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Common Implementation Strategy
for the Water Framework Directive (2000/60/EC)

Guidance Document No. 27

Technical Guidance For Deriving Environmental Quality Standards

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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. Focus is on methodological questions related to a common understanding of the technical and scientific implications of the Water Framework Directive. In particular, one of the objectives of the strategy 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 establishment of the EQSs in the field of water policy. This activity was led by UK and the Joint Research Centre and supported by the Working Group E (WG-E). The Working Group E 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.).

The enclosed 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 to use the 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). 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 2011, accompanying its conclusion, where appropriate, with proposals to identify new priority substances and to set EQSs for them in water, sediment and/or biota.

The Scientific Committee on Health and Environmental Risks (SCHER) adopted its opinion on Technical Guidance for Deriving Environmental Quality Standards in October 2010[1]. The Water Directors endorsed the Guidance during their informal meeting under the Hungarian Presidency in Budapest (26-27 May 2011).

This Guidance Document is a living document that will need continuous input and improvements as application and experience build up in all countries of the European Union and beyond. The Water Directors agreed to make publicly available the Guidance in its current form in order to present it to a wider public as a basis for carrying forward ongoing implementation work.

The Water Directors would like to thank the leaders of the activity and the members of the Working Group E for preparing this high quality document. The Water Directors also commit themselves to assess and decide upon the necessity for reviewing this document in the light of scientific and technical progress and experiences gained in implementing the Water Framework Directive and Environmental Quality Standards Directive.

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[1] http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_127.pdf

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) establishes the maximum acceptable concentration and/or annual average concentration for 33 priority substances and 8 other pollutants which, if met, allows the chemical status of the waterbody to be described as 'good'.

EQSs for the 33 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. They are also referred to as Annex X substances of the WFD.

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 WFD. Compliance with EQSs for Specific Pollutants forms part of the assessment of ecological status (Figure 1-1). EQSs are therefore key tools in assessing and classifying chemical status and can therefore affect the overall classification of a waterbody under the WFD (Figure 1.1). 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 water.

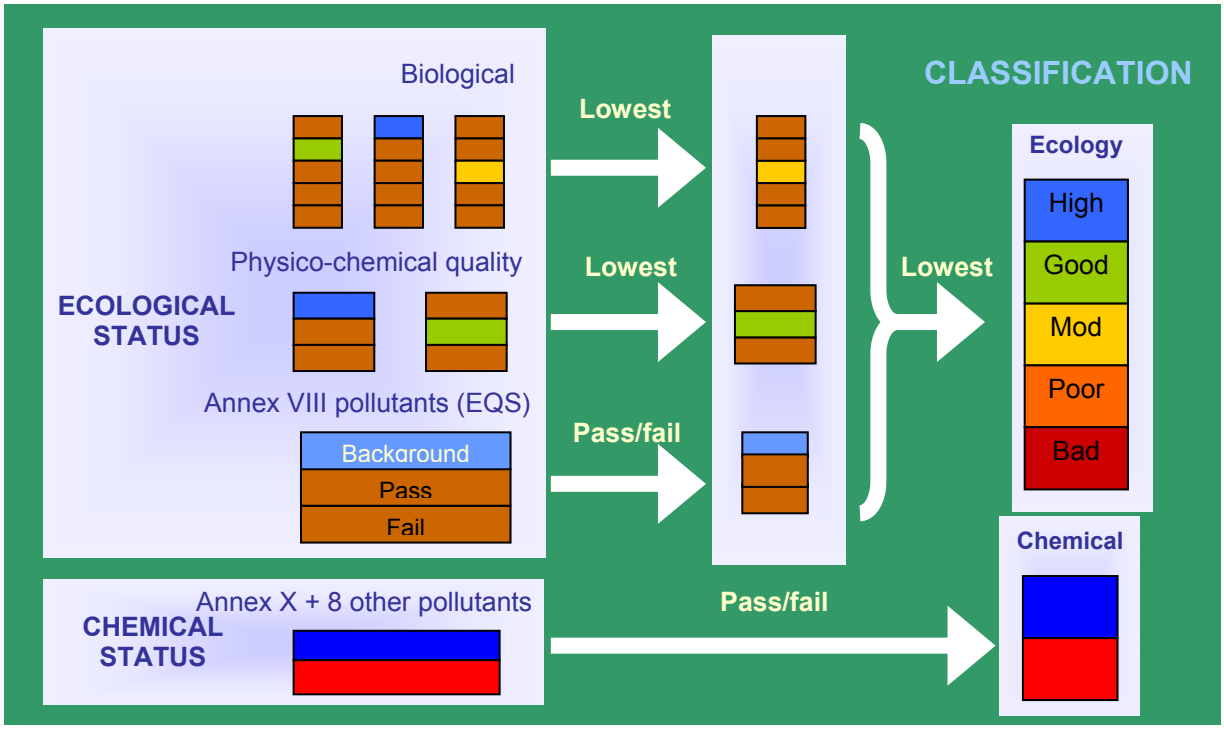


Figure 1.1 Role of EQSs in waterbody classification

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 however the science has since moved on requiring the need for an update of the guidance.

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, when this paradigm is applied to EQS derivation it can lead to unworkable and/or unrealistically low EQS values (CSTEE[2], 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 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 sediment standard may be required alongside, or instead of, the water column EQS, as referred to in the EQS Directive 2008/105/EC (Art 3, para 2). 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. 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.

1.2 Scope of the guidance

This guidance document addresses the derivation of environmental quality standards for water, sediment and biota. It addresses the need for further guidance highlighted above and responds to 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 takes account of the principles highlighted in a SETAC (Society for Environmental Toxicology and Chemistry) workshop on environmental standards that took place in 2006 (SETAC, 2009) so that the latest scientific thinking on setting and implementing environmental standards is reflected.

This guidance applies to the derivation of EQSs for PSs, PHSs and Specific Pollutants. 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. However, it does address some aspects of the way an EQS is implemented, where this has a direct bearing on the way an EQS is derived and expressed, e.g. assessing compliance with an EQS. The guidance does not cover issues relating to sampling and chemical analysis: these are covered by separate guidance on monitoring (EC, 2010).

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

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

based on extensive data sets, and it may even be inadvisable to implement such EQSs**.** This technical guidance does not recommend when uncertainties are so large that an EQS should not be implemented, or used in 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 policymaker 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 policymaker 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 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 in which an EQS is implemented, eg 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), statistical aspects of compliance assessment, availability of suitable analytical methods, the impact of residual uncertainty in the EQS and a threshold for the relevance of a specific pollutant for which an EQS is needed (e.g. exceedance of 50% of the EQS).

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

It is important to highlight some conceptual differences between EQS derivation and the estimation of a PNEC (Predicted No Effect Concentration) from chemical risk assessment or TER (Toxicity Exposure Ratio) for a pesticide. For example:

- the concept of an overall threshold (Sections 2.3 and 2.4) that protects all receptors and routes is a feature of EQS derivation that does not normally apply in chemical risk assessment

- 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 is therefore 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 done in the assessment of the impact of pesticides.

A PNEC derived as part of a risk assessment will provide a key step in the derivation of an EQS and, in some cases, the PNEC from a risk assessment will be identical to the EQS. However, for the reasons outlined above, it will not be sufficient to simply adopt the PNEC as the EQS as a matter of course. Nevertheless, the process of deriving environmental standards is similar to that used in the effects (i.e. hazard) assessment that is required for a risk assessment for chemicals. For the purposes of the WFD, short and long-term effects are of concern, though greater emphasis is placed on risks from long-term or continuous exposure. Authoritative guidance on effects assessment for chemicals has been developed, notably the technical guidance developed for industrial chemicals (now under REACH (ECHA, 2008)) and pesticides under Directive 91/414/EEC. Annex V of the WFD refers directly to the methodology described for the Existing Substances Regulation (ESR) (now under REACH). Furthermore, the guidance for undertaking risk assessment of pesticides allows for short term impacts and recovery. 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 REACH.

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, ie derivation of EQSs for the water column are considered in Section 3, those for 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 and assessing compliance with these EQSs. 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 for the technical advice given here.

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, as a rule by 2015. 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 (2008/105/EC) for the 33 priority substances and 8 other pollutants 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 EQS Directive and applied according with 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 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 can not be classified as either 'Good' or 'High'status, even if the biological quality is 'Good' or 'High' (Figure 1.1).

2.2 Overview of the steps involved in deriving an EQS

Figure 2.1 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.

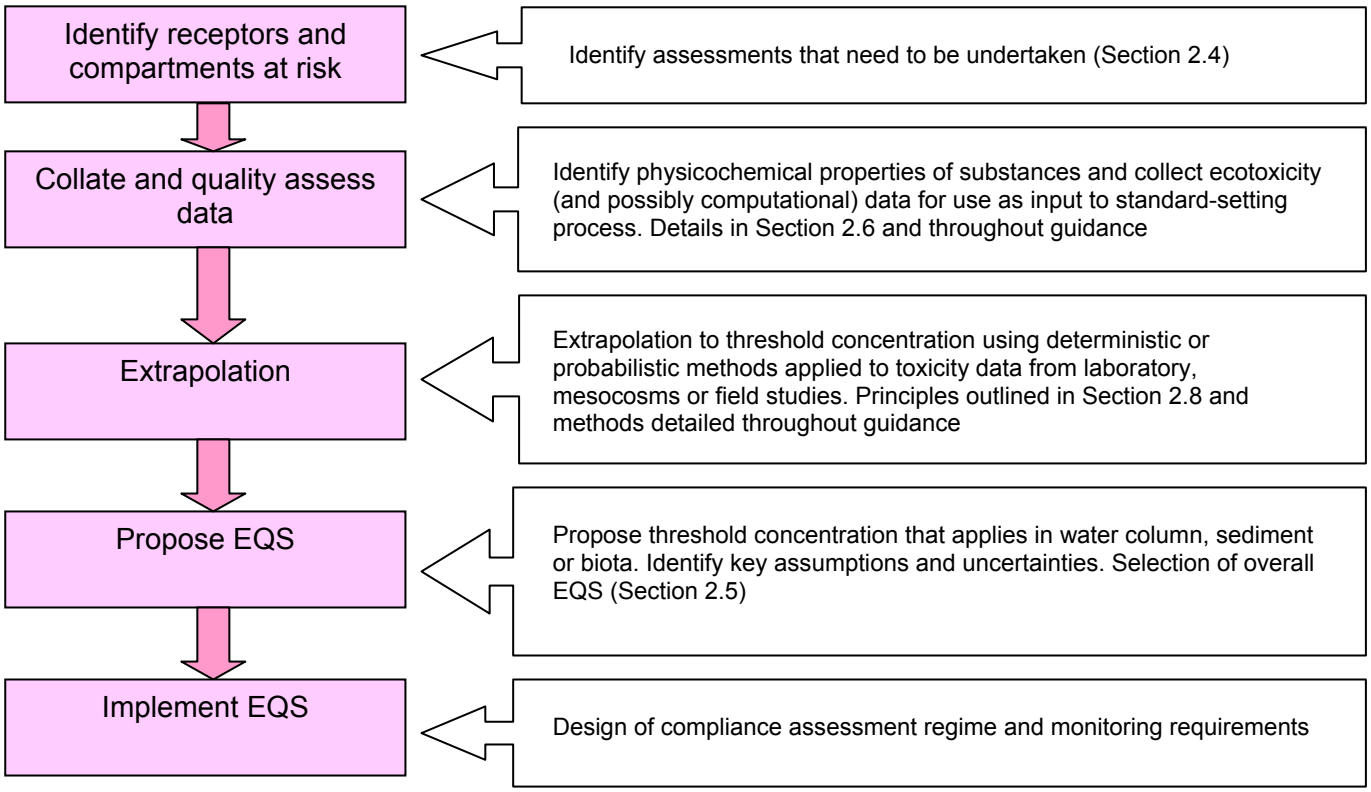


Figure 2.1 Key steps involved in deriving an EQS

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 2.2.

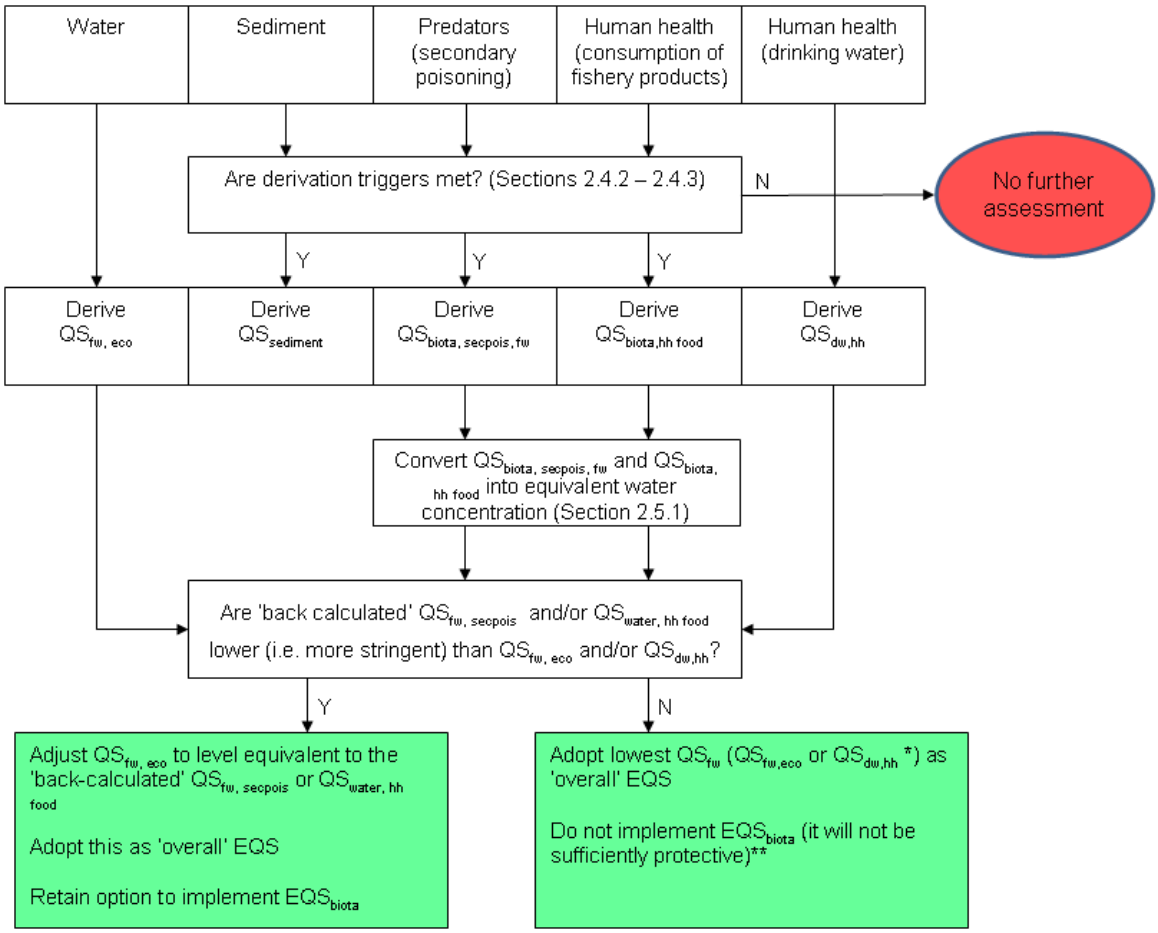
		Environmental compartment		
		Water	**Sediment**	**Biota**
Receptor(s) at risk	**Humans**	**Yes**	**No**	**Yes (consumption of fish products)**
	Sediment dwelling biota	**No**	**Yes**	**No**
	Pelagic biota	**Yes**	**No**	**Yes (secondary poisoning)**
	Top predators (birds, mammals)	**Yes**	**No**	**Yes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

Figure 2.2 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 i.e. if a substance does not bioaccumulate (or doesn't 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 2.3). 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 2.3 and detailed in Section 2.5.

In this way, all relevant protection objectives should be taken into account. 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 2.3 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.



* $QS_{dw,hh}$ 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 2.3 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. 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 Article 3 of the EQS Directive, quality standards shall apply to contaminant concentrations in water, sediments and/or biota. As illustrated in Figure 2-3, **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 to 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 World Health Organization (WHO) could be used, if available, as the basis for the QS derivation, as described in Section 3.9. 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.

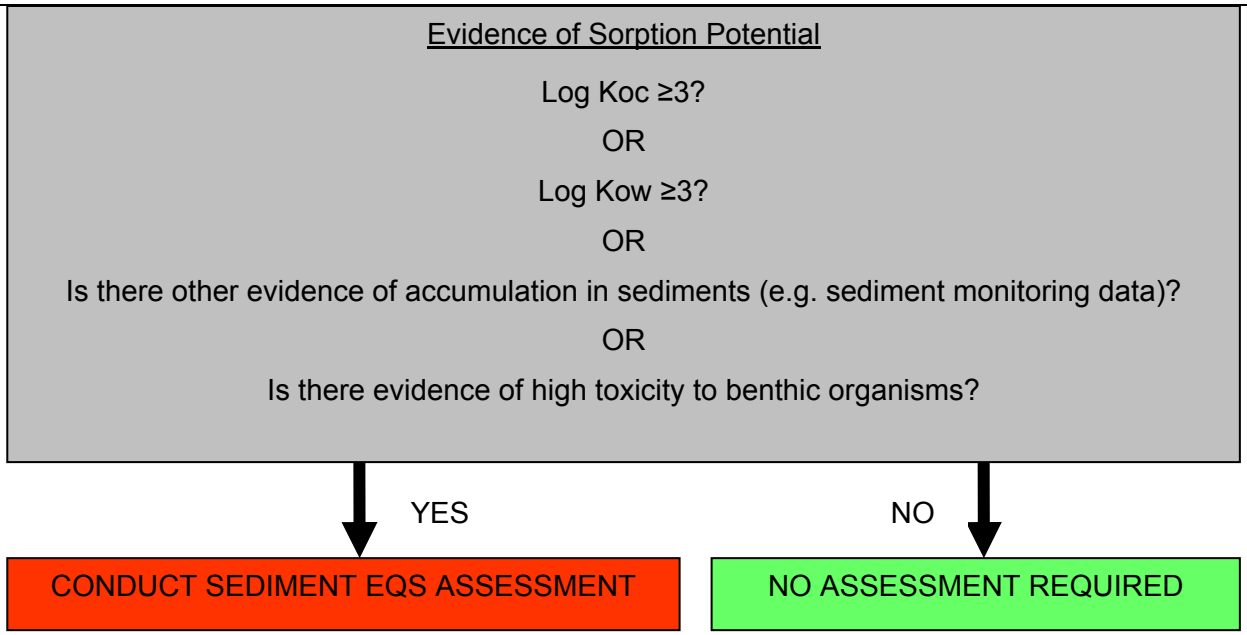
In the derivation of QSs to protect human health two major exposure routes are considered (consumption of fishery products and consumption of drinking water). There may be other routes of exposure, such as exposure during recreation (dermal exposure, ingestion of water). These routes are of minor importance 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 a low salinity, 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 ánd marine waters', but either for freshwaters or saltwaters. As a default, we recommend a salinity of 5‰ as the cutoff unless other evidence suggests a different cutoff is appropriate for a particular location. For instance, Bothnian Sea (inner BalticSea) 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 $l \cdot kg^{-1}$ 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

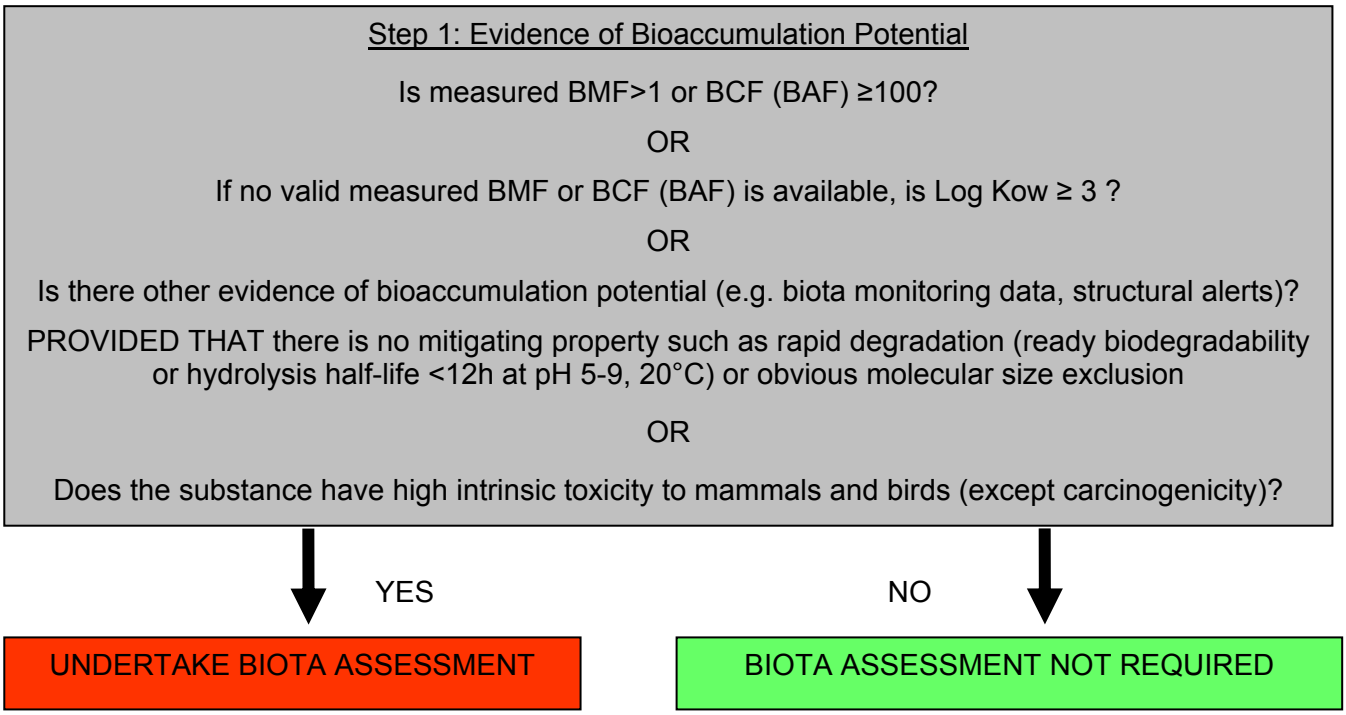
The criteria determining whether or not a biota standard is needed are more complex. A standard would be required if there was a risk of secondary poisoning of predators (e.g. mammals or birds) from eating contaminated prey ($QS_{biota,secpois}$), or a risk to humans from eating fishery products ($QS_{biota,\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

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)[3]. The triggers for derivation of 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.

[3] 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 setting.

2.4.3.1 Protection of predators from secondary poisoning

(1) Organic substances



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 reliable 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 or BCF data are available, the octanol–water partition coefficient (K_{ow}), can be used as a surrogate for bioaccumulation potential. A log K_{ow} of ≥3 would be expected to capture substances with a BCF of ≥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 molecular size can be an indicator of 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 is available in the REACH guidance (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 QSwater, 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[4] 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

[4] 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 evaluation of toxicological data not necessarily reflected in 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 will soon replace the R-phrases in EU chemicals legislation via the Classification, Labelling and Packaging Regulation (2008) (EC, 2008). The conversion between H and R phrases is provided below. Check the status of the R and H phrases. 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 2-3 and further guidance is given in Section 2.5.1. Finally, sediment QSs are dealt with independently of water column and biota standards. This leads to 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 4.7.2. 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 4.7.1.2.

