

SCIENTIFIC OPINION

Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment¹

EFSA Panel on Plant Protection Products and their Residues (PPR)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

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ABSTRACT

The European Food Safety Authority (EFSA) asked the Panel on Plant Protection Products and their Residues (PPR) to develop an opinion on approaches to evaluate the toxicological relevance of metabolites and degradates of pesticide active substances in dietary risk assessment. This opinion identifies the threshold of toxicological concern (TTC) concept as an appropriate screening tool. The TTC values for genotoxic and toxic compounds were found to be sufficiently conservative for chronic exposure, as a result of a validation study with a group of pesticides belonging to different chemical classes. Three critical steps were identified in the application of a TTC scheme: 1) the estimate of the level of the metabolite, 2) the evaluation of genotoxicity alerts and 3) the detection of neurotoxic metabolites. Tentative TTC values for acute exposure were established by the PPR Panel by analysis of the lowest 5th percentiles of No Observed Adverse Effect Levels (NOAELs) used to establish the Acute Reference Doses (ARfD) for the EFSA pesticide data set. Assessment schemes for chronic and acute dietary risk assessment of pesticide metabolites, using the TTC approach and combined (Q)SAR and read across, are proposed. The opinion also proposes how the risk assessment of pesticide metabolites that are stereoisomers should be addressed due to isomer ratio changes reflected in the composition of metabolites. The approach is ready for use, but it is anticipated that on many occasions the outcome of the assessment scheme will be that further testing is needed to reach a firm conclusion

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on the toxicological relevance of the metabolite. However, the benefit of applying the approach is that it will allow prioritisation of metabolites for subsequent testing.

EFSA will develop a Guidance Document based on the results in this opinion.

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KEY WORDS

Pesticide metabolites, dietary exposure, endocrine disruptor, Quantitative Structure Activity Relationship, (Q)SAR, read-across, stereoisomer, Threshold of Toxicological Concern (TTC)

SUMMARY

The use of pesticides in agriculture may lead to a large number of metabolites being present at low levels in food and feed. Progress in analytical methods and their increasing sensitivity results in the detection of a growing number of metabolites in low amounts. The residue definition for dietary risk assessment should include the active substance and all metabolites of toxicological relevance. A comprehensive toxicological dossier is developed for parent compounds, prior to approval of substances for use within EU (Regulation EC (No) 1107/2009), while often only limited information about the toxicological properties of metabolites is available. In light of these considerations, EFSA asked the PPR Panel to develop an opinion on approaches to evaluate the toxicological relevance of metabolites and degradates of pesticide active substances in dietary risk assessment. The need to minimise the use of laboratory animals where possible was highlighted. The Panel was also asked to consider whether the approaches and methodologies developed for pesticide metabolites are applicable to isomer ratio changes of active substances existing as isomer mixtures or which are used as individual isomers.

A key issue is whether a metabolite would have been tested with the parent compound in laboratory species, due to its formation *in vivo*. For those metabolites not so tested, because they are unique to plants or livestock, an alternative approach is necessary. The PPR Panel considered relevant publications in the scientific literature and current applications of non-testing approaches in various regulatory contexts. On this basis four projects were outsourced to evaluate the potential impact of metabolic processes on the toxicity of pesticide metabolites and to explore the reliability of the available computational tools. The PPR Panel has developed a strategy to estimate the dietary exposure to pesticide metabolites. Several exposure scenarios were considered covering various possibilities of metabolite ratio extrapolation and the extent of uses. Case studies illustrate the methods that have been used. It is noted that the choice of the scenario has a considerable impact on the estimate of the metabolite level to be used.

The Panels conclusions on these approaches are as follows:

- The potential impact of structural metabolic changes to parent compounds on the toxicological properties of derived metabolites was analysed for the most relevant chemical classes of active substances listed in Annex 1 of Directive 91/414/EEC. Despite the high level of uncertainty due to the heterogeneity of ADME studies and inadequacy of toxicological data on metabolites, the metabolic pathways are in most cases specific for each chemical group and toxification/detoxification potential cannot be reliably attributed to specific metabolic steps.
- The TTC concept is the most appropriate tool for evaluating the toxicological relevance of pesticide metabolites. The existing TTC values for genotoxic and toxic compounds were found to be sufficiently conservative for chronic exposure by a validation study with groups of pesticides belonging to different chemical classes. These values, based on the assumption of continuous exposure during lifetime, are overly conservative for short term exposure duration. Tentative TTC values for acute exposure were established by the analysis of the lowest 5th percentiles of NOAELs used to establish ARfDs for the EFSA pesticide data set. Three critical steps were identified in the application of the TTC scheme in risk assessment of pesticide metabolites: 1) the estimate of the level of the metabolite, 2) the evaluation of genotoxicity alerts and the 3) detection of neurotoxic metabolites arising from a parent compound with a structural alert not covered by the scheme.
- The evaluation of genotoxicity alerts was addressed in an outsourced project involving the application of several (Q)SAR models using the largest dataset available of active ingredients and metabolites. The results showed individual models to have low sensitivities in identifying genotoxic pesticides, while the same tools applied in combination appeared good identifiers of classified mutagens. The low sensitivity was mainly attributed to the heterogeneity of the

underlying pesticide database. The PPR Panel concluded that the performance of these applied tools is not satisfactory and cannot support, at the present time, the application of solely (Q)SAR approaches to predict the potential genotoxicity of unknown pesticide metabolites.

- The applicability of (Q)SAR tools, grouping and read-across approaches in the evaluation of developmental and neurotoxic effects of pesticide metabolites was addressed by another outsourced project. The predictivity for neurotoxicity of the (Q)SAR models, tested alone or in combination, is currently inadequate to be applied for pesticide metabolites. (Q)SAR tools alone appeared insufficiently reliable to predict developmental effects, due to their low sensitivity and specificity, but a stepwise approach involving (Q)SAR analysis and read-across, resulted in an improvement in the identification of potential developmental toxicants.
- The results of the (Q)SAR projects allowed the PPR Panel to propose the application of computational methods, involving the separate or sequential use of (Q)SAR and read-across in the prediction of genotoxicity and developmental toxicity, to complement the TTC approach in the assessment scheme for pesticide metabolite exposure.
- Estimates of exposure to pesticide metabolites by the Panel are based mainly on residue metabolism studies. These data have also been adapted using a metabolite to parent ratio applied to the available residue end-points from the supervised trials data to give different estimates of exposure for both chronic and acute exposure. The key issue affecting the results is the potential for extrapolating data, encompassing metabolism groupings and the extent of uses. The approaches tested allowed the Panel to propose a dietary exposure tree for pesticide metabolites. However different methodological approaches produce different outcomes and risk managers would need to advise on the level of protection that is desired.
- The scientific principles that underpin pesticide metabolite exposure calculations (above) are also directly relevant to the derivation of conversion factors which are established during the regulatory evaluation of parent compounds in the framework of Regulation (EC) No 1107/2009 when the residue definitions for monitoring and dietary risk assessment differ. The PPR Panel recognises that currently, there is no unambiguous approach to deriving conversion factors and recommends the developing further guidance in this area.
- Chronic and acute assessment schemes are proposed for the risk assessment of pesticide metabolites considering different strategies for mammalian (rodent or laboratory test species) and plant or livestock specific metabolites. A chronic exposure estimate is necessary in all cases, while an acute exposure assessment is needed only when an Acute Reference Dose (ARfD) has been allocated for the parent compound or structural alerts for acute neurotoxicity and developmental toxicity are detected.
- The chronic assessment scheme involves the comparison of chronic exposure with the corresponding threshold values given in the decision tree. Computational tools involving the combination of (Q)SAR and read-across are proposed in the evaluation of an alert for genotoxicity. If the exposure estimate exceeds the identified TTC values, different approaches are proposed for mammalian rodent and plant or livestock metabolites. A weight of evidence approach is recommended to determine if the toxicological profile of rodent metabolites is covered by the data on parent compound. Plant or livestock specific metabolites need to be assessed using an appropriate testing strategy.
- An acute exposure assessment scheme was developed by the PPR Panel. *Ad hoc* acute TTC values of 0.3 µg/kg bw/d for substances with a neurotoxicity alert and 5 µg/kg bw/d for substances allocated in Cramer class II and III were derived. A combination of (Q)SAR and read-across approaches is proposed for the prediction of developmental toxicity.

- Where exposure to a metabolite exceeds the respective TTC value, acute and chronic toxicity testing strategies were proposed by the PPR Panel, considering the need to derive health based limits for human exposure.
- The opinion also proposes how the risk assessment of pesticide metabolites that are stereoisomers should be addressed due to isomer ratio changes reflected in the composition of metabolites. The PPR Panel does not propose that the TTC scheme is used for individual stereoisomers, although the TTC scheme has utility as a screening assessment of the isomer mixture for a metabolite. Further development of (Q)SAR tools would be beneficial, both to predict genotoxicity and to address stereochemistry aspects. Furthermore, metabolism guidelines should require compositional information on stereochemistry to consider the full impact on the dietary risk assessment.

The approaches described in this opinion are ready for use, but it is anticipated that on many occasions the outcome of the assessment scheme will be that further testing is needed to reach a firm conclusion on the toxicological relevance of the pesticide metabolite. However, the benefit of applying the approaches is that it will allow prioritisation of pesticide metabolites for subsequent testing. These approaches should not be used as an alternative to conventional risk assessment for pesticide active substances (parent compounds) themselves occurring as residues in food. They should be assessed prior to authorisation on the basis of dossiers including toxicological tests (Regulation (EC) No 1107/2009).

It is noted that EFSA will develop a Guidance Document based on the results in this opinion.

TABLE OF CONTENTS

| | |
|---|----|
| Abstract | 1 |
| Summary | 3 |
| Table of contents | 6 |
| Background as provided by EFSA | 9 |
| Terms of reference as provided by EFSA | 10 |
| Assessment | 10 |
| 1. Introduction | 10 |
| 1.1. Structure of the Opinion..... | 12 |
| 2. Current approaches to residue definition for pesticides..... | 12 |
| 2.1. Use of terms “pesticide residue” and “residue definition” | 12 |
| 2.2. Residue definitions..... | 13 |
| 2.3. Comparison of residue definitions by EFSA and JMPR..... | 13 |
| 2.4. Current status and future development of analytical methods for establishing residue definitions | 14 |
| 2.5. Relevant metabolites..... | 16 |
| 3. Rationale for the outsourced projects | 16 |
| 4. Impact of metabolic processes on the toxicological properties of pesticide residues..... | 17 |
| 4.1. Evaluation of toxicological profiles of pesticide metabolites in different regulatory contexts (results of outsourced project)..... | 18 |
| 4.2. Evaluation of metabolic pathways with respect to their toxification/detoxification potential for selected groups of pesticides as presented by the contractor..... | 18 |
| 4.3. Conclusions of the PPR Panel..... | 19 |
| 4.3.1. Evaluation of metabolic pathways with respect to their toxification/detoxification potential for selected groups of pesticides..... | 19 |
| 4.3.2. Considerations by the PPR Panel on conjugated and bound residues | 20 |
| 4.3.3. Evaluation of toxicological profiles of pesticide metabolites..... | 20 |
| 5. Threshold of Toxicological Concern (TTC)..... | 21 |
| 5.1. Current applications of TTC concept..... | 24 |
| 5.1.1. Additives: JECFA and EFSA | 24 |
| 5.1.2. Pharmaceuticals: European Medicines Agency (EMA) | 24 |
| 5.1.3. Pesticide metabolites in groundwater Guidance document SANCO/221/2000 rev 10 of 25 February 2003 | 25 |
| 5.1.4. Industrial chemicals (REACH): ECHA..... | 25 |
| 5.2. Applicability of TTC approach in the dietary risk assessment of pesticide metabolites: outcome of the outsourced project..... | 25 |
| 5.2.1. Validation of the developed TTC concept: case studies on metabolites | 27 |
| 5.2.2. Estimation of the exposure | 28 |
| 5.2.3. Results and conclusions of TTC case study presented by the contractor | 28 |
| 5.2.4. Conclusions of the PPR Panel on the TTC case study presented by the contractor | 29 |
| 5.3. Application of TTC approach for acute exposure..... | 30 |
| 5.3.1. Derivation of acute exposure thresholds..... | 30 |
| 5.4. Overall conclusions on TTC | 32 |
| 6. SAR/ (Q)SAR concept..... | 33 |
| 6.1. Characterisation of chemical space..... | 33 |
| 6.2. Performance of (Q)SAR models | 34 |
| 6.3. Read-across | 34 |
| 6.4. (Q)SAR approach in the dietary risk assessment of pesticide metabolites | 34 |
| 6.4.1. Software tools for genotoxicity and carcinogenicity prediction | 34 |
| 6.4.2. Current applications of (Q)SAR approach in predicting mutagenicity and carcinogenicity..... | 35 |
| 6.5. Applicability of (Q)SAR analysis to the evaluation of the toxicological relevance of metabolites of pesticide active substances for dietary risk assessment..... | 36 |
| 6.5.1. Outsourced project by the Joint Research Centre (JRC) | 36 |
| 6.5.2. Framework for assessing the usefulness of (Q)SAR models..... | 37 |

| | | |
|---------|---|----|
| 6.6. | Applicability of (Q)SAR analysis to the evaluation of genotoxicity of pesticide metabolites for dietary risk assessment: case studies | 38 |
| 6.6.1. | Software tools applied | 38 |
| 6.6.2. | Compilation of datasets | 38 |
| 6.6.3. | Characterization of chemical space by Principal Component Analysis (PCA) | 39 |
| 6.6.4. | Performance of the models in predicting mutagenicity | 40 |
| 6.6.5. | Prediction results | 40 |
| 6.7. | Conclusions of the contractor | 42 |
| 6.8. | Conclusion by PPR Panel | 42 |
| 7. | Applicability of (Q)SAR analysis in the evaluation of developmental and neurotoxicity effects of pesticide metabolites: outcomes of outsourced activity | 43 |
| 7.1. | Predictive performance of (Q)SAR/read-across strategy for neurotoxicity | 44 |
| 7.2. | Predictive performance of (Q)SAR/read-across strategy for developmental toxicity | 44 |
| 7.3. | Conclusions of the contractor | 46 |
| 7.4. | Conclusions by the PPR Panel | 46 |
| 8. | Potential exposure to pesticide metabolites in the human diet | 47 |
| 8.1. | Introduction | 47 |
| 8.2. | Types of exposure scenarios | 49 |
| 8.3. | Estimation of metabolite levels | 49 |
| 8.4. | Models used for calculating acute and chronic dietary exposure | 50 |
| 8.5. | Conversion factors for estimating metabolite levels | 51 |
| 8.6. | Results and discussion | 53 |
| 9. | Impact of stereochemistry on the toxicological relevance of pesticide metabolites for dietary risk assessment | 54 |
| 9.1. | Terminology | 54 |
| 9.2. | Introduction | 55 |
| 9.3. | Consequences of stereoisomerism for risk assessment | 56 |
| 9.4. | Data requirements regarding stereochemistry under Regulation (EC) 1107/2009. | 57 |
| 9.5. | Assessment of the toxicological relevance of isomer ratio changes of metabolites | 58 |
| 9.6. | Exposure assessment | 58 |
| 9.7. | Conclusion on applicability of approaches to address relevance of metabolites to isomer ratio changes. | 59 |
| 10. | Critical issues and uncertainties | 60 |
| 10.1. | Critical issues in toxicology | 60 |
| 10.1.1. | TTC for genotoxicity | 60 |
| 10.1.2. | TTC for neurotoxicity | 60 |
| 10.1.3. | TTC for endocrine disruptors | 60 |
| 10.1.4. | New TTC values for acute exposure | 61 |
| 10.2. | Critical issues on exposure | 61 |
| 10.2.1. | Ratio metabolite/parent estimations for estimating the exposure of pesticide metabolites | 61 |
| 10.2.2. | Extent of uses of the pesticide | 61 |
| 10.2.3. | General recommendations on these two issues: | 61 |
| 10.2.4. | Use of residue data for metabolite estimation | 62 |
| 10.2.5. | Acute exposure to metabolites | 62 |
| 10.2.6. | Cumulative and aggregate exposure | 62 |
| 10.3. | Critical issues on stereoisomers | 63 |
| 10.4. | Uncertainties affecting the assessment | 63 |
| 11. | Proposed strategy for assessing the toxicological relevance of pesticide metabolites. | 71 |
| 11.1. | Assessment scheme | 72 |
| 11.1.1. | Assessment scheme for chronic exposure | 72 |
| 11.1.2. | Assessment scheme for acute exposure | 75 |
| 11.2. | Acute and chronic toxicity - Testing Strategy | 76 |
| 11.2.1. | Genotoxicity testing | 76 |
| 11.2.2. | Toxicity testing | 77 |
| 12. | CONCLUSIONS AND RECOMMENDATIONS | 78 |

| | |
|--|-----|
| References | 80 |
| Appendices | 86 |
| A. European Guidelines in international context..... | 86 |
| B. Selected Residue definition examples of pesticides with supporting analytical methods for residue monitoring/enforcement..... | 88 |
| C. EFSA and JMPR residue definitions on pesticides for consumer risk assessment..... | 90 |
| D. Examples of metabolite to parent ratio for 10 pesticides and its dependence on use pattern..... | 112 |
| E. Pesticide metabolite estimations - Case studies..... | 115 |
| F. Checklist in support of assessment of adequacy of (Q)SAR Predictions | 170 |
| G. Acute exposure thresholds..... | 172 |
| Glossary..... | 181 |
| Abbreviations | 186 |

BACKGROUND AS PROVIDED BY EFSA

Annex VI of Council Directive 91/414/EEC of 15 July 1991 concerning the placing on the market of plant protection products⁴⁵ sets out uniform principles for the evaluation and authorisation of chemical plant protection products and the active substances they contain. The likely risk to humans, animals and the environment need to be addressed. Assessment of the risk for the consumer is a major part of this process. This assessment requires the identification of metabolites and of degradates of the active substances present in food commodities.

Metabolites may be produced from plant metabolism in primary and following crops, from microbiological activity in soil, or from livestock metabolism after consumption of feeding stuffs containing residues. Degradates arise from abiotic physical and chemical processes (e.g. photolysis) and from processing before the consumption of plant and animal commodities (e.g. cooking). In practice the consumer is therefore exposed not only to the active substance as applied, but also to a wide range of chemical compounds as a result of metabolic and degradation processes. The number and amount of distinct compounds, defining the residue pattern the consumer is exposed to, may widely differ from pesticide to pesticide depending on many parameters.

One of the outcomes of the evaluation of an application for use of an active substance on a crop is the establishment of two residue definitions, one for monitoring and one for dietary risk assessment. As outlined in the guidance document⁶ on residue definition, adopted by OECD in 2006, the underlying rationales for these two definitions are different. While the residue definition for monitoring has regulatory purposes for the enforcement of the MRLs (Maximum Residue Levels) and must reflect analytical practicalities, the residue definition for dietary risk assessment may be wider, as its purpose is to assess consumer safety, and it should therefore include all metabolites and degradates of toxicological relevance.

In other words, in order to perform an appropriate assessment of the risk for the consumer, the residue definition for dietary risk assessment should be qualitatively and quantitatively representative of the actual toxicological burden. This means that establishment of the residue definition for dietary risk assessment requires not only a decision on which metabolites or degradates, due to their level, may significantly contribute to toxicological effects, but also an assessment of the toxicological endpoints of interest, and related reference values.

A major difficulty stems from the fact that, from the mixture (active substance, its metabolites and degradates) to which the consumer is exposed, only the toxicological properties of the active substance are in practice directly investigated through the range of toxicological studies required by Directive 91/414/EEC. In contrast, very limited information about the toxicological properties of metabolites and degradates is available in the majority of cases, while requests for further toxicological studies are restricted as far as possible to minimise the use of animals in toxicological testing.

In view of the guidance document on residue definition published by the OECD, and in order to ensure consistency and robustness of expert judgement, EFSA considers that all relevant scientific tools need to be reviewed and evaluated so that they can be used optimally in evaluating the toxicological burden of metabolites and degradates.

Following adoption of this opinion, a guidance document will be developed on the establishment of residue definition for dietary risk assessment. This guidance should be a practical instrument, aimed at helping risk assessors and regulatory authorities to adopt such definitions based on a combination of

⁴EC (1991). Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal L 230, 1-290. 19 August 1991

⁵In the course of drafting this opinion, this Directive was replaced by Regulation (EC) No 1107/2009.

⁶OECD, Environmental Directorate (Guidance document on the definition of residue, Series on testing and assessment Nr 63. Series on Pesticides Nr. 31, 10-Oct-2006.

scientific tools. This guidance should also be used for identifying cases where further experimental data are needed.

TERMS OF REFERENCE AS PROVIDED BY EFSA

EFSA asked the PPR Panel to develop an opinion on approaches to evaluating the toxicological relevance of metabolites and degradates of pesticide active substances in dietary risk assessment.

The original terms of reference were extended to address also the issue of isomer conversion and therefore amended as follows:

Regarding possible isomer ratio changes of the active substances existing as isomer mixtures or which is an individual isomer, the PPR Panel is asked to address:

- If the approaches and methodologies developed for pesticide metabolites are applicable to the isomer ratio changes of the active substances existing as isomer mixtures or which are an individual isomer
- To identify if relevant, specific issues of dietary risk assessment applicable to active substances existing as isomer mixtures or which are an individual isomer and develop the respective appropriate assessment methodologies or identification of relevant research need.

ASSESSMENT

1. Introduction

The use of pesticides on food and feed crops may lead to residues in edible parts of the plant and hence results in exposure of the consumer to a mixture of compounds including the active substance and/or its metabolite(s), (OECD, 2009a). The number of metabolites varies from pesticide to pesticide and from none to, in some cases, a large array of metabolites found. In addition progress in analytical methods and their increasing sensitivity results in the detection of a growing number of metabolites at low levels. The term metabolite in this opinion refers to a metabolite or a degradation product of an active substance as defined in Regulation (EC) No 1107/2009⁷ (see Glossary). Metabolites have varying relevance for human exposure depending on their inherent toxicities and levels at which they are found. The process of metabolism or degradation of active compounds may give breakdown products maintaining the active moiety responsible for the biological activity and in some cases for the toxic effects, or alternatively the toxic moiety may be modified to reduce or eliminate toxicity. Also a new toxic moiety may be created with a potentially different mechanism of action.

Therefore metabolism studies in soil, plants, and livestock using radiolabelled active substances are requested prior to the authorisation of plant protection products and the active substance that they contain. The objective of these studies is to identify the nature of terminal residues in food and feed commodities (from plant and animal origin) and quantify them. Depending on a number of factors (e.g. mode and time of application, environmental conditions, nature of the crop), the terminal residues may differ between crops, and between crops and animal products. In addition, the residue pattern in rotational crops frequently differs from that in primary crops, which is often related to the residues in the soil after an aging period and uptake by plants.

A crop metabolism study should be submitted for each crop group for which use is proposed. Similarly, depending on potential exposure of livestock, metabolism studies in livestock, e.g. lactating

⁷Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EC and 91/414/EEC. Official Journal L 309, 1-50. 24 November 2009.

goat and laying hen, should be provided. The independent assessment of individual plant and livestock metabolism studies in conjunction with proposed analytical methods for enforcement of MRLs may lead to different conclusions regarding the residue definition for monitoring and for risk assessment in individual plant and animal commodities. Nevertheless, for reasons of pragmatism it is current practice to try to establish common residue definitions for monitoring and for risk assessment (unless suitable conversion factors can be proposed), covering plant and animal products. As the use pattern of pesticides is an evolving process, residue definitions may need to be re-evaluated periodically with the development of new uses for national registration. These re-evaluations affect not only raw plant commodities, but also processed commodities and products of animal origin which may result in a change in the number of metabolites in the residue definitions. Similarly, the evaluation of compounds under Directive 91/414/EEC (and now under Regulation EC (No) 1107/2009) relying on the “one representative use” concept for approval in the EU. Article 4 paragraph 5 of Regulation in the regulation may lead to conclusions based on the evaluation of only a limited number of the existing range of uses of active substances. When additional uses are assessed, novel metabolites may be identified which affect the residue definition.

A comprehensive toxicological dossier (data requirements according to Regulation (EC) No 544/2011⁸) must be developed for parent compounds prior to approval of substances for use within the EU, including toxicokinetic and metabolism studies in mammals. However, specific toxicity studies may be available on only some metabolites, as these data tend not to be provided for the full range of metabolites found. The extent of testing necessary will depend on whether the metabolite is produced at appreciable levels in laboratory species, where the toxicological effects will reflect, at least in part, the toxicity of the metabolite. In contrast, where a metabolite is unique to plants or livestock, no information on potential toxicity will be available from the toxicity testing of the parent compound. Considering the limited toxicity testing resources worldwide and in order to minimise the use of laboratory animals in toxicological testing, new approaches should be considered for the risk assessment of metabolites, taking into account all the available information and using predictive models based on comparative analyses of hazard data from structurally related compounds. In this context, all available alternative scientific tools, need to be reviewed and evaluated for their applicability in the evaluation of the toxicological profile of metabolites of pesticides, so that they can be optimally applied to derive relevant toxicological (threshold) values.

A new Guidance document on the definition of pesticide residues for monitoring and risk assessment was adopted by OECD in 2006, with a slightly revised version published in 2009. The most recent Guidance documents available on the definition of residues (e.g. FAO, 2002; EC, 1997a, b) were taken into account by the OECD during the drafting of this guidance and the FAO manual dated 2002 was the main document from which the OECD Guidance was developed. The OECD Guidance recommends that the residue definition for consumer risk assessment should include those metabolites which, due to their levels present, significantly contribute to the dietary risk. However it does not present tools to evaluate the toxicological burden of pesticide metabolites.

Marketed pesticides can comprise various types of stereoisomer composition: a single mixture, various different mixtures, or a single isomer. Each case should be handled differently.

With respect to the assessment of isomer mixtures, the OECD Guidance states that “in practice the starting point in authorising plant protection products is the mixture of isomers where all metabolites should be found and taken into account”. This can be translated into: the composition of the mixture of stereoisomers in a technical active ingredient has to be known and linked to the hazard and risk profile of the pesticide under consideration. However, the OECD Guidance identifies several aspects that should be considered in deciding whether isomers need special consideration:

- The type of isomers (enantiomers, diastereomers or cis-trans isomers) should be clarified.
- Stability of the isomers (inter-conversion).
- Level of isomers

⁸Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances. Official Journal L 155, 1-66. 11 June 2011.

- Differences in their toxicological properties

1.1. Structure of the Opinion

In the current chapter and in chapter 2 supported by appendices A-C the scope of the opinion is presented as well as background and current approaches on how definitions of pesticide residues are derived. Chapter 3 describes the rationales for four outsourced projects on different tools not involving animal testing to evaluate the potential toxicological impact of metabolites from pesticides. The toxicological consequence of metabolism of the active substance is presented and discussed in chapter 4. The Threshold of Toxicological Concern (TTC) concept and a proposal for a modified TTC approach (including both chronic and acute exposure thresholds) for application to pesticide metabolites is presented in chapter 5 and Appendix G. The application of Quantitative Structure Activity Relationship (Q)SAR methods, with particular reference to genotoxicity alerts, is discussed in chapter 6 and Appendix F. The applicability of (Q)SAR and read-across methods for evaluating developmental and neurotoxic effects of metabolites is presented in chapter 7. To perform a risk assessment, estimates of consumer intakes of all possible pesticide metabolites are required. Therefore, in chapter 8, supported by Appendices D-E metabolite exposure predictions of laboratory animal metabolites and metabolites that are specific to plants and livestock are presented. Conversion factors for converting residues determined in the residue definition for monitoring to values suitable for a dietary risk assessment are also discussed in this chapter. In chapter 9 the applicability of the approaches and methodologies (as developed for pesticide metabolites) to isomer mixtures is discussed. Critical issues as well as uncertainties related to the different chapters in the opinion are presented in chapter 10. In chapter 11 a strategy for assessing the toxicological relevance of pesticide metabolites is proposed. Finally chapter 12 gives conclusions and recommendations for future approaches and research.

2. Current approaches to residue definition for pesticides

2.1. Use of terms “pesticide residue” and “residue definition”

Many Guidelines, Guidance documents and Regulations covering pesticide residues are available (OECD, 2009a; FAO, 2009, 2012; EC, 1997a, b; Regulation (EC) No 1107/2009⁹), Regulation (EC) No 396/2005¹⁰), that differ to some extent from each other on use of terms describing residues. This opinion relates to Regulation 1107/2009, which stipulates that substances or products produced or placed on the market should not have any harmful effect on human or animal health or any unacceptable effects on the environment. The term pesticide residue in Regulation EC (No) 1107/2009 is defined as “one or more substances present in or on plants and plant products, edible animal products, drinking water or elsewhere in the environment and resulting from the use of a plant protection product, including their metabolites, breakdown or reactions products”¹¹.

Most active substances undergo, after application of the formulated product to crops, chemical and biochemical degradation processes generally leading to an overall reduction in residue levels. At the same time (relevant) metabolites and degradates may be formed. The "residue definition" aims to provide a reasonable description of compounds related to the active ingredient initially applied and

- contributing to toxicological burden when the food items are consumed --> residue definition for risk assessment

⁹Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EC and 91/414/EEC. Official Journal L 309, 1-50. 24 November 2009.

¹⁰Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Official Journal L70, 1-16. 16 March 2005.

¹¹Breakdown or reaction products in this opinion are referred to as degradates.

- and/or being applicable to routine residue analytical methodology --> residue definition for monitoring/enforcement of residues present in the food item at time of harvest or slaughter

The analytical methodology used in developing the residue data for submission of a dossier is usually more complex and demanding than that for routine monitoring (OECD, 2009b) and is more likely to provide direct information on metabolite levels. Bridging between the residue definition for monitoring/enforcement and the residue definition for risk assessment is achieved by applying a so-called conversion factor (see chapter 8). Ideally, the residues trials will generate all possible relevant analytes covering both forms of the residue definition. The selected examples in Appendix B illustrate typical scenarios encountered when the residues of a pesticide are defined. As an example, with haloxyfop-P-methyl, a rapid cleavage of the methyl ester to the free acid and conjugation in plants is observed. The residue definition includes the ester, salts and conjugates, as the available analytical method relies on hydrolysis of total haloxyfop residues and its conversion to either methyl or butyl ester and determination by GC-MS. This technique does not distinguish between *R* and *S* haloxyfop and its esters and conjugates, and therefore "any ratio" is included in the residue definition.

2.2. Residue definitions

The residue definitions for dietary risk assessments thus should consider all residue components of toxicological interest and usually include the parent compound together with all or the main toxicologically relevant metabolites and/or degradation products (OECD, 2009a; FAO, 2009a, 2012). Various factors encompassing exposure potential and relevant toxicity are considered before inclusion of a metabolite in the risk assessment residue definition. Residue definitions for dietary risk assessment at EU level are now being published per compound in EFSA Conclusion reports, and with the EU Commission published review reports associated with Annex I listing when the residue levels (MRLs, HR and STMR) have been included. For both monitoring and risk assessment, a collated source of EU residue definitions (both monitoring/enforcement and risk assessment) can be found on the website of the German Federal Institute of Risk assessment (BfR, 2009). Historically residue definitions for risk assessment have been publicly available only as either national evaluations or evaluations by the Food and Agriculture Organisation of the United Nations/World Health Organisation Joint Meeting on Pesticide Residues (FAO/WHO JMPR).

Residue definitions for monitoring/enforcement are intended to be as simple as possible and often refer only to the active substance itself (OECD, 2009a; FAO, 2009a, 2012). These can be used to indicate exceedences of the MRL of the pesticide and can be analysed and quantified easily by a broad base of national laboratories, ideally using a multi-residue method. For monitoring/enforcement, current MRLs and corresponding residue definitions at EU level are, for example, available from the EU Pesticide database¹² and Regulation (EC) No 396/2005 and its amendments.

2.3. Comparison of residue definitions by EFSA and JMPR

Since food safety nowadays is a global issue, European Guidelines are explained in an international context in Appendix A. Within the framework of the Codex Alimentarius, the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) is the risk assessment body responsible for establishing residue definitions. In the outsourced project on metabolic processes (AGES, 2010) it was noted that the residue definitions across active substances are not always the same, when comparing EFSA conclusions and JMPR reports.

The PPR Panel screened 43 decisions on residue definitions on pesticide active substances for monitoring/enforcement and risk assessment taken by EFSA in the period of 2008-2010 and JMPR to build on the work by AGES and to investigate reasons why the conclusions differ. It was noted that for only 14 compounds did EFSA and JMPR derive the same residue definitions for risk assessment and for 24 compounds EFSA included more metabolites in the definition than JMPR. Selected examples are presented in Appendix C.

¹²EU pesticide database http://ec.europa.eu/sanco_pesticides/public/index.cfm

The risk assessment and residue definitions of active substances were often concluded by JMPR prior to the EFSA assessments, which may have influenced the data package submitted as well as the interpretation of the guidelines followed. Additionally, JMPR tends to consider a wider range of uses than those indicated in the Annex to Regulation (EU) No 540/2011¹³. Over time the emphasis on the relevance of metabolites has increased. This is common to both EFSA and JMPR, however, a more cautious approach seems to be taken in the EU than within Codex, with an associated greater attention to metabolites present at low levels.

The recently adopted OECD Guidance Document on Definition of Residue (OECD, 2009a) provides an overall framework rather than the detailed guidance required to achieve harmonised residue definitions. Once opinion follow on guidance based on the current opinion has been developed by EFSA, the PPR panel suggests that, to promote international harmonisation and since the methodologies discussed are expected to have wide applicability, the OECD are asked to consider whether inclusion of this approach could be a useful addition to their Guidance on Definition of Residues.

2.4. Current status and future development of analytical methods for establishing residue definitions

Toxicological relevance and predicted exposure levels of residues are the primary drivers for whether a metabolite should be included in a residue definition. However, it is also often the case that analytical methods are a constraining factor in decision making on what the residue definitions should be. This is due to the physico-chemical properties of a pesticide and its various metabolites that can affect extraction/clean-up/chromatographic properties and/or detection or a combination of all of them. The choice of analytical method can be influenced by specific methodological factors, and there are a variety of reasons why an analyte or series of analytes can present particular challenges. In some circumstances, it may not be feasible to analyse a parent pesticide and the metabolites of that pesticide simultaneously, especially if the nature of a metabolite is quite different from that of the parent, for example, for triazole pesticides, the parent molecule commonly needs to be analysed by a different method than to the metabolites of interest that include triazole alanine and triazole acetic acid. It may not be possible to determine a pesticide and its metabolites separately; this can be an issue with methods that involve a derivatisation step e.g. for cycloxydim where the parent and metabolites are converted to a glutaric acid derivative, thereby using a common moiety approach.

A different problem for analysis of some pesticides is that a unique hydrolysis step may be required to enable the residues to be extracted and this may have an impact on the recovery of free and conjugated residues; such a hydrolysis step would not be suitable for determination of other pesticide analytes. For those pesticides that are particularly difficult to determine, specifically adapted "single residue methods" or "common moiety methods" should be applied.

Some common moiety approaches may further be problematic because they are not specific to the pesticide that has been applied e.g. for bisdithiocarbamates where residues are converted during analysis to CS₂. All ethyl- and propyl dithiocarbamates, including their metal complexes like mancozeb, are detected through generation of carbon disulfide and assayed by colorimetric or chromatographic methods. It is not possible to distinguish the amount of CS₂ found into parent compounds and metabolites, as this information is lost in the process. Furthermore, other precursors of CS₂ in the sample may contribute to the result. In this case it is not possible to perform a refined risk assessment since different dithiocarbamate pesticides and other substances have different toxicities. Therefore, if there is a choice, specific methods that quantify concentrations of individual compounds of related residues are preferred over common moiety methods. These common moiety methods may also not be ideal for assessing cumulative exposures since whilst they can determine some combinations of residues, the analysis masks the information on residue levels of individual active

¹³Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. Official Journal L 153, 1-186. 11 June 2011.

substances or metabolites that would otherwise be available if specific methods for each analyte had been used.

The generic requirements of an analytical method in terms of performance and to a lesser extent cost can result in compromises on the nature and level of detectable metabolites that can be included at any one time. The various difficulties described and the challenge of being able to determine a large number of pesticides simultaneously are why the international community has derived the two-fold approach in setting different residue definitions (as described in chapter 2.1) in order to circumvent this compromise, and, on occasion, allow the use of different analytical approaches to cover conflicting analytical requirements. The methods for pre-registration of active substances aim to identify and quantify the active substance and metabolites (i) to generate residues data on which consumer dietary exposure assessments are based, and (ii) to support studies on the fate and behaviour of the active substance in foodstuffs, the environment, ecotoxicology and toxicology (EC, 2000). The methods of analysis can use more complex analytical approaches if warranted in order to fully cover the scope of all the components that are in the residue definition for risk assessment. Therefore, during the development of these analytical methods the applicant aims to tailor the methods for various matrix types to all the analytes of potential interest from a risk assessment perspective.

Such a tailored approach to method development is not suitable for the analytical methods for monitoring and enforcement, since the aim of these post-registration methods that are used by monitoring laboratories is to fit preferably into existing multi-residue methods, which reliably detect several hundreds of compounds in a cost effective manner. It is thus not feasible to achieve the concurrent analysis of the large number of potentially applied pesticides together with their toxicologically relevant metabolites and/or breakdown or reaction products. Therefore, the simpler monitoring and enforcement residue definition is used based on a 'marker concept' and MRLs that are set cover only the level of residue for the analyte(s) included in the monitoring and enforcement residue definition. Applicants are requested to demonstrate, whether the components in residue definition for monitoring and enforcement can be analysed reliably using multi-residue methods. To do this, applicants may test existing published multi-residue approaches or they may justify that the method they have proposed uses commonly available laboratory equipment. Ideally, all pesticides would be determinable by multi-residue testing; however this is not always achievable and 'single methods' for particular pesticides need to be developed and used, an approach which tends to add significant additional cost to the monitoring programmes.

The herbicide glyphosate is an example of a compound which has required a "single method" approach; glyphosate, through its amphoteric nature (glyphosate carries both negative and positive charges at physiological pH conditions), escapes conventional extraction and clean-up schemes and has to be analysed by a sequence of tailor-made steps such as ion-exchange extraction and pre- or post-column derivatisation to achieve the necessary limits of detection.

The approach to derive residue definitions for both for monitoring and risk assessment as described earlier is only really possible if a conversion factor (as a multiplication factor) can be proposed to enable the residue level determined in the monitoring to then be converted to a corresponding level for risk assessment purposes. In this way, a risk assessment can be performed covering the components of interest to risk assessment that were not actually analysed in the residue monitoring. Conversion factors are further discussed in chapter 8.5.

Practical experience of working with conversion factors seems to support the view that they are not easy to set, are not available for every crop circumstance, and they are not always used (see chapter 8.5.). The latest advances in the analytical methodology (generic and simple extraction procedures with advanced mass spectrometers with liquid and/or gas chromatography) facilitate simultaneous determination of an increasing number of pesticides and metabolites, without compromising the reliability of the methods. However, conversion factors are still considered necessary because there tends to be a limitation on the total number of compounds analysable in a multi-residue method, the availability of analytical standards for metabolites, the time required to process the information and

the costs of the analysis. Whilst technical developments and research can be harnessed to solve analytical problems and improve the reliability and efficiency of methods of analysis, issues of scale and cost will always need to be addressed. The utility of monitoring programmes in assessing dietary exposures to pesticides, not only considering compliance with MRLs, depends on the ability to evaluate as large a number of pesticide residues as possible at low levels, and to ensure that a full risk assessment covering all relevant metabolites can be performed based on the results obtained. In the future, these risk assessments will also need to cover cumulative exposures.

2.5. Relevant metabolites

This opinion relates to Regulation (EC) No 1107/2009 which stipulates that special attention should be paid to whether the metabolite poses a higher or comparable risk to organisms than the parent substance or if it has certain toxicological properties that are considered unacceptable. The OECD Guidance document (OECD, 2009a) proposes a list of aspects to be considered when deciding on inclusion or non-inclusion in the dietary risk assessment when the different aspects presented in (Table 1) are considered.

