### Risk assessment

#### Individual substance risk assessment

In case a mixture risk assessment has to be performed, please add the headline 9.5.2.1 to the respective section 9.5.2. Conduct a normal risk assessment for each a.s. and afterwards add section 9.5.2.2.

Please note, in contrast to the Aquatic Guidance Document (shortly AGD, cf. EFSA Journal 2013;11(7):3290) the term “individual substance” instead of “single substance” is used in this template (and the tool “AGD\_AquaMix”)1 for semantic reasons – “assessing the single active substances of a mixture” would be like “counting the single keys on a keyboard” which is better expressed as “assessing the individudal active substances of a mixture” (“counting the individual keys on a keyboard”).

To keep the mixture risk assessment concise this template was kept as short as possible. The **tables** below can be filled by **copy & paste** from the **sheet “Final\_conclusion” of the calculation tool**. It is **highly recommended to attach the full calculations** conducted with the tool “AGD\_AquaMix” as excel file and/or document them in an Appendix.

The mixture toxicity risk assessment according to the AGD contains several assessment steps, which are based on the decision scheme as provided by EFSA (2013, section 10.3.11). For an overview of the Steps please be referred to the sheet “overview” in the calculation tool “AGD\_AquaMix”. Further in-depth explanations of the assessment procedure are included in the calculation tool (as well as the FAQ file) and are not repeated here.

#### Mixture toxicity risk assessment

The evaluation of the mixture toxicity risks for aquatic (and sediment-dwelling) organisms was performed in accordance with the “Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15. January 2015) and in EFSA Journal 2013;11(7):3290 (Aquatic Guidance Document, abbreviated as EFSA, 2013). The whole mixture assessment is based on the model of concentration addition (CA) and particularly the decision scheme as provided by EFSA (2013, section 10.3.11) was applied. The calculations were performed with the tool “AGD\_AquaMix”.[[1]](#footnote-1) To keep the assessment concise the focus of this section of this registration report is on the parts of the assessment leading to the most critical results. It does not cover each calculation step for each organism group. Further documentation of the full results is given in the attached mixture risk calculation file / Appendix XXX / following: <add explanation>.

Table 9.5.2.2‑1: Summary of assessment steps considered for the mixture risk assessment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Steps** | **Conclusion on the Steps** | | | |
| **Fish** | **Invertebrates** | **Algae** | **Macrophytes** |
| **Screening 1: ETR trigger (optional Step)** |  |  |  |  |
| **Screening 2: Driver (optional Step)** |  |  |  |  |
| **Step 1: data available?** |  |  |  |  |
| **Step 2: apparent synergism or antagonism?** |  |  |  |  |
| **Step 3: mixture similar or not?** |  |  |  |  |
| **Step 4: ETRmix assessment (ECxPPP)** |  |  |  |  |
| **Step 5: driver available?** |  |  |  |  |
| **Step 6: driver assessment** |  |  |  |  |
| **Step 7: synergism assessment (few data)** |  |  |  |  |
| **Step 8a: ETRmix assessment** |  |  |  |  |
| **Step 8b: RQmix assessment** |  |  |  |  |
| **Step 9: antagonism assessment** |  |  |  |  |
| **Step 10: synergism assessment** |  |  |  |  |

**Overview of the assessment steps:** Table 9.5.2.2‑1 gives a summary of the decisions made for each organism group during the mixture risk assessment with respect to the decision scheme as provided by EFSA (2013, section 10.3.11). A discussion of all details of every decision is not intended in this section of this registration report. Further in-depth justification and explanation for the decisions made are documented in the attached mixture risk calculation file / Appendix XXX / following: <add explanation>. However, at least data for some important steps are documented in this section of this registration report, beginning with the check for (and potentially assessment of) synergistic or antagonistic effects (Step 2 and Steps 7, 9 and 10) as well as the ETRmix or RQmix calculation results for the assessed FOCUS Scenarios (Steps 4 and 8a/b).