(b) For an organic substance, if the log $K_{OW} \geq 3$ 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 4.7.1.2). 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 resource required for QS derivation is 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 which can be very important when interpreting laboratory and field ecotoxicity are water solubility, vapour pressure, photolytic and hydrolytic stability, 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 when derived using EqP, 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 ($K_{susp\text{-}water}$), K sediment – water ($K_{sed\text{-}water}$), 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 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. **All available data for any taxonomic group or species should be considered, provided the data meet quality requirements for relevance and reliability** (Section 2.6.2). This may include data for alien species and even exotic species[5], 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 not 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.

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.

[5] This is because test species not only represent species that occur in European waterbodies but to ensure a range of sensitivities is represented in the dataset with the result that any resulting QS is more likely to protect the range of species sensitivities found in nature.

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 2.4.4). 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 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, 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 reliable, relevant data should be considered valid for use in setting a quality standard.**

2.6.2.1 Reliability

Guidance on the principles of data validation and the aspects to be considered is given in Appendix 1, based on REACH guidance. Data are assigned a score according to the reliability of the study.

Further assessment of data generated or assessed under Community legislation such as Regulations (EC) 793/93 and 1488/94 (existing chemicals) or Directives 91/414/EC (plant protection products) or 98/8/EC (biocides) 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 to ‘Good Laboratory Practice’ (GLP), to international (e.g. OECD) test guidelines and submitted under a regulatory regime may be taken at ‘face value’ without further review. This is because they have already been reviewed by a competent authority and there is a precedent for their acceptability. 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 very short test durations.

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

1. Only data that can be considered as valid 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 in a database with quality scores clearly assigned to each datum. **Only those considered as valid (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 <u>Relevance</u>

A study can be well conducted and fully reported but the test endpoint may have little ecological significance. Studies used for EQS derivation should be those where the test endpoint 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 be faced with 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 to 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 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 no 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, ie 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, eg in the derivation of an SSD, for aggregation of data for the same species and end point 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 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) for estimating 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 (ECHA 2008). 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 Pestide 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 2.6.3) 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, eg polyaromatic hydrocarbons (PAHs) is preferred compared to the QS for individual substances (Section 2.6.5).

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

Some mixtures are intentionally emitted with a known and largely constant composition, but change after entry into environment, for example pesticide and biocide preparations. Other mixtures are released with a partly unknown, reasonably constant composition, but change after entry 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 an 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 published in 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 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 91/414/EEC

Many pesticides currently on the EU market have been reviewed under the Plant Protection Products Directive (91/414/EEC) which includes an assessment of freshwater ecotoxicity data. The data are peer-reviewed by a competent authority, they usually follow standard (OECD) test methods, and are performed to GLP so the studies are fully auditable. Non-regulatory data, ie 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 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 assessment is based on a field margin ditch scenario close to the point of application, which would not normally apply under the WFD: the WFD seeks to provide protection to all waterbodies, including lakes, rivers, transitional and coastal waters.
- The 91/414/EEC assessment makes an allowance for recovery from impacts. This does not feature at all in the Annex V methodology under 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 risk assessment 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.
- Algal toxicity data are dealt with differently under REACH and 91/414/EEC. This can lead to different outcomes when algae are the critical data determining the threshold (Lepper, 2005).
- Under 91/414/EEC, 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 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

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 variation 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 may be as low as 1 or has high as 10000) to extrapolate to a QS, the AF allowing for the uncertainties in the available data. Probabilistic methods adopt species sensitivity distribution (SSD) modelling in which all reliable toxicity (usually NOEC) data are ranked and a model fitted. From this, the concentration protection a certain proportion of species (typically 95%) can be estimated (the HC5).

Laboratory and (where available mesocosm) data are used to derive QSs that account for direct toxicity of chemicals to pelagic and sediment-dwelling organisms. 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. bioassays) or other specific effects that have not been adequately reflected in bird or mammals studies used to derive the NOAELoral (e.g. only 28day 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 increased if data for sensitive taxa are missing when dealing with substances with a specific mode of action like insecticides, herbicides or antibiotics.

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 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 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). 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 unless an 'added risk' approach to compliance assessment is to be adopted (Section 3.5). However, if uncertainties in the extrapolation are largely responsible for the QS being below the background level (e.g. an AF > 50 is required), this must be highlighted in the datasheet as a key uncertainty for the policymaker.

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. Rather, they are transformed from one chemical form to another. 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, can limit growth, survival and reproduction of the organisms. Excess amounts of certain metals, on the other hand, are potentially toxic. Table 2-1 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 2.1 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. Guidance on both bioavailability and backgrounds is provided in more detail in the Sections dealing with specific media (See Section 3.5, 4.7 and 5.2).

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[6] average concentration (AA-EQS) and normally based on chronic toxicity data

and

(ii) a short-term standard, referred to as a maximum acceptable concentration 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 Including aspects of water management and monitoring into the final decision about EQSs

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

[6] 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)

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 policymaker 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 policymaker 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.

2.11.3 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 thepreferred method of EQS expression/compliance assessment with policy makers or river basin managers.

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 exposure, 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 $QS_{biota\ sec\ pois\ fw}$)[7] (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 ($QS_{dh,hh}$) (Section 3.9)

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 (ie biota, hh) 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 particular requirements for deriving 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 exposure 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 to distinguish this value from the QS mentioned in (i)

[7] 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 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 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 3.1.

Table 3.1 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 effect concentrations 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 inform an understanding of 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[8]) 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[9].

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.

8 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 to perform a meaningful statistical comparison.

9 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 not 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 can not be extrapolated to the marine environment and therefore freshwater and marine NOECs can not 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 the 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 modeling ('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 for substances that are released continuously. **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**. In all three methods, 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. These 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. If all methods can be performed, the final $QS_{fw, eco}$ should preferably be based on the results from the SSD method or the model ecosystem-studies, since these entail a more robust approach towards assessing ecosystem effects. It cannot be stated beforehand which method is preferred, the selection of 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 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, both the SSD and mesocosm should include species that are likely to be sensitive. If sensitive species are not available, nor represented in the mesocosm studies, 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 to apply 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 3.2). The procedures for estimating an AA-$QS_{fw,\ eco}$ are the same as the aquatic effects assessment and the calculation of the PNEC (≈ AA-QS_{water}) 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 species tested is a standard test organism (see notes to Table 3.2). A lower assessment factor will be applied to the lowest NOEC derived in long term tests with a relevant test organism.

The algal growth inhibition test of the base set is, in principle, a multigeneration test. However, for the purposes of applying the appropriate assessment factors, the EC50 is treated as a short term toxicity value. The NOEC from this test may be used as an additional NOEC when other long-term data are available. In general an algal NOEC should not be used unsupported by long term NOECs of species of other trophic levels. However if the short term algal toxicity test is the most sensitive of the short term tests, the NOEC from this test should be supported by the result of a test on a second species of algae. The investigations with bacteria (eg 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 3.2 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 3.2.

Table 3.2 Assessment factors to be applied to aquatic toxicity data for deriving a $QS_{fw, eco}$

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels (fish, invertebrates (preferred *Daphnia*) 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 an $QS_{fw, eco}$ from short-term toxicity data.
Variation from a factor of 1000 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 regarded 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. 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.[10]

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 $QS_{fw, eco}$.

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 footnote 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

[10] 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).

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*, the latter in the case of the marine environment, can be considered to fill the gap when data for *Daphnia* are missing. 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. 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, but 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.* (2002).

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) and 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 most in depth analyses of various uncertainties) although others are permissible

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, 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*., 2002). 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, ie the output from an SSD-based QS is considered reliable if the database contains preferably more than 15, but at least 10 NOECs/EC10s, from different species covering at least 8 taxonomic groups. For estimating a $QS_{fw,\ eco}$, the following taxa would normally need to be represented:

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

SSDs for substances with a specific mode of action

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 be 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 and 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.

Testing goodness of fit

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 of < 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.

Calculating the HC5

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:

$$\text{Log HC5} = Xm - k*s$$

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 cu-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;
- knowledge on presumed mode of action of the chemical (covering also long-term exposure). Details on justification could be referenced from structurally similar substances with established mode of action;
- statistical uncertainties around the HC5 estimate, e.g., reflected in the goodness of fit or the size of confidence interval around the 5th percentile, and consideration of different levels of confidence (e.g. by a comparison between the median estimate of the HC5 with the lower estimate (90% confidence interval) of the HC5);
- comparisons between field and mesocosm studies, where available, and the HC5 and mesocosm/field studies to evaluate the level of agreement between laboratory and field evidence.

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 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 effects observed (and all NOECs derived), 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 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 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 (footnote 'f' to Table 3.2), 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 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 statuses 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 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 3.3) are applied to the lowest credible data (critical data) to derive the $QS_{sw, eco}$. The AFs (Table 3.3) 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 3.3).

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: *Cordylophora caspia*, *Eirene viridula*);
- Annelida. e.g. *Neanthes arenaceodentata*.
- Echinoderms (e.g. sea urchins: *Arbacia punctulata*, *Strongylocentrotus purpuratus*, *Strongylocentrotus droebachiensis*, *Echinocardium cordatum*, *Paracentrotus lividus, Psammechinus miliaris*, or asteroids: *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 which is 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 3.3 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 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. Except for substances with intermittent release, as defined in ECHA (2008), under no circumstances should a factor lower than 1000 be used in deriving a $QS_{sw, eco}$ 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 an 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 an 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 can not 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, 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 an $QS_{sw, eco}$

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 $QS_{sw, eco}$ (ie 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 for 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, eg for substances that exert only sub-lethal effects after prolonged exposure. Steroid oestrogens would 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 3.4. 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), if the available data show that interspecies variations are low (standard deviation of the log 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 may be selected to reflect this, or at least to ensure the MAC is not lower than the AA.

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

Table 3.4 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 most sensitive taxonomic group included in 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 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 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 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 50% effect concentration for 5% of the species, not a no-effect concentration for 5% of the species, because the input of the SSD are L(EC)50 values. An AF is therefore needed to extrapolate to the MAC-$QS_{fw,\ eco}$.(to account for the effects to no-effects 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 for 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 24h 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 and 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 at 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 2.4.4.1 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 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 3.5.

Table 3.5 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 three trophic levels of the base set (fish, crustaceans and algae)		1000
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 most sensitive taxonomic group included in 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 most sensitive taxonomic group	50

	included in data set	
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 most sensitive taxonomic group included in 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*, fish). If the standard deviation of the log 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 extrapolation 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 an MAC-$QS_{sw, eco}$.

3.5 Deriving EQSs for metals

Many of the principles outlined below also apply to all naturally occurring substances, including metalloids.

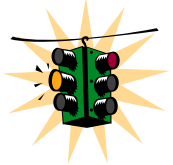
3.5.1 Metal specific mechanisms of action

Advances in our understanding of the physiological processes that control the uptake of inorganic metals and toxicity in aquatic systems 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. Indeed, *bioavailable* metal species (especially free metal ions) have a high affinity for negative binding sites at gills and gill-like surfaces. Some metals, such as copper and zinc, are taken up and eliminated through the sodium, potassium or calcium channels of the cellular membranes, and are often mediated by specific transport systems (e.g. cation ATPases)[11]. Excessive uptake of metal ions can, thereby, cause impairment of the physiological gill functions; the primary toxicity symptom is often an inhibition of active ion transport (Na^+, K^+, Cl^-) that results in ionic imbalances ultimately leading to toxicity (e.g. ICMM fact sheet No. 7; Pagenkopf, 1983; Playle et al, 1992; Di Toro et al., 2001; Grosell et al., 2002; Landner and Reuther, 2004).

The understanding of the interactions between metal species, water characteristics and ionoregulatory impairment of the respiratory organs, as well as acute and chronic toxicity, has formed the basis for metal bioavailability models. The potential for additional toxicity through dietary intake also has been assessed for a range of metals (Cu, Zn, Ni), and the data from laboratory settings (waterborne versus dietborne toxicity, assessment of potential for secondary posioning), mesocosms contaminated with metals (ECI, 2008) and field exposure assessments (Crane *et al*., 2007; Tipping *et al*., 2007) demonstrated that metal EQSs derived from water-only exposures and the application of metal bioavailability models are, at least for the metals investigated, also protective for dietborne exposures as well as of ecosystem structures and functioning.

Research data on metal speciation, metal bioavailability and metal ecotoxicity have been applied in the EU risk assessments for cadmium, zinc, nickel and copper and in the context of the WFD for cadmium (hardness correction)[12]. The models created through such work have allowed a reduction in the intraspecies variability of several orders of magnitude by the normalisation of acute and chronic toxicity data and they adequately predict metal toxicity within a factor of 2.

3.5.2 Generic guidance on setting quality standards for metals in water and sediments



Look Out!

In case of use of bioavailability correction in deriving a QS, the following consideration should be also taken into account:

- Use a QS reference that protects at least 95% of the surface waters instead of 90% in order to follow a precautionary approach.
- Ensure that the use of BLM in upstream parts of a river basin should

[11] Other metals and metalloids may be associated with other uptake mechanisms; for example, arsenic and polonium are often associated with the uptake of phosphorus.

[12] 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.

	not lead to environmental problems in downstream inland-, transitional- or marine waters, either in the water phase and/or in the sediment and/or in biota due to a changes in bioavailability. • Investigate trend monitoring to evaluate the accumulation of pollutants in sediment. • Ensure that the efforts to reduce emissions (source oriented track) by improving techniques are not diminished. • Reconsider the applicability of bioavailability corrections by evaluating the state of play, for instance every 6 years.

The following generic guidance relates to deriving QSs for metals in water and sediments. For guidance on deriving standards for biota and secondary poisoning, see Section 4.6; for more detailed guidance on sediments, see Section 5.2.2, for an explanation of the specific temporary standards used to derive an EQS see appendix 6

The methods used to incorporate 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)).

Figure 3.1 and the text below outline the different steps that allow QSs for metals to be derived for freshwater, marine and benthic compartments in a way that accounts for (bio)availability and background concentrations. The guidance provided is focused on the setting of an AA-EQS, based on chronic ecotoxicity data (NOECs/EC10s) and chronic bioavailability models. A similar approach can nevertheless also be followed when a MAC-EQS is to be derived, based on acute data (EC50s) and acute BLMs.

Because of the differences in iono- and osmoregulatory environments, there may be differences in the toxicity of a substance, and especially of a metal, to freshwater and saltwater species, and it is important to check for such differences. Thus, data should only be pooled if the sensitivity of saltwater species cannot be shown to be significantly different from the sensitivity of freshwater 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.

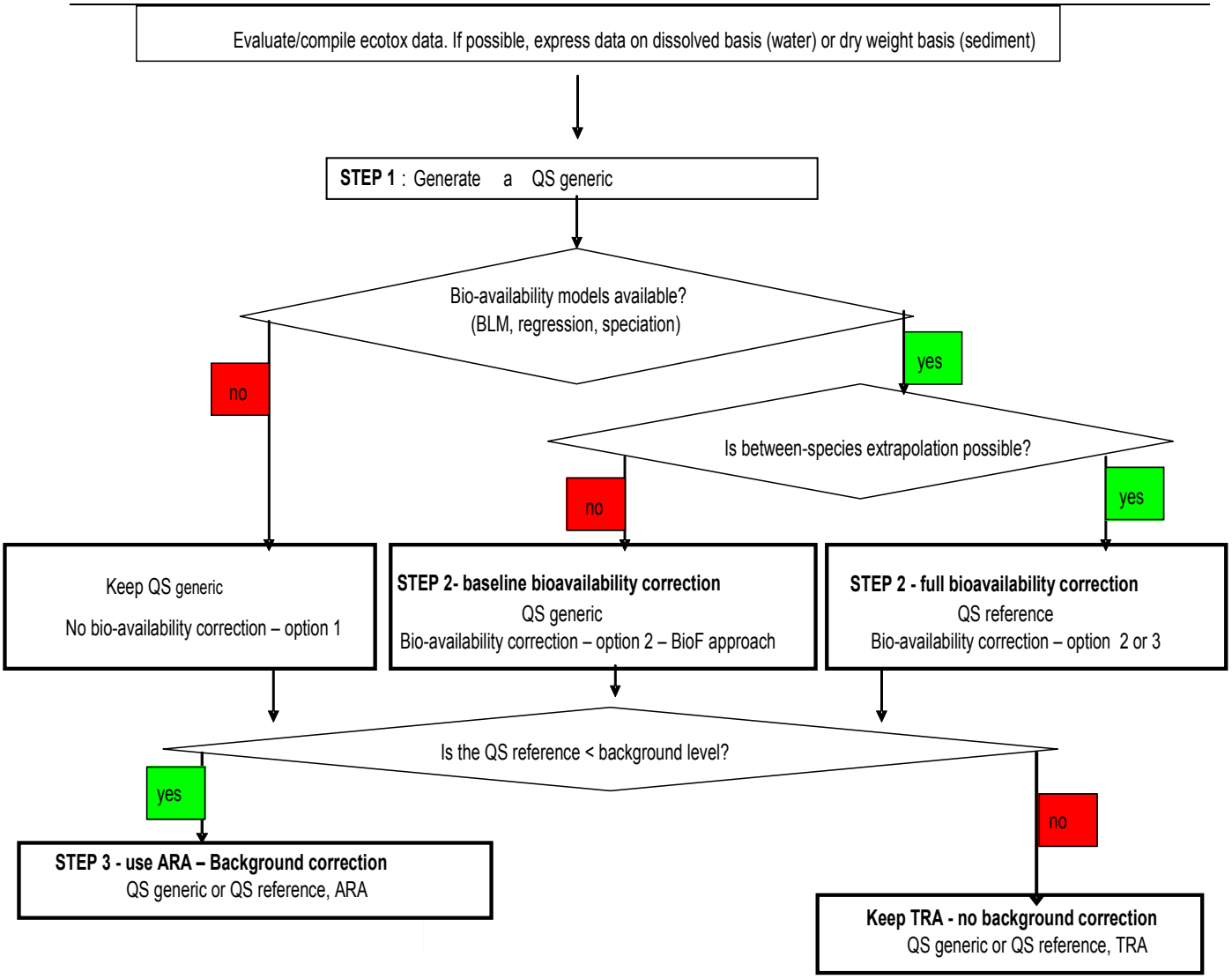


Figure 3.1 Recommended general scheme for deriving QSs and the consideration of bioavailability and background corrections

TRA = total risk approach, ARA = added risk approach (The ARA should not be used in combination with bioavailability correction)

3.5.2.1 Deriving the QS for freshwater

There are three main steps in deriving the QS which are outlined in Figure 3.1. These three steps are the development of a 'generic' QS using ecotoxicity data (Step 1), a QS using bioavailability considerations (Step 2) and a QS accounting for natural backgrounds (Step 3).

The available toxicity data first needs 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 are to be considered as outlined below.

STEP 1

For the water compartment the first step is simply to express the toxicity data on the basis of the dissolved concentration, after filtration using 0.45-µm filters. Any matrix effects related to the filtration of samples should be assessed[13].

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 if possible, taking the following into account:

- For some metals and soluble metal salts (e.g. Zn, Cu) tested in artificial media (and especially when tested in semistatic 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[14].
- For other less soluble metals, however (e.g. lead), an additional step to convert the total concentration into a dissolved fraction is needed. An analysis of relevant solubility products for the relevant metal salts or the ratio of matched dissolved and total metal monitoring data can inform this estimation of dissolved metal concentrations. Solubility products may be found in, for example, the *Handbook of Chemistry and Physics*, 86th edition, CRC Press.
- If test media are natural waters, total concentrations from individual experiments can be recalculated to dissolved concentrations using partition coefficients (taking binding to DOC into account). It has to be borne in mind, however, that the calculated dissolved concentrations for several metals may be uncertain since the partition coefficient (K_p) has been found to vary by several orders in magnitude.

Once data have been collated derive a $QS_{generic,fw}$ based on extrapolation from ecotoxicity data as described in earlier sections. This should be based on conditions of high bioavailability and on a total risk approach (i.e. backgrounds are not accounted for), thereby adopting a reasonable worst-case approach, as outlined below.

STEP 2 - Bioavailability correction

The influence of the key abiotic factors on metal toxicity needs to be investigated and quantified. The simplest (bio)availability correction is the application of **speciation models**. In cases where speciation models (e.g. WHAM (Tipping *et al*., 1991); MINTEQA2, NICCA (Kinniburgh *et al*., 1999) are available, (bio)availability corrections can be considered[15]. For some metals, models have been developed that go beyond metal speciation and these explain the relationships between abiotic factors and metal bioavailability/toxicity. These are toxicity-based models ranging from

[13] The handling of the samples should not affect the dissolved metal fraction in any way; contamination during sampling and filtration should be avoided by using ultra-pure equipment. All laboratory equipment, such as glassware, plastics, etc., must be rinsed with a dilute acid (e.g. 1% HNO_3 solution) and demineralised water before use in order to remove all metals adsorbed. Acidification should be done after filtration. Appropriate quality assurance measures (e.g. procedural blanks, assessment of the matrix effect) are recommended.

[14] In most laboratory test systems, the suspended solids are low and the dissolved to total ratio is very high, typically 95% or greater. Organic particles (e.g. from faeces and food) that appears in the test systems throughout the test, do not significantly affect the dissolved metal concentration in the test when semistatic or flow-through systems are used. Solubility products may be found in, for example, the *Handbook of Chemistry and Physics*, 86th edition, CRC press.

[15] 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).

simple **regression models** (e.g. Cd hardness function) to the more-comprehensive **BLMs**[16] for copper (Santore *et al*., 2001; De Schamphelaere and Janssen, 2002 and 2004; De Schamphelaere *et al*., 2002 and 2003b), nickel (Keithly *et al*., 2004; Hoang *et al*., 2004), silver (Paquin *et al*. 1999) and zinc (Heijerick *et al*., 2002a; Heijerick *et al*., 2002b)) as applied in environmental risk assessments. The use of these models could be considered for deriving QSs under the WFD.

Where toxicity in laboratory experiments is expressed in terms of dissolved metal concentrations and **speciation models**, **chronic regression models** (e.g. Cd hardness correction) or **BLMs** have been developed and validated for the metal/metal compounds of concern, it is recommended that the no observed effect concentrations (NOECs) and/or the effect concentrations for 10% of the tested species (EC10) are expressed on a 'bioavailable' basis (free metal ion concentrations if speciation models are used; normalised dissolved metal concentrations when regression models and BLMs are used).

Bioavailability models should, however, only be applied within their development/validation domains. The ranges applicable to the models, such as those for pH, hardness (H) and DOC, should therefore be specified in the manuals of the models that are used. In other cases, the use of such bioavailability models is allowed on a case-by-case basis only when strong scientific arguments can be formulated to support their application.

For bioavailability to be incorporated into compliance checking, the relevant physicochemical parameters of the investigated site/region (for example pH_{site}, H_{site}, DOC_{site}) affecting metal bioavailability need to be gathered and checked against the applicability domain of the bioavailability model. Site-specific physicochemical parameters are preferred, but if these are not available, information from adjacent sites or similar eco-regions can be used.

The incorporation of (bio)availability into the QS means that compliance monitoring must also be based on (bio)available concentrations. Details are given below.

Implementing a bioavailability based EQS

The following options can be used to correct for availability/bioavailability and for compliance checking (see also Figure 3-1):

Option 1: If there is no relationship between the abiotic factors and toxicity the only viable option is implementing a $QS_{generic,fw}$ as the AA-EQS based, if possible on the lowest species-specific geometric mean EC10s and/or NOECs or SSD approaches as described in Appendix 1. Compliance monitoring is then simply based on dissolved concentrations of metals.

If a bioavailability based approach can be adopted then there are two ways of implementing the QS.

Option 2: The first tier consists of comparing the monitoring results for the dissolved metal from a particular region or site (site-specific C_{TRA}) with the $QS_{reference,fw}$ value.

This $QS_{reference,fw}$ should in principle be protective for all waterbodies that may be monitored. Where possible, the toxicity data should be normalised to a well-defined 'reference' condition that is based on a reasonable worst case (to ensure all waterbodies are protected). Different options are possible to define a reference condition and thus to derive a $QS_{reference,fw}$.

