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SCIENTIFIC OPINION

Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water[1]

EFSA Panel on Plant Protection Products and their Residues[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The EFSA Panel on Plant Protection Products and their Residues (PPR Panel) was tasked to revise the Guidance Document (GD) on Aquatic Ecotoxicology under Council Directive 91/414/EEC (SANCO/3268/2001 rev. 4 (final), 17 October 2002). This scientific opinion of the PPR Panel is the second of three requested deliverables within this mandate. The scientific background for the risk assessment on sediment organisms in edge-of-field surface waters is provided, with reference to benthic ecology and ecotoxicology, available test protocols and current knowledge on exposure and effects of sediment-bound plant protection products (PPPs). The scientific opinion provides approaches on how to derive regulatory acceptable concentrations (RACs) for sediment organisms and exposure to active substances of PPPs and transformation products of these substances, and how to link them in a tiered approach to predicted environmental concentrations (PECs) for the sediment compartment. A list of uncertainties in relation to such approaches is given.

KEY WORDS

pesticides, formulations, metabolites, sediment exposure, benthic organisms, ecotoxicology

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SUMMARY

Sediment Environmental Risk Assessment (ERA) is a combination of exposure and effect assessment. Proposals for these assessments are provided. The opinion describes specific protection goals (SPGs) for sediment-inhabiting organisms based on two options (1) the ecological threshold option (ETO), accepting negligible population effects only, and (2) if applicable, the ecological recovery option (ERO), accepting some population-level effects if ecological recovery takes place within an acceptable time period.

Triggers for sediment ecotoxicity testing are proposed together with a decision scheme for when additional testing may be required. The ecotoxicologically relevant concentrations (ERCs) for sediment organisms are proposed, as this will be influenced by the choice of sediment layer, exposure metric and test duration.

The current Forum for the co-ordination of pesticide fate models and their use (FOCUS) methodology for surface water does not consider the effect of multi-year applications, which could possibly lead to accumulation of pesticides in sediment. To account for this deficit, a proposed methodology for introducing an accumulation factor that can be used until an updated FOCUS methodology becomes available is presented in this opinion. The accumulation factor can be considered a conservative approach, since it does not include any possible transport processes, such as leaching or volatilisation, which may reduce the accumulation in sediment in the real field situation.

A tiered effect assessment approach is proposed for different sediment organisms and how to link regulatory acceptable concentrations (RACs) to predicted environmental concentration (PECs). The assessment of bioaccumulation, biomagnification and secondary poisoning is discussed, as well as the prospect of improving ERA for sediment microorganisms.

Transformation products from active substances also may need to be assessed, as well as mixture toxicity of formulations of plant protection products (PPPs). The opinion gives recommendations on these aspects and discusses issues related to uncertainties of the current and/or proposed ERA approaches.

Soft sediments of edge-of-field ponds, ditches and streams are characterised by a significant horizontal and vertical heterogeneity in physical, chemical and biological properties. The distribution of benthic organisms is patchy and varies among different sediment habitats. Organisms living in (endobenthos) and on (epibenthos) soft sediments cover all trophic levels and different feeding strategies. Benthic organisms comprise microorganisms (bacteria, archaeans, fungi, protozoa), microphytobenthos (algae), rooted macrophytes (vascular plants), meiobenthos (nematodes, targidates, copepods, ostracods, chydorid cladocerans) and macrobenthos (larvae of insects, macrocrustaceans, oligochaetes, molluscs, vertebrates). Although they currently receive little attention in sediment ERA for PPPs, microorganisms (bacteria, archaeans, fungi and protozoans) are integral parts of sediment communities. They play a vital role for metabolic activities and food web interactions and the microbial diversity of sediments is huge.

Internationally accepted protocols to conduct single-species laboratory toxicity tests with typical benthic freshwater species have been developed for a limited number of taxa only. The vast majority of published sediment-spiked laboratory toxicity tests with PPPs concerned tests with insects, *Chironomus* spp., and the crustacean *Hyalella azteca*. Sediment-spiked toxicity tests with PPPs and the oligochaete *Lumbriculus variegatus* (Oligochaeta) and rooted macrophyte *Myriophyllum* spp. have not often been reported until now. In the Organisation for Economic Co-operation and Development (OECD) test protocols artificial sediment is recommended, whereas the United States Environmental Protection Agency (US EPA)/American Society for Testing and Materials (ASTM) International technical guidelines recommend the use of natural sediment. In addition, the OECD and the US EPA/ASTM guidelines differ with respect to the spiking procedure, which may affect exposure conditions in the tests. Standard tests with microorganisms are not included in the current data

requirements for aquatic ERA of PPPs. However, there have recently been repeated calls for improving the consideration of microorganisms in ERA of PPPs. Existing International Organization for Standarization (ISO) tests with microorganisms are of limited use in prospective ERA and more research and method development are needed. From existing information it is still unclear whether microbial communities are more sensitive to PPPs than other organisms and when microbial tests are actually needed. Standardised test systems that are able to provide information that is sufficiently representative of the wide diversity of microorganisms, microbial processes and sediment habitats have not been developed.

The European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues (PPR Panel) recommends the initiation of comparative studies to evaluate and understand differences in OECD and US EPA/ASTM guidelines (e.g. artificial vs. natural sediment; various ageing periods before starting toxicity tests) and the possible consequences for toxicity estimates. For sediment ERA, the PPR Panel also recommends to increase knowledge on (1) the most relevant type of ecosystem (ponds, ditches, streams) in terms of ecological niche for benthic organisms, (2) the most relevant type of edge-of-field aquatic ecosystem in terms of contamination, (3) differences in sensitivity of benthic populations between lentic (ditches and ponds) and lotic (streams) systems, (4) possible differences in benthic communities of edge-of-field surface waters between different regions in Europe (e.g. differences in terms of species composition and life traits), (5) effects of repeated exposure (within one year or over multiple years) on the benthic communities (culmination of effects), and (6) the representativity of standard test species for the field communities in terms of sensitivity and vulnerability.

Since the taxonomic groups that play a major role in providing ecosystem services are the same for the pelagic and sediment compartments, it is advised to adopt the same SPG options for benthic organisms as already developed in the Aquatic Guidance Document (AGD) (EFSA PPR Panel, 2013). This implies that, in general, benthic taxa need to be protected at the population level, except aquatic vertebrates (benthic fish and amphibians) that warrant protection at the individual (to avoid direct mortality and animal suffering) to population level (e.g. chronic effects via reproduction), and microorganisms that need to be protected at the functional group level. The ERO might be applicable to define SPGs in some cases. However, there are several reasons why, for the time being, a prudent approach is required in applying the ERO and thus it is suggested that the ETO is the best option to provide adequate protection of benthic organisms.

Sorption to sediments is likely to reduce the bioavailability of PPPs for many benthic organisms by reducing aqueous concentrations (in overlying and interstitial water). Sorption may, however, increase exposure for benthic fauna, particularly sediment-ingesting organisms. The freely dissolved fraction of PPPs in pore water most likely is the main sediment exposure route for benthic algae, rooted macrophytes and microbes. For benthic animals, both the pore water fraction as well as the particulate-associated fraction may constitute important sediment exposure routes. In particular, dietary exposure can play a role in sediment fauna and phagotrophic protozoans. Furthermore, the specific toxic mode-of-action of PPPs is important to consider when assessing environmental risks of sediment-exposure and selecting benthic test species. The few microcosm and mesocosm studies that focused on the ecological impact of sediment-exposure to PPPs, revealed that compounds that are persistent in sediment may have long-lasting effects on benthic organisms and communities.

This opinion proposes to trigger sediment ERA for PPPs if (1) more than 10 % of the radio-labelled test material can be found in the sediment at or after 14 days after application in the standard water–sediment fate study (OECD Guideline 308), or more than 10 % of the total annual dose of the active ingredient occurs in sediment at the time of maximum PEC_{sed} as assessed by FOCUS modelling, and (2) the chronic No Observed Effect Concentration (NOEC)/EC10 (concentration where 10 % effect was observed/calculated) of *Daphnia* or another relevant pelagic animal species is less than 0.1 mg/L, or the chronic EC50 (concentration where 50 % effect was observed/calculated) of the standard test alga or vascular plant is less than 0.1 mg/L. The current experimental triggers for sediment accumulation should not be replaced by triggers based on properties such as K_{oc} (soil organic carbon

(OC)–water partitioning coefficient) and DegT50 (time taken for 50 % of a substance to disappear in the water–sediment system).

To avoid unnecessary testing with benthic organisms it is furthermore proposed to use chronic toxicity data for pelagic organisms and the equilibrium partitioning (EqP) approach for an initial screening in the ERA for PPPs, but to apply an extrapolation factor of 10 for benthic fauna to cover the possibility of exposure due to sediment ingestion. The predictive value of this modified EqP approach was tested for a limited number of compounds and water-spiked and sediment-spiked tests with *Chironomus*. It is therefore recommended to evaluate the general applicability of this approach for a larger array of PPPs and benthic species.

This opinion proposes to express the PEC_{sed} and RAC_{sed} estimates in terms of (1) total sediment concentration based on dry weight, normalised to either the OC content in the dry sediment or to standard OECD sediment with an organic matter content of 5 %, and (2) the freely dissolved PPP fraction in pore water. Furthermore, it is proposed to use the 0–1 cm sediment layer for PEC_{sed} derivation in the case that benthic fauna and microorganisms are the organisms of concern, while the 0–5 cm sediment layer may be used for rooted macrophytes. The RAC_{sed} derivation should preferably be based on chronic toxicity data using sediment-spiked tests and benthic organisms, not excluding that semi-chronic toxicity data can also be used to derive a RAC_{sed} if an appropriate additional extrapolation factor is used.

The current FOCUS methodology does not consider the effect of multi-year applications that can lead to accumulation in sediment. To account for this deficit it is proposed to include an accumulation factor. The PPR Panel did not revise or evaluate the current exposure assessment in detail but advises to critically evaluate and improve the FOCUS surface water exposure assessment in the future and to develop new sediment scenarios for total content and pore water concentrations.

Bioaccumulation is of particularly high relevance for benthic organisms since the sediment compartment is a sink for substances that may have a high Bioconcentration Factor (BCF), and benthic organisms have a great potential in terms of accumulating toxic substances and in transferring them to higher trophic levels. It is proposed to perform spiked sediment bioaccumulation tests with benthic invertebrates for substances that show significant bioaccumulation in fish tests (BCF > 2 000 L/kg), when the substance is: (i) persistent in sediment (half-life > 120 days in water–sediment fate studies) and log octanol–water partition coefficient (K_{ow}) > 3, or (ii) non-persistent in sediment (i.e. half-life < 120 days in water–sediment fate studies), log K_{ow} > 3 and 10 % or more of the substance found in the sediment (based on water–sediment fate studies) or FOCUS step 2 and/or step 3 modelling (or using another appropriate model). Further guidance on how to incorporate the outcome of invertebrate bioaccumulation studies in the regulatory evaluation of the risks of food chain transfer and secondary poisoning needs to be elaborated. Currently, the risks of biomagnification and secondary poisoning of sediment-bound PPPs are not addressed in a risk assessment scheme. The PPR Panel recommends further development of such a risk assessment scheme based on existing contaminant food web transfer experiments and models. These should include the accumulation from sediments, water and dietary sources into sediment-dwelling invertebrates, fish (primary and secondary consumers), piscivorous birds and mammals and birds and mammals (e.g. bats) preying on emerging adult insects. Guidance for reliable food web modelling is expected to be provided in the future PPR scientific opinion on ecological modelling.

This opinion proposes to adjust the Tier 1 decision scheme based on current data requirements by including additional test organisms (e.g. *Hyalella azteca*) depending on the toxicological mode-of-action of the substance. The Panel asks the Commission to amend the data requirements accordingly. Further considerations on suitable Tier 1 benthic test species for fungicides is required since–at least in terms of acute effects–these substances may be less receptor specific and thus may target vertebrates as well as invertebrates or primary producers.

The PPR Panel proposes to not apply the Geomean approach in the Tier 2 sediment effect assessment based on chronic toxicity data. Stronger scientific underpinning of the concept is needed, using chronic toxicity data for a wide array of sediment organisms and substances that differ in toxic mode-of-action. For the time being, a Weight of Evidence (WoE) approach is proposed if chronic toxicity data are available for additional benthic test species, but the number of data is too low to allow the Species Sensitivity Distribution (SSD) approach. The PPR Panel proposes to develop a transparent decision scheme for the WoE approach, more specifically to develop criteria to lower the default Assessment Factor (AF) to be applied to the lowest valid toxicity value, based on the quality and number of additional toxicity data available.

If sediment toxicity data are available for a sufficient number of benthic species, it is proposed to follow the SSD approach as much as possible according to the criteria described in the AGD (EFSA PPR Panel, 2013). This means that for PPPs toxicity data should be available for at least eight benthic species of the potentially sensitive taxonomic group (most likely arthropods for insecticides; rooted macrophytes for herbicides). For substances for which a specific potential sensitive taxonomic group cannot be identified on basis of the available toxicity data for pelagic organisms, a minimum number of eight toxicity data for at least five different benthic taxonomic/feeding groups may be selected. This may be the case for fungicides with biocidal properties.

The AF of 10 for Tier 1, as given in the uniform principles (Regulation (EC) No 546/2011[4]) for chronic toxicity data, has not been sufficiently validated/calibrated for all types of PPPs and it is not fully clear whether all relevant uncertainties are covered in any case. Calibration should be performed between lower and higher tiers (micro-/mesocosm studies data and, if possible, field data) for sediment organisms. With the reference being the field itself, it is recommended to conduct further investigations in the sediment compartment of edge-of-field surface waters to strengthen the link between results of experimental ERA approaches and the situation in the field, that is, to perform a retrospective evaluation. An important research need is to develop sediment toxicity data sets for benthic organisms and modern PPPs that differ in toxic mode-of-action so that the validity of the tiered approach as proposed in this scientific opinion can be evaluated.

In constructing micro-/mesocosm tests to study population- and community-level effects of sediment exposure to PPPs, field-collected sediment is largely preferred over artificial sediment (in accordance with OECD guidelines). Natural sediments allow the development of a realistic and diverse benthic community, despite the fact that they may be contaminated with unknown background chemicals and difficult to standardise in terms of composition across studies. An important question is whether to use spiked sediment to construct micro-/mesocosm or to follow the traditional approach in constructing micro-/mesocosms with ‘clean’ sediment and to spike the water column with the PPP (water or sediment slurry applications). The PPR Panel considers both designs feasible, but a reasoned case should be presented as to why a specific design is chosen.

The PPR Panel recommends exploring the use of micro-/mesocosm test systems that associate both the aquatic (surface water) and the sediment contamination, which would allow study of more realistic conditions of contamination in water bodies. Such studies would focus on effects of combined exposure routes (i.e. spiked water that simulate the drift entry and spiked sediment that simulate the historical background and the freshly entering PPP). Irrespective of the design of micro-/mesocosm experiments, dynamics in exposure concentrations in the relevant sediment layers should be monitored. This implies that for a proper sediment effect assessment for benthic invertebrates, the dynamics in exposure concentrations in the upper 1 cm of the sediment compartment have to be monitored. For rooted macrophytes a deeper sediment layer (5 cm) may be appropriate. If measuring exposure concentrations in pore water is difficult, prediction on the basis of sediment characteristics

[4] Commission Regulation (EU) No 546/2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

and measured total PPP concentrations is also a possibility. In conducting sediment micro-/mesocosm tests, the PPR Panel advises to always include observations on long-term benthic population and community-level effects. The duration of the study needs to be long enough to cover the duration of the full life cycle of the most sensitive benthic species at risk in order to detect the effects.

The effect assessment for aquatic vertebrates and exposure to PPPs in sediment is a research activity to date. Based on the data requirements and current knowledge, it is not possible to deliver, at this stage, a consolidated ERA scheme. In particular, more research and analysis of data is needed to identify which exposure routes are most relevant, depending on aquatic vertebrate species and substances.

Functional properties of microbes currently have greater potential than structural ones for prospective ERA of PPPs, since effects are easier to interpret as either positive or negative. Recently developed ISO standards for determining effects of chemicals on functional properties related to nitrogen cycling seem to have the highest potential for use in prospective ERA of PPPs.

When the effect estimate for benthic organisms is expressed in terms of initial exposure concentration, it should be plausible that the exposure profile in the sediment toxicity test is realistic worst-case relative to that predicted for field sediments, otherwise these effect estimates cannot be directly used in ERA. If the effect estimates on which the RAC_{sed} is based are expressed in terms of the initial test concentration, it is recommended that the $PEC_{sed;max}$ concentration should be used in ERA to ensure a more realistic worst-case risk assessment. Furthermore, it is recommended to use the $PEC_{sed;max}$ in sediment ERA as a default procedure, and to consider the use of the $PEC_{sed;twa}$ only if field exposure concentrations are demonstrated to be sufficiently variable during a time frame smaller than the duration of the sediment-spiked toxicity test that drives the RAC_{sed}. In addition, it is recommended to develop two types of sediment exposure scenarios, one with low OC (worst-case pore water scenario) and one with high OC (worst-case total content scenario). It seems necessary to develop environmental scenarios for ponds, ditches and streams in the near future to better integrate the physico-chemical and biological properties important for exposure and effect assessment, and to ensure that the ERA for pelagic organisms is not in conflict with that for benthic organisms in the sediment compartment of the same system.

If the relative contribution of the older (e.g. > 1 year) and recent fractions (e.g. latest growing season) in the $PEC_{sed;tot}$ is calculated this knowledge might be considered in a higher tier by (1) using refined-exposure toxicity tests by spiking the sediment in different phases and allowing different ageing periods for the different fractions before using the sediment in sediment-spiked toxicity tests, or (2) using appropriate modelling approaches to better estimate the bioavailable fraction of the $PEC_{sed;tot}$ estimate.

For the chronic ERA of metabolites in the sediment compartment this opinion proposes to follow the same approach as described in the AGD (EFSA PPR Panel, 2013). For the chronic ERA of chemical mixtures, this opinion proposes to follow also the approach described in the AGD (EFSA PPR Panel, 2013) as well as the further developments and recommendations of the scientific opinion (EFSA PPR Panel, 2014). It is acknowledged that more information is needed on the presence and bioavailability of historical pollution and more recent pollution in sediments not only by the product under evaluation but also by other products applied simultaneously or successively in order to take account of possible consequences of multiple stressors in the prospective sediment ERA for PPPs.

Lists of uncertainties related to exposure and effects assessment and the combination of the two are derived (although not exhaustive). In the development of guidance all uncertainties should be weighted for an overall assessment of uncertainty. Based on this overall assessment it can then be decided if a precautionary approach should be applied as stated in Article 1(4) of EU Regulation (EC) No 1107/2009[5].

[5] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

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BACKGROUND AS PROVIDED BY EFSA

Member States' competent authorities were requested by the Director of Sciences of the European Food Safety Authority (EFSA) on 3 July 2006 *via* the Standing Committee on the Food Chain and Animal Health, to send EFSA a priority list of existing Guidance Documents to be revised and proposals for development of new ones. Answers were received from 15 Member States.

Regarding the revision of the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001, rev. 4 final, 17 October 2002), five detailed requests were received (FI, DE, NL, DK, SE) highlighting the importance of liaising with the revision of Annex II and Annex III.

In 2006 and 2007, EFSA has issued six opinions on the Annexes II and III, two of which related to the ecotoxicological studies (EFSA PPR Panel, 2007a) and the fate and behaviour in the environment (EFSA PPR Panel, 2007b). The rapporteur (UK) has taken these opinions on board in the revision of the Annexes, which are currently with the Commission. It should be considered to generally revise the structure and content of the available Guidance Documents.

Member States highlighted the following issues as being particularly important:

- More clarity regarding the data requirements for substances expected to be endocrine disrupters is needed;
- More guidance should be provided regarding the use of $FOCUS_{SW}$ modelling, e.g. on input parameters or the use of Step 4;
- Need for revision in particular with regard to the protection level in adjacent small ditches and main watercourses (in line with the requirements of the Water Framework Directive);
- More integrated development of the assessment of exposure modelling and effects;
- Conceptual consistency between higher tier assessments in aquatic and terrestrial ecotoxicology needed;
- More guidance regarding the assessment of higher tier aquatic studies (assessment of addition of sediments, assessment of quality and quantity of mesocosm studies, assessment of ecotoxicological field studies, trigger levels for higher tier studies);
- Harmonised endpoints for authorisation of plant protection products needed;
- A clear and transparent relationship with the Water Framework Directive is wished for.

The EFSA *PRAPeR Unit* emphasised that the aquatic GD needs to be updated regarding the long-term ERA to take account of the new exposure data that are the outcome of the FOCUS models. The interaction between exposure and effects needs some more guidance. Of course also possible new data requirements in the new regulation that will replace Council Directive 91/414/EEC need to be taken up in the existing GD.

Relevant topics and scientific principles of already existing scientific opinions elaborated by the PPR Panel will also be incorporated into the revised Guidance Document. Further, on-going work in other fora, pertinent to the GD will be closely monitored and taken into account where relevant.

The public was consulted on the existing GD in October – December 2008 and comments and ideas for the revision by stakeholders will be taken into account during the process. Also comments from a risk manager survey performed October – December 2008 are considered. Furthermore, the activity performed under EFSA-Q-2009-00861 to develop specific protection goals will be used as input to this updated mandate.

TERMS OF REFERENCE AS PROVIDED BY EFSA

EFSA tasks its Scientific Panel on Plant Protection Products and their Residues (PPR Panel) to prepare a revision of the Guidance Document on Aquatic Ecotoxicology under Council Directive 91/414/EEC (SANCO/3268/2001 rev.4 (final), 17 October 2002).

The PPR Panel is asked to develop a Guidance Document and two Scientific Opinions, as summarised below:

Guidance Document on tiered risk assessment for aquatic organisms in edge-of-field surface waters (by July 2013).

In particular, the following issues need to be addressed:

- Update the current guidance in view of the new Regulation (EC) No 1107/2009
- Update the current guidance in view of the revised data requirements to Regulation (EC) No 1107/2009
- Develop guidance on first tier aquatic effect assessment
- Develop guidance on higher tier aquatic effect assessment (based on laboratory studies and model ecosystem studies, guidance on design and evaluation of higher tier studies)
- Guidance on appropriate linking of aquatic exposure and effect assessment

This PPR Panel Guidance should be subject to a Public Consultation.

Scientific Opinion of the PPR Panel on the effect assessment for pesticides on sediment organisms in edge-of-field surface waters (31 December 2015)

A scientific opinion will be provided that describes the state of the art of effect assessment for sediment organisms.

In particular the following issues will be addressed:

- Identification of standard test species
- Use of the geometric mean approach when toxicity data for a limited number of additional test species are available
- Use of Species Sensitivity Distribution approach for sediment organisms
- Use of the model ecosystem approach for sediment organisms
- Defining the ecotoxicologically relevant concentrations (ERCs) for acute and chronic risk assessment

Scientific Opinion on the state of mechanistic effect modelling approaches for regulatory risk assessment of pesticides for aquatic organisms (31 December 2017)

A scientific opinion will be provided that describes the state of the art of mechanistic effect modelling in the aquatic environment.

In particular the following state of the art of the following types of models will be addressed (for all aquatic water column and sediment dwelling organisms):

- Describe regulatory questions that can be addressed by effect modelling

- Describe model parameters that need to be included in relevant models and that need to be checked in evaluating the acceptability of effect models
- Describe available effect models for aquatic organisms, in particular
    - Toxic kinetic/toxicodynamic models
    - Mechanistic population models
    - Mechanistic food web models
        - Secondary poisoning
        - Ecosystem models representative for ditches, ponds and streams
- Selection of focal species
- Development of ecological scenarios that can be linked to the regulatory defined water bodies in the climatic zones of Europe

This Scientific Opinion addresses the second part of the Terms of Reference, whereas the first output (the Guidance Document) was already published in July 2013, and the third output (the opinion on mechanistic effect modelling) outlined above will follow later.

ASSESSMENT

1. Introduction

1.1. Context and regulatory background of the opinion

In 2013, the European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues (PPR Panel) published the document '*Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters*' (EFSA PPR, Panel 2013), a revision of the former Guidance Document on Aquatic Ecotoxicology (EC, 2002). The revision was, amongst others, necessary because Regulation (EC) No 1107/2009 and the new data requirements (EC, 2013) place new demands on the effect assessment for pesticides in edge-of-field surface waters. The revised Aquatic Guidance Document (AGD) of the PPR Panel focuses on water exposure to pesticides and aquatic organisms living in the water column, paying limited attention to sediment exposure and risk assessment schemes for typical benthic organisms. In the AGD only a Tier 1 effect assessment procedure for sediment-dwelling organisms on the basis of the 28-day water-sediment test with *Chironomus riparius* or *Lumbriculus* spp. is presented. As mentioned above in the 'Terms of reference as provided by EFSA', it was emphasised that a second deliverable within the PPR Panel mandate of the revision of the former Guidance Document on Aquatic Ecotoxicology would focus on prospective effect assessment procedures for benthic organisms and exposure to pesticides in the sediment compartment. This scientific opinion aims to be this second deliverable.

1.2. Aim and scope of the opinion

For the time being, until EFSA has developed guidance on the effect assessment for pesticides on sediment organisms, the EFSA AGD (EFSA PPR Panel, 2013) recommends to use the 28-day sediment-spiked test with *Chironomus* (OECD, 2004a) for substances with an insecticidal activity and the 28-day sediment-spiked *Lumbriculus* test (OECD, 2007a) for active substances with a fungicidal activity. In addition, a sediment-spiked test with the rooted macrophyte *Myriophyllum* has been proposed as a suitable test for substances with herbicidal activity (Maltby et al., 2010). According to the AGD, the Tier 1 Regulatory Acceptable Concentration (RAC) for the sediment compartment is derived by applying an Assessment Factor (AF) of 10 to the lowest 28-day No Observed Effect Concentration (NOEC)/EC10 (concentration were 10 % effect was observed/calculated) for *Chironomus riparius* and/or *Lumbriculus* spp. In line with the effect assessment on the basis of water exposure as currently used in the AGD, the effect assessment for sediment exposure and rooted macrophytes may be conducted by applying an AF of 10 to the EC50 (concentration were 50 % effect was observed/calculated) derived from a sediment-spiked *Myriophyllum* test. In the risk assessment the toxicity estimates for the Tier 1 sediment test species are compared with the sediment predicted environmental concentrations (PECs) using the Forum for the Co-ordination of pesticide fate models and their Use (FOCUS) exposure assessment methodology (see Chapter 7).

This scientific opinion describes the state-of-the-art of assessment procedures to address effects of plant protection products (PPPs) to sediment-dwelling organisms. Sediment-dwelling, or benthic organisms, are defined here as organisms that, during an important part of their life cycle, have their habitat on (epibenthos) or in the sediment (endobenthos). This opinion predominantly deals with prospective sediment risk assessment within the context of the regulatory framework underlying the authorisation of PPPs in the European Union (EU). Within this context, EFSA is also in the process of updating guidance on risk assessment for pesticides and soil organisms. The usefulness of total concentrations and pore water concentrations of pesticides in soil as metrics for the assessment of ecotoxicological effects on soil organisms was already the subject of an EFSA opinion (EFSA PPR Panel, 2009). Currently, the linking of exposure to effects and possible effect assessment procedures for soil organisms are discussed in an EFSA PPR Panel working group. Where appropriate, the concepts and approaches already developed by EFSA for prospective environmental risk assessments (ERAs) for soil organisms will be considered in the current scientific opinion on sediment organisms. Furthermore, principles for ERA of the sediment compartment developed under the auspices of other European regulatory authorities (e.g. EC, 2011b; ECHA, 2014), as well as those developed by the

Organisation for Economic Co-operation and Development (OECD) (e.g. OECD, 2004a, b, 2007a, 2010a) and regulatory authorities outside the EU (e.g. US EPA, 2000) will be considered as well. In addition, current knowledge on sediment ecotoxicology of pesticides, as published in the scientific literature, will be taken into account.

1.3. Focus and restrictions of the opinion

This scientific opinion will focus on experimental approaches that can be used in the prospective effect assessment for benthic organisms subject to sediment exposure of PPPs in edge-of-field surface waters (ditches, streams and ponds), with special reference to soft sediments (e.g. sandy, loamy, peaty sediments). This means we do not develop separate ERA schemes for organisms on rocky surfaces and in biofilms since we consider that this is sufficiently addressed in the AGD (EFSA PPR Panel, 2013). A later PPR Panel scientific opinion will deal with possible mechanistic effect models that can be used in the aquatic risk assessment for sediment-dwelling organisms. PPPs with microbial active agents have specific data requirements and are not treated in this scientific opinion.

Assessment of risks to organisms is always a combination of an effect assessment and an exposure assessment (EFSA PPR Panel, 2010a). This scientific opinion focuses on the effect assessment. The current exposure assessment for active substance approval is based on FOCUS (2001, 2006, 2007a, b) and is described in greater detail in Chapter 7 of this scientific opinion. The level of protection achieved by the current FOCUS surface water exposure assessment methodology is uncertain since the FOCUS scenarios have not been reviewed during the revision of the AGD by the PPR Panel and no exposure assessment goals have yet been defined for edge-of-field surface waters, including their sediment compartment. Furthermore, these standard FOCUS surface water scenarios were developed as realistic worst-case surface water scenarios for the prediction of PEC_{sw} values. Therefore, it is possible that the corresponding predicted sediment concentrations describe a more or less best-case rather than worst-case situation, since a realistic worst-case exposure in the water column will not occur if PPPs show a fast partitioning to the sediment compartment. Nevertheless, the methodology has been used in regulatory decision making throughout the last few years and there is currently no alternative standardised exposure assessment methodology. Therefore, it is assumed that the $FOCUS_{sw}$ methodology will continue to be used until updated or new methods become available and adopted by the Standing Committee of Plants, Animals, Food and Feed (SCoPAFF) and will replace the existing tools. $FOCUS_{sw}$ is used for approval of active substances at EU level. It is also used in some Member States for product authorisation, but also different exposure assessment procedures may be used.

The data requirements for sediment risk assessment of pesticides mainly focus on freshwater organisms, and the exposure assessment is performed for edge-of-field freshwater ecosystems only. This scientific opinion, however, will evaluate if sediment toxicity data for marine benthic organisms could be used in combination with sediment toxicity data for freshwater benthic species in possible higher-tier assessments.

1.4. Structure of the document

The ecology of sediment flora and fauna is briefly introduced in Chapter 2, as supporting information for Chapter 3 on specific protection goals (SPGs) and Chapter 4 on the current knowledge of exposure and effects of sediment-bound PPPs in edge-of-field surface waters. Chapter 5 deals with the legislative triggers for sediment testing. Chapter 6 deals with defining the ecotoxicologically relevant concentrations (ERCs) for sediment risk assessment. The calculation of PECs for total sediment and pore (interstitial) water based on the FOCUS surface water approach, and adjusted to cover accumulation of the PPP in sediment because of possible multi-year use, is introduced in Chapter 7. Chapter 8 is the main chapter on effect assessment, dealing with different tiers to assess toxicity of sediment-exposure to benthic organisms (section 8.2), as well as bioaccumulation, biomagnification and secondary poisoning (section 8.1). Chapter 9 addresses the linking of exposure to effects in sediment ERA. Chapter 10 focuses on sediment ERA on metabolites and of formulated products with more than one active substance. Chapter 11 deals with the uncertainties occurring in the sediment risk assessment and finally Chapter 12 presents the main conclusions and recommendations of this scientific opinion.

2. Benthic ecology of edge-of-field surface water and available guidelines for test species

2.1. Properties of the sediment compartment

In the scientific literature several papers can be found that describe the ecology of edge-of-field ponds (e.g. Davies et al., 2008a, b; Biggs and Brown, 2010), ditches (e.g. Davies et al., 2008a, b; Brock et al., 2010a; Biggs and Brown, 2010; Verdonschot, 2012) and streams (e.g. Biggs et al., 2007; Davies et al., 2008a, b; Alonso Prados and Novillo-Villajos, 2010; Biggs and Brown, 2010; Wogram, 2010). These papers, however, do not describe in a comparative way the main differences in physical, chemical and biological properties of the sediment compartment of these systems. Therefore, on the basis of the overall descriptions of these surface waters, the differences in properties of the sediment compartment between ponds, ditches and streams were distilled in general terms (see Table 1).

Table 1: Properties of edge-of-field ponds, ditches and streams, with special reference to the sediment compartment

	Ponds	Ditches	Streams
Origin	Natural or man-made	Man-made	Natural but often regulated
Landscape properties	Drain a relatively small area Usually isolated surface waters	Linear water bodies, usually with a straight course, that drain an intermediate size area Often high-density network of ditches interconnected with other surface waters	Linear, often meandering, water bodies that drain a relatively large area Interconnected with other surface waters (e.g. rivers, lakes)
Physical properties sediment compartment	Relatively stable environment hardly influenced by water flow Within-system variability in sediment type and mineral particle size distribution relatively small but between-system variability large in EU because of regional differences in soil properties Relatively high organic matter levels in upper sediment layer of older ponds Highly fluctuating dissolved oxygen concentrations at sediment surface and anoxic conditions in deeper sediment layers	Effect of water flow on sediment transport limited but upper sediment layer mechanically removed periodically to guarantee drainage function Within-system variability in sediment type and mineral particle size distribution relatively small but between-system variability large in EU because of regional differences in soil properties High spatial variability in organic matter content of upper sediment layer (management history) Highly fluctuating dissolved oxygen concentrations at sediment surface and anoxic conditions in deeper sediment layers	Dynamic environment: downstream sediment transport in periods of high water flow Within-system variability in sediment type and mineral particle size distribution relatively large and between-system variability large in EU because of regional differences in soil properties High spatial variability in organic matter levels (pools and riffles; dependent on water flow) Upper sediment layer more often oxygenated
Chemical properties sediment compartment	Owing to relatively long retention time and small catchment, higher chance of long-term exposure to a limited number of PPPs and other hydrophobic pollutants Ionic composition highly	Owing to intermediate retention time and intermediate catchment area, chance of time-variable exposure to more PPPs and other hydrophobic pollutants Ionic composition highly	Owing to relatively short retention time and large catchment chance of repeated short-term exposure to a higher number of PPPs and other hydrophobic pollutants Ionic composition highly

	Ponds	Ditches	Streams
	influenced by soil properties catchment Nutrient levels highly influenced by agricultural practises within catchment	influenced by soil properties catchment Nutrient levels highly influenced by agricultural practises within catchment and ditch cleaning practises	influenced by soil properties catchment Nutrient levels highly influenced by agricultural practises, soil properties within catchment and rainfall events
Biological properties sediment compartment	Benthic food web is mainly fuelled by (decomposing) aquatic primary producers (algae and particularly macrophytes) Bacteria usually play a more important role than fungi in the processing of organic matter Lentic benthic invertebrate community dominated by collectors, filterers and predators Chironomini, Tanypodinae, Orthocladiinae and Oligochaeta (particularly Tubificidae) often abundant in endobenthos Macrocrustaceans (e.g. Amphipoda and Isopoda), Ephemeroptera (e.g. Caenidae) and Mollusca common in epibenthos Microbenthos (e.g. Protozoa) and meiobenthos (e.g. Copepoda and Nematoda) received little attention	Benthic food web is mainly fuelled by (decomposing) aquatic primary producers (algae and particularly macrophytes) Bacteria usually play a more important role than fungi in the processing of organic matter Lotic benthic invertebrate community dominated by collectors, filterers and predators Chironomini, Tanypodinae, Orthocladiinae and Oligochaeta (particularly Tubificidae) often abundant in endobenthos Macrocrustaceans (e.g. Amphipoda and Isopoda), Ephemeroptera (e.g. Caenidae) and Mollusca common in epibenthos Microbenthos (e.g. Protozoa) and meiobenthos (e.g. Copepoda and Nematoda) received little attention	Benthic food web is fuelled by both (decomposing) aquatic primary producers and plant litter of terrestrial origin Compared with ponds and ditches, fungi usually play a, relatively, more important role than bacteria in the processing of organic matter Lotic benthic invertebrate community with a relatively higher contribution of shredders Simuliidae, Tanytarsini and Orthocladiinae more common in sediment habitats with relatively high water flow, as well as representatives of EPT taxa (Ephemeroptera, Plecoptera and Trichoptera) in epibenthos Microbenthos (e.g. Protozoa) and meiobenthos (e.g. Copepoda and Nematoda) received little attention

Edge-of-field ponds, ditches and streams have soft sediments in common. The main features of soft sediments are their fine-grained texture (silt, clay, sand, fine particulate organic matter), the periodic depletion or absence of oxygen and the accumulation of decomposing organic matter, nutrients and other substances. Sediment-inhabiting organisms are adapted to these conditions. In addition, soft sediments are characterised by a significant horizontal and vertical heterogeneity because of physical, chemical and biological processes. The chemical, physical and biological properties of sediment habitats differ because of several interrelated environmental factors, e.g. size and depth of water body, ionic composition of water (e.g. brackish or fresh), type and composition of solid substrates, trophic status, quantity and quality of the supply of organic matter, water movements and other hydrological conditions, general climatic conditions and light availability. This opinion focuses on soft sediments, since the periphyton of hard substrates is largely covered in the earlier AGD (EFSA PPR Panel, 2013).

2.2. General introduction to benthic communities of soft sediments and possible exposure routes to PPPs

This section briefly provides information on sediment-associated (benthic) taxa living in edge-of-field surface waters (ponds, ditches, streams) with a focus on taxa living either in (i.e. endobenthic organisms or endobenthos) or on soft sediments (i.e. epibenthic organisms or epibenthos). More detailed ecological information on benthic organisms is referred to in ecological textbooks (e.g. Smith, 2001; Tachet et al., 2010; Thorp and Covich, 2010) or literature on the use of sediment organisms in ecotoxicology (e.g. Hart and Fuller, 1974; Burton, 1991; Sheahan and Fisher, 2012).

Sediment-inhabiting organisms comprise algae, rooted macrophytes, invertebrates, bacteria, archaeans, fungi and protozoans. The benthic fauna is conventionally divided into three size groups: the macrobenthos (larger than 500 µm), the meiobenthos (size between 50 and 500 µm) and the microbenthos (smaller than 50 µm). Microbenthic organisms (both protozoans and microscopic algae), however, are often assigned to the group of microorganisms (or microbes) together with bacteria, archaeans and fungi.

2.2.1. Benthic primary producers

2.2.1.1. Microphytobenthos

In both lentic and lotic freshwater systems, benthic microalgae and cyanobacteria are significant components of the periphyton on solid surfaces (mainly rocks and stones, wood, macrophytes and invertebrate animals). They may also live in a biofilm on soft sediments when light is available and are then termed microphytobenthos. A benthic biofilm consists of a compact association of algae, microbes and organic and inorganic particles embedded in a mucus matrix. Diatoms (e.g. *Nitzschia* and *Navicula*) often dominate the microphytobenthic community on soft sediments, but also cyanobacteria and chlorophytes may be common. Sediment substrates with their biofilm can be easily disrupted by water flow or animal activity (Van der Grinten, 2004). Through photosynthesis and growth benthic algae contribute to the input of organic matter to the system and the immobilisation of nutrients from the surrounding water. Their abundance and species composition are influenced by many environmental factors, e.g. trophic conditions (e.g. Cattaneo et al., 1997; Veraart et al., 2008), hydrological conditions (Biggs et al., 1998), grazing pressure (Wellnitz and Rader, 2003) and water chemistry and temperature (Brown et al., 2008). In addition, it has been known for decades that communities of benthic algae and cyanobacteria are sensitive to various types of disturbance, e.g. exposure to chemical pesticides (Carder and Hoagland, 1998; Larras et al., 2014). Furthermore, it has been reported that biofilms may increase the bioavailability of the insecticide chlorpyrifos to the endobenthic invertebrate *Chironomus riparius* (Widenfalk et al., 2008b).

It is likely that benthic algae are exposed mainly to PPPs dissolved in the (pore) water fraction. Herbicides, and fungicides with herbicidal properties, are the PPPs that most likely will be toxic to primary producers.

2.2.1.2. Rooted macrophytes

In both lentic and lotic edge-of-field surface waters, soft sediments are often colonised by rooted macrophytes. The sediment is the main source of inorganic nutrients (e.g. nitrogen and phosphorus) for their growth. Rooted aquatic macrophytes fulfil several critical structural and functional roles in aquatic ecosystems. For example, they play an important role in organic matter production, by their root system stabilise sediments and provide oxygen to deeper sediment layers, accumulate and translocate chemicals and provide substrate and habitat for many other aquatic organisms. The majority of the organic matter produced by rooted aquatic macrophytes, however, is not directly grazed by herbivores but enters the benthic decomposer food chain (e.g. Wetzel, 2001).

The main exposure route of sediment-bound PPPs to rooted macrophytes is via the pore water, but because of the water-soluble nature of many herbicidal compounds exposure via overlying water often plays a more important role in the direct toxic effects (see e.g. Burešová et al., 2013).

2.2.2. Microorganisms

In this scientific opinion, ‘microorganisms’ (or ‘microbes’) include single-celled organisms with heterotrophic organotrophic or chemolithotrophic autotrophic lifestyles, which can be bacteria, archaeans, fungi or protozoans. The photosynthetic microorganisms, i.e. the eukaryotic algae and cyanobacteria, are treated above (section 2.2.1 ‘Benthic primary producers’).

In the universal phylogeny of life forms on earth, microorganisms represent more geno- and phenotypic diversity than all other organisms taken together (Pace, 2009). Looking at sediment habitats as a whole, the phylogenetic and functional diversity of microorganisms is extremely high (Nealson, 1997; Findlay, 2010). Collectively, heterotrophic microorganisms are vital for the degradation of organic matter and cycling of nutrients (both as mineralisation and immobilisation) in sediments and soils. Thereby, they also play a major role in transformation and eventual mineralisation of organic pollutants, including chemical pesticides (Semple et al., 2007). In addition, microbial biomass makes an important contribution to trophic transfer of matter and energy in food webs.

The microbial food webs associated with sediments are fuelled by the continuous supply of more or less labile organic matter that is trapped in and on the sediment, either originating from decaying aquatic plants and animals or from terrestrial origin (e.g. leaf litter). A relative estimate suggests a 1 000-fold concentration of bacteria in the sediments compared with that in the water column (Moss, 1980). In many sediment habitats, prokaryotic microbes (bacteria and archaeans) make up a dominating part of the microbial community in terms of both biomass and numbers (Nealson, 1997), whereas communities of eukaryotes (fungi and protozoans) are smaller. The distribution of microorganisms can be quite patchy on a microscale because of spatial and temporal variation in the quantity and quality of the input of potential substrates and nutrients, presence of animals or plants and gradients in chemical and physical conditions. Since many microorganisms have rather specific environmental requirements, different functional groups differ widely in their distribution. The presence and activity in sediments of invertebrates and macrophytes introduce strong gradients and contribute specific habitats where various groups of microbes may thrive. For instance, bioturbation of sediment materials by invertebrates create channels or burrows, which can have substantial impacts on the distribution and activities of different groups of microbes (Ravit et al., 2003; Gilbertson et al., 2012). The roots of macrophytes extend into the sediment, where they can influence microbial communities by adding degradable organic material by exudation and dying of roots and transport oxygen into otherwise anaerobic parts of the sediment (Gribsholt and Christensen, 2002; Oliveira et al., 2010). Gradients in the presence of potential electron acceptors used in microbial respiration or fermentation are other crucial factors for the distribution and activity of different functional groups of microbes. In aerobic parts of the sediment, respiratory processes dependent on O_2 will dominate, whereas in anaerobic parts NO_3^-, Mn(IV), Fe(III), SO_4^{2-} and CO_2 will be used sequentially with more reducing conditions.

There are some general differences in microbial communities between lentic and lotic systems (Findlay, 2010). The latter typically experience larger temporal variation in water movements and transportation of organic and inorganic particulate matter, have higher contribution of leaf litter and therefore higher relative abundance of fungi and have less contribution from anaerobic microbial processes. In shallow lotic systems, microbial biofilms on rocks, wood or macrophytes containing both photosynthetic microalgae and non-photosynthetic microorganisms (Rier et al., 2007) can constitute a large but highly dynamic component of the microbial community (Hudson et al., 1992; Findlay et al., 1993).

Protozoans are united not by phylogeny but by being eukaryotic, unicellular, non-photosynthetic organisms which are not fungi. Based on morphology, most protozoans are ciliates, flagellates or amoebae. A special property of protozoans is that most are phagotrophic, in contrast to the prokaryotes and fungi in which osmotrophic nutrition dominates. Protozoans ingest particulate matter for nutrition and are predators, particularly of bacteria (Epstein, 1997). Mixotrophy is common in several flagellate groups, e.g. dinoflagellates and euglenoids. Mixotrophic species have both autotrophic (photosynthetic) and heterotrophic nutrition. Thus, these flagellate groups overlap between eukaryotic algae and protozoans.

The freely dissolved fraction in pore-water of sediment-associated PPPs most likely is the main exposure route for microorganisms, but dietary exposure might also play a role in protozoans.

2.2.3. Meio- and macrobenthic invertebrates

Soft sediments form food and habitat for many invertebrates and the activity of these animals is normally restricted to the upper layers where decomposing organic matter accumulates and conditions are not too harsh, e.g. because of oxygen depletion.

The meiobenthos, with a size between 50 and 500 µm, comprise nematodes, tardigrades and microcrustaceans (copepods, ostracods and chydorid cladocerans). In addition, early life stages of macroinvertebrates may be part of the meiobenthos. The main food sources for meiobenthos are assumed to be detritus, benthic algae, microbes and microbenthos (Van der Bund, 1994).

The macrobenthos, animals that are retained on a 500 µm sieve, comprise taxa such as insects (particularly larvae of chironomids and nymphs of ephemeropterans), macrocrustaceans (e.g. *Gammarus*, *Asellus*), oligochaete worms and molluscs (particularly bivalves). Their feeding strategies may be diverse, including the consumption of course particulate matter and associated microbes (e.g. shredders, such as *Gammarus* and *Asellus*), collecting fine particulate organic matter and associated microbes (suspension feeders, such as many chironomids, oligochaete worms and bivalve molluscs), grazing the biofilm at the sediment surface (e.g. scrapers, such as snails) or predating other benthic fauna (predators, such as *Sialis* larvae, and chironomids, such as Tanypodinae). In turn, macrobenthos may serve as food for aquatic and terrestrial vertebrates, such as fish and birds (Covich et al., 1999).

Considering exposure routes to PPPs, benthic invertebrates may be subject to potentially high exposure to sediment-bound substances, via all possible uptake routes, i.e. contact with, and ingestion of, contaminated sediment particles, but also contact with pore water and overlaying water. The primary exposure route varies, mainly depending on the properties of the chemical and on the feeding behaviour of the species.

2.2.3.1. Insects

Among aquatic insects, several chironomid taxa are particularly abundant in and on soft sediments. Chironomids form a species-rich family of aquatic dipterans (Insecta), with a Holarctic distribution (Tachet et al., 2010). The biology and ecology of this group has been well described in, for example, Murray (1979) and Armitage et al. (1995). Larvae of Chironomini are omnivorous (eggs, nymph and adult do not feed). They mainly feed on decomposing organic matter, detritus and bacteria that are swallowed with sediment particles (Tachet et al., 2010). Their feeding activity contributes to the mixing of the upper sediment layers. Other aquatic insects that may occur locally in high numbers in and on soft sediments comprise larvae of Ephemeroptera (e.g. representatives of the genera *Caenis*, *Ephoron* and *Hexagenia*) and Neuroptera (e.g. *Sialis lutaria*). The nymphs of *Caenis* spp. feed on detritus and associated microorganisms (see e.g. Elliott and Humpesch, 2010). Early instar nymphs of *Ephoron virgo* feed on fine particulate organic matter at the sediment surface. In later stages they build U-shaped tubes in the sediment and start to filter food, such as detritus and algae, from the water by generating wave-like movements in their burrows with their feathered tracheal gills (Kureck and Fontes, 1996). The American species *Hexagenia limbata* and *Hexagenia bilineata* have a more or less similar habitat and feeding strategy (Waltz and Burian, 2008). The nymphs of these sediment-associated Ephemeroptera are reported to be sensitive to sediment-bound toxicants (e.g. De Haas et al., 2002; Brock et al., 2010b; Harwood et al., 2014). Larvae of *Sialis lutaria* are carnivores and their predominant food organisms are chironomid larvae and oligochaetes in the larger larvae, benthic crustaceans in the smaller larvae and microorganisms and detritus in the first instar larvae (Elliott, 1977). In edge-of-field surface waters many other, predominantly epibenthic, insect taxa may occur, such as representatives of Plecoptera (stoneflies) and Trichoptera (caddisflies). Many of these taxa, however, more often dwell on solid substrates, such as pebbles, course particulate organic matter and macrophytes, and less so in the upper layer of soft sediments.

2.2.3.2. Crustaceans

Benthic crustaceans are predominantly epibenthic. Characteristic meiobenthic Crustacea comprise harpacticoid copepods, ostracods and chydorid cladocerans. Most of the taxa of meiobenthic crustaceans are omnivorous or detritivorous, feeding, for example, on detritus, benthic algae and microorganisms. Chydorids take advantage of the presence of chironomid larvae by feeding on their faecal pellets, presumably digesting the associated microorganisms (Van der Bund, 1994). Characteristic macrobenthic Crustacea comprise Amphipods (e.g. *Gammarus* and *Hyalella*) and Isopods (e.g. *Asellus* and *Proasellus*). Most amphipods are omnivorous, usually feeding on coarse particulate organic material (e.g. decaying leaves and their associated microorganisms), detritus and algae. They are occasional predators of aquatic invertebrates or can feed on dead animals. The biology and ecology of fresh/brackish water crustaceans has been well described in, for example, Smith (2001) and Thorp and Covich (2010).

