

EFSA Guidance on residue definition for dietary risk assessment

First experiences of a Member State



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Overview



- Why do we need this Guidance?
- Our first, second and (draft) final impression
- Case study Triticonazole
- General open questions
- Survival kit

Why do we need this Guidance?

Why do we need this Guidance?



- It allows better quality of dossiers and evaluations
- It allows better predictability for notifiers and Member States
- It saves time and resources of all concerned parties
- It improves confidence of MSs and EFSA in the evaluation of RMS
- It improves communication and exchange between toxicologists and consumer safety assessors
- It means more work but at the end – hopefully less surprises 😊 ...

Our first, second and (draft) final impression
of the Guidance

Our first impression (March 2016, call for comments)



- We recognised the efforts invested (it is easy to complain about the work somebody else has done!)
- We were wondering about:
 - Hazard characterization prior to exposure estimation (single exposure event provoking a genotoxic effect)
 - *Combined exposure* of all metabolites against the TTC of 0.0000025 mg/kg bw/d exposure (unless same specific genotoxic effect known to allow grouping)
 - Consideration of general toxicity for metabolites where combined exposure does not even exceed genotoxicity TTC

Our first impression (March 2016, call for comments)



- General limit of 10% identified solely in urine (no other tissues or plasma) to cover general toxicity of metabolite
- Introduction of relative potency factor, triggering further testing - and this might happen late in the evaluation process
- Lack of clear guidance to structural similarity and grouping in order to define a representative group molecule and avoid unnecessary animal testing
- Sequence of data requirements for general toxicity (increased need for animal testing)
- Again, no harmonisation between GD on metabolites in groundwater and GD for residue definition ☹

Our first impression (March 2016, call for comments)



- Toxicological burden and 75% coverage insufficiently explained
- Practical applicability of conversion factors from metabolism studies (within one crop group) not representing actual GAPs (e.g. metabolism study on apples – representative for tomatoes as well, but different application regimes)
- Practical applicability of conversion factors for crops not covered by metabolism studies (e.g. three crop groups covered) and implication for further crop authorisations (e.g. an additional crop group)

Our second impression (May 2016, TC with MS and EFSA)



- We recognised that:
 - Clarification of genotoxicity prior to exposure estimation is the core element of the Guidance (Module 1)
 - It was not the intention of EFSA to enhance animal testing – re-wording and re-structuring of Step 17 (Module 2)
 - EFSA was ready to further explain unclear issues (e.g. toxicological burden) (Step 18)

Our (draft) final impression (September 2016, publication)



Many issues more clearly presented in the final version...and the list of possible targeted toxicity studies deleted 😊😊😊

- We will get used...
- We have to gain or extend our knowledge (e.g. QSAR, grouping of chemicals)...
- We have to communicate early in the process...
- We have to exercise and further develop the Guidance...
- Still open points included for discussion at the end of the talk

Case study Triticonazole

Case study Triticonazole



- AIR3 Substance
- Seed treatment of wheat as representative use, and beside this, seed treatment on cereals and limited other crops currently authorised (ornamentals) according to our knowledge
- Manageable number of metabolites (no groundwater metabolites)
- Notifier (BASF) very proactive and ready to address the issue appropriately
- AGES and notifier worked in parallel... using different approaches:
 - RMS: comparison of cumulative exposure toward genotoxic and general toxicity TTC
 - Notifier: grouping of metabolites and use of QSAR

