

ECPA position on the Consumer Risk Assessment of Dietary Metabolites

Introduction

In 2012 EFSA published a [Scientific Opinion](#) which serves as a basis for their future guidance document on the establishment of the residue definition to be used for dietary risk assessment.

ECPA supports the development of guidance on the establishment of a procedure to evaluate the potential risk of dietary metabolites to human health and would therefore like to provide a position paper on the consumer risk assessment of metabolites observed in commodities of plant and animal origin.

Summary of Proposal

ECPA presents in this position paper a proposed decision tree for the consumer risk assessment of metabolites observed in commodities of plant and animal origin; case study examples have been provided to demonstrate some of the approaches described in the decision tree.

Metabolites in primary, processed and rotated crops and in livestock are considered herein. In cases where these dietary metabolites are formed in test animals used in the toxicity tests they can be considered covered by the toxicity assessment of the parent substance. This is based on the level of the metabolite found in animals, their physical-chemical characteristics, the toxicological properties of the parent and estimated exposure. When these dietary metabolites are not determined in test animals, a dietary risk assessment can be conducted using non-testing approaches, such as the Threshold of Toxicological Concern (TTC) as proposed in the Scientific Opinion, or using tailored testing strategies.

The Thresholds of Toxicological Concern (TTCs) are considered to be valuable toxicity reference doses when deciding on the extent of data that should be generated for metabolites in plants and livestock. However use of the TTCs should not be mandatory if alternative toxicity reference doses can be proposed. TTCs are robust and conservative values which can reliably be used provided a reasonable level of certainty can be achieved regarding estimated exposure.

With respect to the estimate of exposure to dietary metabolites this is based on data from supervised field trials and/or the appropriate metabolism studies where the data is used to provide suitable metabolite to parent (or marker substance) ratios. These ratios are then applied to the available residue data to provide estimates of metabolite exposure for both chronic and acute exposure.

With respect to the toxicological assessment of dietary metabolites, the extent of scientific knowledge on modes of action and development of extensive databases on chemicals should help in minimizing the use of laboratory animals in toxicological testing. A strong weight of evidence analysis of all data available not only on the parent but also on any structurally-related compounds is recommended before embarking on animal testing. The use of predictive models based on comparative analyses of hazard data from structurally-related compounds can be helpful although it should be recognized that some tools need to be improved to better predict specific endpoints (e.g. reproductive toxicity). Other tools include in vitro methods employing

biochemical tests or other technologies which may complement or replace conventional animal testing, provided the robustness and reliability of these assays are demonstrated.

An assessment of a metabolite using the TTC concept may not necessarily lead to an inclusion of this metabolite into the residue definition for dietary risk assessment; it may not need to be included if the risk relative to parent is low. It is the understanding of ECPA that the EFSA Guidance on the establishment of the residue definition to be used for dietary risk assessment will be complementary to and aligned with the [OECD Guidance on Definition of Residue](#).

