Frequently Asked Questions in the context of aquatic Mixture Toxicity and in relation with the use of the MixTox Tool

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Disclaimer: This FAQ answers common questions on complex areas of the aquatic mixture risk assessment. Please, be aware that for these topics, no strict guidance exists in the EFSA AGD (2013); thus the given answers should be considered only as recommendations from the group which developed the aquatic MixTox tool (i.e. members of authorities from DE, DK, AT, NO and NL).

Content

1. Metabolites

- Question: How metabolites should be included in the aquatic mixture risk assessment?

Metabolites should in general be included in the mixture toxicity risk assessment, unless it was clearly demonstrated that the proposed approach is not relevant/necessary. Such exceptions should be justified by the applicant. Many variables influence this assessment and some structured suggestions on how to proceed are provided below.

1.1. Scheme

Example based on a mixture of two a.s. ("*A*" and "*B*" in following text) and a metabolite from parent A ("*metab.*" in following text)

1- Is the metabolite toxic of equal toxicity (endpoint value within a factor 3 of parent endpoint) or of higher toxicity compared to parent A? Please note that the comparison should be done for the same species.

Yes: Go to 2

No: No further consideration of metabolite(s) needed in the mixture toxicity risk assessment. Conduct a mixture toxicity risk assessment based simply on *A* and *B* using the MixTox Tool.

2- Is the metabolite contributing to the risk with more than 90% compared to the a.s. (i.e. > 90% of risk due to PEC_{metab}/RAC_{metab} + PEC_A/RAC_A attributed to PEC_{metab}/ RAC_{metab} at the most critical/ worst-case FOCUS Step and scenario(s))?

Yes: Conduct a mixture toxicity risk assessment according to step 8 based on the *metab.* and *B* using the MixTox Tool.

No: Go to 3

3- Is the metabolite of parent A only formed in water[1] (e.g. based on fate studies such as hydrolysis and/or phytolysis degradation studies)?

Yes: Go to 4

No or unknown: Go to 6

4- Is the maximum formation rate of the metabolite occurring within the duration[2] of the test performed with the parent (e.g. based on information available from water-sediment degradation tests)?

Yes: Go to 5

No: Go to 6

5- Conduct a mixture toxicity risk assessment based on *A*, *B* and *metab.* according to step 8 in the AGD. If relevant, FOCUS Step 4 PECsw-values considering risk mitigation measures should be used. Please use information provided below under section 1.2, for the expression of the test endpoints. Does the assessment lead to an unacceptable risk?

Yes: Consider if mixture toxicity can be refined using additional data e.g. geomean or SSD.

No: No unacceptable risk identified, no further steps are needed.

6- Conduct a mixture toxicity risk assessment solely based on *A* and *B* according to step 8 in the AGD (i.e. no consideration of *metab.*). If relevant, FOCUS Step 4 PECsw-values considering risk mitigation measures should be used. Please use information provided below under section 1.2, for the expression of the test endpoints. Does the assessment lead to an unacceptable risk?

Yes: Consider if mixture toxicity can be refined using additional data e.g. geomean or SSD.

No: No unacceptable risk identified, no further steps are needed.

[1] A metabolite could be also formed in soil but enter surface waters via runoffs (in this case choose "No" below)

[2] E.g. within 96 h, if fish is relevant for the acute risk assessment

1.2. Further Information

1.2.1. Information on test endpoints (from a.s. studies for MixTox calculation)[3]

- *Option 1*: Use the MixTox Tool to enter the endpoint of *A* expressed in mg parent *A* /L (enter also e.g. the endpoint of *B* expressed in mg parent *B*/L when dealing with a product with 2 a.s./ parents).
  The test endpoint is expressed in mg parent A /L. If the metabolite is not measured during the test performed with the parent A, the test endpoint is derived considering that the toxicity is only attributed to the concentration of the parent (instead of the sum (parent A + metab.), Option 2). This approach is considered as suitable and conservative (however, please note that in general (especially) toxic metabolites should be measured),

  OR

- *Option 2:* Use the MixTox Tool to enter the endpoint of *A* in mg (sum parent *A + metab.)* /L; (enter also e.g. the endpoint of *B* expressed in mg *B*/L when dealing with a product with 2 a.s./ parents).
  The test endpoint is expressed in mg sum (parent A + metab.)/L. If the metabolite is sufficiently measured in the test performed with the parent A, the test endpoint can be calculated based on the average concentrations of parent and metabolite. The calculation of the test endpoint could be done in a similar way as for a product endpoint using the information from Appendix J of EFSA report 2019 (EFSA Supporting publication 2019:EN-1673) (Section 4.1 Case 1: All active substances have been analytically measured).
  Please note, that in Option 2:
  - the proportions of the actives and the metabolites as measured in the test should be regarded for the mixture toxicity assessment;
  - the value of a test endpoint is more accurate and less conservative than in Option 1, because the concentration of a parent plus metabolite will always be higher than the concentration of the parent alone;
  - the risk of mistakes in calculations are higher than in Option 1.