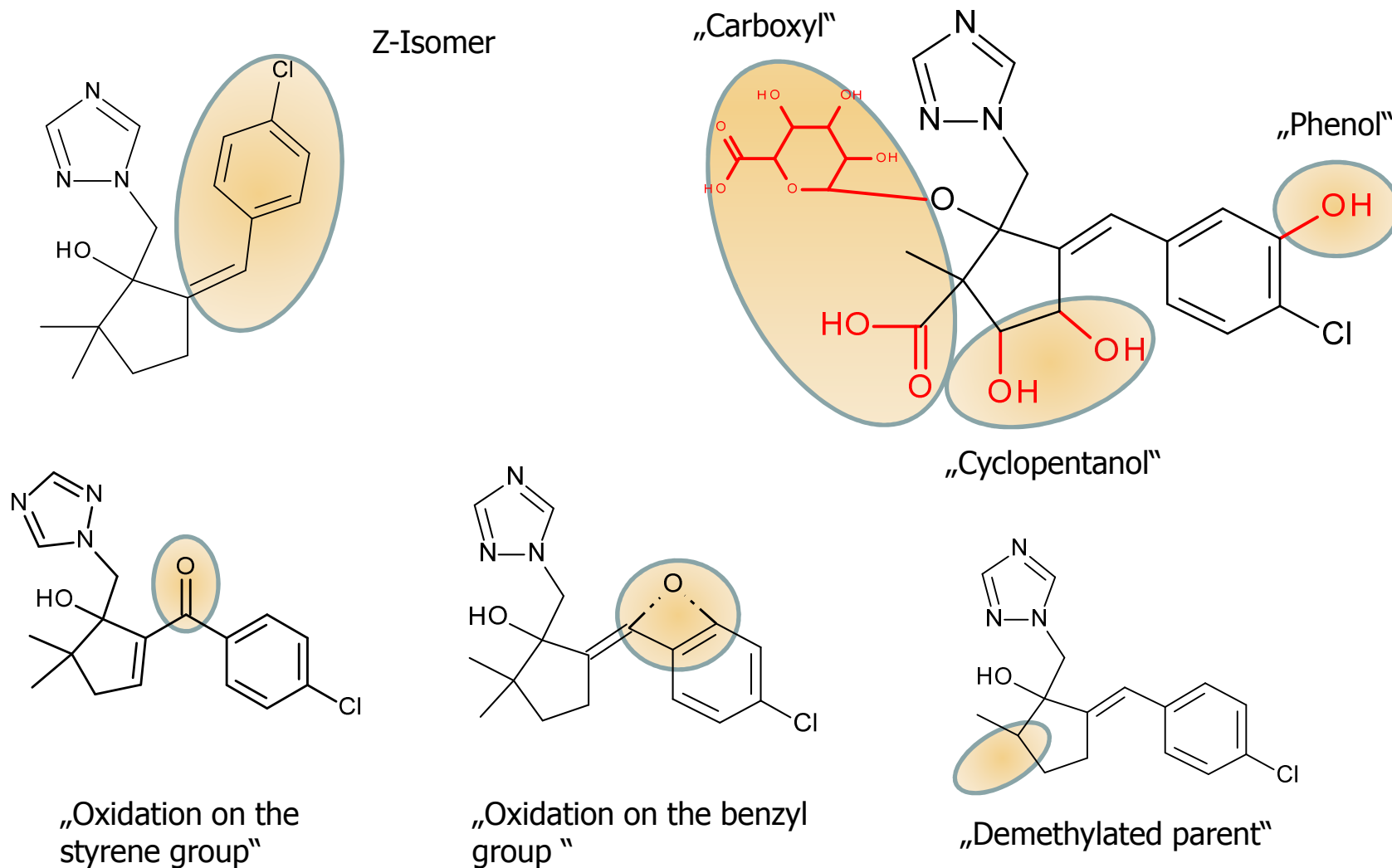
Case study Triticonazole – Notifier's approach



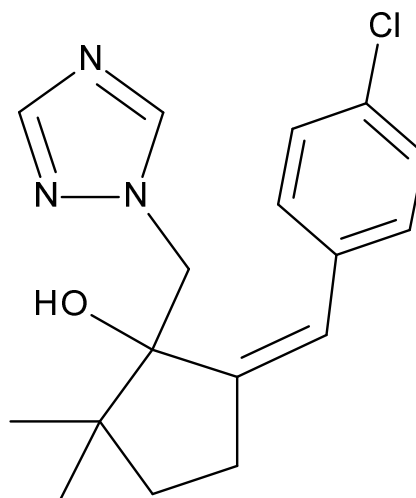
Group No	Name	Metabolites
1	Isomer of parent	M595F014
2	Oxidation and conjugation of/on existing hydroxyl groups	M595F005, M595F006, M595F010
3	Hydroxylation on the chlorobenzene ring	M595F013
4	Hydroxylation on the cyclopentan ring	M595F001, M595F002, M595F007
5 a	Oxidation on the styrene group 1	M595F004-1*
5 b	Oxidation of the styrene group 2	M595F004-2*
6	Oxidation on the benzyl group	M595F015
7	Demethylation	RegNo. 4710773