**Screening Assessment**

In case Screening 1 suggests acceptable or unacceltable risks for particular organism groups, it is possible to further explain the screening assessment results here.

In case Screening 2 suggests the availability of a driver, please consider conducting a normal mixture risk assessment as otherwise this could contradict the decision scheme as provided by EFSA (2013, section 10.3.11). Please, mind that a driver might be only available for some FOCUS Scenarios and for others a full mixture risk assessment may have to be conducted.

<Screening results>

**Step 2:** MDR calculation to check for synergistic or antagonistic effects

In this step the calculated mixture toxicity (ECxmix-CA) is derived and compared to the measured mixture toxicity (studies performed with the plant protection product, ECxPPP) in order to figure out if synergistic or antagonistic effects occur or the assumption of concentration addition (CA) holds ture. The calculation details and further explanations can be found in the calculation tool “AGD\_AquaMix” (sheet Step 2) and are not repeated here.

Note, that the MDR calculation should be based on the same species of a taxonomic group of Tier 1 standard test species. If same species are not available, use species as closely related as possible (e.g. two species of green algae would be acceptable; but combining green algae and diatom or mixing Lemna and Myriophyllum is not acceptable); also use the same endpoint (e.g. only ErC50, or only EbC50).\* Further, it should refer to the same calculation basis; i.e. ECxPPP expressed as "sum of mg a.s./L" has to be used and not ECxPPP expressed as "mg product/L" ("mg product/L" is usually delivered; cf. AGD, 10.3.4.). The tool “AGD\_AquaMix” automatically takes the ECxPPP as "sum of mg a.s./L".

\*Please, note that in later steps (in particular Step 8), ECxmix-CA is not necessarily calculated based on Tier 1 standard test species, but should be based on the **most** **sensitive speciesor Tier 2 A/B data**. These **could be added manually to Table 9.5.2.2‑2**, if necessary, in order to give a comprehensive data overview.

**Copy & Paste remark:** Please note that it is recommended to use the “keep text only” paste option of word. This is accessed by marking all the cells into which you want to paste the text/results, doing a right click on the selection and choosing the symbol  unter copy options. Some formatting has to be redone, though. It should be possible to copy the data from the calculation tool in one go, by beginning the selection with the righthand side next to the last line of the table and dragging the selection up to the line “Fish, acute toxicity1”.

Table 9.5.2.2‑2: Calculated mixture toxicity and MDR for each group of organisms (see sections 10.3.3 and 10.3.4 of EFSA (2013) for more details)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Active substance** | **Concentration (Ci) in formulation  (g a.s./L)** | **Pi** | **ECxi (mg a.s./L)** | **ECxmix-CA  (mg sum a.s. /L)** | **ECxPPP (mg sum a.s./L)** | **MDR2** |
| **Fish, acute toxicity1** | | | | | | | |
| *species sp.* | a.s.1 |  |  |  |  |  |  |
| *species sp.* | a.s.2 |  |  |  |
| *species sp.* | a.s.3 |  |  |  |
| *species sp.* | a.s.4 |  |  |  |
| **Invertebrates, acute toxicity1** | | | | | | | |
| *species sp.* | a.s.1 |  |  |  |  |  |  |
| *species sp.* | a.s.2 |  |  |  |
| *species sp.* | a.s.3 |  |  |  |
| *species sp.* | a.s.4 |  |  |  |
| **Algae** | | | | | | | |
| *species sp.* | a.s.1 |  |  |  |  |  |  |
| *species sp.* | a.s.2 |  |  |  |
| *species sp.* | a.s.3 |  |  |  |
| *species sp.* | a.s.4 |  |  |  |
| **Macrophytes** | | | | | | | |
| *species sp.* | a.s.1 |  |  |  |  |  |  |
| *species sp.* | a.s.2 |  |  |  |
| *species sp.* | a.s.3 |  |  |  |
| *species sp.* | a.s.4 |  |  |  |

1 For a possible inclusion of chronic toxicity please refer to the FAQ document distributed alongside the aquatic mixture toxicity calculation tool “AGD\_AquaMix”.

