Criteria for toxicological characterization of metabolites and testing strategy

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Andrea Terron EFSA, Pesticide Unit

OUTLINE

- Identification of metabolites characterised by the toxicological studies
- Testing strategy for general toxicology endpoints
  - Applicability of TTC for the general toxicity
  - Grouping and read across for general toxicity
  - Testing strategy
- RPF and toxicological burden

IDENTIFICATION OF METABOLITES CHARACTERISED BY TOX. STUDIES

- Default assumption for toxicological characterization
  - Contributing ≥10% (as individual metabolite) of the administered dose in terms of total radioactive material recovered in the urine as detected in the ADME study
    - 10% is intended as the total amount excreted over the collection time.

IDENTIFICATION OF METABOLITES CHARACTERISED BY TOX. STUDIES

- The ADME study design is relevant:
  - Conducted in rat by repeated administration at doses similar to the one applied in the general toxicity studies.
  - Quantification of the metabolite based on
    - Lowest administered dose.
    - Animal sex showing the lowest excreted amount.
  - Different study design is a possibility
    - Justified by considering the nature of the hazard and the toxicological profile of the parent.

IDENTIFICATION OF METABOLITES CHARACTERISED BY TOX. STUDIES

- Deviation from default
  - Mixture of isomers
    - 10% is the sum of the individual isomers; this will apply only to the genotoxicity assessment.
  - Glycoside and glucoronide conjugates of a given metabolites will be summed up with the unconjugated form and compared to the 10%.
  - Metabolites only occurring at high doses in the ADME study can be considered toxicologically characterized if the same dose was used in the toxicology studies from which the parent was characterised.

IDENTIFICATION OF METABOLITES CHARACTERISED BY TOX. STUDIES

- Deviation from default
  - Concentration of metabolite in plasma
    - 10% threshold can be applied.
  - Concentration of metabolite in the bile
    - 10% threshold can be used if systemic exposure is proven i.e. bioavailability study.
  - Limited absorbed active substances (≤80%)
    - 10% should refer to the absorbed dose.
    - Lack of data on metabolite absorption is representing an uncertainty (increased or decreased conservatism).

Some considerations on conjugated metabolites

- The amount of Phase-I metabolites (Aglycones) and Phase-II metabolites (conjugated-Aglycones) may differ depending on the stage of plant growth.
- The same Phase-I metabolite could be present in more than one conjugated form.
- To accurately estimate a total individual Phase-I metabolites:
  - Conjugated metabolites should first systematically be tested-hydrolyzed with different selective enzymes to verify their conjugated nature. Following the acquired knowledge from that test, they should collectively be hydrolyzed at various degrees of HCl until conjugated residues are released and converted to their Phase-I metabolic aglycones.
  - As humans have all kind of sugar hydrolysing enzymes and glucuronidase, plus gastric acid, hydrolysis to the metabolite (Aglycone) can be reasonably assumed even if this re-conversion might not be 100%.
- Less information and experience available on e.g. amino acid or natural acid conjugates

Some considerations on limited absorbed active substances

- Use of the absorbed dose for quantification of metabolites is recognized as uncertainty in the absence of absorption data for the metabolite/s.
- Different approaches have been considered during the calculation of the toxicological burden in order to avoid any potential underestimation of highly absorbable metabolite.
- The approaches tested were making complicated an already complex process.
- The general approach proposed in the guidance is to evaluate the weight of metabolites contributing to the toxicological burden, rather than assessing the specific risk.
- A case by case solution (scientifically justifiable and suitable for risk assessment), by making specific consideration during the risk assessment process after residue definition, was considered a suitable approach and place for correction for absorption.