1.2.2. Information on PECs for mixture toxicity risk calculations

In terms of mixture toxicity risk calculations, the PEC values used for *A* should be the sum of PEC *A* (that dissipates over the time) plus PEC metabolite (that forms over the time).

- For Option 1, use PECmax of substance *A* only, since only the a.s. is considered for endpoint derivation;
- For Option 2, use PECmax for *A* and PECmax for metabolite (for refinements, use PECmax of higher FOCUS Steps).

[3] The considerations presented are not correct if e.g. a surfactant in the formulation PPP would change the formation rate of the metabolite. However, this is not expected and difficult to demonstrate, unless it leads to synergy.

2. Combining FOCUS Steps of different levels, in particular FOCUS Step 4 with lower FOCUS Steps

- Question: How to proceed, if data from a higher FOCUS Step (typically FOCUS Step 4) data are only available for some active substances?

The risk calculations should in principle be performed using the PECsw from the same FOCUS Step (1, 2, 3 or 4) for all a.s. to ensure that the underlying exposure assumptions for the PECsw are the same. However, this is not strictly demanded, but a recommendation.

Likewise, data not yet available from a higher FOCUS Step should be requested even if they are less conservative and were not necessary to demonstrate an acceptable risk for the single a.s.. Although assessing e.g. FOCUS Step 4 for all a.s. in all FOCUS scenarios may become a time-consuming effort, the FOCUS Step 4 data should be provided for all a.s. and all FOCUS scenarios unless it can be clearly demonstrated that a particular FOCUS scenario is considered as worst-case scenario covering all other FOCUS scenarios.

Combining different FOCUS Steps, however, may be reasonable depending on the assessment step considered (cf. remarks below). Still, it is advised to generally use the same FOCUS Step instead of combining different FOCUS Steps in order to be able to follow the scheme 10.3.11 in the AGD – some mixture toxicity assessment steps in the scheme cannot be reasonably calculated with different FOCUS Steps.

Explanation

Combining PEC-values from different FOCUS Steps is considered **not reasonable**:

- for mixture toxicity assessment step 3 (leading to the product mixture assessment, Step 4): The ratio in Step 3 ($ECx_{mix\text{-}CA}$ (a.s. in PPP)/ $ECx_{mix\text{-}CA}$ (a.s. in PEC_{mix})) needs to be accurate for deriving a correct decision in Step 3 (i.e. precise PEC input data). Deviations towards lower or higher values will lead to wrong decisions regarding the questions if product data can be used or not. Thus, combining different FOCUS Steps here is considered not reasonable.
- for mixture toxicity assessment Step 5 (leading to the driver assessment, Step 6) currently FOCUS Step 4 cannot be calculated and included in the assessment. Moreover it is also not recommended to combine different FOCUS Steps, as it may lead to a false decision on a driver. However, a less toxic a.s. will (only) have calculations for a lower FOCUS Step, which should lead to a higher PEC of the respective a.s. and which, consequently, gives a higher TU (eq. 14). In a strict sense it will not be correct as it could mask a driver, but would still be conservative (because mixture toxicity assessment Step 8 would be taken, cf. also FAQ question 4).

Combining PEC-values from different FOCUS Steps is considered **reasonable:**

- primarily for mixture toxicity assessment Step 8, as using data from lower FOCUS Steps should lead to higher $PEC_{sw,max}$ for the respective a.s. Thus, it will give a conservative estimate for the mixture toxicity risk assessment.
- Note that an evident **prerequisite** for combining different FOCUS Steps directly in assessment Step 8 is that **synergism is excluded** (check MDR calculation in Step 2; in case

of a synergism indicated by a MDR > 5[4] but <10, the RA can be based on a calculated mixture toxicity that compares ETR to trigger (AF)/ MDR, as suggested in the AGD 10.3.4). Even if a slight synergism (i.e. MDR borderline to 5, case by case decision) occurs, care should be taken and FOCUS Step 4 data should be requested.