2 MDR = 0.2 – 5 🡪 Go to Step 3 (concentration addition (CA) approximately holds for the calculated mixture);

MDR > 5 🡪 Go to Step 10 (the calculated mixture is more toxic than CA, i.e. synergistic);

MDR < 0.2 🡪 Go to Step 9 (the calculated mixture is less toxic than CA, i.e. antagonistic)

**Step 7, Step 9 and Step 10:** Assessment of possible antagonism/synergism

The Steps 7, 9 and 10 are considered as “in-between” steps (cf. sheet “overview” in the calculation tool “AGD\_AquaMix”); thus, they are already mentioned here instead of using the order in which the steps are numbered in the AGD. In this section discuss e.g. toxic co-formulants, synergism, literature data, mixture toxicity calculation with “unbound endpoints”, differences in the test system, sensitive species … Some further suggestions are given in the mixture toxicity calculation tool “AGD\_AquaMix” in the respective sheets (Step 7, Step 9, Step 10). **If not needed (all MDR = 0.2 – 5) delete this section.**

Please, mind that in the AGD 10.3.4 it is noted that a modified trigger could be used for synergism (e.g. ETR-trigger/MDR or AF\*MDR -> Go to Step 8) to enable an approximate risk assessment. This is particularly important if synergism is identified, but product data cannot be utilized, e.g. because the mixture in the environment (PECmix) differs from the mixture in the product (according to Step 3).

Due to missing data (Step 7)/According to the MDR calculation a discussion regarding antagonism/synergism (Step 9/Step 10) is indicated for fish/invertebrates/algae/macrophytes.

<Add discussion for the respective organism group>

**Step 3** Comparison of mixture composition of PPP and PECmix

Step 3 (also an “in-between” step) is conducted for each PECmix to check if product data can be used for the assessment, when CA applies (i.e. MDR = 0.2 – 5). The calculation details and further explanations can be found in the calculation tool “AGD\_AquaMix” (sheet Step 3) and are not repeated here.

According to Step 3 it has to be checked whether the mixture composition in the formulation is similar to the mixture composition at PECmix (which is the sum of PECi of individual active substances). Therefore, the “ECxmix-CA (a.s. in PPP) / ECxmix-CA (a.s. in PECmix)” ratios were calculated.

In case the ratio is in the range of 0.8–1.2 (mixture similar), the risk assessment can be based on the ECxPPP (Step 4).

In case the ratio is outside the range of 0.8–1.2 (i.e. mixture not similar), the endpoint ECxmix-CA normalized to the mixture composition at PECmix (reported as “ECxmix-CA (a.s. in PECmix)” in EFSA, 2013, and the following parts of this report) has to be used to calculate the risk (Step 5 or Step 8, respectively).

The decision how the mixture risk assessment is conducted depends on the organism group as well as the assessed FOCUS Scenarios. The overall mixture risk assessment results are given in Table 9.5.2.2‑3, in which it is documented if product or calculated endpoint data (according to Step 3) have to be used. Further in-depth justification and explanation are documented in the attached mixture risk calculation file / Appendix XXX.

Please note, if a **different assessment factor or additional data** (e.g. sensitive species, Tier 2 A/B data) are used, Step 3 can be skipped/is not required. In such case, you may insert the sentence given below in this box and delete the rest of this proposed template for Step 3 (text above this box).

**Reasoning**: in these cases using **product data (Step 4, measured risk assesment) may not reflect the risk adequately** since product tests are mostly conducted with standard species and rarely with the most sensitive species within an organism group. Performing the risk assessment based on the measured mixture toxicity (ECxPPP) would only be applicable if the study conducted with the PPP was performed with the most sensitive species tested. If not, this could lead to an underestimation of the risk. Thus, in the cases where additional data (i.e. sensitive species) are available the risk assessment should be conducted according to Step 8.

