

Supporting Information for

***Predictive environmental risk assessment of chemical mixtures: a conceptual framework***

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## CA-based risk quotients for chemical mixtures and their quantitative relationship

The following argumentation focuses on the assessment steps in the environmental risk assessment of chemical mixtures. It therefore assumes that the exposure scenario is defined, i.e. a decision has been made previously on which  $n$  compounds make up the mixture of concern and that the environmental concentrations ( $PECs^1$ ) are known for all individual compounds, either from chemical monitoring studies or from modelling efforts. It is furthermore assumed that “the base set” of toxicity data ( $EC50$  values for algae, daphnids, fish) is available for all mixture components. An assessment factor ( $AF$ ) of 1 000 is applied in this situation, according to the REACH Guidance Document (European Chemicals Agency, 2008) for the calculation of a Predicted No Effect Concentration ( $PNEC$ ).

On the basis of such a set of data, and having the concept of Concentration Addition in mind, the joint toxicity of the mixture may be estimated in two different ways (see Fig. 1):

1. The  $PNEC$  is calculated individually for each mixture component. Afterwards, the  $PEC/PNEC$  ratios of all mixture components are summed up to a final Risk Quotient (termed  $RQ_{PEC/PNEC}$  in the following), of the mixture. That is:

$$RQ_{PEC/PNEC} = \sum_{i=1}^n \frac{PEC_i}{PNEC_i} = \sum_{i=1}^n \frac{PEC_i}{\min(EC50_{\text{algae}}, EC50_{\text{daphnid}}, EC50_{\text{fish}})_i} \times 1/AF \quad (1)$$

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<sup>1</sup> PEC=Predicted Environmental Concentration. It should be noted that exactly the same approaches can be followed when using measured environmental concentrations instead.

2. The Sum of Toxic Units ( $STU$ )<sup>2</sup> is calculated for each trophic level. Afterwards, the  $STU$  for the trophic level with the highest predicted sensitivity to the mixture (maximum  $STU$  of all analysed trophic levels) is multiplied with the Assessment Factor ( $AF$ ) for estimating the risk quotient of the mixture (termed  $RQ_{STU}$  in the following):

$$RQ_{STU} = \max(STU_{\text{algae}}, STU_{\text{daphnid}}, STU_{\text{fish}}) \times AF = \max\left(\sum_{i=1}^n \frac{PEC_i}{EC50_{i,\text{algae}}}, \sum_{i=1}^n \frac{PEC_i}{EC50_{i,\text{daphnid}}}, \sum_{i=1}^n \frac{PEC_i}{EC50_{i,\text{fish}}}\right) \times AF \quad (2)$$

The main conceptual difference between both RQ's is the order in which the single substance assessments and mixture toxicity estimations are made (see Fig. 1): the calculation of the  $RQ_{PEC/PNEC}$  starts with the single substance assessment (the calculation of individual  $PNECs$ ), which is then followed by the mixture assessment (summing up the individual  $PEC/PNECs$ ). This order is reversed when calculating  $RQ_{STU}$ : In the beginning, the mixture toxicity is estimated for each trophic level separately (summing up the individual  $PEC/EC50$  values), and the mixture is then risk-assessed as if it were a single chemical, by basing the final assessment on the most sensitive trophic level.

$RQ_{PEC/PNEC}$  has been initially suggested by Calamari and Vighi (1992). It is usually straight forward to apply and well established in the literature. Unfortunately, it violates fundamental assumptions of  $CA$  and implies a potential risk overestimation (see discussion in the main text and below). This is avoided by applying  $RQ_{STU}$ , which estimates the toxicity of the mixture by