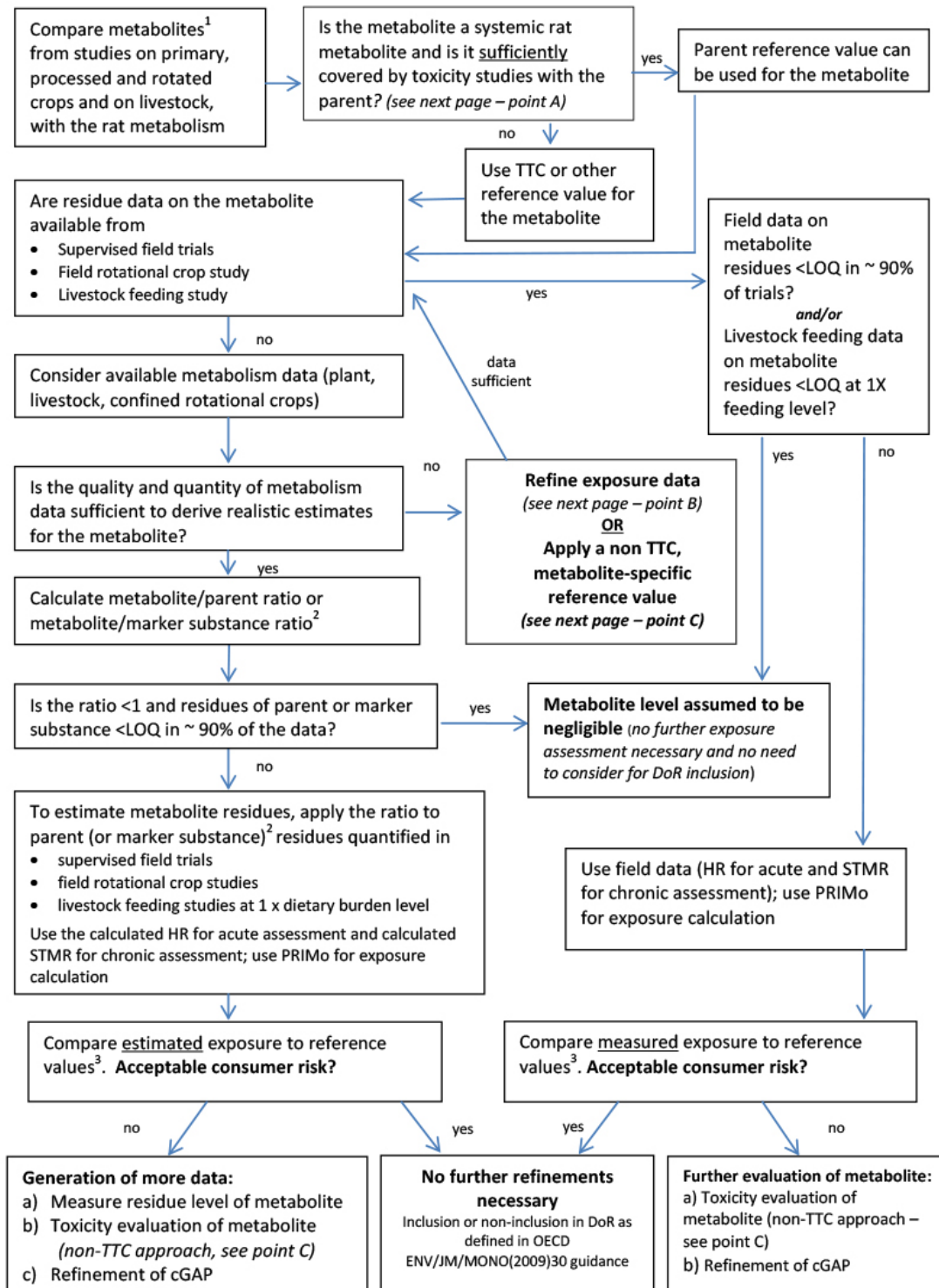
Proposed Decision tree

The proposed decision tree given below is supported by guidance notes (Annexes I & II).

Definitions used throughout the document are provided in Annex III.

References are provided in Annex IV of this document.

Example case studies to support the decision tree are provided in Annex V.



- 1 To determine which metabolites need to be considered see OECD Guidance Metabolism in Crop 501; Metabolism in Rotational Crops 502; Metabolism in Livestock 503; Nature of the Pesticide Residues in Processed Commodities 507
- 2 A marker substance can be any analyte measured in the field trials or livestock, not necessarily the one included in the residue definition for monitoring. If no metabolite/parent or metabolite/marker substance ratio is feasible adjust metabolite level from the metabolism study to field use rate, or 1x feeding level
- 3 Reference values are the TTC thresholds (for genotoxicity, neurotoxicity, Cramer Classes) if applicable, parent ADI/ARFD, metabolite ADI/ARFD, other values derived from literature or read-across

Point A:

Is the metabolite a systemic 'rat' metabolite and is it sufficiently covered by toxicity studies with the parent substance?

- A systemic 'rat' metabolite (or other test species, e.g. mouse, rabbit, dog) is any metabolite found in the body and excreted via urine and/or bile
- If such a metabolite appears at $\geq 10\%$ of the orally dosed parent substance in urine/bile, blood, plasma and organs/tissues, then in principle, its toxicity profile is covered by that of the parent
- If such a metabolite is present at $< 10\%$ of the oral dose, it may also be considered sufficiently covered if the dietary exposure is very low, the toxicological studies were dosed at high levels, and the toxicological profile of the parent substance is considered well characterized
- All conjugates and successor metabolites, found in urine/bile, blood, plasma and in organs/tissues of *rat* should be summed up to identify the total amount of the metabolite

Point B:

Refine exposure data

Plant

- quantify metabolite level in plant commodities originating from residue trials
- re-conduct plant metabolism studies if the original studies are not adequate

Livestock

- conduct livestock feeding studies
- re-conduct livestock metabolism studies if the original studies are not adequate

Point C:

Consumer risk assessment based on non-TTC approach

A non-TTC approach (metabolite-specific reference value) may be used in an alternative risk assessment when uncertain exposure estimates provide an insufficient margin of safety between the exposure estimate and the TTC values.

To determine a reference value based on a non-TTC approach, the metabolite will be assessed for toxicological alerts and compared with known available toxicological data on structurally-related compounds (i.e. the parent, other metabolites or other molecules), making use of the following tools:

- read across ([ECHA practical guide 6](#))
- grouping of metabolites with similar structures ([OECD Guidance on Grouping of Chemicals, Second Edition](#))
- QSAR (use several models to estimate toxicological alerts)

Based on the level of concern identified, further toxicity testing may be considered, possibly with a representative metabolite in case of grouping of metabolites

- Low concern: limited toxicology testing of the metabolite may be needed; define suitable reference values for acute and chronic risk assessment
- High concern: consider in vivo toxicology testing of the metabolite to define metabolite-specific reference values for acute and chronic risk assessment

Conduct a consumer risk assessment using available exposure data and non-TTC (metabolite-specific) reference values to determine the margin of safety

Annex I - Explanatory notes

Supporting Notes - Exposure:

The following notes provide guidance on how exposure can be estimated for the consumer risk assessment of dietary metabolites.

- Consider metabolites in primary and processed crops, rotational crops and livestock commodities
- For exposure estimation give consideration to all EU registered crops
- Use of metabolite/parent ratios for exposure estimation of metabolites
 - Use the most appropriate metabolite/parent ratio (with regard to matrix, PHI, intended use pattern), but the highest ratio, e.g. from two radiolabels. Consider the application regime of the metabolism study (soil, seed treatment, foliar, number of applications, intended PHIs) and align with cGAP of the respective commodity
 - Generally extrapolate ratios only within the same metabolism crop group according to OECD 501 (see *Annex II of this document*); for crop groups not covered by the three primary crop metabolism studies, sensible extrapolations should be allowed, e.g. cereal grains to oilseeds; cereal forage to legumes, leaves of root/fruit crops to leafy vegetables etc.
 - If no ratio for the metabolite/parent or to any marker substance can be established, normalize the metabolite level measured in metabolism studies to the cGAP rate
 - Rotational crop: use the highest ratio from the appropriate plant back interval (PBI)
 - *In future*, extrapolate ratio or metabolite level within succeeding crop categories as per anticipated OECD Guidance Document on Residues in Rotational Crops (*currently in draft*)
- If a metabolite is not found in the metabolism study of a certain crop group the field estimate should be set to zero in all authorized crops of the same crop group
- For metabolites with closely related structures (e.g. hydroxylation at different positions in the same ring) or for metabolites which are related by simple metabolic conversions (e.g. conjugates → free aglycon; carboxylic esters → free acid/alcohol) or are succeeding each other in the metabolic pathway, the combined estimated exposure should be considered and compared to the same relevant reference value.
- For livestock two different routes into animal products have to be considered: (1) by direct formation (from parent) in livestock metabolism and (2) by occurrence in feedstuff and possible transfer into animal products; the levels arising from route (1) can be estimated by normalization of metabolite levels from the livestock metabolism studies to the 1x feeding level (or establish a metabolite/parent ratio and apply to measured parent residues in a feeding study if available); the levels arising from route (2) can be estimated by calculating a livestock dietary burden for this metabolite and deriving the transfer of this

metabolite into edible animal matrices from the livestock metabolism study (e.g. use the transfer of the parent substance as surrogate if feasible)