[16] The BLM mathematically integrates the interaction of a trace metal with solution phase ligands to predict its speciation and its subsequent interaction with receptor sites (the biotic ligand) on the organism (ICMM fact sheet No. 7).

Examples of this may be:

- Use the relevant 10th or 90th percentile (depending on parameter) of the bioavailability parameters in Europe, e.g. if DOC is an important parameter, the DOC level used should correspond to the lower 10th percentile of DOC concentrations found across Europe. Unrealistic scenarios induced by combining parameters (e.g. pH, hardness) need to be avoided.
- Use conditions that apply to a sensitive eco-region or river representative of a reasonable worst case of the area to be protected by the $QS_{reference,fw}$.
- Considering that ecotoxicity tests are usually carried out under conditions that maximise bioavailability, an alternative option would be to use the $QS_{generic, fw}$, (non-normalised QS), as the $QS_{reference, fw}$. This alternative has the disadvantage that the water conditions in ecotoxicity tests are variable and, thus, the actual boundaries of the $QS_{reference, fw}$ water conditions are not well defined or would have to be obtained indirectly from model calculations. However, this option allows a common approach to setting $QS_{reference, fw}$ for metals, irrespective of whether bioavailability models are available or not (see Option 1).
  To avoid the situation in which some EU countries have waterbodies that are unprotected by the $QS_{reference, fw}$, the assessor should also define, when publishing the $QS_{reference, fw}$, the boundaries of the water conditions for which the $QS_{reference, fw}$ is derived. If the physicochemical conditions of a specific river basin fall outside the $QS_{reference}$ protection zone (e.g. DOC and/or pH values of <10th percentile of Europe or the most-sensitive eco-region), but inside the BLM developed/validated boundaries; then to ensure protection of the ecosystem, for each of these sites, a $QS_{site-specific, fw}$ may be derived and assessed against the monitoring data for compliance. If the physico-chemical conditions of the site fall outside of the BLM boundaries the $QS_{generic, fw}$ is applied.

Compliance is achieved when measured concentrations are less than the $QS_{reference, fw}$ value. If the $QS_{reference, fw}$ value is exceeded (bearing in mind that the EQS derived from this value may be expressed as an annual average, in which case several samples taken over the period defined in the standard contribute to the decision about compliance or failure), then a (bio)availability factor (BioF) will be applied to the monitoring data C_{TRA}. The BioF is based on a comparison between the expected bioavailability at the reference site and that relating to site-specific conditions.

Option 3: This is identical to that described in Option 2 except that bioavailability correction is applied to the QS instead of the monitoring data. The end result is the same but Option 3 results in a site-specific QS, which might be preferable in some cases. If the $QS_{reference}$, fw is exceeded then a site-specific QS is derived relevant to the site-specific conditions ($QS_{site-specific, fw}$), which is assessed against the monitoring data for compliance. Effectively, (bio)availability is accounted for in the QS rather than in the monitoring data – the reverse of Option 2.

Options 2 and 3 only differ in that they apply the bioavailability correction to the exposure and effects side of the assessment respectively.

The preferred choice of Options 1 to 3 and practice for site-specific QS and BioF calculations depends on (1) the availability of suitable models (see Criterion 1 below), (2) the extent to which it is possible to read across between species for which a BLM has been developed and species for which a BLM has not been developed (Criterion 2) and (3) preferences from policy/administrative points of view.

Criterion 1: The availability of models

If the (bio)availability correction relates to chemical 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. $[M^{2+}]$) and ecotoxicity (NOECs/EC50s) has been developed, the observed quantitative relationship can be applied to all ecotoxicity data selected for EQS derivation, and a $QS_{reference,\ fw}$ corrected for availability can be derived as described under one of Options 2 or 3.

If models are available that involve bioavailability correction (e.g. BLMs), the models may be species-specific and, therefore, bioavailability correction is only possible if the BLM models have been developed and validated for at least three higher taxonomic groups, including an algal, an invertebrate and a fish species. Bioavailability corrections based on the three species only is considered as the baseline correction. **If read-across of the models to other species cannot be demonstrated, bioavailability corrections can only be carried out for the BLM species and the $QS_{generic,\ fw}$ can not be translated to a $QS_{reference,\ fw}$. Therefore the most-conservative BioF is subsequently used on a metal by metal basis**. The most-conservative BioF or baseline BioF is the ratio of $QS_{generic,\ fw}/QS_{site\text{-}specific,\ fw}$, determined as the highest ratio of the $NOEC_{generic}/NOEC_{site\text{-}specific}$ calculated for the three BLM (regression model) species. This approach is expected to provide the most-conservative implementation of bioavailability. In such cases, bioavailability correction of monitoring data is preferred over adjustments to the toxicity data. For compliance assessment, the bioavailable exposure concentration of the monitoring data value is, therefore, calculated as $C_{TRA} \times$ BioF, and this is compared with the $QS_{generic,\ fw}$ (Option 2).

Criterion 2: BLM read-across between species

Full BLM normalisation of the entire NOEC (for chronic data) dataset is justified and full bioavailability correction can be performed only if models are available (Criterion 1) and if additional quantitative evidence is available to confirm the applicability of the three BLMs to at least three additional taxonomic groups (at least at the level of class, but preferably at the level of phylum, eg Cyanophyta, Protozoa, Mollusca, Rotifera, Insecta, higher plants). The accuracy of the BLM predictions for the additional taxonomic groups should be proven by showing that the model actually decreases the variability in the data for the investigated additional species, otherwise the BLM read-across is not applicable for that species. In such cases, chemical (abiotic) normalisation might be considered (more details are available from the background document). Full BLM normalisation consists of applying the bioavailability model across species of similar trophic levels (e.g. applying the *Daphnia magna* BLM for normalisation of the toxicity data from other invertebrates). The bioavailability model normalises the chronic effects concentrations (NOEC or EC10) of the metal for each species' endpoint, and a normalised $QS_{site\text{-}specific,\ fw}$ (i.e. a site-specific QS) is calculated. This $QS_{site\text{-}specific.\ fw}$ is compared to the monitoring data for compliance checking (Options 3). Alternatively, the $QS_{site\text{-}specific,\ fw}$ can be used to calculate the site-specific BioF. In this case, the BioF full bioavailability correction is calculated as $QS_{reference,\ fw}/QS_{site\text{-}specific,\ fw}$ ($QS_{site\text{-}specific,\ fw}$ calculated from full BLM normalisations). The bioavailable exposure concentration is then calculated as $C_{TRA} \times$ BioF, and this is compared with the $QS_{reference,\ fw}$.

STEP 3 – Accounting for backgrounds: total risk versus added risk approach

In a TRA, no explicit account is taken of natural background levels; this approach accounts for the total dissolved amount of a metal in a waterbody. This means that no distinction is made between the fraction of a metal that is present in a waterbody for natural reasons and the fraction added because of anthropogenic activities.

Preferably, metal QSs should be based on the TRA. However, QS values below natural background levels may be generated if:

(1) The QS has been set to an unrealistically low level simply because of a (too) conservative approach adopted in the QS derivation (i.e. a large AF) to compensate for uncertainties arising from a lack of reliable (eco)toxicological data.

(2) The QS was set using ecotoxicity tests with organisms cultured/tested under conditions of low metal concentrations compared with the surface water background levels (i.e. organisms locally

may have adapted to higher natural concentrations). This may occur, especially for metals with a significant background concentration in relation to the estimated QS.

Setting QSs below the natural background level would result in an EQS that serves little regulatory purpose and is scientifically indefensible. Furthermore, many waterbodies would fail the QS even though there is no risk to biota. A pragmatic way to overcome this problem is

- to evaluate the scope for refining the QS by reducing uncertainty (including making a correction for bioavailability) and/or
- to use the added risk approach (ARA).

To assess the need for applying the ARA, the $QS_{reference,\ fw}$ (or $QS_{generic,\ fw}$) and the background metal concentration in the EU, taken as the 90th percentile value from the FOREGS database (http://www.gsf.fi/foregs/geochem), should be compared. If the 90th percentile background value is higher or similar to the QS, the ARA should be used preferentially. The procedure for determining local 'natural' background levels is described in Section 3.6.

The ARA was discussed for the purpose of setting QSs by Lepper (2005). This approach accounts for natural background concentrations and avoids setting regulatory standards below this background level in a simple manner: a maximum permissible addition (MPA) to the background level of a certain metal is calculated. The MPA is the maximum amount of a metal that may be added to the local background concentration of this metal without adversely affecting the assessed ecosystem. Correct determination of the natural background level is key in this approach, and this may not be easy to achieve. As background concentrations are often estimated from relatively small datasets, the calculation of background concentrations should be an iterative process, reviewing the values when new monitoring data become available.

In the ARA, the $QS_{added,\ fw}$ is derived from toxicity data that are based on the added concentration of the metal in the toxicity tests without the background concentration in the test media. In order to use the ARA, the toxicity data should thus be re-evaluated. From each toxicity study, the background concentrations presen tin test medium or test water should be subtracted from the total measured concentrations in the test. The result of the study (NOEC, EC10) should then be calculated on the basis of these 'added' concentrations. The QS should be derived using these 'background-corrected' NOECs or EC10s and is termed $QS_{added,\ fw}$. Where bioavailability correction is possible an ARA approach will not normally be used – only the TRA approach.

To assess compliance, the background concentration (Cb) can either be added to the $QS_{added,\ fw}$ ($QS = QS_{added,\ fw} + Cb$) or the monitoring data can be corrected for background concentration ($C_{ARA} = C_{TRA} - Cb$). If the $C_{TRA} < QS$ or $C_{ARA} < QS_{added}$, then compliance is demonstrated. If, for example, the background is expressed as total dissolved metal, but the QS is expressed as bioavailable metal, then the two options may not be comparable. These approaches require that the monitoring data (including the background) and the QSs are compared on the same basis: dissolved concentration or the bioavailable metal fraction.

Under specific local geological circumstances (e.g. in mineralised areas), the local background concentration can be substantially higher than the regional background concentration. The ARA may still be used to assess the possible risk related to anthropogenic emissions in such areas. However, the variability of the local background levels can be substantial under such conditions and policymakers will need to decide on a case-by-case basis whether the (generic) QS can still be applied at all (the local natural ecosystem may be different from the generic ecosystem used to derive the QS). In this respect, it should also be noted that the principle of the ARA cannot be stressed infinitely: if possible, an upper limit for the value of the QS + background level ($QS_{ARA,dissolved/bioavailable}$) may be derived. In practice, this upper value may be formed by the calculated predicted no-effect concentrations for secondary poisoning or human health in water ($QS_{fw,\ secpois}$ or $QS_{dw,\ hh}$) that have also to be considered when local background values are (very) high. Another reason for setting an upper value is that, in reality, the relationship between toxicity and natural background concentrations is unknown, and that some populations might in fact live close to their tolerance limit. It should be stressed that this upper value is **not** a maximum

acceptable concentration (MAC-EQS). The MAC-EQS refers to short-term exposures that occur in peaks and in connection with intermittent releases, while the above-mentioned upper limit refers to long-term exposures and to an average concentration (typically based on a year) for the release period.

With data-poor substances, there will often be no information available on the relationship between total and dissolved concentrations, or between abiotic parameters and the dissolved fraction. Therefore, it will not be possible to take bioavailability into account if only total concentrations are given. However, extra effort should always be made to try to take availability into account in the reference ecotoxicity value to which the assessment factor is applied.

The decision to follow the ARA approach will be made after comparing the QS with the background.

Following the ARA, bioavailability can further be considered as in Step 2, but considering only the added fraction at the exposure side (Step 2, Option 2) or the added fraction at the effects site (Step 2, Option 3). Under no conditions should background levels be considered if a total $QS_{reference,\ fw}$ is used.

3.5.2.2 Bioavailability correction for saltwater

Freshwater and marine organisms face very different iono- and osmoregulatory issues related to living in either a very dilute or concentrated salt environment. Differences in iono- and osmoregulatory physiology may also lead to differences in metal accumulation and metal toxicity (Prosser, 1991; Wright 1995; Rainbow, 2002). Despite these apparent physiological differences, it has been shown that marine fish also suffer from osmoregulatory disturbances under metal exposure and, therefore, similar toxicity mechanisms may apply (ECI, 2008).

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, Cu RAR, 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 ($NOEC/EC_{50}$), 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 7.8–8.3), 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[17] defines receiving marine environment scenarios. The model includes DOC values for coastal and open ocean waters of 2.0 and 0.2 $mg \cdot l^{-1}$, respectively. The applicability of 2.0 $mg \cdot l^{-1}$ 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 a standard level of 2.0 $mg \cdot l^{-1}$ DOC is,[18] therefore, to be used for deriving a coastal water $QS_{reference,\ sw}$. Alternatively, and if no bioavailability correction can be carried out, a non-normalised generic QS can be used as $QS_{generic,\ sw}$. This corresponds to the Option 1 or Figure 3-1.

Where the waterbody does not comply with the $QS_{reference,\ sw}$, availability can be accounted for by applying Step 2 (see Figure 3-1). Similar to the procedure described for the freshwater compartment (Section 3.5.2.1), availability can be corrected by several means:

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

[18] If DOC has been added to the test media (e.g. as humic acids), the difference in binding strength of the natural DOC compared with added DOC is to be considered.

Option 2: If the marine $QS_{reference,\ sw}$ is exceeded (Tier 1), then a BioF can be applied to the monitoring data value. The BioF is based on a comparison between the expected availability at the reference site and that relating to site-specific conditions. The bioavailability correction for a site can be performed (Tier 2) as follows:

Calculate the BioF using BioF = $QS_{reference,\ sw}$ (2.0 $mg \cdot l^{-1}$ DOC)/$QS_{site\text{-}specific,\ sw}$ (normalised to the site-specific $mg \cdot l^{-1}$ DOC.

Determine the available dissolved metal concentration at the site, calculated as dissolved metal concentration $\times$ BioF.

Compliance then can be checked as available dissolved metal concentration at the site < $QS_{reference,\ sw}$.

Option 3: If the marine $QS_{reference,\ sw}$ is exceeded (Tier 1), then a marine $QS_{site\text{-}specific,\ sw}$ is derived based on the site-specific DOC concentration (using the empirically observed relationship between the NOECs/EC10s and DOC) and this value is assessed against the monitoring data for compliance (Tier 2).

The DOC correction proposed for the marine environment is a simple 'availability' correction, irrespective of the species considered and 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.

3.5.2.3 Using mesocosm and field data for metals

Similar to deriving a QS for organic substances, high quality mesocosm and field data can be used for QS derivation for metals. The quality criteria to be used are the same as those used for organic substances, but some metal-specific issues are to be considered as outlined in Section 3.6.

If a bioavailability correction can be applied, then QSs normalised to the physicochemistry of the mesocosm/field studies are recommended .

3.6 Estimating background levels of metals

3.6.1 General comments

If the QS is below or close to the natural background level and there is no further scope for reassessing either backgrounds or the derivation of the QS, then the ARA may be applied. The general definition of natural background level is the concentration that is present owing to natural and geological processes only, i.e. the background level with no anthropogenic contribution ('pre industrial' levels). In reality, true pristine areas are rare within Europe, and it must be considered on a case-by-case basis whether a given area represents a pristine condition for a specific metal.

In most areas in Europe, any estimate of a natural background concentration will inevitably include a small contribution from anthropogenic sources because much of Europe's landscape has been altered by man's activities for mineral extraction, agriculture or habitation for millennia and this historical contribution may be obscure. In addition, long-term anthropogenic activities, such as drainage, irrigation and special crops (e.g. conifers creating acid soil conditions), may influence environmental release of metals. This contribution is difficult to quantify and distinguish from what concentrations might have been in the absence of such activities. Finally, contributions from diffuse anthropogenic sources, eg aerial deposition, may be impossible to eliminate entirely.
Therefore, any estimate of a background concentration will more likely be an 'ambient' background concentration rather than a value relating to a purely natural pristine environment.

3.6.2 Estimating backgrounds for freshwater

The natural background concentration is determined by mineral and biological factors. A major contribution to the background concentration will be from weathering of surface geology and any groundwater spring inputs. Therefore, a 'global' natural background level will normally not be meaningful because of the great variation between different regions.

In freshwater, the preferred procedure for assigning a 'natural' background will usually be to determine the concentrations in springs and/or in waterbodies in 'pristine' areas in the given region, e.g. headwaters. Other possibilities are:

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

Pristine waters are scarce and, in practice, mainly restricted to the immediate vicinity of a source. Further downstream, the water will take up the remnants of decaying organic material in the form of DOC. Plants contain substantial amounts of essential elements extracted from the soil that remain present through binding to DOC, thereby causing a natural increase in metal background concentrations. De Schamphelaere et al. (2003b) have measured the natural zinc and copper content bound to DOC. If such bound DOC concentrations measured in practice are taken into account in many surface waters, this natural contribution appears to exceed the mineral contribution described above (see VROM report VEM july 2004, appendix 3 in Dutch).

In other situations, biological depletion may take place, c.f. the great lakes in the USA, but also European mountain lakes with long residence times. In such cases, the natural background concentration might be below the pristine source concentration. This is due to the uptake of essential elements from the upper water layers by organisms which, after death, fall to the deeper regions of the waterbody, thereby taking with them the essential metal. Natural background concentrations may decline in this biological depletion process by over one order of magnitude (e.g. Nriagu *et al*., 1996).

In practice, the input data needed to determine background concentrations in pristine areas by modelling may be inadequate to estimate a reliable value. An alternative pragmatic approach in these cases is to take the 10th percentile dissolved metal concentration of all the monitoring data available for the waterbody or region (after removing sample results with elevated concentrations from known point source discharges or pollution events). If this technique is used, some interpolation of the distribution of values is needed from the laboratory's reporting limit (the 'less than' value) and zero. Using this approach, an example from the Mersey hydrometric area (UK) produces 5th and 10th percentile values of 3.0 and 3.7 $\mu g \cdot l^{-1}$, respectively for dissolved zinc (Figure 3-2).

Further, 'hot spots' may also be located using geological information.

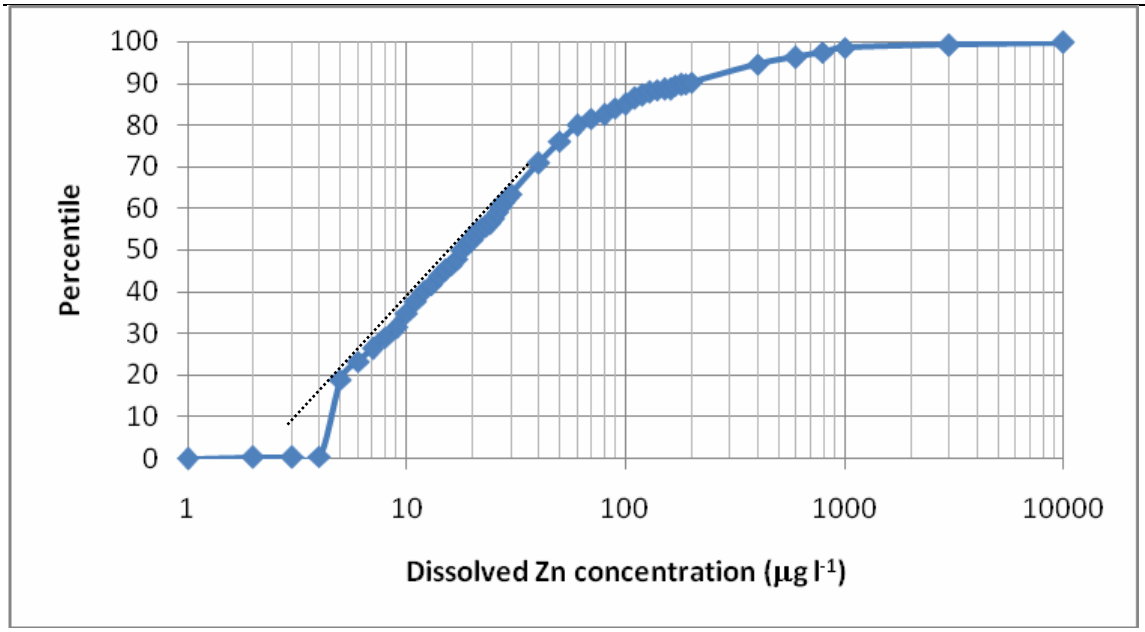


Figure 3.2 Distribution of dissolved zinc concentrations in the Mersey hydrometric area (UK)

A comparison of freshwater background concentrations based on a wider river basin level or more-local hydrometric area is given in Table 3.6. The British Geological Survey (BGS) Geochemical Baseline Survey of the Environment (G-BASE) project data of single measurements taken at small, relatively unimpacted streams are also shown.

Table 3.6 Example freshwater background concentrations based on river basin and hydrometric area levels obtained from different sources

Metal (Dissolved)	FOREGS Ranges (µg·l^{-1})	BGS G-BASE (Median) (µg·l^{-1})	10th Percentile of Monitoring Data (µg·l^{-1})
Cu UK South West England river basin district default	1.45–1.97	1.6	1.8
Tamar hydrometric area specific	<1.97	1.0	0.5
Zn UK South West England river basin district default	2.68–4.00	3.4	3.2
Tamar hydrometric area specific	<2.86	2.0	2.5

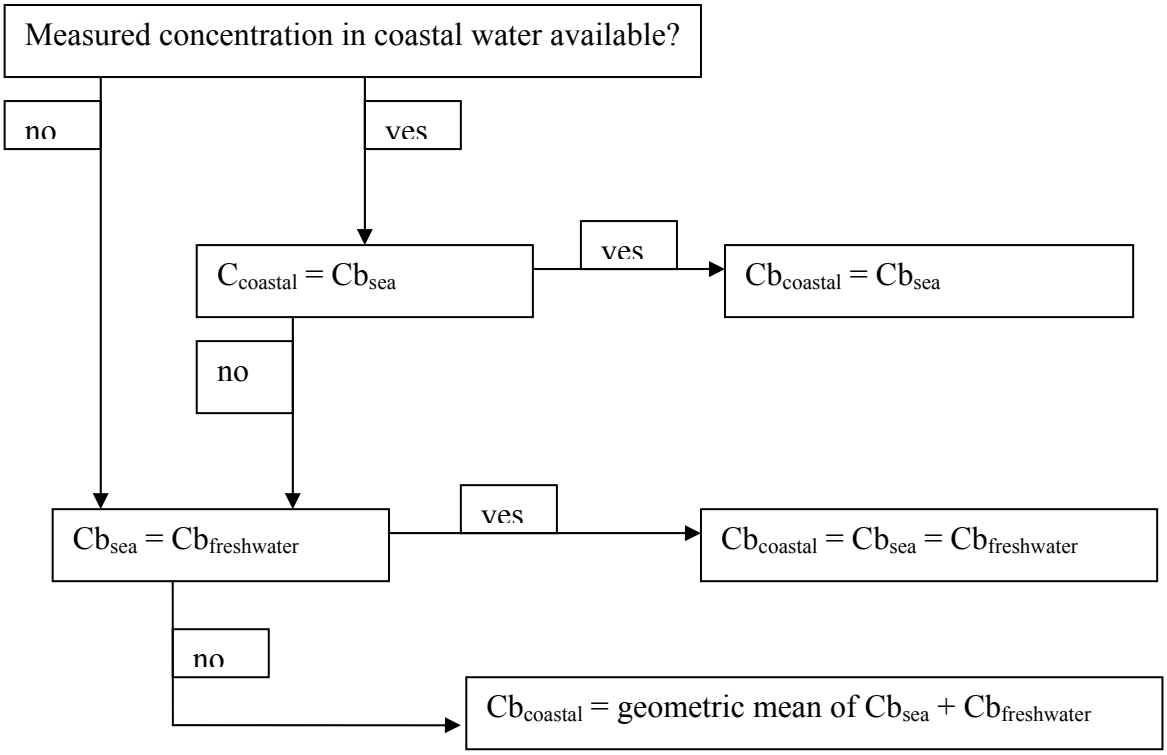
3.6.3 Estimating background concentrations for saltwaters

In saltwater, the concentrations of metals (dissolved) far at sea will normally suffice as natural background levels. Natural background concentrations (Cb) may be higher in coastal waters

because of the natural input from rivers and the settling of particles. The determination of the $Cb_{coastal}$ in such waters may, however, be very difficult because rivers are likely to drain pristine areas as well as areas influenced by anthropogenic inputs, and thus a pragmatic approach is needed. As a starting point (see Figure 3-3), the dissolved metal concentration in the coastal water is compared with the Cb at sea (Cb_{sea}). If these values are equal, then the $Cb_{coastal}$ for the coastal water is set equal to the Cb_{sea}. If there are no measurements in the coastal water or if the concentration is greater than Cb_{sea} then the Cb in freshwater and at sea are compared. If they are the same, it will be reasonable to set the Cb in estuaries and coastal waters equal to those in freshwater and at sea. If the $Cb_{freshwater}$ is different from $Cb_{sea,}$ the geometric mean of the two values may be used for coastal waters. In cases where the concentration in coastal water is between $Cb_{freshwater}$ and Cb_{sea}, the $Cb_{coastal}$ is set equal to the measured value. If the $Cb_{coastal}$ values derived as above create no problems in relation to measured concentrations and compliance, then no further refinement will be necessary. Alternatively, the $Cb_{coastal}$ can be derived as the 10th percentile of concentrations measured in coastal waters draining only relatively uncontaminated areas.