- To combine different FOCUS Steps in the tool technically, follow the described procedure:
    1. Go to "Input PEC" sheet;
    2. For the active substance(s) where e.g. FOCUS Step 4 values are available enter these in the FOCUS Step 4 tables;
    3. For the active substance(s) where e.g. FOCUS Step 4 values are missing, enter the FOCUS Step 2 or 3 values in the FOCUS Step 4 tables.
- Lastly, combining different FOCUS Steps requires that only the $PEC_{sw,max}$ values are used; consideration of FOCUS profiles and PECtwa of the active substances should not be considered in the mixture toxicity assessment unless further guidance is available.

3. Chronic mixture toxicity

- Question: How should the chronic mixture toxicity be included in the assessment?

Background information

How and whether to conduct a chronic mixture toxicity assessment is currently not harmonized between zones and Member States. Only limited specific information for chronic mixture toxicity is provided in the AGD, but the approach developed for acute mixture toxicity was meant to be applied also for chronic (see e.g. section 10.3.4. about MDR and step 1 scheme).

In order to facilitate a chronic assessment, please see the following suggestions

In general, the chronic mixture toxicity assessment can follow the acute approach from the AGD (section 10.3.11). When using a calculated mixture toxicity approach (which is likely as often chronic product data are not available) going directly to the RQ_{mix} (Step 8b) is preferred (after synergism is discussed, i.e. Step 7). This is also due to technical reasons, as the ETR-triggers are hard coded in this tool while the AF for the RQ_{mix} can be adapted (which is necessary for a correct chronic toxicity assessment).

Measured mixture toxicity:

If a chronic formulation test is available, the standard steps of the risk assessment scheme (AGD 10.3.11) should be followed.

A formulation test (chronic) should be delivered if it is not possible to extrapolate the mixture toxicity from data of the a.s. For instance, if a PPP is more acutely toxic than the a.s. by a factor 10, a product test is mandatory (AGD 10.3.2; unless demonstrated that exposure will not occur).

[4] The MDR threshold of 5 is from the current AGD. Please note that this may change in the future due to potential needs of aligmenet with other assessment areas (e.g. a MDR threshold of 3 is proposed in the upcoming EFSA B&M)

Calculated mixture toxicity:

Reliable EC_{10} values should be preferably used also for the mixture toxicity calculations (whenever available) because the NOEC strongly depends on dose-spacing and could already reflect a certain effect level – besides exhibiting several other drawbacks.

In long-term/chronic toxicity tests, typically more diverse biological endpoints are tested than in the acute assessment. Thus, a two-steps approach is reasonable.

- In a first step, the lowest endpoints are combined (e.g. egg production for substance A can be combined with body weight for substance B). This approach based on worst-case assumptions assumes that the population level can be overall affected; it is thus more conservative and regulatory sound. If it leads to an unacceptable risk, a second/ refinement step may be considered.

In a second/ refinement step, comparable endpoints could be combined (e.g. based on example above, combine only endpoints for egg productions or only endpoints for body weight effects). This approach is less conservative but it is more closely related to the concentration addition (CA) model that applies theoritically to similar MoA and thus to specific targets; it could be accepted if supported by a WoE to justify why the different endpoints should not be considered jointly.

4. Assessment based on a driver (Step 5 + 6 as well as Screening 2)

- Questions: Which issues can occur while deriving a driver? How to calculate a driver in case there are data for more than one species? How to implement the simplified mixture toxicity assessment (10.3.7.) in this regard?

Starting point

The **driver** (of toxicity) approach was **introduced to reduce time and effort** in risk assessment. It is based on the calculation of toxic units (TUs, cf. Equation 14 in the AGD). However, based on practical experience with this approach a number of critical issues were raised, which question its usefulness and original purpose. **The approach leads to more questions raised than solved.** The main reason to keep the step 5 on driver in the current tool is because it is part of the scheme in the current AGD.

General issues

First (as minor issue) the driver approach is given in two sections of the AGD, in the decision scheme (AGD section 10.3.11.) but also as screening step in the section on simplified approaches (10.3.7.). This is a regulatory conflict, because in the scheme the product assessment is placed before the driver assessment, whereas in the simplified/screening approach the product assessment is done after the driver assessment. It is not clear what the priority is.

Second (and more important), deriving a driver can be considered as a **deviation from the usual risk assessment tiered approach**, that is to conduct first a conservative risk assessment and then in case of high risk identified, to refine the risk. A screening step should usually be more conservative than the first-tier step. However, the derivation of a driver is never more conservative than conducting a **(full) mixture toxicity risk assessment**, because the later includes the driver plus another substance, which will always lead to a **more conservative** risk assessment (two added risks are always bigger than one (driver) risk) although its significance could indeed be questioned. Thus, with respect to a risk assessment the driver should, in principle, not be a starting point as it is leading to a less conservative assessment.