- Metabolites included in the residue definition for risk assessment should be assessed using their suitable reference values,
 - in most cases using the parent toxicological endpoint where the exposure values for each analyte in the residue definition are added together in the risk assessment, or
 - using separate endpoints for a specific metabolite in a separate risk assessment.
- For metabolites in plant and animal also give consideration to exposure via drinking water (if modelled in groundwater above the 0.75 µg/L threshold; see [SANCO 221/2000](#))
- Use the most current PRIMo model for consumer risk assessment

Supporting Notes - Exposure:

The following notes provide guidance on how a reference value can be determined for the consumer risk assessment of dietary metabolites.

- If the quality and quantity of exposure data are sufficient to derive realistic estimates for the metabolite, TTC-derived values to be used for risk assessment are determined with the help of the following tools (assuming appropriate combination of the tools and taking into account that some tools might be inappropriate to address specific endpoints)
 - Read-across from existing toxicological data on structurally-related compounds
 - Grouping of metabolites with similar structures
 - QSARs (e.g. VEGA, T.E.S.T, Toxtree, OECD Toolbox, DEREK, TopKat)
 - Determination of Cramer class

Example: metabolites of neurotoxic substances which can be demonstrated through structural analysis or experimental data not to be neurotoxic, should be considered to be in Cramer Class III and the TTC values of 1.5 and 5 µg/kg bw/day for the chronic and acute risk assessment, respectively, should be applied.

- If the estimated exposure exceeds the TTC values or if uncertain exposure estimates provide an insufficient margin of safety between the exposure estimate and the TTC values, a reference value based on a non-TTC approach should be derived. The metabolite will be assessed for toxicological alerts and compared with available toxicological data on structurally-related compounds (i.e., the parent, other metabolites and any other tested molecules). The assessment will comprise a weight of evidence analysis to cover hazard identification and characterization over acute and chronic exposure. Each toxicological endpoint should be assessed in order to define the level of concern, *i.e.* the level of confidence that a reference value can be used for human risk assessment based on existing data generated on another compound (e.g. the parent) or that data are insufficient to allow the use of such a value and therefore toxicological studies have to be conducted on the metabolite.
 - Consider the metabolite level in the rodent metabolism study and other available toxicokinetic data
 - Consider structural similarity to parent and other molecules
 - Consider available data on structurally-related compounds: available toxicological data, doses tested, reference doses, hazard potentials, NOAELs

As in the TTC approach, the following tools can be used:

- Read-across from existing toxicological data on structurally-related compounds

- Grouping of metabolites of similar structure
- QSARs (e.g. VEGA, T.E.S.T, Toxtree, OECD Toolbox, DEREK, TopKat)

Examples of Low Concern:

- The metabolite is found at 5% in the rat metabolism study. A compound structurally similar to the metabolite has been tested in a 28-day study, and doses tested were moderately high, the NOAEL was higher than the parent. The parent has no hazard classification.

Examples of High Concern:

- A metabolite is not present in the rat metabolism studies. The metabolite is structurally dissimilar to the parent but similar to a product known to cause developmental toxicity.

Based on this analysis, further in vitro or in vivo testing can be conducted either to lower the level of concern and allow the use of the reference value from another compound, or to determine a reference value by direct testing of the metabolite.

Toxicology testing:

- The study package should be limited to address a specific area of concern. More extensive testing could be required if there is a lack of data and the need to derive a reference value for the metabolite.
- If the metabolite shares structural similarity with the parent the study could be designed to compare the toxicity of the metabolite with the existing parent data and determine if the reference value to be used in the risk assessment should be derived from this study or from the data available on parent.
- The parent product should be included in the study only if there is some reason to suspect that the parent substance data is incomplete or has serious deviations from current guideline requirements. In such a case the results obtained with the comparator (i.e. the parent treated group) cannot be used to determine a new reference value for the risk assessment of the parent.
- If several metabolites have to be tested in vivo in different studies, it may not be appropriate/needed to include the parent product in every study, provided studies are conducted in the same testing facility and at reasonable time intervals.

Genotoxicity:

The applicability of QSAR analysis in the evaluation of genotoxicity alerts in pesticide metabolites has been evaluated in the EFSA [Scientific Opinion](#) 2012. A wide range of sensitivity and specificity was observed with the different QSAR tools. The best performance was found in predicting bacterial mutagenicity. According to the report of [JRC 2010](#), pairwise combinations of these tools could increase the overall sensitivity to a level close to 90%, which is better than the sensitivity of the Ames test for rodent carcinogens based on the extended database of 264 NTP chemicals ([Ashby et al, 1989](#)). Therefore it should be possible to rely on the results of these analyses to detect mutagenicity alerts of metabolites.

In case of a positive alert in QSAR mutagenicity or when the genotoxicity TTC threshold value is exceeded, it is recommended to run 2 in vitro tests, as recommended in the EFSA [Scientific Opinion](#) 2011 on genotoxicity testing strategies. If negative, no follow-up in vivo test is required. In case of positive in vitro results, an appropriate in vivo test should be performed ([EFSA Scientific Opinion 2011](#)). It should also be possible to use the Margin of Exposure approach ([EFSA 2012](#)) which is applicable to impurities in substances added to food or feed.