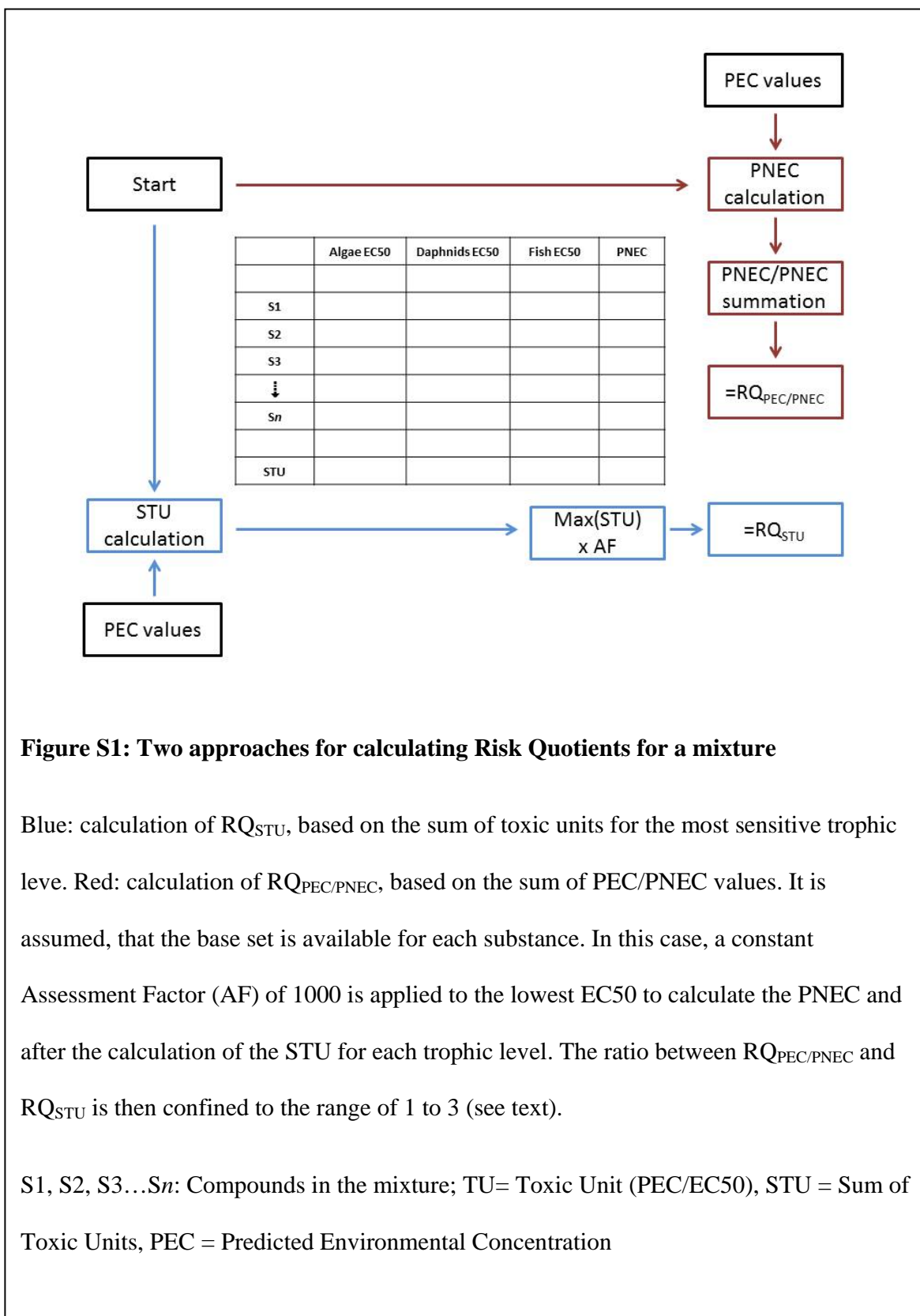
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<sup>2</sup> A Toxic Unit (TU) in this context is defined as the ratio of the concentration of the individual component in the mixture (its  $PEC$ ) and the used toxicity descriptor ( $NOEC$  or  $EC50$ ). Thus, a Toxic Unit is different from a  $PEC/PNEC$  ratio with respect to the  $AF$ : A Toxic Unit is calculated without considering any  $AF$ , while a  $PEC/PNEC$  ratio incorporates an appropriate  $AF$ .

making use of the principle of Concentration Addition in the form of a toxic unit summation for each trophic level. It then assesses the mixture as if it were a single chemical.

$RQ_{PEC/PNEC}$  is easier to apply, as only  $PNEC$  values for the mixture components are needed as ecotoxicological input information, which facilitates a mixture assessment by allowing the re-use of available single substance assessments.  $RQ_{STU}$  on the other hand relies on the availability of the  $EC50$  values for each compound and trophic level.

In view of this situation we argue for a stepwise application of both approaches: starting with the calculation of  $RQ_{PEC/PNEC}$  and only continue with  $RQ_{STU}$  if the data indicate a possible reason for concern (see main text). Obviously, such a strategy makes only sense if  $RQ_{PEC/PNEC}$  is a slightly more conservative measure than  $RQ_{STU}$ , which we will demonstrate in the following.



