receptor cannot be derived because the required data are lacking, this should be flagged.

Again, metals data require additional considerations, and these are covered in Section 2.10.

2.6.2.2   Relevance

A study can be well conducted and fully reported, but the test endpoint may have little ecological significance. The studies used for EQS derivation should be those in which the test endpoints can be related to ecologically significant hazards. For practical purposes, this means effects that can be linked to population sustainability, and particularly:

a. survivorship of adults,

b. time taken to develop (particularly to reach reproductive age),

c. reproductive output.

Most standard test methods include one or more of these endpoints. However, the assessor may face data from studies describing endpoints that do not include direct measurements of survival, development or reproduction, but, rather, describe e.g. behavioural effects, anatomical differences between control and treatment groups, effects at the tissue or sub-cellular level, such as changes in enzyme induction or gene expression. Generally, these are unsuitable as the basis for EQS derivation. However, some other endpoints are relevant. For example, anatomical changes in gonad development that would prevent successful reproduction, or changes in behaviour if the effect described would impair competitive fitness may be relevant. Avoidance reactions may also be relevant if populations are likely to avoid a contaminated habitat where they would normally be present. Further examples are given in Appendix 1.

2.6.3 'Critical' and 'supporting' data

Not all data have an equal influence on QS derivation. **Critical data** are ecotoxicity data (typically NOECs/EC10s or LC/EC50) for sensitive species and endpoints that are used as the basis for extrapolation and hence determine – or strongly influence - the value of the QS. Section 3 details the various approaches for extrapolation, in particular the deterministic and probabilistic methods. Critical data play a key role where a deterministic approach to extrapolation is used (i.e. an AF is applied), because the AF is applied to the lowest credible NOEC/EC10 or LC/EC50 (the critical

datum). If a species sensitivity modelling approach is adopted, a distinction between critical and supporting data does not apply. This is because all the data are used in the model extrapolation and so, all the data can be regarded as critical (as long as they are reliable and relevant).

Supporting data are those data that are not described as critical data. They include data that are not among the most sensitive species/endpoints, studies that have estimated a non-standard summary statistic, e.g. a LOEC is reported but not a NOEC, field or mesocosm experiments that are difficult to interpret, or where a study might be sound but is not fully reported. Supporting data are not used directly for QS derivation when using the deterministic approach, but can help inform the derivation of the QS by, for example, identifying sensitive taxa, determining if freshwater and saltwater datasets can be combined for QS derivation, averaging or aggregating the data in order to identify the critical data, and selecting an appropriate AF. All reliable and relevant data are used when deriving a QS using the probabilistic approach, i.e. SSDs. **Experiments that are clearly flawed should not be used in any way, even as supporting data.**

It is essential that all available toxicity data, both critical and supporting, are subject to rigorous quality assessment and are comprehensively reported as all data may be used, e.g. in the derivation of an SSD, for aggregation of data for the same species and endpoint and for comparison of fresh and saltwater data. Further guidance can be found in Appendix 1.

2.6.4 Data gaps – non-testing methods

A lack of experimental data can lead to high uncertainty in the derivation process, possibly resulting in over-precautionary QSs. Whilst the generation of well-targeted experimental data can be critical in helping reduce uncertainty, it can also be expensive and time-consuming. Under these circumstances there is a useful role for computational methods to fill data gaps, including quantitative structure–activity relationships (QSARs) for predicting toxicity and quantitative structure-property relationships (QSPRs), to estimate physicochemical properties. 'Read across' approaches can also be useful to infer the properties of chemicals for which data are absent, based on the properties of closely related analogues. Such approaches are now recommended in chemical risk assessment; notably chemical regulation activity and the effort to reduce animal testing under REACH may lead to an increased regulatory acceptance of this type of information and new tools for deriving non-test data. The use of QSARs to predict toxicity has been examined in the following European research projects:

- DEMETRA (Emilio Benfenati: Quantitative Structure-Activity Relationships (QSAR) for Pesticides Regulatory Purposes; Elsevier, 2007, ISBN: 978-0-444-52710-3): Prediction of five eco-toxicological endpoints: Acute toxicity trout, daphnia, quail (oral and dietary exposure), and bee

- CAESAR http://www.caesar-project.eu/: Prediction of five toxicological endpoints: Bioconcentration factor, skin sensitisation, carcinogenicity, mutagenicity, developmental toxicity

Detailed guidance on non-testing approaches is given in section 6 but possible applications are briefly summarised below.

2.6.4.1   Predictive models (QSARs, QSPRs)

The most likely application for computational methods is to fill non-critical data gaps (Section 2.6.4) in the dataset for acute aquatic toxicity, especially when a deterministic assessment is to be followed. It is vital that computational methods are used within their legitimate operating domains; further guidance on QSARs and their use is given in Section 6.

2.6.4.2   Analogue approaches

Further non-testing methods include 'read across' and 'category' approaches. The most likely application of read-across is to fill data gaps, when the setting of a QS for mixtures, e.g. polyaromatic hydrocarbons (PAHs) is preferred compared to the QS for individual substances (Section 2.7).

Section 6 outlines another approach for inferring the properties, including ecotoxicological properties, of substances for which data are lacking. Essentially, it uses a category building approach in which chemical analogues are arranged by some physicochemical property (e.g. log Kow) and data from close neighbours are used to fill data gaps by interpolation. The approach can have value in demonstrating that additional AFs are not justified when using data for one substance to derive a QS for another closely related one. However, the following criteria must be met:

- There is a consistent and reliable trend within a category that is relevant to the endpoint of interest (e.g. log K_{ow} increases as ecotoxicity increases)

- If toxicity is the endpoint of interest, reliable measured toxicity is needed to identify the most sensitive trophic group

- Reliable measured data for the endpoint of interest, allowing interpolation to a value for the substance of interest (i.e. where there is a data gap)

- QSARs may be used to support read across but cannot be used to replace measured values

Predictive and analogue methods may be used for generating supporting data but are not suitable for predicting toxicity to be used as critical data. Furthermore, the range of substances to which these models can be applied is limited to chemicals with certain physicochemical and mode of action properties and are not suitable for all substances.

2.7   Calculation of QSs for substances occurring in mixtures

Aquatic organisms may be exposed to a combination of chemicals. The current guidance addresses those mixtures that are known to occur e.g. when they occur in the same product (e.g. many pesticides) or as a result of a particular process (e.g. PAHs following combustion). Some mixtures are intentionally emitted with a known and largely constant composition, but change after their entry into the environment, for example pesticide and biocide preparations. Other mixtures are released with a partly unknown, reasonably constant composition, but that may also change after entering into the environment. In such circumstances an EQS for mixtures of substances may be preferable to deriving EQSs for the individual constituent substances. Section 7 provides guidance on the approaches that can be adopted if a mixture-based approach is preferred.

2.8   Using existing risk assessments

In the interests of economy and consistency, it is sensible to utilise existing assessments, or at least the data on which they are based. As noted in section 1, the effects assessments conducted for chemical and pesticide risk assessments share many of the same principles and practices as those used to estimate a QS. Sections 2.8.1 and 2.8.2 provide guidance on the use of such assessments as a basis for deriving QSs, when they are available.

2.8.1 Risk assessments under Existing Substances Regulations (ESR)

For some industrial chemicals, detailed evaluations and risk assessments will already have been prepared under Regulation (EC) No. 793/93 or Directives 98/8/EC, and then have been published as Risk Assessment Reports (RARs). We recommend that the Predicted No Effect Concentrations

(PNECs) derived from this process are normally adopted as QSs because the assessments and associated data will have undergone thorough peer review. This also promotes consistency between chemical assessment and control regimes.

However, there are some circumstances that could prompt a review of the RAR PNEC, including:

- If new, potentially critical, ecotoxicity data (i.e. sensitive species or endpoints) has become available since the publication of the RAR.

- If there is new evidence for a mode of toxic action that was not considered in the RAR e.g. new evidence of endocrine disrupting properties.

- Where species sensitivity distribution modelling has been used for extrapolation, there can sometimes be finely balanced arguments about the size of the AF applied to the HC5 to account for uncertainty. For example, where the PNEC for a metal is close to natural background levels, this would encourage a review of uncertainties and how best to account for them so that a compliance assessment regime for the EQS can be practically implemented.

2.8.2 Pesticide risk assessments under Regulation 1107/2009

Many pesticides currently on the EU market have been reviewed under the Plant Protection Products Regulation (1107/2009) or its predecessor Directive (91/414/EEC). These reviews include an assessment of freshwater ecotoxicity data. The data are peer-reviewed by a competent authority, and they usually follow standard (OECD) test methods and are performed under GLP; so that these studies are fully auditable. Non-regulatory data, i.e. data that do not conform to GLP and were not covered by the dossier submitted to the regulatory body may also be included in the review. However, some aspects of risk assessment under 91/414/EEC and 1107/2009 are different to the approaches taken under REACH to derive PNECs and on which the derivation of EQSs is based. For example:

- The 91/414/EEC and 1107/2009 assessments are based on a field margin ditch scenario close to the point of application, which would not normally apply under the WFD, which seeks to provide protection to all waterbodies, including lakes, rivers, transitional and coastal waters.

- The 91/414/EEC and 1107/2009 assessments give the possibility to consider the recovery under certain conditions. This possibility does not feature at all in the Annex V methodology under the WFD.

- Under 91/414/EEC the risk is expressed as a Toxicity Exposure Ratio (TER), based on a direct comparison of toxicity values (without assessment factors) to predictions of concentrations in the environment (PEC). Hence, the risk assessment methodology under 91/414/EEC does not use AFs applied to the toxicity side of the risk equation, but to the risk quotient, yielding a TER. However, in the guidance document published for use under 1107/2009, the higher tier studies regulatory acceptable concentrations (RAC) are mentioned and use AF (e.g. on a NOEC from a mesocosm study).

- Algal toxicity data are dealt with differently under REACH and 91/414/EEC. This can lead to different outcomes when the algae study is the critical data that determines the threshold (Lepper, 2005). This hasn't changed in 1107/2009.

- Under 91/414/EEC and 1107/2009, acute toxicity data are never used to extrapolate to chronic toxicity values; risk assessment for chronic exposure is carried out using only chronic toxicity data because this is a minimum requirement for registration.

Although a risk assessment under 91/414/EEC or 1107/2009 should not be used directly to set a QS, the list of endpoints produced for the review process and published on the

internet by the Commission, provides a valuable data set. These data must, however, be supplemented with other ecotoxicity data where they are available, and also meet quality criteria.

2.9 Extrapolation

The derivation of all QSs requires some form of extrapolation from the available data to estimate a threshold that takes account of uncertainties such as inter- and intra-species variations and laboratory to field extrapolation.

Two main approaches are possible, the deterministic and probabilistic methods. Essentially the deterministic approach takes the lowest credible toxicity datum and applies an AF (which, in principle, may be as low as 1 or as high as 10000) to extrapolate to a QS, the AF allowing for the uncertainties in the available data. Probabilistic methods adopt a species sensitivity distribution (SSD) modelling in which all reliable toxicity (usually NOEC) data are ranked and a model fitted. From this, the HCx, which is the concentration at which the EC10 or NOEC will be greater for a certain proportion of species (typically 95%, HC5) can be estimated. A smaller AF (1-5) would then normally be applied to the HC5.

Laboratory data are used to derive QSs that account for direct toxicity of chemicals to pelagic and sediment-dwelling organisms. The probabilistic approach is preferred but, where there are insufficient data for a probabilistic approach, a deterministic approach is adopted (Section 3). Where there are sufficient data, both deterministic and probabilistic approaches to extrapolation will normally be performed (Section 3). Species sensitivity distribution models explicitly account for differences in sensitivity between species but, as Section 3 explains, a further AF is applied to the HC5 arising from model extrapolation to account for 'residual' uncertainties that are not accounted for by the SSD model. In a deterministic approach, larger AFs are typical, depending on the quantity and type of data available.

The purpose of these AFs is to account for the uncertainty that is not accounted for already in the experimental toxicity data or modelling (in the case of a probabilistic assessment). A basic principle of extrapolation is that, where uncertainty is high, larger AFs are necessary. Guidance on the size of these AFs is given in Section 3. The REACH guidance makes clear the possibility of flexibility in the size of the AF but any change from the 'default' AF (either to increase it, making the QS more stringent or to decrease it, making the QS less stringent) should be justified.

Useful lines of evidence that may be used to inform the extrapolation (and possibly influence the size of AF applied) include mode of action data, effects' data from the field, and background concentration data for naturally occurring substances, as outlined below.

2.9.1 Mode of action

If there are indications of adverse effects via endocrine activity (e.g. *in vivo* bioassays) or other specific effects that have not been adequately reflected in bird or mammal studies used to derive the NOAEL$_{oral}$ (e.g. only 28-day studies are available), an additional assessment factor may be considered to cover the anticipated effects.

On the other hand, uncertainty is reduced when there are relevant test endpoints from ecotoxicity studies that are highly relevant to a substance's mode of toxic action. An example would be fish life cycle studies for a chemical that is known to affect the reproductive physiology of vertebrates. Similarly, if a substance has a specific mode of toxic action, and reliable data for taxa that would be expected to be particularly sensitive are available (e.g. data for a range of insects for an insecticide that acts by inhibiting acetyl cholinesterase activity, or data for blue-green algae when dealing with chemicals that have bactericidal properties) then, again, an important aspect of uncertainty is reduced. Under these conditions, a smaller AF than the default value may be justified.

It follows that uncertainty may be <u>increased</u> if data for sensitive taxa are missing when dealing with substances with a specific mode of action like insecticides, herbicides or antibiotics. Under these circumstances, an AF larger than the default may be warranted.

2.9.2 Field and mesocosm data

Annex V of the WFD states that:

> *"…the standard thus derived should be compared with any evidence from field studies. Where anomalies appear, the derivation shall be reviewed to allow a more precise safety factor to be calculated."*

Field data, whilst rarely being suitable as the critical data for deriving a QS, can be used to corroborate (or challenge) the choice of AF. Crane et al. (2007), describe techniques for estimating a field threshold based on chemical exposure and biological data from matched locations and sampling occasions in the field. Field data also have a key role in deriving sediment standards (Section 5.2.1.3). In principle, where there is evidence of a mismatch, this would prompt consideration of the reasons why there is a discrepancy between the QS derived using laboratory data and experience in the field. Given the variability in field data (and indeed in laboratory ecotoxicity data), small differences between a laboratory-based QS and field data should not be given undue weight. We suggest that differences larger than an order of magnitude would, however, warrant further investigation and, if justified, a revision of the AF.

Mesocosm studies such as experimental pond or stream systems can also provide a useful line of evidence when choosing a suitable AF. Mesocosm studies usually employ only a single contaminant stressor but biological impacts seen in the field may be attributable to several stressors, including non-chemical stressors. This can impair interpretation of matched chemical and biological data. However, if a 'one-sided' analysis is undertaken, i.e. calculate the maximum concentration that still permits a good biological quality, the resulting threshold will be a conservative estimate. Analysis of mesocosm or field data may suggest the laboratory-based QS is over-protective (the QS based on laboratory data is lower than the field threshold) and, under these circumstances, Annex V of the WFD would encourage the use of a reduced AF. However, if the laboratory data do not include species that are known to be sensitive to the contaminant, a reduction of the AF cannot be justified.

2.9.3 Background concentrations

Another line of evidence that could affect the final QS is information about background levels for naturally occurring substances, e.g. metals and some organics, which occur widely in nature, e.g. polycyclic hydrocarbons and some cyanides. The size of the AF should not normally result in a QS that is below the natural background level (this is particularly relevant for metals – see 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 policymakers. Under these circumstances, it may be cost-effective to commission new studies to address the uncertainty.

2.10   Dealing with metals

2.10.1 Why metals are different

Unlike most organic substances, metals are neither created nor destroyed by biological or chemical processes; instead, they are only transformed from one chemical form into another one. Because metals are naturally occurring, many organisms have evolved mechanisms to regulate their accumulation and storage. Moreover, some metals are essential nutrients, so, when they are not present in sufficient concentrations, they can limit growth, survival and reproduction of the

organisms. Excess amounts of certain metals, on the other hand, are potentially toxic. Table 1 summarises the essentiality status for some environmentally relevant metals.

These features, along with the fact that metals naturally occur as inorganic forms in environmental compartments (e.g. sediments) and are cycled through the biotic components of an ecosystem, complicate the evaluation of toxicity data for inorganic metal substances and have a major influence on the way we derive QSs for metals.

Table 1 Essentiality of metals and metalloids to living organisms

Essential	Non-essential
Cr, Co, Cu, Fe, Mn, Mo, Ni, Se, Zn	As, Sb, Cd, Pb, Hg, Tl, Ag, Sn

When evaluating toxicity data to derive quality standards for metals, total metal concentrations are not usually directly related to ecotoxicological effects because many abiotic and biotic processes can modify the *availability* of metals, even rendering them unavailable for uptake. This means that the fraction available for uptake and toxicity may be a very small part of the total metal present. Due to several physicochemical processes, metals exist in different chemical forms which might differ in (bio)availability. Thus, the (bio)availability of metals in both laboratory tests and in the 'real' environment may be affected by several physicochemical parameters such as the pH, hardness of water and the dissolved organic carbon (DOC). Organic carbon (OC) and sulphides' levels are key influencing factors for the sediment compartment. As geographically distinct watersheds show distinct geochemical characteristics, the degree to which different aquatic systems can safely accommodate metal loadings will vary. For this reason, ecotoxicity data, derived for the same species and same endpoint may vary widely when artificial/natural waters or sediments are used as test media.

The Water Framework Directive explicitly acknowledges the issues of (bio)availability and naturally occurring concentrations for metals. The Daughter Directive to the WFD on EQSs (2008/105/EC) (EC, 2008) states in Annex I, part B.3:

Member States may, when assessing the monitoring results against the EQS, take into account:

> *(a)   natural background concentrations for metals and their compounds, if they prevent compliance with the EQS value; and*

> *(b)   hardness, pH or other water quality parameters that affect the bioavailability of metals.*

Ideally, the derivation of QSs for metals requires an explicit consideration of (bio)availability using speciation models or, failing that, to utilise dissolved concentrations instead of total concentrations. Background concentrations may also need to be taken into account.

Specific guidance on deriving EQSs for metals is provided in Section 3.5.

2.11   Expression and implementation of EQSs

2.11.1 Accounting for exposure duration

Depending on the release pattern of a chemical and its environmental fate, chemical exposure may occur over long periods - or even continuously - in biota, in sediments, and even in the water column. In the water column, exposure may also occur intermittently for short periods e.g. coinciding with storm events or short periods of chemical use.

In order to cover both long- and short-term effects resulting from exposure, two water column EQSs will normally be required:

(i) **a long-term standard, expressed as an annual[7] average concentration (AA-EQS) and normally based on chronic toxicity data,**

and

(ii) **a short-term standard, referred to as a <u>m</u>aximum <u>a</u>cceptable <u>c</u>oncentration EQS (MAC-EQS) which is based on acute toxicity data.**

Where EQSs are derived for biota and sediment, they are always expressed as a long-term standard. **It is not appropriate to derive a short-term standard for these compartments because exposure will typically be over long periods of time.**

2.11.2 Expression of EQSs for water

The overall EQS for water that is derived as described above is expressed as a dissolved concentration. Water column EQSs may also be expressed as a total (dissolved + particulate) concentration or concentration associated with SPM. In most cases the dissolved concentration will be preferred. However, for substances that are highly adsorbed to suspended matter the EQS might be based on suspended matter concentrations, which can be more appropriate for calculating substance fluxes in river systems. For such substances, this may be preferable to expressing the EQS as a total water concentration because this is dependent on the highly variable suspended matter concentration in water (which is a function of seasonality, turbidity and so on) and so may be highly uncertain. Emission controls are usually based on total concentrations in discharges too. When faced with such situations, the assessor should agree on the preferred method of EQS expression/compliance assessment with policy makers or river basin managers.

2.11.3 Introducing a new EQS – the role of scientific assessment

Although uncertainty is taken into account during extrapolation through the use of modelling and/or AFs applied to critical data, small datasets invariably lead to greater uncertainty in the EQS. Under some circumstances, the policymaker responsible for implementing a standard may decide that a standard is too uncertain to be used in a statutory context, i.e. the policymakers may decide the risks of implementing an imprecise standard outweigh any benefits, or that it is only appropriate to use the EQS in an advisory context. As explained earlier, the role of the scientist deriving an EQS is to advise the policymakers on the nature and importance of unresolved uncertainties, and the steps that could be taken to resolve them (e.g. conducting further ecotoxicity tests), so that decisions about how to implement the standard can be made in an informed way.

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[7] When the exposure pattern for a substance is known to be episodic, e.g. many pesticides, the averaging period may be a shorter period than a year. This is case-specific but is determined by the expected exposure pattern, not toxicology (EC 2000/60/EC)

3 STANDARDS TO PROTECT WATER QUALITY

3.1 General approach

QSs for the protection of **pelagic communities** (organisms inhabiting the water column) are required for all substances. This section covers the protection of freshwater and saltwater pelagic communities from both long-term and short-term exposures, as well as those in transitional waters. In addition, this section also covers the assessment of **risks to human health from drinking water**.

For the water column, four different QS values can be derived:

- A QS based on direct ecotoxicity ($QS_{fw, eco}$ or $QS_{sw, eco}$ (Section 3.2),

- A QS based on secondary poisoning of predators ($QS_{biota\ sec\ pois\ fw}$ or $QS_{biota\ sec\ pois\ fw}$)[8] (Section 4.4),

- A QS based on human consumption of fishery products ($QS_{biota,\ hh\ food}$)[7] (Section 4.5)

and

- A QS for human consumption of drinking water ($QS_{dw,hh}$) (Section 3.7)

As explained in Section 2.4.3, the $QS_{biota,\ sec\ pois}$ and $QS_{biota,\ hh}$ only need to be derived if specific trigger values are met. The lowest of these values is set as the overall EQS, although the drinking water standard is only adopted as an overall standard for waters intended for drinking water abstraction.

As explained in Section 2.5.1, in order to select an overall EQS, it will be necessary to translate biota and human health standards into an equivalent water concentration, so they can be compared directly with other water column QSs. Some jurisdictions may also prefer to assess compliance with these standards by sampling the water column rather than biota. The conversion of biota QSs into their equivalent water column concentrations is covered in Section 4.7.2.

The specific requirements to derive the water column standards for metals are dealt with in Section 3.5.

3.2 Derivation of QSs for protecting pelagic species

3.2.1 Relationship between water column QS and MAC-QS

As explained in Section 2.11, two QSs are required for the water compartment to cover both long-term and short-term exposures to a chemical:

(i) an annual average concentration (QS) to protect against the occurrence of prolonged exposure, and

(ii) a maximum acceptable concentration (MAC-QS) to protect against possible effects from short term concentration peaks. The temporary standard during derivation is termed MAC-QS in order to distinguish this value from the QS mentioned in (i)

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[8] The $QS_{biota,\ sec\ pois}$ and $QS_{biota,\ hh\ food}$ are based on biota standards and are unlikely to be implemented as annual average concentrations in practice. They may be converted to equivalent water concentrations, e.g. to set an overall EQS or to enable compliance assessment using water samples instead of biota sampling.

Whilst derivation of the QS typically employs chronic toxicity data, the MAC-QS always relies on acute data. When data are sparse or the ratio between acute effects and chronic no-effects is narrow, the estimated MAC-QS can sometimes be more stringent than the QS. It is also possible that the effects observed in chronic studies are due to the initial contact with the test substance, rather than to prolonged exposure. In that case it is also reasonable that the MAC-QS and QS are similar. When the MAC-QS is lower than the QS, a further analysis should be presented in which the possible causes are discussed. When acute and chronic critical data for the QS derivation relate to the same species, and the acute L/EC50 is lower (more stringent) than the chronic EC10 or NOEC, the data should be re-evaluated and justified, and/or an EC10 should be derived instead of a NOEC to derive the QS if the statistical analysis to derive the NOEC has insufficient discriminating power. Since the effects of chronic exposure normally occur at lower concentrations than those of acute exposure, MAC-QS values below the QS make little toxicological sense. **Therefore, where the derivation of the MAC-QS leads to a lower value than the QS, the MAC-QS is set equal to the QS for direct ecotoxicity**. This is summarised below in Table 2.

Table 2 Summary of MAC-QS recommendation based on relationship with QS for direct ecotoxicity

Relationship between estimated AA and MAC	Recommendation
MAC-QS < QS	Set MAC-QS equal to AA-QS.
MAC-QS > AA-QS	Derive MAC-QS.

3.2.2 *Preparing aquatic toxicity data*

Aquatic toxicity data are the key inputs to the derivation of water column standards for direct ecotoxicity. Before the assessor can derive QSs the available data must be properly assessed for reliability and relevance. This is because all data contribute to the final outcome, especially when a probabilistic analysis (SSD) is performed. Guidance on data quality assessment is detailed in Appendix 1.

Before starting the extrapolation steps, the following steps are also taken:

- Data are aggregated when there are multiple data for the same species and endpoint (Section 2.6.1.2);

- Analyses are performed to see whether freshwater and saltwater data can legitimately be combined. This is covered in detail in Section 3.2.3.

As an aid to properly understanding the available data, the assessor should plot all the data graphically so that he/she can develop (and communicate) an appreciation of the quantity of data and spread of species and effects over a range of concentrations. A convenient way to do this is to separate acute and chronic data for freshwater and saltwater species, rank EC50s, EC10s or NOECs, and simply plot the cumulative ranks against concentration. This can be achieved simply in Excel (or using the ETX programme (Van Vlaardingen *et al.*, 2004)), ideally identifying the different taxonomic groups by different symbols so any particularly sensitive or tolerant taxa become immediately obvious. This presentation helps the understanding of the acute:chronic ratios. It also identifies outliers and different sensitive groups, especially if groups are given different symbols.

3.2.3 Combining data for freshwater and saltwater QS derivation

3.2.3.1 Organic compounds

In principle, ecotoxicity data for freshwater and saltwater organisms should be pooled for organic compounds, if certain criteria are met. **Where the criteria for combining data are met (see below), the pooled datasets are then used to derive both freshwater and saltwater QSs, but with different assessment factors (see Sections 3.3.1 and 3.3.2).**

The presumption that for organic compounds saltwater and freshwater data may be pooled must be tested, except where a lack of data makes a statistical analysis unworkable. In those cases where there are too few data (either freshwater or saltwater) to perform a meaningful statistical comparison and there are no further indications (spread of the data, read-across, expert judgement[9]) of a difference in sensitivity between freshwater vs saltwater organisms, the data sets may be combined for QS derivation.

To enable a robust comparison, it is important that a comprehensive set of data is included. For compounds with a specific mode of action, this should include particularly sensitive taxonomic group(s). This reinforces the need for a search strategy for ecotoxicological data that is as wide as possible.

Where there are sufficient toxicity data in both the freshwater and saltwater datasets to enable a statistical comparison, the following procedure should be followed. The null hypothesis is that freshwater and saltwater organisms do not differ in their sensitivity to the compound of interest; *i.e.* they belong to the same statistical population:

1. All freshwater data are collected and tabulated (note: this data set contains one toxicity value per species). Next, a logarithmic transformation of each of these toxicity values is performed.

2. All saltwater data are collected and tabulated (note: this data set contains one toxicity value per species). Next, a logarithmic transformation of each of these toxicity values is performed.

3. Using an F-test, determine whether the two log-transformed data sets have equal or unequal variances. Perform the test at a significance level (α) of 0.05.

4. A test for differences between the data sets, e.g. a two-tailed t-test where the data are normally distributed (with or without correction for unequal variances, depending on the results of step 3), is performed. Perform the test at a significance level (α) of 0.05[10].

5. Especially for compounds with a specific mode of action, it is important to identify particularly sensitive taxonomic groups and perform a separate statistical analysis for this specific group. If enough data are available to make a comparison for individual or related taxonomic groups (e.g., insects, crustaceans, arthropods, fish, vertebrates), this may help to determine if there are differences between saltwater and freshwater species.

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[9] Information on a closely related compound(s) may be used ('read across') (See Section 6). The toxicity data of the related compound should not be used, but toxicological information or knowledge may be used to underpin conclusions. Any use of information from related compounds should be well documented. This can be especially useful when differences are expected for a compound, but the dataset is too small to perform a meaningful statistical comparison.

[10] Beware of confounding factors. For example: (i) a specific group of organisms might be more sensitive than other organisms, (ii) over-representation of results from one study or species from a specific taxonomic group in one of the two data sets might cause bias in the results. Results of statistical tests become increasingly meaningful with increasing sample size.

When a significant difference in sensitivity cannot be shown, the two data sets remain combined for QS derivation, and the QS$_{fw, eco}$ and the QS$_{sw, eco}$ are derived using the same data set. However, different extrapolations should be used for the two compartments (detailed in Sections 3.3 and 3.4).

When a difference in sensitivity is demonstrated based on toxicity, the freshwater and saltwater data sets should <u>not</u> be pooled and QSs for both compartments should be derived using the respective data sets separately and the appropriate extrapolation method.

3.2.3.2   Metals

Freshwater and saltwater toxicity data for metals should be separated *a priori*. This is because differences in toxicity between freshwater and saltwater species are likely because of differences in metal speciation and bioavailability as well as (osmo)regulation. Datasets should only be combined when there is no demonstrable difference in sensitivity. If metals effects data are expressed as dissolved metal concentrations, freshwater and saltwater sensitivities can be compared to assess whether they can be combined, as described for organic substances (Section 3.2.3.1).

However, when metal bioavailability correction is being considered for the freshwater QS, such correction cannot be extrapolated to the marine environment and therefore freshwater and marine EC10s or NOECs cannot be combined.

3.3   Deriving a QS$_{fw, eco}$

3.3.1 Derivation of a QS for the freshwater community (QS$_{fw, eco}$)

For the derivation of the QS$_{fw, eco}$ combined toxicity data sets (with one toxicity value per species) of freshwater and saltwater species may be used (see Section 3.2.3), if after evaluation of the freshwater and saltwater toxicity data it appears that these data can be pooled. Where data permit, the QS$_{fw, eco}$ is derived in three ways:

1. deterministic approach: assessment factor applied to the lowest credible datum ('AF method', Section 3.3.1.1)

2. probabilistic approach using species sensitivity distribution modelling ('SSD method', Section 3.3.1.2),

and

3. using results from model ecosystem and field studies (Section 3.3.1.3).

The methodology is consistent with the REACH provisions for effects assessment of the substances that are released continuously. In general, the most reliable extrapolation method for each substance should be used, reflecting the available data (taxonomic representation, quality of data, ecological relevance etc). **If the conditions to use the SSD-method for the derivation of quality standards are met, it should always be used. However, a QS should also be derived using the AF method, and, where valid data exist, also using model ecosystems**.

Remaining uncertainty is taken into account by applying an assessment factor. This implicitly means that the resulting QS, whether it is derived using the AF method, the SSD method, or using model ecosystem studies, are all considered reliable. It is possible, however, that the results differ. This should be covered in the report on the derivation of the QS, with an explanation of possible discrepancies in the results and the reason for choosing the final method.

The method used to select the final QS$_{fw, eco}$ remains subject to expert judgement. The SSD gives a robust estimate of the range of sensitivities to be encountered in an ecosystem, but it is still based on single species data, and species-interactions at the ecosystem level are not covered. In the

case of mesocosm or field studies, it is often not possible to disentangle the exact cause-effect relationships, but they may point to long-term effects on the ecosystem that cannot be shown in single-species laboratory studies (*i.e.* indirect effects, predator-prey interactions). The relevance of the ecosystem structures of the available model ecosystem studies is an important consideration. In any case, the SSD, mesocosm or field data should include species that are likely to be sensitive. In practice, field or model ecosystem studies would be used to inform the size of the AF applied to an HC5 from an SSD or to QS derived using the AF method. Further guidance on this point is given in Section 3.3.1.3. If sensitive species are not available, nor represented in the mesocosm studies or field data, the deterministic approach may still be preferred, because it makes greater allowance for uncertainty.

Rarely, there may not be appropriate data for the water column available, but there are suitable tests with benthic studies (e.g. only sediment tests with chironomids for an insecticide). In such a case it might be considered applying the equilibrium partitioning method (section 5.2.1.2) in a reversed way from how it is usually applied. However, in such a case it must be considered whether exposure to the substance is primarily through the aqueous phase. This means that for highly hydrophobic substances, where food ingestion contributes significantly to the exposure, this approach could not be applied.

3.3.1.1   *Extrapolation using assessment factor method*

For substances with small datasets, the deterministic approach or assessment factor method (AF method) is the only realistic option because the data requirements of the SSD method (Section 3.3.1.2) are too demanding. The quantity and types of data available determines the assessment factors used (Table 3). The procedures for estimating an AA-QS$_{fw, eco}$ are the same as in the aquatic effects assessment and the calculation of the PNEC ($\approx$ AA-QS$_{water}$) as described in the guidance prepared for REACH (ECHA, 2008).

If an assessment factor equal to or higher than 100 is used, this implies a high level of uncertainty and it should always be highlighted in a 'residual uncertainty' paragraph in the technical report describing the derivation of the AA-QS$_{freshwater, eco}$, together with possible ways to reduce this uncertainty (e.g. perform an additional toxicity test for a specific species).

When only short-term toxicity data are available an assessment factor of 1000 will be applied to the lowest L(E)C50 of the relevant available toxicity data, irrespective of whether or not the tested species is a standard test organism (see notes to Table 3). A lower assessment factor will be applied to the lowest EC10 or NOEC derived in long term tests with a relevant test organism.

The algal growth inhibition test of the base set, in principle, is a multigeneration test. However, for the purposes of applying the appropriate assessment factors, the EC50 is treated as a short-term toxicity value. The EC10 or NOEC from this test may be used as an additional EC10 or NOEC when other long-term data are available. In general, an algal EC10 or NOEC should not be used unsupported by long term EC10s or NOECs of species of other trophic levels. However, if the short term algal toxicity test is the most sensitive among the short-term tests, the EC10 or NOEC from this test should be supported by the result of a test on a second algal species. The investigations with bacteria (e.g. growth tests) are regarded as short term tests. Additionally, blue-green algae should be counted among the primary producers due to their autotrophic nutrition, i.e. they assume the same status as green algae.

The assessment factors presented in Table 3 should be considered as general factors that under certain circumstances may be changed. In general, justification for changing the assessment factor could include one or more of the following:

- evidence from structurally similar compounds (Evidence from a closely related compound may demonstrate that a higher or lower factor may be appropriate);

- knowledge of the mode of action (some substances, by virtue of their structure, may be known to act in a non-specific manner);
- the availability of test data from a wide selection of species covering additional taxonomic groups other than those represented by the base-set species;
- the availability of test data from a variety of species covering the taxonomic groups of the base-set species across at least three trophic levels. In such a case the assessment factors may only be lowered if these multiple data points are available for the most sensitive taxonomic group.

Specific comments on the use of assessment factors in relation to the available data set are given in the notes below Table 3.

Table 3 Assessment factors to be applied to aquatic toxicity data for deriving a QSfw, eco

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels (fish, invertebrates (preferred *Daphnia*)[11] and algae) (i.e. base set)	1000 [a)]
One long-term EC10 or NOEC (either fish or *Daphnia*)	100 [b)]
Two long-term results (e.g. EC10 or NOECs) from species representing two trophic levels (fish and/or *Daphnia* and/or algae)	50 [c)]
Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, *Daphnia* and algae) representing three trophic levels	10 [d)]
Species sensitivity distribution (SSD) method	5-1 (to be fully justified case by case) [e)]
Field data or model ecosystems	Reviewed on a case by case basis [f)]

a) The use of a factor of 1000 on short-term toxicity data is a conservative and protective factor and is designed to ensure that substances with the potential to cause adverse effects are identified. It assumes that the uncertainties identified above make a significant contribution to the overall uncertainty. For any given substance there may be evidence that this is not so, or that one particular component of the uncertainty is more important than any other. In these circumstances, it may be necessary to vary this factor. This variation may lead to a raised or lowered assessment factor depending on the available evidence. A factor lower than 100 should not be used in deriving a $QS_{fw, eco}$ from short-term toxicity data.
The use of a factor different from 1000 on short-term toxicity data should not be regarded as normal and should be fully supported by accompanying evidence.

b) An assessment factor of 100 is applied to a single long-term result (e.g. EC10 or NOECs) (fish or Daphnia) if this result was generated for the trophic level showing the lowest L(E)C50 in the short-term tests.

If the only available long-term result (e.g. EC10 or NOECs) is from a species (standard or non-standard organism) which does not have the lowest L(E)C50 from the short-term tests, applying an assessment factor of 100 is not considered as protective of other more sensitive species. Thus, the hazard assessment is based on the short-term data and an assessment factor of 1000 applied.

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[11] "Daphnia" is in this document is generally used to mean small crustaceans

However, the resulting QS based on short-term data may not be higher than the QS based on the long-term result available.

An assessment factor of 100 can also be applied to the lowest of two long-term results (e.g. EC10 or NOECs) covering two trophic levels when such results have not been generated from that showing the lowest L(E)C50 of the short-term tests. This should, however, not apply in cases where the acutely most sensitive species has an L(E)C50 value lower than the lowest long-term result (e.g. EC10 or NOECs) value. In such cases the QS might be derived by using an assessment factor of 100 to the lowest L(E)C50 of the short-term tests

.
c)   An assessment factor of 50 applies to the lowest of **two** long term results (e.g. EC10 or NOECs) covering two trophic levels when such results have been generated covering that level showing the lowest L(E)C50 in the short-term tests. It also applies to the lowest of **three** long term results (e.g. EC10 or NOECs) covering three trophic levels when such results have not been generated from that trophic level showing the lowest L(E)C50 in the short-term tests. This should however not apply in cases where the acutely most sensitive species has an L(E)C50 value lower than the lowest long-term result (e.g. EC10 or NOECs) value. In such cases the QS might be derived by using an assessment factor of 100 to the lowest L(E)C50 of the short-term tests.

d)   An assessment factor of 10 will normally only be applied when long-term toxicity results (e.g. EC10 or NOECs) are available from at least three species across three trophic levels (e.g. fish, Daphnia, and algae or a non-standard organism instead of a standard organism).
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. It may sometimes be possible to determine with high probability that the most sensitive species has been examined, i.e. that a further long-term result (e.g. EC10 or NOECs) from a different taxonomic group would not be lower than the data already available. In those circumstances, a factor of 10 applied to the lowest long-term result (e.g. EC10 or NOECs) from only two species would also be appropriate. This is particularly important if the substance does not have a potential to bioaccumulate. If it is not possible to make this judgment, then an assessment factor of 50 should be applied to take into account any interspecies variation in sensitivity. A factor of 10 cannot be decreased on the basis of laboratory studies.[12]

e)   Basic considerations and minimum requirements as outlined in Section 2.6.1.2.

f)   The assessment factor to be used on mesocosm studies or (semi-) field data will need to be reviewed on a case-by-case basis (see Section 3.3.1.3 for further guidance).

Not all circumstances can be dealt with in these footnotes and specific cases may require specific considerations with respect to the choice of the AF. Any deviation from the scheme should be explained. To help with some questions that might arise, further guidance is offered below:

1.   *The base set (acute data for fish,* Daphnia, *algae*) *is complete, but chronic data are only available for one trophic level of the base set*: This relates to footnotes a and b because we have to decide whether to use an AF of 100 applied to chronic data or 1000 applied to acute data. An AF of 100 is applied to the lowest chronic NOEC or EC10 but (a) it has to be either *Daphnia* or fish and (b) the NOEC or EC10 should be from the same trophic level as that of the lowest acute L(E)C50. If (a) and (b) are not the case, an AF of 1000 is applied to the lowest L(E)C50 and the two results are compared: lowest L(E)C50/1000 versus NOEC (or EC10)/100; the lowest value is selected as $QS_{fw, eco}$.

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[12] However, this only refers to the deterministic approach. If the SSD approach is used, which is also based on laboratory data, a lower assessment factor than 10 can be used (1-5).

2. *The base set is complete, but chronic data are only available for two trophic levels from the base set*: This relates to footnotes b and c. An assessment factor of 50 is applied to the lowest chronic NOEC or EC10, if such chronic data are available from two trophic levels from the base set. The trophic levels of the NOECs and/or EC10s should include the trophic level of the lowest acute L(E)C50. If the trophic level for the lowest acute L(E)C50 is not included in the chronic data (NOECs and/or EC10s) then:
   - an assessment factor of 100 is applied to the lowest NOEC or EC10 if the lowest L(E)C50 is higher than the lowest NOEC or EC10;
   - an assessment factor of 100 is applied to the lowest L(E)C50 if the lowest L(E)C50 is lower than the lowest NOEC or EC10.

3. *The base set is complete and chronic data for each of the trophic levels of the base set are available*: This relates to footnotes c and d. An assessment factor of 10 is applied to the lowest chronic NOEC or EC10 if chronic data are available from all three trophic levels of the base set. The trophic levels of NOECs and/or EC10s should include the trophic level of the lowest acute L(E)C50. If acute toxicity data are available for trophic levels not covered in the chronic toxicity data, and the trophic level of the lowest L(E)C50 is not included in that of the NOECs and/or EC10s then:
   - an assessment factor of 50 is applied to the lowest NOEC or EC10 if the lowest L(E)C50 is higher than the lowest NOEC or EC10;
   - an assessment factor of 100 is applied to the lowest L(E)C50 if the lowest L(E)C50 is lower than the lowest NOEC or EC10.

4. *The base set is not complete, because data are missing:* Although the table refers specifically to *Daphnia*, any reliable data for small crustaceans would be acceptable. In practice, Daphnia data will be the most readily available, but other species, such as *Ceriodaphnia*, *Gammarus*, or *Acartia* (in the case of the marine environment), can be considered to fill the gap. A similar approach can be followed when data for algae or cyanophytes are missing, but macrophyte data are present. If there is evidence that the missing trophic level would not be the potentially most sensitive species (*e.g. Daphnia* in case of a herbicide) or when it can be assumed that the available species are potentially sensitive (*i.e.* insect and *Daphnia* data in case of an insecticide, where algae are missing), the assessment scheme can be followed as if the base set were complete.

5. *Insect growth regulators* For this specific type of pesticides, *Daphnia* may not be the most sensitive species. Within the context of pesticide authorisation, it is advised that insects should be tested when for an insecticide the toxicity to *Daphnia* is low (i.e. 48 h EC50 > 1 mg/L, 21-d NOEC > 0.1 mg/L; EC, 2002). This means that where the presence of acute and chronic data for algae, *Daphnia* and fish normally allows for an AF of 10, in this case additional information from insects is considered necessary.

In line with the REACH guidance (ECHA, 2008), data for bacteria representing a further taxonomic group may only be used if non-adapted pure cultures were tested. Studies with bacteria (e.g. growth tests) are regarded as short-term tests. **Consequently, NOECs or EC10 values derived from bacterial studies may not be used in the derivation of the QS$_{fw, eco}$ using assessment factors. The EC50 values from bacterial tests may be used, but they cannot substitute any of the other trophic levels (acute data on algae, *Daphnia*, fish) for completion of the base set**. 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.

Blue-green algae should be counted among the primary producers due to their autotrophic nutrition (ECHA, 2008). Thus, cyanobacteria (blue-green algae or Cyanophyta) belong to the trophic level of primary producers. **This means that data from (both chronic and acute) tests with cyanobacteria are considered as additional algal data and are treated in the same way (*i.e.* if they represent the lowest endpoint, the AF will be based on cyanobacteria, even when data for green algae are present). They can also be used to complete the base set where there are no algal data**.

When there are indications that a substance may cause adverse effects via disruption of the endocrine system of mammals, birds, aquatic or other wildlife species, the assessor should consider whether the assessment factor would be sufficient to protect against effects caused by such a mode of action, or whether a larger AF is needed (Section 2.9.1).

Use of non-testing methods to reduce uncertainty

Emphasis is placed on experimental toxicity data for deriving an EQS. However, non-testing methods (e.g. QSARs, read-across methods) are also available which can be used to predict toxicity of certain organic chemicals and endpoints. They should not be used to generate critical data to derive an EQS; however, predicted data can play a role in reducing uncertainty and thereby influence the size of AF chosen for extrapolation. Detailed guidance on the use of non-testing methods is given in Section 6.

3.3.1.2   Extrapolation using SSDs

Statistical extrapolation in line with the provisions of the REACH guidance (ECHA, 2008), namely the species sensitivity distribution method (SSD), can be used for the derivation of EQSs for water. Extensive information on the backgrounds and use of SSDs is given in Posthuma *et al.* (2001).

To construct an SSD, toxicity data are log-transformed and fitted to a distribution function from which a percentile (normally the 5th percentile; often referred to as the HC5) of that distribution is used as the basis for an EQS. Several distribution functions have been proposed. The US EPA (1985) assumes a log-triangular function, Kooijman (1987), Van Straalen and Denneman (1989) a log-logistic function, and Wagner and Løkke (1991) a log-normal function. Aldenberg and Slob (1993) and Aldenbeg and Jaworska (2000) further refined the way to estimate the uncertainty of the 95th percentile by introducing confidence levels. The log-normal distribution is a pragmatic choice from the possible range of distributions because its mathematical properties are well-described (methods exist that allow for a more in depth analyses of various uncertainties) although others are permissible. The ETX program (van Vlaardingen et al, 2004) uses the Hazen plotting positions when constructing the SSD $(y=(i-0.5)/n)$.

Data requirements

For estimating a $QS_{fw, eco}$ the input data to the SSD should be quality-assessed chronic NOEC or EC10 data according to the criteria recommended in Section 2.6.2. As for deterministic extrapolation, the data should first be aggregated to one toxicity value per species, and statistical comparisons undertaken to decide if freshwater and saltwater data can be pooled. In practice, the same dataset is used for both the deterministic and probabilistic methods.

Ideally the dataset for an SSD should be statistically and ecologically representative of the community of interest (Posthuma *et al.*, 2001). An EQS should be protective for the wide range of surface waters and communities that can occur within Europe. Given this broad scope of protection of the WFD, the requirements of the REACH guidance with respect to the number of taxa and species to be included in the dataset (ECHA, 2008) are followed, i.e. the output from an SSD-based QS is considered reliable if the database contains preferably more than 15, but at least 10 NOECs/EC10s values, 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 phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.);

   • An order of insect or any phylum not already represented;

   • Algae or Cyanobacteria;

   • Higher plants.

<u>SSDs for substances with a specific mode of action</u>

For a substance exerting a specific mode of action, SSDs should be constructed using:

(a) the entire dataset (i.e. all taxa, so that the relative sensitivities of taxa can be examined) and

(b) only those taxa that are expected to be particularly sensitive (e.g. for a herbicide acting by photosynthetic inhibition, this would be data for higher plants and algae).

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. If there is clear evidence of a 'break' in the distribution between the sensitive species and the other species, or poor model fit, the HC5 should be estimated using only data from the most sensitive group, provided that the minimum number of 10 datapoints is present. If other evidence is available that indicates there might be a specific sensitive group of species, for example, 'read-across' data from a structurally similar substance, this could also be used.

<u>Testing goodness of fit</u>

Different parametric distributions, e.g. log-logistic, log-normal or others may be used. For example, the Anderson–Darling goodness of fit test can be used in addition to the Kolmogorov-Smirnov-test, to help choose a parametric distribution for comprehensive data sets, because it gives more weight to the tails of the distribution. Further details are given in REACH guidance (ECHA, 2008). The following guidance is offered:

Whatever the model fitted to a distribution, results should be discussed with regards to the graphical representation of the species distribution and the different p-values (probability value: the likelihood of wrongly rejecting a statistical hypothesis when it is true) obtained with each test ($p < 0.05$ means a probability < 5%).

The choice of a distribution function other than the log-normal or log-logistic distribution should be clearly explained.

If the data do not fit any distribution, the left tail of the distribution (the lowest effect concentrations) should be analysed more carefully. If a subgroup of species is particularly sensitive and, if there are sufficient data, an SSD may be constructed using only this subgroup. However, this should be underpinned if possible by some mechanistic explanation e.g. high sensitivity of certain species to this particular chemical.

The SSD method should not be used in cases where there is a poor data fit to all available distributions.