TTC values for dietary metabolites:

For genotoxicity: intake of 0.0025 µg/kg bw/day for acute and chronic risk assessment (RA)

For neurotoxicity: intake of 0.3 µg/kg bw/day for acute and chronic RA

Cramer Class III: intake of 5 µg/kg bw/day for acute and intake of 1.5 µg/kg bw/day for chronic RA

Cramer Class I: intake of 30 µg/kg bw/day for acute and chronic RA

Annex II - Crop Groups for Metabolism Studies according to OECD 501, Annex 1

Code	Category	Crops
F	Fruit	Citrus fruit Tree nuts Pome fruit Stone fruit Berries Small fruit Grapes Fruiting vegetables Banana Persimmon
R	Root crops	Root and tuber vegetables Bulb vegetables
L	Leafy crops	Brassica vegetables Leaf vegetables Stem vegetables Hops Tobacco
C/G	Cereal/Grass crops	Cereals Grass and forage crops
P/O	Pulses and oilseeds	Legume vegetables Pulses Oilseeds Peanuts Legume fodder crops Cacao beans Coffee beans
-	Miscellaneous	In general, crops not listed above or not covered by a grouping are considered as miscellaneous and will not normally be accepted as one of the three crop groups. However, if it is proposed to use such a crop to cover one of the three crop groups due to its national/regional importance, applicants are strongly urged to consult with regulatory authorities.

Annex III - Definitions

ECPA:	European Crop Protection Association
EFSA:	European Food Safety Authority
J-TRAG:	Joint Toxicology and Residues Ad-hoc Expert Group (ECPA)
TTC:	Threshold of Toxicological Concern
DoR:	Definition of Residue
RA:	Risk Assessment
HR:	Highest Residues in field trials
STMR:	Supervised Trial Median Residues derived from field trials
PRIMO:	EFSA Pesticide Residues Intake Model
LOQ:	Limit of Quantification
cGAP:	critical GAP, in case of several use patterns, use the highest expected residues
ADI:	Acceptable daily intake
ARfD:	Acute reference dose
NOAEL:	No observed adverse effect level
QSAR:	Quantitative Structure Activity Relationship
VEGA:	Virtual models for the Evaluation of chemical properties within Global Architecture
T.E.S.T:	Toxicity Estimation Software Tool
DEREK:	Deductive Estimate of Risk from Existing Knowledge
Toxtree:	Toxic Hazard Estimation by decision tree approach
TOPKAT:	Toxicity Prediction by Komputer Assisted Technology
NTP:	National Toxicology Program
MNT:	Micronucleus test

Annex IV - Source of references

- **EFSA Scientific Opinion** on the Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment published August 2012
<http://www.efsa.europa.eu/en/efsajournal/doc/2799.pdf>
- **OECD Guideline Document for the Testing of Chemicals - Test Number: 501** – Metabolism in Crops
<http://www.oecd-ilibrary.org/docserver/download/9770101e.pdf?expires=1410192619&id=id&accname=guest&checksum=8EB32551F9702B98F1EBCC3456C32C36>
- **OECD Guideline Document for the Testing of Chemicals - Test Number: 502** - Metabolism in Rotational Crops
<http://www.oecd-ilibrary.org/docserver/download/9770201e.pdf?expires=1422309180&id=id&accname=guest&checksum=B6611ED0D988F2A23DE0B049FA07A80A>
- **OECD Guideline Document for the Testing of Chemicals – Test Number: 503** – Metabolism in Livestock
<http://www.oecd-ilibrary.org/docserver/download/9770301e.pdf?expires=1412871551&id=id&accname=guest&checksum=8BD8E7EE6202FA23E2A686D4501F370D>
- **OECD Guideline Document for the Testing of Chemicals – Test Number: 507** – Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis
<http://www.oecd-ilibrary.org/docserver/download/9770701e.pdf?expires=1412871769&id=id&accname=guest&checksum=865C8F856E30A096FD3F62991326E1BD>
- **OECD Guidance Document for the Definition of Residue ENV/JM/MONO(2009)30**
[http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=ENV/JM/MONO\(2009\)30](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=ENV/JM/MONO(2009)30)
- **How to report read-across and categories** (ECHA practical guide 6)
http://echa.europa.eu/documents/10162/13655/pg_report_readacross_en.pdf
- **Guidance on Grouping of Chemicals, Second Edition** - ENV/JM/MONO(2014)4
[http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2014\)4&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en)
- **EFSA Scientific Opinion** on genotoxicity testing strategies applicable to food and feed safety assessment published October 2012
<http://www.efsa.europa.eu/en/efsajournal/doc/2379.pdf>
- **JRC Scientific Report** on the applicability of QSAR analysis to the evaluation of the toxicological relevance of metabolites and degradates of pesticide active substances for dietary risk assessment published May 2010
<http://www.efsa.europa.eu/fr/search/doc/50e.pdf>
- **Classification** according to chemical structure, mutagenicity to Salmonella and level of carcinogenicity of a further 42 chemicals tested for carcinogenicity by the U.S. National Toxicology Program. Ashby J, Tennant RW, Zeiger E, Stasiewicz S. *Mutat Res.* 1989 Jun;223(2):73-103.
<http://www.ncbi.nlm.nih.gov/pubmed/2662004>

- **EFSA Scientific Opinion** on the statement on the Applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed
<http://www.efsa.europa.eu/en/efsajournal/doc/2578.pdf>

Annex V- Case Study Examples

Case Study Example 1

Case Study based on actual metabolite exposure data

Step 1 - Metabolism

A new fungicide in development had three primary crop metabolism studies conducted on grapes, potatoes and lettuce. In addition a high temperature hydrolysis study and a confined crop rotation study were also completed.

