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SUPERSEDED GUIDANCE - NEWER VERSION AVAILABLE

Transitional Guidance on the Biocidal Products Regulation

Transitional Guidance on mixture toxicity assessment for biocidal products for the environment

May 2014

TRANSITIONAL GUIDANCE

LEGAL NOTE

This document contains Transitional Guidance on 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 (Biocidal Products Regulation, the BPR).

Users are reminded that the text of the BPR is the only authentic legal reference and that the information in this document does not constitute legal advice. Only the Court of Justice of the European Union can give authoritative interpretations on the contents of Union law.

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Transitional Guidance on mixture toxicity assessment for biocidal products for the environment

Publ.date: May 2014
Language: EN

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PREFACE

This Transitional Guidance is to be applied to applications for active substance approval and product authorisation submitted under the Biocidal Product Regulation (the BPR). This document describes the BPR obligations and how to fulfil them.

This Guidance replaces the Technical Notes for Guidance (TNsG) on Data Requirements (EU, 2008a) in support of Directive 98/8/EC (Biocidal Product Directive - BPD).

A "Transitional Guidance" is a document that has been initiated under the "old" Biocidal Products Directive and because it has been finalised before the relevant new Biocidal Products Regulation guidance document has been fully developed, it is being made available as a Transitional Guidance document until such time as the relevant new document is ready for publication.

This Transitional Guidance document has been discussed and supported by the Environmental WG of the Biocidal Products Committee (BPC). The document has not undergone any consultation with the Biocidal Competent Authorities and Accredited Stakeholder Organisations. The document is waiting for inclusion into Volume IV Environment, Part B of the new BPR guidance structure, at which time it will undergo a full consultation procedure.

NOTE to the reader:

This Transitional Guidance will be reformatted when it is incorporated into the New Guidance Structure. When this is completed, the finalised version will be uploaded onto the website of ECHA. No consultation will be made to do this

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List of Abbreviations

Standard term / Abbreviation	Explanation
AF	Assessment Factor
BPD	Biocidal Products Directive. Directive 98/8/EC of the European Parliament and of the Council on the placing on the market of biocidal products
BPR	Biocidal Products Regulation. Regulation (EU) No 528/2012 of the European Parliament and of the Council concerning the making available on the market and use of biocidal products
CA	Concentration addition
CBA	Component-based approaches
EBI	Ergosterolbiosynthesis-inhibting
EC	Effect Concentration
EC_{50}	Median effective concentration
ECB	European Chemicals Bureau
EO	Essential oils
ERA	Environmental risk assessment
ESD	Emission Scenario Document
IA	Independent action
LC_{50}	Median lethal concentration
MAF	Mixture assessment factor
MCR	Maximum cumulative ration
MoA	Mode of action
MSCA/CA	Member State Competent Authority/Compent Authority
NOEC	No observed effect concentration
OECD	Organisation for Economic Cooperation and Development

Standard term / Abbreviation	Explanation
PBO	Piperonyl Butoxide
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration,
POP	Persistent organic pollutant
PT	Product typ
QSAR	Quantitative structure activity relationship
RA	Risk assessment
RMM	Risk Mitigation Measures
RQ	Risk Quotient
$RQ_{Product}$	Risk Quotient of the Product
SoC	Substances of concern
STP	Sewage Treatment Plant
STU	Sum of Toxic Units
TGD	Technical Guidance Document
TM	Technical Meeting
TNsG	Technical Notes for Guidance
TU	Toxic Units
TUS	Toxic unit summation

1. Background

Biocidal products are usually multi-component mixtures of one or more active substances and a range of co-formulants that serve different purposes e.g. anti-foaming agents, stabilisers, pigments, emulsifiers, solvents, or diluents. Therefore the overall ecotoxicity of a biocidal product might be significantly different from that of each individual ingredient(s) alone and hence, needs to be assessed during the product authorisation. Article 19(2) of the Biocidal Products Regulation (BPR, 528/2012 EU) states that *"the evaluation […] shall take into account the following factors: […] (d) cumulative effects, (e) synergistic effects."* This is further elaborated in Annex VI (common principles for the evaluation of biocidal products) which states that the risks associated with the relevant individual components of the biocidal product shall be assessed, taking into account any cumulative and synergistic effects.

However, only very limited details on how mixture effects should be considered during the authorisation of a biocidal product are provided in the current Technical Notes for Guidance on Product Evaluation (TNsG on product evaluation, [27]) and no specific guidance is at hand on how potential combination effects of active substance(s) and other ingredients should be accounted for during the environmental risk assessment of biocidal products.

This guidance document therefore addresses the assessment of the mixture toxicity of products as well as synergistic effects as required by the BPR (and the Biocidal Products Directive (BPD, 98/8/EC) which was replaced in September 2013 by the BPR) by applying a tiered scheme for the adequate consideration of mixture effects during the environmental risk assessment of biocidal products.

1.1 Definitions

At the 47th meeting of representatives of Members States Competent Authorities for the implementation of Directive 98/8/EC concerning the placing of biocidal products on the market (CA-Meeting) in July 2012 a room document was provided regarding the definitions of "mixture toxicity", "synergistic effects" as well as "aggregated exposure" (response to document CA-July12-Doc.5.2.h). According to this, the terms are defined as follows:

- **Mixture toxicity**: refers to the combined toxicity and risk to human and animal health, and the environment, from all relevant substances (see point 3.2.2) in a biocidal product, including their degradation products and regardless of the underlying mechanism(s) of mixture toxicity (non-interactive or interactive joint action) and taking into account the different environmental, occupational and residential mixture(s) which are formed during all life cycle steps relevant under the BPR. 1
- **Synergism/synergistic effect:** an effect or toxicity from a chemical mixture which is greater than that expected from non-interactive joint action because one mixture component influences the toxicity of another. As the default assumption of non-interactive joint action is concentration addition (see point 2), synergistic effects are effects of a mixture which are greater than that predicted by CA by a factor of 5 or more[1].

[1] Value derived from the results of several research projects on the mixture toxicity of wood preservative products as a pragmatic proposal [22-24], but might be higher or lower in some cases. If data are indicating synergistic effects, they should be checked carefully regarding the applied methods for the calculation of the prediction of mixture toxicity, the performance of the tests as well as the tested species. Also the criteria given under point 3.2.3 should be taken into consideration to verify synergistic interactions.

- **Aggregated effects/exposure:** refers to the overall exposure to humans and the environment, to the same substance, by emissions during all life cycle steps relevant under the BPR of different products belonging to the same PT or different PTs.

2. Generic Options for Mixture Toxicity Assessment

A number of ways to include mixture toxicity in risk assessment have been proposed in literature [1]:

A) Applying a specific mixture assessment factor (MAF): safeguarding against mixture effects by means of a special factor, similar to other uncertainty factors in single substance assessment.
B) Bridging or read-across: drawing conclusions from available data from similar products.
C) Component-based approaches (CBA): calculating the expected joint toxicity from the toxicity data for the individual mixture components by applying corresponding prediction models e.g. Concentration addition (CA) and Independent action (IA).
D) Direct experimental testing of the mixture of concern, i.e. the whole product or the environmentally relevant mixture resulting from the use of the product.

These approaches are more or less suitable for mixture toxicity assessment in a legal context in general and for product risk assessment under the BPR in particular. Using a specific safety factor, e.g. the MAF for mixtures has been dismissed, mainly since it would be difficult to scale such a factor for all different kinds of biocide product types. Bridging is considered as a possible way of building a case but there are clear problems with defining "similar mixtures". Hence, the focus of this guideline is on component-based approaches (CBA) and the direct testing of a chemical mixture.

2.1 Component-based approaches (CBA)

By using mathematical models it is possible to calculate the effect that would presumably be caused by a mixture based on knowledge of the toxicities of the individual mixture components. This is referred to as a component-based approach (in mixture risk assessment).

The idea is that by knowing the composition of the mixture under evaluation as well as the hazard profiles for the individual substances of the mixture, it would be possible to predict the effect caused by the mixture without further testing. This is clearly an advantage since it would be impossible to test the vast range of possible mixtures in the environment. Furthermore, the BPR clearly states that unnecessary testing, especially using vertebrate species, should be avoided. A number of methods and models have been suggested in literature for the analysis and assessment of combined effects of substances. However, most of them are based on only two different fundamental concepts for the assessment of the so-called non-interactive joint action (which appears to be the prevalent type of combined effect): Concentration (or Dose) addition (CA) and Independent action (IA), which is sometimes referred to as Response addition (Table 1).

Table 1 The different types of joint action of chemicals and their distinctions

The different types of joint action of chemicals and their distinctions * (after Plackett and Hewlett 1952 [45]; Badot et al 2011 [10])		
Type of combined effect	**Similar joint action**	**Dissimilar joint action**
Non-interactive	Simple similar action Concentration (dose) addition	Simple dissimilar action Independent action, Response addition
Interactive	Complex similar action Synergy, Potentiation (greater than non-interactive effects)	Dependent joint action Antagonism (less than non-interactive effects)

* Interactive joint action denotes the situation where one substance influences the toxicity of another, leading to synergism or antagonism. Such effects cannot be accounted for by CA or IA.

Interactions of components in a mixture can cause either significantly increased (synergistic) or decreased (antagonistic) effects compared with the effects predicted by the reference models (CA, IA, table 1). From the current knowledge such interactions seem to be comparatively rare in general, relatively small and largely confined to mixtures with only few compounds [36]. Furthermore, synergisms are very specific for certain mixtures (compound types, their concentrations and mixture ratios), particular organisms and endpoints. Hence they cannot be incorporated into a general risk assessment scheme, but must be treated on a case-by-case basis. When it comes to pinpointing the causes for synergisms or antagonisms, there are substantial knowledge gaps in the current scientific understanding, e.g. regarding the conditions that might lead to synergistic mixture toxicities or the size that synergisms are likely to be [36] (see point 3.2.3).

Both the concepts, CA and IA, build upon mathematical models that can be reasonably transferred to the current understanding of chemical and physiological interactions. In other words, the mathematical models mirror several properties of how chemicals interact with physiological processes. As explained in further detail below, neither concept makes any in-depth assumptions on biology or physiology, nor requires any details on toxicodynamic or toxicokinetic processes. Moreover, for IA, toxicant effects are assumed to be expressed completely independently from each other, which is hardly the case in reality considering that organisms consist of complex interacting subsystems. Taken together, both concepts represent remarkably simple assumptions. Despite this, they have been shown to produce very accurate predictions of mixture toxicity even on higher levels of biological organization such as algal biological communities [3, 6, 7, 9, 15, 16, 29, and 47].

Even though both concepts can be related to toxicological events, they build upon fundamentally different basic principles which sometimes give different results in terms of the predicted effect level. This distinction is clearly important to discuss in the context of risk assessment.

2.1.1 Concentration Addition (CA)

The concept CA was first formulated by the German pharmacologist Loewe in 1926 [40]. Mathematically the concept can be described as:

$$\sum_{i=1}^{n} \frac{c_i}{ECx_i} = 1 \qquad \text{eq. 1}$$

Where n is the number of components in the mixture, c_i is the concentration of component i in a mixture which elicits x % effect (e.g. 50), and ECx_i is the Effect Concentration (EC) at effect x % for component i (e.g. EC_{50}, correspondingly).

The fraction c/ECx for a compound is termed a "toxic unit" (equation 2). This represents the concentration of a compound scaled to its potency (e.g. the EC_{50}). The size of the toxic unit can be understood as a measure of how much compound i contributes to the mixture effect. A component with a large toxic unit contributes more to the mixture effect than a component with a small toxic unit.

$$TUi = \frac{ci}{ECxi} \qquad \text{eq. 2}$$

If the sum of toxic units (STU) in a given mixture that provokes x% effect equals 1, the mixture behaves according to CA. Under these circumstances any component in the mixture is replaceable by another compound without changing the overall mixture toxicity, as long as the size of the toxic unit of the replacing compound is equal to the toxic unit of the compound being replaced. This interchangeability is usually interpreted as a combination of two things. First the assumption that the compounds in the mixture do not interact, neither on a physico-chemical level nor on toxicodynamic or toxicokinetic processes. Secondly, that the compounds have a similar mechanism of action, e.g. by binding to the same receptor site. Inherently, compounds with same mechanism of action would also have an effect on the same endpoint.