2.2.3.3. Oligochaetes

Oligochaete worms are a major component of endobenthos in freshwater ecosystems (Brinkhurst, 1974), but epibenthic species may also be abundant (e.g. *Dero* spp.). They have a Holarctic distribution. The most commonly found oligochaetes belong to four families: Tubificidae (e.g. *Tubifex* spp., *Limnodrilus* spp.), Naididae (e.g. *Nais* spp.), Enchytraeidae (e.g. *Grania* spp.) and Lumbriculidae (e.g. *Lumbriculus* spp.). Details on oligochaete biology and ecology can be found in, for example, Kaster (1989), Smith (2001) and Thorp and Covich (2010). Most oligochaetes are detritivores that feed on decomposing organic matter from the sediment. Bacteria and algae are other relevant food sources (Wavre and Brinkhurst, 1971; Moore, 1978). Feeding requires the processing of a large amount of sediment particles. This feeding process is called sediment bioturbation: deeper sediments are transported to the surface as faecal pellets, thus providing mixing of the upper layers of sediments. As oligochaetes may exhibit large sizes (up to 15 cm), they locally may cause mixing of the upper ca. 20 cm of the sediment layer (Wang and Matisoff, 1997). The tolerance to sediment grain size varies within species. For instance, *Tubifex tubifex* generally inhabits fine material whereas *Lumbriculus variegatus* may be more tolerant of coarser material, such as quartz sand (Egeler et al., 2006). Tubificids generally tolerate high organic carbon (OC) content and low dissolved oxygen saturation (Pennak, 1989).

2.2.3.4. Nematodes

These animals may reach a length of several centimetres, but most species belong to the meiobenthos. Nematodes are distributed worldwide and are often the most abundant and species-rich meiobenthic taxa in soft sediments. Terrestrial soils and freshwater sediments share many common species. Their biology and ecology has been described in detail in, for example, Smith (2001) and Thorp and Covich (2010). Nematodes have evolved various feeding strategies and have been grouped into bacteria-, algae-, fungi- and plant-feeders, plus omnivores and predators. They play an important role in benthic food webs (Fenchel, 1992; Traunspurger et al., 1997). Their high abundances as well as the high structural and functional biodiversity of nematodes in benthic habitats allow a valid community analysis in relatively small samples. In addition, indirect effects of stressors can be detected as changes in the nematode community structure because of their many roles in the benthic food web (e.g. Brinke et al., 2010).

2.2.3.5. Molluscs

Molluscs (gastropods and bivalves) are ubiquitous in fresh/brackish waters around the globe. They are observed in every type of habitat, from the smallest ponds to the largest rivers. Their biology and ecology has been described in details in, for example, Smith (2001) and Thorp and Covich (2010). Gastropods are commonly found on soft sediments in freshwater (e.g. *Lymnaea stagnalis*) or brackish water (e.g. *Potamopyrgus antipodarum*), but are not tightly bound to this compartment. Most gastropods are herbivorous to omnivorous, feeding by scraping living algae, bacteria and fungi covering the substrate. Living or decaying plant materials and dead animals, as well as detritus, are other important food sources. Benthic bivalve molluscs may be common in freshwater ecosystems (e.g. the genera *Pisidium*, *Corbicula*, *Unio* and *Anodonta*). Bivalves feed on suspended fine organic detritus (Pennak, 1989). Species that burrow, such as Sphaeriidae, can be found up to 25 cm below the sediment surface and rely on organic detritus and associated microorganisms from the sediment, which are placed in suspension in the interstitial water and then filtered (Burton, 1991). Bivalves usually require a high dissolved oxygen content.

2.2.4. Vertebrates

Vertebrates, such as fish, may be closely associated with soft sediments, particularly when they actively forage on benthic invertebrates (e.g. benthic fish-like bream; Persson and Brönmark, 2002). Furthermore, sediments may be used as spawning sites, or upon spawning in water fish eggs may sink to the sediment surface (e.g. zebrafish eggs; Hollert et al., 2003). In addition, fish and amphibians may periodically dwell in the upper sediment layer, e.g. to hide or to overwinter. In this way aquatic vertebrates may become directly (contact) or indirectly (via food) exposed to sediment-bound PPPs.

2.3. Benthic test species used in ecotoxicology and available protocols for sediment-spiked toxicity tests

2.3.1. Primary producers

Under normal growth conditions in freshwater ecosystems, the planktonic algae (e.g. *Selenastrum* spp.) and non-rooted macrophytes (e.g. *Lemna* spp.) used as standard aquatic test species are not in direct contact with the sediment compartment. However, they can be successfully used for toxicity testing of elutriates and interstitial water (Munawar and Weisse, 1989) or even be used in whole-sediment toxicity assays (Munawar et al., 1987; Zhang et al., 2012). In sediment risk assessments, however, it seems more appropriate to conduct tests with benthic algae and rooted macrophytes that under normal growth conditions are more intimately associated with sediments.

2.3.1.1. Microphytobenthos

In spite of the evident importance of microphytobenthos, these organisms have not been used much in ecotoxicology. Currently, no official test guidelines exist for conducting sediment-spiked toxicity tests with benthic algae and cyanobacteria. Nevertheless, efforts have been made in recent years to use microalgae for whole sediment toxicity tests, mainly for retrospective risk assessment purposes. The studies focused on free benthic marine microalgae (e.g. Adams and Stauber, 2004; Moreno-Garrido et al., 2007; Araújo et al., 2010). Studies suggest that sediment exposure of PPPs via interstitial water may affect benthic algae (Magnusson et al., 2013).

Recently, the use of alginate-immobilised microalgae has been suggested as a sediment toxicity test that can be used in retrospective whole-sediment toxicity testing but might also be used in prospective toxicity testing using spiked sediments (Moreno-Garrido et al., 2007; Zhang et al., 2012). For this the current planktonic standard test algae, such as *Pseudokirchneriella subcapitata*, can be used also (Zhang et al., 2012). Although the immobilised algal beads showed slower growth than free cells, growth of algae cells in the beads was high enough to be valid for an appropriate toxicity test. An advantage of using immobilised algal beads in a whole sediment toxicity test is that this overcomes the difficulty of cell counting when using free cells. In whole-sediment toxicity tests using immobilised beads, after exposure one can easily distinguish algal cells from sediment particles with a light microscope (Zhang et al., 2012).

2.3.1.2. Rooted macrophytes

Rooted macrophytes are more closely linked to sediments, and are thus more relevant than *Lemna* species for whole-sediment toxicity testing (Knauer et al., 2008; Maltby et al., 2010). Several species of the dicotyledonous macrophyte *Myriophyllum* spp. have been shown to be suitable for sediment toxicity testing, especially *Myriophyllum aquaticum* and *Myriophyllum spicatum*. An OECD test guideline has recently been proposed for water–sediment tests with *Myriophyllum spicatum* (OECD, 2014). For retrospective risk assessments a standard protocol for a whole-sediment toxicity test with

Myriophyllum aquaticum has been developed (Feiler et al., 2004; ISO, 2010a). Recently, an international ring test was performed to evaluate the precision of the test method proposed for the *Myriophyllum aquaticum* standardised sediment contact test, showing its suitability as a tool to assess the toxicity of sediments (Feiler et al., 2014). When monocot species are required for sediment toxicity testing rooted vascular plants, such as *Elodea* sp. and *Glyceria maxima*, may be used, but standard test protocols do not yet exist for these taxa (Davies et al., 2003; Knauer et al., 2006).

Since protocols for conducting sediment toxicity test with *Myriophyllum* spp. are available it is anticipated that they will be used in the near future to assess the potential effects of sediment-bound PPPs with herbicidal activity. A short description of the properties of these standard test species is given below.

Myriophyllum spicatum is a perennial aquatic macrophyte. Both sexual and vegetative reproduction occurs. *Myriophyllum spicatum* is distributed worldwide. It is found at shallow depths in lakes, ponds, shallow reservoirs, slow flow areas of rivers and streams and brackish water of protected tidal creeks and bays. It tolerates a wide range of water conditions and temperatures (ISSG, 2014). *Myriophyllum spicatum* is also one of the preferred standard test species for rooted macrophytes to evaluate water exposure to PPPs with a herbicidal mode-of-action (EFSA PPR Panel, 2013).

Myriophyllum aquaticum is a perennial species. It exhibits two different leaf forms depending on whether it is growing as a submerged or emergent plant. Almost all individuals are female (male plants are unknown outside of South America), so that no or little sexual reproduction occurs. Fragments are formed mechanically and readily root, ensuring a rapid spreading of the plant. *Myriophyllum aquaticum* is also distributed worldwide and found in freshwater lakes, ponds, streams and canals. It tolerates high nutrient concentrations (ISSG, 2014).

2.3.2. Microorganisms

Despite the importance of microorganisms for the metabolic processes in sediments, standard tests with microorganisms are not required in the aquatic effect assessment for PPPs (EC, 2013). It has been recurrently advocated that the evaluation of effects on microbes in prospective risk assessments of chemical pesticides need to be strengthened, including microbes in sediments (DeLorenzo et al., 2001; Van Beelen, 2003; Puglisi, 2012; Diepens et al., 2014a; ECHA, 2014). Similarly, a previous EFSA scientific opinion proposed two protection goals regarding heterotrophic microbes based on the ecosystem services these organisms provide (EFSA PPR Panel, 2010a; also see Chapter 3): (a) ‘no unacceptable effects on functions of microbial communities’ and (b) ‘no decrease of biodiversity’.

However, concerning microbes, there is a requirement in the European Union (EU) for testing effects on the growth of a pelagic microbial alga (e.g. *Pseudokirchneriella subcapitata*), and for studies of potential effects of the active substance on microbial nitrogen transformation in soil (EC, 2013). Furthermore, since some microbes are able to metabolise and use some chemical pesticides as substrate, data on degradation rates at aerobic conditions in at least three soils and, if applicable, also on potential degradation at anaerobic conditions, are required. In addition, a general requirement states that effects on (micro)biological sewage treatment processes shall be reported where the use of the PPP can give rise to such adverse effects. Along these lines, there are OECD guidelines for testing effects of chemicals on microbial respiration in activated sludge from sewage treatment plants (OECD, 2010b). In the USA, a test building on these OECD guidelines is required (US EPA, 2012b). Otherwise, similar to the EU, the USA requires testing effects of the chemical on a photosynthetic aquatic microbial alga and on microbial activity in soil. In the USA, the required tests concern effects on growth of the cyanobacterium *Anabaena flos-aquae* (US EPA, 2012c) and the general microbial activity in soil measured as respiration (US EPA, 2012a).

Regarding standards for testing effects of chemicals on microbiological processes, OECD developed one on the inhibition of the activity of anaerobic bacteria in, for example, sludge or sediment (OECD, 2007b). International Organization for Standarization (ISO) standards for soil and water quality include several tests based on both functional and structural microbial endpoints that could be relevant from a sediment risk assessment perspective.[6] Examples are *Pseudomonas putida* bacterial growth inhibition test (ISO, 1995), assessing inhibition of nitrification in activated sludge (ISO, 2006), inhibitory effect on the light emission of *Vibrio fischeri* (ISO, 2007), determination of effects of chemicals on nitrogen mineralisation and nitrification in soils (ISO, 2012a) and inhibition of potential nitrification with test of ammonium oxidation (ISO, 2012b). Other ISO standards not directly targeting inhibitory effects are, determination of soil microbial community structure using phospholipid fatty acid (PLFA) analysis (ISO, 2010c), abundance and activity of soil microflora using respiration curves (ISO, 2012c), laboratory assessments for characterising denitrification in soil (ISO, 2015a) and estimation of abundance of selected microbial gene sequences by quantitative real-time polymerase chain reaction (ISO, 2015b). These ISO standards do not directly correspond to any current data requirements for PPPs, but those on functional properties related to nitrogen cycling target more specific functions than the currently used nitrogen mineralisation test in soil and might have the potential to replace that.

For further discussion on the prospects of introducing microbial tests to evaluate the effects of sediment-bound PPPs see section 8.2.5 of this scientific opinion.

2.3.3. Meio- and macrobenthic invertebrates

For several freshwater invertebrates a test protocol is available to conduct sediment-spiked toxicity tests and they comprise different taxonomic groups as described below.

2.3.3.1. Insects

Among those benthic insect groups that predominantly occur in freshwater, *Chironomus* species are the most widely used standard test species in sediment testing. OECD Guidelines 218 (OECD, 2004a) and 233 (OECD, 2010a) describe long-term (28–65 days) and life cycle (44–100 days) tests for, respectively, *Chironomus riparius* (most frequently used in Europe) and *Chironomus acutus* (= *Chironomus tentans*; most frequently used in North America) and *Chironomus yoshimatsui* (frequently used in Japan). These OECD protocols advocate the use of artificial sediments, containing 4–5 % peat. Besides the above-mentioned chronic *Chironomus* tests, several guidelines exist that concern semi-chronic 10-day sediment toxicity tests with *Chironomus dilutus* or *Chironomus riparius* e.g. the Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates ASTM E1706-05[7] (ASTM, 2010a). These semi-chronic tests are usually conducted with *Chironomus dilutus* and natural sediments as part of the regulatory requirements in the USA (US EPA, 2000). The test requirement in Europe concern chronic sediment-spiked toxicity tests with *Chironomus*, following OECD Guideline 218 in particular. An overview of available sediment-spiked toxicity tests conducted with PPPs and *Chironomus riparius* or *Chironomus dilutus* is referred to in Deneer et al. (2013). The guideline ASTM E1706-05 is also used in North America to conduct 10-day toxicity tests with nymphs of ephemeropteran *Hexagenia* spp. (see e.g. Harwood et al., 2014).

Since the long-term OECD Guideline 218 sediment toxicity test with *Chironomus riparius* is commonly used in Europe, a short description of the biology and ecology of this standard test species is given below.

The larvae of *Chironomus riparius* are found in lentic and lotic environments and the species favours eutrophic conditions or conditions with relatively high organic loadings. Under optimal conditions it can reach densities of up to 50 000 individuals/m^2. The red colour of the larvae is caused by haemoglobin which helps them tolerate reduced levels of dissolved oxygen. They construct protective tubes from detritus, algae and other sediment particles with one extremity opened at the sediment

6 http://www.iso.org/iso/home/store/catalogue_tc/catalogue_tc_browse.htm?commid=54366; http://www.iso.org/iso/home/store/catalogue_tc/catalogue_tc_browse.htm?commid=52972

7 http://www.astm.org/Standards/E1706.htm

surface, where they shelter during their whole development (Vos, 2001). Larvae ventilate their tubes with freshwater by undulations of the body, thereby drawing in food (dead organic particles and associated microorganisms and algae) from nearby surface of sediments; thereby reducing activities outside the protective tube, and thus predation risk (Sheahan and Fisher, 2012). The life cycle of *Chironomus riparius* comprises an egg stage, four larval stages and a pupal stage (all in the aquatic environment), in addition to the adult terrestrial stage. Larvae tolerate a wide range of substrates from particle size ranges of > 90 % silt and clay to 100 % sand without detrimental effects on survival or growth (Suedel et al., 1993). Sediment OC content studies have shown good survival and growth of *Chironomus riparius* in natural sediments with an OC content of 0.6–8.8 % (Milani et al., 1996). OC levels of < 0.5 % in artificial sediments have been shown to reduce survival (Suedel and Rodgers, 1994).

2.3.3.2. Crustaceans

Of the benthic crustaceans, the freshwater/estuarine amphipod *Hyalella azteca* is the most widely used standard test species in sediment testing. This species is not indigenous in Europe. The available test guidelines for sediment testing with benthic crustaceans have all been developed and used for regulatory purposes in North America (US EPA, 2000). ASTM E1706-5 (ASTM, 2010a) describes chronic (42-day) and semi-chronic (10-day) tests for *Hyalella azteca* (see also US EPA, 2000). In addition, this ASTM International test guideline can be used to conduct a 10-day sediment-spiked toxicity test with the benthic amphipod *Diporeia* spp. Note, however, that in the scientific literature predominantly toxicity data for the 10-day sediment-spiked test (predominantly using natural sediments) with *Hyalella azteca* are available (Deneer et al., 2013). The sediment toxicity data for freshwater crustaceans generated for regulatory purposes in North America might also be used as additional information in the European risk assessment.

Since benthic crustaceans are very common in estuarine and marine environments, official test guidelines have also been developed for 28-day sediment-spiked toxicity tests with the estuarine amphipods *Leptocheirus plumulosus* (ASTM, 2010b) and *Eohaustorius estuarius* (US EPA, 1996a) and the marine amphipods *Ampelisca abdita* and *Rhepoxynius abronius* (US EPA, 1996a). In addition, official test guidelines are available for 10-day sediment-spiked toxicity tests with these amphipods (ASTM, 2010b; US EPA, 1996a). These test guidelines are mainly used in North America. Sediment toxicity data from sediment-spiked tests with PPPs and estuarine/marine amphipods might be of use in higher-tier effect assessment procedures in sediments of edge-of-field surface waters if benthic arthropods can be identified as a sensitive taxonomic group.

Since the sediment toxicity tests with *Hyalella azteca* are commonly used, particularly in the USA, a short description of the biology and ecology of this standard test species is given below.

Hyalella azteca is found across Central America, the Caribbean and North America. It is a freshwater epibenthic amphipod which lives near the sediment surface, burrowing in sediment and also scavenging on the leaf litter, algae and detritus material on the sediment surface. It is primarily found on the sediment surface and in algal mats and while their primary source of food varies by habitat, they appear to prefer epiphytic algae over detrital organic matter (Wang et al., 2004). However, under laboratory conditions, they may be found often burrowing in sediments (Ingersoll et al., 2005). They have a generation time of approximately 33 days (dependent on temperature) and reach a maximum length of about 7 mm in 120 days (Othman and Pascoe, 2001).

2.3.3.3. Oligochaetes

Of the benthic freshwater oligochaetes, *Lumbriculus variegatus* and *Tubifex tubifex* are the most widely used standard test species in sediment testing. OECD Guideline 225 (OECD, 2007a) describes a 28-day sediment-spiked toxicity test with *Lumbriculus variegatus*. In addition, a standard test protocol for a 28-day sediment contact test to assess bioaccumulation in *Lumbriculus variegatus* has been developed (ASTM, 2010c; US EPA, 2000). For *Tubifex tubifex* a test guideline for a 10-day sediment-spiked toxicity test is available (ASTM, 2010a). Remarkably, in the scientific literature very few sediment toxicity data for these oligochaete standard test species and PPPs can be found (Deneer et al., 2013). This may be because a sediment toxicity value for *Lumbriculus variegatus* became a regulatory data requirement only recently.

A polychaete marine standard test species for which an official test guideline for short- and long-term sediment-spiked tests has been developed is *Neanthes arenaceodentata* (ASTM, 2007).

Since the long-term OECD sediment toxicity test with *Lumbriculus variegatus* is now a data requirement in Europe, a short description of the biology and ecology of this standard test species is given below.

Lumbriculus variegatus is a benthic oligochaete worm occurring in North America, Europe and Asia. It lives in shallow-water ecosystems feeding on organic material and associated microorganisms. Favoured microhabitats include layers of decomposing leaves, submerged rotting logs or sediments at the base of emergent macrophyte vegetation. *Lumbriculus* may also occupy silty sediments from deeper water, but other oligochaetes, such as tubificids, are more common in these habitats. In nature, *Lumbriculus* uses its head to forage in sediments and debris, while its tail end, specialised for gas exchange, often projects upwards. When possible, the worm stretches its tail vertically to the water surface where it forms a right-angle bend and breaks the water surface tension. This posture facilitates gas exchange between the air and the pulsating dorsal blood vessel lying just beneath the epidermis. This respiratory behaviour markedly contrasts with that of tubificid worms, which often undulate their tail ends as they protrude from burrows in sediments well below the water surface. Maximal body size is about 10 cm in length (Brinkhurst, 1974; Brinkhurst and Gelder, 1991; Drewes, 1997). An adult has approximately 150 to 250 segments, each of which has the ability to regenerate into a new individual when separated from the rest of the animal. In most populations, this is the primary mode of reproduction. Such specimens appear as sexually mature hermaphrodites. Although never documented, sexual reproduction in mature worms probably involves copulation and sperm exchange. Then, worms produce transparent cocoons, each containing 4–11 fertilised eggs that undergo direct embryonic development with no larval stage (Drewes and Brinkhurst, 1990). Small worms, about 1 cm in length, emerge from cocoons in about two weeks. Field-collected *Lumbriculus* are often larger than laboratory-reared worms, which are usually 4–6 cm in length and never reach sexual maturity or produce cocoons. Reproduction under laboratory conditions is always by asexual fragmentation, during which a worm spontaneously divides into two or more body fragments. Each surviving fragment then undergoes rapid regeneration of body segments to form a new head end, tail end or both ends. Eventually each fragment grows into a normally sized worm comprising a combination of older and newer segments, representing two or more generations of development. The capacity for a sexual reproduction by fragmentation is matched by the ability to self-amputate in response to injury or other types of noxious stimuli (Lesiuk and Drewes, 1999).

2.3.3.4. Nematodes

Ecotoxicological assessment of aquatic sediments with nematodes generally involves *Caenorhabditis elegans*. Traunspurger et al. (1997), and other subsequent studies (e.g. Höss et al., 1999, 2001; Donkin and Williams, 2009), have demonstrated that this free-living soil species can be useful to assess the toxicity of both the water phase and the whole sediment. A standard test guideline exists for soil and sediment toxicity testing with this nematode (ISO/CD 10872; ISO, 2010b). It is a four-day test. In the scientific literature no sediment toxicity data for PPPs spiked in sediments and *Caenorhabditis elegans* could be found (Deneer et al., 2013).

An overview of *Caenorhabditis elegans* biology is available from Stange (2006). *Caenorhabditis elegans* usually lives less than a month. Its life cycle comprise three development phases: egg, larvae (with four larval instars) and adults. An alternative third instar larval stage exists called the dauer stage; this dormant stage occurs under unfavourable environmental conditions. It is an hermaphrodite that reproduces by selfing or outcrossing, producing from 200 to 1 000 eggs per brood, and one brood every three to four days. *Caenorhabditis elegans* is not tightly bound to the sediment, and thus tolerates a wide range of sediment characteristics, as demonstrated by Höss et al. (1999) for the following particle size distribution (2.5–18 % clay, 25.7–68.2 % silt, 18.7–70.9 % sand) and organic content (2.5–77.1 %). They feed on the bacteria that develop in decaying organic matter.

2.3.3.5. Molluscs

Currently no official test guidelines for sediment-spiked toxicity tests with freshwater molluscs are available. Diepens et al. (2014a) propose to develop a test protocol for the endobenthic freshwater bivalve *Pisidium* sp. or *Sphaerium* sp. Duft et al. (2003a, b) described a chronic toxicity test with the epibenthic snail *Potamopyrgus antipodarum*. To date, a very limited number of sediment toxicity tests have been conducted with PPPs and molluscs (Deneer et al., 2013).

2.3.3.6. Vertebrates

Few official test guidelines are available to test effects of sediment-spiked substances on benthic vertebrates. As a more or less conventional Tier 1 single species test, the ASTM E2591-07 test for the amphibian *Rana pipiens* may be used (ASTM, 2013). Furthermore, a sediment contact assay using zebrafish egg as an offshoot of the original zebrafish embryo assay (DIN 38415-T6) has been developed (Hollert et al., 2003). Since toxicity testing with vertebrates should be minimised because of ethical and legal constraints, an alternative approach might be to use cell line assays of vertebrate species to evaluate potential hazards of sediment-bound contaminants (see e.g. Houtman et al., 2006). An important topic for future research is the evaluation of the ecological significance of cell line assays.

2.3.4. Main differences between existing OECD and ASTM and US EPA guidelines

As discussed by Faber and Bruns (2015), one of the main differences between OECD technical guidelines and the corresponding guidelines from North America is the type of sediment used. In the OECD test protocols artificial sediment is recommended. The United States Environmental Protection Agency (US EPA)/ASTM International technical guidelines recommend the use of natural sediment. In addition, the OECD and the US EPA/ASTM International guidelines differ with respect to the spiking procedure which may affect exposure conditions in the tests. In particular, differences in ageing period of the spiked sediment before introducing the test organisms may influence exposure conditions and, consequently, the comparability of results between toxicity tests conducted according to OECD and US EPA/ASTM International guidelines. The PPR Panel recommends the initiation of comparative studies to evaluate and understand differences in OECD and US EPA/ASTM guidelines (e.g. artificial vs. natural sediment; ageing period before starting sediment toxicity tests) and the possible consequences for toxicity estimates.

3. Specific Protection Goals

3.1. Introduction

Regulation (EC) No 1107/2009 requires a high level of protection of non-target organisms, including sediment-dwelling organisms, but in this document protection goals are described in general terms only. As biodiversity is the general protection goal of the Regulation EC 1107/2009, it is also important to communicate to the risk managers the level of protection of biodiversity as an entity on its own.

In order to allow the development of decision schemes in prospective ERA more precisely defined SPGs are required. In EFSA PPR Panel (2010a), a process is described for defining SPG options for key drivers (main organism groups) covering ecosystem services which could potentially be affected by PPPs. In updating the AGD (see EFSA PPR Panel, 2013) this process to derive SPGs was used to identify the aquatic key drivers for edge-of-field surface waters, as well as their ecological entities to be protected. Since in EFSA PPR Panel (2013) a clear distinction in SPG for pelagic and benthic organisms was not made, additional information on ecosystem services provided by sediment-dwelling organisms, and possible consequences for SPGs, will be presented below.

3.2. Deriving specific protection goals for benthic organisms

In this scientific opinion we focus on the potential impact of pesticides on the ecosystem services provided by benthic organisms in surface waters. In another paper, Diepens et al. (submitted 2015a) performed a similar exercise to derive SPGs to be used in risk assessment schemes for freshwater and marine benthic organisms and sediment exposure to the broader group of organic chemicals. In order to define SPGs for sediment-dwelling (benthic) organisms we used information on the functions and services provided by benthic organisms as presented in Wall (2004) and Covich et al. (2004).

The importance of ecosystem services provided by benthic organisms in edge-of-field and larger surface waters, and the potential impact of pesticides on them, is presented in Table 2. In this table a distinction is made between provisioning services (i.e. products obtained by humans), regulating ecosystem services (i.e. regulating processes beneficial for humans), cultural ecosystem services (i.e. important conditions for humans related to aesthetic, spiritual, educational and recreational values and benefits) and supporting ecosystem services (ecosystem functions that support ecosystem sustainability). These categories of ecosystem services have been proposed by the Millennium Ecosystem Assessment (2005).

Table 2: Estimation of the importance of ecosystem services provided by benthic organisms based on subjective classes in aquatic ecosystems and the potential impact of pesticides on them. Adapted after EFSA PPR (2010a) and Diepens et al. (submitted 2015a)

Millennium Ecosystem Assessment category	Ecosystem service	Edge-of-field surface waters (agricultural landscapes)	Larger surface waters (WFD and Natura 2000)	Benthic key drivers (service providing units)
Provisioning services	Food	+	++	Consumable benthic fish, shellfish and macrophytes
	Fibre, fuel and construction material	+	++	Rooted macrophytes (thatched roofs; peat)
	Genetic resources, biodiversity	++	+++	Benthic species, and their wild relatives, potentially harvested and/or cultured by man
	Biochemical/ natural medicines	+	+	Medical plant extracts and cosmetics from plants and snails
	Ornamental resources	+	+	Aquaria and garden pond rooted macrophytes
	Biological products	+	+	Benthic invertebrates (e.g. chironomids and oligochaete worms) for fish bait and aquaria food
Regulating services	Sediment bioremediation	+++	+++	Bacteria, fungi, rooted macrophytes, bioturbating benthic invertebrates
	Water purification	+++	+++	Bacteria, fungi, rooted macrophytes, bioturbating benthic invertebrates
	Hydrological regulation	+++	+++	Rooted macrophytes
	Air quality regulation	+	+	Rooted macrophytes, benthic algae
	Erosion regulation	++	++	Rooted macrophytes, benthic biofilms
	Pollination	+	+	(Semi-)Aquatic insects that pollinate vascular plants and that have benthic larval stages (e.g. Ephydridae)

Millennium Ecosystem Assessment category	Ecosystem service	Edge-of-field surface waters (agricultural landscapes)	Larger surface waters (WFD and Natura 2000)	Benthic key drivers (service providing units)
	Pest and disease regulation (e.g. control of aquatic species that act as host for parasites and diseases)	+	+	Benthic fish and invertebrates
	Invasion resistance	+	++	All native benthic organisms with similar niche
Cultural services	Education and inspiration (including conservation of biodiversity)	+++	+++	All benthic taxa
	Recreation and ecotourism	++	+++	Benthic vertebrates, rooted macrophytes, benthic algae (e.g. Characeae), benthic invertebrates
	Cultural heritage	++	++	Preservation of surface waters constructed and/or modified by man and their typical biota (e.g. canals, clay and peat excavations)
	Aesthetic values	++	+++	Benthic red list species
	Spiritual and religious value	+	++	All species
Supporting services	Sediment formation and structuring	+++	+++	Bacteria, fungi, rooted macrophytes and bioturbating invertebrates
	Nutrient cycling, decomposition and mineralisation	+++	+++	All benthic organisms
	Photosynthesis	+++	+++	Benthic algae and rooted macrophytes
	Primary and secondary food production, including food web control mechanisms	+++	+++	All benthic organisms
	Provision of habitat and shelter	+++	+++	Rooted macrophytes and benthic biofilms

+ small; ++ intermediate; +++ large

Overall, the benthic species that provide ecosystem services belong to the same main taxonomic groups (microbes, algae, aquatic vascular plants, aquatic invertebrates, aquatic vertebrates) as the pelagic species providing ecosystem services in aquatic ecosystems (see e.g. EFSA PPR Panel, 2010a, 2013). In Table 3, these ecosystem service providing main taxonomic groups (key drivers) are linked to Tier 1 aquatic taxa mentioned in the data requirements (Commission Regulation (EU) 283/2013). The majority of these Tier 1 standard test species concern organisms predominantly occurring in the water column (pelagic organisms), but the rooted macrophytes *Myriophyllum* and *Glyceria maxima*, the insect *Chironomus* ssp. and the oligocheate worm *Lumbriculus* spp. are benthic (sediment-dwelling) organisms that can be used to assess the potential impact of sediment exposure as well.

In general, to ensure ecosystem services, both pelagic and benthic taxa representative for aquatic key drivers need to be protected at the population level. However, it is proposed to protect aquatic vertebrates (fish and amphibians) at the individual (to avoid direct mortality and animal suffering) or population level (e.g. chronic effects via reproduction). Since functional redundancy of microbes is considered to be relatively high and tools to study the impact of pesticide exposure on specific microbial populations are difficult to apply, it is proposed to protect them at the functional group level.

Following the procedure of EFSA PPR Panel (2010a), SPG options for benthic key drivers (main taxonomic groups to be protected) need not be defined only in terms of the ecological entity to be protected (e.g. individual, population, functional group, community) but also in terms of the dimensions attribute (e.g. the measurement endpoints behaviour, survival/growth, abundance/biomass, process), magnitude of tolerable effect (e.g. negligible to large), temporal scale of the tolerable effect (e.g. days to seasons), spatial scale of the tolerable effect (e.g. in-crop, such as rice fields, edge-of-field, watershed) and degree of certainty (which always needs to be high). Options for this are elaborated below.

Table 3: The aquatic key drivers and their ecological entity to be protected as proposed in EFSA PPR Panel (2010a) and the current standard aquatic test species related to these key drivers. The benthic (sediment-dwelling) Tier 1 test species are presented in bold

Key driver (service providing taxonomic group)	Ecological entity to be protected	Tier 1 taxa mentioned in data requirements (Commission Regulation (EU) 283/2013)[8]
Aquatic algae	Populations	Green algae, e.g. *Pseudokirchneriella subcapitata* Other taxonomic groups, e.g. the diatom *Navicula pelliculosa*
Aquatic vascular plants	Populations	Monocots, e.g. *Lemna gibba/minor*, ***Glyceria maxima*** Dicots, e.g. ***Myriophyllum***
Aquatic invertebrates	Populations	Crustaceans: *Daphnia magna*/*pulex*, *Americamysis bahia* Insects: ***Chironomus* ssp. (e.g. *Chironomus riparius*)** Oligochaetes*:* ***Lumbriculus* spp. (e.g. *Lumbriculus variegatus*)**
Aquatic vertebrates	Individuals (in acute risk assessment to avoid visible mortality), populations (in chronic risk assessment)	Fish, e.g. *Oncorhynchus mykiss*
Aquatic microbes	Functional groups	No standard test species

3.3. Specific protection goal options for benthic organisms

In EFSA PPR Panel (2013) two SPG options are presented for aquatic organisms that, in principle, may be used for benthic organisms as well, namely (1) the ecological threshold option (ETO) that accepts only negligible effects on the ecological entity to be protected and (2) the ecological recovery option (ERO) that accepts some population/functional group-level effects if ecological recovery takes place within an acceptable time period. Note, however, that in EFSA PPR Panel (2013) the dimension 'spatial scale' is fixed to edge-of-field surface waters and the dimension 'degree of certainty' always should be high. It seems therefore logical that for benthic organisms in edge-of-field surface waters the SPGs also need to be defined in terms of the dimensions 'ecological entity', 'attribute', 'magnitude' and 'temporal scale'.

In Table 4 a proposal for the ETO option and the risk assessment of sediment exposure to pesticides is presented. A potential problem when defining SPGs for aquatic microbes is that Tier 1 data

[8] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market Text with EEA relevance. OJ L 93, 3.4.2013, p. 1–84.

requirements are not defined for benthic microbes, but the Tier 1 data for microbes and soil risk assessments may be of use here. In addition, for benthic algae Tier 1 data are not required officially but potential risks to benthic algae probably are sufficiently addressed by either the risk assessment procedure used for planktonic algae or that for rooted macrophytes. This, however, is an important topic for future research.

For completeness, and to be in line with EFSA PPR Panel (2013), a proposal for the ERO and the risk assessment of sediment exposure to pesticides is presented in Table 5, although there may be several reasons why the ERO for sediment exposure to pesticides may not often be applicable (see arguments below). Note that in EFSA PPR Panel (2013) the recovery option can be assessed only on basis of higher-tier tests that allow the measurement of recovery rates of affected populations/endpoints to be addressed. These higher-tier tests comprise micro-/mesocosm experiments and/or experimental studies in combination with mechanistic effect models. In deriving ERO–RACs on the basis of micro-/mesocosm tests the AGD proposes to do that on the basis of Effect class 3A of the most sensitive measurement endpoint. An Effect class 3A is defined as a clear short-term effect that does not last longer than eight weeks. Furthermore, in order to derive an ERO–RAC from micro-/mesocosm experiments vulnerable populations should also be present in the test systems. Generally, species that have a high chance of exposure (e.g. low avoidance potential), are sensitive to the pesticide because of specific traits (e.g. poor detoxification mechanism, low elimination rate) and have a low recovery potential (e.g. long generation time and less developed dispersal abilities) are vulnerable. Benthic organisms fulfilling these characteristics are considered vulnerable and should be considered for derivation of ERO–RACs. A conceptual framework to address ecological recovery for any assessed product that falls under the remit of EFSA, an EFSA scientific opinion is under development (EFSA SC, 2016).

Table 4: Proposed SPGs for the ETO for sediment exposure to pesticides in edge-of-field surface waters

Organism group	Ecological entity	Attribute	Magnitude	Temporal scale
Benthic algae	Population	Abundance/biomass	Negligible effect	Not applicable
Rooted macrophytes	Population	Survival/growth Abundance/biomass		
Benthic invertebrates	Population	Abundance/biomass		
Benthic vertebrates	Individual Population	Survival Abundance/biomass		
Benthic microbes	Functional group	Processes (e.g. litter break down)		

Table 5: Possible SPGs for the ERO for sediment exposure to pesticides in edge-of-field surface waters

Organism group	Ecological entity	Attribute	Effect allowable on most sensitive/vulnerable population Magnitude and duration
Benthic algae	Population	Abundance/biomass	Total effect period < 8 weeks irrespective of direct and indirect effects (also for repeated applications; Effect class 3A in micro-/mesocosms) [(a)]
Rooted macrophytes	Population	Survival/growth Abundance/biomass	
Benthic invertebrates	Population	Abundance/biomass	
Benthic vertebrates	No recovery option		
Benthic microbes	Functional group	Processes	Negligible to short-term effects (total effect period < 8 weeks) [(b)]

(a): Usually not possible for vulnerable populations with long life cycles and low dispersal abilities.
(b): More research is needed to characterise relevant recovery periods for microorganisms and microbial processes.

In principle, both the ETO and the ERO, as worked out in the updated AGD (EFSA PPR Panel, 2013) to assess the effect of water exposure to pesticides on aquatic organisms in edge-of-field surface water, may be used to assess the effects of sediment exposure to benthic organisms. If proof can be provided that because of ageing/aged sorption (see Figure 1) the bioavailability of the PPP in sediments shows a relatively fast decrease, the ERO might be applicable for the PPP under evaluation.

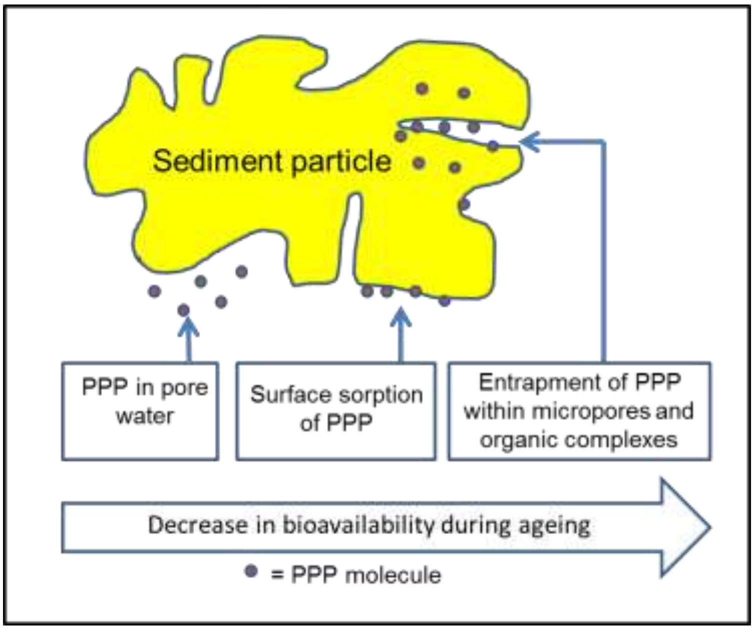


Figure 1: Schematic presentation of processes that play a role in the decrease in bioavailability of a persistent PPP in the sediment compartment (adapted from Semple et al., 2003). For a more detailed description on aged sorption see EFSA PPR Panel (2015a, in preparation)

Note, however, that there are several reasons why, for the time being, a prudent approach is required in applying the ERO to assess the effect of sediment exposure to benthic organisms in edge-of-field surface waters. Reasons for this are:

- Sediment risk assessment is triggered for pesticides that are hydrophobic and persistent in sediment and/or applied frequently, so that chronic exposure regimes in the sediment compartment will be the rule rather than the exception. Since the compounds enter the sediment compartment via the water compartment, potential risks of short-term exposures of PPPs to aquatic organisms is probably sufficiently addressed by the water exposure assessment as described in EFSA PPR Panel (2013).
- The sediment compartment may be a sink for several hydrophobic compounds. Furthermore, in agricultural landscapes different PPPs may be used simultaneously (e.g. tank mixtures) and repeatedly. Therefore, the possibility of background contamination and mixture toxicity in the sediment compartment of edge-of-field surface waters cannot be excluded (see Chapter 10). Note that in the prospective risk assessment PPPs predominantly are evaluated individually, so that possible cumulative and synergistic effects on benthic organisms of sediment exposure to different PPPs will remain unnoticed. Therefore, the ETO is a better option when evaluating individual PPPs. It thus may better address issues of the 'uniform principles' as laid down in Regulation (EC) No 546/2011, which requires that Member States base their authorisation decision on the 'proposed conditions for the use of the plant protection product'. Furthermore, the standard data requirements for PPPs request: 'any information on potentially unacceptable effects of the plant protection product on the environment, on plants and plant products shall be included as well as known and expected cumulative and synergistic effects'.

- The ERO requires either semi-field experiments or modelling. Little experience exists in evaluating concentration–response relationships for sediment exposure derived from (sediment-spiked) micro-/mesocosm experiments. Furthermore, appropriate effect modelling approaches to address the effects of sediment-bound PPPs to benthic organisms is a research activity to date.
- By not accepting population-level effects on benthic algae, rooted macrophytes and benthic invertebrates in edge-of-field surface waters, populations of these taxonomic groups will be protected and propagation of effects to the community-, ecosystem- and landscape-level will be less likely.
- According to Diepens et al. (submitted 2015a), for benthic communities it may be difficult to identify vulnerable key species for each relevant taxonomic group, since many benthic taxa have a high plasticity, fulfil a variety of functions and their vulnerability might change depending on their life stage. To date, the vulnerability of typical benthic taxa to pesticide stress has not received much attention in the scientific literature.

The items mentioned above suggest that the ETO is the best option for providing adequate protection of benthic organisms.

4. Current knowledge of exposure and effects of sediment-bound PPPs

4.1. The occurrence of pesticides in sediments

EU and national agencies responsible for the quality of freshwaters make very few measurements of the concentrations of modern pesticides in freshwater sediments: the majority of effort is directed at monitoring compounds in solution (Warren et al., 2003). This is also partly because of the difficulties and costs of measuring concentrations in a complex matrix such as sediment and because of the lack of regulatory requirements (e.g. Water Framework Directive) to monitor pesticide concentrations in sediments. The Water Framework Directive priority substances suggested for future trend monitoring in sediment concern a limited number of pesticides only, namely alachlor, chlorfenvinphos, chlorpyrifos, endosulfan, isoproturon, tributyltin compounds, trifluralin, dichlorodiphenyltrichloroethane (DDT), aldrin, endrin, isodrin and dieldrin (AMPS, 2004). Most of the pesticides listed are already banned for agricultural use in the EU.

Warren et al. (2003) presented a review on the occurrence, fate and bioavailability of pesticides in the sediment compartment. The majority of pesticides enter the freshwater environment from agricultural sources, through processes such as spray drift, leaching, surface runoff, soil erosion and volatilisation. The extent of losses of agricultural pesticides to edge-of-field surface waters, however, depends on a wide range of factors, including method of application, formulation, weather conditions, soil type and topography, farming practice and crop type and mitigation measures such as buffer strips. Many pesticides associate strongly with natural sedimentary material with the more hydrophobic compounds usually exhibiting the strongest interaction. For many organisms, sorption to sediments is likely to reduce bioavailability by reducing aqueous concentrations (in overlying and interstitial water). Sorption may, however, increase exposure for benthic fauna, particularly sediment-ingesting organisms. In lentic ecosystems (e.g. ponds) contaminated sediments are more or less fixed in space, but sediment movement is clearly an important transport mechanism for many hydrophobic pesticides in fluvial systems (streams and rivers). Degradation of pesticides in bed sediments has, to date, received little study but many banned organochlorine pesticides are still detected in freshwater sediments owing to their high persistence in the environment. Bioturbation can enhance the transport and vertical distribution of pesticides in sediments and is reported to enhance biodegradation of sediment-associated pesticides by producing conditions which stimulate microbial activity (Monard et al., 2008). Pesticide bioavailability is currently not quantitatively well understood and influenced by many factors relating to compound, sediment and organism type, environmental conditions (e.g. temperature, redox) and the age of the residues (Warren et al., 2003, and literature cited; see also Figure 1).

Studies on modern pesticides in sediments indicate that a wide range of compounds may be present in river sediments draining agricultural catchments. In the Humber catchment area (United Kingdom), Long et al. (1998) monitored, in 1995 and 1996, the following PPPs in six tributaries of the Humber catchment area: dimethoate, simazine, atrazine, lindane, diazinon, propanil, carbaryl, linuron, flutriafol, propiconazole, permethrin, cypermethrin, fenvalerate, deltamethrin, trifluralin, propazine, desmetryn, prometryn, terbutryn, fenitrothion, malathion, cyanazine and parathion. The following main conclusions with respect to PPP concentrations in sediments can be derived from the study of Long et al. (1998):

- Sediments can act as 'sink' as well as potential secondary source of PPPs within the fluvial system.
- A wide range of PPPs associated with both the sediment bed and suspended sediment solids could be demonstrated, including both hydrophobic (e.g. pyrethroids) and water-soluble (e.g. triazine herbicides) substances.
- Considerable temporal variation in the concentrations of PPPs in sediments could be demonstrated.
- Different catchments exhibited differences in terms of type, frequency and concentrations of PPPs in sediments, reflecting differences in land-use and seasonal dynamics.
- The high concentrations of PPPs sometimes associated with sediments did not correlate with concentrations measured in the water compartment.
- In general, concentrations of PPPs were higher in association with suspended sediments than bed sediments, although exceptions occurred as well.
- Simultaneous exposure to several PPPs (and other contaminants) in sediments could be demonstrated on all sampling dates and in all tributaries, but on different samplings and localities usually a limited number of PPPs (particularly pyrethroid insecticides) were of high concern from an ecotoxicological point of view.

In Australian streams draining agricultural landscapes Schäfer et al. (2011) monitored 97 pesticides of which 48 could be detected in grab water samples and 27 in sediment samples above their limit of quantitation. In these streams sediment-mediated exposure was considered the main cause of the observed effects. Highest toxicity, in terms of toxic units (TUs), attributed to sediment samples and sediment toxicity, showed a better correlation with the SPEcies At Risk ($SPEAR_{pesticides}$) index than water toxicity (TUs in water samples). Compounds detected in sediments that potentially affected macroinvertebrates were the insecticides carbaryl, chlorpyrifos, diazinon, permethrin and pirimicarb and the fungicides chlorothalonil and iprodione.

Anderson et al. (2012) analysed trends in sediment contamination and sediment toxicity in watersheds of California (USA) for the period from 2008 to 2010. The results (statewide samples) indicate that detection of pyrethroid pesticides (mainly bifenthrin) in sediment increased from 55 % in 2008 to 85 % in 2010. Frequencies of detection and concentrations of organophosphate pesticides in sediment decreased between 2008 and 2010. The relation between amphipod (*Hyalella azteca*) and chemical concentrations in sediments were investigated as well. A strong correlation was found between amphipod mortality and the total concentration of pyrethroid pesticides.

4.2. Sediment toxicity data for pesticides on benthic invertebrates and rooted macrophytes

4.2.1. Laboratory toxicity data

Deneer et al. (2013) published a literature review on sediment-spiked laboratory toxicity data for pesticides to benthic invertebrates and rooted macrophytes, of which a summary is presented below.

In publicly available scientific literature, laboratory sediment toxicity data for 13 organochlorine compounds, 14 pyrethroid insecticides, 14 acetyl-cholinesterase inhibiting insecticides, 16 other types of insecticides, 24 fungicidal substances and 18 herbicides were found. Most open literature papers report semi-chronic (10–13-day) toxicity data relating to either the amphipod *Hyalella azteca*, the chironomid *Chironomus dilutus* and to a lesser extent the chironomid *Chironomus riparius*. Chronic toxicity data (≥ 28 days) derived from sediment-spiked toxicity tests are available for *Chironomus riparius* in particular, and to a lesser extent for *Chironomus dilutus*. Other less frequently studied freshwater benthic invertebrate species in sediment-spiked toxicity tests are *Asellus aquaticus* (Isopoda), *Ephoron virgo* (Ephemeroptera), *Jappa kutera* (Ephemeroptera), *Lumbriculus variegatus* (Oligochaeta), *Potamopyrgus antipodarum* (Mollusca) and *Tubifex tubifex* (Oligochaeta). These less frequently studied species, however, were tested with different compounds hampering an interspecies comparison of sensitivity. For several pesticides (insecticides in particular) toxicity data for estuarine/marine benthic species are incidentally reported (e.g. the amphipods *Ampelisca abdita*, *Corophium volutator*, *Eohaustorius estuarius* and *Leptocheirus plumosus*, the copepods *Amphiascus tenuiremis*, *Microarthridion littorale* and *Paronychocamptus wilsoni*, the polychaete worms *Neanthes arenaceodentata*, *Nereis diversicolor* and *Nereis virens* and the bivalve *Mercenaria mercenaria*). Only a few herbicide studies quantified effects on the rooted macrophyte *Myriophyllum* in standardised sediment exposure experiments. Overall, herbicides and fungicidal compounds appear to be much less toxic to benthic arthropods than insecticidal compounds. Furthermore, the relatively few sediment toxicity data available for benthic test species other than arthropods, and *Hyalella* and *Chironomus* in particular, seem to suggest that for fungicides arthropod species are not necessarily the most sensitive. In the literature (including regulatory documents), results of sediment-spiked toxicity tests for *Lumbriculus variegatus* are very scarce, so it cannot be evaluated whether this benthic test species is a better indicator than the arthropods for risks of sediment exposure to fungicides. For the macrophyte *Myriophyllum aquaticum*, in the few available tests with sediment-spiked herbicides the 10–13-day EC50 values (sediment contact test) were, overall, lower than the sediment toxicity values reported for benthic arthropods (Deneer et al., 2013, and literature cited). These data seem to suggest that the specific toxic mode-of-action of pesticides cannot be ignored when assessing environmental risks of sediment exposure.