The parent compound formed the major part of the residue in all primary crop studies (40 – 90% TRR) with the exception of potato tubers where little parent (<5% TRR and 0.002 mg/kg) had been translocated from the foliage. A total of 14 plant metabolites were identified which were all below 5% TRR and 0.05 mg/kg with the exception of Metabolite 1. This metabolite, a cleavage product of parent i.e. structurally dissimilar, was present only in potato tubers at levels up to 13% TRR and 0.006 mg/kg (parent equivalents). Metabolite 1 was not detected in either the confined crop rotation study or the high temperature hydrolysis study.

An investigation into the mammalian metabolism of the fungicide had demonstrated extensive metabolism but no cleavage of the molecule and therefore Metabolite 1 was not detected. This was also true in the ruminant metabolism study.

Metabolism information showed that residues in crops arising from the use of the fungicide were predominantly parent with the exception of potato tubers where very little parent was present. In tubers the largest component of the residue was Metabolite 1 which was not detected in the rat metabolism studies and hence the toxicological properties of Metabolite 1 were not considered to have been evaluated

Step 2 – Crop Residues

Residues of parent compound in potatoes were <0.01 mg/kg in 15 trials and in one trial it was detected at 0.01 mg/kg; therefore as expected parent was not a good marker compound to estimate residues of Metabolite 1. Consequently, residue analysis for Metabolite 1 was conducted in the potato tubers and residues were <0.005 mg/kg in 14 trials but were 0.011 mg/kg and 0.013 mg/kg in two other trials. These values were consistent with data generated in the USA and in Japan.

Robust and extensive residue analysis had confirmed the presence of Metabolite 1 at levels higher than the parent compound in residue field trials

Step 3 – Toxicological Assessment

The Threshold of Toxicological Concern (TTC) approach was investigated as a possible means of evaluating the hazard of Metabolite 1. From a chemical structure perspective Metabolite 1 is not an organophosphate or carbamate and has no other alerting features. The potential for Metabolite 1 to have any genotoxicity properties was excluded by conducting a range of appropriate tests.

Metabolite 1 was therefore categorized into Cramer class III, for which EFSA opinions propose the appropriate TTC values are 1.5 µg/kg bw/day and 5 µg/kg bw/day for chronic and acute exposures respectively. These are equivalent to:

- **Chronic threshold: 0.0015 mg/kg bw/day**
- **Acute threshold: 0.0050 mg/kg bw/day**

The Threshold of Toxicological Concern approach was used to derive chronic and acute reference values for Metabolite 1 to use in dietary risk assessments

Dietary Risk Assessment

Chronic and acute dietary exposure assessments were conducted assuming that all potatoes contain residues of Metabolite 1 at the highest level detected in field trials (0.013 mg/kg). Results were:

- **Chronic intake: 0.00008 mg/kg bw/day**
- **Acute intake: 0.0020 mg/kg bw/day**

Comparing these intakes to the TTC values above showed intakes of approximately 5.1% of the chronic threshold and 40% of the acute threshold.

Acceptable risk has been demonstrated from dietary exposure to Metabolite 1 in potato tubers

Conclusions

A plant specific metabolite, only present in potatoes, was demonstrated to be acceptable from a dietary risk perspective by:

- 1. Determining residue levels of the metabolite in potato tubers in a full set of residue trials**
- 2. Using the Threshold of Toxicological Concern to assign appropriate hazard values for use in chronic and acute dietary risk assessments**
- 3. Conducting dietary risk assessments which showed acceptable risk**

In future any registrations on other root and tuber crops would need to generate residue data on this metabolite.

Case Study Example 2

Case Study based on grouping of metabolites and toxicological weight of evidence

Metabolites to consider in the evaluation

12 metabolites identified in nature of the residue studies in treated crops, livestock, rotated crop and processed commodities [M1 - M12].

8 metabolites (M1, M2, M4 - M7; M9, M11) were plant metabolites.

2 metabolites M3 and M12 were processing metabolites.

6 metabolites (M2, M4, M7-M10) were hen and/or goat metabolites.

M3, M6, M9, M10, M12 were not detected in the rat or other lab species.

M2 is the major rat metabolite (found at 20%), M7 and M8 at 8% and 11%.

Thus, for M2 and M8, assessment against parent ADI would be possible.

Metabolites selected for field residue analysis

For metabolites M1, M2, M3, and M11 some residue data from field residue supervised trials are available in some crops.

For M1, M2 and M11 quantifiable residues were found.

Residues of M3 were <LOQ in most cases, but it is a compound of special concern.

Metabolite M11 is the conjugate of M3.

For all other metabolites no residue data is available, data from metabolism has to be used.

For the animal metabolites M2, M4, M7, M8, M9, M10 no individual residue data from feeding studies are available. Ratio from metabolism were used and applied to the measured common moiety residues of the feeding study.

Exposure estimates for Metabolites M1-M12

M1, M2 > 1.5 µg/kg bw/d -> relevant and inclusion into residue definition for RA in plants M3 > 1.5 µg/kg bw/d -> conduct of 28-d study and derivation of separate ADI, exposure estimate =14% of this ADI; no inclusion into the residue definition

M4-M12 all individually below 1.5 µg/kg bw/d

M10 even below 0.0025µg/kg bw/d

All 6 livestock metabolites (M2, M4, M7-M10) are covered in the residue definition for animal products since this is based on a common moiety method.

Metabolite Grouping Approach Group A (Metabolites M1, M4): Summed Exposure >1.5 µg/kg bw/d

Metabolites M1 and M4 are closely related in structure and are structural isomers of parent.