*The proposed structures for this residues are uncertain and contain different structural elements, which prevents them from grouping. However the amount of the residue was so low, that no more elaborated structure elucidation could be performed in the study

Case study Triticonazole – Notifier's approach



Case study Triticonazole – Notifier's approach – Z-Isomer



Z-isomer

- Cereals (straw)

Case study Triticonazole – Notifier's approach – Z-isomer



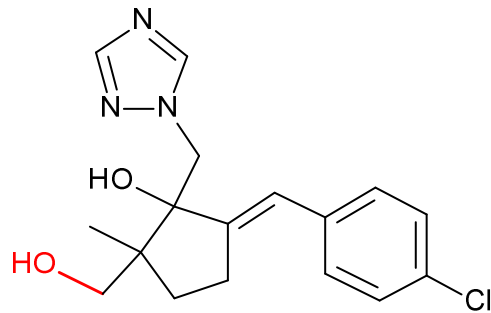
- Current conclusion notifier:
 1. Acute oral LD50 value > 2000 mg/kg bw , negative AMES and in vitro MN assay
 2. Not identified in rat metabolism

1+2 = Cramer Class III threshold of 0.0015 mg/kg bw/d applicable

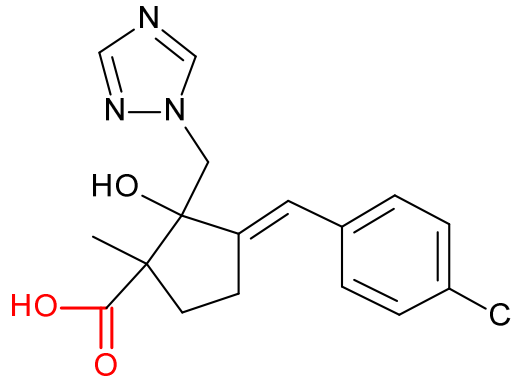
- Current conclusion RMS:
 1. Genotoxicity sufficiently addressed by studies
 2. Not identified in rat metabolism

1+2 = Cramer Class III threshold of 0.0015/0.005 mg/kg bw/d applicable

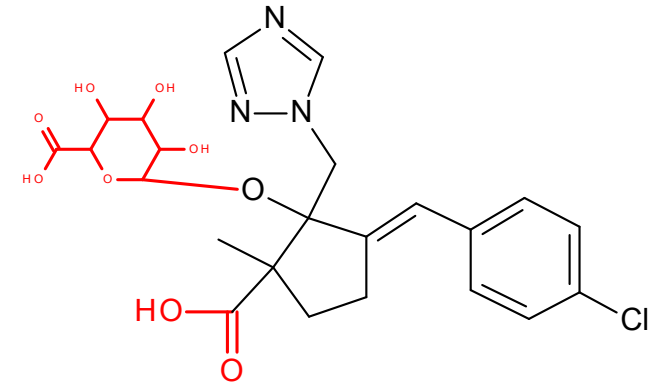
Case study Triticonazole – Notifier's approach – “Carboxyl” group



M595F005



M595F006



M595F010

- Goat (liver, kidney, muscle), rotational crop (wheat straw), cereals (grain, straw)

Case study Triticonazole – Notifier's approach – “Carboxyl” group



- Current conclusion notifier:
 1. None of the three presented a relevant QSAR alert (“specific exercise” including genotoxicity data of triticonazole and metabolites/impurities, showing “in domain” for M595F005 and no structural alert in AMES)
 2. M595F006 and M595F005 are major metabolites in rat

1+2 = Group is covered by rat metabolism, ADI of parent if necessary

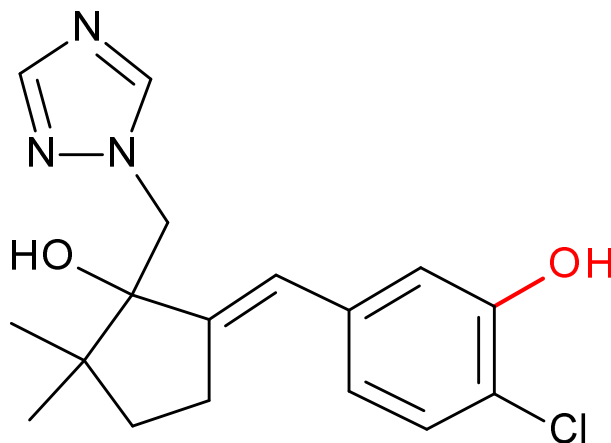
Case study Triticonazole – Notifier's approach – "Carboxyl" group