<u>Calculating the HC5</u>

The method of Aldenberg and Jaworska (2000) is considered most appropriate because it enables the calculation of a confidence interval (normally the 90%-interval) for the HC5. This method is used in the ETX-computer program (Van Vlaardingen *et al.*, 2004).
The HC5 according to Aldenberg and Jaworska is calculated as follows:

   Log HC5 = Xm-k*s

Where:

Xm = mean of log-transformed NOEC and EC10 data

k= extrapolation constant depending on protection level and sample size (according to Aldenberg and Jaworska, 2000)

s=standard deviation of log-transformed data

The extrapolation constant k is taken from Aldenberg and Jaworska (2000). Three values are given for k. The 5%[ile] cut-off value (HC5) is calculated with the median estimate for k and, in addition, the confidence limits are calculated using the upper and lower estimates of k.

The median estimate of the HC5 (sometimes denoted as HC5-50) is used as the basis of the QS. SSD modelling deals explicitly with differences in sensitivity between species. According to the requirements set out above, an SSD can only be constructed when data are plentiful but there may still be some residual uncertainty that needs to be accounted for in the final QS. For this reason, the HC5 is divided by an additional AF:

$$QS = HC5 / AF$$

Choice of AF applied to HC5

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):

- 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;

- the 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 the 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);

- the 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.

3.3.1.3 Use of field and mesocosm studies for derivation of the $QS_{fw, eco}$

Field studies and simulated ecosystem studies such as microcosm and mesocosm experiments (e.g. ponds and streams) are frequently used to assess the environmental risks posed by pesticides. They can be 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 such studies are available, and they fulfil the criteria regarding reliability and relevance as defined below, they may be used either as the basis of $QS_{fw, eco}$ derivation or, when an SSD is used, to help select the size of AF applied to

the HC5. This section specifically deals with the use of mesocosm studies for derivation of the $QS_{fw, eco}$. The use of mesocosm data for derivation of the MAC-QS is addressed in Section 3.4.1.3.

Mesocosms

For more detailed guidance on the conduct and evaluation of micro- or mesocosm studies see e.g. Hill *et al.* (1994), Giddings *et al.* (2002) and De Jong *et al.* (2008). The following criteria should be addressed when assessing mesocosm data:

- Adequate and unambiguous experimental set-up;
- Realistic community;
- Adequate description of exposure patterns, especially in the compartment of interest e.g. water column;
- Sound statistical evaluation;
- Sensitive endpoints that are in accordance with the mode of action of the chemical.

Irrespective of the framework under which the studies were originally conducted, these basic principles apply to all simulated ecosystem studies. However, there may be some features that are of particular importance to QS derivation since the objectives of risk assessment under Council Directive 91/414/EEC or its successor regulation 1107/2009 and QSs under the WFD are not entirely compatible. The following points are particularly important:

1. For $QS_{fw, eco}$ derivation, exposure in the test system must be properly characterised. Therefore, a prerequisite for using a field or mesocosm study is that the concentration of the substance is measured over the course of the experiment so that time-weighted average concentrations (TWA) within a well-defined time window can be calculated for persistent active ingredients.

2. All summary statistics (EC50s, EC10s and NOECs), must be related to the respective TWA concentration. It is not acceptable to use the initial concentration as the basis for assessment unless there is evidence that this level of exposure has been maintained.

3. This means that, for $QS_{fw, eco}$ derivation, mesocosm studies with rapidly dissipating compounds (with half-lives of hours) cannot be used unless steps have been taken to replenish the test substance at intervals consistent with the substance's half-life in the environment. For experiments with a repeated pulse application, it should be evaluated on a case-by-case basis whether long-term exposure can be considered to be maintained.

4. In risk assessment of plant protection products, the potential for recovery following removal of the chemical stressor is normally taken into account. This principle does not apply in QS derivation i.e. a temporary impact is not normally tolerated, especially when deriving a $QS_{fw, eco}$ which is intended to protect against long-term exposure when recovery conditions might never actually occur.

5. The scope of protection of an EQS under the WFD is broader than that of the "acceptable concentration" in the risk assessment of pesticides. The EQS must be protective for all types of surface waters and communities, not just the type covered by a particular mesocosm or field study. We therefore need to assess whether the test system can be considered as representative for the full range of waterbodies that might be subject to pesticide exposure. Higher tier (e.g. mesocosm) studies in the context of the pesticide risk assessment are normally focused on shallow eutrophic waterbodies occurring in the immediate vicinity of agricultural areas. An EQS under the WFD, however, must also assure protection for waterbodies that differ significantly from this paradigm, for instance those with a wide range of flow regimes, subject to point source inputs of plant protection products (e.g. formulation plants), occurring in different climatic zones, or with different trophic status. Preferably, the available (semi-)field data should cover this wide range of water types, but in reality this is not

the case and therefore the guidance presented here should be considered when deciding on the choice of the AF (see below).

6. In general, the more similar the test system is to the field situation, the higher its relevance for risk assessment and EQS setting. Differences between experimental mesocosms and the field can result in either an over- or underestimation of the response of the field ecosystem.

- Species composition: more relevant EC10s or NOECs are likely to arise when the species composition in a mesocosm is representative of that found in the field. This does not mean that the species composition in a micro- or mesocosm experiment should be exactly the same as that in the field; it is more important that a sufficient number of representatives of sensitive taxonomic groups are present, especially taxa that are expected to be sensitive given the substance's mode of action (e.g. insect larvae in a study with an insecticide that acts by disrupting moulting). Maltby *et al.* (2005) showed that taxonomy plays a more important role than habitat and geographical region in predicting the sensitivity of water organisms to pesticides with a specific toxic mode of action. Furthermore, the representativeness of the biological traits of the tested species is important. In general, vertebrates are not incorporated in mesocosm studies. If laboratory data suggest vertebrates belong to the most sensitive group, little weight should be given to a mesocosm study without vertebrates.

- Avoidance and drift: examples are known from the literature (for example, *Gammarus pulex*; see Schulz and Liess, 1999) of organisms that detect and avoid toxic substances by moving to areas with lower concentrations. Sessile organisms cannot avoid exposure. Although avoidance and drift are relevant endpoints, in general, laboratory and mesocosm studies do not accommodate avoidance reactions.

Selecting an AF to apply to a mesocosm EC10 or NOEC

According to the REACH guidance, the AF applied to mesocosm studies or (semi-)field data will need to be reviewed on a case-by-case basis (see footnote 'f' of Table 3), but no guidance is given with respect to the *range* of AFs to be applied. Brock et al. (2008) compared micro/mesocosm experiments for several chemicals in which long-term exposure was simulated. They estimated a geographical extrapolation factor based on the ratio of the upper and lower limit of the 95% confidence interval of NOECs for toxic effects. These factors ranged between 1.4 and 5.4. This suggests that, where there is (a) only a single model ecosystem study, and (b) sensitive taxa are included in the study of a compound with a specific mode of action, an assessment factor of 5 would account for variation in the NOECs. When additional, confirmative mesocosm studies are available, the AF may be lowered. Further discussion around the selection of AFs on mesocosm studies is to be found in Giddings et al (2002).

In determining the size of AF to be applied, the following should be considered:

− What is the overall quality of the micro- or mesocosm study/studies from which the EC10 or NOEC has been derived?
− What is the relationship between the mode of action of the investigated substance and the species represented in the available micro- or mesocosm studies? Are sensitive species represented?
− Do the available micro- or mesocosm studies include vulnerable species or representatives of taxonomic groups (e.g. families, orders) of vulnerable species that are part of the aquatic ecosystems to be protected?
− Do the available micro- or mesocosm studies represent the range of flow regimes that should be protected by the EQS? Consider specific populations of species inhabiting the lotic and lentic water types to be protected.

− How representative are the mesocosm studies: do they represent the range of trophic statutes of waterbodies that should be protected by the EQS?

3.3.2 Derivation of a QS for the saltwater pelagic community (QS$_{sw,\ eco}$)

The QS$_{sw,\ eco}$ protects the saltwater ecosystem from potential chronic toxic effects. For the derivation of the QS$_{sw,\ eco}$ combined toxicity data sets (with one single toxicity value per species) of marine and freshwater species may be used when the provisions for pooling data are met (see Section 3.2.3). As with estimation of the QS$_{fw,\ eco}$, the QS$_{sw,\ eco}$ may be derived by several different approaches:

• a deterministic approach using assessment factors applied to a critical datum,

• a probabilistic approach using SSD modelling, and

• using mesocosm data (although field and mesocosm studies are rarely available for saltwater).

3.3.2.1 Extrapolation using the AF method

The procedures for the marine effects assessment as described in the REACH guidance (ECHA, 2008) are adopted here, i.e. specific AFs for marine effects assessment (Table 4) are applied to the lowest credible data (critical data) to derive the QS$_{sw,\ eco}$. The AFs (Table 4) for deriving the QS$_{sw,\ eco}$ are higher than those used for freshwater. This is justified by the need to account for the additional uncertainties associated with extrapolation for the marine ecosystem, especially the general under-representation in the experimental dataset of specific marine key taxa and possibly a greater species' diversity. As a result, the QS$_{,\ eco}$ is often more stringent than the corresponding standard derived for the freshwater environment.

Even when based on the same set of data, the QS$_{sw,\ eco}$ may differ therefore from the QS$_{fw,\ eco}$. Where data are available for additional marine taxonomic groups, the uncertainties are reduced and so the magnitude of the AF applied to a data set can be lowered (Table 4).

Data from studies with marine test organisms other than algae, crustaceans and fish, and/or having a life form or feeding strategy differing from that of algae, crustaceans or fish can be accepted as additional marine taxonomic groups and will allow a reduction in the AF applied (provided that the toxicity data are reliable and relevant). Marine species from taxa other than algae, crustaceans and fish include:

− Macrophyta, e.g. Sea grass (Zosteraceae);
− Mollusca, e.g. *Mytilus edulis*, *Mytulis galloprovincialis*;
− Rotifers, e.g. *Brachyonus plicatilis*;
− Hydroids, e.g. hydroids as *Cordylophora caspia*, *Eirene viridula*;
− Annelida, e.g. *Neanthes arenaceodentata*;
− Echinoderms, e.g. sea urchins as *Arbacia punctulata*, *Strongylocentrotus purpuratus*, *Strongylocentrotus droebachiensis*, *Echinocardium cordatum*, *Paracentrotus lividus, Psammechinus miliaris*, or asteroids as *Asterias rubens*.

In addition, marine organisms that belong to the taxa algae, crustaceans or fish but have a different life form or feeding strategy than the representatives in the freshwater toxicity dataset can be considered additional marine taxonomic groups and may also allow a reduction in the size of the AF:

− Macro-algae, e.g. *Enteromorpha* sp., *Fucus* sp and *Champia* sp;
− Crustaceans (including crabs) are found in both freshwater and marine water. However, crabs, for example, have a life form and feeding strategy very much different from *Daphnia* sp., which is the test organism nearly always present in the freshwater toxicity data set, or other common

freshwater crustaceans. Thus, such species can be used to reduce the AF where other crustaceans may not. Examples of crabs used in toxicity tests include *Cancer magister*, *Cancer pagurus*, *Carcinus maenas* and *Cancer anthonyi*.

Table 4 Assessment factors to be applied to aquatic toxicity data for deriving a QS$_{sw, eco}$

Data set	Assessment factor
Lowest short-term L(E)C50 from freshwater or saltwater representatives of three taxonomic groups (algae, crustaceans and fish i.e. base set) of three trophic levels	10,000 [a)]
Lowest short-term L(E)C50 from freshwater or saltwater representatives of three taxonomic groups (algae, crustaceans and fish) of three trophic levels, plus two additional marine taxonomic groups (e.g. echinoderms, molluscs)	1000 [b)]
One long-term result (e.g. EC10 or NOEC) (from freshwater or saltwater crustacean reproduction or fish growth studies)	1000 [b)]
Two long-term results (e.g. EC10 or NOEC) from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish)	500 [c)]
Lowest long-term results (e.g. EC10 or NOEC) from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing three trophic levels	100 [d)]
Two long-term results (e.g. EC10 or NOEC) from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish) plus one long-term result from an additional marine taxonomic group (e.g. echinoderms, molluscs)	50
Lowest long-term results (e.g. EC10 or NOEC) from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing three trophic levels + two long-term results from additional marine taxonomic groups (e.g. echinoderms, molluscs)	10 [e)]

Notes

General note

Evidence for varying the assessment factor should in general include a consideration of the availability of data from a wider selection of species covering additional feeding strategies/ life forms/ taxonomic groups other than those represented by the algal, crustacean and fish species (such as echinoderms or molluscs). This is especially the case, where data are available for additional taxonomic groups representative of marine species. More specific recommendations with regard to the issues to consider in relation to the data available and the size and variation of the assessment factor are indicated below.

When there are indications that a substance may cause adverse effects via disruption of the endocrine system of mammals, birds, aquatic or other wildlife species, it should be considered whether the assessment factor would also be sufficient to protect against effects caused by such a mode of action, or whether an increase of the factor would be appropriate.

a) The use of a factor of 10,000 on short-term toxicity data is a conservative and protective factor and is designed to ensure that substances with the potential to cause adverse effects are identified. It assumes that uncertainties identified above make a significant contribution to the overall uncertainty. For any given substance there may be evidence that this is not so, or that one particular component of the uncertainty is more important than any other. In these circumstances, it may be necessary to vary this factor. This variation may lead to a raised or lowered assessment factor depending on the evidence available. A factor lower than 1000 should not be used when deriving a QSsw,eco from short-term toxicity data.
Evidence for varying the assessment factor could include one or more of the following:
- evidence from structurally similar compounds which may demonstrate that a higher or lower factor may be appropriate.

- knowledge of the mode of action as some substances by virtue of their structure may be known to act in a non-specific manner. A lower factor may therefore be considered. Equally a known specific mode of action may lead to a higher factor.
- the availability of data from a variety of species covering the taxonomic groups of species across at least three trophic levels. In such a case the assessment factors may only be lowered if multiple data points are available for the most sensitive taxonomic group (i.e. the group showing acute toxicity more than 10 times lower than for the other groups).
Variation from an assessment factor of 10,000 should be fully reported with accompanying evidence.

b) An assessment factor of 1000 is applied where data from a wider selection of species are available covering additional taxonomic groups (such as echinoderms or molluscs) other than those represented by algal, crustacean and fish species; if data are at least available for two additional taxonomic groups representative of marine species.
An assessment factor of 1000 is applied to a single long-term result (e.g. EC10 or NOEC) (freshwater or saltwater crustacean or fish) if this result was generated for the taxonomic group showing the lowest L(E)C50 in the short-term algal, crustacean or fish tests.
If the only available long-term result (e.g. EC10 or NOEC) is from a species which does not have the lowest L(E)C50 in the short-term tests, applying an assessment factor of 1000 is not regarded as protective of other more sensitive species. Thus, the hazard assessment is based on the short-term data with an assessment factor of 10,000 applied. However, normally the lowest $QS_{sw, eco}$ should prevail.
An assessment factor of 1000 can also be applied to the lowest of the two long-term results (e.g. EC10 or NOEC) covering two trophic levels (freshwater or saltwater algae and/or crustacean and/or fish) when such results (e.g. EC10 or NOEC) have not been generated for the species showing the lowest L(E)C50 of the short-term tests.
This should not apply in cases where the acutely most sensitive species has a L(E)C50-value lower than the lowest long-term value. In such cases the $QS_{sw, eco}$ might be derived by applying an assessment factor of 1000 to the lowest L(E)C50 of the short-term tests.

c) An assessment factor of 500 applies to the lowest of **two** long-term results (e.g. EC10 or NOEC) covering two trophic levels (freshwater or saltwater algae and/or crustacean and/or fish) when such results have been generated covering those trophic levels showing the lowest L(E)C50 in the short-term tests with these species. Consideration can be given to lowering this factor in the following circumstances:
- It may sometimes be possible to determine with a high probability that the most sensitive species covering fish, crustacea and algae has been examined, that is that a further longer-term result (e.g. EC10 or NOEC) from a third taxonomic group would not be lower than the data already available. In such circumstances an assessment factor of 100 would be justified;
- a reduced assessment factor (to 100 if only one short-term test, to 50 if two short-term tests on marine species are available) applied to the lowest long-term result (e.g. EC10 or NOEC) from only two species may be appropriate where:
- short-term tests for additional species representing marine taxonomic groups (for example echinoderms or molluscs) have been carried out and indicate that these are not the most sensitive group, and;
- it has been determined with a high probability that long-term results (e.g. EC10 or NOEC) generated for these marine groups would not be lower than that already obtained. This is particularly important if the substance does not have the potential to bioaccumulate.
An assessment factor of 500 also applies to the lowest of three long-term results (e.g. EC10 or NOEC) covering **three** trophic levels when such results have not been generated from the taxonomic group showing the lowest L(E)C50 in short-term tests. This should, however, not apply in the case where the acutely most sensitive species has a L(E)C50 value lower than the lowest long-term result (e.g. EC10 or NOEC) value. In such cases the $QS_{sw, eco}$ might be derived by applying an assessment factor of 1000 to the lowest L(E)C50 in the short-term tests.

d) An assessment factor of 100 will be applied when longer-term toxicity results (e.g. EC10 or NOEC) are available from three freshwater or saltwater species (algae, crustaceans and fish) across three trophic levels. The assessment factor may be reduced to a minimum of 10 in the following situations:
- where short-term tests for additional species representing marine taxonomic groups (for example echinoderms or molluscs) have been carried out and indicate that these are not the most sensitive group, and it has been determined with a high probability that long-term results (e.g. EC10 or NOEC) generated for these species would not be lower than that already obtained;
- where short-term tests for additional taxonomic groups (for example echinoderms or molluscs) have indicated that one of these is the most sensitive group acutely and a long-term test has been carried out for that species. This will only apply when it has been determined with a high probability that additional long-

term results (e.g. EC10 or NOEC) generated from other taxa will not be lower than the long term results already available.

e) A factor of 10 cannot be decreased on the basis of laboratory studies only. It may be permitted if justified by mesocosm or field data.

3.3.2.2 Extrapolation using the SSD approach for deriving an $QS_{sw, eco}$

In principle, for quality standards referring to saltwater, the same approach as described in Section 3.3.1.2 can be used. **Marine and freshwater toxicity data are combined, unless evaluation of the freshwater and saltwater toxicity data shows that the data cannot be pooled. In such a case, the combined data set can be used to establish a common SSD that is relevant for both freshwater and saltwater effects assessment (Section 3.2.3).**

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. This is analogous to the additional AF of 10 for $QS_{sw, eco}$ derivation in the deterministic method. When only one additional marine taxonomic group (as defined above) is present in the dataset, an AF of 5 is used **in addition to** the AF of 1-5. This is consistent with the provisions of REACH for marine effects assessment where a larger AF is recommended to cover the increased uncertainty resulting from the larger diversity of marine ecosystems and the limited availability of effects data for marine life forms.

When freshwater and saltwater datasets cannot legitimately be combined, constructing an SSD with ecotoxicological data for marine organisms has the same requirements regarding the quantity and quality of input data as described in Section 3.3.1.2. However, taxa that are poorly represented in the marine environment, like insects and higher plants, may be replaced by more typical marine taxa such as, e.g., molluscs, echinoderms, annelids, and specific marine species of crustaceans or coelenterata. This means that the additional marine species are automatically present in this non-combined dataset, and no additional AF is needed in addition to the AF of 1-5 applied to the HC5.

3.3.2.3 Use of simulated ecosystem studies for deriving a $QS_{sw, eco}$.

Saltwater mesocosm or field studies can be used for $QS_{sw, eco}$ derivation and the guidance for the freshwater situation (Section 3.3.1.3) also applies here. Marine mesocosm data often apply solely to small pelagic organisms such as calanoid copepods, and such studies will therefore seriously under-represent many taxa, e.g. benthic epifauna. Thus, it should be taken into account how representative the marine mesocosm study is, when determining the assessment factor to be applied and which standard will be selected as final $QS_{sw, eco}$ (i.e. AF method, SSD method or mesocosm).

Freshwater ecosystem studies could be used for marine effects assessment. However, in such a case an extra assessment factor of 10 should be applied to derive the $QS_{sw, eco}$ in addition to the factor applied in the derivation of the $QS_{fw, eco}$. However, preference may be given to the deterministic or SSD approach, if the laboratory studies do contain additional marine taxonomic groups.

3.4 Deriving a MAC-QS

For deriving a MAC-QS, the REACH guidance for effects assessment of substances with intermittent release is adopted. If enough short-term EC50/LC50 data are available to construct an SSD this extrapolation approach should be used as well as the deterministic approach, as detailed in Section 3.4.1. Relevant mesocosm studies may be available (especially for pesticides) and these can be used to derive the final MAC-EQS, as described in Section 3.4.1.3. Field monitoring

data are unlikely to have a useful part to play in informing the estimation of a MAC-QS because they typically describe changes in biology arising from long-term exposure, so they are more relevant to AA derivation. Any discrepancies in the results obtained with the different extrapolation approaches need to be discussed and the decision for the preferred MAC-QS derivation justified. Predicted data using QSAR models or 'read across' approaches can be used as supporting information, but not as a basis for the derivation of a QS.

Under some circumstances, a MAC-QS may not be justified, e.g. for substances that exert only sub-lethal effects after prolonged exposure. Steroid oestrogens could be one example.

3.4.1 Deriving a MAC-QS for the freshwater pelagic community (MAC-QS$_{fw, eco}$)

3.4.1.1   Extrapolation using the AF method

For exposures of short duration, acute toxicity data are relevant and the AFs to use are given in Table 5. Combined acute toxicity data sets for freshwater and saltwater species may be used, if the data can be pooled (Section 3.2.3). Where there are at least 3 short term tests using species from three trophic levels (base set), an AF of 100 applied to the lowest L(E)C50 is normally used to derive the MAC-QS$_{fw, eco}$. Under some circumstances an AF less than 100 may be justified, e.g.;

For substances which do not have a specific mode of action (e.g. acting by narcosis only) and if the available data show that interspecies variations are low (standard deviation of the log$_{10}$ transformed L(E)C50 values is < 0.5), an AF<100 may be appropriate.

For substances with a specific mode of action, the most sensitive taxa can be predicted with confidence. Where representatives of the most sensitive taxa are present in the acute dataset, an AF <100 may again be justified.

Where there is a good understanding of the relationship between acute and chronic toxicity (e.g. acute: chronic ratios for a range of species), the AF used to estimate the MAC-QS may be selected to reflect this, or at least to ensure the MAC-QS is not lower than the AA-QS.

In no case should an AF lower than 10 be applied to a short-term L(E)C50 value.

Table 5 Assessment factors to derive a MAC-QS$_{fw, eco}$.

Toxicity data	Additional information	Assessment factor
Base set not complete	–	– [a]
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, crustaceans and algae)		100
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, crustaceans and algae)	Acute toxicity data for different species do not have a higher standard deviation than a factor of 3 in both directions[b] OR known mode of toxic action and representative species for the most sensitive taxonomic group included in the data set.	10 [c]

Notes

a) When the base set is not complete, a MAC-QS$_{fw, eco}$ cannot be derived. It should be considered if the base set could be completed with non-testing data (See Section 2.6.). Non-testing data should not be used as critical data in the derivation of the MAC-QS$_{fw, eco}$.

b) To assess the span of the acute toxicity data, all reliable acute toxicity data collected are used, with a minimum of three LC50 or EC50 values, for species representing each of the base set trophic levels (algae, *Daphnia*, fish). If the standard deviation of the $\log_{10}$ transformed L(E)C50 values is < 0.5, an assessment factor of 10 could be applied, otherwise an assessment factor of 100 should be applied.

c) Lowest assessment factor to be applied.

For the specific group of insect growth regulators, acute data do not give information on delayed effects and cannot be used for the derivation of the MAC-QS because the test duration is too short to detect long-term effects of a single peak of exposure. In general, for compounds with a (very) high acute to chronic ratio, the possibility of delayed effects resulting from a single peak should be considered and the chronic data should be consulted.

3.4.1.2   Extrapolation using the SSD approach

The same approach as described in Section 3.3.1.2 can be applied. However, instead of long-term EC10s or NOECs, acute L(E)C50 data are the appropriate input data. Combined acute toxicity data sets for marine and freshwater species may be used, if, after evaluation of the freshwater and saltwater toxicity data, the data can be pooled (Section 3.2.3).

The resulting HC5 refers to a concentration representing 50% or greater effect for 5% of the species, because the input to the SSD are L(EC)50 values. An AF is therefore needed to extrapolate to the MAC-QS$_{fw, eco}$ to account for the EC50 to EC10 extrapolation. 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.

3.4.1.3   Use of simulated ecosystem studies in deriving a MAC-QS$_{fw, eco}$

General guidance regarding the derivation of a QS from micro/mesocosm studies is given in Section 2.9.2. For determining the MAC-QS$_{fw, eco}$, experiments simulating short-term exposure are most relevant.

For substances that do not dissipate quickly, the MAC-QS$_{fw, eco}$ values should be based on measured time weighted average (TWA) concentrations, and biological effects determined over a time span that is representative of the most acute toxicity studies (i.e. 48–96 h). Measurement of

exposure concentrations should take account of both spatial and temporal changes within the mesocosm. Furthermore, it is important to determine which part of the exposure profile is most relevant. For example, if the peak concentration causes the effect, the actual initial concentration in the cosms is relevant, as well as the concentration at various time intervals (hours in the case of rapidly-dissipating compounds). An understanding of the exposure phase that is most relevant to any toxic effects (the Ecologically Relevant Concentration, ERC) is important because it (a) influences how the assessor interprets the mesocosm data and (b) how the resulting MAC-EQS should be expressed (e.g. a 24-hour or a 1-month peak). Such properties must be drawn to the attention of policy makers because it will affect how compliance is assessed, or indeed whether a MAC-EQS for compliance monitoring can be feasibly implemented at all. Such an EQS may still have value for planning purposes.

3.4.1.4 Application of an assessment factor to the threshold concentration from a mesocosm to derive a MAC-QS$_{fw, eco}$

For substances for which the mode of action and/or the most sensitive taxa are known, an assessment factor ranging from 1-5 is applied to the lowest threshold concentrations from the available mesocosms, with the same considerations as given for the derivation of the QS$_{fw, eco}$ (Section 3.3.1.3).

Brock et al. (2006, 2008) compared the outcome of 6 mesocosm studies with the insecticides chlorpyrifos and lambda-cyhalothrin that simulated short-term exposure. They looked at the spread (ratio of the upper to the lower limit of the 95% confidence interval) of the threshold concentrations for toxic effects. The spreads were 2.9 for chlorpyrifos and 2.6 for lambda cyhalothrin. They concluded that for a substance with a specific mode of toxic action, an AF of 3 can be applied, provided that the study is well-performed. This can be lowered depending on the number of available mesocosms.

3.4.2 Derivation of a MAC-QS for the saltwater pelagic community (MAC-QS$_{sw, eco}$)

The MAC-QS for coastal and territorial waters (MAC-QS$_{sw, eco}$) is intended to protect the saltwater ecosystem from potential acute toxic effects exerted by transient exposure to toxic chemicals. These peak concentrations can, for instance, occur in fish farms, in connection with batch effluent releases on the ebb tide, or when a ship is cleaned. For transitional waters, the guidance in Section 2.4.1.1 is relevant.

To derive a MAC-QS for saltwater, the same approach as described for the QS$_{sw, eco}$ can be applied in principle. However, instead of using long-term EC10s and NOECs, acute L(E)C50 data will serve as input data. Combined acute toxicity data sets for marine and freshwater species may be used, if analysis shows that the data can be pooled (Section 3.2.3.).

3.4.2.1 Extrapolation using the AF method

As in the derivation of the QS$_{sw, eco}$, when additional information on the sensitivity of specific saltwater taxonomic groups is available, the additional assessment factor of 10 can be lowered to 5 (one additional marine taxonomic group) or 1 (two or more additional marine taxonomic groups), see Section 3.2 for explanation of what is meant by 'additional marine taxonomic groups'. The AFs to be used when deriving a MAC-QS$_{sw, eco}$ are given in Table 6.

Table 6 Assessment factors to derive a MAC-QS$_{sw, eco}$

Toxicity data	Additional information	Assessment factor
Base set not complete	–	– [a]
At least one short-term L(E)C50 from each of the three trophic levels of the base set (fish, crustaceans and algae)		1000
At least one short-term L(E)C50 from each of the three trophic levels of the base set (fish, crustaceans and algae)	Acute toxicity data for different species do not have a higher standard deviation than a factor of 3 in both directions[b] OR known mode of toxic action and representative species for the most sensitive taxonomic group included in the data set	100
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, crustaceans and algae) + one short-term L(E)C50 from an additional specific saltwater taxonomic group		500
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, crustaceans and algae) + one short-term L(E)C50 from an additional specific saltwater taxonomic group	Acute toxicity data for different species do not have a higher standard deviation than a factor of 3 in both directions[b] OR known mode of toxic action and representative species for the most sensitive taxonomic group included in the data set	50
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, crustaceans and algae) + two or more short-term L(E)C50s from additional specific saltwater taxonomic groups		100
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, crustaceans and algae) + two or more short-term L(E)C50s from additional specific saltwater taxonomic groups	Acute toxicity data for different species do not have a higher standard deviation than a factor of 3 in both directions[b] OR known mode of toxic action and representative species for the most sensitive taxonomic group included in the data set	10 [c]

Notes

a) When the base set is not complete, a MAC-QS$_{sw, eco}$ cannot be derived. It should be considered if the base set could be completed with non-testing data (See Section 6). Non-testing data should not be used as critical data in the derivation of MAC-QS$_{sw, eco}$.

b) To assess the span of the acute toxicity data, all reliable acute toxicity data collected are used, with a minimum of three LC50 or EC50 values, for species representing each of the base set trophic levels (algae,

Daphnia[13], fish). If the standard deviation of the $\log_{10}$ transformed L(E)C50 values is < 0.5, an assessment factor of 10 should be applied, otherwise an assessment factor of 100 should be applied.

c) Lowest assessment factor to be applied.

3.4.2.2 Extrapolation using SSD approach

The same approach as described in Section 3.3.1.2 can be applied. However, instead of long-term NOECs and EC10s, acute L(E)C50 data (one value per species) are the appropriate input data. Combined acute toxicity data sets for marine and freshwater species may be used, if after evaluation of the freshwater and saltwater toxicity data, the data can be pooled (Section 3.2.3). This would result in the same HC5 for freshwater and saltwater assessments, but, given the greater uncertainties in extrapolating for the marine environment, a larger AF is required than that used to deal with residual uncertainty in the freshwater MAC-QS.

For the MAC-QS$_{fw,eco}$, the default AF to be used on the HC5 is 10. However, when the datasets for fresh- and saltwater are combined, for a MAC-QS$_{sw, eco}$ derivation an additional assessment factor of 10 is used to deal with residual uncertainty, resulting in a total AF of 100. In line with the derivation of the QS$_{sw, eco}$, when one typically marine taxonomic group is present in the dataset, an additional AF of 5 is used on top of the default AF of 10 and when two typically marine taxonomic groups are present, no additional assessment factor is necessary. When separate datasets are used to calculate an SSD for MAC-QS derivation, it follows that the necessary amount of data for marine taxa are available to calculate an SSD, and an additional AF on top of the default AF of 10 is no longer necessary.

3.4.2.3 Use of simulated ecosystem studies in deriving a MAC-QS$_{sw, eco}$

For the derivation of the MAC-QS$_{sw, eco}$ the highest initial concentration in a simulated ecosystem study that caused no ecologically relevant effects may be used. Further guidance regarding the derivation of the MAC-QS from micro/mesocosm studies is given in Section 2.9.2. Freshwater mesocosms should not be used in the derivation of a MAC-QS$_{sw, eco}$.

3.5 Deriving EQSs for metals

3.5.1 Principles of metal toxicity – availability and bioavailability

There have been major advances in our understanding of the physiological processes that control the uptake of inorganic metals and toxicity in aquatic systems. These indicate that, for most metals (e.g. Cd, Cu, Zn, Ni, Pb, Ag), the primary target tissues are 'respiratory organs (gills or gill-like structures)' at the interface between the organism and the waterbody. Bioavailable metal species (especially free metal ions) have a high affinity for negative binding sites at gills and gill-like surfaces (Luoma et *al.*, 2011).

Metal toxicity is strongly affected by water chemistry, through its effects on (bio)availability. The understanding of the interactions between metal species, water characteristics, and the ionoregulatory/respiratory system of aquatic organisms, has led to the development of several models linking metal bioavailability to toxicity in freshwaters ('Biotic Ligand Models', BLMs). The potential for additional toxicity through dietary intake has also been assessed for a range of metals (Cu, Zn, Ni). The data from laboratory settings (waterborne versus dietborne toxicity, assessment of potential for secondary poisoning), mesocosms contaminated with metals (ECI, 2008) and field exposure assessments (Crane et *al.*, 2007; Stockdale et al. 2010, Peters et al. 2014) demonstrated

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[13] "Daphnia" in this document is generally used to mean small crustaceans

that metal EQSs derived from water-only exposures and the application of metal bioavailability models suggests that, at least for the metals that were investigated, water-only exposures are also protective of exposure via the diet.

Without consideration of (bio)availability, intraspecies variability of several orders of magnitude can be seen in estimates of ecotoxicity with metals (e.g. EC, 2008c). If it is not dealt with properly, this obviously would undermine confidence in any resulting QS. Bioavailability models allow us to explain much of the variation in ecotoxicity between the tests done under different conditions, achieving predicted chronic toxicity of several metals within a factor of 2 in experimental data for specific water chemistry conditions (Van Sprang et al., 2009; Peters et al., 2014; Peters et al., 2016). Research data on metal speciation, metal bioavailability and metal ecotoxicity have been applied in the EU risk assessments for cadmium, zinc, and nickel; for copper in a voluntary risk assessment under the Existing Substances Regulation; and WFD EQSs for cadmium (hardness correction)[14], lead (DOC correction) and nickel (full bioavailability correction).

Therefore, where adequate understanding exists, it is strongly recommended to incorporate bioavailability in the derivation of QSs for metals.

3.5.2 Overview of guidance on setting quality standards for metals in water

The following guidance relates to deriving QSs for metals in water. QSs for metals in water should be derived in the dissolved phase[15], as required by the EQS Directive[16] (for more details, see Section 3.5.3.1). For guidance on estimating water standards for metals based on standards for biota to protect humans or wildlife via the food chain, see Section 4; for more detailed guidance on sediments, see Section 5.2.2.

The methods used to incorporate the availability/bioavailability corrections will depend on the availability of data and models and metal-specific considerations (e.g. importance of metal–DOC binding in aquatic systems, and availability of a metal-specific biotic ligand model (BLM)). When available, the use of the BLM model is preferred to other methods.

Figure 5 and the following sections outline the different steps that allow QSs for metals to be derived for freshwater and saltwater compartments in a way that accounts for (bio)availability. The guidance provided is focused on deriving an AA-QS, based on chronic ecotoxicity data (NOECs/EC10s) and chronic bioavailability models. A similar approach can nevertheless also be followed when a MAC-QS is to be derived, based on acute data (EC50s) and acute BLMs.

Because of the differences in iono- and osmo-regulated environments, there may be differences in the toxicity of a metal to freshwater and saltwater species. Availability corrections for freshwater cannot currently be directly translated to saltwater conditions; therefore, pooling of freshwater and saltwater data should be avoided when availability corrections have been applied.

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[14] Chronic biotic ligand models (BLMs) have been built and validated in the laboratory and in the field for several metals (Zn, Ni, Cu and to some extent Cd) and the models allow the prediction of chronic metal toxicity in a wide range of waters worldwide. Acute BLMs are available for a much wider range of metals, although they have not been widely validated in Europe.

[15] Directive 2008/105/EC amended by Directive 2013/39/EU requires that EQSs for metal be derived in the dissolved fraction. EQSs based on total metal can be highly inaccurate because (a) the total metal fraction rarely has a clear link to toxicity (b) water chemistry conditions have a marked impact on availability and toxicity, which would be 'masked' if assessments of risk (i.e. compliance with the EQS) are based on total metal concentrations. EQSs based on dissolved metal provide a better assessment of risk if a bioavailability-based approach (like those set out in Section 3.5.3.2) is not available. It follows that risk assessment (compliance with the EQS) should be based on the dissolved metal rather than total metal concentrations.

[16] Directive 2008/105/EC as amended by Directive 2013/39/EU.
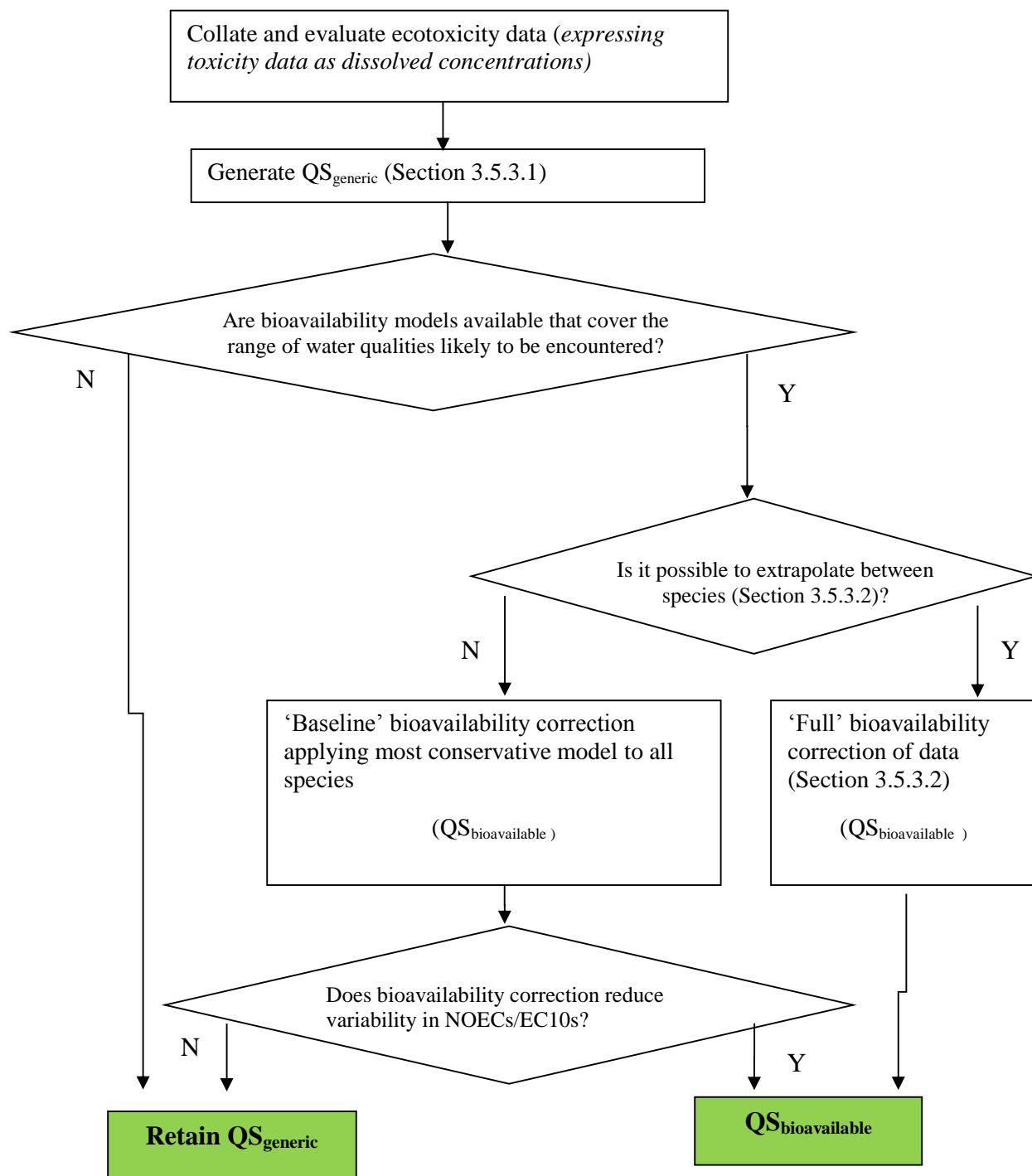
Figure 5: Recommended general scheme for deriving QSs for metals

3.5.3 Deriving the QS for freshwater

There are essentially two different approaches, which may be taken when deriving the QS, as outlined in Figure 5. These two approaches are the development of:

(1) a 'generic' QS using ecotoxicity data without explicit consideration of the bioavailability (Section 3.5.3.1),

and

(2) a "bioavailable"-QS that does incorporate bioavailability considerations (Section 3.5.3.2).

For both approaches, the available toxicity data first need to be compiled and evaluated (See Section 2.6.2.). The quality criteria to be used are the same as those used for organic substances, but some metal-specific issues need to be considered as outlined below and detailed in Section 3.5.5.

3.5.3.1   Generic EQS

This approach is adopted where there is no underlying understanding of the factors affecting bioavailability of the metal of interest, or it is insufficiently developed to be incorporated in the derivation of a QS.

For the water compartment, the first step is simply to express the toxicity data on the basis of the dissolved concentration. Ideally, test data will be expressed as dissolved concentrations of metal. If dissolved concentrations in the test media are not given, the relationship between the total and dissolved metal concentrations in ecotoxicity media should be checked. For some metals in fully soluble metal salt form (e.g. $Zn(NO_3)_2$ or $Cu(NO_3)_2$) tested in artificial media (and especially when tested in semi-static or flow-through systems), no additional conversion into a dissolved fraction has to be applied because there is evidence that all the metal is in solution[17].

This situation does not always apply if tests are performed in natural waters and may be different for other metals. For other, less soluble, metals (e.g. lead, iron, tin, etc.), we cannot assume that all the metal will be in solution. An additional step is therefore needed to convert the total concentration into a dissolved fraction. This would involve analysing relevant solubility products for the metal salts, or the use of existing data on the ratio of matched dissolved and total metal monitoring data to inform the estimation of dissolved metal concentrations. Solubility products may be found in, for example, the *Handbook of Chemistry and Physics*, 86th edition, CRC Press, 2005. Nevertheless, there are potential uncertainties in these calculations that should be understood and detailed. If this approach is adopted, the original (total) concentrations and estimated dissolved concentrations should be presented together.

Although dissolved concentrations from individual experiments in natural waters can be recalculated from total concentrations using partition coefficients (taking binding to suspended particulate matter into account), the calculated dissolved concentrations of several metals may be uncertain since the partition coefficient (K_p) has been found to vary by several orders of magnitude. **The use of partition coefficients to estimate dissolved concentrations of metals is therefore discouraged, unless the Kp refers to the specific water type in the test. The use of measured dissolved metal test concentrations is preferred.**

In practice, tests performed, e.g. according to OECD test guidelines, would be carried out under conditions of high (bio)availability, but, only tests undertaken under conditions (pH, hardness) that

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[17] In most laboratory tests, suspended solids are low and typically >95% of the metal is in solution. Organic particles e.g. from faeces or uneaten food are not thought to significantly affect the dissolved metal concentration when semi-static or flow-through test systems are used.

are within the normal physiological range of the test organisms should be used. The tests in which the added DOC is in excess of 2 mg/l should not be used.

3.5.3.2   (Bio)-availability correction - deriving QS$_{bioavailable}$ for metals

This approach is adopted where there is a clear understanding of the factors affecting metal speciation and (bio)availability. The influence of the water chemistry on metal toxicity can be significant so it should be quantified where this is practical.

It is recommended to consider the following five steps in deriving a QS$_{bioavailable}$. Detailed guidance on each of these steps follows below.

STEP	ACTIVITY
1	Derive QS$_{generic}$
2	Select suitable bioavailability model
3	Test suitability of preferred model across species
4	Normalise ecotoxicity data to a reference water chemistry and derive HC5
5	Derive QS$_{bioavailable}$

STEP 1 - DERIVE A GENERIC QS:

The first step is to derive a QS$_{generic}$, as described in earlier sections of this guidance without any consideration of (bio)availability. As mentioned previously, this QS$_{generic}$ should be derived in the dissolved phase. The methodology for deriving a generic QS for metal in water is similar to deriving a QS to protect pelagic species against any other type of substances, and should follow the recommendations of section 3.3.1. This QS generic provides a 'starting point' for subsequent refinements to account for (bio)availability using one of the possible approaches (Step 2). In the absence of appropriate bioavailability correction, the QS$_{generic}$ is retained as the final QS.

STEP 2 – CHOOSE BIOAVAILABILITY MODEL:

Options for correcting for (bio)availability include (a) speciation models that predict the effects of water chemistry on metal speciation (b) empirical models that relate toxicity to water chemistry and (c) Biotic Ligand Models that encompass both abiotic and biotic factors determining metal toxicity. Where available, BLMs are preferred over other methods for taking into account bioavailability. When this is not possible, the use of speciation models or of empirical regression represent the next best options:

a) The first availability correction is the application of **speciation models** (e.g. WHAM (Tipping *et al.*, 1991; Tipping *et al.*, 2011); MINTEQA2, NICCA (Kinniburgh *et al.*, 1999). Where these models are available for the metal of interest, availability corrections can be considered[18], so long as there is a clear link between the metal speciation and its availability/toxicity (i.e. predictions of metal species concentrations match experimental effects) (Vink, 2002 and 2009).
If the correction relates only to <u>chemical</u> availability (e.g. speciation modelling), it is not organism-specific because it applies to the medium in which all organisms are living. In such cases, if a quantitative relationship between the parameter (e.g. [M^{Z+}]) and ecotoxicity

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[18] Most often this is the free metal ion, but it should be noted that the free ion is not necessarily the best predictor for all metals, and other metal species, such as neutral species (e.g. AgCl, HgS) and anionic species (e.g. SeO^{2-}, AsO$_4^{2-}$), may contribute to the observed toxicity (Campbell, 1995).

(EC10s (or NOECs)/EC50s) has been developed, the observed quantitative relationship can be applied to all ecotoxicity data selected for EQS derivation, and a QS$_{(bio)available}$ can be derived.

b) For some metals, empirical models have been developed which directly relate water chemistry conditions to the metal bioavailability/toxicity. These toxicity-based models range from simple limiting functions to single or multiple parameter **regression models** (e.g. Brix et *al*., 2017). When using such models, it is important to ensure that they are applied within the appropriate model applicability domain and validation ranges. Specifically, this means that the water chemistry conditions from the biological test data that support the model are relevant to the water chemistry conditions over which the model is being applied (Brix et *al*., 2017).

c) More advanced mechanistic or semi-mechanistic models have also been developed for some metals, and these models are typically known as Biotic Ligand Models (BLMs). Comprehensive chronic BLMs are currently available for copper (De Schamphelaere and Janssen, 2004a, 2004b and 2006; De Schamphelaere et *al*., 2003), nickel (Deleebeeck et *al*., 2007a, 2007b, 2008, 2009a, 2009b), zinc (Heijerick et *al*., 2002a; Heijerick et *al*., 2002b), and now also for lead (De Schamphelaere et *al*., 2014; Nys et *al*., 2014).

A QS$_{bioavailable}$ provides an ecologically and environmentally relevant metric by which potential metal risks can be assessed. As long as appropriate supporting water chemistry data are available, the QS$_{bioavailable}$ can be used to assess compliance, characterise waterbodies and identify those at unacceptable risk (EC, 2011). Further guidance on using such EQSs for these purposes is provided separately.

Background understanding – what do Biotic Ligand Models do and how can they be used?

Introduction

BLMs can be used to describe the toxicity of a metal as a function of water chemistry. Specifically, BLMs can be used to predict the chronic ecotoxicity of a metal in a waterbody, in the form of a dissolved metal concentration, if the physico-chemistry of that waterbody is known.

In general terms, a biotic ligand model combines equilibrium-functions for different species of the metal, between the metal ion and complexes with abiotic ligands (binding sites, e.g. metal-DOC complexes), and between the metal and complexes with biotic ligands (binding sites, e.g. sodium channel proteins in the gills of fish). The BLM model predicts the concentration of each metal-species in a complicated system of metal complexes that includes complex binding to biota, DOC and inorganic ligands. The model further includes the competitive binding of other metal ions to these binding sites (e.g. Ca^{2+}, Mg^{2+} etc.).

Metal bioavailability is <u>always</u> linked to the water chemistry. However, the physico-chemical conditions that determine a metal bioavailability will not necessarily be the same for different species. The result is that different water chemistry conditions can give rise to different species sensitivity rankings, i.e. the most sensitive species under one set of conditions, may be different under another set of conditions. Whilst low DOC concentrations will always lead to higher bioavailability conditions, this is not true for the other water chemistry parameters, such as pH and hardness, which also control metal bioavailability. Whilst some species, such as crustacea, may be most sensitive under very soft water conditions, with very low calcium concentrations and hardness, other species, such as algae, may be most sensitive under high pH conditions. Consequently the relative sensitivity of the various species within an SSD can differ appreciably between hard waters and soft waters.