Based on the occurrence of both metabolites in the same metabolic pathways in plant, livestock and rat, these metabolites are placed into a common assessment group A. Consequently, studies performed with any metabolite of group A are representative for the entire group.

Toxicity: QSAR analysis predicted a limited alert for genotoxicity in vitro with in silico metabolic activation for M1 and M4 which is considered of no relevance as M1 was tested negative for in vitro genotoxicity (AMES, MNT). Comparable acute toxicity after oral application in mice and rats to parent was observed for M1 and no potential for eye- or dermal irritation was seen. By weight of evidence M1 is considered as being of lesser toxicity after subchronic exposure than parent which by definition is then also true for M4.

In conclusion, M1 and M4 are considered to be not of higher toxicity than parent.

Therefore the human health based reference values for parent also apply to M1 and M4.

Metabolite Grouping Approach Group B (Metabolites M2, M5): Summed Exposure >1.5 µg/kg bw/d

Metabolites M2 and M5 are closely related in structure and differ by side-chain de-amidation. Based on the occurrence of both metabolites in the same metabolic pathways in plant and rat, these metabolites are placed into a common assessment group B. M2 occurs in rat at >10% dose, being a major metabolite. Thus, the toxicological properties of M2 and M5 are considered to be covered by parent.

Toxicity: No relevant structural alerts were identified for M2 but a limited genotoxicity alert for M5 which is considered non-relevant as no evidence for genotoxicity was observed in vitro (AMES). M2 is a major rat metabolite and the toxicological properties of group B metabolites covered by parent. In conclusion M2 and M5 are considered to be part of rat metabolism and their toxicological properties covered by parent. Therefore the human health based reference values for parent also apply to M2 and M5.

Metabolite Grouping Approach Group C (Metabolite M11): Exposure < 1.5 µg/kg bw/d

M11 is a conjugated metabolite found in carrot only. No structural similarity to any other metabolite is apparent; therefore M11 is placed into a single metabolite assessment group C.

Toxicity: QSAR analysis predicted a limited alert for genotoxicity in vitro with in silico metabolic activation which is considered of no relevance as there is no evidence for genotoxicity for M11 in vitro (AMES, MNT). Therefore M11 is considered to be of no genotoxicological concern and the reference value of 1.5µg/kg bw/d for compounds of Cramer Class III can be applied.

Metabolite Grouping Approach Group D (Metabolite M12): Exposure < 1.5 µg/kg bw/d

M12 is formed during processing. No structural similarity to any other metabolite is apparent; therefore M12 is placed into a single metabolite assessment group D.

Toxicity: QSAR analysis predicted a limited alert for genotoxicity in vitro with in silico metabolic activation which is considered of no relevance as M12 was tested negative for in vitro genotoxicity (AMES, MNT). Acute toxicity in mice revealed comparable oral toxicity to parent. M12 is considered to be of no genotoxicological concern and the human health based reference value of 1.5µg/kg bw/d for compounds of Cramer Class III can be applied.

Metabolite Grouping Approach Group E (Metabolites M6, M7, M8):

Summed Exposure <1.5 µg/kg bw/d

M6, M7 and M8 are ring opened metabolites of parent. M6 is only found in plant metabolism and M7 as well as M8 are major rat metabolites. Whereas ring hydrolysis can be an initial step in the metabolism of parent in plant, leading to the formation of M6, the metabolic route in rat is different. In rat, ring-dealkylation is the preferred reaction prior to ring-hydrolysis, thereby bypassing the formation of M6 leading to M7 and M8. However, the rat is able to metabolize M6 to M7 and M8. Thus, based on close structural similarity and a predicted common metabolic pathway, M6, M7 and M8 are placed into the common metabolite group E.

Toxicity: QSAR analysis predicted a limited alert for genotoxicity in vitro with in silico metabolic activation for all metabolites which is considered of no relevance as M6 was tested negative for in vitro genotoxicity (AMES, MNT) and M7, M8 are major rat metabolites. The acute toxicity after oral application of M6 to rats is comparable to parent. Overall the toxicological properties of M6, M7 and M8 are considered covered by parent based on lack of genotoxicity in vitro as well as the combined group exposure in rat metabolism.

Thus, the reference value for parent also applies to M6, M7 and M8.

Metabolite Grouping Approach Group F (Metabolites M9 and M10):

Summed Exposure <1.5 µg/kg bw/d

M9 and M10 are ring-opened metabolites of parent and determined in plant, livestock and soil (photolysis) but not in rats, likely due to the ready elimination of precursor metabolite M7 in rat urine. Although M9 and M10 could potentially be formed in rat from M7 by de-amidation, a common metabolic process in rat, both metabolites were placed into a separate metabolite group F as proposed by the RMS.

Toxicity: QSAR predicted a limited alert for genotoxicity in vitro after in silico metabolic activation which is considered of no relevance based on in vitro genotoxicity data for M9 indicating non-mutagenicity (AMES, MNT). Structurally, metabolites M9 and M10 fit into the definition for Cramer Class III compounds according to the TTC concept. Thus, the reference value of 1.5µg/kg bw/d for compounds of Cramer Class III can be applied.

Metabolite Grouping Approach Group G (Metabolite M3): Exposure > 1.5 µg/kg bw/d

M3 is a metabolite determined in plant, hydrolysis and soil. Although structural similarity is given to all metabolites, most apparent to M9 and M10, M3 does not occur in the rat and was therefore assessed individually in a single metabolite group G as proposed by the RMS.