Guidance is given in OSPAR (2004) on ambient metal concentrations measured in the waters of the Convention area. However, these data should be interpreted with care when deriving coastal background values. Indeed, the ranges presented for the different metals refer to open ocean ranges which are usually lower in value than those for near and on the shelf (e.g. for Cd and Cu).

It is important to note that preference should be given to values reflecting natural background concentrations for coastal zones, and that some might be found in the literature (e.g. see Laane et al., 1992 for the North Sea; Landing et al., 1995 for the Atlantic Ocean, the UK National Marine Monitoring Programme 2004 http://www.jncc.gov.uk/pdf/nmmp2ndreport.pdf; and ICME, 1996).



Alternatively, $Cb_{coastal}$ = the 10th percentile of concentrations measured in coastal waters draining only relatively uncontaminated areas. If the concentration in coastal waters is between Cb_{sea} and $Cb_{freshwater}$ then $Cb_{coastal} = C_{coastal}$

Figure 3.3 Determining the natural background concentration of a metal in coastal waters;
$C_{coastal}$ = concentration measured in coastal water, $Cb_{coastal}$ = natural background concentration in coastal water, Cb_{sea} = natural background concentration at sea, $Cb_{freshwater}$ = natural background concentration in freshwater; concentrations refer to the dissolved metal

3.7 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 (including the background concentration in the culture medium), **any ecotoxicity study not supported by analytical data (i.e. endpoint concentrations reported as nominal values) would automatically be excluded from the most reliable studies**. Nominal concentrations will usually[19] overestimate the final concentration. Therefore, if the

[19] Except for essential metals (nutrients may be added to the test waters) and if natural waters are used as test waters (the metal concentrations in the natural waters may substantially contribute to the dissolved metal concentration).

lowest effect concentration is a nominal value, then the study should not be discarded unless there are other reasons to invalidate it.

2. *Total versus dissolved metal concentrations in test media*: Measured data on the dissolved fraction (0.45 µm) are required in order to obtain the most reliable toxicity test data. 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 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.

3. *Culture conditions*: If the test organisms have been cultured in conditions that are outside the natural background concentration ranges (see Section 3.6), such data should be discarded from the high quality database and, at best, may only be considered as supporting evidence when selecting the assessment factor[20].

4. *Chelators*: Data from studies in which the test media contain artificial chelators (e.g. EDTA) should be excluded from EQS derivation, except in algal tests where small amounts of chelators (EDTA (can be replaced by natural DOC)) are unavoidable.

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 if corrections for bioavailability are carried out. The aquatic medium used should be characterised by DOC concentration, hardness, pH, alkalinity, presence of complexing agents, such as humic acids and EDTA, and any other specific parameters of importance to 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 the missing data from known physicochemical parameters (e.g. estimate alkalinity from Ca and alkalinity relationships (Adams et al., 2008)) or to use default values derived from other studies using standard test media or from historic monitoring data for natural waters (Santore et al., 2002). 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.

   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 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.

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.

[20] This is especially relevant under the TRA.

7. *Read-across and QSAR*: If ecotoxicity data are lacking for a specific metal or metal compound, read-across of ecotoxicity data from other inorganic compounds of the same metal should be considered. The basic assumption is that the bioavailable metal ion is responsible for toxicity. Ecotoxicity data for simple soluble metal salts, therefore, can be combined on condition that the metal ion alone is responsible for the effects observed for all of the metal salts considered (e.g. $CuSO_4$, $CuCl_2$). Toxicity data measured for all soluble metal salts should, therefore, be used and the effects data (NOECs/EC_{10}s or EC_{50}s) should be expressed as the dissolved (bioavailable) metal ion concentration (µg $M \cdot l^{-1}$).

   The development of QSAR methods for metals and inorganic metal compounds has not been as actively pursued as for organic substances. However, for some inorganic substances, predicting toxicity from chemical properties may be relevant. In this respect, quantitative ion character–activity relationships (QICARs) and quantitative cationic activity relationships (QCARs) have recently been developed (Ownby and Newman, 2003; Walker et al., 2003).

8. *Combining freshwater and saltwater toxicity data*: As explained in Section 3, **freshwater and saltwater data for metals should generally not be pooled if availability corrections have been applied**.

9. *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. Chronic data for metals exhibiting this behaviour should be treated with caution. Greater reliance may need to be placed on field data for such metals.

10. *Estimating bioaccumulation (for back-calculating water concentrations from biota standards)*: Section 4.7.2 details how to determine the relevant experimental bioconcentration factor (BCF) or bioaccumulation factor (BAF) data for metals.

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

3.8.1 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[21]. 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).

Discrepancies between total and dissolved concentrations may only become evident for very hydrophobic substances, ie 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 $EQS_{water,dissolved}$.

[21] 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 $mg \cdot l^{-1}$ 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.

As explained in Section 2.11 some Member States may have a preference to undertake monitoring using total 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 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 total 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). For 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 (Kp,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;
- $EQS_{water,dissolved}$ is the value of dissolved concentration in water, mostly directly derived from the toxicity or bioaccumulation tests;
- $K_{p,susp}$ = partition coefficient to suspended matter ($l \cdot kg^{-1}$), which might be estimated as the product of the K_{oc} value for the substance ($l \cdot kg^{-1}$) and the organic carbon content (f_{oc}) of suspended matter (EU default from TGD (EU 2003) 0.1);
- C_{SPM} = concentration of suspended matter ($mg \cdot l^{-1}$; 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 $mg \cdot l^{-1}$ for freshwaters and 3 $mg \cdot l^{-1}$/L for marine waters and for example, the annual average TOC content of the Rhine in the Netherlands is about 4mg l^{-1}, however, under deviating 'local' environmental conditions other values need to be applied); and
- 10^{-6} is = a conversion factor to convert 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 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 algorithms to calculate the concentration in SPM from the dissolved concentration in water and vice versa are as follows:

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

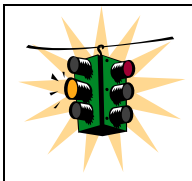
where:

– EQS_{SPM} = quality standard for water referring to the substance concentration in SPM (EU TGD (EU 2003) default 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);

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



Look Out!

The approach chosen in this guidance in case of the absence of a drinking water standard is based on human toxicity. This implies that the precautionary principle and organoleptic aspects such as smell, taste and colour are overlooked. For the production of drinking water these elements play an important role. This means that for some substances there is need for specific measures to limit the risks because of concerns for the potability of drinking water in respect of taste and odour as a consequence of exposure (Commission Recommendation 2001/838/EC).

3.9.1 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 standards are adopted, e.g. EU drinking water standards from Drinking Water Directive 98/83/EC and the World Health Organization (WHO) drinking water standards. These drinking water standards are used to set the $QS_{dw,\ hh}$ for those water bodies used for the abstraction of drinking water ($QS_{dw,\ hh}$). A treatment factor should be applied to the drinking water standard so that the $QS_{dw,\ hh}$ relates to the 'raw' water (i.e. it is an 'environmental' standard). Drinking water standards and treatment processes used to achieve them should be taken into account in determiing 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 standards are available (either DWD or WHO standards) a standard for drinking water abstraction from surface water may be derived by the procedure described in Section 3.9.2.

3.9.2 $QS_{dw, hh}$ for drinking-water abstraction

A QS for the abstraction of drinking water ($QS_{dw,hh}$) needs to be derived as follows (see also Figure 3-4)[22]:

1. If an EU drinking water standard (from Directive 98/83/EC) or a WHO drinking water standard is available, follow the procedure described below. If both the WHO and EU have a drinking water standard and the values are different, the WHO drinking water standard is preferred,.because it is health-based.

    - If the 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.

    - If the drinking water standard 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 removal efficiencies 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.

$$QS_{\text{dw, hh}} = \frac{drinking\ water\ \text{standard (98/83/EC)}}{F_{\text{not removable by treatment}}} \qquad \text{(A)}$$

2. If neither an EU or WHO drinking water standard is available,, follow the procedure described below:
    - A provisional drinking water standard is calculated according to equation B.

[22] 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

$$QS_{\mathrm{dw,\,hh}} = \frac{0.1 \cdot TL_{\mathrm{hh}} \cdot bw}{uptake_{\mathrm{dw}}} \qquad \text{(B)}$$

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 0.1 of the human toxicological standard (TL_{hh}) is allocated to 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.

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

If the compound of interest is potentially carcinogenic[23], 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 an $QS_{dw,\,hh}$ need not be derived and 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 $F_{\text{not removable by treatment}}$.
  2. The $QS_{dw,\,hh}$ is then calculated using equation A.

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

[23] No guidance is given on how to establish the potential carcinogenicity of a compound, but the assessor should check the appropriate R phrases. 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.

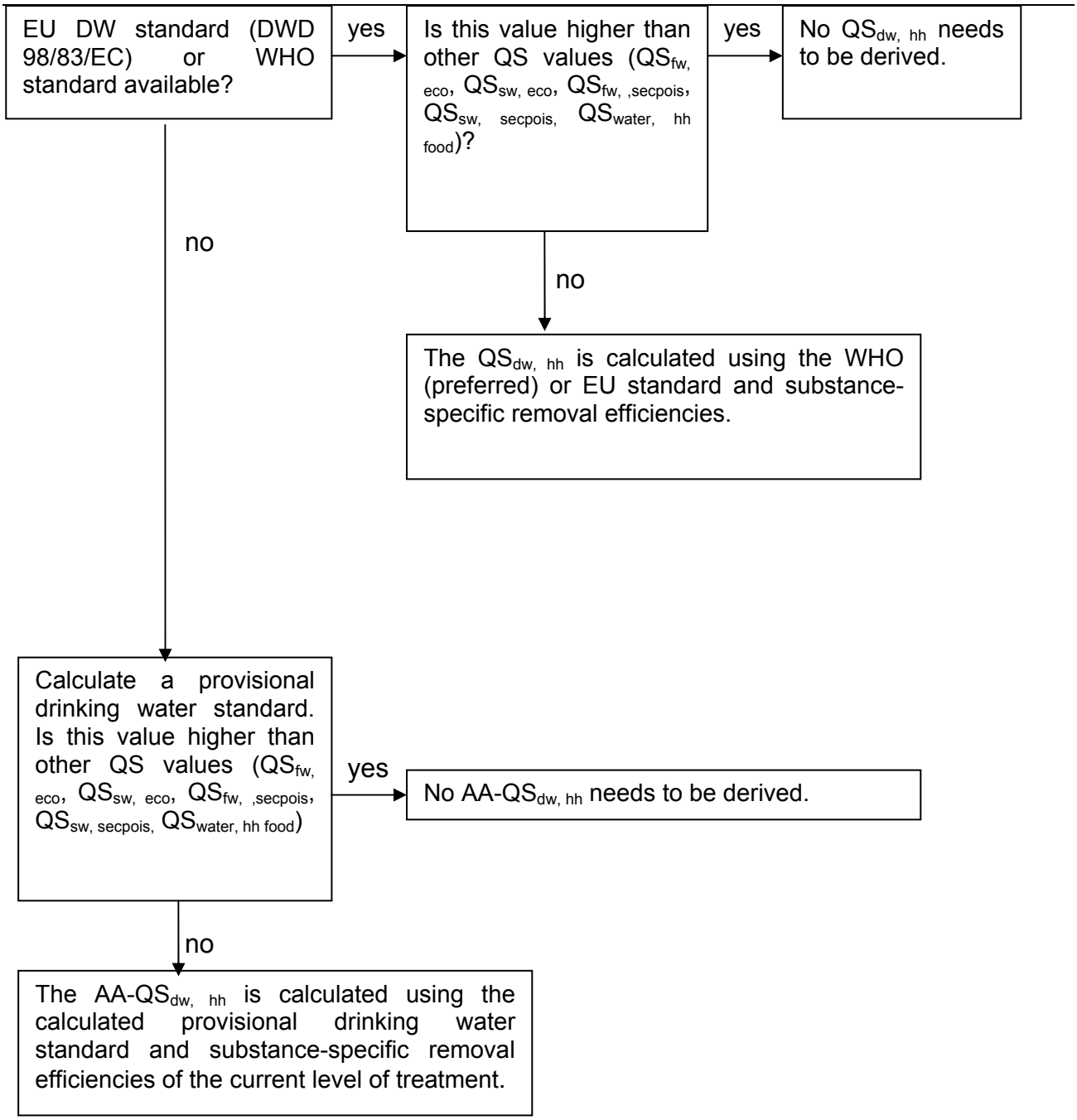


Figure 3.3 Schematic overview of the derivation of the quality standard for drinking water abstraction from surface water ($QS_{dw, hh}$)

4 DERIVATION OF BIOTA STANDARDS

4.1 Introduction

One of the factors leading to unmanageable water column standards is the very low concentrations that may be estimated for some substances, especially those with very low water solubility or 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 may be required alongside, or instead of, the water column EQS. This is typically the case for hydrophobic substances, and biota standards have been proposed 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 line with the requirements of the EQS Directive, these biota standards are presented as possible alternatives to a water column standard.

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. 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 $QS_{biota,hh}$ and $QS_{biota,secpois}$are derived, see Appendix 6). 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. Possible approaches for the future are set out in Appendix 4, but these will need to be developed and tested before they can be adopted as formal guidance. **At present, biota standards developed for birds and mammals are assumed to be sufficiently protective for benthic and pelagic predators**.

The process for deriving and using biota standards to meet these protection goals is illustrated in Figure 4-1. In principle, to derive a biota standard, the assessor must estimate an acceptable level of chemical input when it occurs in the organism's food. Standard toxicity tests are available that estimate a no observed adverse effect level (NOAEL) or a no observed effect concentration ($NOEC_{oral}$) and these values are used to derive a predicted no-effect concentration for the ingestion of food ($PNEC_{oral}$) (taking account of variations between studies, species and test endpoints). Extrapolation from $NOEC_{oral}$ data to a $PNEC_{oral}$ (equivalent to a QS_{biota}) is detailed in Section 4.4.

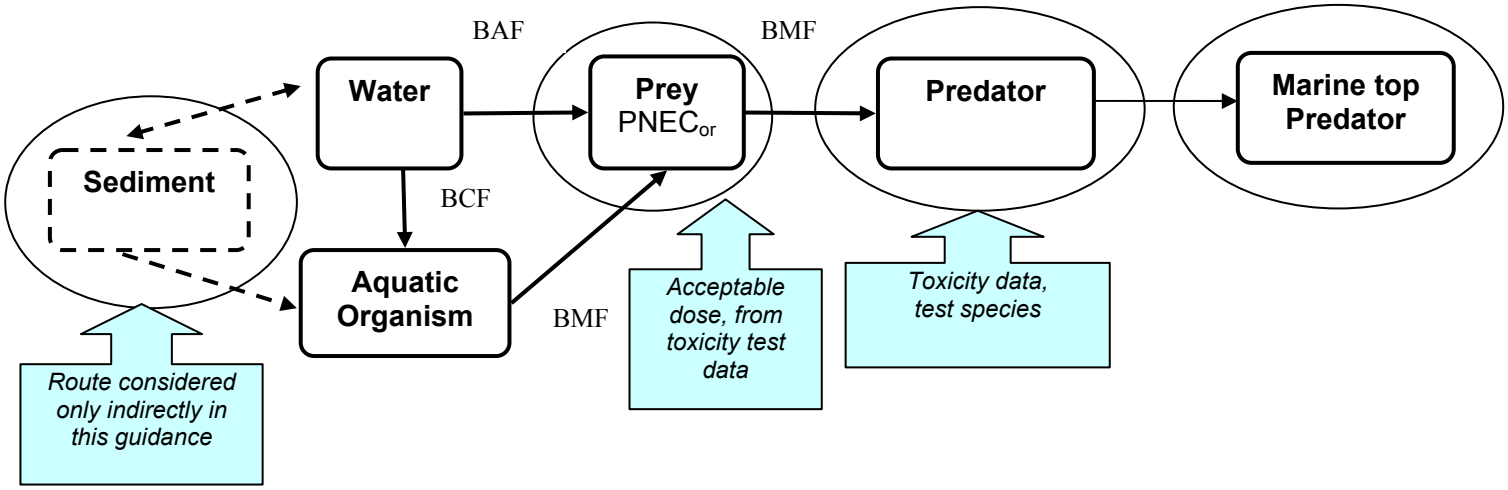


Figure 4.1 Steps involved in deriving a biota standard

Biota standards are preferably expressed as a concentration in an organism – corresponding to the prey items that may form the diet of top predators (including humans). Following the CSTEE (2001, 2004) opinion, biota quality standards are preferably expressed as biota concentrations 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 also helpful when selecting an overall EQS (Section 2.5), so that standards can be compared on the same (mass/volume) basis.

Whilst a biota standard could, in principle, be converted into the equivalent water concentration (one that is predicted to give rise to the critical concentration in biota), there are technical disadvantages with this approach for highly hydrophobic substances (those identified as B or vB according to Annex XIII of REACH). The translation to an equivalent water concentration depends on a good understanding of the bioconcentration, bioaccumulation and biomagnification processes from water and through the food web which 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:

- 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 species are prefereable. The QS should be expressed in terms of g/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. If lipid normalisation is possible and scientifically justified (i.e. the substance primarily accumulates in lipids), all data should be lipid normalised to a standard lipid content of 5% (ECHA, 2008).

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, residues in animals and plants effectively integrate exposure over a period of time and, in any case, 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 time. Greater emphasis should be placed on the spatial design of sampling schemes.

4.4 Deriving a biota standard to protect against the secondary poisoning of predators

Secondary poisoning is concerned with toxic effects at higher trophic levels of the food chain which result from the ingestion of contaminated aquatic organisms from lower trophic levels.
In accordance with Romijn *et al*. (1993) and following the paradigm used under TGD (EC, 2003) and REACH (ECHA, 2008), we will define our food chain with its trophic levels as water –BCF→ aquatic organisms –BMF_1→ fish → fish-eating predator for freshwater ecosystems. For marine ecosystems, however, another trophic level may be introduced: water –BCF→ aquatic organisms –BMF_1→ fish –BMF_2→ fish-eating predator → top predator (where BCF is the bioconcentration factor and BMF is the biomagnification factor). This is illustrated in Figure 4.1.

A QS expressed as the concentration found in prey tissue which should protect predators from secondary poisoning ($QS_{biota,secpois}$) is often referred to as the diet-based approach. In terms of deriving the standard, only one extrapolation step, from food to predator (Figure 4.1), is necessary. Extrapolation to take account of possible differences in sensitivity between species is covered in detail in Section 4.4.4.

4.4.1 Identifying the critical data

Few data for an oral route of exposure are available for organisms other than birds and mammals. Whilst scientific and data developments may allow us to assess risks to 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 $QS_{biota,\ secpois}$ with the corresponding water concentration is significant lower than a QS for protecting pelagic species.

If relevant ecotoxicological information (e.g. fish feeding studies) can be found in the literature or can be produced for supporting sound QSs, the same approach developed for bird and mammals can be used for pelagic fish species.

The general methodology to derive a $QS_{biota,\ secpois}$ is based on the simple food chain described above and assumes that all species at a certain trophic level contain similar concentrations of pollutants. In addition, it assumes 100% reliance on a particular prey item. This assumption is appropriate where EU-wide standards are required (e.g. for Priority Substances and Priority Hazardous Substances) and to promote consistency in approaches across Member States for Annex VIII substances (Specific Pollutants) of the WFD. However, if a site-specific assessment is required, these assumptions may be refined as described in Appendix 4. The lowest reference concentration is used to derive a $QS_{biota,\ secpois}$ for predators. For substances with a high potential to biomagnify within food chains, it is important that the $QS_{biota,\ secpois}$ be applied to the appropriate aquatic trophic level to protect all predators feeding. Application of the $QS_{biota,\ secpois}$ at that level will also protect wildlife feeding at lower trophic levels. Monitoring should be based on the sampling and analysis of tissues from the prey 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.

4.4.2 Data requirements

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 more-sensitive endpoints (i.e. give rise to lower $NOEC_{oral}$ values) than survival endpoints.

As secondary poisoning effects rarely become manifest in short-term studies, results from long-term studies establishing long-term NOECs are strongly preferred. A QS derived where no chronic effects data are available is subject to high uncertainty and this 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. Effects 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. These may be used after correction for daily food intake, as described in Section 4.4.3. 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 $QS_{biota,\ secpois}$ may not be protective. On the other hand, by applying a higher assessment factor than needed, the QS 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 of 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.

Further guidance on bird and mammalian toxicity data and their evaluation is provided in the REACH guidance (ECHA, 2008) and in the European Food Safety Authority guidance document (EFSA, 2007).

4.4.3 Expressing toxicological endpoints as a concentration in food

Mammalian or avian toxicity data may be expressed as NOECs relating to concentration in food ($NOEC_{oral}$, expressed in units of $mg \cdot kg^{-1}$ food) or as no observed adverse effect levels relating to dose ($NOAEL_{oral}$, expressed in units of $mg \cdot kg^{-1}$ $bw \cdot d^{-1}$). For the standard derivation of EQSs for secondary poisoning, the results need to be expressed as the concentration in food because this is the basis of the adopted risk model. The general rule for the conversion is that the concentration in food is equal to the daily dose multiplied by the body weight (bw) divided by the daily food intake (DFI), or

$$NOEC_{oral} = NOAEL_{oral} \frac{bw}{DFI}$$

where:

- $NOEC_{oral}$ = no observed effect concentration ($mg \cdot kg^{-1}$ food);
- $NOAEL_{oral}$ = no observed adverse effect level [$mg \cdot kg^{-1}$ $bw \cdot d^{-1}$];
- DFI = daily food intake (g $food \cdot d^{-1}$); and
- bw = body weight (g).

Table 4.1 presents a guide with a standard set of conversion factors that can be used to promote internal consistency when converting concentrations from dose into diet for mammals. The guide should be used only in the absence of more specific data from the study itself or other sources. For example, a chicken (Gallus domesticus) typically consumes around one eighth of its body weight per day, and so the conversion factor in this case would be 8 kg bw·d·kg–1 food. It should be noted that the conversion factors for young birds and mammals might differ from those for adults. For avian reproduction studies, a default factor of 10 can be used as a conversion factor (i.e. bw/DFI = 10) (see Appendix 6 of EFSA, 2008). For this conversion to be valid, no food avoidance should have occurred in the study. Recommendations from EFSA (2008) should be

considered as indicative. REACH guidance (ECHA, 2008) should be followed rather than EFSA (2008).