According to **Step 3** it has to be checked whether the mixture composition in the formulation is similar to the mixture composition at PECmix (which is the sum of PECi of individual active substances). However, a different assessment factor or additional data have been used in the a.s. assessment. Therefore, it is not appropriate to use product data and not necessary to apply Step 3.

**Conclusion:**

🡪 Go directly to Step 8 for fish/invertebrates/algae/macrophytes (even if mixture is similar, Step 3 is not possible / reasonable).

**Step 5:** Driver detection

In Step 5 (also an „in-between” step) toxic units (TUs) and sum of TUs are calculated to determine if one substance can be considered a driver concerning a particular FOCUS Scenario. The calculation details can be found in the calculation tool “AGD\_AquaMix” (sheet Step 5) and are not repeated here.

However, mind that it might be possible to skip TU calculations and go directly to Step 8. Step 8 includes the toxicity of the driver and the toxicity of other substances and, thus, always leads to more conservative ETR / RQmix-values for mathematical reasons. This can be of importance if e.g. sensitive species data (other than species used in MDR calculations) are available and used in the assessment of the driver and the driver assessment is refined to a large extent, but data on fewer species are available for non-driver substances.

Note: In the frame of a revision of the AGD, the usefulness of this TU approach should be reconsidered. At this stage, it is perceived as adding more complexity rather than facilitating the MixTox assessment (as initially intended). However, since the TU approach is currently included in the AGD it has to be retained for the time being in this mixture risk assessment.

The decision how the mixture risk assessment is conducted depends on the organism group as well as the assessed FOCUS Scenarios. The overall mixture risk assessment results are given in Table 9.5.2.2‑3, in which it is documented if a driver of risk is available according to Step 5. Further in-depth justification and explanation are documented in the attached mixture risk calculation file / Appendix XXX. Further information for the availability of a driver for the risk assessment of particular FOCUS Scenarios is given in Table 9.5.2.2‑3 (FOCUS Step 1 – 3) and Table 9.5.2.2‑4 (FOCUS Step 4) below.

**Step 4 and Step 8a/b:** Mixture risk assessment based on measured and/or calculated mixture toxicity

In these Steps (4 and 8 a/b) the final conclusions on the risk assessment are given. The calculation details and further explanations can be found in the calculation tool “AGD\_AquaMix” (sheet Step 4 and sheet Step 8) and are not repeated here.

Decisions taken when using the scheme as provided by EFSA (2013, section 10.3.11) lead to several assessment steps, which give a conclusion on the risk of a mixture or provide additional information important for the assessment. In the tables below the conclusions of different steps of the mixture risk assessment are summarized for the FOCUS Scenarios of concern. Information is reported on:

1. Step 2 and associated steps, in which the question is answered if concentration addition can be assumed (i.e. a standard mixture toxicity risk assessment is appropriate) or if an antagonism/synergism is possible (more detailed information on Step 2 is given above). This is important to derive if further, specific considerations have to be made in the risk assessment. Associated steps are Step 7, Step 9 and Step 10.
2. Step 5, in which the question is answered if there is a driver of risk and if so, which of the substance can be classified as driver (more detailed information on Step 5 is given the attached mixture risk calculation file / Appendix XXX). This is reported as additional information in case an assessment based on a driver would also be possible; for comparative reasons the table always lists the results from the mixture risk assessment according to Step 4 and/or Step 8. For an assessment based on a particular a.s. (the driver) please be referred to the individual a.s. risk assessment.
3. Step 4 with the assessment results for cases where product data are usable (according to Step 3) and the risk assessment can be carried out based on the measured toxicity.
4. Step 8 (a/b) with the assessment results for cases where product data are not usable (according to Step 3) and the risk assessment has to be based on calculated mixture toxicity.