4.2.2. Micro-/mesocosm experiments

Micro- and mesocosms containing a benthic community can be used to study the long-term effects, including latency, of sediment-associated pesticides on populations of benthic species, the recovery of affected benthic species and the interactions between species. To date, however, only a few studies are available that specifically address the impact of pesticide exposure to endo- and epibenthic invertebrates and rooted macrophytes (e.g. Fletcher et al., 2001; Rand, 2004; Roessink et al., 2006; Pablo and Hyne, 2009; Brock et al., 2010b). In all these outdoor micro-/mesocosm studies the pesticides were applied to the water compartment. Furthermore, the treatment-related responses observed for benthic invertebrates and rooted macrophytes were expressed in terms of (initial) concentrations in the water column. If dynamics in sediment concentrations were also measured, in most studies this was not carried out in different sediment layers and in both solid and aqueous phases. In the open literature, we found only one example of a sediment-spiked outdoor microcosm/mesocosm experiment with a focus on estuarine/marine test systems and responses to a contaminant mixture (copper, pyrene and DDT) on the colonisation of the sediment compartment by benthic macroinvertebrates (Balthis et al., 2010).

Despite some limitations in the freshwater micro-/mesocosm experiments mentioned above, these studies clearly reveal that hydrophobic pesticides that quickly sorb to sediments may have a pronounced and sometimes long-lasting effect on benthic organisms and communities. For example, in the experimental ditch study of Brock et al. (2010b), the hydrophobic insecticide lufenuron was applied to ditch sections that covered 33 %, 67 % and 100 % of the ditch surface area. The benthic community was mainly impacted in the insecticide-treated sections and recovery of the impacted benthic population (e.g. chironomids and the ephemeropteran *Caenis horaria*) was very slow in treated ditch sections. In a follow-up experiment it was demonstrated that the colonisation by chironomids of trays containing lufenuron-contaminated sediment, compared with colonisation of an experimental ditch not contaminated with this insecticide, was negatively impacted by this insecticide (see Figure 2).

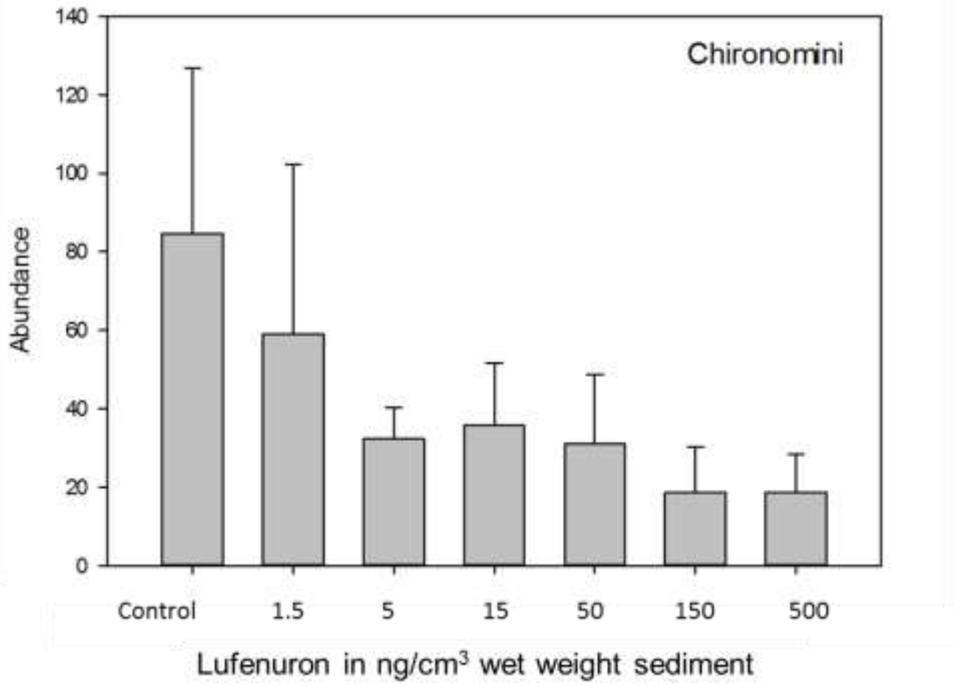


Figure 2: Abundance of larvae of Chironomini in trays with approximately 475 cm^3 wet weight sediment containing different initial concentrations of the insecticide lufenuron (in ng/cm^3 wet weight). The trays (with a surface area of 160 cm^2 were placed for 10 weeks in an experimental ditch not contaminated with lufenuron. After the colonisation period of 10 weeks the lufenuron concentration in the sediment was approximately 70 % of the initial concentration. A NOEC of 5 ng lufenuron/cm^3 wet weight sediment could be calculated (T.C.M. Brock, unpublished data). The figure was prepared by the PPR WG.

4.3. Current knowledge on effects of pesticides on sediment microorganisms

Chemical pesticides and other organic pollutants can influence microorganisms in two different ways: (i) the compound may be toxic and negatively impact metabolic activity and growth in a microbe; and (ii) in microbes exhibiting tolerance to a compound, it may act as a growth substrate and, hence, stimulate growth of those organisms. Thus, while general toxicity test systems using single microbial species or strains can give information about specific toxicity mechanisms or degradation pathways, they are less suitable in prospective risk assessment for investigating potential effects on microbes in general (see sections 2.3.2 and 8.5 for a more detailed discussion about test systems and their inherent potentials and limitations). Another consequence of the possible dual effects of pesticides on different metabolic and functional groups of microbes, is that spiking with pesticides in aquatic meso- or microcosms—including sediments—commonly results in increases in microbiological parameters (Laursen and Carlton, 1999; Lopez et al., 2002; Downing et al., 2004; Huang et al., 2010; Negroni et al., 2010). In addition, concerning pesticide interactions with heterotrophic microorganisms, degradation studies have been considered highly desirable and received much attention, since degradation can potentially lead to production of toxic metabolites (see Chapter 10), which is a critical issue to consider in a risk assessment.

A substantial number of studies in meso- or microcosms and using a multitude of different microbial endpoints have demonstrated that pesticide addition can have effects on both structural and functional properties of heterotrophic microbial communities in sediments (e.g. Svensson and Leonardson, 1992; Melendez et al., 1993; Dahllöf et al., 1999; Widenfalk et al., 2004, 2008a; Hwang et al., 2005; Negroni et al., 2010; Huang et al., 2010) and reviews on the subject have been published (DeLorenzo et al., 2001; Puglisi, 2012; Diepens et al., 2014a). Increasingly advanced molecular methods for studies of microbes in their natural environments are continuously developed and such methods have been employed in studies of pesticide effects on structural properties of sediment microbial communities (e.g. Cordova-Kreylos et al., 2006; Widenfalk et al., 2008b; Lin et al., 2012; Dimitrov et al., 2014). Despite the detailed information that these methods provide, the recent studies of Lin et al. (2012) and Dimitrov et al. (2014) failed to demonstrate significant effects of two fungicides on the structure of total bacterial and fungal communities in sediments.

A systematic literature search and analysis of response of microorganisms to pesticides was supported by EFSA (Puglisi, 2012). The obtained records were separated into a terrestrial and an aquatic lot. The aquatic lot contained 42 studies, of which 30, 10 and 8 looked at herbicides, insecticides and fungicides, respectively. With respect to endpoints, 33, 14 and 12 studies reported effects on biomass, activity and structure, respectively. In the general data analysis and presentation of the aquatic lot, studies in sediments were lumped with those in other aquatic compartments. The analysis and presentation also lumped the photosynthetic algae with the heterotrophic microorganisms, so effects on these two groups could not be evaluated separately in the brief summary below. The aquatic data set was relatively small and comparisons were also hampered by a quite wide spectrum of pesticides, test organisms, types of sediments considered, experimental set-ups as well as chosen endpoints in the retrieved studies (in total, 254 different endpoints were recorded in this review). Nevertheless, it was shown that with regard to endpoints relating to activity and biomass, a no-effect outcome or stimulation of (at least some groups of) microorganisms was principally as likely as inhibition. This general pattern was valid for fungicides, herbicides as well as insecticides, although it has to be kept in mind that the specific endpoints may have differed among the pesticide classes. Cases with significant effects on activity and biomass were recorded for all three classes. Puglisi (2012) also noted that recovery of parameters to original conditions—from both increases and decreases—was sometimes reported. However, it was restricted to four of the 30 studies on herbicides, which is too few for drawing general conclusions on the likeliness of recovery. Regarding effects on endpoints related to community structure, a majority of the cases showed significant changes, but these data originated in the fewest number of studies (12), compared with biomass or activity endpoints.

Regarding potential adverse effects of PPPs on aquatic microbial communities, effects of fungicides on natural fungal communities is of particular interest. Dijksterhuis et al. (2011) isolated several fungi from aquatic habitats in the Netherlands. In single species tests on agar media, they showed that the lowest effect concentration inhibiting fungal growth was lower than the regulatory acceptable concentration based on acute HC5 (hazardous concentration of 5 % of the species) values derived from Species Sensitivity Distributions (SSDs) constructed with non-microbial aquatic taxa (e.g. algae, macrophytes, invertebrates). Based on their results and the important role of fungi in aquatic ecosystems, they concluded that current regulatory procedures are not protective enough and suggested that more research and development is needed on toxicity testing with non-target aquatic fungi. There are currently no standardised tests with aquatic fungi. There is an ISO test on effects of pollutants on mycorrhiza fungi (ISO/TS 10832:2009); however, these fungi are not prominent in aquatic ecosystems.

Meso- and microcosm experiments have shown that pesticide additions can also result in indirect effects on various organisms. For example, the results of Sumpono et al. (2003) from wastewater treatment ponds imply that addition of the herbicide diuron led to death and decomposition of photosynthetic microorganisms, which in turn led to increased production and biomass of total bacteria. Similarly, the study of Staley et al. (2011) showed that altered phytoplankton and periphyton communities because of exposure to the herbicide atrazine can indirectly stimulate the abundance of a faecal indicator bacterium (*Escherichia coli*) in sediments. It is not unexpected that pesticides can have indirect effects on sediment biota and it has often been advocated that assessments need to consider trophic interactions in food webs (Pratt et al., 1997; DeLorenzo et al., 2001). In addition, the response of sediment microorganisms to pesticide exposure depends to some extent on environmental conditions, e.g. supplies of inorganic nutrients, quantity and quality of organic matter, salinity and metals (Pratt and Barreiro, 1998; Garcia-Ortega et al., 2011; Muturi et al., 2013; Magbanua et al., 2013).

Post-exposure monitoring studies also give some evidence that exposure to chemical pesticides can affect sediment microbes. For instance, high pesticide concentrations were correlated with low total bacterial abundance in harbour sediment (Vezzuli et al., 2003). However, it is problematic to identify direct toxic effects from monitoring studies, because of the possibility of adaptation of microbial communities as a response to long-term exposure to pesticides. Thus, de Lipthay et al. (2003) reported that phenoxy acid contamination led to higher abundances of *Pseudomonas* bacteria and microbial phenoxy acid degraders in sediments of a shallow freshwater aquifer. Similarly, Dahllöf et al. (2001) demonstrated adaptation (induced tolerance or other community changes), in the microbial functional diversity, to an organotin (tributyltin; previously used in anti-fouling paint for ships hulls, now banned) in a mesocosm experiment in a marine sediment. Another important aspect in retrospective monitoring studies is that environmental variables also influence the properties of sediment microbial communities. Schäfer et al. (2011) found that in southeast Australian streams, densities in several microbial groups were more strongly correlated with environmental variables than with concentration of pesticides. Likewise, Cordova-Kreylos et al. (2006) reported that spatial variation in two indicators of overall microbial community structure were more strongly correlated with environmental differences than to pollutants (including six pesticides) in Californian salt marsh sediments. These two studies highlight that when assessing pesticide effects on sediment microbes in retrospective investigations, availability and inclusion of appropriate unexposed control sediment habitats is critical.

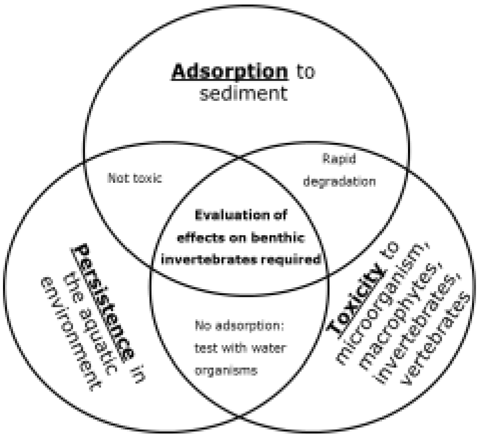
In conclusion, assessing potential effects of pesticides on microorganisms is a highly complex undertaking. A fair number of studies on effects on sediment microbes have been published, but because of the wide range in, for example, pesticidal compounds, test organisms or communities, types of sediments, experimental set-ups and selection of endpoints, few firm general conclusions on the response of sediment microbial communities to pesticides can currently be drawn. Moreover, toxicity tests with single species of microorganisms have big limitations for revealing probable responses to pesticide exposure in natural conditions, although community-based assays for microorganisms have partly solved these limitations.

5. Trigger for sediment testing

Appropriate trigger schemes are required to decide for which compounds additional sediment toxicity testing should be mandatory. At the same time the trigger schemes should not be overly conservative and lead to unnecessary testing and costs. In this section the existing approach for aquatic habitats (EFSA PPR Panel, 2013) will be discussed and compared with other existing or proposed approaches in sediment risk assessment of contaminants.

5.1. Overview of existing approaches

Most existing or proposed schemes for triggering risk assessment of contaminants in sediments are based on a combination of compound-specific and environmental properties, such as sorption behaviour and persistence of the active substance in sediment, mode-of-action and the toxicity to sediment-dwelling organisms. These principles were illustrated by a conceptual scheme of triggers for the assessment of sediment toxicity in a paper by Maund et al. (1997) (Figure 3).



Circles describe the three characteristics that should be evaluated, and overlap between the circles indicates the decision-making process for combination of those attributes. Adapted from Maund et al. (1997)

Figure 3: Theoretical basis for defining triggers for sediment toxicity studies with benthic invertebrates based on Maund et al. (1997).

Maund et al. (1997) proposed the following quantitative trigger levels (as summarised in Diepens et al. (submitted 2015a): (1) adsorption: K_{oc} (soil organic carbon (OC)–water partitioning coefficient) $\geq$ 1 000; (2) persistence in the environment: laboratory aerobic soil half-life time $\geq$ 30 days; and (3) toxicity: 48-hour median effect concentration (EC50) to *Daphnia* < 1 mg/L or a 21-day NOEC < 0.1 mg/L.

During a recent workshop on sediment risk assessment (ECHA, 2014) it was concluded that 'Sediment assessment should be triggered by a combination of specific factors instead of a single trigger. Triggering should include elements such as exposure routes not covered by the pelagic assessment, interstitial water concentrations, bioavailability (including sediment ingestion), partitioning and persistence.'

In the current risk assessment for PPPs another approach is taken, based on experimental data to be submitted during the approval procedure according to EC Regulation 1107/2009. In the former and recently updated AGDs for this regulation (EC, 2002; EFSA PPR Panel, 2013) the need to perform sediment toxicity tests is triggered by the outcome of mandatory water–sediment dissipation studies conducted with radio-labelled substances according to OECD Guideline 308 (OECD, 2002). Sediment toxicity studies are triggered when (i) the water–sediment study indicates that > 10 % of the applied radioactivity is present in the sediment at or after day 14 and (ii) the outcome of a chronic *Daphnia* test (or another comparable study with insects) results in an EC10 (or NOEC) < 0.1 mg/L. This trigger value is similar to the value proposed by Maund et al. (1997). As reported in the European Commission guidance document (EC, 2002), the toxicity trigger (EC10 (or NOEC) < 0.1 mg/L) was set to prevent unnecessary testing with substances of low toxicity to invertebrates. This value was chosen because on the basis of data from monitoring studies it is unlikely that higher concentrations will often occur in surface waters.

The use of toxicity values from *Daphnia* tests as a trigger for requiring tests on sediment-dwellers was also mentioned in Annex II, Point 8.2.7 of Directive 91/414/EEC.[9] A review that compared toxicity data for *Daphnia* and sediment-dwellers supports the approach (Streloke et al., 2002). In this review, effects data from chronic *Daphnia* tests and spiked water *Chironomus* tests were compared. It showed that the trigger of NOEC < 0.1 mg/L addresses most of the insecticides but also a large number of herbicides and fungicides. For compounds which do not reach the '10 % trigger' but are applied more

[9] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p.

than once during the season, due consideration should be given to the potential for accumulation of residues in the sediment and additional toxicity testing. Exposure triggers based on the water–sediment study are more difficult to apply to such use patterns because in the water–sediment study typically only a single application is made (EC, 2002).

In a recent review by Diepens et al. (submitted 2015a) comparison was presented of decision schemes and triggers for sediment toxicity testing in different European regulations (see Table 6). In addition to the afore-mentioned EC Regulation 1107/2009, these included the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation, the Biocidal Products Regulation (BPR), and the veterinary medicinal product residues (VMPR) (Table 6). Under the REACH regulation the information requirements are linked to the annual production and usage volumes of chemicals. Sediment-specific ecotoxicity data are only mandatory for chemicals above 100 (fate data) and 1 000 tonnes per year (ECHA, 2013).

Approaches followed are not consistent among the different European regulations. Under the REACH, and the BPR some of the elements proposed by Maund et al. (1997) are included, such as criteria for sorption (K_{oc} and K_{ow} (octanol–water partition coefficient)) and Predicted No Effect Concentration for sediment toxicity ($PNEC_{sediment}$). Criteria for persistence in the environment are not explicitly included as triggers under these regulations. Extrapolation of sediment toxicity from aquatic endpoints using the equilibrium partitioning (EqP) method for compounds with a log K_{ow} < 5, and a modified EqP by using an extrapolation factor of 10 for compounds with a log K_{ow} > 5, is included in the REACH, BPR and VMPR regulations.

Table 6: Criteria that are currently applied to trigger sediment toxicity testing as described in existing EU regulations and directives, as well as guidelines belonging to these regulations. Source: Adapted from Diepens et al. (submitted 2015a).

Regulation	Trigger	Reference
Regulation EC/1107/2009 concerning the placing of PPPs on the market	Sediment toxicity tests with benthic organisms are required if in the water–sediment fate study > 10 % of the applied radioactivity of the parent compound is present in the sediment at or after day 14, and the chronic toxicity value (EC10 or NOEC) derived from the 21-day Daphnia test (or another comparable chronic toxicity tests with a relevant crustacean or insect) is < 0.1 mg/L. In addition, compounds applied more than once, with a potential for accumulation of residues in the sediment should be given consideration for sediment testing as well (SANCO, 2002)	EC (2002) EFSA PPR Panel (2013)
Regulation EC/1907/2006[10] concerning REACH	ANNEX X (1 000 tonnes or more): 9.5.1 Long-term toxicity to sediment organisms: Long-term toxicity testing shall be proposed by the registrant if the results of the chemical safety assessment indicate the need to investigate further the effects of the substance and/or relevant degradation products on sediment organisms. The choice of the appropriate test(s) depends on the results of the chemical safety assessment. A log K_{oc} or log K_{ow} of ≥ 3 is used as a trigger value for sediment effects assessment: • log K_{ow} > 3: at least a screening assessment using the EqP method has to be performed.	ECHA (2008)

[10] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (OJ L 396, 30.12.2006).

Regulation	Trigger	Reference
	• log K_{ow} 3–5: the screening assessment using EqP based on pelagic data is considered appropriate. • log K_{ow} > 5 or a correspondingly high adsorption or binding behaviour: a more comprehensive sediment assessment is needed. If using the EqP approach, the RQ (risk quotient) is increased by an extra factor of 10 to take into account possible uptake via ingestion of sediment. If this RQ > 1, then a study, preferably long-term, with benthic organisms using spiked sediment is recommended. For substances that are highly insoluble and for which no effects are observed in aquatic studies, the application of the EqP method is not possible. In this case, at least one spiked sediment test has to be performed	
Directive 98/8/EC concerning the placing of biocidal products on the market (BPD)[11]	A log K_{oc} or log K_{ow} of ≥ 3 can be used as a trigger value for sediment effects assessment. For substances with a log K_{ow} > 5, the RQ (based on EqP) is increased by an extra factor of 10 to take possible uptake via ingestion of sediment into account. Tests with benthic organisms using spiked sediment are likely to be necessary if, using the EqP method, the PEC/PNEC ratio > 1	EC (2003, 2008)
Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products (BPR)[12]	Effect studies with sediment-dwelling organisms (marine or estuarine) are required for specific biocides (with direct releases to water), such as product type 21 (PT-21; anti-fouling products). (ECHA, 2013, Table page 207) When accumulation of an active substance in an aquatic sediment is indicated or predicted by environmental fate studies, the impact on a sediment-dwelling organism should be assessed. Testing might be required...if the risk assessment for sediment based on the equilibrium partition method indicates a possible risk to the benthic compartment (ECHA, 2013, page 118)	ECHA (2013)
Directive 2001/82/EC with amendments Veterinary medicinal products Referring to the Guidance (VICH, 2004)	If the RQ for a tested aquatic invertebrate is ≥ 1 it is recommended to consider the $PEC_{sediment}/PNEC_{sediment}$ ratio. The $PNEC_{sediment}$ is calculated using EqP. This method uses the $PNEC_{aquatic}$ invertebrate and the sediment/water partitioning coefficient as input. If the RQ is ≥ 1, then testing of sediment organisms is recommended. For substances with a log K_{ow} > 5, the RQ is increased by an extra factor of 10 to take account of possible uptake via ingestion of sediment. If the RQ is > 1, then a study, preferably long-term, with benthic organisms using spiked sediment is recommended	EC (2004a)[13] VICH (2004)

In the prospective ERAs for pesticides in the USA sediment toxicity tests with freshwater, marine or estuarine organisms are a conditional requirement (US EPA, 2007). The trigger system used by the

[11] Directive 98/8/EC, available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:31998L0008
[12] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products (BPR).
[13] Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products (Official Journal L 311, 28/11/2001 p. 1–66). Amended by Directive 2004/28/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/82/EC on the Community code relating to veterinary medicinal products (Official Journal L 136, 30/4/2004 p. 58–84).

EPA includes the likelihood of aquatic exposure, sorption to sediments, persistence in sediment and exposure conditions. The decision scheme applied is shown in Figure 4.

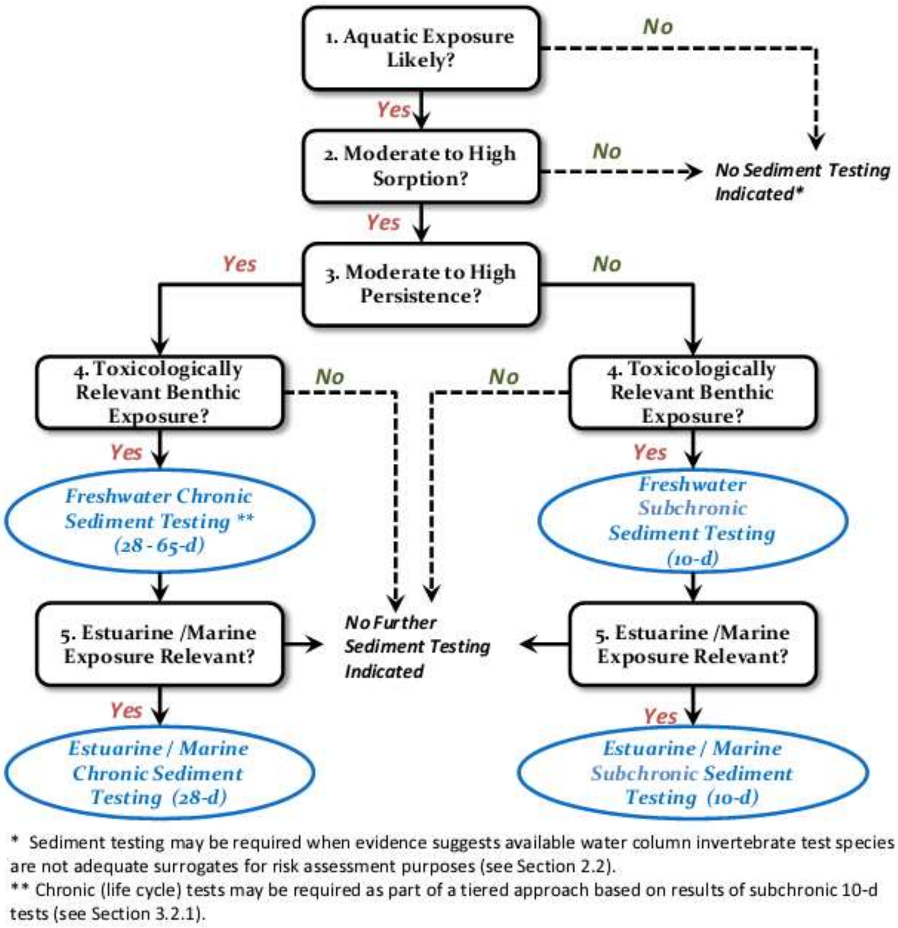


Figure 4: Conceptual framework for requesting sediment toxicity data by US EPA, for approval of pesticides in the USA. Source: Brady (2014). Copied with permission. The legend in the figure refers to the Brady (2014) and not to this document.

In the USA, semi-chronic (10-day) tests with freshwater sediment organisms are required when the half-life of the pesticide in the sediment is ≤ 10 days in either aerobic soil or aquatic metabolism studies and if any of the following conditions are fulfilled: (i) the soil partition coefficient (K_d) is ≥ 50; (ii) the log K_{ow} is ≥ 3; or (iii) the K_{oc} ≥ 1 000. Further tests with estuarine/marine species are required if the product is intended for direct application to the estuarine or marine environment or when relevant exposure can be expected because of runoff, erosion, expected use or mobility pattern. Chronic sediment tests (28–65 days) are required when the half-life of the pesticide in the sediment is > 10 days (derived from aerobic soil or aquatic metabolism studies), when the estimated environmental concentration (EEC) in sediment is > 0.1 of the acute LC50/EC50 values and the above-mentioned triggers for sorption are exceeded[14]. Brady (2014) further explained that the K_d trigger of 50 was chosen to represent 80 % sorption of the chemical to sediments with an OC content of 2 %, and that it was important to note that exposure to benthic invertebrates would still be expected to some extent for chemicals with K_d, K_{oc} and K_{ow} values below the numeric triggers. For the European PPP regulation this approach is less relevant, as the experimental data are available for the

[14] Trigger criteria derived from data requirement table 40 CFR Part 158 Subpart G (Terrestrial and aquatic nontarget organisms data requirements table) of US EPA (2007). URL: www.law.cornell.edu/cfr/text/40/158.630 (accessed 26 November 2014).

expected accumulation in sediment and expected exposure conditions are provided by the $FOCUS_{sw}$ scenarios and models, allowing prediction of the development of PEC_{sed} values over time.

5.2. Predictability of accumulation in sediment in edge-of-field waters

Most of the decision and trigger schemes discussed in the previous sections start with the predicted chemical fate derived from sorption or persistence of the specific compounds. Some of the schemes (REACH, BPD/BPR) rely mainly on sorption or hydrophobicity parameters for neutral compounds (K_{oc} or K_{ow}). The inclusion of a trigger for K_d, as in the US regulation for pesticides, allows for the inclusion of sorption mechanisms other than OC partitioning, which is of importance for, for example, more polar or ionisable compounds. The trigger scheme in the PPP regulation is the only scheme that makes use of experimentally determined accumulation in the sediments in combination with toxicity data. In Appendix A data from existing dossiers were used to compare the measured fraction of compounds accumulated in sediments with the predicted distribution, based on sorption (K_{oc}) and persistence in the environment (DegT50). The modelling exercise was executed to evaluate to what extent the accumulation in sediments can be predicted reliably from these properties. A dynamic model was used for a water–sediment system with the dimensions and characteristics of the OECD Guideline 308 test (OECD, 2002).

In Figure 5 the predicted distribution to sediments during 180 days is presented for several combinations of K_{oc} (50–1 000) and DegT50 (10–300 days). The predicted values did not match measured values very well (see Figures 1–7 in Appendix A). The total explained variance was 50 % with some large deviations, which was attributed to uncertainties and inconsistencies in experimental values and nature and composition (e.g. OC content) of the sediments used in the experiments. It was concluded that it is not possible to establish a link between sorption constants and expected maximum residues of PPP active agents in sediments, except for compounds that are highly persistent in sediment. Using worst-case assumptions (no degradation) for, for example, compounds that are persistent in sediment it was demonstrated (see Figures 1–3 in Appendix A) that compounds with K_{oc} values greater than 50 L/kg would already meet the criteria when the trigger is set to 10 % accumulation in the sediments. That is not in line with current trigger values of 1 000 L/kg for the K_{oc} value in some of the schemes. Using this K_{oc} value of 1 000 L/kg for compounds persistent in sediment would be equivalent to more than 75 % accumulation in the sediments. However, compounds that are non-persistent in sediment are unlikely to reach these values. For non-persistent compounds combinations of K_{oc} and DegT50 could principally be used to estimate the maximum residues in sediment (Figure 5). The question remains how to estimate the DegT50 in the water–sediment system before doing the actual tests. Therefore, we conclude that the current triggers in the PPP regulation based on experimental distribution data (OECD, 2002) should not be replaced by triggers related to predicted accumulation in sediments. The current experimental triggers integrate both accumulation and degradation processes.

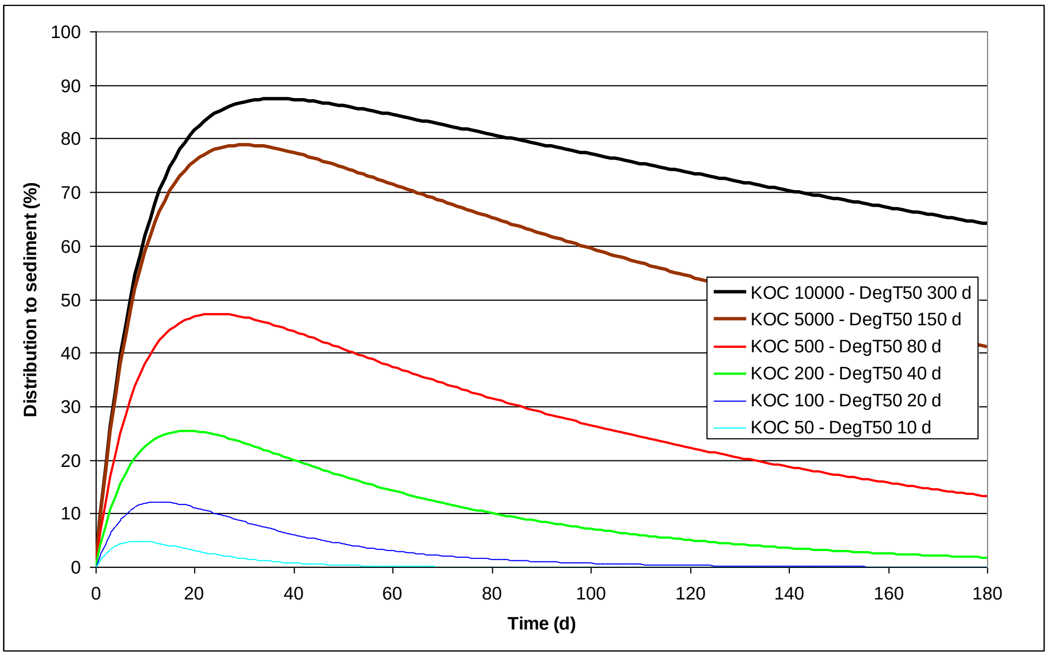


Figure 5: Calculation of time-dependent residues in sediment at different K_{oc} and DegT50 (Corg: 5 %, bulk density (bd): 0.8 L/kg, $r_{wat\text{-}sed}$: 3.5). The figure was prepared by the PPR WG.

5.3. The equilibrium partitioning approach

From the data presented in Table 6 it appears that several Regulations/Directives consider EqP as a screening tool to assess potential risks to sediment organisms by using chronic toxicity data for pelagic organisms. In principle, according to the EqP theory, a RAC_{sed} can be estimated using chronic toxicity data derived for pelagic organisms or directly using the chronic $RAC_{sw;ch}$ (indicative for the threshold option). The concept of EqP is based on the work of Di Toro et al. (1991), which assumes that the partitioning of a chemical between sediment and pore water can be represented by a simple equilibrium equation (1):

$$C_{soc} = C_{pw} \times K_{oc} \qquad (1)$$

C_{soc} is the concentration of the chemical in the sediment per unit mass of OC (µg/kg OC). C_{pw} is the concentration of the chemical in pore water (µg/L), and K_{oc} is the partition coefficient of the chemical to sediment OC (L/kg OC). When replacing C_{pw} with a concentration in surface water (C_{sw}) and assuming that this concentration C_{sw} corresponds to the $NOEC_{sw}$ or RAC_{sw} derived for pelagic water organisms (on basis of water toxicity tests), the C_{soc} becomes the $NOEC_{OC,EqP}$ or $RAC_{OC,EqP}$ (i.e. expressed in unit of OC, mg/kg OC). A short-coming of the EqP approach is that it neglects sediment ingestion as a relevant uptake pathway, as it only represents transfer occurring through passive partitioning between organic matter, water and lipids. Indeed, it has been shown that this approach is inadequate for several polychlorinated biphenyls (PCBs) and the mayfly *Hexagenia* sp. (Selck et al., 2012), the marine amphipod *Corophium volutator* (Diepens et al., submitted 2015b, the marine polychaete worm *Arenicola marina* (Besselink et al., 2012) and the freshwater oligochaete *Lumbriculus variegatus* (Leppänen and Kukkonen, 1998), for chlorpyrifos and the annelid *Lumbriculus variegatus* (Jantunen et al., 2008) and for several organochlorine pesticides and the marine copepod *Chasmagnathus granulata* (Menone et al., 2004). These organisms accumulated up to two orders of magnitude higher concentrations of organic substances than EqP predicts. Therefore, to be protective this should be taken into account. Following the recommendation by Diepens et al. (submitted 2015a it is proposed in the PPP risk assessment for benthic fauna to always derive a toxic threshold that is a factor 10 lower than originally calculated by means of the EqP approach (also used in REACH, for biocides and pharmaceuticals with log $K_{oc}/K_{ow} > 5$), as in equation (2):

$$NOEC_{oc;modEqP} = (NOEC_{sw}*K_{oc})/10 \text{ or } RAC_{oc;modEqP} = (RAC_{sw}*K_{oc})/10 \qquad (2)$$

As the standard sediment contains, on average, 2.5 % OC (OECD Guideline 218), this has to be taken into account to express the final NOEC in terms of total sediment:

$$NOEC_{sed;modEqP} = NOEC_{oc,EqP}/40 \text{ or } RAC_{sed;modEqP} = RAC_{oc,EqP}/40 \qquad (3)$$

It is proposed in sediment ERA for PPPs and benthic fauna that may become exposed by ingestion of food, to always use as an initial screening the modified EqP approach as described above, to avoid unnecessary tests with benthic invertebrates and vertebrates. For organisms that primarily are exposed via pore water (e.g. plants and microorganisms) the EqP as such (without the extrapolation factor of 10) may be used. The validity of the modified EqP approach is explored in the next section by evaluating the results of water-spiked water–sediment and sediment-spiked water–sediment toxicity tests with *Chironomus* sp.

5.4. Comparing water-spiked and sediment-spiked toxicity test data

In order to learn more about the predictive value of the modified EqP approach (using the extra AF of 10), toxicity data for *Chironomus riparius* from water-spiked and sediment-spiked water–sediment tests are compared (Table 7). To do so, the effect concentrations in the water ($NOEC_{sw}$ in mg/L) were recalculated in terms of sediment concentrations using the modified EqP concept ($NOEC_{sed,EqP}$ in mg/kg sediment).

The $NOEC_{measured}/NOEC_{calculated}$ ratios, as presented in Table 7, are all above 1, which shows that when using the modified EqP concept, the estimated toxicity is generally higher if derived from the water phase in a water-spiked test ($NOEC_{calculated}$) than from the sediment phase in a water–sediment-spiked test ($NOEC_{measured}$).

In conclusion, the correctness of the modified EqP method as a realistic worst-case approach was verified for a number of substances (mostly fungicides and insecticides) (Table 7). This evaluation shows that using the modified EqP approach (i.e. using an extra factor of 10 to account for dietary intake in cases of invertebrates and fish) for recalculating effects values in milligrams per litre into sediment concentrations enables a conservative first tier assessment. Indeed, this assessment shows that exposure through water is more often the worst-case exposure than exposure through sediment when comparing *Chironomus riparius*. If this assessment indicates an unacceptable risk (toxicity exposure ratio > 10), performing more appropriate chronic sediment-spiked water–sediment tests is necessary.

The data presented in Table 7 also reveal that it may be considered acceptable to use data of chronic water-spiked water–sediment tests with relevant species to estimate a corresponding endpoint for the sediment compartment, by using the modified EqP approach. For *Chironomus riparius* results of water-spiked water–sediment tests are, at present, more frequently available than data of sediment-spiked tests.

Table 7: Comparison of 28-day NOEC data from water–sediment tests with *Chironomus riparius* for various substances as derived from spiked sediment (mg/kg) or spiked water (mg/L), usually as nominal concentrations (data derived from Umwelt Bundesamt (UBA) and EU databases, including International Union of Pure and Applied Chemistry Footprint database)

Substance	$NOEC_{sediment}$ = $NOEC_{measured}$ mg/kg sediment	$NOEC_{sw}$ mg/L	K_{oc} mg/kg OC	$NOEC_{sed,EqP}$ = $NOEC_{calculated}$ mg/kg sediment	Ratio $NOEC_{measured}$/ $NOEC_{calculated}$
	sediment-spiked test		water-spiked test		
Fungicide					
Bixafen	20	0.0156	3 869	0.151	132
Boscalid	23.2	1	772	1.93	12
Myclobutanil	6.07	3.02	518	3.87	1.57
Fenpropidin	80	1	3 808	9.52	8.4
Isopyrazam	56	≥ 1	2 400	6.0	≤ 9.3
Difenoconazole	10	0.015	3 760	0.141	71
Picoxystrobin	5	0.0625	898	0.140	35
				6.06	
Insecticide					
Beta-cyfluthrin	0.200	0.0002	100 000	0.05	4
Gamma-cyhalothrin	0.0126	0.000046	60 000	0.0069	2
Metaflumizone	1.610	0.00256	30 700	0.19	8
Bifenthrin	0.040	0.00032	236 610	0.019	2
Chlorantraniliprole	0.005	0.0025	330	0.002	2.5
Fipronil	0.0002	0.0001	727	0.000182	1.1
Spinetoram	0.0972	0.00075	3 681	0.0069	14

5.5. Comparing environmental risks for water and sediment compartments

In order to investigate whether surface water (pelagic) organisms are likely to be more at risk than sediment organisms, effect assessments were compared with PEC values for overlying water and sediment compartments calculated according to FOCUS step 2. This comparison of the risk for pelagic organisms on the basis of Tier 1 RAC_{sw} values and in sediment on the basis of chronic NOECs derived from sediment-spiked *Chironomus* tests for 13 PPPs can be found in Appendix B. In summary, the outcome of this analysis indicates that if the risk is acceptable for pelagic organisms using the Tier 1 RAC_{sw} and PEC_{sw}, then a sediment risk assessment would generally not be of relevance, assuming that *Chironomus riparius* is a representative sensitive benthic species. However, if this risk to pelagic organisms based on the Tier 1 RAC_{sw} and PEC_{sw} is considered as unacceptable then a sediment risk assessment may be required. This evaluation, however, ignores the fact that toxicity data for benthic standard test species, other than *Chironomus riparius*, may be required in sediment effect assessment, since *Chironomus riparius* will not be the most sensitive benthic standard test species for all toxic mode-of-actions (see section 8.2.1).

5.6. Proposed trigger and decision scheme

The dossier data used in the modelling study described in Appendix A demonstrated that under the current PPP regulation for 24 of the 26 compounds the > 10 % distribution criterion is exceeded. Although this is not an exhaustive survey, the outcome is expected as the trigger can be met already for substances with sorption constants (K_{oc}) above 10 L/kg. The PPR Panel proposes to extend the current experimental triggers in the PPP regulation by also addressing repeated applications. In order to avoid unnecessary testing, the use of the modified EqP as an initial effect assessment tier for benthic fauna is proposed.

The current text of the AGD (EFSA PPR Panel, 2013) states the following triggers for sediment testing:

'Water/sediment study showed > 10 % of applied radioactivity at or after day 14 present in the sediment and chronic Daphnia test (or other comparable study with insects) EC10 (or NOEC) < 0.1 mg/L. For the time being, the guidance as given in the former SANCO guidance (2002) should be followed. This might be revised in the future, depending on the PPR Panel opinion on sediment effect assessment under development (EFSA-Q-2012-00959)'.

Considering the issue of possible accumulation in the sediment because of repeated PPP application it is recommended to adopt the triggers for sediment testing as follows:

"Water/sediment study showed > 10 % of applied radioactivity at or after day 14 present in the sediment; or FOCUS STEP 2, or if available STEP 3 (or other appropriate exposure model), modelling for total annual dose applied, results in > 10 % residues in sediments at the time of the maximum PEC_{sed}; and chronic *Daphnia* test (or with another relevant species) EC_{10} (or NOEC) < 0.1 mg/L. Note that for herbicides (and for some fungicides) the other relevant species may be algae or aquatic vascular plants and that the EC_{50} value < 0.1 mg/L derived from water-only (e.g. algae, *Lemna*) or a water-spiked toxicity test with sediment (e.g. *Myriophyllum spicatum*) is the relevant trigger for sediment testing".

The data presented in Table 7 and by Diepens et al. (submitted 2015a) suggest that the modified EqP approach (using an extra AF of 10) may be a cost-effective screening approach for PPPs to estimate sediment no-effect levels for benthic fauna. However, it should be realised that for specific chemical groups (e.g. high molecular weight substances and micelle-forming chemicals) the (modified) EqP may not always be appropriate for a realistic worst-case prediction of effect thresholds for sediment organisms (ECHA, 2014). However, PPPs usually do not belong to these chemicals groups. It is recommended that for a larger array of PPPs than presented in Table 7 the general applicability of the modified EqP is investigated.

In Figure 6 the decision scheme of the proposed trigger for sediment testing of PPPs is presented. For compounds with affinity to sediments and that are likely to cause long-term exposure and effect on benthic fauna we propose to apply a modified EqP (with an additional AF of 10 as described in the previous section) to derive an estimate of the chronic sediment RAC ($RAC_{sed;modEqP}$) from the aquatic $RAC_{sw;ch}$ (threshold option). Note that for plants the EqP approach without the additional AF of 10 for dietary uptake will suffice to derive a $RAC_{sed;EqP}$ that in ERA should be compared with the PEC_{sed}. For compounds where OC-based partitioning is not applicable, equivalent K_d values should be used. The PECs to be applied should be derived from the FOCUS modelling (see Chapter 7). Special attention should be given to the fact that for an appropriate linking of fate and effects the PEC_{sed} and $RAC_{sed;modEqP}$ (or for plants the $RAC_{sed;EqP}$) are expressed in concentrations that are normalised to sediments with the same OC content (e.g. 5 % OC as used in FOCUS scenarios or 2.5 % OC usually used to express sediment toxicity derived from spiked tests).

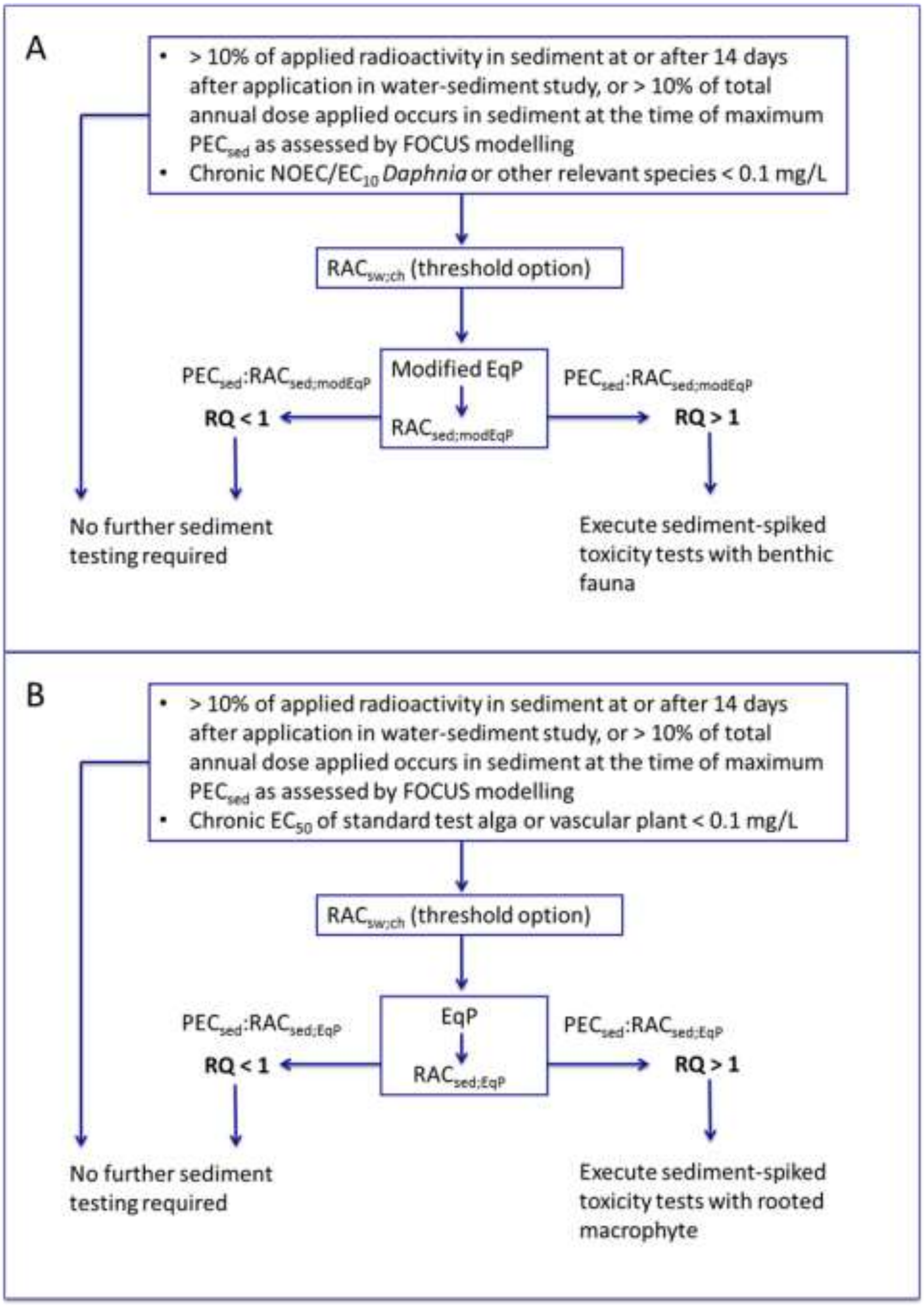


Figure 6: Decision scheme for conducting sediment-spiked toxicity tests for PPPs and benthic fauna (panel A) and benthic algae and rooted plants (panel B). The figure was prepared by the PPR WG.

In Chapter 8 proposals are given for the selection of appropriate test species in sediment effect assessment as well as for criteria to decide when additional assessment of bioaccumulation and biomagnification is required.

6. Ecotoxicologically relevant concentrations for sediment risk assessment

6.1. The ecotoxicologically relevant concentration concept

The interface between the exposure and the effect assessment of pesticides is defined as the type of concentration (e.g. concentration in overlying water, pore water or total sediment; peak or TWA concentration) that gives an appropriate correlation to ecotoxicological effects, and is referred to by EFSA PPR Panel (2005) and Boesten et al. (2007) as the ERC. A clear definition of the ERC is important to facilitate communication between experts involved in the risk assessment, as their respective view may differ to the angle with which exposure in the field and in ecotoxicity tests is approached.

In sediment risk assessment, the ERC concept needs to be consistently applied so that the predicted exposure concentration in the sediment compartment (PEC_{sed}) and regulatory acceptable concentrations for sediment organisms (RAC_{sed}) can be compared as readily as possible. It is important that, within the same risk assessment scheme (addressing the same specific protection goal), the type of ERC used to express the 'C' in the PEC_{sed} estimates should not be in conflict with the ERC used to express the 'C' in the RAC_{sed} estimates, in the sense that a realistic to worst-case risk assessment can be performed. The question is not so much 'What is the appropriate ERC for each species–pesticide combination?' but rather 'Which ERC to select within an ERA scheme that likely will result in a realistic to worst-case risk assessment?', e.g. a pore water concentration PEC (in micrograms per litre) should not be linked to the EC10 expressed in micrograms per kilogram dry mass.

From a theoretical point of view it will be the internal concentration at the target site of the organism that is the most appropriate ERC. In most tests underlying the registration procedure, however, body burdens of pesticides in the test organisms are not measured. Consequently, the 'C' in the PEC and RAC estimates usually refers to external exposure concentrations/contents. This scientific opinion focuses on benthic organisms that dwell on and in the sediment compartment of edge-of-field surface waters. In defining the ERC, important considerations include the specific habitats in the sediment compartment where the benthic organisms at risk live or temporarily dwell (e.g. water–sediment interface for epibenthos and deeper sediment layers for endobenthos) and the bioavailable fraction in that environment (e.g. the freely dissolved and/or particulate associated fraction).