Table 4.1 Conversion factors for converting NOAELs (dose) from mammalian toxicity studies into NOECs (concentration)

Species	**Age/study**	**Conversion Factor** (bw/DFI) (ECHA, 2008; EC, 2003)	**Conversion Factor** (bw/DFI) (EFSA, 2008)
Rat (Rattus norvegicus	>6 weeks	20	
Rat (Rattus norvegicus)	<6 weeks	10	
Rat	28 and 90days		10
Rat	Two generation study first mating[a]		12.5
Rat	Two generation study overall (females)[a]		8.33
Mouse (Mus musculus)	28 and 90days	8.3	5.0
Vole (Microtus spp)		8.3	
Rabbit (Oryctolagus cuniculus)		33.3	
Dog (Canis domesticus)	Adult/all	40	40
Monkey (Macaca spp)		20	
Chicken (Gallus domesticus)		8	

[a] The first mating value for a two-generation study should be used for assessment when effects (general or on reproduction) are seen to relate to the pre-mating phase of the first mating, or effects are seen only in male F0 parents at any time. For all other aspects of a two-generation study, the overall conversion figure should be used.

NOECs derived from NOAELs in this way are assumed to be equivalent to directly measured NOECs.

4.4.4 Extrapolation to derive a $QS_{biota,\ secpois}$

Two approaches can be followed to determine this quality standard for biota. These approaches are briefly described here with further detail provided in the following sections.

The first is the standard approach from the TGD (EC, 2003; ECHA, 2008). In this methodology, the concentration in the diet of the toxicity test is the basis for the quality standard in biota. The extrapolation from diet to biota comprises the interspecies variation, differences in exposure duration, as well as the difference in caloric content of the diet of laboratory animals and the diet of fish-eating birds or mammals (EC, 2003).

In the second approach, the dose rather than the diet concentration, is used as a starting point (EFSA, 2008), which helps to minimise bias relating to different food intake rates between laboratory and field situations. A group of key species should represent all the organisms at risk from secondary poisoning. Information on body weight, dietary composition and feeding rate by predators are necessary to select those species most likely to experience the highest exposures to contaminants through the aquatic food web. By definition, if these are protected (and the assumptions are correct) other species will also be protected.

4.4.4.1 Derivation of QSbiota, secpois according to the standard approach in REACH

The quality standard that describes the threshold concentration of a substance in the food of a predator, $QS_{biota,\ secpois}$ ($\approx PNEC_{oral}$, in $mg \cdot kg^{-1}$ food), is derived by applying appropriate assessment factors (AF_{oral}; see Table 4.3) to the selected NOEC oral for each species. 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, use the same species and test conditions and report the same key endpoints. It may be that a test with a 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 may be applied to the most sensitive endpoint.

Table 4.2 Assessment factors for the extrapolation of mammalian and bird toxicity data into $QS_{biota,\ secpois}$ (EC, 2003)

TOX_{oral}	Duration of test	AF_{oral}
$NOEC_{oral,birds}$	chronic	30
$NOEC_{oral,mammals}$	28 days	300
	90 days[a]	90
	chronic	30

[A] for consideration of reproduction studies

Since monitoring in biota in the marine compartment is preferably performed at the level of fish rather than e.g. seals, the $QS_{biota,\ secpois}$ for the marine compartment should include BMF_2 (cf. figure 4-1 in section 4.3. Therefore:

$$QS_{biota,secpois,\ fw} = \frac{TOX_{oral}}{AF_{oral}}$$

$$QS_{biota,secpois,sw} = \frac{TOX_{oral}}{AF_{oral} \cdot BMF_2}$$

The final value for the $QS_{biota,\ secpois}$ is selected by comparison of the different values for the tested species and choosing the lowest resulting values (EC, 2003; Lepper, 2005). If sufficient data are available, there is no reason why a probabilistic approach to extrapolation (ie an SSD approach) should not be used. However it should be noted that in the applied assessment factor the factor of 10 to extrapolate from the lowest chronic NOEC values to the $QS_{biota,\ secpois}$ is already included and that when applying a statistical extrapolation, the NOECs need ony to be converted from subacute

(28d; factor 10) and subchronic (90d; factor 3) to chronic and from laboratory diet to fish or mussels (all data; factor 3). For the application of a species sensitivity distribution (SSD), data should be available for a minimum of 10 species. The dataset should include both birds and mammals and should also include wildlife-relevant predatory species of both birds and mammals. For further considerations, the assessor is referred to Section 3.2.4.2.

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 and acute and long term toxicity (ie 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' $QS_{biota,\ birds}$ by applying a large (precautionary) AF of 3000 to the lowest reliable lethal concentration for 50% of the individuals (LC50) value (ECHA, 2008, section R.10.8.2). If the resulting 'tentative' $QS_{biota,\ birds}$, is lower than the $QS_{biota,\ mammals}$ then, given the lack of information on relative sensitivities between birds and mammals, the uncertainties should be highlighted in the datasheet.

4.4.4.2 Derivation of QSbiota, secpois according to the refined approach using key species

If it is possible to identify the key indicator wildlife species in the ecosystem the following approach can be used to derive the $QS_{biota,\ secpois}$. The key species is defined as the most susceptible species on the basis of its ratio of body and daily food intake and its position in the trophic chain (the latter only of the substance is subject to significant biomagnification). The NOEC for the key indicator wildlife species can then be calculated from the lowest reliable NOAEL from laboratory studies using information on body weight (bw) and daily food intake (DFI) for these species as indicated below:

$$NOEC_{wildlife} = NOAEL_{laboratory} * (bw_{wildlife}/DFI_{wildlife})$$

Only the mammals NOAEL is used to extrapolate to mammalian wildlife species. Similarly, only the avian NOAEL is used to extrapolate to avian wildlife species. Then the $QS_{biota,\ secpois}$ is derived from the $NOEC_{wildlife}$ in this case using the assessment factors from Table 4.4. In this table the extra factor of three for the difference in caloric content between laboratory food and a diet based on fish and/or mussels is omitted.

Table 4.3 Assessment factors for the extrapolation of mammalian and bird toxicity data into $QS_{biota,secpois}$ in a refined assessment

TOX_{oral}	**Duration of test**	**AF_{oral}**
$NOEC_{oral,\ birds}$	Chronic	10
$NOEC_{oral,\ mammals}$	28days [a]	100
	90days	30
	Chronic	10

[a] Note: The AF of 3 accounting for extrapolation from laboratory to field is omitted because the method already takes the dietrary intake differences between laboratory and field into account

The resulting AF should allow for interspecies variation in sensitivity to account for differences in toxicity. A factor of 10 accounting for interspecies variation is appropriate for this purpose. An additional AF of 3 to 10 is applied when exposure periods are not truly chronic (ie subchronic to chronic extrapolation).

The same considerations as in the standard approach may be applied with regard to the use of acute avian data and data treatment for the same species. For application of the SSD method the

same considerations as in the standard approach are valid with the exception that in this case the input data should be based on dose and not diet concentrations.

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

The QSbiota, hh food is intended to protect all humans against adverse health effects from consuming contaminated fishery products. Dealing with risks to human health from substances in drinking water is covered in Section 3.9. Like the biota standards for protecting predators, the standards described here are expressed in terms of body residues in food items.
No internationally recognised approach exists for determining the uptake of contaminants from fishery products by humans. However, several EU Directives (Council Directives 91/414/EEC and 97/57/EC) specifically deal with the risks to humans from several classes of organic contaminants, such as dioxins, dioxin-like polychlorinated biphenyls (PCBs) – PCB congeners that exhibit toxicological properties similar to dioxins – and polyaromatic hydrocarbons (PAHs) (Council Regulation (EC) No 1881/2006 of 19 December 2006), and metals, such as lead, cadmium and mercury (Council Regulation (EC) No 78/2005, amending Regulation 466/2001), via edible aquatic species, such as fish, molluscs, crustaceans and cephalopods. Therefore, when legislation has already led to the derivation of standards, the $QS_{biota,\ hh\ food}$ should refer to the maximum allowable concentration in $\mu g \cdot kg^{-1}$ wet weight in the specific tissue or sampling material.

Where no established $QS_{biota,\ hh\ food}$ value exists, the procedure described in Lepper (2005) is recommended. It assumes that the uptake of a substance from fishery products does not exceed 10% of the relevant threshold level (TL), estimated from experimental data and expressed in $\mu g \cdot kg^{-1}$ bw$\cdot d^{-1}$ for humans. For practical purposes, the acceptable daily intake (ADI), tolerable daily intake (TDI) or $NOAEL_{oral}$ (the latter divided by an assessment factor) provides such an estimate. The $QS_{biota,\ hh,\ food}$ (expressed as $\mu g \cdot kg^{-1}$) is calculated using defaults for human bw (70 kg) and for the consumption of fishery products (0.115 kg$\cdot d^{-1}$) as follows:

$$QS_{biota,\ hh\ food} = \frac{0.1 \cdot TL \cdot 70}{0.115}$$

This approach does not specifically consider possible sensitive groups, such as the developing foetus or subpopulations that consume more fishery products than the European average. However, the assumption that fishery products make up no more than 10% of the threshold level value (0.1·TL) at the European average level of compound uptake provides a margin of safety.

4.6 Metals

The approach described above for secondary poisoning and human consumption of fishery products, whereby NOEL, NOAELs for secondary poisoning and ADI, TDI or a comparable human threshold values for fishery products are used, is also applicable to metals. After the quality standard in biota has been derived, it should be compared to the background levels of metals in biota. The definition of the natural background level for metals in biota is as for in water, and the same types of difficulties exist when determining the level. In general, the considerations concerning natural background levels in biota are as for water (see Section 3.5).
Preferably, measurements of metals in biota should be taken from species living close to springs or far at sea. It should be recognized 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 Monitoring compliance with biota standards

4.7.1 Biota monitoring

Procedures for species monitored through international conventions for inland, transitional, coastal and marine waters already exist, e.g. Helsinki Commission (HELCOM), OSPAR, International Commission for the Protection of the Rhine (ICPR). A separate background document summarises current monitoring programmes in Europe, and detailed guidance on the sampling and analysis of chemical residues in biota and sediments is the objective of another guidance document that is being prepared by the Chemical Monitoring Activities Working Group (EC, 2010).

4.7.1.1 Selection of species for monitoring

The primary aim of existing biota monitoring programmes is to assess environmental concentrations through long-term surveillance monitoring but, in principle, species that are already used in existing national or international monitoring programmes should be used for biota monitoring. The choice of particular species is not specified here, but certain criteria should ideally be met:

- The choice of species monitored should depend on the identified protection goal (e.g. humans, top predators).
- The standard in biota refers to a trophic level that is defined by the simple food chain (Section 4.4).
- The sampled organisms need to be potential food for predatory organisms or humans.

To provide an unbiased sample, the use of bulk samples of many individuals is recommended. Furthermore, those life-cycle stages that are most likely to be consumed by predators should be preferred and/or the organisms need to be of a size that is relevant to predator species. Large animals have fewer predators and analysis of these individuals may not provide any useful additional information about predator exposure. However, if the species selected is not high enough in the food chain, the outcome from monitoring could be underprotective for biomagnifying substances (if the concentration of a biomagnifying substance is close to the biota standard at lower trophic level, the concentration would exceed the biota standard at higher trophic levels for such substances). If selection of such a representative species is not possible from the point of view of standard organisms to be monitored in routine monitoring programmes, the biota standard should be adjusted to the appropriate trophic level of the monitored species .

4.7.1.2 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 when an EQS is lower than three times the LOQ_{25} (limit of quantification). In this case, it is not always possible to quantify some substances 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/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 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.

4.7.1.3 Sampling

The sampling frequency, sampling methods, sample preservation and cleanup should follow the guidelines already defined in the WFD monitoring guidance (EC, 2010). Although there are greater unit costs associated with collecting samples and performing the analysis for biota than for water, the sampling frequency is lower than for water.

4.7.2 Converting QSs expressed as biota concentrations into equivalent water concentrations

4.7.2.1 Organics

Normally, the $EQS_{biota}$is expressed as a body residue. It follows that monitoring is also performed in biota. The biota standard ($\mu g \cdot kg^{-1}_{diet}$) could, however, be converted into a water column concentration standard ($QS_{fw,secpois}$ or $QS_{sw,\ secpois}$ in $\mu g \cdot l^{-1}$), e.g. 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 uses the threshold in prey (QS_{biota}) and bioaccumulation data (BCF, BMF and/or trophic magnification data) of the substance concerned. Effectively, the back calculation to a water concentration is equivalent to estimating the PEC_{oral} in chemical risk assessment. As explained below, it is necessary to account for the longer food chains in the marine environment where it concerns the secondary poisoning route, by incorporating not only biomagnification in the prey of predators (BMF_1, as for freshwater), but also in the prey of top predators (BMF_2). This does not apply to the EQS derivation for human fish consumption as here, fish is the species consumed by the 'top predator' (humans). However, the BMF_2 is also needed to set the EQS_{biota} for the marine environment because it is unacceptable to monitor at the trophic level of the marine predators, such as seals, that serve as food for the top predators, such as killer whales and polar bears. This leads to a different value for QS_{biota} for freshwater and QS_{biota} for saltwater where it concerns secondary poisoning, which is explained in the next section.

There are important issues involved in expressing the biota standard as a concentration in prey or as as a concentration in water and these are summarised in Table 4-5.

Table 4.4 Considerations in expressing a biota standard as a concentration in prey or in the water column

	Conversion into a water-column QS	Expression of the standard as body residue
Selection of a suitable 'matrix' for monitoring	• Easy (Daughter Directive text currently requires whole water for organics) • Analytical sensitivity issues likely (see below)	• Need to decide on appropriate trophic level, and species and tissue for monitoring (whole body or specific organ?)
Uncertainty in deriving EQS	• Uncertainty in BCF/BMF or BAF used in converting into water-column standard	• Uncertainty concerning AFs applied to TOX_{oral} and TDI and BMF_2 (only for the marine environment) • Uncertainty in converting into water-column standard eliminated
Comparison with other water-column EQSs	• Direct comparison possible	• Different matrix so cannot compare directly
Availability of data	• Requires toxicity data from feeding studies and BCF and BMF, or BAF	• Requires only toxicity data from feeding studies and BMF_2 (only for the marine environment)
Analysis	• Consistent with existing practice • $QS_{fw,\ secpois}$ or $QS_{sw.\ secpois}$ or $QS_{water,\ hh\ food}$ often < LOQ • Individual sample costs < biota sample costs, but method development required to achieve required sensitivity • Several samples needed per year	• Method development (e.g. cleanup) may be required to deal with biological matrix • Individual sample costs > water sample costs, but only infrequent sampling needed (requested actually 1/year, but 3 to 4 times/year seems more reasonable)
Relevance to water quality classification	• Need high quality data on food webs and the identification of the correct trophic level • Existing classification rules can apply, e.g. QA/QC Directive, but with high uncertainties and, therefore, low confidence that failure has actually occurred, in part because of sampling uncertainties that come with spot samples	• High – biota residue effectively integrates exposure over long time periods • Need high quality data on food webs and the identification of the correct trophic level for sampling the correct species

Where a QS_{biota} (in general) is to be converted into QS_{water}, experimental BCF and BMF data, or a field derived BAF, are required. The water concentration value is calculated as follows:

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

The term bioaccumulation refers to transfer mechanisms of hydrophobic contaminants by both bioconcentration (accumulation via media) and biomagnifications (accumulation via food). Normally, the combined effects of each step are combined in a multiplicative approach. Therefore, the BAF may be calculated as:

$$BAF = BCF \cdot \prod_{i=1}^{n} BMF_i$$

where the number of BMFs depends on the trophic level or position of the organism in the food web. According to REACH Guidance (ECHA, 2008), a simple food web is assumed that consists of water –BCF→ aquatic organisms –BMF_1→ fish → fish-eating predator. As indicated above, for marine top predators, an additional BMF in prey of top predators (BMF_2) should be applied. Therefore:

$$QS_{fw,\sec pois}(\mu g / l) = \frac{QS_{biota,\sec pois,\ fw}(\mu g / kg)}{BCF(l / kg) \cdot BMF_1}$$

$$QS_{sw,\sec pois}(\mu g / l) = \frac{QS_{biota,\sec pois,\ sw}(\mu g / kg)}{BCF(l / kg) \cdot BMF_1}$$

There are ways in which uncertainty in the calculation can be reduced:

1. The field BAF value for the correct trophic level should be used.
2. The laboratory BCF value is multiplied by the field BMF.

Ideally, the BMFs should be based on measured data. In general, the most reliable data on biomagnification originate from trophic magnification studies. In such studies, the levels of contaminants in several species in an ecosystem are measured and expressed as a function of the trophic level. The trophic level is mostly derived from stable nitrogen isotope ratios and a regression is made between contaminant concentration and trophic level. The contaminant values should preferably be normalised to the fraction in the organisms that contains the substance, e.g. lipids.

The advantage of this method is that it takes into account magnification along the whole food chain and it is not subject to the rather arbitrary choice of two species for which a BMF is calculated. The BMF_1 may be deduced from the increase in (lipid-normalised) concentration of the contaminant over one trophic level in a simple pelagic food chain. 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 $\delta^{15}N$ (based on stable nitrogen isotope ratios) per trophic level. The value of the FWMF or TMF can be taken as the BMF over one trophic level, equivalent to BMF_1 in a pelagic food chain. Care must be taken that the regression is based on trophic level rather than $\delta^{15}N$. If this is not the case, a correction for the increase of $\delta^{15}N$ per trophic level has to be applied.

For the marine environment, an extra BMF is included. In this case, poikilotherms (invertebrates and fish) and homeotherms (seabirds and mammals) should be distinguished. As the first group is related to the first BMF for fish, the second group is representative for the biomagnification in predating birds and mammals. Thus, BMF_2 should preferably be extracted from a study that describes such a food chain. In general, the biomagnification in homeotherms is larger than that in poikilotherms and, thus, BMF_2 is generally larger than BMF_1.

If it appears that the FWMF or TMF is not significantly greater than one, it is reasonable to conclude that there is no significant biomagnification, and both values for BMF may be set to one. If the value for FWMF or TMF is significantly below one, trophic dilution is indicated. For the pelagic food chain, BMF_1 then equals one, as the BMF value represents biomagnification from small fish to predatory fish, while the metabolic capacity in fish is assumed to be uniform and the BCF will mostly refer to fish. For the marine environment, not only the top predators, but also the predators that feed on fish should be protected. Therefore, even if trophic dilution occurs from predator to top predator, this step in the food chain is then superfluous as both refer to toxicity of mammals and birds. In this case, BMF_2 has to be set equal to one as well.

Other sources of information are BMFs from field or laboratory studies. Care should be taken in interpreting these values because they only represent one link in the food chain and may not represent the overall biomagnification potential of a substance. A BMF is restricted to the ratio between the concentrations in the predator and in its prey or food in the case of a laboratory study.

The availability of biomagnification data is limited; therefore, the default BMF values given in Table 4-6 (EC, 2003) may be necessary. However, a reliable experimental BCF value is always preferred above the log K_{ow} to estimate the BMF value because it takes the metabolism of the substance into account, which is an important parameter in food web accumulation.

Table 4-5 Default BMF values for organic substances

log K_{ow} of substance	BCF (fish)	BMF_1	BMF_2
<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 conversion from a biota standard into an equivalent water concentration can introduce uncertainty, especially for highly lipophilic substance (i.e. BCF >2000). Generally, substances with a BCF of 500 or less can be converted into an equivalent water concentration with reasonable confidence. Where it is necessary to convert a biota QS into an equivalent water-column concentration, the uncertainties involved in making the extrapolation may be taken into account by performing the conversion for extreme BAF values as well as using 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. The worked examples for hexachlorobenzene (HCB) and lindane below show that for HCB the biota QS is likely to be the critical QS regardless of the uncertainties of the extrapolation, whereas in the case of lindane there is uncertainty as to whether the biota QS or the water QS is the critical QS.

HCB example

QS_{water}	13 ng·l^{-1} (EQS Substance data sheet, 2005)
$PNEC_{oral}$	16.7 □g·kg^{-1}(EQS Substance data sheet, 2005)
BAF	52,300 L·kg^{-1} (mean value; 26 experimental fish BAF values, min 8130, max 550,000, median 51,900) (Arnot and Gobas, 2006)

$$EQS_{water} = \frac{EQS_{biota}}{BAF}$$

Extrapolated QS_{water}

Calculated with median BAF	0.3 ng·l^{-1}
Calculated with minimum BAF	2 ng·l^{-1}
Calculated with maximum BAF	0.03 ng·l^{-1}

Lindane example

QS_{water}	20 ng·l^{-1} (EQS Substance data sheet, 2005)
$PNEC_{oral}$	33 □g·kg^{-1}
BCF	1300 (selected in the EQS datasheet, min 220, max 2200) (EQS Substance datasheet, 2005)[24]
BMF	A BMF of 1 was assumed according to the TGD (EC, 2003)

$$EQS_{water} = \frac{EQS_{biota}}{BCF.BMF}$$

Extrapolated QS_{water}

Calculated with selected BCF	25 ng·l^{-1}
Calculated with minimum BCF	150 ng·l^{-1}
Calculated with maximum BCF	15 ng·l^{-1}

4.7.2.2. Metals

If a secondary poisoning risk (to birds and mammals) from metals is identified (Section 2.4.3.1), or a risk for human fish consumption then the methodology described in Section 4.4 for the derivation of the $QS_{biota,secpois}$ or Section 4.5 for the derivation of the $QS_{biota, hh food}$ should be followed.

Where toxicological information on critical body (or organ/tissue) levels is lacking, BCFs or BAFs may be used to estimate metal accumulation in animals relative to the concentration in water.

There are added complexities when selecting an overall EQS because BCFs used to back-calculate to a water concentrationmay depend on water concentration. For naturally occurring substances, such as metals, many species regulate their internal concentrations through (1) active regulation (2) storage or (3) a combination of active regulation and storage over a wide range of environmental exposure conditions. Although these homeostatic control mechanisms have evolved largely for essential metals, they are not entirely metal specific and will, to some extent, apply to non-essential metals. A list of metals and metalloids classified by their essentiality to organisms is given in Table 4-7 (ICME, 2001)

[24] Note that the example for lindane used here follows the EQS datasheet (2005), but does not use a BAF value, or apply a BMF value. Use of a BAF value (e.g from Arnot and Gobas, 2006) results in the biota QS being the critical QS.

Table 4-6 Metals and metalloids classified by essentiality to living organisms

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

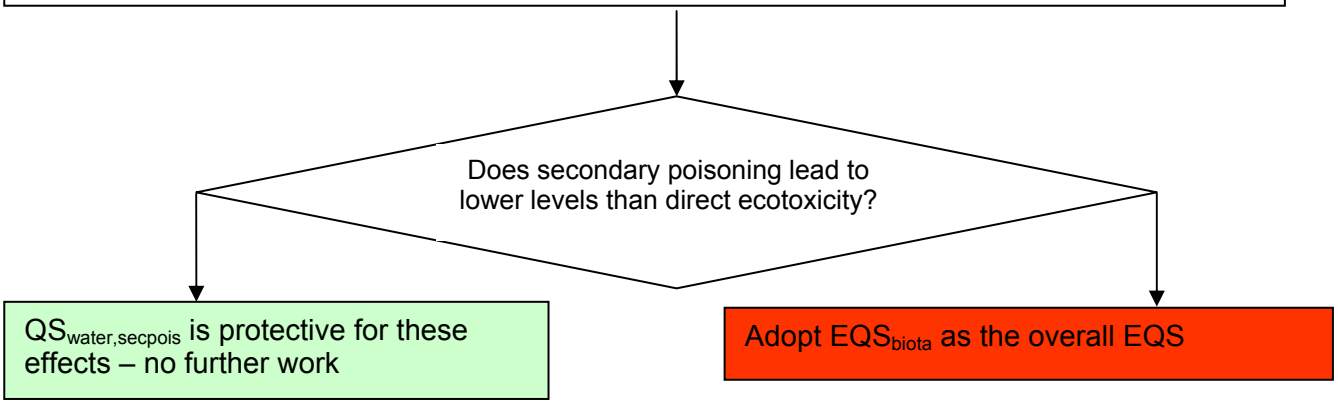
At low metal concentrations organisms accumulate essential metals (and often non-essential metals via the same uptake mechanisms) more actively in order to meet their metabolic requirements. At higher concentrations organisms with active regulation mechanisms even limit their uptake by the extraction of excess metals (ECHA, 2008). As a consequence metal concentrations in tissue based on a range of exposure concentrations may be quite similar yet the BCFs/bioaccumulation factors (BAFs) are variable, even showing an inverse relationship with external metal concentrations (ie higher BCFs at lower exposure concentrations and lower BCFs at higher exposure concentrations). This means that the use of BCF values for metals must be performed with care.