2.1.2 Independent action

First described by Bliss in 1939 [13], the concept IA, like CA, assumes that all mixture components have effect on the same integrating endpoint. However, in contrast to CA, IA assumes that the mixture components do not share a common mechanism of action. IA assumes that the components act on different subsystems (e.g. tissues, cells, molecular receptors) of an exposed organism, without any overlap. These affected subsystems must evidently affect the observed endpoint, but independently of each other. Like CA, IA assumes that there are no interactions between the mixture components. The expected mixture effect can thereby be calculated according to the mathematical concept of joint probability of independent events (equation 3).

$$E(c_{mix}) = 1 - \prod_{i=1}^{n} [1 - E(c_i)] \qquad \text{eq. 3}$$

According to this equation, n is the number of components, $E(c_i)$ denotes the effect that component i has (on its own, if applied singly) at concentration c, which is the component's concentration in the mixture. This annotation of the IA-equation applies if the effect is scaled 0-1 where 1 means 100 % effect (e.g. 100% mortality). The total concentration of the mixture is called c_{mix}, and $E(c_{mix})$ is thereby the IA-predicted effect of the whole mixture.

In line with what is stated above and the mathematical concept, Independent action of the individual compounds in a mixture is commonly interpreted as the compounds having dissimilar mechanisms of action.

2.1.3 Applicability of the models in hazard assessment

Deciding which model would be most accurate in predicting the effect of a given mixture may be difficult and highly dependent on the availability of detailed information on the mechanism of action of the single components. However, such information is rarely at hand, and for most mixtures the very strict requirements of both CA and IA of total similarity or dissimilarity of toxic action is hardly met in reality. It is generally recognized that CA may be used as the default concept of choice for a number of reasons [36]. This discussion is only described very briefly here. A more comprehensive overview can be found in the State of the Art Report on Mixture Toxicity by Kortenkamp and co-workers [36] and the EU-Commission's Expert Panel's opinion on mixture toxicity assessments (SCHER, SCCP, SCENHIR, 2012 [50]).

By comparing predicted mixture toxicity to actual tested mixtures it has been shown that for most tested mixtures, CA predicts higher mixture toxicity than IA, and CA is much less likely to underestimate the effect of a given mixture [1]. For a precautionary predictive risk assessment regime it would not be appropriate to use a concept where there is a potential of underestimating the risk. Furthermore, it could be shown, that CA is also applicable for mixtures composed of strictly dissimilarly acting compounds, especially as the difference of predicted effects between CA and IA are usually small [18], at least when studying integrating endpoints such as mortality. Finally, for pragmatic reasons, CA is much more applicable since it can be used with single datapoints or single substance data, such as EC_{50}- or NOEC-values whereas IA requires more detailed effect information, typically in the low effect range.

As recommended by the EU Commission in 2012 [19] on the basis of numerous scientific reports and opinions (EU Commissions report on the State of the art on mixture toxicity [36], the EU-Commission´s Expert Panel (SCHER, SCCP, SCENHIR, 2012 [50]) as well as several other publications, CA is the preferred concept for estimating mixture toxicity from chemical mixtures, at least in the absence of adequate mode of action information. Moreover, by using CA, the currently available data for active substances can be used without major alterations since EC_{50}- and NOEC- values can be used as input data for the various models building upon the CA concept (see point 3.3) and additional testing is minimized. Furthermore, the CA concept is likely to not underestimate the risk from the evaluated mixture.

2.2 Whole mixture testing

In certain cases, whole mixture testing may be the only viable option (see points 3.2.2, 3.2.3 & 3.3.4, Figures 1-5). This situation may occur when it is suspected that a component in the mixture acts as a synergist, and may cause an interactive type of joint action for which CA (or IA for that matter) is an invalid assumption (see above, table 1). Whole mixture testing could be used in such situations.

Another cause for choosing to perform whole mixture testing would be that even higher tier effect modeling predicts unacceptable risk (see point 3.3, Figures 1-5). It should be noted, however, that it is stated in the BPR that unnecessary vertebrate testing should be avoided and the employed strategy for refinement of the risk assessment should acknowledge this by choosing invertebrate or algal species to demonstrate the applicability of the concept (and extrapolate to vertebrate/fish). Therefore, testing should always be the last option. If testing is conducted, the most sensitive species as indicated by the single substance data should be tested.

If the whole mixture testing approach is chosen, careful consideration should be taken to determine the most relevant mixture to be tested ("relevant mixture") on a case-by-case basis and it is recommended that the test design is agreed with the Competent Authority before tests are conducted. In some cases, where the environment is directly exposed to the formulated product, testing of the product might be useful. However, in most cases,

the environment is exposed to a mixture that is different from the original composition of the product. For a few product types it might be expected, that all components end up in the environment, but in different relative amounts than given in the original product composition. For the vast majority of the product types it can be assumed, that the composition changes radically before release into the environment with regard to both the ratio and the concentration of the mixture components, leading to an environmental mixture which is considerably qualitatively different from the product, for instance after solvents have evaporated. For certain product types, e.g. PT08 (wood preservatives) or 21 (antifoulings), leachate testing might then be indicated and a useful risk assessment option. For other product types it might be adequate to calculate and design a mixture depending on expected environmental fate and behaviour of the various components, before performing whole mixture testing ("surrogate mixture", see point 3.2.2 & 3.3.4).

3. Tiered Approach

Based on the existing generic options for mixture assessment described in the literature (see above) a tiered approach for the mixture assessment in the environmental risk assessment (ERA) of biocidal products was developed.

This approach accommodates (i) different data situations, acknowledging that the initially available data might be quite different for the various product types covered by the BPD / BPR, (ii) optimises resource usage, (iii) limits biotesting as far as possible and (iv) ensures adequate protection of the environment. It mainly builds on using component-based approaches (CBAs) based on the concept of Concentration addition (CA) for mixture toxicity prediction, which is either approximated by summing up PEC/PNEC ratios or implemented as sums of Toxic Units (STU). These component-based approaches should be complemented by the direct biotesting of the product or the ecologically relevant mixture only where essential ("relevant mixture ", see points 3.2.2 & 3.3, Figures 1-5). This is already stressed in the BPR (Annex III), because it reduces the need for further (vertebrate) testing and also facilitates the re-use of existing data for individual (active) ingredients, a factor likely to be increasingly important in the future as the BPR will request data sharing between applicants. However, the direct testing of the mixture of concern should be regarded as a straight forward approach for the assessment of the mixture toxicity in principle, especially if synergistic interactions are indicated (see point 2, 3.2.3), although there might be limitations (see point 3.3.4). The reason for preferring whole mixture data is that such data capture any interactions that may occur between the mixture components e.g. synergistic effects as well as contributions from compounds that have not been considered in the mixture toxicity predictions or for which ecotoxicity information is lacking (e.g. formulation additives, see point 3.2.2). If such data are available and sufficient for a comprehensive risk assessment, the ERA will be based on the mixture as whole, comparable to the ERA for single substances (see point 3.3, Figure 1).

In the following the terms "mixture" and "relevant mixture " are used for the product itself and the ecologically relevant mixture, respectively.

Note
The competing concept of Independent action (IA) was assessed as not being suitable for incorporation into a tiered approach without explicit confirmatory studies, as it might otherwise lead to an underestimation of the actual environmental risk, especially when assessing mixtures with components present below effect levels. In addition, IA would lead to higher data demands compared to CA. However, if the applicant can prove that IA adequately describes the toxicity of a given product by submitting appropriate data, e.g. information about the MoAs and the concentration-response relationships of the mixture components, these data should be taken into account for mixture toxicity assessment and assessed according to expert judgment.

3.1 Requested input data for a component-based approach

The minimum requested set of data for a component-based assessment consists of (i) reliable and complete information on the product composition, (ii) basic data[2] for all ingredients on which it can be decided whether a substance has to be regarded as a relevant substance to be included in mixture assessment (see point 3.2.2, Annex 1) as well as (iii) at least the PEC/PNEC ratios for the compounds identified as relevant substances and (iv) information on the occurrence of synergistic interactions between the product components (see point 3.2.3).

In the following the consecutive tiers of the approach are described, which are also depicted in decision trees in Figures 1-5 for a better traceability. Case studies applying the tiered approach on products from different product types (PTs) can be found in Annex 4.

3.2 Screening Step

3.2.1 Identification of the concerned environmental compartments

Sufficient appropriate data have to be submitted from which it can be decided whether an exposure of environmental compartments can be expected from the application of the product and if so, which environmental compartments are likely to be at risk. Thereby, the procedure is similar to the procedure applied for single substances by taking into account the general principles described in the Technical Guidance Document [28] and the Technical Notes for Guidance on Product Evaluation [27] as well as the related Guidance Documents and Emission Scenario Documents.

If the data provided reveal that an exposure of environmental compartments to the products and its components is unlikely, e.g. due to a negligible exposure as the product is only applied in completely closed systems, no ERA and consequently no mixture assessment has to be performed.

In case an exposure of the environment due to the application of the product is possible, it has to be checked whether there is a direct release of the product or a release of a modified mixture into environment and if so, which components are likely to be released. Also the physico-chemical properties of the product components influencing the environmental fate have to be evaluated. It is possible that for some of the mixture components the intrinsic substance properties indicate that the environment is unlikely to be significantly exposed to these substances. In such a situation qualitative argumentation may be submitted to demonstrate that environmental exposure in a particular compartment would be negligible. Such argumentation should be supported by appropriate data. Examples may include very rapid degradation or dissipation (e.g. by volatilisation and rapid photochemical oxidation in air, see also point 3.2.2, Annex 1).

A definition of a time-scale for indirect releases is only possible on a case-by-case basis and should be discussed in relation with the respective Emission Scenarion Document (ESD) as this information is highly dependent on the actual use of the biocidal product.

[2] Ecotoxicological-, Fate and Behaviour- as well as relevant physico-chemical end-points should be derived for the product components based on available information (e.g. laboratory studies, material safety data sheets, EU or International chemical reviews, QSARs. etc). All reasonable efforts should be made to submit the most up to date and reliable information and this should be detailed in the submission, along with any letters of access that may be required. As only semi-quantitative data are needed for this purpose, e.g. QSAR-estimates, hazard classification data from classification and labeling according to the CLP Regulation (EC) No 1272/2008, censored toxicity data (e.g. from limit tests) or simple exposure estimates should be sufficient in the first step.

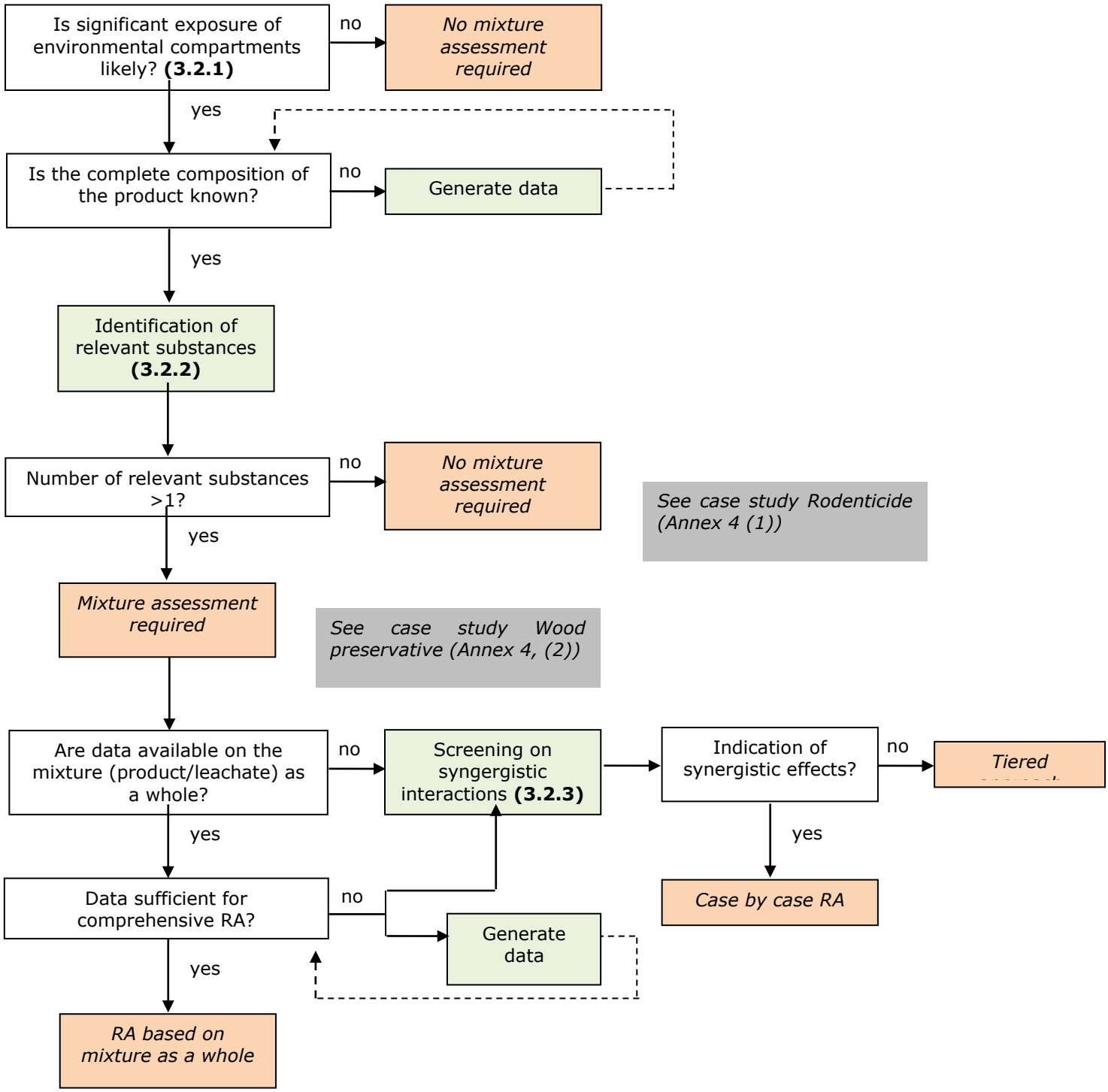

Figure 1: Decision tree for the Screening Step (point 3.2, RA **= risk assessment).**