- Current conclusion RMS:
 1. Times AMES and Times CA: negative but "out of domain"
 2. Vega (Caesar, SarPy, ISS, KNN): negative, but low to moderate reliability
 3. "Specific exercise" not yet included in the provided documents
 4. M595F005: only in rat faeces (15% in males and 24% in females after repeated low dose)
 5. M595F006 in rat urine 11% in females and 2% in males; 22% in females faeces, 34% in males faeces
 6. Rat metabolism bile study = high (total) excretion via bile (88-95% in 48 hours), metabolites not identified
 7. Goat metabolism study:
 - a) M595F006 measured at 23% in liver, 57% TRR in kidney and 15% TRR in muscle. It makes 12% of TRR in bile
 - b) M595F010 measured at 20% of TRR in liver and 70% of TRR in bile

1+2+3+4+5+6+7= M595F005 and M595F006 are present to a sufficient amount in rat metabolism (>10% trigger) to contribute to observed toxicity. M595F010 is glucuronide of M595F006. All three metabolites are covered by toxicity studies with parent. No additional information on genotoxicity necessary. Reference values of parent can be applied

Case study Triticonazole – Notifier's approach – "Phenol" group



M595F013

- Cereals (straw, hay)

Case study Triticonazole – Notifier's approach – “Phenol” group



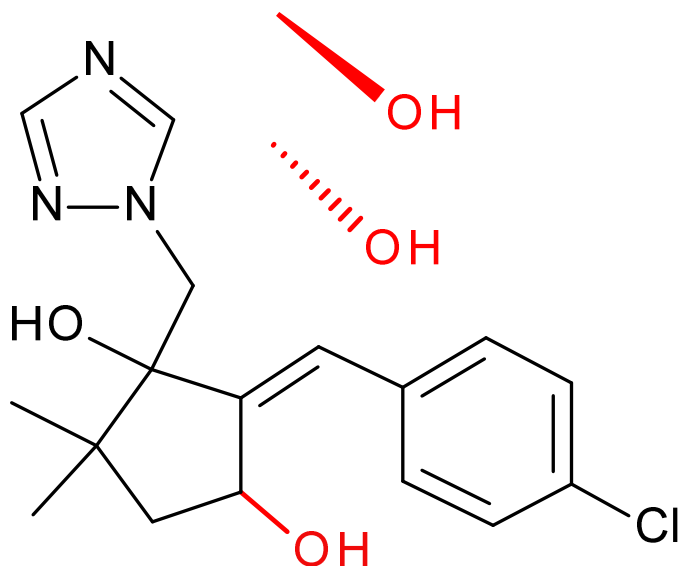
- Current conclusion notifier:
 1. No relevant QSAR alert, but “out of domain”
 2. Not found in rat metabolism but hydroxylation of the phenyl-ring as a metabolic step occurs in metabolic pathway of triticonazole

1+2 = Genotoxicity cannot be excluded, TTC of 0.0000025 mg/kg bw/d to be applied

- Current conclusion RMS:
 1. Times AMES negative, Times CA positive; both “out of domain”
 2. Vega (Caesar, SarPy, ISS, KNN): negative, but low to moderate reliability