The text below sets out the steps to be used to select an overall EQS for metals:

1. **Derive standards in biota**
- Derive the $QS_{biota,\ secpois\ fw}$ or the $QS_{biota,\ secpois\ sw}$ following guidance in Sections 4.4 and 4.5.
- Compare the derived value to background levels of the substance for biota and ensure that the QS_{biota} > background concentration in biota (Section 4.6).

2. **Estimate biomagnification (BMF) and bioconcentration (BCF, BAF)**
- Collect (preferably) field-determined BAF data, e.g. of fish and molluscs, in which both internal and external metal concentrations have been reported.
- Determine the relationship between internal and external concentrations for the metal for several species (e.g. fish, molluscs). The $QS_{fw,\ secpois}$ or $QS_{sw,\ secpois}$ and $QS_{wataer,\ hh\ food}$ should be included in the range of internal concentrations (biota concentrations) or, alternatively, the $QS_{fw,\ eco}$ or $QS_{sw,\ eco}$ should be included in the range of external concentrations (water concentrations).
- Collect any relevant data that can be used to assess the bioavailability/bioaccessibility of tissue-associated metal.
- Determine the BMF relevant to the food chain considered.

3. Compare tissue concentration or water concentrations for the routes of direct ecotoxicity and secondary poisoning
- Compare the QS_{biota}/BAF or QS_{biota}/(BCF·BMF) with $QS_{water,eco}$ using a BAF or BCF and BMF that is determined at an internal (biota) concentration equal to the QS_{biota} or, alternatively, compare the $QS_{water,eco}$·BAF or $QS_{water,eco}$·BCF·BMF with the QS_{biota} using a BAF or BCF and BMF that is determined at an external (water) concentration equal to the $EQS_{water,eco}$. A prequisite is that the relationship between internal and external concentrations should be well determined, otherwise this approach cannot be followed.
- If a specific BAF or BCF is not available, a worst case approximation can be made using a BAF or BCF determined at a concentration in water *lower* than the $QS_{water,eco}$.



For metals, BCF values may be obtained in a variety of ways:

- In cases where there is evidence of concentration dependency of BCFs (i.e. the BCF is higher at lower environmental levels), regression models based on the observed inverse relationship should be used to derive the most-appropriate BCF value for the prey organisms considered (Brix et al., 2001; Efroysmen et al., 2001, McGeer et al., 2003, DeForest et al., 2007).

- Where regression lines cannot be calculated, BCFs may be obtained either by calculating species-specific arithmetic means from BCF studies using environmentally relevant metal concentrations in the test media or by using BAFs observed in the field (Lepper, 2005).

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.

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 may act as a source of contaminants to particle feeders through resuspension (eg 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 alongside or, instead of, 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 makes 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 5-1). Further detail on each of these steps, eg 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.

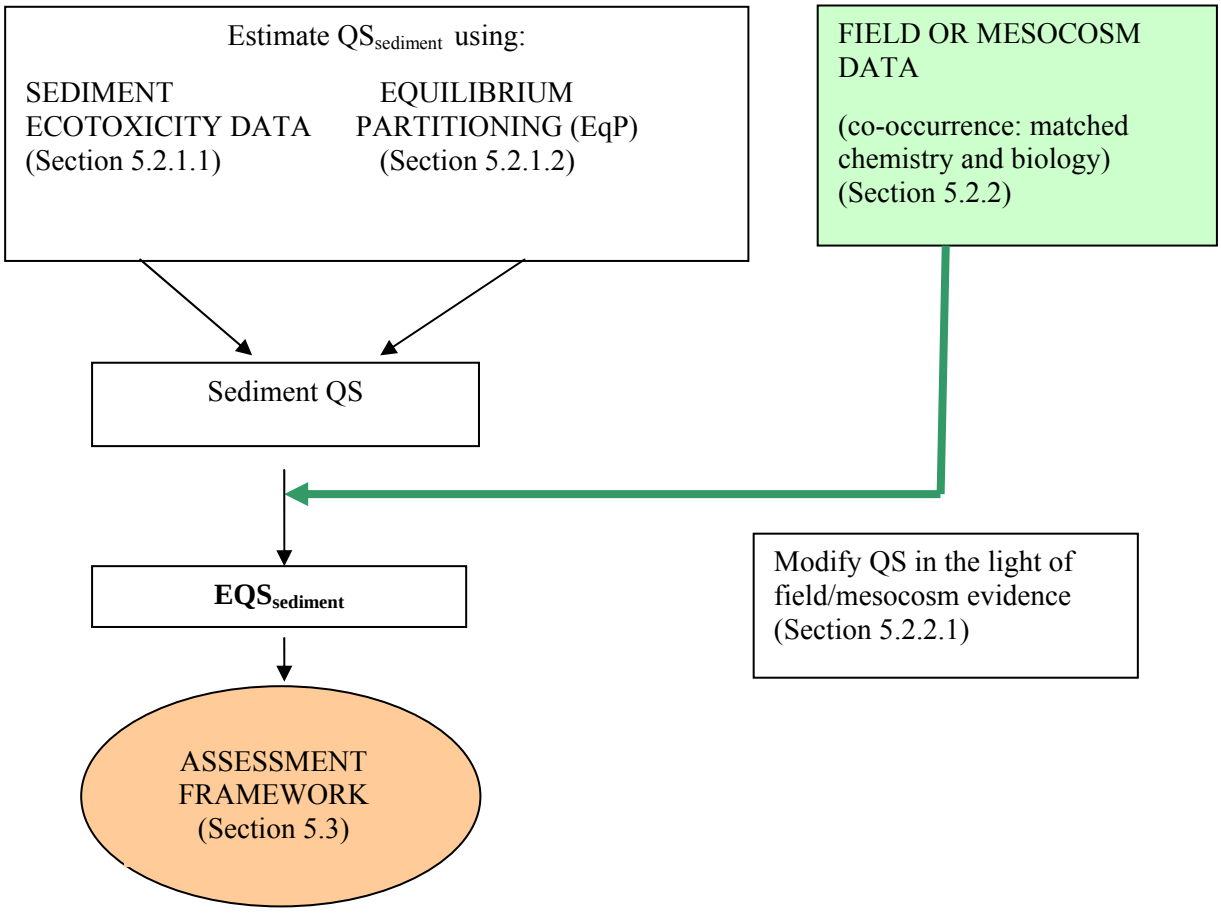


Figure 5.1 Overview of process for deriving a sediment standard

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 (ie 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 normalizing 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.

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

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}	
$\text{TEST RESULT}_{\text{EU standard test}}$	Test result expressed in EU standard sediment	mg kg_{dw}^{-1}	
$\text{TEST RESULT}_{\text{test sed}}$	Test result expressed in EU standard sediment		
$F_{\text{oc, EU standard sed}}$	Organic carbon content (w/w) of EU standard sediment	kg kg^{-1}	0.05
$F_{\text{oc, test sed}}$	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 $QS_{\text{sediment, fw eco}}$ or $QS_{\text{sediment, sw eco}}$ is determined using the assessment factors (AFs) in Table 5-1, 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 5.1 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 is 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 ($C_{effect\text{-}water}$).

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 Kcomp-water

In the EqP method outlined in ECHA guidance, the 'dimensionless'partition co-efficient Ksed-water is used in untis of m3m-3. This parameter is also called a total compartment-water partition coefficient. It is calculated according to the equations given in REACH guidance (ECHA, 2008) R.16, 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 sediemtns rather than suspended matter and sediment standards based on suspended matter characteristics bear more relevance to the water column than do standards based on sediment characteristics. The default values for compartment specific characteristics (Faircomp, RHO solid etc) from the REACH (ECHA, 2008) should be used; their values are listed in the table below the equations.

$$K\mathrm{p}_{\mathrm{sed}} = F\mathrm{oc}_{\mathrm{sed}} \times K_{\mathrm{oc}} \qquad 2$$

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

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

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

Description:

Parameter	Description	Unit	Default value
1000	conversion factor from m^3 to litre	L m^{-3}	1000
$Cporew_{sed}$	total concentration in pore water of sediment	mg m^{-3}	
$Ctotal_{sed}$	total concentration in sediment	mg m^{-3}	
$Fair_{sed}$	fraction air in sediment	m^3 m^{-3}	0
Foc_{sed}	weight fraction of organic carbon in sediment	kg kg^{-1}	0.05
$Fsolid_{sed}$	fraction solids in sediment	–	0.2
$Fwater_{sed}$	fraction water in sediment	m^3 m^{-3}	0.8
H	Henry's law constant	Pa m^3 mol^{-1}	
$K_{air\text{-}water}$	air-water partition coefficient	m^3 m^{-3}	
K_{oc}	partition coefficient between organic carbon and water	L kg^{-1}	
Kp_{sed}	partition coefficient solid-water in sediment	L kg^{-1}	
$K_{sed\text{-}water}$	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
RHOsolid	density of the solid phase	kg_{solid} m_{solid}^{-3}	2500
TEMP	environmental temperature	K	285

Calculation of $QS_{sediment,\ fw\ EqP}$ or $QS_{sediment,\ sw\ EqP}$

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

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

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

$$CONV\text{sed} = \frac{RHO_{\text{sed}}}{F\text{solid}_{\text{sed}} \times RHO\text{solid}} \quad (7)$$

$$QS_{\text{sediment, EqP, dw}} = CONV\text{sed} \times QS_{\text{sediment, EqP, ww}} \quad (8)$$

Description (some of the variables are listed in the previous table):

Parameter	Description	Unit	Default value
1000	conversion factor from m^3 to litre	L m^{-3}	1000
*CONV*sed	conversion factor for sediment concentration wet-dry weight sediment	$kg_{ww} \cdot kg_{dw}^{-1}$	
*F*solid$_{sed}$	fraction solids in sediment	–	0.2
$K_{sed\text{-}water}$	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
*RHO*solid	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 5.2.

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

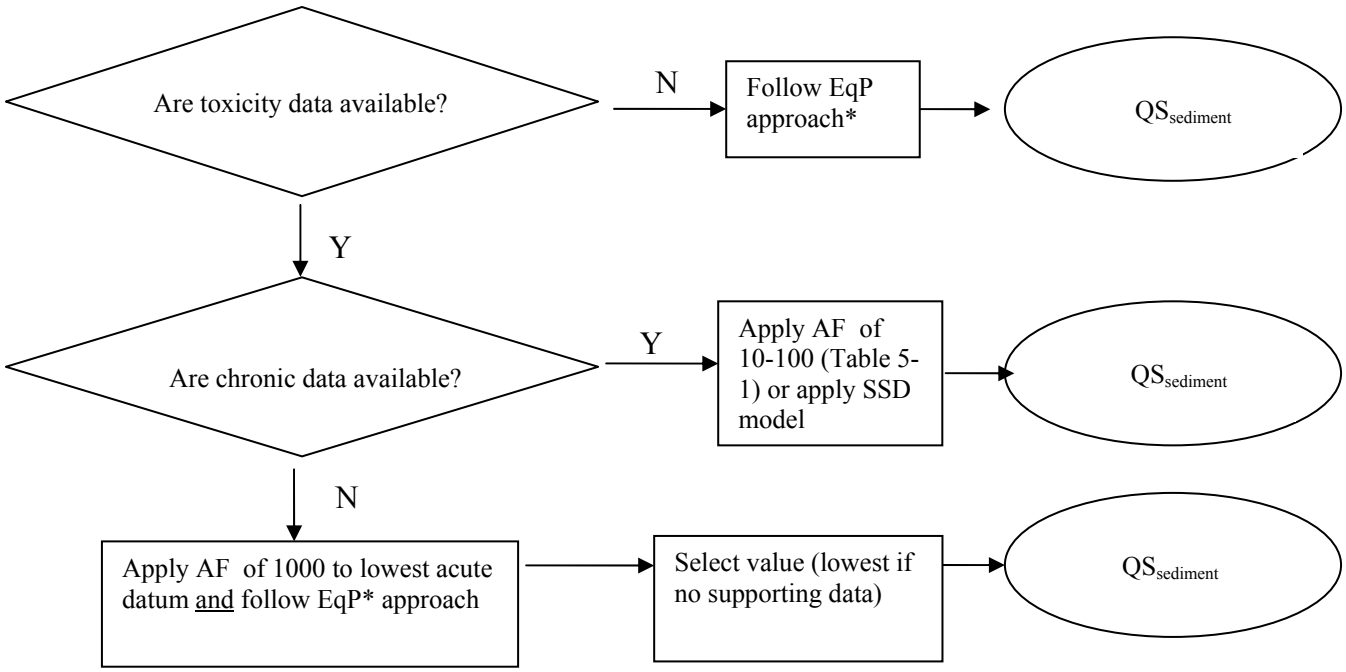
geometric mean K_{OC} will be used in the above equation. For highly lipophilic substances (Kow > 5), equilibrium may not be achieved, so a correction for exposure through food was introduced in the TGD (EC, 2003). For such substances, an additional AF of 10 is recommended.

Reliance on EqP alone involves several important assumptions such as equilibrium among phases, 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 5.2 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, r^2=0.89,s.e.=0.45
Non hydrophobics	$\log K_{OC}=0.52*\log K_{OW}+1.02$	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, carbamates, esters, phenylureas, phosphates, triazines, triazoles, uracils	$\log K_{OC}=0.40*\log K_{OW}+1.12$	n=21, r^2=0.51,s.e.=0.34
Alcohols, organic acids	$\log K_{OC}=0.39*\log K_{OW}+0.50$	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.=0.451
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	$\log K_{OC}=0.47*\log K_{OW}+1.08$	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=0.60,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

The process for using laboratory toxicity data and the EqP approach in deriving a $QS_{sediment}$ is summarised in Figure 5.2.



* apply additional AF of 10 if log Kow >5

Figure 5.2 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 2.8.2) 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 anomolies 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 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 5-1).

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 5.2.1.3.3).
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) (E.C. 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 definition 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 10^{th} 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 50^{th} percentile of concentrations (dry weight) associated with a biological effect and the 15^{th} 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 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 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 sulfide binding to metals is not present.

5.2.2.2 Accounting for background concentrations in sediments

The methodology described for considering metals in the pelagic- water compartment - using an added risk approach where needed (Section 3.5.2.1) - can also be applied to the sediment compartment.

The definition of the natural background levels for metals in metals is similar to that for water. Again, the default procedure will be to search for baseline levels in pristine or close to pristine areas. Unlike the situation for water, the analysis of deeper, undisturbed bed sediments, combined with radio-isotopic techniques, may allow one to estimate historical ambient concentrations, and thus to judge 'pre-industrial' levels.

Other possibilities are to:

- To gather information from national or international databases, for example, FOREGS Geological Baseline Programme (http://www.gsf.fi/foregs/geochem)

- Geological modelling, to estimate the contribution from erosion

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, modeled 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 target species (fish or mammals), $QS_{sediment}$ 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 approach 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 5-3).

Table 5.3 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 test 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 3.3) 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 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[25]. 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 5-3)[26].

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 exceedence 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.

[25] 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.

[26] Nevertheless, the framework is not mandatory; local authorities may disregard this framework and manage directly to recover a quality matching the standard

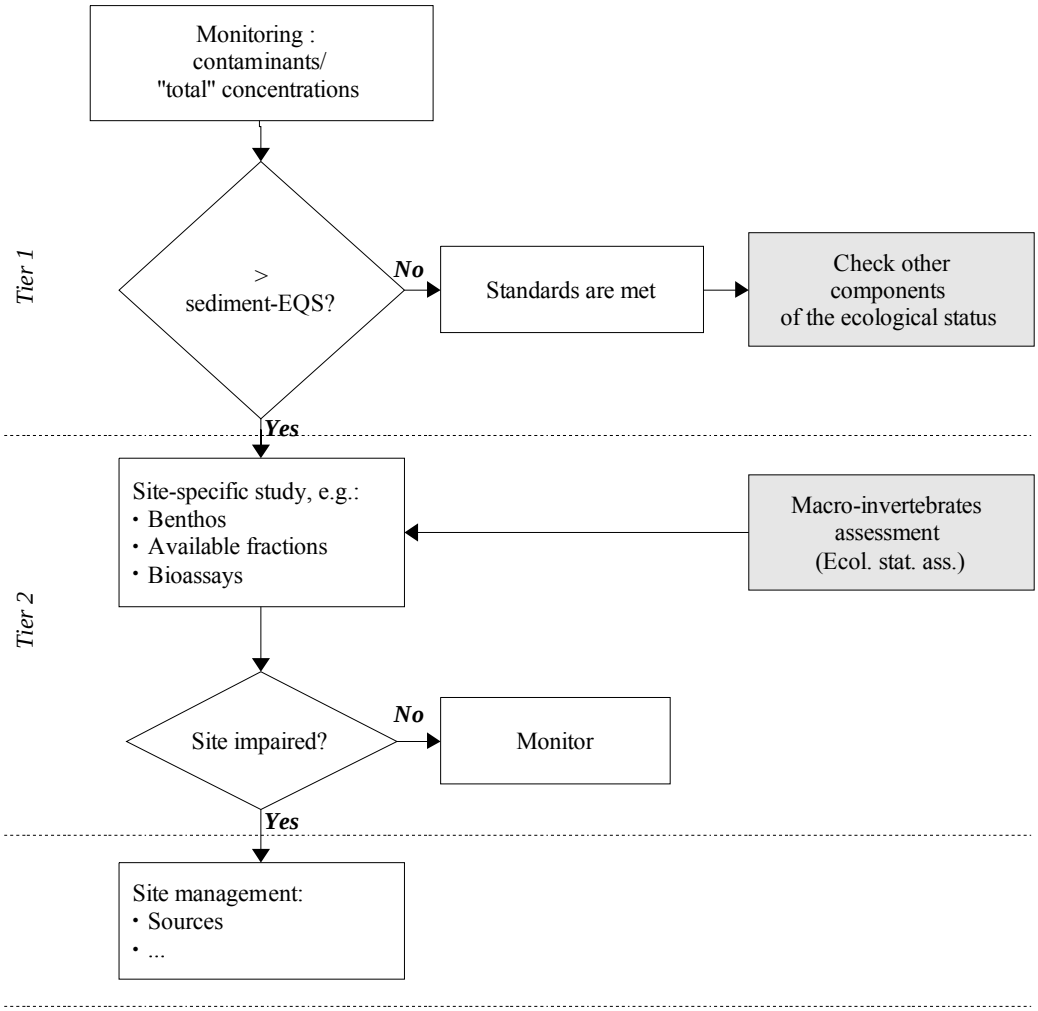


Figure 5.3 Tiered assessment framework for sediments

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)[27] or direct measurements in organisms. In this case, measured concentrations should be compared with the $QS_{fw,\ eco}$ or $QS_{sw,\ eco}$ (Table 5-4).

Table 5.4 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 e.g. “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 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 5-5 illustrates the large difference in MS solubility products. This means that the presence of FeS and MnS indicates that MS, with solubility product lower then 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.

[27] 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 5.5 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_2S (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 and one mole of SEM silver reacts 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 regionale 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 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 this 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 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 6.1 illustrates a scheme for deciding how non-testing methods may be deployed for EQS derivation.

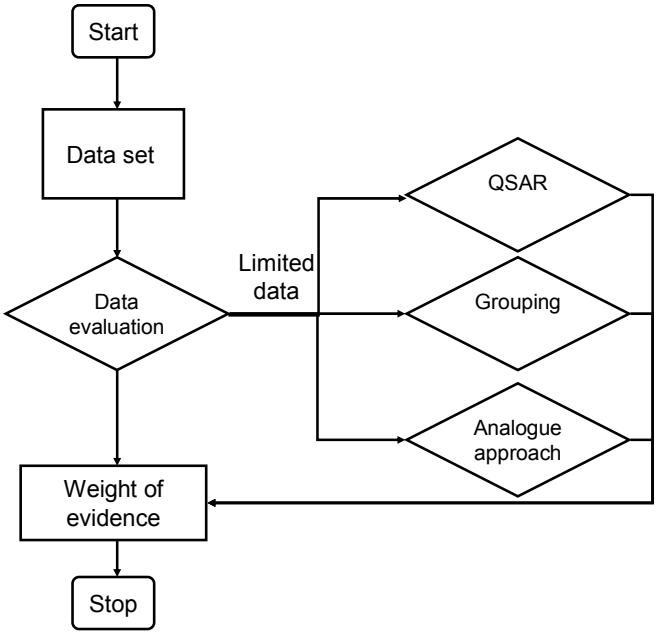


Figure 6.1: Application of non-testing methods

6.1 Grouping of substances / category approach

A chemical category is a group of chemicals whose physicalchemical 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 using 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 6.2 shows the procedure for category development.

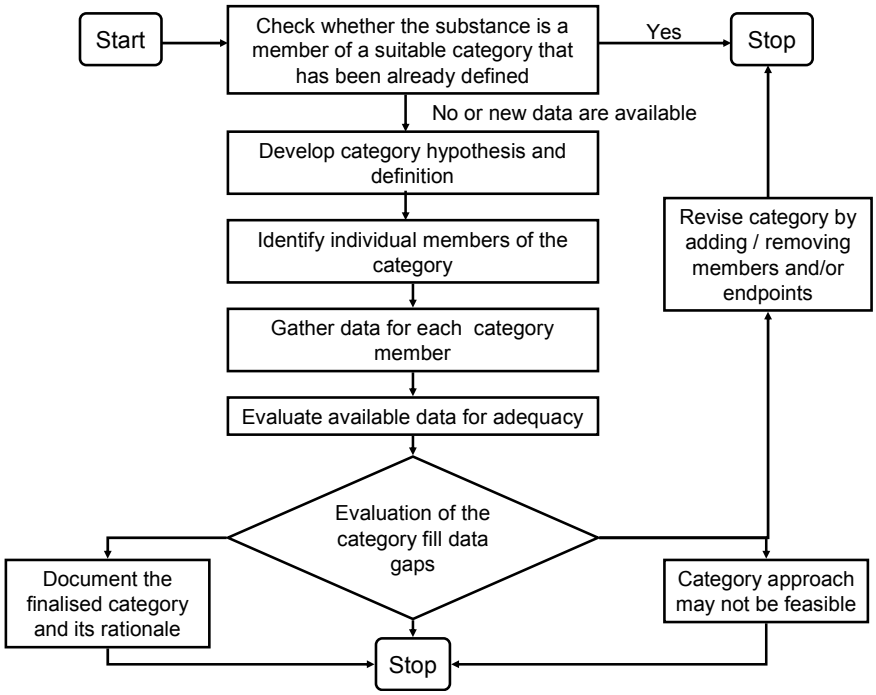


Figure 6.2. Stepwise procedure for category development

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 the substances and endpoints covered. Although the chemical structure is usually the starting point, a category definition could also refer to a group of chemicals related by a mechanism of action (e.g. non-polar narcotics) or a particular property. For each member of the category, relevant data should be gathered and 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).