The exposure assessment for sediment is currently based on the FOCUS scenarios which will continue to be used until updated or new methods become available and adopted by the SCoPAFF. The exposure assessment, which is explained in more detail in Chapter 7 of this opinion, considers the entry routes spray drift and vapour drift during application together with input via runoff, soil erosion and drainage after application. The scenarios cover major agricultural areas in Europe for pond, ditch and stream habitats. For all surface water bodies sediment layers were defined and the concentrations in sediment are part of the regular output of the FOCUS software packages.

6.2. Selecting the appropriate sediment layer

Selecting the appropriate sediment layer may be an important ERC consideration. Pesticides used in agriculture usually enter edge-of-field surface waters via the water column (e.g. spray drift, drain pipes, surface runoff). Particularly for lipophilic compounds that easily sorb to sediment particles, after a single exposure event the fluffy upper sediment layer initially may have much higher concentrations than deeper sediment layers (see e.g. Figure 7).

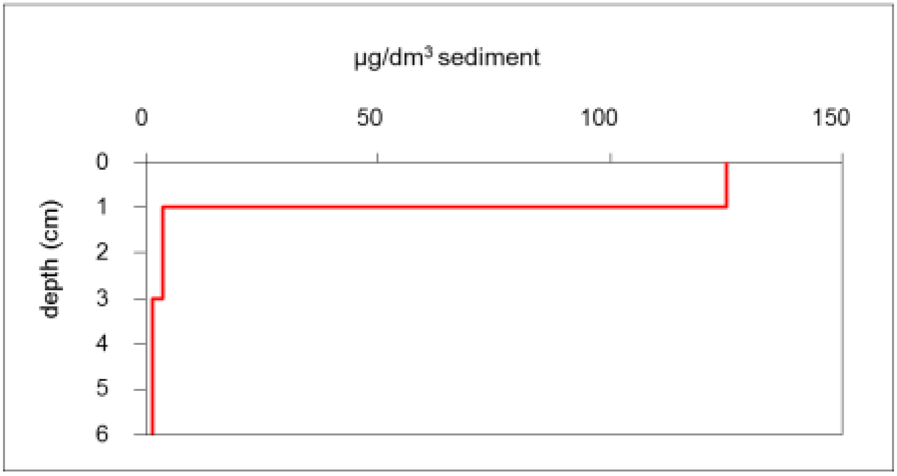


Figure 7: Stratification of the insecticide chlorpyrifos in different sediment layers seven days after a single spray drift application (nominal concentration 44 µg/L) to an experimental ditch (data derived from Crum and Brock, 1994; Brock, 2013). The figure was prepared by the PPR WG.

Note also that many benthic invertebrates can be found in the upper sediment layer because of more favourable food and oxygen conditions; therefore, this layer may be considered for a realistic worst-case ERC consideration.

It can be argued that for different types of benthic organisms PECs for different sediment layers need to be calculated because of differences in habitat occupied. According to the European Chemicals Agency (ECHA, 2014) a complicating factor is the heterogeneity of the sediment compartment and this also accounts for the exposures in the top sediment layer. Mobile epibenthic organisms in particular may have the ability to detect and avoid contaminated patches of sediment and, as a consequence, relatively healthy benthic communities may exist in sediments that have 'escape' areas. Spatial heterogeneity of pesticide exposure in standard sediment-spiked toxicity tests is less of a problem since in these tests the pesticide is mixed thoroughly through the sediment (e.g. OECD, 2007a, 2010a).

In developing risk assessment schemes for soil organisms, EFSA PPR Panel (2010b) already addressed ERC considerations with respect to stratification of pesticides in soil. The procedures developed for soils may be of value for sediment exposure assessment. With respect to soil depth, EFSA PPR Panel (2010b) proposed to assess concentrations averaged over 0 to 1 cm, 0 to 2.5 cm, 0 to 5 cm and 0 to 20 cm. The depth of 20 cm was selected since, under conventional tillage, soils of agricultural fields are mixed up to 20 cm depth periodically, e.g. by ploughing. This, of course, is not applicable for sediments.

In line with the pesticide exposure assessment in soil, however, calculation of PECs in the sediment compartment for the top 1, 2.5 and 5 cm seem good options, since these depths take into account different microhabitats where epibenthic and endobenthic invertebrates and microbes dwell. For a realistic worst-case exposure assessment for macrophytes, the 5 cm sediment layer may be selected for PEC calculation, since their roots may even occupy deeper layers. Using a 1 cm depth layer as a conservative scenario for other organisms is considered a reasonable and feasible approach.

However, although using a 1 cm depth layer as a conservative approach is feasible and realistic in most cases, this may not represent a realistic worst-case scenario for some epibenthic species exposed to pesticide-bound particulates entering through runoff events or to suspended particulates from sediment remobilisation/resuspension. Indeed, such species can be partly exposed to pesticides via food uptake through the highly contaminated thin top layer, i.e. to pesticides bound on particulates that sediment at the interface water/sediment (e.g. top 1 or 2 mm). In such a case, from the exposure side, the PEC_{sed} expressed as concentration of the top 1 cm layer would be an underestimation of the real exposure (e.g. in this example, 5- to 10-fold).

Considering effect assessment, either the test species is tested under experimental conditions using water and sediment, or water only. In the first case, the test can be performed by spiking the water or spiking the sediment. In the case of water-spiked water-sediment tests the exposure concentration in the upper sediment layer initially will be higher than that in deeper layers. Consequently, the NOEC/EC10 value in a water-spiked water-sediment test, when expressed in terms of measured and/or predicted exposure concentration in the sediment, will depend on the sediment layer that is considered. In case of a sediment-spiked water-sediment test, this is less a problem since the pesticide is mixed homogeneously in the sediment (e.g. depth of the sediment layer of 1.5 to 3 cm for the Sediment-Water Chironomid Life-Cycle Toxicity Test, using Spiked Sediment; OECD, 2010a). Therefore, knowledge on how exposure–effect relationships have been assessed in sediment toxicity tests is important, since using different methods will lead to different toxicity values that may not always be readily linked to the PEC_{sed} value of, for example, the upper 1 cm sediment layer in the field.

6.3. Selecting the appropriate exposure metric

For benthic organisms there are typically two main routes of exposure to pesticides, i.e. aqueous exposure (microbes, plants and animals) and food or ingestion exposure (particularly animals). According to EFSA PPR Panel (2009) and ECHA (2014) for non-ionic organic chemicals (the majority of pesticides) the most appropriate metric for bioavailability in soils and sediments appears to be the 'freely dissolved pore water concentration' rather than the total sediment concentration, particularly for compounds with a log $K_{ow} < 5$. Two pragmatic approaches have regularly been used to support that the concentration in pore water is usually a more precise exposure metric: changes in effect over time (ageing; see Figure 1) and the usual normalisation of effects on basis of OC content observed in various sediment types. Nevertheless, for several sediment-dwelling invertebrates, ingestion of polluted sediment material may add to toxicant accumulation and/or overall toxicity (e.g. Leppänen and Kukkonen, 2000; Lu et al., 2004; Sormunen et al., 2008a, b; Maul et al., 2008).

OECD Guideline 218, the sediment–water *Chironomus* test using spiked sediment, specifies that as a minimum the concentrations in overlying water, pore water and total sediment (sum of aqueous and solid fractions) should be measured (OECD, 2004a). Effect concentrations should be expressed as concentrations in total sediment, based on dry weight, at the beginning of the test. Similarly, the sediment–water *Chironomus* life cycle test using spiked water or spiked sediment (OECD Guideline 233), specifies the same minimum set of measurements in overlying water, pore water and total sediment (OECD, 2010a). Although effect concentrations should be expressed as a concentration in the total sediment at the start of the test, OECD Guideline 233 does not explicitly specify on what basis the L(E)Cx (lethal effect concentration) and NOEC values should be expressed.

OECD Guideline 225 (*Lumbriculus* toxicity test using spiked sediment) specifies that the concentration in total sediment and overlying water should be verified through measurement, although the guideline also outlines a method for isolation and subsequent measurement of the chemical in pore water. Effect concentration should be expressed in milligrams per kilogram sediment on a dry weight basis (OECD, 2007a).

The US EPA OPPTS 850.1735 Guideline (whole sediment acute toxicity invertebrates, freshwater) states that 'Concentrations of spiked chemicals may be measured in total sediment, interstitial water and overlying water...', but does not specify on what basis effect concentrations should be expressed, other than 'In some cases it may be desirable to normalise sediment concentrations to factors other than dry weight, such as organic carbon for non-ionic organic compounds or acid volatile sulphides for certain metals.' (US EPA, 1996b).

EFSA recently published a scientific opinion on the assessment of exposure of organisms to pesticides in soils (EFSA PPR Panel, 2010b). This report advocates that the ERC for soil organisms should be reported both in concentration units of mass of pesticide per mass of dry soil, and in parallel as a concentration in pore water. It seems likely that the rationale behind the advocated use of both measures of exposure in soil (insufficient knowledge on the importance of various routes of uptake) would also hold for sediment and benthic organisms. This would strongly suggest that toxicity data and PECs generated for sediment organisms should also be reported on the basis of both pore water concentrations and sediment mass (preferably normalised to OC content of the dry sediment). This is obviously not yet in line with OECD and EPA guidelines, where the most common suggestion is to report effect concentrations on the basis of sediment mass only.

If the use of pore water concentrations in the risk assessment seems appropriate, the necessary information could be extracted from the current FOCUS software scenarios, although not as part of the standard output delivered by the FOCUS tools (see Chapter 7 of this opinion).

The use of pore water concentrations as the basis for calculation of effect concentrations, however, may introduce some new uncertainties. Sappington (2013) points out that caution should be taken when using calculated instead of measured pore water concentrations, since slight differences in quality of OC may result in large differences between assumed and actually occurring partitioning in sediments. According to Xu et al. (2007) it is the free concentration in pore water that is essentially independent of sediment conditions, and this free concentration would therefore be preferred as the basis for calculation of effect concentrations. However, analytical error in measured pore water concentrations tends to increase with chemical hydrophobicity, resulting in increased uncertainty (Sappington, 2013) and expressing risk on the basis of total sediment concentrations only may avoid complications resulting from analytical uncertainty in measured pore water concentrations.

6.4. Selecting the appropriate exposure duration

Another important ERC topic is the choice of the time-window for the exposure estimate to use in the risk assessment, i.e. whether the peak exposure estimate (e.g. $PEC_{sed;max}$) or the TWA exposure estimate (e.g. $PEC_{sed;twa}$) is most appropriate to compare with the RAC_{sed}, and, if the TWA approach is appropriate, what should be the time-window of this TWA PEC. Hydrophobic and slowly degrading pesticides in particular will result in long-term sediment exposure, although the bioavailability of these PPPs may decrease in time (Figure 1).

Risks due to short-term exposure of sediment organisms most likely will be covered by the risk assessment schemes for typical water column organisms, since (1) peak concentrations of these hydrophobic pesticides in the water column may be relatively high and trigger acute risks to pelagic and epibenthic water organisms and (2) most typical sediment-dwelling species are taxonomically related to typical water column species. Consequently, risk assessment schemes for PPPs and sediment-dwelling organisms should focus on chronic effects due to long-term exposure in the sediment compartment.

Unfortunately, the current FOCUS surface water scenarios do not consider the effect of multi-year applications, which could lead to accumulation of these types of substances in sediment even if the surface water concentrations are hardly effected. In order to address this problem, in Chapter 7 of this opinion a pragmatic solution is presented based on a simple accumulation factor that should be multiplied with the maximum sediment concentration in order to compensate for the FOCUS deficit. The FOCUS surface water scenarios do not consider the effect of a gradual decrease in bioavailability of PPPs in the sediment compartment because of ageing, the gradual 'burrial' of previous PPP inputs to deeper sediment layers or the annual removal of the sediment layer when edge-of-field ditches are cleaned. Consequently, the accumulation factor to address multi-year use of PPPs may result in a relatively worst-case approach to derive PEC_{sed} values.

In standard sediment toxicity tests the pesticide is introduced only once during spiking, and the toxicity estimate is often expressed in terms of initial (or nominal) exposure concentration. Consequently, under these test conditions another requirement for a realistic to worst-case risk assessment is that the rate of dissipation or the decrease in bioavailability of the test compound in the spiked sediment of the toxicity test should not be faster than that predicted for the sediment in the relevant edge-of-field surface water. For further guidance on this topic in chronic risk assessments see also Brock et al. (2010c) and EFSA PPR Panel (2013, chapter 9).

In sediment risk assessment schemes of the US EPA, not only true chronic (28–60-day) sediment toxicity tests, but also semi-chronic 10-day tests with sediment invertebrates may be used if aerobic soil or aerobic aquatic metabolism half-life of the test compound is ≤ 10 days (Brady, 2014). In fact, in the open literature and for pesticides more semi-chronic (usually 10-day L(E)C50 values) than chronic sediment toxicity data for benthic invertebrates can be found (Deneer et al., 2013). In order to use these semi-chronic toxicity data in sediment risk assessment schemes, an option may be to extrapolate these data to chronic NOEC/EC10 values. From the literature review conducted by Deneer et al. (2013) it appears that the results of semi-chronic 10-day L(E)C50 values for pesticides and *Hyalella azteca* and *Chironomus dilutus/riparius* usually do not deviate more than a factor of 5 from corresponding NOEC values from chronic tests with these sediment invertebrates (duration ≥ 28 days), although exceptions also exist. On basis of sediment toxicity data for benthic invertebrates and a wider array of organic chemicals, Diepens et al. (submitted 2015a) propose to apply an AF of 5 to 10 to extrapolate an HC5 (hazardous concentration to 5 % of the species) derived from a SSD constructed with L(E)C50 values from semi-chronic sediment toxicity tests in order to estimate the corresponding chronic HC5.

6.5. Proposal for ERC in sediment ERA for PPPs

Considering the current practise of sediment toxicity testing as laid down in OECD and US EPA test protocols, the PPR Panel proposes to express the PEC_{sed} and RAC_{sed} estimates at least in terms of total sediment concentration based on dry weight, either normalised to OC content in the dry sediment or normalised to standard OECD sediment with an organic matter content of 5 % (which approximates to 2.5 % OC on dry weight basis). In addition, the PPR Panel advocates the expression of PEC_{sed} and RAC_{sed} estimates in terms of the freely dissolved PPP fraction in pore water. To assess the risks of sediment exposure to benthic organisms, it is proposed to use the 0–1 cm sediment layer for PEC_{sed} derivation in case benthic fauna are the organisms of concern, while the 0–5 cm sediment layer may be used if rooted macrophytes are assessed. Furthermore, it is proposed to consider the accumulation of individual PPPs because of multi-year use in the PEC_{sed} estimate. In addition, considering the long-term exposure regimes of PPPs that accumulate in sediments, the RAC_{sed} derivation should preferably be based on chronic toxicity data using sediment-spiked tests and benthic organisms, not excluding that semi-chronic toxicity data also can be used to derive a RAC_{sed} if an appropriate additional extrapolation factor is used.

7. Exposure assessment

7.1. Introduction

PPP exposure assessment for the sediment compartment of the aquatic environment in the EU is currently based on the FOCUS methodology (FOCUS, 2001). This is done for approval of active substances at EU level. It is also used in some Member States for product authorisation, but other exposure assessment procedures may also be used. The principle of this methodology is extensively described in the guidance on tiered risk assessment for PPPs for organisms in surface waters (EFSA PPR Panel, 2013). However, the FOCUS methodology has not been reviewed by the PPR Panel of EFSA during the revision of the guidance document on aquatic ecotoxicology and the overall level of protection for approval of active substances at EU level is therefore not clear. Nevertheless, the methodology has been used in regulatory decision making throughout the last years and there is currently no alternative standardised exposure assessment methodology. EFSA PPR Panel (2013) therefore assumes that the FOCUS surface water methodology will continue to be used until updated or new methods that can replace the existing tools become available and adopted by the SCoPAFF.

The FOCUS Surface Water Modelling Working Group defined a step by step procedure for the calculation of predicted environmental concentrations in surface water (PEC_{sw}) (FOCUS, 2001). The procedure consists of four steps, whereby the first step represents a very simple approach using simple kinetics, and assuming a loading equivalent to a maximum annual application, for calculating PEC_{sed} (maximum values, actual concentrations and TWA concentrations). The second step is the estimation of concentrations taking into account a sequence of loadings, and the third step focuses on more detailed modelling taking into account realistic ‘worst case’ amounts entering surface water via relevant routes (runoff, spray drift, drainage). The third step considers substance loadings as foreseen in step 2, but it also takes into account the range of possible use patterns. The use patterns are, therefore, related to the specific and realistic combinations of crop, soil, weather, field topography and aquatic bodies adjacent to fields. The fourth step accounts for risk mitigation measures. Originally, the FOCUS steps were defined as different tiers but the experience with the scenarios showed that the PECs at step 3 and step 4 could be higher than respective PECs at step 2. Therefore, in this document the FOCUS methodology is considered a stepped rather than a tiered approach.

It is important to notice that none of these steps were designed to describe realistic worst-case scenarios for sediments, since FOCUS (2001) considers only the pelagic water compartment. The FOCUS methodology is nevertheless recommended to be used also for sediment risk assessment provided that the sediment concentrations are corrected for possible accumulation after multi-year applications. Assuming that the current exposure scenarios really represent worst-case conditions for the overlying water, they cannot represent worst-case conditions for sediment at the same time.

Within the stepped FOCUS approach initial steps 1 and 2 calculations were developed to represent ‘worst-case loadings’ and ‘loadings based on sequential application patterns’, respectively, but should not be specific to any climate, crop and topography or soil type. FOCUS (2001) considered the assumptions at both steps 1 and 2 as very conservative. Spray drift values are essentially based on drift data calculated from Biologiche Bundesanstalt für Land- und Forstwirtschaft (BBA, 2000) and an estimation of the potential loading of PPPs to surface water via runoff, erosion and/or drainage. However, EFSA PPR Panel (2015b) concluded that recent research showed considerably higher spray drift values than the numbers used by FOCUS (2001), particularly at short distances (0–3 m). This loading represents any entry of PPP from the treated field to the associated water body at the edge of the field. Step 3 requires the use of the mechanistic models Pesticide Root Zone Model (PRZM), MACRO and TOXic substances in Surface WAters (TOXSWA).

In terms of metabolites, already at steps 1 and 2, concentrations can be calculated not only for the active compound but also for metabolites formed in the soil before runoff/drainage occurs. The user must define the properties of the metabolite, including its maximum occurrence in soil and the ratio of the molecular masses of parent compound and metabolite.

The fate of metabolites formed in the water body can also be taken into consideration at steps 1 and 2. The formation will be calculated in a similar way based on the maximum occurrence of the metabolite in water–sediment studies. It is recommended to update the FOCUS tools to deliver the pore water concentration for the top 1 cm sediment layer.

7.2. Description of the different steps

7.2.1. Step 1

The FOCUS (2001) scenario properties on step 1 were based on existing concepts within the EU and Member States. In step 1 a water column of 30 cm overlying a sediment of 5 cm depth with 5 % OC (density: 0.8 $g \cdot cm^3$) is considered, but only the upper 1 cm of the sediment is used when calculating the distribution between water and sediment layer. However, the official output which is presently delivered by the model considers a depth of 5 cm, i.e. a dilution with a factor of 5 compared with the 1 cm used for calculating the water sediment distribution. Sediment concentrations at a specific depth horizon have to be calculated manually according to the following equation (7.1):

$$PEC_{sed,i} = \frac{PEC_{sed,FOCUS}\ 5\,cm}{d_i} \qquad (7.1)$$

$PEC_{sed,i}$: predicted sediment concentration (µg/kg) over a depth of i cm

$PEC_{sed,FOCUS}$: FOCUS STEP 1 sediment concentration (µg/kg)

d_i: depth of sediment layer[cm]

The scenario furthermore considers sediment with 5 % OC, selected in order to comply with existing risk assessment approaches within the EU: Note that existing ecotoxicity testing guidelines for sediment-dwelling organisms (e.g. current OECD Guidelines 218, 219, 225, 233; OECD, 2004a, b, 2007a, 2010a) use sediment with different OC content. The standard sediment in these OECD guidelines has a 4–5 % peat content which corresponds to an OC content of approximately 2.5 %. Further equations and parameter settings can be found in EFSA PPR Panel (2013).

At step 1, inputs of spray drift, runoff, erosion and/or drainage are evaluated as a single loading to the water body and 'worst-case' surface water and sediment concentrations are calculated. The loading to surface water is based on the number of applications multiplied by the maximum single use rate, except for compounds with a short half-life in sediment–water systems. If three times the degradation half-life (3 × DegT50) (combined water + sediment) is less than the time between individual applications, there is no potential for accumulation in the sediment–water system and the maximum individual application rate is used to derive the maximum PEC. For first order kinetics the value of 3 × DegT50 is comparable to the DegT90 value. Considering runoff loadings, while pesticide mass is entered into the stagnant 30 cm water, the runoff water is not. This implies that exposure caused by runoff entries will be estimated in a conservative way by step 1.

Four crop groups (arable crops, vines, orchards and hops) representing different types of application technology and aerial applications are separated into different drift classes for evaluation at steps 1 and 2. Drift values have been calculated at the 90th percentile from BBA (2000). No drift is assumed when the substance is incorporated or applied as granules or as a seed treatment. However, EFSA PPR Panel (2004) concluded that dust drift may occur for such applications and provided computational procedures to estimate this route.

The loading to the water body from combined runoff/erosion/drainage is set at 10 % of the application for all scenarios.

On the day of application, drift entries are assumed to be present in the water phase only in order to obtain a conservative peak concentration. One day later the compound is assumed to be distributed between water and sediment according to equation 7.2.

The run-off/erosion/drainage entry is distributed instantaneously between water and sediment at the time of loading according to the K_{oc} of the compound in order to simulate the process of deposition of eroded soil particles containing PPPs. In this way compounds are distributed directly between sediment and water according to equation 7.2.

$$\text{Fraction of compound in sediment} = 1 - \frac{W}{(W + (S_{eff} \cdot bd \cdot foc \cdot K_{oc}))} \qquad (7.2)$$

where: W = mass of water (30 g)

S_{eff} = mass of sediment available for partition (0.8 g)

foc = mass fraction of OC in sediment (0.05 g/g)

K_{oc} = sorption coefficient related to OC (L/kg)

bd = bulk density of the sediment (kg/L)

As a consequence of the direct water and sediment distribution, concentrations for both compartments are calculated with the same dynamics (see Figure 8).

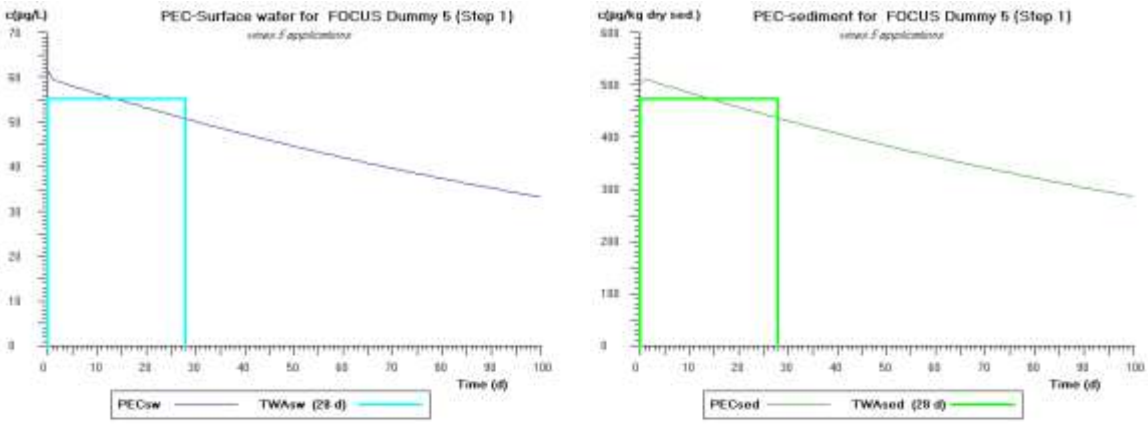


Figure 8: FOCUS step 1 simulation for FOCUS Dummy 5 (K_{oc}: 860 L/kg). The figure was prepared by the PPR WG.

Step 1 estimates the total sediment concentration, but does not output the pore water concentrations. If concentrations in pore water are to be used for the risk assessment they have to be calculated by the user according to the following equation, which describes the equilibrium partitioning based on two phases, pore water and the solid sediment matrix.

$$C_{pw} = \frac{C_{sed}}{\frac{P}{bd} + Koc\ foc} \qquad (7.3)$$

C_{pw}: pore water concentration (mg/L)

C_{sed}: sediment concentration (mg/kg)

foc mass fraction of OC in sediment (0.05 g/g)

K_{oc} OC partition coefficient (L/kg)

bd = bulk density of the sediment (kg/L)

P: porosity in sediment (L/L)

7.2.2. Step 2

The surface water properties of step 2 are defined by FOCUS (2001) identically as in step 1. Thus, a static water body with a water depth of 30 cm, overlying a sediment of 5 cm depth (density: 0.8 g cm^{-3}) with 5 % OC is assumed. Similar to step 1, only 1 cm of sediment is used when calculating the partitioning between water and sediment. When calculating PEC_{sed} a depth of 5 cm is used, i.e. a dilution factor of 5 compared with the 1 cm used for the water sediment distribution. Sediment concentrations at different depths have to be calculated according to equation 7.1

At step 2 the width of the water body is not defined and all entries are calculated in a similar way based on a percentage of the application rate in the treated field. In addition, the same ratio (10:1) is defined to reflect the proportion of a treated field from which PPPs are lost to surface water.

However, at step 2 inputs of spray drift and the combined load caused by runoff, erosion and drainage are evaluated as a series of individual loadings comprising drift events (number, interval between applications and rates of application) followed by a loading representing a combined load by runoff, erosion and drainage event four days after the final application. Note that only runoff mass is entered into the stagnant 30 cm water, so no runoff water is added. This implies that peak exposure events caused by runoff entries will be estimated in a conservative way by step 2. Degradation rates are assumed to follow first-order kinetics in soil, surface water and sediment and the exposure assessor has the option of using different degradation rates in surface water and sediment.

In order to prevent multiple worst-case assumptions for multiple application patterns, FOCUS (2001) defined different individual drift percentiles dependent on the total number of applications per season, which represent the overall 90th percentile. Unfortunately, the procedure may result in lower predicted concentrations for multiple applications than for individual applications with the 90th drift percentile. The software automatically calculates for both situations so that the user can select the higher value of the two. The Panel proposes to update the methodology to calculate drift values.

Drift inputs are loaded into the water column where they are subsequently distributed between water and sediment according to the K_{oc} of the active substance. However, the process of adsorption to sediment at step 2 is assumed to take longer than one day (which was assumed at step 1).

In contrast to step 1, the amount of PPP that enters the soil at step 2 is corrected for crop interception. For each crop, several interception classes have been defined depending on the crop stage. Crop interception will decrease the amount of PPP that reaches the soil surface and thus ultimately enters the surface water body via runoff/drainage.

Four days after the final application, a combined load caused by runoff, erosion and drainage is added to the water body. This loading is a function of the residue remaining in soil after all of the treatments (g/ha) and the region and season of application. The different runoff/erosion/drainage percentages applied at step 2 have been calibrated by FOCUS against the results of step 3 calculations as described in FOCUS (2001). The user selects between two regions (northern EU and southern EU according to the definitions given for crop residue zones in the SANCO Document 7525/VI/95-rev.7; SANCO, 2001) and three seasons (March to May, June to September and October to February).

In common with step 1, the runoff/erosion/drainage entry is distributed between water and sediment at the time of loading according to the K_{oc} of the compound. As a consequence, water and sediment concentrations are again calculated with the same dynamics (see Figure 9).

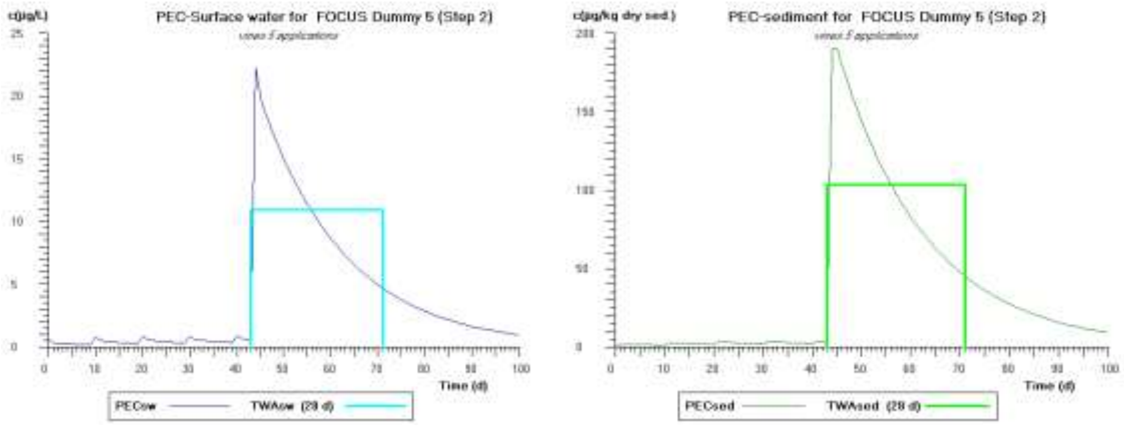


Figure 9: FOCUS step 2 simulation for FOCUS Dummy 5 (K_{oc}: 860 L/kg). The figure was prepared by the PPR WG.

Similar to step 1, step 2 does not provide pore water concentrations in sediment. If concentrations in pore water should be used for the risk assessment they have to be calculated using equation 7.3 as for step 1 simulations.

7.2.3. Step 3

For step 3 a selection of scenarios is defined based on a number of broad data sets that covered all areas of the European Community in 2001 (15 Member States). According to FOCUS (2001) they should consider representative realistic worst-case situations and should take into account all relevant entry routes to a surface water body, as well as considering all appropriate target crops, surface water situations, topography, climate, soil type and agricultural management practices. However, owing to the lack of comprehensive databases that characterise most of these agro-environmental parameters at a European level, when the scenarios were defined (1997–2001), they were not selected in a rigorous, statistically based manner. Instead a pragmatic approach was adopted, using very basic data sources together with expert judgement. All scenarios are represented by specific field sites for which monitoring data were available. Table 8 shows the inherent agro-environmental characteristics of the scenarios.

Table 8: Inherent agro-environmental characteristics of the surface water scenarios (from FOCUS surface water report (2001) Table 3.2–6)

Scenari [(a)]	Meteo-rological station	Mean spring and autumn temperature (°C)	Mean annual rainfall (mm)	Mean annual recharge (mm)	Slope (%)	Soil
D1	Lanna	< 6.6	600–800	100–200	0–0.5	Clay with shallow groundwater
D2	Brimstone	6.6–10	600–800	200–300	0.5–2	Clay over impermeable substrate
D3	Vredepeel	6.6–10	600–800	200–300	0–0.5	Sand with shallow groundwater
D4	Skousbo	6.6–10	600–800	100–200	0.5–2	Light loam over slowly permeable substrate
D5	La Jailliere	10–12.5	600–800	100–200	2–4	Medium loam with shallow groundwater
D6	Váyia, Thiva	> 12.5	600–800	200–300	0–0.5	Heavy loam with shallow groundwater
R1	Weiherbach	6.6–10	600–800	100–200	2–4	Light silt with small organic matter
R2	Valadares, Porto	10–12.5	> 1 000	> 300	10–15	Organic-rich light loam
R3	Ozzano, Bologna	10–12.5	800–1 000	> 300	4–10	Heavy loam with small organic matter
R4	Roujan	> 12.5	600–800	100–200	4–10	Medium loam with small organic matter

(a): D, Drainage, R, Runoff scenario.

Inputs to surface water bodies from spray drift are incorporated as an integral part of all of the scenarios based on the same tables as for the previous tiers (BBA, 2000). In addition to spray drift the scenarios are characterised by either runoff/erosions (R) or drainage (D) entries.

For each location a maximum of two water body types is defined as shown in Table 9.

Table 9: Water bodies associated with scenarios (from FOCUS, 2001)

Scenario	Inputs	Water body type(s) [(a)]
D1	Drainage and drift	Ditch, stream
D2	Drainage and drift	Ditch, stream
D3	Drainage and drift	Ditch
D4	Drainage and drift	Pond, stream
D5	Drainage and drift	Pond, stream
D6	Drainage and drift	Ditch
R1	Run-off and drift	Pond, stream
R2	Run-off and drift	Stream
R3	Run-off and drift	Stream
R4	Run-off and drift	Stream

(a): All ditches and streams are assumed to have a length of 100 m, a width of 1 m and a variable, but minimum depth of 30 cm whereas the ponds are defined by surface water areas of 30 m × 30 m together with a depth of 100 cm.

For calculating substance entries into the surface water and the time-dependent concentration in the surface water bodies, different computer models are used. The currently recommended models (FOCUS, 2001) are MACRO for estimating the contribution of drainage, PRZM for the estimation of the contribution of runoff (added to the water phase) and erosion (added to the sediment phase), TOXSWA for the estimation of the final PECs in surface waters and Surface WAter Scenario Help (SWASH) for the estimation of spray drift entries and as the overall user shell.

To facilitate the calculation of exposure concentrations at step 3 level, SWASH is used. It is an overall shell (user interface) combining all models involved in step 3 calculations. The main functions of the shell are:

- maintenance of a central PPP properties database,
- provision of an overview of all step 3 FOCUS runs required for use of a specific PPP on a specific crop,
- calculation of spray drift deposition onto various receiving water bodies and
- preparation of input for the models MACRO (drainage entries), PRZM (runoff/erosion entries) and TOXSWA (fate in surface water).

When working with MACRO and PRZM the user cannot enter application dates directly. Instead this is done by a similar pesticide application timer (PAT) which uses an application window as input. PAT then attempts to select appropriate application dates.

MACRO, as well as PRZM, calculate the fraction of the dose being intercepted by the crop canopy. In both models the user can select the application methods of ground spray, air-blast, granular, incorporated and aerial. Interception is assumed zero for both granular and incorporated applications.

The TOXSWA model describes the behaviour of PPPs in a water body at the edge-of-field scale, i.e. a ditch, pond or stream adjacent to a single field. It calculates PPP concentrations in both the water and the sediment layers. In the water layer, the PPP concentration varies in the horizontal direction (varying in sequential compartments), but is assumed to be uniform throughout the depth and width of each compartment. In the sediment layer, the PPP concentration is a function of both horizontal and vertical directions.

TOXSWA considers four processes: (i) Transport, (ii) Transformation, (iii) Sorption and (iv) Volatilisation. In the water layer, PPPs are transported by advection and dispersion, while in the sediment, diffusion is included as well. The transformation rate covers the combined effects of hydrolysis, photolysis (in cases where this is accounted for in the experimental setup used to derive this parameter value) and biodegradation and it is a function of temperature. Sorption to suspended solids and to sediment is described by the Freundlich equation. Sorption to macrophytes is described by a linear sorption isotherm but this feature is not used in the TOXSWA model. PPPs are transported across the water–sediment interface by advection (upwards or downwards seepage) and by diffusion. In the FOCUS surface water scenarios, transport across the water–sediment interface takes place by diffusion only.

The water level in the water body varies in time, but it is assumed to be constant over the length of the water body. However, as the model cannot handle low water levels close to zero, a minimum water depth of 30 cm was defined for every stream and ditch scenario.

Similar as at steps 1 and 2, sediment concentrations are provided in the standard output files over a depth of 5 cm. However, the model internally uses several thin sediment layers with following dimensions:

- 0 mm to 1 mm
- 1 mm to 2 mm
- 2 mm to 3 mm
- 3 mm to 4 mm
- 4 mm to 6 mm
- 6 mm to 8 mm
- 8 mm to 10 mm
- 10 mm to 15 mm
- 15 mm to 20 mm
- 20 mm to 30 mm
- 30 mm to 40 mm
- 40 mm to 50 mm
- 50 mm to 70 mm
- 70 mm to 100 mm

However, in order to use the concentrations in these internal layers additional graphical output has to be requested before the start of the TOXSWA simulation and additional special post-processing tools have to be developed. This additional graphical output also contains pore water concentrations, so there is no need to use equation 7.3 at step 3.

Compared to simulations at steps 1 and 2, the dynamics of concentrations in water and sediment is different, since the model is using several sediment compartments and the distribution to sediment is simulated more realistically based on a kinetic approach that accounts for concentration gradients in neighbouring phases (see Figure 10 for example results).

Even though TOXSWA considers suspended sediment particles in the water phase, complexation based on dissolved OC is not taken into consideration. However, that can be considered conservative for the sediment layer as it may increase concentrations in the water phase.

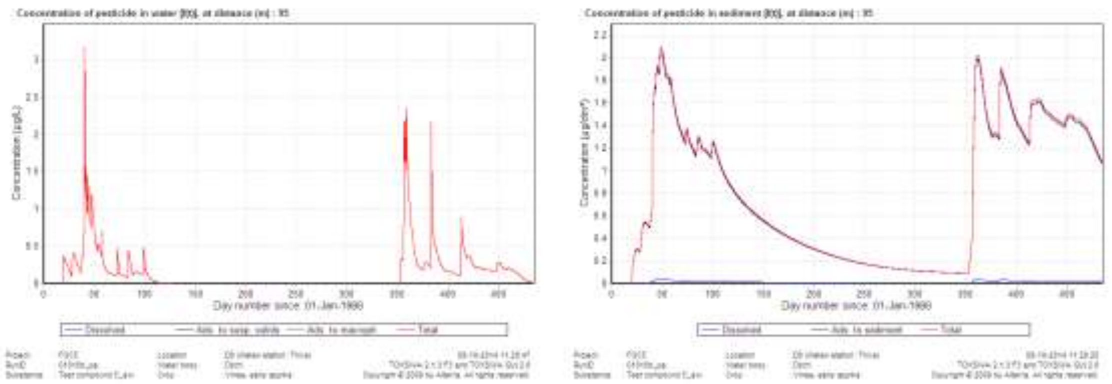


Figure 10: FOCUS step 3 D6-ditch simulation for FOCUS Dummy 5 (K_{oc}: 860 L/kg). The figure was prepared by the PPR WG.

7.2.4. Step 4

Step 4 simulations are usually performed according to the results of the FOCUS group on Landscape and Mitigation Measures in Ecological Risk Assessment (FOCUS, 2007a, b).

Similar to the other steps, also for Step 4 a software tool (Surface Water Assessment eNabler (SWAN)) is recommended by FOCUS, which is available and developed on behalf of European Crop Protection Association. Further interpretation of the mitigation of runoff in the FOCUS surface water scenarios is described by FOCUS in its Landscape and Mitigation report (FOCUS, 2007a).The software modifies the input and output files of the step 3 models TOXSWA and PRZM to consider drift and runoff buffer zones. The standard exposure reduction factors for runoff (water volume and PPP mass in runoff water) and erosion (eroded soil and PPP mass sorbed to eroded soil) are given by FOCUS (2007a, b).

SWAN can also handle drift reduction due to the use of more advanced nozzle techniques (low drift nozzles). In addition to the entry routes considered in the first three steps the exposure via air for volatile substances, using the recommendations of the FOCUS air group (FOCUS, 2008), can be considered.

The effect of drift buffer zones (i.e. no-spray buffer zones) can be considered in SWAN for distances up to 100 m from the surface water body. The model considers the same reduction rates as in the FOCUS SWASH tool and both are based on BBA (2000). It should be noted that whilst SWAN can be used to parameterise drift buffer zones up to 100 m and the effects of low drift nozzles can be combined with drift buffer zones to reduce spray drift inputs still further, FOCUS (2007a) prescribes a ceiling on spray drift mitigation. This prescription is that spray drift cannot be mitigated such that the mass per unit area reaching the water body surface is < 5 % of the mass, calculated using the FOCUS defined baseline distance for that crop (1–6 m), i.e. the ceiling for spray drift mitigation is 95 %.

As at step 3, when a use pattern includes multiple applications, it can also be necessary to simulate a single application as well as multiple applications at step 4, to ensure that appropriate peak concentrations are generated and available for use in the risk assessment. The need for this procedure is reduced when the extent of implemented spray drift mitigation increases.

7.3. Considering accumulation in sediment

The current FOCUS methodology for surface water does not consider the effect of multi-year applications, which could possibly lead to accumulation of pesticides in sediment. Reason for this deficit is that the original intention of FOCUS was to develop realistic worst-case scenarios for the water compartment where accumulation was not expected to be important. As no water body was considered being static and without outflow, significant accumulation in the water phase would not occur even if multiple applications of compounds that are persistent in sediment were simulated. However, in sediments the situation can be completely different, especially for strongly adsorbing pesticides where the calculation of an additional $PEC_{sed,\ max\text{-}accu}$ may be necessary. As FOCUS does not provide such a value in any of the present FOCUS tiers a pragmatic solution would be the use of a simple accumulation factor that has to be multiplied with the maximum sediment concentration and then added to the standard FOCUS concentrations. The procedure follows the methodology described in EFSA PPR Panel (2012) for soil concentrations. It can be considered a worst-case approach as apart from degradation it does not consider any other loss processes (e.g. leaching, volatilisation). The calculation of the accumulation factor is described in equation 7.4.

$$PEC_{sed,accu} = PEC_{sed,FOCUS} + PEC_{sed,\max,FOCUS}\frac{X}{1-X} \quad (7.4)\ (a)$$

$$X = \exp\left(-\frac{365\ln(2)\ f}{DegT50}\right) \qquad (7.4)\ (b)$$

$$f = \exp\left(\frac{-E}{R}\left[\frac{1}{T_{arr,scen}+273.15}-\frac{1}{T_0+273.15}\right]\right) \qquad (7.4)\ (c)$$

$PEC_{sed,accu}$:	predicted sediment concentration including accumulation (µg/kg)
$PEC_{sed,FOCUS}$:	predicted concentration in sediment according to FOCUS (µg/kg)
$PEC_{sed,max,FOCUS}$:	maximum concentration in sediment according to FOCUS (µg/kg)
f:	temperature correction factor (–)
DegT50 water/sediment system:	degradation in water/sediment at reference temperature (days)
$T_{arr,scen}$:	Arrhenius-weighted average concentration of the scenario (°C)
T0:	Reference temperature during the degradation study (20 °C)
E:	Arrhenius activation energy, (kJ/mol)
R:	Gas constant (kJ/mol/K)

In the equation above, $PEC_{sed,FOCUS}$ represents the standard environmental concentrations as provided by FOCUS at the different steps. It could alternatively be a maximum concentration or an actual concentration sometime after the maximum or a TWA. Information about which concentrations shall be used for the risk assessment can be found in the Chapter 9 of this scientific opinion. The DegT50 in water/sediment is a key parameter when calculating the accumulation factor. It is current practice to use a default of 1 000 days if no experimental values can be obtained for a certain compound. However, for some substances (e.g. bixafen) it was shown that half-lives longer than 1 000 days may occur.

As the FOCUS scenarios are characterised by different temperatures, special correction factors can be obtained for each scenario (Table 10). These factors have been calculated with the default Arrhenius Activation Energy (EFSA PPR Panel, 2008). For illustration purposes only, the table presents some examples of the resulting accumulation factors X/(1–X) dependent on the FOCUS scenario and different DegT50 under normalised conditions. Finally, FOCUS simulations were performed based on three imaginary active substances with realistic properties (an insecticide, a herbicide and a fungicide). The active substances used for the calculations were designed to cover the most relevant range of input parameters. The calculations demonstrate the effect of the proposed new ecotoxicologically relevant sediment depth and the new accumulation factor (see Appendix C).

Table 10: Temperature correction factor f and accumulation factors X/(1–X) for some combinations of FOCUS scenario and DegT50 under normalised conditions (20 °C)

Scenario	Meteorological station	correction factor *f*	DegT50: 10 days	DegT50: 100 days	DegT50: 300 days	DegT50: 1 000 days
D1	Lanna	0.366	0.000	0.656	2.766	10.307
D2	Brimstone	0.424	0.000	0.520	2.326	8.831
D3	Vredepeel	0.483	0.000	0.418	1.989	7.694

D4	Skousbo	0.400	0.000	0.571	2.492	9.390
D5	La Jailliere	0.526	0.000	0.359	1.791	7.026
D6	Thiva	0.841	0.000	0.135	0.969	4.218
R1	Weiherbach	0.483	0.000	0.418	1.989	7.694
R2	Porto	0.660	0.000	0.232	1.343	5.503
R3	Bologna	0.679	0.000	0.219	1.294	5.336
R4	Roujan	0.662	0.000	0.231	1.337	5.485

7.3.1. A preliminary comparison of maximum concentrations in surface water and pore water

In order to explore whether an acute risk assessment for benthic organisms would be covered by the acute risk assessment as described in the AGD (EFSA PPR Panel, 2013), maximum concentrations in the water compartment and the maximum concentration in pore water were compared using a few examples. The Panel is aware that further investigations on this topic have to be performed since multi-year and multiple applications within one year were not included in this exercise.

These FOCUS step 3 simulations were performed with two compounds which are characterised by extremely different behaviour (K_{foc} = 0, DegT50 in water: 1 000 days, DegT50 in sediment 1 000 days and K_{foc} = 1 024 000 L/kg, DegT50 in water: 0.76 days, DegT50 in sediment). In order to stimulate high pore water concentrations the compounds were incorporated into the soil (not sprayed).

Figures 11 and 12 present results for the R1 pond and R1 stream scenario. However, similar results can be obtained also for the other FOCUS scenarios.

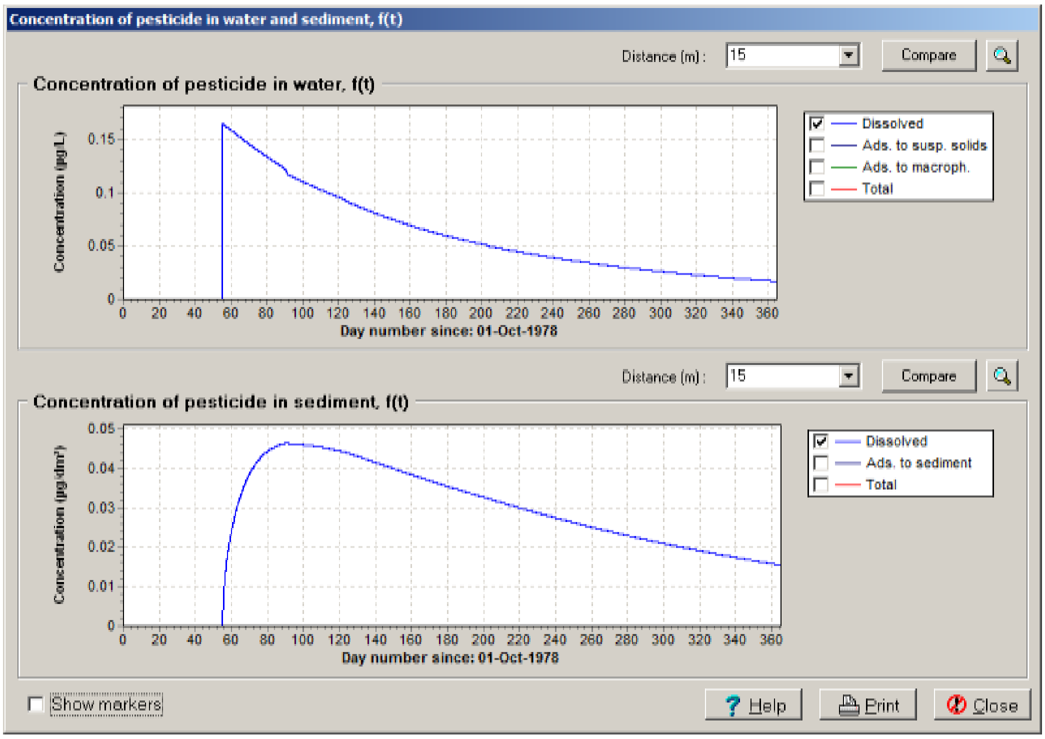


Figure 11: FOCUS step 3 R-pond scenario: Concentration in free water (top) and pore water over 5 cm (bottom) for a compound with K_{foc} = 0 (screenshot, sediment concentration in dm^3 of bulk sediment). The figure was prepared by the PPR WG.

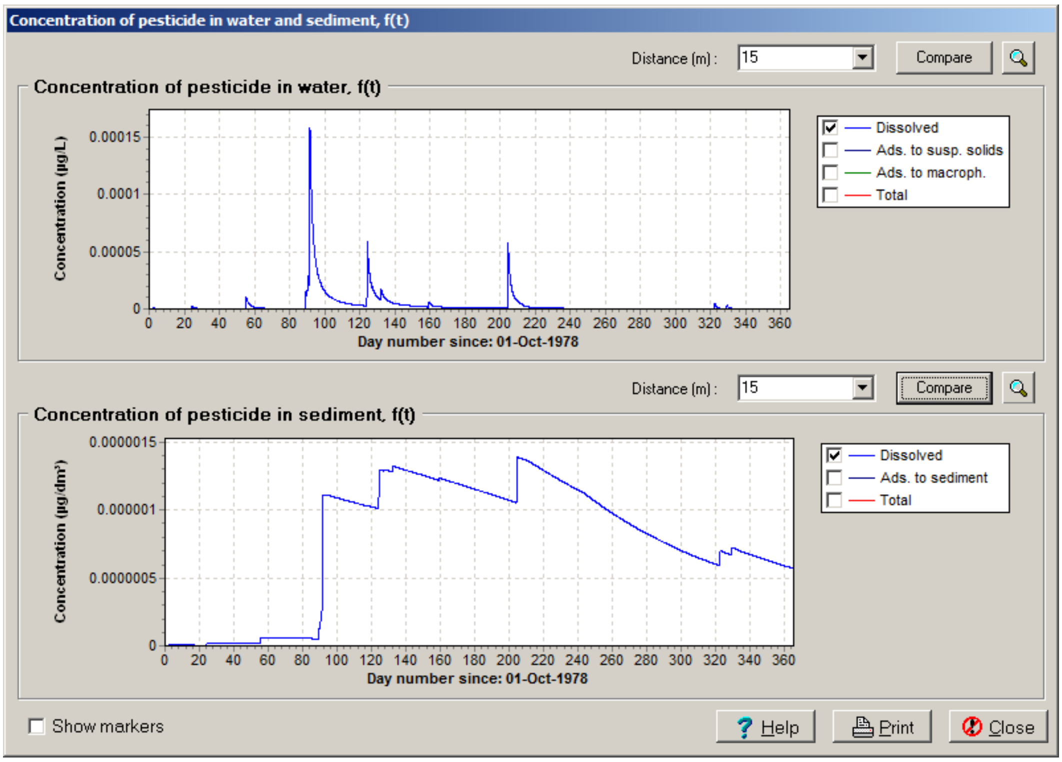


Figure 12: FOCUS step 3 R-stream scenario: Concentration in free water (top) and pore water over 5 cm (bottom) for a compound with K_{foc} = 1 024 000 L/kg (screenshot, sediment concentration in dm^3 of bulk sediment). The figure was prepared by the PPR WG.