As a result of these differences in sensitivity between different species, it is not appropriate to assume that tests performed in synthetic waters will always reflect conditions of very high bioavailability. Laboratory tests will typically not contain any added organic matter, which tends to favour bioavailability compared to many natural waters. However, the pH and hardness conditions of the tests also need to be considered. Many typical test waters contain moderate levels of hardness and have a circumneutral pH. Consequently, these standardised synthetic test waters

will not necessarily provide the conditions that maximise metal bioavailability which could potentially be encountered for any of the tested organisms. Organisms such as algae whose sensitivity is governed largely by pH may need to be tested under high pH conditions in order to reflect the conditions under which these species are most sensitive, whereas fish and invertebrates may need to be tested in very soft, acidic, waters in order to reflect the conditions under which these species are most sensitive.

Using BLMs to derive a QS

Preferably, it should be possible to normalise the entire ecotoxicity database with the BLMs. If there are sufficient data, an SSD can be constructed for a specific set of water chemistry conditions. This effectively removes a large component of the intra-species variability that is due to differences in the water chemistry in the different tests (see Figure below). This approach can be used to establish the EQS$_{bioavailable}$.

Where BLMs are sufficiently well developed and validated to enable the recalculation of the entire SSD for each individual water chemistry, a range of site-specific HC5 values can be calculated for a range of water chemistry conditions, which may be encountered within a region, or even across Europe. Where it is not possible to normalise the entire SSD to the specific water chemistry in question, an alternative approach needs to be taken towards the derivation of the reference EQS$_{bioavailable}$. One possible approach is to select the most sensitive test result for each individual species for inclusion in the SSD, although this is likely to result in combining test results related to very different water chemistry conditions. These steps are explained in more details in Section 3.5.2.2 of this guidance.
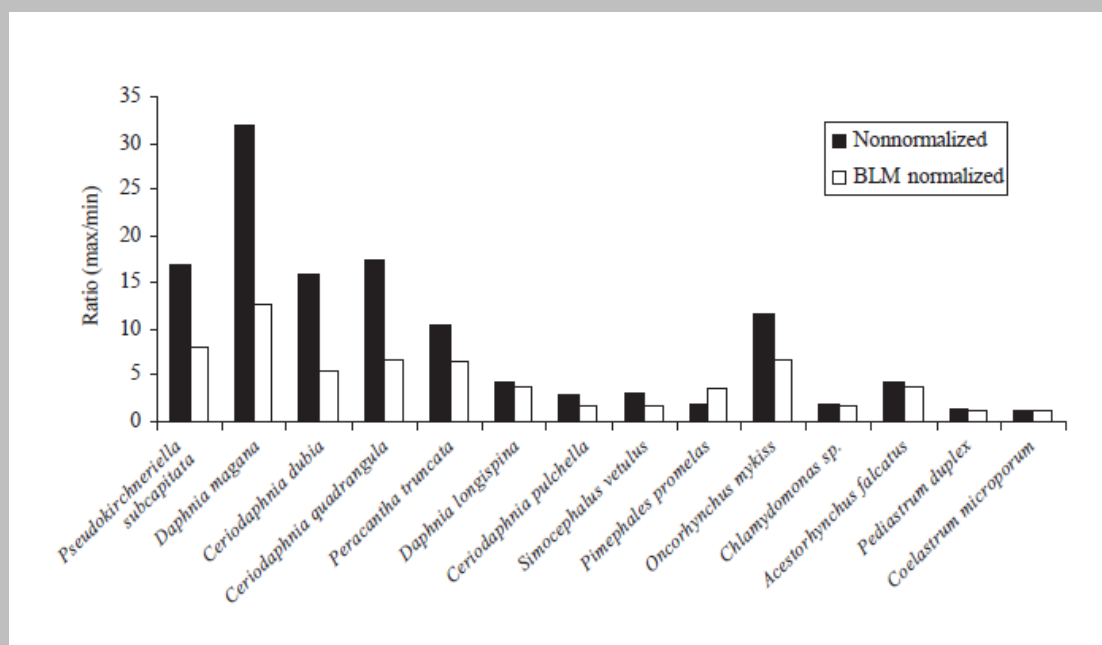
Intra-species variability in sensitivity to nickel, expressed as max/min ratios of the EC10/NOECs without adjusting for bioavailability (black bars), and BLM-normalised (open bars) to the River Rhine water chemistry conditions using the chronic nickel BLMs (EC, 2008).

Valid water chemistry conditions for using the BLMs

(Bio)availability models have validation domains, i.e. the water chemistry conditions over which they have been shown to work. The ranges of pH, hardness and DOC should therefore be specified in the EQS dossier of the metal and in the user manuals of the models that are used. The validation boundaries of the BLMs represent the extremes of water quality conditions at which the validation of the chronic tests was undertaken.

A common problem in BLM development, and arguably one of the reasons for the existence of the validation ranges, is that the species typically used for BLM development (i.e. species commonly used in laboratory ecotoxicity testing) are not tolerant to all the natural water conditions. For example, many crustacea and snails will not perform adequately in control waters of very low hardness or pH. Equally, they will not be present in naturally low hardness waters. The validated ranges of the BLMs will never cover all EU waters because there are fundamental difficulties in performing standard ecotoxicity tests in waters that are outside those conditions which are physiologically acceptable to the test organisms, i.e. it will not be possible to deliver acceptable control performance. In addition, these types of natural waters (at the extremes of pH or low hardness) often have very specific ecological assemblages, which are not always more sensitive to metal exposures than more typical "mid-range" waters.

The inclusion of <u>validation ranges</u>, explicitly linked to the ecotoxicity data from which the $EQS_{bioavailable}$ is derived is a transparent recognition of potential uncertainties, not usually necessary with other (non-metal) EQSs.

Where toxicity in laboratory experiments is expressed in terms of dissolved metal concentrations and **speciation models**, empirical **models** (e.g. Cd hardness correction) or **BLMs** have been developed and validated for the metal/metal compounds of concern, and then we can follow the steps below to derive a $QS_{bioavailable}$.

STEP 3 – CHECK SUITABILITY OF PREFERRED MODEL ACROSS SPECIES:

The bioavailability correction should only be applied if it results in a general decrease in the intra-species variance in the EC10 and NOEC values, or if it is is able to explain the observed differences in toxicity as a function of water chemistry conditions. This requires making bioavailability corrections to the available ecotoxicity data using one defined water chemistry. The choice of water chemistry is not critical at this stage, but it should be the one that gives rise to high metal bioavailability (typically low DOC). A practical approach would be to use a water chemistry associated with high bioavailability as described in Step 4.

The models for bioavailability correction **may be species-specific and, therefore, bioavailability correction is only possible if the BLM models have been developed and validated for at least** one species in each **three** trophic levels**, including an** alga**, an invertebrate**, **and a fish species**. Bioavailability correction based on the three species from these three trophic levels only is considered as the baseline correction. Preferably, the model should be applicable to other species as well. **If read-across of the models to other species cannot be demonstrated** (by applying the species-specific bioavailability models to other species from the same trophic level), **bioavailability corrections can only be carried out for the BLM species and the QS_{generic}** cannot **be translated into a** <u>general</u> $QS_{bioavailable}$. In these circumstances, the most critical of the bioavailability corrections (i.e. the one leading to the greatest availability) for the three BLM (regression model) species is subsequently used.

Full BLM normalisation of the entire EC10 and NOEC (for chronic data) dataset is justified, and full bioavailability correction can be performed only if additional evidence is available to confirm the applicability of the three BLMs to at least three additional taxonomic groups from other phyla, e.g. <u>Cyanophyta, Protozoa,</u> Mollusca, Rotifera, Insecta, Angiospermae. In other words, it is assumed that predictions on e.g. *Daphnia* also give reasonably accurate predictions of the toxicity for other invertebrate taxa.

The accuracy of the BLM predictions for the additional taxonomic groups can be demonstrated by showing that the model actually decreases the variability in the data for the investigated additional species, or that the model is able to explain the differences in toxicity as a function of water chemistry conditions. If the BLM read-across is <u>not</u> applicable for that species, the BLM should not be used and one of the other availability models (Step 2) considered instead. This avoids the

problems that could arise if different models were used to account for bioavailability between different species.

STEP 4 – NORMALISE ECOTOXICITY DATA TO A REFERENCE WATER CHEMISTRY AND DERIVE HC5:

This normalisation step is critical because the different water chemistries across the ecotoxicity tests can have a considerable influence upon toxicity. Normalising all the chronic ecotoxicity data in a metal dataset to the same water chemistry conditions is equivalent to undertaking all of the tests in the same water chemistry conditions. Differences in bioavailability are then distinguished from observed differences in sensitivity between species. In order to derive a QS that is sufficiently protective in a wide range of conditions, one must therefore normalise the ecotoxicity data to the single water chemistry that results in the higher level of bioavailability.

If there are sufficient data to construct an SSD (Section 3.3.1.2)[19], we recommend the toxicity data are recalculated (using the chosen bioavailability model) for a range of water chemistries that are found across Europe, and SSDs constructed for this range of water chemistries. This gives rise to a distribution of HC5 values from which the 'reference water' can be chosen (and hence the HC5 that will form the basis of the $QS_{bioavailable}$). Guidance on the selection of a suitable reference water is given below.

STEP 5 – DERIVE QS$_{BIOAVAILABLE}$ FOR HIGH (BIO)AVAILABILITY CONDITIONS:

The $QS_{bioavailable}$ should be protective for the majority of water bodies that may be encountered across Europe. We therefore have to decide what water chemistry conditions the data are to be normalised to in order to ensure that the $EQS_{bioavailable}$ is protective.

The conditions underlying the $QS_{bioavailable}$ should be reflective of a reasonable worst-case condition – i.e. high bioavailability for the metal being considered. It is important to recognise that this will vary between metals, but the aim is to identify a condition that gives rise to high bioavailability and is therefore protective of all other water chemistry conditions that are likely to be encountered in the region of interest (Europe[20]), irrespective of what they are.

To ensure that there is just one $QS_{bioavailable}$ that is applicable to the whole of Europe, it is necessary to have an understanding of the abiotic conditions that are likely to result in the greatest metal bioavailability, and thus represent the most critical conditions to metal exposures. This understanding is relatively easily gained using repeated use of the BLMs to different water chemistry conditions. To illustrate this, Figure 6 gives an example, from the Nickel EQS Dossier (EC, 2010), of the variation in calculated HC5 for nickel under differing water chemistry conditions.

We recommend the use of a water chemistry that ensures protection of 95% of waterbodies in the most vulnerable (i.e. high bioavailability) region as the basis for the $QS_{bioavailable}$. This will avoid the errors and bias associated with extreme values, which are over-dependent on the quantity of available data.

In order to define the water chemistry condition for the $EQS_{bioavailable}$, the following steps are suggested:

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[19] If there are insufficient data, a QS is estimated using the deterministic method (Section 3.3.1.1). In practice, for metals where a BLM has been developed, there should normally be sufficient data for an SSD.

[20] The same procedure may be used to derive a QS for a Specific Pollutant but in this case the region of interest will be smaller, usually a country. For the purposes of this guidance we refer to 'Europe' throughout.

a) Use the Geochemical Baseline Database FOREGS (http://weppi.gtk.fi/publ/foregsatlas/ForegsData.php) that provides water chemistry in surface waters in unimpacted areas across Europe.

b) Use the chosen bioavailability model (Steps 1-3) to calculate a range of site-specific HC5 values across Europe. This will result in a list of waterbodies with their corresponding 'vulnerability' to the metal of interest.

c) From this, identify the most vulnerable region (i.e. the region – typically a country or large administrative region - associated with occurrence of the lowest median HC5 values).

d) Locate any additional water chemistry data (as a minimum, DOC, pH and hardness or [Ca]) for the vulnerable region identified in stage (c). For example, freshwater regulatory monitoring data are becoming more readily available and can be accessed through National Agency websites or through European-wide repositories such as EIONET.

e) Repeat step (b) for the region of interest.

f) Once these data have been processed, frequency distributions of the resulting median estimate HC5 values can be plotted for each location in the region of interest. The 5th percentile of the distribution of median HC5 values of all the locations can then be determined. It is **_this_** HC5 that is the basis of the $QS_{bioavailable}$.

At step (f), it is advisable to compare the 5th percentile HC5 from the region with the highest bioavailability with the one obtained for the whole of Europe (step (b). If these are close, the latter value is preferred because it is based on a much larger body of evidence than would be available for the most critical region alone.

At step (d), it is possible that insufficient data are available for the region of interest to make a reliable estimate of the 5th percentile HC5. We recommend that data should give a good level of spatial coverage without major gaps. If sufficient data cannot be found, the $EQS_{bioavailable}$ should be based on the 5th percentile of the entire European database (from step (a).

The $QS_{bioavailable}$ should be accompanied by the range of physico-chemical conditions within which the chosen bioavailability model is valid. The final QS is reliant on the usual decisions around the size of the assessment factor to be used, as for any other substance (Section 3.3.1). As explained below, the $QS_{bioavailable}$ is expressed as a dissolved metal concentration.

What is an $EQS_{bioavailable}$?

The $EQS_{bioavailable}$ is a total dissolved metal concentration which is highly bioavailable and which does not make any allowance for background in its derivation. It is derived, initially, as the normalised, median estimate of the HC5 for a specific set of water chemistry conditions – one that is reflective of high bioavailability conditions. The HC5 selected should be protective of 95% of waters in the region (country) shown to have the highest bioavailability of that particular metal. By definition, it will also be protective of almost all other waters in Europe within the validated range of the BLMs.
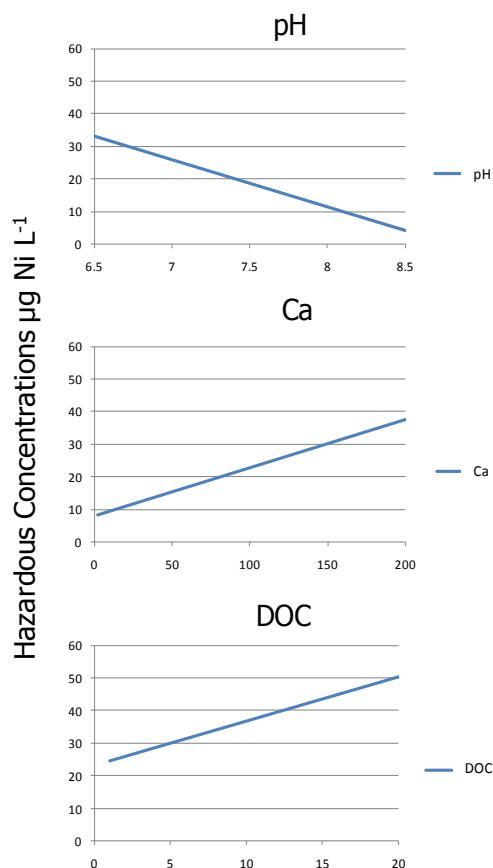
Figure 6 Representation of changes in the ecotoxicity of dissolved nickel using the bio-met bioavailability tool.

Results are expressed as an HC5, for pH, calcium (Ca mg l-1) and dissolved organic carbon (DOC mg l-1). Individual parameters were varied while the o other two parameters remained constant (pH 7, Ca 120 mg l-1, DOC 2 mg l-1) (from: EC, 2010)

3.5.4 Implementing a EQS$_{bioavailable}$ – consideration of backgrounds

In the field, many aquatic organisms are able to adapt to elevated concentrations of some essential metals, like Zn or Cu. This means that at some locations there may be a significant natural background to which the organisms are tolerant, and which has little or no toxicological impact. The background is likely to vary from place to place and therefore it cannot be anticipated as part of the EQS derivation. For this reason, in the approach for deriving a QS$_{bioavailable}$ described here, a total risk approach (TRA) is adopted that makes no explicit allowance for background metal levels. Although vital to the proper implementation of the EQSs for metals, these considerations go beyond the derivation of the EQS (the purpose of this guidance). Guidance on the determination of background levels, and accounting for these when implementing the metal EQS, is provided in a separate guidance document that focusses on how EQSs for metals should be used to assess risk, and the supporting data that is required.

However, the following points are worth considering because they could affect the derivation of an EQS for a metal.

There are two circumstances in which natural backgrounds might result in failure of an EQS. First, if natural background concentrations are locally elevated, and second, if the EQS is set unrealistically low, i.e. below typical natural background levels. In the latter case, many water

bodies could fail the EQS. The failures in both cases would occur despite there being little risk to local aquatic communities. It is therefore important to try avoiding a too conservative approach when deriving the EQS, e.g. a large AF to compensate for, e.g. uncertainties involved in extrapolating from laboratory to field conditions.

If a proposed EQS is expected to lead to widespread EQS failure due to natural background concentrations of the metal, the first step should be to check the scope for refining the EQS by reducing uncertainty, before the proposed EQS is adopted as the final EQS. If this is not possible, the natural background will need to be taken account of when assessing compliance, as allowed for in the EQSD. This is covered in the separate guidance on implementation, mentioned above.

3.5.5 Bioavailability correction for saltwater

Freshwater and marine organisms face very different iono- and osmo-regulatory issues related to living in either a very diluted or concentrated salt environment. Differences in iono- and osmo-physiological regulations may also lead to differences in metal accumulation and metal toxicity (Wright 1995; Rainbow, 2002). For these reasons we would normally expect different sensitivities of marine and freshwater organisms to metals.

Marine BLMs are in their infancy, but, as for freshwater, the influence of DOC binding, metal speciation and metal 'availability' on metal toxicity to marine organisms has been demonstrated for some metals (e.g. Smolders et $al.$, 2004; ECI, 2008). The data show that metals binding to organic ligands can reduce metal toxicity to marine organisms, so an availability correction may be needed. Therefore, if experimental data allow the assessor to derive a quantitative relationship between DOC and ecotoxicity (EC10 and NOEC/EC_{50}), and this significantly reduces intraspecies variability, this equation can be used to normalise all marine ecotoxicity data.

In marine waters (coastal and open sea), hardness, pH and alkalinity do not play a role because coastal/open sea waters are characterised by high pH (typically, between 7.8 and 8.3), high salinity (35‰) and high ionic strength. Unlike the inorganic composition of marine waters, DOC levels may vary considerably between marine waterbodies. The MAMPEC model[21] defines receiving marine environment scenarios. The model includes DOC values for coastal and open ocean waters of 2.0 and 0.2 mg·l^{-1}, respectively. The applicability of 2.0 mg·l^{-1} DOC as a reasonable worst case for coastal waters was further confirmed from an extensive literature search (see Cu RAR, 2008). A DOC normalisation of the ecotoxicity data to "full availability" may be used for deriving a coastal water $QS_{bioavailable,sw}$. Alternatively, and if no bioavailability correction can be carried out, a non-normalised generic QS can be derived ($QS_{generic, sw}$). The DOC correction proposed above for the marine environment is a simple 'availability' correction, irrespective of the species considered. It is therefore not necessary to demonstrate the applicability of the DOC correction for a wide range of species.

For estuarine waters, salinity, alkalinity or total carbonate also should be considered, if possible.

3.5.6   Using mesocosm and field data for metals

High quality mesocosm and field data can be used to support QS derivation for metals, similar to deriving a QS for organic substances (Section 2.9.2) However, if a bioavailability correction is applied in the EQS, then mesocosm or field NOECs should be normalised to the physico-chemical conditions of the mesocosm or field site before determining whether the proposed $QS_{bioavailable}$ is likely to be adequately protective.

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[21] Standard model employed for the risk assessment of antifouling paints in marine environments.

3.5.7 Data requirements for deriving QSs for metals

As for organic substances, aquatic toxicity data to be used for the setting of water (sediment/biota) quality criteria for metals are evaluated as described in Appendix 1. However, the following metal-specific aspects need to be considered:

1. *Measured versus nominal test concentrations*: Because it is important to understand the true exposure concentrations, **any ecotoxicity study not supported by analytical data (i.e. endpoint concentrations reported as nominal values) would** not be regarded as reliable unless there is evidence that all or most of the metal is in solution, in which case a nominal concentration may be acceptable. In all other respects, the criteria used to quality-assess ecotoxicity data apply.

2. *Total versus dissolved metal concentrations in test media*: Measurements of dissolved metal concentrations are critical to the assessment of sparingly soluble metals (particles and precipitation may occur) and in the use of natural waters as the test media (adsorption to suspended solids may occur). If only total metal measured data are available, it may be possible, in some cases, to estimate the dissolved fraction from published solubility constants for the principal anions present, e.g. sulphate or carbonate, and/or suspended solids/water partitioning coefficients. However, such manipulations would need to be reflected in the data quality assessment.

3. *Culture conditions*: If the test organisms have been cultured in conditions that are outside the natural background concentration ranges of these organisms[22], such data should have a reliability score of 3, with an accompanying explanation.

4. *Chelators*: Data from studies in which the test media contains artificial chelators (e.g. EDTA) should be excluded from EQS derivation (i.e. reliability score of 3), except in algal tests where small amounts of chelators (EDTA) are unavoidable in order to ensure adequate availability of iron during the test.

5. *Test medium characteristics:*

For water: Considering the strong influence of water physicochemistry on metal toxicity, the physicochemical conditions in a test should be adequately described, especially with regard to the identified drivers of bioavailability, i.e. DOC concentration, hardness, pH, alkalinity, presence of complexing agents, such as humic acids and EDTA, and any other specific parameters of importance to the bioavailability/toxicity of the metal in question. Where all the physicochemical data have not been reported for a test and are important for speciation models, it may be possible to estimate some of the missing data from known physicochemical parameters (e.g. estimate alkalinity from Ca and alkalinity relationships (Peters *et al*., 2014)). The physicochemical parameters should not only be measured at the beginning of the test because the factors may change, e.g. because of food addition, but at one other occasion over the test period as well.

Metal–DOC equilibrations: The kinetics of metal–DOC binding in aqueous and sediment test media may require an equilibration period between the metal and test medium prior to exposing the organisms. This is to allow full metal–OC binding in a way that is representative of natural

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[22] A new guidance document under development will provide insights on how to determine natural background concentrations.

environments (e.g. Kim et al., 1999). Where the kinetics for reaching equilibrium conditions for binding to OC, etc., are known to be slow and may affect the test outcomes, reviewing the details of the test design may provide additional information on the reliability of the data, particularly for any extreme values.

6. *Oxidation state*: Many metals have more than one oxidation number, which poses several complications. Firstly, chemical characteristics, and thus toxicity, can vary markedly between different oxidation states. Consequently, the oxidation number of the trace element(s) in a given substance must be known. This is not necessarily a trivial problem, as mixed oxidation states can occur. Secondly, some oxidation states may be unstable in specific or all environmental compartments, meaning that distinct changes in bioavailability may occur during even a short-term toxicity assay (e.g. Cr(III)/Cr(VI)). In such cases, it may be necessary to derive a separate EQS for each of the relevant oxidation states.

7. *Combining freshwater and saltwater toxicity data*: As explained in Section 3.2.3, freshwater and saltwater toxicity data for metals should be separated *a priori*. Datasets should only be combined when there is no demonstrable difference in sensitivity.

8. *Interpreting biological effects*: Metals can exhibit physical toxic effects (e.g. smothering by metal precipitates) as well as effects caused by systemic toxicity. Some metals (e.g. Fe, Al) precipitate over short timescales compared with the duration of chronic toxicity tests, making the data difficult to interpret. It may be necessary to make distinctions between chemical toxicity and adverse physical effects in the quality assessment of the studies. Chronic data for metals exhibiting these physical effects should be treated with caution. Greater reliance may need to be placed on field data for such metals.

3.6 Assessing compliance with a water-column EQS for organic compounds

3.6.1 Option to translate an EQS for dissolved water into an equivalent EQS for total water and/or suspended particulate matter

Standard laboratory toxicity and bioconcentration tests contain low levels of total organic carbon (TOC) in the test system[23]. As a result, the resulting EQSs refer to **dissolved concentrations**. It follows that compliance assessment with a water column EQS should ideally be based on the sampling and analysis of the dissolved fraction. This is similar to the way the PNEC is used according to the TGD (Part 2, Section 2) (EC, 2003) and REACH (R.16) (ECHA, 2008 and updated 2016).

Discrepancies between total and dissolved concentrations may only become evident for very hydrophobic substances, i.e. K_p values in excess of 10000 l.kg^{-1} or K_{oc} values for linear partitioning into amorphous organic matter in excess of 100000 l.kg^{-1}. This will generally only be found for substances with a log K_{ow} above 6. Thus, for compounds with log K_p<4 (or, if this value is not available, log K_{ow} <6), the $EQS_{water, total}$ is equivalent to the $EQS_{water,dissolved}$.

Some Member States may have a preference to undertake monitoring using <u>total</u> water samples, incorporating both the dissolved fraction and the chemical that is sorbed onto suspended particulate matter (SPM), or the SPM fraction only. The fraction found on SPM is likely to be

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[23] OECD guidelines for the acute and chronic daphnid test, the fish early life stage test and short-term fish embryo and sac-fry stage tests, the fish juvenile growth test, the chironomid test, and the bioconcentration test with fish, all set a maximum level of 2 mg.l^{-1} to the TOC content. In most laboratory studies, however, the TOC content will not reach this level, which means that in practice toxicity results reflect dissolved concentrations.

particularly important for hydrophobic substances. To allow for this option, guidance is provided here on converting the water column standard as derived for the dissolved concentration (the final EQS value) into an equivalent <u>total</u> concentration in water (EQS$_{water,total}$) that corresponds to the quantity of the substance that is in true solution plus any of the substance sorbed to SPM. In some cases, laboratory tests include significant levels of SPM (OECD test guidelines permit some SPM). In such cases, the dissolved concentration must first be determined (Step 1). Only then can the EQS$_{water,total}$ be estimated (Step 2).

Step 1 – Estimation of EQS$_{water,dissolved}$

If no organic carbon content is present, the concentration is assumed to be fully dissolved and this step can be omitted. The derived quality standard should then be considered to refer to the dissolved concentrations (EQS$_{water, dissolved}$). If organic carbon is measured in the critical toxicity studies, the dissolved concentration (C$_{water, dissolved}$) can be calculated from the total concentration in critical ecotoxicity experiments (C$_{test water, total}$) and the total organic carbon content in these experiments (TOC$_{test water}$) as follows, where K$_{oc}$ is in l.kg^{-1} and TOC$_{test water}$ is in mg.l^{-1}.

$$C_{water,dissolved} = C_{test water, total} \cdot \frac{1}{1 + K_{oc} \cdot TOC_{test result} \cdot 10^{-6}}$$

In this case, the concentrations are corrected for organic carbon, including DOC that limits the substance's (bio)availability.

This equation may be used for laboratory toxicity or bioconcentration data but could also be used to convert data from a mesocosm study or a field bioaccumulation study. Where an EQS has been derived using an SSD approach, it is useful to examine all studies that lie around or below the HC.

Step 2 – Estimation of EQS$_{water,total}$

For highly hydrophobic compounds the final derived EQS (which is an EQS$_{water, dissolved}$) should be corrected using the default concentration of suspended matter (C$_{SPM}$) and the partition coefficient to suspended matter (K$_{p,susp}$).

$$EQS_{water,total} = EQS_{water, dissolved} \cdot (1 + K_{p,susp} \cdot C_{SPM} \cdot 10^{-6})$$

where:

- EQS$_{water,total}$ = quality standard for the total concentration in water;
- EQS$_{water,dissolved}$ = value of dissolved concentration in water, mostly directly derived from the toxicity or bioaccumulation tests;
- K$_{p,susp}$ = partition coefficient to suspended matter (l·kg^{-1}), which might be estimated as the product of the K_{oc} value for the substance (l·kg^{-1}) and the organic carbon content (f$_{oc}$) of suspended matter (EU default from TGD (EU 2003) 0.1);
- C$_{SPM}$ = concentration of suspended matter (mg·l^{-1}; For several water types like large rivers the SPM content is reasonably constant, and a default value has been proposed for this type of river. EU defaults are 15 mg·L^{-1} for freshwaters and 3 mg·L^{-1} for marine waters and for example, the annual average TOC content of the Rhine in the Netherlands is about 4 mg L^{-1}, however, under deviating 'local' environmental conditions other values need to be applied); and
- 10^{-6} is = the conversion factor from mg into kg.

A further refinement is to base compliance monitoring on the analysis of the SPM instead of the unfiltered water samples. This is because hydrophobic substances are more likely to be sorbed to SPM than to be freely dissolved in the water column. For the purpose of comparing the analyses of SPM to the derived water column EQS, guidance is provided below on how to convert the water column EQS into an EQS based on SPM (EQS$_{SPM}$).

When the EQS for an organic chemical is expressed as a dissolved concentration in water (referred to as $EQS_{water,dissolved}$ in this section) , a corresponding concentration in SPM may be calculated and used as a surrogate standard. This should be done for hydrophobic organic substances whose partition coefficient triggers exceed those given above.
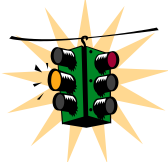
The equation to calculate the concentration in SPM from the dissolved concentration in water and vice versa is as follows:

$$EQS_{SPM} = EQS_{water, dissolved} \cdot K_{p,susp}$$

where:

– EQS_{SPM} = quality standard for water referring to the substance concentration in SPM; according to the EU TGD (EU, 2003); by default water has an organic carbon content of 10%;

– $EQS_{water, dissolved}$ = quality standard for water referring to the dissolved concentration; and

– $K_{p, susp}$ = substance-specific partition coefficient for SPM–water (e.g. f_{oc}, K_{oc} or any valid experimental value);

3.7   Deriving quality standards for water abstracted for drinking water ($QS_{dw, hh}$)

	Look Out! Standards set under the WFD apply in water bodies, including water bodies used for drinking water abstraction. At the tap, the standards set in the Drinking Water Directive apply. To ensure coordination between the DWD and the WFD, Article 7 of the WFD requires Member States not only to meet the objective of good status in water bodies used or intended for drinking water abstraction, but also to ensure that under the water treatment regime applied, the resulting water will meet the requirements of the Drinking Water Directive. This may mean that more stringent EQS need to be set, as described in section 3.7.2. It may also be appropriate to take into account organoleptic aspects such as smell, taste and colour.

3.7.1 Overview

In addition to potential exposure through the consumption of fishery products (see Section 4.5), a second route for human exposure to substances in water is through drinking water. The WFD therefore requires quality standards to protect humans against this route of exposure.

In principle, existing drinking water thresholds (e.g. EU drinking water standards in the Drinking Water Directive 98/83/EC, and World Health Organization (WHO) drinking water guideline values[24]) provide the basis for the derivation of the $QS_{dw, hh}$ for those water bodies used for the abstraction of drinking water ($QS_{dw, hh}$). Standards in the Drinking Water Directive take into account toxicological data, but also other considerations (e.g. chemical-analytical considerations, acceptability to consumers, or the ability to achieve values lower than the WHO guideline values in practice in Europe, justified on the basis of the precautionary principle). The WHO guideline values represent concentrations that do not result in any significant risk to health over a lifetime of consumption. The approach chosen in this guidance focuses preferentially on the toxicological

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[24] The WHO guideline values are not called standards because they are not legally binding.

effects of substances in drinking water. A treatment factor should be applied to the drinking water threshold so that the $QS_{dw, hh}$ relates to the 'raw' water (i.e. it is an 'environmental' standard). Drinking water thresholds and treatment processes used to achieve them should be taken into account in determining quality standards for water abstraction resources. This should have regard to Article 7 of the WFD with reference where appropriate to simple treatment.

WFD (Article 7(2) and (3)) and DWD (Article (4) require Member States to prevent any deterioration of the present quality of water intended for human consumption or any increase in the pollution of waters used for the production of drinking water.

If no existing drinking water thresholds are available (either DWD standards or WHO guideline values) a standard for surface water abstracted to produce drinking water may be derived using the procedure described in the next Section (3.7.2).

3.7.2 $QS_{dw, hh}$ for drinking-water abstraction

A QS for the abstraction of drinking water ($QS_{dw,hh}$) needs to be derived as follows (see also Figure 7)[25]:

1.   If an EU drinking water standard (from Directive 98/83/EC) or a WHO drinking water guideline value is available, follow the procedure described below. If both the WHO guideline value and EU drinking water standard exist but the values are different, the WHO drinking water guideline value is preferred.

   o   If the drinking water threshold is less stringent than the other QS_{water} values already derived (i.e. $QS_{fw, eco}$, $QS_{sw, eco}$, $QS_{fw, ,secpois}$, $QS_{sw, secpois}$, $QS_{water, hh food}$), it could be decided that a $QS_{dw, hh}$ need not be derived.

   o   If the drinking water threshold is more stringent than the other QS_{water} values already derived (i.e. $QS_{fw, eco}$, $QS_{sw, eco}$, $QS_{fw, ,secpois}$, $QS_{sw, secpois}$, $QS_{water, hh food}$), the $QS_{dw, hh}$ is derived as follows:
       •   Substance-specific <u>removal efficiencies</u> are estimated. This may require consultation with drinking water experts. The removal efficiency is expressed as the *fraction (F) not removable by treatment*.
       •   The $QS_{dw, hh}$ is then calculated using equation A.

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[25] High treatment factors reflect the need for a high removal rate. Even where highly effective treatment is already in place, relying on this to compensate for contamination is not the most sustainable approach. Drawbacks include: (i) higher treatment costs; (ii) higher energy consumption and carbon footprints; (iii) compromise of the multiple barrier principle - i.e. an inadequate margin of safety between pollutant concentrations in raw water and drinking water, such that treatment failure could lead to exceedance of maximum acceptable concentrations in drinking water. For this reason, Art. 7(3) WFD requests that "Member States shall ensure the necessary protection for the bodies of water identified with the aim of avoiding deterioration in their quality in order to reduce the level of purification required in the production of drinking water."

Therefore, in line with the combined approach laid down in the WFD, when deriving EQS for water abstracted for drinking water using treatment factors, Member States should in parallel strive to reduce pollution in the raw water body (e.g. as part of the Programmes of Measures) to reduce the treatment required to reliably meet the drinking-water standards. At a local level, the process of planning the (combined) control measures for the drinking-water supply system, which determine the treatment factors, calls for cooperation between the drinking-water sector experts and the authorities that manage the raw water bodies

$$QS_{dw,hh} = \frac{drinking\ water\ threshold}{F_{not\ removable\ by\ treatment}} \quad \text{(A)}$$

2. If neither an EU drinking water standard nor WHO guideline value is available, follow the procedure described below:

    - A provisional drinking water QS is calculated according to equation B.

$$QS_{dw,hh} = \frac{0.2 \cdot TL_{hh} \cdot bw}{uptake_{dw}} \quad \text{(B)}$$

Use a human body weight (bw) of 70 kg and a daily uptake of drinking water (uptake$_{drw}$) of 2 litres (ECHA, 2008). By default, a fraction of 0.2 of the human toxicological threshold (threshold level human health, TL$_{hh}$) is allocated to the intake of the substance via drinking water. This default may be adapted, but this should only be done when sufficiently underpinned data (e.g. total diet studies and total coverage of possible intake routes) are available demonstrating that either a higher or lower value is justified. The value for TL$_{hh}$ should be the acceptable daily intake (ADI) or tolerable daily intake (TDI) if these are available, a reference dose (RfD), or a benchmark dose.

If no ADI or TDI is available, the TL$_{hh}$ could be calculated from the NOAEL$_{min}$ (the lowest no observed adverse effect level value from a review of mammalian toxicology data) using equation C. However, before deriving a TDI or an ADI from a NOAEL, a human toxicologist should be consulted in any case.

$$TL_{hh} = \frac{NOAEL_{min}}{100} \quad \text{(C)}$$

If the compound of interest is potentially carcinogenic[26], the TL$_{hh}$ is equal to the concentration corresponding to an additional risk of cancer for 1×10^{-6} (for 70 years exposure).

- If the (provisional) drinking water standard is less stringent than the other QS$_{water}$ values already derived (i.e. QS$_{fw, eco}$, QS$_{sw, eco}$, QS$_{fw, secpois}$, QS$_{sw, secpois}$, QS$_{water, hh food}$), it could be decided that a QS$_{dw, hh}$ need not be derived and that no further work is required.
- If the QS$_{dw,hh}$ calculated using equation B is more stringent than the other AA-QS$_{water}$ values already derived (i.e. QS$_{fw, eco}$, QS$_{sw, eco}$, QS$_{fw, ,secpois}$, QS$_{sw, secpois}$, QS$_{water, hh food}$), the QS$_{dw, hh}$ is derived as follows:
  1. The removal efficiency of the substance is estimated. This may require consultation with drinking water experts. The removal efficiency is expressed as $F_{not\ removable\ by\ treatment}$.
  2. The QS$_{dw, hh}$ is then calculated using equation A.

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[26] No guidance is given on how to establish the potential carcinogenicity of a compound, but the assessor should check the appropriate R phrases (DSD) or Hazard statements (CLP/GHS). No guidance is available on how to estimate a concentration that corresponds to an excess cancer risk of 10^{-6}. Therefore, a human toxicologist should be consulted.

For metals, the same approach as described here is followed.

| EU DW standard (DWD 98/83/EC) or WHO guideline value available? | → yes → | Is this value higher than other QS values ($QS_{fw, eco}$, $QS_{sw, eco}$, $QS_{fw, secpois}$, $QS_{sw, secpois}$, $QS_{water, hh food}$)? | → yes → | No $QS_{dw, hh}$ needs to be derived. |

no ↓ (below first box)

no ↓ (below second box)

The $QS_{dw, hh}$ is calculated using the WHO (preferred) or EU standard and substance-specific removal efficiencies.

| Calculate a provisional drinking water standard. Is this value higher than other QS values ($QS_{fw, eco}$, $QS_{sw, eco}$, $QS_{fw, secpois}$, $QS_{sw, secpois}$, $QS_{water, hh food}$) | → yes → | No AA-$QS_{dw, hh}$ needs to be derived. |

no ↓

The AA-$QS_{dw, hh}$ is calculated using the calculated provisional drinking water standard and substance-specific removal efficiencies of the current level of treatment.

Figure 7: Overview of the derivation of the quality standard for drinking water abstracted from surface water ($QS_{dw, hh}$)

4 DERIVATION OF BIOTA STANDARDS

4.1 Introduction

For many substances, an EQS based on their concentration in the water column is appropriate. The derivation of EQSs in water is covered in Section 3. However, if substances pose a significant risk through indirect toxicity (i.e. secondary poisoning resulting from food-chain transfer), or their analysis is more feasible in other environmental matrices, such as biota and/or sediments, then a biota standard may be required alongside, or instead of, the water column EQS. This is typically the case for, but not restricted to, hydrophobic substances having very low water solubility or a tendency to bioaccumulate through the food web.

Biota standards were set for hexachlorobenzene, hexachlorobutadiene and mercury and its compounds in the Daughter Directive to the WFD on EQSs (2008/105/EC), establishing concentration limits in prey tissue (fish, molluscs, crustaceans and other biota). In Directive 2013/39/EC, the set of biota standards was extended to include brominated diphenylethers, fluoranthene, benz[*a*]pyrene and associated PAHs, dicofol, PFOS, dioxins and dioxin-like compounds, hexabromocyclododecane (HBCDD) and heptachlor (epoxide). For some compounds, Directive 2013/39/EC also gives the equivalent water column standard[27]. Where no such water-based standard is given, the EQS-directive gives Member States the option to develop an EQS for an alternative matrix in order to adapt the assessment of compliance in order to fit more closely with the local monitoring strategy.

This chapter describes the derivation of biota standards. The steps involved in deriving biota standards for the protection goals described in Section 4.2 are outlined in the scheme below (Figure 8).

4.2 Protection goals

The WFD requires biota EQSs to protect:

1. Humans from adverse effects resulting from the consumption of chemical-contaminated food (fish, molluscs, crustaceans, etc.).
2. Predators and top predators, such as birds and mammals, from risks of secondary poisoning brought about by consuming toxic chemicals in their prey.
3. Benthic and pelagic predators (e.g. predatory fish) that may also be at risk from secondary poisoning.

This section provides guidance for dealing with the first two protection goals (for which the temporary standards $QS_{biota, hh food}$ and $QS_{biota,secpois}$ are derived. The methodology applies to biota standards for freshwater (inland waters) and marine (transitional, coastal and territorial waters) ecosystems. Currently, technical guidance for benthic and pelagic predators (the third protection goal) is not well-developed. **At present, biota standards developed for birds and mammals are assumed to be sufficiently protective for benthic and pelagic predators**.

This means that the guidance must cover biota standards to (a) protect wildlife against the risks of secondary poisoning and (b) to protect humans against the risks of eating contaminated fishery products. These are dealt with separately in Sections 4.4 and 4.5, respectively. It cannot be assumed that a biota standard protecting the human food chain will automatically protect wildlife, or vice-versa.

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[27] This is a concentration in water, which by uptake and biomagnification through the food web, would reach a tissue concentration equivalent to the biota EQS.

Biota standards are preferably expressed as a concentration in the prey that may form the diet of predators (including humans). For the marine environment, the food chain underpinning the biota standard is extended with an additional step to protect the marine top predators such as polar bears and orcas. The extra biomagnification step in the diet of the top predators should be accounted for in the biota standards for marine waters.

The CSTEE (2004) expressed the opinion that biota quality standards are preferably expressed as concentrations in biota and assessment is based on direct assessment and monitoring of biota. However, some Member States may wish to retain an option to sample and analyse only water column samples. Translation of the biota standard to a water column threshold is therefore helpful. The water column equivalent concentration is also required when selecting an overall EQS (Section 2.5), so that standards can be compared on the same (mass/volume) basis.

Secondary poisoning (Section 4.4)
Determine critical food item in the freshwater or marine food chain
Section 4.4.3

Freshwater	Marine water
TMF(lipid)<0.8 or TMF(dry weight)<1.0?	TMF(lipid)<0.8 or TMF(dry weight)<1.0?
Yes? Calculate risk limit for bivalves	Yes? Calculate risk limit for bivalves
No? Calculate risk limit for fish	No? $BMF_{b/m}$(lipid)<0.7 or $BMF_{b/m}$(dry weight)<1.1?
	Yes? Calculate risk limit for fish
Section 4.4.3.1	No? Calculate risk limit for birds and mammals
	Section 4.4.3.2

Data selection (Section 4.4.4) and
expression on a basis of energy normalized diet concentrations (section 4.4.5)

Oral dose	Diet concentration
Calculate normalized concentration with body weight and daily energy expenditure estimated from body weight	Normalize to energy content of the diet
Section 4.4.5.1	Section 4.4.5.2

Conversion of endpoints to concentrations in critical food item
Section 4.4.6

Extrapolation of acute and subchronic endpoints to chronic toxicity
Apply assessment factor for study duration
Section 4.4.7

Deriving a quality standard for secondary poisoning
Extrapolation to the required protection level of the ecosystem
AF 10 to lowest value or AF 1-5 to species sensitivity distribution
Section 4.4.8

$QS_{biota, sec pois, fw}$ for critical food item	$QS_{biota, sec pois, sw}$ for monitored food item

Final EQS_{biota}	Human consumption of fishery products
	$QS_{biota, hh food}$
	Section 4.5

Calculate concentration in other species to be monitored with TMF (BMF) and lipid/dry weights ratios	Calculate concentration in water with BAF corresponding to critical food item. Compare with standards for other routes
Section 4.6.1	Section 4.6.2

Figure 8: Steps involved in deriving a biota standard

The translation from a biota standard to an equivalent water concentration depends on a good understanding of the bioconcentration, bioaccumulation and biomagnification processes from water and through the food web. However, there might be disadvantages with this approach for highly bioaccumulative substances (those identified as B or vB according to Annex XIII of REACH[28]) because estimates of bioconcentration, bioaccumulation and biomagnification can be uncertain for such substances.

4.3   Expression of a biota standard

There are several options for expressing a biota standard depending on the methodology used to derive it. A biota standard may refer to (EC, 2014):

- A specific species or group of species;

- A surrogate matrix for a particular species (e.g. eggs, pellets, etc.);

- A specific group of food (diet products from aquatic ecosystems).

Any of these is acceptable, but prey (food) species are preferred. The QS should be expressed in terms of µg/kg (wet weight) of the whole organism. Since hydrophobic organic chemicals tend to accumulate in body lipids, experimental residue data are sometimes expressed in terms of a lipid-normalised concentration. For other substances, such as metals, a normalisation to dry weight could be more appropriate. If is possible and scientifically justified (e.g. the substance primarily accumulates in lipids), all data should be **normalised** to a standard lipid **(or dry weight) fraction** (Section 4.4.2 and 4.4.3).

Because some substances biomagnify throughout the food chain, higher concentrations are usually observed at higher trophic levels. **These higher trophic levels are more relevant for both secondary poisoning of birds and mammals and for human fish consumption, and, therefore, a normalisation to trophic level should be performed as well, if possible** (Section 4.4.1 and 4.4.3).

For water column standards, protection against long-term exposure is addressed by expressing the standard as an average over a fixed time (usually a year). Although a biota standard is also intended to protect against prolonged exposure, sampling of biota is likely to be rather infrequent. Unlike water standards, there is likely to be greater variability in exposure between sites than there is over the time. Greater emphasis should be placed on the spatial design of sampling schemes. Further guidance on sampling and interpretation of data to assess compliance with a biota EQS is provided separately (EC, 2014). This guidance focuses on the <u>derivation</u> of biota standards.

4.4   Deriving a biota standard to protect wildlife from secondary poisoning

As explained in Section 4.2, biota standards may be set to protect humans from consumption of contaminated fishery products or to protect wildlife from exposure via the food chain. **This section describes the latter objective, i.e. derivation of quality standards for secondary poisoning for food chains in the aquatic environment.** The scientific basis for the methodology is presented in more details elsewhere (Verbruggen 2014).

In the previous European guidance document (EC, 2011) two other methods were described. One is the diet-based approach (also used in the EU TGD (EC, 2003) and REACH guidance (ECHA,

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[28] Substances with a bioconcentration factor (BCF) > 2000 L/kg are considered bioaccumulative (B) and substances with a BCF > 5000 L/kg are considered very bioaccumulative (vB). Also other information, such as biomagnification factors (BMF), trophic magnification factor (TMF), bioaccumulation factor (BAF), or biological half-lives could be used to reach these conclusions.

2008) and the other is a dose-based approach. The approach described here is based on the same principles as the previous guidance, but it accounts for the energy content of the food items and, as a result, default assessment factors to convert from laboratory diet to natural diet in the field are avoided.

The guidance described here replaces that described previously (EC, 2011). A rationale for preferring the method presented here over the two old methods is given in the underlying report (Verbruggen, 2014).

4.4.1 Description of relevant food chains

4.4.1.1 Freshwater food chain

The routes for secondary poisoning that were included in the previous EQS-TGD (EC, 2011) are consistent with those in the TGD (EC, 2003) and the REACH guidance (ECHA, 2008) where the food chain in freshwater ecosystems is defined as:

water → aquatic organisms → fish → fish-eating predator

The predators are mostly birds or mammals, although feeding studies for large predatory fish may be used as the basis for the standard if these are available (EC, 2011).

The transfer of a chemical from the environment and up the food chain can be described by a combination of bioconcentration and biomagnification. The bioconcentration factor (BCF) is the ratio between the concentration of a substance in the organism compared to that in water, so by considering exposure only through water and not via food. These BCF values are determined in laboratory experiments. The biomagnification factor (BMF) is the ratio between concentrations in an organism and its diet, usually determined from field studies, which includes exposure via water and food simultaneously.

A biomagnification factor (BMF) is supposed to express the ratio between the chemical concentration in a consumer and the concentration in its diet and, as a consequence, it only covers one trophic level (TL) (e.g. a predatory fish eating a small fish). However, for substances that biomagnify throughout the food chain, the only species that are in thermodynamic equilibrium with the water phase are the species at the base of the food chain, which are primary producers, i.e. algae and plants (e.g. Kelly *et al.*, 2007; Borgå *et al.*, 2012; Burkhard *et al.*, 2013). The fish in the simplified food chain shown above are then only primary consumers, eating plant material and occupying trophic level 2. These are usually not the fish eaten by birds, mammals or humans, which are generally larger and belong to trophic level 3.5 to 4 (EC, 2014; Moermond and Verbruggen 2013).