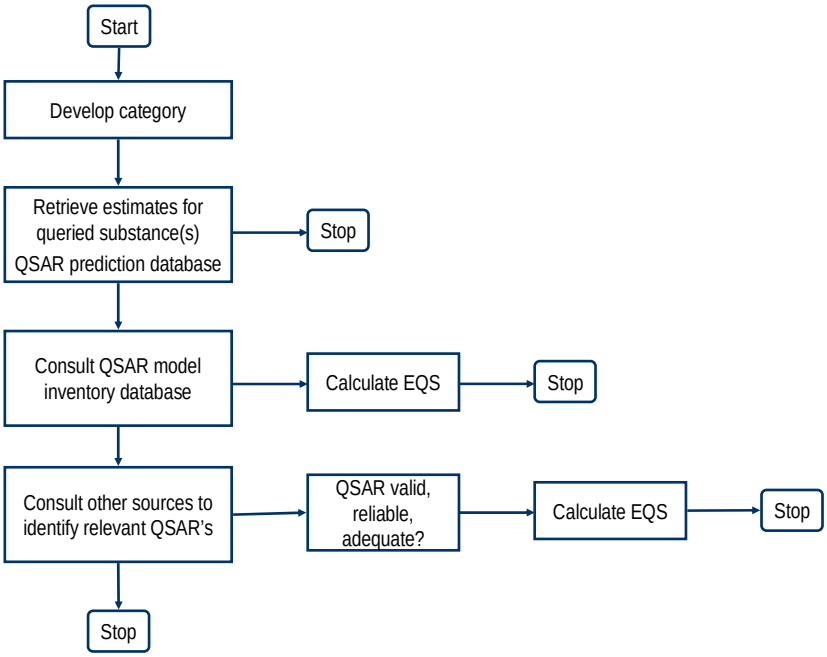


Figure 6.3 Stepwise approach to applying QSARs

There are various publically 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. Failing this, others models can be searched for in the literature, external databases and tools.

Harmonised templates such as the QSAR Model Reporting FormatQMRF 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 6.4 describes the application of the analogue approach.

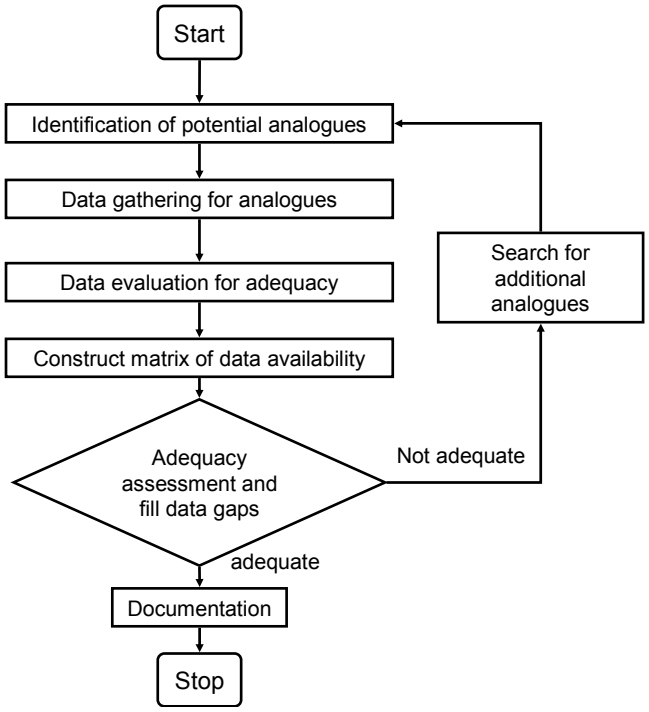


Figure 6.4. Stepwise procedure for the analogue approach

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 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 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, ie 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 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).

$TEQ = \Sigma_n (TEF_i * c_i)$

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 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 (eg PETROTOX) to fill data gaps as 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 can not be used for EQS setting as it requires the recalculation taking 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 fraction 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/). 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 ($g \cdot mol^{-1}$);
- 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 ($Pa \cdot m^3 \cdot mol^{-1}$);
- water solubility: S_w ($mg \cdot L^{-1}$), 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 ($L \cdot kg^{-1}$). For organic substances, the partition coefficients normalised to organic carbon are preferred: K_{oc} ($L \cdot kg^{-1}$). 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 1).

Table 1. 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 Table :

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 0) 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 2). The structural formula of the compound is also placed in this table.

Table 2. 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 ($g \cdot mol^{-1}$)		
Melting point (°C)		
Boiling point (°C)		
Vapour pressure (Pa)		
Henry's law constant ($Pa \cdot m^3 \cdot mol^{-1}$)		
Water solubility ($mg \cdot L^{-1}$)		
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 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. $K_{p,\ susp\text{-}water}$

For organic substances, the value of $K_{p,\ susp\text{-}water}$ is derived from the K_{oc} value and the fraction organic carbon of suspended matter used within the EU ($Foc_{susp,TGD}$), 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.

$$K_{p,\ susp-water} = K_{oc} \times Foc_{susp,\ TGD} \quad (1)$$

If site-specific data for suspended matter are available these can be used directly as well and might be preferred. The value for $K_{p,\ susp\text{-}water}$ for metals is derived from experimental data. The geometric mean value is calculated from the valid $K_{p,\ susp\text{-}water}$ 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 $mg \cdot L^{-1}$ 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 0 provides more detail.

3. After evaluating a study, the results of the study are summarised by entering it into the data table (see Sections 0 and 0).
   - 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 0 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 0 and 0), 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 an organic carbon content of 5% (Section 0). In the toxicity 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 a standard sediment (Section 0).
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 0.

A1.3.2.1. Study quality: validity codes

Studies are quality assessed according to the scheme developed by Klimisch *et al*. (1997). The quality codes assigned are:

1 = reliable without restrictions: 'studies or data...generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline...or in which all parameters described are closely related/comparable to a guideline method.'

2 = reliable with restrictions: 'studies or data...(mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.'

3 = not reliable: 'studies or data...in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g., unphysiologic pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment.'

4 = not assignable: 'studies or data....which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.).'

In general, when a test has fundamental shortcomings, it should be classified as not reliable (3). This applies to situations where the test was incubated too long (e.g. for algae), the

oxygen content was too low, control mortality was too high, solubility of the test substance was exceeded (see Section 0 for more detail), a co-solvent or emulsifier has been used in high concentrations (see Section 0), pH was out of the appropriate range (see Section 0 for specific guidance), the light used had an unrealistic UV intensity, the identity of the substance is not clear (see Section 0 for more guidance), or the actual concentrations are unknown because of significant but unquantified losses.

If the experiment is carried out in a medium that is not the natural habitat of the tested species, these tests are generally not reported rather than being classified as not reliable (see Section 0 for more guidance).

When a study contains useful toxicity information, but it cannot be used directly for derivation of EQSs, it is still tabulated. Examples are 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 0 for more detail). The test can then still be classified as reliable or reliable with restrictions.

A1.3.2.2. Acute and chronic studies

A chronic toxicity study is defined 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 definition 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 common 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 and most ELS tests for fish, but also for other species such as amphibians (FETAX test) 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. 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.

A1.3.2.3. 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’.

A1.3.2.4. 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 0) the concentrations of the solvent, emulsifier or dispersant should not exceed 100 mg/L^{-1} (or 100 µL/L^{-1} or 0.01%).

A1.3.2.5. 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 0).

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[28]. 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.

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 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.

A1.3.2.6. 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.

[28] '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.

A1.3.2.7. 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'.

A1.3.2.8. 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 NOEC or L(E)C50, the result of that study should be tabulated as NOEC ≥ or L(E)C50 >, followed by the value of the highest test concentration. The test result should be reported in the toxicity data tables. The result itself cannot be used in calculations of EQSs. However, it is valuable information that a species from this taxon (or trophic level) has been tested and that it was not sensitive to the toxicant at a known concentration. It may therefore have a useful supporting role. For example: when NOEC values for algae, *Daphnia* and fish are found, of which one is a 'NOEC ≥ ' value, and this value is not the lowest effect concentration, an assessment factor (AF) of 10 may be applied, whereas the AF would have been 50 if the study had been rejected.

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

A1.3.2.9. Quality Assurance

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 confidentiality, original study reports are often unpublished and may not be accessible.

Studies do not need to have been performed under a formal quality assurance scheme, such as Good Laboratory Practice (GLP). The reported description of a study and comparison with results from comparable studies and organisms, should provide all information necessary to assess its quality.

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. The most frequently used guidelines for ecotoxicological studies are summarised in this section, although others may also be reported.

- 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 guideline version from 1984 mentions both biomass (sometimes called growth) and growth rate as endpoints. From studies based on the OECD 201 - 1984 guideline, the value for the growth rate is preferred, because this is the more relevant parameter (European Commission (Joint Research Centre), 2003a). However, if only growth is presented, this value can be used as well. The result for the endpoint biomass (growth) is generally somewhat lower than the growth rate and can therefore be considered as a conservative value.

N.B. This guideline was revised in 2006. Endpoints derived from a study conducted following the revised (2006) are valid.

- 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 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.
- 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.
- 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 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.
- 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 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 220: Enchytraeid Reproduction Test. The 14-d range finding test from this guideline in which mortality is recorded is an acute test. The definitive test that lasts for 6 weeks is a chronic test. In this test the number of offspring is recorded as well as the mortality of the parent animals, which are only exposed for three weeks and are thereafter removed from the system.
- OECD Revised Proposal for a New 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).

- FETAX (Frog Embryo Teratogenesis Assay *Xenopus*): This test is a rather short test of 96 hours duration, possibly extended with a few hours, if the larvae have not reached a certain developmental stage. However, considering the sensitive endpoints (next to mortality also development and malformation) and the sensitive life stage (embryonic stages), this test can be considered as chronic for the derivation of EQSs.
- EPA. Ecological Effects Test Guidelines. OPPTS 850.1735. Whole sediment acute toxicity invertebrates, freshwater. Draft, 1996. This test can be used as a chronic test for species such as *Hyalella azteca*.

In addition to tests on birds (OECD guidelines 205 and 206), 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 top 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

A1.3.3. Aquatic toxicity data tables

The following subsections (Sections 0 to 0) 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[29] 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.

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

[29] 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).

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 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[30]. 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 are included in the saltwater tables. The division between freshwater, brackish water and seawater on the basis of salinity is given in Table . 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 3: 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 4 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

[30] However, because of the qualitative nature in which salinity is used in EQS derivation, this definition and its inherent accuracy are not relevant.

possible. For example, for a reproduction study with Oncorhynchus mykiss, 60 days (post-hatch) is noted rather than ‘2 months’.

Table 4: Used abbreviations for exposure times.

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 . Their use in EQS derivation is described in the third and fourth columns of this table.

Table 5. 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[31]	No	▪ Omit if NOEC is also available from same experiment ▪ Else: tabulate value; may be valuable as additional information
acute test	NOEC	No [a]	▪ Tabulate value; may be valuable as additional information
acute test	TLm	Yes	▪ Tabulate as LC50 [b]
Chronic test	EC10 or LC10	Yes	▪ Tabulate value
Chronic test	EC50 or LC50	No [a]	▪ Tabulate value; may be valuable as additional information
Chronic test	ECx (x < 10)	No	▪ Omit if NOEC is also available from same experiment ▪ If more than one ECx value is available, try to establish an EC10 from a reliable dose-response relationship ▪ Else: tabulate value; may be valuable as additional information
Chronic test	ECx (10 < x < 20)	Yes	▪ Omit if NOEC is also available from same experiment ▪ If more than one ECx value is available, try to establish an EC10 from a reliable dose-response relationship ▪ Tabulate value if the ECx is the lowest effect concentration measured. Calculate NOEC = ECx/2 (TGD guidance) and tabulate this NOEC [c]
Chronic test	ECx (x ≥ 20)	No	▪ Tabulate value; may be valuable as additional information ▪ If more than one ECx value is available, try to establish an EC10 from a reliable dose-response relationship
Chronic test	LOEC	No	▪ Omit if NOEC is also available from same experiment ▪ Else: (i) if percentage effect is known, see ECx in this table for further guidance ▪ Else: (ii) if percentage effect is unknown: tabulate value; may be valuable as additional information
Chronic test	MATC - single value, no further information	Yes	▪ Omit if NOEC is also available from same experiment ▪ Else, if no further information is available, calculate NOEC = MATC/√2 (TGD guidance) and tabulate this NOEC [d]
Chronic test	MATC - reported as a range	Yes	▪ Omit if NOEC is also available from same experiment ▪ Else, if no further information is available, tabulate the lowest value of the range as NOEC [e]
Chronic test	MATC – spacing factor is given [f]	Yes	▪ Omit if NOEC is also available from same experiment ▪ Else, if no further information is available, calculate NOEC = MATC/√(spacing factor)[f] and tabulate this NOEC [g]

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

Test type	Criterion	Use in EQS derivation?	Action
Chronic test	NOEC	Yes	▪ Omit LOEC if it is also available from same experiment

Notes to Table 5.

a) For toxicity tests with algae and *Lemna* sp., both the EC50 and the EC10 or NOEC are used in the EQS derivation, if available.

b) A footnote should be added to the toxicity data table stating that the TLm is used as LC50.

c) A footnote should be added to the toxicity data table stating that the NOEC is calculated as ECx/2.

d) A footnote should be added to the toxicity data table stating that the NOEC is calculated as MATC/√2.

e) A footnote should be added to the toxicity data table stating that the lowest value of the MATC range is taken as NOEC.

f) The spacing factor is the factor of difference between two subsequent testing concentrations employed in the toxicity experiment.

g) A footnote should be added to the toxicity data table stating that the NOEC is calculated as MATC/√(spacing factor).

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

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

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

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 (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

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 0 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: $mg \cdot kg^{-1}$, $\mu g \cdot kg^{-1}$

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: $mg \cdot kg^{-1}$, $\mu g \cdot kg^{-1}$

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. This hampers 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 0 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.

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 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 4 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 4 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 to 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 Section 0 for 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 0 for 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 0 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 $mg \cdot L^{-1}$, the geometric mean of NOECs for mortality = 3.1 $mg \cdot L^{-1}$ and there is a single EC10 value for growth of 0.67 $mg \cdot L^{-1}$. The geometric mean value of 0.49 $mg \cdot L^{-1}$ 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 0) 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

For each of the selected avian or mammalian toxicity studies, the test result is expressed as a $NOEC_{oral}$ in $mg \cdot kg_{food}^{-1}$. No observed adverse effect concentrations (NO(A)ELs, expressed on a basis of $mg \cdot kg_{bw}^{-1} \cdot d^{-1}$), are converted into $NOECs_{oral}$ (in $mg \cdot kg_{food}^{-1}$) using the following equations (Eqs. 3 and 4), with the conversion factors from Table or a suitable factor for the daily food intake for any other species:

$$NOEC_{\text{bird}} = NOAEL_{\text{bird}} \cdot CONV_{\text{bird}} \quad (3)$$

$$NOEC_{\text{mammal,food\_chr}} = NOAEL_{\text{mammal,oral\_chr}} \cdot CONV_{\text{mammal}} \quad (4)$$

Table 6. Conversion factors from NOAEL into NOEC for several species.

Species	Common name	Conversion factor ($bw \cdot DFI^{-1}$)
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 ($g \cdot d^{-1}$).

A1.4. BIOCONCENTRATION AND BIOMAGNIFICATION DATA

A1.4.1. Data collection

The literature should be searched for bioconcentration (BCF) and biomagnification (BMF) studies 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 and BMF 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, 1996). 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 BMF studies, which are

mostly derived from field studies. Apart from the analysis, a reliable BMF study requires that the prey and predator species 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 (Section 0).

A1.4.3. Bioaccumulation data tables

The following subsections (Sections 0 to 0) 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. The following subsections have titles identical to the column headings in the data tables.In the following sections, it is assumed that fish are the test organism most frequently encountered in BCF studies. However, BCF studies with molluscs 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

See Section A1.3.3.2.

A1.4.3.3. Test substance

Clearly report what compound is used. If a radiolabelled compound is used, 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, 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.

A1.4.3.12. 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 metals, BCF data are invalid.

A1.4.3.13. Bioaccumulation

Unit: $L \cdot kg^{-1}$.

Here, the value of the BCF or BMF is denoted. The basis for the BCF 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 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.

BCF values used for triggering and calculating the routes of secondary poisoning and human consumption of fishery products should be whole body BCFs, expressed in $L \cdot kg^{-1}$. This allows for variation since these BCFs are not normalised to lipid or fat content, which dominates accumulation. The EQS derivation is dependent on the available studies. In most 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.

A1.4.3.14. 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.

A1.4.3.15. 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.

If the method cannot be described easily, a footnote to the table can be entered.

A1.4.3.16. 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.

A1.4.3.17. 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 summarised in the data table (Section 0) calculate the geometric mean values per species. Of these values per species, the most reliable should be taken unless they are equally reliable, in which case the geometric mean of several BCFs is selected. For metals, BCF values should not be used. Instead, BMF data should be used or an assessment as described in the main guidance.

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 ≥3.

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:

$$\log BCF_{\text{fish}} = 0.85 \times \log K_{\text{ow}} - 0.70 \quad (5)$$

For substances with a log K_{ow} higher than 6, a parabolic equation can be used (Eq. 6):

$$\log BCF_{\text{fish}} = -0.20 \times \log K_{\text{ow}}{}^2 + 2.74 \times \log K_{\text{ow}} - 4.72 \quad (6)$$

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. Further discussion can be found in REACH guidance, Chapter R.11 (ECHA, 2008).

A1.4.4.3. BMF – experimental data

Experimental BMF values generally originate from field studies. From the valid BMF studies summarised in a BMF data table, the geometric mean value is calculated.

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 7. In this table, BMF_1 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_2 value. This BMF_2 is a value for biomagnification in the prey of top predators.

The most relevant values for BMF_1 are those for biomagnification from small to 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. 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. For comparison, the default values from Table can be used. 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_2 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. However, besides data for the marine environment, other data for biomagnification from fish to fish-eating birds and mammals should be considered as well.

Table 7 Default BMF values for organic substances.

log K_{ow} of substance	BCF (fish)	BMF_1	BMF_2
<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 Table 7 also shows (ranges of) BCF values. However, if one or more experimental BCF data are available, the BCF values from the tables are not needed. If there is no experimental BCF value, the numbers from Table cannot be regarded as guidance, because they represent ranges instead of single values. In such a case, it is better to estimate the BCF from the log K_{ow}. This procedure is described in Section A1.4.4.2. The results are broadly consistent with the ranges presented in Table 7.

A1.5. TOXICOLOGICAL DATA FOR THE PROTECTION OF HUMANS

A1.5.1. Threshold limits

A human toxicological threshold value 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 threshold values 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. 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 (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 8: 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
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/

Source name and publisher	Available at
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
BCF	bioconcentration factor
BMF	biomagnification factor
bw	body weight
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
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
*K*oc	organic carbon adsorption coefficient
*K*ow	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
NOEC	no observed effect concentration
NOEL	no observed effect level
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
TLm	median tolerance limit; also encountered as median threshold limit
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	$kg \cdot kg^{-1}$	0.05
$Foc_{susp,TGD}$	weight fraction of organic carbon in suspended matter as defined in the TGD	$kg \cdot kg^{-1}$	0.1
R	gas constant	$Pa \cdot m^3 \cdot mol^{-1} \cdot K^{-1}$	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 9: 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 ºC). 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 ºC 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. 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 is certainly feasible if parameters for more specific interactions are taking into account.
>
> '*Domain*

An extensive description of the domain is given in Table [32]. 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%)[33] range from 0.35 to 1.0 log units for the different models. The standard errors are indicated in Table [5] for each 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).'

[32] The number of the table refers to that given in this annex and not the table number in the TGD.

[33] For clarification, the standard error is equal to σ.

Table 10. 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 11. 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.

(End of citation)

The QSARs in Table 3 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)	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 0**
Proposed MAC-EQS for [freshwater] [µg.L^{-1}] **Proposed MAC-EQS for [marine waters] [µg.L^{-1}]**		**See section 0**

3.2 Specific Quality Standard (QS)

Protection objective*	Unit	Value	Comments
Pelagic community (freshwater)	[µg.l^{-1}]		See section 0
Pelagic community (marine waters)	[µg.l^{-1}]		
Benthic community (freshwater)	[µg.kg^{-1} $_{dw}$]		e.g. EqP, see section 0
	[µg.l^{-1}]		
Benthic community (marine)	[µg.kg^{-1} $_{dw}$]		
	[µg.l^{-1}]	-	
Predators (secondary poisoning)	[µg.kg^{-1} $_{biota\ ww}$]		See section 0
	[µg.l^{-1}]	(freshwaters) (marine waters)	
Human health via consumption of fishery products	[µg.kg^{-1} $_{biota\ ww}$]		See section 0
	[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	$DT_{50\ (type\ of\ water)}$= d	

6 AQUATIC ENVIRONMENTAL CONCENTRATIONS

Estimated concentrations

Compartment	Predicted environmental concentration (PEC)	Master reference
Freshwater		
Marine waters (coastal and/or transitional)		
Sediment		
Biota (freshwater)		
Biota (marine)		
Biota (marine predators)		

Measured concentrations

Compartment	Measured environmental concentration (MEC)	Master reference
Freshwater		
Marine waters (coastal and/or transitional)		
WWTP effluent		
Sediment		
Biota		
Biota (marine predators)		

EFFECTS AND QUALITY STANDARDS

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**
$MAC\text{-}QS_{fw, eco}$	*Gender species* / d or h EC_{50} : $mg.l^{-1}$		$\mu g.l^{-1}$
$MAC\text{-}QS_{sw, eco}$			$\mu g.l^{-1}$
$QS_{fw, eco}$	*Gender species* / 21d NOEC : $mg.l^{-1}$		$\mu g.l^{-1}$
$QS_{sw, eco}$			$\mu g.l^{-1}$
$QS_{sediment, fw, EqP}$	-	EqP	- $\mu g.kg^{-1}_{ww}$ - $\mu g.kg^{-1}_{dw}$

$QS_{sediment,\ sw\ EqP}$	-	EqP	- $\mu g.kg^{-1}_{ww}$ - $\mu g.kg^{-1}_{dw}$

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 : $mg.kg^{-1}_{biota\ ww}$		-- $\mu g.kg^{-1}_{biota\ ww}$ corresponding to -- $\mu g.L^{-1}$ (freshwater) -- $\mu g.L^{-1}$ (marine waters)

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	-- $mg.kg^{-1}_{biota\ ww}$		-- $\mu g.kg^{-1}_{biota\ ww}$ (-- $\mu g.L^{-1}$)

Human health via consumption of drinking water		**Master reference**
Existing drinking water standard(s)	$\mu g.L^{-1}$ (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 actual BCF values; the situation is similar for BAF, however there are much less published values.

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APPENDIX 4: INVESTIGATION OF FURTHER METHODOLOGIES TO IMPROVE THE PROTECTION OF PREDATORS AGAINST SECONDARY POISONING RISK

A4.1. Introduction

In Section 4 (Derivation of Biota Standards) only the protection of top predators' birds and mammals species is considered against the secondary poisoning risk. However the CSTEE (2004) expressed their concerns on the fact that the exposure of chemicals through the food chain is not only relevant for secondary poisoning in birds and mammals, but also for aquatic invertebrates and fish.

Few data assessing the oral route toxicity are currently available for organisms other than birds and mammals. However some relevant ecotoxicological information can be found in the literature or can be produced, as strongly recommended by the CSTEE, for the very limited number of chemicals selected as priority substances.

In order to improve the development of quality standards for the protection of predatory organisms some further methodologies to assess secondary poisoning are discussed.