Toxicity: QSAR predicted a limited alert for genotoxicity in vitro after in silico metabolic activation which is considered of no relevance due to available genotoxicity studies with M3 indicating non-mutagenicity in vitro and in vivo (AMES; MNT). Toxicity after repeated oral gavage for 28-days of M3 was adequately addressed in a standard regulatory short-term toxicity study in Wistar rats. A LOAEL of 20 mg/kg bw/d was determined. No effects were observed at the NOAEL of 7.5 mg/kg bw/d, the lowest dose tested. Supporting information indicates a reversible mode of action for the findings at LOAEL as observed after acute intra peritoneal

exposure to M3 in Fischer rats at dose levels comparable to the 28-day study in Wistar rats. It can be assumed that there is a threshold for the effects which has been adequately established to be above the NOAEL of 7.5 mg/kg bw/d. Appropriate safety factors to extrapolate from short-term or chronic studies have been used (acc. to ECHA a factor of 3 for subacute to subchronic studies, and a factor of 2 for sub-chronic to chronic extrapolation). For inter- and intra-species variation a total safety factor of 100 is assumed.

In conclusion, the acceptable daily intake (ADI) of M3 is 12.5µg/kg bw/d. Exposure is 14% of this ADI.

Case Study Example 3

Case Study based on

- TTC approach with actual metabolite exposure data [Metabolite M9]
- Toxicological weight of evidence [Metabolites M13+M14]
- Negligible exposure of metabolite [Metabolites M2, M3, M4, M5, M6, M7, M8, M11, M12]
- Grouping of metabolites [Metabolites M1, M10, and M15 were not measured but were grouped with M2/M3, M11/M12, and M9/M13, respectively]
- Exposure estimate using metabolite-specific toxicity reference dose [Metabolites M13+M14]

15 metabolites were identified in the nature of the residue studies in treated crops, livestock, rotated crop and processed commodities [M1 - M15]. Of the 15 metabolites:

- Ten metabolites (M1 - M3; M9 - M15) were plant metabolites.
- Ten metabolites (M1 - M9, and M13) were hen and/or goat metabolites. M12 was transient in hen and goat.
- Three metabolites (M10, M12, M14) were not detected in the rat or other lab species.
- None of the metabolites were quantified at >10% in rat urine or bile.

Given the low levels of residues in the rat urine and bile, the metabolites were not sufficiently covered by the risk assessment for parent. Therefore, for all 15 metabolites, exposure estimates were needed.

- Metabolites M4 - M8 were metabolites found only in livestock matrices. Livestock feeding studies were not conducted based on the low livestock dietary burdens and the low transfer of residues to edible tissues, milk and eggs observed in the livestock metabolism studies. Based on the expectation that metabolite residues would be <LOQ at 1X feeding level, M4 - M8 were proposed as not relevant for residue definitions.

For the remaining 10 plant metabolites, there were not enough data available from the nature of the residue studies to estimate exposure so field residue data were generated.

- Seven metabolites M2, M3, M9, M11, M12, M13, and M14 were selected for analysis in field residue supervised trials for treated and rotated crops.
- Metabolite M1 was not quantified in field samples because the evaluation of relevancy for M2 and M3 can be extrapolated to the chemically similar M1. Metabolites M2 and M3 are hydroxylated forms of M1. The three metabolites are seen at similar levels in the metabolism studies. If needed, formal extrapolation could be made using metabolism study ratios of M1 to M2 and/or M3.
- Metabolite M10 was not quantified in field samples because the evaluation of relevancy for M11 and M12 can be extrapolated to the chemically similar M10. Metabolite M11 is the acid form of the amide, M10, on

the pathway to the deacidified M12. Formal extrapolation could be made using metabolism study ratios of M10 to M11 and/or M12.

- Metabolite M15 was not quantified in field samples. However, because M15 is the deacidified form of M9 and the methylated form of M13, the evaluation of relevancy for M9 and M13 extrapolates to the chemically similar M15.

Once the field residue data were available, the data were evaluated to determine if the metabolite was relevant to the residue definitions.

- Residues of metabolites M2, M3, M11, and M12 were not quantifiable (<0.01 mg/kg) in more than 98% of all field trials samples. Based on the observation of negligible residues in food commodities, M2, M3, M11, and M12 were proposed as not relevant for residue definitions.
- Because M1 is chemically similar to M2 and M3 and was seen at similar levels in the metabolism studies, M1 was proposed as not relevant for residue definitions.
- Because M10 is chemically similar to M11 and M12, M10 was proposed as not relevant for residue definitions.

Metabolite M9, exposure assessment using the TTC as the toxicity endpoint

- Metabolite M9 was not genotoxic in a reverse bacterial mutagenicity assay. Nor was it genotoxic in an in vitro chromosome aberration test in human peripheral blood lymphocytes. This test was conducted in such a way that both structural and numerical chromosome aberrations could be detected. In the rat single-dose metabolism study, M9 was detected at levels below 1% of the applied dose. M9 does not contain organophosphate or carbamate toxicophores. Since it cannot be determined if M9 would fall into Cramer Class I or Class III, the Threshold of Toxicological Concern (TTC) for Cramer class III substances was used for the chronic reference dose. Since the data for the parent do not lead to the need for an Acute Reference Dose, no ARfD is proposed for M9.
- Exposure from consumption was estimated from treated and rotational crop field data, processing data and an estimate of <0.01 mg M9 residues/kg in livestock commodities. M9 residues were not detected in environmental fate laboratory studies at >5% applied radioactivity so exposure via drinking water is negligible. The highest calculated chronic exposure for M9 was 0.0007 mg/kg bw/day (French toddler), less than 45% of the Cramer Class III TTC.
- Exposure from inhalation was estimated using treated tobacco field data. The inhalation exposure estimate was 0.00077 mg/kg bw/day, <5% of the Cramer Class III TTC.
- Based on the exposure estimates being less than the Cramer Class III TTC, M9 was proposed as not relevant for residue definitions.