Table 1: Aspects to consider when deciding on inclusion or non-inclusion of metabolites in the dietary risk assessment (excerpt from OECD Guidance document, 2009a)

| More likely to be included | Less likely to be included |
|---|---|
| Parent compound is highly toxic | Parent compound has low toxicity relative to expected exposures |
| Metabolite/degradate likely to be found in commodities that are human food | Metabolite/degradate found in only one matrix at 10-20% of the total residue (unless that matrix is a major human food) |
| Metabolite/degradate levels in magnitude of residue studies exceed those expected from metabolism studies | Metabolite/degradate present at very low levels (in mg/kg) |
| Metabolite/degradate is not formed through metabolism in rats | Metabolite/degradate structure is similar to innocuous chemicals |
| Parent compound was non-detectable, but metabolites were found in high levels in metabolism studies | Metabolite/degradate occurs predominantly in animal feeds rather than commodities that are human foods |
| | Hydrophilic metabolites less toxic than the parent compound |
| Considerations for drinking water: | Considerations for drinking water: |
| Environmental degradate is persistent | Environmental degradate is short-lived |
| Environmental degradate has low soil binding potential | Environmental degradate has high soil binding potential |
| Degradate is detected in water monitoring studies | Degradate is not detected in terrestrial field dissipation studies |

The OECD Guidance document does not explain in detail how to assess the toxicological burden of metabolites. This is therefore addressed in the following chapters.

3. Rationale for the outsourced projects

The establishment of the residue definition for the purpose of consumer risk assessment involves a decision on which metabolites are of toxicological concern.

As only limited information on the toxicological properties of metabolites is usually available, EFSA considers that assessment methods and alternative scientific tools, not involving animal testing, need to be evaluated. Such a consideration and/or the development of these approaches are needed to optimise the consistency and robustness of the evaluation of the toxicological profile of the metabolites from plant protection products. EFSA outsourced four activities to develop this task:

- The metabolic pathways and degradation processes may modify the toxicological properties of pesticide active substances. Due to the lack of experimental toxicological data on metabolites, the EU peer review of pesticides used a case by case approach in the evaluation of toxicological relevance of metabolites. Factors such as the presence of the metabolite in *in vivo* metabolism studies and structural similarity have been used as possible indicators of toxicity similar to parent compound. The aim of the outsourced project was the assessment of the scientific evidence, regarding the possible influence of metabolism on the toxicity of pesticides, see chapter 4.
- The Threshold of Toxicological Concern (TTC) approach is based on the principle of establishing a human exposure threshold value for chemicals, below which there is a very low probability of an appreciable risk to human health. The TTC approach allows identification of threshold values for chemicals of unknown toxicity considering only their structure and toxicity data on chemicals sharing broadly similar structural characteristics. The outsourced project was aimed at assessing the usefulness of the TTC approach for pesticide metabolites, while taking into account the legal framework of Directive 91/414/EEC (now Regulation (EC) No 1107/2009), see chapter 5.
- Applicability of (Q)SAR analyses - the basic assumption of (Quantitative) Structure Activity Relationship (Q)SAR analysis in risk assessment is that biological activity of a chemical depends on its intrinsic properties and can be directly predicted from its molecular structure and inferred from properties of similar compounds whose activities are known. The outsourced project was aimed at exploring the applicability of computational methods in the evaluation of toxicological relevance of pesticide metabolites with a focus on genotoxicity alerts, see chapter 6.
- Applicability of (Q)SAR analysis for developmental toxicity and neurotoxicity - in order to refine a proposed assessment scheme for acute effects involving the TTC approach (which is based on long term effects), and with regard to identifying metabolites that would need to be considered in this scheme, a project was outsourced to evaluate if (Q)SARs can be used to identify pesticides having developmental and/or neurotoxic effects. Experience has shown that these are generally the critical endpoints following short term exposure, see chapter 7.

It is noted that none of the outsourced projects sought to specifically address the possible impact of stereoisomerism since this part of ToR (Terms of Reference) was added at a later stage. The PPR Panel, building on the outsourced projects, the OECD guidance document, and its own research will in this opinion present approaches on how to evaluate the toxicological relevance of metabolites of pesticide active substances, including stereoisomers, in dietary risk assessment.

4. Impact of metabolic processes on the toxicological properties of pesticide residues

To review current knowledge on the importance of metabolic processes to the toxicology of pesticides, the project “Impact of metabolic and degradation processes on the toxicological properties of residues of pesticides in food commodities” was outsourced to the Austrian Agency for Health and Food Safety (AGES). The contractor addressed two main issues related to the impact of metabolism in the evaluation of the toxicological relevance of metabolites of pesticide Active Substances for Dietary Risk Assessment: a) the criteria applied for evaluating pesticide metabolites in different regulatory contexts; b) evaluation of metabolic pathways with respect to their toxification/detoxification potential for selected groups of pesticides (AGES, 2010).

4.1. Evaluation of toxicological profiles of pesticide metabolites in different regulatory contexts (results of outsourced project)

AGES screened scientific literature and guidance documents in order to evaluate different approaches for handling metabolites on the basis of their toxicity. The criteria applied for evaluating the toxicological profiles of pesticide metabolites were also considered by analysing decisions on metabolites made by EFSA and JMPR between 2006 and 2009.

The contractor noted differences between EFSA and JMPR on the inclusion of metabolites in the residue definition. It was observed that no clear criteria are available to support the decision of when the toxicity of metabolites is considered covered by the studies on the active substances, on the basis of their concentration in body fluids. Decisions are made on a case by case basis, based on expert judgement.

As a conclusion of their review, AGES suggested some criteria to be applied in the evaluation of pesticide metabolites:

- A metabolite occurring in rat or livestock metabolism studies at > 10% in body fluids needs to be assumed as present in sufficient amounts to contribute to the overall toxicological profile, as considered for pharmaceuticals.
- Metabolites and their precursors/intermediates found in livestock (ruminants, poultry) should be considered as present in rat metabolism even if not measured, based on the assumption that warm blooded animals have comparable metabolism.
- Conjugates of metabolites found in plants should not be automatically assumed to be of no concern, since they can be cleaved to release free unconjugated metabolites.

These conclusions are discussed by the PPR Panel in chapter 4.3.

4.2. Evaluation of metabolic pathways with respect to their toxification/detoxification potential for selected groups of pesticides as presented by the contractor.

AGES analysed the potential impact of the structural changes to the parent compounds during metabolism on the toxicological properties of the derived molecules through a comparison of the toxic effects of the metabolites and parent compounds. All of the active pesticide compounds entered on CIRCA (424) were considered, and 11 chemical classes of pesticides were selected on the basis of common metabolic pathways, representativeness in the chemical group (at least four active substances), number of active substances in Annex 1 of Directive 91/414/EEC and number of new compounds. The chemical groups selected were: sulfonylureas, triazoles, aryloxyphenoxy-herbicides (FOPs), chloroacetamides, strobilurines, dinitroanilines, benzimidazoles, neonicotinoids, carboxylic acids and amides, cicarboximides and macrocyclic lactons, containing 56 parent compounds and their metabolites. All the data available on the ADME studies and on the toxicological properties of the active substances and metabolites were collected and evaluated.

The descriptions of the ADME studies in the evaluated DARs were very heterogeneous and not sufficiently detailed. The large majority of studies available on metabolites were acute toxicity studies giving information on LD₅₀ and on some clinical observations. Data on subchronic or developmental studies were available only in a few cases. In addition, the studies on parent compounds and metabolites were carried out in different species (rat or mice) or strains, and/or in different experimental conditions. The presence of more than one metabolic step between an active compound and its metabolites in some cases impaired the attribution of a toxification potential to a specific metabolic reaction.

Despite these constraints, and that this exercise to compare the toxicity of the metabolites and parent compounds has a high level of associated uncertainty, some general conclusions were drawn by the contractor:

- It is not possible to attribute a toxification/detoxification potential to a specific metabolic step, although a number of metabolic steps were identified as probably not causing higher toxicity of metabolites, as shown for several compounds (simple demethylation of the ring or side chain, simple hydroxylation of the ring system without any cleavage of the ring, hydroxylation of another ring position than the parent, conjugation of metabolite with amino acids).
- The metabolic pathways are in most cases specific to the chemical groups.
- The toxification/detoxification depends on the toxicological profile of the parent compounds: a detoxification reaction in one case could be a toxification step in another case.

As a general suggestion, the contractor proposed a revision of the study design and harmonisation of criteria for the selection of radiolabel positions and of kinetic parameters for metabolites in ADME studies.

The contractor also suggested the replacement of acute toxicity studies with metabolite testing using subchronic studies: the 90 days rat study OECD TG 408 (OECD, 1998) was recommended. The use of animals of the same species, strain, age and sex as used in the toxicity studies of the active substance was also recommended, in order to avoid potential differences in detoxification processes.

4.3. Conclusions of the PPR Panel

4.3.1. Evaluation of metabolic pathways with respect to their toxification/detoxification potential for selected groups of pesticides.

The PPR Panel considers that the outcomes of the case studies on the potential impact of structural changes to parent molecules on the toxicological profiles of derived compounds, although related to a limited number of chemical groups, reflect the inadequacy of the available database on toxicokinetics and on toxicological profiles of pesticide metabolites.

The PPR Panel outlines the need for adequate toxicokinetic data which should be used to help in study design in order to improve the efficiency of toxicity testing of pesticide metabolites. The new OECD Guidance document on toxicokinetics OECD TG 417 (OECD, 2010), meets the recommended criteria: it addresses the main requirements to obtain data on absorption, distribution, metabolism, excretion and potential bioaccumulation and to provide useful information for planning and evaluating the toxicity studies and for understanding the mode of action of the compounds. Specific recommendations are made on the use of the same animal species and strain for ADME and toxicological studies on metabolites. Studies on the possible effects on enzyme induction/inhibition are considered. In line with TG 417, the PPR Panel also recommends the use of physiologically-based pharmacokinetic (PBPK) modeling as an approach to be considered in the assessment of ADME processes. PBPK models are quantitative descriptions of the absorption, distribution, metabolism and excretion (ADME) of chemicals in biota based on interrelationships among key physiological, biochemical and physicochemical determinants of these processes. PBPK models are applied in pharmaceutical research and health risk assessment in facilitating the prediction of inter-individual, interspecies and route-to-route differences in dose metrics based on physiological and physicochemical properties.

ADME processes usually have considerable complexity where physiological, physico-chemical and metabolic processes contribute to the fate of a compound in the organism. PBPK models tend to

simplify and separate the processes in a multi-compartment model, where the compartments represent predefined organs or tissues (or groups of these) connected by a stream of body fluids.

Recently a guidance document on "Principles of Characterizing and Applying PBPK Models in Risk Assessment" has been developed within the framework of the IPCS project on the harmonisation of approaches to the assessment of risk from exposure to chemicals (IPCS, 2010)

4.3.2. Considerations by the PPR Panel on conjugated and bound residues

The detoxification mechanisms of exogenous organic compounds in higher plants lead to different products, conjugates and bound (also known as non-extractable) residues, with different toxicological properties. Pesticide conjugate metabolites are formed by reaction of parent compounds or their phase I metabolites with endogenous substrates like glutathione, sulphate or sugars. In general, some conjugates can readily be cleaved. Due to possible release of unconjugated product, conjugates should be considered as potentially bioavailable as their unconjugated products. As a consequence they should be included in the evaluation of toxicological relevance.

The number of conjugate types is extensive, the most frequently reported being glycosides, sulphates and metabolites resulting from glutathione conjugation. Limited information is available on the stability of conjugates to enzymatic and hydrolytical attack in the gastrointestinal tract of humans and livestock species (DEFRA, 2007). The outcomes of a project carried out with twelve different β -D-glucosides, as the most common type of conjugate, confirmed the differences in behaviour related to the different functional chemical groups and glycosidic linkages (DEFRA, 2007), suggesting that the evaluation of toxicological relevance should be done on a case-by-case basis.

The situation with bound residues is more complex than for conjugates. Bound residues covers a range of molecules:

- covalently bound but otherwise intact metabolites;
- metabolites that are physically encapsulated within the macromolecular matrix of plant and animal tissues;
- transitory simple molecules, such as malate, pyruvate and others which are used to produce the plethora of biomolecules in the plant.

An approach to distinguish the covalently bound metabolites and the endogenous labeled biomolecules that have become naturally incorporated has to be found. Whilst these types are considered "bound residues", it is generally agreed (Sanderman, 2004) that only the covalently bound or physically encapsulated but intact molecules are of toxicological concern. The criterion to apportion the total radioactive residue (TRR) which is unextractable between the randomly labeled biomolecules and the other bound metabolites is therefore to use hydrolytic or enzymatic cleavage to liberate the metabolites. Where no metabolite can be cleaved, the residual TRR tends to be considered as not bioavailable and is not taken into account for toxicological relevance. The data generated by more sophisticated studies (DEFRA, 2007) suggests that routine chemical approaches to release bound residues from unextractable TRR may over-estimate bioavailability of bound residues. It is recognised that the available research on bioavailability of xenobiotics is currently limited.

4.3.3. Evaluation of toxicological profiles of pesticide metabolites.

The PPR Panel, taking into account the comments/suggestions made by the contractor on the criteria for evaluating toxicological profiles of metabolites as well as the current approach applied by EFSA in peer review of pesticides, concludes that:

- EFSA decisions on the toxicity of pesticide metabolites should be made on a case by case basis considering different factors and not only the concentration of metabolites in body fluids.
- Due to the wide heterogeneity of available data it is difficult to establish a standardised procedure in the evaluation of relevance of metabolites to the overall toxicological profile. A pragmatic approach needs to be followed, on a case-by-case basis, taking into account several factors such as the presence of the metabolite as an intermediate in the rodent metabolic pathway, the similarity of the chemical structure of the metabolite and the parent compound, and the structural similarity of the metabolite to known classes of toxic compounds.
- The data on livestock metabolites have mainly qualitative significance considering the restricted number of tested animals: a ruminant metabolism study can be carried out on a single animal. The metabolism studies in livestock are carried out under different exposure conditions and using different dose ranges than rat studies. Additionally, differences in metabolic pathways and in the extent of metabolism can be envisaged in different species. The information on the intermediates in the metabolic pathway of the rodent could be of importance in the evaluation of the relevance of the livestock metabolites, and this should be done case-by-case using a weight of evidence approach.
- The PPR Panel considers that an improved understanding of the behaviour of pesticide conjugates in the gastrointestinal tract of human and livestock species is needed, e.g. the gut of ruminants with its specialised stomach anatomy and physiology, and associated microflora is quite different to that of rats and humans, and the impact of this on the dietary risk assessment could benefit from further study.
- The PPR Panel considers that a case-by-case approach to the evaluation of conjugates and bound residues needs to be adopted, that takes account of the experimental methods used in investigating these types of residues. Reasonable effort should be made in terms of methodological approaches to demonstrate that residues are bound prior to concluding that they are not of relevance to the risk assessment. Where metabolites are found as conjugates, it is usually reasonable to conclude that they are bioavailable and the estimation of exposure to metabolite should be based on the sum of the free and conjugated residues. If specific evidence is available to support a different approach for conjugates, then this should be assessed on its scientific merit.

5. Threshold of Toxicological Concern (TTC)

The Threshold of Toxicological Concern (TTC) approach was considered by the PPR Panel, as a tool for providing scientific advice about possible human health risks from low levels of exposure to metabolites of pesticides. The TTC approach is also the subject of a separate opinion from the EFSA Scientific Committee (EFSA, 2012) related to the relevance and reliability of the TTC concept for application more general application in the food and feed area.

The TTC approach is based on a fundamental principle of toxicology, that toxicity depends upon dose and duration of exposure¹⁴. The TTC is based on a number of generic structural and other characteristics, where exposure threshold values could be established for chemicals below which there is no appreciable risk to human health. The TTC approach therefore enables the identification of the relevant threshold value for a chemical of unknown toxicity considering only its structure and a toxicity database on chemicals sharing broadly similar structural characteristics. It should be noted that the TTC exposure values are derived using a probabilistic approach.

¹⁴For an explanation on the ongoing discussions regarding low-dose toxicity, see chapter 5.4.

The first scheme for predicting the hazard of chemicals based on their structure was developed by Cramer et al., (1978). Three different chemical classes were proposed, with increasing order of oral toxicity, based on whether the compound is a normal constituent of the human body, its potential reactivity and the nature of functional groups present in the molecule. A decision tree using a series of structure-related questions was applied to allocate the chemicals into one of the three classes. The logic of the sequential questions was based on contemporary knowledge on toxicity and on metabolic pathways in different mammalian species.

The Cramer classification scheme was further developed and revised following extensive analyses of available chronic oral toxicity data (Munro 1990; Munro et al., 1999; Kroes et al., 2004). The distribution of NOELs (No Observed Effect Levels) for chronic effects were plotted for each Cramer class and the 5th percentile of each distribution was used to derive a threshold value, with a 95% probability that the NOEL of an unstudied compound allocated to the same class would be higher than the value derived. TTC values were obtained for the Cramer classes by dividing the 5th percentile NOELs by the default uncertainty factor of 100 (to give TTC values of 30, 9, 1.5 µg/kg bw/day) and multiplying by a default body weight of 60 kg (1800, 540 and 90 µg/person/day for classes I, II and III respectively). Regarding neurotoxicity, the overall distribution of NOELs for organophosphates (OPs), the most potent compounds in the neurotoxicity database, was around one order of magnitude from the distribution of NOELs for other compounds in Cramer class III. A TTC value for OP compounds (0.3 µg/kg bw/day or 18 µg/person/day) was proposed and a step to identify a structural alert for OPs was introduced into the decision tree (Kroes et al., 2004). The TTC values for the Cramer classes were developed for application to chemicals with no structural alerts for genotoxicity. A TTC value for substances with a structural alert for genotoxicity (0.15 µg/person/day or 0.0025 µg/kg bw/day) was established by linear extrapolation from the TD₅₀ values obtained from animal cancer studies to a risk of 1 in 10⁶, through the analysis of an expanded version of the Carcinogenic Potency Database (CPDB, US FDA, 1995; Gold and Zeiger, 1997) using the results from genotoxicity tests and structural alerts for genotoxicity. A number of high potency genotoxic compounds (aflatoxin-like, N-nitroso-, azoxy-compounds), as well as certain other very potent compounds (steroids, polyhalogenated dibenzo-p-dioxins and dibenzofurans) may still be of concern at the TTC of 0.15 µg/person/day and were therefore excluded from the TTC scheme (Cheeseman et al., 1999; Kroes et al., 2004). All metals and inorganic chemicals were also excluded since they were not covered by the database.

The science behind the TTC approach was critically examined by the EFSA Scientific Committee (EFSA, 2012) in order to evaluate whether the human exposure threshold values, for cancer and non-cancer endpoints, are sufficiently conservative. The information on the toxicological database sources used, and the types of endpoints that determined the NOELs were considered. In addition an assessment of the original published papers and reports referenced in the database on the substances in the lowest 10th percentile of the distribution of NOELs for classes I and III was carried out, in order to assess the quality of the studies and whether the NOELs identified were appropriate.

The EFSA Scientific Committee concludes that, where a conservative estimate on human exposure is available, the TTC values for compounds with genotoxic alerts, with anti-cholinesterase activity, and compounds classified in Cramer class I and III are sufficiently conservative to be applied in risk assessment of substances of unknown toxicity present at low levels in food. The TTC value for Cramer class II substances, derived from toxicological data on only a few compounds, is not well supported by the presently available databases. The suggestion of the EFSA Scientific Committee is to treat substances that would be classified in Cramer Class II as if they were Cramer Class III substances.

The EFSA Scientific Committee, following the analysis of substances classified for reproductive and developmental toxicity under the EU legislation, also considered the TTC values for Cramer class I and III sufficiently protective for these effects (EFSA, 2012).

The EFSA Scientific Committee considered the situation with regard to substances that may have endocrine-mediated toxicity since the TTC approach might not be applicable to such substances due to uncertainty about low-dose effects (Kroes et al., 2004; Cheeseman et al., 1999). The Scientific Committee also identified steroids as a group that includes some potent carcinogens. The Scientific Committee overall concludes (details in footnote¹⁵) that in most situations where the TTC approach could be applied there would be no *a priori* knowledge that a substance has endocrine mediated activity. The Scientific Committee recommends that if there are data showing that a substance has endocrine mediated toxicity, then the risk assessment should be based on those data, rather than using the TTC approach, as would be the case for adverse data on any other endpoint.

The SC TTC opinion does not provide any specific recommendations on which computational genotoxicity tool could be used.

The Scientific Committee is not confident about the general applicability of available proposals for adjusting TTC values for short term exposure to substances with structural alert for genotoxicity. It is recommended to address the issue of less than chronic exposure case-by-case by considering the margin between the appropriate TTC value (without any adjustment for duration of exposure) and the estimated dietary exposure.

The Scientific Committee concluded that the TTC approach should not be used for the following (categories of) substances: high potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds, benzidines, hydrazines), inorganic substances, metals and organometallics, proteins, steroids, substances with a high potential for bioaccumulation, nanomaterials, radioactive compounds, and mixtures of substances containing unknown chemical structures. A revision and refinement of the TTC scheme, based on the updated databases and on the advances in knowledge is recommended for the future.

The EFSA Scientific Committee opinion on TTC concludes that the TTC values provide adequate assurance of protection of sensitive subpopulations, with the exception of young infants under the age of 6 months, where careful consideration in the application of TTC is needed. The TTC values should be expressed in µg/kg bw/day for comparison with exposure for the respective age groups.

The Scientific Committee noted that the current TTC values for non-cancer endpoints are applicable to chronic exposure, as, with the exception of the TTC value for organophosphate and carbamate structure, they were derived from databases that do not address effects from acute exposure. The

¹⁵From EFSA 2012: "Intensive discussions are also taking place within the European Union under the aegis of the Community Strategy for Endocrine Disruptors, which is addressing the key requirements of further research, international co-operation, communication to the public, and appropriate policy action. A draft of the measures concerning specific scientific criteria for the determination of endocrine disrupting properties in relation to human health impacts is anticipated to be ready by the end of 2013. These measures are required, in particular, for the legislation governing REACH and the Plant Protection Products Regulation, but the intention is to develop a systematic approach for the identification and assessment of endocrine disruptors which can be applied across the different pieces of EU legislation. The general concept should be consistent and should ensure that endocrine disruptors are dealt with in a consistent and co-ordinated manner across the EU (EC, 2011a)."

"Regarding the issue of substances that may have endocrine-mediated toxicity, the Scientific Committee concludes as follows:

- a. In most situations where the TTC approach might be applied, there would be no *a priori* knowledge that a substance has endocrine activity.
- b. If there are data showing that a substance has endocrine activity, but the human relevance is unclear, then these data should be taken into consideration, case-by-case, in deciding whether or not to apply the TTC approach.
- c. If there are data showing that a substance has endocrine-mediated adverse effects, then, as would be the case for adverse data on any other endpoint, the risk assessment should be based on the data, rather than the TTC approach.
- d. In view of the extensive work, currently ongoing, to develop an EU-wide approach for defining and assessing endocrine disruptors, once that approach is finalised it will be necessary to consider any impact it may have on the use of TTC approach.
- e. In the meantime, the Scientific Committee recommends that untested substances, other than steroids, can be evaluated using the TTC approach recommended in this opinion."

Scientific Committee considered that there is insufficient information to enable any recommendations for a reliable/appropriate means of adjusting the TTC values for shorter durations of exposure. However, there is no scientific reason why this should not be possible, with a suitable database. In cases where TTC values are exceeded the use of a refined approach for exposure assessment and/or chemical specific toxicity data on a case-by-case basis was recommended. A software package¹⁶ is available to estimate toxic hazard of chemicals by applying a decision tree approach, including the original Cramer rulebase, with extensions, and the TTC decision tree of Kroes et al., (2004).

The Scientific Committee recommends that when the TTC approach is applied to substances with closely related structures and to which there is co-exposure, it may be appropriate to sum their exposures, as would be done in a cumulative risk assessment on substances with the same mode of action.

5.1. Current applications of TTC concept

The PPR Panel reviewed the current fields of application of the TTC approach in different regulatory contexts.

5.1.1. Additives: JECFA and EFSA

The TTC approach was adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to evaluate flavouring substances in 1997 (FAO/WHO, 1997) and has since been modified several times (FAO/WHO, 1999, 2006, 2009). The JECFA decision tree scheme places chemicals into Cramer structural classes and then makes decisions on the need for toxicity data based on whether or not intakes under the expected conditions of use will exceed the threshold of toxicological concern for the relevant structural class. A further threshold (1.5 µg/person/day US FDA threshold of regulation based on carcinogenic risk) is applied at the final step of the scheme for substances for which no toxicity data are available to provide a threshold with an adequate margin of safety. If this 1.5µg/person/day threshold is not exceeded by the estimated intake, then it is concluded that no data are required for such substances (which have passed earlier steps in the decision tree), provided that they do not contain structural alerts for genotoxicity. An evaluation of the data on the application of the TTC approach between 1999 and 2006 on approximately 1800 flavouring substances allowed JECFA (FAO/WHO, 2006) to confirm the applicability of the TTC approach for flavouring agents and also to consider its application to other substances present in the diet in small amounts. It was emphasised that the TTC approach should be used only in conjunction with a conservative estimate of dietary exposure.

The same procedure for the evaluation of flavouring substances was adopted by the EU Scientific Committee on Food (SCF, 1999) and has subsequently been used by EFSA in modified form since 2004 for the evaluation of several thousand substances on the EU Register of Flavouring Substances¹⁷.

5.1.2. Pharmaceuticals: European Medicines Agency (EMA)

The European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) proposes the use of a “threshold of toxicological concern” (TTC) for genotoxic impurities (EMA, 2006). The TTC refers to a threshold exposure level to compounds that does not pose a significant risk for carcinogenicity or other toxic effects. The EMA Guideline recommends a TTC of 1.5 µg per person per day for all but a highly potent subset of compounds (aflatoxin-like, N-nitroso-, azoxy-, steroids, polyhalogenated dibenzo-p-dioxins and dibenzofurans). This threshold corresponds to an incremental 1 in 10⁵ lifetime risk of cancer, a risk level that the EMA considers justified because of the benefits derived from pharmaceuticals.

¹⁶TOXTREE version 2.5.0 August 2011 Ideaconsult Ltd., Bulgaria – available via the EC Joint Research Centre website at [http://ecb.jrc.ec.europa.eu/\(Q\)SAR/\(Q\)SAR-tools/](http://ecb.jrc.ec.europa.eu/(Q)SAR/(Q)SAR-tools/)

¹⁷http://ec.europa.eu/food/food/chemicalsafety/flavouring/database/dsp_search.cfm

The Guideline indicates that a TTC value higher than 1.5 µg per day may be acceptable in situations where the anticipated human exposure will be short-term, for the treatment of life-threatening conditions, when life expectancy is less than 5 years, or where the impurity is a known substance and human exposure will be much greater from other sources. This is based on a weight-of-evidence approach taking account of the profile of genotoxicity results. A reduction by a factor 10 was proposed for the acceptable daily intake of genotoxic impurities for short term exposure in pediatric and young adult patients. The acceptable limits for daily intake of genotoxic impurities are 5, 10, 20, and 60 µg/day for duration of exposure of 6-12 months, 3-6 months, 1-3 months, and less than 1 month, respectively. For a single dose, an intake of up to 120 µg is acceptable. When more than one genotoxic impurity is present in the drug substance, the TTC value of 1.5 µg/day can be applied to each individual impurity provided the impurities are structurally unrelated. In case of structural similarity, it can be assumed that the impurities act by the same genotoxic mode of action and have the same molecular target and thus might exert effects in an additive manner. In such a situation, the sum of the genotoxic impurities limited to 1.5 µg/day is recommended (Muller et al., 2006).

Genotoxicity testing is not obligatory when a potential genotoxic impurity is controlled at the TTC level, or if the testing batch of drug substance with the impurity at a level of $\leq 0.05\%$ is negative in a genotoxicity battery. The Guidelines recommend an expert scientific review of the synthetic route and the chemical reactions and conditions involved to identify compounds of special concern. This review should include an evaluation of structure-activity relationships (SAR) for genotoxicity. The absence of structural alerts based on a well-performed assessment (e.g. through the application of commonly used SAR assessment software including DEREK and MCASE) is sufficient to conclude that the impurity is of no concern with respect to genotoxicity. Compounds showing positive alerts not present in the active substance need to be tested with a bacterial gene mutation test. A negative bacterial gene mutation test overrules the structural alert (EMA, 2010).

5.1.3. Pesticide metabolites in groundwater Guidance document SANCO/221/2000 rev 10 of 25 February 2003 (EC, 2003)

The strategy for the assessment of the relevance of pesticide metabolites in ground water as described in the guidance document includes a TTC approach for those metabolites that are considered not relevant (in the sense of point C 2.5.1.2 of the annex VI of the Directive 91/414/EEC and of the Directive 98/83/EC¹⁸ regulating the quality of water intended for human consumption) after tiered hazard screening steps. As a pragmatic approach to limit the requirement for animal tests, it is accepted that such non-relevant metabolites can be present in groundwater up to a concentration of 0.75 µg/l. Assuming a consumption of 2 liters of water per day, this corresponds to the threshold of 1.5 µg/person/day set by the US FDA for the 'Threshold of Regulation'.

5.1.4. Industrial chemicals (REACH): ECHA

Some application of the TTC approach is envisaged in the REACH regulation, but limited to cases where there are only a few exposure scenarios that are well characterised. The REACH regulation states the need for non-testing methods and considers the possibility of waiving tests on the basis of exposure considerations. A TTC value could be applied to waive some specific tests (e.g. repeated dose or reproductive toxicity) where it can be demonstrated that there is no significant human exposure.

5.2. Applicability of TTC approach in the dietary risk assessment of pesticide metabolites: outcome of the outsourced project.

Within the frame of a commissioned project "Applicability of thresholds of toxicological concern in the dietary risk assessment of metabolites, degradation and reaction products of pesticides (CRD,

¹⁸Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Official Journal L 330, 32-50. 5 December 1998.

2010), the contractor Chemicals Regulation Directorate (CRD) UK considered the strengths and weaknesses of the TTC scheme (Kroes et al., 2004) for its application to pesticide metabolites.

In order to validate the existing TTC values for use with pesticide metabolites the TTC values should ideally be compared against experimental data for pesticide metabolites. However, for most PPP metabolites the available toxicity database is limited and it is unlikely that sufficient toxicity data would ever be available for metabolites and transformation products to adequately validate the TTC values in this way. The choice was to use pesticide active substances for which a comprehensive database is available to derive robust ADI values. It was felt that this approach was justified given the similarity of chemical structures and of potential toxic effects.

100 active substances were selected at random from a list of 500 compounds that were evaluated under the Directive 91/414/EEC. The list included a mixture of chemical classes covering existing and new active substances. ADI values were identified for each substance. The range of ADIs covered four orders of magnitude, from >1 mg/kg bw/d to 0.00008 mg/kg bw/d.

Cramer classifications were determined for each of the 100 active substances by importing the chemical structure into a software package loaded with the Cramer rules decision tree¹⁹.

A (Q)SAR system (DEREK Version 11) was used to generate predictions for genotoxicity for each of the 100 active substances. The results showed that the overall reliability of the DEREK prediction was low. A key concern was that genotoxicity alerts were not triggered for 12 compounds with evidence of positive results in different *in vitro* genotoxicity assays.

The effect of using a different program to predict genotoxicity was investigated applying the modules in Toxtree. The level of predictivity for the Toxtree software is not better than DEREK and a relatively poor concordance between the two programs was also observed. Combining DEREK with Toxtree did not improve the predictivity. Considering that the EU criteria for classifying a compound as genotoxic require positive results *in vivo*, only 5 chemicals of the 100 active substances selected for the validation exercise based on *in vitro* results had positive results in *in vivo* tests and for all an alert of some genotoxic event was triggered in DEREK (even if not necessarily on the same endpoint), although not matching the specific endpoint described in the individual studies.

On the basis of these results, the TTC validation exercise was carried out following a tiered approach considering two steps before the application of the Toxtree software.

1st Step Genotoxicity: Any genotoxicity alert in DEREK resulted in allocation of a threshold of 0.0025 µg/kg bw (0.15 µg/person/day).

2th Step Neurotoxicity: For this exercise a neurotoxicity trigger was set for all cholinesterase inhibitors (OPs and N-methylcarbamates), compounds acting on the sodium channel (pyrethroids) and other insecticides with a structure associated with a neurological mode of action, such as neonicotinoids. For substances meeting the criteria for this trigger the TTC threshold would be 0.3 µg/kg bw (18 µg/person/day).

Substances that did not reach the genotoxicity and neurotoxicity triggers were allocated to a Cramer Class using the Toxtree software.

The TTC exercise with 100 active substances resulted in allocation of 95 compounds to Cramer class III, 2 to Cramer class II and 1 to Cramer class I. The TTC approach was protective compared with the ADI for 96/100 compounds. For 25/100 substances, the ADI that had been established was more than

¹⁹(Toxtree version 1.51, Ideaconsult Ltd., Bulgaria – available via the EC Joint Research Centre website at [http://ecb.jrc.ec.europa.eu/\(Q\)SAR/\(Q\)SAR-tools/](http://ecb.jrc.ec.europa.eu/(Q)SAR/(Q)SAR-tools/))

1000-times greater than the TTC threshold which would have applied to that substance. Only 4 active substances in the dataset of 100 had ADIs below the respective TTC value identified (Table 2).

Table 2: Comparison of ADI and TTCs

| | TTC Threshold (µg/person/d) | TTC Threshold (µg/kg bw/d) | No. of substances with an ADI below applicable TTC threshold | Compounds (Ratio: ADI/TTC) |
|----------------------------|-----------------------------|----------------------------|--|---|
| | | | Total no. of substances considered = 100 | |
| Genotoxicity alert | 0.15 | 0.0025 | 0 | |
| Neurotoxicity alert | 18 | 0.3 | 0 | |
| Cramer class III | 90 | 1.5 | 3 | Aviglycin (0.67) Haloxypyr-R (0.43) Amitrole (0.67) |
| Cramer class II | 540 | 9 | 1 | 1-MCP is a gas and deriving the ADI involved many assumptions and uncertainties (0.1) |
| Cramer class I | 1800 | 30 | 0 | |

The ADIs for two compounds were established incorporating additional uncertainty factors (due to limitations in their databases) and the other two substances had a ten-fold gap between the NOAEL and the LOAEL in the study used to establish their ADI. In addition 1-MCP is a gas and the application of the TTC approach was probably inappropriate. The ADI/TTC ratio for the other three compounds ranged from 0.43 - 0.67, which, in view of the discussion above, is considered inconsequential given the overall level of uncertainty involved.

5.2.1. Validation of the developed TTC concept: case studies on metabolites

A modified TTC approach for application to metabolites of plant protection products was proposed by CRD.

The adaptation of the TTC concept involved two aspects:

Genotoxicity: Any compound with a structural alert for genotoxicity was considered to be a potential genotoxic carcinogen and was allocated a TTC of 0.15 µg/person/day (0.0025 µg/kg bw/d). Although this clearly gives false positives, its use as a screening approach was considered appropriate. For any compound with predicted exposures above the threshold further considerations are needed

Neurotoxicity: Considering that ADIs for some neurotoxic compounds are in the range of the low ADIs usually set for OPs, it was initially proposed to include metabolites and transformation products of all active substances with a neurotoxic mode of pesticidal action in the OP TTC of 0.3 µg/kg bw/day. Three groups of compounds were evaluated in more detail: N-methylcarbamates, pyrethroids and neonicotinoids.

Like OPs the N-methylcarbamates are cholinesterase inhibitors but have a more transient effect. ADIs for two N-methylcarbamates are below the non-neurotoxic TTC value for Cramer class III of 1.5 µg/kg bw/day – both oxamyl and carbofuran have ADIs of 1 µg/kg bw/day. It was therefore decided to include metabolites and transformation products of N-methylcarbamates in the neurotoxic TTC grouping.

Data on pyrethroids and neonicotinoids were analysed separately on the basis of the derivation of the reference doses from neurotoxic (mainly tremors in dogs) or non-neurotoxic end-points and taking into account the presence of an α -cyano group. None of the pyrethroids or neonicotinoids have ADIs that would not be covered by the Cramer class III TTC value of 1.5 $\mu\text{g/kg bw/day}$. Overall, there is no reason to include pyrethroids or neonicotinoids in the neurotoxic TTC grouping.

The TTC approach developed was evaluated carrying out 15 case studies. The compounds for the case studies were selected based on the availability of toxicity data for their metabolites and with the intention of covering a representative range of pesticides that have been evaluated under Directive 91/414/EEC and of the possible scenarios:

- Few metabolites - predominant residue is parent;
- Few metabolites - predominant residue is not parent;
- Many metabolites;
- Profile of metabolites changes with Pre-Harvest Interval (PHI);
- Profile of metabolites changes with crop;
- Novel metabolites seen in animal transfer studies;
- Active substances of low, medium and high toxicity.

5.2.2. Estimation of the exposure

Suitable estimates of exposure levels of metabolites need to be derived, to compare against the established thresholds of toxicological concern, in order to determine whether the need to consider a metabolite further from a toxicological perspective can be ruled out (i.e. on the basis of structure and exposure levels alone).

Since, for a large number of pesticides, the levels of metabolites are not analysed directly in quantitative residues trials, the contractor proposed an approach for estimation of metabolite levels that made best use of the data available in the residues regulatory data package.

Metabolism data for representative crops were taken from the Draft Assessment Reports (DAR) for each active substance. For each commodity/harvest interval/application rate considered (or combinations thereof) the ratio between the level of each metabolite (mg/kg or % Total Radioactive Residues [TRR]) and the parent compound in each of the plant metabolism studies was determined.

The supervised trials median residue (STMR) levels for the parent compound were determined from the available residues trials data, conducted according to Good Agricultural Practice (GAP) supporting a particular crop use. Reference was made to DAR and FAO/WHO Joint Meeting on Pesticide Residues (JMPR) evaluations. The STMR for each metabolite was then determined using the median level of parent compound found in the trials and the expected ratio of metabolite to parent from the relevant metabolism studies. Long-term (chronic) intakes (NEDIs) for ten UK consumer groups were calculated using “high level” (97.5th percentile) rather than average consumption data based on long term consumption patterns and the median residue found in a food commodity. Since the UK model allows intakes to be calculated for ten different consumer groups within the UK population, intakes were reported for the adult consumer group and the critical consumer group only (i.e. the group of consumers that gave the highest intake for a particular crop/residue combination).

5.2.3. Results and conclusions of TTC case study presented by the contractor

None of the metabolites had structures identified as belonging to classes of compounds of special concern that cannot be considered using a TTC approach (e.g. dioxins, metal containing or N-nitroso compounds). The metabolites were allocated TTC categories based on the following criteria:

- Assume Cramer Class III (with a TTC of 1.5 µg/kg bw/day) unless there were data/information to the contrary;
- DEREK predictions for genotoxicity (negative or positive) were assumed to be reliable and any alert would result in a TTC of 0.0025 µg/kg bw/day unless there were sound reasons to ignore the alert (e.g. test data or the identical alert was triggered in a related compound which had negative data);
- Metabolites of the cholinesterase inhibitors in the group of 15 were allocated a TTC of 0.3 µg/kg bw/day unless there were data to show the compound was not a potent cholinesterase inhibitor;

Exposure estimates were compared with the allocated TTC. If the highest estimated exposure for a consumer group was below the TTC, the transformation product was considered to be “not relevant”. If one or more of the estimates was above the applicable TTC, the transformation product was considered potentially relevant and would merit further consideration.

Out of a total 79 metabolites, 63 were considered non-relevant as exposure estimates were below the allocated TTC. Of the 15 active substances considered, 9 had one or more metabolites that exceeded the allocated TTC. Of the 16 metabolites that exceeded the allocated TTC, 9 compounds had a structural alert for genotoxicity. For the 7 remaining compounds (9%), the data available did not allow further considerations. The applied TTC scheme appears appropriate for the assessment of metabolites, degradation and reaction products. Two critical issues were identified in the application of the TTC scheme:

- The identification of structural alerts for genotoxicity is the first step in the TTC scheme. The software tools applied in the TTC case studies (DEREK and Toxtree) showed poor predictivity for genotoxicity, giving both false positives and false negatives, when the outcome of the analysis was compared with the available experimental genotoxicity data.
- A neurotoxic metabolite arising from a parent compound lacking a structural alert for neurotoxicity (e.g. organophosphate) would not be covered by the proposed scheme.