The trophic magnification factor (TMF) is defined as the average increase in contaminant concentration over each trophic level in the food chain. It is thus a measure of the biomagnification factor for one trophic level. The following example food chain helps explain:

water → algae → daphnids → small fish → predatory fish → fish-eating predator

	TL1	TL2	TL3	TL4	TL5
BCF	TMF	TMF	TMF	$BMF_{b/m}$	

Based on research with hexachlorobenzene (Moermond and Verbruggen, 2013) it appears that the BAF for predatory fish at TL4 can be explained by considering the magnification at each of the

three steps in the food chain between algae (TL1) and the predatory fish (TL4). So the BAF for the TL4 fish is TMF^3, reflecting the theory of trophic magnification (Burkhard *et al*, 2013).

As a reasonable estimate for substances that accumulate (biomagnify) throughout the food chain, fish that occupy trophic level 4 are selected as basis for the biota standard.

There may be several reasons to look at the bioaccumulation potential of species other than fish (for example mussels or crustaceans). For example, if metabolism is more efficient at higher trophic levels, such as for polycyclic hydrocarbons in fish, aquatic organisms from lower trophic levels accumulate the substance to a higher concentration than fish. This process is called biodilution (e.g. Wan *et al.*, 2007). Also, for substances that do not biomagnify, but have other mechanisms of accumulation, such as metals, species in lower trophic level of the food chain may have higher bioaccumulation potential. For example, a recent analysis showed that uranium accumulates in aquatic plants, bivalves and fish to comparable levels (Van Herwijnen and Verbruggen, 2014). In these cases, accumulation in aquatic organisms other than fish is at least as relevant:

water → aquatic organisms → predator

In conclusion, the food item that will determine the final value for the quality standard in biota is not only dependent on the energy contents of the food items, but also on the bioaccumulation characteristics of the substance through the food chain This is why we need to select the critical food item when deriving a biota standard for secondary poisoning (Section 4.4.3).

4.4.1.2   Marine food chain

For marine ecosystems, the same routes are identified as for freshwater ecosystems, but a further trophic level has been added to account for the longer food chains that exist in the marine environment. This is the level of the top predators that feed on the marine fish-eating predators (like sharks, polar bears or some cetaceans). The marine food chain thus becomes (EC, 2003):

water → aquatic organisms → fish → fish-eating predator → top predator

	TL1-3	TL4	TL5		TL6
BCF		TMF^3			$BMF_{b/m}$

In the case that other aquatic organisms are more relevant, these aquatic organisms are used instead of predatory fish:

water → aquatic organisms → predator → top predator

Although this additional step is also described in the previous version of the EQS-TGD (EC, 2011), no such difference has been made for biota quality standards in the new European Directive 2013/39/EU, in which the same biota standard has been set for fish from both freshwater and other surface waters. Nevertheless, in order to protect the additional trophic level of marine top predators, such a differentiation between biota standards for fresh and marine surface water is

necessary if the substance biomagnifies in birds and mammals. This should be accounted for by the inclusion of a biomagnification factor in the biota standard that describes this biomagnification from fish or other aquatic organisms to birds and mammals.

In the marine environment, the fish-eating predator is, similar to the freshwater compartment, usually a bird or mammal. As for the freshwater compartment, the risk assessor should investigate which of the food items is critical for the quality standard in biota. This means for the marine food chain that as well as establishing acceptable concentration limits in aquatic organisms such as fish and molluscs, the concentration limits in predators of these (fish-eating birds and mammals, e.g. seals) have to be established to protect top predators (section 4.4.3.2). **A consequence of this additional step is that separate biota standards for freshwater and marine waters may be necessary** and, for biomagnifying substances, the biota standard in marine systems will usually be more stringent.

4.4.2 Characteristics of different food items

When deriving a biota standard, it is necessary to identify the food item (i.e. the point in the food chain) in which the EQS will be expressed. To select the food item in a food chain that is most relevant for secondary poisoning, both the energy content and bioaccumulation parameters should be collected for several food items. Section 4.4.3 provides further detail.

If bioaccumulation parameters are normalized to the lipid fraction, as is usually done for hydrophobic substances, the lipid fraction also needs to be known. If bioaccumulation parameters are expressed on a dry weight basis, as is usually done for most metals, dry weight fraction should be known instead. The lipid normalisation for organic substances and the dry weight normalisation for metals could be considered as default approaches. However, there might be substances for which a different normalisation is more appropriate, such as protein normalisation for perfluorinated compounds (PFCs). In general, the choice of normalisation is determined from a review of the literature available for a substance.

For fish, a default lipid fraction of 5% has been suggested (ECHA, 2014; OECD, 2012). A reasonable default for small birds and mammals is 10% (Hendriks *et al*, 2001; Hendriks *et al.* 2005). These default values for lipid fraction are consistent with the values for dry weight fraction and energy content for fish and vertebrates (Smit, 2005; EFSA, 2009), taking into account the standard energy contents for lipids, carbohydrates, and proteins of respectively 37, 17, and 17 kJ/g (90/496/EEC).

There is no standard default lipid fraction for bivalves, another important food item in both the freshwater and marine ecosystems. A default dry weight fraction of 8.3% and an energy content of 19.3 kJ/g_{dw} (Smit, 2005; EFSA, 2009) would lead to a lipid fraction of 1% in bivalves. This seems to be a reasonable value for freshwater and marine mussel species (Bruner *et al*, 1994; Lazzara *et al.*, 2012; Pleissner *et al.*, 2012) and so this default value is proposed here. Data for bivalves, fish, and mammalian and avian vertebrates are summarised in Table 7.

Information on lipid fraction for food items other than fish, bivalves or small vertebrates, or information on protein fraction is not yet readily available. Some default data for protein fraction have been used for food web modelling: 10% for invertebrates, 18% for fish and 21% for birds and mammals (Hendriks *et al*. 2005). These defaults could be used if bioaccumulation parameters are protein-normalised.

Table 7 Energy content, moisture fraction and lipid fraction for food items addressed in risk assessment schemes for aquatic food webs

Food item	Energy content [kJ/g$_{dw}$]	Moisture fraction [%]	Lipid fraction [%]
Bivalves	19	92	1
Fish	21	74	5
Vertebrates	23	68	10

4.4.3 Selection of the critical food item

4.4.3.1 Freshwater food chain

The food item that is critical in the food chain needs to be identified first. This will be the food item that contains the highest energy normalised concentration at a certain water concentration. The birds or mammals that feed on this food item are exposed to the highest concentration in their diet. The 'critical' food item is dependent on the relative ratio of the concentration of a substance in different food items, and thus on the bioaccumulation characteristics of a substance throughout the food chain.

The concentration ratios in different food items are described by the bioaccumulation parameters such as the biomagnification factor (BMF), which is the concentration ratio between an organism and its food, or the trophic magnification factor (TMF), which is the average increase in concentration per trophic level[29], determined by regression over several trophic levels (e.g. Burkhard *et al.*, 2013). As described in Section 4.4.2, bioaccumulation parameters such as BMF and TMF are mostly normalized to lipid fraction for hydrophobic substances, dry weight for metals or sometimes the protein fraction for perfluorinated compounds.

Primary consumers occupying trophic level 2 are often considered as a reference level in trophic magnification (e.g. Borgå *et al.*, 2012). For the freshwater and marine aquatic food web, mussels belong to this trophic level. The energy-normalised concentration for a contaminant in mussels is.

$$C_{energy\,normalized,\,mussel}\,[mg/kJ] = \frac{C_{mussel}}{energy\,content_{dw,\,mussel} \cdot (1 - moisture\,content_{mussel})}$$

One then need to estimate what would be the corresponding concentration in fish at TL=4. Fish at trophic level 4 differ by two trophic levels from mussels and other aquatic species feeding on algae and plants. Therefore, normalised concentrations in fish are higher than in mussels by a trophic magnification factor raised to the power 2. For hydrophobic substances, at a certain concentration in mussels, the concentration in fish belonging to trophic level 4 from the same food web then becomes:

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[29] The trophic level can be estimated from stable nitrogen isotope ratios. Food web magnification factors (FWMFs) or trophic magnification factors (TMFs) are based on the slope of the regression of the logarithm of the concentration versus trophic level. The trophic level is calculated assuming an enrichment of 2 to 5‰ (usually 3.4 or 3.8‰) for δ^{15}N (based on stable nitrogen isotope ratios) per trophic level. Care must be taken that the regression is based on trophic level rather than δ^{15}N. If this is not the case, a correction for the increase of δ^{15}N per trophic level has to be applied.

$$C_{\text{energy normalized, fish}} [\text{mg/kJ}] = \frac{C_{\text{mussel}} \cdot TMF^2}{\text{energycontent}_{\text{dw, fish}} \cdot (1 - \text{moisturefraction}_{\text{fish}})} \cdot \frac{\text{lipidfraction}_{\text{fish}}}{\text{lipidfraction}_{\text{mussel}}}$$

If the TMF is used for the pelagic food chain (i.e. up to fish), it must include only data for aquatic species, i.e. excluding birds and mammals. For substances that are not normalised to lipid fraction but to dry weight fraction (1-moisture fraction), this equation becomes simpler:

$$C_{\text{energy normalized, fish}} [\text{mg/kJ}] = \frac{C_{\text{mussel}} \cdot TMF^2}{\text{energycontent}_{\text{dw, fish}} \cdot (1 - \text{moisturefraction}_{\text{mussel}})}$$

Based on the default data presented in Table 7, it follows that, at equal water concentrations, mussels have higher energy normalised concentrations than fish at trophic level 4 (a) if the TMF is smaller than 0.8 ($\sqrt{0.69}$) for hydrophobic substances partitioning into lipids or (b) if TMF is smaller than 1.0 ($\sqrt{1.09}$) for substances that are better normalised to dry weight fraction, such as metals. This is in accordance with the general perception that, if biodilution occurs (i.e. TMF significantly lower than one, or BAF for invertebrates is higher than BAF for fish), invertebrates are the most critical food item (e.g. for PAHs). For substances with a higher TMF, we would normally expect fish to have higher energy-normalised concentrations in their flesh than invertebrates such as mussels.

From these equations and the values from Table 7, it follows that fish at an equal trophic level as bivalves, i.e. solely herbivorous fish at TL~2, and possibly even some fish at intermediate trophic levels, have higher energy normalised concentrations of lipophilic substances than bivalves. This is because bioaccumulation is largely into the lipids of an organism, and bivalves have a low ratio of lipid to dry weight fraction compared to fish.

This calculation assumes that concentrations normalised to lipid fraction are perfectly explained by the trophic magnification factor. However, for biodiluting substances, there will generally be a difference in metabolic capacity between fish and invertebrates, leading to a lower concentration in fish compared to invertebrates, even when they occupy the same trophic level. In these cases, the differences in metabolism might result in a bioaccumulation trend that is not continuous over the food chain, in contrast with biomagnification due to hydrophobic partitioning. This has indeed been observed for PAHs. Strong biodilution of PAHs usually occurs if trophic accumulation over the whole ecosystem, including invertebrates and fish, is considered (Wan *et al.*, 2007; Nfon *et al.*, 2008; Takeuchi *et al.*, 2009). On the other hand, in a more recent food web study with PAHs (Wang *et al.*, 2012), no biodilution was observed in 24 species of fish from a lake, which spanned 2.4 trophic levels.

Although there is a sharp decrease in concentration of biodiluting substances from invertebrates to fish, there is no such decline between different fish species occupying different trophic levels. Therefore, differences in metabolism because of different taxonomy are more important than trophic level. **This leads to the conclusion that invertebrates – instead of fish – are indeed the critical food item for substances that are subject to biodilution. For such substances, the EQS should not be expressed in fish but in invertebrates.**

4.4.3.2   Marine food chain

For the marine environment another step in the food chain should be considered, to include marine top predators that consume fish-eating mammals and birds. The concentration in these birds and

mammals could be calculated by the concentration in fish or other aquatic organisms by an extra biomagnification factor ($BMF_{b/m}$, a BMF for birds and mammals):

$$C_{\text{energy normalized, b/m}} \, [\text{mg/kJ}] = \frac{C_{\text{mussel}} \cdot TMF^2 \cdot BMF_{b/m}}{\text{energycontent}_{\text{dw, b/m}} \cdot (1 - \text{moisturefraction}_{b/m})} \cdot \frac{\text{lipidfraction}_{b/m}}{\text{lipidfraction}_{\text{mussel}}}$$

$$C_{\text{energy normalized, b/m}} \, [\text{mg/kJ}] = \frac{C_{\text{mussel}} \cdot TMF^2 \cdot BMF_{b/m}}{\text{energycontent}_{\text{dw, b/m}} \cdot (1 - \text{moisturefraction}_{\text{mussel}})}$$

$BMF_{b/m}$ thus describes the accumulation from fish, or other aquatic organisms, to birds or mammals. Such a factor has to be determined experimentally from field studies in which homeotherms are included. If the trophic magnification factor is merely based on birds and mammals as predators (i.e. TMF is not merely reflecting the accumulation in the aquatic food chain up to fish), this TMF can be used as a measure of $BMF_{b/m}$. If experimental data are lacking, modelling of the biomagnification potential (e.g. as done in Kelly *et al.*, 2007) is an alternative.

At a $BMF_{b/m}$ higher than 0.7 on a lipid weight basis, the mammalian and avian vertebrates will be the food item leading to the highest concentration for lipophilic substances. For substances that are normalised to dry weight fraction, a $BMF_{b/m}$ higher than 1.1 on a dry weight basis will cause the mammalian and avian vertebrates to contain the highest energy-normalised concentration. This means that for substances that are not easily metabolised by birds and mammals compared to fish, the extra step in the food chain will most likely determine the final quality standard for secondary poisoning in the marine food chain.

4.4.4 Data selection – toxicity studies

Only toxicity studies reporting on dietary and oral exposure are relevant because the pathway for secondary poisoning deals exclusively with uptake through the food chain. Studies that assess effects on developmental or reproductive endpoints are likely to be critical studies because these tend to be sensitive endpoints (i.e. give rise to lower $NOEC_{\text{oral}}$ values) than survival endpoints.

As secondary poisoning effects rarely become manifest in short-term studies, results from long-term studies are strongly preferred. A QS derived in the absence of chronic effects data is subject to high uncertainty and this uncertainty must be flagged in the datasheet. The minimum duration for the study requirements is dependent on the characteristics of the chemical and the lifespan and life-stage of the test species. Toxicity data should ideally relate to tests of 90 days duration or longer (this would result in an AF of 90 or lower according to the TGD and REACH guidance). However, many mammalian toxicity data are generated from 28-day studies. The risk of selecting a study with an insufficient length of exposure as the critical datum could underestimate the potency of a compound, and therefore the $QS_{\text{biota, secpois}}$ may not be protective. On the other hand, by applying a higher assessment factor than needed, the $QS_{\text{biota, secpois}}$ may be over protective.

As toxicity data for wildlife species are not normally available, it will be necessary to extrapolate threshold levels from toxicity data using laboratory test species to wildlife species. If studies are available for wildlife species as well as for conventional laboratory test species, both should be included in the assessment.

Most data for an oral route of exposure are available for birds and mammals. Whilst scientific and data developments may allow us to assess risks to other aquatic predators in the future, in the meantime we must adopt biota standards for birds and mammals, assuming these values provide adequate protection to other taxa that might be at risk from secondary poisoning (e.g. predatory fish). This assumption might only be valid if the secondary poisoning of predators is the most-

sensitive route and if the water concentration estimated from a $QS_{biota, secpois}$ is significantly lower than a QS derived to protect pelagic species.

If relevant ecotoxicological information (e.g. fish feeding studies) can be found, the same approach developed for birds and mammals can be used for pelagic fish species. Although it is not currently practical to develop separate quality standards for the protection of pelagic predators, it is useful to assess whether or not the quality standard for biota is likely to be protective of exposures via food and whether or not the quality standard for water is likely to be protective of exposures via the water. It may be necessary to review this position if information becomes available suggesting that combined exposures (i.e. from both the water and food) lead to greater risks. Under these circumstances, the quality standards may not be protective and a review may be warranted.

4.4.5 Endpoints based on energy normalised diet concentrations

There are two ways in which an energy-normalised concentration may be estimated. Both are presented here. Which method should be followed is dependent on the information available. If a complex or undefined diet is used, the energy content and moisture fraction might be unknown. In such a case, the method to use the dose and daily energy expenditure may be more useful (Method A: Section 4.4.5.1). If only diet concentrations are given and no information on food consumption is available, a dose cannot be calculated, and method B to normalise the diet to energy content could be used (Section 4.4.5.2).

Recent comparisons show that the two ways of calculating a concentration on the basis of energy content yield similar results. If data for both methods of calculations are available, it might be helpful to perform both methods and compare their outcomes.

4.4.5.1   Method A - Input parameters daily dose and body weight

If the endpoint of a toxicity test is expressed as a daily dose (e.g. mg/kg$_{bw}$/day), this could be expressed as a diet concentration normalised to the energy (caloric) content of the food. This accounts for the amount of food an animal has to consume to meet its energy requirements, referred to as its daily energy expenditure (DEE).

For both birds and mammals, the daily energy expenditure (DEE; kJ/d) under field conditions is strongly correlated with the body weight (bw; kg) (Crocker *et al.*, 2002). In other words, small animals expend relatively more energy than larger animals. For animals in a toxicity study, the body weight is mostly known and the daily energy expenditure for birds and mammals (under field conditions) can be estimated from these weight data (Crocker *et al.*, 2002). The final regressions between DEE and bw that are presented and recommended for use by DEFRA (2007) are:

Mammals:

$$\log DEE\,[kJ/d] = 0.8136 + 0.7149 \cdot \log bw\,[g] \text{ for 46 mammal species}$$

This group excludes marine and desert eutherians as well as non-eutherians. The regression for the group of all 115 mammal species estimates a DEE that is only about 20% lower.

Birds:

$$\log DEE\,[kJ/d] = 1.032 + 0.6760 \cdot \log bw\,[g] \text{ for 44 bird species}$$

This regression is for the group of passerine species. The data set for all 134 bird species gives a regression which results in estimates that are about 95% of the estimated DEE for passerine species. Note that in these equations the body weight is given in g instead of kg. The diet concentration on an energy basis (mg/kJ) can now be calculated as:

$$C_{\text{energy normalized}}\,[\text{mg/kJ}] = \text{dose} \cdot \frac{\text{bw}}{\text{DEE}}$$

The dose in this equation is a toxicological endpoint such as the NOAEL, LOAEL, LD50 or similar, expressed as daily dose in mg/kg_{bw}/d. The body weight (bw) is expressed in kg. The DEE can be considered as the energy a bird or mammal must extract from the food under field conditions. With low assimilation efficiency the amount of food consumed will be higher, but this will also lower the effective dose of the chemical taken up by the organism.

4.4.5.2   *Method B – Input parameters diet concentrations and energy content of diet*

If only diet concentrations are given, and no information on food consumption is available, a dose cannot be calculated. In such a case, dietary concentrations could be normalised to the energy and moisture fraction of the specific diet from the study, if these are known:

$$C_{\text{energy normalized}}\,[\text{mg/kJ}] = \frac{C_{\text{diet}}\,[\text{mg/kg}_{\text{fw}}]}{\text{energycontent}_{\text{diet,dw}} \cdot (1 - \text{moisturefraction}_{\text{diet}})}$$

$$= \frac{C_{\text{diet}}\,[\text{mg/kg}_{\text{dw}}]}{\text{energycontent}_{\text{diet,dw}}}$$

The diet concentration (C_{diet}) here is a toxicological endpoint, such as the NOAEC, LOAEC, LC50 or similar, expressed in mg/kg_{fw} or mg/kg_{dw}. The energy content is expressed in kJ/kg_{dw}, the moisture fraction is the amount of water as a fraction of the total diet fresh weight. Energy content values for different types of diets are tabulated in the literature, including fodder that is often used in laboratory studies (Smit, 2005; EFSA, 2009) and values for fat, carbohydrates, and proteins (90/496/EEC). For guidance, these and some other common dietary constituents are tabulated below (Table 8). Of course, if a specific diet with known energy content is provided or can be retrieved, this value should be used instead of the default values.

Table 8 Energy content and moisture fraction for food items used in toxicity tests

Food item	Energy content [kJ/g_{dw}]	Moisture fraction [%]
Commercial fodder	15.1	8.0
Grass and cereal seeds	18.4	14.7
Fruit	14.8	83.9
Fish	21.0	73.7
Terrestrial vertebrates	23.2	68.4
Fat	37	
Carbohydrates	17	
Proteins	17	
Fibre	8	

4.4.6 Conversion of endpoints to concentrations in critical food item

To derive risk limits (thresholds) for secondary poisoning, the energy normalised endpoints of the toxicity tests should be converted into threshold concentrations in the prey that is considered as the critical food item in the food chain (Section 4.4.3). With the energy content of a specific type of food (fish, mussels etc.) the concentration in that food can be calculated from the energy normalised diet concentration (in mg/kJ):

$$C_{\text{fooditem}}\,[\text{mg/kg}_{ww}] = C_{\text{energy normalized}}[\text{mg/kJ}] \cdot \text{energy content}_{\text{fooditem,dw}} \cdot (1 - \text{moisture fraction}_{\text{fooditem}})$$
$$= C_{\text{energy normalized}}[\text{mg/kJ}] \cdot \text{energy content}_{\text{fooditem,fw}}$$

With this equation, concentrations for each type of food can be calculated. The concentration in a range of potential food items can be estimated, providing a very flexible way of selecting the most critical route to derive the final quality standards for biota. If for example, aquatic vegetation appears to have much higher BAFs than fish or mussels, the energy content and moisture fraction for aquatic vegetation can be used instead of those for fish and mussels. In this way, many types of diet that might be consumed by birds and mammals can be selected as the critical food item for secondary poisoning on which to base the quality standard for biota.

4.4.7 Extrapolation of acute and subchronic endpoints to chronic toxicity

Many studies performed with birds or mammals are not full chronic studies. To be able to use all mammalian and avian toxicity data, assessment factors are used for subchronic, subacute, and acute toxicity studies in regulatory frameworks. No clear distinction is made between acute and chronic toxicity data, as in the case of direct toxicity for aquatic and benthic species. The use of acute toxicity studies is not encouraged, but might be necessary if no other data are available. The assessment factors that should be applied to a mammalian or avian NOEC to account for a limited exposure time instead of a full chronic study are presented in Table 9. Because we do not need to allow for the energy content of the food, the factors shown below omit the additional factor of 3 that is applied in current European guidance documents (ECHA, 2010; EC, 2011) to account for the differences in energy content.

Table 9 Assessment factors to be applied to account for limited exposure time in the toxicity studies compared to assumed life-time exposure in the field

Reason for assessment factor	Specific case	Assessment factor	Applicable to
Study duration	Chronic study	1	Chronic NOEC for birds or mammals
	Subchronic study	3	NOEC from 90-d study for mammals
	Subacute study	10	NOEC from 28-d study for mammals
	Acute study	100	LC50/LD50 from acute study for birds

There may be more than one chronic study for the same species. Under these circumstances, the assessor should select the more sensitive study. Data from two different toxicological studies should only be merged if they have been conducted according to a similar guideline, used the same species and test conditions and reported the same key endpoints. It may be that a test with shorter exposure duration reports a more sensitive endpoint than the test with longest exposure duration. In such a case, the assessment factor corresponding to the longest exposure time might be applied to the most sensitive endpoint, even if it is from a study with a shorter exposure time.

A type of study that is not covered in the table is a study in which mammals (e.g. rats, mice or rabbits) are exposed over ten days or more in the gestation period (teratogenicity study, e.g. OECD test guideline 414: Prenatal Development Toxicity Study). Although involving short-term exposure, an assessment factor of 3 is used because the compound is administered during a critical phase in embryonic development.

In the selection of the final assessment factor, consideration must be given to all available data for the same species to reflect all endpoints and test durations of the available studies (Section 4.4.8).

4.4.8 Deriving a quality standard for secondary poisoning - extrapolation to the required protection level of the ecosystem

One value is selected per species after the application of the assessment factor for the study duration in the former step (Section 4.4.7). If an assessment factor is used to derive the quality standard for secondary poisoning, the lowest chronic toxicity value for the set of species is selected. The same data set with entries for all tested species is also used if there are sufficient data to construct an SSD and the HC5 estimated from the SSD is used as basis for the $QS_{biota, secpois}$.

If there are not many species available, the $QS_{biota, secpois}$ will be derived by applying an assessment factor of 10 to the lowest value selected (Table 10). Even with data for only one bird or mammal, the $QS_{biota, secpois}$ is derived from this single study with an assessment factor of only 10[30]. To construct an SSD, data should be available for a minimum of 10 species, including wildlife-relevant predatory species of both birds and mammals (e.g. kestrel and mink). An assessment factor of 1 to 5 should then be applied to the HC5 to account for remaining uncertainty.

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[30] For comparison, the assessment factor to be applied for direct ecotoxicity to aquatic or benthic species is 100 if there is only one chronic NOEC available. In those cases, at least three species are necessary to lower the assessment factor to 10.

Table 10 Assessment factors to extrapolate from laboratory toxicity studies to different protection levels

Reason for assessment factor	Specific case	Assessment factor	Applicable to
Protection level	$QS_{biota, secpois}$	10	Lowest chronic value
		1-5	HC5 of all chronic values

If chronic NOECs for both birds and mammals are available, the lower of the toxicity values is used in the secondary poisoning assessment. In many cases, only acute toxicity data for birds will be available. Although there is no predictable link between acute and long-term toxicity (i.e. a substance that is of low acute toxicity will not necessarily be of low long-term reproductive toxicity), a pragmatic approach in the absence of a chronic study is to derive an 'indicative' standard from the lowest reliable lethal concentration for 50% of the individuals (LC50) value (ECHA, 2008, section R.10.8.2). If the resulting 'tentative' standard for birds is lower than the standard for mammals then, given the lack of information on relative sensitivities between birds and mammals, the uncertainties should be highlighted in the datasheet.

The final value derived for the critical food item in the fresh water food chain can be considered as the quality standard in biota for freshwater ($QS_{biota, sec pois, fw}$):

$$QS_{biota,secpois,fw} \text{ [mg/kg]} = \frac{\text{lowest chronic value or HC5}}{AF}$$

A biota standard for the water compartment is preferably expressed as a critical concentration in aquatic organisms, such as fish or bivalves. For the marine environment, the highest trophic level of the top predators will feed on birds and mammals. Birds and mammals are unsuitable for environmental monitoring, both for practical and ethical reasons so, even if these birds and mammals are the critical food item (Section 4.4.3), they cannot be taken forward as the basis for a biota standard. Therefore, the critical concentrations in birds and mammals should be recalculated to a corresponding concentration in the prey organisms lower in the food chain that can be monitored routinely, usually fish[31]. The biota standard for the marine environment ($QS_{biota, sec pois, sw}$) should then be derived by dividing the final value for birds or mammals by the $BMF_{b/m}$ to arrive at a $QS_{biota, sec pois, sw}$ in fish, followed by a proper lipid or dry weight normalisation between birds/mammals and fish.

$$QS_{biota,secpois,sw} \text{ [mg/kg]} = \frac{\text{lowest chronic value or HC5}}{AF \cdot BMF_{b/m}} \cdot \frac{\text{lipid/dry weight fraction}_{fish}}{\text{lipid/dry weight fraction}_{b/m}}$$

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[31] As an exception, eggs of marine birds could be used as matrix for biota monitoring. In such case, the biota standard does not need to be corrected for the lower trophic level of the biota standard compared to the critical food item. However, normalisation to lipid or dry weight content need still be taken into account.

4.5 Protection of humans against adverse health effects from consuming contaminated fisheries products

Section 4.2 explains that biota QS may be derived to protect wildlife or humans. The $QS_{biota, hh food}$ is intended to protect humans against adverse health effects from consuming contaminated fishery products. The risks to human health arising from substances in drinking water are covered in Section 3.7. Like the biota standards for protecting predators, the standards described here are expressed in terms of residues in food items. In this section, "EU Food Limit" refers to the regulatory standards set by the European food legislation.[32]

4.5.1 General approach to deriving the QS $_{biota, hh food}$

The EU Food Limit, where it exists, is adopted as the $QS_{biota, hh food}$ without further assessment. If no EU Food Limit exists, the derivation of a $QS_{biota, hh food}$ will require undertaking a toxicological assessment based on the following factors:

1.   the toxicity of a substance (based on a tolerable daily intake, TDI, acceptable daily intake, ADI, or reference dose.

2.   the amount of fish consumed each day and

3.   the proportion of the diet that comes from fishery products.

The formula for the calculation of the $QS_{biota, hh}$ food where no EU Food Limit exists is available in section 4.5.3

4.5.2 Fish consumption in the human diet

The default for daily fish consumption by humans under REACH is 0.115 kg d^{-1} (ECHA, 2016). This value was adopted from the former TGD (EC, 2003). However, the exact origin of this default is not clear. It is most likely based on the highest yearly consumption observed for a European member state in a survey in 1992, but the value is not in line with data reported for other countries in that survey and better documented data from more recent food consumption surveys are available from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011), using the most recent update of September 15th 2015.

The 95th percentile of the daily intake of fish and seafood by adults from the general public in Europe is a rational basis for the $QS_{biota, hh food}$. To estimate this value, the 95th percentile of consumption of fishery products by adults from the general public for 16 countries reported in the EFSA database was weighted according to the number of adult inhabitants for each country. For each country, the most recent estimates for consumption of fishery products by the general adult population were used. The number of adult inhabitants (>20 years of age) was retrieved from Eurostat. The resulting value is 0.114 kg·d^{-1} per person, the equivalent value based on reported body weights is 0.00163 $kg_{fish} \cdot kg^{-1}_{bw} \cdot d^{-1}$. These values are almost identical to the former value of

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[32] At the time of writing this TGD, several regulatory limits (called maximum levels or ML) in seafood for the protection of human health are established by regulation Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. No maximum residue level (MRL) in seafood has been set so far (Regulation 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC).

0.115 kg·d^{-1} per person with an average body weight of 70 kg, but the values presented here are traceable and well documented (Smit *et al*, in preparation).

Therefore, unless there are food basket data to estimate actual daily fish consumption, we recommend a default of 0.115 kg d^{-1} in combination with a body weight of 70kg. This is equivalent to a daily consumption of 1.6 g fish kg^{-1} body weight.

4.5.3 *Contribution to diet from fishery products*

Chemical exposure via food can come from a variety of sources (e.g. fish, vegetables, meat, etc.). To correct for the intake of contaminants via other sources, we need to consider the contribution made by fishery products to the total intake of the chemical under consideration. In such a food basket approach, actual data on contaminant levels in different food sources are combined with consumption data to estimate the relative importance of each food source. Since this is a time-consuming exercise which requires expert knowledge, we recommend a starting assumption in which a default value for the uptake of a substance from fishery products is used.

The default allocation factor of 20% is a conservative value to protect humans from adverse health effects caused by consuming contaminated fish and seafood. If the $QS_{biota,\ hh\ food}$ appears to be the critical QS when derived on the basis of this default (i.e. it is lower than QSs for other receptors when compared on the same basis, i.e. in the same matrix, e.g. as a concentration in water), substance-specific allocation factors based on food-basket data should be applied where possible. The level of 20% is based on the default allocation factor for the establishment of the WHO guidelines for drinking water quality (WHO, 2011), which changed from 10% to 20%.

The $QS_{biota,\ hh,\ food}$ (expressed as µg·kg$^{-1}_{biota}$) is then calculated as follows from the TL_{hh} (expressed as µg·kg$^{-1}_{bw}$·d^{-1}):

$$QS_{biota,hh\ food} = \frac{0.2 \cdot TL_{hh}}{0.00163}$$

Once a $QS_{biota,hh\ food}$ has been estimated, we need to decide whether secondary poisoning of wildlife or for protection of human health should 'drive' the biota standard. To do this, the $QS_{biota,\ hh\ food}$ should be compared with the $QS_{biota,\ secpois}$ (Section 4.4).

This is a simple step when the EU Food limit is adopted as the $QS_{biota,hh\ food}$ - the more stringent threshold (the one with the lower value) is used as the overall biota QS.

Where the toxicologically-based formula is used, the decision about which receptor (human health or wildlife) drives the overall biota standard is more complex. The recommended steps are as follows:

If the resulting $QS_{biota,\ hh\ food}$ using the above formula is higher than the $QS_{biota,\ secpois}$, further refinement is not needed and the $QS_{biota,\ secpois}$ will be taken forward as the EQS_{biota}. This is because the default assumptions used to estimate the $QS_{biota,\ hh\ food}$ tend to be conservative. If the $QS_{biota,\ hh\ food}$ is more critical (i.e. lower) than the $QS_{biota,\ secpois}$[33], some further assessment is necessary.

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[33] Note that for biomagnifying compounds, the $QS_{biota,\ secpois,\ sw}$ may lower than the $QS_{biota,\ secpois,\ fw}$ because an additional biomagnification step is taken into account. This is not the case for the $QS_{biota,\ hh\ food}$. Whether or not the $QS_{biota,\ hh\ food}$ is critical may thus differ between freshwater and marine compartments.

This refinement is as follow: Firstly, check if the $QS_{biota, hh\ food}$ calculated using the default assumptions is lower than the EU Food Limit (if one is available). In this case, the $QS_{biota, hh\ food}$ should be refined using actual data for fish consumption and allocation factor in place of the defaults, described earlier. If this confirms that fish is the major uptake route, there is no need to keep to the default in which it is assumed that that other routes contribute 80% of the consumed chemical. In this case, the $QS_{biota, hh\ food}$ should be calculated using a higher allocation factor than the default 20% but not exceed 60%[34]. Such a food basket approach is in line with the approaches of WHO (2011) and EFSA (2010, 2011). It is advised to use the default allocation factor of 20% as a minimum level. It is possible that actual data for fish consumption indicate that the contribution of fish to the total intake is less than the 20% default. However, adapting the allocation factor to a lower value would lead to the contradictory situation that strict standards are applied while at the same time, the effect of those standards on the protection of humans is limited, because other food sources contribute much more to the exposure.

After conducting this refined assessment, the revised $QS_{biota\ hh\ food}$ is again compared to the $QS_{biota\ secpois}$ and the more stringent would be adopted as the overall QS_{biota}. If an EU Food Limit exists and it is lower than the refined $QS_{biota\ hh\ food}$, then this would be adopted.

Finally, if the $QS_{biota, hh, food}$ leads to a back-calculated water-based $QS_{water, hh\ food}$ (see Section 4.6.2) that is lower than the $QS_{fw, eco}$ or $QS_{sw, eco}$, (i.e. the human health food chain is the critical receptor) it may be helpful to refine the $QS_{biota, hh, food}$ using a food basket approach (if that has not already been done). When such an approach is not feasible due to a lack of reliable data on the intake of a particular compound via different routes, it may be worthwhile to perform a "reality check" and to identify the main routes of exposure of a substance on the basis of substance characteristics and emission patterns. For example, substances with a high BCF, K_{ow} or K_{oc} may in general give limited exposure through drinking water and/or leaf vegetables.

The following diagrams illustrate the decision-making process to decide whether the $QS_{biota, hh\ food}$ or the $QS_{biota, secpois}$ drive the overall biota standard.

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[34] The use of a weighted percentile to estimate the default fish consumption should be protective for adults, infants and adolescents (https://www.efsa.europa.eu/en/food-consumption/comprehensive-database) based on the proposed default consumption of 1.6 g/kg bw/day, as explained in Section 4.5.2. However, toddlers and other children have a fish consumption that is about 1.5 X higher (2.43 g/kg bw/day; Smit *et al*, in preparation). If it is considered necessary to accommodate all these groups, the allocation factor may be raised but should not exceed 60%.

Figure 9: Selection of biota standard

Does an EU Food Limit exist?

Y

N

Is EU Food Limit < $QS_{biota\ secpois}$?

Undertake toxicological assessment to calculate $QS_{biota,\ hh\ food}$ (estimated using defaults)

N

Y

Adopt $QS_{biota\ secpois}$

Adopt EU Food Limit as QS

Is $QS_{biota,\ hh\ food}$ < $QS_{biota\ secpois}$?

$\bar{Y}$

N

Review default assumptions about fish consumption and % allocation due to fishery products in diet

Adopt $QS_{biota\ secpois}$

Are the required data available?

$\bar{Y}$

N

Refine $QS_{biota,\ hh\ food}$ using food basket data

Keep $QS_{biota,\ hh\ food}$ (estimated using defaults)

4.6 Transfer of biota standards to equivalent levels in other matrices

A separate guidance on biota monitoring under the Water Framework Directive was completed in 2014 (EC, 2014). This guidance summarises current monitoring programmes in Europe, and detailed guidance on the sampling and analysis of chemical residues in biota. Other procedures for species monitored through international conventions for inland, transitional, coastal and marine waters also exist, e.g. Helsinki Commission (HELCOM), OSPAR, and the International Commission for the Protection of the Rhine (ICPR).

In this section, we describe how the $QS_{biota, \text{ sec pois, fw/sw}}$ can be 'translated' into an equivalent concentration in the tissues of a different trophic level i.e. a group of species (Section 4.6.1) or non-biotic environmental matrix (e.g. water) (Sections 4.6.2) that will be monitored to assess compliance with the $QS_{biota, \text{ sec pois}}$. These conversions are done by dividing the biota standards by the relevant bioaccumulation parameters, discussed in the sections below.

A consistent use of the bioaccumulation parameters is important, and all calculations should be expressed on the basis of the default parameters as presented in Section 4.4.2 (e.g. on basis of 5% lipids for fish). Because the biomagnification parameters are normalised, usually to lipid weight or dry weight content, the ratio of the lipid or dry weight contents between the two types of food items also needs to be taken into account when these parameters are used.

4.6.1 Conversion of biota standard into another species suitable for monitoring

The quality standard could be expressed as a concentration in a group of species that is considered more suitable for environmental monitoring than a species at trophic level 4. In these cases, the quality standards for biota as determined by the procedure in Sections 4.4 and 4.5 can be recalculated in the food item that will be monitored by applying the relevant bioaccumulation parameters. The relationships between the quality standard for biota and the species that will be monitored are presented below.

The quality standard for mussels, derived from a biota standard in fish can be calculated as:

$$QS_{biota,mussel} = \frac{QS_{biota,fish}}{TMF^2} \cdot \frac{\text{lipid/dry weight fraction}_{mussel}}{\text{lipid/dry weight fraction}_{fish}}$$

More in general, e.g. for lower trophic level fish, this can be written as:

$$QS_{biota,monitored species} = \frac{QS_{biota,fish}}{TMF^{(4-TL_{monitored species})}} \cdot \frac{\text{lipid/dry weight fraction}_{monitored species}}{\text{lipid/dry weight fraction}_{fish}}$$

If the substance does not biomagnify, only the normalisation to lipid or dry weight content will be required.

4.6.2 Converting a biota standard into an equivalent water concentration

The biota standard can be converted into a water column concentration standard ($QS_{fw,secpois}$, $QS_{sw, secpois}$ or $QS_{fw/sw, hh food}$ in $\mu g \cdot l^{-1}$) for comparison with other water column standards (see Section 2.5) to select an overall EQS, or to fit in with national monitoring regimes that use only water sampling.

This conversion needs to allow for the biomagnification of the substance of interest in the food chain. Bioconcentration factors (BCF) will not be a useful parameter for the bioaccumulation of biomagnifying substances. Rather, field-derived bioaccumulation factors (BAF) for the proper trophic level are more relevant, as has been shown for hexachlorobenzene (Moermond and Verbruggen, 2013) and mercury (Verbruggen *et al.*, 2015). **Therefore, to analyse the bioaccumulation potential of strongly biomagnifying substances, bioaccumulation factors are preferred over laboratory bioconcentration factors.**

water — BAF → predatory fish → predator

The predatory fish referred to in this scheme should belong to trophic level 4 (Section 4.4.1). The biota EQS effectively applies at this level. In selecting the BAF values to estimate the equivalent concentration in water, geometric mean values for BAF at trophic level 4 could be used. A correlation between log BAF and trophic level is very useful to determine which BAF is associated with trophic level 4. Finally, an assessment of all bioaccumulation data including BAF, BMF, TMF, and BCF values, as was done in the example for hexachlorobenzene, is recommended (Moermond and Verbruggen, 2013). At the same time, for substances that do not biomagnify, BAF will not be dependent on trophic level and will be approximately equal to the laboratory derived BCF (Burkhard *et al.*, 2013).

If reliable bioaccumulation factors are missing, the bioaccumulation factor at trophic level 4 can be estimated from the bioconcentration factor and trophic magnification factor, if reliable values for these parameters are available:

$$BAF(TL = 4) = BCF \cdot TMF^3$$

If reliable experimental bioaccumulation data are not available, the BAF at upper trophic level might also be estimated by QSAR. The programme BCFBAF within the EPISuite 4.11 calculates a BAF value for the upper trophic level for fish with a lipid content of 10.7%. After lipid normalisation such a value can be used. For hexachlorobenzene for example, it yields the same value as the selected BAF value for trophic level 4 based on experimental BAFs (Moermond and Verbruggen, 2013). The same routine could be used to estimate the biomagnification for each trophic level if an equivalent biota standard is required for other species (Section 4.6.1).

Finally, the water standard corresponding to the biota standard can be calculated from the selected BAF value:

$$QS_{water, biota} = \frac{QS_{biota}}{BAF}$$

There are added complexities for metals and some other substances, because BCFs and BAFs used to calculate a water concentration may depend on exposure concentration in water. At low metal concentrations, organisms accumulate essential metals and often non-essential metals via the same uptake mechanisms to a higher extent in order to meet their metabolic requirements. At higher concentrations organisms with active regulation mechanisms limit their uptake of metals (ECHA, 2008). As a consequence, the BCFs/BAFs are variable, showing an inverse relationship with external metal concentrations (i.e. higher BAFs and BCFs at lower exposure concentrations). This means that the use of BAF and BCF values for metals must be performed with care.

For metals, BAF and BCF values may be obtained in a variety of ways. In cases where there is evidence of concentration dependency (i.e. the BAF/BCF is higher at lower environmental levels), regression models based on the observed inverse relationship should be used to derive the most appropriate BAF/BCF value for the prey organisms considered (Brix *et al.*, 2001; Efroysmen *et al.*, 2001, McGeer *et al.*, 2003, DeForest *et al.*, 2007). The BAF and BCF for metals, and also for other substances for which the BAF/BCF is concentration dependent, could be described by the following equation:

$$\log(BAF/BCF(C_w)) = a \cdot \log(C_w) + b$$

If BAFs or BCFs are available for more species, a value could be selected for each group of species (e.g. fish or molluscs). This can be done by assuming a similar concentration dependency for the whole group of species. The slope (a) is then the same for all species, while the intercept (b) is different for each species. The BAF for each group is then selected by taking an average value for the intercept in combination with the generic slope. See for an example the assessment of bioaccumulation of uranium (Van Herwijnen and Verbruggen, 2014). With BAF/BCF=C_{biota}/C_w the water concentration corresponding to the biota standard will be equal to:

$$QS_{water,biota} = 10^{\frac{\log(QS_{biota}) - b}{a - 1}}$$

Where regression lines cannot be calculated, BAFs or BCFs may be obtained by calculating geometric means for each group of species from BCF studies using environmentally relevant metal concentrations in the test media or by using BAFs observed in the field. Where there is a choice of BCF or BAF values, the use of BAF is preferred because it considers not only uptake via water, but also exposure via food or sediments, and is therefore considered to be ecologically more relevant than BCF values. BAFs are also more relevant in cases where there is an indication for concentration dependent bioaccumulation and BCFs are determined at concentrations that are substantially higher than ambient concentrations.

4.7 Implementation issues

4.7.1 *Accounting for background concentrations of metals in biota*

The approach described above for secondary poisoning and human consumption of fishery products, whereby NOELs or NOAELs are used for secondary poisoning, and ADI, TDI or a comparable human threshold are used for fishery products, is also applicable to metals.

To assess compliance with a biota standard for a metal it may be necessary to take account of background levels in the environment, otherwise, there is a risk of false positives. Just as defining

a background for metals in water can be challenging, the definition of the natural background level for metals in biota faces the same types of difficulties. Section 3.5 refers to estimating background levels of metals in the environment. While detailed guidance lies outside the scope of EQS derivation, in principle, it would involve measurements of metals in biota taken from species living close to springs or far at sea. It should be recognised that biota may take up metals from the water as well as from particulate matter in water, including plankton, or from the sediment. In general, measurements in biota living in water where metal levels are elevated in either the sediment or the water should not be used for the determination of the natural background level of the substance in biota. The background concentration in biota is species-specific and is further influenced by organisms' age/size and the local food habits. Therefore, background concentrations for biota should always be reported with species age or size and origin.

4.7.2 Biota monitoring to infer water concentrations

Some Member States may prefer to monitor compliance with EQSs expressed as water concentrations from residues in biota, i.e. to use biota for inferring concentrations in water. This might apply, for example, when analytical sensitivity is inadequate to quantify the EQS in water. In addition, because of dilution effects and a decrease in the solubility of hydrophobic pollutants and metals in transitional, coastal and marine waters, it is expected that low concentrations might occur in these systems.

Biota and sediments are able to integrate the pollutant concentrations over a period of time (usually months but not years), while water is more variable, and, in the case of sea water, levels can be related to the tide period as well as the main current or predominant wind direction during the sampling. If biota sampling is used in this way, there must be a good correlation between levels of the contaminants in the organism and in the surrounding water so that the biota concentration can be used to estimate the water concentration with confidence. For example, mussels (*Mytilus edulis*, *Mytilus galloprovincialis*) are likely to be a favoured genus in the marine environment because of the existence of historical datasets.

5. STANDARDS TO PROTECT BENTHIC (SEDIMENT DWELLING) SPECIES

5.1 Introduction

Sediments can act as a sink for chemicals through sorption of contaminants to particulate matter, and they may act as a source of contaminants to particle feeders through resuspension (e.g. by dredging or natural events) or back to the water phase by desorption. The derivation of sediment EQSs is particularly relevant for hydrophobic substances and some metals (see 2.4.2). EQSs for sediments are used instead of, or alongside, EQSs for other compartments to assess the status of water bodies. EQSs for sediments are required to protect benthic (sediment-dwelling) species.

Sediments are a major sink for historic pollutants and changes in bioavailability of such contaminants make compliance assessment more complex than in other compartments. As with other standards, major sources of uncertainty in standard derivation should be highlighted in the technical datasheet dealing with sediment EQSs, along with suggestions on how they might be ameliorated. Section 5.3 provides further suggestions to policy makers on how sediment quality can be assessed and how to identify where management measures may be warranted.