In these examples, independent of the scenario, the K_{oc} and the water body (stream, pond), the peak concentrations in the water phase were higher than the corresponding peak concentrations in sediment. This was found to be true also for ditch scenarios (not presented in figures).

Further investigation is recommended to analyse the effect of accumulation and multiple applications.

7.4. How worst case are the current FOCUS scenarios for sediment?

As the intention of FOCUS clearly was the development of worst-case scenarios for surface water it can even be concluded that—assuming they really represent worst-case surface water scenarios—they cannot represent worst-case conditions for sediment at the same time. However, EFSA PPR Panel (2013) assumes that the FOCUS surface water methodology will continue to be used until updated or new methods become available. Therefore, to account for this deficit it is recommended to use the accumulation factor presented in this opinion until new developments are available. The accumulation factor can be considered a conservative approach since it does not include any possible transport processes, such as leaching, resuspension or volatilisation, which may reduce the accumulation in sediment in the real field situation.

However, the obvious disadvantage of the FOCUS scenarios—that they represent worst-case situations for the free water—may turn into an advantage in sediment assessments if concentrations in pore water are used for the risk assessment. At steps 1 and 2 these concentrations can be obtained based on equation 7.3. For steps 3 and 4 pore water concentrations can be directly taken from the existing model output.

Overall, the Panel recommends to develop two types of sediment exposure scenarios, one with low OC (worst-case pore water scenario) and one with high OC (worst-case total content scenario).

8. Effects assessment

8.1. Bioaccumulation, biomagnification and secondary poisoning

Bioaccumulation of contaminants from different sources (water, diet, sediment) may lead to the building up of internal exposure concentrations in tissues or specific organs that may exceed critical levels and result in direct toxic effects. Bioaccumulation may proceed over long periods even when external concentrations are low. In addition, feeding and predation on contaminated preys may lead to food web transfer and—for highly bioaccumulative compounds—to biomagnification and direct and indirect effects at higher trophic levels. Knowledge of bioaccumulation and food web transfer of PPPs in aquatic invertebrates, fish and predators is important for a proper understanding of potential direct and indirect effects. Understanding bioaccumulation has become one of the critical considerations in the evaluation of the safety of new chemicals in many national or international regulations. In the PPP regulation (EC, 2009) and the old and revised AGDs of the PPR Panel (EFSA PPR Panel, 2013) this is also included.

The history of the usage and later in time the bans and restrictions of DDT is one of the best documented cases worldwide of bioaccumulation in aquatic and terrestrial organisms, food web transfer, biomagnification in top predators and effects on the reproduction of birds of prey (Hunt and Bisschoff, 1960; Carson, 1962; Peakall and Kiff, 1988). This and similar observations for other persistent chlorinated pesticides and polyaromatic compound classes (e.g. dioxins, PCBs) have led to international legislation, such as the Stockholm Convention on Persistent Organic Pollutants (UNEP, 2001). The evaluation of new chemicals with respect to their persistency (P), bioaccumulative potential (B) and toxic potency (T), the so called PBT profile, is an important step in risk assessment under the different regulatory frameworks in Europe. Compounds can be classified as PBT or vPvB (very persistent and bioaccumulative) depending on the exceedance of specific cutoff criteria (Table 11). Examples are REACH (EC, 2006), BPR (EC, 2012) and former BPD, and the PPP regulations (EC, 2009). In other OECD countries similar programmes are in place.

The definitive assessment of the bioaccumulation potential is usually based on the Bioconcentration Factor (BCF) in an aquatic species. For initial screening purposes the log K_{ow} is used as indicator in various regulations. For a substance to be considered as bioaccumulative, the trigger value for the BCF is set at > 2 000 L/kg in all European regulations, for the B-assessment (see Rauert et al., 2014). Cutoff criteria for bioaccumulative potential under different regulatory regimes are summarised in Table 11.

In Table 12 the most common metrics and their definitions used in the assessment of the aquatic bioaccumulation and biomagnification potential in Europe (REACH, BPR and former BPD), North America (US EPA, Environment Canada) and OECD countries are presented. In terrestrial studies and other regulatory schemes sometimes slightly different definitions may be in use.

Bioaccumulation is the net result of exposure of the organism to a contaminant from various sources over time. This represents the balance between the fluxes into the organism and the losses through protective processes such as biotransformation (ability to metabolise the compound) and elimination (Landrum et al., 1995), as well as reproduction losses and dilution due to growth. It is quantified with the so called BCF, describing the aqueous uptake pathway and the Bioaccumulation Factor (BAF), describing both aqueous and dietary uptake (further explained in Table 12). Factors that can have an effect on the bioavailability and bioaccumulation can be categorised into physical, chemical and biological. The physical and chemical factors include the properties of the contaminants (i.e. hydrophobicity, K_{ow}) and the characteristics of the surrounding environment (water, pore water and sediment characteristics, temperature, redox, ageing). Biological factors include feeding behaviour, dietary assimilation efficiency, ventilation, growth, reproduction and biotransformation. For rooted macrophytes, partitioning to roots and shoots, translocation between roots and shoots and growth dilution is important (Diepens et al., 2014b). For benthic invertebrates and fish living in or close to the sediment, ingestion of sediment constitutes a potential additional pathway for uptake of contaminants in addition to uptake from (pore) water and direct dietary sources. This is especially relevant for compounds with a high sorption and dietary assimilation efficiency, such as very hydrophobic compounds (log K_{ow} > 5; Carbonell et al., 2000). For such compounds usually additional bioaccumulation tests are recommended with sediment-dwelling or -inhabiting invertebrates exposed to spiked sediments (e.g. following OECD Guideline 315; OECD, 2008).

Biomagnification in aquatic invertebrates and fish depends on many factors, such as, for example, food web relationships, dietary preferences and toxicokinetics at different trophic levels and compound hydrophobicity (K_{ow}) (Gobas and Morrison, 2000). The octanol–air partitioning coefficient (K_{oa}) is also relevant for air breathing top predators, such as piscivorous mammals and birds (Kelly et al., 2007).

Table 11: Cutoff criteria for the assessment of bioaccumulative potential of compounds

Toxic Substances Control Act—New Chemicals Program PBT Policy (TSCA, 1999)	May be bioaccumulative Bioaccumulative	Fish BCF or BAF ≥ 1 000 L/kg, or log K_{ow} > 4.2 Fish BCF or BAF ≥ 5 000 L/kg, or log K_{ow} > 5.0
Canadian Environmental Protection Act (CEPA, 1999)	Bioaccumulative	BAF or BCF ≥ 5 000 L/kg, or log K_{ow} ≥ 5.0
UNEP Stockholm Convention (2001)	Bioaccumulative	BCF or BAF > 5 000 L/kg, or log K_{ow} > 5.0
REACH (EC, 2006), BPR (EC, 2012), PPP Regulation (EC, 2009)	Bioaccumulative (B) Very bioaccumulative (vB)	BCF > 2 000 L/kg BCF > 5 000 L/kg
EFSA PPR Panel (2013)—AGD	Trigger for requirement of fish bioaccumulation test (OECD Guideline 305-I- Aqueous bioconcentration fish test)	Not rapidly degraded in water (< 90 % loss in 24 hours) and log K_{ow} > 3 or other indications of bioconcen-tration (for instance monitoring data in biota or structural alerts)
	Trigger for requirement of dietary fish bioaccumulation test (OECD Guideline 305-III Dietary exposure bioaccumulation fish test)	Not rapidly degraded in water (< 90 % loss in 24 hours) and log K_{ow} > 6

Modelling of bioaccumulation and food web transfer has evolved during the last decades and several models are capable of providing order of magnitude predictions of environmental concentrations, biomagnification and transfer in food webs (MacKay and Fraser, 2000; Gobas and Morrison, 2000; Carbonell et al., 2000; Brooke and Crookes, 2007; Imhoff et al., 2004, Sormunen et al., 2008b). An overview (not comprehensive) of existing models is provided by De Voogt and van Hattum (2003) and Sormunen et al. (2008b). Available bioaccumulation models range from those being highly complex and data hungry (e.g. Aquatox v2.1) to relatively simple models for which only a limited number of compound properties and biological data need to be provided (e.g. Foodweb v2.0, OMEGA and GEMCO). Arnot and Gobas (2003, 2004, 2006) reported a generic relationship to predict BAFs as a function of K_{ow}, biological parameters (bodyweight, lipid content, trophic position) and possible biotransformation. Although these models have been validated to a limited extent and still exhibit a large variation between predicted and measured BCFs and BAFs, their simplicity and limited data requirements may favour their application in situations where only order of magnitude predictions or relative ranking is required for initial or first tier assessments. An updated version of the BAF–BCF model is currently included in the most recent versions of Episuite (US EPA, 2014a), which provides BCFs and BAFs for fish of different size corrected for biotransformation.

Table 12: Common metrics and definitions used for assessment of bioaccumulation and biomagnification potential (EU Reach, US EPA, OECD)

Bioconcentration factor	BCF, L/kg wet weight (ww)	Ratio of the steady state chemical concentrations in an aquatic water-respiring organism (C_B, g chemical/kg ww) and the water (C_W, g chemical/L) determined in a controlled laboratory experiment in which the test organisms are exposed to a chemical in the water (but not in the diet) From kinetic studies, e.g. OECD Guideline 305, the steady state BCF can also be derived from the ratio of the uptake rate constant (k_1) of the elimination rate constant (k_2)	$BCF = C_B/C_W$ $BCF = k_1/k_2$
Bioaccumulation factor [(a)]	BAF, L/kg ww	Ratio of the steady state chemical concentrations in an aquatic water-respiring organism (C_B, g chemical/kg ww) and the water (C_W, g chemical/L) determined from laboratory or field data in which sampled organisms are exposed to a chemical in the water and in their diet	$BAF = C_B/C_W$
Biomagnification factor [(b)]— laboratory based	BMF, kg ww/ kg ww	Ratio of the steady state chemical concentrations in a water- or air-respiring organism (C_B, g chemical/kg ww) and in the diet of the organism (C_D, g chemical/kg dry) determined in a controlled laboratory experiment in which the test organisms are exposed to chemical in the diet (but not the water or air)	BMF = CB/CD
Biomagnification factor—field based	BMF, kg ww/ kg ww	Ratio of the steady state chemical concentrations in a water- or air-respiring organism (C_B, g chemical/kg ww) and in the diet of the organism (C_D, g chemical/kg ww) determined from field data in which sampled organisms are exposed to chemical in air, water and diet	$BMF = C_B/C_D$
Trophic magnification factor or food web magnification factor	TMF or FWMF, unit less	The average factor by which the normalised chemical concentration in biota of a food web increases per trophic level. The TMF is determined from the slope (m) derived by linear regression of logarithmically transformed normalised chemical concentration in biota and trophic position of the sampled biota	$TMF = 10^m$
Biota-sediment accumulation factor—field based	BSAF kg dry/kg dry or kg OC/kg lipid	Ratio of steady state concentration in a specific organism (C_B g chemical/kg dry weight or lipid) and in the sediment (C_S g chemical/kg dry weight or OC), derived from field data	$BSAF = C_B/C_S$
Octanol–water partition coefficient	K_{ow}, unitless	Ratio of the chemical concentrations in 1-octanol (CO) and water (CW) in an octanol–water system that has reached a chemical equilibrium	$K_{ow} = C_O/C_W$
Octanol–air partition coefficient	K_{oa}, unitless	Ratio of the chemical concentrations in 1-octanol (C_O) and air (C_A) in an octanol–air system that has reached a chemical equilibrium	$K_{oa} = C_O/C_A$

(a): Also referred to as field BCF.
(b): Referred to as bioaccumulation factor in some terrestrial studies.
Source: adapted from Spacie and Hamelink (1985), TGD (2003), Gobas et al. (2009) and ECHA (2012).

8.1.1. Current requirements for PPPs in relation to bioconcentration and bioaccumulation

In Table 13 the current data requirements for PPPs in relation to bioconcentration and bioaccumulation are summarised. The data requirements (Commission Regulation (EU) 283/2013 and 284/2013) prescribe consideration of the risk of bioconcentration and results of a fish bioconcentration test (OECD Guideline 305), for compounds that are not rapidly degraded in water (< 90 % loss in 24 hours) and with a log K_{ow} > 3 or when other indications for bioconcentration potential are present. For strongly hydrophobic substances (log K_{ow} > 6), a dietary test is recommended (OECD, 2012) since testing via aqueous exposure may become increasingly difficult and the exposure via the food for those substances becomes the predominant route of exposure (OECD Guideline 305 part 2). A dietary fish test has been developed to determine uptake by ingestion, yielding a BMF (OECD, 2012). Note that there may be some inconsistencies in the data requirements (see note 2 in Table 13).

Table 13: Regulations and data requirements for PPPs regarding bioconcentration and bioaccumulation

Regulation	Data requirements
Commission regulation (EU) No 546/2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of **PPPs**	Section C 2.5.2.2 ‘Where there is a possibility of aquatic organisms being exposed, no authorization shall be granted if ….. the maximum bioconcentration factor (BCF) is **greater than 1000** for plant protection products containing active substances which are readily biodegradable or **greater than 100** for those which are not readily biodegradable… unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on the viability of exposed species (predators) occurs — directly or indirectly — ….’
Commission Regulation (EU) No 283/2013[15] of 1 March setting out the data requirements for **active substances** (in force since January 2014 and replacing com. regulation 544/2011[16])	Section 8.1.3. Active substance bioconcentration in prey of birds and mammals ‘For active substances with a log Pow [(a)] > 3, an assessment of the risk posed by bioconcentration of the substance in the prey of birds and mammals shall be provided.’ 8.2.2.3. Bioconcentration in fish ‘The bioconcentration of the substance, shall be assessed where: — the log Pow is greater than 3 (see point 2.7) or there are other indications of bioconcentration, and — the substance is considered stable, that is to say there is less than 90 % loss of the original substance over 24 hours via hydrolysis [(b)] (see point 7.2.1.1).’
Commission Regulation (EU) No 284/2013 of 1 March setting out the data requirements for **PPPs** (in force since January 2014 and replacing com. regulation 546/2011)	Refers to Part A of the Annex to Regulation (EU) No 283/2013

(a): Pow old symbol for n-octanol water partitioning coefficient Kow.
(b): Inconsistencies: degradation is only evaluated through hydrolysis; biodegradation is not included whereas it was included in the (EU) regulation 546/2011 (readily biodegradable).

[15] Commission Regulation (EU) No 283/2013 of 1 March setting out the data requirements for active substances, in accordance with the Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.
[16] Commission Regulation (EU) No 544/2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products.

- In the AGD (EFSA PPR Panel, 2013), the evaluation of risks for secondary poisoning is proposed for a fish-eating bird or mammal. It follows the methodology of the Technical Guidance Document (TGD, 2003) and is based on theoretical BMFs, derived from fish BCFs. (e.g. for BCF < 2 000 or log K_{ow} < 3: BMF = 1, with a BMF > 1 being a supportive indication of high bioaccumulation. The RAC_{sp} (regulatory acceptable concentration for secondary poisoning) is calculated from the No Observed Adverse Effect Level (bird or mammal), BCF_{fish}, BMF, a factor for the consumption rate and an AF. When the RAC_{sp} is below the 21-day TWA PEC_{sw} (PEC for surface water) then a higher-tier assessment including modelling is prescribed (section 7.6.3 of EFSA PPR Panel, 2013). Overall, for birds and mammals eating fish, the risk is usually addressed through secondary poisoning and considers only BCF in fish). This possibly underestimates the risk and should be improved.
- In the AGD (EFSA PPR Panel, 2013), the evaluation of risks for secondary poisoning is also recommended for fish-eating fish (e.g. pike (*Esox lucius*)) but no recommendation is provided; it only refers to the former guidance document on aquatic ecotoxicology (EC, 2002, section 5.7.4). There is a need for food web modelling developed for predatory and prey fish that considers not only BCF but also BAF (accumulation through water and diet). This should be developed.

8.1.2. Current approach in REACH

For chemicals registered under REACH, data requirements depend on the quantities which are manufactured or imported per year. While for substances below 10 tonnes/year, only a basic data set is mandatory, at a tonnage of ≥ 100 tonnes/year, a bioaccumulation study in an aquatic organism, preferably on fish, should be considered. The bioaccumulation potential needs to be considered in relation to long-term effects and environment hazard classification. For the majority of non-ionised organic substances, classification may be based initially on the log K_{ow} if no reliable measured fish BCF is available. For the 'B' part of the PBT/vPvB assessment, such screening information is considered and can be supplemented by other data and information (e.g. non-standardised tests, literature, Quantitative Structure–Activity Relationships (QSARs), read-across from structurally related substances or grouping approaches) (see Rauert et al., 2014). In the chemical safety assessment of substances under REACH, the fish BCF and BMF values are used for the secondary poisoning assessment for wildlife, as well as for human dietary exposure. An invertebrate BCF can be used to model a food chain based on consumption of sediment worms or shellfish. A predicted BCF may be used for first tier risk assessment. If the PEC/PNEC ratio based on worst-case BCF or default BCF indicates potential risks at any trophic level, the BCF/BMF can be refined if needed. A Weight of Evidence (WoE) procedure can be used for expert judgement on the available data and to decide on the need for additional testing.

8.1.3. Need for refinement of bioaccumulation assessment

In a critical paper by Gobas et al. (2009) based on the outcome of a SETAC Pellston Workshop ('Science-based guidance and framework for the evaluation and identification of PBTs and POPs', January 2008, Florida, USA) further improvements for assessment criteria for bioaccumulative substances (including biomagnification) were proposed. The commonly used fish BCF was considered a less adequate indicator for bioaccumulative potential and biomagnification in fish, especially for poorly soluble substances and compounds for which no equilibrium conditions are reached within the normal duration of the test period. The authors proposed that additional determination of laboratory- or field-derived BMFs should be mandatory for registration of PBTs and persistent organic pollutants, as well as the inclusion of the TMF for the assessment of biomagnification.

8.1.4. Recommended approach for bioaccumulation testing with benthic organisms

In this section, recommendations are given for the assessment of the potential for bioaccumulation, biomagnification and secondary poisoning of sediment-bound contaminants. Currently only limited data are available on benthic invertebrate bioaccumulation studies with PPPs in existing dossiers and literature.

Bioaccumulation is of particularly high relevance for benthic organisms since they may take up environmental contaminants via different uptake routes (e.g. overlying and interstitial waters, suspended or sedimented particles). Since they are exposed to all these uptake routes, they have a great potential in terms of accumulating toxic substances and in transferring them to higher trophic levels. Furthermore, the sediment compartment is a sink for substances being persistent and/or with high BCF, and bioaccumulation processes are often slow. Thus, benthic organisms may be exposed not only acutely but also chronically, which is likely to lead to significant uptake.

In terms of food chain transfer, studying bioaccumulation in different invertebrates is highly relevant since an invertebrate BAF can be used to model a food chain based on, for example, a fish consuming a sediment worms.

Among benthic invertebrates, oligochaetes are a relevant group of species to study bioaccumulation via different uptake routes. OECD Guideline 315 is a test on bioaccumulation in sediment-dwelling benthic oligochaetes (OECD, 2008). It consists of two phases, usually a 28-day uptake phase and an elimination phase of a maximum duration of 10 days. The uptake rate constant (k_s), the elimination rate constant (k_e) and the kinetic bioaccumulation factor ($BAF_K = k_s/k_e$) are calculated. Besides, the worm lipid content, the sediment total OC content and the residue level in worms at the end of the elimination phase are useful for the interpretation of the results.

Hyalella azteca could be also used to study bioaccumulation, although its relevance for sediment toxicity testing has been questioned (Wang et al., 2004; Borgmann et al., 2005) and no standardised test method (e.g. OECD) is available. The species has been used in various experimental bioaccumulation studies, which confirms its potential as test species for bioaccumulation.

In bioaccumulation tests with benthic invertebrates, consideration should be given to the lipid content of the test organisms under consideration. The usually assumed lipid content of 5 % of the body weight for fish is not applicable for invertebrate species which often have a much lower lipid content (Rauert et al., 2014). The PPR Panel acknowledges that there is much discussion about normalisation to lipid content in experimental and modelling studies and recommends that this should be considered in further detail in the future opinion on effect models. The formation of possible relevant metabolites during bioaccumulation tests should also be considered.

8.1.5. Triggers for spiked sediment bioaccumulation tests with benthic invertebrates

In the current regulation (EU, No 283/2013), there is no trigger for bioaccumulation testing with benthic invertebrates. A preliminary proposal is presented below. In the data requirements for aquatic ERA, fish bioaccumulation tests need to be performed for substances with log Kow >3. This information is used as a starting point and is based on a BCF that accounts only for aqueous uptake. However, the recommended descriptors for bioaccumulation in benthic invertebrates are BAF and BSAF, since these include all uptake pathways. Therefore, considerable uncertainties may be involved in the extrapolation from information on BCF in fish to BAF/BSAF in invertebrates. These uncertainties are related to gaps in knowledge around this approach (e.g. related to bioavailability, biotransformation) and need to be addressed further in the development of guidance for sediment ERA.

For substances that show significant bioaccumulation in fish tests (BCF > 2000 L/kg wet weight normalised to 5 % lipid content as in OECD 305 (OECD, 2012)), additional bioaccumulation testing with benthic invertebrates exposed to spiked sediments may be required depending on the combination of triggers for persistence and sorption. Indeed in some cases, a risk for chronic exposure to significant quantities of the compound in sediment cannot be excluded. More specifically, the following triggers are proposed:

- Persistence in sediment: half-life degradation (> or < 120 d in the water- sediment fate study) based on the criterion for persistence in freshwater sediments and used in different European substance regulations (PPPs, REACH, BPR) (Rauert et al., 2014),
- Sediment partitioning: 10 % or more of the substance distributed into the sediment in the water- sediment fate study or with FOCUS calculation (or other appropriate model).
- Lipophilicity: K_{ow}, i.e. log K_{ow} > 3 as this is similar as in the current regulation for aquatic organisms (EU Regulation No 283/2013).

It is recommended to perform spiked sediment bioaccumulation tests with benthic invertebrates for substances that show significant bioaccumulation in fish tests (BCF > 2 000 L/kg), when the substance is persistent in sediment (half-life > 120 days in the water–sediment fate study) and log K_{ow} > 3.

For other substances (i.e. half-life < 120 days in the water–sediment fate study), log K_{ow} > 3 and sediment partitioning equivalent to 10 % or more of the substance distributed into the sediment in the water–sediment fate study or with FOCUS calculation (or other appropriate model).

We also recommend requiring spiked sediment bioaccumulation tests with benthic invertebrates when the triggers are not exceeded but a concern is raised based on, for example, read across information from other substances or other ‘expert judgement’.

Further guidance on how to incorporate the outcome of invertebrate bioaccumulation studies in the regulatory evaluation of the risks of food chain transfer and secondary poisoning needs to be elaborated, along the lines indicated in the next section.

8.1.6. Recommendations to develop a risk assessment scheme suitable for benthic organisms for food chain modelling

As discussed above, risk based on the uptake route via water only may result in an underestimation since other key routes that are not taken into account, may be of relevance (e.g. dietary uptake) and could be addressed using BAF (accumulation through water and diet). Therefore, it is recommended to further develop an ERA scheme for biomagnification in the future, as mentioned in the AGD. This is also particularly relevant for sediment risk assessment. Additional guidance for reliable food chain/food web modelling is needed and will be provided in the future PPR scientific opinion on ecological modelling.

In the context of the current scientific opinion for sediment organisms, consideration of food webs should always account for benthic species (e.g. oligochaete worms, larvae of chironomids) as presented in Figure 13. This may be food chains with, for example, the following steps:

- fish-consuming benthic organisms (e.g. oligochaetes) (so called fish primary consumers);
- fish-eating birds or mammals, or predatory (fish-eating) fish (so called fish secondary consumers);
- birds or mammals eating the primary consumer and predatory fish (see figure below).

As well as:

- emerging adults of benthic insects (e.g. chironomids) being then preyed on by terrestrial species, i.e. birds or mammals (e.g. bats).

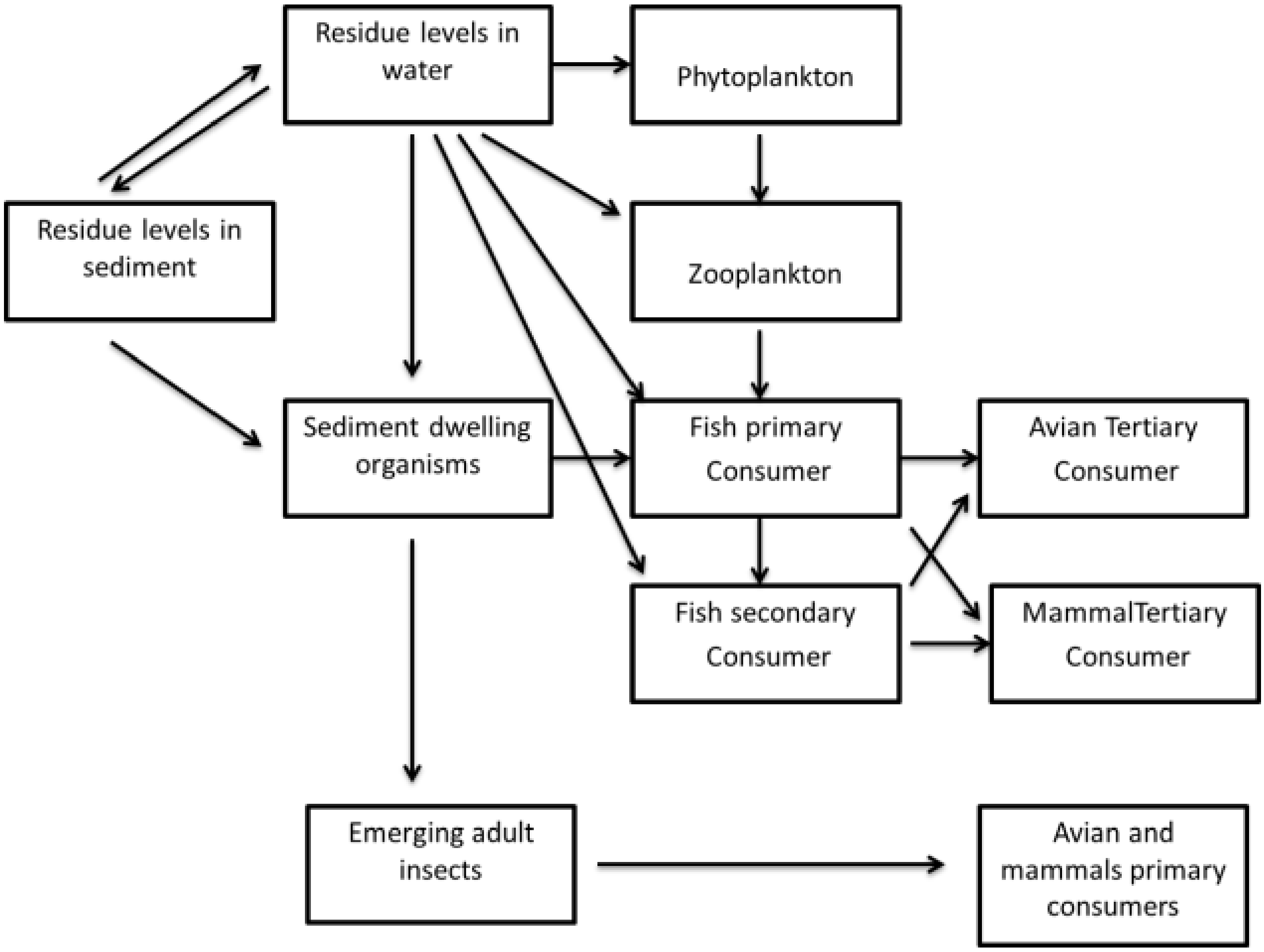


Figure 13: Schematic representation of a food web model for sediment risk assessment. The figure was prepared by the PPR WG.

8.2. Assessing toxicity to benthic organisms

The AGD (EFSA PPR Panel, 2013) focuses on effect assessment schemes for PPP exposure via the water compartment. In this scientific opinion a tiered effect assessment scheme for exposure via the sediment is proposed. Triggers for sediment studies take into account the potential for exposure via the sediment and the potential for toxicity, as described in Chapter 5.

8.2.1. Tier I. Effect assessment based on standard test species

In the context of sediment risk assessment, the use of spiked sediment water–sediment tests is recommended for assessing chronic toxicity of substances accumulating in sediment. Test protocols that may be used in the Tier 1 chronic effect assessment for benthic invertebrates and macrophytes are presented in Table 14. This is especially relevant if the EqP approach (modified or not according to the group of organisms)—used as a screening step—does not exclude risks for benthic organisms (see Chapter 5).

Table 14: Standard chronic protocols for sediment spiked toxicity tests on freshwater invertebrates and macrophytes

Species/test system	Duration	Endpoint	Effect	Reference
Insects *Chironomus* spp.	20–28 days for *Chironomus riparius* and *Chironomus yoshimatsui*; 28–65 days for *Chironomus dilutus*	Immobility, growth, emergence and development time	ECx, NOEC, LOEC	OECD Guideline 218 (2004)
	Two generations: ca. 44 days for *Chironomus riparius*, ca. 100 days for *Chironomus dilutus* (extension of OECD Guideline 218)	Immobility, growth, emergence and development rate, sex ratio, fecundity, fertility	ECx, NOEC, LOEC	OECD Guideline 233 (2010a)
Amphipod *Hyalella azteca*	10 days	Survival (growth optional)	ECx, NOEC, LOEC	ASTM E1706 (2010a) [(a)]
	(28–) 42 days	Survival, growth and reproduction	ECx, NOEC, LOEC	ASTM E1706, 2010a; US EPA (1996b, 2000) [(a)]
Oligochaete *Lumbriculus* spp.	28 days	Survival, growth, reproduction, faecal pellet production rate	ECx, NOEC, LOEC	OECD Guideline 225 (2007a)
Tubifex tubifex [(b)]	10 days			ASTM E1706 (2010a) [(a)]
Rooted macrophytes *Myriophyllum* sp.	14 days	Shoot length and weight	EC50, NOEC, LOEC	OECD Guideline 239 (2014) [(c)]

(a): Adaptation of the ASTM/US EPA test protocols may be required to align them as much as possible to the OECD test guidelines with respect to the ageing period of the spiked sediment before introducing the test organisms.
(b): For Tubifex tubifex, the test guideline available is only for a 10-day semi-chronic sediment-spiked toxicity test (ASTM E1706-5; ASTM, 2010a), but could be adapted for a chronic test (see below).
(c): OECD Guideline 239 is a water–sediment test with Myriophyllum spicatum. It can be adapted for testing spiked sediment; however, a ring test was performed with data from spiked water tests but not for spiked sediment tests.

When sediment toxicity testing is triggered, available toxicity data from the aquatic risk assessment for pelagic organisms and water exposure for the substance under evaluation should be collected and considered, so that test species of the potentially most sensitive taxonomic groups are selected to address the risk for sediment-dwelling organisms.

The following decision scheme is proposed to select the appropriate benthic test species:

1) Do the acute and chronic laboratory toxicity tests (and semi-field tests if available) indicate that aquatic arthropods (insects and/or crustaceans) exposed via water are consistently more sensitive (at least one order of magnitude) than other taxonomic groups?

   No: Go to 2

   Yes: Sediment-spiked toxicity tests with *Chironomus riparius* (or another OECD Chironomus species, OECD Guideline 218) and *Hyalella azteca* are proposed in the Tier 1 effect assessment procedure. The lowest EC10/NOEC value is used in the effect assessment (RAC_{sed} = EC10/10)

2) Do the acute and chronic laboratory toxicity tests (and semi-field tests if available) indicate that aquatic primary producers (algae and/or macrophytes) exposed via water are consistently more sensitive (e.g. one order of magnitude) than other taxonomic groups?

   No: Go to 3

   Yes: A sediment-spiked toxicity tests with the dicotyledonous rooted macrophyte *Myriophyllum spicatum* or *Myriophyllum aquaticum* is proposed in the Tier 1 effect assessment procedure. In case of the latter species, the emerged form should be used since it was shown for *Myriophyllum aquaticum* that the sensitivity of the plant depends on its heterophylly (Ebke et al., 2013). In addition, a sediment-spiked toxicity test with *Chironomus riparius* (or another arthropod species such as *Hyalella azteca*) or *Lumbriculus variegatus* is proposed in the Tier 1 effect assessment procedure. The lowest value of EC50 (macrophyte, with a preference for a growth endpoint) or EC10/NOEC (invertebrate) is used in the effect assessment (RAC_{sed} = EC50/10 (macrophyte) or EC10/10 (invertebrate)).

3) Do the acute and chronic laboratory toxicity tests (and semi-field tests if available) indicate that aquatic vertebrates (e.g. fish, amphibians) exposed via water are consistently more sensitive (e.g. one order of magnitude) than other taxonomic groups?

   No: Go to 4

   Yes: A sediment-spiked toxicity tests with an appropriate aquatic vertebrate is proposed (for more details, see section 8.2.3). In addition, a chronic sediment-spiked toxicity test with *Chironomus riparius* (or another arthropod species such as *Hyalella azteca*) or *Lumbriculus variegatus* is proposed in the Tier 1 effect assessment procedure. The lowest (estimated) chronic EC10/NOEC value is used in the effect assessment and the RAC_{sed} = EC10/10.

4) Substance for which the criteria in 1 to 3 do not apply and likely with fungicidal/biocidal properties. Two sediment-spiked toxicity tests, one with a soft-bodied organism (e.g. *Lumbriculus variegatus* or *Tubifex tubifex*) and a second benthic standard test species other than Oligochaeta are proposed in the Tier 1 effect assessment procedure. The selection of the second test species should be motivated based on available toxicity data for pelagic organisms. Note that, if the pelagic toxicity data indicate that the most sensitive taxonomic group (e.g. a mollusc) is not represented in the set of standard benthic test species, it may be necessary to conduct a sediment-spiked toxicity test with a non-standard benthic representative of the potential sensitive taxonomic group (e.g. a benthic mollusc). The lowest chronic EC10/NOEC value is used in the effect assessment and the RAC_{sed} = EC10/10.

8.2.1.1. Sediment ERA for benthic algae and rooted macrophytes

If primary producers are targeted in sediment ERA, it is assumed that the toxicity in the sediment compartment is exclusively exerted via pore water exposure (in contrast to benthic animals which may be exposed via pore water and ingested sediment particles). In Chapter 7, it is reported that the peak concentrations of PPPs are higher in surface water (free water) than in sediment pore water (see section 7.3.1). In case a TWA PEC cannot be used in the risk assessment for primary producers (e.g. algae), the effect concentrations for primary producers tested in water are compared with the $PEC_{sw;max}$, and the risk would be triggered by the surface water concentration rather than the pore water concentration. However, long-term exposure concentrations may become higher in the pore water than in the overlying water, a phenomenon that may be important for rooted macrophytes in particular. A pragmatic approach might be to compare in the first instance the EC50 (preferably using the growth endpoint (EFSA PPR Panel, 2013)) for primary producers derived from water exposure or water-spiked water-sediment tests directly to the $PEC_{sed;pw}$. This approach overcomes the problems linked to sediment rooted macrophyte tests as highlighted by Diepens et al. (2014b). They showed that an exposure period of 28 days might not be sufficient for sediment-spiked toxicity tests with macrophytes, as the uptake from sediment and translocation to shoots appears to be a slow chemical- and species-specific process; equilibrium was thus only reached later than the 28 days test period for the compounds and macrophyte species studied by Diepens et al. (2014b). By contrast, when the overlying water was spiked, 28 days were sufficient for chemicals studied by Diepens et al. (2014b) since they more rapidly translocated from shoot to root than the other way around. However, one issue is that the duration of the standard test with macrophytes is only of 7 to 14 days. It is therefore necessary to evaluate if the effect assessment based on the recent OECD test guideline for rooted macrophytes (OECD, 2014), and application of an AF 10 to the EC50 (preferably using the growth endpoint) of the standard test species sufficiently protects populations of a wider array of macrophytes under field conditions.

8.2.1.2. Deriving regulatory acceptable concentrations

Table 15 summarises how to derive regulatory acceptable concentrations (RAC_{sed}) for each group of species.

Table 15: Endpoints available from sediment toxicity tests

Taxonomic group	Species/test system	Duration	Chronic endpoint [(a)]	Regulatory acceptable concentration
Insects	*Chironomus* spp.	20–28 days	EC10 (NOEC)	EC10 (NOEC)/10
Amphipod	*Hyalella azteca*	(28–)42 days	EC10 (NOEC)	EC10 (NOEC)/10
Oligochaete	*Lumbriculus* spp.	28 days	EC10 (NOEC)	EC10 (NOEC)/10
Macrophyte	*Myriophyllum* sp.	14 days	EC50	EC50/10

(a): The endpoints concern the most sensitive and ecologically relevant (sub-lethal) effects for each test species.

8.2.1.3. Other information, limitations and recommendations

It should be noted that for benthic oligochaetes, information on the sensitivity of the proposed species is scarce (only few toxicity studies are available using *Tubifex tubifex* and *Lumbriculus* sp.). Therefore, their relevance to test effects of substances with fungicidal activity is difficult to determine at this stage. The relevance of the benthic test species for the field communities in terms of sensitivity and vulnerability should also be explored in terms of representativity.

Appropriate alternative test species may be used if an internationally accepted guideline is available and/or if this guideline can be adopted easily to be in accordance with a proper chronic sediment test. For example, for *Tubifex tubifex*, for which only a semi-chronic test guideline exists, the guideline can be adapted to address the test requirement of a chronic sediment-spiked toxicity test (e.g. by aligning the test protocol to that of the chronic sediment-spiked *Lumbriculus* sp. toxicity test) as has been done for *Gammarus* sp. in the adapted water exposure test.

For sediment toxicity tests, the concentrations in the pore water, the overlying water and the bulk sediment are all of relevance for epibenthic test species such as, for example, *Chironomus* sp. and *Hyalella azteca*. Ideally, the sediment concentrations over the entire course of the toxicity experiment are measured.

For spiked sediment test design with organisms other than microbes (see section 8.2.5), the sediment is spiked first and the test species are introduced after a stabilisation period, and this stabilisation period should be harmonised if using both OECD and US EPA/ASTM test protocols (to avoid differences in exposure concentrations due to different ageing periods). Ideally, the food provided to the test organisms should be spiked with the test compound as well, although it is realised that technically it can be challenging, particularly for carnivorous test species. Alternatively, field-collected sediment with a high enough food content for the total test period could be used instead of standard OECD sediment which has lower nutritional value.

The key endpoints from the spiked sediment studies should be given in milligrams of active substance (a.s.) per kilogram of dry sediment normalised to 2.5 % OC. Note that the chronic ASTM tests use field-collected sediments, thus normalisation for OC content would also be necessary. When analytically and technically feasible the toxicity should also be expressed in terms of pore water concentration.

The derivation of an EC10 rather than a NOEC is preferred; therefore, the range of concentrations tested should be suitable.

Substances with fungicidal mode-of-action are usually not receptor specific and thus may target a vertebrate as well as an invertebrate or a primary producer (e.g. strobilurin acts on mitochondria and thus would act on vertebrates as well as on invertebrates and plants). This 'non-specificity' is particularly true for acute effects but for long-term effects—which are the main concern in sediment risk assessment—it cannot be excluded that different mechanisms are triggered. There is a need for further knowledge on such mechanisms being specifically related to fungicides.

8.2.2. Tier II. Effect assessment based on sediment-spiked toxicity tests with standard and additional test species

8.2.2.1. Introduction

As outlined in section 8.2.1, the Tier 1 test species of benthic invertebrates and plants that are recommended for chronic standard sediment-spiked toxicity testing are limited to the insect *Chironomus riparius* (or another chironomid recommended by OECD, such as *Chironomus dilutus*), the crustacean *Hyalella azteca*, the oligochaete *Lumbriculus variegatus*, and the rooted macrophytes *Myriophyllum spicatum* or *Myriophyllum aquaticus*. Following the Tier 1 decision scheme presented in section 8.2.1, at least for two standard test species chronic sediment-spiked toxicity data should be delivered when sediment testing is triggered. However toxicity data from water–sediment tests performed with spiked water can also be used since it was shown that this enables a conservative assessment (see Chapter 5). To avoid unnecessary testing with invertebrates and vertebrates (amongst others for animal welfare reasons), it may be sufficient to use water exposure toxicity data and the modified EqP concept as much as possible to estimate effects of sediment exposure to benthic fauna.

In accordance with the tiered effect assessment procedures described in the EFSA AGD (EFSA PPR Panel, 2013), laboratory single species tests with additional test species of the potentially sensitive taxonomic group(s) may be used to refine the effect assessment. Protocol tests for sediment-spiked benthic test species, others than the Tier 1 test species mentioned above, are described in section 2.3. Furthermore, more or less standardised sediment-spiked toxicity tests conducted with estuarine/marine benthic taxa (e.g. crustaceans and polychaetes) may be available. An overview of benthic test species for which protocol tests are available is given in Table 16.

Additional information on sediment-spiked toxicity tests with benthic species can be obtained by mining the literature or by conducting sediment-spiked toxicity tests with non-standard benthic species following the available test protocols (for related standard test species) as far as possible. Guidance for assessing the reliability of the additional toxicity tests is provided in EFSA (2011). According to EFSA PPR Panel (2013), higher-tier risk assessments for edge-of-field surface waters could consider supplementary information from the open literature on relevant marine/estuarine species, unless there is evidence of significant differences in sensitivity between freshwater and marine/estuarine species that would preclude combining effects data. However, combining toxicity data for freshwater and marine/estuarine benthic species would first require demonstration of taxonomic and ecological relevance to edge-of-field surface water.

For any effect assessment approach using sediment-spiked toxicity data for additional test species the most sensitive endpoint that is toxicologically and ecologically relevant should be used.

Considering test protocols for sediment-spiked toxicity tests with benthic macroinvertebrates, a pragmatic distinction can be made in semi-chronic toxicity tests for macroinvertebrates (test duration usually 10 days and a focus on EC50 and LC50 values) and chronic toxicity tests (test duration usually ≥ 28 days and a focus on ECx or NOEC values). Ideally, a chronic toxicity test should cover the whole life cycle, or at least the most sensitive life stage, of the test organism. In addition, a chronic test should focus on sub-lethal endpoints, such as growth and reproduction. For tests with benthic micro-/mesofauna to be considered as chronic shorter test duration may be appropriate, depending on the duration of the life cycle of the test organism. For example, in tests using the nematode *Caenorhabditis elegans*, the chronic effect endpoint reproduction can be studied in a four-day test. Protocol tests with vascular plants have a duration of 7 to 14 days.

The data requirements in the EU concern chronic toxicity tests, with a focus on EC10/NOEC endpoints. In the USA, however, the data requirements for macroinvertebrates in sediment ERA comprise both semi-chronic (usually 10-day) and chronic tests and often concern LC50 or EC50 endpoints. For this reason the data mined from the literature often cannot be readily compared.

Table 16: Overview of benthic test species for which protocols are available for the conduct of sediment spiked toxicity tests

Test species	Long-term (chronic) test guideline	Semi-chronic test guideline
Chironomus spp. (insect)	28–65-day tests; OECD Guideline 218 (OECD, 2004a) 44–100-day life cycle test; OECD Guideline 233 (OECD, 2010a)	10-day test; ASTM E1706 (ASTM, 2010a)
Hexagonia spp. (insect)	–	10-day test; ASTM E1706 (ASTM, 2010a)
Hyalella azteca (crustacean)	(28–)42-day test; US EPA (1996b, 2000) and ASTM E1706 (ASTM, 2010a)	10-day test; ASTM E1706 (ASTM, 2010a)
*Diporei*a spp. (crustacean)	–	10-day test; ASTM E1706 (ASTM, 2010a)
Leptocheirus plumulosus (estuarine crustacean)	28-day test; US EPA (2001) and ASTM E1367 (ASTM, 2010b)	10-day test; US EPA (1996a) and ASTM E1367 (ASTM, 2010b)
Eohaustorius estuarius (estuarine crustacean)	28-day test; US EPA (1996a)	10-day test; US EPA (1996a) and ASTM E1367 (ASTM, 2010b)
Ampelisca abdita (marine crustacean)	28-day test; US EPA (1996a)	10-day test; US EPA (1996a) and ASTM E1367 (ASTM, 2010b)
Rhepoxynius abronius (marine crustacean)	28-day test; US EPA (1996a)	10-day test; US EPA (1996a) and ASTM E1367 (ASTM, 2010b)
Corophium volutator (estuarine/marine crustacean)	–	10-day test; ISO 16712 (ISO, 2005)
Lumbriculus variegatus (oligochaete worm)	28-day test; OECD Guideline 225 (OECD, 2007a)	–
Tubifex tubifex (oligochaete worm)	–	10-day test; ASTM E1706 (ASTM, 2010a)
Neanthes arenaceodentata (estuarine/marine polychaete worm)	20–28-day test; ASTM E1611 (ASTM, 2007)	10-day test; ASTM E1611 (ASTM, 2007)
Caenorhabditis elegans (nematode worm)	Four-day test; ISO 10872 (ISO, 2010b)	–
Myriophyllum spicatum (vascular plant)	14 days; OECD Guideline 239 (OECD, 2014)	–
Myriophyllum aquaticum (vascular plant)	Seven days; ISO 16191(ISO, 2010a)	–

8.2.2.2. Normalising sediment toxicity data for chronic Tier 2 effect assessment

The current OECD test protocols (e.g. OECD 2004a, b, 2007a, 2010a) advocate the use of artificial sediment, containing 4–5 % peat (approximately 2.5 % OC). In the literature to obtain additional toxicity data derived from sediment-spiked tests, sediments predominantly are used that deviate from the standard OECD sediment. For example, in sediment toxicity tests conducted with marine/estuarine aquatic invertebrates, usually field-collected sediments differing in organic matter content are used. In addition, field-collected sediment is, in most cases, also used in the ASTM and US EPA protocols (US EPA, 1996a). All protocols, however, require the determination of the OC content in the sediment, enabling the recalculation of effect concentrations on the basis of either OC content or standard OECD sediment (with approximately 2.5 % OC). To allow a comparison of sediment toxicity data from different sources, where possible and relevant, we recommend to standardise the sediment toxicity either in terms of micrograms per kilogram of dry weight OECD standard sediment with an OC content of 2.5 % (a procedure usually followed in the EU) or on basis of sediment OC content (a procedure often followed in North America). When analytically and technically feasible the toxicity should also be expressed in terms of pore water concentration.

8.2.2.3. Geomean and Weight of Evidence approaches

In the EFSA AGD (EFSA PPR Panel, 2013) the Geomean approach is a Tier 2 option that may be used in the risk assessment. This approach can be used if, for taxa of the potentially most sensitive taxonomic group(s), more toxicity data are available than required for the Tier 1 assessment but less than required for the Species Sensitivity Distribution approach (see also EFSA PPR Panel, 2006). When using the Geomean approach in the acute effect assessment for water organisms, the geometric mean L(E)C50 value is calculated using all available L(E)C50 values for different species belonging to the same taxonomic group (e.g. crustaceans, insects or oligochaete worms) and characterised by a comparable measurement endpoint (e.g. mortality and immobilisation) and test duration (e.g. 48 hours and 96 hours). The lowest geometric mean L(E)C50 value for the different taxonomic groups thus obtained is selected and the same AF normally used in the acute Tier 1 effect assessment (i.e. 100) is applied in the $RAC_{sw;ac}$ derivation. In the acute effect assessment for water organisms and pesticides the Geomean approach is relatively straight forward to use since acute L(E)C50 data for different species of the same taxonomic group usually concern a similar measurement endpoint and are obtained from tests with similar duration. In addition, for water organisms, the validity of the Tier 2 Geomean approach could be calibrated with the ETO–$RAC_{sw;ac}$ values derived from micro-/mesocosm experiments with insecticides (Van Wijngaarden et al., 2014). The validity of the Geomean approach in the chronic effect assessment for water organisms, however, could not be investigated because of a limited amount of chronic toxicity data and the diversity of sub-lethal endpoints (e.g. growth, biomass, emergence, reproduction) used in chronic toxicity testing, even for species within the same taxonomic group. Thus, it remains to be investigated whether the Geomean procedure as currently used in the acute effect assessment for pesticides can be used for chronic toxicity data.