If this procedure reveals that an exposure of environmental compartments is likely due to the application of the product, it has to be checked, in the next steps, whether a mixture assessment is required for the product. For this purpose the complete composition of the product will be required (see point 3.1 and 3.2.2).

3.2.2 Identification of relevant substances

The default approach is that all ingredients originally present in the biocidal product are considered as *a priori* relevant for a mixture risk assessment. Qualitative or quantitative argumentation taking into account, *e.g.,* the composition of the mixture to which the environment is exposed, or expected relative contribution to an additive mixture effect, may be employed to demonstrate that some ingredients can be safely disregarded whilst still allowing an adequate assessment of the risk of the mixture. Further guidance on this is given below.

Any component based approach requires that all "relevant" components of a mixture are included in the assessment, i.e. biologically active chemicals that are present at sufficiently high concentrations and are contributing to the overall toxicity of the respective mixture [1]. Obviously, if relevant compounds besides the active substance(s) are not considered in a component-based mixture toxicity assessment, the calculated risk will be an underestimation of the actual risk of the biocidal product. It is, however, impossible to provide a general estimate of the magnitude of such an underestimation, as this depends on the concentration and toxicity of the compounds that are not included in the assessment. Therefore, special care has to be taken to ensure that all relevant ingredients are included in a component-based assessment of a biocidal product.

If there is no confidence that all relevant substances are included in the assessment or if no (ecotoxicological) information is at hand for some of the ingredients, the only effect assessment options are either the direct biotesting of the substances, for which no information is available or the direct biotesting of the biocidal product and/or the resulting environmental mixture, respectively. The direct testing of the "relevant mixture" should be regarded as a straight forward approach for the assessment of the product toxicity in principle, especially in cases where synergistic interactions are indicated (see point 3.2.3, 3.3.4).

Likewise, in case of testing a "surrogate mixture", i.e. a mixture supposed to represent the product because it is impossible to test the product as it is (see above & point 3.3.4), it has to be ensured that all relevant substances are included in this mixture.

What are 'Relevant Substances' in a typical biocidal product?[3]

The following substances are regarded as relevant for mixture assessment:

1) Active substances.
2) Substances of concern (SoC).
3) Active substances from other PTs. However, it should be considered under which conditions exemptions are possible (e.g. substances contained in Annex I of the BPR or substances contributing only to a very limited extent to the overall toxicity of the mixture, see Annex 1, 2 and 4).
4) Other ingredients which do not fall under one of the aforementioned categories but might be relevant for mixture assessment like e.g. known synergists should be considered as well case by-case.

Article 3 (f) of the BPR defines that "(...) substance of concern means any substance, other than the active substance, which has an inherent capacity to cause an adverse effect, immediately or in the more distant future, on humans, in particular vulnerable groups, animals or the environment and is present or is produced in a biocidal product in

[3] For identification and exact definition of SoCs reference is made to ongoing discussions within the so-called SoC- working group. The definition of relevant substances should be reviewed after finalisation of the discussion on SoC and amended accordingly.

sufficient concentration to present risks of such an effect. Such a substance would, unless there are other grounds for concern, normally be:

- a substance classified as dangerous or that meets the criteria to be classified as dangerous according to Directive 67/548/EEC, and that is present in the biocidal product at a concentration leading the product to be regarded as dangerous within the meaning of Articles 5, 6 and 7 of Directive 1999/45/EC, or
- a substance classified as hazardous or that meets the criteria for classification as hazardous according to Regulation (EC) No 1272/2008, and that is present in the biocidal product at a concentration leading the product to be regarded as hazardous within the meaning of that Regulation,
- a substance which meets the criteria for being a persistent organic pollutant (POP) under Regulation (EC) No 850/2004, or which meets the criteria for being persistent, bio-accumulative and toxic (PBT) or very persistent and very bio-accumulative (vPvB) in accordance with Annex XIII to Regulation (EC) No 1907/2006.".

A discussion on SoCs is currently on-going. The investigation whether the substances identified as relevant for mixture assessment by the workshop participants are already covered by this definition of SoCs as well as the further elaboration of this topic are the task of the SoC-Working group. Dependent on the decisions made by the group this chapter should be reviewed and amended accordingly.

Nevertheless, a first draft proposal for the identification of relevant substances for mixture assessment has been developed based on the discussion at the workshops and the revision of the preliminary guidance document. This proposal can be found in Annex 1.

It has to be emphasised again, that special care has to be taken to ensure that all relevant substances are included in a component-based assessment of a biocidal product, because otherwise the risk for environment resulting from the application of the product may be underestimated. If no or not sufficient (ecotoxicological) information is at hand for all ingredients (see Annex 1), to decide whether a substance is relevant for mixture assessment the only effect assessment option is the direct biotesting either of the respective substance(s) or of the biocidal product and the resulting environmental mixture, respectively. If a mixture cannot be assessed in its entirety, because of e.g. insoluble pigments or other ingredients making a direct testing of the product unfeasible, it is also possible to assess generic mixtures of the relevant substances („surrogate mixture", see above & point 3.3.4). If the assessment reveals, that there are several relevant substances (>1) contained in the product, a mixture assessment is required for the respective product under consideration. It has to be checked then, whether mixture data, i.e. product tests or leachate toxicity tests, are already available and whether these data are sufficient for a comprehensive environmental risk assessment (ERA). If such data are at hand and are sufficient for a comprehensive ERA, the risk assessment (RA) will be based on the mixture data as a whole, comparable to the ERA for single substances (see point 3.3.4).

If the available mixture data are not sufficient for RA, there are several options to continue: The first option is to provide the missing data for the RA, whereas the second option is to proceed with the tiered approach. If it can be concluded from the available mixture data, that no synergistic interactions are likely to occur between the product components, it can be proceeded as described under point 3.3. If this conclusion is not possible, it is recommended to continue with the next step of the tiered approach. It is also recommended to continue with the next step of the tiered approach if mixture data are lacking (see point 3.2.3).

3.2.3 Screen on synergistic interactions

Synergistic interactions describe the combined effect of two or more substances as stronger than expected from non-interactive joint action because the mixture components are influencing each other's toxicity (see point 2.1). The interactions may vary according to the relative concentration level and the biological targets as well as the route(s), timing and duration of exposure (including the biological persistence of the mixture components). Several different types of interactions are described in literature [20, 31, 50, 55]:

- Chemical-chemical interactions: chemicals are reacting together directly to form another compound or a complex which is more toxic (or less toxic) than the parent compounds or enhances (or weakens) their toxicity
- Toxicokinetic interactions: chemicals modifying the absorption, distribution or elimination of others or chemicals competing for active transport mechanisms (uptake, clearance) leading to an increase (or decrease) in the internal dose of a compound compared to the level that occur if no interactions occurred.
- Metabolic interactions: chemicals modifying the metabolism of other mixture components due to e.g. enzyme induction, enzyme inhibition or saturation of an enzyme by the presence of other substrates.
- Toxicodynamic interactions: interactions between the biological responses resulting from exposure to the individual chemicals, e.g. resulting from similar targets (e.g. ligand-receptor interaction).

Concentration addition (CA), as well as Independent action (IA), is based on the assumption that the compounds in a mixture do not interact, neither chemically nor in their toxicokinetic / toxicodynamic phases (see point 2.1, ref. 1, 2, 4, 55). Although cases where the observed mixture toxicity deviated significantly from the expected additivity, indicating synergisms, are comparatively rare in general and for biocides in particular [1, 4] , several examples can be found in the literature (see Annex 3). In this context is has to be distinguished between intended synergisms, i.e. the intended use of synergists (e.g. PBO) in products to enhance the efficacy of the a.s. in the target-organisms, and un-indented synergistic interactions between the product components. In both cases a careful evaluation of the available data is indispensable for the risk assessment process (see below).

Synergism is mainly reported for mixtures with a few (usually two) compounds, which is exactly the situation that is relevant for many biocidal product, which contain typically two or more active ingredients. For biocides they are mainly described for antifouling substances (e.g. 37, 60) and essential oils (EOs) in combination with pyrethrins and other insecticides (e.g. 33, 48, 56) as well as for ergosterolbiosynthesis-inhibting (EBI) fungicides in combination with pyrethroid insecticides, organophosphates or neonicotinoids (17, 43, 51, 55, see also Annex 3). For example, the combination of zinc-pyrithione and copper shows a clearly higher toxicity than predicted by CA in a range of bioassays such as diatoms, worms or amphipods, partially due to the formation of copper-pyrithione by trans-chelation of zinc-pyrithione with copper [11, 37]. Mixtures of organophosphates and carbamates (insecticides) were consistently more toxic to fish than predicted by CA, despite their similar mechanisms of action [39]. This is most likely caused by the inhibition of organophosphate biotransformation to their inactive dicarboxylic acid derivates by carbamates. Further examples can be found in Annex 3.

Synergism is, besides other factors, highly dependent on: (i) the ratio and (ii) the concentrations of the mixture components, (iii) the presence of other chemicals, (iv) the species in which synergism is to be expected as well as (v) the mode of action of the substances [34, 38, 39, 58], these factors should be taken into account when deciding whether synergism is relevant for a product under consideration. Furthermore, it should

be taken into account whether there are direct emissions to water and soil or whether a modified mixture is introduced into the environment. For additional effective substances such as synergists in a product formulation, independent sources of information, e.g. from the intentional use aspects would need to be considered.

It is therefore proposed that sufficient and reliable data has to be submitted from which it can be decided whether synergistic interactions are unlikely to occur between the product components. The following aspects should be considered within the decision-making process:

- Are known or indented synergists or components declared as synergists present in the product?
- Are substances present in the product which are contained in one of the tables in Annex 3? For the substances in the tables in Annex 3 potential synergistic effects are reported in the peer-reviewed literature. These publications should be seen as indications for possible synergisms of the shown substances and be taken into account during the decision making process. However, they should be analysed in more detail for this purpose, e.g. regarding the tested concentrations, mixture ratios and the concentration-dependence of interaction as well as the tested organisms and endpoints.
- Are synergisms known or reported elsewhere in literature for one of the product components? If so, for which group of organisms, endpoints and concentrations (incl. number and ratios) are synergistic effects reported? Which conclusions can be drawn from these data for the product under consideration? Are the deviations from CA covered by the AF applied on the single substance data according to TGD [28] or are the data sufficient to derive an additional assessment factor to cover the observed synergistic interactions as suggested by [17], [20] and [55]? For which compartments are the synergistic effects reported? Are these likely to be at risk due to the application of the product?
- Are there any structural similarities for one or more of the product components with known synergists ("structural alerts" e.g. methylenedioxyphenyl group, piperamides, furanocoumarins, [12, 42, 48])?
- Can one or more product components significantly enhance the uptake of other components [50]?
- Can one or more product component inhibit significantly the excretion/clearance of other components [50]?
- Do one or more of the product components exert their toxic action via the formation of an active metabolite(s) and may one or more of the components induce the metabolising enzymes that may be involved in the formation of these active metabolites [50]?
- Can two or more product components act on different enzymes in an important metabolic pathway [50]?
- Can two or more product components act on different elements of cellular protection mechanisms or cellular repair mechanism [50]?