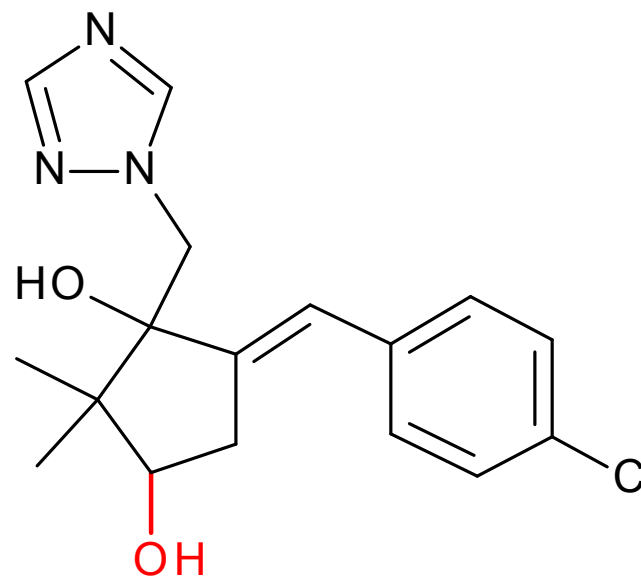
1+2 = additional QSAR information is necessary; otherwise TTC of 0.0000025 mg/kg bw/d to be applied

Case study Triticonazole – Notifier's approach – "Cyclopentanol" group



M595F001

M595F002



M595F007

- Cereals (forage, grain, hay and straw), rotational crop (wheat straw)

Case study Triticonazole – Notifier's approach – "Cyclopentanol" group



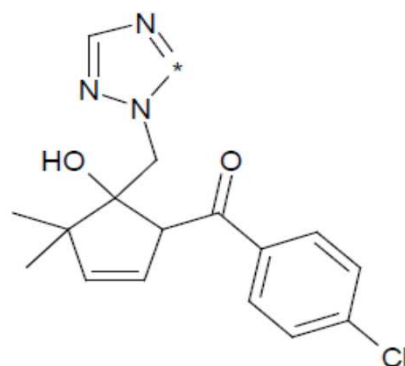
- Current conclusion notifier:
 1. No relevant QSAR alert for M595F007 ("specific exercise" including genotoxicity data of triticonazole and metabolites/impurities, showing "in domain" and no structural alert in AMES)
 2. Acute oral LD50 value > 2000 mg/kg bw and negative AMES available for trans-diol M595F002
 3. All three metabolites have similar LogP values presenting single toxicological group

1+2+3 = Cramer Class III threshold of 0.0015 mg/kg bw/d applicable

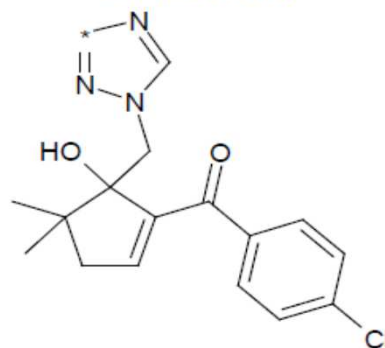
- Current conclusion RMS:
 1. Times AMES and Times CA: negative but "out of domain"
 2. Vega (Caesar, SarPy, ISS, KNN): negative, but low to moderate reliability
 3. "Specific exercise" not yet included in the provided documents
 4. AMES assay not sufficient to cover genotoxicity

1+2+3+4 = either additional information on QSAR necessary or *in vitro* MN assay;
Otherwise TTC of 0.0000025 mg/kg bw/d to be applied

Case study Triticonazole – Notifier's approach – "Oxidation on the styrene group"



Or isomer



M595F004-1 and -2 (tentative structures)

- Cereals (straw, hay)

Case study Triticonazole – Notifier's approach – "Oxidation on the styrene" group



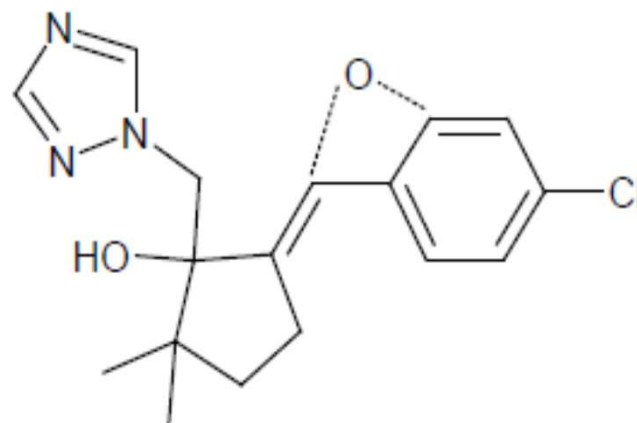
- Current conclusion notifier:
 1. No relevant QSAR genotoxicity alert, but "out of domain"
 2. While M595F004-1 has a structural alert for α,β – unsaturated carbonyl group, M595F004-2 does not have this alert
 3. Two structures cannot be grouped together

