Risk assessment for the combinations of a.s. in the formulation

Following the dilution and spraying of the formulated product, much of the formulation constituents are likely to be lost by volatilisation. Therefore, shortly after application of a formulated product, aquatic organisms are mainly exposed to the active substances present in the formulation. In addition, as demonstrated in the short-term studies (see below) there are no indications for interactions of the active substances (no synergisms or additional toxicity occurs due to the co-formulants) given that the formulation does not cause an (unexpected) increased toxicity compared to the active substances.

According to the new EFSA Scientific Opinion (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is used as proposed by EFSA (see Table 10.2.1-4).

To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($LC_{50}/EC_{50\ mix\text{-}CA}$) by the measured mixture toxicity ($LC_{50}/EC_{50\ PPP}$). Ecotoxicity studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined when an increased/decreased mixture toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, *i.e.* if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

The calculated MDR values are between 0.2 and 5 for each organism except fish and daphnia (see Table 10.2-4), indicating that the formulation does not cause an (unexpected) increased toxicity compared to the active substances for these organisms. No synergisms or additional toxicity occurs due to the co-formulants. The apparent antagonism for fish and daphnia (toxicity of the formulation lower than expected) can be explained by the fact that endpoints for individual active substances are "higher than" values.

Table 10.2-4: Summary of results obtained in the studies with the formulated product PPP and comparison of calculated and measured mixture toxicity

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Measured toxicity of PPP (converted to be a.i. based) ($LC_{50\ PPP}$ or $EC_{50\ PPP}$)	Calculated mixture toxicity[a] $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Model deviation ratio (MDR = $EC_{50\ mix\text{-}CA}$ / $EC_{50\ PPP}$)
O. mykiss	LC_{50}, acute, 96 h	1.97	0.236	0.120
D. magna	EC_{50}, acute, 48 h	2.87	0.192	0.0670
P. subcapitata	E_rC_{50}, short-term, 72 h	0.184	0.101	0.550
L. gibba	E_rC_{50}, short-term, 7 d	0.0166	0.0245	1.48
M. spicatum	E_rC_{50}, short-term, 14 d	0.0249	0.0203	0.813

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of AAA (*i.e.* 100 g/L*)* and BBB (*i.e.* 5 g/L) within the formulation and the product density (*i.e.* 0.989 g/cm^3).

It is necessary to check whether the mixture composition in the formulation study giving the measured mixture toxicity ($LC_{50}/EC_{50\ PPP}$) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the $LC_{50}/EC_{50\ PPP}$ with the relative proportion at the PEC_{mix} is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, $LC_{50}/EC_{50\ mix\text{-}CA}$ is calculated for the mixture composition of the a.s. at the PEC_{mix} and is compared with the estimate calculated for the formulation.

The calculated factors fall outside 0.8-1.2 for each organism (see Table 10.2-5), indicating that the mixture composition in the formulation study giving the measured mixture toxicity is not similar to the mixture composition at the PEC_{mix}.

Table 10.2-5: Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PPP)[a] $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Calculated mixture toxicity (a.s. in PEC_{mix})[b] $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Factors ($EC_{50\ mix\text{-}CA}$ (a.s. in PPP)/$EC_{50\ mix\text{-}CA}$ (a.s. in PEC_{mix}))
O. mykiss	LC_{50}, acute, 96 h	0.236	0.349	**0.676**
D. magna	EC_{50}, acute, 48 h	0.192	0.284	**0.676**
P. subcapitata	E_rC_{50}, short-term, 72 h	0.101	0.0233	**4.34**
L. gibba	E_rC_{50}, short-term, 7 d	0.0245	0.00331	**7.42**
M. spicatum	E_rC_{50}, short-term, 14 d	0.0203	0.00403	**5.03**

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of AAA (*i.e.* 100 g/L*)* and BBB (*i.e.* 5 g/L) within the formulation and the product density (*i.e.* 0.989 g/cm^3).

[b] The mixture toxicity of the formulation was re-calculated based on the mixture composition at the PEC_{mix} for AAA (*i.e.* 0.00185 mg/L at Step 3) and BBB (*i.e.* 0.001025 mg/L at Step 3).