The assessment of the possible interaction requires expert judgement and hence needs to be considered on a case-by-case basis in a weight-of-evidence approach. If there are any indications of synergistic effects, which cannot be explained by the available data or are not manageable by e.g. additional safety factors, the only option is the direct testing of the product or of the ecologically relevant mixture for a comprehensive environmental risk assessment as synergisms are not predictable with the available methods in a systematic fashion, especially under the data situation given for biocidal products and their components [1, 2, 20, 21, 55].

If there are no indications for synergistic effects, it is recommended to proceed with the next step of the tiered approach (see point 3.3).

3.3 Tiered assessment scheme

According to point 3.1 the minimum requested set of data for a component-based assessment consists of (i) reliable and exhaustive information on the product composition, (ii) basic data for all ingredients on which it can be decided whether a substance has to be regarded as a relevant substance to be included in mixture assessment (see point 3.2.2) as well as (iii) at least the PEC/PNEC ratios for the compounds identified as relevant substances and (iv) information on the occurrence of synergistic interactions between the product components (see point 3.2.3).

The assessment scheme is based on a series of four tiers that begins with simple and conservative screening steps and moves to higher tiers as necessary (Figure 2):

- Tier 1: PEC/PNEC-Summation,
- Tier 2: modified Toxic Unit Summation (TUS)separated for trophic levels,
- Tier 3: standardToxic Unit Summation (TUS) separated for trophic levels,
- Tier 4: Experimental testing.

Each of the higher tiers involves a less conservative and more accurate assessment than the previous tiers but requires also more resources, including additional exposure and toxicity data. Two different approaches for the Toxic unit summation are proposed to acknowledge the fact, that not for all relevant substances of a biocidal product homogenous data sets are available.

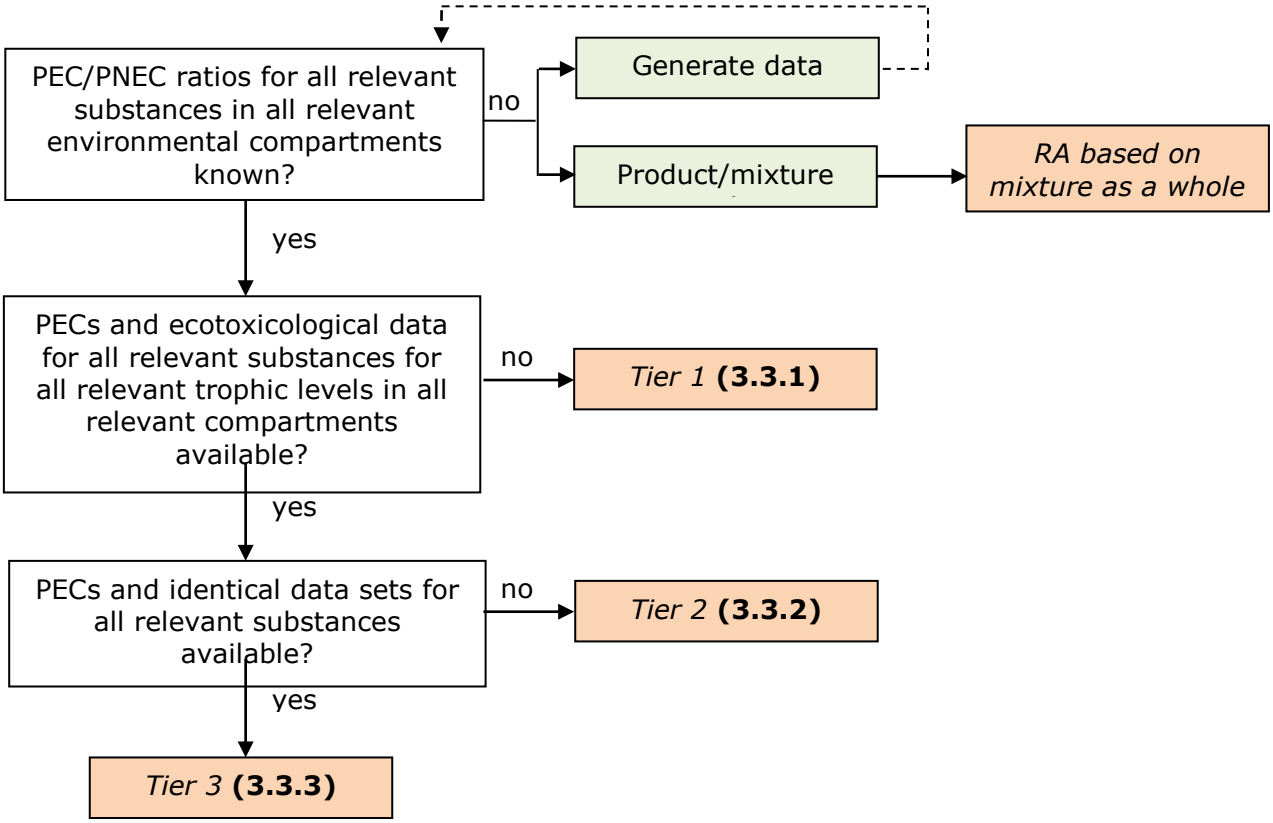

Figure 2: Decision tree for the tiered approach (point 3.3, PEC **= Predicted Environmental Concentration,** PNEC **= Predicted No Effect Concentration,** RA **= risk assessment).**

The tiers must not be performed step by step for a respective product, e.g. in case the data for tier 3 are available in the beginning, the assessment can be started with tier 3 (see Figure 2). Dependent on the data availability for all of the product components which were identified as relevant substances in the previous steps (see point 3.2.2) it should be proceeded with the different tiers of the assessment scheme: If at least the PEC/PNEC-ratios are available for all relevant substances for all relevant compartments and scenarios it is recommended to start with tier 1. If ecotoxicological data and PEC-values are available for all relevant substances for all relevant trophic levels for all relevant compartments and for all relevant scenarios it is recommended to start with tier 2. In case identical ecotoxicological data sets are available for all relevant substances for all relevant species and all relevant compartments, it is recommended to start with tier 3 (see Figure 2).

As outlined above, the tiered approach is based on the concept of Concentration addition (CA, point 2). For the practical application of CA in a regulatory context, a number of different approaches have been suggested in the literature [1]. For pragmatic reasons, these CA-based regulatory approaches usually include simplifying or additional assumptions, and hence they deviate more or less from the principal assumptions that are inherent to the original concept of CA. As a result, such CA-based approaches may differ with regard to both the suitability for specific assessment purposes and the quantitative mixture toxicity estimates that are derived from their application. Several types of pragmatic deviations or simplifications are at hand, for which four are relevant for the use of CA-based approach in biocidal products authorisation:

- No strictly identical (eco-)toxicological endpoint (selection of test species, exposure conditions, exact testing criteria and methodology) for all relevant substances,
- Use of NOEC- instead of EC_x-values,
- Assessment factors included in the single substance toxicity data (e.g. PNEC),
- Assumption of parallel concentration response curves for all mixture components.

As input data, the original concept of CA requires effect concentrations that refer to the same biological effect in the same species under identical test conditions. For the regulatory use as developed here, however, pragmatic simplifications and assumptions are unavoidable. This refers to the merging of data for different test conditions, endpoints and species and to the use of NOEC values as surrogate for quantitative estimates of low effect concentrations. In any case, the potential additional errors that may be introduced by such deviations from the original concept should be made transparent and where possible, should be removed in a stepwise manner [1].

3.3.1 Tier 1

If the PEC/PNEC ratios are available for all relevant ingredients, the risk quotient of the product can be simply estimated by their sum:

$$RQ_{\text{Product}} = \sum_{i=1}^{n} \left(\frac{PEC}{PNEC} \right)_i \qquad \text{eq. 4}$$

Summing up PEC/PNECs is mentioned in the Technical Notes for Guidance as one option for biocidal product assessment (ECB, 2002 [27]). However, it should be pointed out that eq. 4 is fundamentally different from the concept of Concentration addition (CA), as the PNECs from the various compounds might be based on data from completely different endpoints and species. Hence eq.4 violates one of the fundamental assumptions of CA, that all individual toxicity data refer to same biological endpoint and organism. Consequently, the use of PEC/PNEC sums derived from a set of different species and

endpoints are only recommended for first-tier CA assessment in the opinion on mixture toxicity assessment as put forward by the EU scientific committees [50]. It can be proven that eq. 4 provides a conservative approximation of CA [4]. Furthermore, it is a major advantage of the PEC/PNEC sum (eq. 4) that it can be applied even if different amounts of data are available for the different compounds in the product, for example when an extended data set including chronic ecotoxicity data is at hand for the active ingredient, but only base-set data are available for the other substances of concern. For a more detailed discussion on the use of PEC/PNEC sums see [4].

Should eq. 4 indicate reasons for concern ($RQ_{Product}$ >1), the following options exist:

(i) a refinement of the PEC- and/or PNEC-values by providing additional information on the exposure and/or hazard characterisation of the compounds, especially those that dominate the sum of PEC/PNECs,

(ii) continue with tier 2 , i.e. the application of CA in the form of a modified Toxic Unit Summation for each trophic level separately if homogenous data sets for the relevant substances are notavailable,

(iii) continue with tier 3, i.e. the application of CA in the form of the standard Toxic Unit Summation for each trophic level separately(in cases where homogenous data sets are available for all relevant substances),

(iv) direct testing of the mixture of concern (tier 4),

(v) the definition of effective Risk Mitigation Measures (RMM).

If the aforementioned options are not applicable the only remaining option is the non-authorisation of the product (Figure 3).

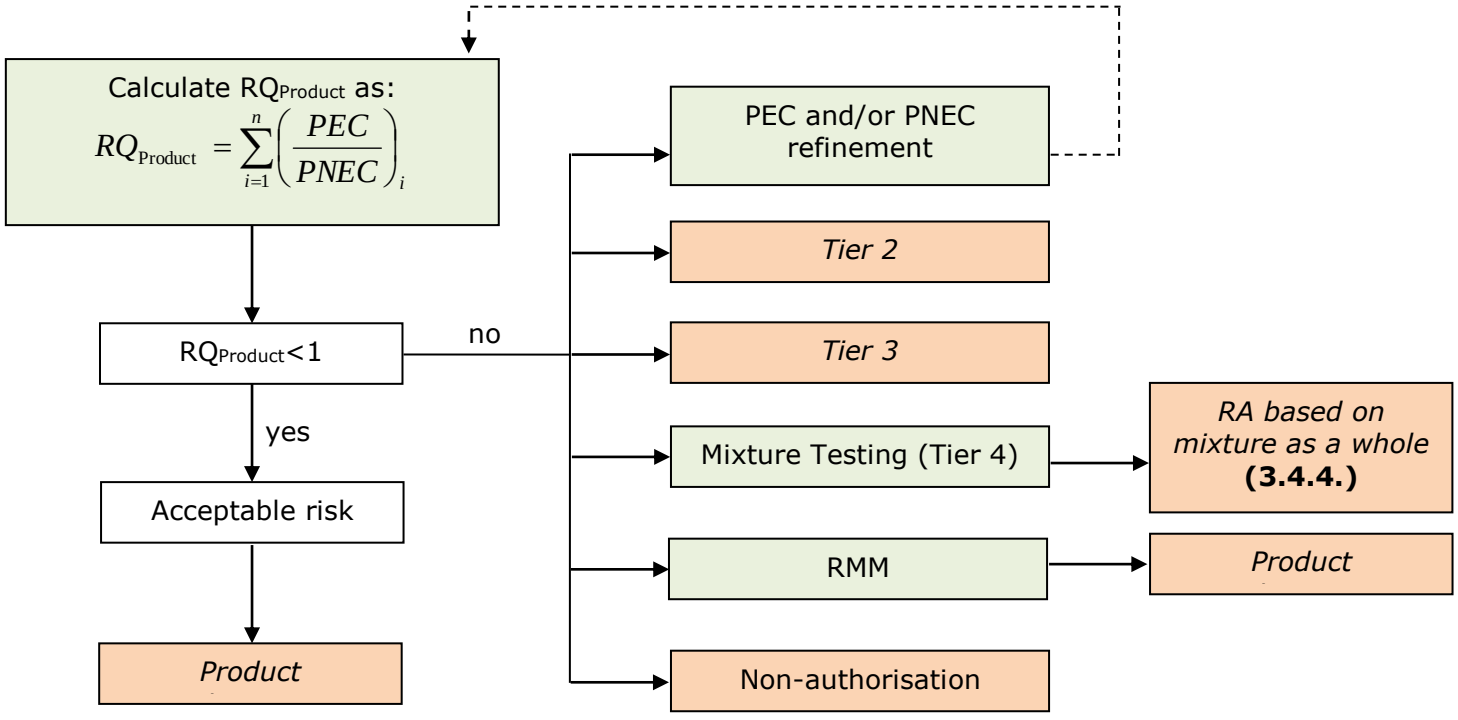

Figure 3: Decision tree for tier 1 (PEC/PNEC-Summation, point 3.3.1, PEC **= Predicted Environmental Concentration,** PNEC **= Predicted No Effect Concentration,** RA **= risk assessment,** RMM**= Risk Mitigation Measures,** $RQ_{Product}$ **= Risk Quotient of the Product).**

The refinement in tier 2 and tier 3 consists of looking separately at the combined risk from all relevant substances towards each separate trophic level, by calculating the Sum of Toxic Units (STU) for each trophic level. Two approaches are presented: First, a modified Toxic Unit Summation (TUS) which can take into account varying data sets for the relevant substances (tier 2) and secondly, the standard TUS as described by Backhaus and Faust [4] for cases where homogeneous data sets are available for all relevant substances.