The problem identified for the Geomean approach based on chronic toxicity data for water organisms also exists for the application of the Geomean approach in sediment effect assessment. Consequently, for the time being, the PPR Panel proposes to be prudent in the use of the Geomean approach in the chronic effect assessment for pesticides. In future, the use of the Geomean approach in the chronic effect assessment based on chronic toxicity data for sediment organisms will be revisited once additional information is available and it is shown that the concept is suitable for chronic data. Instead, for the time being a WoE approach is proposed (see below).

The Geomean approach, however, might be used in the effect assessment based on semi-chronic toxicity data such as the 10-day toxicity data for benthic invertebrates of the potentially sensitive taxonomic group(s). In order to derive an appropriate RAC_{sed} based on the Geomean approach and semi-chronic toxicity data, a higher AF than that used in the chronic effect assessment (currently 10) is required since the 10-day tests deliver semi-chronic toxicity data and often address the mortality endpoint. An AF of 100 might be used, as that currently in the Tier 2 effect assessment for pelagic water organisms, when applying the Geomean approach. However, in case of large differences in semi-chronic toxicity values, e.g. if the endpoint of the most sensitive species is below the Geomean–RAC_{sed} based on semi-chronic L(E)C50s and application of an AF of 100 for all the tested species of the most sensitive taxonomic group, the use of a WoE approach in the effect assessment is more appropriate (see below).

In case additional toxicity data are available but the SSD approach cannot be used, the WoE approach can be used. In this approach the toxicity value of the most sensitive benthic species, irrespective of the endpoint, and a lower overall AF is used. According to EFSA PPR Panel (2006), the overall AF can be interpreted as follows: $AF_{overall} = AF_{species} \times AF_{other}$ where $AF_{species}$ is the AF to allow for uncertainty due to variation in sensitivity among species (e.g. all invertebrates) and AF_{other} is intended for other sources of uncertainty. The contribution of both elements is not defined but it seems reasonable to maintain as a default approach the assumption from EC (2002) that for acute toxicity data (but in this case, also for semi-chronic), the AF_{spec} and AF_{other} have a more or less equal weight (so 10 for both AF_{spec} and AF_{other} = 10 × 10 = 100 for the $AF_{overall}$). Consequently, based on the number of semi-chronic toxicity data for different benthic test species the $AF_{overall}$ to be applied to the semi-chronic 10-day L(E)C50 of the most sensitive benthic test species of the relevant taxonomic group may vary from a value larger than 10 up to 100 and the more additional toxicity data available the lower the $AF_{overall}$ might be. Further guidance on selecting a lower AF when additional species toxicity data are available is presented in EFSA PPR Panel (2006). Note, however, that if enough sediment toxicity data are available to conduct effect assessments using either semi-chronic or chronic toxicity data for benthic species, it is proposed to select the effect assessment based on chronic toxicity data.

A WoE approach might also be used if additional chronic toxicity data for the potentially sensitive taxonomic group(s) are available, and there are fewer toxicity data than the required minimum for the SSD approach (toxicity data for at least eight different benthic species). It is assumed that in the chronic ERA the AF_{spec} and AF_{other} do not have an equal weight since, amongst others, the uncertainty of the acute to chronic extrapolation is already addressed. Further more, the AF should be larger than the AF of 3 used in the chronic SSD approach, Therefore, we propose that the $AF_{overall}$ to be applied to the chronic toxicity value of the most sensitive benthic test species of the relevant taxonomic group may vary from 4 up to 10. The more additional chronic toxicity data are available the lower the AF might be.

8.2.2.4. Example how to use the Geomean and WoE approach

An example how the Geomean and WoE approaches might be used to derive the Tier 2 RAC_{sed} using sediment toxicity data is presented below for a pyrethroid insecticide, I_{pyr} (Table 17). Benthic arthropods (crustaceans and insects) can be considered the potentially most sensitive taxonomic group for insecticides (see EFSA PPR Panel, 2013). Even though this insecticide is an imaginary compound these data are based on, more or less, realistic sediment toxicity data for several pyrethroids with a similar toxicity profile. The proposed Tier 1 test species for the sediment effect assessment are *Chironomus riparius* and *Hyalella azteca* (see section 8.2.1). Applying an AF of 10 to the 28-day EC10 value of the most sensitive standard test species results in a RAC_{sed} for I_{pyr} of 0.225 µg/kg dry weight standardised sediment. An additional 65-day EC10 value is available for *Chironomus dilutus*, so that for three benthic arthropod taxa chronic toxicity data are available. If, for example, on basis of the WoE approach, and the fact that for one additional species a valid chronic EC10 value is provided, it is decided to lower the overall AF of 10 to 9, and to apply this AF of 9 to the lowest chronic toxicity value; this results in a Tier 2 WoE RAC_{sed} of 0.250 µg/kg standardised sediment (Table 17).

Additional sediment toxicity data comprise 10-day LC50 values for two insect taxa and three crustaceans, giving a total of five benthic arthropod taxa for which semi-chronic toxicity data are available. In case it is decided to lower the overall AF from 100 to 65 (since five semi-chronic toxicity data for benthic arthropods are available) and applying this AF of 65 to the lowest 10-day LC50 of 12.8 µg/kg, results in an alternative Tier 2 WoE RAC_{sed} for I_{pyr} of 0.197 µg/kg standardised sediment (Table 17). Alternatively, the Geomean LC50 is lowest for the three crustaceans. Applying an AF of

100 to the Geomean 10-day LC50 for benthic crustaceans results in a Tier 2 Geomean-RAC$_{sed}$ for I$_{pyr}$ of 0.353 µg/kg standardised sediment.

The Tier 1 and Tier 2 RAC$_{sed}$ values presented above for this example data set do not deviate much. In the sediment effect assessment, by using semi-chronic toxicity data it may be an option to always use both the Geomean and WoE approaches and to select the lowest Tier 2 RAC$_{sed}$ thus obtained. However, a Tier 2 WoE RAC$_{sed}$ based on chronic toxicity data is given more weight in the effect assessment than a Tier 2 RAC$_{sed}$ based on semi-chronic toxicity data, resulting in a final Tier 2 RAC$_{sed}$ of 0.250 µg/kg.

Note that in the example presented in Table 17, the overall AF used in the WoE approach is tentative. The PPR Panel proposes to develop a transparent decision scheme for the WoE approach, more specifically to develop criteria on how much the AF$_{overall}$ should be to lowered and then applied to the lowest valid toxicity value, based on the quality and number of additional toxicity data available.

Table 17: Sediment-spiked toxicity data in µg/kg dry weight sediment (normalised to 5 % organic matter) for benthic arthropods and the insecticide I$_{pyr}$ and the derived Tier 1 and Tier 2 RAC$_{sed}$ values. The Tier 2 RAC$_{sed}$ values are based on the WoE and Geomean approaches

Species	**Toxicity endpoint**	**Toxicity (µg/kg)**	**Tier 1 RAC$_{sed}$ (µg/kg)**	**Tier 2 RAC$_{sed}$ (µg/kg)**	
				WoE	**Geomean**
Chronic toxicity data					
Chironomus riparius (Insecta; Chironomidae)	28-day EC10 (emergence)	12.8			
	28-day EC10 (biomass)	2.25	2.25/10 = 0.225	2.25/9 = 0.250 (three chronic toxicity data)	Not applicable to chronic data
Chironomus dilutus (Insecta; Chironomidae)	65-day EC10 (emergence)	9.4			
Semi-chronic toxicity data					
Chironomus dilutus (Insecta; Chironomidae)	10-day LC50 (mortality)	65.8	Not applicable		Geomean LC50 Insecta = 70.8 70.8/100 = 0.708
Hexagenia sp. (Insecta; Ephemeroptera)	10-day LC50 (mortality)	76.0	Not applicable		
Hyalella azteca (Crustacea; Amphipoda)	10-day LC50 (mortality)	22.0	Not applicable		Geomean LC50 Crustacea = 35.3
Corophium volutator (Crustacea; Amphipoda)	10-day LC50 (mortality)	12.8	Not applicable	12.8/65 = 0.197 (five semi-chronic LC50s)	35.3/100 = 0.353
Eohaustorius estuarius (Crustacea; Amphipoda)	10-day LC50 (mortality)	155.0	Not applicable		

8.2.2.5. The Species Sensitivity Distribution approach

In the AGD (EFSA PPR Panel, 2013) the SSD approach is adopted as a Tier 2 effect assessment approach if for at least eight different species appropriate toxicity data are available. In the chronic effect assessment scheme the SSD is constructed with chronic EC10/NOEC values of the sensitive taxonomic group(s), and the calculated HC5 is used to derive a Tier 2 RAC$_{sw}$ by applying an AF of 3.

In the sediment ERA and the derivation of the Tier 2 RAC_{sed} based on chronic sediment-spiked toxicity data a similar methodology may be used. The selection of the relevant taxonomic group to incorporate in the SSD may be based on the decision scheme for selecting appropriate Tier 1 test species (e.g. arthropods for pesticides with insecticidal properties and different taxonomic groups for fungicides with biocidal properties).

In this scientific opinion we propose to follow the SSD approach as much as possible according to the criteria described in the AGD (EFSA PPR Panel, 2013). This means that for PPPs characterised by a specific toxic mode-of-action, at least for eight species of the potentially sensitive taxonomic group (most likely benthic arthropods for insecticides; rooted macrophytes for herbicides) toxicity data should be available. Note, however, that the endpoint of the toxicity estimate (e.g. EC10) used in the SSD can differ between species in terms of type and duration. For PPPs for which a specific potential sensitive taxonomic group cannot be identified on basis of the available toxicity data for pelagic organisms, a minimum number of eight toxicity data for at least five different taxonomic/feeding groups may be selected. This may be the case for fungicides with biocidal properties.

To derive a RAC_{sed} based on the SSD approach, the SSD should preferably be constructed with chronic EC10/NOEC data addressing the most sensitive sub-lethal endpoints for each species.

Another approach may be to use semi-chronic data (e.g. 10-day L(E)C50 values) separately to construct a semi-chronic SSD and to calculate a corresponding semi-chronic HC5. The RAC_{sed} may be estimated by applying an AF of 15 to this semi-chronic HC5, assumed that a factor of five covers the extrapolation of a semi-chronic HC5 to a chronic HC5 and a factor of three to cover the remaining uncertainty in deriving a RAC_{sed} from a chronic HC5. The factor of five to extrapolate a semi-chronic HC5 to a chronic HC5 for PPPs is proposed, since it seems to cover, in most available cases, the difference between 10-day L(E)C50 values and chronic EC10/NOEC values for the same benthic invertebrate species and PPP (see the review on sediment toxicity data for PPPs presented in Deneer et al. (2013)). A more recent publication dealing with sediment-spiked toxicity tests with the pyrethroid bifenthrin and the crustacean *Hyalella azteca* (Anderson et al., 2015) also reveals that the factor of five is sufficient for a realistic worst-case extrapolation of 10-day LC50 values to 28-day EC10 values.

8.2.2.6. Example how to use the SSD approach

Currently there is no data set available containing enough toxicity data for a single PPP derived from sediment-spiked tests for benthic species to evaluate the proposal described above. For this reason an example data set for the fungicide $F_{organotin}$ is constructed that illustrates how the SSD approach might be used to derive the Tier 2 RAC_{sed}. Even though this example fungicide is an imaginary compound these data are based on, more or less, realistic sediment toxicity data of several organotin fungicides and biocides with a similar toxic mode-of-action.

For the example fungicide $F_{organotin}$, sediment-spiked chronic toxicity data are available for, in total, eight freshwater and estuarine/marine benthic macroinvertebrates. The chronic toxicity data set comprises representatives of Insecta, Crustacea, Oligochaeta, Polychaeta, Mollusca and Echinodermata. In the chronic data set the Tier 1 standard test species proposed for PPPs with a fungicidal/biocidal mode-of-action (see section 8.2.1) are also represented, namely *Tubifex tubifex* (28-day NOEC = 25.5 µg/kg dry weight sediment) and *Chironomus riparius* (28-day EC10 = 85.0 µg/kg dry weight sediment). Applying an AF of 10 to the 28-day NOEC of *Tubifex tubifex* results in a Tier 1 RAC_{sed} of 2.55 µg/kg dry weight sediment. However, for organotin compounds it has been demonstrated that snails, in particular, are amongst the most sensitive taxa (see e.g. Duft et al., 2003b). For this reason it seems logical for this case to also consider the snail *Potamopyrgus antipodarum* as an additional Tier 1 standard test species (28-day EC10 = 2.76 µg/kg dry weight sediment), resulting in an alternative Tier 1 RAC_{sed} of 0.276 µg/kg dry weight sediment.

The SSD constructed with the (estimated) chronic NOEC/EC10 values available is presented in the upper panel of Figure 14. The Anderson–Darling test for normality was accepted at all levels for the

SSD, indicating than the curve fitted the toxicity data well. The median HC5 value that could be derived from the curve was 2.736 µg/kg dry weight sediment (normalised to 2.5 % OC). Applying an AF of three to the median HC5 derived from the SSD curve results in a Tier 2 SSD–RAC_{sed} of 0.912 µg/kg dry weight sediment for benthic invertebrates and example fungicide $F_{organotin}$. Note that this Tier 2 SSD–$RAC_{sed;ch}$ is lower that the Tier 1 RAC_{sed} when based on the standard species *Chironomus riparius* and *Tubifex tubifex* (2.55 µg/kg), but approximately a factor of three higher when also considering *Potamopyrgus antipodarum* as an additional Tier 1 test species (0.276 µg/kg).

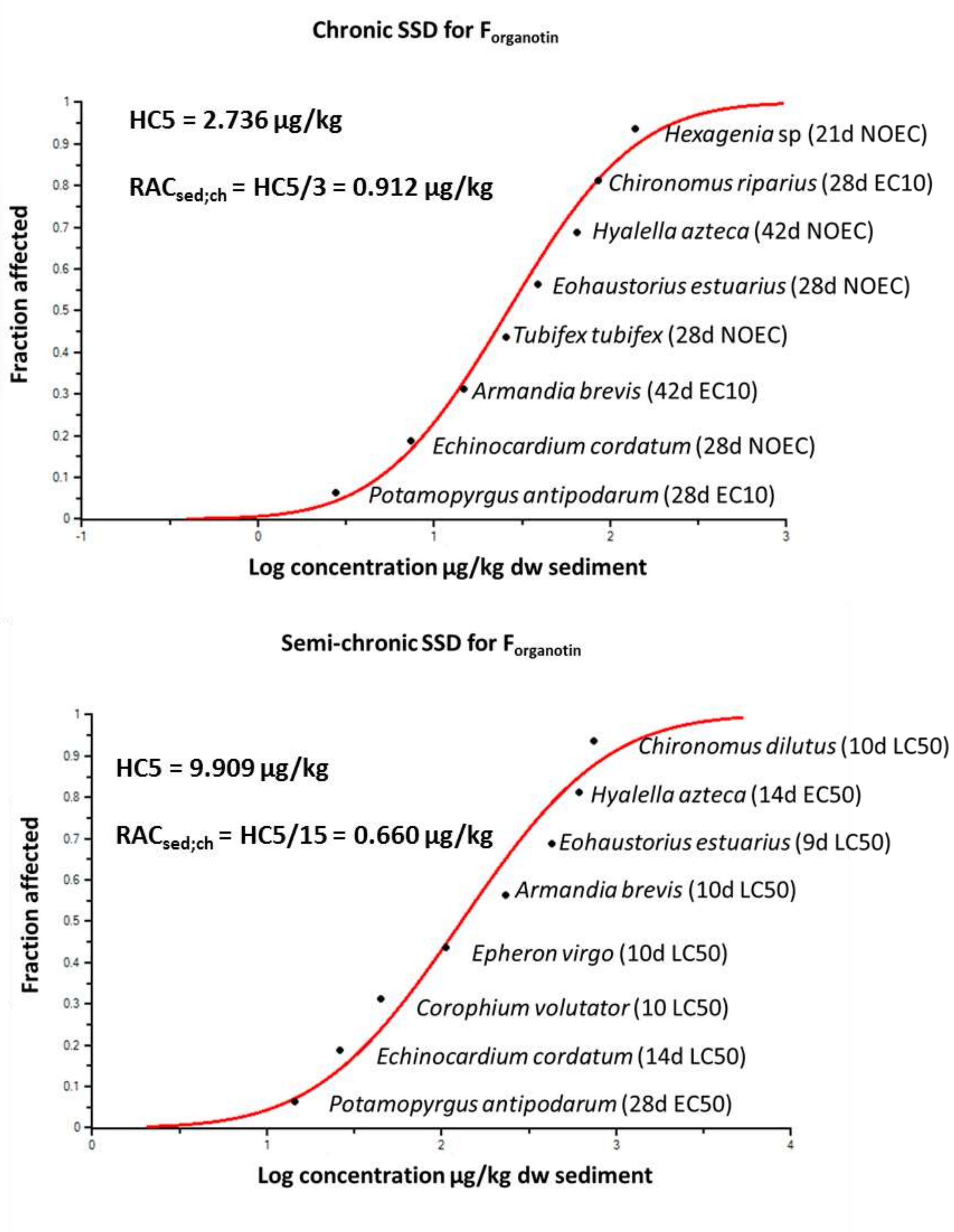


Figure 14: SSD curves for fungicide $F_{organotin}$ constructed with chronic NOEC/EC10 values (upper panel) and semi-chronic L(E)C50 values (lower panel for freshwater and estuarine/marine benthic invertebrates (toxicity data expressed in µg/kg dry weight sediment, normalised to 2.5 % OC). The figure was prepared by the PPR WG.

For the example fungicide $F_{organotin}$, semi-chronic toxicity data are available for, in total, eight freshwater and estuarine/marine benthic macroinvertebrates belonging to five orders/families (Insecta, Crustacea, Polychaeta, Mollusca and Echinodermata), allowing the SSD approach to be applied (Figure 14, lower panel). The Anderson–Darling test for normality was accepted at all levels, indicating than the curve fitted the toxicity data well. The median HC5 value that could be derived from the curve was 9.909 µg/kg dry weight sediment (normalised to 2.5 % OC). Applying an AF of 15 to the median HC5 derived from the semi-chronic SSD curve results in a Tier 2 SSD-RAC_{sed} of 0.660 µg/kg dry weight sediment for benthic invertebrates and example fungicide $F_{organotin}$. Note that in this example the alternative estimate of the Tier 2 SSD–RAC_{sed} is lower that the Tier 2 SSD–RAC_{sed} based on chronic sediment toxicity data (the preferred option) and more than a factor of two higher when *Potamopyrgus antipodarum* is also considered as an additional chronic Tier 1 test species (0.276 µg/kg).

It might be argued that for a RAC_{sed} derivation to be used in the ERA for freshwater benthic organisms, marine/estuarine toxicity data should be used only if they concern a taxonomic group also present in freshwater ecosystems. In the example data set for the fungicide $F_{organotin}$, however, one toxicity value concerned a taxonomic group not typical for freshwater ecosystems, i.e. the echinoderm *Echinocardium cordatum*. Deleting this species results in a chronic data set that is too small to apply the SSD approach. The WoE approach described above, however, may be applied in this case.

The example for fungicide $F_{organotin}$ presented above suggests that the differences in derived RAC_{sed} values between Tier 1 and Tier 2 are relatively small, and that a Tier 1 effect assessment based on the prescribed test species (e.g. *Lumbriculus variegatus* or *Tubifex tubifex* and *Chironomus riparius*) may not always provide sufficient protection to benthic organisms. Diepens et al. (submitted 2015a) came to the same conclusion when evaluating the sediment toxicity data for the biocide tributyltin. This observation may be a consequence of the specific example data set and the properties of organotin compounds. An important research need is to develop sediment toxicity data sets for benthic organisms and modern PPPs that differ in toxic mode-of-action so that the validity of the tiered approach as proposed in this scientific opinion can be evaluated.

8.2.3. Tier III. Effect assessment based on micro-/mesocosm studies

8.2.3.1. Introduction

The information provided in the AGD (EFSA PPR Panel, 2013, section 9.3) on model ecosystem experiments is largely relevant in the context of sediment risk assessment. However, owing to the nature of the sediment compartment and specific features of sediment exposure-mediated effects, some considerations need special attention when conducting micro-/mesocosm studies to address environmental risks of sediment exposure.

8.2.3.2. Generalities

The advantages of micro- and mesocosm studies over the other types of experimental higher-tier studies (e.g. additional laboratory toxicity tests to construct SSDs; refined exposure studies) are:

- Ability to integrate more or less realistic exposure regimes with the long-term assessment of endpoints at higher levels of biological integration (population- and community-level effects), and to study intra- and inter-species interactions and indirect effects in a more or less realistic community.
- A higher number of species and ecological groups are exposed for which population/community level dose–response relationships may be obtained.
- Since micro-/mesocosm tests can be performed for a relatively long time, they may be used to assess, for example, latency of effects, population and community recovery, culmination of effects.

- Owing to increased control over confounding factors, causality between exposure to a sediment-bound contaminant and effects is easier to demonstrate in micro-/mesocosms than in field monitoring studies. In addition, micro-/mesocosm experiments allow the study of different contaminant levels, replication and real controls (contaminant not present), which normally is not possible in a field study.

However, micro- and mesocosm experiments present a number of limitations, e.g.:

- Although community level tests have been developed to increase ecological realism, they cannot fully account for the natural complexity of ecosystems. These systems typically lack the presence of top predators and after elimination a realistic recolonisation by certain species (e.g. semivoltine or univoltine species that do not have aerial stages) may be hampered in isolated test systems.
- The biological and environmental conditions in a specific micro-/mesocosm study represent only one of the many possible conditions for sediment communities. Therefore, they should preferably simulate typical conditions in order to derive a relevant sensitive endpoint for a risk assessment. On this basis, the spatial–temporal extrapolations to account for the variability in the effect assessment are expected to be covered by applying an AF.

8.2.3.3. Specific features of sediment risk assessment using mesocosm studies

Micro-/mesocosm studies generally enable the evaluation of more realistic exposure patterns than those used in laboratory tests, for example linked to differences in terms of:

- Duration: Micro- and mesocosm studies present some advantages over other test systems, particularly for sediment ERA, since communities in the field are exposed to long-term sediment contamination. Furthermore, they include more appropriate physico-chemical and biological factors that are affecting the bioavailability of contaminants in sediment such as bioturbation and ageing effects.
- Exposure matrix: Exposures through overlying water and sediment (including pore water) are all of relevance. In addition, sediment-spiked micro-/mesocosm test systems can be designed to consider the predicted PEC_{sed}, including the plateau-level due to multiple applications, i.e. $PEC_{sed,accu,max}$. This could then account for either multiple applications within a year or over multiple year of use (i.e. background contamination). If well defined in advance, the effects of specific or combined PPP exposure routes (and PPP combinations) can be studied as well in micro-/mesocosm test systems.
- Spatial heterogeneity: Spatial variability of exposure concentrations may be higher in sediments than in overlying water. However, an experimental design aiming at a realistic spatial variability in sediment exposure within test systems is always a compromise with the aim of uniform conditions between replicate test systems.

Performing sediment micro-/mesocosms with contaminated sediment is mostly aimed at determining direct and indirect biological effects on the various biological levels of organisation. In addition, it could be helpful to gain more experience in the use of spiked sediment for studying the bioaccumulation and biomagnification of the chemicals through the food web.

8.2.3.4. Recommendations to perform micro-/mesocosm studies for sediment risk assessment

If micro- and mesocosms are to be used more routinely in the higher-tier risk assessment of contaminated sediment, further guidance need to be developed on how to design, conduct and interpret such studies. Further guidance is, for example, needed for:

- Type of sediment (natural vs. artificial) to use: Field-collected sediment is largely preferred over artificial (OECD) sediment for the development of a realistic and diverse benthic community despite the fact that they may be contaminated due to unknown background chemicals and difficult to standardise in terms of composition across studies.

- Type of spiking: An important question at stake is whether to use spiked sediment to construct micro-/mesocosm or to follow the traditional approach in constructing micro-/mesocosms with 'clean' sediment and to spike the water column with the PPP (water or sediment-slurry applications). The PPR Panel considers both designs feasible, but a reasoned case should be presented why a specific design is chosen. Irrespective of the design, dynamics in exposure concentrations in the relevant sediment layers, and in overlying water, should be monitored.
- An alternative design can be to spike both the water column and the sediment in order to study effects of PPP under most realistic environmental conditions (i.e. spiked water that simulate the drift entry and spiked sediment that simulate the historical background PPP).
- Type of community: A well-established community including sensitive species should be used, i.e. with natural interactions between individuals and populations since this can influence the response to the toxicant as shown in aquatic mesocosm experiments by, for example, Knillmann et al. (2012a, b). Note that when using a spiked sediment design, the impact of PPP exposure in sediment on the colonisation of benthic organisms may be the focus of the study. Using the traditional approach in constructing micro-/mesocosms with clean sediment and spiking the PPP in water may better allow determining its impact on an already established, undisturbed benthic community.
- Duration: Further information can be provided if the study is performed over a long period of time since some effects may be observable only long after exposure as shown for aquatic mesocosms (Woin, 1998 Liess and Beketov 2011, 2012). As already described in section 4.2.2, persistent PPPs that quickly sorb to sediments may have long-lasting effects on benthic invertebrates (see also Figure 2). The PPR Panel therefore advises to always include observations on long-term benthic population and community-level effects. The duration of the study needs to be long enough to cover the duration of the full life cycle of the relevant benthic species at risk.
- Exposure routes to consider and compartments to monitor for fate of the substance: Appropriate sampling of overlying water, sediment (not only bulk but also relevant sediment layers) to measure dynamics in PPP concentration in total sediment (e.g. in µg/g OC) and, when possible, pore water. If measuring in pore water is difficult, prediction on the basis of sediment characteristics and measured total PPP concentrations is also a possibility. For benthic invertebrates, it is proposed to assess the exposure concentration in the upper 1 cm and for rooted plants in the upper 5 cm, and the concentration in this sediment layer should be used to express the concentration–response relationship for sediment organisms.

Owing to the fact that, until now, effect concentrations of micro-/mesocosm tests are usually expressed in terms of (initial) concentrations in the water column and not in terms of sediment concentrations, it is difficult to perform an appropriate sediment risk assessment. Note that in the end, RAC_{sed} values have to be compared with the PEC_{sed}. In conclusion, if adopting a water-spiked design to study the impact of PPP exposure on benthic organisms this implies that for a proper sediment effect assessment the dynamics in exposure concentrations in the upper 1 cm of the sediment compartment have to be monitored for benthic invertebrates. For rooted macrophytes a deeper sediment layer (5 cm) may be appropriate.

8.2.3.5. Evaluating effects and interpreting results

Comprehensive information is provided in the AGD (EFSA PPR Panel, 2013, sections 9.3.2 and 9.3.3).

The sediment risk assessment focuses on assessment of chronic toxicity (see section 2.3 on specific protection goal options for benthic organisms). In the AGD (EFSA PPR Panel, 2013), effect assessment schemes for both the ETO and the ERO are provided. In principle, both options can also be considered in sediment ERA. However, in the ERO option as operationalised in the AGD (EFSA PPR, 2013) the effects on the most sensitive measurement endpoint should not be longer than eight weeks. Thus, because of the chronic exposure regimes in sediment, the recovery option most likely is less feasible since the chance is high that when effects occur they will be long term. Guidance for the evaluation of concentration–response relationships of populations and communities statistically (using, for example, multivariate analysis) and ecologically (using, for example, traits (e.g. Liess and Beketov, 2011) can be found the AGD (EFSA PPR Panel, 2013, section 9.3.2.5). Guidance for interpretation of concentration–response relationships by means of effect classes can be found in section 9.3.5.3 of the AGD (EFSA PPR Panel, 2013). Information on how knowledge on minimum detectable differences might be used in the interpretation of treatment-related effects of PPPs in micro-/mesocosm experiments can be found in Brock et al. (2015).

8.2.3.6. Further considerations to perform micro-/mesocosm studies for sediment risk assessment

8.2.3.7. Simulating remobilisation

Some substances can be both persistent in sediment and subject to remobilisation under some circumstances which may exert an increased toxicity to water or epibenthic organisms (but may also be considered a local dissipation route for benthic species on the longer-term), especially in flowing waters. Knowledge on potential effects of increased exposure due to remobilised sediment is lacking and may be important for stream communities in particular. In principle, it is possible to study this phenomenon in appropriately designed stream mesocosms.

8.2.3.8. Risk refinements using micro-/mesocosms with contaminated sediment

First tier studies for surface water ERA do not include sediment and usually consider worst-case exposure conditions (e.g. constant exposure); therefore, a large component of the refinements in the tiered approach is attributed to decreased exposure to the compound when using micro-/mesocosms, in particular because of the presence of sediment. This may not be the case for sediment risk assessment if the compound does not degrade in sediment and/or its bioavailability does not substantially decrease in time. Note that in sediment ERA, sediment is present in all tiers, including the Tier 1 laboratory tests, and that the focus of sediment ERA is on chronic exposures. Thus, using micro-/mesocosms with contaminated sediment, the refinements possible in terms of exposure most likely are of a much lesser extent than in similar tests with a focus on water organisms and exposure in overlying water. However, sediment-spiked micro-/mesocosm tests may be required to calibrate/validate our lower tier approaches and they allow studying population-level effects (including possible recovery if a decrease in bioavailability, e.g. due to dissipation and ageing, plays a role).

8.2.4. Effect assessment on vertebrates in sediment

The effect assessment for vertebrates in sediment is only briefly mentioned here, as it is considered by the working group as an item for the future. Indeed, based on data requirements and current knowledge, it is not possible to propose/deliver, at this stage, a consolidated ERA scheme. However, we provide a few considerations below.

For vertebrates, sediment exposure may occur via different routes (contact, pore water, food and ingestion of particulates):

- The risk due to exposure to pore water may be estimated by considering (chronic) toxicity data for fish that address effects of water exposure and information on the duration of the period in which the vertebrate is in contact with sediment. In addition, the risk occurring by contact could be assessed in a first step by the EqP (see below), and if needed with, for example, the sediment contact assay for zebrafish eggs. However, few data are available at this stage on this test (DIN 38415-T6 further developed by Hollert et al., 2003) or on the amphibian *Rana pipiens* 10-day test (ASTM, 2013) as they are relatively new and not yet used in sediment

ERA for PPPs. Therefore, there is no information on the relevance of these tests with respect to the protectiveness for vertebrates. Further research is needed to validate these tests.

- The risk due to food intake can be addressed through bioaccumulation studies and with appropriate food web models (line for future research).
- The importance of the risk due to ingestion of sediment while feeding may be negligible but is not well known, although it is not the main route of exposure. In a first step, this risk is assessed by using the modified EqP. If it can demonstrated that the uptake of sediment particles is minor, the EqP as such could be used.

More research and analysis of data is needed to identify which exposure routes are most relevant, depending on aquatic vertebrate species and substances.

In case the aquatic risk assessment identifies fish or amphibians as the most sensitive group of organisms, assessing the risk of sediment-related fish species (e.g. eels) could be carried out using the existing tests on standard species living in surface water. The effect concentrations for water could thus be (i) compared with pore water concentrations ($PEC_{sed;pw}$) or (ii) recalculated into sediment concentration through the EqP approach (modified EqP for adult species, for example, or without the extra extrapolation factor of 10 for fish eggs, for example) and then compared with $PEC_{sed;tot}$. However, if this approach indicates an unacceptable risk, a more appropriate sediment risk assessment based on sediment-spiked toxicity tests could become necessary as for other groups of organisms. Indeed, there may be some circumstances where vertebrates should be tested in the sediment ERA. For example, if a substance accumulates in sediment and a chronic endpoint of fish tests drives the risk in surface waters, then an appropriate scheme and/or vertebrate testing for sediment would need to be performed, considering also animal welfare.

Further considerations on the sensitive life stages are also needed. If the aquatic risk assessment indicates that exposure before and during hatching is critical, then the results of a fish full life cycle (FFLC) test could be used (since eggs are exposed to the free water) for the sediment risk assessment. The $RAC_{sw;ch}$ thus obtained could be directly compared with the $PEC_{sed;pw}$, or recalculated into sediment concentration through the EqP and then compared with PEC_{sed} since the mechanism of uptake for eggs occurs only through membrane diffusion. When toxic effects are acting specifically on the first life stages but a FFLC test can/was not run, a toxicity test with fish egg (e.g. sediment contact assay with fish eggs) could be performed.

8.2.5. Effect assessment on sediment microorganisms

8.2.5.1. Effect assessment for microorganisms

There are as yet no standardised and validated tests for determining effects of chemical pesticides on heterotrophic microorganisms in sediments for use in prospective risk assessment (see section 2.3.2). Similar to the situation for vertebrates, based on data requirements and current knowledge, it is not possible to present a consolidated ERA scheme for sediment microorganisms. Regarding scientific investigations, widely different approaches are used for testing effects of pollutants on sediment microorganisms (Van Beelen, 2003; and references therein). Studies focusing on retrospective risk assessment employ laboratory tests with microorganisms for evaluating toxicity of polluted sediments (often using various biosensors, e.g. Farré and Barceló, 2003; Girotti et al., 2008) or monitor differences in microbial communities between polluted and unpolluted sediments (de Lipthay et al., 2003; Vezzuli et al., 2003; Schäfer et al., 2011). Information from such studies is useful when establishing sediment quality guidelines and pollutant concentration thresholds.

Test organisms can vary from single strain cultures of fungi or bacteria (Johnson et al., 2009; Dijksterhuis et al., 2011) to complex communities in field samples used in meso- or microcosm experiments (further discussed below), which have substantially higher environmental relevance. The single-species test approach is hampered by the quite low representativity of one species to the vast microbial geno- and phenotypic diversity of sediment systems. In addition, the overwhelming majority of microorganisms currently cannot be cultured as pure culture isolates, but rather can be studied only in the context of more or less complex natural communities (e.g. Wagner, 2004). In line with this, an ECHA report from a workshop on sediment ERA (ECHA, 2014), concluded that effects assessment should, when possible, evaluate the impact on the ecosystem/community structure, not on single species.

Another factor that has probably contributed to the better developed toxicity tests with single strains of photosynthetic aquatic microalgae (eucaryotes or cyanobacteria) than those with heterotrophic microorganisms is that tests with the former are more straight-forward, since it can reasonably be assumed that the organisms cannot directly degrade the test substance. In addition, it is easier to identify aquatic microalgae at the species level.

8.2.5.2. Investigating community-level properties of sediment microorganisms

An accumulating literature advocates that community-level tests using micro- or mesocosms of natural sediments offer the most environmentally relevant and resource-efficient way to assess effects of PPPs and other pollutants on microorganisms (Puglisi, 2012; Diepens et al., 2014a; ECHA, 2014). With this approach, longer-term chronic effects induced by pesticides can be determined, covering taxonomic and functional shifts in microbial communities. Endpoints in community level testing can be divided into those related to function (activity or processes), biomass (total biomass, or for taxonomic or functional groups) or structural properties (community structure or diversity). Diepens et al. (2014a) argue that a combination of endpoints relating to functioning (enzyme activity, functional genomics) and microbial composition (targeting ribosomal RNA) may offer a more complete overview of the effects of sediment-bound toxicants on microbial communities. Examples of such an approach are provided by Widenfalk et al. (2008a) and Dimitrov et al. (2014), who studied the responses of benthic microbial communities to pesticide exposure.

Measuring effects on community-level functions in laboratory microcosms can be comparatively straight-forward, following similar approaches as for the tests of effects of PPPs on microbial assemblages of sewage treatment processes and on microbial nitrogen transformation in soil, already included in the EU data requirements. Endpoints have included, for example, respiration, total bacterial production, potential (anaerobic) denitrification and potential (aerobic) nitrification (Svensson and Leonardsson, 1992; DeLorenzo et al., 1999; Laursen and Carlton, 1999; Enrich-Prast, 2006; Pesce et al., 2006; Milenkovski et al., 2010). It can be assumed that various steps and processes involved in nitrogen cycling and mineralisation are highly relevant endpoints for assessing effects of pollutants, since nitrogen is an essential nutrient but also since nitrogen transformation processes are vital in water purification in both the active sludge process and in constructed wetlands. Of the two most widely studied assays, potential nitrification is considered as generally more sensitive to pollutants (Pell et al., 1998), since the aerobic ammonia-oxidising bacteria mediating the first step is a comparatively small group of fastidious, slow-growing lithotrophs. For potential denitrification, on the other hand, the community response is dependent on a much larger fraction of the microbial community, since many different bacterial groups have the ability to denitrify. However, more research is needed to determine which functional endpoints of sediment microbial communities are most sensitive to exposure to PPPs.

Regarding endpoints for microbial community structure or diversity, several types of advanced molecular methods to describe structural as well as functional properties of microorganisms in their natural environments are available, and progress in this area is fast. One established method is PLFA analysis, where the PLFA profile of a sample gives a picture of the microbial community structure. Analyses of PLFA have been used to demonstrate changes in microbial community structure of sediments and stream water after pesticide exposure (Chinalia and Killham, 2006; Littlefield-Wyer et al., 2008; Widenfalk et al., 2008a). Nucleic acid-based molecular methods have higher potential to give detailed information on the structure and diversity of microbial communities and specific phylogenetic or functional groups. Thus, investigations of pesticide effects on aquatic microbial assemblages have employed denaturing or temperature gradient gel electrophoresis, terminal restriction fragment length polymorphism, fluorescence *in situ* hybridisation and, more recently, 454 pyrosequencing (Chinalia and Killham, 2006; Pesce et al., 2006; Stachowski-Haberkorn et al., 2008; Pesce et al., 2009; Tadonléké et al., 2009; Vercraene-Eairmal et al., 2010; Lin et al., 2012; Aguayo et al., 2014; Dimitrov et al., 2014).

Besides structural properties, modern metagenomic approaches can also provide information on functional properties of sediment microbes. For example, Fang et al. (2014) reported the relative abundance of various biodegradation genes (the number of specific biodegradation genes hit tags over the total number of tags in each sample) in freshwater and marine sediments.

8.2.5.3. Interpretation of microbial test outcomes

For several reasons, the translation of pollutant-related community-level effects into actual risk with respect to defined protection goals is still a challenge. To begin with, because chemical pesticides can induce inhibiting as well as stimulating effects in different microorganisms, it is not uncommon that addition of pesticides to sediments in mesocosm experiments results in increases in microbiological process parameters (see section 4.3). Thus, a no-effect outcome in a functional endpoint of a pesticide challenge to a sediment community is a net response, and not proof that no populations have been affected in either a negative or a positive way. Regarding measurements of chronic effects, functional redundancy—i.e. if organisms mediating a certain function is inhibited or eliminated, other organisms take over and fill the niche—can also obscure effects on sub-populations within functional groups and their processes, and thereby complicate the interpretation of test outcomes. In the specific protection goals for benthic microbes the defined ecological entity is functional group and the attribute is processes (see section 3.3).

Another confounding factor when interpreting micro- or mesocosm studies mimicking chronic exposure to pesticides is that the functional and structural microbial traits of unpolluted sediments are not constant over time and also varies substantially with type of sediment. Temporal variation in microbial parameters is caused by fluctuations in the input of potential substrates, presence of higher organisms (animals, macrophytes and algae) and chemical and physical conditions. The variation can be caused by human interventions as well, such as, for example, recurring clearance of ditches. The need for better knowledge and understanding regarding baseline, or reference conditions of sediment biota, i.e. the natural or pre-disturbance conditions, was emphasised in the ECHA report Principles for Environmental Risk Assessment of the Sediment Compartment (ECHA, 2014). This report concluded that ‘Even if a significant change in an ecological endpoint can be attributed to sediment contaminants, more thorough assessments are required to truly understand the potential consequences of this change to other components of the ecosystem’. Owing to the high background variation in sediment properties, for standardised testing, selection of the most relevant sediment type(s) is also an issue. Standardisation by using artificial sediment is not a satisfactory solution for community-based tests on microorganisms, since the community cannot be separated from the matrix of a natural sediment.

Interpretation of the outcomes of microbial community-based tests is generally more straight-forward for functional than for structural endpoints. While for the former, a rate decrease in, for example, nitrogen mineralisation or a specific enzymatic reaction can be considered as an adverse effect, for the latter, a changed structure pattern (e.g. disappearance of some operational taxonomic units but appearance of others) cannot be directly translated into potential risk. Similarly, the recent ECHA report (ECHA, 2014) on sediment ERA concluded that it is difficult to include properties relevant to the impact assessment (biodiversity, species richness, endemism, etc.) in prospective risk assessment, because of the variety of systems that need to be covered. This may be easier in retrospective risk assessment.

8.2.5.4. Conclusions and recommendations from ECHA workshop

Besides the conclusions and recommendations discussed previously in this section, of the proceedings from the ECHA workshop ‘Principles for Environmental Risk Assessment of the Sediment

Compartment' in May 2013 (ECHA, 2014), other main conclusions and recommendations of this report and with relevance for microbial ERA of PPPs were:

- Microbial communities are responsible for crucial ecosystem functions. 'However, toxicity testing with such communities poses a number of scientific challenges that need to be resolved by future research'. There is a need for sediment microbial effects guidelines/testing protocols.

- Microbial processes should be included in effect assessments for sediments.

- There is little scientific information available on the effects of contaminants on microbial functions or on the interaction between microbes, other organisms and contaminants in sediments. More research should be performed to evaluate the importance of including these types of tests and endpoints in a sediment risk assessment.

8.2.5.5. Possible strategies for introduction of sediment microbial tests

One possibility to strengthen the risk assessment coverage of interactions of PPPs with microbial communities could be to expand the tests on nitrogen mineralisation and on degradation rates of the PPP in soil to also include sediments. If the nitrogen mineralisation test in sediments does not fail and the substance is degraded in sediments, this would imply that the sediment microbial community retain fundamental traits related to biodegradative functions when challenged with the PPP. In that case it could be relevant to perform the tests under both aerobic and anaerobic conditions. Comparative studies in representative soils and sediments, with PPPs differing in toxic mode-of-action, are needed to determine whether introducing such tests for sediments can increase the level of protection.

The ISO quality standards that target microbial properties for determining soil and aquatic habitat quality are potentially useful in prospective risk assessment for sediments (see section 2.3.2). However, a common feature of these ISO standards is that they are 'instant', and designed primarily for retrospective risk assessment (comparison of test outcomes for different samples as a tool to estimate soil or aquatic environmental quality). Thus, for determining 'chronic' effects on microbial communities (including population shifts and indirect effects) in prospective risk assessment, additional development and standardisation are needed on how to conduct the longer-term micro- or mesocosm experiments that are needed.

8.2.5.6. Conclusions effect assessment microorganisms

In conclusion, although it is possible that tests on animals, plants and microalgae to some extent may also show the potential for adverse effects on microorganisms in sediments, there is no doubt that development of standardised tests of effects at least on functional microbial properties could improve the prospective risk assessment of PPPs. However, developing and introducing standardised test systems that are able to provide information that is sufficiently representative of the wide diversity of microorganisms, microbial processes and sediment habitats still involve several future challenges. It is still difficult to say whether microbial communities are more sensitive to PPPs than other organisms and when microbial tests are actually needed. Many of the questions that need to be answered are still mainly a research activity and Deneer et al. (2013) concluded that it has to be discussed with risk managers whether the potential risks of pesticide exposure to aquatic microorganisms need to be specifically addressed by introduction of additional tests.

9. Linking exposure to effects in sediment ERA

9.1. Introduction

A crucial step in the ERA for sediment organisms is the linking of the PEC_{sed} estimates (Chapter 7) to the RAC_{sed} estimates (Chapter 8). In Chapter 6, dealing with the ERCs for sediment ERA, it is suggested to select not only the pore water concentration but also the total sediment concentration as a metric in the PEC_{sed} and RAC_{sed} estimates. If using pore water as metric, $PEC_{sed;pw}$ and $RAC_{sed;pw}$ values expressed in micrograms per litre are obtained. When expressing the PEC_{sed} and RAC_{sed} in terms of total sediment concentration, it is recommended normalising the concentration either to a specific OC content of the sediment, such as the standard OECD sediment ($PEC_{sed;tot}$ and $RAC_{sed;tot}$ in µg/kg dry weight sediment), or to express this concentration in terms of OC content of the sediment ($PEC_{sed;oc}$ and $RAC_{sed;oc}$ in µg/g OC in dry sediment). In addition, it is suggested to select the 0–1 cm, and 0–5 cm sediment layers when deriving field exposure estimates, and to use the PPP concentration of the 0–1 cm sediment layer to obtain realistic worst-case $PEC_{sed;pw}$, $PEC_{sed;tot}$ or $PEC_{sed;oc}$ estimates for benthic invertebrates, while that of the 0–5 cm layer may be used in ERAs for rooted macrophytes. Since rooted macrophytes do not consume sediment the $PEC_{sed;pw}$ and $RAC_{sed;pw}$ estimates seem most relevant for these plants. Furthermore, the focus is on a chronic sediment ERA, since it is triggered when a longer-term exposure of the PPP is expected in the sediment compartment. The appropriate sediment layer for the $PEC_{sed;pw}$ estimate for microorganisms is a research activity to date. For the time being the 0–1 cm sediment layer is proposed.

9.2. When to use the peak or TWA PEC in sediment ERA

In principle, for a PEC_{sed} estimate in chronic ERA, either the peak concentration (maximum) or the TWA concentration in (normalised) total sediment and/or pore water can be used to compare with a RAC_{sed} (expressed in terms of a comparable exposure metric). In the text below, when referring to PEC_{sed} and RAC_{sed} estimates, this may be either for pore water or the concentration in total sediment (normalised to a fixed OC content or expressed in terms of sediment OC content).

In ERA the $PEC_{sed;max}$ or $PEC_{sed;twa}$ should be lower than the RAC_{sed}. In Chapter 6 it is recommended that the effect estimate derived from sediment toxicity tests should be expressed in terms of TWA or mean exposure concentrations during the test. However, in current sediment toxicity tests the effect estimate (such as EC10 and NOEC) is usually expressed in terms of initial exposure concentration. When the effect estimate is expressed in terms of initial exposure concentration, it should be plausible that the exposure profile in the toxicity test is realistic worst-case relative to that in the field, otherwise these effect estimates cannot be directly used in ERA. Furthermore, if the effect estimates on which the RAC_{sed} is based are expressed in terms of the initial test concentration, it is recommended that the $PEC_{sed;max}$ concentration should be used in ERA to assure a more realistic worst-case risk assessment.

If using the $PEC_{sed;twa}$ in the risk assessment, the time window for this field exposure estimate should be equal to or shorter than the time window for the chronic effect estimate that drives the risk (i.e. the duration of tests delivering the critical chronic EC10 values that drive the RAC_{sed}). In addition, proof of reciprocity in longer-term toxicity tests should be provided in order to use the $PEC_{sed;twa}$ in the risk assessment. Reciprocity refers to Haber's law, which assumes that toxicity depends on the product of concentration and time. Note that if the PPP is relatively persistent in the sediment compartment, which usually is the case when a sediment ERA is triggered, the $PEC_{sed;max}$ and the $PEC_{sed;twa}$ will not differ much. Consequently, a pragmatic approach might be to consider the use of the $PEC_{sed;max}$ in sediment ERA as a default procedure, and to consider the use of the $PEC_{sed;twa}$ only if field exposure concentrations are demonstrated to be sufficiently variable during a time frame smaller than the duration of the sediment-spiked toxicity test that drives the RAC_{sed}.

9.3. How to link the PEC_{sed} of the current FOCUS approach to the RAC_{sed} derived from different tiers

Currently, the PEC_{sed} derivation for sediment ERA is based on the FOCUS methodology adjusted for multi-year sediment accumulation (Chapter 7). The FOCUS exposure scenario is constructed to allow a realistic worst-case exposure assessment for pelagic organisms, but not necessarily for benthic organisms. In addition, the FOCUS exposure scenario is characterised by a sediment compartment with a 5 % OC content. In contrast the OECD standard sediment used in Tier 1 sediment-spiked toxicity tests is characterised by a 5 % organic matter content, corresponding to approximately 2.5 % OC.

On basis of the EqP theory the predicted/measured exposure in sediment with a higher OC content will be lower in pore water, but higher in total sediment. Consequently, the $PEC_{sed;pw}$ estimate using the FOCUS exposure scenario is, relatively, a best case when compared with the $RAC_{sed;pw}$ estimate based on standard OECD sediment-spiked toxicity test, but the $PEC_{sed;tot}$ estimate using FOCUS is, relatively, a worst case when compared with the $RAC_{sed;tot}$ estimate using standard OECD tests. A way forward would be to develop two types of scenarios, one with low OC (worst-case pore-water (pw) scenario) and one with high OC (worst-case total content scenario). These sediment scenarios should be developed as an integral part of the environmental scenarios for the edge-of-field surface waters. Note, however, that toxicity values for PPPs derived from sediment-spiked toxicity tests as reported in the literature and in PPP dossiers predominantly are expressed in terms of total sediment concentrations and rarely in terms of pore water concentrations (see e.g. Deneer et al., 2013). Consequently, in the majority of sediment ERAs for PPPs the $PEC_{sed;tot}$ and the $RAC_{sed;tot}$ are used, which seems to result in a relatively conservative approach. From a scientific view it may be argued that in the sediment ERA of individual PPPs the same metric should be used in both the exposure and effect estimate, e.g. by normalising the PEC_{sed} and RAC_{sed} (e.g. by expressing these values in terms of μg/g OC in dry sediment).