5.2.4. Conclusions of the PPR Panel on the TTC case study presented by the contractor

The PPR Panel concludes that the TTC approach seems to be widely held as scientifically valid in all regulatory areas where it has been considered, as a tool for providing scientific advice about possible human risk for low level exposure. The application of the TTC approach is dependent on the quality of the underlying database and on an estimate of human exposure to the chemical in the field of application, for which there is confidence that it is not an underestimate. Where TTC approaches have not been accepted for regulatory purposes it was generally because of concerns that the databases used to derive the thresholds do not adequately cover the classes of chemicals under consideration.

The TTC approach has been applied to only a limited extent in the toxicological evaluation of pesticides and their metabolites, although recent chemoinformatic analyses of TTC datasets reported in an EFSA funded study (Bassan et al., 2011), showed that the TTC databases are adequately representative of the different pesticide classes and confirmed their potential use in risk assessment.

The PPR Panel considers that the TTC scheme proposed in the CRD report, as a result of a validation process and of specific case studies, would be a good starting point to develop a decision tree for the evaluation of the toxicological relevance of metabolites. The critical steps identified in the TTC scheme were considered by the PPR Panel.

5.2.4.1. Genotoxicity prediction

The first step in the TTC decision tree involves the assessment of genotoxic potential. The validation exercise performed in the TTC outsourced project applied non-testing tools in the evaluation of genotoxicity alerts. Two (Q)SAR tools (DEREK and Toxtree) applied alone or in combination showed a low predictive performance. However, this exercise suffered from the small datasets used and the heterogeneity of the available genotoxicity data.

The predictability of genotoxicity using software tools was further explored with the outsourced (Q)SAR project, extending the analysis to more software tools and to more extensive datasets (see chapter 6).

5.2.4.2. TTC for neurotoxicity

Neurotoxic compounds were subject to a specific consideration in the CRD project. Compounds, other than OPs, with structural alerts for a neurotoxic mode of action (i.e. N-methylcarbamates, pyrethroids, neonicotinoids) were evaluated in detail. For a few compounds belonging to the methylcarbamate chemical class the ADI was below the TTC for Cramer class III. The PPR Panel therefore decided to include metabolites of N-methylcarbamates in the neurotoxic TTC grouping.

Although to date no examples are known of neurotoxic metabolites arising from non-neurotoxic pesticides, the possibility that this might occur cannot be excluded. It is important to note that these metabolites might not be covered by the proposed TTC approach, unless the toxicophore formed during metabolism has already been characterised. The use of (Q)SAR tools, grouping and read-across approaches in identifying neurotoxic effects of pesticides was further addressed in an ad hoc study (see chapter 7).

5.3. Application of TTC approach for acute exposure

The TTC approach was designed to be applied in risk assessment of chronic exposure: the TTC values were derived from chronic studies and were based on the assumption of continuous lifetime exposure. Research on residues of pesticides in individual fruits and vegetables revealed random occurrences of comparatively high residue levels. Some individuals who consume significant amounts of such foods will occasionally eat the “hot” commodity unit, but this will occur only infrequently (Hamilton and Crossley, 2004). This gave rise the need to consider an approach for acute exposure assessment. The TTC concept could in principle be suitable for these situations as well. However, when using the chronic TTC values to assess acute dietary risk (which may be overly conservative, see EFSA, 2012), estimates of short term intake in many cases exceeded these TTC values. This was confirmed also for pesticide metabolites in a number of ad hoc studies carried out by the PPR Panel (see chapter 8 and Appendices E).

5.3.1. Derivation of acute exposure thresholds

The estimates of metabolite exposure performed in the context of the current opinion by way of case studies (see Appendix E, also discussed in chapter 8), expanded the work the CRD contractor had done in the outsourced TTC project on chronic exposure metabolite estimation, by also considering the potential for metabolite levels as a result of acute exposure. Since the chronic TTCs could be considered to be overly conservative for assessment of acute exposure (EFSA, 2012) it was concluded that if both chronic and acute exposure estimates for metabolites were relatively low, and below the chronic TTC thresholds, it could be proposed that no further toxicological assessment of the metabolites would be needed. In this way a ‘screen’ using the chronic TTC values would be adequate to propose an assessment scheme by comparing all intake values calculated for metabolites with the TTC values. However, the case studies on metabolite estimations showed that estimations were markedly higher for the acute exposure assessments (in the PPR Panel case studies, Appendix E and discussed below) which necessitated a more specific approach for acute considerations in a TTC assessment scheme. Due to the different parameters in the metabolite estimations, it was not possible to derive a consistent factor between an acute and chronic exposure result for a particular metabolite.

For example, for dimethoate the acute metabolite estimates (for the critical consumer) were 1.2 – 2.6 x higher than the corresponding chronic metabolite estimate intakes, whereas for azoxystrobin the acute metabolite estimates (for the critical consumer) were 2.7–27 x higher than the corresponding chronic metabolite estimate intakes. A difference in acute and chronic exposure of at least an order of magnitude can be realistically expected. This therefore triggered the need for extension of the TTC approach to cover the acute exposure situation for pesticide metabolites, in the scope of this opinion.

In order to tackle the issue of acute exposures to pesticide metabolites, acute TTC values for the assessment of pesticide metabolites were derived from pesticide NOAELs used for deriving ARfDs (i.e. short term NOAELs).

As a first step in the process, pesticide active substances for which an ARfD had been established were extracted from the EFSA MRL database (status 6th May 6 2010).

In this internal EFSA database all pesticides for which dietary reference values (ADI and ARfD) have been established are listed. For each substance, the information provided includes the value of the ADI/ARfD, uncertainty factor applied, source of reference value (e.g. EFSA, JMPR, etc.), year of decision, functional category (e.g. insecticide, herbicide, etc.).

In total, 406 ARfDs were established for 267 different active substances. Since for several active substances there was more than one ARfD, as a second step the value considered as most relevant was selected using the following priority: EFSA (PRAPeR), Commission (Standing Committee on the Food Chain and Animal Health (SCoFCAH), European Community Co-Ordination (ECCO)), JMPR, Draft Assessment Report (DAR), EU Member State, so that there was only one ARfD for each active substance. For identical sources the most recent entry was selected.

Substances for which a LOAEL had been used to establish the ARfD (in total 5 substances) were excluded from the analysis, as the TTC approach should be based on NOAELs only.

Substances classified as genotoxic (i.e. all categories of germ cell mutagenicity) according to Regulation (EC) No 1272/2008²⁰ were excluded (in total 4 substances).

As a result, 258 substances remained for further analyses.

Following the Kroes scheme that provides specific thresholds for substances having a structural alert suggesting neurotoxicity, 41 substances belonging to the chemical classes of either carbamates or organophosphates were analysed separately from the remaining 217 active substances.

The SMILES codes of the 217 non-neurotoxic substances were inserted in ToxTree v.2.10 for allocation into the three Cramer classes. Almost all of the 215 were assigned to Cramer Class III (2 were assigned to Cramer Class II). Based on these results and on the recommendations in the scientific opinion of the Scientific Committee on application of TTC (EFSA, 2012) questioning the adequacy of the database for Cramer Class II compounds, it was concluded that only one acute TTC threshold was necessary for pesticide metabolites without a structural alert for neurotoxicity.

The distributions of NOAELs for the two groups of compounds were used to identify the respective 5th percentile values (i.e. the points on the distributions where 5% of substances had lower NOAELs and 95% had higher NOAELs) (see tables 1 and 2 in Appendix F).

The 5th percentile NOAELs were then divided by 100 (i.e. applying the default safety factor) to give “acute exposure thresholds” of 0.295 g/kg bw/d for the organophosphate/carbamate group (virtually

²⁰ Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Official Journal L 353, 1-1355. 31 December 2008.

identical to the “chronic” TTC value of Kroes et al., 2004 for this group of compounds) and of 5.125 g/kg bw/d for the remaining group of substances.

The PPR Panel agreed that the current genotoxicity threshold of 0.0025 µg/kg bw/d (0.15 µg/person/d) should be retained in the “acute TTC scheme” for substances with structural alerts for genotoxicity.

Thus, the PPR Panel recommends “acute exposure thresholds” for pesticide metabolites of 0.0025 µg/kg bw/d for metabolites with a structural alert for genotoxicity, of 0.3 µg/kg bw/d for substances having structures suggesting neurotoxicity (AChE inhibition) and of 5.0 µg/kg bw/d for all other metabolites.

5.4. Overall conclusions on TTC

The PPR Panel concludes that the TTC approach is the most appropriate available tool in the evaluation of the toxicological relevance of pesticide metabolites associated with dietary exposure, for which there are few or no relevant toxicity data. This approach should not be used as alternative to conventional risk assessment for the evaluation of pesticide active substances (parent compounds) themselves occurring as residues in food. They should be assessed prior to authorisation on the basis of dossiers including toxicological tests (Regulation (EC) No 1107/2009).

The PPR Panel noted that increasing numbers of studies address effects of chemical substances at low doses, with many of these studies referring to endocrine active substances or endocrine disruptors. According to the low-dose hypothesis, these substances may cause adverse effects at low doses but not necessarily at all higher doses. They do not therefore follow the classical (or “monotonic”) dose-response curve, showing a greater likelihood of an adverse effect at higher doses. Alternatively they may show a different kind of dose-response curve, e.g. a U-shaped curve with responses both at low- and high-dose levels but not in intermediate ranges. Such a dose-response curve is termed a non-monotonic dose-response curve. Such findings challenge current concepts in chemical risk assessment including the TTC approach. On 14–15 June 2012 (one week before the adoption of the current opinion) EFSA hosted Scientific Colloquium N°17 on low dose response in toxicology and risk assessment²¹. Some key scientific questions and next steps in terms of methodological requirements, research gaps, appropriate testing strategies and methods, and the use of predictive tools were identified, but as yet no scientific consensus has been reached on the validity of the low-dose hypothesis.

The PPR Panel concludes that for the time being, untested substances, other than steroids and several other categories of substances as concluded by the Scientific Committee²², could be evaluated using the TTC approach. However, if there are data indicating that a substance may have endocrine-mediated adverse effects, then the risk assessment should be based on the data, rather than the TTC approach. Once the EU-wide approach for defining and assessing endocrine disruptors is finalised it will be necessary to consider any impact it may have on the use of the TTC approach.

The TTC approach seems to be widely held as scientifically valid in all regulatory areas where it has been considered, see 5.1 However the application of this approach is dependent on the quality and relevance of the underlying toxicity database, and a reliable estimation of the exposure to the chemical in the respective field of application. The PPR Panel considers that the TTC values for genotoxic (0.0025 µg/kg bw/day) and toxic compounds (0.3 µg/kg bw/day for OP compounds, 1.5 µg/kg bw/day for Cramer class II and III and 30 µg/kg bw/day for Cramer class I) are sufficiently conservative for the evaluation of the toxicological relevance of metabolites, as a result of a validation process with groups of pesticides belonging to different chemical classes (CRD project). However TTC values

²¹ <http://www.efsa.europa.eu/en/events/event/120614.htm>. The outcome of the colloquium will be summarised in a report to be published in the autumn of 2012.

²² The Scientific Committee concluded that the TTC approach should not be used for the following (categories of) substances: high potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds, benzidines, hydrazines), inorganic substances, metals and organometallics, proteins, steroids, substances with a high potential for bioaccumulation, nanomaterials, radioactive compounds, and mixtures of substances containing unknown chemical structures.

based on the assumption of continuous lifetime exposure were considered overly conservative for acute exposure. Tentative TTC values for acute exposure were established by the PPR Panel by the analysis of the lowest 5th percentiles of NOAELs used to establish ARfD for the EFSA pesticide data set with values of 0.3 µg/kg bw/d for substances with a neurotoxicity alert and 5 µg/kg bw/d for substances allocated in Cramer class II and III.

The TTC scheme proposed in the CRD's project work was considered by the PPR Panel as a suitable starting point to develop a decision tree for acute and chronic exposure to metabolites. Three critical steps identified in the TTC scheme were considered by the PPR Panel: a) the estimate of the level of the metabolite, b) the evaluation of genotoxicity alert that was addressed by the use of (Q)SAR approach; c) the potential neurotoxicity of metabolites derived from non-neurotoxic parents that was addressed by an ad hoc project exploring the use of computational methods.

The PPR Panel considers that since there is, to date, no consensus on when a compound should be defined as an endocrine disruptor, risk managers have the following options with respect to applying the TTC approach, 1. not to use the approach until it is clear how to assess endocrine disruptor activity, 2. to use the TTC approach, but re-evaluate the applicability when there is consensus on how to assess endocrine disruptor activity. EFSA's SC recommended the latter option. In addition, the PPR Panel notes that at present there is no tool available for assessing the relevance of metabolites in a consistent way and that applying the approach described in the present opinion will improve the risk assessment even if it has to be adapted at a later date in the light of ongoing discussions on endocrine disruptors.

6. SAR/ (Q)SAR concept

Computational methods, including (Q)SAR ((quantitative) structure activity relationships) and read across were considered by the PPR Panel as potential tools in assessing the toxicological relevance of pesticide metabolites in order to limit the need for toxicity testing in animals.

SARs and (Q)SARs, collectively referred to as (Q)SARs, are theoretical models that are used to predict in a qualitative or quantitative manner the physicochemical, biological, toxicological properties and environmental fate of compounds from a knowledge of their chemical structure.

The basic assumption for the application of (Q)SAR analysis in risk assessment is that the biological activity of a chemical depends on its intrinsic nature and in principle can be directly predicted from its molecular structure and inferred from the properties of similar compounds whose activities are known.

More specifically, SAR is a qualitative relationship between a molecular structure or substructure and a specific biological activity, or the modulation of a biological activity imparted by another substructure. A substructure associated with the presence of a biological activity is called a structural alert.

A (Q)SAR is a mathematical model (often a statistical correlation) relating one or more parameters derived from a chemical structure to a quantitative measure of a property or activity. (Q)SARs are quantitative models yielding continuous or categorical results. The parameters used in a (Q)SAR model are also called (molecular) descriptors. A molecular descriptor is a structural or physicochemical property of a molecule, or a part of a molecule, which specifies a particular characteristic of the molecule and is used as an independent variable in the (Q)SAR model.

6.1. Characterisation of chemical space

The characterisation of chemical space is the first step in the evaluation of the adequacy of a (Q)SAR model as a predictive tool for a specific group of compounds.

The chemical space of a dataset (or inventory of chemicals) is defined as the ranges of physicochemical properties and structural features covered by the chemicals in the dataset. The characterisation of the chemical space is relevant in the evaluation and application of computational models for the following reasons:

- because a model should be applied to chemicals within its applicability domain; outside of its applicability domain, a model is unlikely to yield reliable predictions;
- it is useful to compare the chemical space of the test set with that of the training set when the predictive performance of a model is assessed by challenging it with an independent (external) test set.

Principal Component Analysis (PCA), a multivariate statistical method, is used to reduce complex multi-dimensional datasets to simpler lower dimensional datasets, minimising the loss of information.

6.2. Performance of (Q)SAR models

The predictive performance of (Q)SAR models is generally assessed by the evaluation of the number of compounds correctly identified as positive or negative²³. Two parameters are considered to define the performance: the sensitivity and the specificity. The sensitivity expresses the percent of positive compounds correctly predicted and is calculated by the formula: Number of true positive (TP) compounds/(Number of true positive compounds + Number of false negative (FN) compounds). The specificity expresses the percent of correctly identified negative compounds and is calculated by the formula: Number of true negative (TN) compounds/(Number of true negative compounds + false positive compounds). The accuracy is defined as (TP+TN)/(TP+FN+TN+FP).

6.3. Read-across

A chemical category is a group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a similar pattern, usually as a result of structural similarity (OECD, 2007a). The grouping approach represents a move away from the traditional substance-by-substance evaluation to a more robust approach based on a family of related chemicals. Within a chemical category, data gaps may be filled by read-across, trend analysis and (Q)SARs (van Leeuwen et al., 2009).

By its very nature, the grouping and read-across approach is an ad hoc, non-formalised approach based on a number of steps including expert choices. As with (Q)SARs (ECHA, 2010a) estimated properties obtained by the grouping and read-across approach need to be assessed in terms of their adequacy, and the justification needs to be clearly documented according to an accepted format (ECHA, 2010b). Critical issues in chemical category formation and read-across are the quality of the underlying experimental data for the analogues and definition of (chemical and/or biological) similarity (Jaworska and Nikolova-Jeliazkova, 2009).

6.4. (Q)SAR approach in the dietary risk assessment of pesticide metabolites

The PPR Panel addressed in more detail the potential use of computational methods in the evaluation of genotoxicity to complement the TTC approach.

6.4.1. Software tools for genotoxicity and carcinogenicity prediction

Genotoxicity and carcinogenicity prediction is featured in a wide range of commercial and freely available software tools, as reviewed by Serafimova et al. (2010). The scientific literature relating to the *in silico* prediction of genotoxicity and carcinogenicity is substantial, with more than 100 papers dedicated to (Q)SARs.

²³This is only correct for categorical (Q)SARs having only two categories (positive or negative). Different parameters have to be used for (Q)SARs with multiple categories and (Q)SARs which give a quantitative result (e.g. a NOAEL).

A number of papers are devoted to the comparison of the performances of different models, including software models: many of them report the results of evaluation studies for prediction of carcinogenicity.

The outcome of a series of external prediction exercises performed by various investigators with three models: MultiCase, TOPKAT, and Derek were summarised (Benigni and Bossa, 2008). The common characteristic of these studies is that the chemicals to be predicted were different from those used in the training sets by the model developers, and were performed independently. It was found that the predictions for external chemicals vary considerably both in terms of overall accuracy and in terms of relative proportions of true and false positives.

A factor which contributes to reduced model performance is the quality of the underlying mutagenicity data: inconsistent data interpretation or the lack of quality assurance may contribute to incorrect predictions made by *in silico* systems. When using computational models for regulatory purposes, the predictions of genotoxicity and carcinogenicity should not be based on the use of any single model alone, but on a “weight of evidence” approach including information from all available sources ((Q)SARs, read across, *in vitro* test methods). A number of studies in the literature (e.g. Contrera et al., 2007) support the usefulness of computational tools, applied in batteries that combine high sensitivity models (to minimise false negatives) with high specificity models (thereby minimising false positives).

6.4.2. Current applications of (Q)SAR approach in predicting mutagenicity and carcinogenicity

6.4.2.1. European Chemical Agency

The REACH regulation²⁴ and associated guidance foresee the application of (Q)SARs in a number of ways (ECHA, 2008), mainly to:

- provide information for use in priority setting procedures;
- guide the design of an experimental test or testing strategy;
- improve the evaluation of existing test data;
- provide mechanistic information (which could be used, for example, to support the grouping of chemicals into categories);
- fill data gaps for classification and labelling and for risk assessment.

6.4.2.2. Danish Environmental Protection Agency

To address the problem of classification under Directive 67/548/EEC and Regulation (EC) No 1272/2008 for the large number of existing substances in the EU not included in the list of harmonised classification and labelling of hazardous substances (Annex VI of Regulation (EC) No 1272/2008), the Danish EPA published an advisory list for self-classification of dangerous substances²⁵. The list of suggested hazard classifications was derived by using predictions from (Q)SAR models obtained or developed by the Danish EPA for the following endpoints: acute oral toxicity, skin sensitisation, mutagenicity, carcinogenicity. MultiCASE software was used for genotoxicity.

Five different models predicting genotoxicity *in vivo* were applied for the screening by Danish EPA. The data for the training sets were obtained from papers available in the scientific literature. The

²⁴Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Official Journal L 33, 1-278. 29 May 2007.

²⁵http://www.mst.dk/English/Chemicals/assessment_of_chemicals/The_advisory_list_for_selfclassification/

performance varied with the model applied: the sensitivity and specificity range from 0.30-0.73 and from 0.37-0.93, respectively. For a substance regarded as a probable mutagen it needs to be positive in at least two models, accepting only predictions where no significant deactivating fragment was detected. If positive results from one or more genotoxicity tests were available, this would overrule negative predictions obtained with the models.

6.4.2.3. European Medicines Agency (EMA)

The application of structure-activity relationships (SARs) is considered in EMA Guidelines for genotoxicity evaluation of impurities in pharmaceuticals. The absence of a structural alert based on a well-performed assessment (e.g. through the application of commonly used software tools including DEREK and MULTICASE²⁶) is sufficient to conclude that the impurity is of no concern with respect to genotoxicity. Compounds showing positive alerts not present in the active substance need to be tested with a bacterial gene mutation test. A negative bacterial gene mutation test overrules the structural alert. Structural alerts are also used in the context of the TTC approach.

An approach proposed by Muller et al., (2006) in the application of computational models for genotoxicity prediction, uses a five class scheme to help decide whether an impurity possesses a high level of risk and should, therefore, be controlled at very low levels of daily intake:

- Class 1 Impurities known to be both genotoxic (mutagenic) and carcinogenic;
- Class 2 Impurities known to be genotoxic (mutagenic), but with unknown carcinogenic potential;
- Class 3 Alerting structure, unrelated to the structure of the active ingredient and of unknown genotoxic (mutagenic) potential;
- Class 4 Alerting structure, related to the active ingredient;
- Class 5 No alerting structure or sufficient evidence for absence of genotoxicity.

It was demonstrated that DEREK for Windows can be successfully used as a first step for the identification of structural alerts for genotoxicity in the above scheme (Dobo et al., 2006). In a retrospective analysis of some 272 compounds, the implementation of this strategy gave an overall concordance of 92% for compounds in classes 1, 2, 4 and 5 with 67 (25%) of compounds falling into class 3, that would require further investigation.

6.4.2.4. Assessment of the equivalence of technical materials of substances regulated under Council directive 91/414/EEC

The use of validated (Q)SAR models to predict toxic effects, including mutagenicity, is considered in the assessment of toxic hazard of impurities for the evaluation of equivalence of technical materials for substances regulated under Directive 91/414/EEC.

6.5. Applicability of (Q)SAR analysis to the evaluation of the toxicological relevance of metabolites of pesticide active substances for dietary risk assessment.

6.5.1. Outsourced project by the Joint Research Centre (JRC)

An outsourced activity was carried out by the Joint Research Centre (JRC) Ispra, to explore the applicability of computational methods in the evaluation of the toxicological relevance of metabolites of pesticide active substances (JRC, 2010). An extensive review on the available computational

²⁶ MULTICASE was not part of the current evaluation (see Chapter 6)

models with emphasis on (Q)SARs focusing on toxicological endpoints (acute and repeat-dose toxicity, including organ and system specific toxicities; genotoxicity and carcinogenicity; developmental and reproductive toxicity; immunotoxicity, endocrine-related effects) and on Absorption, Distribution, Metabolism and Excretion (ADME) was produced (Mostrag-Szlichtyng and Worth, 2010).

On the basis of the literature review, it was concluded that some available software tools (e.g. TOPKAT and MCASE) are useful for predicting acute toxicity in categorical terms (e.g. in terms of Globally Harmonised System, GHS, classifications).

The availability of (Q)SAR models for the prediction of chronic toxicity endpoints is very limited.

Since a large number of potential targets and mechanisms are associated with repeat dose effects, it is unlikely that any single model or software tool will be capable of making reliable predictions for all chemicals of interest to dietary risk assessment due to limitations of the underlying database.

The modeling of organ-specific and system-specific effects represents a developing field.

Only a few (Q)SAR studies have focused on the effects of chemicals on the central nervous system, in some cases through the modelling of *in vivo* toxicity. Among the commonly used software tools, Derek for Windows v.11 includes the structural alerts: organophosphate (for direct and indirect anticholinesterase activity), *N*-methyl or *N,N*-dimethylcarbamate (for direct anticholinesterase activity) and gamma-diketones (for neurotoxicity).

The availability of (Q)SARs for reproductive and developmental toxicity (excluding models related to endocrine activity) is limited as a result of the diversity and biological complexity of the endpoints, and the scarcity of data suitable for modelling. Available models are potentially useful as a means of supporting hazard identification and priority setting, but not for use in risk assessment.

A large number of (Q)SAR tools have been developed for ADME prediction, mainly for pharmaceutical purposes and for specific ADME properties (e.g. blood/brain barrier permeability, human intestinal absorption, placental permeability). However, their applicability in the dietary risk assessment of chemicals other than drugs is either poor or not established.

6.5.2. Framework for assessing the usefulness of (Q)SAR models

In their report (JRC, 2010) the JRC introduces a conceptual framework for the use of (Q)SARs that is comprehensively described in the REACH guidance on Information Requirements and Chemical Safety Assessment (ECHA, 2008).

According to the framework developed for REACH for using (Q)SAR models instead of experimental data these models have to be documented appropriately, must be scientifically valid, applicable to the chemical(s) of interest and relevant for the purpose they are used for in order to be considered adequate.

In order to be considered as scientifically valid OECD (2007e) established the principle that any (Q)SAR model used needs to have a defined endpoint, an unambiguous algorithm, a defined applicability domain, appropriate measures of goodness-of-fit, robustness and predictivity and should, if possible, be associated with information on mechanistic interpretation.

As a next step in assessing the adequacy of a model it needs to be verified if the chemical(s) of interest are within the applicability domain of the model. Only for chemicals that are within the applicability domain of a chosen model can reliable results be achieved. Therefore it needs to be assessed if the descriptor values of the chemical are within the predefined ranges and if it contains any structural fragments that are not “known” to the model, any predefined mode/mechanism of action and

information on likelihood of transformation/metabolites and the characteristics of such transformation products.

In order to demonstrate the adequacy of a (Q)SAR estimate generated by a valid and applicable model some additional considerations are needed, namely if the model endpoint is relevant for the regulatory purpose.

Relevance of models predicting directly a regulatory endpoint is self-evident (e.g. (Q)SARs designed to predict LD₅₀ values). For (Q)SARs focusing on mechanistic endpoints, extrapolation from the modeled endpoint (e.g. nucleophilic reactivity towards DNA or proteins) to the regulatory endpoint (e.g. mutagenicity) needs to be made.

Overall, any prediction needs to be assessed within the context of the regulatory purpose and taking into account other relevant information applying a weight of evidence approach.

The JRC has provided in their report a checklist with 10 questions that can give useful support in assessing the adequacy of (Q)SAR models. This checklist is inserted in Appendix F to this opinion.

6.6. Applicability of (Q)SAR analysis to the evaluation of genotoxicity of pesticide metabolites for dietary risk assessment: case studies

In the JRC project, case studies were carried out on the potential applicability of (Q)SAR approaches in predicting genotoxicity with the aim of integrating (Q)SAR and the TTC approach.

6.6.1. Software tools applied

The software tools were selected by JRC on practical grounds, taking into account their in-house availability, as well as budgetary and procurement constraints for the acquisition of new licenses.

The software tools applied included:

- DEREK, a rule-based system combining toxicological knowledge and expert judgment;
- CAESAR, LAZAR, TOPKAT, HazardExpert and ToxBoxes, based on statistical methodologies;
- Toxtree, a hybrid tool implementing both expert rules and statistical methodologies.

6.6.2. Compilation of datasets

The ability to predict genotoxicity and carcinogenicity was based on the application of the various software tools to three datasets consisting of 185 pesticides, 1290 heterogeneous chemicals, and 113 heterogeneous classified mutagens.

6.6.2.1. Compilation of an “internal” pesticides dataset

The chemical space of pesticides was represented by two datasets for which chemical structures were available:

- a) **CRD pesticides dataset:** initially consisting of 135 parent compounds from the CRD TTC study (see chapter 5) including 100 parent compounds used by the CRD to develop the TTC scheme, 15 to validate it and 20 metabolites. This was reduced to 128 after removal of structures that cannot be handled by computational tools (e.g. salts, organometal compounds);

- b) **AGES pesticides dataset:** initially consisting of 67 parent compounds from the AGES study (see chapter 4). This was reduced to 57 compounds after removal of compounds in common with the above-mentioned TTC dataset.

The total number of case study structures, including CRD (128) and AGES compounds (57), was 185. Experimental data for carcinogenicity were available for 104 compounds (45 active, i.e. carcinogenic and 59 inactive, i.e. non-carcinogenic). Information on mutagenic activity was available for 181 substances, but only 11 of the compounds showed some evidence of genotoxicity in the Ames test, of which only 5 compounds (etridiazole, carbendazim, dichlorvos, thiobencarb, parathion-methyl) were associated with test data that might result in regulatory classification.

6.6.2.2. Compilation of “external” datasets

- a. **Heterogeneous dataset:** The DSSTox Carcinogenic Potency Database (CPDB) contains the results of cancer and Ames mutagenicity tests on 1547 chemicals (pharmaceuticals, natural compounds in the average diet, air pollutants, food additives and pesticide residues). From the initial database, the following compounds were excluded: inorganics (60), organometal compounds (44), compounds for which structures were not available, macromolecules, i.e. polymers, proteins, DNA, or other large biomolecular species (3) and formulations/mixtures (75). Since computational tools cannot handle certain structures (e.g. salts), these were also excluded, resulting in the removal of a further 36 substances, thereby leaving 1290 chemicals in the CPDB database. Carcinogenicity data were available for 1288 substances: 717 compounds were active (i.e. carcinogenic) and 571 were inactive (i.e. non-carcinogenic). Mutagenicity data, obtained using the Ames test, were available for 748 of the 1290 DSSTox molecules: 368 compounds were positive (i.e. mutagenic) and 380 negative (i.e. non-mutagenic).
- b. **Dataset of classified mutagens:** A series of 601 classified compounds was considered, comprising 594 substances extracted from the ex-ECB Claslab database and 7 substances added after comparing the ex-ECB database to Annex VI of CLP²⁷ (personal communication, J.J.A. Muller). From this, 113 substances that had been classified as mutagens (Muta. Category 2 R46 and Muta. Category 3 R68) during the EU harmonised classification process (the corresponding GHS classifications are Muta. 1B and Muta 2, respectively) were considered suitable for the analysis.

6.6.3. Characterization of chemical space by Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was applied to reduce complex multi-dimensional datasets to simpler lower dimensional datasets, while minimising the loss of information (variance in the data). Trends and patterns can be more easily identified by using the Principal Components (PCs), which are linear combinations of the original descriptors. The “meaning” of each PC can be derived from the loadings of the original descriptors on the PCs. For the purpose of this study, a range of easily interpretable descriptors (constitutional descriptors, functional group counts and molecular properties) were used. As a result, the chemical space was built from a combination of physicochemical properties and sub-structural features. PCA reduced the dimensions of 35 molecular descriptors to 5 representative PCs.

The JRC study found that the chemical spaces of the pesticides (pesticides database including 821 compounds on the EU list of Plant Protection Products for which the structures were available) and the CPDB dataset were overlapping, thereby supporting the usefulness of CPDB when assessing the applicability of (Q)SARs to pesticides as well as other chemicals. The CRD dataset was broadly representative of the chemical space of the pesticide inventory, but lacking a number of structural

²⁷ CLP is the Regulation on classification, labelling and packaging of substances and mixtures (EC 1272/2008). This Regulation aligns previous EU legislation on classification, labelling and packaging of chemicals to the GHS (Globally Harmonised System of Classification and Labelling of Chemicals).

classes. Moreover, the use of a broader dataset increased the coverage of structural space, thereby providing a more extensive and robust analysis.

6.6.4. Performance of the models in predicting mutagenicity

The performance of all of the models applied was assessed by the analysis of correct and wrong predictions. The number of compounds identified as true or false positive (TP, active and predicted as active and FP not active but predicted as active) and true and false negative (TN, not active and not predicted as active and FN, active but not predicted as active) was determined. Sensitivity was calculated as $TP/(TP+FN)$ and specificity as $TN/(TN+FP)$. The accuracy was defined as $(TP+TN)/(TP+FN+TN+FP)$.

6.6.5. Prediction results

6.6.5.1. Prediction results for CRD-AGES dataset

The results on genotoxicity prediction for the internal dataset including CRD and AGES pesticides are reported in Table 3. A 100% sensitivity was observed with ToxBboxes, although only 4/11 active compounds were correctly predicted and 7/11 were classified as equivocal. The lowest sensitivity was observed with Lazar: 0.45. The sensitivity for the other applied tools ranged between 0.50 and 0.64.

The specificity ranged between 0.57 and 0.87. The better performance of the model in identifying inactive compounds is related to the high percentage of non-genotoxic compounds included in the training and test datasets.

Carcinogenicity prediction for this dataset is very poor: the range of sensitivity values is 0.31- 0.58. The specificity is higher, ranging between 0.53 and 0.84.

Table 3: Genotoxicity prediction for the CRD-AGES dataset

| Number of compounds: 185 Experimental values available: 181 Exp. active compounds: 11 Exp. inactive compounds: 170 | | | | | | | | | | | |
|---|-------------|-----|----|----|----|----|-------------|------|------|------|------|
| SOFTWARE | STATISTICS* | | | | | | | | | | |
| | TP | TN | FP | FN | EQ | ND | SP | SE | CONC | 1-SE | 1-SP |
| CAESAR | 7 | 129 | 40 | 4 | 1 | 0 | 0.76 | 0.64 | 0.76 | 0.36 | 0.24 |
| Derek | 6 | 148 | 22 | 4 | 1 | 0 | 0.87 | 0.60 | 0.86 | 0.40 | 0.13 |
| HazardExpert | 5 | 95 | 71 | 5 | 5 | 0 | 0.57 | 0.50 | 0.57 | 0.50 | 0.43 |
| Lazar (Kazius/Bursi) | 7 | 127 | 41 | 4 | 0 | 2 | 0.76 | 0.64 | 0.75 | 0.36 | 0.24 |
| Lazar (Toxbenchmark) | 5 | 127 | 41 | 6 | 0 | 2 | 0.76 | 0.45 | 0.74 | 0.55 | 0.24 |
| TOPKAT | 7 | 121 | 48 | 4 | 0 | 1 | 0.72 | 0.64 | 0.71 | 0.36 | 0.28 |
| ToxBboxes | 4 | 112 | 22 | 0 | 43 | 0 | 0.84 | 1.00 | 0.84 | 0.00 | 0.16 |
| Toxtree (Benigni-Bossa) | 6 | 117 | 53 | 5 | 0 | 0 | 0.69 | 0.55 | 0.68 | 0.45 | 0.31 |

TP – true positives; TN – true negatives; FP – false positives; FN – false negatives; EQ – compounds predicted as equivocal; ND – the number of compounds that were not handled by the software; SP – specificity; SE – sensitivity; CONC – overall concordance; 1-SE – false negative rate; 1-SP – false positive rate

6.6.5.2. Prediction results for DSSTox dataset

The performance of the applied models for genotoxicity prediction was best for the external DSSTox Carcinogenic Potency Database (CPDB) dataset, including mutagenic and non-mutagenic compounds, classified on the basis of the results from the Ames test. The sensitivity values ranged between 0.66 and 0.93. The specificity values ranged from 0.61 to 0.93. ToxBboxes showed the highest sensitivity

and specificity. This outcome is expected considering that the training dataset used to develop the applied (Q)SAR models is based on the same genetic endpoint, *in vitro* mutagenicity with Ames test.

6.6.5.3. Prediction Results for classified mutagen dataset

The results for genotoxicity prediction for the external dataset of classified mutagens are reported in table 4. The highest sensitivity (0.87) was obtained with Toxtree, using the *in vivo* micronucleus rulebase, followed by HazardExpert (0.77). The lowest sensitivity (0.44) was obtained with ToxBoxes which is a model optimised to predict Ames mutagenicity.

This result is expected because the dataset includes a large majority of compounds classified as genotoxic based on the *in vivo* micronucleus test.

To improve the sensitivity of the applied models various pairwise software combinations were tested. If either tool in the combination gave a positive result then the overall prediction was considered positive. A reduction of the false negative rate was obtained, with the lowest value of 8 % for the combined use of Toxtree and Derek.

Table 4: Genotoxicity prediction for the classified mutagen dataset

| Software (used alone) | ND | EQ | TP | SE | FN | 1-SE | No TS |
|--|----|----|-----|------|----|------|-----------|
| Toxtree (genotoxic carcinogenicity) | 0 | 0 | 86 | 0.76 | 27 | 0.24 | NA |
| Toxtree (<i>in vivo</i> micronucleus) | 0 | 0 | 98 | 0.87 | 15 | 0.13 | NA |
| Toxtree (genotoxic carcinogenicity or <i>in vivo</i> micronucleus) | 0 | 0 | 98 | 0.87 | 15 | 0.13 | NA |
| TOPKAT | 1 | 0 | 65 | 0.58 | 47 | 0.42 | 43 |
| CAESAR | 1 | 0 | 82 | 0.73 | 30 | 0.27 | 48 |
| HazardExpert | 0 | 5 | 82 | 0.77 | 25 | 0.23 | Not known |
| Lazar (Kazius/Bursi) | 0 | 0 | 65 | 0.58 | 48 | 0.42 | 58* |
| Lazar (Toxbenchmark) | 0 | 0 | 56 | 0.50 | 57 | 0.50 | 60* |
| Lazar (Kazius/Bursi or Toxbenchmark) | 0 | 0 | 69 | 0.61 | 44 | 0.39 | 74* |
| Derek (mutagenicity or chromosome damage) | 0 | 2 | 81 | 0.73 | 30 | 0.27 | NA |
| ToxBoxes | 0 | 27 | 38 | 0.44 | 48 | 0.56 | Not known |
| Software (used in combination) | | | | | | | |
| Toxtree or CAESAR | 0 | 0 | 101 | 0.89 | 12 | 0.11 | 48 |
| Derek or CAESAR | 0 | 0 | 96 | 0.85 | 17 | 0.15 | 48 |
| Derek or Lazar | 0 | 0 | 92 | 0.81 | 21 | 0.19 | 74* |
| Derek or TOPKAT | 0 | 0 | 89 | 0.79 | 24 | 0.21 | 43 |
| Toxtree or Lazar | 0 | 0 | 102 | 0.90 | 11 | 0.10 | 74* |
| Toxtree or Derek | 0 | 0 | 104 | 0.92 | 9 | 0.08 | NA |
| HazardExpert or CAESAR | 0 | 0 | 94 | 0.83 | 19 | 0.17 | ≥ 48 |

Test set of 113 classified mutagens; ND – not determined; EQ – compounds predicted as equivocal; TP – true positives; SE – sensitivity; FN – false negatives; 1-SE – false negative rate; No TS – number of chemicals already in the training set of the model (where applicable); NA – not applicable

*: For Lazar it is not important whether a substance is in the dataset used to build the model, since an instance-based prediction is generated by a local model built from data that exclude the query chemical

6.7. Conclusions of the contractor

A conceptual framework for the evaluation of the adequacy of (Q)SAR models in the context of dietary risk assessment has been developed. A checklist was proposed and applied to select software models for prediction of genotoxicity of pesticides. See 6.5.2 and Appendix F.

An extensive review of the potential applicability of computational methods in the evaluation of the toxicological relevance of pesticide metabolites reveals that the usefulness of models for the prediction of chronic toxicity endpoints is very limited, while some available software tools are useful for predicting acute toxicity in categorical terms.

A number of (Q)SARs tools for reproductive and developmental toxicity and for endocrine disruptors have been developed as a means of supporting hazard identification and priority setting.

A large number of (Q)SARs tools have been developed for ADME prediction, mainly for specific ADME properties (e.g. blood/brain barrier permeability, human intestinal absorption). While it is difficult to give firm conclusions on the applicability of such tools, it is clear that many have been developed with pharmaceutical applications in mind, and as such might not be applicable to other types of chemicals (this would require further research investigation). On the other hand, a range of predictive methodologies have been explored and found promising, so there is merit in pursuing their applicability in the field of food safety.

The (Q)SAR case studies focussed on the applicability of several software tools for predicting genotoxicity of pesticide metabolites. The results of these studies, using the largest dataset available of active ingredients and metabolites, show a wide range of sensitivity from 0.45-1.00 and specificity from 0.57-0.93. The accuracy of the prediction is related to the training set data applied, as demonstrated by the high performance of ToxBoxes and Toxtree in detecting chemicals positive in the Ames test or in the *in vivo* micronucleus test, respectively.