3.3.2 Tier 2

If ecotoxicological data and PEC-values are available for all relevant substances for all relevant trophic levels in all relevant compartments and for all relevant exposure scenarios, but the amount of available data varies from substance to substance, the risk quotient of the product can be calculated by applying the following equation:

$$RQ_{\text{Product}} = \max \sum_{i=1}^{n} \left(\frac{PEC}{ECx / AF} \right)_i \qquad \text{eq. 5}$$

where EC_x[4] is the effect concentration that affects x% of the exposed organisms and is calculated for each trophic level and each relevant substance, separately. The AF is the same Assessment factor used for calculating the PNEC of the respective substance (see tier 1). This means that for each substance, the same AF is used consistently.

In this tier the trophic levels of the respective compartment are assessed separately, e.g. separate risk-ratios are calculated for all relevant substances for algae, dahnids and fish. In this approach it is preferred to compare the same types of endpoints, e.g. chronic effects for same trophic level. However, if chronic data are not available for all substances acute effects can be included in the calculation as well. This method is a more realistic approach than tier 1, as it combines effects for each trophic level; however it requires several more data and calculations. The difference to tier 3 is that the same data sets for all relevant substances might not be available and hence, it would not make sense to use a common AF for all relevant substances as used in eq. 6.

Note

The modified toxic unit equation (eq. 5) should be used with caution. It gives the opportunity to include different types of effect values and AFs, which in itself is a violation of the CA assumption of similar endpoints. For each substance, the AF used to derive the substance PNEC is used to calculate the RQ for the different trophic levels, regardless of whether the effect concentrations are similar to that used for the PNEC derivation of the substance. For example, if the PNEC for substance X is based on a fish NOEC and an AF 100, and you only have an EC_{50} for e.g. algae, the AF used to calculate the contribution of substance X's toxicity towards algae would also be 100. Since you cannot know whether the chronic toxicity towards algae would be higher than towards fish, this represents some uncertainty. On the other hand, if the data set had contained chronic data for both fish and algae, the overall AF would be lower (less conservative). Furthermore, if a higher AF is used on those endpoints that are acute, regardless of the AF used for the PNEC derivation of the substance, the basis for tiers 1 and 2 are not longer the same and hence tier 2 might not represent a meaningful refinement.

If there are acute endpoint values with low AFs in the equation, the uncertainty they bring to the resulting $RQ_{Product}$ should be considered. If an RQ for a trophic level is close to 1 and a low acute endpoint value with a low AF is included in the STU for that trophic level, the uncertainty might be too high and extra justification or a higher tier might be warranted.

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[4] lowest EC_{50}-, LC_{50}- or NOEC values for the same endpoint and *preferably* (not necessarily) the same exposure setting and the same species.

It is recommended to use tier 2 in cases where identical data sets are not available for all relevant substances and hence a standard toxic unit summation (TUS, tier 3) is not possible, because a common overall AF (the prerequisite of the TUS) cannot be applied. Tier 2 is similar to the Standard TUS regarding separate evaluation of each trophic level and the use of the RQ for the trophic level which is most at risk (the highest RQ). The only difference between the two tiers is that tier 2 gives the opportunity to use different AFs for each relevant substance (eq. 5). If identical data sets are available for all relevant substances and hence a common AF can be used, the two tiers give the exact same result. An example of the application of equation 5 can be found in Annex 4.

Therefore, going from tier 2 to tier 3 is only a refinement option, when additional data are provided for the relevant substances for which less data are available. Tier 2 is applied in the first place, when identical data sets are not available for all relevant substances and the TUS-approach is not possible as no common AF can be used. To do a Standard TUS with substances with dissimilar data sets, it would be necessary to disregard some of the data and only use what is common for all substances, e.g. acute data. If chronic data for some substances are disregarded and only acute data with a common AF of 1000 are used (i.e. it is pretended that for some substances the data sets are smaller than they actually are), it is likely to end up with higher RQ's and a more conservative result than in tier 1.Hence, tier 3 would not be a refinement if applied to these unbalanced data. This is the reasoning behind proposing tier 2, i.e. a modified toxic unit approach, where it is possible to take into account the differing data sets and AFs for the different substances. In case identical data sets are available, the tier 2 calculations would be identical to the tier 3 (TUS) calculations.

If in Tier 2 (eq. 5) the criterion for an acceptable risk for the environment is still not met, i.e. $RQ_{Product} > 1$, the following options exist:

(i) a refinement of the PEC- and/or EC_x-values by providing additional information on the exposure and/or hazard characterisation of the compounds,

(ii) the application of CA in the form of the standard toxic unit summation (TUS) for each trophic level separately (tier 3). In cases where homogenous data sets are already available for all relevant substances, it is recommened to start the assessment directly with tier 3 (see above & Figure 2).

(iii) direct testing of the mixture of concern (tier 4), or

(iv) the definition of effective Risk Mitigation Measures (RMM).

If the aforementioned options are not applicable the only remaining option is the non-authorisation of the product (Figure 4).

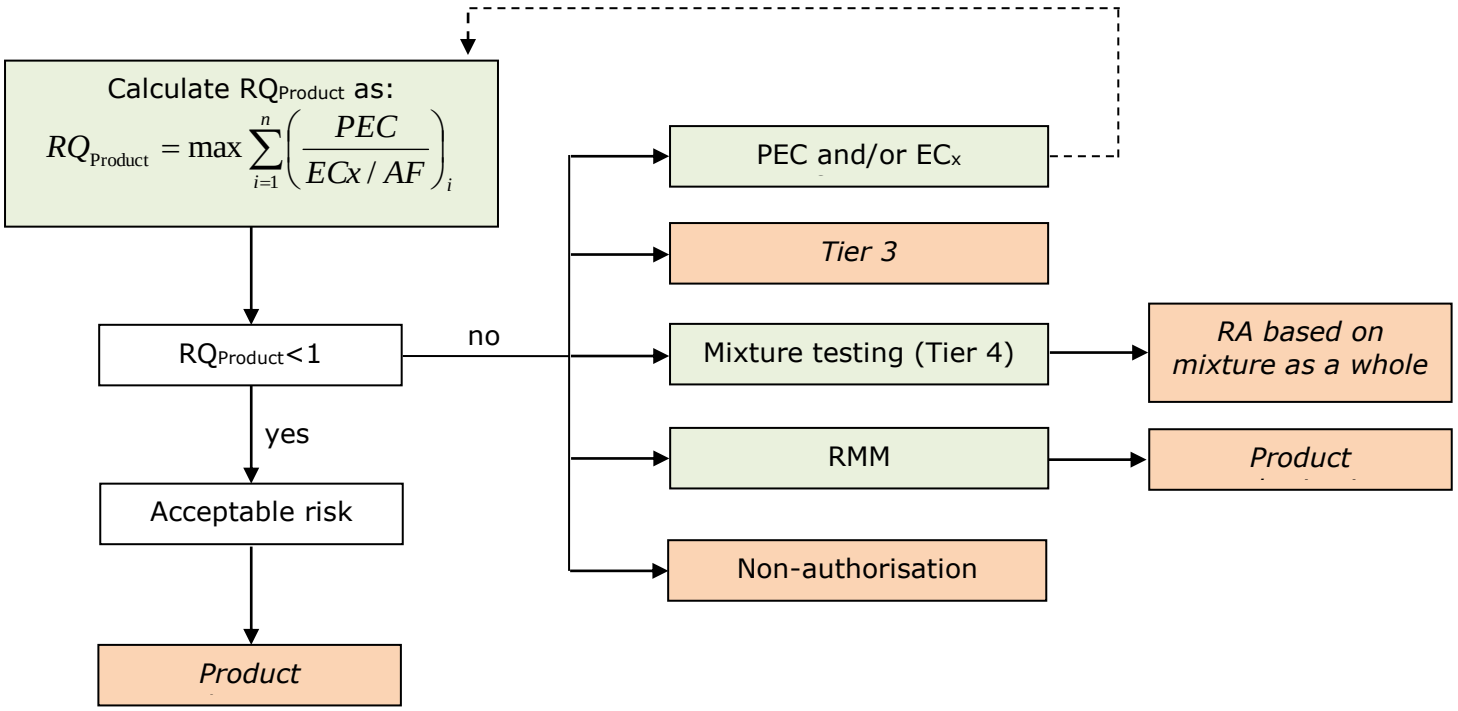

Figure 4: Decision tree for tier 2 (Modified Toxic Unit Summation, point 3.3.2, AF = Assessment Factor, EC_x = Effect Concentration that provokes an x%-effect in the exposed organisms, PEC = Predicted Environmental Concentration, RA = risk assessment, RMM= Risk Mitigation Measures, $RQ_{Product}$ = Risk Quotient of the Product).

3.3.3 Tier 3

In case identical ecotoxicological data sets are available for all relevant substances for all relevant species and all relevant compartments, the risk quotient for the product can be assessed by calculating the sum of Toxic units (STU) for each trophic level / group of organisms and every of *m* ecotoxicological endpoints (e.g. daphnia immobility, fish mortality, algae growth) separately for every component *i* of the mixture (equation 6):

$$RQ_{Product} = \max(STU_{endpoint\,1}, STU_{endpoint\,2}, \ldots, STU_{endpoint\,m}) \times AF$$

$$RQ_{Product} = \max\left(\sum_{i=1}^{n} \frac{PEC_i}{ECx_{i,j}}, \sum_{i=1}^{n} \frac{PEC_i}{ECx_{i,j}}, \ldots, \sum_{i=1}^{n} \frac{PEC_i}{ECx_{i,m}} \right) \times AF \quad \text{eq 6}$$

AF denotes the resulting assessment factor according to TGD [28], as used for calculating the PNEC of the respective substance (see tier 1). PEC/EC_x is a toxic unit.

Of the calculated STU, one for each endpoint, the highest is used for calculating the risk quotient. The assessment factor is selected depending on the amount of available data according to the rules set up in the TGD [28].

Equation 6 is only a rearrangement of equation 5, in that the AF is placed outside the brackets, allowing same AFs to be used for each involved substance. If a common AF is used for all substances, the equations 5 and 6 give identical results. In equation 6 the maximum STU is calculated first (toxic unit = PEC/EC_x) before it is multiplied by an AF. In equation 5, these two steps are combined.