The respective assessment conclusions are presented in Table 9.5.2.2‑3 (FOCUS Step 1 – 3) or Table 9.5.2.2‑4 (FOCUS Step 4). The assessment is based either on the exposure-toxicity ratio (ETRmix) or the risk quotient (RQmix); the ETRmix and RQmix are compared to respective trigger values: 1/10 or 1/100 for the ETRmix (depending on the species assessed) and 1 for RQmix. Values below the trigger indicating a high risk are marked in **bold**. For the sake of a concise assessment the results below are limited to the organism groups most at risk. Further in-depth justification and explanation for other organism groups are documented in the attached mixture risk calculation file / Appendix XXX.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Table 9.5.2.2‑3: Summary of the mixture toxicity risk assessment (related to Steps 2, 4, 5 and 8a/8b) for fish/invertebrates/algae/macrophytes, for FOCUS Step 1 – 3 for application of <formulation> in <crop> (use/use group) | | | | | |
| **FOCUS Scenario** | **Synergism or Antagonism? (e.g. Step 2)** | **Driver?  (Step 5)** | **ETRmix-PPP  (Step 4)** | **ETRmix-CA  (Step 8a)** | **RQmix  (Step 8b)** |
| **Step 1** |  |  |  |  |  |
| **Step 2** |  |  |  |  |
| **N-Europe** |  |  |  |  |
| **S-Europe** |  |  |  |  |
| **Step 3** |  |  |  |  |
| **D1/ ditch** |  |  |  |  |
| **D1/ stream** |  |  |  |  |
| **D2/ ditch** |  |  |  |  |
| **D2/ stream** |  |  |  |  |
| **D3/ ditch** |  |  |  |  |
| **D4/ pond** |  |  |  |  |
| **D4/ stream** |  |  |  |  |
| **D5/ pond** |  |  |  |  |
| **D5/ stream** |  |  |  |  |
| **D6/ ditch** |  |  |  |  |
| **R1/ pond** |  |  |  |  |
| **R1/ stream** |  |  |  |  |
| **R2/ stream** |  |  |  |  |
| **R3/ stream** |  |  |  |  |
| **R4/ stream** |  |  |  |  |

Table 9.5.2.2‑4: Summary of the mixture toxicity risk assessment (related to Step 4/8a/8b) for fish/invertebrates/algae/macrophytes, based on FOCUS Step 4 calculations for application of <formulation> in <crop> (use/use group)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **FOCUS Step 4** | **Scenario** | **ETRmix-PPP or ETRmix-CA or RQmix** | | | | | | | | |
| **Nozzle reduction** | **Vegetative strip [m]** | **None** | **None** | **None** | **None** | **None** | **5** | **10** | **15** | **20** |
| **No spray buffer [m]** | **None,  default** | **5** | **10** | **15** | **20** | **5** | **10** | **15** | **20** |
| **None** | **D1 Ditch** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **D1 Stream** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **D2 Ditch** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **D2 Stream** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **D3 Ditch** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **D4 Pond** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **D4 Stream** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **D5 pond** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **D5 stream** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **D6 Ditch** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **R1 pond** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **R1 stream** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **R2 stream** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **R3 stream** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |
| **None** | **R4 stream** |  |  |  |  |  |  |  |  |  |
| **50%** |  |  |  |  |  |  |  |  |  |  |
| **75%** |  |  |  |  |  |  |  |  |  |  |
| **90%** |  |  |  |  |  |  |  |  |  |  |

1. Sometimes also termed “Aquatic MixTox Tool”. The current version can be found on the EFSA Knowledge Junction platform, DOI:  [10.5281/zenodo.4593675](https://doi.org/10.5281/zenodo.4593675) ([Aquatic Mixture Toxicity Tool and additional Information | Zenodo](https://doi.org/10.5281/zenodo.4593675)) [↑](#footnote-ref-1)