Metabolites M13/M14, exposure assessment using a metabolite-specific toxicity endpoint

- Metabolite M14 is a glucose-conjugate of M13 formed in plants. When the glucose conjugate M14 is consumed by livestock, it is metabolized to M13. Because M14 metabolizes to M13 in livestock and is expected to similarly metabolize to M13 in humans, combined residues of (M13 + M14) in food and feed items were used to estimate exposure, and toxicity tests on M13 were used to determine toxicity for the combined residue.
- The weight of evidence is that M13 is not of concern for genotoxicity. M14 was not genotoxic in a reverse bacterial mutagenicity assay. Nor was it genotoxic in an in vitro chromosome aberration test in human peripheral blood lymphocytes. This test was conducted in such a way that both structural and numerical chromosome aberrations could be detected. Since EU modelling suggests groundwater levels of M13 exceed 0.75 µg/L in some scenarios, a 28-day repeated dose toxicity rat feeding study was conducted. M13 did not show any signs of toxicity in rats up to the highest doses tested, which were equivalent to 1157 mg/kg bw/day for male rats and 1265 mg/kg bw/day for female rats. A chronic reference dose (ADI)

of 0.39 mg/kg bw/day was derived from the NOAEL of 1157 mg/kg bw/day by applying the typical inter- and intra-species adjustment factor of 100x, plus extra adjustment factors of 3x and 10x since a 28-day study is being used instead of a 90-day study and for extrapolation to a chronic exposure scenario from an sub-acute study. Since the data for the parent do not lead to the need for an Acute Reference Dose, no ARfD is proposed for (M13 + M14).

- Exposure from the consumption of food was estimated from treated and rotational crop field data, processing data, and an estimate of residues in livestock commodities from livestock feeding on commodities containing both parent and M13/M14 residues. The highest calculated chronic exposure for (M13 + M14) was 0.0024 mg/kg bw/day (French toddler) which is equal to 0.6% of the proposed ADI.
- Exposure to (M13 + M14) from the consumption of drinking water was estimated. M14 residues were not detected in environmental fate laboratory studies at >5% applied radioactivity so exposure via drinking water is negligible. Exposure to M13 from drinking water was estimated using the maximum modelled concentration in EU groundwater for adults, toddlers and infants. The highest calculated chronic exposure for M13 from consumption of water was 0.4564 µg/kg bw/day = 0.0005 mg/kg bw/day (infant) which is equal to 0.12% of the proposed ADI.
- Exposure from inhalation was estimated using treated tobacco field data. The inhalation exposure estimate was 0.000135 mg (M13 + M14)/kg bw/day = 0.03% of the proposed ADI.
- Based on the exposure estimates being less than the proposed ADI, **M13 and M14 were proposed as not relevant for residue definitions.**
- **Because M15 is chemically similar to M9 and M13, M15 was proposed as not relevant for residue definitions.**

Case Study Example 4

Published Case Study based on

- TTC approach with actual metabolite exposure data
- Toxicological weight of evidence
- Grouping of metabolites
- Exposure estimate using metabolite-specific toxicity reference dose

Utilizing relative potency factors (RPF) and threshold of toxicological concern (TTC) concepts to assess hazard and human risk assessment profiles of environmental metabolites: A case study

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Abstract

There is currently no standard paradigm for hazard and human risk assessment of environmental metabolites for agrochemicals. Using an actual case study, solutions to challenges faced are described and used to propose a generic concept to address risk posed by metabolites to human safety. A novel approach built on the foundation of predicted human exposures to metabolites in various compartments (such as food and water), the threshold of toxicological concern (TTC) and the concept of comparative toxicity was developed for environmental metabolites of a new chemical, sulfoxaflor (X11422208). The ultimate aim was to address the human safety of the metabolites with the minimum number of in vivo studies, while at the same time, ensuring that human safety would be considered addressed on a global regulatory scale. The third component, comparative toxicity, was primarily designed to determine whether the metabolites had the

same or similar toxicity profiles to their parent molecule, and also to one another. The ultimate goal was to establish whether the metabolites had the potential to cause key effects – such as cancer and developmental toxicity, based on mode-of-action (MoA) studies – and to develop a relative potency factor (RPF) compared to the parent molecule. Collectively, the work presented here describes the toxicology programme developed for sulfoxaflor and its metabolites, and how it might be used to address similar future challenges aimed at determining the relevance of the metabolites from a human hazard and risk perspective.

Sulfoxaflor produced eight environmental metabolites at varying concentrations in various compartments – soil, water, crops and livestock. The MoA for the primary effects of the parent molecule were elucidated in detail and a series of *in silico*, *in vitro*, and/or *in vivo* experiments were conducted on the environmental metabolites to assess relative potency of their toxicity profiles when compared to the parent. The primary metabolite, X11719474 found in soil, crops and, potentially, at low concentrations, in groundwater, was the most extensively studied, with genetic, acute, short-term rat and dog, rodent reproductive and developmental toxicity studies, and MoA studies conducted. These data supported that the toxicity profile for X11719474 was limited to liver effects via the same MoA as the parent and, overall, X11719474 was significantly less toxic than parent. Subsequently, the comparative toxicology programme was extended to cover all metabolites of sulfoxaflor. Based on structure (i.e., similarity of metabolite structures to one another), toxic effects in comparison with parent (i.e., consistency of the toxicity profiles and confidence in terms of ability to read across), residue compartment (e.g., crop, soil, water) and predicted level of exposure, fewer studies were required for establishing safety of these metabolites compared to X11719474. For example, for some metabolites with very low predicted environmental concentrations only genotoxicity testing was required. For some metabolites with low predicted concentrations, for example only present in liver, a TTC approach was utilized.

This strategy of comparative assessment utilizing MoA data, relative potency, hazard characterization, read-across, predicted exposure and TTC provided a robust database, which minimized animal use, comprehensively assessed the hazard and human risk presented by these metabolites.

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