Lastly (and most important), the main argument for the driver calculation is to reduce time and effort, but a full mixture toxicity risk calculation can be conducted easily, e.g. if programmed tools are available, and the **driver approach needs further considerations regarding its regulatory**

implementation. For instance, in the AGD scheme (10.3.11.), it is written that the driver in assessment step 5 should be based on the TUs "calculated for the formulation". However, this is scientifically questionable since the TU approach depends on the "assumed" mixture composition, which is not necessarily the composition of the formulation. Indeed, the correct "assumed" composition could either be the composition of the product/ formulation or the composition at the PEC. For a scientifically sound risk assessment the TU calculation/ driver assessment should rather be based on the composition at PECmix for each FOCUS scenario (this could be named "driver of risk", not only "driver of toxicity"). The effort for conducting such a driver calculation increases substantially. However, this will be the composition to which non-target organisms are exposed to in the environment and not the composition of the formulation. Therefore, this is what is implemented in the calculation tool. The fact that this option is preferred in regulation has been clarified in an email exchange to EFSA by some member states as a topic for further discussion.

Overall, the derivation of a **driver is not only one simple calculation**. It **adds more complexity** to the risk assessment instead of simplifying it and is even (slightly) less conservative.

Specific issues

a) **Different results for different FOCUS scenarios**

There are further points which make the driver assessment approach more complex. In case the driver is a borderline case (90% in FOCUS Step 1), it can occur that the driver holds only for some (but not all) FOCUS scenarios in FOCUS Step 2 and 3. Thus there will be some scenarios with a driver and others without. In this regard, the driver approach can be considered obsolete as it would be more **conservative and simpler to always do the calculation of a (full) mixture toxicity risk assessment (e.g. Step 8b)** with the tool.

b) **Inclusion of higher tier and sensitive species information**

Furthermore, it is interesting to note that there will be often refinements or higher FOCUS Steps available for the driver (since of most concern), but not for the non-driver. This can lead to **different results depending on which data are used.** This issue is not (well) reflected in the AGD. For example, how to deal with the situation of a substance being a driver according to Tier 1 data but not anymore when considering additional/ Tier 2 data? Or how to deal with the opposite situation: a substance being a driver when considering additional (more sensitive species)/ Tier 2 data, but not according to standard data? Should only data of the same species or also between species be compared?

A similar question was asked to us during the update of this FAQ. The main points for an ad-hoc solution could be summarized as follows:

In case of data available for more than one species only for one active substance (substance A) and only standard data for an active substance B: deriving the driver could be based either on using the standard species only or on using the most sensitive species and the standard species. The AGD lacks of clarity about a preferred option. Although the tool automatically considers the additional data (in this example: the sensitive species data[5]), our proposal is to **calculate twice the TU/driver**, i.e. with standard species only (for substances A and B) and additionally with the sensitive species (for substance A) and the standard species (for substance B). In this sense, create two excel files/tables, and **consider these options**:

1. If the substance A is a **clear driver** (i.e. clearly more toxic), the **species selection should not matter** much and both calculations should point to the existence of a driver. However, a small uncertainty remains, as data are missing for a full comparison on all

[5]Mentioned in the tool in the calculation details of step 5: "*The calculations are based on the additional data (e.g. sensitive species), if entered. Otherwise, the standard Tier 1 data are taken*".

species and also the ranking of species in terms of sensitivity could vary according to the substance tested.

2. If it is **dependent on the species** (e.g. the substance A is classified as a driver if based on the sensitive species/additional data but not if based on the standard data only), there are two possibilities:
   a. Declare that there is no clear driver (i.e. there are indications, but the issue cannot be fully resolved due to species differences). To finalize the assessment a **mixture toxicity calculation based on sensitive species and standard species** should be conducted (step 8) as pragmatic solution.
   b. **Generate the missing data** for non-standard (sensitive) species for the other substance B to enable a comprehensive assessment.

   => Option b has the advantage of producing further clarification and would be scientifically preferred. Furthermore, there may also be the case that substance A has sensitive species X and substance B has sensitive species Y; in that case, **having all data would be desirable for a full weight-of-evidence**. However, usually it will be easier to proceed with option a. This has the advantage of being conservative considering the information available (and with respect to the alterative, which would be to declare a driver). It is a pragmatic solution with respect to the given knowledge.

In conclusion, the specific issues are solvable if more guidance is provided. However, they demonstrate that deriving a driver increases the risk assessment complexity beyond its original purpose (of simplification). This could be avoided if the driver assessment is replaced by the more conservative calculation of a (full) mixture toxicity risk assessment (e.g. step 8b in the tool).