1+2+3 = Genotoxicity cannot be excluded, TTC of 0.0000025 mg/kg bw/d to be applied

- Current conclusion RMS:
 1. No detailed information on QSAR models or platforms yet included in the provided documents

1 = either additional information on QSAR necessary or genotoxicity studies; otherwise TTC of 0.0000025 mg/kg bw/d to be applied

Case study Triticonazole – Notifier's approach – "Oxidation on the benzyl group"



M595F015 (tentative structure)

- Cereals (straw)

Case study Triticonazole – Notifier's approach – "Oxidation on the benzyl group"



- Current conclusion notifier:

1. No relevant QSAR alert ("specific exercise" including genotoxicity data of triticonazole and metabolites/impurities, showing "in domain" and no structural alert in AMES)

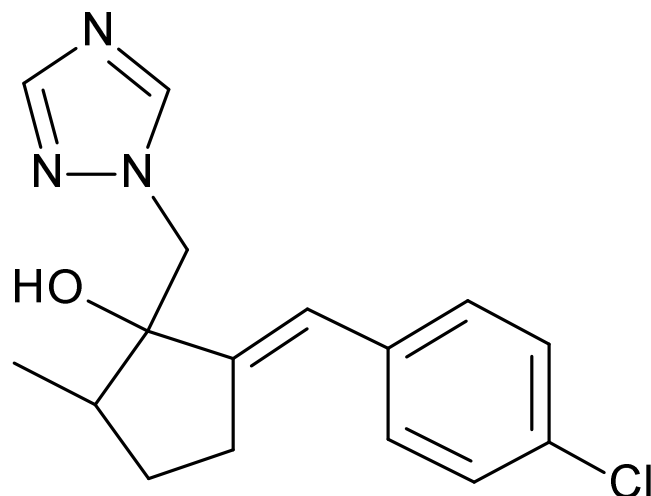
1 = TTC of 0.0000025 mg/kg bw/d to be applied

- Current conclusion RMS:

1. No detailed information on QSAR models or platforms yet included in the provided documents

1 = either additional information on QSAR necessary or genotoxicity studies; otherwise TTC of 0.0000025 mg/kg bw/d to be applied

Case study Triticonazole – Notifier's approach – "Demethylation of the parent"



RegNo 47010773

- Cereals (straw, hay) – artefact metabolite?

Case study Triticonazole – Notifier's approach – “Demethylation of the parent”



- Current conclusion notifier:
 1. No relevant QSAR alert
 2. Negative AMES assay

1 + 2 = Cramer Class III threshold of 0.0015 mg/kg bw/d applicable

- Current conclusion RMS:
 1. Times AMES negative and Times CA positive, but both “out of domain”
 2. Vega (Caesar, SarPy, ISS, KNN): negative, but low to moderate reliability
 3. Negative AMES assay

1 + 2 + 3 = either additional information on QSAR necessary or *in vitro* MN assay;
Otherwise TTC of 0.0000025 mg/kg bw/d to be applied

Case study Triticonazole – RMS approach



Adobe Acrobat
Document

Case study Triticonazole – Conclusion RMS



- Without metabolites M595F005, M595F006 and M595F010 (which are considered covered by the parent) no exceedance of any (genotoxicity, acute or chronic) combined TTC
- Additionally, grouping as done by the notifier would be acceptable
- If QSAR analysis or further genotoxicity assessment still followed as supporting information (not considered necessary by AGES):
 - QSAR should be extended for models where the molecules are within the domain (not necessary if combined genotoxicity TTC not exceeded)
 - AMES test might be sufficient for an impurity below 1% in technical material but is not sufficient for exclusion of genotoxicity of metabolites

Case study Triticonazole – Conclusion RMS



- It is acceptable that a metabolite's toxicity can be considered sufficiently covered by studies with parent even if 10% of administered dose is not achieved for metabolite in urine of one sex in the RLD group

BUT

high amount of the metabolite is identified in faeces and high (total) excretion via bile is measured. And metabolite *identified* to high amount in livestock tissue and bile