**Figure S1: Two approaches for calculating Risk Quotients for a mixture**

Blue: calculation of  $RQ_{STU}$ , based on the sum of toxic units for the most sensitive trophic level. Red: calculation of  $RQ_{PEC/PNEC}$ , based on the sum of  $PEC/PNEC$  values. It is assumed, that the base set is available for each substance. In this case, a constant Assessment Factor (AF) of 1000 is applied to the lowest EC50 to calculate the PNEC and after the calculation of the STU for each trophic level. The ratio between  $RQ_{PEC/PNEC}$  and  $RQ_{STU}$  is then confined to the range of 1 to 3 (see text).

S1, S2, S3...Sn: Compounds in the mixture; TU= Toxic Unit ( $PEC/EC50$ ), STU = Sum of Toxic Units, PEC = Predicted Environmental Concentration

### Quantitative Relations between $RQ_{PEC/PNEC}$ and $RQ_{STU}$

We will show in the following that  $RQ_{PEC/PNEC}$  always provides an equal or higher risk estimate than  $RQ_{STU}$  and that the maximum factor between both quotients equals the number of considered trophic levels, i.e. 3 if the “base set data” are available for all mixture components.

As illustrated in Fig. 1, the total matrix of toxic units that is available as a basis for calculating  $RQ_{PEC/PNEC}$  or  $RQ_{STU}$  is composed of data for  $n$  mixture components times three trophic levels.  $RQ_{PEC/PNEC}$  and  $RQ_{STU}$  each sum up selected subsets of these data. In principle a third mixture risk quotient  $RQ_{total}$  could be determined by summing up all of the  $TUs$ , for all components and all trophic levels:

$$RQ_{total} = \left( \sum_{i=1}^n \frac{PEC_i}{EC50_{i, \text{algae}}} + \sum_{i=1}^n \frac{PEC_i}{EC50_{i, \text{daphnid}}} + \sum_{i=1}^n \frac{PEC_i}{EC50_{i, \text{fish}}} \right) \times AF = (STU_{\text{algae}} + STU_{\text{daphnid}} + STU_{\text{fish}}) \times AF \quad (3)$$

$RQ_{total}$  is toxicologically not meaningful and is therefore not suggested for any practical purposes. However, for the analysis of possible quantitative differences between  $RQ_{PEC/PNEC}$  and  $RQ_{STU}$ ,  $RQ_{total}$  serves the purpose of defining the theoretical upper limit for any mixture RQ that is calculable from the given data.

$RQ_{total}$ , as defined in eq. 3, is the sum of three partial sums of toxic units (STU), calculated for each of the three trophic levels and multiplied by the constant AF. Each of these partial sums results from  $1/3^{\text{rd}}$  of all the available TUs.  $RQ_{STU}$ , as defined in eq. 2, is the highest out of these three partial sums, i.e. the STU for the most sensitive trophic level, multiplied by AF. This means that the STUs for the other two trophic levels must be smaller or at most equal to the value used for calculating  $RQ_{STU}$ . Hence, it follows that  $RQ_{total}$  cannot be larger than three times the value of  $RQ_{STU}$ :

$$RQ_{total} \leq 3 \times \left[ \max(STU_{algae}, STU_{daphnid}, STU_{fish}) \times AF \right] = 3 \times RQ_{STU} \quad (5)$$

The alternative risk indicator  $RQ_{PEC/PNEC}$  is calculated by always using the lowest of each of the three available EC50 values for every compound, divided by AF for estimating the PNECs (Eq. (1)). As well as  $RQ_{STU}$ ,  $RQ_{PEC/PNEC}$  also sums up 1/3<sup>rd</sup> of all the available TUs, but in contrast to  $RQ_{STU}$ , these may origin from different trophic levels. The definition of  $RQ_{PEC/PNEC}$  given in Eq. (1) can be re-arranged to:

$$RQ_{PEC/PNEC} = \sum_{i=1}^n \frac{PEC_i}{PNEC_i} = AF \times \sum_{i=1}^n \max \left( \frac{PEC_i}{EC50_{i,algae}}, \frac{PEC_i}{EC50_{i,daphnid}}, \frac{PEC_i}{EC50_{i,fish}} \right) \quad (6)$$

This shows that  $RQ_{PEC/PNEC}$  is based on summing up the maximum TU of each compound. Hence  $RQ_{PEC/PNEC}$  is the highest RQ that is possible to obtain from using only 1/3<sup>rd</sup> of the available TUs, under the condition that the calculation includes one and no more than one TU value per mixture component. Consequently  $RQ_{PEC/PNEC}$  is always higher than or equal to  $RQ_{STU}$ , but smaller or at least not larger than  $RQ_{total}$ :

$$RQ_{total} \geq RQ_{PEC/PNEC} \geq RQ_{STU} \quad (7)$$

Combining Eqs. (5) and (7) gives:

$$RQ_{total} \geq RQ_{PEC/PNEC} \geq RQ_{STU} \geq 1/3 \times RQ_{total} \quad (8)$$