5.2 Derivation of sediment standards

The derivation process is based on that used for effects assessment under REACH (ECHA, 2008) but with an additional consideration of field or mesocosm data. This enables different lines of evidence (sediment toxicity tests, aquatic toxicity tests in conjunction with equilibrium partitioning (EqP) and field/mesocosm studies) to be used to generate the final standard (Figure 10). Further detail on each of these steps, e.g. the use of Equilibrium Partitioning, is provided in the following sections. The temporary standards used in the derivation of sediment standards are explained in Appendix 6.
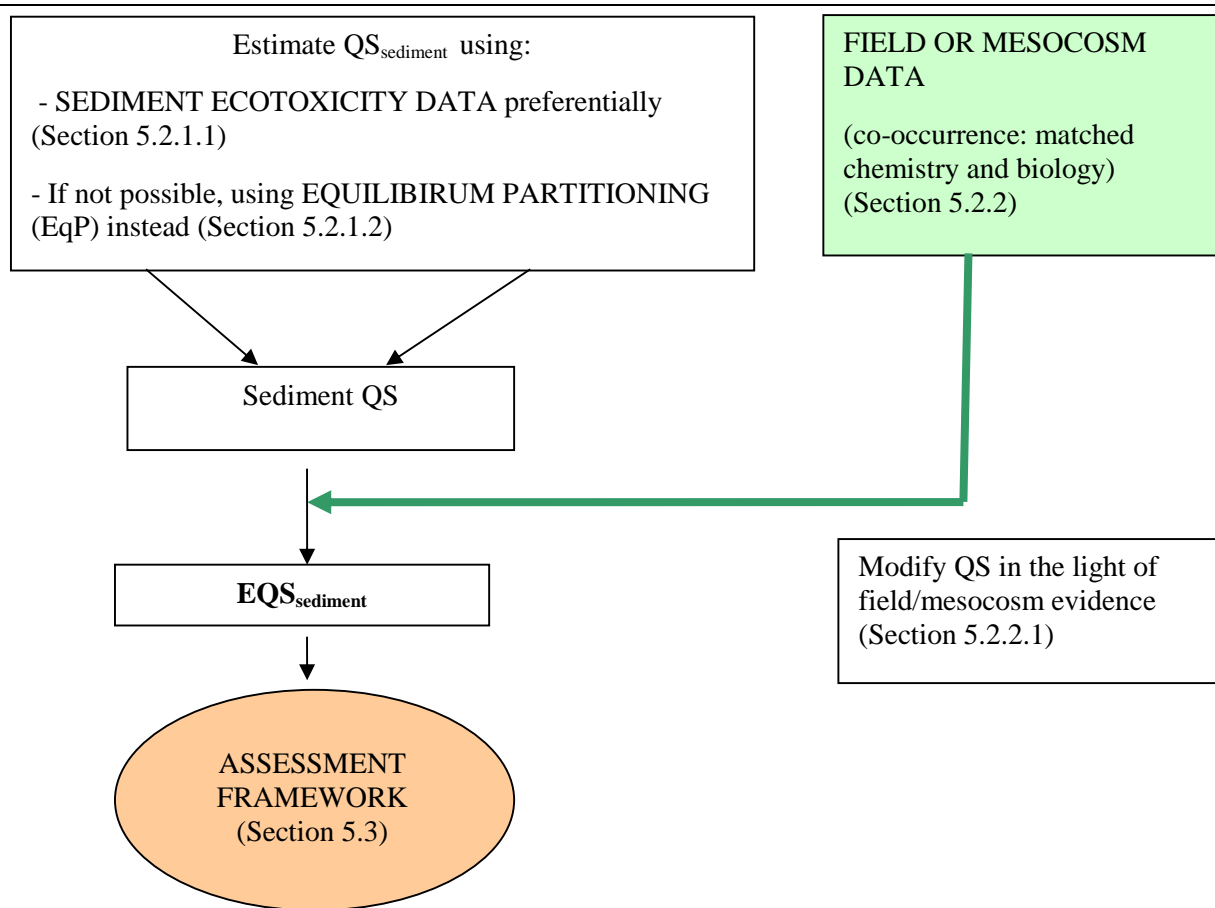
Figure 10: Overview of process for deriving a sediment standard

5.2.1 Derivation of EQS$_{sediment}$ for the protection of freshwater benthic organisms

Data used for the derivation of EQS for sediment can include:

(i)   ecotoxicity data from experiments with benthic organisms (Section 5.2.1.1);
(ii) water column ecotoxicity data used in conjunction with equilibrium partitioning (Section 5.2.1.2);
(iii) empirical field or mesocosm data (e.g. co-ocurrence of benthos and chemical contamination in the field (Section 5.2.1.3).

Where sediment ecotoxicity data are available, option (i) is preferred over option (ii) because of the assumptions and uncertainties inherent in the equilibrium partitioning approach (detailed in Section 5.2.1.2).

5.2.1.1   Use of sediment toxicity data to derive quality standards

Most sediment laboratory toxicity data are based on the use of spiked sediments in which clean sediment has been deliberately contaminated in the laboratory and test organisms introduced to this spiked sediment. Most tests have been performed according to OECD, ASTM or USEPA guidelines using benthic invertebrates (e.g. *Chironomus riparius* OECD 218 - chironomid test/spiked sediment / growth and emergence). Other test species may be used but details on the test conditions must be reported and the data should be assessed for reliability and relevance as described in Section 2.6.2. Further guidance, specific to sediment toxicity tests, is to be found in Appendix 1.

Test data in which availability of the contaminant is maximised are preferred. Maximising exposure should lead to the derivation of more protective values and decrease the uncertainty associated with EQS (i.e. reflect a 'worst case' scenario). In the EU, a 'standard sediment' has a default organic carbon (OC) content of 5% and for organic chemicals a normalisation of toxicity data to this standard sediment is preferred for the derivation of the EQS$_{sediment}$.

For substances for which the bioavailability is dependent on the organic carbon content of the sediment, the variability introduced by the presence of toxicity values generated at different organic carbon concentrations can be accounted for by normalising each (valid) toxicity test result (LC50, EC50, EC10, NOEC) to organic carbon and then express all results in sediment with a standard organic carbon content. The resulting sediment standard can be recalculated to any organic carbon content measured in the field. The organic carbon content of the EU standard sediment is 5%, equal to that used in the TGD, REACH and EUSES.

$$\text{TEST RESULT}_{EU\ standardsed} = \frac{\text{TEST RESULT}_{test\ sed}\ x\ F_{oc,EU\ standardsed}}{F_{oc,\ test\ sed}}$$

Parameter	Description	Unit	Default Value
TEST RESULT	Outcome of toxicity experiment with benthic organism, expressed as EC50, LC50, EC10, LC10, NOEC etc	mg kg$_{dw}$$^{-1}$	
TEST RESULT $_{EU\ standard\ test}$	Test result expressed in EU standard sediment	mg kg$_{dw}$$^{-1}$	
TEST RESULT$_{test\ sed}$	Test result expressed in EU standard sediment		
F$_{oc,\ EU\ standard\ sed}$	Organic carbon content (w/w) of EU standard sediment	kg kg^{-1}	0.05
F$_{oc,\ test\ sed}$	Organic carbon content (w/w) of the experimental sediment	kg kg^{-1}	

Results of long-term toxicity tests with sediment organisms are preferred for deriving sediment standards due to the generally long-term exposure of benthic organisms to sediment bound substances. If such studies are available, a QS$_{sediment,\ fw\ eco}$ or QS$_{sediment,\ sw\ eco}$ is determined using the assessment factors (AFs) in Table 11, applied to the lowest credible datum. The assessment factors are based on those used within the REACH guidance (ECHA, 2008) and applied as follows:

QS$_{sediment}$ [mg/kg] (dry weight) = lowest NOEC or EC10 [mg/kg] /AF (range 100 – 10)

Table 11 Assessment factors applied to spiked sediment tests (ECHA, 2008)

Available data	Assessment factor
One long term test (NOEC or EC10)	100
Two long term tests (NOEC or EC10) with species representing different living and feeding conditions	50
Three long term tests (NOEC or EC10) with species representing different living and feeding conditions	10

If only results from short-term tests with sediment-dwelling organisms are available, an assessment factor of 1000 is applied to the lowest reliable value. In situations where only short-term test data are available, a QS should also be derived using the Equilibrium Partitioning approach (See Section 5.2.1.2). The lowest value would be proposed as the $QS_{sediment}$ in these situations.

In principle, the species sensitivity distribution (SSD) modelling approach (Section 3) can be applied to sediment toxicity data rather than the deterministic (AF) approach. In practice however, the minimum data requirements for an SSD will rarely be met, except perhaps for a few well-studied metals. Guidance on the use of SSD for the derivation of sediment thresholds has not been included within the REACH guidance (ECHA, 2008) however the approach was used within the Voluntary Risk Assessment undertaken on copper (ECI, 2008).

5.2.1.2   Equilibrium Partitioning

If no reliable sediment toxicity data are available, Equilibrium Partitioning (EqP) can be used to estimate the $QS_{sediment, fw EqP}$ or the $QS_{sediment, sw EqP}$

EqP is a mechanistic approach developed by Di Toro *et al*. (1991) for deriving sediment quality guidelines. Assuming the toxicity of a non-ionic organic chemical in sediment is proportional to its concentration in water, then the concentration of this chemical in sediment that will cause toxicity can be estimated if the relationship between the chemical concentration in the pore water and that in sediment is understood.

The partitioning of a chemical between sediment and pore water can be represented by a simple equilibrium equation:

$$C_{SOC} = C_{PW} \times K_{OC}$$

C_{SOC} is the concentration of the chemical in the sediment per unit mass of organic carbon, C_{PW} is the concentration of the chemical in pore water, K_{OC} is the partition coefficient of the chemical to sediment organic carbon). The C_{PW} can be replaced with the chemical concentration in water associated with a biological effect in the water column ($C_{effect-water}$).

Replacing CPW by the $QS_{fw, eco}$ or the $QS_{sw, eco}$ (Section 3) will yield a $QS_{sediment, fw EqP}$ or the $QS_{sediment, sw EqP}$. For EqP calculations, the equations outlined in the REACH guidance and EUSES will be used.

Calculation of $K_{comp-water}$

In the EqP method outlined in ECHA guidance, the 'dimensionless' partition coefficient $K_{sed-water}$ is also used in units of $m^3 m^{-3}$. This parameter is also called a total compartment-water partition coefficient. It is calculated according to the equations given in REACH guidance R.16 (ECHA, 2016), which are presented here for the sediment compartment only. Note that EqP to the bulk-sediment compartment is performed within the current EQS guidance, while REACH guidance uses suspended matter characteristics. This is done for several reasons: the REACH standard organic carbon content of suspended matter is relatively high (viz 10%) for most sediments; compliance checking will be performed with sediments rather than suspended matter and sediment standards based on suspended matter characteristics bear more relevance to the water column than standards based on sediment characteristics. The default values for compartment specific characteristics (Faircomp, RHO solid, etc.) from REACH (ECHA, 2008) should be used; their values are listed in the table below the equations.

$$Kp_{sed} = Foc_{sed} \times K_{oc} \qquad\qquad 2$$

$$K_{\text{sed}-\text{water}} = \frac{C\text{total}_{\text{sed}}}{C\text{porew}_{\text{sed}}} \qquad\qquad 3$$

$$K_{\text{sed-water}} = F\text{air}_{\text{sed}} \times K_{\text{air}-\text{water}} + F\text{water}_{\text{sed}} + F\text{solid}_{\text{sed}} \times \frac{K\text{p}_{\text{sed}}}{1000} \times RHO\text{solid} \qquad\qquad 4$$

$$K_{\text{air}-\text{water}} = \frac{H}{R \times TEMP} \qquad\qquad 5$$

Description:

Parameter	Description	Unit	Default value
1000	conversion factor from m^3 to litre	L m^{-3}	1000
$C\text{porew}_{\text{sed}}$	total concentration in pore water of sediment	mg m^{-3}	
$C\text{total}_{\text{sed}}$	total concentration in sediment	mg m^{-3}	
$F\text{air}_{\text{sed}}$	fraction air in sediment	m^3 m^{-3}	0
$F\text{oc}_{\text{sed}}$	weight fraction of organic carbon in sediment	kg kg^{-1}	0.05
$F\text{solid}_{\text{sed}}$	fraction solids in sediment	–	0.2
$F\text{water}_{\text{sed}}$	fraction water in sediment	m^3 m^{-3}	0.8
H	Henry's law constant	Pa m^3 mol^{-1}	
$K_{\text{air-water}}$	air-water partition coefficient	m^3 m^{-3}	
K_{oc}	partition coefficient between organic carbon and water	L kg^{-1}	
$K\text{p}_{\text{sed}}$	partition coefficient solid-water in sediment	L kg^{-1}	
$K_{\text{sed-water}}$	partition coefficient between sediment and water	m^3 m^{-3}	
R	gas constant	Pa m^3 mol^{-1} K^{-1}	8.314
RHO_{sed}	bulk density of wet sediment	kg_{ww} m^{-3}	1300
$RHO\text{solid}$	density of the solid phase	kg_{solid} m_{solid}^{-3}	2500
$TEMP$	environmental temperature	K	285

Calculation of QS$_{\text{sediment, fw EqP}}$ or QS$_{\text{sediment, sw EqP}}$

The calculation of the QS for sediment by equilibrium partitioning according to the REACH guidance R.10 (ECHA, 2008) is given below.

- The QS$_{\text{sediment,fw EqP}}$ is calculated for freshwater sediments according to EqP from the QS for aquatic organisms, QS$_{\text{fw, eco}}$ using Eqs 6 and 8 or in the case of marine sediment, from QS$_{\text{sw, eco}}$

- When the $QS_{sediment}$ has been calculated using EqP and log Kow >5 for the compound of interest, $QS_{sediment}$ is divided by 10. This correction factor is applied because EqP only considers uptake via the water phase. Extra uncertainty due to uptake by ingestion of food should be covered by the applied assessment factor of 10.

$$QS_{sediment,EqP,ww} = \frac{K_{sed-water}}{RHO_{sed}} \times QS_{fw,eco} \times 1000 \qquad 6$$

$$CONVsed = \frac{RHO_{sed}}{Fsolid_{sed} \times RHOsolid} \qquad 7$$

$$QS_{sediment,EqP,dw} = CONVsed \times QS_{sediment,EqP,ww} \qquad 8$$

Description (some of the variables are listed earlier):

Parameter	Description	Unit	Default value
1000	conversion factor from m^3 to litre	$L\ m^{-3}$	1000
$CONVsed$	conversion factor for sediment concentration wet-dry weight sediment	$kg_{ww}.kg_{dw}^{-1}$	
$Fsolid_{sed}$	fraction solids in sediment	–	0.2
$K_{sed-water}$	partition coefficient between sediment and water	$m^3\ m^{-3}$	
$QS_{sediment,\ EqP,\ dw}$	dry weight quality standard for sediment based on equilibrium partitioning	$mg\ kg_{dw}^{-1}$	
$QS_{sediment,\ EqP,\ ww}$	wet weight quality standard for sediment based on equilibrium partitioning	$mg\ kg_{ww}^{-1}$	
$QS_{fw,\ eco}$	quality standard for direct ecotoxicity on freshwater aquatic organisms	$mg\ L^{-1}$	
RHO_{sed}	bulk density of wet sediment	$kg_{ww}\ m^{-3}$	1300
$RHOsolid$	density of the solid phase	$kg_{solid}\ m_{solid}^{-3}$	2500

Experimentally determined values for K_{OC} are preferable. These K_{OC} values may be derived from standardised tests (e.g. OECD Guideline 106) or from other studies published in scientific literature. Koc values equation (van Vlaardingen and Verbruggen, 2007). Examples of QSPRs for

defining the relationship between Kow and Koc for different substance groups are provided in Table 12.

The EqP approach assumes that phases are at equilibrium, and thus exposure through pore water determined by the HPLC method (OECD guideline 121) should be considered as estimates of the real Koc values and consequently, these values are not used as experimental values. Because K_{OC} values may vary widely and no value for Koc can be considered as the most reliable value, the geometric mean of all valid K_{OC} values is calculated, including one value estimated from K_{OW}. This geometric mean K_{OC} will be used in the above equation. For highly lipophilic substances (Kow > 5), equilibrium may not be achieved, so a correction for exposure through food was introduced in the TGD (EC, 2003). For such substances, an additional AF of 10 is recommended.

Reliance on EqP alone involves several important assumptions such as equilibrium among phases, and similar sensitivities among pelagic and benthic species. In a risk assessment scenario, potential sediment risks indicated by EqP would trigger further sediment toxicity testing. This is not always possible in QS derivation so any QS_sediment that is based on EqP (or indeed a small toxicity test dataset) carries a high degree of uncertainty that must be highlighted in the datasheet for consideration by policymakers.

Table 12 QSPRs for soil and sediment sorption for different classes (Sabljic *et al*, 1995)

Chemical class	Equation	Statistics
Predominantly hydrophobics	$\log K_{OC}=0.81*\log K_{OW}+0.10$	n=81, r²=0.89,s.e.=0.45
Non hydrophobics	$\log K_{OC}=0.52*\log K_{OW}+1.02$	n=390, r²=0.63,s.e.=0.56
Phenols, anilines, benzonitriles, nitrobenzenes	$\log K_{OC}=0.63*\log K_{OW}+0.90$	n=54, r²=0.75,s.e.=0.40
Acetanilides, carbamates, esters, phenylureas, phosphates, triazines, triazoles, uracils	$\log K_{OC}=0.47*\log K_{OW}+1.09$	n=216, r²=0.68,s.e.=0.43
Alcohols, organic acids	$\log K_{OC}=0.47*\log K_{OW}+0.50$	n=36, r²=0.72,s.e.=0.39
Acetanilides, carbamates, esters, phenylureas, phosphates, triazines, triazoles, uracils	$\log K_{OC}=0.40*\log K_{OW}+1.12$	n=21, r²=0.51,s.e.=0.34
Alcohols, organic acids	$\log K_{OC}=0.39*\log K_{OW}+0.50$	n=13, r²=0.77,s.e.=0.40
Amides	$\log K_{OC}=0.33*\log K_{OW}+1.25$	n=28, r²=0.46,s.e.=0.49
Anilines	$\log K_{OC}=0.62*\log K_{OW}+0.85$	n=20, r²=0.82,s.e.=0.34
Carbamates	$\log K_{OC}=0.37*\log K_{OW}+1.14$	n=43, r²=0.58,s.e.=0.451
Dinitroanilines	$\log K_{OC}=0.38*\log K_{OW}+1.92$	n=20, r²=0.83,s.e.=0.24
Esters	$\log K_{OC}=0.49*\log K_{OW}+1.05$	n=25, r²=0.76,s.e.=0.46
Nitrobenzenes	$\log K_{OC}=0.77*\log K_{OW}+0.55$	n=10, r²=0.70,s.e.=0.58
Organic acids	$\log K_{OC}=0.60*\log K_{OW}+0.32$	n=23, r²=0.75,s.e.=0.34
Phenols, benzonitriles	$\log K_{OC}=0.47*\log K_{OW}+1.08$	n=24, r²=0.75,s.e.=0.37
Phenylureas	$\log K_{OC}=0.49*\log K_{OW}+1.05$	n=52, r²=0.60,s.e.=0.34
Phosphates	$\log K_{OC}=0.49*\log K_{OW}+1.17$	n=41, r²=0.73,s.e.=0.45
Triazines	$\log K_{OC}=0.30*\log K_{OW}+1.50$	n=16, r²=0.32,s.e.=0.38

The process for using laboratory toxicity data and the EqP approach in deriving a QS_sediment is summarised in Figure 11.

* apply additional AF of 10 if log Kow >5
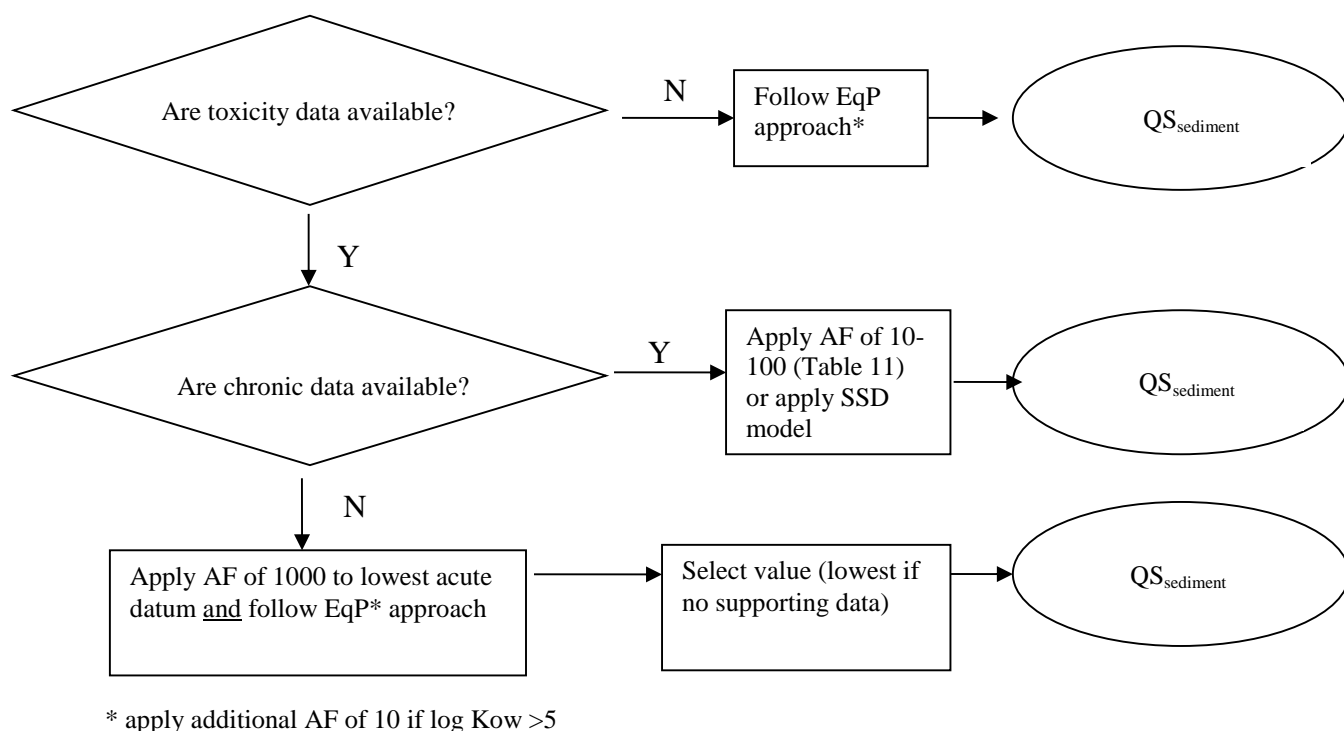
Figure 11: Process for the derivation of a QS_sediment

5.2.1.3   *Use of field or mesocosm data*

Role of field and mesocosm data

Field and/or mesocosm data should be considered, where available, in the derivation of the QS_sediment. This approach is consistent with the guidance for water column QSs (Section 2.9.2) and with Annex V of the WFD where it states that *"… the standard thus derived should be compared with any evidence from field studies. Where anomalies appear, the derivation shall be reviewed to allow a more precise safety factor to be calculated…"*

It should be borne in mind that laboratory experiments are likely to result in high levels of chemical availability because spiked sediments are rarely aged. This is in contrast with field or mesocosm data where chemical exposures are more likely to be closer to equilibrium. For these reasons, we would expect a bias in laboratory data toward higher toxicity (and more stringent standards). Lower toxicity under field conditions could reflect the real effect of ageing that should be accounted for, if possible, in standard setting.

In the absence of useful corroborating evidence from the field or mesocosms, the QS that is derived from chronic toxicity data is retained. If this is not possible, the lowest of the QSs derived based on the EqP approach or short-term toxicity data is taken as an interim standard (Figure 10).

Types of field and mesocosm data

Mesocosm studies may be available which have generated NOEC/EC10 data. Effect concentrations may also be available from field studies. If such tests are considered reliable the results can be used in the derivation of the QS_sediment (Section 5.2.1.3.).

A number of empirical approaches that link biological responses of benthos to chemical contamination in the field have been described (Batley *et al.*, 2005). They are based primarily on field data, in which matched sediment chemistry and biological effects data are analysed using various statistical approaches to relate chemical concentrations to the frequency of biological effects. Further details on these analyses are to be found in the following sources:

- Threshold effect level (TEL) / probable effect level (PEL)(Smith, Mc Donald *et al.*, 1996), effect range low (ERL) – effect range medium (ERM) (Long, Mc Donald *et al.,* 1995)
- Screening level concentration (SLC) (EC, 1992; Persaud, Jaagamugi *et al.,* 1993)
- Logistic regression modelling (LRM) (Field, Mc Donald *et al.*, 1999; Field, MacDonald *et al.*, 2002). The LRM approach focuses on establishing the probability of adverse effect as a function of sediment chemical concentration. As this relationship is continuous, this approach can be used to define sediment standards associated with any desired probability of impact. For practical purposes the 10th percentile is the preferred cut-off; this also corresponds to the ERL (see below)
- Field-based species sensitivity distribution (Kwok *et al.*, 2008)

For the purposes of QS derivation, field thresholds referring to concentrations where biological effects are unlikely to occur (sometimes referred to as 'threshold effect levels' (TEL), 'effect range low' (ERL) or 'no-effect level' (NEL, in the SLC approach)) are preferred over thresholds associated with a significant biological impact (e.g. 'probable effects level', PEL). The definitions of ERL or TEL specify that not more than 20-25% of samples should display a toxic effect.
If a field threshold has not been calculated, one of the approaches referred to above can be applied to matching chemistry and biological data, e.g.:

- ERL is the 10th percentile of the distribution of concentrations (dry weight) associated with an effect in a database matching chemistry and ecotoxicological tests applied to sediments collected from the field.
- TEL is the geometric mean of the 50th percentile of concentrations (dry weight) associated with a biological effect and the 15th percentile of the no-effects set.

None of these approaches should be used without a thorough assessment of the reliability of the data and their relevance. Entries associated with an effect for a given chemical are relevant if the concentration for this chemical is at least 2-fold above the background (McDonald *et al.,* 1996).

Application of the field/mesocosm data within QS$_{sediment}$ derivation

Reliable data arising from field/mesocosm studies can be used to influence the derivation of the QS$_{sediment}$ as follows:

1. If the TEL or ERL, or mesocosm NOEC/EC10, is higher than, or equal to the QS$_{sediment, eco}$, derived based on available ecotoxicity data, either the latter is used as the EQS$_{sediment}$ or there may be a case for reducing the size of the AF applied to the laboratory data, but only if the field or mesocosm data are reliable and relevant to a wide range of European (or national, in the case of Specific Pollutants) conditions.

2. If the TEL or ERL is lower than the QS$_{sediment}$ derived based on ecotoxicity tests, there might be a case for increasing the size of the AF if the field or mesocosm data are reliable.

3. If the TEL or ERL is higher than, or equal to, the value calculated by applying the equilibrium partitioning, the latter is used for the derivation of the EQS$_{sediment}$.

4. If the TEL or ERL is lower than the value calculated by applying equilibrium partitioning, the former value is used with an assessment factor (AF) to derive a sediment QS. The AF value would be set at 5.

5.2.2 *Metals and the need to cope with bioavailability issues*

Where possible, consideration should be given to those factors that affect the availability (and hence toxicity) of contaminants in sediment. Natural sediments used in ecotoxicological tests contain different binding ligands, which restrict the mobility of metals. As a consequence, this may also influence the availability and the toxicity of metals to sediment dwelling organisms. Major binding ligands for cations in the aerobic layer of sediments are iron and manganese oxyhydroxides (FeOOH and MnOOH), carbonates and organic carbon (OC). In anoxic sediments, bioavailability of metals can also be controlled by the formation of stable complexes with sulphide. The environmental fate of metals present in anionic forms is dominated by different sorption properties. For metals that have a high affinity to bind to these ligands, it is worthwhile exploring whether a relationship can be established between the observed toxicity levels and the presence of one or more of the ligands. If so, the toxicity of a metal in sediments can then be normalised towards a standard or a specific local condition.

5.2.2.1 *Use of data from direct (spiked) toxicity tests*

The approach previously described in section 5.2.1.1 will be applied to the set of data constituted on the basis of the following requirements. See also the list of general requirements in section 2.

- *Sediment:* For deriving sediment QSs from direct sediment toxicity data, information on the sediment chemistry is needed for data interpretation, especially if bioavailability corrections are carried out. In the latter case artificial sediments used in studies should be characterised (e.g. particle size, pH of pore water, organic matter (OM), cation exchange capacity (CEC)/anion exchange capacity (AEC), as well as iron and manganese oxides). If natural sediment is used, SEM (Simultaneously Extracted Metals) and AVS (Acid Volatile Sulphides) concentrations should be measured.
- *Metal-OC equilibrations*: The kinetics of metal-DOC binding in aqueous and sediment test media may require an equilibration period between the metal and test medium prior to exposing the organisms. This is to allow full Me-OC binding in a way that is representative of natural environments (e.g. Ma *et al.*, 1999). Where the kinetics for reaching equilibrium conditions for binding to OC etc are known to be slow and may affect the test outcomes, reviewing the details of the test design may provide additional information on the reliability of the data, particularly for any extreme values.
- *Metal-sediment equilibration*: After spiking the water-sediment system with the test substance, an equilibrium period is crucial to ensure partitioning of the substance between the water-phase and solid-phase. For metals and inorganic metal compounds, the concentration of the test substances should be measured in the overlying water of semistatic and static sediment toxicity tests, and the testing preferably initiated only when the overlying water concentration reaches stable concentrations (this can be more than 2 months for metals). If these criteria are not met, the tests cannot be assigned Q1.

If a relationship with OC can be discerned, the same normalisation as above (section 5.2.1.1) will also be applicable to metals. In addition, for metals toxicity values are preferred, originating from tests carried out under aerobic conditions, with low acid-volatile sulphide (AVS) levels (e.g. < 1.0 µmol AVS/g dry wt or tests with artificial sediments). These sediments could be considered as realistic "worst cases" for aerobic sediments, since ferric- and sulphide binding to metals is not present.

5.2.2.2 *Accounting for background concentrations in sediments*

See Section 3.5.4

5.2.2.3   Equilibrium partitioning

When using the EqP approach for metals, measured Kd values for sediment/suspended solids from freshwater, estuarine and marine waterbodies respectively can be used. Preference is given to Kd values derived from field measurements and not laboratory sorption or toxicity experiments. However, large variations in Kd are often observed even among different field-based measurements and therefore, for freshwater sediments, the QS derived from EqP may be refined by using Kds, modelled from WHAM speciation models (Tipping, 1994). It should be noted however that the only solid phase that can be estimated by WHAM is organic carbon. Before using this approach, the validity of organic carbon determined WHAM Kd values should be checked, as other factors may contribute to partitioning.

5.2.3 Dealing with bioaccumulated/biomagnified substances

For some very hydrophobic organic substances such as polychlorobiphenyls (PCBs) polychlorodibenzo-dioxins (PCDDs) or furans (PCDFs), the protection of sediment-dwelling organisms may not be the key objective. Direct toxic effects may arise at concentrations far above the concentrations of concern for predators located at higher levels in food webs, such as predatory fish or mammals. In this case, biota standards should be set. Nevertheless, sediment standards might also be useful, for management or monitoring purposes, as long as they fulfil the trigger criteria set out in Section 2.4.2.

When sediment is the primary source of exposure for the target species (fish or mammals), the $QS_{sediment}$ for such substances should be derived from the QS_{biota}.. Available exposure models range from very simple ones, based on BSAFs (accumulation factors from sediment to biota), to food-web models (Section 4). BSAFs are not recommended, as published values are highly variable. Moreover, studies on uncontaminated areas tend to yield higher BSAFs (Burzynski, 2000) than studies on contaminated sites. Food-web modelling would thus be more appropriate but are more appropriately applied at local or regional scales, yielding site-specific or region-specific EQSs. For this reason, this step is not relevant for substances for which a Europe-wide EQS is sought.

5.2.4 Protection of saltwater benthic organisms

The same approaches as that described for freshwater sediments are recommended for the derivation of $QS_{sediment}$ for marine waters. Marine and freshwater sediment toxicity data may be pooled unless it can be documented that differences in toxicity exists between freshwater and saltwater sediments. Further refinements of the process for deriving sediment standards for metals are given in Section 5.3.

5.2.4.1   Spiked sediment (ecotoxicity) testing

In principle the same approach as that outlined in Section 5.2.1.1 with regard to sediment of inland surface waters is adopted. However, larger assessment factors may apply depending on the quality and quantity of toxicity data available (Table 13).

Table 13 Assessment factors for derivation of the QS$_{sediment, sw\ eco}$ based on the lowest available NOEC/EC10 from long-term tests (ECHA, 2008)

Available test results	Assessment factor [a)]
One acute freshwater or marine test (L(E)C50)	10000 [b)]
Two acute tests including a minimum of one marine test with an organism of a sensitive taxa (lowest L(E)C50)	1000 [b)]
One long term freshwater sediment test	1000
Two long term freshwater sediment tests with species representing different living and feeding conditions	500
One long term freshwater and one saltwater sediment test representing different living and feeding conditions	100
Three long term sediment tests with species representing different living and feeding conditions	50
Three long term tests with species representing different living and feeding conditions including a minimum of two tests with marine species	10

[a)] The general principles of notes (c) and (d) as applied to data on aquatic organisms (Table 3) shall also apply to sediment data. Additionally, where there is convincing evidence that the sensitivity of marine organisms is adequately covered by that available from freshwater species, the assessment factors used for freshwater sediment data may be applied. Such evidence may include data from long-term testing of freshwater and marine aquatic organisms, and must include data on specific marine taxa.

[b)]   If an indicative QS$_{sediment, sw\ eco}$ is calculated with short-term toxicity data, an alternative EQS must be calculated using the equilibrium partitioning approach (see section 5.2.1.2). The final value is selected by expert judgement, taking all available information into account.

As other combinations of data could occur (van Vlaardingen and Verbruggen, 2007), the following additional guidance is offered:

- an assessment factor of 500 is applied if only one long-term marine but no freshwater test is available;

- If two long-term tests with marine species representing different living and feeding conditions are available, but there are no freshwater tests, an assessment factor of 100 is applied;

- an assessment factor of 1000 might only be applied to a short-term toxicity test if the lowest value available is for a marine species.

5.2.4.2 Other derivation approaches

The derivation approaches described in Section 5.2.1 also apply to marine and coastal sediments. The standards selected should refer to marine or coastal environments.

5.2.5 Derivation of sediment QS for transitional waters

The same derivation approaches described in Section 5.2.1 and 5.2.4 also apply to sediment in transitional waterbodies.

Specific data for transitional waters will probably be lacking in most cases. To decide whether a freshwater or saltwater sediment QS is the most appropriate for a particular location, the most convenient approach would be to assess the diurnal range of salinities, decide whether the considered ecosystem (in a transitional waterbody) is closer to a freshwater system or to a saltwater system, and apply the corresponding QS.

5.3   Using sediment QS that are subject to high uncertainty

5.3.1 Overview

Sediment standards allow the assessment of good status alongside standards for other compartments. The following guidance suggests how we might assess situations where the sediment standard fails. A simple pass/fail approach to assessment is not always appropriate, especially as residual uncertainties in sediment standards can be high making compliance assessment difficult. For this reason, we recommend a tiered assessment framework in which decisions to take remedial measures use sediment standards as only one of a number of lines of evidence. A similar framework has been adopted by OSPAR for monitoring of marine sediments[35]. Member States or Basin Authorities can either implement directly remediation measures or apply either tier.

Detailed advice on monitoring lies outside the scope of this guidance. However, if policymakers deem that formal assessments of compliance using an $EQS_{sediment}$ are necessary, a tiered assessment framework is recommended that uses evidence to corroborate any risks indicated by exceedances of the $EQS_{sediment}$ (Figure 12)[36].

In this framework, chemical analysis at Tier 1 provides a 'face value' assessment of compliance. This should use an $EQS_{sediment}$ that has been based on data simulating worst-case conditions for availability (Section 5.2.1.1). EQS exceedance would trigger a more detailed assessment (i.e. Tier 2) that accounts for bioavailability or uses biological data to assess whether the benthic community is actually impaired or not. If no risks are expected after accounting for bioavailability, or the biological community was not impaired – even though an EQS exceedance is indicated – any further action might be restricted to further monitoring instead of more costly risk reduction measures. On the other hand, demonstrable impacts coupled with EQS exceedances would be good evidence for a need for risk reduction.

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[35] Final report of the OSPAR/ICES Workshop on the Evaluation and Update of Background Reference Concentrations (BRCs) and Environmental Assessment Criteria (EACs) and How These Assessment Tools Should Be Used in Assessing Contaminants in Water, Sediment and Biota (February 2004), presented to ASMO as ASMO 04/4/5 Add 1.

[36] Nevertheless, the framework is not mandatory; local authorities may disregard this framework and manage directly to recover a quality matching the standard.
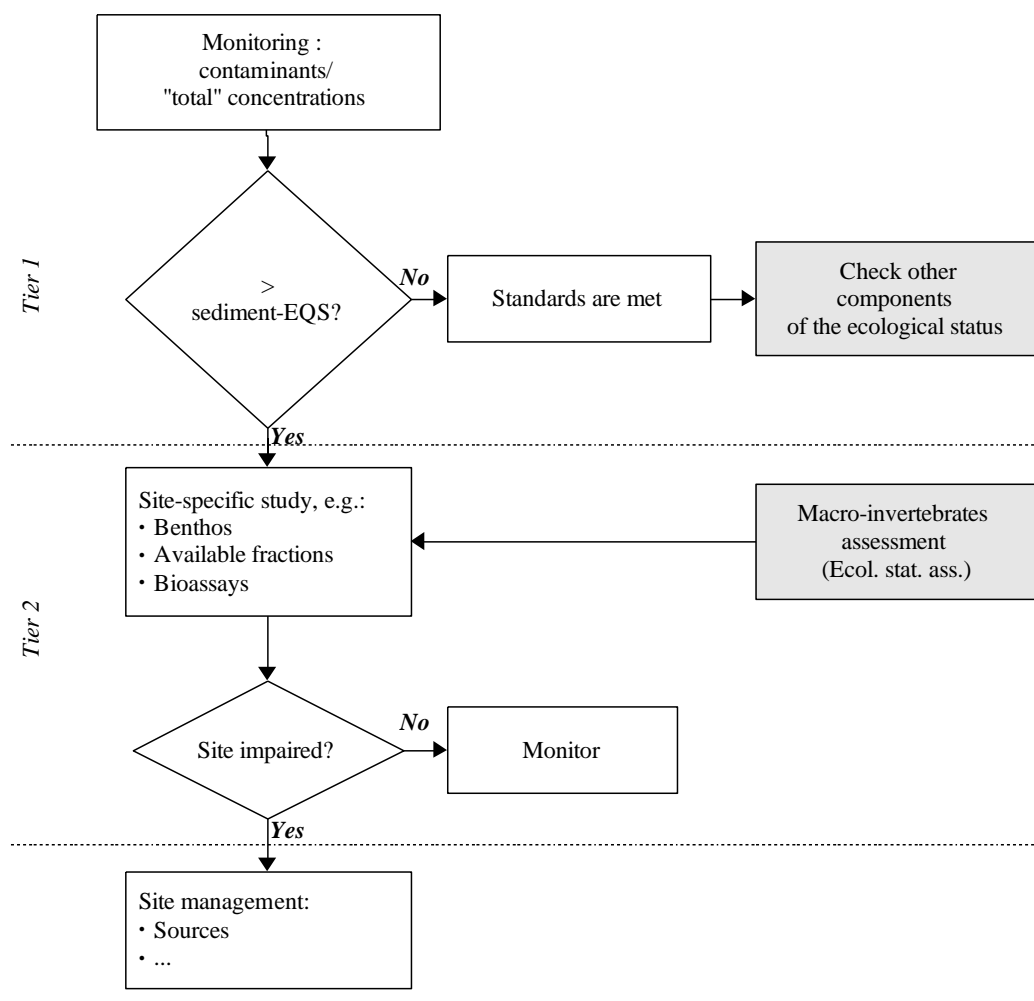
Figure 12: Tiered assessment framework for sediments

There are several possible approaches for the second tier, depending on the factors most likely to affect the risks posed by a particular substance. These might include assessment of the bioavailable fraction (Section 5.3.2), benthic community assessment or even bioassays conducted *in situ* or *ex situ*. While benthos assessment and bioassays may provide valuable additional information, they can be difficult to use and should be considered as options, to be selected on a case by case basis.

5.3.2 Assessing the bioavailable fraction

This assessment seeks to refine the exposure concentration to which sediment-dwelling organisms are exposed. One possible way to estimate the bioavailable fraction is to measure the extractable fraction in amorphous organic matter by extraction with a solid sorbent (e.g. Tenax ®) for a set time (e.g. 6h) (Cornelissen, Rigterink *et al.*, 2001). This extraction is based on differences in contaminant desorption kinetics between amorphous organic carbon and hard carbon. The concentration in amorphous organic matter is then related to the freely dissolved concentration in pore water (N'Guyen *et al.*, 2005; Schüürmann *et al.*, 2006). These Tenax ® extractable concentrations are highly related to concentrations in organisms (Landrum, Robinson *et al.*, 2007). The concentrations extracted from amorphous organic matter could be compared directly with the sediment quality standards.

Another approach could be to estimate the bioavailable fraction through porewater sampling with SPME (solid phase micro-extraction) or POM (poly-oxy-methylene)[37] or direct measurements in organisms. In this case, measured concentrations should be compared with the $QS_{fw, eco}$ or $QS_{sw, eco}$ (Table 14).

Table 14 Interpretation of bioavailability measurements

Method	Exposure concentration compared to
SPME	Water EQS
POM	Water EQS
Tenax ®	Sediment EQS
Organism	Biota EQS

For metals, several methods for measuring bioavailability are under development, such as "Diffusive Gradients in Thin-films" (DGT) (Cornu & Danaix, 2006), "Sediment or Fauna Incubation Experiment" (SOFIE) (Duester, Vink & Hirner, 2008), and "Simultaneously Extracted Metals – Acid Volatile Sulphides" (SEM-AVS). In the EU risk assessments for cadmium, zinc, and nickel, and in the voluntary industry risk assessments for copper and lead, the SEM-AVS concept has been employed.

For metals the anoxic sediment could be of greatest concern as these tend to be depositional, clayey sediments where metals accumulate. In these sediments, bioavailability of metals can be controlled by the formation of stable complexes with sulphide. More erosional sediments that are oxic and have larger grain sizes have no or very low AVS, but also rarely have metal contamination (Burton *et al.*, 2007).

The binding strength of the metal sulphide (MS) is inversely related to its solubility product and therefore, metals characterised by the lowest MS solubility product (K_{sp}) will have the highest affinity for sulphides. The MS solubility products, described in Table 15 illustrates the large difference in MS solubility products. This means that the presence of FeS and MnS indicates that MS, with solubility product lower than the ones of MnS and FeS are formed by preference, may actually displace the less stable FeS and MnS and are less vulnerable to oxidation.

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[37] For a detailed review, see ICES (2008). Report of the Working Group on Marine Sediments in Relation to Pollution (WGMS). Copenhagen, International council for the Exploration of the Sea**:** 64.

Table 15 Solubility products of metal sulphides

Metal sulphide	Log K[(a)]	Log K[(b)]
MnS (s)	-19.15	- 13.50
FeS (amorphic)	-21.80	-
FeS (s)	-22.39	-18.10
NiS (s)	-27.98	-
ZnS (s)	-28.39	-24.70
CdS (s)	-32.85	-27.00
PbS (s)	-33.42	-27.50
CuS (s)	-40.94	-36.10
Ag$_2$S (s)		-50.10
HgS	-57.25	-52.70

a   Di Toro *et al*, 1990
b   Stumm and Morgan, 1981

Based on field validation data, it has been demonstrated that the fraction of metals bound to sulphides in the sediment, and thus sequestered in the solid phase of sediments, is not available for exposure to benthic organisms via the pore water route and toxicity to benthic organisms and can be estimated from SEM-AVS (Simultaneously Extracted Metals – Acid Volatile Sulphides) measurements.

The basic concept behind the SEM-AVS approach is that the Acid Volatile Sulphides (AVS) present in the sediment reacts with the Simultaneously Extracted Metals (SEM). SEM and AVS are operationally defined parameters. AVS (Acid Volatile Sulphides) are those sulphides that are extracted by cold extraction (1 M HCl) of sediments. SEM (Simultaneously Extracted Metals) is the term used for those metals that are liberated under the conditions of the AVS analysis (ICMM fact sheet No. 10).

The SEM-AVS concept has been shown to be predictive of the toxicity of those metals having a high affinity for AVS: e.g. Cd, Cu, Pb, Ag, and Zn. For Ni, field data exist that support the SEM-AVS concept, but as laboratory studies did not constitute a test of this theory further research is still ongoing. For metals with lower sulphide solubility products, the applicability of the SEM-AVS approach has still to be demonstrated and may be questionable. Thus, the SEM-AVS approach cannot be used at this time for metals other than those referred to above.

As several factors influence metal availability, the SEM-AVS approach could be used as a line of evidence in the weight of evidence to predict the absence of metal toxicity, i.e. when SEM-AVS ratio is <1.

Metals act in a competitive manner when binding to AVS. Applying the principles of competitive displacement kinetics, the SEM-AVS model can be made metal-specific. The procedure assigns the AVS pool to the metals in order of their solubility products. For example, ranked from the lowest to the highest solubility product the following sequence is observed for these six metals: SEMHg SEMAg, SEMCu, SEMPb, SEMCd, SEMZn and SEMNi. This means that mercury has the highest affinity for AVS, followed by silver, copper, lead, cadmium, zinc and nickel until the AVS is exhausted. The remaining SEM is that amount present in excess of the AVS and potentially available.

For divalent metals, one mole of SEM will react with one mole of AVS. For silver the stoichiometric relationship differs slightly, one mole of SEM silver reacting with two moles of AVS.

When applying the SEM-AVS concept to compliance checking, consideration is to be given to seasonal and vertical variations on AVS measurements. It is therefore recommended to assess the SEM and AVS in the same sample and to sample sediments for SEM and AVS measurements preferably in spring and from the upper 5 to 10 cm (AVS lowest in spring and upper sediment layer) or on a regional scale to take the 10th percentile of available AVS.

For more background information on the SEM-AVS concept the reader is referred to the risk assessment made under the EU Existing Substance Regulation for Cd, Zn and Ni and the voluntary risk assessments for Cu and Pb that have been discussed by the Technical Committee for New and Existing substances.

6. LIMITATIONS IN EXPERIMENTAL DATA – USE OF NON-TESTING APPROACHES

Several databases of physicochemical and biological effects data are available, and data have also been published in the literature. However, the number of tested chemicals with reliable test data remains small compared to regulatory inventories of interest [Netzeva *et al*, 2007]. Data gaps may be filled by commissioning physical, degradation or ecotoxicological studies, but this is not always possible.

A lack of data reflects a lack of knowledge about the properties or effects of a substance and gives rise to uncertainty. The conventional way to respond to this uncertainty is to apply larger AFs, but this can result in very low QSs that cannot be implemented in practice. In some cases, it may not be possible to derive a QS due to the lack of data. If that uncertainty can be reduced, the need for such large AFs may be reduced accordingly. If carefully chosen, the use of a relevant and reliable non-testing method can provide additional information, which can lower the overall uncertainty and result in the use of a smaller AF. Non-testing methods will not be useful in all circumstances however.

Three non-testing approaches to filling data gaps are recognised. These are:

- Grouping methods (Section 6.1);

- QSARs (Section 6.2);

- Analogue approach / read-across (Section 6.3).

Non-testing methods may be used under REACH to fill data gaps, provided that:

- The model used is shown to be scientifically valid;

- The model used is applicable to the chemical of interest;

- The prediction is relevant for the regulatory purpose (in this case, EQS derivation);

- Appropriate documentation on the method and the result is given (e.g. by using the QSAR Model Reporting Format recommended by the European Commission).

All assessments using non-testing methods should be reviewed and updated as new information is generated, and as experience in forming and assessing non-testing methods is continually growing. Figure 13 illustrates a scheme for deciding how non-testing methods may be deployed for EQS derivation.
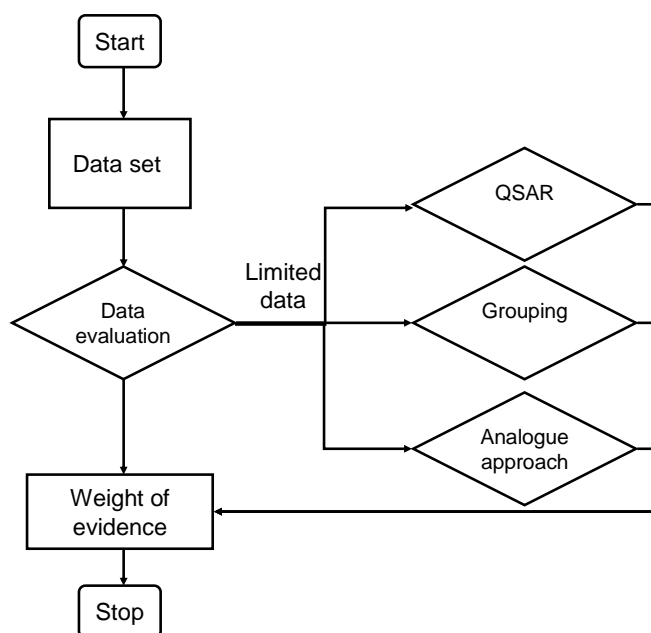
Figure 13: Application of non-testing methods

6.1   Grouping of substances / category approach

A chemical category is a group of chemicals whose physico-chemical properties, fate and behaviour and mammalian or environmental toxicological properties, are likely to be similar or follow a regular pattern as a result of structural similarity, e.g. PAHs or another shared characteristic.