With regard to the mixture risk assessment EFSA further states that if the toxicity of the mixture is largely explained by the toxicity of a single active substance, a sufficient protection level might be achieved by simply basing the RA on the toxicity data for that single 'driver'.

Regarding PPP, no active substance is clearly driving the acute risk for and algae and *Myriophyllum spicatum*. The studies performed with the formulated product PPP do not reflect the toxicity of one particular active substance, as the formulation toxicity – endpoint recalculated to each active substance concentrations – does not come for 90 % (of more) from the toxicity per fraction of a single a.s. (TU_i) (see Table 10.2-6). Regarding fish, daphnia and *Lemna gibba*, the risk assessments based on single-substance toxicity data for AAA, AAA and BBB respectively are sufficient given that they were identified as the drivers of the mixture toxicity.

Table 10.2.-6: Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s.

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in PPP) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	**Calculated toxicity per fraction of PPP (based on each a.s.) $(1/TU_i)$[a]**	**Deviation from mixture toxicity (1-$EC_{x\ mix\text{-}CA}$ x $(1/EC_{x\ mix\text{-}CA} - TU_i)$) [%]**
O. mykiss	LC_{50}, acute, 96 h	0.236	AAA: 0.236 BBB: 2100	**AAA: 99.99%** BBB: 0.01%
D. magna	EC_{50}, acute, 48 h	0.192	AAA: 0.192 BBB: 6132	**AAA: 100.00%** BBB: 0.00%
P. subcapitata	E_rC_{50}, short-term, 72 h	0.101	AAA: 0.218 BBB: 0.188	AAA: 46.2% BBB: 53.8%
L. gibba	E_rC_{50}, short-term, 7 d	0.0245	AAA: 2.43 BBB: 0.0248	AAA: 1.0% **BBB: 99.0%**
M. spicatum	E_rC_{50}, short-term, 14 d	0.0203	AAA: 0.0563 BBB: 0.0317	AAA: 36.0% BBB: 64.0%

[a] TU_i is defined as the concentration of the i^{th} a.s. at the $EC_{50\ PPP}$ (re-calculated to the sum of a.s.) divided by the respective single-substance toxicity ($EC_{50\ a.s.}$). This is calculated based on the nominal contents of AAA (*i.e.* 100 g/L) and BBB (*i.e.* 5 g/L) within the formulation and the product density (*i.e.* 0.989 g/cm^3).

For a mixture RA based on calculated mixture toxicity, the ETR is calculated by dividing PEC_{mix} by the calculated mixture toxicity assuming CA ($EC_{50\ mix\text{-}CA}$). As determined here above, the relevant $LC_{50}/EC_{50\ mix\text{-}CA}$ is calculated for the mixture composition of the a.s. at the PEC_{mix}. This assessment has to be carried out for each endpoint and exposure scenario separately. If the standard acceptability criteria based on first-tier data (i.e. standard laboratory data) and worst case PEC_{mix} are met ($ETR_{mix} \leq$ ETR trigger value), the risk from a CA action of the mixture is considered acceptably low. If the relevant trigger value is not met, further available refinement options regarding both exposure and effect assessment might be checked on a case-by-case basis.

The refinement is conducted by taking into account FOCUS PECsw values for AAA (Step 3) and BBB (Step 3 and Step 4) (see Table 10.2-7).

Table 10.2.-7: Mixture risk assessment based on calculated mixture toxicity and PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity* (a.s. in PEC_{mix}) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	**PEC_{mix}**	**$ETR_{mix\text{-}CA}$ ($PEC_{mix}/LC_{50\ PPP}$ or $EC_{50\ PPP}$) (Trigger = 0.1)**