Several tools were good identifiers of Ames mutagenicity (typical sensitivities of 0.80-0.93; typical false negative rates of 0.07-0.20). Furthermore, some of these tools were good identifiers of classified mutagens (highest sensitivities of 0.73-0.87; lowest false negative rates of 0.13-0.27). Pairwise combinations of these tools could increase the overall sensitivity (to about 0.90) and reduce the false negative rate (to about 0.10). The software tools or combinations of them can be optimised with the aim of increasing the sensitivity, reducing the number of false negatives.

6.8. Conclusion by PPR Panel

A checklist was proposed and applied to select software models for prediction of genotoxicity of pesticides. The panel considers the questions in the checklist as valid ones, but did not use the tool itself.

The PPR Panel concludes that the performance of the (Q)SAR tools applied individually, in the prediction of genotoxicity of the pesticide dataset, involving parent compounds and metabolites tested in the CRD and AGES case studies, is unsatisfactory (resulting in too many false positives and false negatives). The low sensitivity of the applied tools (between 0.45 and 0.64) could be attributed to the heterogeneity of the compounds in the dataset set. In addition the experimental data available to test the performance of the (Q)SAR tools are heterogeneous, including results on different genotoxic endpoints. Several tools were good identifiers of Ames mutagenicity with a sensitivity range of 0.80-0.93, some of them are also good identifiers of classified mutagens (sensitivities: 0.73-0.87). The range of sensitivity and specificity values derived from the case study on the classified mutagen dataset applying (Q)SAR tools alone or in combination, is in the range of those described in the scientific literature. The results on the classified mutagen dataset confirms the usefulness of applying a battery of (Q)SAR tools to increase the level of predictivity.

The usefulness of (Q)SAR tools in the prediction of endocrine disruptor activity was not investigated because of lack of a clear definition and availability of test results.

Overall, these outcomes, although not conclusive considering that one of the most commonly used software tool (MULTICASE) in genotoxicity prediction (EMA, 2006) was not explored in this exercise, do not support a proposal for the application of a (Q)SAR approach alone to predict the potential genotoxicity of unknown pesticide metabolites. This conclusion is in agreement with the considerations reported in the EFSA SC opinion on genotoxicity testing strategies (EFSA, 2011b).

However, the PPR Panel recommends that the application of integrated approaches including combined (Q)SAR models and read-across is explored in future studies. The use of read-across implies the availability of a robust database comprising the main genotoxic endpoints.

Further research is needed to develop batteries, including (Q)SAR models for each critical genotoxic endpoint, with the aim of increasing the sensitivity, and reducing the number of false negatives. Two important challenges faced by (Q)SAR models for genotoxicity prediction of pesticide metabolites are the diversity of compound structural space including the differences between stereoisomers and the multiplicity of structural alerts that can produce the same effect. The development of mechanistic SARs and the possibility of expanding the applicability domain could increase confidence in the predictions made by *in silico* models allowing improvements in the future use of (Q)SAR model combinations in the prediction of genotoxicity.

The outcome of the (Q)SAR project allows the PPR Panel to propose the application of computational methods, involving separate or sequential use of (Q)SAR and read-across, as a complement to the TTC approach in the assessment scheme for pesticide metabolite exposure. If the analysis predicts genotoxic activity, then the metabolite is by default considered as genotoxic and the choice of further testing to prove otherwise rests with the applicant. If the analysis is negative, further testing is still required because of the probability of false negatives.

The use only of computational tools, (Q)SAR and read across, should not be employed in the evaluation of pesticide active substances themselves (parent compounds) occurring as residues in food. They should be assessed prior to authorisation on the basis of the results of a battery of tests using a stepwise approach.

7. Applicability of (Q)SAR analysis in the evaluation of developmental and neurotoxicity effects of pesticide metabolites: outcomes of outsourced activity

Within the EU peer review of active substances, the need to establish an Acute Reference Dose (ARfD) on the basis of adverse effects exerted early in repeat dose toxicity studies, is most commonly triggered by either developmental or, albeit to a lesser extent, neurotoxic effects. A similar conclusion was reached by Solecki et al., (2010) in a recent review of on ARfD setting within the EU.

The outcome of the (Q)SAR project carried out in preparation of the opinion (see Chapter 6) suggested that computational tools could be used to explore developmental and neurotoxic alerts.

EFSA therefore commissioned a further project at the JRC in which computational tools were evaluated for their suitability in excluding developmental and neurotoxic effects of pesticides with the aim of possibly including them for refinement of a draft assessment scheme for metabolites.

A stepwise strategy was followed in which (Q)SAR tools were used in an initial step for the identification of potentially neurotoxic active chemicals and a subsequent step, based on grouping and read-across was applied to discriminate between true and false negatives generated by the (Q)SAR analysis.

7.1. Predictive performance of (Q)SAR/read-across strategy for neurotoxicity

A dataset for neurotoxicity, including 40 positive and 21 negative substances was provided by EFSA. Twenty-one substances among the positives belonged to different chemical classes, carbamates, neonicotinoids and pyrethroids, which are expected to show neurotoxic effects. Organophosphates were not included in the dataset because the neurotoxic mechanism of action of these compounds is also well known in humans. In addition, such compounds are readily identified from their structure alone.

The available software tools for predicting neurotoxicity were considered. The large majority are commercial and some are related to prediction of blood-brain (BB) barrier penetration and are not directly relevant to the current project. Five (Q)SAR models were applied as a first step: Derek Nexus, HazardExpert, Pass, ADME Predictor (probability of blood-brain barrier permeability predicted as low or high), and Accord (quantitative linear regression model for predicting the blood-brain barrier penetration as log BB). The performance, in terms of negative predictivity, of the individual models was low: the best performing tool was Derek with a specificity of 100 %, a negative predictivity of 43% and a false negative rate of 74%. The use of two-model combinations increased the negative predictivity to 48% (for the combination Derek and Pass), but this was also associated with an increased false negative rate (84%).

The read-across approach was not used for the neurotoxicity prediction due to the lack of a suitable reference database.

7.2. Predictive performance of (Q)SAR/read-across strategy for developmental toxicity

Three sets of data were considered for the predictivity of developmental toxicity:

A) A dataset of pesticides provided by EFSA including:

- 37 pesticides positive for developmental effects, identified by considering the substances for which the ARfD was based on developmental toxicity and selecting early onset specific malformations in rat and/or rabbit at maternally non-toxic doses considered for the establishment of an ARfD;
- 39 pesticides negative for developmental effects, with no adverse effects observed in valid development tests with rat and/or rabbit at doses up to those associated with maternal toxicity;

B) An extended dataset of 135 substances, which comprised the EFSA dataset of pesticides and an additional group of compounds classified for developmental toxicity, provided by RIVM;

C) A dataset derived from the US EPA's ToxRefDB.

The performance of seven (Q)SAR models suitable for developmental toxicity prediction was evaluated, in terms of negative predictivity (see Glossary) in order to exclude the metabolites from acute exposure assessment (Table 5). The results based on the EFSA dataset suggest that Derek, TOPKAT and PASS are the best stand alone tools in terms of their negative predictivity, although these models cannot be considered suitable for use on their own, due to their low negative predictivity ranging from 49-55%. Analysis of a larger dataset, US EPA's ToxRefDB (in this case the substances were considered positive for any developmental adverse effect when the Low Effect Level (LEL) was lower than the maternal LEL), which included different categories of substances, showed that negative predictivity (87%) and false negative rate (37%) were best with Leadscape. The evaluation of the predictive performance of batteries of developmental toxicity models showed that the best results were obtained with HazardExpert combined with PASS, with a specificity of 100%, a negative predictivity of 41% and a false negative rate of 74%.

Table 5: Predictive performance of developmental toxicity models used alone against EFSA test set (taken from JRC, 2011)

| | Derek | | Caesar | | TOPKAT | | Leadscope | | Hazard Expert | | PASS (embryo-toxicity) | | PASS (teratogenicity) | |
|--|-------|----|--------|----|--------|----|-----------|----|---------------|----|------------------------|----|-----------------------|----|
| | A* | B | A | B | A | B | A | B | A | B | A | B | A | B |
| % of chemicals | | | | | | | | | | | | | | |
| sensitivity | 14 | 27 | 73 | 66 | 51 | 53 | 33 | 45 | 61 | 49 | 35 | 70 | 32 | 69 |
| specificity | 97 | 97 | 24 | 24 | 57 | 57 | 48 | 48 | 73 | 73 | 59 | 59 | 62 | 62 |
| concordance | 57 | 47 | 48 | 54 | 54 | 54 | 40 | 46 | 66 | 53 | 47 | 67 | 47 | 67 |
| negative predictivity | 54 | 35 | 47 | 21 | 55 | 33 | 39 | 22 | 53 | 24 | 49 | 45 | 49 | 44 |
| positive predictivity | 83 | 96 | 48 | 68 | 53 | 75 | 42 | 74 | 79 | 89 | 45 | 81 | 44 | 81 |
| false negative rate | 86 | 73 | 27 | 34 | 49 | 47 | 67 | 55 | 39 | 51 | 65 | 29 | 68 | 31 |
| false positive rate | 3 | 3 | 76 | 76 | 43 | 43 | 52 | 52 | 27 | 27 | 41 | 41 | 38 | 38 |
| No of chemicals (A 76, B 135 in total) | | | | | | | | | | | | | | |
| TP | 5 | 26 | 27 | 63 | 18 | 48 | 10 | 39 | 11 | 24 | 13 | 68 | 12 | 66 |
| TN | 38 | 38 | 9 | 9 | 21 | 21 | 13 | 13 | 8 | 8 | 23 | 23 | 24 | 24 |
| FP | 1 | 1 | 29 | 29 | 16 | 16 | 14 | 14 | 3 | 3 | 16 | 16 | 15 | 15 |
| FN | 32 | 70 | 10 | 33 | 17 | 43 | 20 | 47 | 7 | 25 | 24 | 28 | 25 | 30 |
| ND | 0 | 0 | 1 | 1 | 4 | 7 | 19 | 22 | 47 | 75 | 0 | 0 | 0 | 0 |

*A = EFSA dataset of 76 pesticides for which ARfD was based on developmental effects; B = Expanded EFSA dataset of 135 chemicals.

As a second step of the project, the suitability of the read-across approach was explored. The consistency of this approach depends on the selection of appropriate analogues and on the availability of reliable experimental data. OECD (Q)SAR toolbox was used for grouping the compounds and the EPA's ToxRefDB database was selected to perform the read-across exercise for developmental toxicity. The aim of this exercise was to refine the predictions resulting from the application of (Q)SAR. Chemicals can be defined as active, inactive or inconclusive, as a result of a number of expert choices in the read-across procedure. The predictive performance of this tool cannot be evaluated because the outcomes could be different on the basis of different choices, but it could be considered in a step-wise approach combined with the use of (Q)SAR tools. Table 6 (Table 9.1 of the JRC report, 2011) shows the possible outcome of a proposed stepwise strategy carried out with the EFSA extended dataset (135 substances) and involving the application of the PASS model for teratogenicity, then grouping and read-across using the OECD Toolbox and US EPA's ToxRefDB database. The overall outcome shows that read-across increases the positive predictivity of (Q)SAR analysis (to 90%), allowing better discrimination of true and false negatives generated by the use of (Q)SAR.

Table 6: Possible outcome of applying the stepwise assessment strategy (taken from EC, 2011)

| Step | Entering | Predicted positive | Predicted negative | Not predicted | Filtered out | Proceeding to next step |
|-------------------------|----------------|--------------------|--------------------|---------------|-----------------------|-------------------------|
| 1. Existing data | 135 96P,39N | | | 43 | 43 (adequate data) | 92 72P,20N |

| | | | | | | |
|--|----|-----------------|---------------|----|--------------|----|
| 2. (Q)SAR model (PASS teratogenicity) | 92 | 63 | 29 | 0 | 63 | 29 |
| 3. Read-across (OECD Toolbox) | 29 | 5 | 9 | 15 | 15 (no data) | |
| Totals | | 68 62TP, 6FP | 9 8TN, 1FN | 58 | | |

7.3. Conclusions of the contractor

No individual (Q)SAR model or combination of models appears to be adequate to predict the neurotoxic potential of pesticide metabolites, based on the outcome of the exercise carried out with a limited dataset comprising 40 positive and 21 negative pesticides.

The application of a stepwise approach including (Q)SAR models and read-across for the prediction of the developmental toxicity of pesticide metabolites appears to be promising.

The key step for future development of this strategy is the establishment of a searchable structural database including high quality toxicological data on pesticide parent compounds. In addition, considering that developmental toxicity is a complex process involving short term and long term effects associated with acute and/or chronic exposure, classification of the events could help in the identification of acutely toxic substances.

A stepwise assessment scheme based on the combined use of (Q)SAR and read-across was proposed. The general stepwise assessment scheme based on the use of existing data and non-testing methods in the report from the contractor (see reference list). A first step involves the use of (Q)SAR models, alone or in combination, for identifying developmental toxicants. A second step includes a further evaluation of the compounds predicted as negatives by (Q)SAR using a read-across approach.

7.4. Conclusions by the PPR Panel

The PPR Panel concludes that the predictivity for neurotoxicity of the (Q)SAR models, tested alone or in combination, is currently inadequate to be applied in the evaluation of the toxicological relevance of metabolites. A similar conclusion was reached with the application of DEREK in the TTC case study performed by CRD, where a more reliable prediction of neurotoxicity was obtained using the known mechanism of action of pesticide active substances.

It is not possible to use a read-across approach to predict neurotoxic effects at the present time, due to the lack of reference databases for this effect.

(Q)SAR tools alone are not sufficiently reliable to predict developmental effects, due to their low negative predictivity. The read-across exercise performed by the contractor as part of a stepwise approach, using the US EPA's Toxicity Reference Database (ToxRefDB), which includes data on developmental toxicity for more than 300 pesticides, resulted in an improvement in the identification of potential developmental toxicants and non-developmental toxicants. The PPR Panel considers that a combined approach including (Q)SAR and read across, as proposed by the contractor (see chapter 11), could be a preliminary step before the application of the TTC scheme, in order to evaluate if an acute exposure assessment is required. No clear criteria could be derived for the application of the proposed scheme, because the read-across approach is not an automatic procedure; unlike the (Q)SAR tools it is very dependent on the selection of the reference database and a number of expert choices involved.

Research is needed to further develop the use of (Q)SAR tools, by classifying the different endpoints associated with developmental toxicity. In addition the development of an appropriate database on pesticides, would allow improvement in the use of the read-across approach.

8. Potential exposure to pesticide metabolites in the human diet

8.1. Introduction

In order to evaluate the relevance of pesticide metabolites it is necessary to have conservative exposure assessments, which take into account high exposure scenarios.

The potential for exposure to metabolites in food and feed may vary depending on active substance and residue specific factors as well as the exposure scenarios being considered. In this opinion chronic and acute exposure scenarios considering metabolic profiles in different crops and various intended uses of the pesticide are presented.

Residue levels of pesticides and metabolites in plant and livestock depend on several factors related to Good Agricultural Practice (GAP) i.e. mode and time of application, applied dose, number of applications and the pre-harvest interval (PHI), but also on environmental conditions and nature of the crop or livestock. The pesticide may be converted into metabolites to different degrees quantitatively and also qualitatively, due to differences in metabolic pathways across different species. When estimating the exposure to the metabolites with limited data available there is a need to extrapolate between different crops and potentially between different crop metabolism groups and to consider the extent of uses that should apply. The levels of metabolites in various crops depend on many factors, especially pre-harvest interval, type of crop and part of plant considered, and therefore the relative amounts of metabolite(s) in relation to parent seem to follow complex rather than easily predictable patterns. This is also reflected in the practice of setting the conversion factors, for applying to the level of residue in the residue definition for monitoring to one for use in risk assessment, as presented in this chapter (chapter 8.5) and demonstrated in Appendix D.

The estimation of exposure to metabolites has been tested by the Panel, building on the exposure work presented in the outsourced project (CRD, 2010), by way of case studies, encompassing scenarios for primary crop, rotational crop and livestock on six pesticides, namely azoxystrobin, bitertanol, boscalid, dimethoate, napropamide, and prohexadione calcium. The case studies are presented in Appendix E.

In Figure 1 an Exposure tree describes the different steps to consider when estimating exposure to the metabolites of a pesticide. The different steps in the Exposure tree are explained in detail in this chapter and illustrated in the case studies, see Appendix E.

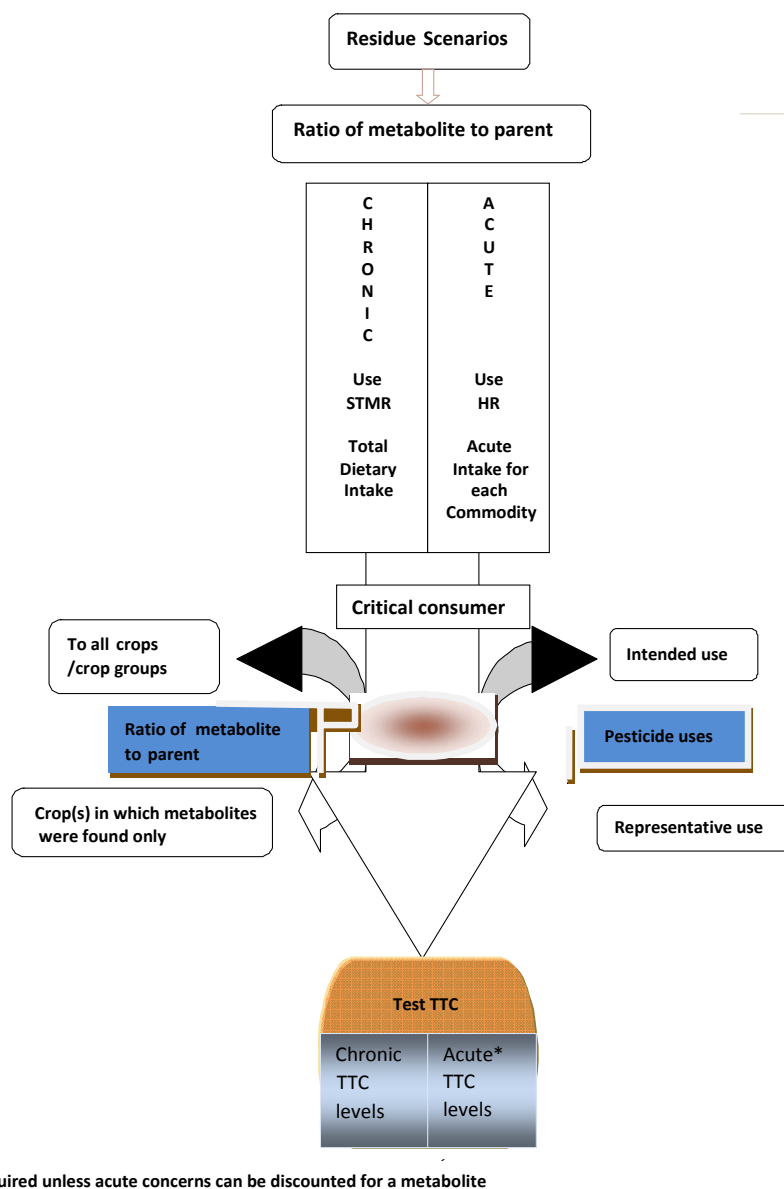


Figure 1: Assessment scheme for chronic exposure

Residue scenario: Metabolites may be qualitatively and quantitatively different in various residue scenarios i.e. in primary crops, rotational crops or in animal products, see case study Appendix E

Ratio of metabolites: Only limited data on levels of metabolites are usually available. Therefore consider the level of metabolite to a level of parent as a ratio for use in estimating chronic and/or acute exposure, see chapter 8.3

Chronic and acute exposure estimation: For chronic intake calculations of metabolites use STMR of the parent and for acute use HR of the parent in conjunction with the metabolite ratio, or where necessary alternative methodology discussed, see chapter 8.3

Critical consumer: Calculate the exposure for various consumer groups, including young children and other vulnerable groups, and deduce the highest result across all of the consumer groups, representing the 'critical consumer'.

Pesticide uses: Ratio of metabolite to parent may differ with PHI, crops and crop groups. The extent of pesticide use on different crops may also differ. These factors influence intake estimate considerably, see case study Appendix E

Test TTC: Compare the intake estimates with relevant TTC, see Appendix E and G

8.2. Types of exposure scenarios

Relevant exposure scenarios for assessment of consumer risk for pesticide residues are explained in the triazole opinion (EFSA, 2009a, chapters 3.5 to 3.10). The reader is referred to this opinion for a detailed description. In summary; scenario 1 refers to the actual exposure (i.e. from the patterns of usage that actually occur in practice), during a short (i.e. 24 hours) time span; scenario 2 to the actual exposure during a chronic (i.e. lifetime) time span; scenario 3 to acute (i.e. 24 hours) exposure relevant for MRL-setting (i.e. a theoretical exposure where the residue of the compound/commodity combination under evaluation is at the level of the MRL); and scenario 4 to chronic (i.e. lifetime) exposure relevant for MRL-setting assessed at the level of the STMR.

In this opinion, we are focussing on scenario 3, acute exposures relevant for MRL setting and scenario 4, chronic exposures relevant for MRL setting, since there are limited data available on metabolites for the monitoring situation (actual exposure scenario). For practical purposes, the relevant residue definition typically used is that for monitoring and enforcement purposes rather than the residue definition for dietary risk assessment. In EU monitoring, although it is not possible to generalise, metabolites are only included for a fairly small sub-set of the overall pesticides included in the monitoring schemes.

During the risk assessment process, a chronic exposure estimate is always made for parent compounds. A specific chronic exposure estimate for the metabolites is therefore also needed in the process of evaluating their toxicological relevance. An acute exposure estimate for parent compounds is done if the compound has acute toxicity, and the possibility of acute toxicity of the related metabolites should also be considered (see chapter 10.2.5).

The dietary exposure of the consumer to metabolites varies with potential future intended uses (see Fig. 1). Three metabolite ratio scenarios are considered to be relevant when considering exposure estimates. These are (i) application of the ratio to the crop in which the metabolite was found (ii) application of the ratio to the crop in which the metabolite was found to all of the relevant crops in the same metabolism grouping (iii) application of the ratio (highest ratio) in which the metabolite was found to all crops/crops groups. In this opinion we will address scenarios i and iii because they will provide the lowest and the highest possible exposure estimates. Different options for estimating exposure are taken into account that aim to reflect the possibility of extrapolation of data between different crops, and the extent of uses being considered. These options (A, B, C and D) and examples of rotational crops and livestock exposure scenarios are further discussed in the case studies presented in Appendix E. The choice of scenario depends on the level of protection that a risk manager wishes to apply and as such is outside the remit of EFSA. Since the TTC approach requires the use of conservative estimates of exposure, a pragmatic way forward could be to focus on the scenario that involves a number of conservative assumptions, whilst also giving an estimate between the extremes.

8.3. Estimation of metabolite levels

Crop Supervised Trials Median Residues (STMR) and Highest Residue (HR) values derived from supervised residues trials studies conducted at GAP rates and timings tend to be available as key residue values for the parent residue. Whilst these studies are recognised as giving a quantitative determination of levels of residues of parent at harvest (for risk assessment and MRL setting purposes), the levels of metabolites are harder to obtain since metabolites are only analysed in field trials if a registrant is considering their potential inclusion in the proposed residue definitions. At most a few metabolites tend to be sought in the residues trials. Plant metabolism studies provide more qualitative information on the presence of metabolites, but these studies involve treating only a small number of plants and as such the results are generally regarded at best as 'semi-quantitative'. Therefore for the case studies presented here (see Appendix E), the residue level of metabolites was calculated by applying the metabolite to parent ratios as determined in the plant metabolism studies to the STMR and HR for the parent residues from the supervised residues trials. Where parent is not identified in the metabolism data, e.g. in the case of extensively metabolised pesticides, a ratio of

metabolite to parent cannot be derived. In such cases the level of metabolite can be indicated by making an adjustment to the level of the metabolite found in the metabolism study according to the rate of the metabolism study in relation to the GAP rate. For example if the metabolism study is at '2 x' the relevant GAP rate, then the estimated level of metabolite for the case study would be half that found in the metabolism study.

The range of metabolites were evaluated to consider those that were present in an amount below or above the TTC threshold values, when classifying the metabolites as 'mammalian' (found in laboratory animal metabolism, usually rats) or plant or livestock specific (not found in the rat). This was done to consider whether 'mammalian' (laboratory animal) metabolites could be compared to the TTC level or toxicological reference values (ADI or ARfD) for the parent pesticide, and to consider whether plant or livestock specific metabolites could be efficiently handled by assessing the metabolites by way of the Cramer Class structural characterisations that can be readily performed using publicly available software²⁸. 'Mammalian' metabolites were classified as such if they were found at any level in laboratory animal metabolism studies, in the urine, blood or bile of the test species (rat). Metabolites that were found only in rat faeces were not included since it is possible that these metabolites are formed by intestinal microorganisms. Plant or livestock specific metabolites are those metabolites which were found only in the plant (or livestock) metabolism and were not, as far as the identification work conducted in plant and livestock (typically hen or goat) and rat could conclude, found also in the rat metabolism.

The case studies aimed to consider a range of different active substances with different characteristics: well metabolised versus little metabolism; variable metabolic profile according to crops treated; compounds of apparent high (e.g. ADI 0.001 mg/kg bw/d), moderate (e.g. 0.04 mg/kg bw/d), and low (e.g. 0.2 mg/kg bw/d) toxicity. The case studies also evaluated some examples considering metabolites found in rotational crops and livestock, particularly covering novel metabolites not seen in the primary treated crop. This was done to address pesticide residues in crops grown in rotation after the primary crop that has been treated. Carry over of residues in the soil can then be taken up in the rotational crop. Additionally, crops used as feed may also give rise to residues in livestock commodities, such as milk and meat. Monitoring programmes do not always take metabolites that are specific to livestock or the rotational crop situation into account, although efforts to improve the range of analytes being covered in animal products monitoring programmes appear to be underway. The availability and cost of analytical standards for 'novel metabolites' can be a factor influencing their inclusion in the monitoring.

In terms of extent of uses, it is not always possible to know the full range of future intended uses. Residues data are more readily available for the DAR representative uses, although it is likely that an exposure assessment for the DAR assessment is an underestimation of the potential exposures in view of future uses to be considered. In the case studies this is illustrated by using different metabolite to parent ratios with different extent of uses focusing on the extremes of approaches (Appendix E).

The least and most conservative approach of metabolite to parent ratio and extent of uses are used in the case studies to demonstrate the impact of the different approaches.

8.4. Models used for calculating acute and chronic dietary exposure.

The same methods that are used to calculate the exposure to parent compounds were employed by the PPR Panel to calculate theoretical chronic and acute exposure to pesticide metabolites in the case studies for this opinion. The following paragraphs briefly describe the methodology, however the reader is referred to the reference list for a more detailed explanation.

WHO developed a simple model to calculate chronic exposure to a pesticide (IEDI; International Estimated Daily Intake). The basis of the model is the multiplication of average food consumption

²⁸ e.g. TOXTREE version 2.5.0 August 2011 Ideconsult Ltd., Bulgaria – available via the EC Joint Research Centre website at [http://ecb.jrc.ec.europa.eu/\(Q\)SAR/\(Q\)SAR-tools/](http://ecb.jrc.ec.europa.eu/(Q)SAR/(Q)SAR-tools/)

levels by average concentrations in the relevant foods, and summing the contribution from all relevant foods. The total mean dietary exposure (summed over all commodities on which the pesticide is used) is then compared to a chronic (long-term) toxicological reference value (ADI) (WHO, 1997a, 2009).

In general, EU Member States and EFSA follow the WHO approach of using average consumption estimates for chronic assessment, however some Member States have introduced additional refinements to the risk assessment calculation, also considered appropriate by WHO on the use of best available consumption data and tailored consumption data sets. An example of such a refinement is chronic exposure assessment for high level consumers (WHO, 1997a). In the United Kingdom, the usual practice is to base the calculation for the two highest contributing food groups on the 97.5th percentile consumption recorded, while for the other food groups the average intake figures are used (EFSA, 2007). As discussed in a recent EFSA opinion (EFSA, 2009), further evaluation is needed on the merits of using various forms of above average consumption data.

To calculate acute exposure, WHO developed a model called the International Estimated Short Term Intake (IESTI). The model assumes that a consumer may eat a large portion (high level consumer at the top end of the distribution curve) of a food which may contain higher residues than the composite sample which was derived from supervised field trials. The acute assessment is conducted for each commodity separately as it is considered unlikely that a consumer will eat two or more different commodities in large portion weight within a short period of time and that those commodities have the highest level of the same pesticide (WHO, 1997b). EU Member States and EFSA follow the WHO approach for acute exposure assessment although the current European practice takes into account only the amendments adopted by the 2002 and 2003 JMPR Meetings and no later amendments (EFSA, 2007).

These further amendments and in particular the question of the level of the default variability factors have been the subject of review and continuing consideration in the EU (EFSA, 2005; 2007).

The primary method for conducting risk assessments in Europe and to compare consumer intakes based on various EU national consumption data is through the use of the EFSA PRIMo model for chronic and acute risk assessment (rev. 2.0)²⁹.

The PPR Panel used the UK model and approach to calculating the chronic exposure to estimate the exposure to metabolites in the case studies for this opinion, since the starting point for the methodology for the exposure calculations was the outsourced project on TTC (see chapter 5) which was carried out by a contractor from the UK (CRD, 2010). The UK model for chronic exposure (the UK NEDI, National estimate of daily intake) is different to the generally used EU PRIMo assessment, due to the use of the 97.5th percentile values, as explained above. However, the impact of this difference on the conclusions of the use of the TTC approach is minor, since the Panel found that the acute exposure is the critical issue. The NESTI (National Estimated Short Term Intake) calculations were performed following the current EU practice (see above), and using the UK model (since the UK model conducts the same acute exposure calculations as those done in the EU PRIMo assessment). The UK consumption spreadsheets³⁰ were thus used as a model approach covering, in accordance with PRIMo, a range of consumers, including vulnerable sub-groups such as the elderly and young children.

8.5. Conversion factors for estimating metabolite levels

Conversion factors are discussed in this opinion, as the scientific principles that underpin the derivation of such factors are directly relevant to metabolite exposure calculations. Conversion factors

²⁹ In the context of evaluation of temporary MRLs, EFSA created a European food consumption database by collecting all the consumption data already available at Member State level (national diets) and at international level (i.e. the GEMS/Food WHO diets WHO diets). It was named EFSA PRAPeR database (EFSA, 2007a) after the name of the unit within EFSA who set-up this database (PRAPeR: Pesticide Risk Assessment and Peer Review). Version 2 of the EFSA model has been renamed EFSA PRIMo (Pesticide Residue Intake Model) database (EFSA, 2008b and 2008c).

³⁰ UK NEDI and NESTI spreadsheets are available at <http://www.pesticides.gov.uk/approvals.asp?id=1687>

are multiplication factors which may be established when the residue definitions for monitoring and risk assessment differ, but relate to the same toxicological endpoint. Conversion factors are thus applied to monitoring data to take into account exposure to metabolites that are not measured during the monitoring.

A conversion factor for converting the residue definition for monitoring into one for risk assessment is derived by dividing the value of the measured residue for risk assessment by the value of the measured residue for enforcement. This should be done for each crop; for each pair of residues for each residue trial (using data sets with comparable and suitable GAPs) and a mean value across all these trials derived. Likewise the same principles can apply to livestock feeding studies. In this opinion the reverse factor is used (metabolite to parent as a ratio) for estimating metabolite levels. Additionally the assessment of metabolite to parent in this chapter and in the case studies (Appendix E) considers the levels of metabolites individually, whereas conversion factors are proposed to address the presence of a number of metabolites where needed.

Metabolism studies, supervised trials as well as feeding studies show that metabolism of parent in plants and livestock depends on various factors related to the GAP and the crop or commodity analysed. When estimating metabolite exposure or deciding which conversion factor to choose, it is thus important to consider different use patterns of the compound, such as timing of application, since this has an effect on the levels present and the ratio of metabolite to parent. The optimal aim is to cover the practical conditions of use of the pesticide, and thus representative trials data are preferred (rather than relying on metabolism data alone) for setting conversion factors.

This is illustrated in Appendix D, where the metabolite to parent ratio is presented for different uses of six pesticides. As observed, these ratios change over time, with the type of crop and with the part of the plant considered.

The variability may be due to natural spatial variability (e.g. of degradation rates), number of applications, but also to variability of application techniques that lead to uneven distribution which cannot be avoided (e.g. furrow or band application of granules).

The expected behaviour would be that the metabolite to parent ratio will increase with time as the metabolic process advances and parent levels decline. However, the experimental results show that the ratio can be maintained or even diminish. One possible reason is successive applications of the active substance. Appendix D also shows that some pesticides degrade to several metabolites (e.g. malathion, kresoxim-methyl, flonicamid) and the comparison of ratios of each metabolite to parent shows which one is the most important in terms of prevalence (see the malathion example in Appendix D).

As such, use of conversion factors for estimating residue metabolite levels are subject to a number of uncertainties (see also chapter 10). The type of supporting data at the time of setting a conversion factor usually places constraints on how a conversion factor should be used, and these are typically stated in the EU end-points. These constraints usually relate to the crop or crop groups that the conversion factor covers, possibly by extrapolation from one species to others. The opinion extent of extrapolation from one crop to another is critical in the case studies of this opinion when estimating the likely potential intake of metabolites that have not necessarily been determined in the residues trials.

The success of the approach in setting two different residue definitions depends on the reliability and availability of conversion factors. In practice conversion factors tend not to have been used by monitoring bodies in Member States, or by EFSA, since they are not readily available for all pesticide and commodity combinations. The lack of widespread use of conversion factors could also possibly be due to recognition of the uncertainties in the way they are set and serve to estimate an upper potential theoretical exposure level for residues of dietary interest based on the determination of marker

components. Further guidance on the use of conversion factors by monitoring authorities is not yet available.

8.6. Results and discussion

The exposure scenarios considered in this opinion are acute exposure and chronic exposure relevant for MRL setting. These scenarios are relevant for the residue definition for dietary risk assessment, where metabolites are specifically considered (EFSA, 2009a). The method for estimating metabolites as illustrated in the Exposure tree (Figure 1) has been tested out in case studies applied using the TTC scheme as presented in Chapter 11.

The metabolite estimations in the case studies demonstrate significant consumer exposures to a range of pesticide metabolites.

The different methods for estimating exposure, where the extent of extrapolation of the metabolite ratio and extent of uses being considered varied, produced notably different results. The range of metabolite intake values obtained, span a very wide range. Few metabolite estimations were below the genotoxicity threshold of $< 0.0025 \mu\text{g/kg bw/day}$ (only for adults when considering the primary crop situation) and quite a large number of metabolite intake levels estimated were above Cramer class I of $> 30 \mu\text{g/kg bw/day}$, especially for the exposure option that considered the most extreme assumptions (option D³¹ - widespread extrapolation of the highest metabolite ratio and widest extent of uses being considered).

Since the chronic TTCs were considered to be conservative for use in acute exposure (EFSA, 2012) it was concluded that if both chronic and acute exposure estimates for metabolites were relatively low and below the chronic TTC thresholds, it could be proposed that no further toxicological assessment of the metabolites would be needed. In this way a 'screen' using the chronic TTC values would be adequate to propose an assessment scheme by comparing all intake values calculated for metabolites with the TTC values. However, the intake estimations were markedly higher for the acute exposure assessments (in the PPR Panel case studies, Appendix E) which necessitated a more specific approach for acute considerations in a TTC assessment scheme. It is worth noting that the results for the acute estimates of metabolite exposure are only presented (in Appendix E) for the highest commodity intakes, and there will be lower intakes for other commodities that could pass a 'TTC screening approach' more easily than the worst case commodities that are presented in the case study. Due to the different parameters in the metabolite intake estimations, it was not possible to derive a consistent factor between an acute versus chronic exposure result for a particular metabolite. For example, for dimethoate the acute metabolite estimates (for the critical consumer) were 1.2 – 2.6 x higher than the corresponding chronic metabolite estimate intakes, whereas for azoxystrobin the acute metabolite estimates (for the critical consumer) were 2.7-27 times higher than the corresponding chronic metabolite estimate intakes. A difference in acute and chronic exposure by at least an order of magnitude can be realistically expected. There was a much higher proportion of chronic exposure estimates for metabolites below the 'neurotoxicity' threshold of $0.3 \mu\text{g/kg bw/day}$ than could be concluded for the acute exposure situation. Additionally these metabolites were typically above the 'genotoxicity' threshold of $< 0.0025 \mu\text{g/kg bw/day}$. Depending on the exposure options being considered (especially excluding the most extreme approach, option D), a number of metabolites were below the $1.5 \mu\text{g/kg bw/day}$ threshold level even when only considering chronic exposures. However when the extreme set of assumptions was applied (widespread extrapolation of the highest metabolite ratio and widest extent of uses being considered), only a limited number of metabolites were below the $1.5 \mu\text{g/kg bw/day}$ threshold level. The TTC values for chronic exposure are based on effects that are often not relevant for acute exposure. Hence, in view of the notably higher intake results typically observed for the acute exposure results compared to chronic exposure, the PPR Panel developed an approach to derive acute TTC levels (chapter 5.3.1) for pesticide metabolites to enable a more suitable comparison of the acute exposure estimates for the metabolites in a TTC assessment scheme.

³¹ See chapter 8.2 ratio metabolite scenario iii

In the PPR panel case studies (Appendix E) a second step of comparisons of the estimated metabolite intakes to TTC levels was made to consider the newly proposed acute TTC levels proposed (chapter 5.3.1). This analysis, unsurprisingly showed a greater proportion of metabolites below the new proposed acute threshold value of 5.1 µg/kg bw/day, than were previously assessed (at the first step comparison stage) when a number of the metabolites were found to have acute exposures above the (chronic TTC) level of 1.5 µg/kg bw/day. As an example, for one exposure option C and the critical consumer group 53% of the metabolite intake estimates were between 0.3 and 5 µg/kg bw/day, whereas only 17% were between 0.3 and 1.5 µg/kg bw/day. Therefore this tailored approach, using more relevant TTC levels differentiating between acute and chronic effect will have practical use within the assessment schemes (Chapter 11) in identifying the metabolites that require further specific toxicological testing. The various TTC levels used by the PPR Panel for both the chronic and acute assessments are stated in Table 2 of Appendix E.

The results of the case studies and Cramer Class Toxtree (CCT) assessments show that metabolites are mostly in the same structural class, Cramer Class III (CCIII) suggesting a presumption (in the absence of specific toxicity data for the metabolites) of significant toxicity. The case studies show that of the 47 primary crop metabolites considered in the case studies, only six were not CCIII (across two pesticide substances, of the six, five were CCI and only one was CCII). Therefore the results demonstrate that structural class assignation using Toxtree does not provide a particularly useful method of differentiating further between metabolites that either do or do not require further assessment as part of a TTC decision tree approach. It is noted that the EFSA Scientific Committee (EFSA, 2012) in their work on the TTC approach for application more generally in the food and feed area, have suggested treating substances that would be classified in Cramer Class II as if they were Cramer Class III substances. This is reflected in the work of the second step of comparisons performed in the PPR Panel case studies (Appendix E), and in the TTC levels used by the PPR Panel for both the chronic and acute assessments (as stated in Table 2 of Appendix E).

The case examples covering the rotational crop and livestock situations demonstrate that exposures to metabolites via these routes, although seemingly ‘indirect’, should not be automatically discounted. The case studies also show the practical difficulties that can be encountered in trying to consider these metabolite exposures, although the data availability and specific residue situations for the primary treated crops can also affect the ease with which levels of the primary crop metabolites can be estimated. The uncertainties in these metabolite estimations are further discussed in Chapter 10. The case studies confirm that metabolites from the rotational crop and livestock situation can be and should be considered as the exposure levels can be significant. It does not automatically follow that metabolites found in the rotational crop or livestock species do not need to be further considered if they have been identified in the primary crop, as exposure levels can be quite different, and it is recommended that an assessment should be made taking account of the specific residues data.