The assessment of chemicals using a category approach differs to the assessment of chemicals on an individual basis. The effects of the individual chemicals within a category are assessed on the basis of the evaluation of the category as a whole, rather than being based on measured data for any one particular substance alone. For a substance (a category member) that lacks data for a particular endpoint (e.g. there are no chronic aquatic toxicity data), the data gap can be filled in a number of ways, including read-across from one or more other category members. If the similarity of category members is very high, e.g. for PAHs with the same number of rings, it may only be necessary to use data from one category member and read-across principles to adequately characterise the category member for which data is lacking.

In an ideal situation a category would include all potential members of the category (e.g. all homologues in a series), but this ideal situation will be difficult to achieve in practice. The successful use of a category approach should lead to the identification and characterisation of the hazards for all the members of the category, irrespective of their production volume / exposure.

A chemical category should be described by a matrix consisting of the category members and the relevant endpoints e.g. BCF, log K_{ow}. In some cases, an effect can be present for some but not all members of the category, and then sub-categories should be built (e.g. the 16 hydrocarbon 'blocks' used for hydrocarbons in PETROTOX). Figure 14 shows the procedure for category development.
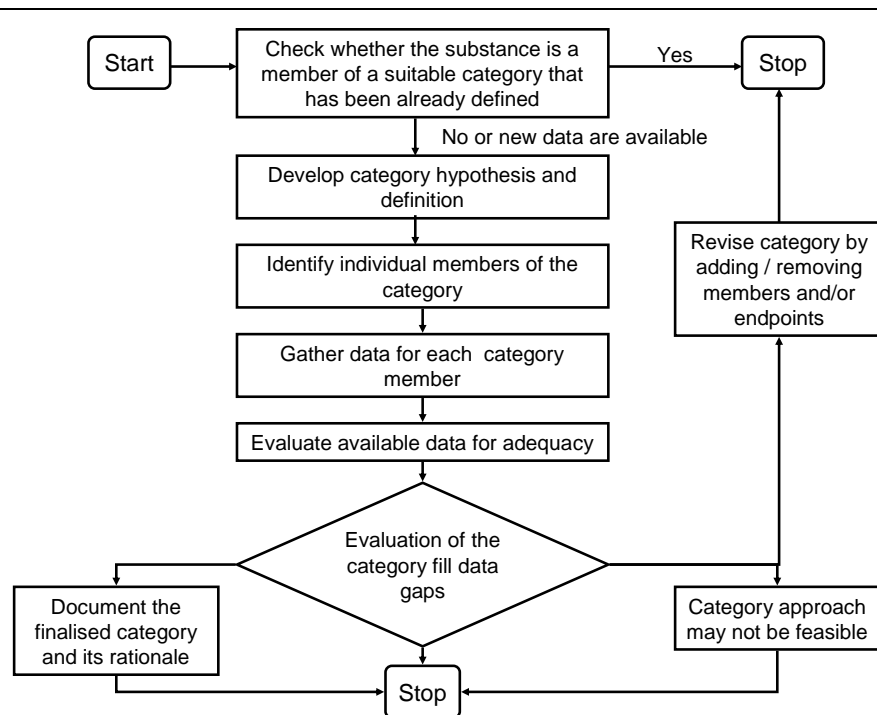
Figure 14: Stepwise procedure for category development

Before considering whether to develop a category for a group of substances, the first step should be to determine whether the chemicals of interest are named members of a category that has already been evaluated. The category definition should list all of covered substances and endpoints. Although the chemical structure is usually the starting point, a category definition could also refer to a group of chemicals related by a common mechanism of action (e.g. non-polar narcotics) or a particular property. For each member of the category, relevant data should be gathered, and the quality assessed as described in Section 2.6.2.

A matrix of data (category endpoints vs. members) should be constructed with the category members arranged in a suitable order (e.g. according to ascending log K_{OW}). The ordering of the members should reflect any trends or progression seen within the category. The cells of the matrix should indicate whether data are available or missing. Categories may be revised by adding or removing member(s) and endpoint(s).

The finalised category should be documented. A category may be revised subsequently in the light of new data or experience.

6.2   QSARs

The chemical category and Quantitative Structure-Activity Relationship (QSAR) concepts are strongly connected. A QSAR is a quantitative (mathematical) relationship between a numerical measure of chemical structure, or a physicochemical property, and an effect/activity e.g. acute toxicity to the waterflea, *Daphnia magna*. QSARs often taken the form of regression equations, and can make predictions of effects/activities that are either on a continuous scale (e.g. reproductive output) or on a categorical scale (e.g. mortality).

For a given category endpoint, the category members are often related by a trend (e.g. increasing, decreasing or constant) in a particular effect, and a trend analysis can be carried out using a model based on the data for the members of the category.

Similarly, a Quantitative Activity-Activity Relationship (QAAR) is a mathematical relationship, but between two biological endpoints, which can be in the same or different species. QAARs are based on the assumption that knowledge about the mechanism or mode of action, obtained for one

endpoint, is applicable to the same endpoint in a different species, or to a similar endpoint in the same species, since the main underlying processes are the same (e.g. partitioning, reactivity, enzyme inhibition). QAARs are less widely used than QSARs but also provide a means of performing trend analysis and filling data gaps.

Thus, a chemical category can be seen as a set of internal QSARs (and possibly also internal QAARs) for the different endpoints. Data gaps can also be filled by an external QSAR model, where the category under examination is a subcategory of the wider QSAR (Netzeva *et al.,* 2008).
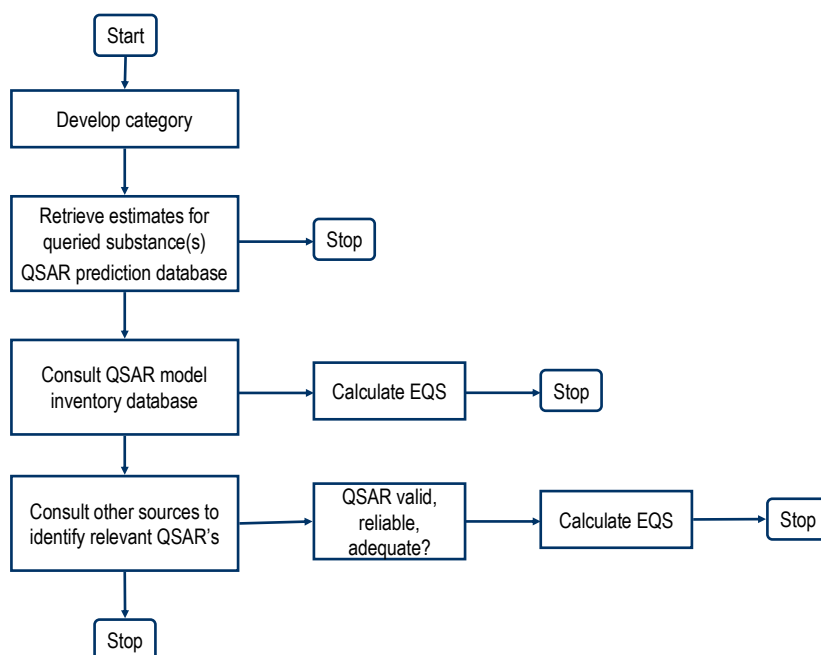
Figure 15: Stepwise approach to applying QSARs

There are various publicly and commercially available computational tools and databases available to predict data endpoints (Bassan and Worth, 2008). Information regarding QSAR software tools for regulatory purposes is available on

http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/qsar-tools

If relevant QSAR prediction databases do not include predictions for the particular substance(s) of interest, relevant QSAR models can be searched for in the QSAR database. If this fails, other models can be searched for in the literature, external databases and tools.

Harmonised templates such as the QSAR Model Reporting Format (QMRF) and the QSAR Prediction Reporting Format (QPRF) should be used to document the results. The QMRF is a harmonised template for summarising and reporting key information on QSAR models, including the results of any validation studies. The information is structured according to the OECD (Q)SAR validation principles. The QPRF is a harmonised template for summarising and reporting substance-specific predictions generated by QSAR models.

QSARs are suitable for identifying a substance as potentially PBT/vPvB. BIOWIN, BCFWIN and ECOSAR are thought to be reliable models for these assessments. However, mammalian toxicity QSARs are presently not sufficiently reliable for use in estimating secondary poisoning QS. Although they have a place in supplementing experimental ecotoxicity data, sole reliance on QSARs in ECOSAR for estimating acute or chronic toxicity, and the subsequent use of these results for deriving a QS, is not recommended because of the tendency for ECOSAR to

underestimate toxicity for the types of substances prioritised or proposed for QS derivation, sometimes by a substantial amount.

6.3   Analogue approach / read-across

If it is not possible to associate the compound of interest with any existing category, similar compounds may be identified by performing a similarity assessment procedure, as described below. Figure 16 describes the application of the analogue approach.
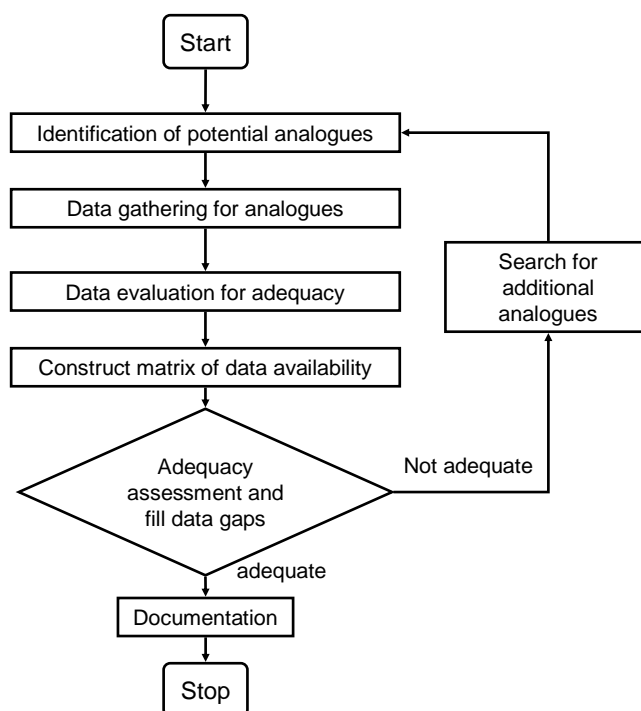
Figure 16: Stepwise procedure for the analogue approach

Computational tools, e.g. Toxmatch http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/qsar-tools or the OECD Toolbox should be used for analogue selections in combination with electronic substructure searching using molecular similarity indexes (e.g. the Tanimoto similarity index or Hellinger distance [atom environments]) and other molecular descriptors [e.g. log K_{ow}]). For each analogue, relevant data should be gathered, and the quality assessed as described in Section 2.

The decision about whether data from an analogue can be used to fill a data gap depends largely on expert judgement at present. Wherever possible, the relevance of the read-across should be evaluated in the light of the known or suspected mode of action. If the read-across from an analogue is suitable, the approach should be documented according to an appropriate format.

The OECD Toolbox was used to identify suitable analogues for indeno(1,2,3-cd)pyrene by Crane *et al.* (2008). They concluded that read-across using a weight of evidence approach and all relevant measured and estimated values for physical and eco-toxicological properties could be a valuable approach for deriving QSs, if measured data are available for interpolation to the substance and endpoint(s) of interest, or if a reliable trend with low variability exists.

The *de minimis* dataset for reliable read-across consists of:

a) For endpoints that incorporate an assessment of potency (dose-effect): Evidence of a consistent and reliable trend within a category of relevance to the endpoint of interest (e.g. a monotonic increase in log Kow with an increase in measured BCF and toxicity).

b) Consistent and reliable measured values to identify the most sensitive trophic group, if toxicity is the endpoint of interest.

c) Reliable measured data for the endpoint of interest that allow interpolation to a value for the substance of interest.

d) QSAR estimates may be useful in a weight of evidence role for supporting read-across, but should not be used to replace the measured values identified in a – c above.

7.   CALCULATION OF QS FOR SUBSTANCES OCCURRING IN MIXTURES

For well-defined mixtures, i.e. those with a well defined qualitative and quantitative composition, the toxic unit (TU) approach (e.g. Altenburger and Greco, 2009) may be used to calculate the EQS. A Toxic Unit (TU) is defined as the ratio of the exposure concentration to the effect concentration for a specific medium (e.g. water). A TU for each constituent$_i$ in a substance / group of substances should be calculated as,

$$TU_i = \frac{C_{w,i}}{QS_i}$$

Cw,i   Concentration in water of the constituent i

QSi   PNEC for the constituent i

To estimate the toxicity of the mixture, the TU_i values for all constituents in the mixture/group of substances are summed.

$$TU_{mixture} = \Sigma TU_i$$

When the $TU_{mixture}$ equals one or is greater than one, the mixture is expected to be above the threshold (ie QS).

EQSs may be defined for grouped substances that exert a similar mode of action and may be expressed according to the concept of Toxic Equivalent [TEQ] concentrations in environmental samples. The Toxic Equivalency Factor [TEF] is the fraction of the PNEC of constituent$_i$ divided by the lowest PNEC measured or calculated for a constituent that belongs to the group of substances being considered (Di Toro, 2000).

TEQ = Σ_n (TEF$_i$*c$_i$)

TEFi   Toxic Equivalency factor for constituent i

Ci   concentration of constituent i

The TU concept is equivalent to the Toxic Equivalency Factors (TEFs) for PCB's, PCDD's and PCDF's for humans and wildlife which were agreed by the World Health Organization (WHO) in 1997 and have been revised for dioxin-like compounds by the WHO in 2005, including criteria to take substances into the TEQ concept (Van den Berg *et al.*, 1998, 2006)

Some substances are of unknown or variable composition, complex reaction products or biological materials (UVCBs). The variability in composition can be large and unpredictable. Methods for assessing UVCBs are still under development but current approaches focus on the identified constituents, where assessment can be limited by a lack of data. However, some UVCBs, like petroleum substances, can be assessed using the hydrocarbons block method and the use of non-testing methods (e.g. PETROTOX) to fill data gaps, as it is demonstrated for the case study of gasolines (McGrath, 2005).

PETROTOX (CONCAWE) is a tool to assess aquatic toxicity hazard of complex petroleum and related substances; it:

- includes a library of about 1500 individual hydrocarbons, grouped in 16 hydrocarbon blocks, with details on their physical chemical properties and estimated PNECs;

- predicts toxicity of substances to different aquatic organisms (based on the Narcosis Target Lipid Model);

- assesses impact of composition/test design on toxicity results; and

- estimates Predicted No-Effect Concentrations (PNECs) needed as input to environmental risk assessments of petroleum substances using the Hydrocarbon Block Method;

- estimates HC5 of the individual components needed as input to environmental risk assessments of petroleum substances using the Hydrocarbon Block method.

Petrotox estimates the HC5 for the different components and hydrocarbon blocks of the original petroleum product prior to any treatment that occurs prior to discharge. As the shift of the hydrocarbon block composition is not taken into account, the estimated HC5 cannot be used for EQS setting because it requires a recalculation that takes into account the hydrocarbon block composition in the receiving environment. To estimate the toxicity of hydrocarbon mixtures in environmental samples, the HC5 of all the components present in a hydrocarbon block and subsequent calculation of the Toxic Unit (TU) is required. An EQS for hydrocarbon mixtures may be set by grouping them into hydrocarbon blocks.

The PETROTOX model uses QSAR modelling to predict the toxicity of the different fractions. In an alternative approach to derive quality standards for total petroleum hydrocarbons, a fraction-based approach was used to calculate the internal concentrations in organisms exposed to spiked sediments. This calculation was based on partitioning of the different fractions between sediment, oil, pore water and the lipids of membranes. The toxicity observed in these spiked sediments for six benthic species was related to the calculated membrane concentrations. HC5 could thus be based on these internal membrane concentrations (Verbruggen *et al*, 2008). The observed values are lower than the QSAR estimates from the PETROTOX model.

When establishing EQSs for UVCBs such as petroleum products separate values should be defined for different fractions or blocks of the mixture. In compliance checking the concentrations of these individual fractions should be measured and a concentration addition approach should be applied to assess the effect of the total mixture in the environment.

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APPENDIX 1: DATA COLLECTION, EVALUATION AND SELECTION

Contents

A1.1. INTRODUCTION

This background document covers the collection of data that may be used to derive Environmental Quality Standards (EQSs), and its evaluation and selection for actual use in EQS derivation.

To promote consistency, it also gives guidance on the presentation and reporting of data. The topics covered are physicochemical data (Section 2), toxicity data (Section 3), bioconcentration and biomagnification data (Section 4) and toxicity data for the protection of humans (Section 5).

This background document is based on that provided in Van Vlaardingen and Verbruggen (2007)

A1.2. PHYSICOCHEMICAL DATA

A1.2.1. Data collection

A1.2.1.1. Identity

The following data on substance identity are collected:

- IUPAC name
- structural formula
- CAS registry number
- EINECS number
- chemical formula
- SMILES code

IUPAC name, CAS registry number, EINECS number and chemical formula are primarily derived from the ESIS database (JRC website http://esis.jrc.ec.europa.eu/ https://ec.europa.eu/jrc/en/scientific-tools). A structural formula can also be obtained here for a great number of compounds. If a structural formula cannot be obtained from the ESIS database, EPI Suite software can be used (US EPA, 2007b) or handbooks can be consulted, e.g. Tomlin (2002) for pesticides or more general handbooks like Mackay *et al.* (2006). The SMILES code is generated by EPI Suite software. If the compound of interest is not available in the EPI Suite database, the SMILES code can be generated using chemical drawing software, e.g. ChemSketch (ACD/Labs, 2006).

A1.2.1.2. Physicochemical properties

Physicochemical parameters should be collected for each compound for which EQSs are being derived. These parameters provide information on the behaviour of the compound in the environment. Data on the following parameters are collected (name, symbol, unit):

- molecular weight: M_w (g·mol^{-1});
- melting point: T_m (°C);
- boiling point: T_b (°C);
- vapour pressure: P_v (Pa), experimental melting point and boiling point can be useful for estimation of the vapour pressure;
- Henry's law constant: H (Pa·m^3·mol^{-1});
- water solubility: S_w (mg·L^{-1}), experimental melting point can be useful for the estimation of the solubility from log K_{ow};
- dissociation constant: pK_a (-);
- *n*-octanol/water partition coefficient: K_{ow} (-);
- sediment/water partition coefficient: K_p (L·kg^{-1}). For organic substances, the partition coefficients normalised to organic carbon are preferred: K_{oc} (L·kg^{-1}). For metals, field-based partition coefficients (K_p) for suspended matter are searched.

The following steps should be followed to collect physicochemical data:

1. The following databases and estimation methods are used to retrieve or calculate data on physicochemical parameters (Table 16).

Table 16 Sources and estimation methods to be screened for physicochemical parameters

Parameter	Sources/methods
M_W	Mackay, EPI Suite, SPARC, IUCLID
T_m	Mackay, EPI Suite, IUCLID
T_b	Mackay, EPI Suite, SPARC, IUCLID
P_v	Mackay, EPI Suite, SPARC, IUCLID
H	Mackay, BioLoom, EPI Suite, SPARC, IUCLID
S_w	Mackay, EPI Suite, SPARC, IUCLID
pK_a	Mackay, BioLoom, SPARC, IUCLID
K_{ow}	BioLoom, Mackay, EPI Suite, SPARC, IUCLID
K_{oc}	Mackay, BioLoom, Sabljić, EPI Suite, IUCLID
K_p (metals)	Sauvé, Bockting, scientific literature

References to the sources and programs mentioned in the above TableTable :

   Mackay   = Mackay *et al.* (2006);

   EPI Suite = US EPA (2007b);

   SPARC   = SPARC online calculator (Karickhoff *et al.*, 2007);

   IUCLID   = International Uniform Chemical Information Database (European Commission (Joint Research Centre), 2007);

   Bioloom   = BioByte including internet database (BioByte, 2004);

   Sabljić   = Sabljić and Güsten (1995) cited in European Commission (Joint Research Centre). (2003b) or
                Sabljić *et al.* (1995).

   Sauvé   = Sauvé *et al.* (2000)

   Bockting = Bockting *et al.* (1992)

2. Scientific literature. For all of the listed parameters, the open literature may be searched if a reliable estimate is lacking or if the number of reliable or relevant data is very low. This might be most applicable to K_p values for metals (see Annex).

3. Contact people from environment agencies in other countries asking if they have access to specific information on ecotoxicological toxicity data (see Section A1.3.1) and/or physicochemical data and are willing to share those data.

4.   The industry parties involved in production or use of the compounds under investigation are invited to submit relevant studies, making clear these will be treated as public literature.

A1.2.2. Data evaluation and data tables

All retrieved literature is read and evaluated to assess its relevance and reliability. Important aspects for evaluation are discussed in the annex.

After evaluating a study, the results of the study are summarised by entering these into a data table (Table 17). The structural formula of the compound is also placed in this table.

Table 17 Overview and default table structure for identity and physicochemical parameters listed for each compound.

Properties	Value	Reference
IUPAC Name		
Structural formula		
CAS number		
EINECS number		
Chemical formula		
SMILES code		
Molecular weight (g·mol^{-1})		
Melting point (°C)		
Boiling point (°C)		
Vapour pressure (Pa)		
Henry's law constant (Pa·m^3·mol^{-1})		
Water solubility (mg·L^{-1})		
pK_a		
n-Octanol/water partition coefficient (log K_{ow})		
Sediment/water sorption coefficient (log K_{oc})		
Sediment/water sorption coefficient (log K_p)		
Suspended matter/water partition coefficient		

A1.2.3. Data selection

A1.2.3.1. K$_{ow}$

The K_{ow} value that is selected for use in EQS derivation is preferably the experimental value (MlogP) presented by BioLoom (BioByte, 2004). This value is assigned the highest quality in the underlying database (MedChem). Only if this database does not give a value or when careful considerations lead to a different selection, the selected (log) K_{ow} value is then the average value of all reliable values determined by the shake flask, slow stirring or generator column method, for which guidance is given in the annex. K_{ow} values estimated using the HPLC method are indirect

estimates of octanol/water partitioning and are therefore not regarded as most reliable. They should not be used when more reliable data are available.

When no, or only unreliable, experimental data on K_{ow} are available, the selected data should be calculated with a QSPR programme. The use of the K_{ow} values obtained with the ClogP program (BioByte, 2004) is preferred.

A1.2.3.2. K_{oc}

For the selection of the K_{oc} value, experimentally determined values should be retrieved. These K_{oc} values may be derived from standardised tests (e.g. OECD guideline 106; OECD, 2000) or from other studies published in scientific literature. K_{oc} values determined by the HPLC method (OECD guideline 121; OECD, 2001) should be considered as estimates of the real K_{oc} values and consequently, these values are not used as experimental values. Because K_{oc} values may vary widely and no value for K_{oc} can be considered as the most reliable value, the geometric mean of all valid K_{oc} values is calculated, including one value estimated from K_{ow}. This geometric mean K_{oc} will be used as the selected value in EQS derivations (Otte *et al.*, 2001).

A1.2.3.3. $K_{p, \text{susp-water}}$

For organic substances, the value of $K_{p, \text{susp-water}}$ is derived from the K_{oc} value and the fraction organic carbon of suspended matter used within the EU ($Foc_{\text{susp,TGD}}$), applying Eq. 1. Note that the fraction of organic carbon is equal to 0.1 in this case (the EU standard): the outcome of this equation triggers EQS_{sediment} derivation and should be uniform within Europe.

$$K_{p, \text{susp–water}} = K_{oc} \times Foc_{\text{susp,TGD}} \tag{1}$$

If site-specific data for suspended matter are available these can be used directly as well and might be preferred. The value for $K_{p, \text{susp-water}}$ for metals is derived from experimental data. The geometric mean value is calculated from the valid $K_{p, \text{susp-water}}$ values summarised in the table containing physicochemical properties (see Annex); this value is used in EQS derivations. If experimental data on K_p for metals are lacking, the data gap is reported and its possible solution suggested.

A1.2.3.4. Water solubility

The selected value for the water solubility may be calculated from the geometric mean of all valid values for the water solubility. Values below 10 mg·L^{-1} determined with the shake-flask method should be considered as unreliable. For these poorly soluble compounds, the geometric mean of the generator column and slow-stirring is the value to be used.

A1.2.3.5. Vapour pressure

In general, the guidance in Table 1 of the annex can be used to determine which values for the vapour pressure are reliable. However, if results from different methods deviate significantly from each other, only the methods with a direct analysis of the compound should be used, e.g. the gas saturation method. Complementary to this, the data from GC retention times may be used if there are not enough reliable data. If no experimental data are available, the estimate from EPI Suite can be used (US EPA, 2007b).

A1.2.3.6. Henry coefficient

The validity of values for the Henry coefficient should be considered on a case-by-case basis. When no reliable experimental values are available, the Henry coefficient can be estimated from the quotient of the vapour pressure and the water solubility, provided that reliable values are

available for both parameters. If this is not the case, the estimate from EPI Suite can be used (US EPA, 2007b).

A1.3. TOXICITY DATA

A1.3.1. Data collection

To collect toxicity data for a compound the following steps are recommended:

1. Environment agencies in other countries are consulted by sending out an e-mail enquiry, in which they are asked if they have access to specific information on toxicity data and/or physicochemical data (see Section 2.1.2) and are willing to share those data.
2. Industry parties involved in production or use of the compound under investigation are invited to submit relevant studies, which will be treated as public literature.
3. The on-line literature systems Current Contents and TOXLINE are screened.
4. It is important to perform a retrospective literature search. The reference lists of publications or reports obtained should be carefully checked for related studies that have been published at earlier dates. A hard copy of each study that is deemed relevant should be obtained.
5. The ECOTOX database from the US EPA (US EPA, 2007a) is searched for relevant ecotoxicological studies. A copy of all studies retrieved from the search results is requested. Other national or organisational databases may also be searched.
6. The IUCLID database is searched for the compound of interest (European Commission (Joint Research Centre), 2007).
7. The availability of OECD SIDS documents or EU risk assessment reports is checked.
8. The database of the Japanese National Institute of Technology and Evaluation (NITE) is searched.
9. For pesticides, public assessment reports are available online at several locations. The following websites are recommended:
   UK Pesticides Safety Directorate (PSD): http://www.pesticides.gov.uk/psd_evaluation_all.asp
   US EPA: http://www.epa.gov/pesticides/reregistration/
   Health Canada: http://www.pmra-arla.gc.ca/english/pubs/reeval-e.html
   EU Pesticides Database: http://ec.europa.eu/sanco_pesticides/public/index.cfm
10. A further search may be performed in libraries.
11. If no or very few data are found in the steps described above, an additional internet search can be performed on the chemical name and CAS number of the compound using established search engines.

In principle, all ecotoxicological studies are evaluated for usefulness in EQS derivation. Studies from which one of the endpoints LC50, EC50, LC10, EC10 or NOEC can be calculated using data presented by the author(s) are also used. Studies that show results in a graph of good quality that can be used to extract raw data are also relevant.

Ecotoxicity studies conducted in freshwater, seawater, brackish water, groundwater (usually no data) and sediment are relevant. Whether or not data on secondary poisoning should be collected is dependent on whether an assessment is required (see main guidance) some trigger values. In the case that secondary poisoning should be assessed, toxicity data for birds and mammals should be collected, screening the appropriate sources described above. In the case of toxicity to birds, acute 5-day studies generating LD50 values should be collected too.

A1.3.2. Data evaluation and data tables

An outline of the general procedure of the evaluation of the toxicity data is given below.

1. All retrieved literature is read and evaluated with respect to its relevance and reliability.
2. Each study should be assigned a quality code. Section A1.3.2.1 provides more detail.

3. After evaluating a study, the results of the study are summarised by entering it into the data table (see Sections A1.3.3 and A1.3.4).
   - Toxicity data on freshwater organisms and on marine organisms are placed in separate tables.
   - Data on aquatic and benthic species are separated into acute and chronic data, with a separate table for each category (see Section A1.3.2.3 for more guidance).
   - Toxicity data on birds and mammals are placed in separate tables. If many data are available, a distinction can be made between studies with oral (gavage) and dietary (food) exposure.
4. Each row of the toxicity data table contains a test result for one species, endpoint and summary statistic. The columns of the toxicity data table contain the various study parameters. Columns should be filled as completely as possible. When there is no value for a given parameter, the table cell is filled with 'n/d'.
5. All references of toxicity studies should be included.
6. In the toxicity data tables, all tested species are clustered according to taxonomic groups (see Sections A1.3.3.1 and A1.3.4.1), usually: fish, amphibians, crustaceans, insects, molluscs, annelids, macrophytes, algae, birds, mammals.
7. For benthic toxicity data for organic compounds, recalculate toxicity test results to standard sediment with organic carbon content of 5% (Section A1.3.4.14). In the toxicity data table on benthic data, both the test result in the test sediment (expressed as a dry weight concentration) as well as the test result in standard sediment (expressed as a dry weight concentration) are reported. For metals, tests should not be normalised to standard sediment (Section A1.3.4.14).
8. Finally, a new table of selected toxicity data is created in which toxicity data are aggregated to one toxicity value per species. The table will contain the data that are used for the actual EQS derivation. Guidance to compile this table is given in Section A1.3.6.

A1.3.2.1. Study quality

Studies **that might influence an EQS must be** quality assessed. The assessment may be performed according to the scheme developed by Klimisch *et al.* (1997) or CRED (Moermond et al, 2016; and Kase et al. 2016). The Klimisch system is a long-established one that is also used in other chemical assessment regimes, but CRED offers the ability to further assess relevance of aquatic ecotoxicity data in addition to the reliability criteria and is recommended to be applied for the critical studies in a dataset.

Either method may be used to quality-assess data to be used in EQS derivation, CRED is recommended for: (a) potentially critical or contentious studies and (b) where studies are borderline reliable with restrictions or regarded as 'not reliable'. When using CRED in such cases, it is important to use the CRED template (Appendix 4) for recording judgements of reliability and relevance. This helps promote transparency in the conclusions about the reliability and relevance and in general about the defensibility of a particular study.

For further details on the quality assessment methods, see Appendix 4. The reliability codes assigned using both Klimisch and CRED are:

1 = reliable without restrictions

2 = reliable with restrictions:

3 = not reliable

4 = not assignable

In general, when a test has fundamental shortcomings, it should be classified as not reliable (3).

Additionally, CRED offers a similar structured evaluation method for assessing the relevance, see Appendix 4.

When a study contains useful toxicity information, but it cannot be used directly for derivation of EQSs, it is still tabulated. Examples might be a NOEC value from a short-term test, or a value higher than the highest tested concentration or lower than lowest tested concentration (see Section A1.3.2.9 for more detail). The test can then still be classified as reliable or reliable with restrictions.

A1.3.2.2. Quality Assurance

Studies do not need to have been performed under a formal quality assurance scheme, such as Good Laboratory Practice (GLP) or do not need to be OECD validated or ISO certified, but should follow generally accepted good scientific principles. The reported description of a study and comparison with results from comparable studies and organisms, should provide all information necessary to assess its quality. Guidance on quality assessment of data is provided in Section A1.3.2.1.

Toxicity studies originate from various sources, which are tracked as much as possible to the original source. The two key sources are (i) publications in scientific journals and (ii) original study reports that have not been published elsewhere. The latter category has been in the minority since, for reasons of data protection, original study reports are often unpublished and may not be accessible.

A1.3.2.3. Acute and chronic studies

A chronic toxicity study is defined for the purpose of EQS derivation as a study in which:

    (i)   the species is exposed to the toxicant for at least one complete life cycle, or
    (ii)   the species is exposed to the toxicant during one or more sensitive life stages.
This is in line with REACH guidance, which states that NOECs from chronic/long-term studies should preferably be derived from full life-cycle or multi-generation studies (ECHA, 2008). True chronic studies cover all sensitive life stages.

Unfortunately, no clear guidance is provided on individual studies, whether these are to be considered as chronic studies or as acute studies. What is considered chronic or acute is very much dependent on 1) the species considered and 2) the studied endpoint and reported criterion.

For most common species, toxicity studies with fish are considered acute if mortality is determined after 96 hours (standard acute test) or after 14 days (prolonged acute toxicity test). The most commonly accepted as chronic toxicity tests for fish are early life-stage tests (ELS), in which eggs or larvae are exposed and the effects on hatching, malformation and growth are considered. Reproduction studies (ideally over a full life cycle, see OECD, 2008) and most ELS tests for fish, but also for other species such as amphibians (OECD Tests no.231 and 241) or echinoderms, can be considered as chronic toxicity studies. For daphnids, the standard exposure time for acute toxicity is 48 hours, but with regard to chronic toxicity, there is a factor of three difference between the tests with *Daphnia magna* (21 days) and *Ceriodaphnia dubia* (7 days), the latter having a much shorter reproduction time.

For algae, the standard exposure time is 72 hours. In this time, the algae regenerate several times. Since the cells are at different stages of their life cycles it is necessary to ensure that they are exposed at least once over an entire cell cycle. However, the EC50 of this test is considered as acute, while the NOEC or EC10 of the same test is regarded as a chronic value.

For any short-term test, it should be considered if the partitioning of the test substance between the water phase and the organisms can be assumed to have reached a steady state. This would usually not be a problem, except for tests with duration from a few minutes to hours. Even if effects are observed, the toxicity may have been underestimated if test duration was too short to reach steady state. This will depend on the characteristics of the test system as well as on the test

substance. Ideally, the authors have discussed this issue in the paper. Otherwise, a comparison of different test durations may be helpful when evaluating this aspect (e.g. as done in Fröhner et al. 2002 for *Vibrio fischeri*).

A1.3.2.4. Comparison of toxicity value with water solubility

In principle, toxicity studies that have been conducted at concentrations above the water solubility should not be used. However, depending on the uncertainty in the estimate of the water solubility, test results (L(E)C50, NOEC, EC10) that are ≤2 times the estimated value might be included. The factor of 2 is a rather arbitrary value; when experimental data show that the variation in the estimate of the water solubility is lower, it should be lowered accordingly. When the variation in the estimate of the water solubility is higher than a factor of 2, it may be increased to a factor of 3 (maximum). Toxicity studies showing results above the water solubility receive a footnote stating: 'test result above water solubility'.

A1.3.2.5. Use of co-solvents, emulsifiers and dispersants

Sometimes, the solubility of a compound is so low that a solvent, emulsifier or dispersant is used to prepare suitably concentrated stock solutions of the test substances. Such vehicles may not be used to enhance the solubility of the test substance in the test medium, and in any case the compounds used for this purpose may not be toxic to the tested species. Therefore, a control with the vehicle (solvent control) used should be incorporated in the set-up of the test. According to several OECD test guidelines for aquatic toxicity testing (see Section A1.3.2.10) the concentrations of the solvent, emulsifier or dispersant should not exceed 100 mg/L^{-1} (or 100 µL/L^{-1} or 0.01%).

A1.3.2.6. pH of test water and pK_a and ionisation of test compound

When a test has been performed according to a guideline, the pH should be within the required range and, if not, the test validity should be reviewed, e.g. for effects on organism health or test substance hydrolysis.

In some cases, the compound itself may alter the pH strongly. In such cases, it should always be checked whether any observed toxicity might be caused by this change in pH. If so, the test must not be used because the buffering capacity of the environment will usually prevent such a pH effect in the field. For compounds containing functional groups with acidic or basic properties, the pK_a value(s) should be reported in the table with physicochemical properties (Section A1.3.2.10).

Attention should be paid to possible relationships between pH and toxicity of the tested compound, for example, due to a reduced availability (speciation, precipitation, hydrolysis, etc.) of the test compound. The toxicity of a compound may be influenced by its degree of ionisation[38]. As a rule, hydrophobicity, and consequently bioaccumulation and toxicity, will increase with decreasing ionisation. The degree of ionisation of a compound in a toxicity test is determined by several factors:

– the pK_a (s) of the test compound,
– the concentration of the test compound,
– pH of the test compartment (water, sediment),
– the buffering capacity of the test-matrix.

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[38] 'Degree of ionisation' as used in this section expresses the ratio of the number of charged molecules over the total number of neutral and charged molecules at a given concentration and at a given pH.

In practice, a compound's potential to ionise (pK_a in physicochemical table) should be checked. The presence of one or more pK_a value(s), or ionisable group(s), triggers attention for pH effects in the toxicity studies. If toxicity test results show that toxicity is dependent on the pH of the test medium, the results are rejected if the pH falls outside the range of what can be expected naturally.

Test results should be rejected when the toxicity in a given study is not caused by the compound alone, but also by a pH change. Hence, results from tests with ionisable compounds performed in buffered media (providing sufficient buffering capacity) are more reliable than those performed without a buffer. Studies that explicitly measure pH after addition of the toxicant are most useful in this respect.

A1.3.2.7. Purity and identity of the test substance

In some tests the identity of the test substance is largely unknown, or the purity of the test substance is very low. Depending on the nature of the impurities present, if these have been identified at all, a minimum purity of 80% is required, unless it is known that the impurities do not cause any toxic effects by themselves and do not influence the toxicity of the substance of interest. When the purity of the tested compound is <90%, the test result should be corrected for purity. For pesticides, toxicity should be expressed in terms of the concentration of active ingredient.

A1.3.2.8. Toxicity studies performed in other media

Benthic species are sometimes tested in a water-only system. In such cases the data are still tabulated, but for organisms that normally live in the sediment and not on the surface of the sediment, the test should be assigned the code 'invalid'.

A1.3.2.9. Dealing with toxicity values higher or lower than range of test concentrations

If the highest concentration in a toxicity test is not high enough to determine the EC10, NOEC or L(E)C50, the result of that study should be tabulated as EC10 or 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 particular 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. In the case of NOEC <, an attempt should be made to calculate the EC10, if possible.

A1.3.2.10. Use of toxicity tests performed according to established guidelines

International guidelines exist for performing toxicity studies for many species. If such protocols are followed and the requirements for the study are met, the results from such studies are generally reliable. Quality data do not, however, have to conform to formal test guidelines, not even if a formal test guideline exists for a particular test organism. The most frequently used guidelines for ecotoxicological studies are summarised in this section, although others may also be reported.

Algae and plants

• OECD guideline 201: Alga, Growth Inhibition Test. The EC50 from this 72-h algae test is considered an acute value, the NOEC or EC10 a chronic value.

The 1984 version of the test guideline includes both growth rate and growth as test endpoints. Growth rate is calculated as the logarithmic increase of biomass during the test period, whereas the effect on growth is calculated as the area under the growth curve (sometimes also indicated as biomass or biomass integral). Growth rate is the more robust endpoint (European Commission (Joint Research Centre), 2003a). However, if only values for growth (biomass integral) are available, these can be used. The result for the biomass integral ('growth' in the 1984 version of the OECD guideline) is generally lower than the growth rate and can therefore be considered as a conservative value, but this is purely due to mathematical reasons (not using log-transformed numbers in the calculations of the integral).

The guideline was revised in 2006 (with a correction of annex 5 on non-linear regression analysis in 2011) and this version specifies growth rate as the scientifically sound endpoint, but offers the option to calculate the yield if needed for regulatory purposes. Yield is the absolute difference in cell numbers over the test duration, similar to the growth endpoint in the 1984 version. However, the effect on yield is not calculated based the area under the growth curve, but as the relative change in cell numbers compared to the control. From studies conducted according to OECD (2006/2011), growth rate is the preferred endpoint. For more information on this issue please also refer to chapter 6.4 in Ratte et al. (1998).

When the growth rate ErC10 and ErC50 are not reported, these values should be re-calculated based on the raw data. Resulting values can be pooled to derive one value per species.

For deriving the AA-EQS, the ErC10 as well as the NOEC can be used. For reliable estimates of ErC10 (i) the concentration-response curve needs to be consistent with a sigmoidal concentration-response relationship and (ii) sufficient concentrations should be used to define the ErC10 with an appropriate level of confidence, i.e. according to OECD 201 the concentration series should preferably cover the range causing 5-75 % effect.

If it is not possible to recalculate the ErC10 because of missing data or estimates of the ErC10 are not reliable, preference should be given to the NOEC. Due to typical spacing of test concentrations (spacing factor <3.2 according to OECD 201), NOECs based on growth rate or yield are often identical. Pooling of NOECs for either growth rate or yield from different studies on the same species might be justified for AA-EQS derivations.

• OECD guideline 221: Lemna sp. Growth Inhibition Test. For this 7-d test with duckweed the same considerations can be made as for the algal test (OECD 201): the EC50 from this test is considered an acute value, the NOEC or EC10 a chronic value. Both chronic and acute data should be retrieved from the test. The preferred endpoints are growth rate (based on frond number) or biomass (dry weight, fresh weight or frond area).

• OECD guideline 238: Sediment-Free Myriophyllum Spicatum Toxicity Test. This 14-day test examines effects on the submerged aquatic plant Myriophyllum spicatum growing in a sediment-free test system. Endpoints are growth of shoot length, of lateral branches and roots, development of fresh and dry weight, increase of whorls. Both average specific growth rate (r) and yield (y) are determined and then used to express ErC50 and EyC50, respectively. As with the algal and the

Lemna test guideline, the EC50 from this test is considered an acute value, the NOEC or EC10 a chronic value.

Invertebrates

•   OECD guideline 202: Daphnia sp., Acute Immobilisation Test. For the derivation of EQSs for water, only the EC50 from this 48-h acute toxicity study is considered. The endpoint is immobility, as indicated by the inability to swim after agitation.

•   OECD guideline 211: Daphnia magna Reproduction Test. This is a chronic test with water fleas. The most important endpoint is the number of young per female (both young and parent alive). Other endpoints are the survival of the parent animals and time to production of first brood. Additionally, parameters such as growth (e.g. length) of the parent animals, and possibly intrinsic rate of increase are useful endpoints. When the male production in the young has been recorded, the sex ratio can be used as an indication of endocrine disruption (OECD, 2012).

•   OECD guideline 242: Potamopyrgus antipodarum Reproduction Test. This guideline is designed to assess effects of prolonged exposure to chemicals on the reproduction and survival of the freshwater mudsnail Potamopyrgus antipodarum. Because the recorded endpoints are reproduction and survival during 28 days, the test can be regarded as chronic. The NOEC or the EC10 from this test can be used for the derivation of AA-EQSs.

•   OECD guideline 243: Lymnaea stagnalis Reproduction Test. This guideline is designed to assess effects of prolonged exposure to chemicals on the reproduction and survival of the freshwater snail Lymnaea stagnalis (the Great Pond Snail). Because the recorded endpoints are reproduction, growth and survival during 28 days, the test can be regarded as chronic. The NOEC or the EC10 from this test can be used for the derivation of AA-EQSs.

Fish

•   OECD guideline 203: Fish, Acute Toxicity Test. For the derivation of EQSs for water, only the LC50 from this 96-h acute toxicity study is considered. The recorded endpoint is mortality.

•   OECD guideline 204: Fish, Prolonged Toxicity Test: 14-day Study. This study is also considered as an acute toxicity study, and consequently, in most cases, only the LC50 is used for the derivation of EQSs. Note: Following the OECD Council decision, the Test Guideline 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014.

•   OECD guideline 236: Fish Embryo Acute Toxicity (FET) Test. In this test, newly fertilised zebrafish eggs are exposed to the test chemical for a period of 96 hrs. The following apical endpoints as indicators of lethality are recorded daily: coagulation of fertilised eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac, and lack of heartbeat. For the derivation of MAC-EQSs for water, the LC50 from this 96-h acute toxicity study is considered.

•   OECD guideline 210: Fish, Early-life Stage Toxicity Test. This test with fish is a chronic test which covers the life cycle of fish from eggs to free feeding juvenile fish. The recorded endpoints are mortality at all stages, time to hatch, hatching success, length, weight and any morphological or behavioural abnormalities.

•   OECD guideline 212: Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages. In the guideline it is stated that this test can be used as a screening test for chronic toxicity. Especially for species that cannot be kept under laboratory circumstances for a period long enough to perform a full early-life stage (ELS) test, this test can be a useful alternative. Because the sensitive life stages from egg to sac-fry are covered in this test, it can be considered a chronic test. However, it is

expected to be less sensitive than the full ELS test. The same endpoints are recorded as for the full ELS test.

• OECD guideline 215: Fish, Juvenile Growth Test. Because the recorded endpoint is growth during 28 days and the criterion is the NOEC or EC10, the test can be regarded as chronic.

• OECD guideline 234: Fish Sexual Development Test. The test is in principle an enhancement of Fish ELS-Test (OECD test no. 210), where the exposure is continued until the fish are sexually differentiated. Two core endpoints are measured as indicators of endocrine-associated developmental aberrations, the VTG concentrations and sex ratios (proportions of sex) determined via gonad histology. The Test Guideline should be seen in the context of the "OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals" (for further details, please refer to OECD GD no. 150, OECD 2012b). Different from VTG, sex ratio is considered as a biomarker endpoint as well as an apical endpoint. For assessing the relevance of the different endpoints in the context of EQS derivation refer to chapter A1.3.3.14.

• OECD guideline 240: Medaka Extended One Generation Reproduction Test (MEOGRT). In comparison to the aforementioned fish tests, this test is a more comprehensive test based on exposure over multiple generations. Besides the main endpoints survival and growth, suspected endocrine disrupting chemicals are assessed by measuring the following endpoints: vitellogenin expression, phenotypic secondary sex characteristics (SSC) as related to genetic sex, and evaluating histopathology. Its application should be seen in the context of the "OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals" (for further details, please refer to OECD GD no. 150, OECD, 2012b). For assessing the relevance of the different endpoints in the context of EQS derivation refer to chapter A1.3.3.14.

The following test guidelines or test methods are used as screening tests or at least should only be used as supporting information with regard to EQS-derivation:

• OECD guideline 229: Fish Short Term Reproduction Assay. This bioassay serves as an in vivo reproductive screening assay and its application should be seen in the context of the "OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals", included in Annex 1.4 of the Guidance Document no. 150 (OECD, 2012b). The biomarker endpoints vitellogenin (VTG) and secondary sexual characteristics are measured in males and females as indicators of endocrine activity of the test chemical. Apical endpoints survival and fecundity are included in this assay. Gonads histopathology examination is considered optional. Given the focus on the identification of endocrine disrupting chemicals, and less on determining key apical endpoints, the test guideline is considered to be of low relevance regarding EQS-derivation.

• OECD guideline 230: 21-day Fish Assay. This bioassay serves as an in vivo screening assay for certain endocrine modes of action and its application should be seen in the context of the "OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals" (for further details, please refer to OECD GD no. 150, OECD 2012b). The biomarker endpoints vitellogenin (VTG) and secondary sexual characteristics are measured. This assay, however, does not include apical endpoint fecundity. For the same reasons given above, the study is considered to be of low relevance for EQS-derivation.

• OECD Guidance document on the Androgenised Female Stickleback Screen (series Series on Testing and Assessment No. 148): This Androgenised Female Stickleback Screen (AFSS) describes a 21-day in vivo assay for identifying endocrine active chemicals with (anti)androgenic activity in fish using female sticklebacks (*Gasterosteus aculeatus*). The biomarker endpoints spiggin are measured. Its application should be seen in the context of the "OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals" (for further details, please refer to OECD GD no. 150, OECD 2012b).

Amphibians

•   OECD guideline 231: Amphibian Metamorphosis Assay (AMA). This 21-day test with the African clawed frog (*Xenopus laevis*) is intended to screen substances, which may interfere with the thyroid system of vertebrate species. Endpoints measured include developmental stage, length and histopathology evaluations of the thyroid gland.

•   OECD guideline 241: The Larval Amphibian Growth and Development Assay (LAGDA). This Test Guideline with the African clawed frog (*Xenopus laevis*) is more comprehensive than the Amphibian Metamorphosis Assay (AMA) and its application should be seen in the context of the "OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals" (for further details, please refer to OECD GD no. 150, OECD, 2012b). The test is designed to assess early development, metamorphosis, survival, growth, and partial reproductive maturation. The test enables measurement of endpoints that allows for diagnostic evaluation of suspected endocrine disrupting chemicals or other types of developmental and reproductive toxicants.

•   FETAX (Frog Embryo Teratogenesis Assay Xenopus): This test is a rather short test of 96 hours duration, with the possibility of prolongation by a few hours, if the larvae have not reached a certain developmental stage. However, considering the short test duration as compared with organism's average life-span, the study endpoints (mortality, development and malformation) are considered rather as acute for the derivation of EQSs, albeit the endpoints development and malformation may indicate the presence of chronic effects (see chapter A1.3.2.2.). In any case, OECD 231 and 241 have a higher relevance with regard to chronic effects on amphibians.