- No mechanistic or animal studies necessary in this case

Case study Triticonazole – Conclusion RMS



- 1,2,4 triazole and triazole-alanin have their own reference values (outcome of the peer review expected soon) and separate risk assessment
- None of the non-TDM metabolites to be included in the residue definition since stopped at Step 11 (true?)
- What if some metabolites (e.g. animal commodities), although passing TTC, are major according to the definition ($\text{TRR} \geq 10\%$)?
- If metabolites are candidates for RD, which reference values if no specific data (data not necessary since they passed TTC)?
 - ➡ Reference values from parent? Or acute and chronic TTC values?
- Hypothetical case: if reference values of parent are lower than TTC values? (TTC precedes estimation of potency)

Case study Triticonazole – Proposal for residue definition



If we stop at Step 11 (combined acute and chronic TTCs not exceeded):

BASF:

Residue definition in plant matrices for risk assessment	Triticonazole
Residue definition in animal matrices for risk assessment:	Triticonazole

AGES:

Residue definition in plant matrices for risk assessment	Triticonazole
Residue definition in animal matrices for risk assessment:	Triticonazole

Case study Triticonazole – Proposal for residue definition



If no exit at Step 11 (although chronic and acute TTC not exceeded but major metabolites to be considered):

Plant metabolites in food or processing:

- Parent only (no major metabolites)

Plant metabolites in feed:

- Dietary burden calculation for the sum of parent and major metabolites did not exceed the trigger of 0.004 mg/kg bw/d (Step 15)

Conclusion = residue definition for food and feed (cereals) = Triticonazole

Case study Triticonazole – Proposal for residue definition



If no exit at Step 11...

Major metabolites in animal commodities (Steps 16 – 18):

M595F006 (15% TRR in goat muscle, 57% in goat kidney and 23 % in goat liver) and **M595F010** (20% in goat liver)

Calculation of the toxicological burden:

Goat meat:

Triticonazole (1x3x1) + M595F006 (1x15x1) = 18 = 100%

Triticonazole = 16.7%

M595F006 = 83.3%

Goat kidney:

Triticonazole (1x1x1) + M595F006 (1x57x1) = 58 = 100%

Triticonazole = 1.7%

M595F006 = 98.3%

Goat liver:

Triticonazole (1x15x1) + M595F006 (1x23x1) + M595F010 (1x20x1) = 58 = 100%

Triticonazole = 25.8%

M595F006 = 39.7%

M595F010 = 34.5%

Case study Triticonazole – Proposal for residue definition



If no exit at Step 11...

AGES proposal for residue definition:

Residue definition in plant matrices for risk assessment	Triticonazole
Residue definition for goat (meat, kidney, liver) for risk assessment:	Sum of triticonazole, M595F006 and M595F010, expressed as triticonazole

General open questions

Guidance document – Open questions



- Practical applicability and acceptance of structural similarities, simple structural changes and grouping – how to select a “lead group metabolite”?
- Need for a database for common metabolites with an unique identifier?
- Simultaneous exposure to common metabolites works in theory only – how to address?
- Practical applicability/reliability of relative potency factors with tiny data set for metabolites (Step 18, Table 1)

Guidance document – Open questions



- For metabolites ending at step 11 (“dead-end-street”, “no concern” after acute and chronic TTC calculation): are they, in spite of missing link to Module 3 (Residue definition), considered for RD if e.g. they are major metabolites in animal commodities?
- How to adapt > 4N studies, if only these available (e.g. seed treatment)?
- Practical applicability of conversion factors: a) within one crop group with different GAPs and b) for crop not covered by available metabolism studies (questions from slide 9)?

Guidance document – Open questions



- Do we need special consideration for cases where metabolites pass acute/chronic TTC but where reference values of parent are lower than TTC values?
- More details on decision which metabolites to be included into 75% toxicological burden (e.g. Step 18, Table 1: M01 has been included, M04 not – both having relative toxicological burden of 14%)

Survival kit

Survival kit



- Permanent dialogue between RMS and the notifier is a must, EFSA can be also consulted
- Improved communication and exchange between toxicologists and consumer safety assessors
- Evaluation of the dossier begins in the pre-submission meeting(s) – insist on them
- Communication should not end with the dossier submission
- Science-based and justified requirements by RMS, even at the very last stage, should be followed