The Standard Toxic Unit Summation (TUS) is a more strict application of CA, than PEC/PNEC summation (Tier 1) and the Modified TUS (tier 2), and requires that same

species and endpoint are used for the different mixture components. For example: daphnia acute test data are combined with other daphnia acute test data and fish reproduction data with fish reproduction data etc. This leads to a calculated risk quotient for a given environmental compartment that is based on the most sensitive organism group for the evaluated mixture. A prerequisite for using the standard toxic unit summation (TUS) is that the ecotoxicological dataset for the evaluated mixture is balanced for all relevant substances, i.e. data from a specific endpoint can only be used if there are data for the same endpoint for all relevant substances. For example, the availability of only the base set of acute toxicity for all substances would enable a common AF on the effect concentrations. Likewise, similar chronic data for all relevant substances would allow using a reduced AF.

If there are chronic data available for some substances, but not for others, the dataset would be unbalanced and those data could not be used since that would violate the assumption of similar endpoints. In that case, the extra chronic data would have to be disregarded and only the common acute data could be used. The problem in this case is that in tier 1 (PEC/PNEC summation), imbalanced data sets are not an issue as the PNECs can be derived using different AFs. To disregard chronic data and hence use a higher AF for a substance in tier 3 than in tier 1 could in some cases be more conservative, and it can result in an RQ in tier 3 which is higher than in tier 1. Therefore, a modified toxic unit summation approach could be considered for cases with unbalanced data sets (tier 2, see above), or further data have to be provided for the relevant substances for which less data are available

The maximum STU indicates which endpoint for which species is expected to be most sensitive to the biocidal product in question and is hence used for the final assessment, i.e. by applying the corresponding AF according to TGD [28] the RQ for the product is calculated.

It can be proven that the risk quotient that results from summing up PEC/PNECs (eq. 4) is always equal or higher than the maximum STU according to eq. 6 [5], provided that the same data is used as a basis for the PEC/PNEC summation and Toxic unit summation. Their precise relationship depends on the ecotoxicological profiles of the compounds in the mixture. In case of dissimilar profiles, the ratio between the application of eq. 4 and 6 approaches the theoretical maximum of *m* (number of considered endpoints). If the compounds have almost the same ecotoxicological profiles (which can be expected e.g. for a mixture of simple organic solvents), then the risk quotients from both equations become identical.

Note
The maximum ratio between the risk quotients (RQs) of tier 1 and tier 3 of *m* (number of species-specific ecotoxicological endpoints) provides a convenient decision criterion on whether the detailed data collection or production in order to conduct a refined assessment based on the RQ of tier 3 (eq. 6) might influence the regulatory outcome: if the RQ of tier 1 is higher than *m*, the RQ of tier 3 will always be above 1, i.e. indicate reason for concern. In such cases it is not constructive to proceed with the tiered approach and alternatives such as the direct testing of the product /or the ecologically relevant mixture or effective Risk Mitigation Measures should be taken into account. In case the aforementioned options are not applicable the only remaining option is the non-authorisation of the product.

Employing eq. 6 requires that data for all relevant compounds are available for all endpoints, as it would otherwise be impossible to determine the maximum of all organism- and endpoint-specific STUs and an appropriate overall assessment factor (AF). This makes an application of equation 6 – although it most closely follows the conceptual idea of CA – rather demanding.

A risk quotient exceeding one might be caused by the mixture toxicity overestimation that results from the application of CA to a mixture of not entirely similarly acting compounds. Details on how to estimate this possible overestimation are provided by Junghans and colleagues [35] and Backhaus and colleagues [4]. The direct testing of the biocidal product or the ecologically relevant mixture might provide additional insight, given that a substantial risk overestimation by CA is possible, which depends on the number of involved compounds, their toxicity and ratio in the mixture. Otherwise there would be a clear indication for a reason for environmental concern, which would call for appropriate risk management strategies

If the tiers still indicate an unacceptable risk for environment, the only risk assessment option is the direct biotesting either of the biocidal product, if there is a direct release of the product into environment or of the ecologically relevant mixture in case the composition of the product changes radically before release to environment as the ultimate option for clarification (tier 4). If the direct biotesting of the mixture of concern, i.e. the product and/or the ecologically relevant mixture is not possible and other options such as a further refinement of the single substance data or the definition of effective RMMs are not applicable, the only remaining option is the non-authorisation of the product (Figure 5).

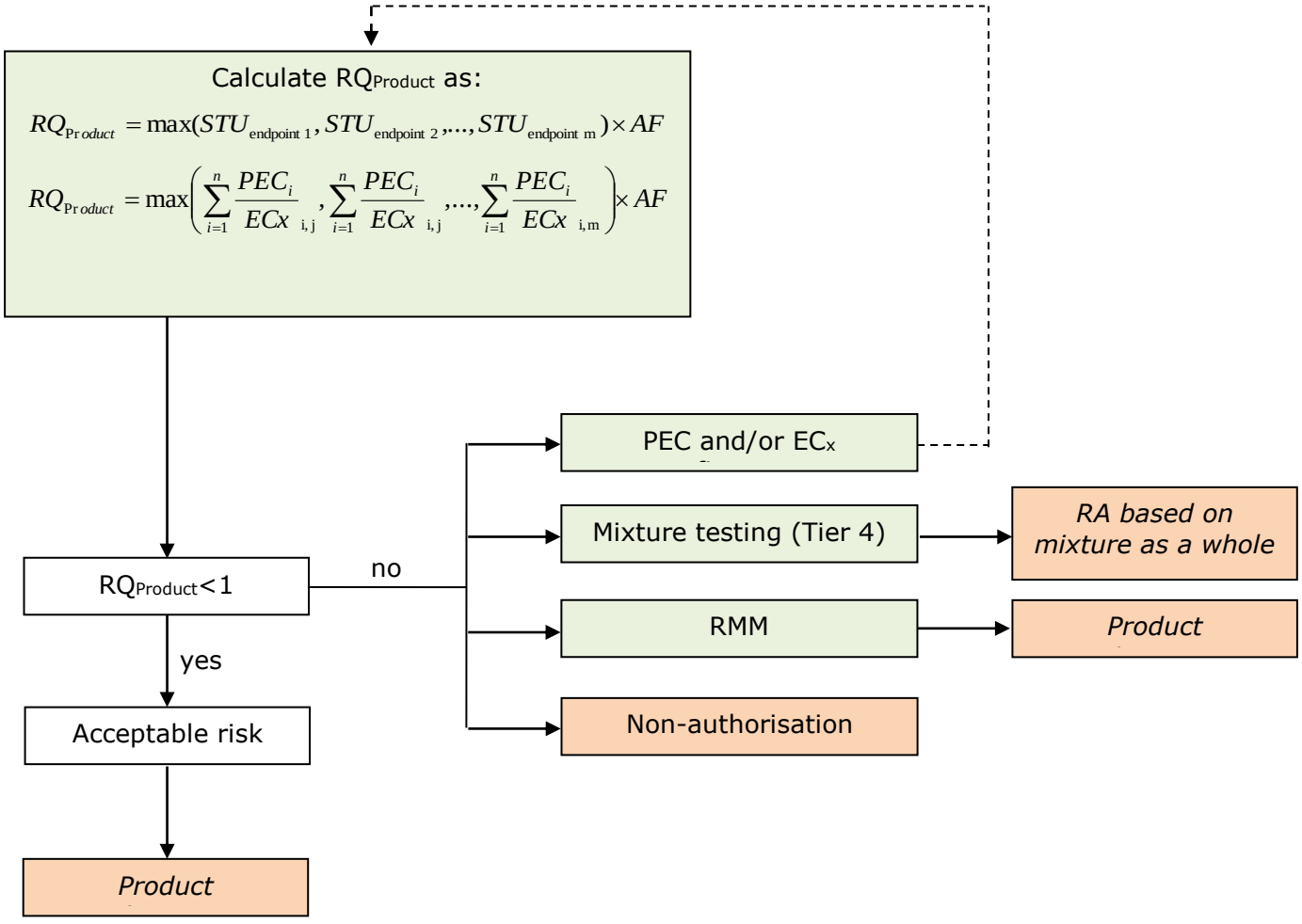

Figure 5: Decision tree for tiers 3 and 4 (Standard Toxic Unit Summation and Mixture Testing, point 3.3.3, AF **= Assessment Factor,** EC_x **= Effect Concentration that provokes an x%-effect in the exposed organisms,** PEC **= Predicted Environmental Concentration,** RA **= risk assessment,** RMM**= Risk Mitigation Measures,** $RQ_{Product}$ **= Risk Quotient of the Product,** STU **= Sum of Toxic Units).**

3.3.4 Tier 4

If data on the whole "relevant mixture", i.e. product tests or tests with the ecologically relevant mixture are not already available, they should only be employed in situations, where well-founded suspicions for synergistic interactions require clarification, or as last option where results of predictive modelling (tiers 1-3) indicate unacceptable risks for environment (see also point 2.2). In these cases the most sensitive species from the single substance data should be tested.

Effects Assessment

Direct testing of the whole product is straight forward in principle, as it does not require any specific methodology and can hence use the same experimental outline as the tests of an individual chemical.

However, the BPR states that tests with vertebrate animals can only be conducted as a last resort, i.e. when alternative testing and assessment methods have been exhausted. Furthermore the testing of a biocidal product for its chronic toxicity might be of only limited informative value (although it is feasible, as it could shown by Coors et al., 2012 [24]). The composition of the product might change already during the exposure in the biotest system, as the different chemicals might have a different stability and distribution between the different compartments in the test (e.g. biota, headspace, aqueous media, soil, sediment). Changes in the chemical composition of the initial mixture are most likely even more pronounced if environmental fate and distribution processes are taken into consideration. Such processes can be accounted for by testing the ultimate, environmentally relevant mixture instead of the original product. For example, it might be more relevant to test the leachate of a wood preservative than the original product. It could be shown in two research projects for several test organisms, that the leachates are clearly less toxic than the original product [20], providing an opportunity to lower the risk for a respective product by providing leachate toxicity data. The validity of the toxicity data for the risk assessment then strongly depends on a thorough definition of the underlying exposure scenario. However, there are currently also no agreed guidelines at hand in the EU for the testing of such "realistic" mixtures (e.g. leachates).

If the solubility of the product or the environmentally relevant mixture in water is low or reduced, the OECD Guidance document No. 23 on Aquatic Toxicity Testing of Difficult Substances and Mixtures [42] should be followed. If it is technically not feasible to test the mixture in its entirety, because of e.g. insoluble pigments or other ingredients making a direct testing of the product unfeasible, it is also possible to assess generic mixtures of the relevant substances („surrogate mixture") by combining the substances identified as relevant for mixture assessment in a ratio similar to that of the product or the ecologically relevant mixture.

By experimental testing of a given mixture of substances, both effect concentrations and NOEC values can be determined in the same way as this is usually done for single substances. Therefore, no knowledge either about the composition of the mixture e.g. nature, number or concentration ratio of the components must be known for testing nor toxicity data for the individual components or their mechanism of action.

When testing mixtures the procedures applied are similar to the procedures applied for single substances by taking into account the general principles described in the Technical Guidance Document [28) and the Technical Notes for Guidance on Product Evaluation [27] as well as the related Guidance Documents and Emission Scenario Documents. Risk quotients for mixtures can be derived from such experiments if in the exposure situation in the environment the concentration ratio of mixture components is comparable to that in the experiments. However, it should be kept in mind, that due to distribution and transformation processes in the environment, the mixture to which the non-target

organisms may be exposed is only conditionally comparable with the original composition of the product [1]. But, as such mixture data encompass any effects due to interactions that may occur between the mixture components, e.g. synergistic interactions as well as contributions from compounds that have not been considered in the mixture toxicity predictions or for which ecotoxicity information is lacking (e.g. formulation additives), the risk assessment will be based on the mixture as a whole if the data are available, rather than on the sole prediction of the mixture toxicity by using the concept of Concentration addition or pragmatic approaches of this concept, i.e. PEC/PNEC-summation.

Note
It is difficult to suggest a generally applicable testing scheme for products and ecologically relevant mixtures also in terms of the test design (i.e. species, test duration, test concentrations etc.). Therefore, it is recommended to assess each mixture carefully and base decisions regarding testing on expert judgement in agreement with the Competent Authority.

Exposure Assessment

According to the Technical Notes for Guidance on Product Evaluation (TNsG, chapter 5.2 Risk assessment for products, [27]) the calculation of a PEC for the whole product is possible if there is a direct release of the product to environmental compartments:

"For products for which a direct exposure of a given compartment is possible, test results with whole products can be taken into account. A PEC and a PNEC can be derived for the whole product as for single substances and a corresponding risk characterisation can be performed for the product:

$(PEC/PNEC)_{product} = PEC_{product}/PNEC_{product}$

The approach is usually not possible throughout a risk assessment for all compartments."