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity* (a.s. in PEC_{mix}) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	**PEC_{mix}**	**$ETR_{mix\text{-}CA}$ ($PEC_{mix}/LC_{50\ PPP}$ or $EC_{50\ PPP}$) (Trigger = 0.1)**
P. subcapitata	E_rC_{50}, short-term, 72 h	0.0233	0.00288[a]	**0.124**
		0.0823	0.00195 (CZ)[b]	0.0237
M. spicatum	E_rC_{50}, short-term, 14 d	0.00403	0.00288[a]	**0.713**
		0.0159	0.00195 (CZ)[b]	**0.123**
		0.0176	0.00194 (winter cereals, 10 m BZ, CZ)[c]	**0.110**
		0.0235	0.00189 (winter cereals, 10 m BZ, BE)[d]	0.0803
		0.0255	0.00188 (winter cereals, 20 m BZ, CZ)[e]	0.0737

* The mixture toxicity of the formulation was re-calculated based on the mixture composition at the corresponding PEC_{mix}.

[a] Sum of the $PEC_{SW\ MAX}$ of the individual active substances contained in the formulation on the basis of **FOCUS Step 3 PEC values (D2 ditch, winter cereals)** for AAA (*i.e.* 0.00185 mg/L) and BBB (*i.e.* 0.001025 mg/L).

[b] Sum of the $PEC_{SW\ MAX}$ of the individual active substances contained in the formulation on the basis of **FOCUS Step 3 PEC values (R3 stream, winter cereals)** for AAA (*i.e.* 0.00182 mg/L) and BBB (i.e. 0.000134 mg/L). Highest values for scenarios other than D1 and D2 (which are considered not relevant for the Central Zone). The R3 stream scenario for autumn use in winter cereals is the only scenario within the GAP of PPP providing an ETR above the trigger at FOCUS Step 3 when excluding the D1 and D2 scenarios.

[c] Sum of the $PEC_{SW\ MAX}$ of the individual active substances contained in the formulation on the basis of **FOCUS Step 3 PEC values (R3 stream, winter cereals)** for AAA (*i.e.* 0.00182 mg/L) and **FOCUS Step 4 PEC values (10 m BZ, R3 stream, winter cereals)** for BBB (i.e. 0.0000115 mg/L). Highest values for scenarios other than D1 and D2 (which are considered not relevant for the Central Zone).

[d] Sum of the $PEC_{SW\ MAX}$ of the individual active substances contained in the formulation on the basis of **FOCUS Step 3 PEC values (R3 stream, winter cereals)** for AAA (*i.e.* 0.00182 mg/L) and **FOCUS Step 4 PEC values (10 m BZ, R1 stream, winter cereals)** for BBB (*i.e.* 0.0000700 mg/L). Highest value in Belgium (for relevant scenarios R1, D3, D4).

[e] Sum of the $PEC_{SW\ MAX}$ of the individual active substances contained in the formulation on the basis of **FOCUS Step 3 PEC values (R3 stream, winter cereals)** for AAA (*i.e.* 0.00182 mg/L) and **FOCUS Step 4 PEC values (20 m BZ, R3 stream, winter cereals)** BBB (i.e. 0.0000600 mg/L). Highest values for scenarios other than D1 and D2 (which are considered not relevant for the Central Zone).

Values in bold exceed the trigger

Unacceptable risk to algae and *Myriophyllum spicatum* is expected from the exposure to the combined active substances following proposed uses of PPP taking into account FOCUS Step 3 PEC_{sw} values for scenarios other than D1 and D2 (situation "a" in Table 10.2-7). This is not considered to be indicative of a real risk for aquatic plants from florasulam in the countries of the Central and Southern European Zones. Indeed the D1 scenario is representative of climatic conditions of Northern Europe (Scandinavian countries) and the D2 scenario represents <1% of the drained cereal growing land in Europe.

Taking into account FOCUS Step 3 PEC_{sw} values for AAA and FOCUS Step 4 PEC_{sw} values resulting from a 20 m buffer zone for BBB (in both cases for scenarios other than D1 and D2) the risk in winter cereals is acceptable for both algae and *Myriophyllum spicatum* exposed to the combined active substances following proposed uses of PPP (situation "e" in Table 10.2-7).

In Belgium, the risk is acceptable considering a 10 m buffer zone taking into account FOCUS Step 3 PEC_{sw} values for both AAA and BBB given that only scenarios R1, D3 and D4 are relevant (situation "d").