Hence, it immediately follows that  $RQ_{PEC/PNEC}$  and  $RQ_{STU}$  cannot differ by more than a factor of 3:

$$1 \leq \frac{RQ_{PEC/PNEC}}{RQ_{STU}} \leq 3 \quad (9)$$

## Examples

Table 1 in the main text provides an example of how the two risk quotient relate to each other for a mixture of benzene and  $K_2Cr_2O_7$ , two compounds with a different ecotoxicological profile. For this mixture,  $RQ_{PEC/PNEC}$  provides a clearly higher risk estimate of 0.43, while  $RQ_{STU}$  provides a risk estimate of 0.24.

A similar analysis for a mixture of benzene and chlorbenzene, which both have an almost identical ecotoxicological profile (they are both mainly toxic to fish) yields the same risk quotient of 0.4, independent on whether  $RQ_{PEC/PNEC}$  or  $RQ_{STU}$  is used (see table S1 below).

## References

Calamari D, Vighi M. 1992. A proposal to define quality objectives for aquatic life for mixtures of chemical substances. *Chemosphere* 25: 531-542.

European Chemicals Agency. 2008. Chapter R.10: Characterisation of dose [concentration]-response for environment. In: *Guidance on information requirements and chemical safety assessment*.

European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), *Aquatic Hazard Assessment II*, Technical Report 91, 2003

**Table S1: Example of the relationship between  $RQ_{STU}$  and  $RQ_{PEC/PNEC}$  using a simple 2-compound mixture of Benzene and Chlorbenzene**

	<b>Benzene</b>	<b>Chlorbenzene</b>
EC50 (algae)	3202 $\mu\text{mol/L}$	1407 $\mu\text{mol/L}$
EC50 (daphnids, acute)	4117 $\mu\text{mol/L}$	2900 $\mu\text{mol/L}$
EC50 (fish, acute)	0.85 $\mu\text{mol/L}$	5.63 $\mu\text{mol/L}$
resulting PNEC (AF=1000)	0.85 nmol/L	5.63 nmol/L
PEC	0.2 nmol/L	1 nmol/L
<b><math>RQ_{PEC/PNEC}</math></b> (sum of PEC/PNECs)	$RQ_{PEC/PNEC} = \frac{PEC_{Benzene}}{PNEC_{Benzene}} + \frac{PEC_{Chlorbenzene}}{PNEC_{Chlorbenzene}} = 0.41$	
<b><math>RQ_{STU}</math></b> (sum of Toxic Units)		
algae	$= \frac{0.2 \text{ nmol/L}}{3202 \mu\text{mol/L}} + \frac{1 \text{ nmol/L}}{1407 \mu\text{mol/L}} = 0.77 \times 10^{-6}$ $\text{sum of TUs} \times AF = 0.77 \times 10^{-3} \text{ (STU}_{\text{Algae}})$	
daphnids	$= \frac{0.2 \text{ nmol/L}}{4117 \mu\text{mol/L}} + \frac{1 \text{ nmol/L}}{2900 \mu\text{mol/L}} = 0.39 \times 10^{-6}$ $\text{sum of TUs} \times AF = 0.39 \times 10^{-3} \text{ (STU}_{\text{Daphnids}})$	
fish	$= \frac{0.2 \text{ nmol/L}}{0.85 \mu\text{mol/L}} + \frac{1 \text{ nmol/L}}{5.63 \mu\text{mol/L}} = 0.41 \times 10^{-3}$ $\text{sum of TUs} \times AF = 0.41 \text{ (STU}_{\text{Fish}})$	
Final $RQ_{STU}$	$RQ_{STU} = \max(\text{STU}_{\text{algae}}, \text{STU}_{\text{crustaceans}}, \text{STU}_{\text{fish}}) = 0.41$	

Toxicity data were collected from ECETOC's EAT-5 database (ECETOC, 2003). Exposure data are arbitrary figures. RQ = risk quotient, STU = sum of toxic units, PNEC = predicted no effect concentration, PEC = predicted environmental concentration, TU=PEC/EC50, AF = Assessment Factor (1 000, according to (European Chemicals Agency 2008))