Sediment species: benthic invertebrates and rooted macrophytes

•   OECD guideline 239: Water-Sediment *Myriophyllum Spicatum* Toxicity Test. This 14-day test examines effects on the submerged rooted aquatic plant *Myriophyllum spicatum* growing in a water-sediment test system. The measured quantitative variables include growth of shoot length and development of fresh and dry weight, and the measured qualitative variables include presence or not of chlorosis and necrosis or growth deformities. Both average specific growth rate (r) and yield (y) are determined and then used to express ErC50 and EyC50, respectively. As with the algal and Lemna test guideline, the EC50 from this test is considered an acute value, the NOEC or EC10 a chronic value.

•   OECD guideline 235: Chironomus sp., Acute Immobilisation Test. The test method is based on the 48-h Daphnia Acute Immobilisation Test (OECD test no. 202) and is designed to complement existing Test Guidelines for chironomid chronic toxicity assays (TG 218, 219 and 233). The EC50 from this 48-h acute toxicity study is used for the derivation of MAC-EQSs.

•   OECD guideline 218: Sediment-Water Chironomid Toxicity Test Using Spiked Sediment. This is a chronic toxicity study with a chironomid species. The measured endpoints are the total number of adults emerged and the time to emergence. Additionally, larval survival and growth after a ten-day period are recommended endpoints.

•   OECD guideline 219: Sediment-Water Chironomid Toxicity Test Using Spiked Water. This test is similar to OECD guideline 218. However, for reasons of stability of the test concentrations, the OECD 218 is preferred. If a test with spiked water is available this test should always be accompanied by a determination of actual concentrations in the sediment.

•   OECD guideline 225: Sediment-Water *Lumbriculus* Toxicity Test Using Spiked Sediment. This 28-day static test examines effects of prolonged exposure of the endobenthic oligochaete Lumbriculus variegatus to sediment-associated chemicals. The test organism burrows in the

sediment and ingests sediment particles below the sediment surface. This ensures exposure of the test organisms to the test substance via all possible uptake routes (e.g. contact with, and ingestion of contaminated sediment particles, but also via porewater and overlying water). The recorded endpoints are survival, reproduction (as increase of worm numbers) and the biomass (dry weight). The NOEC or the EC10 from this test can be used for the derivation of AA-EQSs.

•   OECD guideline 233: Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment. This test is an extension of the existing OECD test guideline 219 or 218 using a spiked-water exposure scenario or a spiked sediment scenario, respectively. Measured endpoints for both 1st and 2nd generations include: total number of adults emerged, development rate, sex ratio of fully emerged and alive adults. Endpoints restricted to the 1st generation include: number of egg ropes per female and fertility of the egg ropes. The NOEC or the EC10 from this test can be used for the derivation of AA-EQSs.

EPA. Ecological Effects Test Guidelines. OPPTS 850.1735. Whole sediment acute toxicity invertebrates, freshwater. Draft, 1996. Endpoints are survival and growth (10–28 days). This test can be used as a chronic test for species such as *Hyalella azteca*. Note: Meanwhile, a revised test method recommended by Environment Canada is available (for further details, see Report EPS 1/RM/33, published in January 2013).

Terrestrial vertebrates

•   OECD guideline 205: Avian Dietary Toxicity Test. This test can be used as an acute toxicity test with birds for the assessment of secondary poisoning.

•   OECD guideline 206: Avian Reproduction Test. This test can be used as a chronic toxicity test with birds for the assessment of secondary poisoning, because the exposure duration is at least 20 weeks.

In addition to tests on birds (OECD guidelines 205, 206 and 223), the OECD has a series of guidelines of toxicity tests with mammals for use in human health risk assessment. These data might also be used in the derivation of EQSs (secondary poisoning of predators) provided that the test endpoints relate to the effects at the population level of the species. The following OECD guidelines are most important in this respect:

•   OECD guideline 401: Acute Oral Toxicity

•   OECD guideline 407: Repeated Dose 28-day Oral Toxicity Study in Rodents

•   OECD guideline 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents

•   OECD guideline 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents

•   OECD guideline 414: Prenatal Development Toxicity Study

•   OECD guideline 415: One-Generation Reproduction Toxicity Study

•   OECD guideline 416: Two-Generation Reproduction Toxicity

•   OECD guideline 443: Extended One-Generation Reproductive Toxicity Study

A1.3.3. Aquatic toxicity data tables

The following subsections (Sections A1.3.3.1 toA1.3.3.18) discuss the data to be reported in the aquatic toxicity data tables. The parameters are treated in the same order as they appear in the default toxicity data table. The following subsections have titles identical to the column headings in the data tables.

A1.3.3.1. Species

All available toxicity data for a given compound are ordered by test organism. Species are grouped in taxonomic groups. Species names are reported in Latin. Taxonomic groups are shown in bold font, species names are shown in italic font. Species names within a taxon are listed in alphabetical order. For example:

Bacteria

Pseudomonas putida

Algae

Chlorella vulgaris

Pseudokirchneriella subcapitata

Scenedesmus acuminatus

Crustacea

Daphnia pulex

A1.3.3.2. Test organism information

The most relevant properties of the test organism are mentioned in this column; e.g. age, size, weight, life stage or larval stage. Toxicity data for organisms of different ages, size, life stage, etc., are presented as individual entries (i.e. one entry in each row) in the data table.

A1.3.3.3. Chemical analysis

This column reports whether the test compound is analysed during the experiment. Y (Yes) is entered in this column when the compound has been analysed. When no analysis for the test compound is performed, N (No) is entered in this column.

In some cases, the test compound is analysed, but the test results (L(E)C50, EC10, NOEC) are not calculated from the measured concentrations. If the test result is based on nominal concentrations, this is mentioned in a footnote to this study: 'Test result based on nominal concentrations'. This is valid when measured concentrations are close to initial concentrations (drop in concentration <20% over exposure period) and 'Test result based on nominal concentrations, measured concentrations were >80% of nominal' is noted.

If the test compound is analysed, but not used for the test results and there is considerable change in the concentration during the test (>20% loss of test compound), the test result should be recalculated using *actual* concentrations. In such cases, a footnote should mention that test results are recalculated to actual concentrations.

In static or renewal tests, when samples are analysed at different points of time, the mean of the measured values is used. When the initial concentration is not measured and one or more samples during the test are, a mean of the initial nominal and the measured concentration(s) is used. In general, taking the average of start and end concentrations slightly overestimates the average concentration during the whole experiment, while the geometric mean underestimates the concentration. For calculating the mean concentration during the course of a static experiment, the best assumption is an exponential decay of the concentration in time. In continuous flow experiments, the concentrations are usually reported as mean measured values and, here, no further calculations are necessary.

A1.3.3.4. Test type

The following test types are distinguished:

S	static system
Sc	static system in closed bottles or test vessels
R	renewal system (semistatic)
F	flow-through system
CF	continuous flow system
IF	intermittent flow system

A1.3.3.5. Test compound

– This column can be deleted when the compound under consideration has only one structural molecular configuration.
– If the tested compound is a metal, the tested metal salt should be reported here.
– If the tested compound is a stereoisomer[39] or consists of a mixture of isomers, the name of the tested molecule(s) should be reported here. For some stereoisomers it might be appropriate to derive individual EQSs. The stereoisomers dieldrin and endrin are an example of such a case.
– If the tested compound is a structural isomer, the individual compounds, in general, have different physicochemical and toxicological properties and each compound will be the subject of a separate EQS derivation (e.g., anthracene and phenanthrene).
– Formulated products (e.g. biocides, pesticides) should be reported here.

A1.3.3.6. Purity

Unit: %

The purity of the test compound expressed as percentage is reported in this column. Alternatively,

the following abbreviations may be entered for the designation of chemical purity.

ag	analytical grade
lg	laboratory grade
rg	reagent grade
tg	technical grade
fp	formulated product

Here, the first four have a relatively high purity, while technical grade is in general somewhat less pure. When the purity of the test compound is expressed only by an abbreviation, this abbreviation is reported. However, a purity expressed as percentage is preferred.

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[39] Stereoisomers: geometric isomers (*cis-* and *trans-*isomers or *E-* and *Z-*isomers), optical isomers (+- and –-isomers or *R-* and *S-*isomers) and conformational isomers (e.g. chair and boat structures in cyclohexane ring structures).

A1.3.3.7. Test water

In this column, the test water or medium is reported using abbreviations. Choose from the following list. A footnote to the test may be added if further description of the test medium is needed.

am   artificial medium, such as media used for bacterial and algal tests, artificial seawater

dw   de-ionised water, dechlorinated water or distilled water

nw   natural water, such as lake water, river water, sea water, well water

rw   reconstituted water: (natural) water with additional salts

rtw   reconstituted tap water: tap water with additional salts

tw   tap water

A1.3.3.8. pH

If possible, measured pH values should be reported. If a pH range is given, this range is reported.

A1.3.3.9. Temperature

Unit: °C

In this column the temperature at which the test is performed should be reported, preferably a measured temperature. If a temperature range is given, the range is reported.

A1.3.3.10. Hardness

Unit: mg $CaCO_3 \cdot L^{-1}$

This column is shown in tables showing data from freshwater experiments, not for marine water. The hardness of the test water should be reported here. If the hardness of an artificial medium is not reported, but the composition of the medium is reported, the hardness should be calculated. Recalculation should be performed by summing the molar concentrations of all calcium (Ca) and magnesium (Mg) salts and expressing the result as $CaCO_3$ in units of $mg \cdot L^{-1}$.

A1.3.3.11. Salinity

Unit: ‰

This column is only shown in tables showing data from saltwater experiments, and it replaces the column for hardness in the freshwater tables. In practice salinity may be determined by recalculating the measured chloride ion only to total salinity, using the assumption that the total amount of all components in the oceans is constant. The average salinity of seawater is around 35‰ (roughly 35 g of salts per litre of seawater). The unit of salinity might also be found expressed in parts per *thousand* (ppt) as w/w. To derive the salinity expressed in ppt the following conversion can be applied:

–   when only chloride ions (Cl^-) have been measured, the salinity can be recalculated to ‰ from the chloride concentration using: S(ppt) = 1.80655 × chloride concentration (ppt), in which S = salinity

– psu = practical salinity units[40]. One psu roughly equals one ppt (‰). Seawater has a salinity of approximately 35 psu ≈ 35 ‰ = 35 g.kg^{-1}.

Animals living (and tested) in brackish water environments are not placed in separate tables, but these data are included in the saltwater tables. The division between freshwater, brackish water and seawater on the basis of salinity is given in Table 18. The division in these categories is rather arbitrary and depends on the source used. For the division between freshwater and brackish water, the value of 0.5‰ is defined in the Water Framework Directive (European Commission, 2000).

Table 18 Classification of water according to salinity

Water type	Salinity (‰)
Freshwater	<0.5
brackish water	0.5–30
Seawater	30–40

A1.3.3.12. Exposure time

The duration of exposure to the toxicant in the toxicity experiment is given in this column. The abbreviations listed below in Table 19 can be used. A rule of thumb is to stick to the most common expression of test duration in case of standardised tests (e.g. OECD or ISO tests) where this is possible. For example, for a reproduction study with Oncorhynchus mykiss, 60 days (post-hatch) is noted rather than '2 months'.

Table 19 Classification of water according to salinity

Test duration in	Abbreviation	
Minutes	min	
Hours	h	
Days	d	
Weeks	w	
Months	mo	
Years	y	

A1.3.3.13. Summary statistics

The summary statistics commonly encountered in ecotoxicological tests are summarised in Table 20. Their use in EQS derivation is described in the third and fourth columns of this table.

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[40] However, because of the qualitative nature in which salinity is used in EQS derivation, this definition and its inherent accuracy are not relevant.

Table 20 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	MATC[41]	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 [h]
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
Chronic test	LOEC	No	▪ Omit if NOEC is also available from same experiment ▪ Else: (i) if percentage effect is known and LOEC > 10 and < 20% effect: NOEC can be calculated as LOEC/2 ▪ 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

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

Test type	Criterion	Use in EQS derivation?	Action
			NOEC [g]
Chronic test	NOEC	Yes	▪ Omit LOEC if it is also available from same experiment [h]

Notes to Table 20.

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/$\sqrt{2}$.

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/$\sqrt{}$(spacing factor).

h) In new guidance on biocidal products regulation (BPR) (ECHA 2015) it reads "the choice between the NOEC or ECx point estimates is subject of continuing debate. OECD (1998) favours the use of an ECx. Extensive information on the implications of either choice for test set-up and statistical evaluation is given by OECD (2006)." Please note that substances with a flat dose-response relationship may cause significant effects well below the (reliable) EC10. In these cases, the choice between NOECs or EC10 may have a strong influence on the resulting AA-EQS and a reason for preferring one over the other. This reasoning should consider the case-specific concentration-response data but also general considerations regarding the choice of EC10 or NOEC values given in this TGD and/or current changes in related TGDs.

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 A1.3.6).

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.

A1.3.3.14. Test endpoint

The list below shows some relevant endpoints:

> growth (weight, length, growth rate, biomass)
>
> number (cells, population)
>
> mortality
>
> immobilisation
>
> reproduction
>
> hatching (rate, time, percentage)
>
> sex ratio
>
> development (egg, embryo, life stage)
>
> malformations (teratogenicity)
>
> proliferation (cells)
>
> filtration rate
>
> carbon uptake (algae)
>
> reburial (of e.g. certain crustacean species)

This list is not exhaustive. In general, only those endpoints that have consequences at the population level of the test species are considered relevant (see main guidance). Toxicity test results based on endpoints of whose relationship to effects at the population level is uncertain are not included in the toxicity data tables. Some examples of endpoints where effects at population level are unclear include:

> blood or plasma protein levels
>
> histopathological endpoints (depending on evidence level)
>
> organ weights (e.g. hepatosomatic index, gonadosomatic index)
>
> mRNA induction
>
> endpoints determined *in vitro* tests
>
> behavioural responses (e.g. swimming behaviour, antenna motility, etc.)
>
> coloration

However, it should be noted that these endpoints might be reconsidered when a definite correlation or causal relationship with population sustainability can be established.

A1.3.3.15. Value

Unit: $mg \cdot L^{-1}$, $\mu g \cdot L^{-1}$.

The unit in which the results of toxicity tests are expressed is optional. For reasons of comparison and to avoid errors, the same unit is used throughout all aquatic toxicity data tables in one report. In general, values are expressed in two or three digits. At most, four significant digits are reported. However, further calculation with these data may be necessary: averaging, dividing the values by an AF, use of the results in species sensitivity distributions (SSDs), etc.

Toxicity data for metal compounds are always expressed in quantities of the cation, not the salt. For example, a test performed with $CoSO_4 \cdot 7H_2O$ is expressed as Co^{2+}. Test results are

recalculated if necessary. A similar approach is followed for all charged substances with a non-toxic counterion.

A1.3.3.16. Validity

This column contains a number (1, 2, 3 or 4) indicating the quality of the study. Section A1.3.2.1 describes the background of the quality scoring system.

A1.3.3.17. Notes

This column contains references to footnotes that are listed below the toxicity data tables. Numbers are used to refer to footnotes.

A1.3.3.18. Reference

The reference to the study from which data are tabulated has the following format:

1 author	Bringmann, 1956
2 authors	Bringmann and Kühn, 1976
3 or more authors	Bringmann *et al.*, 1977

If two or more studies have the same citation, distinguish between the different studies by adding a character to the year, e.g. 1980a. All cited references are listed in a reference list.

A1.3.4. Sediment toxicity data tables

The following subsections (Sections 3.4.1 to 3.4.18) discuss the parameters that are reported in the toxicity data tables on acute and chronic toxicity data for benthic species. The parameters are treated in the same order as they appear in the default toxicity data table. The following subsections have titles identical to the column headings in the data tables.

A1.3.4.1 Species

See Section A1.3.3.1. for guidance on reporting data on species.

A1.3.4.2. Test organism information

See Section A1.3.3.2.

A1.3.4.3. Sediment type

In this column, list the sediment type: e.g. fine sandy or organic rich, muddy.

A1.3.4.4. Chemical analysis

See Section A1.3.3.3.

A1.3.4.5. Test compound

See Section A1.3.3.5.

A1.3.4.6. Purity

See Section A1.3.3.6.

A1.3.4.7. pH

Report the pH or the range of pH values, of the test sediment in this column.

A1.3.4.8. Organic carbon

Unit: %

In this column the weight percentage of organic carbon in the sediment is reported. When the percentage organic matter (om) is given, recalculation to percentage organic carbon (oc) is necessary according to Eq. 2:

$$\% \; om = 1.7 \times \% \; oc \qquad (2)$$

This is the general conversion between organic matter and organic carbon used throughout the whole process of deriving EQSs. The value of 1.7 is derived from the TGD (based on standard soil in the TGD containing 2% oc or 3.4% om).

A1.3.4.9. Temperature

See Section A1.3.3.9.

A1.3.4.10. Exposure time

See Section A1.3.3.12.

A1.3.4.11. Summary statstic

Extensive information on the summary statistics is given in Section A1.3.3.13. ECx data are treated in the same way as ECx data for aquatic species.

A1.3.4.12. Test endpoint

See Section A1.3.3.14.

A1.3.4.13. Result for test sediment

Unit: mg·kg^{-1}, µg·kg^{-1}

The unit in which the results of toxicity tests are expressed is optional. For reasons of comparison and to avoid errors, the same unit is used for all benthic toxicity data tables. This column shows the result as obtained in the experiment, expressed in weight per kg dry weight of the test sediment (i.e. *not* recalculated to standard sediment). For further guidance, see Section A1.3.3.15.

A1.3.4.14. Result for standard sediment

Unit: mg·kg^{-1}, µg·kg^{-1}

The unit in which the results of toxicity tests are expressed is optional. For reasons of comparison and to avoid errors, the same unit is used for all benthic toxicity data tables. This column shows the result recalculated into weight per kg of standard sediment (dry weight).

The bioavailability of compounds in sediment is influenced by properties like organic matter content, pH, etc. The variation in these parameters hamper direct comparison of toxicity results obtained for the same substance in different sediments. To make results from toxicity tests conducted in different sediments more comparable, results should be normalised using relationships that describe the bioavailability of the compound in sediment. Results are converted

into a standard sediment, defined as having an organic carbon content of 5% (w/w, see Section A1.3.4.8).

Organic compounds

For non-ionic organic compounds, it is assumed that bioavailability is determined by organic matter content only.

Recalculation to standard sediment is possible with the software program EUSES (European Union System for the Evaluation of Substances; European Commission, 2004).

Metals

In general, toxicity data for metals should not be normalised to a standard sediment. For EQS derivation, all reliable toxicity results with metals to benthic organisms are grouped in the appropriate data table without normalisation.

A1.3.4.15. Validity

This column contains a number (1, 2, 3 or 4), indicating the quality of the study summarised. Section A1.3.2.1 describes the background of the quality scoring system.

A1.3.4.16. Notes

See Section A1.3.3.17.

A1.3.4.17. Reference

See Section A1.3.3.18.

A1.3.5. Bird and mammal toxicity data tables

When secondary poisoning is assessed, results from toxicity studies with birds and mammals are tabulated in separate tables. Data on bioconcentration and biomagnification should be collected as well. For information on the collection of these parameters, see Section A1.4. An expert on human toxicology should be consulted when interpretation of toxicity tests with mammals is complex, e.g. multiple dosing.

A1.3.5.1. Species

See Section A1.3.3.1.

A1.3.5.2. Test organism information

See Section A1.3.3.2. If the body weight of the test species is reported, it should be entered in this column. Body weight is important for estimating the daily energy expenditure (DEE) of an organism, which is required for deriving a biota QS based on secondary poisoning (specifically the calculation of the energy-normalised diet concentration (Section 4.4.5)).

A1.3.5.3. Product or substance

Toxicity studies on birds or mammals may also be carried out with formulations or products rather than individual substances. Report the name of the substance, product of formulation that has been used in this column.

A1.3.5.4. Purity or active ingredient content

In the case that a product (or formulation) is tested, report the content of active ingredient (a.i.) present in the product, expressed in %. If the purity of the active ingredient (used in formulation) is also known, report this in a footnote.

If a single substance has been applied in the test, report the purity of the tested compound in this column.

A1.3.5.5. Application route

Relevant are those toxicity tests in which the animals are dosed orally. This might be achieved via a direct method (intubation, gavage) or by dosing via the food or water.

A short list of application routes is given below:

- intubation or gavage
- capsule
- diet
- water or feeding solution

A1.3.5.6. Vehicle

A carrier used to dose the test substance to the test animals (e.g. corn oil) is reported here.

A1.3.5.7. Test duration

The value in this column reports the total duration of the test. The abbreviations listed in Table 19 can be used. This column should also be filled in when the test duration is equal to the exposure duration. The test duration might be longer than the exposure time, which is reported in the next column (Exposure time). For example, in the acute avian dietary toxicity test, in which the exposure lasts 5 days, but the minimal recommended test duration is 8 days.

A1.3.5.8. Exposure time

The duration of exposure to the toxicant in the toxicity experiment is expressed in this column. The abbreviations listed in Table 19 can be used.

A1.3.5.9. Summary statistics

Short term toxicity tests will either yield an LC50 ($mg \cdot kg_{food}^{-1}$) or an LD50 ($mg \cdot kg_{bw}^{-1} \cdot d^{-1}$ in the case of repetitive dosing). Long-term toxicity tests will generally result in a NOEC (no observed effect concentration in diet; $mg \cdot kg_{food}^{-1}$), or a NOEL (no observed effect level in a dosing study; $mg \cdot kg_{bw}^{-1} \cdot d^{-1}$). Results from long-term toxicity tests may also be reported as a NOAEL (no observed adverse effect level), which is the no observed adverse effect level. However, the effects generally observed for the derivation of the NOEC/NOEL are adverse on the organisms.

A1.3.5.10. Test endpoint

The toxicological parameter for which the test result is obtained is tabulated here. Screening for clinical parameters at haematological, histopathological or biochemical level is common in these types of tests. However, secondary poisoning only aims at taking into account effects at the population level.

The list below shows only some of the relevant endpoints:

body weight

egg production

eggshell thickness

hatchability

hatchling survival

mortality

reproduction (e.g. litter size, teratogenic effect, malformation, gestation duration…)

viability (percentage of viable embryos per total number of eggs)

A1.3.5.11. Value from repetitive oral dosing studies

Unit: $mg \cdot kg_{bw}^{-1} \cdot d^{-1}$.

See also Sections 4.4.5 and A1.3.7.2 for guidance on data handling.

From short term toxicity experiments with repetitive dosing on consecutive days (5 d LD50 for birds) and long-term oral dosing studies, a value expressed in $mg.kg_{bw}^{-1}.d^{-1}$ is obtained. The results from such studies (*viz.* LD50 and NO(A)EL) are reported in this column.

A1.3.5.12. Value from diet studies

Unit: $mg.kg_{food}^{-1}$.

See also Section 4.4.5 for guidance on data handling.

The results of toxicity tests in which the substance of interest is administered via the food are expressed in $mg.kg_{food}^{-1}$. The results of dietary studies (*viz.* LC50 or NOEC values) are reported in this column.

A1.3.5.13. Validity

This column contains a number (1, 2, 3 or 4), indicating the quality of the study summarised. Section A1.3.2.1 describes the background of the quality scoring system.

A1.3.5.14. Notes

See Section A1.3.3.17.

A1.3.5.15. Reference

See Section A1.3.3.18.

A1.3.6. Data selection

A1.3.6.1. Aquatic compartment

One value per species and endpoint is selected for use in the assessment. Where multiple data are available for the same species/endpoint, individual toxicity data may be aggregated using the same principles as those in Chapter R.10 of the REACH Guidance (ECHA, 2008):

1. Identify particularly sensitive species and/or endpoints that may be lost upon averaging data to single values.
2. Investigate multiple values for the same endpoint on a case-by-case basis and seek to explain differences between results.

3. Where valid data show high variation that can be explained, grouping of data is considered, e.g. by pH ranges. If an effect of test conditions is expected to be the cause of variation in toxicity values (hardness of test water, life stage of the test animal, etc.), averaging of data per species should not be performed.

4. Data used for EQS derivation should be selected on the relevance of test conditions (pH, hardness, etc.) to the field.

5. If the variation in test results of different life stages of a test animal is such that averaging data would cause significant underprotection of sensitive life stages, only the data for the most sensitive life stage should be selected. In other words, it is important that sensitive life stages are protected.

6. Calculate the geometric mean of multiple comparable toxicity values for the same species and the same endpoint. This applies to both acute and chronic data.

7. If multiple toxicity values or geometric means for different endpoints are available for one species, the most-sensitive endpoint is selected as long as it is relevant to population sustainability. If multiple valid toxicity data for one species are left that cannot be averaged, the *lowest* value is selected.

> Example: There are values (of NOECs or EC10 values) for three different endpoints, derived from several chronic studies with *Daphnia magna.* The geometric mean of NOECs for reproduction is 0.49 mg·L^{-1}, the geometric mean of NOECs for mortality = 3.1 mg·L^{-1} and there is a single EC10 value for growth of 0.67 mg·L^{-1}. The geometric mean value of 0.49 mg·L^{-1} for reproduction is selected for use in EQS derivation.

8. If differences in the chemical form of the test compound (congeners, stereoisomers, etc.) are the cause of variation in toxicity values for a test species, data should not be averaged. In these cases, the *lowest* reliable toxicity datum is selected and separate EQSs should be derived for each chemical form.

9. Particular steps have been developed for metals to account for variations in the toxicity of different metal species. These are explained in Section 4 of the main guidance.

10. Limitations of toxicity data should be explained, for example, when toxicity results are not valid at low pH. Explanation for these types of limitations should be reported in the datasheet in the section dealing with key assumptions and uncertainties.

A1.3.7. Data treatment

A1.3.7.1. Combining freshwater and marine datasets for EQS derivation

1. To derive EQSs for transitional, coastal and territorial waters, toxicity datasets of marine and freshwater species are normally combined because current marine risk assessment practice suggests a reasonable correlation between ecotoxicological responses of freshwater and saltwater biota (i.e. the same datasets can be used interchangeably for freshwater and saltwater effects assessment). Where this is not justified based on the available evidence (i.e. there is a clear difference in the sensitivity of the freshwater and saltwater biota), EQSs for inland surface waters and transitional, coastal and territorial waters must be derived on the basis of distinct datasets for freshwater and marine organisms. Toxicity data for freshwater organisms and marine organisms are combined *before* EQS derivation for the aquatic compartments. If there are doubts as to whether organisms from both environments show similar sensitivity, differences may be tested in the following way: All freshwater data that are

going to be used for EQS derivation are collected (note: this dataset contains one toxicity value per species, see Section A.1.3.6.1) and the $\log_{10}$ value of each of these toxicity values is calculated.

2. Repeat the above step for all marine toxicity data.
3. Test whether the two log-transformed datasets have equal or unequal variances using an *F*-test. Perform the test at a significance level (α) of 0.05.
4. A two-tailed *t*-test, with or without correction for unequal variances as determined in point 3, is performed to test for differences between the datasets. Perform the test at a significance level (α) of 0.05.
5. When using a statistical test, be aware of some confounders. For example: (i) a specific group of organisms might be more sensitive than other organisms; (ii) over-representation of data from one study or species from a specific taxonomic group in one of the two datasets might cause bias. Results of a *t*-test become increasingly meaningful with increasing sample size.

If the null hypothesis is supported, the datasets may be combined. This procedure must not be applied to metals. For metals, the freshwater and saltwater datasets must always be kept separate.

A1.3.7.2. Conversion of data on birds and mammals

Section 4.4 provides guidance on the derivation of QSs covering secondary poisoning of wildlife. This allows for differences in the energy content of prey items in the field and the diets provided in laboratory studies. In this methodology, toxicity data expressed as dietary concentrations or dose rates are converted to energy-normalised concentrations expressed as mg/kJ. If the available data do not allow for the calculation of energy-based diet concentrations, the methodology set out below should be followed.

For each of the selected avian or mammalian toxicity studies, the test result is expressed as a $NOEC_{oral}$ in mg/kg food. If the test result is expressed as a dose (e.g. in mg/kg bw/day) and it is not possible to convert to a dietary concentration (using the reported daily food intake and body weight), the following equations (equations 3 and 4) should be used, in conjunction with the default conversion factors (CONV) from Table 21. For species other than those listed in Table 21, a conversion factor should be selected that is based on similarity to the feeding characteristics of one of the listed species.

$$NOEC_{bird} = NOAEL_{bird} \cdot CONV_{bird} \qquad (3)$$

$$NOEC_{mammal,\,food\_chr} = NOAEL_{mammal,\,oral\_chr} \cdot CONV_{mammal} \qquad (4)$$

Table 21 Conversion factors from NOAEL into NOEC for several species

Species	Common name	Conversion factor (bw·DFI^{-1}) (ECHA, 2008)
Canis domesticus	Dog	40
Macaca sp.	Macaque species(monkey)	20
Microtus spp.	Vole species	8.3
Mus musculus	House mouse	8.3
Oryctolagus cuniculus	European rabbit	33.3
Rattus norvegicus (>6 weeks)	Brown rat	20
Rattus norvegicus (≤ 6 weeks)	Brown rat	10
Gallus domesticus	Chicken	8

bw = body weight (g); DFI = daily food intake (g·d^{-1}).

A1.4. BIOCONCENTRATION AND BIOMAGNIFICATION DATA

A1.4.1. Data collection

The literature should be searched for bioconcentration (BCF), bioaccumulation (BAF) and biomagnification (BMF or TMF) factors if a biota EQS is triggered (see Section 2 of the main guidance). Useful data sources for BCF values are the physicochemical properties and environmental fate handbook (Mackay *et al.*, 2006) and ECOTOX (US EPA, 2007a). The BCF, BAF, BMFand TMF data should be tabulated separately.

A1.4.2. Data evaluation and data tables

In principle, the evaluation of bioaccumulation data follows the evaluation for toxicity. All retrieved literature is read and evaluated with respect to its relevance and reliability. The most relevant BCF studies are those performed with fish, but studies performed with molluscs are important for secondary poisoning as well. The BCF data for other species should be carefully checked because they are prone to experimental errors, e.g. accumulation may not reflect uptake, but adsorption to the outside of the organism. For this reason, BCF values for algae are rarely reliable. A reliable BCF study should be similar in experimental set-up to the updated OECD guideline 305 (OECD, 2012)). At least the concentration of the (parent) compound in the aqueous phase, and in fish, has to be measured at several time points. No specific guidance is available for BAF, BMF and TMF values, which are mostly derived from field studies. Apart from the analysis, a reliable field study requires that all the prey and predator species and water samples originate from the same area and from the same period in time. After evaluating a study, the results of the study are summarised by entering it into the appropriate data table.

A1.4.3. Bioaccumulation data tables

The following subsections discuss the parameters that are to be reported in the bioaccumulation data tables. The parameters are treated in the same order as they appear in the default bioaccumulation data table. In the following sections, it is assumed that fish are the test organism most frequently encountered in bioaccumulation studies. However, studies with molluscs and other species may also be found. These data are relevant, as the food chain water → mollusc (→ fish) → mollusc/fish-eating bird or mammal is also important.

A1.4.3.1. Species

See Section A1.3.3.1.

A1.4.3.2. Test organism information

In addition to properties such as age, weight and length (Section A1.3.3.2), two other parameters are also very important. These are trophic level (for field studies) and lipid content (in the case of lipophilic organic chemicals) or dry weight content (for other substances, such as metals). These parameters are used to normalise the data (to either lipid content or dry weight). If trophic level is not reported, it might be possible to estimate it from any stable isotope analyses of the biota samples.

A1.4.3.3. Test substance

Clearly report what compound is used. If a radiolabelled compound is used in a bioconcentration study, it should be reported in this column of the bioaccumulation data table. For organic compounds that have one or more isomers, the specific isomer (or mixture of isomers) used in the test is reported, e.g. diastereomers, *cis*/*trans* conformation, *o*, *m*, *p* substitution, formulations, etc.

A1.4.3.4. Substance purity

See Section A1.3.3.6.

A1.4.3.5. Chemical analysis

A column in the bioaccumulation data table is included that gives information on the analysis of the aqueous phase/biological matrix. However, as the determination of the water and biota concentration is a prerequisite of any good BCF study, this column should give information on how the concentration is determined, e.g. GC-FID or GC-MS (gas chromatography coupled to a flame ionisation detector or a mass spectrometer, respectively) and HPLC-UV (high-performance liquid chromatography). Where a radiotracer is used in a bioconcentration study, the method of detection is important. Liquid scintillation counting (LSC) measures total radioactivity, including the parent compound and metabolites. HPLC used in combination with radiodetection can be used to resolve only the parent compound.

A1.4.3.6. Test type

See Section A1.3.3.4.

A1.4.3.7. Test water

See Section A1.3.3.7.

A1.4.3.8. pH

See Section A1.3.3.8.

A1.4.3.9. Hardness/Salinity

See Sections A1.3.3.10 and A1.3.3.11.

A1.4.3.10. Temperature

See Section A1.3.3.9.

A1.4.3.11. Exposure time

In this column, the times of the uptake phase and, if carried out, the depuration phase are listed. If both phases are determined, the exposure time and depuration time are listed as two separate time spans: e.g. 14 + 14 d. These columns refer to laboratory conditions, so they are not applicable to field studies.

A1.4.3.12. Time of sampling

For field-derived parameters, samples should be taken in the same period. Therefore, sampling times for water and biota should be recorded.

A1.4.3.13 Sampling area

For field studies giving rise to BAFs and BMFs, samples of biota and water should be taken from the same location, which should be documented.

A1.4.3.14 Exposure concentration

The concentration at which the bioaccumulation study is performed is given in this column table. This is important because guidelines require that the concentration meets some conditions. For example, according to the OECD guideline 305 (OECD, 1996), the highest aqueous concentration should be about one hundredth of the acute LC50 or the acute LC50 divided by an appropriate acute-to-chronic ratio, while the lowest concentration should preferably be a factor of ten below the highest concentration, but at least ten times above the limit of detection in the aqueous phase. As explained in the main guidance (Section 2), the exposure concentration can have a major influence on BCF values.

For BAF values, the exposure concentration is equally important because it sis the basis of the BAF calculation. By tabulating the exposure concentrations, any particularly low or high values can be more easily detected. It also helps identify any evidence of concentration dependency of the BAF.

A1.4.3.15. Bioaccumulation

Unit: $L \cdot kg_{ww}^{-1}$ (BCF, BAF), kg_{ww}/ kg_{ww} (BMF, TMF) or kg_{lw}/ kg_{lw} or kg_{dw}/ kg_{dw} in case normalised organism concentrations are available.

Here, the value of the BCF, BAF, BMF or TMF is denoted. The basis for the BCF or BAF value is the ratio of the concentration in wet weight (ww) of the organism, mostly fish, divided by the water concentration. The unit of the BCF and BAF is $L \cdot kg_{ww}^{-1}$; if the BCF is normalised to dry weight or lipid weight, this should be explicitly indicated with a note describing the origin of the value. If it is possible to normalise the data, BCF and BAF values should also be given for lipid or dry weight content (Section 4.4.2). These values should be used for triggering and calculating the routes of secondary poisoning and human consumption of fishery products. The EQS derivation is dependent on the available studies. In older BCF studies, fat content is often not reported. It is preferable to include such studies because, otherwise, risks to predators and humans may be overlooked.

A1.4.3.16. Biological matrix

In this column in the table, it is reported what part of the organism the BCF has been determined for. Possibilities are, for example, whole fish ww, whole fish dw, edible parts, non-edible parts viscera, etc.

For BMF, two different parts of the body of prey and predator may be monitored. These may have different accumulation characteristics, so attention should be given to the reliability of any resulting BMF, especially if the data have not been normalised to lipid or dry weight content.

A1.4.3.17. Method

The method used to calculate the bioaccumulation value is reported in this column. Basically, the method can be based on equilibrium concentrations or on kinetics, including the uptake and depuration rate constants (k_1 and k_2). With equilibrium concentrations (noted as equilibrium), the BCF is determined as the quotient of the concentrations in organisms, mostly fish, and water at equilibrium. When the kinetic constants (k_1/k_2) are used to calculate the BCF, the BCF is calculated as the quotient of uptake rate (k_1) and depuration rate (k_2), mostly determined independently during an uptake and a depuration phase (k_1, k_2 independent). However, in some studies, k_2 is first determined from the depuration phase and k_1 estimated from the data of the uptake phase, with this value of k_2 implied to take the non-linearity of the uptake into account (k_1 implied by fitted k_2). A further possibility is that k_1 and k_2 are fitted simultaneously by a non-linear regression model.

The latter method is preferred because it takes all the data into account. Fitting should be performed on both un-transformed and transformed data and any effect of transformation should be reported. If the method cannot be described easily, a footnote to the table can be entered.

A1.4.3.18. Notes

Additional notes may include information on the analysis, the basis of the BCF value (dry weight or lipid weight) or the method used to determine the BCF.

A1.4.3.19. Reference

See Section A1.3.3.18.

A1.4.4. Data selection

A1.4.4.1. BCF – experimental data

Aquatic compartment

From the valid studies calculate the geometric mean values per species. If geometric mean BCF values are available for several species, the geometric mean per taxon is calculated. The values for fish and mussels are used for comparison with the trigger values and listed in the summary table.

A1.4.4.2. BCF – calculation method

Aquatic compartment

When a BCF cannot be derived on the basis of experimental data, a BCF may be calculated as described below for substances whose log K_{ow} value is ≥2.

For substances with a log K_{ow} of 2–6, the following linear relationship (Eq. 5), as developed by Veith *et al.* (1979), can be used:

$$\log BCF_{fish} = 0.85 \times \log K_{ow} - 0.70 \quad (5)$$

For substances with a log K_{ow} higher than 6, a parabolic equation can be used (Eq. 6):

$$\log BCF_{fish} = -0.20 \times \log K_{ow}{}^2 + 2.74 \times \log K_{ow} - 4.72 \qquad (6)$$

Because of experimental difficulties in determining BCF values for such substances, this mathematical relationship has a higher degree of uncertainty than the linear one (Eq. 5). Both relationships apply to compounds with a molecular weight of less than 700. For a discussion on both relationships see REACH R.7c (p. 19-21) (ECHA, 2008).

A1.4.4.3. BMF – experimental data

Experimental BMF and TMF values generally originate from field studies. Laboratory derived BMF values derived according to the OECD 305 test guideline cannot be used for this purpose, because these were derived in the absence of simultaneous aqueous exposure. Due to the fact that field studies are non-standard by nature, calculating a geometric mean BMF might not be justified and a value might be selected based on expert judgement. Additional information from BAF-studies may be used to select a BMF that together with the BCF would cover the BAF-values encountered in the field (see e.g. motivation in Moermond and Verbruggen 2013). This final BMF is, complimentarily to the BCF and BAF, used for comparison with the trigger values and listed in the summary table (see Section 2.4.3).

The most relevant values for BMF are those for biomagnification from small into larger fish (either fresh or marine water). These larger fish then serve as food for predators such as otters and herons, or seals in the marine environment. As fish at trophic level 4 is 3 three levels above the trophic level that is in equilibrium with the water phase, BMF_{fish} should thus include three trophic magnification steps. Such a BMF does not represent a single predator-prey relationship. Three trophic levels will not be included in a BMF from fish to fish. If no reliable estimate of the BAF at trophic level 4 can be generated, an alternative might be to use the trophic magnification factor instead of a BMF. To account for magnification over several trophic levels, the value $TMF^{number\ of\ trophic\ levels}$ could be used (see Section 4.6). Data for biomagnification from other small species such as crustaceans to fish might be useful as well, but care must be taken that in the further assessment of secondary poisoning, BCF and BMF values are consistent.

Another group of prey that might be relevant to the route of secondary poisoning are mussels. If mussels are directly consumed by birds or mammals, and a BCF value for mussels is available, a biomagnification step would be absent. However, there are also several common fish species that feed on mussels. In such a case BMF data on accumulation from mussels to fish would be relevant.

For the marine environment a further biomagnification step is considered by introducing the $BMF_{b/w}$ value. This step refers to the biomagnification from fish to small mammals and birds. For the marine environment, a good example is the biomagnification from fish to seals. The latter species then serve as prey for top predators such as polar bears and killer whales. Besides data for the marine environment, other data for biomagnification from fish to fish-eating birds and mammals should be considered as well.

A1.4.4.4. BMF – calculation method

When a BMF cannot be derived on the basis of experimental data, a BMF may be estimated using log K_{ow} data as described in Table 22. In this table, BMF_{fish} is a value for the biomagnification in the prey of predators for the freshwater environment. For the marine environment, an additional biomagnification step is included, which is reflected in the $BMF_{b/m}$ value. This $BMF_{b/m}$ is a value for biomagnification in the prey of top predators.

Table 22 Default BMF values for organic substances.

log K_{ow} of substance	BCF (fish)	BMF$_{fish}$	BMF$_{b/m}$
<4.5	<2000	1	1
4.5–<5	2000–5000	2	2
5–8	>5000	10	10
>8–9	2000–5000	3	3
>9	<2000	1	1

The second column of this table shows (ranges of) BCF values. These values are meant to help select default BMF values if experimental BCF data are available.

The programme BCFBAF within the EPISuite 4.11 could also be used to estimate BMF/TMF values for hydrophobic substances in the pelagic environment. This could be done by comparing the BAF values calculated at different trophic levels after lipid normalisation of the BAF (lipid contents are 10.7%, 6.85% and 5.98% for the upper, middle and lower trophic levels, respectively).

A1.4.4.5. BAF – Experimental data

The derivation of standards for secondary poisoning and human health should be based on a comprehensive evaluation of BCF, BMF and BAF-values. In general, preference is given to the use of BAFs instead of using the product of BCF and BMF, because the BAF is based on field samples and includes all possible uptake routes (Moermond and Verbruggen 2013). For a valid BAF, however, insight into the corresponding concentrations in water is needed and the BAF should be valid for the appropriate trophic level. This can for example be done by a regression of BAF values as a function as trophic level. Depending on the type and validity of information, it sometimes may be more appropriate to rely on the combination of BCF and BMF (e.g. if aqueous concentrations in the field are uncertain). In such case it could aslo be considered to use the BAF values for hydrophobic substances calculated by the programme BCFBAF within the EPISuite 4.11 after normalisation for lipid content (lipid contents are 10.7%, 6.85% and 5.98% for the upper, middle and lower trophic levels, respectively).

A1.5. TOXICOLOGICAL DATA FOR THE PROTECTION OF HUMANS

A1.5.1. Threshold limits

A human toxicological threshold may be needed for EQS derivation in two cases:

− in the derivation of the QS$_{hh\ food,water}$ (consumption of fishery products)
− in the derivation of the QS$_{dw,water}$ (drinking water)

The human toxicological thresholds that can be used are the ADI (acceptable daily intake) and TDI (tolerable daily intake). The US ATSDR uses the term MRL (minimum risk level) while the US EPA uses the term RfD (reference dose). The basis for the human-toxicological threshold levels is in principle a NO(A)EL from a mammalian toxicity study, which is useful if established threshold levels are unavailable. However, the NOAEL is not a human toxicological threshold limit and an AF (typically 100) must be used. To derive a TDI or ADI from a NOAEL a human toxicologist should be consulted.

Effect data are the relevant NOAEL, ADI, TDI values identified in the human health section of risk assessments according to Council Regulation (EEC) No. 793/93 or Council Directive 91/414/EEC (or its successor regulation 1107/2009). The ADI or TDI values adopted by international bodies such as the World Health Organization may also be used. Where a threshold level cannot be given, unit risk values corresponding to an additional risk of, for example, cancer over the whole life of 10^{-6} (one additional cancer incident in 10^6 persons taking up the substance concerned for 70 years) may be used, if available.

A list of organisations or frameworks that have published human toxicological threshold limits is presented in Table 23 (extracted from Hansler *et al.*, 2006). In general, it is advised to take the most recent value and consult a human toxicologist on the final choice of the value. If a clear value is reported in a European risk assessment report, this should be used.

Table 23 Sources for the retrieval of human toxicological threshold limits

Source name and publisher	Available at
HSDB (NLM/NIH)	http://toxnet.nlm.nih.gov/
ATSDR Toxicological Profiles (ATSDR)	http://www.atsdr.cdc.gov/mrls/index.html (MRLs) http://www.atsdr.cdc.gov/mrllist_12_05.pdf
CEPA Priority Substances Assessments (Environment- & Health-Canada)	http://www.cen-rce.org/eng/projects/cepa/
CICAD (IPCS)	http://www.inchem.org/pages/cicads.html
EHC (WHO/IPCS)	http://www.inchem.org/pages/ehc.html
ESIS (ECB)	http://ecb.jrc.it/esis/
HSG (WHO)	http://www.inchem.org/pages/hsg.html
IARC Monographs (WHO)	http://monographs.iarc.fr http://www.inchem.org/pages/iarc.html
ICSC (IPCS-EU)	http://www.inchem.org/pages/icsc.html
IRIS (US-EPA)	http://cfpub.epa.gov/ncea/iris/index.cfm
JECFA Monographs (WHO/FAO)	http://www.inchem.org/pages/jecfa.html
JMPR Monographs (WHO/FAO)	http://www.inchem.org/pages/jmpr.html
WHO/FAO (pesticides)	http://www.fao.org/docrep/W3727E/w3727e00.HTM
MPC_{human} values for the derivation of SRC_{human}	http://www.rivm.nl/bibliotheek/rapporten/711701025.pdf

Source name and publisher	Available at
NTP (NIH-NIEHS)	http://ntp-server.niehs.nih.gov/
OEHHA Toxicity Criteria Database (Cal-EPA)	http://www.oehha.org/risk/chemicalDB/index.asp
SIDS (OECD-UNEP)	http://www.chem.unep.ch/irptc/sids/OECDSIDS/sidspub.html
TERA (TERA)	http://www.tera.org/ITER
DWQG (WHO)	http://www.who.int/water_sanitation_health/dwq/guidelines/en/
Umwelt-Online	http://www.umwelt-online.de/recht/gefstoff/g_stoffe/adi.htm

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A1.7. ABBREVIATIONS, VARIABLES AND DEFAULT VALUES

ACD	Advanced Chemistry Development
ADI	acceptable daily intake
AF	assessment factor
ag	analytical grade
a.i.	active ingredient
am	artificial medium
ATSDR	Agency for Toxic Substances and Disease Registry
BAF	bioamplification factor
BCF	bioconcentration factor
BMF	biomagnification factor
BMF_{fish}	biomagnification factor from the bottom of the food chain into trophic level 4 fish
$BMF_{b/m}$	biomagnification factor from fish, or other aquatic organisms, to birds or mammals

bw	body weight (in kg)
CAS	Chemical Abstracts Service
CEPA	Canadian Environmental Protection Act
CF	continuous flow system
CICAD	concise international chemical assessment document
ClogP	log octanol/water partitioning coefficient, calculated by software program BioLoom
d	days
DEE	daily energy expenditure (in kJ/d)
DFI	daily food intake
dw	de-ionised water, dechlorinated water or distilled water
	dry weight
DWQG	drinking-water quality guidelines
EC	effect concentration
	European Commission
ECHA	European Chemicals Agency
ECB	European Chemicals Bureau
ECx	effect concentration at which an effect of x% is observed, generally EC10 and EC50 are calculated
EEC	European Economic Community (replaced by EU)
EHC	environmental health criteria
EINECS	European inventory of existing commercial chemical substances
ELS	early life stage
EPA	Environmental Protection Agency
EPI	estimation programs interface
EPICS	equilibrium partitioning in closed systems
EqP	equilibrium partitioning
EQS	environmental quality standard
ESIS	European Chemical Substances Information System
EU	European Union
EUSES	European Union System for the Evaluation of Substances
F	flow-through system
FAO	food and agriculture organisation
FETAX	frog embryo teratogenesis assay *Xenopus*

GC	gas chromatography
GC-MS	gas chromatography–mass spectrometry
GC-FID	gas chromatography–flame ionisation detection
GLP	good laboratory practice
h	hours
HPLC	high-performance liquid chromatography
HSDB	hazardous substances databank
HSG	health and Agency for Research on Cancer
ICSC	international chemical safety cards
IF	intermittent flow system
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
Koc	organic carbon adsorption coefficient
Kow	octanol/water partition coefficient
LCx	effect concentration at which x% lethality is observed, generally LC50 and LC10 are calculated
LD50	dose that is lethal to 50% of the tested animals
lg	laboratory grade
LSC	liquid scintillation counting
LOEC	lowest observed effect concentration
MATC	maximum acceptable toxicant concentration
MCI	molecular connectivity indices
MlogP	log octanol/water partitioning coefficient, measured value selected by software program BioLoom
min	minutes
mo	months
MPC	maximum permissible concentration
MRL	minimum risk level

mRNA	messenger ribonucleic acid
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NOAEL	no observed adverse effect level (usually corresponds to about 10% effect)
NOEC	no observed effect concentration (usually corresponds to about 10% effect)
NOEL	no observed effect level (usually corresponds to about 10% effect)
NTP	National Toxicology Program (United States)
nw	natural water, such as lake water, river water, sea water, well water
oc	organic carbon
OECD	Organisation for Economic Co-operation and Development
OEHHA	office of environmental health hazard assessment
om	organic matter
OPPTS	office of prevention, pesticides and toxic substances
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
ppt	parts per thousand or parts per trillion
psu	practical salinity unit
QS	quality standard
QSAR	quantitative structure–activity relationship
QSPR	quantitative structure property relationship
R	renewal system
RfD	reference dose
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
SIDS	screening information dataset
SMILES	simplified molecular input line entry system
sp.	species
SPARC	SPARC performs automatic reasoning in chemistry

SRC$_{human}$	human toxicological serious risk concentration
susp	suspended particulate matter
SSD	species sensitivity distribution
TDI	tolerable daily intake
TERA	toxicology excellence for risk assessment
tg	technical grade
TGD	Technical Guidance Document
TL	trophic level
TLhh	threshold level, human health
TLm	median tolerance limit; also encountered as median threshold limit
TMF	trophic magnification factor
tw	tap water
UNEP	United Nations Environment Programme
US	United States
UV	ultraviolet
w	weeks
WAF	water accommodated fraction
WHO	World Health Organization
ww	wet weight
y	years

List of defaults and variables.