Another potential problem in linking of $PEC_{sed;tot}$ estimates to $RAC_{sed;tot}$ estimates is the observation that the bioavailable fraction of a PPP that is persistent in the sediment compartment may decrease in time because of ageing (see Figure 1). Since the PEC_{sed} estimate as proposed to use in sediment ERA (see Chapter 7) considers accumulation in sediment due to multi-year use of the PPP, the fraction of the PPP that has been present in the sediment compartment for longer time may be characterised by a relatively low bioavailability, while the fraction recently adsorbed to the sediment may be more bioavailable. In contrast, in laboratory toxicity tests with benthic organisms, the time between spiking of sediment and starting the test usually is no longer than 7–10 days. If the relative contribution of the older (e.g. > 1 year) and recent fractions (e.g. latest growing season) in the $PEC_{sed;tot}$ is calculated this knowledge might be considered in a higher tier by (1) using refined-exposure toxicity tests by spiking the sediment in different phases and allowing different ageing periods for the different fractions before using the sediment in sediment-spiked toxicity tests or (2) using appropriate modelling approaches to better estimate the bioavailable fraction of the $PEC_{sed;tot}$ estimate. The experimental approach to address ageing of persistent PPPs in the sediment is a very costly and labour-intensive approach, particularly in higher-tier effect assessments, e.g. SSD approach or sediment-spiked microcosm tests. The modelling approach to address ageing of persistent PPPs in sediment, therefore, seems more promising in prospective sediment ERA. In order to develop and verify these models, however, experiments will be required.

In conclusion, adopting the adjusted FOCUS approach, as described in Chapter 7, to estimate the $PEC_{sed;tot}$ and linking this estimate to the $RAC_{sed;tot}$ derived on basis of standard sediment-spiked OECD toxicity tests will result in a, relatively, worst-case ERA for individual PPPs. Nevertheless, normalising the PEC_{sed} and RAC_{sed} estimates to a specific sediment OC content is highly recommended. In the near future it seems necessary to develop environmental scenarios for ponds, ditches and streams to better integrate the physico-chemical and biological properties important for exposure and effect assessment, and to assure that the ERA for pelagic organisms is not in conflict with that for benthic organisms in the sediment compartment of the same system.

10. Metabolites risk assessment and mixture toxicity for sediment

10.1. How to link the PEC_{sed} to the RAC_{sed} derived for metabolites

10.1.1. Introduction

In this section metabolites and degradation products are referred to as residues.

In principle, the risk to sediment dwellers from exposure to residues can be addressed in the same way as required for the parent compound (see Chapter 5). However, there are several questions that need to be addressed before the decision can be made to conduct a sediment ERA for residues. For example, important questions are: 'When is a risk assessment for metabolites in the sediment compartment needed?' and 'Is the risk from metabolites addressed by the risk assessment of the parent active substance?'

To address these questions, the PPR Panel is of the opinion that the risk assessment of residues in the sediment compartment should follow the same approach as the risk assessment of residues in the water compartment and thus readers are referred to the AGD (EFSA PPR Panel, 2013). The AGD states that for metabolites, that are formed and/or accumulate in the sediment, which at any time account for more than 10 % of the parent compound added to the water–sediment system, or between 5 and 10 % at two or more occasions, or more than 5 % at the end of the study, an ERA for the metabolite is always required. For a more detailed definition of metabolites, and what an ecotoxicologically relevant metabolite is, please refer to section 10.2 in the AGD (EFSA PPR Panel, 2013). The difference between effect assessments for water and sediment metabolites mainly relates to the character of the test data available for sediment species, e.g. duration of tests (see section 8 on effect assessment).

The outline for assessing the environmental risk of individual metabolites is described below. The assessment of environmental risks of metabolites that are formed and/or accumulate simultaneously in sediment possibly along with the original parent substance should be addressed following the advice regarding combined toxicity.

10.1.2. Non-testing methods and the definition of toxophores

In order to minimise the need for testing that may be unnecessary, it is allowed to estimate metabolite toxicity using non-testing methods. However, this approach requires an assessment of the presence of the toxophore, i.e. an assessment if the active part (toxophore) of the molecule is present or not. Please refer to section 10.2.3 in the AGD (EFSA PPR Panel, 2013) for details. It is noted, that the non-testing approach for deriving toxicity data for relevant sediment species is hampered by the lack of valid models.

10.1.3. Exposure assessment

As for the parent compound, exposures from relevant residues are calculated using the current FOCUS methodology, adjusted for multi-year sediment accumulation (see Chapter 7). For many metabolites experimentally determined physico-chemical properties will not be available. Application of reviewed QSARs and read-across methods is recommended in this case. Please refer to section 10.1 in the AGD (EFSA PPR Panel, 2013) for details. Derivation of proper (bio)degradation rate constants of metabolites is expected to be a considerable source of uncertainty.

10.1.4. Effect assessment

The same decision scheme as proposed for the parent substance (see Chapter 8) to select the appropriate benthic test species is proposed for the effect assessment of relevant residues. However, please note that fewer ecotoxicity data are available for metabolites and often these data only comprise acute toxicity tests for standard pelagic aquatic organisms. If, for the same metabolite, an ERA is performed for the water compartment, the available data from the pelagic organisms may be used to assess the RAC for sediment organisms by using the (modified) EqP method (dependent on toxic mode-of-action). Alternatively, these data can be used in order to decide on additional and potentially most sensitive, standard sediment species that should be tested. If sediment toxicity data is available for the parent this should also be considered in selecting the test species for the metabolite.

It should also be assessed if the metabolite appears in sufficient quantity in the sediment toxicity test with the parent compound so that the test may also be adequate for assessing the potential effect of the metabolite. Guidance on this assessment is found in section 10.2.5 of the AGD (EFSA PPR Panel, 2013), with the exception on guidance related to degradation by photolysis, as this is not relevant for the sediment compartment.

If in the sediment toxicity test with the parent compound the metabolite is formed in quantities of more than 5 % of the parent compound (in terms of mean concentration over the test), the responses in the toxicity test may also be expressed in terms of the mean concentration of the metabolite. This toxicity value may be used as a worst-case estimate in sediment ERA of the metabolite. If the potential effects of the metabolite are not sufficiently addressed by the effect assessment of the parent compound and the toxophore is still present in the metabolite, or it is unclear if the toxophore is present in the metabolite, then one chronic ecotoxicity test with the metabolite and a relevant benthic test species should be performed. This species may be selected based on information on the most sensitive pelagic species identified from the parent substance ERA (see Chapter 8). If by following this procedure, potential risks to sediment-dwelling organisms are identified, higher-tier refinements may be considered (see Chapter 8).

If an assessment indicates that the toxophore is no longer present (see section 10.2.3 in the AGD (EFSA PPR, 2013)) it, in the first instance, may be assumed that the chronic toxicity of the metabolite is equal to the toxicity of the parent compound for all first tier taxonomic groups of benthic organisms. In this way the risk can be assessed using parent compound RAC_{sed} data and metabolite exposure data for the sediment compartment. If this procedure triggers potential risks to benthic organisms, either adequate non-testing methods may be used to estimate effects (see section 10.2.8 in the AGD (EFSA PPR Panel, 2013)) or, if no adequate non-testing methods are available, the RAC_{sed} may be estimated (i) using available toxicity data for pelagic test species (using, dependent on the toxic mode-of-action of the compound, the EqP or modified EqP approach) or (ii) the RAC_{sed} may be based on a sediment-spiked chronic ecotoxicity test with the most relevant standard benthic test species (informed by the most sensitive pelagic species identified from the parent substance risk assessment (see Chapter 8)). If by following these procedures potential risks to sediment-dwelling organisms are triggered, higher-tier refinements may be considered (see Chapter 8).

10.1.5. Linking exposure to effects in sediment ERA

If preliminary ERAs indicate potential concerns then, as for parent molecules, risk refinement is possible either by refining effect concentrations or by refinement of the exposure concentration.

If higher-tier studies have been conducted with the a.s., or a relevant formulation, these studies may also have assessed the risk from the metabolites. It is advised that if a higher-tier study, for example, a mesocosm study is being carried out, appropriate chemical analysis of both the parent and their metabolites should be conducted so that an assessment of both the exposure and effects of any metabolites can be made.

For exposure refinements, please see Chapter 7.

10.1.6. Other issues

The environmental risk for bioaccumulation, biomagnification and secondary poisoning of the metabolites in sediment should also be addressed if the log K_{ow} trigger is exceeded (see section 8.1). If valid non-testing methods exist, calculated log K_{ow} estimates are accepted in order to assess effects from secondary poisoning (see AGD (EFSA PPR Panel, 2013) Please note that in most cases metabolites are expected to more polar than the parent compound (Boxall et al., 2004).

10.2. Considering toxicity of mixtures in the sediment ERA

10.2.1. Introduction

The Regulation (EC) No 1107/2009 requires in Article 29 that ‘interaction between the active substance, safeners, synergists and co-formulants shall be taken into account’ in the evaluation and authorisation. This explicitly refers to marketed PPPs, which are, by origin, technical mixtures containing one to several a.s., plus, typically, several co-formulants. Furthermore, the standard data requirements for PPPs (Commission Regulation (EU) No 284/2013) do request ‘any information on potentially unacceptable effects of the plant protection product on the environment, on plants and plant products shall be included as well as known and expected cumulative and synergistic effects’. Furthermore, in the ‘uniform principles’ as laid down in Regulation (EC) No 546/2011 it is required that Member States base their authorisation decision on the ‘proposed conditions for the use of the plant protection product’. While the AGD (EFSA PPR Panel, 2013) has addressed this issue for pelagic organisms, the focus in this section, is on addressing the risk to benthic organisms from combined toxicity. Further developments and recommendations on how to address ERA of mixtures are found in the scientific opinion on terrestrial plants (EFSA PPR Panel, 2014).

Where possible the risk assessment of combined toxicity in the sediment compartment may follow the same approach as the risk assessment of combined toxicity in the water compartment, i.e. focusing on chronic assessments. For further details, refer to the AGD (EFSA PPR Panel, 2013).

Products are normally segregated before they reach the sediment compartment, as the different components have different dissipation pattern before they reach the sediment. Once substances reach the sediment compartment, natural processes, such as sedimentation, bioturbation and substance diffusion, will cause the substances to be buried in the sediment. Often, the biological degradation of buried substances will be impaired by the lack of oxygen in the deeper layers of the sediment. This is the reason that sediment often act as a sink for persistent substances including metabolites, although their bioavailability may decrease in time because of ageing (see Chapter 3).

In line with the single substance ERA in the sediment compartment, the assessment of environmental risks in sediment from combined toxicity will focus on long-term effects (see Chapter 6).

Ideally, ERAs of combined toxicity of substances in sediment address all concerns, including multiple stress due to historical pollution and simultaneous and successive application of different PPPs (e.g. informed by plant protection programmes characterised by intensive PPP use and the fate and behaviour of the individual substances in sediment). However, the number and character of pollutants in sediment varies and is often unknown. Moreover, the additional risk from this pollution may be affected by changes in bioavailability because of processes such as ageing, bioturbation and stratification, re-suspension and transport of sediments in flowing waters and clearance of edge-of-field surface waters (e.g. the periodical removal of the upper sediment layer of ditches). Other environmental stressors (e.g. hydrodynamic stress and oxygen depletion due to eutrophication) may also affect the potential impacts of exposure to PPPs.

The lack of knowledge regarding the presence and bioavailability of pollutants in the sediment compartment of edge-of-field surface waters makes it difficult to assess the potential combined effects on benthic organisms of new and existing pollution. Consequently, more information is needed in order to take account of possible consequences of multiple stressors in the prospective sediment ERA for PPPs. To this end, it should be noted that a more holistic catchment based ERA for PPP’s may be required in prospective ERA (e.g. EFSA SC, 2016).

Note that this scientific opinion has its focus on prospective ERA procedures for sediment organisms in edge-of-field surface waters to support the science behind Regulation (EC) No 1107/2009 which focus on risk assessment of single substances and related products. Consequently, the proposed assessment of risk of combined products (as described below) will not include other types of pollutants, but only address the risk from PPPs under evaluation according to good agricultural practice used.

10.2.2. Exposure assessment

In a first screening step sediment exposure estimates of single compounds/active substances (PEC_i) are calculated using the current FOCUS methodology, adjusted for multi-year sediment accumulation (see Chapter 7). For PPPs that contain more active ingredients and/or the ERA should focus on more toxic substances (e.g. active ingredient and major metabolites), the total exposure concentration of the mixture (PEC_{mix}) is, in this first step, calculated as the simple sum of the PEC_i values of the n (number of components) individual components (per default: a.s.) by:

$$PEC_{mix} = \sum_{i=1}^{n} PEC_i$$

It should be carefully checked whether metabolites of (eco)toxicological relevance have to be included into the PEC_{mix} or not, or if any safeners, synergists and co-formulates are relevant. For an initial screening approach, it is assumed that the maximum PEC_{sed} of all a.s. present in the formulation will occur at the same moment and are not separated in time (i.e. worst-case PEC_{mix}). In a subsequent step, more detailed consideration of the predicted exposure patterns in time can be undertaken to identify a more 'realistic worst-case' PEC_{mix} decisive for a refined mixture ERA, i.e. PEC_{mix} will change over time and that can be reflected in the risk assessment. For exposure refinements, please see Chapter 10 in the AGD (EFSA PPR Panel, 2013).

10.2.3. Effect assessment

First it has to be assessed which sediment toxicity data are available for a given product and its active ingredients that can be used in a product risk assessment. As product sediment toxicity data seldom will be available (e.g. sediment-spiked toxicity tests with *Chironomus riparius*), it may often be the case that sediment ERA of the combined toxicity of active ingredients within the same product is addressed by a 'calculated mixture toxicity' approach as detailed in section 10.3.3 of the AGD (EFSA PPR Panel, 2013). If, however, product sediment toxicity data are available, these data should also be used in a risk assessment (see below).

In accordance with the recommendations in the AGD (EFSA PPR Panel, 2013) on how to calculate mixture toxicity, the concentration addition model (CA model) is proposed here[17]:

$$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

Equation x

where:

n: number of mixture components

i: index from 1…n mixture components

pi: the Ith component as a relative fraction of the mixture composition (note: Σ pi must be 1)

[17] If use of independent action for mixture toxicity calculation is required (please see AGD (EFSA PPR, 2013) for more guidance).

ECxi: concentration of component i provoking x% effect (pragmatically, NOECi may be inserted, too).

The general guidance provided in sections 10.3.8 and 10.3.11 of the AGD (EFSA PPR Panel, 2013) in order to decide on the suitability of a risk assessment based on calculated mixture toxicity is also recommended for sediment risk assessment. In view of the typically scarce availability of sediment chronic toxicity studies with formulations (of more than one a.s.), a higher uncertainty regarding potential synergistic effects is obvious. The counter-checking calculated and measured mixture toxicity as suggested in sections 10.3.4 and 10.3.11 of the AGD (EFSA PPR Panel, 2013) might be possible only in rare cases. It is therefore recommended to carefully conduct this exercise based on available toxicity data (for the given formulation and the a.s. comprised therein) for pelagic species. If there are no indications for obvious synergistic effects, a sediment risk assessment based on calculated mixture toxicity is considered adequate.

Further, the CA model can make use of another endpoint (e.g. ECx, NOEC) from existing data by use of the concept of TUs[18]. As detailed in sections 10.3.7 and 10.3.11 of the AGD (EFSA PPR Panel, 2013), using the concept of TUs is recommended in order to identify the most sensitive group of organisms and/or identify ‘drivers’ of mixture toxicity/risk (i.e. the toxicity of the mixture is largely explained by the toxicity of a single a.s.). The TU of a mixture has been defined as the sum of TU of each individual chemical of that mixture:

$$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

It should be emphasised that for both calculating the ECx–CA or applying the TU approach, calculations should refer to the same endpoint of the same test species. For risk assessment, the most sensitive standard test species should be selected for the ERA and the normal AF of 10 should be applied to derive a RAC_{sed} for the given mixture.

The combined toxicity of a product approach may be based on the (modified) EqP approach (dependent on the toxic mode-of-action and the organisms at risk) as this method is valid for all single components of the combined assessment (see Chapter 5). If a risk assessment based on mixture toxicity using the (modified) EqP approach indicates a risk from an intended use, a refined risk assessment using the most critical effect endpoint from all sediment effect studies available for each relevant substance is required. In the situation that no sediment data exist for an active substance it may be an option to request new experimental data (see Table 14).

If a refined assessment based on existing or new active substance data cannot address the risk from combined toxicity for a given group of organisms, sediment toxicity studies with the product may have to be generated. It is recommended to follow the same decision scheme as for the actives substances (see Chapter 8), by assessing the product data from the water compartment (mainly acute toxicity data) in order to decide on which type of organisms should be tested in order to address the risk. If a Tier 1 effect assessment is insufficient to address the risk, a Tier 2 effect assessment (e.g. WoE approaches or SSD) or Tier 3 mesocosm study may be conducted (in line with Chapter 8 of this scientific opinion). The recommendations provided in section 10.3.8 of the AGD (EFSA PPR Panel, 2013) for a mixture risk assessment based on the ‘calculated mixture toxicity’ using higher-tier data should be followed accordingly.

[18] “the ratio between the concentration (i.e. c_i) of a mixture component and its toxicological acute (e.g. EC50) or chronic (e.g. long-term NOEC) endpoint”.

10.2.4. Linking exposure to effects in sediment ERA

The proposed procedure for the assessment of risk to sediment living organisms from a PPP reflects the availability of relevant data and is in accordance with the procedure in the AGD (10.3.8)) (EFSA PPR Panel, 2013) both regarding 'Simplified approaches for mixture risk assessment' and 'Risk assessment based on calculated mixture toxicity'. It is recommended to follow the decision scheme in section 10.3.11 of the AGD, considering the sediment focus (long-term effect data, etc.), i.e. the ERA will often be based on TU.

11. Addressing uncertainties

As stated in the AGD (EFSA PPR Panel, 2013), uncertainties should be addressed at different stages of the risk assessment, i.e. when (i) refining a risk assessment, (ii) characterising the overall risk and (iii) extrapolating to field situations. These aspects have been well described in the AGD and are summarised below in sections 11.1 to 11.3. However, it should be added that for the sediment risk assessment, the consistency of the tiered approach is unclear since the requirement that lower tiers are more conservative than higher tiers has not yet been evaluated. The internal consistency of the tiered approach should be evaluated for substances that differ in toxic mode-of-action.

It should be kept in mind that, according to Article 1(4) of the EC 1107/2009, 'Member States shall not be prevented from applying the precautionary principle where there is scientific uncertainty as to the risks with regard to... the environment posed by the plant protection products to be authorised in their territory'.

11.1. Approaches for characterising uncertainty in higher-tier assessments

Regulation (EC) No 1107/2009 lists under Annex II criteria for approval of a.s., safeners and synergists under 3.8 Ecotoxicology, point 3.8.1 '...The assessment must take into account the severity of effects, the uncertainty of the data, and the number of organisms groups...'. Uncertainties in the data must be thus considered.

The AGD (EFSA PPR Panel, 2013) also reports: 'Regulation (EC) No 546/2011 states that no authorisation shall be granted unless it is clearly established that no unacceptable impact occurs. The term 'clearly established' implies a requirement for some degree of certainty. First tier assessments use standardised scenarios and decision rules which are designed to provide an appropriate degree of certainty, but this assumption has to be evaluated. Higher-tier assessments are less standardised, and so the degree of certainty they provide has to be evaluated case-by-case.' In sediment ERA, however, Tier 1 effect assessments have not yet been calibrated, neither with higher-tier nor with field information. Consequently, it is uncertain whether the Tier 1 procedure provides sufficient protection, despite its standardisation, i.e. it is unclear if the selected standard test species and test protocols in combination with the Tier1 AF are sufficient to guarantee no unacceptable impact.

Methods for characterising uncertainty can be grouped into two main types: qualitative and quantitative methods:

- Qualitative evaluation of the uncertainties affecting the outcomes should be performed on every tier of the effect and exposure assessments, using a systematic tabular approach (e.g. as in Table 40 of the AGD (EFSA PPR Panel, 2013)). In assessments with multiple lines of evidence, the uncertainties affecting each line of evidence should be evaluated separately. As well as evaluating each individual source of uncertainty, it is also essential to give an indication of their combined effect.
- Quantitative evaluation should be performed in cases where qualitative evaluation of uncertainty is not sufficient to determine whether it is clearly established that no unacceptable impact will occur. Such quantitative methods can be either deterministic (i.e. that generate deterministic quantitative estimates of impact for a range of possible scenarios) or probabilistic (i.e. that give numeric estimates of the probabilities of different outcomes).

11.2. Risk characterisation and weight-of-evidence assessment

Risk characterisation is the final step of ERA. At this point, all relevant information or lines of evidence that have been gathered are used to produce an overall characterisation or description of the risk. This will further require an evaluation of the uncertainties associated with each line of evidence. A systematic tabular approach (e.g. as in Table 41 from the AGD (EFSA PPR Panel, 2013)) can be used to do so. If the overall characterisation is expressed qualitatively rather than quantitatively, great care should be taken to describe the outcome and its uncertainty as clearly as possible.

11.3. Uncertainties in extrapolating to real field situations

Some sources of uncertainties are not considered in the previous section. These include the uncertainty related, for example, to (i) simultaneous exposure to different pesticides, (ii) multi-year sequential pesticide exposure to different PPPs, (iii) combined effects between the PPP and environmental stressors as hydrodynamic stress and, for example, unfavourable temperature and (iv) sensitive and vulnerable benthic species that are generally not used in test systems as they are difficult to culture. The uncertainties mentioned here are relevant for all tiers in ERA and need to be considered when extrapolating test procedures.

11.4. Uncertainties more specific for sediment risk assessment

Some types of uncertainties in the aquatic ERA are listed in Appendix G of the AGD, which presents three examples of qualitative assessment for uncertainties in Tiers 1, 2 and 3. Listed below are some uncertainties that are more specific for sediment risk assessment.

11.4.1. Examples of uncertainties related to the testing for ecotoxicological effects

The main categories of uncertainties in sediment ERA concern:

- Over- or underestimation of effects depending on type of sediment (artificial vs. natural) and food in test: Effects can be overestimated when the tests are performed with artificial sediment (as the degradation of the substance will be lower than in natural sediment) unless the effect concentrations are expressed as mean concentrations during the test; artificial sediment may also represent a more stressful environment (e.g. lower microbial activity), which may overestimate the toxic effects. Effects can be underestimated if food provided to the test organisms is not spiked with the test compound.
- Identification of vulnerable key species for each relevant taxonomic group: Many benthic taxa have a high plasticity, fulfil a variety of functions and their vulnerability might change depending on their life stage.
- Sediment-spiked test with rooted macrophytes: To date little experience is available in the conduct of sediment-spiked toxicity tests with rooted macrophytes. Whether the EC50 value of a 7–14 day tests with *Myriophyllum* and the application of an AF of 10 provides a sufficient level of protection for rooted macrophytes is uncertain. There are indications that test duration should be longer since uptake of PPPs by roots may be a much slower process than uptake by shoots.
- Relevance of selected standard test species for PPPs that differ in toxic mode-of-action: When testing sediment exposure of PPPs, the relevance of benthic oligochaetes for PPPs in general and of *Chironomus* for substances other than insecticides is questionable.
- Representativity of the standard test species: The representativity of the test species for the field communities in terms of sensitivity and vulnerability should be explored.
- Semi-chronic toxicity data: Appropriate extrapolation of semi-chronic to chronic data, e.g. 10-day LC50 to 28-day NOEC, needs to be evaluated for a wider array of benthic organisms and PPPs.

- Relative importance of different exposure routes for different groups of organisms: The contribution of water exposure (e.g. freely dissolved fraction in pore water or surface water for epibenthic species) and dietary exposure of sediment-associated PPPs is difficult to assess experimentally in sediment toxicity tests.
- Data availability: Limited ecotoxicological information and/or tests are available for an appropriate sediment risk assessment for benthic invertebrates and rooted macrophytes and even more so for benthic vertebrates and microorganisms (also for evaluating the validity of the tiered approach):
  - for benthic vertebrates very few official guidelines are available and these are hardly used;
  - for microorganisms, what is the sensitivity of different phylogenetic and functional groups in sediments to PPPs, also compared with the sensitivity of other commonly tested organisms? When are microbial tests actually needed to ensure protection?

Other uncertainties related to sediment ERA concern:

- For invertebrates, the top 1 cm layer is selected as relevant layer of sediment for PEC_{sed}. However, for epibenthic species the highly contaminated thinner top layer (e.g. 1 or 2 mm) may be of higher relevance, although such sharp gradient of contamination may be present for only a short duration in natural sediments;
- lipid normalisation in various types of organisms for bioaccumulation testing;
- relevant triggers for the persistence and Koc to perform bioaccumulation tests (based on a theoretical exercise (Appendix 1);
- the extrapolation from information on BCF in fish to BAF/BSAF in benthic invertebrates. Invertebrates tend to a lower capacity for biotransformation. These uncertainties are further related to gaps in knowledge with respect to bioavailability and toxicokinetics and need to be addressed in the further development of guidance for sediment ERA;
- contribution of the toxicity because of parent compound and metabolites in chronic tests and long-term contamination;
- consequences of differences in design in test guidelines (e.g. OECD and ASTM test protocols) for comparability of results;
- ageing, sedimentation and dynamics in bioavailability/bioaccessibility of PPPs in sediments, and consequences for effect and exposure assessments;
- which sub-lethal endpoints to select in chronic sediment toxicity tests with additional test species (WoE and SSD approach);
- underestimation of potential food chain transfer and secondary poisoning in sensitive predatory species;
- analytical uncertainties in terms of accuracy and precision in measuring pore water and total sediment concentrations;
- differences in affinity of PPPs to different types of organic matter/sediment.

11.4.2. Examples of uncertainties related to the extrapolation of effects to the field situation

The following points indicate that a number of uncertainties will underestimate the real risks in the field, e.g.:

- Field contamination by pesticides other than the pesticide under consideration, e.g. 'historical' background and contamination due to simultaneous or successive applications of various pesticides (i.e. mixture toxicity), while in prospective sediment ERA the focus usually is on the environmental risks of single substances.

- Benthic organisms may be affected by multiple stressors (e.g. a substance combined with environmental stressors), while in prospective sediment ERA the focus usually is on the environmental risks of the single substance rather than on multiple stressors.
- The test guidelines (OECD Guidelines 218 and 219; OECD, 2004a, b) recommend to expose the organisms to only one specific spiked compartment (water or sediment); although both compartments may be monitored, effects in the tests may be underestimated compared with the field situation since, in the field, they result from simultaneous exposure to multiple routes of uptakes (i.e. surface water and sediment compartment).
- Remobilization of substances strongly absorbed to particulates (e.g. during runoff events, bioturbation) under some circumstances, which may exert an increased toxicity to pelagic or epibenthic organisms but may also be considered a local dissipation route, especially in lotic systems.
- Repeated exposure within a year or over multiple years may have effect on the ecological responses (sensitivity, adaption, species interactions) and/or composition of the benthic community (culmination of effects), especially on the epibenthic species since they are most exposed to fluctuating concentrations.

Other types of uncertainties may overestimate the real risks in the field. They relate, for example, to the following points:

- In the sediment compartment of edge-of-field surface waters bioavailability/bioaccessibility of the historical pollutants may decrease in time because of ageing, strong or irreversible sorption, sedimentation or burial or removal of surface sediment (e.g. management practices of water bodies such as dredging) and this is not taken into account in PEC_{sed} estimation on basis of the FOCUS modelling approach currently used. In contrast, in recently spiked sediments of laboratory sediment toxicity tests the bioavailability of the test item is relatively high, particularly compared with the PEC_{sed} that takes into account the multi-year accumulation factor as proposed in this scientific opinion.
- The microbial activity and nutritional value for benthic organisms in natural sediments may be larger that that of artificial OECD sediment.
- In laboratory sediment toxicity tests, usually the whole sediment layer is well mixed and homogeneously spiked while in the field sediments may show a larger spatial and temporal variability in microhabitats and exposure conditions.
- Exposure avoidance is limited in laboratory sediment toxicity tests while it may play a role in the field.

The lack of knowledge regarding the presence and bioavailability of pollutants in the sediment compartment of edge-of-field surface waters makes it difficult to assess the potential combined effects on benthic organisms of new and existing pollution. Consequently, more information is needed in order to take account of possible consequences of multiple stressors in the prospective sediment ERA for PPPs. To this end, it should be noted that a more holistic catchment based ERA for PPP's may be required in prospective ERA (e.g. EFSA SC, 2016).

Some uncertainties may either under- or overestimate the risk depending on the type of ecological systems, e.g.:

- Most relevant type of benthic community in terms of ecological niche. For example, ponds may have relatively more pelagic species and relatively less epibenthic species when compared with streams. Epibenthic species in streams are more often associated with coarser substrates (e.g. sand, pebbles) but may be exposed to resuspended sediments.

- Most relevant type of ecosystem in terms of contamination: soft sediments of lentic edge-of-field waterbody may be of higher relevance than that of lotic systems as loamy and detritus-rich sediments (high content of organic matter) are likely to have higher levels of contamination than sandy or coarser sediments, and not subject to processes of remobilisation/local dissipation of pesticide-bound particulates.

11.4.3. Examples of uncertainties related to the current exposure scenarios

- The current status of the FOCUS surface water scenarios leads to significant uncertainty in the risk assessment. It is claimed that the FOCUS scenarios represent a worst-case situation for the aquatic environment. Apart from the fact that it has never been evaluated in detail, the intention of FOCUS was the development of worst-case scenarios for surface water. Please note that, assuming that the scenarios really represent worst-case surface water scenarios, they cannot also represent worst-case conditions for sediments.
- The current exposure assessment based on FOCUS surface water was not revised or evaluated in detail and thus there are uncertainties regarding total content and pore water concentrations.
- The current FOCUS methodology for surface water does not consider the effect of multi-year applications which could possibly lead to accumulation of pesticides in sediment. Although it is recommended to account for this deficit by using an accumulation factor, it may add uncertainty in the calculation. This is because it does not include any possible transport processes, such as leaching or volatilisation, which may reduce the accumulation in sediment in the real field situation. Otherwise the accumulation factor does not consider the effect of kinetic (aged) sorption and could even be underestimated.
- As bioavailability is currently not totally understood because it is influenced by compound and environmental properties (e.g. temperature, redox), but also by the organism type, the current computer models are able to only estimate the bioavailability to a limited extent.
- The exposure calculations do not consider the sedimentation over time (i.e. 'burying' of contaminated sediment) and degradation of pesticides in bed sediments which has, up to now, received little study.
- The exposure calculations do not consider the fact that differences between catchments may exist in terms of type, frequency and concentrations of PPPs in sediments, reflecting differences in land-use and seasonal dynamics.
- Bioturbation enhanced biodegradation of sediment-associated pesticides by producing conditions which stimulate microbial activity (e.g. see Monard et al., 2008) is not considered in the exposure calculations.

11.4.4. Examples of uncertainties related to the linking exposure to effects

- Discrepancies in OC content of the sediment in the FOCUS scenario (i.e. about 5 % OC content) and in standard OECD test sediment (i.e. about 2.5 % OC content). This will result in an overconservative approach when using total sediment concentration and in an underconservative approach when using pore water estimates (i.e. for plants and microbes) in comparing PECs and RACs.
- Uncertainties in differences in the concentration profile with depth, time or temperature. Whether these differences will lead to more or less conservative assessments cannot be given here as it strongly depends on the toxicity test and the FOCUS scenario.
- Uncertainties also related to suspended and re-suspended sediment particles.

11.4.5 Conclusions regarding uncertainties

The list above concerning uncertainties may not be exhaustive and in the development of guidance all uncertainties should be weighted for an overall assessment. Based on this overall assessment it can then be decided if a precautionary approach should be applied as stated in Article 1(4) of EU Regulation (EC) No 1107/2009.

CONCLUSIONS AND RECOMMENDATIONS

- Soft sediments of edge-of-field ponds, ditches and streams are characterised by a significant horizontal and vertical heterogeneity in physical, chemical and biological properties. The distribution of benthic organisms is patchy and varies among different sediment habitats.

- Organisms living in (endobenthos) and on (epibenthos) soft sediments cover all trophic levels and different feeding strategies. Benthic organisms comprise microorganisms (bacteria, archaeans, fungi, protozoa), microphytobenthos (algae), rooted macrophytes (vascular plants), meiobenthos (nematodes, targidates, copepods, ostracods, chydorid cladocerans) and macrobenthos (larvae of insects, macro-crustaceans, oligochaetes, molluscs, vertebrates).

- Although they currently receive little attention in sediment ERA for PPPs, microorganisms (bacteria, archaeans, fungi and protozoans) are integral parts of sediment communities. They play a vital role for metabolic activities and food web interactions and the microbial diversity of sediments is huge.

- Internationally accepted protocols to conduct single-species laboratory toxicity tests with typical benthic freshwater species have been developed for a limited number of taxa only. The vast majority of published sediment-spiked laboratory toxicity tests with PPPs concerned tests with insects, *Chironomus* spp., and the crustacean *Hyalella azteca*. Sediment-spiked toxicity tests with PPPs and the oligochaete *Lumbriculus variegatus* (Oligochaeta) and the rooted macrophyte *Myriophyllum* spp., where not often reported till now. In the OECD test protocols artificial sediment is recommended, whereas the US EPA/ASTM technical guidelines recommend the use of natural sediment. In addition, the OECD and the US EPA/ASTM guidelines differ with respect to the spiking procedure which may affect exposure conditions in the tests. The PPR Panel recommends to initiate comparative studies to evaluate and understand differences in OECD and US EPA/ASTM guidelines (e.g. artificial vs. natural sediment; various ageing periods before starting toxicity tests) and the possible consequences for toxicity estimates.

- For sediment ERA, the PPR Panel recommends to increase knowledge on (1) the most relevant type of ecosystem (ponds, ditches, streams) in terms of ecological niche for benthic organisms, (2) the most relevant type of edge-of-field aquatic ecosystem in terms of contamination, (3) differences in sensitivity of benthic populations between lentic (ditches and ponds) and lotic (streams) systems, (4) possible differences in benthic communities of edge-of-field surface waters between different regions in Europe (e.g. differences in terms of species composition and life traits), (5) effects of repeated exposure (within one year or over multiple years) on the benthic communities (culmination of effects) and (6) on the representativity of standard test species for the field communities in terms of sensitivity and vulnerability.

- Since the taxonomic groups that play a major role in providing ecosystem services are the same for the pelagic and sediment compartments, it is advised to adopt the same SPG options for benthic organisms as already developed in the AGD (EFSA PPR Panel, 2013). This implies that, in general, benthic taxa need to be protected at the population level, except aquatic vertebrates (benthic fish and amphibians) that warrant protection at the individual (to avoid direct mortality and animal suffering) to population level (e.g. chronic effects via reproduction), and microorganisms that need to be protected at the functional group level.

- The ERO might be applicable to define specific protection goals in some cases. However, there are several reasons why, for the time being, a prudent approach is required in applying the ERO and thus it is suggested that the ETO is the best option for providing an adequate protection of benthic organisms.
- Sorption to sediments is likely to reduce the bioavailability of PPPs for many benthic organisms by reducing aqueous concentrations (in overlying and interstitial water). Sorption may, however, increase exposure for benthic fauna, particularly sediment-ingesting organisms.
- The freely dissolved fraction of PPPs in pore water most likely is the main sediment exposure route for benthic algae, rooted macrophytes and microbes. For benthic animals, both the pore water fraction as well as the particulate-associated fraction may constitute important sediment exposure routes. In particular, dietary exposure can play a role in sediment fauna and phagotrophic protozoans.
- The specific toxic mode-of-action of PPPs should be considered when assessing environmental risks of sediment-exposure and selecting benthic test species.
- The few microcosm and mesocosm studies that focused on the ecological impact of sediment-exposure to PPPs, revealed that compounds that are persistent in sediment may have long-lasting effects on benthic organisms and communities.
- Standard tests with microorganisms are not included in the current data requirements for aquatic ERA of PPPs. However, there have recently been repeated calls for improving the consideration of microorganisms in ERA of PPPs. Existing ISO tests with microorganisms are of limited use in prospective ERA and more research and method development are needed.
- From existing information it is still unclear whether microbial communities are more sensitive to PPPs than other organisms and when microbial tests are actually needed. Standardised test systems that are able to provide information that is sufficiently representative of the wide diversity of microorganisms, microbial processes and sediment habitats have not been developed.
- This opinion proposes to trigger sediment ERA for PPPs if (1) more than 10 % of the radio-labelled test material can be found in the sediment at or after 14 days after application in the standard water–sediment fate study (OECD Guideline 308), or more than 10 % of the total annual dose of the active ingredient occurs in sediment at the time of maximum PEC_{sed} as assessed by FOCUS modelling and (2) the chronic NOEC/EC10 of *Daphnia* or another relevant pelagic animal species is less than 0.1 mg/L, or the chronic EC50 of the standard test alga or vascular plant is less than 0.1 mg/L.
- To avoid unnecessary testing with benthic organisms it is proposed to use chronic toxicity data for pelagic organisms and the EqP approach for an initial screening in the ERA for PPPs, but to apply an extrapolation factor of 10 for benthic fauna to cover the possibility of exposure due to sediment ingestion. The predictive value of this modified EqP approach was tested for a limited number of compounds and water-spiked and sediment-spiked tests with *Chironomus*. It is recommended to evaluate the general applicability of this approach for a larger array of PPPs and benthic species.
- Additional spiked sediment tests are triggered when the (modified) EqP approach indicates a potential unacceptable risk.
- This opinion concludes that the current experimental triggers for sediment accumulation should not be replaced by triggers based on environmental properties such as K_{oc} and DegT50.
- This opinion proposes to express the PEC_{sed} and RAC_{sed} estimates in terms of (1) total sediment concentration based on dry weight, normalised to either the OC content in the dry sediment or to standard OECD sediment with an organic matter content of 5 % and (2) the freely dissolved PPP fraction in pore water.

- To assess the risks of sediment exposure to benthic organisms, it is proposed to use the 0–1 cm sediment layer for PEC_{sed} derivation in case benthic fauna and microorganisms are the organisms of concern, while the 0–5 cm sediment layer may be used for rooted macrophytes.
- The RAC_{sed} derivation should preferably be based on chronic toxicity data using sediment-spiked tests and benthic organisms, not excluding that also semi-chronic toxicity data can be used to derive a RAC_{sed} if an appropriate additional extrapolation factor is used.
- The current FOCUS methodology does not consider the effect of multi-year applications that can lead to accumulation in sediment. To account for this deficit it is proposed to include an accumulation factor.
- The PPR Panel did not revise or evaluate the current exposure assessment in detail but advises to critically evaluate and improve the FOCUS surface water exposure assessment in the future and to develop new sediment scenarios for total content and pore water concentrations.
- Bioaccumulation is of particularly high relevance for benthic organisms since the sediment compartment is a sink for substances that may have a high BCF, and benthic organisms have a great potential in terms of accumulating toxic substances and in transferring them to higher trophic levels.
- It is proposed to perform spiked sediment bioaccumulation tests with benthic invertebrates for substances that show significant bioaccumulation in fish tests (BCF > 2 000 L/kg), when the substance is: (i) persistent (half-life > 120 days in sediment) and log K_{ow} > 3, (ii) non-persistent (i.e. half-life < 120 days in sediment), log K_{ow} > 3 and 10 % or more of the substance found in the sediment (based on water–sediment studies) or FOCUS step 2 and/or step 3 modelling (or using another appropriate model). Further guidance on how to incorporate the outcome of invertebrate bioaccumulation studies in the regulatory evaluation of the risks of food chain transfer and secondary poisoning needs to be elaborated. Currently, the risks of biomagnification and secondary poisoning of sediment-bound PPPs are not addressed in the risk assessment schemes. The PPR Panel recommend further development of such a risk assessment scheme based on existing contaminant food web transfer experiments and models. These should include the accumulation from sediments, water and dietary sources into sediment-dwelling invertebrates, fish (primary and secondary consumers), piscivorous birds and mammals and birds and mammals (e.g. bats) preying on emerging adult insects.
- The PPR Panel recommends normalising the internal concentrations of PPPs in benthic organisms to lipid content in experimental and modelling studies. This should be considered in further detail in the future opinion on effect models. The formation of possible relevant metabolites during bioaccumulation tests should also be considered.
- This opinion proposes to adjust the Tier 1 decision scheme based on current data requirements by including additional test organisms depending on the toxicological mode of action of the substance. The Panel asks the Commission to amend the data requirements accordingly.
- Further knowledge on mechanisms involved in chronic effects of fungicides is desirable since—at least in terms of acute effects—these substances may be less receptor specific and thus may target vertebrates as well as invertebrates or primary producers.
- The PPR Panel proposes to not apply the Geomean approach in the sediment effect assessment based on chronic toxicity data. Stronger scientific underpinning of the concept is needed, using chronic toxicity data for a wide array of sediment organisms and substances that differ in toxic mode-of-action.
- For the time being a WoE approach is proposed if chronic toxicity data are available for additional benthic test species, but the number of data is too low to allow the SSD approach. The PPR Panel proposes to develop a transparent decision scheme for the WoE approach, more specifically to develop criteria to lower the default AF to be applied to the lowest valid toxicity value, based on the quality and number of additional toxicity data available.

- If sediment toxicity data are available for a sufficient number of benthic species, it is proposed to follow the SSD approach as much as possible according to the criteria described in the AGD (EFSA PPR Panel, 2013). This means that for PPPs toxicity data should be available for at least eight species of the potentially sensitive taxonomic group (most likely benthic arthropods for insecticides; rooted macrophytes for herbicides). For substances for which a specific potential sensitive taxonomic group cannot be identified on basis of the available toxicity data for pelagic organisms, a minimum number of eight toxicity data for at least five different taxonomic/feeding groups may be selected. This may be the case for fungicides with biocidal properties.

The AF of 10 for Tier 1, as given in the uniform principles (Regulation (EC) No 546/2011) for chronic toxicity data, has not been sufficiently validated/calibrated for all types of PPPs and it is not fully clear whether all relevant uncertainties are covered in any case.

Calibration should be performed between lower and higher tiers (micro-/mesocosm studies data and if possible field data) for sediment organisms.

- With the reference being the field itself, it is recommended to conduct further investigations in the sediment compartment of edge-of-field surface waters to strengthen the link between results of experimental ERA approaches and the situation in the field, that is, to perform a retrospective evaluation.
- An important research need is to develop sediment toxicity data sets for benthic organisms and modern PPPs that differ in toxic mode-of-action so that the validity of the tiered approach as proposed in this scientific opinion can be evaluated.
- In constructing micro-/mesocosm tests to study population and community-level effects of sediment exposure to PPPs, field-collected sediment is largely preferred over artificial (OECD) sediment. Natural sediments allow the development of a realistic and diverse benthic community, despite the fact that they may be contaminated with unknown background chemicals and difficult to standardise in terms of composition across studies.
- An important question is whether to use spiked sediment to construct micro-/mesocosm or to follow the traditional approach in constructing micro-/mesocosms with ‘clean’ sediment and to spike the water column with the PPP (water or sediment slurry applications). The PPR Panel considers both designs feasible, but a reasoned case should be presented why a specific design is chosen.
- The PPR Panel recommends exploring the use of micro-/mesocosm test systems that associate both the aquatic (surface water) and the sediment contamination, which would allow study of more realistic conditions of contamination in water bodies. Such studies would focus on effects of combined exposure routes (i.e. spiked water that simulate the drift entry and spiked sediment that simulate the historical background and the freshly entering PPP).
- Irrespective of the design of micro-/mesocosm experiments, dynamics in exposure concentrations in the relevant sediment layers should be monitored. This implies that for a proper sediment effect assessment for benthic invertebrates, the dynamics in exposure concentrations in the upper 1 cm of the sediment compartment have to be monitored. For rooted macrophytes a deeper sediment layer (5 cm) may be appropriate. If measuring exposure concentrations in pore water is difficult, prediction on the basis of sediment characteristics and measured total PPP concentrations is also a possibility.
- In conducting sediment micro-/mesocosm tests, the PPR Panel advises to always include observations on long-term benthic population and community-level effects. The duration of the study needs to be long enough to cover the duration of the full life cycle of the most sensitive benthic species at risk in order to detect the effects.

- The effect assessment for aquatic vertebrates and exposure to PPPs in sediment is a research activity to date. Based on the data requirements and the current knowledge, it is not possible to deliver, at this stage, a consolidated ERA scheme. In particular, more research and analysis of data is needed to identify which exposure routes are most relevant, depending on aquatic vertebrate species and substances.
- Functional properties of microbes currently have larger potential than structural ones for prospective ERA of PPPs, since effects are easier to interpret as either positive or negative. Recently developed ISO standards for determining effects of chemicals on functional properties related to nitrogen cycling seem to have the highest potential for use in prospective ERA of PPPs.
- When the effect estimate is expressed in terms of initial exposure concentration, it should be plausible that the exposure profile in the sediment toxicity test is realistic worst-case scenario relative to that predicted for field sediments, otherwise these effect estimates cannot be directly used in ERA.
- If the effect estimates on which the RAC_{sed} is based are expressed in terms of the initial test concentration, it is recommended that the $PEC_{sed;max}$ concentration should be used in ERA to assure a more realistic worst-case risk assessment.
- It is recommended to use the $PEC_{sed;max}$ in sediment ERA as a default procedure, and to consider the use of the $PEC_{sed;twa}$ only if field exposure concentrations are demonstrated to be sufficiently variable during a time frame smaller than the duration of the sediment-spiked toxicity test that drives the RAC_{sed}.
- It is recommended to develop two types of sediment exposure scenarios, one with low OC (worst-case pore water scenario) and one with high OC (worst-case total content scenario). It seems necessary to develop environmental scenarios for ponds, ditches and streams in the near future to better integrate the physico-chemical and biological properties important for exposure and effect assessment, and to assure that the ERA for pelagic organisms is not in conflict with that for benthic organisms in the sediment compartment of the same system.
- If the relative contribution of the older (e.g. > 1 year) and recent fractions (e.g. latest growing season) in the $PEC_{sed;tot}$ is calculated this knowledge might be considered in a higher tier by (1) using refined-exposure toxicity tests by spiking the sediment in different phases and allowing different ageing periods for the different fractions before using the sediment in sediment-spiked toxicity tests or (2) using appropriate modelling approaches to better estimate the bioavailable fraction of the $PEC_{sed;tot}$ estimate.
- For the chronic ERA of metabolites in the sediment compartment, this opinion proposes to follow the same approach as described in the AGD (EFSA PPR Panel, 2013).
- For the chronic ERA of chemical mixtures, this opinion proposes to follow also the approach described in the AGD (EFSA PPR Panel, 2013) as well as the further developments and recommendations of the scientific opinion (EFSA PPR Panel, 2014). It is acknowledged that more information is needed on the presence and bioavailability of historical pollution and more recent pollution in sediments not only by the product under evaluation but also by other products applied simultaneously or successively in order to take account of possible consequences of multiple stressors in the prospective sediment ERA for PPPs.
- Lists of uncertainties related to exposure and effects assessment and the combination of the two are derived (may not be exhaustive). In the development of guidance all uncertainties should be weighted for an overall assessment of uncertainty. Based on this overall assessment it can then be decided if a precautionary approach should be applied as stated in Article 1(4) of EU regulation (EC) No 1107/2009.

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Appendix A. Linking the sorption constant to the maximum residue in the sediment phase

Introduction

In order to estimate the maximum level of pesticides in sediment their sorption constants (e.g. K_{oc}) is often used as an initial trigger for further testing (e.g. REACH regulation). However, the sorption constant is for a couple of reasons no optimum parameter to address the problem. This is mainly because the maximum level found in the environment is the result of several parameters and processes such as the entry mode into the surface water (e.g. via spray drift or run-off), the degradation in water and sediment, and the distribution between the two phases, which is not only driven by the thermodynamic parameter sorption constant but also by kinetics. Finally, the whole K_{oc} concept itself does not hold for all types of pesticides.

Instead of using the sorption constant, the result of water/sediment studies also could be used for triggering further studies because they directly provide the desired parameter, ‘maximum occurrence in the sediment phase’. Especially, they cover the processes previously mentioned because the interaction between degradation in and distribution between the phases is adequately considered. But the test also gives valid information for ‘unusual’ substances that cannot be assessed based on the K_{oc} concept because, for example, sorption to clay particles is not negligible.

The aim of the following analysis was to find possible links between sorption constants and maximum occurrences in order to get an idea about the consequences of different triggers based on sorption for maximum concentrations in the sediment phase.

Methodology

First, existing dossier information about sorption constants (K_{oc} or K_{foc}) was collected together with the results of water sediment studies (maximum occurrence in the sediment phase and total system half-life). This information is summarised in Table A1.

Table A1: Maximum occurrence in the sediment phase for some pesticides (dossier information, EFSA database)

Substance	DegT50 (days)	K_{OC} (L/kg)	Maximum occurrence (%)	Day of max.
Chlormequat chloride	3.75	168	63	7–30
Glyphosate	87	1 435	55	14
Cyprodinil	142	2 277	87	14
Mepiquat	32.5	890	56	14
Fludioxonil	575	132 100	80	177
Pyraclostrobin	28	9 304	50–60	2–14
Iprodione	30	700	79	100
Fenhexamid	10.9	475	47	7
Chlorpyrifos	36.5	8 151	3–26	100
Pyrimethanil	80	301	50–70	14–30
Azoxystrobin	205	589	90	0
Spinosad	173	35 024 000	60–70	30–58
Imidacloprid	129	225	10–32	14–60
Lambda-Cyhalothrin	12	157 450	30–70	1
Ethephon	2.8	2 540	5	4–30
Indoxacarb	6	6 450	50–80	1
Thiabendazole	4	7 344	30–71	181
Trifloxystrobin	2.4	2 377	10–42	1
Bifenthrin	161	236 610	88–95	14
Myclobutanil	626	517	65–85	105
Etofenprox	13.3	17 757	63–70	1
Dimethomorph	38	348	53–68	1
Triflumuron	6.4	2 967	48	1
Bupirimate	42.5	1 882	20	120
BetaCypermethrin	17	156 250	51	1
ZetaCypermethrin	17	156 250	45	1

The table demonstrates that there is no obvious correlation between the maximum occurrence of a compound in the sediment phase of the test and its sorption constant. This is also shown in Figure A1, which shows a random distribution without any tendency. Based on this data collection no meaningful link between the sorption constant and the expected maximum residue in sediment can be established.