General principles

- Toxicological assessment of metabolites is primarily understood to enable a *quantitative and qualitative comparison* of the toxicity profile of the metabolite(s) with the parent substance
- The assessment scheme is proposing
  - Use of the TTC approach
  - Elements of grouping and read-across
  - Testing

TTC ASSESSMENT FOR GENERAL TOXICOLOGY ENDPOINTS

- Exposure of all non characterized metabolites can be summed up and compared to the specific TTC values
  - Optional step.
  - Exposure should be reliably estimated.
  - Uncertainties linked to the exposure should be minimized.
- Exposure M1/Cramer Class TTC for M1+ Exposure M2/Cramer Class TTC for M2+ Exposure M3/Cramer Class TTC for M3 +……=
  - **≥ 1 Concern**; specific hazard and/or comparative risk assessment will be conducted.
  - **<1 No concern**; no further assessment is necessary.

General principles

- Grouping can be used for the selection of representative substance/s to be tested and read-across.
- Grouping criteria and/or selection of representative substance/s should be substantiated
  - Criteria for similarity e.g. structural similarities.
  - Compile toxicity data for analogous chemicals.
  - Support the proposed toxicity mechanism by comparative mechanistic data.
  - Identification of the critical effect.

General principles

- Enhanced 28-day (OECD TG 407) or a 90-day (OECD TG 409) would be appropriate in order to enable a comparative assessment to the parent or to derive reference values for the metabolite.
- Alternative approaches as an option
  - **Mechanistic understanding of the toxicity observed with the parent (or group lead compound).**
  - **Comparative mechanistic studies.**
  - **Convincing toxicological assessment using all available data.**
  - **Case by case e.g. scientifically justifiable and suitable for risk assessment**

Sub- acute vs. sub chronic

- The choice of the study should be based on:
  - Toxicological profile of the parent.
  - Design of the study from which the reference dose of the parent was derived.
  - Comparative (to the parent) dose selection.
  - Comparative protocol i.e. species, strain, number of animals, general experimental conditions.
- Set of specific reference value(s) for metabolites qualitatively different from parent or group lead compound
  - Apply additional UF for treatment duration
    - **Sub-acute to sub-chronic (3)**
    - **Sub-chronic to chronic (2)**

Relative Potency Factor (RPF)

- Will be used to express the different potencies of all members of the group relative to that of an index compound e.g. between the metabolite(s) and the group lead compound.
- Ratio between the NOAEL/LOAEL/BMD of the parent (or group lead compound) and the NOAE/LOAEL/BMD of the metabolite(s).
- RPF for the parent or group lead compound will be equal to one.

Carcinogenicity-associated hazards

- For parent compounds considered carcinogens
  - Default assumption is that the same hazard would be applied to the metabolite.
  - It is assumed that the application of the RPF and additional UF for the study duration will cover the risk.

Parent with no DART precedents

- Metabolite is qualitatively similar to the parent
  - No hazard for DART endpoints is expected.
- Metabolite is qualitatively different from the parent
  - Apply an additional UF of 10 when establishing reference dose of the metabolite.
  - Test for DART endpoints.

Parent with DART precedents

- Metabolite is qualitatively similar to the parent
  - Same hazard for the metabolite is assumed.
  - Test for DART endpoints.
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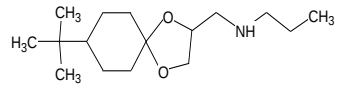
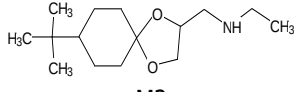
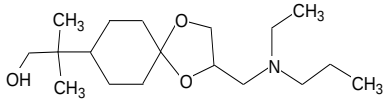
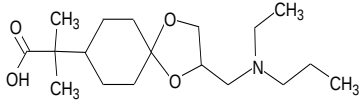
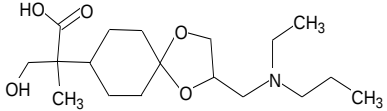
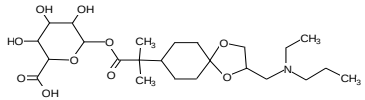
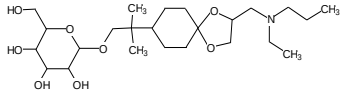
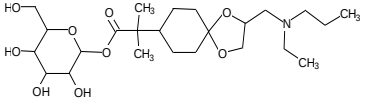
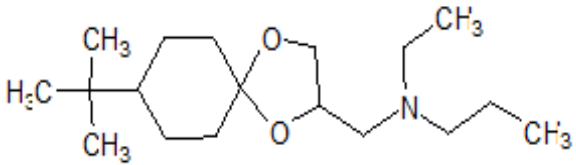
CASE STUDY SPIROXAMINE; TOXICOLOGICAL ASSESSMENT