That means that currently:

- $PEC_{product}$ can be calculated for the first receiving compartment if direct release of the whole product takes place. In this case $PEC_{product}$ can be calculated in the same way as any $PEC_{a.s.}$. The amount of product used will be taken into account in the respective equations in the ESDs. A risk assessment based on the tested mixture (product) is possible.
- There is no agreed methodology available to calculate $PEC_{product}$ if there is an indirect release into environmental compartments e.g. via STP or by distribution between water and sediment, as no partition coefficients for products are available. In this case, a risk assessment based on the tested mixture (product) is currently not possible.
- There is no agreed methodology available to calculate $PEC_{product}$ if there is direct release of a part of the product e.g. as a leachate into environmental compartments. Even if it is possible to analyse all relevant substances in a leachate, the composition of the leachate often vary with time. In this case, a risk assessment based on the tested mixture (leachate) is currently not possible.

It is recommended to review the relevant emission pathways of the application of the biocidal product with regard to direct releases before doing mixture testing.

A more comprehensive summary of the scientific background of the mixture toxicity assessment and the outlined strategy can be found in Altenburger et al. (2012) [1] and Backhaus et al. (2012, [5]).

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Appendix 1. Draft Proposal for the identification of relevant substances for mixture assessment

What are 'Relevant Substances' in a typical biocidal product?

The discussions during the Workshops on mixture toxicity assessment raised the following issues regarding relevant substances:

- Relevant substances for mixture assessment cannot be restricted to active substances (a.s.) of biocidal products only;
- Relevance relates to effects to non-target organisms in environmental risk assessment and not to the purpose of the products use;
- In case of lack or insufficiency of data conceptually there is always the product testing as the ultimate option to gain a satisfactory assessment answer;
- The definition may refer to substances of concern (SoC) as used in BPD and BPR or it may relate to an understanding of relevance in general.
- There may be other components to be considered by default that do not fall under the current SoC definition.

There was a broad agreement among the vast majority of attendees on regarding SoCs as relevant for the calculation of the mixture toxicity. The detailed discussion on SoCs takes place at the moment in the PA&MRFG based on documents prepared by UK and DK and will be continued also at TM -level[5].

It was agreed at the follow-up workshop at TM III/2012 to regard the following substances as relevant for mixture assessment:

1) Active substances.
2) Substances of concern.
3) Active substances from other PTs. However, it should be considered under which conditions exemptions are possible (e.g. substances contained in Annex I of the new regulation or substances contributing only to a very limited extent to the overall toxicity of the mixture, see below & Annex 5).
4) Other ingredients which do not fall under one of the aforementioned categories but might be relevant for mixture assessment like e.g. known synergists should be considered as well on a by-case basis.

Therefore, it is proposed that active substances and SoCs have to be regarded as relevant for mixture assessment *per se*. For all other product components ecotoxicological, fate and behaviour as well as relevant physico-chemical endpoints should be derived based on available information (e.g. laboratory studies, material safety data sheets, EU or international chemical reviews, QSARs etc.) from which it can be decided whether a product component has to be regarded as relevant substance for the mixture toxicity assessment. It is the applicants responsibility to make all reasonable efforts to submit the most up to date and reliable information and this should be detailed in the submission, along with any letters of access that might be required. As only semi-quantitative data are needed for this purpose, e.g. QSAR-estimates, hazard classification data from classification and labeling according to the CLP Regulation (EC) No 1272/2008, data from limit tests or screening studies as well as simple exposure estimates should be sufficient in this first step.

[5] For identification and exact definition of SoCs please refer to the ongoing discussion at the PA&MRFG- and TM-level (SoC- working group). The definition of relevant substances should be reviewed after finalisation of the discussion on SoC at TM- and PA&MRFG-level and amended accordingly.

In addition, a research project funded by the German Federal Environment Agency [24] revealed, that the calculation of the relative toxic units (TU) of the single product components as also recommended by Backhaus & Faust (2012) [4] might be a helpful tool to decide whether *active substances from another PT, or other ingredients which are neither a.s. nor SoC* must be regarded as relevant substance for mixture assessment together with the a.s. and the SoC, provided that toxicity estimates comparable to those of the a.s. and SoC are available for these substances.

The calculation of the individual TU (for each trophic level separately) is based on the concentration of the substances in the product (c*i*) and the available toxicity estimates, i.e. equi-effective concentrations of the single substances, such as the EC_{50}-values ($EC_x i$,[6] equation I):

$$TUi = Ci/EC_x i \qquad \text{eq.I}$$

Hence, the calculation of the TU is independent of any biological testing of the products or the leachates and can therefore be done ahead of experimental investigations to indentify the relevant mixture components and target the testing where relevant [24]. Finally, the relative TU (rel TU) is calculated as depicted in equation II and indicates how much each mixture component contributes to the overall expected toxicity (see also Annex 2 & Annex 4):

$$\text{rel } TUi = (TUi/\Sigma TU)/100 \qquad \text{eq.II.}$$

As the relative TU depends on the overall composition of the product, the concentration of the respective substance in the product as well as their toxicity, no threshold values can be given for the rel TU. Therefore, the decision on whether a substance is relevant for the assessment of the mixture toxicity is subject to expert judgement.

Another possibility to assess the influence of the individual components on the overall toxicity of the mixture is the Maximum Cumulative Ration (MCR) as proposed by industry:


MCR proposal

Inert compounds (e.g. water, non-soluble pigments) are chemicals that do not show any toxic effects, even at excessive concentrations and do not interact with other chemicals present. Hence, they do not have an impact on the mixture toxicity assessment and can be ignored, as both concepts assume that they do not contribute to the overall toxicity of the product, unless there are indications that they influence the toxicity of the other mixture components [3].

However, inert compounds need to be clearly differentiated from compounds that are not an active ingredient *per se*, (i.e. they are not inherently toxic to exposed organisms), but still are biologically active. Piperonyl butoxide (PBO) for example would fall into this group, as the compound itself is not biocidal, but increases the toxicity of other biocides e.g. pyrethrins, pyretroids or carbamates by inhibiting their cytochrome P450-driven metabolisation [32, 41,53]. Such "synergists" might lead to serious toxicity and risk underestimations, and hence have to be considered specifically in a case-by-case manner (see point 3.2.3).

[6]lowest EC_{50}- or LC_{50}-values for the same endpoint and *preferably* (not necessarily) the same exposure setting and the same species.

Also diluents, (lipophilic) organic solvents and surfactants like e.g. naptha may influence the toxicity of a mixture by enhancing the bioavailability of the active substance(s) [24, 27] and should therefore be regarded carefully.

It is possible that certain properties of the compound in question mean that the environment is unlikely to be significantly exposed to that substance. In such a situation qualitative argumentation may be submitted by the Applicant to demonstrate that environmental exposure in a particular compartment would be negligible. Such argumentation should be supported by appropriate data. Examples may include very rapid degradation or dissipation (e.g.by volatilisation and rapid photochemical oxidation in air) or negligible exposure e.g. when only used in completely closed systems.

It has to be emphasised again, that special care has to be taken to ensure that all toxic ingredients are included in a component-based assessment of a biocidal product, because otherwise the risk for environment resulting from the application of the product is underestimated. If no or not sufficient (ecotoxicological) information is at hand for all ingredients, to decide whether a substance is relevant for mixture assessment the only effect assessment option is the direct biotesting either of the respective substance(s) or of the biocidal product and the resulting environmental mixture, respectively. If a mixture cannot be assessed in its entirety, because of e.g. insoluble pigments it is also possible to assess generic mixtures of the relevant substances ("surrogate mixture", see point 2.2 & 3.3.4).

Appendix 2. Sample calculation relative Toxic Unit

Underlying data for the substances indentified as relevant for mixture toxicity assessment:

Substance	Active substance	Preservative	Solvent
Content in the product [w/w%]	0.5	6.5	83.1
Algae (E_rC_{50}(72h)	0.0052 mg/L	9 mg/L	695 mg/L
Daphnid (EC_{50}(48h)	0.003 mg/L	5 mg/L	700 mg/L
Fish (LC_{50}(96h))	0.0012 mg/L	7 mg/L	850 mg/L

Based on these data the TU are calculated for all three product components according to equation I (TU*i* = C*i*/EC_x*i*):

Substance	Active substance	Preservative	Solvent	ΣTU
TU Algae	96.2	0.72	0.12	97.0
TU Daphnid	166.7	1.30	0.12	168.1
TU Fish	416.7	0.93	0.10	417.7

Finally, the relative TU are calculated according to equation II (rel TU*i* = (TU*i*/ΣTU)/100):

Substance	Active substance	Preservative	Solvent
Relative TU Algae	99.13	0.74	0.12
Relative TU Daphnid	99.16	0.77	0.07
Relative TU Fish	99.76	0.22	0.02

According to this calculation only the active substance has to be regarded as relevant for mixture toxicity assessment as the a.s. accounts for more than 99% of the toxicity of the mixture in algae, daphnid and fish in this theoretical example.

Appendix 3. Synergisms

1. Intended Synergisms

Synergistic interaction reported for	Organisms for which synergism are reported	Reference
Bacillus thuringiensis Berliner & Endosulfan	Cotton boll worm (*Helicoverpa armigera*)	46
Copper & Formaldehyd	Micro-organisms	49, 54,
Copper & isothiazolone	Micro-organisms	49
Formaldahyd & Isothiazolone	Micro-organisms	49
Propiconazol & λ-cyhalothrin	Honeybee (*Apis mellifera*)	43
Copper & CPT	Bacteria (*Vibrio fischeri*)	59
Copper (pyrithione)& ZPT	Bacteria (*Vibrio fischeri*), Diatoms (*Thalassiosira pseudomona*), polychaete larvae (*Hydroides elegans*), amphipods (*Elasmopus rapax*), brine shrimp (*Artemia salina*)	11, 38, 60
Copper & Dithiocarbamates	Ciliates (*Colpidium campylum*)	14, 57, 59,
Copper & Diuron	Bacteria (*Vibrio fischeri*), marine algae (*Chaetoceros gracilis*), brine shrimp (*Artemia salina*)	37, 38, 60
Copper & Irgarol	Bacteria (*Vibrio fischeri*), marine algae (*Chaetoceros gracilis*)	37, 60
Copper & Sea Nine 211	Bacteria (*Vibrio fischeri*)	60
Copper & Ziram	Bacteria (*Vibrio fischeri*)	60
Deltamethrin & Carbaryl	Snail (*Lymnaea acuminata*)	25
Diuron & ZPT	Marine algae (*Chaetoceros gracilis*), brine shrimp (*Artemia salina*)	37, 38
Diuron & cadmium	marine algae (*Chaetoceros gracilis*)	37
Dithiocarbamates & heavy metals	*not reported*	57
EBI-fungicides & insecticides (pyrethroids, organophosphates, neonicotinoids)	Microorganisms *(Vibrio fischeri), i*nvertebrates *(Daphnia magna, Apis mellifera)*	17, 44, 51, 55
Isoproturon & Cypermethrin & Difufenican	*not reported*	55
Isoproturon & Cypermethrin & Pendimethalin	*not reported*	55
Isoproturon & Cypermethrin & Trifluralin	*not reported*	55
Isoproturon & Fenvalerate &	*not reported*	55

Pendimethalin		
Isoprotuon & Delthamethrin & Diflufenican	*not reported*	55
Irgarol & Cadmium	Marine algae (*Chaetoceros gracilis*)	37
Irgarol & Diuron	Bacteria (*Vibrio fischeri*), green algae (*Selenastrum capricornotum*), marine algae (*Chaetoceros gracilis*) crustaceans (*Daphnia magna*)	30, 37
Irgarol & TCMTB	Bacteria (*Vibrio fischeri*), green algae (*Selenastrum capricornotum*), crustaceans (*Daphnia magna*)	30
Irgarol & Chlorthalonil	Bacteria (*Vibrio fischeri*), green algae (*Selenastrum capricornotum*)	30
Irgarol & DCF	Bacteria (*Vibrio fischeri*), crustaceans (*Daphnia magna*)	30, 60
Thiacloprid & Tebuconazole	Bees (*Apis mellifera*)	51
ZPT & Irgarol	Marine algae (*Chaetoceros gracilis*)	37
ZPT & cadmium	Marine algae (*Chaetoceros gracilis*)	37
Zinc pyrithione & Ziram	Bacteria (*Vibrio fischeri*)	60
Irgarol & TCMTB & Dichlofluanid	Green algae (*Selenastrum capricornotum*), crustaceans (*Daphnia magna*)	30
Zinc pyrithione & Copper pyrithione & Chlorothalonil	Brine shrimp (*Artemia salina*)	38
Zinc pyrithione & Copper pyrithione & Chlorothalonil	Nrine shrimp (*Artemia salina*)	38

2. Un-intended Synergisms

For the substances depicted in the table potential synergistic effects are reported in the peer-reviewed literature. These publications should be seen as indications for possible synergisms of the shown substances and be taken into account during the decision making process. However, they should be analysed in more detail for this purpose, e.g. regarding the tested concentrations, mixture ratios and the concentration-dependence of interaction as well as the tested organisms and endpoints.