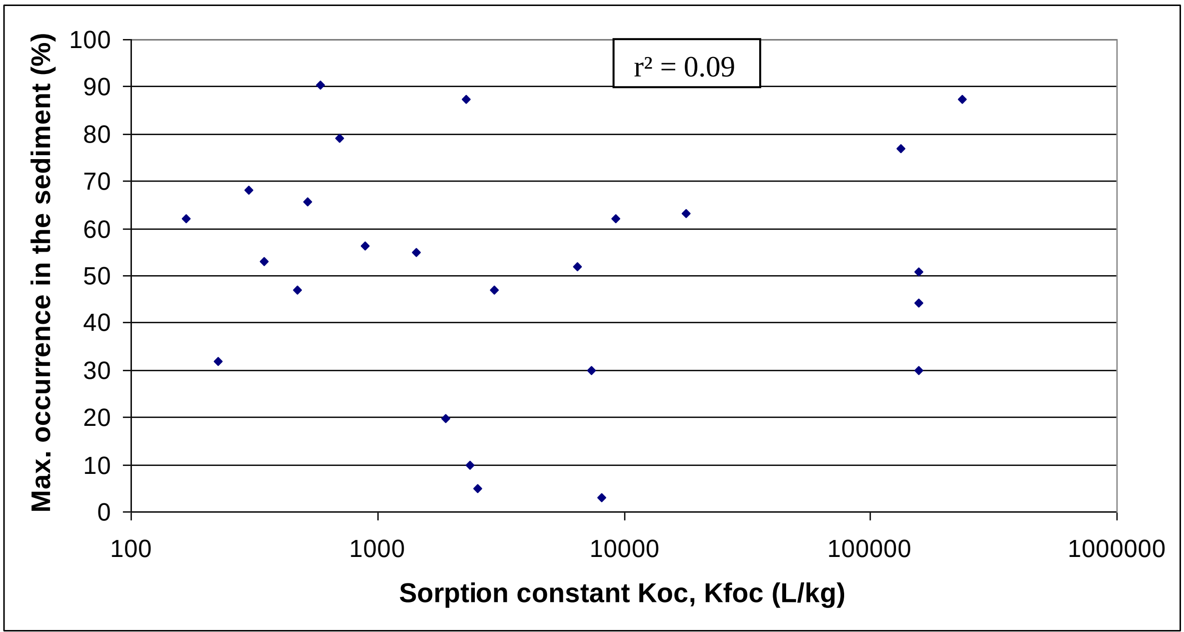

Figure A1: Correlation between sorption constants and maximum occurrence in the sediment phase. The figure was prepared by the PPR WG.

To improve the situation simple models were used to estimate maximum occurrences based on the information given in Table A1.

Persistent compounds

At least for those substances for which degradation in the system can be totally neglected, the equilibrium distribution of the compounds should be a good descriptor for the maximum occurrence in the sediment phase. This distribution can be calculated based on the following equation:

$$EQ_{sed} = \frac{r_{wat-sed}}{r_{wat-sed} + bd_{sed}\ f_{OC}\ Koc} \qquad (1)$$

EQ_{sed}: equilibrium fraction in sediment (–)

$r_{wat\text{-}sed}$: depth ratio of water to sediment layer (–)

bd_{sed}: bulk density in sediment (kg/L)

f_{OC}: fraction of OC in sediment (–)

Koc: sorption constant related to OC (L/kg)

Non-persistent compounds

Calculation of maximum occurrences simply based on equilibrium conditions without taking into account any kinetics is not meaningful as there is a competition between distribution and degradation processes and the maximum residue in sediment will always depend also on the total system half-life. To consider both processes for the estimation of the maximum residue in the sediment layer a simple model is used which combines degradation and partitioning by assuming that both processes follow first-order kinetics. The model is following the recommendation for the fitting of water-sediment studies according to FOCUS kinetics (FOCUS, 2006).

The model algorithms are presented in the following equations. Degradation is considered as being dependent on an overall degradation rate and the amounts in the respective compartments (water: F2, sediment, F3) (Figure A2). The distribution between the compartments is considered by two processes—sorption and desorption—represented by a sorption and desorption rate constant, together with the respective substance amounts in the two compartments. Due to the distribution term it is impossible to describe the concentrations by an analytical expression. Instead, the underlying differential equations (see (2) and (3)) have to be solved by numerically.

$$\frac{dM_{wat}}{dt} = -k_{\deg} M_{wat} - k_{sorp} M_{wat} + k_{des} M_{sed} = -F2 - F1 + F4 \qquad (2)$$

$$\frac{dM_{sed}}{dt} = -k_{\deg} M_{sed} - k_{des} M_{sed} + k_{sorp} M_{wat} = -F3 - F4 + F1 \qquad (3)$$

k_{deg}:	total system degradation rate (d – 1)
k_{des}:	desorption rate (d – 1)
k_{sorp}:	sorption rate (d – 1)
M_{wat}:	residue in the water phase (%)
M_{sed}:	residue in the sediment phase (%)

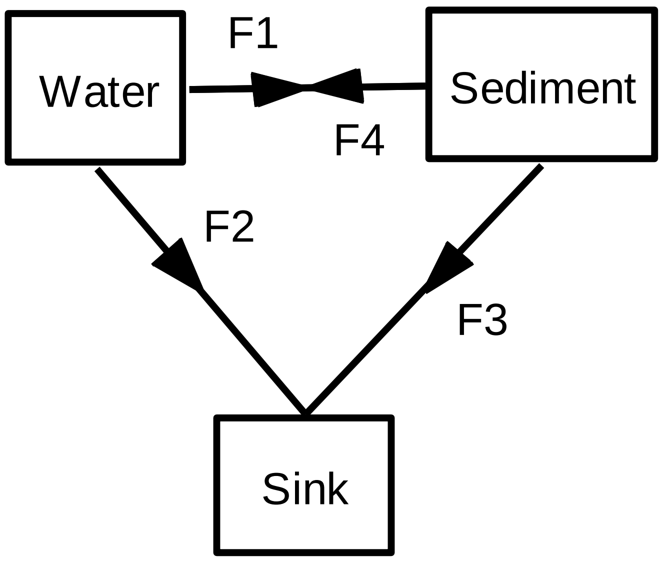

Figure A2: Flow chart of the model used to estimate maximum residues in sediment for non-persistent compounds. The figure was prepared by the PPR WG.

The rate constant k_{deg} can be easily transferred into the respective DegT50 using the following equation:

$$k_{\deg} = \frac{\ln(2)}{DegT_{50}} \quad (4)$$

So far the model does not contain the parameter sorption constant (K_{oc}). However, based on equation (1) the K_{oc} value can be transferred into EQ_{sed} which is linked to the two rate constants for sorption and desorption according to the following equation

$$EQ_{sed} = \frac{k_{sorp}}{k_{sorp} + k_{des}} \quad (5)$$

If time-dependent residues in water and sediment are calculated based on the model, there is still one degree of freedom left, which is the ratio between the degradation and partitioning, which is linked to the question how fast equilibrium condition is reached for a respective persistent compound. In all calculations it was assumed that equilibrium is reached after 10 days.

Results

Persistent compounds

Figure 3 shows the equilibrium distribution of the residue in the sediment layer for very persistent compounds. For the analysis it was assumed that the soil bulk density (*bd)* was 0.8 kg/L and the ratio of the depths of water and sediment layer was 3.5.

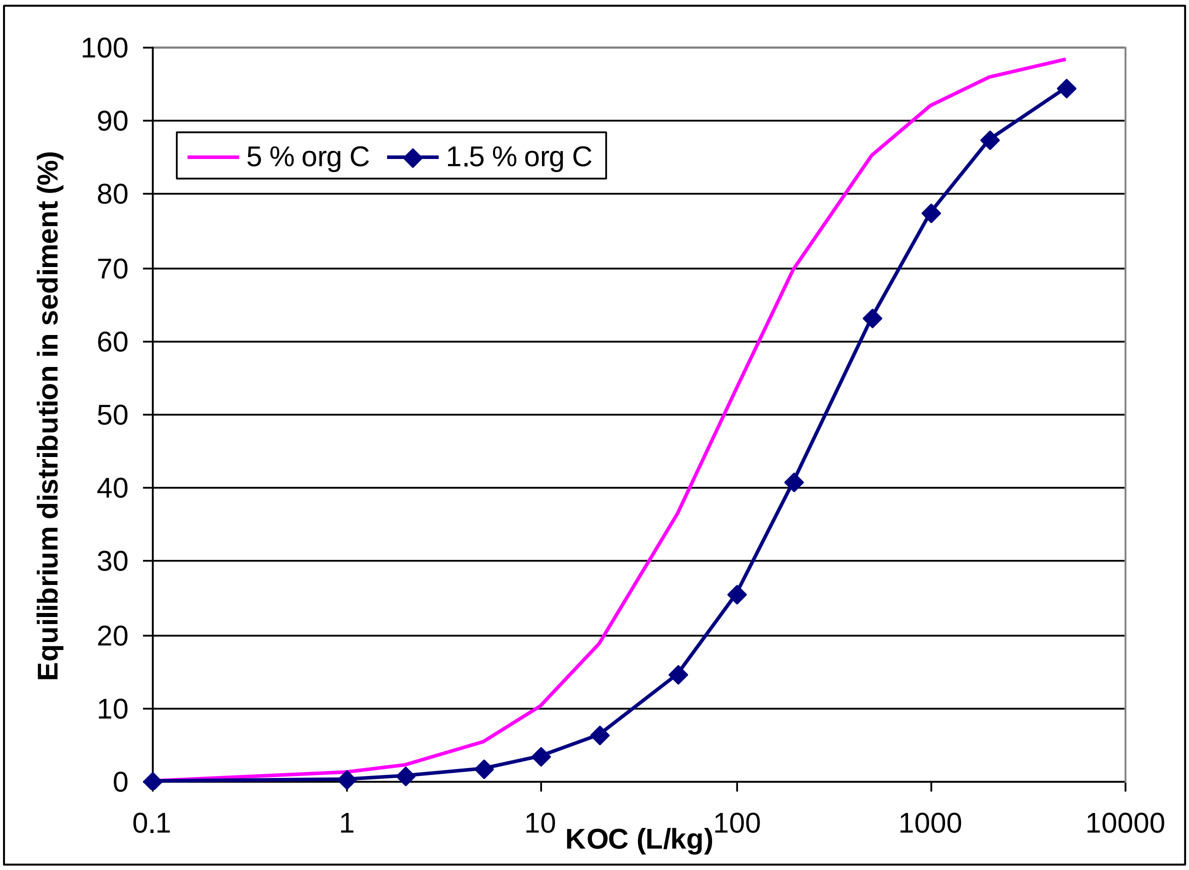

Figure A3: Equilibrium distribution of residues in sediment dependent on K_{oc} and the sediment OC content (sediment bulk density bd: 0.8 L/kg, ratio of water and sediment depth $r_{wat\text{-}sed}$: 3.5). The figure was prepared by the PPR WG.

The figure shows that all substances persistent in sediment with K_{oc} values above 50 L/kg can be expected to distribute to the sediment above 10 %. Distribution of about 90 % can be expected from compounds having K_{oc} values of about 2 000 L/kg. Theoretically, these distributions are expected to occur after an infinite amount of time (so practically at the end of the study).

The curve could be used as a conservative trigger for maximum possible residues in sediment. If maximum residues of 10 % should be triggered assuming equilibrium conditions, compounds with K_{oc} values above 50 L/kg would already meet the criteria.

However, compounds that are non-persistent in sediment will hardly reach these values.

Non-persistent compounds in sediment

For compounds non-persistent in sediment the results are more complex because the degradation in the system principally prevents the system from reaching the theoretical equilibrium distribution. Furthermore, the permanent degradation in the system will always end up with maximum residues during the study instead of at the end.

The following two figures show two extreme situations with regard to pesticide properties and the distribution between water and sediment: the time dependent residues for a strong sorbing (K_{oc} = 2 000 L/kg) and slow degrading (DegT50 = 100 days) compound is presented in Figure 4 whereas Figure 5 shows the same results for a faster degrading compound (DegT50 = 20 d) but only moderately sorbing compound (K_{oc} = 100 L/kg). The theoretical maximum residues (no degradation) can be calculated to be 96 % and 53 % for the compounds shown in Figure 4 and Figure 5, respectively.

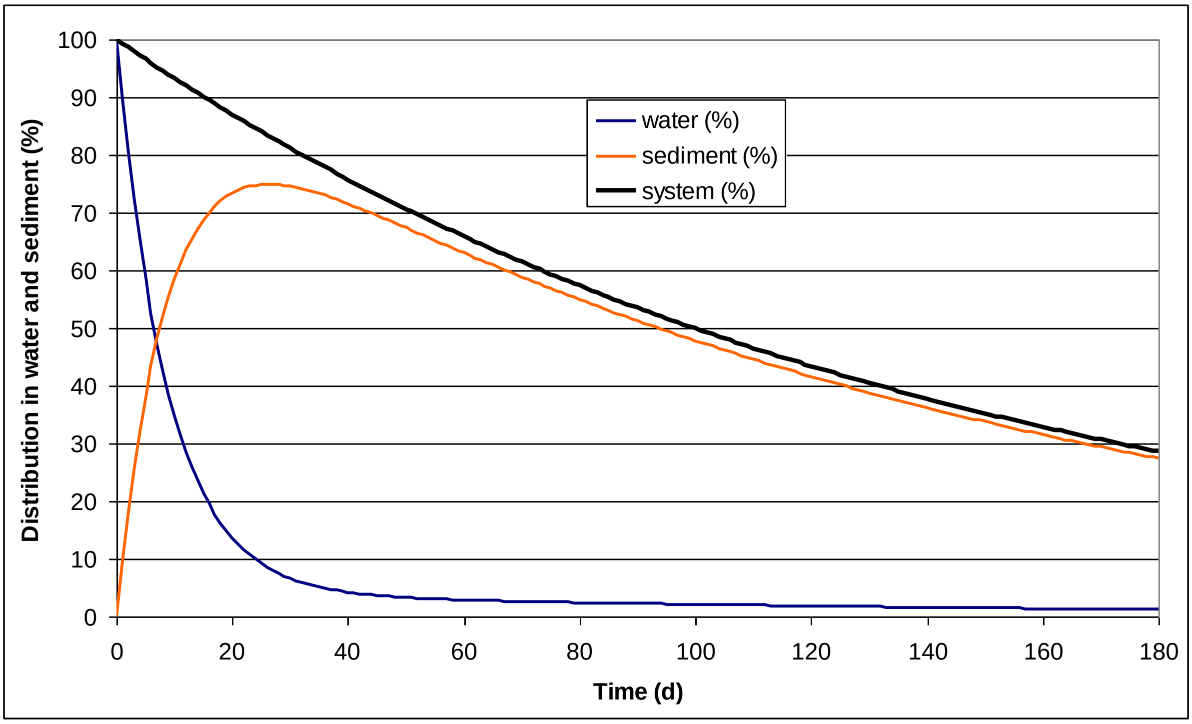

Figure A4: Calculation of time dependent residues in water and sediment (K_{oc}: 2000 L/kg, DegT50: 100 d, Corg: 5%, bulk density (bd): 0.8 L/kg, $r_{wat\text{-}sed}$: 3.5). The figure was prepared by the PPR WG.

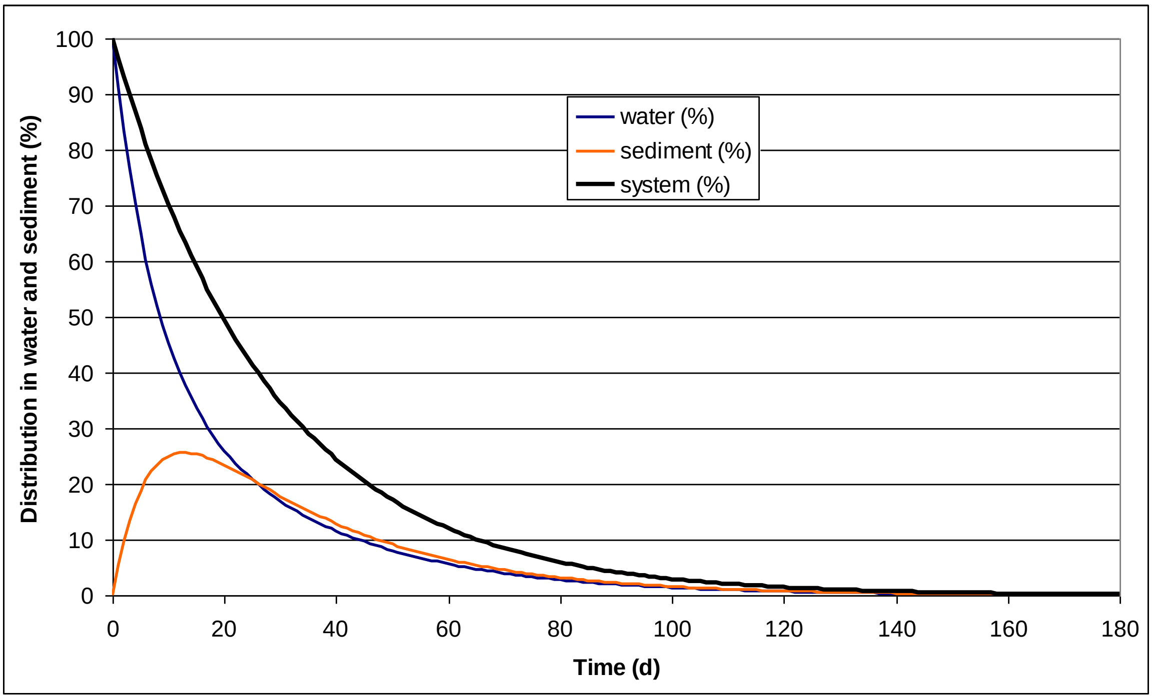

Figure A5: Calculation of time dependent residues in water and sediment (K_{oc}: 100 L/kg, DegT50: 20 days, Corg: 5%, bd: 0.8 L/kg, $r_{wat\text{-}sed}$: 3.5). The figure was prepared by the PPR WG.

Figure 6 presents the time dependent residues in water for a couple of substances with different properties. It shows that the time for reaching the maximum residue in sediment ranges from about 5 days to 50 days dependent on the properties of the compounds. Especially for fast degrading compounds the difference to the thermodynamic equilibrium is significantly lower.

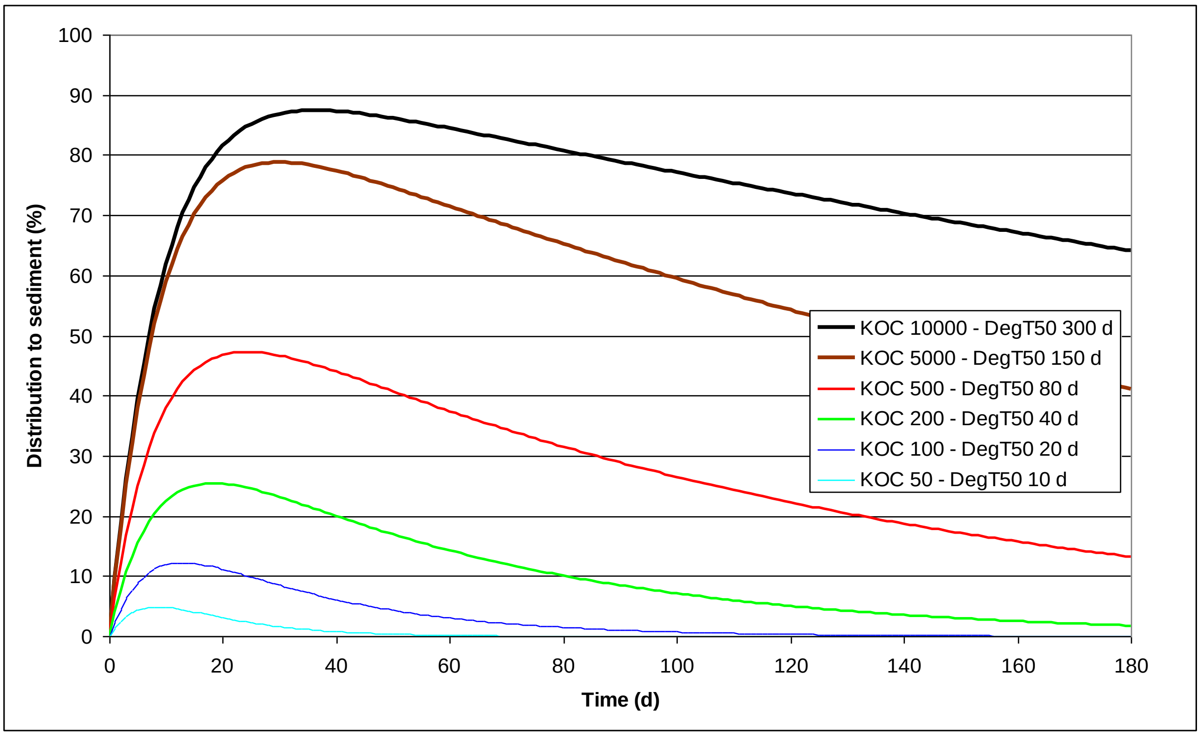

Figure A6: Calculation of time dependent residues in sediment dependent on K_{oc} and DegT50 (Corg: 5%, bulk density (bd): 0.8 L/kg, $r_{wat\text{-}sed}$: 3.5). The figure was prepared by the PPR WG.

Comparison with dossier information

Though the results presented so far seem to be reasonable the simulated curves are based on different simplifications which necessarily lead to deviations compared to respective experimental data. Therefore, in the final step the model was checked against experimental data as listed in Table A2.

Table A2: Comparison of maximum occurrences in the sediment phase for some pesticides in experiment (dossier information, EFSA database) and simulation

Substance	DegT50 (days)	K_{OC} (L/kg)	Experimental max. residues (%)	Simulated max. residues (%)	Day of peak	Day of peak
Chlormequat chloride	3.75	168	63	6.5–12	7–30	4
Glyphosate	87	1 435	55	63–65	14	25
Cyprodinil	142	2 277	87	73–80	14	29
Mepiquat	32.5	890	56	44–53	14	17
Fludioxonil	575	132 100	80	94	177	42
Pyraclostrobin	28	9304	50–60	53–55	2–14	15
Iprodione	30	700	79	40–50	100	16
Fenhexamid	10.9	475	47	22–30	7	9
Chlorpyrifos	36.5	8 151	3–26	58–60	100	18
Pyrimethanil	80	301	50–70	38–58	14–30	24
Azoxystrobin	205	589	90	58–75	0	33
Spinosad	173	35 024 000	60–70	85	30–58	31
Imidacloprid	129	225	10–32	36–59	14–60	29
Lambda-cyhalothrin	12	157 450	30–70	37	1	9
Ethephon	2.8	2 540	5	13–14	4–30	3
Indoxacarb	6	6 450	50–80	24	1	6
Thiabendazole	4	7 344	30–71	18–19	181	4
Trifloxystrobin	2.4	2 377	10–42	12–13	1	2
Bifenthrin	161	236 610	88–95	84	14	31
Myclobutanil	626	517	65–85	60–81	105	43
Etofenprox	13.3	17 757	63–70	39	1	10
Dimethomorph	38	348	53–68	33–49	1	18
Triflumuron	6.4	2 967	48	23–25	1	6
Bupirimate	42.5	1 882	20	55–61	120	19
Beta-cypermethrin	17	156 250	51	44	1	12
Zeta-cypermethrin	17	156 250	45	44	1	12

There are significant differences between the experimental and simulated residues. However, as shown in Figure A7 ($r^2 = 0.50$) the situation at least improved compared to the initial situation in Figure 1 ($r^2 = 0.09$).

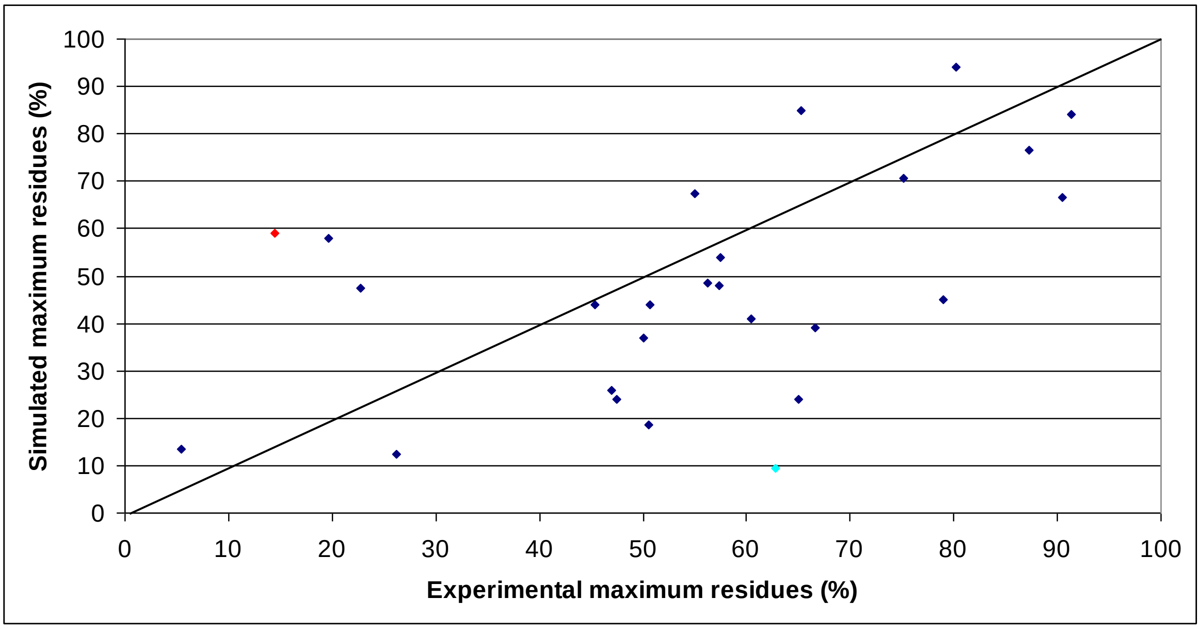

Figure A7: Correlation between sorption constants and maximum occurrence in the sediment phase. The figure was prepared by the PPR WG.

Obviously, the model can only explain about 50 % of the situation. There are several reasons to explain the situation. Some extreme deviations are caused by inconsistencies in the experimental database: it is doubtable that a compound (chlormequat chloride, see blue square in Figure A7) distribute into sediment at a level of 63 % after 7–30 days if the DegT50 is less than 4 days. The simulated level of 6.5–12 % much better fits with that DegT50 value. On the other hand a compound with a K_{oc} of 8151 L/kg and a DegT50 of 36.5 days (chlorpyrifos, see red square in Figure 7) was found to distribute into sediment at a level of only 3–26%. Again the simulated level of 58–60 % would much better describe the properties of the compound.

Apart from these individual outliers there is a tendency that the model underestimates the distribution in sediment by about 10%. The simplest explanation for this bias is the selection of Corg in sediment (average distribution based on two simulations with 1.5 % and 5%) which did actually not meet the composition of Corg in the experimental water-sediment tests exactly. A second explanation which would increase the simulated maximum residues would be to reduce the time of 10 days for reaching equilibrium conditions.

Conclusion

Apart from compounds that are very persistent in sediment it is not possible to establish a link between sorption constants and expected maximum residues in sediment.

The curve presented in Figure 1 which assumes equilibrium conditions could be used as a conservative trigger for maximum possible residues in sediment. If the trigger is set to 10 % compounds with K_{oc} values above 50 L/kg would already meet the criteria. That is not in line with current trigger values of 1000 L/kg for the K_{oc}-value.

However, compounds that are non-persistent in sediment will hardly reach these values.

For non-persistent compounds combinations of K_{oc} and DegT50 could be principally used to estimate the maximum residues in sediment. The question remains how to estimate the DegT50 in water-sediment before doing the respective study.

Appendix B. Comparing environmental risks identified in surface water and in sediment compartments

In the section below, the environmental risks calculated for both surface water and sediment compartments are compared in order to identify if surface water organisms are more likely at risk than sediment organisms. To do so, the PEC_{sw} and PEC_{sed} used were those calculated using the same exposure scenario, usually according to Focus Step 2. For surface water, the lowest value of either $RAC_{sw;ac}$ or $RAC_{sw;ch}$ was selected. For sediment, the RAC_{sed} was exclusively based on data from the *Chironomus* sp. test, using preferably data from the spiked sediment water-sediment study (OECD 219; OECD, 2004b). When no such data is available, then data from spiked water tests (OECD 218; OECD, 2004a) were taken and recalculated, using the modified equilibrium partitioning as described in section 5, in order to convert the values expressed in L into values expressed in kg of sediment, so that they can be compared to the PEC_{sed}.

The outcomes of this comparison (Table B1 below) show that based on a Tier 1 assessment, the potential risk is often (with the exception of Chlorantranilprole in the examples of the table below) higher (up to 4 orders of magnitude) in the surface water compartment than in the sediment compartment. This means that if the risk assessment was limited to a Tier 1, the aquatic risk assessment would then be often protective for the sediment compartment and a specific risk assessment for the sediment compartment would in many cases not be needed. However in the tiered approach, if a risk is indicated at lower tier, then it can be refined using methods specific and adapted to the case. Therefore, although it is shown that the risk is generally higher in the surface waters, once this risk is considered as unacceptable and must thus be refined, then each compartment must be evaluated independently since higher-tier options in both compartments differ.

As a summary, the outcomes of this analysis indicate that if the risk is acceptable at Tier 1 in surface waters, then a sediment risk assessment would generally not be of relevance if *Chironomus riparus* is a representative sensitive benthic species, but if the risk is considered as unacceptable at Tier 1 in surface waters then a sediment risk assessment is indicated.

It should be pointed out that this analysis presents some limitations:

In surface waters, a range of tests on different groups of organisms are performed and the lowest endpoints are used to derive the RAC. By contrast, in sediment ERA, fewer test species can be used, and up to now, most tests are performed with *Chironomus* sp exposed over a period of 28 days (either exposed through spiked water or through spiked sediment). Thus, it may not be always the most appropriate representative of the most sensitive group of species (e.g. oligochaetes may be more appropriate for the testing of fungicides) and thus may not deliver RACs relatively as low as for the aquatic ERA.

Chironomus, as an epibenthic species, is exposed to multiple routes of uptakes simultaneously but the tests are not designed accordingly since the guidelines (OECD 218 and 219; OECD, 2004a, b) recommend to subjecting the organisms only to one specific route of exposure (i.e. via water or via sediment).

Organisms are fed with uncontaminated food during the tests. Therefore compared to the field situation, there may be an underestimation of toxic effects.

Using the modified equilibrium partitioning (when no data from spiked sediment water-sediment test are available) may lead to an over-estimation of the real risk (see section 5).

In sediment testing, effect concentrations are usually expressed in terms of nominal/initial measured concentrations whereas in water testing they are either expressed as mean measured or kept constant, which may introduce a bias in the comparison. Similarly, the use of data for water-sediment tests produced either by spiking the water or spiking the sediment may be also shifted since the rate of dissipation and bioavailability may differ between water and sediment compartments.

Table B1: Comparison of Tier 1 risks for surface water and sediment compartments (Ratios between RAC (Tier 1) and PECsw or PECsed (usually based on Focus Step 2)

Substance	PEC_{sw} µg/L	PEC_{sed} µg/kg)	RAC_{sw} µg/L (Endpoint)	RAC_{sed} µg/kg (Endpoint)	RAC_{sed} EqP µg/kg (Endpoint & K_{oc})	Ratio sw RAC/ PEC	Ratio sed RAC/ PEC	Comparative risk: surface water versus sediment
Fungicide								
Bixafen	1.76	63.06	0.46 (NOEC fish prolonged 4.6 µg/L)	2 000 (NOEC chiro 20 mg/kg)	15 (NOEC 15.6 µg/L; K_{oc} = 3 869; EqP 150 µg/kg)	0.261	317 (0.24 EqP)	1 200-fold more risk in sw
Boscalid	15	98	12.5 (fish ELS: NOEC 125 µg/L)	2 320 (NOEC = 23.2 mg/kg	192 (NOEC = 1 mg/L: EqP = 1 925 µg/kg K_{oc} = 772)	0.78	24 2 (EqP)	30-fold more risk in sw (2.5-fold more risk in sw with EqP)
Myclobutanil	0.012	0.14	2.40 (*M. bahia* acute flow through: LC50 240 µg/L)	607 (NOEC = 6.07 mg/kg)	391 (NOEC = 3.02 mg a.s./L; EqP 3 910; K_{oc} = 518)	200	4 335 (3 142 EqP)	20 fold more risk in sw (15 fold more in sw with EqP)
Fenpropidin	9.23	323	5.4 µg/L (acute endpoint: 0.54 mg/L)	4 000 (NOEC 40 mg/kg)	952 (NOEC:1 mg/L; K_{oc}= 3808; EqP = 9.52)	1.7	12.3 (3 EqP)	7-fold more risk in sw
Isopyrazam	2.85	64.7	0.287 (fish NOEC: 2.87 µg/L)	5 600 (NOEC 56 mg/kg)	> 6 (NOEC > 1 mg/L ; K_{oc} 2 400; EqP > 60)	0.1	86 (> 0.1 EqP)	860-fold more risk in sw
Insecticide								
Beta-cyfluthrin	0.0615	6.83	0.000 0023 (*H. azteca*: 0.23 ng/L)	20 (NOEC 200 µg/kg)	(NOEC 0.2 µg/L K_{oc} 100 000)	3.7×10^{-5}	2.8	100 000-fold more risk in sw
Gamma-cyhalothrin	0.01	1.66	0.000 004 46 (Gammarus 0.000446 µg/L)	1.26 (NOEC:12.6 µg/kg)	0.686 (NOEC 0.046 µg/L; K_{oc} = 60 000; EqP: 6.86 µg/kg)	0.000 44	0.76 (0.41 EqP)	1 700-fold more risk in sw (similar with EqP)
Beta-cypermethrin	0.32	34.2	0.000 15 µg/L (*M. bahia* NOEC 28 d: 0.0015 µg/L)		1.95 (NOEC =0.06 µg/L; K_{oc} = 130 031;	0.000 46	0.05: EqP	100-fold more risk in sw (using EqP)

Substance	PEC_{sw} µg/L	PEC_{sed} µg/kg)	RAC_{sw} µg/L (Endpoint)	RAC_{sed} µg/kg (Endpoint)	RAC_{sed} EqP µg/kg (Endpoint & K_{oc})	Ratio sw RAC/ PEC	Ratio sed RAC/ PEC	Comparative risk: surface water versus sediment
					EqP = 19.5 µg/kg)			
Bifenthrin	0.08	24.3	0.0011 *(D. magna* acute: 0.11 µg/L)	4 (NOEC: 40 µg/kg)	19 (NOEC = 0.32 µg /L; K_{oc} = 236 610; EqP = 189 µg/kg)	0.013	0.17 (0.78 EqP)	10-fold more risk in sw (60-fold more risk in sw with EqP)
Chlorantranilprole	7.18	20.5	0.116 *(D. magna* acute: 11.6 µg/L)	0.5 µg/kg (NOEC: 5 µg/kg)	0.206 (NOEC: 2.5 µg/L; K_{oc} = 330; EqP = 2.06 µg/kg)	61.9	41 (100 EqP)	Similar risk in sw and sed
Metaflumizone	0.29	31.1	0.115 *C. variegatus* (ELS) NOEC ≥ 1.15 µg a.s./L	161 (NOEC chiro: 1 610 µg/kg)	19.6 (NOEC: 2.56 µg/L; K_{oc} = 30 700; EqP 196 µg/kg)	0.003	5.2	1 730-fold more risk in sw
Spinetoram	1.32	25.94	0,00624 *(D. magna* chronic: 0,0624 µg/L)	9.72 (NOEC chiro: 97.2 µg/kg)	0.69 (NOEC chiro: 0,75 µg/L; Koc = 3682[1]; EqP 6,9 µg/kg)	0.0047	0.375	80-fold more risk in sw
Herbicide								
Pendimethalin	10.1	844	0.63 µg/L FLC fish: NOEC: 6.3 µg/L		64 (NOEC ≤ 0.138 mg/L; K_{oc} = 18 550; EqP- 639 µg/kg)	0.06	0.075 (EqP)	Similar risk in both compartments (eqP)

1: Geomean of the two values: XDE-175-J: K_{oc} = 2661; XDE-175-L: K_{oc} = 5096.

Appendix C. FOCUS PEC-sediment calculations to obtain trigger values for the risk assessment

Introduction

FOCUS simulations were performed based on three imaginary active substances with realistic properties (an insecticide, a herbicide and a fungicide). The active substances used for the calculations were designed to cover the most relevant range of input parameters. Physicochemical properties that were used for performing the step1, 2 and 3 exposure calculations are shown in Table C1.

The simulations address a herbicide, fungicide and an insecticide. The same example compounds were used as in the aquatic guidance document.

Table C1: Physicochemical properties of example substances used in the case studies

Property	Herbicide	Fungicide	Insecticide
Crop	Spring cereals	Winter cereals	Apples
Number of applications	1	1	2
Application rate (kg/ha)	0.02	0.75	0.07/0.105
Time between applications (d) (step 2)	–	–	30
Season of application (step 2)	Spring	Spring	Spring and summer
Crop growth stage(s) at application date	BBCH 32–37	BBCH 32	BBCH 10/BBCH 69–71
Molar mass (g/mol)	400	225	250
Water solubility (mg/L) at 20 C	3000	13	600
Saturated vapour pressure (mPa) at 20 C	1e-7	0.5	1e-5
DegT50 at 20 C, pF = 2 in top soil (d)	20	50	100
DegT50 in water (d) at 20 C	150	10	5
DegT50 in sediment (d) at 20 C	100	20	100
DegT50 in total system	150	20	100
K_{OC} (L/kg) for soil	40	1700	170
K_{OM} (L/kg) for soil	23	1000	100

Results of the PEC simulations

Calculations were performed for FOCUS Tier 2 and Tier 3. Table C2 shows the results of the simulations dependent on the scenario. Column 6 shows the original FOCUS calculation whereas the column 7 presents the concentration including the suggested accumulation in sediment after long-term applications. All results based on 5 cm sediment depth (FOCUS standard). The final column represents the new parameter 'maximum residue in sediment as recommended by EFSA'. It is independent on the sediment depth because the residues of all sediment layers are summed up.

Table C2: Results of FOCUS PEC_{sed} calculations over 5 cm for the three example compounds

Mitigation	Location	Water body	DegT50 (d)	Corr factor (–)	PECmax (µg/kg)	PEC, max,akku (µg/kg)	Max residue in sed (%)
Fungicide	Step 2 N	Ditch	20	0.366	148.97	150.438	66.5573
Fungicide	Step 2 S	Ditch	20	0.366	272.06	274.740	67.7924
Fungicide	Step3 D1	Ditch	20	0.366	4.704	4.750	0.3806
Fungicide	Step3 D1	Stream	20	0.366	2.864	2.892	0.0154
Fungicide	Step3 D2	Ditch	20	0.424	9.869	9.915	0.5980
Fungicide	Step3 D2	Stream	20	0.424	8.063	8.101	0.0188
Fungicide	Step3 D3	Ditch	20	0.483	2.584	2.590	4.9269
Fungicide	Step3 D4	Pond	20	0.4	0.680	0.684	12.3838
Fungicide	Step3 D4	Stream	20	0.4	0.185	0.186	0.0271
Fungicide	Step3 D5	Pond	20	0.526	0.593	0.594	13.7484
Fungicide	Step3 D5	Stream	20	0.526	0.050	0.050	0.0252
Fungicide	Step3 D6	Ditch	20	0.841	0.958	0.958	0.4564
Fungicide	Step3 R1	Pond	20	0.483	1.039	1.041	6.7397
Fungicide	Step3 R1	Stream	20	0.483	2.928	2.935	0.0605
Fungicide	Step3 R3	Stream	20	0.679	3.078	3.079	0.0644
Fungicide	Step3 R4	Stream	20	0.662	3.804	3.805	0.0253
Insecticide	Step 2 spring N	Ditch	100	0.366	14.26	23.615	10.3499
Insecticide	Step 2 spring S	Ditch	100	0.366	23.31	38.602	12.4830
Insecticide	Step 2 summer N	Ditch	100	0.366	12.90	21.363	8.0253
Insecticide	Step 2 summer S	Ditch	100	0.366	15.44	25.569	8.8512
Insecticide	Step3 D3	Ditch	100	0.483	2.216	3.142	1.7327
Insecticide	Step3 D4	Pond	100	0.4	1.087	1.708	2.3122
Insecticide	Step3 D4	Stream	100	0.4	0.807	1.268	0.0067
Insecticide	Step3 D5	Pond	100	0.526	0.779	1.059	1.6216
Insecticide	Step3 D5	Stream	100	0.526	0.790	1.074	0.0050
Insecticide	Step3 R1	Pond	100	0.483	0.484	0.686	2.6218
Insecticide	Step3 R1	Stream	100	0.483	0.650	0.922	0.2178
Insecticide	Step3 R2	Stream	100	0.66	0.418	0.515	0.0898
Insecticide	Step3 R3	Stream	100	0.679	1.166	1.421	0.3290
Insecticide	Step3 R4	Stream	100	0.483	0.645	0.914	0.1127
Herbicide	Step 2 N	Ditch	150	0.366	0.29	0.630	5.0417
Herbicide	Step 2 S	Ditch	150	0.366	0.51	1.107	5.0496
Herbicide	Step3 D1	Ditch	150	0.366	0.177	0.384	0.0910
Herbicide	Step3 D1	Stream	150	0.366	0.099	0.216	0.0037
Herbicide	Step3 D3	Ditch	150	0.483	0.021	0.038	1.5540
Herbicide	Step3 D4	Pond	150	0.4	0.018	0.036	4.5293
Herbicide	Step3 D4	Stream	150	0.4	0.006	0.012	0.0042
Herbicide	Step3 D5	Pond	150	0.526	0.012	0.020	5.2700
Herbicide	Step3 D5	Stream	150	0.526	0.005	0.008	0.0057
Herbicide	Step3 R4	Stream	150	0.662	0.092	0.136	0.0188

In the table below respective results are given for the top cm layer. Columns 4 and 5 show the total contents in sediment with and without accumulation, whereas column 6 and 7 show the respective results for pore water.

Table C3: Results of FOCUS PEC_{sed} calculations over 1 cm for the three example compounds

Mitigation	Location	Water body	PECmax (µg/kg)	PEC, max,akku (µg/kg)	PEC_{pw}, max (µg/L)	PEC_{pw}, max,akku (µg/L)
Fungicide	Step 2 N	Ditch	744.85	752.1883	8.6863	8.7719
Fungicide	Step 2 S	Ditch	1360.3	1373.7018	15.8636	16.0198
Fungicide	Step3 D1	Ditch	20.9994	21.2063	0.1059	0.1069
Fungicide	Step3 D1	Stream	11.358	11.4699	0.0503	0.0508
Fungicide	Step3 D2	Ditch	55.6225	55.8843	0.3192	0.3207
Fungicide	Step3 D2	Stream	46.2376	46.4552	0.2624	0.2636
Fungicide	Step3 D3	Ditch	12.9182	12.9470	0.0680	0.0682
Fungicide	Step3 D4	Pond	3.4019	3.4236	0.0138	0.0139
Fungicide	Step3 D4	Stream	0.8239	0.8292	0.0031	0.0031
Fungicide	Step3 D5	Pond	2.9571	2.9609	0.0120	0.0120
Fungicide	Step3 D5	Stream	0.2502	0.2505	0.0009	0.0009
Fungicide	Step3 D6	Ditch	4.7903	4.7904	0.0229	0.0229
Fungicide	Step3 R1	Pond	5.1515	5.1630	0.0218	0.0218
Fungicide	Step3 R1	Stream	12.847	12.8756	0.0570	0.0571
Fungicide	Step3 R3	Stream	13.9393	13.9419	0.0643	0.0643
Fungicide	Step3 R4	Stream	18.5456	18.5499	0.0948	0.0948
Insecticide	Step 2 spring N	Ditch	71.3	118.0747	7.7081	12.7648
Insecticide	Step 2 spring S	Ditch	116.55	193.0099	12.6000	20.8659
Insecticide	Step 2 summer N	Ditch	64.5	106.8137	6.9730	11.5474
Insecticide	Step 2 summer S	Ditch	77.2	127.8452	8.3459	13.8211
Insecticide	Step3 D3	Ditch	10.5101	14.9004	0.6061	0.8593
Insecticide	Step3 D4	Pond	4.1701	6.5515	0.1988	0.3123
Insecticide	Step3 D4	Stream	3.6732	5.7709	0.1746	0.2743
Insecticide	Step3 D5	Pond	2.6218	3.5635	0.1193	0.1622
Insecticide	Step3 D5	Stream	3.0544	4.1515	0.1567	0.2130
Insecticide	Step3 R1	Pond	1.9738	2.7983	0.0876	0.1242
Insecticide	Step3 R1	Stream	3.1665	4.4892	0.1717	0.2434
Insecticide	Step3 R2	Stream	2.0398	2.5130	0.1073	0.1322
Insecticide	Step3 R3	Stream	5.5869	6.8087	0.3178	0.3873
Insecticide	Step3 R4	Stream	3.1502	4.4661	0.1713	0.2429
Herbicide	Step 2 N	Ditch	1.45	3.1480	0.5273	1.1447
Herbicide	Step 2 S	Ditch	2.55	5.5361	0.9273	2.0131
Herbicide	Step3 D1	Ditch	0.4151	0.9012	0.0687	0.1491
Herbicide	Step3 D1	Stream	0.1987	0.4314	0.0305	0.0662
Herbicide	Step3 D3	Ditch	0.1062	0.1906	0.0164	0.0294
Herbicide	Step3 D4	Pond	0.0332	0.0677	0.0043	0.0088
Herbicide	Step3 D4	Stream	0.0186	0.0379	0.0025	0.0051
Herbicide	Step3 D5	Pond	0.027	0.0459	0.0034	0.0058
Herbicide	Step3 D5	Stream	0.0166	0.0282	0.0022	0.0037
Herbicide	Step3 R4	Stream	0.4591	0.6826	0.0822	0.1222

GLOSSARY AND ABBREVIATIONS

AF	Assessment Factor
AGD	Aquatic Guidance Document
AMRAP	Aquatic Macrophyte Risk Assessment for Pesticides, SETAC Europe, 2009 2nd SETAC Europe Special Science Symposium, Brussels, Belgium, 2009–09–17/2009–09–18
a.s.	active substance
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
BPD	Biocidal Product Directive
BPR	Biocidal Products Regulation
BSAF	Biota-sediment accumulation factor
CA	concentration addition
DDT	dichlorodiphenyltrichloroethane
$DegT50_{water/sediment\ system}$	Description of time taken for 50 % of substance to disappear in the water-sediment system according to OECD 308 as a result of transformation processes including mineralisation and formation of metabolites but considering also the formation of bound residues
ECHA	European Chemicals Agency
ECx	Concentration where x % effect was observed/calculated
EEC	estimated environmental concentration
EqP	equilibrium partitioning
ERA	Environmental Risk Assessment
ERC	ecotoxicologically relevant concentration
ERO	ecological recovery option
ETO	ecological threshold option
ETR	Exposure-Toxicity Ratio
EU	European Union
Exposure profile	The course of time of the concentration on a relative concentration scale (an effect study is usually carried out at different concentration levels but with the same exposure profile).
FFLC test	Fish Full Life Cycle test
FOCUS	FOrum for the Co-ordination of pesticide fate models and their USe
HCx	Hazardous concentration for x % of the species of a SSD
ISO	International Organization for Standarization
LOEC	Lowest Observed Effect Concentration
Metabolite	Any metabolite or a degradation product of an active substance, safener or synergist, formed either in organisms or in the environment (thus including

	also oxidation products which may have a larger molecular mass than the parent substance) (EFSA, 2012c).
NOEC	No Observed Effect Concentration
OC	organic carbon
OECD	Organization for Economic Co-operation and Development
PAT	pesticide application timer
PBT	The evaluation of new chemicals with respect to their persistency (P), bioaccumulative potential (B) and toxic potency (T)
PCB	polychlorinated biphenyl
PEC	predicted environmental concentration
PLFA	phospholipid fatty acid
PNEC	Predicted No Effect Concentration
PPP	plant protection product
PPR Panel	EFSA Panel on Plant Protection Products and their Residues
PRZM	Pesticide Root Zone Model
(Q)SAR	(Quantitative) Structure–Activity Relationship
RAC	regulatory acceptable concentration
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RQ	risk quotient
SCoPAFF	Standing Committee on Plant Animal Food and Feed
SED	sediment
SETAC	Society for Environmental Toxicology and Chemistry
SPG	specific protection goal
SSD	Species Sensitivity Distribution
SW	surface water
SWAN	Surface Water Assessment eNabler
SWASH	Surface WAter Scenario Help
TMF	trophic magnification factor
TOXSWA	TOXic substances in Surface WAters
TU	toxic unit
TWA	time-weighted average
US EPA	United States Environmental Protection Agency
VMPR	veterinary medicinal product residues
vPvB	very persistent and bioaccumulative
WFD	Water Framework Directive
WoE	Weight of Evidence