9. Impact of stereochemistry on the toxicological relevance of pesticide metabolites for dietary risk assessment

9.1. Terminology

Molecular asymmetry – a lack of a mirror plane within the structure of a molecule - is a frequent and well known phenomenon among organic substances. This asymmetry, which can be caused by different stereogenic centers, leads to the formation of so-called stereoisomers. Stereoisomers are isomeric molecules that have the same molecular formula and sequence of bonded atoms (constitution), that differ *only* in the three-dimensional orientations of their atoms in space (see Glossary for an extensive list of stereochemical terms). It is estimated that 25% of agrochemicals possess an asymmetric centre or other stereogenic element in their molecular structure that gives rise to a number of stereoisomers. A considerable number of molecules carry more than one stereogenic centre. The total number of possible stereoisomers is then equal to 2^n where n is the number of centres of asymmetry. Stereoisomers can be divided in enantiomers and diastereomers. Enantiomers are two stereoisomers that are mirror images of each other. Diastereomers are isomeric molecules that are not

mirror images. Whereas enantiomers show the same physico-chemical properties (except the direction of deflection of the angle of polarised light) they have in general different biological properties. Diastereomers have different physico-chemical properties and are expected to have different biological properties. Diastereomers can often be separated by conventional (non-enantioselective) chromatographic techniques, but the separation of enantiomers calls for a so-called “chiral column” which, if successful, separates enantiomers through diastereomeric interactions.

9.2. Introduction

From around the time of the mid-20th century, the development of new synthetic organic pesticide molecules was focused on controlling target pests or weeds and, the stereochemistry of the molecules attracted less attention or was largely ignored. This was also due to the fact that an understanding of the biochemical processes (on mode of action and toxicity) and the analytical and chemical-synthetic techniques was far less developed.

In recent decades, the various stereochemical aspects of synthetic organic pesticides have attracted considerable attention from the pesticide industry as well as regulatory authorities and academia for different reasons. The industry relaunched older pesticides that were mixtures of stereoisomers with differing biological properties as enantioenriched or enantiopure compounds (“chiral switch”) combining lower use rates with a claim of reduced residues in crops and lower environmental impact. Furthermore, progress in asymmetric synthesis of pesticides led to a decrease in costs of production of enantiopure active ingredients (e.g. racemic metolachlor → metolachlor-S, Mannschreck and von Angerer, 2009).

For example, cypermethrin was introduced in 1968 as the unresolved isomeric mixture but subsets of isomers of this substance have their own ISO common names: alpha-cypermethrin, beta-cypermethrin, theta-cypermethrin and zeta-cypermethrin are considered different active substances.

The insecticide indoxacarb (one chiral carbon atom and two possible enantiomers) was introduced in the mid-1990s and the ISO common name refers to the *S*-enantiomer only, which carries the insecticidal activity – the *R* enantiomer is insecticidally inactive. This led to the situation, that when manufactured as the racemate (both enantiomers in equal ratio), the first technical materials contained only approx. 50% of active ingredient. In time, new manufacturing processes led to more enantioenriched technical materials with up to 920 g/kg of indoxacarb (FAO, 2009b; The Netherlands 2005).

The “chiral switch” also stimulated the scientific interest of academia, aimed at better understanding the processes involved when chiral molecules undergo metabolism in biota and in the environment.

There are separate reviews on the stereochemistry of agrochemicals and pharmaceuticals. In an IUPAC paper (Kurihara et al., 1997) chirality within chemical classes of pesticides is presented.

Pesticide stereoisomers can differ to some extent in their desired biological activity, and in their fate in living organisms. The ratio of the (stereo)isomers within an active substance consisting of a mixture may change due to metabolism, to environmental degradation or to processing, as a result of preferential degradation (see glossary) and/or conversion. As a consequence the resulting mixture can have significantly different properties compared to the original active substance. The risk assessment can be biased if the endpoints valid for the active substance are used for the mixture resulting from preferential degradation and/or conversion, if such preferential degradation and/or conversion is significantly different in the species of interest (e.g. plants, rats, humans).

A review on residue studies performed with single isomer active substances indicated that the terminal residue in plants can be composed of more than one isomer, i.e. isomerisation occurred when the active substance was metabolised in the plant. One example among several is the strobilurin fungicide fluoxastrobin, defined solely as the (*E*)-isomer. Upon application of the technically pure fluoxastrobin (>98% *E*-isomer) to wheat the isomer ratio in the residue at harvest had changed to approximately

80% *E*- and 20% *Z*-isomer in grain, and 70% *E*- and 30% *Z*-isomer in straw. Consequently, metabolites of fluoxastrobin were also present in their isomeric forms. (The United Kingdom, 2003).

Additionally, a change of the ratio of isomers in the plant residue compared to the applied active substance can occur when the rate of metabolism is different for the individual isomers. A study with the conazole fungicide bromuconazole in wheat analysed the plant residues at harvest for its two diastereomeric pairs of enantiomers. The active substance initially applied was composed of a ratio of the two diastereomers of approximately 50:50. Results of the composition of the final bromuconazole residue indicated a shift in the ratio had occurred towards one diastereomer, leading to a ratio of about 70: 30 in straw and grain, and of 92: 8 in wheat chaff (Belgium, 2009).

Conditions of crop processing (pH, temperature etc.) may also lead to a change in the ratio of isomers. In studies on the residue behaviour of the carbamate fungicide benthiavalicarb conducted with pure material of the variant benthiavalicarb-isopropyl (*R*-L diastereomer), different pH-values were demonstrated to have had an impact on the generation of isomers of benthiavalicarb-isopropyl. The rate of isomerisation from benthiavalicarb-isopropyl into the *S*-L diastereomer tended to increase with a decrease of the pH-value. Processing studies confirmed that, even though not present above the LOQ in the raw commodities, the *S*-L diastereomer was found in tomato processed products and in raisins at significant levels (Belgium, 2004; 2007).

Information on stereochemistry (composition of stereoisomers) of metabolites is missing for most stereoisomeric pesticides. This is not always considered a gap in knowledge, as shown by a recent scientific report on “Applicability of physicochemical data, (Q)SARs and read-across in Threshold of Toxicological Concern assessment”, (Bassan, 2011) where stereoisomers were considered as duplicate structures and the stereoisomer issue was disregarded (“The stereo specificity of the structures, which usually emphasises the differences between the racemate and the individual pure enantiomers is assumed not significant in the common *in silico* procedure and therefore was not taken into account.”).

However, the well known example of the glutamic acid derivative thalidomide shows that correct knowledge on stereochemistry can be crucial in a risk assessment. With thalidomide, the *S*-enantiomer of the drug seems to intercalate into DNA in guanine-cytosine-rich regions, whereas the *R*-form does not. This intercalation is considered as the mechanism of the teratogenic activity of the *S*-enantiomer only, but as the *R* is converted *in vivo* to its antipode, administering pure *R*-enantiomer would not prevent the teratogenic effect.

A review of the published scientific literature indicates that it is not possible to make generalisations about the extent to which preferential metabolism or differential toxicity of isomeric forms of pesticides occurs. Whilst examples of differential metabolism and/or toxicity do exist in the literature for xenobiotics and pharmaceuticals, (see Report on Differential Metabolism of Chiral Compounds³²), it is not possible to draw relevant generic conclusions from the literature that can aid the regulatory assessment of specific active substances. Therefore, where appropriate, these issues need to be addressed within tailored pesticide submissions.

9.3. Consequences of stereoisomerism for risk assessment

When pesticide active ingredients composed of mixtures of stereoisomers were replaced by their enantiopure analogues, regulatory authorities were confronted with the need for a comparative risk assessment. When considering dietary exposure to chiral metabolites, a theoretical worst case situation would be when the prevalence of a particular stereoisomer in mammals – upon which the toxicological reference values are based - is not reflected in plants. It would be of particular concern if the ‘opposing’ isomer, that that was not well represented in the mammalian species, was the predominant stereoisomer in plants. Assuming that the contribution to the toxicological burden arises solely from the enantiomer formed in plants and the enantiomer formed in the rat does not contribute at all, dietary

³²<http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=11787&FromSearch=Y&Publisher=1&SearchText=chiral&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description>

risk might be underestimated. In such a situation and neglecting stereoisomerism, the metabolite in plant would be judged as covered by the metabolism in rodents, whereas a thorough stereochemical analysis would show that it is not the case, with the result that the contribution of that metabolite to the overall toxicological burden is not taken into account at all.

Currently most metabolism studies do not include stereoselective analytical approaches, as the need for this is not currently stated in guidelines. The complexity in risk assessment with pesticide stereoisomers mainly arises from limitations encountered in extending the metabolism studies in mammals (e.g. the ADME study), in the environment and in plants and livestock to cover all possible stereoisomers encountered in the course of these metabolic processes. The complexity of enantioselective analytical methods, the need for enantiopure analytical standards, or analytical standards of known enantiomeric composition, and the potential for new chiral centers to be introduced during the metabolism (from prochiral centers) illustrates the formidable task a company has to face when a thorough elucidation of the stereochemistry in these studies is attempted.

Typically toxicity and metabolism studies are conducted with a certain defined composition of stereoisomers, and comparative studies considering the outcomes, when different test substances with various isomeric compositions are used, tend not to be available. Toxicity studies are often conducted on the (racemic) mixture so in such a situation it is not possible to deduce if one isomer is more toxic than the other. It has generally not been intended to ask for further toxicological studies unless such studies are clearly justified, such as when the diastereomer ratio of a technical pesticide is significantly changed and no longer covered by the already submitted hazard data. Concerns regarding the need to understand the differences in toxicity are heightened when there is a preferential metabolism in plants (or livestock animal tissues) compared to the mammalian species in which the toxicity testing has been performed, and when an estimation of exposure indicates there are potential issues of concern for risk assessment. However in considering this, it is problematic that metabolism studies do not identify the stereochemical nature of metabolites, and thus a situation arises, where neither the toxicological knowledge nor the evidence for preferential metabolism of the isomers are sufficient to allow an overall assessment. The general assumption in the evaluation of the toxicological reference values of mixtures of stereoisomers is, that the hazard characterisation and associated end points cover the main relevant processes including any preferential degradation and conversion in the laboratory animals. However this does not fully resolve the issue of needing to properly cover the risk assessment when it is known that isomers can degrade in different ways and the metabolism studies do not address the isomeric form of metabolites.

Independent of the consideration of the different isomers as active components or major impurities of an active substance, they are applied to the crops and released to the environment as plant protection products, resulting in exposure of humans and non-target organisms. Therefore, all isomers in the mixture need to be considered in an appropriate risk assessment.

The current opinion does not address the active substances but focuses on toxicological relevance of metabolites in dietary risk assessment.

9.4. Data requirements regarding stereochemistry under Regulation (EC) 1107/2009

The current data requirements already establish that the substance tested should match the technical specification (including its isomeric composition) and that formation and effects of metabolites, degradation and reaction products should be investigated. This does not exclude the case when metabolites are isomers of the active substance. The information provided must be sufficient to permit an evaluation to be made on the nature and extent of the risks for man, an assessment of the fate and behaviour of the active substance in the environment, and the identification of non-target species likely to be at risk from exposure to the active substance, its metabolites, degradation and reaction products, where they are of toxicological or environmental significance.

Neither OECD nor EU residues guidelines on metabolism (OECD, 2007b, c, d; EC, 2011) require that the stereochemical nature of metabolites be specifically investigated.

The recent update of the OECD Guideline 417 on Toxicokinetics (OECD, 2010) does not cover stereoisomers explicitly. Where stereochemistry is not addressed, the toxicological reference values are considered to cover the effects of the actual mixture of stereoisomers. However, this means that possible racemisation and preferential degradation/formation processes are not considered. Disaggregation to be able to apportion contributions of individual stereoisomers to overall effects is not really feasible.

When comparing the data submitted for a possible replacement of an active ingredient registered as a mixture of stereoisomers with an enantiopure or enantioenriched compound for a comparative dietary risk assessment, two main approaches used by companies have emerged: a bridging approach and the submission of a new, complete dossier. Such approaches can contribute to case by case evaluation of the isomer issue although experience has shown that, data supporting a more refined assessment are often not available.

The material used in toxicity studies (tox batch) and plant and livestock studies must have a similar stereoisomer composition; the use only of a mixture of stereoisomers in combination with achiral “cold” (non-radiolabelled) and “hot” (radiolabelled) analytical techniques, however, prevents a detailed analysis of preferential metabolism or possible interconversion in plants. Therefore, the PPR Panel recommends inclusion of stereochemistry aspects in metabolism studies.

In order to apply any approach to assess stereoisomers, the data requirements should be adapted in order to be able to *identify* stereoisomers in the different compartments (animals, plants, soil).

9.5. Assessment of the toxicological relevance of isomer ratio changes of metabolites

In principle, the tools intended for the assessment of metabolites (i.e. TTC and (Q)SAR) could be used to assess pesticide isomers. It should be emphasised that the use of the TTC and (Q)SAR approaches does not prejudice the data requirements for active substances as listed in Regulation (EC) No 1107/2009.

The TTC approach, proposed for the acute and chronic risk assessment of pesticide metabolites, considers a classification scheme based on generic structural characteristics. Only two chemical classes (Cramer class I and III) are recommended by the EFSA Scientific Committee (EFSA, 2012). The TTC approach assumes a minimal value that in principle would be applicable to stereoisomers. The (Q)SAR approach, proposed by the PPR Panel in identifying critical toxicological alerts (genotoxicity, developmental toxicity), generally does not currently include stereochemical descriptors. Despite these considerations, in practice, the PPR Panel does not propose that the TTC scheme is used for individual stereoisomers due to the need to consider cumulative exposures (see chapter 9.6), although the TTC scheme has utility as a screening assessment of the isomer mixture for a metabolite. Regarding future possibilities for non-testing approaches, a further development of (Q)SAR tools would be beneficial to address stereochemistry aspects.

Differences in toxicity and metabolism between stereoisomers may not be the same in humans and in other species, such as in the experimental mammals used in the toxicity studies. However, this interspecies difference is not expected to be any greater than other interspecies differences in toxicity and metabolism. The usual safety or assessment factors may therefore be considered as adequate for predicting the likely response of humans to mixtures of isomers from the results of experimental studies, provided the isomeric composition is the same. A difficulty however remains if there is a significantly different metabolism that leads to a predominance of one of the isomers in the tissues of livestock animals or plants when considering the consumer exposure to pesticide metabolites.

9.6. Exposure assessment

In order to carry out an exposure assessment with regard to metabolite stereoisomers, the plant metabolism studies and/or residue trials (including the analytical methods used) would have to reflect

the stereoisomerism of metabolites; at present, this is rarely the case. Without this information an appropriate exposure assessment with regard to metabolite stereoisomers cannot be done.

In some cases, the estimation of dietary exposure based on plant metabolism studies and residue studies is possible through a comparison of single stereoisomer pesticide data with data on the same pesticide formulated as mixture of stereoisomers ('bridging').

If metabolism studies (both ADME and residue metabolism studies) were to better identify the stereochemical nature of the main metabolites then this would initially provide for a better assessment as to whether there are significant interspecies differences in metabolism of isomeric compounds. If there are none then further consideration of isomers is not needed in the dietary risk assessment since the already established toxicological end-points are valid.

As the different stereoisomers of metabolites have identical molecular formulae, the cumulative exposures of the stereoisomers should be considered. It is recognised that different isomers can have different toxicities. However, in the absence of toxicity data on different isomeric forms, co-exposure should be calculated so that the exposures of the individual isomers of the metabolite in question should be summed when used in the TTC scheme, as for a cumulative risk assessment on substances with the same mode of action.

9.7. Conclusion on applicability of approaches to address relevance of metabolites to isomer ratio changes.

Isomers can be selectively metabolised, resulting in a higher ratio of toxic compounds. *In principle* the tools intended for the assessment of metabolites (i.e. TTC and (Q)SAR) could be used to assess individual pesticide isomers. However, *in practice* stereochemistry is lost in the way in which underlying datasets are coded to be used in (Q)SAR. For TTC, if the stereoisomer mixture is present below the threshold level it is irrelevant to further separate the isomers. So the method can be used to conclude on whether or not it is important to look in more detail at isomeric effects. If the stereoisomer mixture is present above the threshold level, the assessment scheme in Chapter 11 applies. Due to potential cumulative exposures, the TTC approach is not proposed for individual stereoisomer assessment, and the TTC assessment scheme only has utility as a screening assessment of an isomer mixture for a metabolite. It is emphasised that non-testing strategies are only intended for use on stereoisomer metabolites, not for their parent compounds.

For the estimation of dietary exposure to individual stereoisomers, information is needed on their relative concentration. Guidelines on metabolism (concerning laboratory animals, plant and livestock) should therefore cover the need for some quantitative information on stereochemical aspects. In addition, specific stereoisomers should be included in residue trials on a case by case basis. The panel recognises that, for the time being, it is not possible to suggest a generic strategy that will cover all situations.

On the basis of the preceding subchapters, and to assess whether additional information is needed, the following check list is proposed:

- Check whether the composition of the isomer mixture tested in plant metabolism studies and mammalian ADME studies was sufficiently characterised to allow comparison of individual stereoisomers formed during metabolism.
- The stability of a chiral metabolite and its further metabolism and conjugation steps should be evaluated using chemistry and biochemistry expert judgment. Enantiomers may racemise under certain chemical conditions, whereas diastereomers usually epimerise. In such a way, the metabolic pathway of a precursor pesticide molecule which is usually 2-dimensional and disregards stereochemistry could be enhanced by taking the spatial arrangement of the metabolites into account.

- Are there any indications or studies to show that there is preferential metabolism of the stereoisomers of the active ingredient in plants leading to different amounts of residues or distribution of plant metabolites or even conversion of one stereoisomer into the other? Note that metabolic processes may convert an achiral parent compound into chiral metabolites.
- Are there any toxicity studies or bridging studies available using single isomers allowing characterisation of the hazard of single stereoisomers? Note that if there is no indication of preferential metabolism in plants and livestock compared to laboratory animals, there is no need to separate the individual stereoisomers in the risk assessment and the usual TTC scheme can be applied. When preferential metabolism is likely or expected, dietary exposure estimates should be made for single stereoisomers based on plant metabolism studies and/or residue trials representing a reasonable GAP. These estimates (summed when appropriate, see chapter 9.6) can be compared to toxicological reference values for specific stereoisomers, if available. Otherwise, assume that each individual stereoisomer is responsible for the total toxicity of the mixture and compare to an adjusted ADI or ARfD based on the isomer ratio in the parent compound.

10. Critical issues and uncertainties

In this chapter, the most critical issues in the areas of toxicology, estimating metabolite exposure, and stereoisomers are discussed. In addition, the uncertainties affecting the risk assessment are listed and discussed.

10.1. Critical issues in toxicology

10.1.1. TTC for genotoxicity

The TTC value for genotoxic compounds, derived by Kroes et al., (2004) was considered sufficiently conservative to be applied in EFSA work provided the structures already designated to be high potency carcinogens are excluded from the TTC approach (EFSA, 2012). The evaluation of structural alerts for genotoxicity is a critical issue in the application of the TTC scheme. The TTC opinion of the SC does not provide any recommendations on which genotoxicity prediction tools could be used. The PPR Panel explored some of the available tools as alternatives to testing in order to develop an assessment scheme for pesticide metabolite exposure. Based on the results of the outsourced projects (see chapter 5 and 6) and of the conclusions of the EFSA SC TTC opinion on genotoxicity (EFSA, 2012), the PPR Panel proposes a tiered approach for genotoxicity evaluation involving computational tools ((Q)SAR and/or read-across) and testing.

10.1.2. TTC for neurotoxicity

Neurotoxic compounds were subject to specific consideration in the process of validation of the TTC approach for chronic exposure. The PPR Panel considers that the neurotoxicity of metabolites is adequately covered by the modified TTC approach including metabolites and degradates of organophosphates (OP), and *N*-methyl carbamates in the neurotoxic TTC grouping. However neurotoxic metabolites arising from non-neurotoxic parent compounds would not be covered by the proposed scheme, unless the toxicophore formed during metabolism has already been characterised. Currently, the decisive factor in the acute exposure assessment of potential neurotoxic metabolites is the mechanism of action of the parent (see chapters 7 and 11). The computational approaches, (Q)SAR tools, grouping and read-across, explored in an ad hoc study, do not currently allow improvement of the prediction of neurotoxic alerts.

10.1.3. TTC for endocrine disruptors

There is ongoing debate regarding the definition and assessment of endocrine disruptors. The PPR Panel concludes that for the time being, untested substances, other than steroids and several other

categories of substances as concluded by the Scientific Committee³³, could be evaluated using the TTC approach. However, if there are data indicating that a substance may have endocrine-mediated adverse effects, then the risk assessment should be based on the data, rather than the TTC approach. Once the EU-wide approach for defining and assessing endocrine disruptors is finalised it will be necessary to consider any impact it may have on the use of the TTC approach.

10.1.4. New TTC values for acute exposure

The TTC concept, developed for chronic exposure, was also considered appropriate, in principle, for risk assessment of acute exposure. TTC values estimated for lifetime exposure were considered overly conservative for short term exposure. The PPR Panel addressed this critical issue in the risk assessment of pesticide metabolites by developing a TTC assessment scheme for acute exposure through the analysis of the available database on pesticides following the same procedure established for chronic exposure (see chapters 5 and 11).

10.2. Critical issues on exposure

The most critical issues identified when estimating the dietary exposure of pesticide metabolites to the consumer are explained below.

10.2.1. Ratio metabolite/parent estimations for estimating the exposure of pesticide metabolites

The metabolism of the parent substance in different crops and food commodities is a dynamic process. There may be more than one metabolite to parent ratio if trials or feeding studies are available for parent and metabolite covering more than one PHI, which are relevant to the GAP, when considering residues in an the individual crop or commodity. Additionally, more than one metabolite ratio can be derived if the metabolite is found in more than one crop or commodity or if more than one pesticide application is made relevant to the GAP, for example, if data are available based on radiolabelling in more than one radiolabel position prior to treatment. The difference in results for a metabolite in a related species could be due to a real difference in metabolism or due to analytical constraints affecting the identification of metabolites in different studies. Different scenarios considering the most and least conservative approach to extrapolating metabolite to parent ratios across different crops are illustrated in Fig 1 and presented in the case study in Appendix E.

Field trials and feeding studies, when such studies are available and the data are adequate, should be used in preference to metabolism studies when calculating the ratio metabolite to parent. Where limited data are available on the metabolites in the field trials or feeding studies, the data can also be used to confirm the validity of the approach based on applying the metabolite ratio from the metabolism study.

10.2.2. Extent of uses of the pesticide

The extent of uses varies with initially considered uses (representative uses) to intended uses (MRL PROFile³⁴ considerations). It may also vary between Member States and when introducing new uses, novel metabolites might occur. Both the extent of uses and the ways in which metabolite ratios are handled affect the outcome of the dietary intake estimate (see case studies, Appendix E).

10.2.3. General recommendations on these two issues:

It will be necessary for risk managers to consider the levels of protection needed and give consideration to the relevant factors to use in calculations for exposure estimation of pesticide

³³The Scientific Committee concluded that the TTC approach should not be used for the following (categories of) substances: high potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds, benzidines, hydrazines), inorganic substances, metals and organometallics, proteins, steroids, substances with a high potential for bioaccumulation, nanomaterials, radioactive compounds, and mixtures of substances containing unknown chemical structures.

³⁴PROFile UserGuide for the Pesticide Residue Overview File (PROFile) in the scope of Article 12 in Reg. EU 396/2005 <http://www.efsa.europa.eu>

metabolites; most notably the extent to which metabolite ratios should be extrapolated versus the extent of uses, and whether always to use the highest metabolite ratio, if there is more than one.

When estimating consumer exposure to pesticide metabolites it is recommended that an assessment should start with the most conservative approach for both the ratio metabolite to parent and for the extent of crop uses. The uncertainties related to these aspects are discussed in chapter 8.5.

A tiered approach for estimation of exposure will be further addressed in the Guidance.

10.2.4. Use of residue data for metabolite estimation

The approach to metabolite estimation aims to make the best use of available residue data, however, the data available were not designed with the intention of obtaining quantitative metabolite estimates. Since plant metabolism studies use only a few plants and livestock metabolism studies can involve one animal, these studies are usually at best regarded as semi-quantitative. There are many uncertainties with the approaches used in estimating the metabolite exposures as is seen in the variation in the results for the case studies and different exposure options (Appendix E) and as stated in chapter 8.

10.2.5. Acute exposure to metabolites

In order to evaluate the toxicological relevance of metabolites for dietary risk assessment it is necessary to have conservative exposure assessments which take into account high exposure scenarios to provide the data for applying the TTC approach. The PPR Panel agreed that chronic exposure assessment should always be performed and acute dietary exposure assessment should be done when an ARfD is allocated to the parent compound and/or the presumption that the metabolites may have acute toxic properties is plausible (see chapters 5.3. and assessment schemes in 11.1.).

To obtain conservative exposure assessments, an estimation of the metabolite levels in the commodities is necessary. This can be obtained using metabolite to parent ratios as determined in the plant metabolism studies.

Three metabolite ratio scenarios are considered to be relevant for performing exposure estimates. During these exposure estimations there is a need to extrapolate between different crops and potentially between different crop metabolism groups and to consider the extent of uses that should apply (see chapter 8.2.). The choice of scenario depends on the level of protection that a risk manager wishes to apply and as such is outside the remit of EFSA.

In the case studies estimating the levels of metabolite exposure arising from the use of different pesticides performed by the Panel (Appendix E), only the commodity that gave the highest intake estimate for the most critical consumer was taken into account to illustrate a conservative exposure scenario for acute exposure and a worst case assessment. However, the case studies (Appendix E) demonstrated that the metabolite exposure estimates were significantly higher for acute exposure compared to chronic exposure.

The TTC approach may also be considered to assess the toxicological relevance of metabolites of pesticide active substances associated with acute dietary exposure. However, the TTC approach was designed to be applied in risk assessment for chronic exposure as the current TTC values are derived from a database that addresses chronic toxicity, see chapter 5.3. Application of the chronic TTC thresholds for acute exposure is therefore overly conservative (see EFSA, 2012). In order to tackle the issue of acute exposure, the PPR Panel introduced new acute thresholds for assessment of the relevance of pesticide metabolites associated with acute toxicity in this opinion, see chapter 5.3.

10.2.6. Cumulative and aggregate exposure

A metabolite of a pesticide coexists with the parent pesticide and other metabolites of that pesticide. If the TTC approach is to be applied to a group of substances with closely related structures and to which there is a co-exposure, it may be appropriate to sum their exposures, as would be done in a cumulative

risk assessment on substances with the same mode of action (EFSA, 2012). The TTC scheme applied to metabolites in this opinion is used to screen for metabolites that may require further toxicological testing so that an appropriate decision can be made, based on toxicology and exposure, on a compounds inclusion in the residue definition. Once a compound is included in the residue definition for risk assessment the principle of cumulative assessment is met since the levels of all the constituents are summed in order to account for their overall relevance to the risk assessment. Different metabolites of a pesticide can have varying degrees and types of toxicities, some of which are more closely related to the toxicity of the parent pesticide than for other metabolites, where frequently, the metabolite is less toxic than parent due to detoxification processes. When using an individual metabolite estimate of exposure within the TTC scheme to decide on whether further toxicity testing is needed, the approach perhaps becomes more uncertain when metabolite structures are very similar and this additional uncertainty should be taken into account in reaching any conclusions based on the outcome of the TTC approach. A possible course of action is to sum the exposure contribution of very similar individual metabolites before using the TTC scheme, as is proposed here for consideration of different stereoisomers of metabolites (see chapter 9.6). Additionally, uncertainties due to the potential for aggregate exposures arising from non-food exposures to the metabolites may also need to be taken into account if aggregate exposures for all routes and sources cannot be estimated (EFSA, 2012).

10.3. Critical issues on stereoisomers

The dietary risk assessment could be done using the approaches as described in this opinion, provided the scope of metabolism studies in laboratory animals, plants and livestock was extended to also generate data on the identity and relative amounts of metabolite stereoisomers formed and degraded. It is noted that advanced biochemical and analytical knowledge would be required to be able to take account of these stereochemical aspects.

In principle, these tools – (Q)SAR, TTC approach – are based on models where the stereochemistry of metabolites is disregarded or lost in the course of the translation of the structure into a computer readable code. Similarly, the set of molecules the SAR is based upon has been treated in the same way and the stereochemical information is lost. However, this is not necessarily the case with all models and some models – usually more expensive ones – do exist which include stereochemical descriptors as well.

In order to fully cover stereoisomerism of pesticide metabolites in the TTC approach, the TTC underlying databases would need to be revised for stereoisomerism. Also (Q)SAR models should be extended to represent stereoisomers as well³⁵.

However, in practice, due to the potential for cumulative exposures and since isomers have identical molecular formulae, it is not proposed by the PPR Panel to consider individual stereoisomers in the TTC scheme. The latter should only apply to the mixture of isomers in the form of a screening assessment (see chapter 9.6.).

10.4. Uncertainties affecting the assessment

All risk assessments are subject to uncertainty. It is important to characterise the degree of uncertainty associated with risk estimates, so that it can be taken into account in risk management (Madelin, 2004; Codex, 2007).

EFSA have previously stated that it is efficient to use a tiered approach to analyse uncertainties (EFSA, 2006). Each individual source of uncertainty may be analysed at one of three levels: qualitative, deterministic or probabilistic. Note that it is not necessary to treat all uncertainties in an assessment at the same level; on the contrary, it is likely to be more efficient to quantify only the most substantial uncertainties. Initially, all significant uncertainties may be analysed qualitatively, using any

³⁵ It is noted that the upcoming version 3 of the OECD (Q)SAR toolbox will include the possibility to assess three-dimensional structures.

available evidence regarding the magnitude of the uncertainties and expert judgement on how they may affect the assessment. While the qualitative assessment is subjective and does not use any formal or quantitative methodology, it may be sufficient, if the outcome is clear enough for risk managers to reach a decision (i.e. if it is clear from the qualitative assessment that the uncertainties would not alter the risk management conclusion). Otherwise, those uncertainties that appear critical to the outcome may be analysed quantitatively, either deterministically or probabilistically. Quantitative analysis could include sensitivity analysis, where the values selected for a variety of input parameters or datasets are varied and the degree to which this influences the risk estimates is evaluated.

A qualitative evaluation of the uncertainties affecting the approaches recommended in this opinion is provided in Table 6 (below). This was constructed by starting with the corresponding table in the EFSA (2009a) opinion on cumulative assessment of triazoles and adapting it to the needs of the present opinion. The first column of the table summarises the uncertainties identified by the Panel. For some uncertainties, more detailed discussion is provided in the text preceding the table. The second column of the table contains the Panel's assessment of the potential impact of the uncertainties on risk assessment outcomes. It is important to note that, because this opinion provides guidance that might be applied to a number of future assessments, Table 6 provides a general evaluation of the potential impact of the uncertainties, and indicates the range of impacts that each uncertainty might have over a series of assessments. The evaluation of impact for a specific assessment may differ, depending on the details of the case in hand, e.g. the amount and quality of data available. Therefore, it is recommended that Table 6 be considered as a generic evaluation, which should be reviewed and if appropriate revised by assessors who are conducting and interpreting individual assessments in the future.

It should be noted that in the context of this opinion, the focus is in particular on the scenarios for MRL setting (not the monitoring data scenarios). Therefore none of the uncertainties in relation to monitoring data were maintained in the table. In addition the uncertainties related to food consumption for instance consumption surveys, food conversion factors and the extrapolation of consumption data are the same as for the uncertainties for the cumulative risk assessments, for more details see EFSA (2009a).

Inclusion or non-inclusion of metabolites in the residue definition for risk assessment will impact on the concentration levels going into the dietary risk assessment. In some cases, there will also be an impact on the toxicological endpoints (ADI/ARfD), when it is considered to be necessary to derive separate endpoints for the metabolite(s).

The toxicological relevance of metabolites is evaluated by using non-testing methods providing threshold values for cancer and non-cancer endpoints. A combined use of TTC approach and (Q)SAR models is proposed for acute and chronic risk assessment. Uncertainties affecting the different components of this approach are discussed below and summarised in Table 6.

The TTC approach is based on the assignment of TTC values for metabolites on basis of chemical structure. The TTC concept is a probability-based screening tool and it does not offer complete certainty. The derivation of the TTC values for cancer and non-cancer endpoints are based on frequency distributions: they are not established on the lowest value in each of the distributions but on a point close to the lowest value.

The TTC value for substances with a structural alert for genotoxicity, derived by linear extrapolation from the TD₅₀ values in animal cancer studies could give rise to less than one in a million lifetime risk of cancer. This approach is extremely conservative assuming that all biological processes involved in the generation of tumors at high dosages are linear over a 500,000 fold range of extrapolation.

The TTC values for non genotoxic substances, proposed in the assessment schemes for acute and chronic exposure to pesticide metabolites, are derived by taking the lower 5th percentile values of the distribution of the available NOELs used for calculation of ADI and/or ARfD considering an

uncertainty factor of 100 (interspecies UF 10 x intraspecies UF 10). Thus the probability of any appreciable non-cancer risk to human health from exposure to substances below the TTC values is low, but not zero (between 0 and 5%). Specifically, for between 0 and 5% of metabolites assessed in this way, the ADI and/or ARfD would (if assessed) be below the TTC.

TTC underlying database for chronic exposure: The reference database used for derivation of TTC values for chronic exposure includes well-validated toxicological data for defined chemical structures, covering a large number of food additives, industrial chemicals and pesticides. In all, the database contained 2941 NOELs from studies conducted on the 613 substances, and from these the most conservative (lowest) NOEL for each substance was used in the TTC calculation. The uncertainty in this step is associated with the limited representation of the new pesticide classes in the database.

The reference database of NOELs applied to evaluate the TTC for OPs and carbamates includes 82 neurotoxic compounds and 52 developmental neurotoxicants. In addition a database of ADIs for OPs and carbamates (93 ADIs for 59 OPs and 27 ADIs for 14 carbamates) was used by the EFSA Scientific Committee, which provides increased confidence in the proposed TTC value for inhibitors of AChE.

TTC Underlying database for acute exposure: An internal EFSA database including all pesticides, for which dietary reference values have been established (406 ARfDs for 267 different active substances), was applied by the PPR panel for derivation of TTC values proposed for acute exposure including Cramer classes and TTC for neurotoxic compounds.

Derivation of NOEL values from animal studies: The lowest available NOEL is taken for each substance. This dose level is the greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable effect³⁶. The NOEL is not necessarily a no-effect dose, it could reflect a dose level where effects are too small to be detected in that particular study, but the size of the possible effect at the NOEL remains unknown. The 'True' NOEL could be lower (if more studies were done) or higher (due to dose spacing or error in the existing studies). It has been estimated that the uncertainty could be between 5 and 10% (EFSA, 2012). 5% is for the continuous responses, 10% is for the quantal responses. It is noted that in the future, NOELs will be replaced by BMDLs. This will reduce the uncertainty (EFSA, 2009b).

Derivation of the TTC: The NOELs used to calculate the TTC are derived through the analysis of the available NOELs used for calculation of ADI and/or ARfD taking the lower 5th percentile values of the distribution of NOELs. Based on this analysis, there is a 95% confidence that a compound of unknown toxicity and structure consistent with a particular chemical class/chemical group is adequately covered.

The NOEL values considered for the extrapolation of the TTC values for chronic exposure for Cramer class I (0.5-10,000 mg/kg bw day) and Cramer class III (0.05 - 1000 mg/kg bw day) vary by up to 5 orders of magnitude.

The cumulative distribution of NOELs for OPs and carbamates differs by one order of magnitude from the distribution of non-OP neurotoxicants ranging from 0.005 and 10 mg/kg bw/ day. The NOEL values considered by the PPR panel for the extrapolation of the TTC values for acute exposure vary by 3 orders of magnitude (0.1-200 mg/kg bw/ day).

The probability of an underestimation of the risk when using the 5th percentile of the cumulative distribution of NOELs is 0-5 %. This is consistent with the results in our case study, where the ADI for 4% of tested pesticides was below the TTC value.

³⁶ Note that the lowest NOEL rather than the NOAEL is used, i.e., the lowest detectable effect, which may be lower than the lowest NOAEL for an adverse effect.

Interspecies extrapolation: Default assessment factors allow for variability in extrapolating toxicity data from laboratory animals to an average representative healthy human. A factor 10 as a product of two factors, 4.0 and 2.5 for differences in toxicokinetics and toxicodynamics respectively, was applied to derive TTC. This factor could be over- or underconservative on the basis of differences in ADME or in sensitivity between laboratory animals and humans.

Intraspecies extrapolation: Default assessment factors allow for variability in functional genetic polymorphisms and sensitivity between potentially susceptible subgroups. A factor 10 was applied to calculate the TTC. This factor is considered overconservative with the exception of specific cases, such as neonates and infants before the age of 6 months, specifically analysed in the Scientific Committee TTC opinion (EFSA, 2012). The conversion of the TTC value as mg/kg bw/d allows for comparison with exposure estimates for different age groups.

(Q)SAR models in the acute and chronic assessment schemes are proposed to evaluate alerts for genotoxicity and in combination with read-across to identify developmental toxicants. (Q)SAR models are designed to make substance specific predictions of defined toxicological end-points. The uncertainty for the application of these tools is not quantified, but could be inferred from the range of sensitivities and specificities derived from case studies. Sensitivity expresses the proportion of positive compounds correctly predicted, while specificity expresses the proportion of correctly identified negative compounds.

(Q)SAR approach in evaluation of genotoxicity alert: The sensitivity of (Q)SAR tools for genotoxicity ranges between 0.73 and 0.93, on the basis of the genotoxic endpoint considered. (Q)SAR models are available to identify Ames mutagenicity with a sensitivity range from 0.80 to 0.93, while the sensitivity to detect classified mutagens is lower (0.73-0.87). The sensitivity of the applied tools in our case study is lower (between 0.45 and 0.64). Based on these results, the proportion of false negatives for genotoxic alert based on (Q)SAR alone is in the region of 50%, but the proposed scheme including a battery of (Q)SAR models and read across should improve the performance of the assessment. The specificity of applied (Q)SAR models in our case study ranges between 0.57 and 0.87, suggesting a similar proportion of false positives.

(Q)SAR approach in identifying developmental toxicants: The performance of (Q)SAR tools alone are not sufficiently reliable to predict developmental effects, due to the low negative predictivity ranging from 49-55%. The proposed stepwise assessment scheme based on the combined use of (Q)SAR and read-across should increase the accuracy of the classification of tested compounds.

Read-across is a non-formalised approach for compound comparison, based on the availability of robust databases and on a number of steps including expert choices. Only few case studies are available on the use of this approach and are insufficient to quantify the uncertainty of the process.

Non TTC approach: The metabolites exceeding the TTC values are evaluated case by case using a different approach for mammalian (laboratory animals, such as rodents) metabolites and for plant and livestock specific metabolites. In this case, more toxicity data, as for the parent compound, will be required to make an assessment. In such a situation, the uncertainty will decrease.

Weight of evidence approach: This approach, applied to evaluate mammalian metabolites detected in laboratory species is based on the analysis of the available toxicokinetic and toxicity data in order to establish if the toxicity of a metabolite is covered by the toxicity of the parent compound. If this is the case, the risk assessment is performed using the reference values of the parent.

Targeted testing: Mammalian (laboratory animals, such as rodents) metabolites not covered by the toxicological data of parent compounds and, plant or livestock specific metabolites need to follow a testing strategy. The strategy is decided case by case using a tiered approach based on the comparison on the toxicological profile of the metabolite and the parent compound. The lowest NOAEL is considered to derive the ADI and ARfD. The 'True' NOEL could be lower (if more studies were done)

or higher (due to dose spacing or error in the existing studies). It has been estimated that the uncertainty could be between 5 and 10%.

Low-dose toxicity/Endocrine disruptors: There is no consensus as yet on when a compound should be defined as an endocrine disruptor, and the applicability of the TTC approach to such substances has been questioned due to uncertainty about low-dose effects (Kroes et al., 2004). If the TTC approach is used, then its applicability should be re-evaluated when there is consensus on how to assess endocrine disruptor activity.