Synergistic interaction reported for	**Organisms for which synergism are reported**	**Reference**
Bacillus thuringiensis Berliner & Endosulfan	Cotton boll worm (*Helicoverpa armigera*)	46
Copper & Formaldehyd	Micro-organisms	49, 54,
Copper & isothiazolone	Micro-organisms	49
Formaldahyd & Isothiazolone	Micro-organisms	49
Propiconazol & λ-cyhalothrin	Honeybee (*Apis mellifera*)	43

Copper & CPT	Bacteria (*Vibrio fischeri*)	59
Copper (pyrithione)& ZPT	Bacteria (*Vibrio fischeri*), Diatoms (*Thalassiosira pseudomona*), polychaete larvae (*Hydroides elegans*), amphipods (*Elasmopus rapax*), brine shrimp (*Artemia salina*)	11, 38, 60
Copper & Dithiocarbamates	Ciliates (*Colpidium campylum*)	14, 57, 59,
Copper & Diuron	Bacteria (*Vibrio fischeri*), marine algae (*Chaetoceros gracilis*), brine shrimp (*Artemia salina*)	37, 38, 60
Copper & Irgarol	Bacteria (*Vibrio fischeri*), marine algae (*Chaetoceros gracilis*)	37, 60
Copper & Sea Nine 211	Bacteria (*Vibrio fischeri*)	60
Copper & Ziram	Bacteria (*Vibrio fischeri*)	60
Deltamethrin & Carbaryl	Snail (*Lymnaea acuminata*)	25
Diuron & ZPT	Marine algae (*Chaetoceros gracilis*), brine shrimp (*Artemia salina*)	37, 38
Diuron & cadmium	marine algae (*Chaetoceros gracilis*)	37
Dithiocarbamates & heavy metals	*not reported*	57
EBI-fungicides & insecticides (pyrethroids, organophosphates, neonicotinoids)	Microorganisms *(Vibrio fischeri), i*nvertebrates *(Daphnia magna, Apis mellifera)*	17, 44, 51, 55
Isoproturon & Cypermethrin & Difufenican	*not reported*	55
Isoproturon & Cypermethrin & Pendimethalin	*not reported*	55
Isoproturon & Cypermethrin & Trifluralin	*not reported*	55
Isoproturon & Fenvalerate & Pendimethalin	*not reported*	55
Isoprotuon & Delthamethrin & Diflufenican	*not reported*	55
Irgarol & Cadmium	Marine algae (*Chaetoceros gracilis*)	37
Irgarol & Diuron	Bacteria (*Vibrio fischeri*), green algae (*Selenastrum capricornotum*), marine algae (*Chaetoceros gracilis*) crustaceans (*Daphnia magna*)	30, 37
Irgarol & TCMTB	Bacteria (*Vibrio fischeri*), green algae (*Selenastrum capricornotum*), crustaceans (*Daphnia magna*)	30
Irgarol & Chlorthalonil	Bacteria (*Vibrio fischeri*), green algae (*Selenastrum capricornotum*)	30

Irgarol & DCF	Bacteria (*Vibrio fischeri*), crustaceans (*Daphnia magna*)	30, 60
Thiacloprid & Tebuconazole	Bees (*Apis mellifera*)	51
ZPT & Irgarol	Marine algae (*Chaetoceros gracilis*)	37
ZPT & cadmium	Marine algae (*Chaetoceros gracilis*)	37
Zinc pyrithione & Ziram	Bacteria (*Vibrio fischeri*)	60
Irgarol & TCMTB & Dichlofluanid	Green algae (*Selenastrum capricornotum*), crustaceans (*Daphnia magna*)	30
Zinc pyrithione & Copper pyrithione & Chlorothalonil	Brine shrimp (*Artemia salina*)	38
Zinc pyrithione & Copper pyrithione & Chlorothalonil	Nrine shrimp (*Artemia salina*)	38

Appendix 4. Case Studies

(1) PT14, Rodenticide

1. Screening Step

1.1 Identification of the concerned environmental compartments

The ready-to-use baits (wax blocks) are used for the control of rats and mice indoors and outdoors (in and around buildings, open areas, waste disposal sites) and in sewers in secure and tamper resistant covered applications (bait stations, other secured coverings).

The use in the sewer system may lead to contamination of surface waters and sediment through sewage water and STP. No or significantly lower contamination of surface water is expected from the other proposed uses of the product.

The exposure of soil organisms to the product by direct contamination of soil may occur following use in and around buildings. It is also possible that soil may become exposed following the spreading of sewage sludge from a sewage treatment plant that has been exposed to the product used in sewers.

There is also a risk for primary and secondary poisoning of non-target organisms.

→ An exposure of the environment towards the product is likely (surface water, sediment, soil).

1.2 Identification of Relevant Substances

The composition of the product is given in table 1.

Table 1: Composition of the biocidal product.

Ingredient	Content in the formulation [w/w%]	classification	Relevant substance
Active substance	0.005	T+; R26/27/28, Repr. Cat. 1; R61, T; 48/23/24/25, N; R50/53	X
Flour	60.88	*not classified*	-
Paraffin	26.80	*not classified*	-
Cereals	6.00	*not classified*	-
Sugar	3.00	*not classified*	-
Co-formulant	2.38	*not classified*	-
Colouring agent	0.68	*not classified*	-
Co-formulant	0.195	*not classified*	-
Preservative	0.04	*not classified*	-
Aroma	0.02	*not classified*	-

X: substance relevant for mixture assessment; -: substance not relevant for mixture assessment

The biocidal product contains no substances of concern or other ingredients bearing an environmental classification or otherwise a potential hazard for environment.

Beside the active substance, the product contains a preservative. This substance is notified as an active substance under the BPD for several PTs and should therefore be

considered as a relevant substance for mixture toxicity assessment (see 3.2.2). However, the preservative is not classified for environment and from the available ecotoxicological data it can be concluded, that the preservative is less toxic for environmental organisms than the a.s.

Furthermore, a comparison of the toxic units of the a.s. and the preservative according to equations I and II (Annex 1), for the aquatic and the soil compartment revealed, that mainly the a.s. is the risk driver of the product toxicity for the aquatic compartment (see Table 3 & 4).

Table 2: Toxicity data for the a.s. and the preservative for the aquatic and the soil compartment.

Substance	Active substance	Preservative
Content in the product [w/w %]	0.005	0.04
Aquatic compartment		
Algae (E_rC_{50}(72h), [mg/L]	0.51	480
Daphnid (EC_{50}(48h), [mg/L]	0.52	982
Fish (LC_{50}(96h)), [mg/L]	0.064	>1000
Soil compartment		
Earthworm (LC_{50}(14d), [mg/kg dw]	>100	>5000

Table 3: Relative toxic units (individual TU in % of the sum of TU) for the a.s. and the preservative with regard to aquatic organisms.

	Active substance	Preservative
Algae	99.16	0.84
Daphnid	99.58	0.42
Fish	99.95	0.05

Table 4: Relative toxic units (individual TU in % of the sum of TU) for the a.s. and the preservative with regard to soil organisms.

	Active substance	Preservative
Earthworms	13.8	86.2

Based on expert judgment it can be concluded that the preservative is not a relevant substance for mixture assessment.

→ Besides the active substance, no other ingredients bearing an environmental classification or otherwise a potential hazard for environment are contained in the product according to the composition provided by the applicant and the material safety data sheet.

1.3 Screen on synergistic interactions

→ There are no indications for synergistic effects for the product or its constituents in the literature.

1.4Conclusion

→ Consequently, the environmental risk assessment for the product is based on the active substance and no mixture assessment is needed.

(2) PT08, Wood preservative

1. Screening Step

1.1 Identification of the concerned environmental compartments

→ The screening step revealed that an exposure of environment is likely. According to the intended use of the product and the applied RMMs only an exposure of the soil compartment is likely.

1.2 Identification of Relevant Substances

→ Besides the four active substances, no other ingredients bearing an environmental classification or otherwise a potential hazard for environment are contained in the product according to the composition provided by the applicant and the material safety data sheet.

1.3 Screen on synergistic interactions

→ There are no indications for synergistic effects for the product or its constituents in the literature.

1.4Conclusion

→ Consequently, the environmental risk assessment for the product is based on the four active substances and a mixture assessment is needed.

2. Tiered assessment scheme

Table 5: Available terrestrial ecotoxicity data and PECs for soil for the four a.s. contained in the product.

a.s.	Effect concentration [mg/]			AF	PNECsoil	PECsoil	PEC/PNEC
	Plants	Earthworms	Microorganisms		[mg/kg]	[mg/kg]	
1	EC_{50} = 30.0	LC_{50} = 800	EC_{50} = 120.0	1000	0.03	0.01	0.33
2	EC_{50} = 5.0	NOEC = 0.05	EC_{50} = 7.0	50	0.001	$8.5*10^{-5}$	0.085
3	EC_{50} = 22 NOEC = 5.0	NOEC = 0.4	NOEC = 6.0	10	0.04	0.035	0.875
4	NOEC = 1.0	NOEC = 20.0	EC_{50}= 30.0	50	0.02	0.01	0.50

red: values used for PNEC-derivation

a) Tier 1

$$RQ_{\text{Product}} = \sum_{i=1}^{n}\left(\frac{PEC}{PNEC}\right)_i$$

$RQ_{product}$ = 0.33 + 0.085 + 0.875 + 0.50 = 1.79

→ unacceptable risk for environment

b) Tier 2

$$RQ_{\text{Product}} = \max \sum_{i=1}^{n}\left(\frac{PEC}{ECx/AF}\right)_i$$

Plants

a.s.	PEC	Effect concentration	AF	EC_x/AF	PEC/(EC_x/AF)
	[mg/kg]	[mg/kg]			
1	0.01	EC_{50} = 30.0	1000	0.03	0,333
2	$8.5*10^{-5}$	EC_{50} = 5.0	50	0.1	0,00085
3	0.035	NOEC = 5.0	10	0.5	0,07
4	0.01	NOEC = 1.0	50	0.02	0,5

$RQ_{product}$ = 0.33 + 0.00085 + 0.07 + 0.5 = 0.904

→ acceptable risk for environment

Earthworms

a.s.	PEC	Effect concentration	AF	EC_x/AF	PEC/(EC_x/AF)
	[mg/kg]	[mg/kg]			
1	0.01	LC_{50} = 800.00	1000	0.8	0.0125
2	$8.5*10^{-5}$	NOEC = 0.05	50	0.001	0.085
3	0.035	NOEC = 0.4	10	0.04	0.875
4	0.01	NOEC = 20	50	0.4	0.025

$RQ_{product}$ = 0.125 + 0.085 + 0.875 + 0.025 = 0.9975

→ acceptable risk for environment

Microorganisms

a.s.	PEC	Effect concentration	AF	EC_x/AF	PEC/(EC_x/AF)
	[mg/kg]	[mg/kg]			
1	0.01	EC_{50} = 120	1000	0.12	0,083
2	$8.5*10^{-5}$	EC_{50} = 7.0	50	0.14	0,00060
3	0.035	NOEC = 6.0	10	0.6	0,058
4	0.01	NOEC = 30	50	0.6	0,017

$RQ_{product}$ = 0,083 + 0,00060 + 0,058 + 0,017 = 0.16

→ acceptable risk for environment

→ *highest* $RQ_{earthworm}$ *= 0.9975*

→ *acceptable risk for soil for all three trophic levels*

→ *no need to proceed with Tier 3 or 4*

→ *analogous procedure for all other relevant compartments*

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