Group A (parent similar metabolites):M01, M02, M05, M06, M07, M19, M40, M44

 M1	 M2	 M5
 M6	 M7	 M19
 M40	 M44	

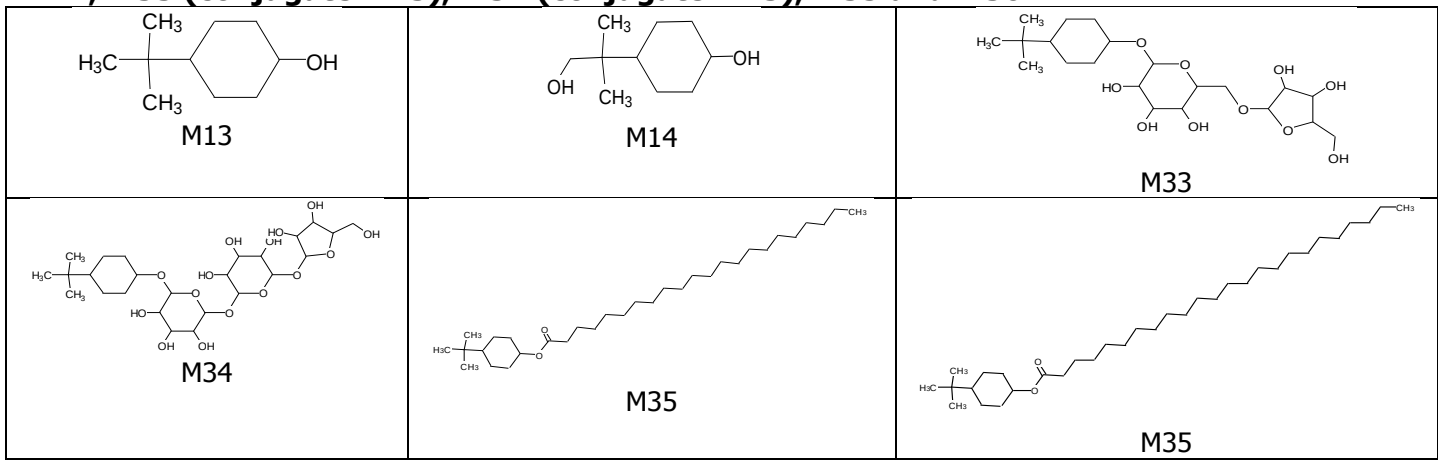


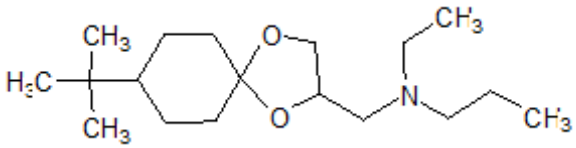
spiroxamine

CASE STUDY SPIROXAMINE; TOXICOLOGICAL ASSESSMENT

Group B

M14, M33 (conjugate M13), M34 (conjugate M13), M35 and M36





spiroxamine

CASE STUDY SPIROXAMINE TOXICOLOGICAL ASSESSMENT

- M13 is group lead compound for M33, M34 and M14.
- M33 and M34 are sugar conjugates, likely resulting in M13 after hydrolysis
- Parent ADI based on a dog study
  - A 28-day and a 90-day study in rat is available
  - A 28-day study in rat is considered suitable for characterization of M13
- Spiroxamine is proposed for classification (cat.2) for developmental toxicity.
  - Tiered approach should be considered for the metabolites
  - First characterize the hazard for potential waiving of testing for developmental toxicity