Additional sources of uncertainty affect the residues and exposure assessment of metabolites, as discussed below.

Extrapolation of metabolite estimations from the metabolism studies in which the metabolite was found to other commodities (or animals), and extent to which extrapolation is taken account of: Metabolism studies are not available for every species for which there is an expected exposure (based on GAP). Typically one or a small number of metabolism studies are available to cover a crop group. If a number of crop groups are needed to cover the representative or intended uses the range of metabolism studies available is extended although the studies are limited (for example, if metabolism is not markedly different, then three studies could be adequate to cover all crop uses). Usually livestock metabolism studies are available for hen and goat. The uncertainty is associated with the expectation that interspecies differences in the qualitative nature and quantitative levels of metabolites can occur that mean that extrapolation gives incorrect predictions and also that there can be interspecies similarities which mean that extrapolation would be more reasonable. The extent to which extrapolation is taken account of in the metabolite exposure estimations considerably affects the outcomes of the exposure assessment of metabolites (see chapter 8.6 and Appendix E).

Limits to knowledge of the full range of extent of uses: During the EU review of active substances, the initially evaluated uses covered in the Draft Assessment Reports (DAR) tends to be for a limited number of representative uses. The supporting data in the DAR only need to be for those crop uses. Further uses may be covered in the EU-RMS MRL assessment reports and EFSA reasoned opinions supporting MRL proposals; however the detail available in the MRL assessments tends not to be as detailed as the DAR assessments. Initial reviews and proposals for Annex 1 listing are reassessed periodically. The extent to which uses beyond the initial representative uses are accounted for considerably affects the outcomes of the exposure assessment of metabolites.

Limitations in the metabolism studies per species: Metabolism studies tend to be regarded as semi-quantitative only since each study uses only a limited number of individual plants or animals, one or a small number per dose. The numbers per species within a study (or number of studies per species) may also be increased (to a limited extent) if more than one label is being studied, for example a study may have one or up to three labelled positions. Further replication beyond these needs does not usually occur. The uncertainty in the context of the current opinion is that the metabolite estimations proposed on the basis of metabolite ratios in the metabolism studies are quantitative assessments. Therefore the metabolism studies are being used for more quantitative purposes than their originally intended use which is primarily to consider the qualitative nature of the metabolism to elucidate a metabolic pathway.

Limitation of the metabolism/field trials to estimate the optimum time to measure concentrations of metabolites and parent: Regulatory studies cover different time points which show that formation of metabolites and the ratio of metabolite to parent is a time dependant process, that is also affected by pesticide specific factors (e.g. metabolism) and less pesticide specific factors (e.g. weathering and ageing). Selection of time points in the metabolism and field trials studies to optimally and fully characterise this dynamic process is subject to uncertainty.

Limitation of the metabolism studies to identify all possible metabolites: The application rate in metabolism studies is expected to be broadly representative of expected exposure (in relation to GAP).

It depends on the resultant levels of metabolites in the crop parts or animal matrices of interest which will be pesticide dependant, coupled with the analytical challenges, as to whether identification of all of the metabolites is possible. OECD Guidelines 501, 502 and 503 (OECD, 2007a; b; c) guide on the extent to which metabolites should be characterised and identified in regulatory studies and it is not expected that metabolites present at too analytically challenging levels would need to be identified. Related to this is whether differences seen in metabolism studies (e.g. livestock versus plants versus the rat) are real differences in metabolism or whether it is due to analytical constraints/design of studies/extent of identification work in each study.

Measurement uncertainties in pesticide concentrations: Analytical measurement is subject to measurement uncertainty, a part of which is considered to be sampling uncertainty for pesticide residues work. Overall measurement uncertainty for pesticide residues analysis is estimated to be around +/- 50% (EC, 2011a).

Handling of data below the LOQ or LOD: In the case studies (Appendix E), if residues were < LOQ, the metabolite estimations were calculated assuming a residue value at the LOQ level, which is worst case. Alternative approaches, which are also subject to uncertainty, would be assuming a level of zero, or an estimated level between zero and the LOQ, see EFSA (2010).

Omission of contribution of processing or concentration in edible or non-edible parts: Since residues can be preferentially removed with inedible peel, or residues may be altered in nature, reduced (or concentrated) during home or industrial processing operations, further uncertainties are introduced by not considering the complexity of these elements.

Use of default variability factors in default assessments: There is uncertainty regarding the level of the variability factors to use in acute assessment (as a multiplication factor to apply to the composite residue value to ensure that exposure to the highest residues in individual commodity units is considered). Since the approach used in the case studies (Appendix E) and this opinion uses deterministic methodology the choice of variability factor considerably affects the outcome of the assessment. Default values that are used are intended to be conservative.

Use of high level dietary assessment values: the case studies (Appendix E) use deterministic assessments. The case studies use 97.5th %ile consumption data for acute exposure assessments, and total dietary intakes for chronic exposure assessment are based on the methodology which includes the 97.5th %ile consumption values for the commodities giving the highest intakes (and mean consumption for the remaining commodities). Use of high level consumption intake values is common in food intake assessment (EFSA, 2011a); for chronic assessments average data are also used (see section 8.4). When selecting an approach, the conservatism of the assessment and the uncertainties of the consumption data for the various point estimates (average, 95th %ile, 97.5th %ile, 99th %ile) can be considered (the reliability of high percentiles is discussed in EFSA, 2011a). This relates to the number of subjects used to calculate the high percentiles and for some commodities, the number of subjects may be not be sufficient for a fully statistically robust assessment. A higher degree of certainty is attained with more frequently consumed commodities. The case studies have calculated the results for the highest commodity estimate (acute) and critical consumer subgroup (chronic and acute exposure). There will also be other lower representative exposures (commodities consumed less and/or by specific consumer groups) that the case studies do not focus on. Intentionally, this TTC work has used a conservative estimate of exposure.

Uncertainties regarding cumulative and aggregate exposures: See section 10.2.6. This opinion and case studies (Appendix E) has considered methodology for individual metabolites. Since metabolites may have a common mode of action to related metabolites and parent, the implications regarding cumulative, and aggregate exposures where possible, should be considered. If related metabolites are handled in the TTC scheme individually then there is an underestimation of cumulative exposure. If similar individual metabolites, with unknown toxicity, are summed before using the TTC scheme then there may be an overestimation of cumulative exposure. It is proposed here that different

stereoisomers of metabolites should not be separately evaluated via the TTC scheme (see section 9.6). The uncertainties regarding cumulative exposure should be taken into account when, following the TTC scheme, a decision is being taken on whether further toxicological testing is not necessary.

Table 7: Summary evaluation of influence of uncertainties on assessing the relevance of pesticide metabolites

| Source of uncertainty/variability | Direction & magnitude |
|---|--|
| Toxicology | |
| TTC approach | |
| Representativeness of databases used to derive TTC values | |
| Acute TTC – based on 406 ARfDs for 267 different substances, all pesticides. | - / + |
| Chronic TTC – based on 2941 NOELs for 613 substances, including food additives and industrial chemicals as well as pesticides, with limited representation from newer classes of pesticides for which toxicity is more likely to be overestimated | - / ++ |
| TTC for OPs and Carbamates – based on a database of pesticides only (59 PO and 14 carbamates). | - / + |
| Endpoints used in TTC approaches | |
| Derivation of NOEL values from animal studies. The lowest available NOEL is taken for each substance. The ‘true’ NOEL could be lower (if more studies were done) or higher (due to dose spacing or error in the existing studies).. | - / + |
| The TTC for genotoxicity was derived by linear extrapolation of TD ₅₀ s from animal cancer studies to a one in a million lifetime risk of cancer. This assumes that all biological processes involved in the generation of tumors at high dosages are linear over a 500,000 fold range of extrapolation. While it cannot be ruled out that this might apply to some chemicals, it is expected to be very conservative in most cases. | ● / +++ |
| TTC derivation | |
| Acute – NOELs vary by 3 orders of magnitude. Based on the method of deriving the TTC, 0-5% of substances are expected have NOELs below the TTC (leading to underestimation of risk), while 95-100% are expected to have higher NOELs (overestimating risk). | - / ++ |
| Chronic – NOELs vary by up to 5 orders of magnitude. | -- / +++ |
| OPs and Carbamates – NOELs vary by 4 orders of magnitude. | - / ++ |
| (Q)SAR/read-across approach | |
| (Q)SAR for Genotoxicity alert – The prediction of genotoxic alerts may generate false negative and false positive rates up to around 50% when based on (Q)SAR alone, based on sensitivity and sensitivity estimates, but the proposed scheme including a battery of (Q)SAR models and read should improve performance to some extent. | - / ++ |
| (Q)SAR for developmental toxicity – a low negative predictivity is expected ranging from 49-55%; combination with read-across seems to improve the accuracy of predictivity | - / ++ |
| NON-TTC approach | |
| Weight of evidence approach: analysis of the available toxicokinetics and toxicity data in order to establish if the toxicity of a metabolite is covered by the toxicity of the parent compound. | - / + |
| Targeted testing: The strategy is decided case by case using a tiered approach based on the comparison on the toxicological profile of the metabolite and the parent compound. | - / + |
| Default uncertainty factors | |
| Interspecies extrapolation uncertainty factor. A factor of 10 is used. For a small proportion of chemicals, humans could be more than 10x more sensitive than animals, leading to underestimation of risk, but for most chemicals, the factor of 10 will overestimate the sensitivity of humans and therefore also the risk. | - / ++ |
| Intraspecies extrapolation uncertainty factor – the standard factor of 10 is considered to be over-protective with the exception of specific cases, such as neonates and infants under 6 months. | - (infants up to 6 months) + (rest of population) |
| Low-dose toxicity, endocrine disruptors | See text |
| Isomers | |

| Source of uncertainty/variability | Direction & magnitude |
|--|------------------------------|
| Stereochemical nature of metabolites – metabolism studies (ADME, and plant and livestock metabolism) do not currently address the nature and levels of stereoisomers. The extent of concerns that the dietary risk assessment cannot be fully covered for specific pesticide situations is subject to uncertainty due to this gap in knowledge. It is estimated that it is unlikely that one stereoisomer will be present at more than 10 times the other one. The current opinion recommends that further metabolism information should be available to better assess metabolites that exist as isomers to enable the impact on the overall dietary assessment to be assessed. | --/++? |
| Residues, exposure | |
| Extrapolation of metabolite estimations from the metabolism studies in which the metabolite was found to other commodities (or animals), and extent to which extrapolation is taken account of (which considerably affects the outcomes of the exposure assessment of metabolites). | --/++ |
| Uncertainty in knowing the extent of uses, and extent to which uses beyond the initial representative uses are accounted for (which considerably affects the outcomes of the exposure assessment of metabolites). | --/● |
| Limitation that a metabolism study is based on a low number of plants (or livestock) and in any one species there is usually only one or two metabolism studies. | - / + |
| Limitation of the metabolism/field trials to estimate the optimum time to measure concentrations of metabolites and parent. | --/++ ? |
| Limitation of the metabolism studies to identify all possible metabolites. | --/++ |
| Uncertainty in whether differences seen in metabolism studies (e.g. livestock versus plants versus the rat) are real differences in metabolism or whether it is due to analytical constraints/design of studies/extent of identification work in each study. | -/+ |
| Measurement uncertainties in pesticide concentrations. | ● |
| Handling of data below the LOD or LOQ. If these are assumed to be equal to LOD or LOQ then this will overestimate exposure and risk. The effect is expected to be larger in chronic assessments as these are based on median residues, whereas acute exposure is dominated by high positive values. | ++ (chronic) ● (acute) |
| Omission of potential contribution of processing Data on the effect of processing (e.g. peeling, canning, cooking) on residues are rather limited, incomplete and frequently based on a limited number of measurements. Processing considerations have therefore not been included in the case studies in this opinion. | - / + |
| Concentrations in edible and non-edible parts of commodities may differ, and could cause over- or underestimation of intakes if the non-edible parts were included in the residue analysis. | - /++ |
| Treatment of unit-to-unit variation (e.g. choice of variability factor) in acute assessments. Use of default values. | - -/++ |
| Case studies for metabolite level estimation use high level dietary intake values in deterministic consumer assessment models (Appendix E, use of 97.5 th %ile consumption data for acute exposure assessments, and total dietary intakes for chronic exposure assessment are based on the methodology which includes the 97.5 th %ile consumption values for the commodities giving the highest intakes (and mean consumption for the remaining commodities)). The statistical reliability of such high percentiles relates to the number of subjects used in calculating them. A higher degree of certainty is attained with more frequently consumed commodities. | -/+ |
| Uncertainties regarding cumulative exposure (and aggregate exposures if aggregate exposures for all routes and sources cannot be estimated). If related metabolites are handled in the TTC scheme individually then there is an underestimation of cumulative exposure. If similar individual metabolites, with unknown toxicity, are summed before using the TTC scheme then there may be an overestimation of cumulative exposure (10.2.6) | --/++ |
| Food consumption data. See EFSA (2009) for explanation of factors influencing the uncertainty, such as use of old survey data. Here, a tentative overall assessment of the uncertainties related to food consumption data is given. | -/+ |
| OVERALL UNCERTAINTY | |
| The overall impact of all the uncertainties should be evaluated case by case for each assessment. Firstly, the assessor should review all the individual uncertainties above, and adjust the evaluations as appropriate to the considerations relevant for their assessment, including the amount and quality of data used. Secondly, the assessor should consider all the uncertainties together and form a subjective judgement of the overall uncertainty affecting the assessment | ? |

| Source of uncertainty/variability | Direction & magnitude |
|--|-----------------------|
| outcome. This should not be done by any simple summation of the symbols for individual uncertainties, but by using expert judgement to consider the overall impact, taking account of any potential dependency between individual uncertainties. The overall conclusion should be expressed using the same symbols and scale as for the individual uncertainties and accompanied by a narrative explanation of the reasoning used by the assessor in reaching their overall judgement. | |

Key to symbols in Table:

- +++ uncertainty is causing over-estimation of exposure (or the ratio of exposure to reference dose) expand by about 100x
- ++ uncertainty is causing over-estimation by about 10x
- + uncertainty is causing over-estimation by about 5x
- the effect of this uncertainty is less than +/- 50%.
- uncertainty is causing under-estimation by about 5x
- - uncertainty is causing under-estimation by about 10x
- - - uncertainty is causing under-estimation by about 100x

The above is a general assessment of the uncertainties. The overall uncertainty will vary between specific assessments.

Once the risk managers have indicated their preference as to the appropriate exposure scenario that needs to be addressed, the overall proposed toxicological risk assessment of pesticide metabolites is considered to be conservative.

Although some steps of the TTC approach, as described above, implicate a probability (0-5%) of an underestimation of the risk, a number of factors taken together: the application of conservative safety factors for inter and intraspecies extrapolation; the use of alternative approaches for specific categories of compounds (e.g high potency carcinogens, EDs); and a special consideration for oversensitive subpopulations, provide an assurance that the assessment of risk using the TTC approach is conservative.

The use of (Q)SAR tools to evaluate genotoxicity alert and to predict developmental toxicity is associated with a high level of uncertainty and therefore it currently only proposed for use as a tool for priority setting to decide on testing pesticide metabolites with unknown chemical structure.

It is highlighted that in practice the uncertainty assessment needs to be done specifically taking account of case by case circumstances, and consequently may give different results. If a conservative approach is not assured, the uncertainty assessment can be used to identify the critical areas that need further refinement.

11. Proposed strategy for assessing the toxicological relevance of pesticide metabolites.

The PPR Panel concludes that the TTC approach is the most appropriate tool in the evaluation of the toxicological relevance of metabolites of active substances associated with chronic dietary exposure. The TTC values established for genotoxic and toxic compounds based on the Cramer et al., (1978) scheme and recently confirmed in the EFSA Scientific Committee opinion (EFSA, 2012), were considered sufficiently conservative in a case study carried out with a group of pesticides belonging to the main chemical classes.

The first step of the chronic assessment scheme involves the prediction of genotoxicity. There are currently no standardised criteria on how to do this. The PPR Panel explored by case studies the potential use of (Q)SAR analysis for the prediction of genotoxicity. The performance of the (Q)SAR tools applied was not satisfactory for a small pesticides dataset (CRD-AGES dataset). This outcome could be attributed to the heterogeneity of the genotoxicity data and to excess of negative compounds in the dataset. The same models applied to a large dataset of compounds classified as mutagens

showed a higher predictivity, confirming the usefulness of applying a battery of complementary (Q)SAR models. Future research should investigate whether the predictivity of the (Q)SAR approach can be improved by applying other models not explored in the case study (e.g. MULTICASE), the combined use of (Q)SAR models and read-across, by using reference databases on genotoxic endpoints. In conclusion, at the present time there is little added value in using non-testing methods for the prediction of genotoxicity. If a compound is predicted negative, further testing is required to confirm the conclusion. However, the PPR Panel considers the approach promising and encourages further research in this area.

The application of TTC requires a suitable estimation of exposure. TTC values were established for chronic exposure and are overly conservative for short term exposure (EFSA, 2012). The estimates of acute and chronic exposure to pesticide metabolites differ by orders of magnitude, when assessed for different pesticides or food commodities. The PPR Panel addressed this issue with two different approaches: the evaluation of potential use of (Q)SAR/read across scheme in predicting acute effects of pesticides (neurotoxicity/developmental toxicity) and the development of tentative TTC values for acute exposure by the analysis of the lowest 5th percentiles of NOAELs applied to establish ARfDs for the EFSA pesticide data set. The outcomes of the case studies show that the (Q)SAR models, tested alone or in combination are at present inadequate to predict neurotoxicity. The PPR Panel proposes to further explore a stepwise approach, considering the use of (Q)SAR tools and read-across in the identification of developmental toxicants.

11.1. Assessment scheme

Before embarking on the application of a TTC decision tree, the need to perform both chronic and acute exposure assessment has to be established. The exposure assessment must be based on the toxicological profile of the compounds. For the parent compound, a chronic exposure assessment is always performed and an acute exposure assessment is performed when an Acute Reference Dose (ARfD) has been established. In the same way, for metabolites, a chronic exposure estimate will always be done; and, whether an acute estimate is needed must also be considered. As for parent, the PPR Panel considers that an acute assessment should always be performed for a metabolite when an ARfD is allocated to the parent compound. In addition, the decision for the acute exposure assessment should be based on a weight of evidence approach using non testing methods. Specific endpoints were considered particularly relevant to acute effects, such as neurotoxicity and developmental toxicity. The metabolites of neurotoxic parent compounds are considered for the acute exposure assessment. A stepwise approach, considering the use of (Q)SAR tools and read-across, should be applied to identify possible developmental toxicants to be included in the acute exposure assessment.

Due to the identical molecular formulae of different isomers, it is considered that co-exposure of the different isomeric forms of a metabolite should be considered in the TTC scheme in a cumulative form of assessment (see chapter 9.6 (isomers) and 10.2.6 (cumulative and aggregate exposures)). In this way the usual metabolite testing scheme applies using a total level of a metabolite (combined level of various isomeric forms that can be obtained from residues metabolism or supervised trials data using non-chiral analytical approaches). A more refined assessment for individual stereoisomers, if specific estimations of exposure could be predicted, would depend on the availability of specific toxicity data for the various isomeric forms, and as such this refinement is outside the scope of the TTC scheme. See chapter 9.6 for further details of this non-TTC form of assessment.

11.1.1. Assessment scheme for chronic exposure

An assessment scheme related to chronic exposure modified to be applied in the risk assessment of pesticide metabolites is proposed considering different strategies for mammalian (rodent or laboratory test species), plant or livestock specific metabolites.



* **Exclusion categories**; high potency carcinogens (cohort of concern: aflatoxin-like compounds, N-nitroso-compounds, azoxy-compounds, benzidines, hydrazines), inorganic substances; metals and organometallics; proteins, steroids; substances known/predicted to bioaccumulate; nanomaterials, radioactive substances; mixture

** If exposure of infants < 6 months is in range of TTC, consider if TTC is applicable

Figure 2: Assessment scheme for chronic exposure

First step

The first step in the decision tree approach is the identification and evaluation of possible structural alerts for genotoxicity. This step involves the exclusion of high potency carcinogens (cohort of concern: aflatoxin-like compounds, N-nitroso-compounds, azoxy-compounds, benzidines, hydrazines), inorganic substances; metals and organometallics; proteins, steroids; substances known/predicted to bioaccumulate; nanomaterials, radioactive substances, substance mixtures and then apply a TTC of 0.0025 µg/kgbw/d.

If the exposure estimate exceeds this TTC value a different approach is considered for mammalian (rodent) metabolites than for plant or livestock metabolites.

All the data available on genotoxicity of the parent compound and on *in vivo* and *vitro* metabolism studies have to be considered in order to evaluate the genotoxic potential and the toxicokinetics of the compound.

The genotoxic potential of major rodent metabolites can be considered to be covered by the tests performed on the parent. Taking into account data from metabolism studies (e.g. metabolite not identified but precursor of a metabolite identified), the genotoxic potential of minor rodent metabolites and plant or livestock metabolites needs to be further assessed.

The (Q)SAR approach, through the use of a combination of different computational tools and read-across approaches, can be considered as the first step in the evaluation of an alert for genotoxicity of pesticide metabolites. A positive result is predictive of genotoxic activity. The compounds with a positive alert are considered as genotoxic. It rests with the applicant to provide experimental data, following the proposed testing strategy (see chapter 11.2) to confirm or refute the genotoxicity prediction. Negative results for a genotoxic alert need to be further explored using a weight of evidence approach considering other computational tools such as read-across and testing.

Second step

The next step is related to the neurotoxicity alert, including organophosphate and carbamate toxicophores.

It is proposed that mammalian (laboratory species) metabolites exceeding the TTC value should be evaluated applying the weight of evidence approach by a comparison of the toxicokinetics and toxicity data in order to establish if they could be considered covered by the data on the parent compounds. Plant or livestock specific metabolites must be tested.

Third step

This step concerns compounds without the neurotoxicity alert relating to the organophosphate and carbamate toxicophores, and it is based on the allocation of the compound into Cramer class I or III and on the comparison of exposure with the corresponding thresholds. If the exposure estimate exceeds the identified TTC values a different approach is considered for mammalian (rodent) and plant or livestock specific metabolites. A weight of evidence approach is adopted to establish if the toxicological profile of mammalian metabolites (laboratory species) is covered by the data on parent compound. In this case the risk assessment is performed using the reference values of the parent compound. In the case of stereoisomers, the isomer composition has to be compared with the parent compound in order to define the strategy for risk assessment, using the reference values, and the need for testing. Plant or livestock specific metabolites need to follow an appropriate testing strategy (see chapter 11.2).

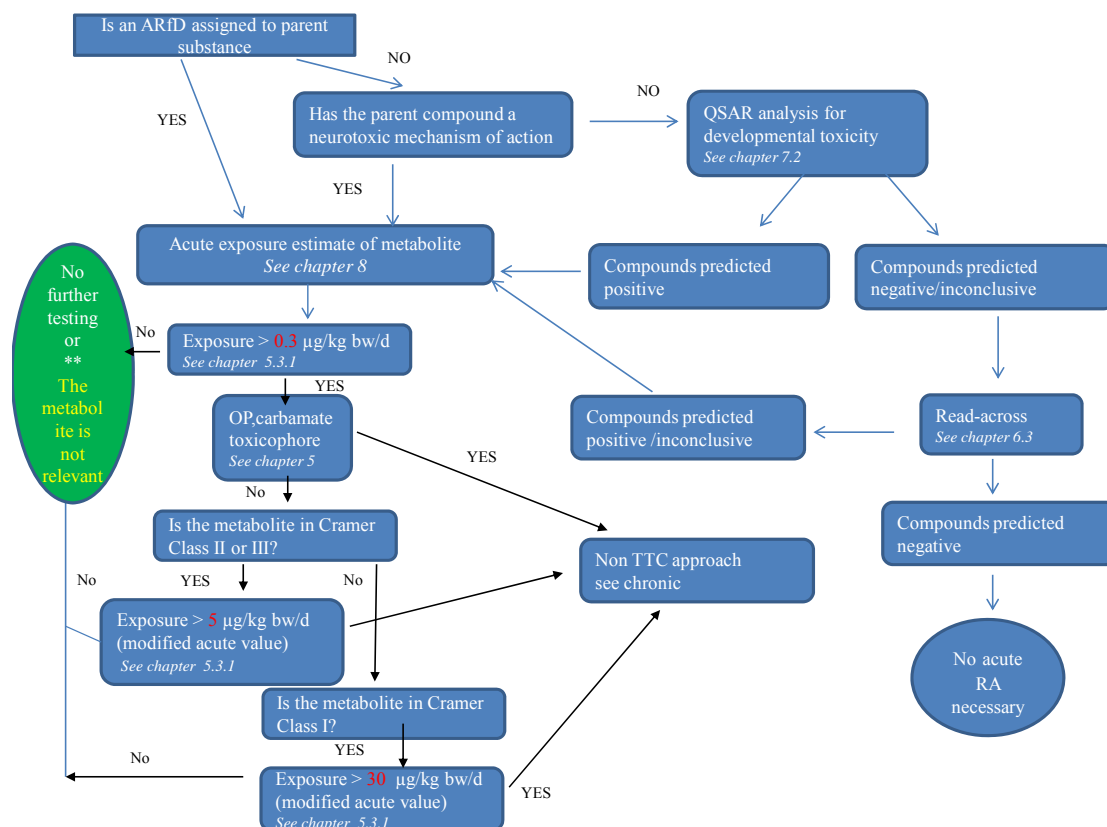
11.1.2. Assessment scheme for acute exposure

Since every assessment starts with the assessment of chronic toxicity, and within this genotoxic compounds are already excluded, only non-genotoxic metabolites are considered for acute exposure assessment. The non-threshold mechanisms of a large number of genotoxic compounds and the wide interindividual mutagen sensitivity in human populations (EFSA, 2011b) mean that adjustment of the current TTC level for genotoxic compounds (0.0025 µg/kg bw/d; 0.15 µg/person/d) for exposure duration is not justified.

The acute exposure assessment is always performed when an Acute Reference Dose (ARfD) has been allocated for the parent compound and for metabolites of neurotoxic parent compounds. A stepwise approach, considering the use of (Q)SAR tools and read-across, should be applied to identify possible developmental toxicants to be included in the acute exposure assessment. A first step involves the use of (Q)SAR models, alone or in combination, for identifying developmental toxicants. A second step involves a further evaluation of the compounds predicted as negatives by (Q)SAR using a read-across approach. For the compounds predicted as negatives by the combined approach no acute risk assessment is necessary.

The acute assessment scheme involves the comparison of acute exposure, with the corresponding threshold values following the Cramer decision tree. Ad hoc TTC values for short term exposure derived from pesticide NOAELs applied for calculation of ARfDs are adopted: 0.3 µg/kg bw/d for substances with neurotoxicity alert and 5 µg/kg bw/d for substances allocated to Cramer class II and III. The same TTC value established for chronic exposure (30 µg/kg bw/d) was adopted for chemicals allocated in Cramer class I. A non-TTC process, involving a weight of evidence approach and testing should be applied if the exposure estimate exceeds the identified TTC values. A weight of evidence approach is adopted to establish if the toxicological profile of mammalian metabolites (laboratory species) is covered by the data on parent compound. In this case the risk assessment is performed using the reference values of the parent compound. Plant or livestock specific metabolites need to follow an appropriate testing strategy.

In the case of stereoisomers, the isomer composition has to be compared with the parent compound in order to define the strategy for risk assessment, using the reference values, and the need for testing.



** If exposure of infants < 6months is in range of TTC, consider if TTC is applicable

Figure 3: Assessment scheme for acute exposure

11.2. Acute and chronic toxicity - Testing Strategy

11.2.1. Genotoxicity testing

The evidence from the results of international collaborative studies and the large databases that are currently available for the assays leads to the conclusion that no single test can detect all genotoxic substances. The strategy for genotoxicity testing of chemical compounds is a stepwise approach based on a combination of assays in order to assess effects on three major endpoints of genetic damage associated with human disease: gene mutation, structural chromosomal aberration (clastogenicity) and numerical chromosomal aberration (aneuploidy).

Regulation (EU) 544/2011 that lays down the data requirements for authorisation of pesticide active substances requests that three *in vitro* tests (bacterial assay for gene mutation, combined tests for structural and numerical chromosomal aberrations and a test for gene mutations in mammalian cells) must always be performed. If all the results of *in vitro* tests are negative, at least one *in vivo* test must be done with the demonstration of exposure. An *in vitro* positive result needs to be confirmed *in vivo*.

The PPR Panel, taking into account the updated information on the performance of *in vitro* and *in vivo* tests and in line with the conclusions of the EFSA Scientific Committee on genotoxicity testing strategy (EFSA, 2011b), proposes two *in vitro* tests for the first tier of testing for metabolites of pesticide active substances. First, the bacterial reverse mutation assay and second, the *in vitro* micronucleus test which fulfils the basic requirement to cover the three genetic endpoints (gene mutation, structural chromosomal aberration (clastogenicity) and numerical chromosomal aberration).

A compound negative in all the *in vitro* assays can be anticipated to be negative also *in vivo*.

The *in vivo* follow up will be considered case-by-case, through the evaluation of the spectrum of genotoxic events observed *in vitro*, the data on toxicokinetics, on bioavailability and on potential target organs.

11.2.2. Toxicity testing

The testing strategy has to combine the need to derive health based limits for human exposure, the cost and time required to conduct and evaluate the toxicity studies and the trend to reduce the use of laboratory animals. The testing strategy for pesticide metabolites when the TTC values are exceeded is different for mammalian (laboratory species) metabolites and plant or livestock specific metabolites and needs to be established case by case. The toxicological and toxicokinetics data and the information on mode of action of the parent compound provides the basis for the approach to the testing of metabolites.

A weight of evidence approach based on different factors (e.g. similarity of chemical structure, major metabolite) is necessary to establish if the toxicity of a mammalian (laboratory species) metabolite is covered by the toxicity of the parent compound. If this is the case, the risk assessment is performed using the reference values of the parent. Mammalian metabolites not covered by the toxicological data on parent compounds and, plant or livestock specific metabolites need to follow a testing strategy. As a first step, a parallel 28-day oral toxicity study in rats on the parent compound and on the metabolite is suggested, using the same strain of laboratory animals and the same experimental conditions. The study needs to include detailed clinical observations and specific analyses such as, FOB, thyroid hormone measurements, histopathology of reproductive organs (test in compliance with OECD TG 407, OECD, 1995). The results of this study will enable comparison of the toxic profile of both compounds in order to address the following questions: a) is the metabolite less, equally or more toxic than the parent? b) are there toxicological alerts for specific effects? If the toxic profile of the metabolite is sufficiently similar to that of the parent, the risk assessment can be performed using the reference values of the parent. If not, and if specific alerts are detected, targeted toxicity studies may be required, case by case, to better establish the toxic profile of the metabolite and to enable establishment of reference values.

Targeted toxicity studies could be for example:

- a. acute neurotoxicity in rodents
- b. repeated neurotoxicity in rodents (only for chronic assessment)
- c. developmental toxicity study
- d. 2-generation reproductive toxicity study in rats (extended one-generation study) (only for chronic assessment)
- e. carcinogenicity study (only for chronic assessment)

For active substances, which are classified for reproductive toxicity (category 1b or 2 for fertility or developmental toxicity), it must be shown by an appropriate test or other convincing evidence that the metabolite does not qualify for the same classification.

For active substances, which are classified as category 2 carcinogens, convincing evidence must be provided that the metabolite will not lead to any risk of carcinogenicity. This may be done by appropriate carcinogenicity testing, by the provision of mechanistic evidence (e.g. absence of the likely mechanistic effect leading to carcinogenicity with the parent molecule, such as target organ pathology, hormonal-dependent proliferation, or metabolism of thyroid hormones) or by a convincing toxicological assessment taking into consideration all available data.

Further testing could be required dependent on the outcome of ongoing discussions on criteria for endocrine disruption properties.

12. CONCLUSIONS AND RECOMMENDATIONS

Based on the non-testing tools reviewed in this opinion, the PPR Panel has developed assessment schemes on both chronic and acute toxicity for pesticide metabolites as described in chapter 11. The approach is ready for use, but it is anticipated that on many occasions the outcome of the assessment scheme will be that further testing is needed to reach a firm conclusion on the toxicological relevance of the metabolite. However, the benefit of applying the approach is that it will allow prioritisation of metabolites for subsequent testing. This approach should not be used as an alternative to the conventional risk assessment for the evaluation of pesticide active substances (parent compounds) themselves occurring as residues in food. They should be assessed prior to authorisation on the basis of dossiers including toxicological tests (Regulation (EC) No 1107/2009).

It is noted that EFSA is planning on developing a Guidance Document based on the suggested approaches in this opinion.

In order to further develop the assessment schemes the PPR Panel makes the following recommendations, (indicating the chapters to which the recommendations relate).

Chapter 4

- The PPR Panel outlines the need of adequate toxicokinetic data which are critical to improving the efficiency of toxicity testing of pesticide metabolites. The PPR Panel recommends the harmonisation of criteria for the selection of radiolabel positions and of kinetic parameters for metabolites considering the possible enantiomers in ADME studies. The PPR Panel recommends the use of physiologically-based pharmacokinetic (PBPK) modeling as an approach to be considered in the assessment of ADME processes in order to simplify and separate the metabolic processes in a multi-compartment model.
- The PPR Panel recommends a case-by-case approach to the evaluation of conjugates and bound residues in plants.

Chapter 5

- The PPR Panel recommends the TTC approach as the most appropriate tool in the evaluation of the toxicological relevance of pesticide metabolites associated with dietary exposure, for which there are few or no relevant toxicity data available. The TTC approach should however not be used for the following (categories of) substances: high potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds, benzidines, hydrazines), inorganic substances, metals and organometallics, proteins, steroids, substances with a high potential for bioaccumulation, nanomaterials, radioactive compounds, and mixtures of substances containing unknown chemical structures. In addition, once the EU-wide approach for defining and assessing low-dose effects or endocrine disrupters are finalised it will be necessary to consider any impact they may have on the use of the TTC approach.
- The existing chronic TTC values applied were validated to cover evaluation of the toxicological relevance of pesticide metabolites.
- The PPR Panel recommends the use of “acute exposure thresholds” for pesticide metabolites, of 0.3 µg/kg bw/d for substances having structures suggesting neurotoxicity (AChE inhibition) and of 5 µg/kg bw/d for all other pesticide metabolites.

Chapter 6

- The PPR Panel recommends that the application of integrated computational approaches including combined (Q)SAR models and read-across should be explored in future studies for the evaluation of genotoxicity alerts. The use of read-across implies the availability of a robust database on pesticides classified for the main genotoxic endpoints.

Chapter 7

- The PPR Panel outlines that further research is needed to optimise the use of (Q)SAR tools, by classifying the different endpoints associated with developmental toxicity. In addition the development of an appropriate database on pesticides considering the developmental endpoints, would allow improvement in the use of the read-across approach.

Chapter 8

- The PPR Panel developed an approach to estimate the exposure to metabolites. However, the outcome of the assessment was found to vary widely depending on the starting assumptions. The PPR Panel recommends that risk managers choose the approach they consider most suitable, thereby defining the level of protection they consider adequate.
- The success of the approach in setting two different residue definitions as well as the estimation of exposure to metabolites depends on the reliability and availability of conversion factors. Guidance on the derivation of conversion factors and their use by monitoring authorities should be developed.
- Rotational crop and livestock derived exposures to metabolites, although seemingly ‘indirect’ routes of exposure, should not be automatically discounted, as the exposure levels can be significant. It is recommended that an assessment should be made taking account of the specific residue data.

Chapter 9

- In order to cover stereoisomerism of pesticide metabolites, the PPR Panel recommends, the extension of (Q)SAR models to represent stereoisomers as well.
- Information is needed on the relative concentration of individual stereoisomers in order to be able to properly assess the estimation of dietary exposure to metabolites that exist as isomers. Guidelines on metabolism (concerning laboratory animals, plant and livestock) should therefore cover the need for some quantitative information on stereochemical aspects. In addition, specific stereoisomers should be included in residue trials on a case by case basis.

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APPENDICES

A. EUROPEAN GUIDELINES IN INTERNATIONAL CONTEXT.

Food safety is an issue that should be addressed at a world-wide level since foods are imported and exported not only within the European Union, but also beyond European borders. In order to achieve consensus on the safety of foods, dietary risk assessment methodology should be harmonised on a global basis as far as is possible. Globally, two organisations are actively involved in formulating guidance for dietary risk assessment: the Organisation for Economic Co-operation and Development (OECD) and the United Nations (Codex Alimentarius; FAO and WHO).

The OECD is a world-wide organisation whose mission is to contribute to the development of the world economy. Since its foundation in 1960, 31 countries have become members of the organisation. Of the current 27 EU-Member States, 20 MS were also members of OECD in October 2010. In addition, the European Commission participates in the OECD work.

The Codex Alimentarius Commission (CAC) was created in 1963 by FAO and WHO to develop Food Standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Program. The main purposes of this Program are protecting health of the consumers and ensuring fair trade practices in the food trade, and promoting coordination of all food standards work undertaken by international governmental and non-governmental organisations. Codex is funded by the FAO and the WHO and has 180 member governments, including all 27 EU Member States and the European Community as a member organisation.

The Codex Alimentarius (Latin for "food code") is the result of the work of the Commission and its around 20 technical committees: a collection of internationally adopted food standards, guidelines and codes of practice. Codex standards and guidelines and further information material are available on the Codex website (www.codexalimentarius.net).

Status of OECD and FAO/WHO guidance documents and guidelines within the EU.

Among other tasks, the OECD has developed (and continues to develop) the 'Guidelines for the Testing of Chemicals'. These Guidelines are a collection of the most relevant internationally agreed test methods used by government, industry and independent laboratories to determine the safety of chemicals and chemical preparations, including pesticides and industrial chemicals. Guidelines, once accepted, are mandatory among the OECD member states with respect to the Mutual Acceptance of Data, while Guidance Documents may supplement test guidelines and are advisory in nature. In time, most Test Guidelines are integrated by the EC into the EU legislation. In 2003, the OECD initiated work to develop harmonised Test Guidelines and Guidance Documents on pesticide residue chemistry. The Guidance Document on definition of residue is one of the products of this initiative (OECD; 2009).

Codex standards are voluntary and therefore non-binding. A government can adopt its own level of protection, e.g. go beyond or stop short of Codex. If a government chooses a higher level of protection, and in the event of a trade dispute (by way of World Trade Organisation Disputes Panel), it may be required to justify the sanitary measure corresponding to its chosen level of protection on scientific, health, or other legitimate grounds. In many countries, most food legislation is already consistent with Codex. In the EU, Codex MRLs are implemented as import MRLs, if it can be shown that they present no risk to the European consumer. Furthermore, guidelines on dietary risk assessment published by the World Health Organisation (WHO) should be taken into account in EU standard setting.

The Codex Committee responsible for pesticide MRL setting is the Codex Committee on Pesticide Residues (CCPR). CCPR is advised by a scientific expert body, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). JMPR consists of the FAO Panel of Experts on Pesticide Residues in

Food and the Environment (residue evaluation) and the WHO Core Assessment Group (toxicological evaluation). JMPR is primarily responsible for performing the risk assessments upon which CCPR and ultimately the CAC base their risk management decisions, and to further develop the risk assessment methodology. Part of JMPR's work concerns the establishment of residue definitions. FAO panel members of JMPR contributed actively in the development of the OECD Guidance Document on definition of residue.

As 20 of the 27 EU MS have already given their agreement to the OECD Guidance Document, the current EFSA opinion (and follow on guidance) document is intended to be complimentary to the guidance of OECD. The OECD Guidance Document states, that 'The residue definition for risk assessment should include metabolites and degradates of toxicological concern irrespective of their source' and 'Metabolites/degradates with higher potential exposures and toxicities are more likely to be included in the dietary assessment' and some general indications are given as to how to assess their toxicity (para 20 + 21). However, this guidance provides an overall framework rather than detailed criteria required to achieve harmonised residue definitions. In addition, the use of alternative strategies such as non-testing methods are not addressed in this OECD guidance document. Therefore, additional guidance is needed.