On one hand, if relevant chronic toxicity data, expressed in terms of the concentration of the chemical in food to which the test subjects were exposed, is available for sediment and pelagic predators e.g. aquatic invertebrates and fish, then a secondary poisoning assessment based on the diet approach set for birds and mammals top predators can be followed, see Section 4. On the other hand, if toxicological data, related to tissue residues in the considered organisms, are available, taking into account all exposure routes for different sediment and pelagic predators, the so-called Critical Body Burden (CBB) approach can be applied for organics as well as for metals. The advantages and disadvantages of this approach are discussed below.

In addition, for the very few data rich cases, the Species Sensitivity Distribution (SSD) approach can be used for both the diet approach and critical body burden approach, to derive an EQS.

Finally, the fish predator is presented as a case study to investigate the potential to derive an EQS based on the previous approaches.

A4.2. Diet Approach

A diet based approach, similar to the one adopted to protect Birds and Mammals Top Predators and in which the concentrations of contaminants in the food of the organisms to be protected are compared against acceptable concentrations in the organisms food, derived from feeding studies, may offer considerable potential for the development of quality standards for the protection of other predatory organisms. A key advantage of this approach is that currently many of the available and relevant chronic toxicity data are expressed in terms of the concentration of the chemical in food to which the test subjects were exposed.
Where this approach is taken it is important that the matrix which is analysed for the assessment of compliance against the quality standard is representative of the food of the organisms to be protected. The species receiving the greatest exposure will be the species with the highest food ingestion rate relative to its body weight and feeding at the highest trophic level(s).

The information presented in Figure 1 indicates that the food ingestion rates, when expressed as a percentage of the organism’s body weight consumed per day, are highest for small organisms, and are higher for small birds than for small mammals.

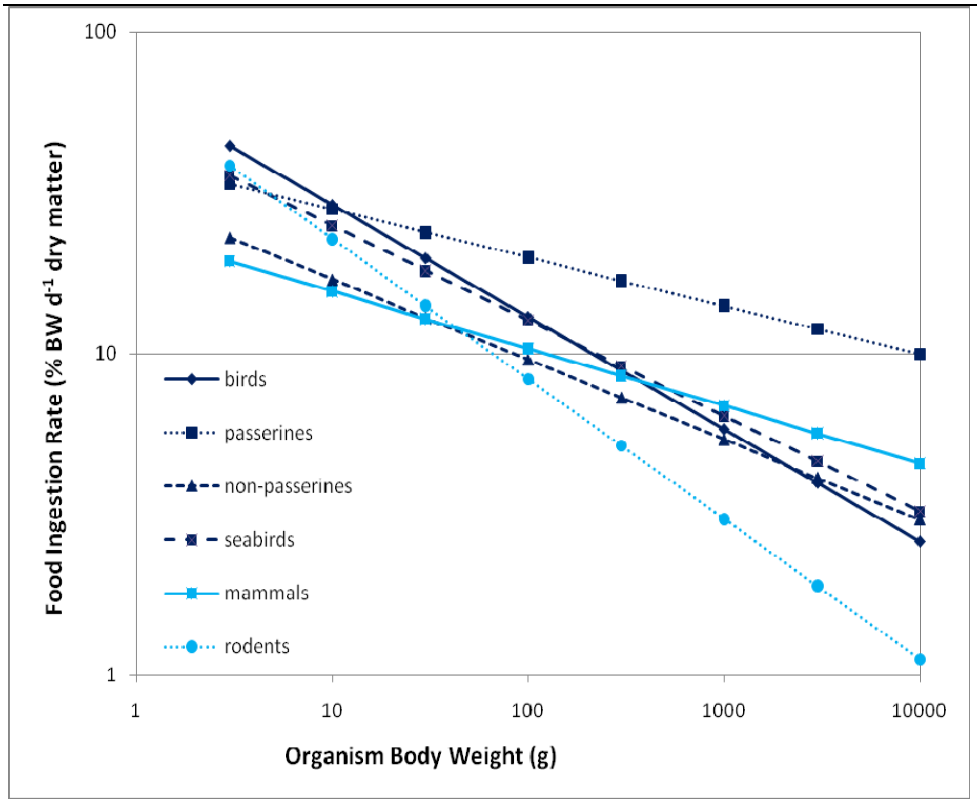

Figure 1 Variation in food ingestion rates, expressed as dry matter and as a percentage of organism body weight per day. Food ingestion relationships from USEPA (1993).

The diet based approach is considered to be a practical option for a relatively large number of substances which may require quality standards deriving for the protection of secondary poisoning.

For the description of the methodology to derive an EQS according to the diet approach please refer to the general and refined approach for birds and mammals top predators in Section 4 of the guidance.

Consideration of mixed diet

If a mixed diet must be considered, the daily food intake rate for food item is not simply achieved by applying the respective fraction as a factor to the respective DFI for a "pure" diet. Instead, the DFI has to be adjusted to reflect the actual contribution of each food item to the daily energy expenditure (DEE) of the indicator species. Starting from a given diet composition in terms of fresh weight, first, the energy content of 1g of the mixed diet (fresh weight) is calculated, taking into account the fractions of individual food items and their respective specific energy contents. Using this figure, DFI_{total}, i.e. the required amount of the mixed diet to reach the DEE of the indicator species can be determined.

$$DFI_{total} = \frac{DEE}{\sum_i PD_i \cdot FE_i \left(1 - \frac{MC_i}{100}\right)\left(\frac{AE_i}{100}\right)}$$

In which:
DFI_{total} = Daily Food Intake rate of total mixed diet (g fresh weight/d)
DEE = daily energy expenditure of the indicator species (kJ/d)
PDi = Fraction (percentage in diet) of food item [i] in mixed diet (related to fresh weight)
FEi = Food energy of food item [i] in mixed diet (kJ/dry g)
MCi = Moisture content of food item [i] in mixed diet (%)

AEi = Assimilation efficiency of food item [i] in mixed diet (%)

The actual DFI_i for one food item [i] in the mixed diet (g fresh weight/d) is then achieved by multiplying DFI_{total} by PDi the fraction for the respective food item.
If the food composition is given in terms of dry weight, the same calculation is applied to achieve DFI_{total}, but the DFIi have to be recalculated to fresh weight to be compliant with the derivation of an $EQS_{biota.TopPredators}$.

Further refinement of the assessment factors

The TGD (2003) highlighted some specific considerations that need to be made in selecting an AF for predators.

- CCME (1998) contains wildlife data on body weight and daily food ingestion rates for 27 bird and 10 mammalian species. In addition, Schudoma *et al*. (1999) derived the mean body weight and daily food intake for the otter. The currently available set on wildlife *bw/DFI* ratios ranges from 1.1 to 9 for birds and from 3.9 to 10 for mammalian species. Comparison of these wildlife conversion factors with the values given in Table 4.4 for laboratory species (8.3 – 40) shows that the wildlife species often have a lower *bw/DFI* ratio than laboratory animals. The difference can be up to a factor 8 for birds and 10 for mammals.
- The interspecies variation, however, should comprise more than just the *bw/DFI* differences between species, e.g. the differences in intrinsic sensitivity. The protective value of the "normal" interspecies variation factor may therefore be questionable in case of predators.
- On top of that, many predator species are characterised by typical metabolic stages in their life-cycle that could make them extra sensitive to contaminants in comparison with laboratory animals (e.g. hibernation or migration). Similar to the *bw/DFI* differences, also this aspect goes beyond the 'normal' interspecies variation.

The Table 4.5 gives AF values corresponding to an AF of 10 for the interspecies variations, (excluding the AF of 3 which take into account differences in ingested dose between the test and wildlife species) and an AF ranging from 3 to 10 for the subchronic to chronic extrapolation.

It should be noticed that in the only study found that examined the use of uncertainty factors for the development of wildlife criteria (U.S. EPA, 1995), a value ranging from 1 to 100 is applied to account for uncertainties when extrapolating toxic effect across species (based on the analysis that 91% of 246 separate interspecies NOAEL ratios for wildlife were less than or equal to a factor of 100) and a value ranging between 1 to 10 is applied to account for the subchronic to chronic extrapolation. In the U.S. EPA (1995) document some guidance is given to select the most appropriate assessment factors on a case-by-case basis. Basically to set the AF for interspecies variation the experts consider the physicochemical, toxicokinetic and toxicodynamic properties of the chemical of concern and the amount and quality of the available data. Selection of the subchronic to chronic assessment factor includes consideration of the amount of time required for the chemical to reach equilibrium in the tissues.

The Canadian Council of Ministers of the Environment adopted the same strategy in their guidelines (CCME, 1998) and proposed an AF ranging from 10 to 100 for the interspecies variations and an AF of 10 for the subchronic to chronic extrapolation.
According to these studies there are still some possibilities to refine the AFs for $EQS_{biota\text{-}TopPredators}$ derivation by increasing the knowledge on the interspecies sensitivity at a site or at EU level and on the toxicokinetic properties of the tested substances.

Finally it should be mentioned that further refinement of the interspecies variation should include more information on the intrinsic and the metabolic stages (e.g. hibernation or migration) sensitivities of the organisms intended to be protected.

Protection of fish predators: Case Study

There are currently a number of standard tests for assessing the potential effects of chemicals on fish, in terms of both their direct toxic effects and their uptake and potential for food-chain transfer.

However, these tests do not usually determine the various effect levels (e.g. NOEC, EC10, etc.) relating to the food exposure so there is currently insufficient information to derive a specific quality standard for pelagic predators. Food ingestion rates for fish assumed within the AQUAWEB model (Arnot and Gobas 2004) range from <0.1, for large fish, up to approximately 15 percent body weight per day, for very small fish (on a wet weight basis). Assuming the food to be 90% moisture the food ingestion rates on a dry weight basis are an order of magnitude lower (i.e. less than 2% body weight per day), the data are shown in Figure 3. These food ingestion rates are much lower than those assumed for birds and mammals, when expressed on a dry weight basis (see Figure 1). This might indicate that quality standards derived for the protection of small piscivorous birds are also likely to provide adequate protection for piscivorous fish when exposed by food ingestion. However differences in species sensitivity and trophic level of the food basket must also be considered.

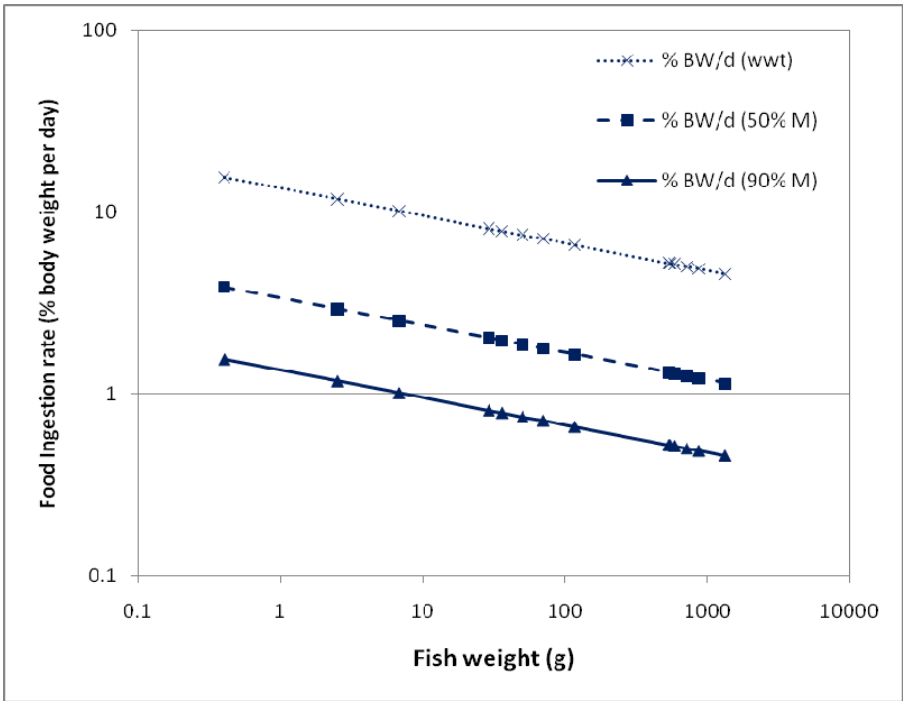

Figure 2 Variation in food ingestion rates for fish, expressed as wet weight (%bw/d (wwt)) and as dry matter assuming 50% (%bw/d (50% M)) and 90% (%bw/d (90% M)) moisture content of the food. All expressed as a percentage of organism body weight per day. (Data from Arnot and Gobas, 2004).

It is not currently considered to be practical to develop separate quality standards for the protection of pelagic predators because of the lack of data. A first approach is to assess if the quality standard for biota is likely to be protective of exposures via the food, and the quality standard for water is likely to be protective of exposures via the water. It may be necessary to review this position should information become available suggesting that where combined exposures occur, from both the water and food, the available quality standards may not be protective and adequate information is available for their derivation and implementation.

A4.3. The critical body burden (CBB) or critical body residues (CBR) approach

The approach of relating ecological toxicity with external concentrations (in this case water values) has some disadvantages for highly hydrophobic substances that do not show toxicity below their solubility values and for substances that tend to bioaccumulate through the food web. For this reason it may be more convenient to change scale of the x-axis when measuring dose and effects and to use concentration in the organisms. In addition measuring concentrations in biota provides indication on the specific bioavailability of a chemical and an integrated estimation of the environmental exposure routes and duration and a strong causality link between acquired dose and biological effect (Meador, 2006). Finally by comparing both metrics in the same experiment it is possible to estimate toxicity and BCF reducing the number of animal tests. Table 4 summarises the main characteristics of this approach when compared with the measurement of water concentrations.

Table 12. Tissue versus water concentration measurements

Tissue concentration	Water concentration
Direct measure of accumulated dose	Indirect measure
Indication of specific bioavailability	Does not consider bioavailable concentration
Integration of exposure routes	Biomagnification not included
Integration of exposure duration	Variable on exposure dynamics (pulse, seasonal, etc.)

The use of Critical Body Burden (CBB) or Critical Body Residue (CBR) - the molal tissue concentration (mmol/kg) of a toxic chemical able to produce a toxic effect, i.e. mortality, reduced growth, reduced reproduction- has been recently promoted by various stakeholders for European risk assessment (see TGD RIP 3.3 and RIP3.2, Chapter R.7B, Appendix 7.8-4), for use in the derivation of environmental quality standards under the Water Framework Directive and in the process of adding substances to the Stockholm Convention on POPs. Under this Convention, CBRs are recommended as a means to compare with environmental concentrations.

This approach was originally proposed after the determination that the tissue concentration of many chemicals with the same mode of toxic action[34] was relatively constant for a defined level of toxicity (McCarty, 1986). McCarty and Mackay (1993) reviewed the CBB approach distinguishing by several model of action, i.e. narcotic, excitatory agents, acetylcholinesterase (AChE) inhibitors, reactives/irritants, central nervous system (CNS) seizure agents, aryl hydrocarbon (Ah) receptor agonists, etc. and between polar and nonpolar compounds. However, even though experimental data supported the application of the CBB approach they cautioned that not all mode of action may support it.

In a recent review, Barron *et al*. (2002) found that experimental data available showed a high variability in tissues residues associated with adverse effects, both within and between chemicals. In addition, dependence on pH, temperature and metabolism showed that the applicability of the CBB approach was not as widely as initially thought. Verhaar *et al*. (1999) showed also that with receptor mediated toxicity the approach did not work. Furthermore, Schuler *et al*. (2004) showed that the CBB approach is not able to deal with substances that form toxic metabolites.

[34] Mode of toxic action is defined as a common set of physiological and behavioural signs that characterize a type of adverse biological response and it can be divided into specific and non-specific. This later is generally referred as the narcosis mode of action (Meador, 2006)

However the inherent advantages of the CBB approach, the great variability range for some substances may exclude the use of one concentration value by model of action and probably a more suitable approach would consists on developing dose-response curves based on tissue concentration like in water and then using the Species Sensitivity Distribution (SSD) approach to arrive at a definition of an EQS. Probably the best approach is to consider case by case. If evidence shows that, for example LR50 (Lethal Residue for 50% mortality), is approximately constant for several species then one value could be used. On the contrary, if the chemical compound shows variable potency between species then the SSD (see below) is the most appropriate method for selecting the tissue residue that will protect the more sensitive species.

Fundamentally, the approach to follow with CBB is the same than using ambient exposure concentrations and both can be combined to produce more results from a single test. In fact, Landrum *et al*. (2004, 2005) provide a methodology for calculating LR50 and MLR50 (mean lethal residues). The main departure from standard toxicity tests (time period ≤96 hours) is that to characterise acute CBR tests should be conducted for a sufficient period of time (7-10 days) to assure that steady-state conditions have been attained. In addition, the same methods to estimate dose-response curves from standard tests (Scholze *et al*., 2001) can also be applied to obtain ER_x (Effective Residues at x proportion) or LR_x (Lethal residue at x proportion). In a similar fashion NOER (No Observed Effect Residue) may be calculated. However, at present stage, we recommend the derivation of a CBB guidance to standardise its application by defining the methodology, the necessary tests as well as the representative species that should be considered. Whereas this already exists for standard toxicity tests an effort is necessary in this case. The coupling with already developed standard toxicity tests is also recommended to reduce animal testing and to obtain already the right conversion values between tissue concentration and water concentration avoiding the high uncertainty of this conversion using the standard approach.

Finally, toxic effects of metabolites should be considered before applying the CBB approach to decide whether to monitor or not the metabolites. In addition, CBB approach does not work for compounds that do not bioaccumulate but cause only a toxic response. These compounds will be eliminated quickly from the organisms and therefore a dose-response curve would not exist or measured concentration will tend to be too low. In this case, probably the food intake would be a more adequate approach. For compounds where exposure and response are separated by long periods of time, i.e. mutagenic chemicals, CBB is not adequate.

Critical Body Residue approach for dietborne metal

As some metals bioaccumulate significantly in metal specific target organs, for example: liver for lead, kidney for cadmium, brain for mercury and eggs for selenium (Beyer *et al*. 1996), it is recommended to identify Critical Organ/Tissue Residues for relevant species instead of the overall CBR proposed for organic compounds. This approach would involve the comparison of measured metal concentrations in the organs of animals with critical established concentrations for the selected organs.

However, in order to apply this approach, relevant indicator species and organs/tissues of these species that are sensitive to the analysed metal would need to be identified and critical concentrations for the organs need to be defined. Afterwards, levels should be monitored. As for the case of organics, it has to be stressed that the interpretation of such data might be hampered by the fact that internal concentrations may result from exposure at different sites. This problem can be overcome by choosing appropriate indicator species foraging and living constantly in a local habitat e.g mussels. The possibility to directly link measured concentrations in organs of indicator organisms to environmental concentrations prevailing in their habitat does increase the relevance of such analyses.

As before, it has to be stressed that due to animal welfare concerns and in order to be in line with the new REACH legislation, the CBB approach should be minimised in vertebrates organisms and should be avoided in top predators.

A4.4. The species sensitivity distribution (SSD) approach

In data rich cases a species sensitivity distribution (SSD) approach using chronic toxicity data for a range of predators might be used in order to estimate an HC5 (Hazardous Concentration for 5% of species). The data requirements for such an approach (i.e. a sufficient number of species will have been tested in long-term tests) are currently unlikely to be fulfilled for many, if not all, substances. This should consider issues such as the applicability of different species, minimum data sets for the use of a species sensitivity distribution in the derivation of a $PNEC_{oral}$ for consumers (variability of species tested, test duration and endpoint, number of data, etc.) and the identification of suitable'representative prey' organisms.

However, an example of how this might be done extracted from Environment Agency Report (2008) is provided below to illustrate its application.

No Observed Effect Concentrations (NOECs) are reported in the draft lead Risk Assessment Report (RAR). Values that are 'greater than' are unbounded NOECs and their use in an SSD is conservative because the true NOEC will be higher.

According to Figure 4 the lognormal model fit meets all normality and goodness-of-fit statistics at the 1% level. The HC5 (50%) is 28.13 mg Pb kg^{-1} wwt and the HC5 (90%) is 10.43 mg Pb kg^{-1} wwt. Only one, unbounded, value of >25 mg Pb kg^{-1} wwt falls marginally below the HC5 (50%), suggesting that the HC5 (50%) is a robust threshold.

If bird and mammal SSDs are constructed separately, the bird HC5 (50%) is 23 mg Pb kg^{-1} wwt and the HC_5 (90%) is 6.72 mg Pb kg^{-1} wwt, and the mammal HC_5 (50%) is 50.7 mg Pb kg^{-1} wwt and the HC5 (90%) is 5.7 mg Pb kg^{-1} wwt. A log-normal model meets all normality and goodness-of-fit statistics at the 1% level for both SSDs. This suggests that, for some substances at least, it may be possible to use an SSD approach in the effects assessment. Whilst it is likely that an assessment factor would be considered for application to the HC5, a lower one might be applied than when using the lowest reported NOEC. Two tests with low NOEC values have been reported for the American Kestrel, which would be considered as a relevant species for wildlife assessment.

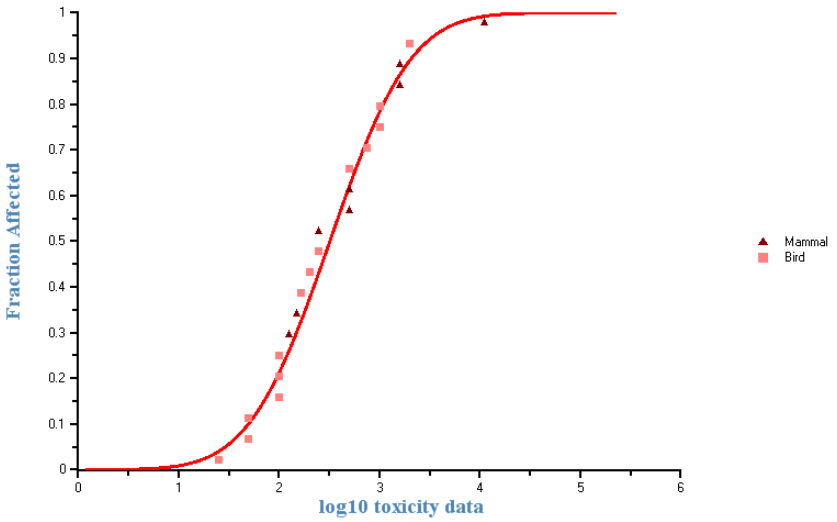

Figure 3. SSD based on mammal and bird oral toxicity data (Peters and Crane, 2008).

Further background information on the use of species sensitivity distributions can be found in the report from the Avian Effects Workshop held in Woudschoten (Hart *et al.* 2001), the publication of

Posthuma et al. (2002) and Section R 10.2.4 of Chapter R 10 of RIP 3.2-2 of the TGD in support of the New EU Chemicals Legislation (REACH).

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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
$NOEC_{site\text{-}specific}$	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
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)
TMF	trophic magnification factor
TL	threshold level
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 BMF2 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 (only BMF_1) as the top predator (i.e. human) is identical for fresh and marine (has the same trophic position). Is this clear from the guidance?
MAC-$QS_{fw, eco}$	MAC-$QS_{sw, eco}$	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			

Freshwater	Saltwater	short description	REMARK
$QS_{generic, fw, eco}$	$QS_{generic, sw, eco}$	uncorrected standard for ecosystem	
$QS_{reference, fw, eco}$	$QS_{reference, sw, eco}$	standard for ecosystem for reference conditions	
$QS_{site\text{-}specific, fw, eco}$	$QS_{site\text{-}specific, sw, eco}$	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 BMF2 is applied for saltwater
$EQS_{sediment, fw}$	$EQS_{sediment, sw}$		

APPENDIX 7: LEADERS OF THE ACTIVITY / MEMBERS OF THE EXPERT GROUP

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