Symbol	Description of variable	Unit	Value
AF	assessment factor	–	1–5
bw	human body weight	kg$_{bw}$	70
Foc$_{standard sediment,TGD}$	fraction of organic carbon in standard sediment as defined in the TGD	kg·kg^{-1}	0.05
Foc$_{susp,TGD}$	weight fraction of organic carbon in suspended matter as defined in the TGD	kg·kg^{-1}	0.1
R	gas constant	Pa·m^3·mol^{-1}·K^{-1}	8.314
TEMP	environmental temperature	K	285

ANNEX TO APPENDIX 1: DATA EVALUATION OF PHYSICOCHEMICAL DATA

1. Evaluation of the vapour pressure for use in EQS derivation

An OECD guideline exists for the experimental determination of the vapour pressure of a compound (OECD guideline 104; OECD, 1995b). In this guideline several methods are discussed, each with its own range of applicability. The following table presents information from the guideline, which specifies what method is suitable for which compound.

Table 24 Domain of applicability of different methods for the determination of vapour pressure.

Method	Suitable for liquids	Suitable for solids	Recommended range
Dynamic method	low melting	yes	10^3-10^5 Pa
Static method	Yes	yes	10-10^5 Pa
Isoteniscope	Yes	yes	10^2-10^5 Pa
Effusion method	Yes	yes	10^{-3}-1 Pa
Gas saturation method	Yes	yes	10^{-5}-10^3 Pa
Spinning rotor method	Yes	yes	10^{-4}-0.5 Pa

In the dynamic method (Cottrell's method), the boiling point of a compound is determined at various pressures between about 10^3 and 10^5 Pa. In the static method, the vapour pressure is determined at one specified temperature by means of a manometer (e.g. 25 ℃). The isoteniscope method is based on the same principle as the static method. In the effusion method the weight loss of the compound is measured. This can be done directly by measuring the mass of the remaining substance or by analysing the volatilised amount by gas chromatography (GC). In the proposed update of guideline 104 (OECD, 2002), isothermal gravimetry is added for the effusion method. The weight loss is then determined at different temperatures and an extrapolation to 20 or 25 ℃ can be made. The range of vapour pressures that can be determined with this method is 10^{-10} to 1 Pa. The gas saturation method makes use of a column containing a carrier material supporting the substance, through which an inert gas is passed. The concentration of the substance in this carrier gas is then determined, usually by gas chromatography (GC). The last method is the spinning rotor method, where the retardation of a spinning ball due to the friction with the gas phase is measured.

In general, the methods that make use of an analysis of the substance, for example, by gas chromatography, are less prone to errors due to impurities than the other methods. The OECD guideline does not mention this explicitly. However, degassing of more volatile compounds prior to the determination of the vapour pressure also enhances the reliability of the determination.

The retention time in gas chromatography can be used to estimate the vapour pressure of a compound. Although this is not a direct determination of the vapour pressure, it generally gives rather accurate results and is applicable to substances with a very low vapour pressure. In addition to this, the vapour pressure can be estimated by the programme MPBPwin, which is incorporated in EPI Suite (US EPA, 2007b). The programme makes use of three estimation methods, which are the Antoine method, the modified Grain method and the Mackay method. All three methods make use of the boiling point for their estimation of the vapour pressure. Also, the melting point of the

compound is a necessary parameter for the estimation. Both boiling and melting point can be estimated by the programme, but experimental values can also be entered if known. For solids, the result of the modified Grain method is presented as the preferred value, while for liquids this is the mean of the Antoine method and the modified Grain method. A value for the vapour pressure can also be estimated by SPARC (Karickhoff *et al.*, 2007), which has a mechanistic thermodynamic basis. In the data tables, both estimated values are reported as well.

2. Henry coefficient

No general accepted guideline exists for the determination of the Henry coefficient. However, several methods exist to determine the Henry coefficient experimentally.

In the batch stripping method, gas is bubbled at a known rate through a solution of the compound in water. The Henry coefficient is calculated with a mass balance from the decrease in the aqueous concentration. The concentration in air is generally not measured. This method works well for fairly volatile compounds with Henry coefficients higher than 2.5 and occasionally down to 0.25 $Pa \cdot m^3 \cdot mol^{-1}$ (Mackay *et al.*, 2000).

One common method, very similar to the batch stripping method, is the gas stripping method in which a gas is bubbled through the aqueous solution and both the aqueous concentration and the gas concentration are determined. The technique was applied to chlorobenzenes, PAHs, and PCBs in a range from 0.018 to 276 $Pa \cdot m^3 \cdot mol^{-1}$ (Ten Hulscher *et al.*, 1992).

A method for highly volatile compounds (i.e. higher than 120 $Pa \cdot m^3 \cdot mol^{-1}$) is the equilibrium partitioning in closed systems (EPICS) method. With this method a known volume of solute in water solution is equilibrated with air in sealed vessels. The headspace air concentrations are measured. The method has a high precision (Mackay *et al.*, 2000). A number of other headspace analysis techniques that are used, are slightly different from the EPICS method, in some techniques not only the headspace but both phases are analysed (Mackay *et al.*, 2000).

A method for less volatile compounds is the wetted-wall method. In this method the solute is equilibrated between a thin flowing film of water and a concurrent air flow in a vertical column. Both phases are measured. The method has been applied to pesticides and other less volatile compounds, but no recommended range is given (Mackay *et al.*, 2000). In the handbook (Mackay *et al.*, 2006), values for polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), and two pesticides are tabulated using this method. The values for PCBs and PAHs range from 0.91 to 74.3 $Pa \cdot m^3 \cdot mol^{-1}$. One of the pesticides (alachlor) has a much lower Henry coefficient of $8.43 \cdot 10^{-4}$ $Pa \cdot m^3 \cdot mol^{-1}$. This is in agreement with the method being suitable for less volatile compounds.

Also the Henry coefficient is sometimes related to retention times (Mackay *et al.*, 2000). However, results obtained using this method should be considered as an estimate. Another estimation that is often used for the Henry coefficient is the quotient of vapour pressure and solubility. This method works quite well for substances that have a solubility of less than 1% in water. The Henry coefficient can also be calculated by a bond contribution method as included in EPI Suite (US EPA, 2007b). These estimated values should be included in the data table.

3. Evaluation of the water solubility for use in EQS derivation

For the experimental determination of the water solubility, an OECD guideline is available (OECD guideline 105; OECD, 1995c), in which two methods are discussed. These methods are the flask method (shake-flask) and the column elution method (generator column). The flask method can be used for compounds with a solubility higher than 10 $mg \cdot L^{-1}$. Below that value, colloid formation will overestimate the true aqueous solubility and in that case the column elution method should be used, which prevents this phenomenon.

Apart from the methods proposed in the OECD guideline, the water solubility of poorly soluble liquid compounds can be accurately determined by means of the slow-stirring method. The reliability of the slow-stirring method applied to liquid substances can be considered as equivalent to that of the column elution method. Only few examples are available of the use of this method for the determination of the solubility, mostly for hydrocarbons and phthalate esters (Tolls *et al.*, 2002; Letinski *et al.*, 2002; Ellington, 1999). This method is often used to prepare saturated solutions of hydrocarbon mixtures (oil products) in water (water accommodated fractions or WAF), by which information on the solubility of a mixture is given (Schluep *et al.*, 2002).

Estimates of the water solubility can be made by two different programmes included in EPI Suite (US EPA, 2007b). These programmes are WSKOWwin, which estimates the solubility from log K_{ow}, and WATERnt, which is a fragment method for water solubility independent of log K_{ow}. Experimental values for log K_{ow} and melting point can be entered in WSKOWwin if available. Otherwise WSKOWwin will use the default values (experimental or calculated) from EPI Suite for these parameters. Another estimation method for the water solubility is the calculation performed by SPARC (Karickhoff *et al.*, 2007), which has a mechanistic thermodynamic basis. These estimated values are reported as well in the data tables.

4. Evaluation of K_{ow} values for use in EQS derivation

Several methods are available for the experimental determination of log K_{ow}. In the OECD guidelines, two methods are available and further there is one draft guideline. The first method is the shake-flask method (OECD guideline 107; OECD, 1995a). This method works well for log K_{ow} values in the range between -2 and 4 (occasionally up to 5), but is impossible to use with surface-active materials. For these materials, a calculated value (using BioLoom; BioByte, 2004) or an estimate based on individual *n*-octanol solubility and water solubility should be provided, preferably in mutually saturated *n*-octanol and water (Sijm *et al.*, 1999; Li and Yalkowsky, 1998a; Li and Yalkowsky, 1998b).

The second method is the HPLC method. Values of log K_{ow} in the range between 0 and 6 can be estimated using high performance liquid chromatography (OECD guideline 117; OECD, 2004). The HPLC method is not applicable to strong acids and bases, metal complexes, surface-active materials or substances which react with the eluent. The HPLC method is less sensitive to the presence of impurities in the test compound than is the shake-flask method. Nevertheless, in some cases impurities can make the interpretation of the results difficult because peak assignment becomes uncertain. For mixtures which give an unresolved band, upper and lower limits of log K_{ow} should be stated.

Before deciding on what procedure to use, a preliminary estimate of the log K_{ow} should be obtained from calculation (see the annex to OECD guideline 117), or where appropriate from the ratio of the solubilities of the test substance in the pure solvents. Still, the HPLC method should be regarded as an estimation method of the log K_{ow}, because it does not directly measure the distribution of a compound between octanol and water.

Another method that determines the distribution of a compound between *n*-octanol and water directly, but whose reach extends beyond the range of the shake-flask method, is the slow-stirring method (draft OECD guideline 123; OECD, 2003). With this method, log K_{ow} values up to 8.2 can be accurately determined, making it suitable for highly hydrophobic compounds. This method prevents the formation of micro droplets of *n*-octanol in the aqueous phase, which results in an overestimation of the water concentration and, consequently, an underestimation of the log K_{ow} value. For the same reason, the shake-flask method can only be used up to log K_{ow} values of around 4 and definitely not higher than 5.

Another method that is not mentioned in OECD guidelines is the generator-column technique. Although this technique is most frequently used for the determination of the water solubility, it is occasionally used for the determination of log K_{ow}. Because the supporting material silica, saturated with *n*-octanol containing the compound, is held in a column, the formation of micro

droplets is excluded. For this reason, the results from this technique can be considered equivalent to results obtained with the slow-stirring method. In general, good correlation exists between the slow-stirring method and the generator-column technique, within the experimental error of both methods. However, only a limited number of studies is available that makes use of this technique, primarily for chlorinated biphenyls and dibenzodioxins (e.g. Tewari *et al.*, 1982; Miller *et al.*, 1984; Doucette and Andren, 1987; Doucette and Andren, 1988; Hawker and Connell, 1988; Shiu *et al.*, 1988; Li and Doucette, 1993; Yeh and Hong, 2002).

Except from experimental determination, log K_{ow} values can also be calculated with a QSAR programme. The log K_{ow} values calculated with ClogP (BioByte, 2004) and EPI Suite (US EPA, 2007b) are always presented for comparison. Both programmes are based on a fragment contribution method. Besides this, SPARC (Karickhoff *et al.*, 2007) is a third estimation programme for the log K_{ow} that is frequently used. This programme is not based on a fragment contribution but has a mechanistic thermodynamic basis.

5. Evaluation of K_{oc} values for use in EQS derivation

The organic carbon normalised partition coefficient (K_{oc}) is calculated or directly retrieved from literature for all valid adsorption studies collected. The sediment type that underlies these partition coefficients is reported in the table. The organic carbon content is also reported. The method to determine the K_{oc} most accurately is the OECD guideline 106 (OECD, 2000). All K_{oc} values that are determined with a method similar to this guideline can be regarded as reliable. However, the TGD also allows K_{oc} values to be derived from field studies or simulation studies. Therefore, whether or not a sorption study is reliable remains subject to expert judgement.

The K_{oc} may also be calculated. Estimation of K_{oc} from K_{ow} is the preferred route, following the QSAR method described in the TGD (cited in the next section). A short description of the use of the method is given after the citation.

Citation from TGD, part III (European Commission (Joint Research Centre), 2003b):

'The models are based on linear regression analysis and log K_{ow} as descriptor variable. It should be noted that all models are developed assuming an equilibrium state. For certain classes of chemicals, e.g. anilines and carbamates, this assumption is not correct, because the sorption to soil is irreversible due to the formation of bonded residues. Improvements of the more specific models are certainly feasible if parameters for more specific interactions are taking into account.

'*Domain*

An extensive description of the domain is given in Table 25[42]. The description is made in terms of chemical structures as well as in terms of log K_{ow} ranges.

'*Accuracy*

The standard errors of the estimates (± 2σ range = 95%)[43] range from 0.35 to 1.0 log units for the different models. The standard errors are indicated in **Error! Reference source not found.**[37] for ach model. A cross-validation has not been performed yet. External validation is not possible, because all available data have been used to generate the models (Sabljić *et al.*, 1995 cited in: European Commission (Joint Research Centre), 2003a).'

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[42] The number of the table refers to that given in this annex and not the table number in the TGD.

[43] For clarification, the standard error is equal to σ.

Table 25 Domain of the sorption models (Sabljić et al., 1995 cited in: European Commission (Joint Research Centre, 2003a)

Model	X-variable domain log K_{ow} in log units	Chemical domain	Substituents or Warnings
Hydrophobics	1–7.5	All chemicals with C, H, F, Cl, Br, and I atoms	
Nonhydrophobics	(−2.0)–8.0	All chemicals that are not classified as hydrophobics	Overestimated *n*-Alkyl Alcohols (0.9 log units) Organic Acids (0.55 log units) Underestimated Amino-PAHs (1–2 log units) Aliphatic Amines (1–2 log units) Alkyl Ureas (1.0–1.5 log units)
Phenols	1.0–5.0	Phenols	Cl, Br, CH_3, OH, NO_2, CH_3O
		Anilines	Cl, Br, CH_3, CF_3, CH_3O, NMe
		Benzonitriles	Chlorinated
		Nitrobenzenes	Cl, Br, NH_2
Agricultural	(−1.0)–8.0	Acetanilides	
		Carbamates	
		Esters	
		Phenylureas	
		Phosphates	
		Triazines	
		Uracils	
Alcohols, acids	(−1.0)–5.0	Alcohols	Alkyl, Phenalkyl, OH
		Organic Acids	All
Acetanilides	0.9–5.0	Anilides	CH_3O, Cl, Br, NO_2, CF_3, CH_3
Alcohols	(−1.0)–5.0	Alcohols	Alkyl, Phenalkyl, OH
Amides	(−1.0)–4.0	Acetamides	F, Cl, Br, CH_3O, Alkyl
		Benzamides	NO_2, NMe
Anilines	1.0–5.1	Anilines	Cl, Br, CF_3, CH_3, NMe, N, NMe_2
Carbamates	(−1.0)–5.0	Carbamates	Alkyl, Alkenyl, Cl, Br, NMe, CH_3O

Model	X-variable domain log K_{ow} in log units	Chemical domain	Substituents or Warnings
Dinitroanilines	0.5–5.5	Dinitroanilines	CF_3, Alkyl-SO_2, NH_2SO_2, CH_3, *t*-Bu
Esters	1.0–8.0	Phthalates	Alkyl, Phenyl, Cl
		Benzoates	Alkyl, Phenyl, NO_2, OH, Cl, NH_2
		Phenylacetates	Alkyl, Phenalkyl
		Hexanoates	Alkyl
		Heptanoates	Alkyl
		Octanoates	Alkyl
Nitrobenzenes	1.0–4.5	Nitrobenzenes	Cl, Br, NH_2
Organic Acids	(–0.5)–4.0	Organic Acids	All
Phenols	0.5–5.5	Phenols	Cl, Br, NO_2, CH_3, CH_3O, OH
		Benzonitriles	Cl
Phenylureas	0.5–4.2	Phenylureas	CH_3, CH_3O, F, Cl, Br, Cycloalkyls, CF_3, PhO
Phosphates	0.0–6.5	All Phosphates	
Triazines	1.5–4.0	Triazines	Cl, CH_3O, CH_3S, NH_2, N-Alkyl
Triazoles	(–1.0)–5.0	Triazoles	Alkyl, CH_3O, F, Cl, CF_3, NH_2

Table 26 QSARs for sediment sorption for different chemical classes (Sabljić et al., 1995 cited in European Commission (Joint Research Centre), 2003a)

Chemical class	Equation	Statistics
Predominantly hydrophobics	$\log K_{oc} = 0.81 \log K_{ow} + 0.10$	$n=81$, $r^2=0.89$, s.e.=0.45
Nonhydrophobics	$\log K_{oc} = 0.52 \log K_{ow} + 1.02$	$n=390$, $r^2=0.63$, s.e.=0.56
Phenols, anilines, benzonitriles, nitrobenzenes	$\log K_{oc} = 0.63 \log K_{ow} + 0.90$	$n=54$, $r^2=0.75$, s.e.=0.40
Acetanilides, carbamates, esters, phenylureas, phosphates, triazines, triazoles, uracils	$\log K_{oc} = 0.47 \log K_{ow} + 1.09$	$n=216$, $r^2=0.68$, s.e.=0.43
Alcohols, organic acids	$\log K_{oc} = 0.47 \log K_{ow} + 0.50$	$n=36$, $r^2=0.72$, s.e.=0.39
Acetanilides	$\log K_{oc} = 0.40 \log K_{ow} + 1.12$	$n=21$, $r^2=0.51$, s.e.=0.34
Alcohols	$\log K_{oc} = 0.39 \log K_{ow} + 0.50$	$n=13$, $r^2=0.77$, s.e.=0.40
Amides	$\log K_{oc} = 0.33 \log K_{ow} + 1.25$	$n=28$, $r^2=0.46$, s.e.=0.49
Anilines	$\log K_{oc} = 0.62 \log K_{ow} + 0.85$	$n=20$, $r^2=0.82$, s.e.=0.34
Carbamates	$\log K_{oc} = 0.37 \log K_{ow} + 1.14$	$n=43$, $r^2=0.58$, s.e.=0.41
Dinitroanilines	$\log K_{oc} = 0.38 \log K_{ow} + 1.92$	$n=20$, $r^2=0.83$, s.e.=0.24
Esters	$\log K_{oc} = 0.49 \log K_{ow} + 1.05$	$n=25$, $r^2=0.76$, s.e.=0.46
Nitrobenzenes	$\log K_{oc} = 0.77 \log K_{ow} + 0.55$	$n=10$, $r^2=0.70$, s.e.=0.58
Organic acids	$\log K_{oc} = 0.60 \log K_{ow} + 0.32$	$n=23$, $r^2=0.75$, s.e.=0.34
Phenols, benzonitriles	$\log K_{oc} = 0.57 \log K_{ow} + 1.08$	$n=24$, $r^2=0.75$, s.e.=0.37
Phenylureas	$\log K_{oc} = 0.49 \log K_{ow} + 1.05$	$n=52$, $r^2=0.62$, s.e.=0.34
Phosphates	$\log K_{oc} = 0.49 \log K_{ow} + 1.17$	$n=41$, $r^2=0.73$, s.e.=0.45
Triazines	$\log K_{oc} = 0.30 \log K_{ow} + 1.50$	$n=16$, $r^2=0.32$, s.e.=0.38
Triazoles	$\log K_{oc} = 0.47 \log K_{ow} + 1.41$	$n=15$, $r^2=0.66$, s.e.=0.48

n is the number of data, r^2 is the correlation coefficient and s.e. the standard error of estimate.

The QSARs in Table 26 are from a report cited in the TGD, but they can also be found in the public literature (Sabljić *et al.*, 1995). In principle, the appropriate QSAR should be chosen on basis of this table. For many compounds with polar groups attached, a separate QSAR is available for that particular chemical class. In general, these QSARs do not deviate very much from the QSARs for larger subsets of chemical classes. However, if there is doubt about which QSAR to use, for example, due to the presence of more than one functional group, it is often most convenient to use the more general QSARs, in particular the QSAR for non-hydrophobic chemicals. This QSAR, together with the QSAR for predominantly hydrophobic compounds provides a reasonable estimate of the K_{oc} for most compounds.

The K_{oc} can also be estimated with an HPLC method (OECD guideline 121; OECD, 2001). As the title of the method indicates, this is no direct determination of the K_{oc} but an estimate based on another property (retention in HPLC). Also, the estimation routine PCKOCwin, which employs a calculation method based on molecular connectivity indices (MCI), may be used to estimate the K_{oc}. PCKOCwin is embedded in the EPI Suite software (US EPA, 2007b). Both methods can aid in the decision by means of an independent estimation, in the case that the interpretation of the estimation method based on log K_{ow} according to the TGD is difficult. Both the estimated value from molecular connectivity and values estimated with the HPLC method, if any available, should be reported.

6. Evaluation of K_p values for metals for use in EQS derivation

Adsorption of metals to the solid fraction of sediment or particulate (suspended) matter is dependent on many variables such as cation exchange capacity, organic matter content and clay content, pH, redox potential, etc. In contrast to organic compounds, there is no estimation method to predict metal–solids partitioning in environmental compartments from compound properties. Thus, partition coefficients for metals have to be determined in and retrieved from experimental studies.

The K_p values are collected from all valid studies reporting metal partition coefficients.

Relevant studies are those that report K_p values for sediment or suspended matter (or K_d values) determined in *field* samples. Batch adsorption studies, performed in the laboratory, are a second type of potentially relevant studies. An established data source of metal K_p values for bulk compartments (sediment, suspended matter) does – to our knowledge– not exist. A few references that are of interest are Sauvé *et al.* (2000) and Bockting *et al.* (1992), although values of the latter have been criticised (Koops *et al.*, 1998). Due to the heterogeneity of adsorbents encountered in various compartments, K_p values for metals usually show a high variation. Since normalisation is generally impracticable, selection of the K_p value(s) to be used in equilibrium partitioning (EqP) needs careful consideration.

APPENDIX 2: PROFORMA FOR EQS DATASHEET

NAME OF THE SUBSTANCE

1 Chemical identity

Common name	
Chemical name (IUPAC)	
Synonym(s)	
Chemical class (when available/relevant)	
CAS number	
EU number	
Molecular formula	
Molecular structure	
Molecular weight (g.mol^{-1})	

2 Existing evaluations and Regulatory information

Annex III EQS Dir. (2008/105/EC)	Not Included / Included
Existing Substances Reg. (793/93/EC)	Not applicable / Liste No
Pesticides (91/414/EEC or its successor regulation 1107/2009)	Not included in Annex I / Included in Annex I
Biocides (98/8/EC)	Not included in Annex I / Included in Annex I
PBT substances	Conclusions / Not investigated
Substances of Very High Concern (1907/2006/EC)	Yes / No
POPs (Stockholm convention)	Yes / No
Other relevant chemical regulation (veterinary products, medicament, ...)	Information / No
Endocrine disrupter	Available information / Not investigated

3 Proposed Quality Standards (QS)

3.1 Environmental Quality Standard (EQS)

QS for -- is the "critical QS" for derivation of an Environmental Quality Standard

Add any comment on possible residual uncertainty.

	Value	Comments
Proposed AA-EQS for [matrix] [unit] Corresponding AA-EQS in [water] [µg.L^{-1}]		**Critical QS is QS--.** **See section 7**
Proposed MAC-EQS for [freshwater] [µg.L^{-1}] **Proposed MAC-EQS for [marine waters] [µg.L^{-1}]**		**See section 7.1**

3.2 Specific Quality Standard (QS)

Protection objective*	Unit	Value	Comments
Pelagic community (freshwater)	[µg.l^{-1}]		See section **7.1**
Pelagic community (marine waters)	[µg.l^{-1}]		
Benthic community (freshwater)	[µg.kg^{-1} $_{dw}$]		e.g. EqP, see section **7.1**
	[µg.l^{-1}]		
Benthic community (marine)	[µg.kg^{-1} $_{dw}$]		
	[µg.l^{-1}]	-	
Predators (secondary poisoning)	[µg.kg$^{-1}_{biota\ ww}$]		See section **7.2**
	[µg.l^{-1}]	(freshwaters) (marine waters)	
Human health via consumption of fishery products	[µg.kg$^{-1}_{biota\ ww}$]		See section **7.3**
	[µg.l^{-1}]	(freshwaters) (marine waters)	
Human health via consumption of water	[µg.l^{-1}]		

4 Major uses and Environmental Emissions

4.1 Summary of Uses and Quantities

4.2 Summary of Estimated Environmental Emissions

5   Environmental Behaviour

5.1   Environmental distribution

		Master reference
Water solubility (mg.l^{-1})	at 20°C	
Volatilisation		
Vapour pressure (Pa)	at 20°C	
Henry's Law constant (Pa.m^3.mol^{-1})		
Adsorption	**The range - is used for derivation of quality standards.**	
Organic carbon – water partition coefficient (K$_{OC}$)	$K_{OC} =$ -	
Suspended matter – water partition coefficient(K$_{susp-water}$)	-	
Bioaccumulation	**The BCF value - on fish is used for derivation of quality standards.**	
Octanol-water partition coefficient (Log Kow)		
BCF (measured)		

5.2   Abiotic and Biotic degradations

		Master reference
Hydrolysis	DT$_{50}$= d at °C (distilled water) DT$_{50}$= d at °C (salt water)	
Photolysis	DT$_{50}$=	
Biodegradation	DT$_{50 \text{ (type of water)}}$= d	

6　Aquatic environmental concentrations

6.1　Estimated concentrations

Compartment	Predicted environmental concentration (PEC)	Master reference
Freshwater		
Marine waters (coastal and/or transitional)		
Sediment		
Biota (freshwater)		
Biota (marine)		
Biota (marine predators)		

6.2　Measured concentrations

Compartment	Measured environmental concentration (MEC)	Master reference
Freshwater		
Marine waters (coastal and/or transitional)		
WWTP effluent		
Sediment		
Biota		
Biota (marine predators)		

7 Effects and Quality Standards

7.1 Acute and chronic aquatic ecotoxicity

ACUTE EFFECTS			Master reference
Algae & aquatic plants (mg.l^{-1})	**Freshwater**	*Gender species* / d or h EC_{50} :	
	Marine	*Gender species* / d or h EC_{50} :	
Invertebrates (mg.l^{-1})	**Freshwater**	*Gender species* / d or h EC_{50} :	
	Marine	*Gender species* / d or h EC_{50} :	
	Sediment	*Gender species* / d or h EC_{50} :	
Fish (mg.l^{-1})	**Freshwater**	*Gender species* / d or h EC_{50} :	
	Marine	*Gender species* / d or h EC_{50} :	
	Sediment	*Gender species* / d or h EC_{50} :	
Other taxonomic groups		*Gender species* / d or h EC_{50} :	

CHRONIC EFFECTS			Master reference
Algae & aquatic plants **(mg.l^{-1})**	**Freshwater**	*Gender species* / d NOEC :	
	Marine	*Gender species* / d NOEC :	
Invertebrates **(mg.l^{-1})**	**Freshwater**	*Gender species* / d NOEC :	
	Marine	*Gender species* / d NOEC :	
	Sediment	*Gender species* / d NOEC :	
Fish **(mg.l^{-1})**	**Freshwater**	*Gender species* / d NOEC :	
	Marine	*Gender species* / d NOEC :	
	Sediment	*Gender species* / d NOEC :	
Other taxonomic groups		*Gender species* / d NOEC :	

Tentative QS$_{water}$	Relevant study for derivation of QS	Assessment factor	Tentative QS
MAC-QS$_{fw, eco}$	*Gender species* / d or h EC$_{50}$: mg.l^{-1}		µg.l^{-1}
MAC-QS$_{sw, eco}$			µg.l^{-1}
QS$_{fw, eco}$	*Gender species* / 21d NOEC : mg.l^{-1}		µg.l^{-1}
QS$_{sw, eco}$			µg.l^{-1}
QS$_{sediment, fw, EqP}$	-	EqP	- µg.kg^{-1}$_{ww}$ - µg.kg^{-1}$_{dw}$
QS$_{sediment, sw EqP}$	-	EqP	- µg.kg^{-1}$_{ww}$ - µg.kg^{-1}$_{dw}$

7.2 Secondary poisoning

Secondary poisoning of top predators		Master reference
Mammalian oral toxicity	Species / Oral / duration / Endpoint NOAEL : mg.kg$^{-1}_{bw}$.d^{-1} NOEC : mg.kg$^{-1}_{biota\ ww}$ (CF=)	
	Species / Oral / duration / Endpoint NOAEL : mg.kg$^{-1}_{bw}$.d^{-1} NOEC : mg.kg$^{-1}_{biota\ ww}$ (CF=)	
Avian oral toxicity	Species / Oral / 14 d EC 50 : mg.kg$^{-1}_{bw}$.d^{-1} NOEC : mg.kg$^{-1}_{biota\ ww}$	

Tentative QS$_{biota}$	Relevant study for derivation of QS	Assessment factor	Tentative QS
Biota	NOEC : mg.kg$^{-1}_{biota\ ww}$		-- µg.kg$^{-1}_{biota\ ww}$ corresponding to -- µg.L^{-1} (freshwater) -- µg.L^{-1} (marine waters)

7.3 Human Health

Human health via consumption of fishery products		Master reference
Mammalian oral toxicity	Species / Oral / duration / Endpoint NOAEL : mg.kg$^{-1}_{bw}$.d^{-1} NOEC : mg.kg$^{-1}_{biota\ ww}$ (CF=)	
CMR		

Tentative QS$_{biota,\ hh}$	Relevant study for derivation of QS$_{biota,\ hh\ food}$	Assessment Factor	Tentative QS$_{biota,\ hh\ food}$

| **Human health** | -- mg.kg^{-1}$_{biota\ ww}$ | | -- µg.kg^{-1}$_{biota\ ww}$ (-- µg.L^{-1}) |

Human health via consumption of drinking water		**Master reference**
Existing drinking water standard(s)	µg.L^{-1} (preferred regulatory standard)	Directive 98/83/EC
Any guideline		

8.   Identification of issues relating to uncertainty in relation to the QSs derived

9.   Identification of any potential implementation issues in relation to the QSs derived

10.   Bibliography, Sources and supportive information

APPENDIX 3: BIOCONCENTRATION, BIOMAGNIFICATION AND BIOACCUMULATION

Accumulation is a general term for the net result of absorption (uptake), distribution, metabolism and excretion (ADME) of a substance in an organism. Information on accumulation in aquatic organisms is vital for understanding the fate and effects of a substance in aquatic ecosystems. In addition, it is an important factor when considering whether long-term ecotoxicity testing might be necessary. This is because chemical accumulation may result in internal concentrations of a substance in an organism that cause toxic effects over long-term exposures even when external concentrations are very small. Highly bioaccumulative chemicals may also transfer through the food web, which in some cases may lead to biomagnification.

The change in concentration of a chemical in biota (C_b) over time can be described as:

$$\frac{dC_b}{dt} = k_{upt} \cdot C_w + k_{food} \cdot C_{food} - k_{dep} \cdot C_b - k_{exc} \cdot C_b - k_{met} \cdot C_b$$

where C_w and C_{food} represent the concentrations of the chemical in the water column and in the food; and the subscripts *upt*, *dep*, *exc* and *met* refer to uptake, depuration, excretion and metabolism, respectively (Gobas *et al.*, 1988).

Bioconcentration refers to the accumulation of a substance, dissolved in water, by an aquatic organism. The bioconcentration factor (BCF) of a compound is defined as the ratio of the concentration of the chemical in the organism and in water at equilibrium.

$$BCF = \frac{C_b}{C_w}$$

The uptake of a chemical from water is a passive diffusion process across the skin or gill membrane, similar to oxygen uptake. Several factors affect this uptake, such as the physicochemical characteristics of the compound, the characteristics of the receptor and the environmental conditions. For example, Boese (1984) demonstrated that decreasing oxygen level in the water accelerated the accumulation of contaminants in the body of clams.

Bioconcentration is normally related to the octanol-water partition coefficient of the compound and the lipid fraction in tissues of the organism (Van der Oost *et al.*, 2003). Several log-linear correlations exist between the logarithm of the octanol-water partition coefficient and the BCF (e.g.: Devillers *et al.*, 1996; Hawker and Connel, 1985, 1986).

The existence of equilibrium between the concentration of the chemical in the organism and the concentration in the water is not easy to assess. For example, for rainbow trout Vigano *et al.* (1994) measured a time range between 15 and 256 days to reach equilibrium after exposure to different concentrations of PCBs.

Biomagnification refers to the accumulation of substances via the food chain. It may be defined as an increase in the (fat-adjusted) internal concentration of a substance in organisms at successive trophic levels in a food chain. The biomagnification factor is defined as the ratio between the uptake of a contaminant from food and its removal by depuration (*dep*), excretion (*excr*) and metabolism (*meta*)(Sijm *et al.*, 1992),

$$BMF = \frac{k_{food}}{k_{dep} + k_{excr} + k_{meta}}$$

The uptake from food can be also defined as:

$$k_{food} = F_F \cdot eff_F$$

where F_F is the quantity of food ingested per unit mass per unit time and eff_F is the efficiency of uptake of the chemical from food.

The BMF can also be expressed as the ratio of the concentration in the predator and the concentration in the prey:

BMF = C_o/C_d

where BMF is the biomagnification factor (dimensionless)

C_o is the steady-state chemical concentration in the organism (mg/kg)

C_d is the steady-state chemical concentration in the diet (mg/kg)

Russell *et al.* (1999) demonstrated that significant biomagnification is not observed for values of log K_{ow} lower than 5.5. Moreover, Fisk *et al.* (1998) observed a high potential to accumulate along aquatic food webs for chemicals with log $K_{ow} \approx 7$.

Laboratory experiments demonstrated that digestibility and absorption of food are critical parameters controlling the BCFs in fish (Gobas *et al.* 1999). Furthermore, Opperhuizen (1991) found that biomagnification accounts for a more important fraction of accumulation of chemicals for larger fish than for smaller fish, which is probably due to a decrease in gill ventilation volume while the relative feeding rate is almost the same.

The term **bioaccumulation** refers to uptake from all environmental sources including water, food and sediment. The bioaccumulation factor (BAF) can be expressed for simplicity as the steady-state (equilibrium) ratio of the substance concentration in an organism to the concentration in the surrounding medium (e.g. water). Normally, it is evaluated using a multiplicative approach. Therefore, the Bioaccumulation Factor (BAF) may be calculated as:

$$BAF = BCF \cdot \prod_{i=1}^{n} BMF_i$$

where the number of biomagnifications factors depends on the trophic level or position of the organism in the food web.

In a recent review, which recommends the use of a high-quality field derived BAF, Arnot and Gobas (2006) analysed 392 scientific literature and database sources which included 5317 BCFs and 1656 BAFs values measured for 842 organic chemicals in 219 aquatic species. Their results indicate that 45% of BCF values are subject to at least one major source of uncertainty and that measurement errors generally result in an underestimation of the actual BCF values; the situation is similar for BAF, however there are much less published values.

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APPENDIX 4: THE 'CRED' METHOD FOR ASSESSING RELIABILITY AND RELEVANCE OF ECOTOXICITY DATA

CRED stands for Criteria for Reporting and Evaluating ecotoxicity Data. Two publications and one user-friendly excel tool are available that describe the CRED evaluation method:

1) Moermond et al (2016) explains how to apply the method

2) Kase et al (2016) provides a comparison of the CRED and Klimisch method

3) The CRED Excel tool is to be found in supporting information to Moermond et al. (2016)

In the following section the main findings are summarised to support risks assessors in EQS derivation and choice of methods:

1) CRED guidance

When deriving threshold concentrations of chemicals in the environment, it is necessary to evaluate the reliability and relevance of ecotoxicity studies. Such evaluation is often subject to expert judgment, which may introduce bias and decrease consistency when risk assessors evaluate the same study. The Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) project attempts to address this problem.
Moermond et al (2016) explain how CRED aims to improve the reproducibility, transparency, and consistency of reliability and relevance evaluations of aquatic ecotoxicity studies. The CRED evaluation method is presented along with CRED includes a set of 20 reliability and 13 relevance criteria, accompanied by extensive guidance Risk assessors who participated in ring test of the method when used in comparison to the Klimisch method evaluated the CRED evaluation method to be more accurate, applicable, consistent, and transparent than the often used Klimisch method Kase et al. (2016). The CRED evaluation method is accompanied by reporting recommendations for aquatic ecotoxicity studies, with 50 specific criteria divided into 6 categories: general information, test design, test substance, test organism, exposure conditions, and statistical design and biological response reported is more likely to be considered for regulatory use, and proper reporting may also help in the peer-review process.".(Moermond et al. 2016)

2) Klimisch and CRED method comparison

The regulatory evaluation of ecotoxicity studies for environmental risk and/or hazard assessment ofchemicals is often performed using the method established by Klimisch and colleagues in 1997. A new evaluation method was developed to address some limitations of this method: Criteria for Reporting and Evaluating ecotoxicity Data (CRED). The CRED evaluation method aims at strengthening consistency and transparency of hazard and risk assessment of chemicals by providing criteria and guidance for assessing the reliability and relevance of aquatic ecotoxicitystudies.
A ring test among 75 risk assessors from 12 countries compared and characterized the differences between the CRED and Klimisch evaluation methods. Results show that the CRED evaluation method provides a more detailed and transparent evaluation of reliability and relevance than the Klimisch method. Ring test participants perceived it to be less dependent on expert judgement, more accurate and consistent. For these reasons it is offered as an alternative or supplementary method for the assessment of ecotoxicological data.

3) CRED excel tool

The CRED excel tool can be downloaded at:

HTTP://ONLINELIBRARY.WILEY.COM/STORE/10.1002/ETC.3259/ASSET/SUPINFO/ETC3259-SUP-0002-SUPPDATA-S2.XLSX?V=1&S=EFCD3202C75BAE26696D16C515EEE5DB604BFEEF

CRED combination
method 160915 SI Ex

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APPENDIX 5: GLOSSARY

5P-COV	5th percentile cut-off value; the 5th percentile of a species sensitivity distribution.
AA-EQS	annual average environmental quality standard
ADI	acceptable daily intake
AF	assessment factor
AF_{oral}	assessment factor applied in extrapolation of $EQS_{biota.Predators}$
ARA	added risk approach
AVS	acid volatile sulphide
B	bioaccumulative
BAF	bioaccumulation factor
BCF	bioconcentration factor
BioF	bioavaiability factor
BMF	biomagnification factor
bw	body weight
CONV	conversion factor from NOAEL into NOEC
CSTEE	Scientific Advisory Committee on Toxicity and Ecotoxicity of Chemicals of the European Commission
Cb	background concentration
C_{ARA}	concentration of dissolved metal monitored at a site excluding the background concentration
C_{SPM}	concentration of suspended matter
C_{TRA}	concentration of dissolved metal monitored at a site
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DFI	daily food intake ($kg_{Food (FW)}.d^{-1}$)
dw	dry weight
EC	European Commission
EC_x	effect concentration for X% of the individuals in a toxicity test
EFSA	European Food Safety Authority
EQS	environmental quality standard
EU	European Union
f_{oc}	fraction of organic carbon
FWMF	food web magnification factor
GLP	Good Laboratory Practice
H	hardness
HC_5	hazardous concentration for 5% of the species (based on the SSD)
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
HELCOM	Helsinki Commission: Baltic Marine Environment Protection Commission
Hg	mercury
ICES	International Council for the Exploration of the Sea
ICME	International Council on Metals and the Environment
ICPR	International Commission for the Protection of the Rhine
K_{ow}	octanol–water partition coefficient
K_{oc}	organic carbon adsorption coefficient
K_p	partition coefficient
$K_{p,susp}$	partition coefficient to suspended matter
LC_{50}	lethal concentration for 50% of the individuals in a toxicity test
log K_{ow}	logarithm (base 10) of the octanol–water partition coefficient
LOEC	lowest observed effect concentration
LOQ	limit of quantification
M	metal
MAC	maximum acceptable concentration
MPA	maximum permissible addition

MS	metal sulphide
$NOAEL_{oral}$	no observed adverse effect level, direct oral dosing tests
NOEC	no observed effect concentration
$NOEC_{oral}$	no observed effect concentration in a toxicity test, feeding tests
$NOEC_{reference}$	reference no observed effect concentration based on a worst-case approach
$NOEC_{site-specific}$	site-specific no observed effect concentration based on local physicochemical conditions
OCP	organochlorine pesticide
OECD	Organisation for Economic Development
OSPAR	Commission for the Protection of the Marine Environment of the North-East Atlantic
PAH	polyaromatic hydrocarbon
PBDE	polybrominated diphenylether
PBT	persistent, bioaccumulative and toxic
PCB	polychlorinated biphenyl
PEC	predicted environmental concentration
PFOS	perfluorooctane sulfonate
PHS	priority hazardous substance
PNEC	predicted no-effect concentration
$PNEC_{oral}$	predicted no-effect concentration for the ingestion of food
$PNEC_{biota}$	predicted no-effect concentration in biota
$PNEC_{secpois}$	predicted no-effect concentration for secondary poisoning
$PNEC_{hh}$	predicted no-effect concentration for the protection of human health
PPP	plant protection product
PS	priority substance
QCAR	quantitative cationic activity relationships
QICAR	quantitative ion character–activity relationships
QS	temporary quality standards, defined during derivation. An overview of temporary standards can be found in Appendix 6
QSAR	quantitative structure–activity relationship
QSPR	quantitative structure-property relationship
RA	risk assessment
RAR	risk assessment report
RBSP	river-basin specific pollutant
REACH	Registration, Evaluation and Authorisation of Chemicals
RfD	reference dose
SEM	simultaneously extracted metals
SETAC	Society for Environmental Toxicology and Chemistry
SOP	standard pperating procedure
SPM	suspended particulate matter
SSD	species sensitivity distribution
TDI	tolerable daily intake
TGD	Technical Guidance Document (EC 2003)
TL	trophic level
TLhh	threshold level, human health
TMF	trophic magnification factor
TOC	total organic carbon
TOX_{oral}	$NOEC_{oral,bird}$ or $NOEC_{oral,mammals}$ or LC_{50} (as indicative value and not for EQS derivation) in $kg.kg_{food (FW)}^{-1}$
TRA	total risk approach
$uptake_{dw}$	daily uptake of drinking water
UVCB	substances of unknown or variable composition, complex reaction products or biological materials
vB	very bioaccumulative
vPvB	very persistent, very bioaccumulative
WFD	Water Framework Directive

ww	wet weight

APPENDIX 6: OVERVIEW OF TEMPORARY STANDARDS FOR EQS DERIVATION

Freshwater	Saltwater	short description	REMARK
TEMPORARY STANDARDS, DURING DERIVATION (QS)			
$QS_{fw, eco}$	$QS_{sw, eco}$	direct ecotoxicity	
$QS_{dw, hh}$		drinking water	standard for saltwater and freshwater is identical
$QS_{biota, secpois, fw}$	$QS_{biota, secpois, sw}$	secondary poisoning expressed in biota	sp standard *in* biota is NOT identical for fresh and salt since $BMF_{b/m}$ is applied for saltwater
$QS_{fw, secpois}$	$QS_{sw, secpois}$	secondary poisoning expressed in water	
$QS_{biota, hh food}$		human consumption of fishery products, expressed in biota	hh standard *in* biota is identical for fresh and salt
$QS_{water, hh food}$		human consumption of fishery products, expressed in water	this standard is equal for fresh and marine water
$MAC\text{-}QS_{fw, eco}$	$MAC\text{-}QS_{sw, eco}$	standard for short term exposure protective for the ecosystem	
$QS_{sediment, fw, eco}$	$QS_{sediment, sw, eco}$	sediment, based on sediment toxicity data (expressed in dry weight)	
$QS_{sediment, fw, EqP}$	$QS_{sediment, sw, EqP}$	sediment, based on EqP, expressed in dry weight sediment	
$QS_{sediment, fw, field}$	$QS_{sediment, sw, field}$	sediment standard, adjusted for field or mesocosm data	
SPECIFIC TEMPORARY STANDARDS IN METAL QS DERIVATION			
$QS_{generic, fw, eco}$	$QS_{generic, sw, eco}$	uncorrected standard for ecosystem	
$QS_{reference, fw, eco}$	$QS_{reference, sw, eco}$	standard for ecosystem for reference conditions	

Freshwater	Saltwater	short description	REMARK
$QS_{site\text{-}specific, fw, eco}$	$QS_{site\text{-}specific, sw, eco}$	site specific standard for ecosystem	
$QS_{added, fw, eco}$	$QS_{added, sw, eco}$	standard for the ecosystem following added risk approach – added part only	
FINAL SELECTED STANDARDS (EQS)			
$AA\text{-}EQS_{fwr}$	$AA\text{-}EQS_{sw}$	selected overall standard for water compartment	
$MAC\text{-}EQS_{fw}$	$MAC\text{-}EQS_{sw}$	selected overall standard protective for short term exposure	
$EQS_{biota, fw}$	$EQS_{biota, sw}$	selected overall standard in biota	secpois standard *in* biota is NOT identical for fresh and salt since $BMF_{b/m}$ is applied for saltwater
$EQS_{sediment, fw}$	$EQS_{sediment, sw}$		

APPENDIX 7: MEMBERS OF THE EXPERT GROUP RESPONSIBLE FOR REVISIONS TO EQS –TGD 2015-16

Member State / Organisation	Individuals
United Kingdom - Environment Agency	Paul Whitehouse (**chair**)
European Commission	Teresa Lettieri Stephanie Schaan
Denmark - Environmental Protection Agency	Henning Clausen
Netherlands **National Institute for Public Health and Environment (RIVM)** **Dutch Ministry of Transport, Public Works, and Water Management (RWS)**	Eric Verbruggen Peter van Vlaardingen Caroline Moermond Els Smit Dorien ten Hulscher
EUROMETAUX **ECI** **IZA** **Nipera**	Annalisa Bortoluzzi Katrien Delbeke Frank Van Assche Chris Schlekat
France - INERIS	Sandrine Andres
Germany Umweltbundesamt	Dieter Schudoma Dieter Veltwisch Joachim Heidemeier Edda Hahlbeck Friederike Vietoris Peter Heininger Volker Mohaupt
Italy – IRSA-CNR	Stefano Polesello Sarah Valsecchi
WCA- Environment	Graham Merrington Adam Peters
Switzerland	Robert Kase Marion Junghans