The current document aims to fill this gap, and will after finalisation within EFSA also be presented to OECD and JMPR for their consideration.

B. SELECTED RESIDUE DEFINITION EXAMPLES OF PESTICIDES WITH SUPPORTING ANALYTICAL METHODS FOR RESIDUE MONITORING/ENFORCEMENT

This table illustrates the complexity of different scenarios that may be encountered when deciding on residue definitions. Residue definitions are from Regulation (EC) No 396/2005 and its amendment, where available.

| Pesticide, and its residue definitions | Scenario | Comments | Anal. Methodology |
|--|--|---|---|
| Haloxfop-P-methyl: Sum of haloxyfop, its esters, salts and conjugates expressed as haloxyfop (sum of <i>R,S</i> isomers, any ratio) | Expression of the residue in terms of the parent compound | Even if the residue consists mainly of a metabolite, the residue should be expressed in terms of the parent pesticide after molecular weight adjustment. If the parent compound can exist as an acid or its salt or a base or its salt, the residue is preferably expressed as free acid (e.g. RCOOH) or free base (e.g. RNH ₂) | A GC-MS method for haloxyfop, its esters, salts and conjugates (after hydrolysis) is available -The method is based on hydrolysis of total haloxyfop residues and subsequent methylation or butylation for non-enantioselective GC-MS analysis. |
| Benomyl and thiophanate-methyl: Benomyl and thiophanate-methyl both degrade to carbendazim. benomyl: sum of benomyl and carbendazim, - carbendazim: carbendazim - thiophanate-methyl: sum of thiophanate-methyl and carbendazim, expressed as carbendazim; overall: sum of benomyl, carbendazim, and thiophanate-methyl, expressed as carbendazim | Metabolites arising from different sources of quickly metabolised or instable parent compounds | In cases where the pesticides are unstable, the residue definition has to be based on the stable common moiety, here carbendazim. | Carbendazim is included in the multimethod and separated by HPLC. |
| Glyphosate: The main metabolite of glyphosate in soybean and in some glyphosate-tolerant corn varieties is aminomethyl phosphonic acid (AMPA). 2004 JMPR concluded that AMPA was of no greater toxicological concern than its parent compound. Definition of glyphosate residue for monitoring/enforcement glyphosate Definition of glyphosate residue (for dietary risk assessment estimation of dietary intake): sum of glyphosate and AMPA, expressed as glyphosate. | Residue definition in case of polar metabolites: | It is not always necessary to include hydrophilic metabolites even if they are major in terms of quantitative occurrence into the residue definition (e.g. hydroxylation or conjugation to a hydrophilic moiety is a common mechanism of detoxification). | Various validated residue analytical methods available. Typically, precolumn derivatisation in combination with HPLC-MS/MS is used and simultaneously detects AMPA and Glyphosate in various matrices. |

| Pesticide, and its residue definitions | Scenario | Comments | Anal. Methodology |
|--|--|---|--|
| Bitertanol: bitertanol in animal commodities: - for MRL setting: bitertanol - for dietary risk assessment: sum of bitertanol, p-hydroxybitertanol, and the acid-hydrolysable conjugates of p-hydroxybitertanol, expressed as bitertanol. | Separate residue definitions for risk assessment and for enforcement | With bitertanol, separate residue definitions for MRL setting and for risk assessment seemed justified. For enforcement, bitertanol is the target compound, for risk assessment together with hydroxymetabolite and conjugates expressed as bitertanol. | Bitertanol is covered with the multimethod and can be analysed by GC or LC with MS detection depending on actual matrix. |
| Chlorothalonil: for enforcement purposes: - commodities of plant origin: chlorothalonil - for commodities of animal origin: 4-OH-2,5,6-trichloroisophthalonitrile, expressed as chlorothalonil equivalents. | Separate residue definitions for plant and for animal commodities | Quite often separate residue definitions need to be established for plant and for animal commodities for risk assessment as well as for enforcement. | The parent is covered with the multimethod and determined by GC, the hydroxymetabolite is not. |
| Cypermethrin: Sum of isomers (racemic) Metalaxyl and metalaxyl-M: Metalaxyl and metalaxyl-M (metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers)) | Pesticides present as enantiomers diastereomers cis-trans isomers | Single mixture of isomers: residue definitions do not specify isomer ratio Different mixtures of isomers are used: risk assessment is based on sum of effects | Cypermethrin is included in the multimethod. GC separates 4 cypermethrin diastereomers which are then summed to “Cypermethrin sum of isomers” or “Metalaxyl and metalaxyl-M (metalaxyl including other mixtures of constituent isomers including metalaxyl-M (sum of isomers))” GC and HPLC multimethod does not allow to separate rac and metalaxyl-M. |

C. EFSA AND JMPR RESIDUE DEFINITIONS ON PESTICIDES FOR CONSUMER RISK ASSESSMENT

The decisions taken by EFSA and JMPR on the three evaluation criteria on metabolites (metabolism investigated in plants and livestock, toxicological studies, and availability of analytical methods) are reported but not assessed. JMPR Evaluation reports and EFSA conclusion reports (46) from 2008 and 2010 were screened for 'concluded' residue definitions in plant and animal products. 14 pesticides had the same residue definitions and for 24 compounds EFSA included more metabolites than JMPR. 11 examples are presented in the table below. The examples are presented as they were reported at the time of writing this opinion, at the time of publishing the decisions may be different

| Captan | | | | |
|------------------------|---|--|--------------------------|--|
| Residue definitions | EU ^{37,38} | Comments on availability of toxicological studies on metabolites and analytical methods | JMPR ^{39,40,41} | Comments on availability of toxicological studies on metabolites and analytical methods |
| | | The metabolites THPI (tetrahydrophtalimide), 3-OH THPI and 5 OH-THPI are found in rat in significant amounts. For THPI several studies were conducted (acute, oral, genotox, developmental, structure activity relationship) and as per worst case, the same toxicity as parent is concluded. For OH THPI and 5 OH-THPI no studies were submitted, but it was decided that they are most probably of same toxicity as THPI since they are just hydroxylated THPI metabolites. Analytical methods for monitoring are available for foods of plant origin, but not for food of animal origin. | | One developmental toxicity study of metabolite THPI is presented but was considered inadequate to determine the contribution of THPI to the developmental toxicity of captan Analytical methods are available for determination of THPI, 3-OH THPI and 5-OH-THPI |
| Risk assessment plants | Sum of captan and (THPI ⁴²) expressed as captan | The metabolism of captan in plants has been described in fruits (tomatoes, apples) and leafy crops (lettuce). Captan forms the major part of residue and only one metabolite, THPI has been identified as contributing significant (10-15% of captan levels) to the toxicological burden. The levels of THPI are drastically increased by processing involving a heating step. Information on the behaviour of captan under processing conditions should be further investigated by degradation studies under representative hydrolytic conditions. | Captan | Metabolism studies on tomatoes apples, lettuce and apples are presented. Captan is considered to be the major component of the residue in plants but may be hydrolysed to THPI during preparation of samples for analysis, frozen storage (especially of homogenised samples), and processing of the raw agricultural commodity. |
| Monitoring plants | | | | |

³⁷ Conclusion on the peer review of captan. EFSA Scientific Report (2009) 296, 1-90

³⁸ Annex 1_PRAPeR and JMPR decisions, AGES report 2010

³⁹ JMPR Evaluation 2000 (R)

⁴⁰ JMPR Report 2004 (T)

⁴¹ JMPR Report 2007 (T)

⁴² THPI= 1,2,3,6-tetrahydrophtalimide

| Captan | | | | |
|--------------------------------|--|---|--------|---|
| Risk assessment animals | Sum of THPI, 3OH-THPI ⁴³ and 5OH-THPI ⁴⁴ expressed as captan | The metabolism of captan has been investigated in lactating goats and laying hens. The substance is extensively metabolised in both animals and was not found in any edible tissue. The metabolic pattern is rather similar to that observed in plants, with additional metabolites in animal tissues, consisting in hydroxylated forms of THPI (3-OH THPI, 5-OH THPI, and 4,5-diOH HHPI 45 These metabolites should not be present above the LOQ of monitoring analysis, but existing feeding studies should be evaluated to confirm that expectation. | Captan | Metabolism studies on lactating goats and laying hens are presented. Metabolite THPI was shown to be the major part of recovered radioactivity in tissues of livestock, nevertheless, this metabolite has not been included in the residue definition for food of animal origin (no explanation given by JMPR). |
| Monitoring animals | | | | |

⁴³ 3-OH THPI = *cis/trans*-3-hydroxy-1,2,6-trihydrophthalimide

⁴⁴ 5-OH THPI = *cis/trans*-5-hydroxy-1,2,6-trihydrophthalimide

⁴⁵ (4,5-dihydroxyhexahydrophthalimide)

| Chlorpyrifos-methyl | | | | |
|--------------------------------|--|---|---------------------|---|
| Residue definitions | EU ^{46,47} | Comments on availability of toxicological studies on metabolites and analytical methods No toxicological study on metabolites has been described Analytical methods available for parent in plant and animal matrices, but not for the metabolite TCP. | JMPR ⁴⁸ | Comments on availability of toxicological studies on metabolites and analytical methods Data from repeated dose studies show that TCP is about 10 times less toxic than the parent compound. Analytical methods available for chlorpyrifos-methyl, not discussed for metabolite TCP |
| Risk assessment plants | Chlorpyrifos-methyl + TCP ⁴⁹ + conjugates expressed as chlorpyrifos-methyl For stored grain: sum of chlorpyrifos-methyl and demethyl chlorpyrifos-methyl | Metabolism studies in leafy vegetables, fruiting vegetables, stored grain (chlorpyrifos-methyl) and fruits, cereals, oilseeds (chlorpyrifos) The rationale for the definition of residue is that it has not been possible to exclude TCP as the data on the toxicological relevance of this metabolite could not be assessed owing to late provision. In addition it is considered that the metabolite identification data from cabbages cannot be extrapolated to other crops, particularly those where the treated foliar portion may form part or all of the consumable entity. It was felt necessary to establish this Residue Definition because chlorpyrifos-methyl is registered in a number of Member States on other crop groups which would not be covered by the current supported Representative Use. Note: No conclusion report from EFSA yet. | Chlorpyrifos-methyl | Two studies were conducted in plants using chlorpyrifos methyl (tomato and cereal grains) and four with chlorpyrifos (citrus, cabbage, peas and radish). Even though TCP can be a significant part of the residues in plant and animals treated with chlorpyrifos-methyl, it is also a major metabolite formed following the application of chlorpyrifos. As a consequence, TCP is not considered as a specific residue marker of the use of chlorpyrifos-methyl. TCP lacks the phosphate ester moiety, responsible for the cholinesterase inhibiting capacity of chlorpyrifos-methyl. Data from repeated dose studies show that TCP is about 10 times less toxic than the parent compound. |
| Monitoring plants | Chlorpyrifos-methyl | No metabolite included in the residue definition for monitoring, no clear explanation given. | Chlorpyrifos-methyl | Also, TCP levels in crops and animal products are generally not higher than those of the parent compound. As a consequence the Meeting agreed that dietary human exposure to this metabolite is not considered of toxicological concern. |
| Risk assessment animals | Chlorpyrifos-methyl + TCP + conjugates expressed as chlorpyrifos-methyl | Animals covered in metabolism studies: Laying hens, lactating goat, and sheep. RMS considers that with the available information TCP must not be excluded from the residue definition. For many relevant matrices, parent chlorpyrifos or chlorpyrifos-methyl does not contribute significantly to residue, whereas unidentified metabolites constitute the vast majority of it. These unidentified metabolites (polar metabolites) have been characterised | Chlorpyrifos-methyl | The current residue definition for |

⁴⁶ Circa Chlorpyrifos methyl addendum residues March 2003

⁴⁷ Circa Chlorpyrifos methyl endpoint complete March 2005

⁴⁸ JMPR Evaluation 2009 (Evaluated for the Periodic Review Programme of the Codex Committee on Pesticide Residues)

⁴⁹ TCP: 2,4,5-trichloropyridinol

| Chlorpyrifos-methyl | | | | |
|---------------------------|---------------------|---|---------------------|--|
| | | as TCP conjugated with glucose and malonic acid. Since these metabolites hydrolyse to TCP under basic conditions it is possible to monitor them using this analyte. | | chlorpyrifos-methyl in plant and animal commodities, for both enforcement and dietary risk assessment purposes is: Chlorpyrifos-methyl (fat-soluble). The Meeting agreed to confirm this residue definition of chlorpyrifos-methyl: Chlorpyrifos-methyl. |
| Monitoring animals | Chlorpyrifos-methyl | No metabolite included in the residue definition for monitoring, no clear explanation given. | Chlorpyrifos-methyl | |

| Cyromazine | | | | |
|-------------------------|--|---|-----------------------|---|
| Residue definitions | EU ^{50,51} | Comments on availability on toxicological studies on metabolites and analytical methods | JMPR ^{52,53} | Comments on availability on toxicological studies on metabolites and analytical methods |
| | | Two plant/environmental metabolites (1-methyl-cyromazine and melamine) were also identified in the rat metabolism. Based on an open literature review, EU concluded 2008, that the toxicity of the metabolites is covered by the reference values of the parent compound. Adequate methods are available to monitor all compounds given in the respective residue definitions in food/feed of plant and animal origin. | | Based on toxicological data on melamine (from open literature) JMPR 2006 concluded that melamine is less toxic than the parent compound. Analytical methods are available to analyse cyromazine and melamine in plant and animal products. |
| Risk assessment plants | Cyromazine plus melamine metabolite, expressed as cyromazine (leafy crops, fruits) | Metabolism has been studied in leafy crops, (lettuce, celery) and fruits (tomato). Parent cyromazine and metabolite melamine represent the major part of the TRR, accounting for 37.1-74.0% and 10.9-45.4% respectively. Melamine is considered to have the same toxicological profile as the parent. Though it was mentioned that consumer exposure to melamine may be possible through other sources (plastic, colorant, flame retardants, veterinary drugs.) the decision to include melamine in the residue definition was taken with regard to the high melamine residue levels observed in treated crops. | Cyromazine | Cyromazine is the major compound found in all crops, with the exception of mushroom, where melamine can be present at levels higher than cyromazine. Melamine is the main (>10%) metabolite found in all crops and most animal products. It is known that cyromazine is not the only source of melamine in agriculture and in the environment and that melamine can be a component in fertilizers and is used in a variety of manufacturing processes, including plastics. Data provided by the manufacturer have shown that, with the exception of Switzerland, the residue definition in most countries in all foods is cyromazine. |
| Monitoring plants | Cyromazine (for leafy crops and fruit crops only) | Residue definitions set for fruit and leafy crop only. | Cyromazine | |
| Risk assessment animals | Not set | Animal dietary burden remain unknown and the validity of the animal metabolism study performed without the parent compound only, remains uncertain. There is no need to propose a residue definition to set MRL for animal products based on intended use lettuce and tomato. | Cyromazine | Based on the present knowledge and for practical purposes, the residue definition for cyromazine for enforcement purposes for food of plant and animal origin should continue to be cyromazine. The definition for cyromazine in food of plant and animal origin, for dietary |
| Monitoring animals | Not set | | Cyromazine | |

⁵⁰ Conclusion on the peer review of cyromazine. EFSA Scientific Report (2008) 168, 1-94

⁵¹ Annex 1_PRAPeR and JMPR decisions, AGES report 2010

⁵² JMPR Report 2006 (T)

⁵³ JMPR Evaluation 2007 (R)

| | | | | |
|------------|--|--|--|--------------------------------|
| Cyromazine | | | | |
| | | | | intake purposes, is cyromazine |

| Diflubenzuron | | | | |
|------------------------|--|--|--------------------------|---|
| Residue definitions | EU ^{54,55} | Comments on availability on toxicological studies on metabolites and analytical methods | JMPR ^{60,61,62} | Comments on availability on toxicological studies on metabolites and analytical methods |
| | | Metabolites: PCA ⁵⁶ , CPU ⁵⁷ , DFBA ⁵⁸ and DFBMA ⁵⁹ were questioned. DFBA was considered of equal toxicity to the parent and PCA is evidently of toxicological concern. Not enough data for the others. Sufficient analytical methods as well as methods and data relating to physical, chemical and technical properties are available to ensure that quality control measurements of the plant protection product are possible. | | Studies on the toxicological profile of CPU, DFBA and PCA have been performed. CPU is considered to be of toxicological relevance. Analytical methods available for residue determination in crop trials. |
| Risk assessment plants | For fruit crops after foliar application (provisional): diflubenzuron. For mushrooms after soil application (provisional): (1) DFBA (2) Sum of diflubenzuron + CPU + PCA expressed as PCA | Metabolism of diflubenzuron was investigated in apples and oranges after foliar application and in mushrooms after soil treatment. Whereas diflubenzuron was only metabolised to a very small extent in fruits, metabolism in mushrooms was extensive. The residue definition for risk assessment is provisional. Following the finalisation of the toxicological evaluation of the metabolites CPU and PCA the residue definition should be reconsidered. | Diflubenzuron | No metabolites have been included since diflubenzuron is a surface residue when applied to the aerial parts of the plant and does not degrade or translocate. For mushroom, JMPR mentioned, that DFBA is the main residue. Attending to the fact that DFBA is not a residue of particular toxicological concern, the intake of mushroom is quite low all around the world, and analytical methods for diflubenzuron, DFBA and CPU are quite laborious, it has not been included in the residue definition neither monitoring nor risk assessment. |
| Monitoring plants | For fruit crops after foliar application: diflubenzuron, For mushrooms after soil application: DFBA | Diflubenzuron accounted for 95-97% of the TRR in fruits and levels of PCA, CPU and DFBA were very low. Diflubenzuron is not a suitable indicator for residues in mushrooms, in which DFBA accounted for 91% of TRR. Although DFBA is a common metabolite for several active substances, it was regarded as suitable indicator for | Diflubenzuron | |

⁵⁴ Conclusion on pesticide peer review of Diflubenzuron. EFSA Scientific Report (2009) 332, 1-111

⁵⁵ Annex 1_PRAPeR and JMPR decisions, AGES report 2010

⁵⁶ PCA = 4-chloroaniline

⁵⁷ CPU = 4-chlorophenylurea

⁵⁸ DFBA = 2,6-difluorobenzoic acid

⁵⁹ DFBMA = 2,6-difluorobenzamide

⁶⁰ JMPR Report 2001

⁶¹ JMPR Report 2002

⁶² JMPR Evaluation 2002

| Diflubenzuron | | | | |
|--------------------------------|---|--|---------------|--|
| | | diflubenzuron residues in mushrooms. | | |
| Risk assessment animals | Provisional: Sum of diflubenzuron + CPU + PCA + 4-PCAA expressed as PCA | Metabolism studies on dairy cattle and laying hens showed a low transfer of diflubenzuron residues into tissues, milk and eggs. Diflubenzuron, CPU, PCA and PCAA ⁶³ were identified. PCAA is not found in rat, no conclusion is possible since no studies are available. As the toxicological evaluation of the metabolites is not finalised yet, all metabolites were included in a provisional residue definition for risk assessment | Diflubenzuron | No metabolites have been included into the residue definition for food of animal origin, since the major part of the TRR (metabolism studies on livestock) was shown to be the parent. |
| Monitoring animals | Diflubenzuron and CPU expressed as diflubenzuron | It was decided to include diflubenzuron and CPU in the risk assessment for monitoring in animal matrices, as they were regarded as suitable indicators for diflubenzuron residues. | Diflubenzuron | |

⁶³ PCAA= 4-chloroacetanilide

| Fenpropimorph | | | | |
|-------------------------------|--|---|--------------------------------|--|
| Residue definitions | EU ^{64,65} | Comments on availability on toxicological studies on metabolites and analytical methods. The metabolites BF-421-15 and BF-421-106 were major metabolites in plants, but also major rat metabolites. Therefore the experts agreed that their toxicity was covered by the reference values of the parent. Methods are available to monitor all compounds given in the respective residue definitions in food/feed of plant and animal origin. | JMPR ^{66,67,68,69,70} | Comments on availability on toxicological studies on metabolites and analytical methods. No toxicological studies on metabolites have been described JMPR Analytical methods for fenpropimorph in plants (bananas) and the metabolite BF 421-2 in animal (goats) products were reported. |
| Risk assessment plants | Sum of fenpropimorph, metabolite BF 421-1 ⁷¹ (free and conjugated) and BF-421-10 ⁷² , expressed as fenpropimorph | Fenpropimorph was the most abundant constituent of the residue in cereal hay, straw and grain, bananas as well as in sugar beet leaves and roots after foliar spraying of fenpropimorph. In the plant metabolism studies metabolites BF 421-1, its conjugate and BF 421-10 occur above 10% and are included in the residue definition. | Fenpropimorph | No metabolite has been included in the residue definition for plant products (cereals, melons and bananas), since the latest study submitted (bananas) show that parent fenpropimorph is the major part of the residues. No metabolite has been included into the residue definition for food of plant origin |
| Monitoring plants | Fenpropimorph | Fenpropimorph is as an appropriate indicator compound | Fenpropimorph | |
| Risk | Sum of | The metabolism was investigated in lactating goats and laying | Sum of B421-2 | On the basis of the metabolism studies on rats |

⁶⁴ Conclusion on the peer review of fenpropimorph. EFSA Scientific Report (2008) 144, 1-89,

⁶⁵ Annex 1_PRAPeR and JMPR decisions, AGES report 2010

⁶⁶ JMPR Evaluation 1995 (R)

⁶⁷ JMPR Evaluation 1999 (R)

⁶⁸ JMPR Report 2001 (T)

⁶⁹ JMPR Report 2004 (T)

⁷⁰ Annex 1_PRAPeR and JMPR decisions, 2010

⁷¹ BF421-1 = 2-methyl-2-(4-{(2*RS*)-3-[*cis*-2,6-dimethylmorpholin-4-yl]-2-methylpropyl}phenyl)propan-1-ol

⁷² BF-421-10 = 2,6-dimethylmorpholine

| Fenpropimorph | | | | |
|-----------------------------------|--|---|--|--|
| assessment and monitoring animals | fenpropimorph and BF 421-2, expressed as fenpropimorph | hens. The main residues are fenpropimorph, BF-421-2 and BF 421-3 ⁷³ . The residue for monitoring and risk assessment was defined as the sum of fenpropimorph and BF 421-2. BF 421-3 was not include in the residue definition because its limited impact in term of consumer safety, In the lactating goat metabolism study, BF 421-1 and BF 421-2 occur >10% . BF 421-1 is not included in the residue definition because is the precursor of BF 421-2. | ⁷⁴ expressed as fenpropimorph | and lactating goats (reviewed 1995), it is agreed that BF 421-2 can be used as a marker compound for risk assessment and monitoring in animal products enforcement purposes. |

⁷³ BF 421-3: 2-methyl-2-(4-((2RS)-3-[cis-2-methy-6-hydroxymethyl-morpholin-4-yl]-2-methylpropyl}phenyl)propanoic acid

⁷⁴ BF-421-2 = fenpropimorph carboxylic acid = 2-methyl-2-{4-[2-methyl-3-(cis-2,6- dimethylmorpholin-4-yl)propyl]phenyl}propionic acid.

| Fludioxonil | | | | |
|------------------------|--|---|---|--|
| Residue definitions | EU ^{75,76} | Comments on availability on toxicological studies on metabolites and analytical methods | JMPR ^{77,78} Fludioxonil. | Comments on availability on toxicological studies on metabolites and analytical methods |
| | | <p>A full assessment of the toxicological relevance of fludioxonil metabolites has not been performed.</p> <p>Metabolites are however considered as covered by toxicological profile of the parent and included them all into the residue definition, without a separate risk assessment.</p> <p>Adequate methods are available to monitor all compounds given in the respective residue definition</p> | | <p>Studies of acute oral toxicity and genotoxicity with a range of plant metabolites of fludioxonil showed that these metabolites are of low acute oral toxicity and are not genotoxic.</p> <p>Adequate multi- and single-residue methods exist for both gathering data in supervised trials and processing studies and for the monitoring and enforcement of fludioxonil.</p> |
| Risk assessment plants | Sum of fludioxonil and its metabolites, which can be oxidised to metabolite CGA 192155 ⁷⁹ | <p>Metabolism was studied for seed treatment -cereals (wheat) and for foliar treatment, -fruits (grapes and peach); fruiting vegetables (tomatoes); bulb vegetables (onion); leafy vegetables (lettuce). The plant metabolism of the compound proceeds through oxidative processes of the pyrrole ring. After foliar treatment, the parent fludioxonil represents major constituent of the residue. Many metabolites are formed but in small amounts < 3% TRR. After seed treatment uptake and translocation of fludioxonil from the treated seed is low <0.02 mg/kg.</p> <p>For risk assessment foliar application and seed treatment the residue definition should include all metabolites containing CGA to cover potential uses of fludioxonil in other commodities not addressed during the peer review.</p> | Fludioxonil. | <p>The metabolism of fludioxonil in and on plants (grape, tomato, peach, green onion and head lettuce) after foliar and seed treatment (potato, rice, wheat, cotton, soya) is adequately understood. Generally, the residue concentrations resulting from seed treatment were too low to permit extraction and identification.</p> <p>The numerous studies of foliar application indicate a similar metabolic pathway, showing fludioxonil as the main component of the residue. The pathway is characterised by the generation of a large number of metabolites and proceeds mainly through oxidation. Each metabolite represents < 10% TRR.</p> |

⁷⁵ Conclusion on the peer review of fludioxonil. EFSA Scientific Report (2007) 110, 1-85,

⁷⁶ Annex 1_PRAPeR and JMPR decisions, AGES report 2010

⁷⁷ JMPR Report 2004 (T,R)

⁷⁸ JMPR Evaluation 2006 (R)

⁷⁹ CGA 192155 = 2,2-difluorobenzo[1,3]dioxole-4-carboxylic acid

| Fludioxonil | | | | |
|---|--|--|---|--|
| Monitoring plants | Fludioxonil | Metabolism in fruit crops and leafy vegetables after foliar application does not result in metabolite formation adding a significant contribution to the toxicological burden. | Fludioxonil | |
| Risk assessment and monitoring animals | Not required (In case of use extension leading to significant livestock exposure, sum of fludioxonil and its metabolites, which can be oxidised to metabolite CGA 192155 | Metabolism studies in livestock are not required because residues in wheat grain and straw are below the LOQ and grapes are not used for production of feed items. The major metabolic pathway is oxidation at position 2 of the pyrrole ring; two minor pathways are oxidation at position 5 of the pyrrole ring and hydroxylation of the phenyl ring. However, metabolism studies were conducted in lactating goats and laying hens. The proposed residue definition is the sum of fludioxonil and all metabolites containing CGA can be adopted in case of use extension leading to significant livestock exposure. | Fludioxonil and its metabolites determined as CGA 192155, calculated as fludioxonil | The results of the studies of metabolism in goats and hens were similar. A feeding study on ruminant showed that fludioxonil and its metabolites (converted via oxidation to CGA 192155), found in milk were: >0.01mg/kg at dosing level of 5.5 mg/kg. |

| Hexythiazox | | | | |
|------------------------|---------------------|---|--|---|
| Residue definitions | EU ^{80,81} | Comments on availability on toxicological studies on metabolites and analytical methods | JMPR ⁸² | Comments on availability on toxicological studies on metabolites and analytical methods |
| | | Data gaps were identified for the toxicological properties of metabolite PT-1-3. Hexythiazox residues in plant commodities with high acid content and high water content can be determined by multi-residue method or single methods. | | Toxicological studies on metabolites are described. The metabolite PT-1-3 is of greater acute toxicity than hexythiazox., Analytical methods available for the determination of residues of (hexythiazox and PT-1-3) in target crops. For animal matrices no analytical methods or validation data was submitted |
| Risk assessment plants | Hexythiazox | Hexythiazox is a racemic mixture of enantiomers, but the possible preferential metabolism/degradation of each constituent isomer in plants was not investigated and not considered. Metabolism in plants was investigated in the fruit plant group (on grape, citrus, pear and apple) and in tea, using foliar applications of hexythiazox and experimental design representative of the supported uses. The metabolism was similar in all crops investigated and hexythiazox was seen to undergo a limited metabolism in plants. Processing studies were provided and processing factors were calculated for the parent compound in citrus and grape commodities. However, little information was provided on the transfer of the metabolite PT-1-3, seen to be the major compound of the residues under sterilisation conditions (48% TRR). Considering that PT-1-3 is more acutely toxic than the parent additional toxicological information is required on its toxicological relevance and on its possible transfer and level in processed commodities | Sum of hexythiazox and all metabolites containing the PT-1-3 moiety ⁸³) expressed as hexythiazox | The metabolism of hexythiazox following foliar application was investigated in the crop group of fruits on apples, citrus, grapes and pears. In addition one study on tea was submitted, which is representative of leafy crops. In field rotation studies, radish tops (0.046 mg/kg) and sorghum stover (0.012 mg/kg) total hexythiazox residues, determined as PT-1-3 for analysis, were found after 30 days. No data on the ratio between hexythiazox and all residues converted to PT-1-3 under field conditions were submitted. Taking into account a possible deviations in the rate of metabolism under field conditions, the Meeting agreed to define the residue definition for intake purposes as “sum of hexythiazox and all metabolites containing the trans-5-(4-chlorophenyl)-4-methyl-2-oxothiazolidinemoiety (PT-1-3), expressed as hexythiazox” to cover all of the residue of toxicological concern. |
| Monitoring plants | Hexythiazox | | Hexythiazox | The combined quantities of metabolites were at levels of less than 10% of the hexythiazox levels in all samples analysed. Parent hexythiazox is a representative marker for hexythiazox residues in all plant commodities, the residue definition for enforcement purposes in plant commodities is parent hexythiazox only. |

⁸⁰ Circa DAR 2006

⁸¹ Circa 2009 Conclusion on the peer review of teflubenzuron, EFSA Scientific Report (2009)

⁸² JMPR Evaluation 2009 (Evaluated for the Periodic Review Programme of the Codex Committee on Pesticide Residues)

⁸³ PT-1-3Trans 5-(4-chlorophenyl)-4-methyl-2-oxothiazolidinemoiety

| Hexythiazox | | | | |
|--------------------------------|----------------------|---|--|--|
| | | Within the scope of representative uses the residue definition as parent hexythiazox only is supported by plant studies on grape, citrus, pear and apple both for monitoring and risk assessment. | | |
| Risk assessment animals | No residues expected | Animal metabolism Lactating ruminant (goat), poultry (hen) Feeding studies and studies on metabolism indicated that residues derived from representative uses of hexythiazox would not exceed 0.01 mg/kg for any residue species in animal-derived products. | Sum of hexythiazox and all metabolites containing the PT-1-3) expressed as hexythiazox | Animal metabolism studies in rats, lactating goats and laying hens. The metabolism results in a higher percentage of hydrolysed metabolites with hexythiazox being found at levels or even below the LOQ. In addition, no analytical methods for the parent substance alone are available, as well as livestock feeding studies analysed for single substances instead of the total residues determined as PT-1-3. In view of these factors the residue definition (risk assessment and enforcement) for hexythiazox in animal matrices is sum of hexythiazox including all metabolites hydrolysable to PT-1-3, expressed as hexythiazox. |
| Monitoring animals | Not discussed. | Not discussed. Not proposed and not required considering the supported uses. | — | |

| Imazalil | | | | | |
|-------------------------------|--|---|-----------------------------------|---|--|
| Residue definitions | EU ⁸⁴ | Comments on availability on toxicological studies on metabolites and analytical methods No toxicological studies on metabolites have been described. Adequate methods are available to monitor all compounds given in the respective residue definitions in food/feed of plant and animal origin | JMPR ^{85,86,87,88,89,90} | Comments on availability on toxicological studies on metabolites and analytical methods No toxicological study on metabolites or degradates has been described. The analytical methods for melons are suitable for regulatory purposes | |
| Risk assessment plants | Sum of imazalil and metabolite R014821 ⁹¹ | Metabolism in plants has been investigated post harvest application (orange, apple), seed treatment (wheat) and foliar application (tomato). Imazalil was found to be the major constituent of the residues. Metabolite R014821 was observed in significant amounts (>10% of TRR) after storage in an apple metabolism study. It is not possible to conclude on R014821 toxicological relevance. Due to the significant proportion of that is observed when the length of storage is increasing, and in view of future representative uses, it is concluded that the residue definition for risk assessment should be sum of imazalil and R014821 expressed as imazalil. | Imazalil | Post harvest use in melons. The potential metabolite formed by O-dealkylation was not determined but it would be expected to be negligible, because it is known not to be produced extensively in fruits and any significant level of it would appear on the chromatogram. The recoveries and limits of determination were >90% and 0.05 mg/kg respectively in all studies. No metabolite has been included into the residue definition for food of plant and animal origin. | |
| Monitoring plants | Imazalil | Imazalil was found to be the major constituent of the residues. The residue definition for monitoring was therefore limited to imazalil. | Imazalil | No distinction is made between residue definition for monitoring and risk assessment. | |
| Risk assessment | Imazalil and total identified | Metabolism studies conducted on goat showed imazalil to be extensively and almost completely metabolised by | Not set or discussed | Note: The latest residue evaluation and | |

⁸⁴ Conclusion on the peer review of imazalil. EFSA Journal 2010; 8(3):15261

⁸⁵ JMPR, Report 1994

⁸⁶ JMPR, Evaluation 1996

⁸⁷ JMPR Evaluation 1994 (R)

⁸⁸ JMPR Report 2000 (T)

⁸⁹ JMPR Report 2001 (T)

⁹⁰ JMPR Evaluation 2005 (T)

⁹¹ R014821 = (RS)-1-(2,4-dichlorophenyl)-2-imidazol-1-yl-ethanol

| Imazalil | | | | |
|---------------------------|---|--|----------------------|---|
| animals | characterised metabolites | animals. Thus, imazalil is not a sufficient marker for the residues in ruminant matrices. | | toxicological evaluations by JMPR is from 1994 and 2005 respectively. |
| Monitoring animals | Open: Imazalil + FK284 ⁹² and/or FK772 ⁹³ (to be confirmed by notifier) | A validated method of analysis for monitoring for products of animal origin (ruminants) including the parent imazalil and metabolites FK284 and/or FK772 should be provided. | Not set or discussed | |

⁹² FK284 = (RS)-3-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]imidazolidine-2,4-dione

⁹³ FK772 = (RS)-3-[2-(2,4-dichlorophenyl)-2-(2,3-dihydroxypropoxy)ethyl]imidazolidine-2,4-dione

| Metaflumizone* | | | | |
|------------------------|--|---|--|--|
| Residue definitions | EU ⁹⁴ | Comments on availability on toxicological studies on metabolites and analytical methods | JMPR ⁹⁵ | Comments on availability on toxicological studies on metabolites and analytical methods |
| | | <p>Toxicological studies for metabolites have been described and not considered to be toxicologically significant</p> <p>Analytical methods available for metaflumizone E and Z isomers and metabolite M320I04 in plant matrices.</p> <p>Analytical methods available for metaflumizone E and Z isomers in animal matrices.</p> | | <p>Toxicological studies of metabolites have been described and not considered to be toxicologically significant. However the Meeting was aware, that the metabolite M320I04⁹⁶ may arise in processed products from acidic raw agricultural commodities in concentrations that may be of interest for dietary intake estimation. This should be taken into account for future uses.</p> |
| Risk assessment plants | Sum of metaflumizone E and Z isomers and metabolite M320I04, expressed as metaflumizone (molecular weight conversion for metabolite to parent is x 1.75) | <p>Metabolism studied in oilseeds/pulses (cotton), fruiting crop (tomato), leafy crop (white cabbage)</p> <p>The main residues found in the crop metabolism studies are the E and Z isomers of metaflumizone. In tomatoes, the metabolite M320I04 constituted up to 16%TRR in the fruits. This metabolite was also found in other studies (cottonseed and cabbage, 16-17%TRR). Other metabolites were found in amounts less than 10 % of metaflumizone.</p> <p>The potential concerns for instability of the Z isomer of metaflumizone over freezer storage and possible conversion in stored samples to metabolite M320I04 which can occur quickly</p> | Metaflumizone, sum of E ⁹⁷ -isomer and Z ⁹⁸ -isomer | <p>Plant metabolism studies were performed on white cabbage (0 to 7 days PHI), tomato (0 and 7 daysPHI) and cotton (21 days PHI) using the benzonitrile- and trifluoromethoxyphenyl-U-14C-labelled metaflumizone The main residues found in the crop metabolism studies are the E- and Z-isomers of metaflumizone. In tomatoes, the metabolite M320I04 constituted up to 16%TRR in the fruit.</p> |
| Monitoring plants | Sum of metaflumizone E and Z isomers and metabolite M320I04, expressed as | <p>Therefore, on this basis, it is proposed that the residue definition should include the E and Z isomer of metaflumizone and also metabolite M320I04. Based on the smaller size molecule of the metabolite, the overall quantitative expression of the residue should include a molecular weight conversion.</p> | Metaflumizone, sum of E ⁹⁹ -isomer and Z ¹⁰⁰ -isomer | <p>Definition of the residue for compliance with MRLs and estimation of dietary intake for plants and animals: Metaflumizone, sum of E-isomer and Z-isomer.</p> |

⁹⁴ Circa DAR 2008

⁹⁵ JMPR Evaluation 2009 , * New compound

⁹⁶ M320I044- {2-oxo-2-[3-(trifluoromethyl) phenyl]ethyl} benzonitrile

⁹⁷ E-isomer: (E)-2'-[2-(4-cyanophenyl)-1-(a,a,a-trifluoro-m- tolyl)ethylidene]-4- (trifluoromethoxy)carbanilohydrazide

⁹⁸ Z-isomer (Z)-2'-[2-(4-cyanophenyl)-1-(a,a,a-trifluoro-m- tolyl)ethylidene]-4- (trifluoromethoxy)carbanilohydrazide

⁹⁹ E-isomer: (E)-2'-[2-(4-cyanophenyl)-1-(a,a,a-trifluoro-m- tolyl)ethylidene]-4- (trifluoromethoxy)carbanilohydrazide

¹⁰⁰ Z-isomer (Z)-2'-[2-(4-cyanophenyl)-1-(a,a,a-trifluoro-m- tolyl)ethylidene]-4- (trifluoromethoxy)carbanilohydrazide

| Metaflumizone* | | | | |
|--|--|---|-----|---|
| | metaflumizone (molecular weight conversion for metabolite to parent is x 1.75) | | | |
| Risk assessment animals | Sum of metaflumizone E and Z isomers (poultry) | Metabolism studies from goat and hen. Both the goat and hen metabolism studies highlight the E and the Z isomers of metaflumizone to be among the expected residues to be found across the various matrices, if residues were at a level that they would be found in animal products. | -“- | The metabolism of metaflumizone has been studied in laboratory rats, goats and hens. The main residues found in the farm animal metabolism studies are the E- and Z-isomers of metaflumizone. Both isomers should be included in the residue definition |
| Monitoring animals | Sum of metaflumizone E and Z isomers (poultry) | | -“- | |

| Prothioconazole | | | | |
|------------------------|--|--|-------------------------|--|
| Residue definitions | EU ^{101,102} | Comments on availability on toxicological studies on metabolites and analytical methods | JMPR ^{103,104} | Comments on availability on toxicological studies on metabolites and analytical methods |
| | | <p>Prothioconazole-desthio is a major metabolite in plant and rats (17.7% in urine or faeces, not clearly stated). It is more toxic than the parent prothioconazole. All other questioned metabolites are structurally closely related to prothioconazole-desthio and consist mainly of hydroxylated forms of this compound.</p> <p>Sufficient analytical methods are available to ensure that at least some quality control measurements of the plant protection product are possible.</p> | | <p>Studies on metabolites have been described with respect to their toxicological profile. Data indicate that, except for prothioconazole-desthio and prothioconazole-sulfonic acid, they are not toxicologically relevant metabolites.</p> <p>The manufacturer provided validated methods for determination of residues in plants, animal tissue, milk and soil samples.</p> |
| Risk assessment plants | Sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety) expressed as prothioconazole-desthio. | <p>The metabolism has been fully investigated in cereals, oilseeds, rotational crops as well as in livestock and mainly proceeds through oxidative reactions. In most plant parts and animal tissues, the major compound of the metabolic pattern is prothioconazole-desthio, which is more toxic than the parent compound.</p> <p>Given the complex plant and animal metabolic pattern and to reflect adequately the toxicological burden the consumer is exposed to, the residue definition for risk assessment in all commodities is the sum of prothioconazole-desthio and all metabolites</p> | Prothioconazole-desthio | <p>Prothioconazole desthio has been chosen for the residue definition for plants since it represents the major part of the residues in plants, corresponding analytical methods are available and the toxicological relevance of this substance.</p> <p>Prothioconazole sulfonic acid has been considered by JMPR to be of toxicological relevance but was not taken into consideration (no justification given, maybe the low level of TRR attributed to the compounds). The multiple prothioconazole desthio structural isomers were not include in the residue definition since individuals represented < 10 % in plant matrices</p> |
| Monitoring plants | Prothioconazole-desthio | The need for monitoring the parent compound was not considered necessary as its toxicity and its | Prothioconazole-desthio | |