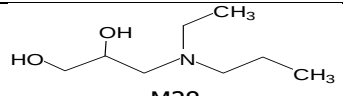
CASE STUDY SPIROXAMINE TOXICOLOGICAL ASSESSMENT

- M14 is very similar to M13
- M35 and M36 are esters of M13
  - Hydrolysis should be demonstrated
  - If hydrolysis occurs, the two resulting alcohols can be grouped based on chemical similarity of the moiety and represented by group lead M13
  - The resulting acids need to be assessed separately as well as the two esters if hydrolysis cannot be demonstrated

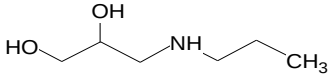
CASE STUDY SPIROXAMINE TOXICOLOGICAL ASSESSMENT

Group C - aminodiol

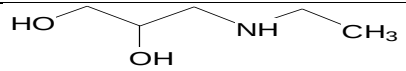
M28, M30, M31



M28



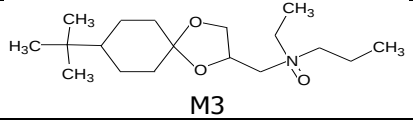
M30



M31

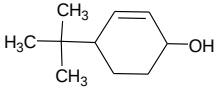
Group D – oxide

M03

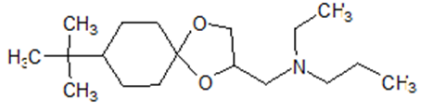


M3

Group E – M37



M37



spiroxamine

TOXICOLOGICAL BURDEN

- Candidate metabolites for inclusion into RD have to be screened for their individual impact.
- The relative contribution of a candidate metabolite to the tox. burden is considered as a suitable measure to assess their relevance.
- The testing strategy should allow for the application of the RPF or specific RV.

TOXICOLOGICAL BURDEN

- The toxicological burden is the sum of the those candidate metabolites not excluded before.
- They are weighted by their RPF within the group and by the ratios of RVs (RRV) between the groups.
- After normalising to 100%, the contribution of each metabolite can be calculated.

Use of the RPF for the assessment of the toxicological burden considering two groups of substances with a different toxicological profile. Groups are assumed as based on chemical structure similarity and to be qualitatively similar in their toxicological profile and that metabolites were tested for their potency

Group A: Metabolites qualitatively similar to the parent Reference value for parent (target liver) = 0.1 mg/kg bw per day		**Group B: Metabolites qualitatively different to the parent** Reference value for group B lead metabolite M03 (target kidney) = 0.2 mg/kg bw per day	
Parent RPF_A=1 M01 RPF_A=0.5 M02 RPF_A=1.5	Parent: 10% TRR M01: 15% TRR M02: 10% TRR Total: 35% TRR	M03 RPF_B=1 M04 RPF_B=1 M05 RPF_B=1	M03: 18% TRR M04: 15% TRR M05: 12% TRR Total: 51% TRR
Ratio of reference values (RRV)			
RRV group A/group A = 1		RRV group A/group B = 0.5	
Toxicological burden = (ΣRPFi x %TRRi x RRVi)			
Parent + M01 + M02 + M03 + M04 + M05 (1x10x1) + (0.5x15x1) + (1.5x10x1) + (1x18x0.5) + (1x15x0.5) + (1x12x0.5) = 55 set to 100%			
Relative toxicological burden of potentially relevant compounds (%):			
Parent: 18 % M01: 14 % M02: 27 % M03: 16 % M04: 14 % M05: 11 %			
Residue definition			
Parent + M01 + M02 + M03 representing 75% of the toxicological burden			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FOR YOUR ATTENTION
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