¹⁰¹ Conclusion on the peer review of prothioconazole. EFSA Scientific Report (2007) 106, 1-98

¹⁰² Annex 1_PRAPeR and JMPR decisions, AGES report 2010

¹⁰³ JMPR, Report 2008

¹⁰⁴ JMPR, Evaluation 2008

| Prothioconazole | | | | |
|--------------------------------|--|--|--|---|
| | | occurrence in plants are lower. Prothioconazole-desthio has been chosen since it represents the major part of the residues in plant. | | |
| Risk assessment animals | Sum of prothioconazole-desthio and all metabolites containing the 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2H-1,2,4-triazole moiety) expressed as prothioconazole-desthio. | Given the complex metabolic pattern in livestock, and similarly to plant products, the residue definition proposed for risk assessment in animal commodities is the sum of prothioconazole-desthio and all metabolites | Sum of prothioconazole-desthio, prothioconazole-desthio-3-hydroxy, prothioconazole-desthio-4-hydroxy and their conjugates expressed as prothioconazole-desthio | For risk assessment purposes, the extensive metabolism of this compound justifies the inclusion of hydroxylated prothioconazole-desthio metabolites and the corresponding conjugates. |
| Monitoring animals | Sum of prothioconazole-desthio and its glucuronide conjugate, expressed as prothioconazole-desthio. | The need for including the glucuronide conjugate in the residue definition results from the fact that the free metabolite was not found in milk and cannot therefore act as a valid marker compound. | Prothioconazole-desthio | Prothioconazole-desthio is included into the residue definition for monitoring purposes only because it is the major part of the residual radioactivity and there is a residue analytical method available. |

| Pyrimethanil | | | | |
|-------------------------------|-------------------|--|-------------------------|--|
| Residue definitions | EU ¹⁰⁵ | Comments on availability on toxicological studies on metabolites and analytical methods Conjugate forms of hydroxylated pyrimethanil were also present in the rat metabolism and their own toxicity can be therefore considered as covered by the toxicological dossier of the parent compound Adequate methods are available to monitor all compounds given in the respective residue definition | JMPR ^{106,107} | Comments on availability on toxicological studies on metabolites and analytical methods No toxicological study on metabolites has been described Analytical methods on the quantitative determination of pyrimethanil in a variety of crops and for the determination of pyrimethanil and metabolites 2-(4-hydroxyanilino)-4,6-dimethylpyrimidine and 2-anilino-4,6-dimethylpyrimidine in bovine commodities |
| Risk assessment plants | Pyrimethanil | The metabolism of pyrimethanil was examined after foliar as well as soil application on carrots representing the crop group 'root vegetables', on tomatoes, apples and grapes, representing the crop group 'fruits' and on lettuce representing the crop group 'leafy vegetables'. Pyrimethanil was shown to be the major part of the total residues present in the edible part of the tested commodities. Metabolites consisting in conjugated forms of hydroxylated pyrimethanil were identified, but always at much lower levels than the parent compound even for PHI as long as 42 days.- | Pyrimethanil | Data for the foliar application of pyrimethanil as a suspension concentrate formulation (SC) to a variety of fruit, vegetable, and nut crops. Additionally, supervised trial data reports were received for the post-harvest treatment of citrus, pome fruit and cherries. The major component of the residue on numerous plant commodities, from the foliar application of pyrimethanil, is pyrimethanil. Minor amounts of hydroxylated pyrimethanil derivatives are found, generally < 10% each of the total residue. The Meeting concluded that the residue definition for both enforcement and dietary exposure for plant commodities is pyrimethanil |
| Monitoring plants | Pyrimethanil | | Pyrimethanil | |

¹⁰⁵ Conclusion on the peer review of pyrimethanil, *EFSA Scientific Report* (2006) 61, 1-70

¹⁰⁶ JMPR, Report 2007* New compound

¹⁰⁷ JMPR, Evaluation 2007

| Pyrimethanil | | | | |
|--------------------------------|---|---|---|--|
| Risk assessment animals | No residues expected at measurable level under practical conditions | The metabolism of pyrimethanil has been investigated in lactating cow. No sign of accumulation was observed. Pyrimethanil itself could not be identified in any of the tissues investigated. Taking into account usual nutritional practices of livestock, the possible highest exposure of animals to residues of pyrimethanil was calculated to be in the range of 0.004, 0.01, 0.007 and 0.006 mg/kg bw/d for dairy cattle, beef cattle, poultry and pigs respectively. A feeding study in dairy cow was carried out at dose levels largely in excess of these expected exposures, demonstrating that residues of pyrimethanil and its metabolites C 614 276 and C 614 2778 in cattle tissues are not expected under normal conditions. | For milk, sum of pyrimethanil and 2-anilino-4,6-dimethylpyrimidin-5-ol, expressed as pyrimethanil For livestock tissues (excluding poultry) is the sum of pyrimethanil and 2-(4-hydroxyanilino)-4,6-dimethylpyrimidine, expressed as pyrimethanil. | In livestock (cow) commodities, pyrimethanil is not found following oral administration of the compound. The major metabolites are 2-(4-hydroxyanilino)-4,6-dimethylpyrimidine and 2-anilino-4,6-dimethylpyrimidin-5-ol, in kidney and milk, respectively. The Meeting concluded that the residue definition for both enforcement and dietary exposure considerations for milk is the sum of pyrimethanil and 2-anilino-4,6-dimethylpyrimidin-5-ol, expressed as pyrimethanil and for livestock tissues (excluding poultry) is the sum of pyrimethanil and 2-(4-hydroxyanilino)-4,6-dimethylpyrimidine, expressed as pyrimethanil |
| Monitoring animals | No residues expected at measurable level under practical conditions | | For milk, sum of pyrimethanil and 2-anilino-4,6-dimethylpyrimidin-5-ol, expressed as pyrimethanil For livestock tissues (excluding poultry) is the sum of pyrimethanil and 2-(4-hydroxyanilino)-4,6-dimethylpyrimidine, expressed as pyrimethanil. | |

D. EXAMPLES OF METABOLITE TO PARENT RATIO FOR 10 PESTICIDES AND ITS DEPENDENCE ON USE PATTERN

Ratios of metabolite to parent are determined for different pesticides classified according to the pre-harvest interval (PHI). The table is constructed according to PHI for the different pesticides and several parameters that can affect the ratio of metabolite to parent are also presented, such as, crop type, part of the plant analysed, dose of pesticide and number of applications. The data presented (from DARs of the active substance)* in the table are mean values of those obtained in the supervised field trials carried out in different EU Member States to represent field and cultural practice variability. The results obtained at different places over the time aim to represent the commercial practice and the variation of residues under different conditions.

| MALATHION | | | | PHI (days) | | |
|---|--------------|------------------|----------------------|-------------------|----------|----------|
| Plant group | Crops | kg a.s/ha | max no. Appl. | 0 | 1 | 3 |
| Ratio Malaoxon/Malathion | | | | | | |
| Berry | Strawberry | 1.5 | 4 | 0.02 | 0.11 | 0.10 |
| Ratio DMM¹⁰⁸/Malathion | | | | | | |
| Berry | Strawberry | 1.5 | 4 | 0.04 | 0.41 | 0.28 |
| Ratio MMCA¹⁰⁹/Malathion | | | | | | |
| Berry | Strawberry | 1.5 | 4 | 0.29 | | 1.72 |
| Ratio MDCA¹¹⁰/Malathion | | | | | | |
| Berry | Strawberry | 1.5 | 4 | 0.27 | | 0.33 |

| BUPIRIMATE | | | | PHI (days) | | | | | |
|--|--------------|------------------|----------------------|-------------------|----------|----------|----------|--------------|--------------|
| Plant group | Crops | kg a.s/ha | Max no. Appl. | 0 | 3 | 5 | 7 | 10~11 | 14~15 |
| Ratio Ethirimol/Bupirimate | | | | | | | | | |
| Berry | Strawberry | 0.25-0.27 | 4 | 37.02 | 13.44 | 17.71 | 8.74 | 6.56 | 0.21 |
| CYROMAZINE | | | | | | | | | |
| Ration Melamine/Cyromazine | | | | | | | | | |
| Leaf vegetables | Lettuce | 0.23-0.27 | 4 | 0.09 | | | 0.46 | | 0.84 |
| Fruiting vegetables | Tomatoes | 0.56-6.2 | 4~19 | 0.59 | | | 0.72 | | 0.74 |
| DICAMBA | | | | | | | | | |
| Ratio 5OH-Dicamba¹¹¹/Dicamba | | | | | | | | | |
| Pasture | Pasture | 0.46-0.52 | 1~2 | 0.088 | 0.03 | | 0.05 | 0.18 | 0.04 |

¹⁰⁸ DMM: desmethyl-malathion

¹⁰⁹ MMCA: monocarboxylic acid-malathion

¹¹⁰ MDCA: dicarboxylic acid-malathion

¹¹¹ 5OH-Dicamba: 5 hydroxy-dicamba

| FLONICAMID | | | | PHI (days) | | | | | |
|---|-------------------|-----------|---------------|------------|------|------|-------|-------|-------|
| Plant group | Crops | kg a.s/ha | Max no. Appl. | 0 | 3 | 6~7 | 10~14 | 18~21 | 28~35 |
| Ration TFNG¹¹²/Flonicamid | | | | | | | | | |
| Fruit | Apples | 0.07 | 3 | 0.11 | | 0.17 | 0.25 | 0.33 | 0.33 |
| Fruit | Peach / Nectarine | 0.07 | 2 | 0.11 | 0.14 | 0.17 | 0.25 | | |
| Tuberculs | Potatoes | 0.07 | 2 | | 1.00 | 1.00 | 1.00 | | |
| Cereals | Wheat (grain) | 0.07 | 2 | | | | | 20.00 | 33.33 |
| Cereals | Wheat (straw) | 0.07 | 2 | | | | | 2.00 | 1.67 |
| Ration TFNA¹¹³/Flonicamid | | | | | | | | | |
| Fruit | Apple | 0.07 | 3 | 0.22 | | 0.50 | 0.50 | 0.67 | 0.67 |
| Fruit | Peach/ Nectarine | 0.07 | 2 | 0.11 | 0.14 | 0.17 | 0.13 | | |
| Tuberculs | Potatoes | 0.07 | 2 | | 1.00 | 1.00 | 1.00 | | |
| Cereals | Wheat (grain) | 0.07 | 2 | | | | | 2.00 | 4.00 |
| Cereals | Wheat (straw) | 0.07 | 2 | | | | | 0.29 | 0.33 |

| KRESOXIM-methyl | | | | PHI (days) | | | | | | |
|---|---------------------------|-----------|---------------|------------|-------|-------|-------|-------|-------|------|
| Plant group | Crops | kg a.s/ha | Max no. Appl. | 0 | 20~22 | 27~29 | 33~36 | 41~43 | 48~50 | 58 |
| Ratio BF490-2¹/Kresoxim | | | | | | | | | | |
| Fruit | Grape | 0.15 | 3 | 0.05 | 0.18 | 0.25 | 0.27 | 0.29 | | |
| Fruit | Pear | 0.1 | 4&8 | 0.28 | 1.00 | 1.00 | 1.00 | 1.00 | | |
| Fruit | Apple | 0.1-0.15 | 8&12 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | |
| Cereals | Wheat (Whole Plant) | 0.025 | 2 | 0.01 | | | | | | |
| Cereals | Wheat (Ears) | 0.025 | 2 | | | | 0.26 | 0.50 | 0.33 | |
| Cereals | Wheat (Grain) | 0.025 | 2 | | | | 1.00 | 0.82 | 0.71 | 1.00 |
| Cereals | Wheat (Straw) | 0.025 | 2 | | | | 0.29 | 0.07 | 0.08 | 0.17 |
| Cereals | Wheat (Rest of the plant) | 0.025 | 2 | | | | 0.16 | 0.14 | 0.14 | |
| Cereals | Barley (Whole Plant) | 0.025 | 2 | 78.57 | | | | | | |
| Cereals | Barley (Ears) | 0.025 | 2 | | | | 0.14 | | | |
| Cereals | Barley (Grain) | 0.025 | 2 | | | | 0.26 | 0.34 | 0.38 | |
| Cereals | Barley (Straw) | 0.025 | 2 | | | | 0.09 | 0.09 | 0.09 | |

¹¹² TFNG: N-[[4-(trifluoromethyl)pyridin-3-yl]carbonyl]glycine or N-(4-trifluoromethylnicotinoyl)glycine

¹¹³ TFNA: 4-(trifluoromethyl)pyridine-3-carboxylic acid or 4-trifluoromethylnicotinic acid

| KRESOXIM-methyl | | | | PHI (days) | | | | | | |
|--------------------------------------|----------------------------|------------|---------------|------------|-------|-------|-------|-------|-------|------|
| Plant group | Crops | kg a.s./ha | Max no. Appl. | 0 | 20~22 | 27~29 | 33~36 | 41~43 | 48~50 | 58 |
| Cereals | Barley (Rest of the plant) | 0.025 | 2 | | | | 0.12 | | | |
| Ratio BF490-9 ¹ /Kresoxim | | | | | | | | | | |
| Fruits | Grape | 0.15 | | 0.05 | 0.14 | 0.19 | 0.22 | 0.23 | | |
| Fruits | Pear | 0.1 | | 0.37 | 1.38 | 1.17 | 1.17 | 1.09 | | |
| Fruits | Apple | 0.1-0.15 | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | |
| Cereals | Wheat (Whole Plant) | 0.025 | 2 | 0.04 | | | 0.37 | 0.50 | 0.42 | |
| Cereals | Wheat (Ears) | 0.025 | 2 | | | | 1.00 | 0.82 | 0.71 | 1.00 |
| Cereals | Wheat (Grain) | 0.025 | 2 | | | | 0.23 | 0.17 | 0.07 | |
| Cereals | Wheat (Straw) | 0.025 | 2 | | | | 0.25 | 0.08 | 0.08 | 0.08 |
| Cereals | Wheat (Rest of the plant) | 0.025 | 2 | | | | 0.23 | 0.17 | 0.07 | |
| Cereals | Barley (Whole Plant) | 0.025 | 2 | 0.04 | | | | | | |
| Cereals | Barley (Ears) | 0.025 | 2 | | | | 0.14 | | | |
| Cereals | Barley (Grain) | 0.025 | 2 | | | | 0.26 | 0.34 | 0.38 | |
| Cereals | Barley (Straw) | 0.025 | 2 | | | | 0.10 | 0.08 | 0.07 | |
| Cereals | Barley (Rest of the plant) | 0.025 | 2 | | | | 0.22 | | | |

* EU evaluations in the forms of the Draft Assessment Report (DAR) (compiled by the Rapporteur Member State to support the evaluation for the inclusion of active substances on Annex 1 of Council Directive 91/414/EEC. These are The United Kingdom, 2009; Finland, 2004; The Netherlands, 2007; 2009; Hellas, 2007; Denmark, 2007; France, 2005; Belgium, 1997; 2010.

E. PESTICIDE METABOLITE ESTIMATIONS - CASE STUDIES

Compounds for case study evaluation

Case examples are presented here for estimating the levels of metabolite exposure arising from the use of different pesticides. To consider a TTC assessment of metabolites reliable exposure data need to be available. These case studies show the practical challenges presented by the types of residues data that tend to be available and discuss possible approaches and show intake results for various outcomes that can be considered.

Six different pesticides were evaluated in the case studies: azoxystrobin, bitertanol, boscalid, dimethoate, napropamide, and prohexadione calcium. The examples were selected to cover a range of possible active substance related profiles and residue situations, as follows:

- Many metabolites;
- Few metabolites - predominant residue is parent;
- Few metabolites - predominant residue is not parent;
- Profile of metabolites changes with Pre-Harvest Interval (PHI);
- Profile of metabolites changes with crop;
- Availability of residues data for both the DAR representative uses and also wider uses e.g. based on the residues data sets considered at the time of MRL setting;
- Active substances of low, medium and high toxicity (based on ADI);
- Active substances either acutely toxic or not (based on whether an ARfD is set);
- Novel metabolites seen in livestock studies;
- Novel metabolites seen in rotational crop metabolism.

Metabolite level estimation methods

The primary emphasis of the case study work was to consider the metabolite exposure levels for the primary crop situation since this is applicable for all pesticide uses and is expected to cover the principal sources of consumer exposure to pesticide metabolites. However both the rotational crop and animal products (based on consumption of treated crops by livestock) exposure routes are also expected to be relevant depending on pesticide specific considerations. Therefore, the case study also considered a small number of examples to demonstrate these situations. For each active substance compound, the residues data sets were considered, summarising the data available on metabolites identified in the primary crop metabolism studies and considering the analytes determined in the residues trials.

With regard to metabolism data, results were summarised showing the mg/kg levels of parent and metabolite in different crops and different crop fractions to enable a comparison of metabolite to parent ratios to be made across different crops. Commodities that were of direct consumer relevance as well as non consumed commodities, such as potato foliage, were covered since the possibility of metabolites being found across different crop species was an issue for consideration. Calculation of a specific metabolite ratio was only possible if the metabolite and parent were found at a determinable level in the commodity fraction being considered. Where parent was not identified in the metabolism

data, e.g. in the case of an extensively metabolised pesticide, a ratio of metabolite to parent could not be derived. In such a case the level of metabolite was estimated by making an adjustment to the level of the metabolite found in the metabolism study according to the rate of the metabolism study in relation to the GAP (Good Agricultural Practice – the recommended use) rate. For example if the metabolism study was at twice the relevant GAP rate, then the estimated level of metabolite for the case study was half that found in the metabolism study.

The usually derived end-points, the supervised trials median residue (STMR) and the highest residue (HR) were taken from summaries of the residues trials. Most frequently, only the parent was analysed in supervised trials, however where relevant the residue values from the trials were selected for metabolites. For example in the case of the parent pesticide dimethoate, the metabolite omethoate was also analysed in the residues trials for all of the crop uses. As such residues end-points were collated on this metabolite as well as for the parent dimethoate.

Therefore for the case studies presented here, the residue level of metabolites was calculated by applying the metabolite to parent ratios as determined in the plant metabolism studies to the STMR (for chronic assessment) and HR (for acute assessment) for the parent residues from the supervised residues trials.

More than one metabolite ratio was derived if the metabolite was found in more than one crop or commodity analysed in metabolism studies or if more than one pesticide application was made in the metabolism studies. In radiolabelled metabolism studies there may be more than one experimental treatment for various reasons e.g. application timing, method or radiolabel position. When an extrapolation of metabolite ratios to different crops was considered (please see the various exposure options considered below), this was done using the highest ratio to apply to the other crops, if more than one ratio was identified. If a metabolite ratio could not be derived (if parent was not found in the same situation as the metabolite found in the metabolism study) then the estimated residue level for the metabolite (according to an adjustment for the dose rate of the metabolism study in relation to the expected GAP) was instead extrapolated to different crops where an extrapolation approach was being applied. For bitertanol for example, an estimated residue level approach was taken for wheat although a ratio approach could only be considered for apple since parent was found in the apple metabolism but not the wheat grain metabolism data.

In most cases either the application of a ratio to the parent STMR or HR from the residues trials or an estimation of a metabolite level from the metabolism study was derived. Where the metabolite level was estimated from the metabolism study only one residue level was established. Taking a simplistic approach, the same level was used in the chronic and acute exposure estimation in the case studies. However, a higher level for acute exposure assessment could be considered. Where a specific metabolite to parent ratio could be derived this enabled separate estimates of metabolite STMR and HR levels for respective chronic and acute dietary exposure. When quantitative residues field studies were available where the metabolite had been analysed in the trials, the STMR and HR for the metabolite were used in preference of applying ratios from the metabolism studies.

Data sources and extent of uses considered

Two main data sources were used for each active: 1) the EU evaluations in the form of the Draft Assessment Report (DAR) (compiled by the Rapporteur Member State to support the evaluation for the inclusion of active substances on Annex 1 of Council Directive 91/414/EEC) together with the supporting residues and the GAP form for the DAR uses; 2) the MRL assessments (MRL assessment reports prepared by the EU Rapporteur Member States (RMS) under Article 12 of MRL Regulation 396/2005) where these were available. Some provisional MRL assessment reports were available, and for most substances they provide a better approximation of the extent of intended uses than the DARs.

Different exposure options considered in the cases studies

An initial consideration of the data suggested that both extent of uses being considered (a limited number of uses (e.g. DAR representative uses) versus wider uses (such as at the time of MRL setting) and the ways in which the metabolite ratios were extrapolated could significantly affect the outcome of the metabolite exposure calculations. As discussed in chapter 8, it was proposed that the case studies should evaluate the various extremes of these situations. As such four different ‘exposure’ options were considered for each of the compounds and metabolites.

Option A: metabolite estimation for specific crop(s) in which the metabolite was found only (in radiolabelled metabolism studies) and a limited number of crop uses.

As such only the DAR representative uses were considered and the metabolite estimation was only considered for the particular crop species in which the metabolite was identified. For example, if the metabolite was found in the rice metabolism and not barley metabolism, then the metabolite estimation was not made for uses of barley, if the representative use was barley and not rice.

Option B: metabolite estimation for specific crop(s) in which the metabolite was found only (in radiolabelled metabolism studies) and wider crop uses considered.

Where available, data considered for MRL setting purposes were used encompassing a wider range of crops that may better approximate the extent of intended uses. As above the metabolite estimation was only specifically done for the particular crop species in which the metabolite was identified.

Option C: metabolite estimation for all crops by extrapolating the ratio for the metabolite in question across all the crops and a limited number of crop uses.

As such only the DAR representative uses were considered. Either the ratio was extrapolated to other crops or otherwise, an estimated level for the metabolite extrapolated.

Option D: metabolite estimation for all crops by extrapolating the ratio for the metabolite in question across all the crops and wider crop uses considered.

Where available, data considered for MRL setting purposes were used encompassing a wider range of crops that may better approximate the extent of intended uses. As above, either the ratio was extrapolated to all of the crops or otherwise, an estimated level for the metabolite extrapolated to all of the uses being considered.

Intake calculations using consumer exposure models and comparison of results to different TTC levels.

‘Metabolite STMR’ data were used to assess total chronic dietary exposures using the UK NEDI¹¹⁴ spreadsheet model. This assessment uses the 97.5th percentile consumption level of consumers only for the two commodities which give the highest intakes, and for the remaining commodities, the mean consumption of the total population group is used. These contributions are summed to give a total high-level dietary intake.

The acute estimations were made using the ‘metabolite HR’ and the UK NESTI spreadsheet model on an individual commodity basis, according to the standard international approach. For the case study results (Tables 3 to 14), only the commodity that gave the highest NESTI is included in the results. It is recognised that there would be other commodity intakes that give lower acute dietary exposure estimations for the same metabolite.

¹¹⁴ UK risk assessment models <http://www.pesticides.gov.uk/approvals.asp?id=1687>

Using both the chronic and acute exposure models, the dietary exposure of ten different UK consumer subgroups, infants, toddlers, young children of various ages, adults, vegetarians and elderly consumers, was calculated. Of these the critical consumer was commonly infant, toddler or 4-6 year old child for both chronic and acute exposure assessment. The results for the critical consumer and also adults as another representative consumer subgroup are covered in the results tables (Tables 3 to 14 covering the various exposure scenarios).

The use of the UK spreadsheet tools for dietary estimation of the metabolites as a model approach to these case studies is further discussed in Chapter 8.

During the development of this opinion there were two different occasions of comparing the intake values with TTC values:

First step: Since the chronic TTCs were considered to be conservative for consideration of acute exposure (EFSA, 2012) it was thought that if both chronic and acute exposure estimates for metabolites were relatively low and below the chronic TTC thresholds, that it could be proposed that no further toxicological assessment of the metabolites would be needed. In this way a screen using the chronic TTC values would be adequate to propose an assessment scheme by comparing intake values calculated for metabolites with the TTC values. This first step considered a comparison of both chronic and acute exposure estimates with toxicological threshold values of 0.0025 µg/kg bw/d, 0.3 µg/kg bw/d 1.5 µg/kg bw/d, 9 µg/kg bw/d and 30 µg/kg bw/d when assuming a body weight of 60 kg. The results were categorised into different classification intervals according to the Cramer Classes (I, II, and III) and other thresholds that were considered relevant. These classification intervals are summarised in Table 1. See chapter 5 for a further discussion of these classes.

Since, following the first step, it was found that depending on the exposure options, quite frequently the TTC levels were exceeded by the metabolite exposure levels, it was concluded that an onward assessment scheme would need to be tailored differently for the purposes of chronic and acute assessment. Therefore, TTC levels were developed and proposed that were considered relevant to the assessment of acute exposure (section 5.3.1). Therefore, a second step of comparing intake values with TTC values was proposed to cover this situation.

Second step: The chronic intake values were compared with the relevant chronic TTC values, and the acute intake values were compared with the newly proposed acute TTC values for pesticide metabolites. At this time, since it was concluded that CCII compounds should be grouped with CCIII (EFSA, 2012), the TTC value for CCII was excluded from this consideration. The TTC levels and classification intervals used for this second step comparison are summarised in Table 2. See chapter 5 for a further discussion of these classes (section 5.3.1 for acute TTC values).

The colour scheme included in the below tables (Tables 1 and 2) are also applied to the results expressed in Tables 3 to 14. This merely serves as a visual aid as the numeric values of intake determine the classification interval to which the metabolite is assigned. In addition, the relevant intervals ('Int' - see Tables 1 and 2) are stated in addition to the use of the colour scheme.

Table 1: TTC Classification intervals proposed for comparison of metabolite exposure for first step comparison

| | Classification intervals | Classification intervals (Intake in µg/kg bw/day) | Upper level threshold (µg/person/day basis) | Upper level categorisation of threshold | Presumption of toxicity (according to threshold) | Cramer et al., 1978 |
|-------------------------------|--------------------------|---|---|---|--|---|
| High to low toxicity ↓ | Int 1 | <0.0025 | 0.15 | 'genotoxicity' | serious | |
| | Int 2 | 0.0025 up to 0.3 | 18 | 'neurotoxicity' | serious | |
| | Int 3 | 0.3 up to 1.5 | 90 | Cramer Class III | serious | No strong presumption of safety |
| | Int 4 | 1.5 up to 9 | 540 | Cramer Class II | moderate | Less innocuous than those of Class I but not suggestive of toxicity |
| | Int 5 | 9 up to 30 | 1800 | Cramer Class I | low | Simple structures, low order of oral toxicity expected |
| | Int 6 | ≥30 | - | - | low | - |

Table 2: TTC Classification intervals proposed for comparison of metabolite exposure for second step comparison

| | Classification intervals | Classification intervals (Intake in µg/kg bw/day) | Upper level threshold (µg/person/day basis) | Upper level categorisation of threshold | Presumption of toxicity (according to threshold) | Cramer et al., 1978 |
|-------------------------------|--------------------------|---|---|--|--|--|
| High to low toxicity ↓ | <u>Chronic</u> | | | | | |
| | Int C1 | <0.0025 | 0.15 | 'genotoxicity' | serious | |
| | Int C2 | 0.0025 up to 0.3 | 18 | 'neurotoxicity' | serious | |
| | Int C3 | 0.3 up to 1.5 | 90 | Cramer Class II/III | serious | No strong presumption of safety |
| | Int C4 | 1.5 up to 30 | 1800 | Cramer Class I | low | Simple structures, low order of oral toxicity expected |
| | Int C5 | ≥30 | - | - | low | - |
| High to low toxicity ↓ | <u>Acute</u> | | | | | |
| | Int A1 | <0.0025 | 0.15 | 'genotoxicity' | serious | |
| | Int A2 | 0.0025 up to 0.3 | 18 | 'neurotoxicity' | serious | |
| | Int A3 | 0.3 up to 5.1 | 306 | all that are not neurotoxic, or genotoxic or otherwise CCI | serious | No strong presumption of safety |
| | Int A4 | 5.1 up to 30 | 1800 | Cramer Class I | low | Simple structures, low order of oral toxicity expected |
| | Int A5 | ≥30 | - | - | low | - |

Other methodological considerations

The following principles were applied to the case studies, which are explained for transparency:

- The potential for refinements due to processing of food prior to consumption were not considered in the case studies.
- If the residue end-point in the residue trials was <LOQ, the LOQ value was used for metabolite estimations. This could lead to a potential overestimation of metabolite levels. It is noted that for napropamide all of the residues trials results were <LOQ for the parent compound. The metabolite ratios were then applied to the LOQ level to give estimated metabolite residue levels.
- For the MRL data sets, if there was more than one data set e.g. US data for Import tolerance as well as an EU use, the highest STMR or HR was used in the case study.
- If the MRL assessment reports has used $\frac{1}{2}$ the MRL where a specific STMR value is not available then this approach was also used in the case study.
- Only identified metabolites were included (since following EU guidelines it is expected that significant metabolites need to be identified or otherwise this is a data gap that needs to be filled).

Other relevant exposure scenarios- approaches to case studies for rotational crop and livestock metabolites

In order to address other exposure scenarios, aside from direct treatment of primary crops, and to consider the practical data challenges arising from the residues data for these situations, a couple of case examples were extended to metabolites found in crops grown as rotational crops or found in animal products.

The rotational crop situation needs to be considered for the presence of metabolites after the pesticide has been used directly to treat a primary crop. The example of azoxystrobin was considered in the case study since there was a good range of metabolism data representing the rotational crop situation. The parent compound azoxystrobin was not present in every commodity matrix considered and therefore the approach taken was to calculate the metabolite levels found in the metabolism study according to the judgement that the studies were at an approximately three-fold exaggerated rate. The values for the 200 days after treatment (DAT) timing of replanting the rotational crop were used for metabolite exposure assessment. Residues were lower for the 365 DAT timing (and were not used in the case study), and the data for 30 DAT (usually tested to consider replanting after crop failure) was less relevant for azoxystrobin where application of the pesticide is to an established crop. The case study focussed on metabolites that were not found in the primary crop situation to look at the exposure of novel metabolites. An exception to this was metabolite M42 since this metabolite was found in both primary and rotational crops and seemed to be the most prevalent metabolite in the rotational crop situation. Residue levels seen in the straw (rotational crop straw samples) were not extrapolated to other crops since crops for direct human consumption are harvested fresh. When rotational crop metabolite levels were extrapolated, residues data for radish tops and wheat forage were used to extrapolate directly to consumed crops. The 'limited uses' scenarios (options A and C) were not really applicable to rotational crops in the case study since it is usually necessary to consider a range of possible rotational crops.

The rotational crop situation was also considered for boscalid since it is known that carry over of the parent residue boscalid in rotational crops can add to consumer intakes from the primary crop. However boscalid is only metabolised to a limited extent. The only 'metabolite' identified for boscalid in rotational crop metabolism was the metabolite M510F61, a sugar conjugate of the parent compound. This shows how the metabolite exposure considerations are strongly dependent on pesticide specific factors.

Boscalid was considered in terms on livestock specific metabolites since seven metabolites were formed in livestock that were not formed in primary or rotational crops. This was approached in the following way: the data of the feeding studies covered parent and metabolite F01; the data on these analytes produced some variable results, but generally supported the 1:1 ratio seen in the metabolism studies for parent: metabolite F01. Therefore the metabolite ratio approach was applied to estimate the levels of F01 and other livestock specific metabolites. The 'STMR' and HR values from the MRL assessment reports were used for animal products to estimate parent levels (from residues data covering the sum of parent and F01), to which the metabolite ratios were applied, since parent boscalid was found in all of the animal product matrices in the livestock metabolism studies.

Results

First step comparison: comparison of both chronic and acute intake estimates for pesticide metabolites with chronic TTC values

The results for each of the metabolites for the six pesticides considered are expressed in Tables 3 and 4 for primary crop metabolites. The metabolite structures together with the structure for the parent compound, their assignation according to Cramer Class (Toxtree evaluation) and designation as either a 'mammalian' metabolite (laboratory animal metabolites, usually rat) or 'plant or livestock specific' metabolites are provided in Tables 15 to 20.

An overall 'trend' is that the metabolite estimations tended to be higher if moving from option A to B to C to D. However, there may be exceptions to this general rule due to residue data specific factors. In addition the wider uses considered (for MRL setting) may have resulted in more limited uses than originally intended by the registrant. For example, for dimethoate the DAR risk assessments were stated as provisional due to data gaps and potential risk concerns. The draft MRL consideration that was used for the case study indicated some critical decisions for the MRL setting process on both an acute and chronic assessment basis. For example wheat was one of the DAR uses, but the MRL assessment no longer included wheat as a supported use.

The results show that the choice of exposure option (A, B, C or D) being considered is a key determinant in the intake assessment of the metabolite. The difference is so noticeable that the classification interval of a metabolite can change according to which exposure option is being addressed.

The levels of metabolite intake overall span a very wide range. There were very few metabolite estimations that were $< 0.0025 \mu\text{g/kg bw/day}$, in interval 'Int 1' and only for adults when considering the primary crop situation and quite a significant number of metabolite intake levels estimated were $> 30 \mu\text{g/kg bw/day}$ in interval 'Int 6', especially for exposure option D. The values for option D are particularly high when there are a large number of wider uses, and when a metabolite ratio (metabolite/parent) is particularly high.

The results for the levels of metabolite intake for acute exposure assessment are typically considerably higher than the corresponding levels of metabolite intake for chronic exposure assessment. The margin of difference between chronic and acute exposure depends on active substance and residues data factors.

The values for adult are considerably lower than the corresponding critical consumer intakes reflecting the need to consider consumption data for various sub-population groups as represented by the case studies.

Considering primary crop metabolites only, option C (an option between the two extreme options A and D) and only reporting on the critical consumer results:

- Chronic assessment: 26% are under the 0.3 threshold (intervals 'Int 1' and 'Int 2' together), 53% are between 0.3 and 1.5 thresholds (interval 'Int 3'), and 21% are above 1.5 (intervals 'Int 4', 'Int 5' and 'Int 6' together) (of the 10 metabolites that are above 1.5, 6 are between 1.5 and 9 (interval 'Int 4'), 3 in the range of 9-30 (interval 'Int 5'), and 1 is >30, (interval 'Int 6')).
- Acute assessment, but comparing with chronic TTCs: 4% are under the 0.3 threshold (intervals 'Int 1' and 'Int 2' together), 17% are between 0.3 and 1.5 thresholds (interval 'Int 3'), and 79% are above 1.5 (intervals 'Int 4', 'Int 5' and 'Int 6' together) (of the 37 metabolites that are above 1.5, 25 are between 1.5 and 9 (interval 'Int 4'), 11 in the range of 9-30 (interval 'Int 5'), and 1 is >30 (interval 'Int 6')).

The corresponding intake results for livestock metabolites are in Tables 7 and 8. These show that different metabolites can, depending on pesticide specific factors, arise in the livestock situation at levels that are relevant to assessing consumer exposures. For boscalid, the example covered here, the compound was stable in plant systems and only in the livestock studies were a range of further metabolites formed (in total seven). As such consumer exposure to metabolites formed in livestock, albeit by a non direct route, should not be overlooked. This case example has shown how this can be approached from a methodological perspective. Total chronic dietary assessment of livestock metabolites was considered taking account of the possibility of finding residues in any consumable animal products. The acute dietary assessments presented here are focussed on the critical commodity which tended to be milk, and occasionally kidney.

The only 'metabolite' identified in the rotational crop situation for boscalid was the metabolite M510F61, a sugar conjugate of the parent compound. The results for the rotational crop situation for azoxystrobin (Tables 5 and 6) show the potential for different metabolites to be formed at levels that are still of relevance to consumer exposure assessment. For this case study example with azoxystrobin, the assessment included M42 which was the most prevalent metabolite in rotational crops. Despite this, the exposure to the metabolite was higher for the primary crop assessment. This conclusion of a higher exposure in the primary crop may not be typical. Clearly there can be novel metabolites in the rotational crop situation that are not seen in the primary crop. The case study on azoxystrobin shows how the metabolite assessment can be made for rotational crop metabolites.

Considering the potential for further 'screening' according to metabolite structure and whether found in the 'mammalian' metabolism (laboratory animal metabolism, usually rat).

Metabolites were further evaluated to consider those that were higher or lower than threshold values, when classifying the metabolites as 'mammalian (M)' or 'plant (P) or livestock specific (L)' (see Tables 15 to 20). 'Mammalian' metabolites were classified as such if they were found at any level in the urine, blood or bile of the test species (rat). Metabolites that were only found in rat faeces were not included since it is possible that these metabolites are formed by intestinal microorganisms. 'Plant or livestock specific' metabolites are those metabolites which were only found in the plant (or livestock) metabolism and were not, as far as the identification work conducted in plant, livestock (typically hen or goat) and rat could conclude, found also in the rat metabolism. The idea was that mammalian metabolites could be considered using the TTC threshold for the parent compound and that plant (or livestock species) specific metabolites could be evaluated according to TTC level according to

Cramer Class assessment. Therefore for all of the metabolites considered in the case studies Cramer Class structural classifications were assigned using the Toxtree software (version 1.6).

The results of the case studies show that metabolites are in the usually in the same structural class, CCIII. This (CCIII) is the classification that commonly applies to pesticide compounds. The case studies show that of the 47 primary crop metabolites considered in the case studies, only six were not CCIII (across two pesticide substances, of the 6 five were CCI and one was CCII). For the case study metabolites considered for rotational crops (azoxystrobin) and livestock (boscalid) all of the metabolites were CCIII (10 and 7 metabolites respectively). Therefore the results show that categorising into 'mammalian' and 'plant or livestock specific' metabolites and onward structural class assignation using Toxtree does not provide a particularly useful method of differentiating further between metabolites that either do or do not require further assessment as part of a TTC decision tree approach.

Second step comparison: comparison of chronic intakes with chronic TTC values and comparison with acute intakes with the newly proposed acute TTC values.

For the 47 primary crop metabolites considered in this case study, considering option C (an option between the two extreme options A and D) and only reporting on the critical consumer results:

- Acute assessment, and comparing with the proposed acute TTCs: 4% are under the 0.3 threshold (intervals 'Int A1' and 'Int A2' together), 53% are between 0.3 and 5.1 thresholds (interval 'Int A3'), and 43% are above 5.1 (intervals 'Int A4' and 'Int A5' together) (of the 20 metabolites that are above 5.1, 19 are between 5.1 and 30 (interval 'Int A4') and 1 is >30 (interval 'Int A5')).

This indicates that a greater number of metabolites are within the newly proposed acute threshold of 5.1 µg/kg bw/day, than were previously assessed (at the first step comparison stage) when a number of the metabolites were found to have acute exposures above the chronic TTC level of 1.5 µg/kg bw/day. For comparative purposes, the first step comparison previously made for these 47 primary crop metabolites:

- Acute assessment, but comparing with chronic TTCs: 4% are under the 0.3 threshold (intervals 'Int 1' and 'Int 2' together), 17% are between 0.3 and 1.5 thresholds (interval 'Int 3'), and 79% are above 1.5 (intervals 'Int 4', 'Int 5' and 'Int 6' together) (of the 37 metabolites that are above 1.5, 25 are between 1.5 and 9 (interval 'Int 4'), 11 in the range of 9-30 (interval 'Int 5'), and 1 is >30 (interval 'Int 6')).

Tables of intake results for metabolite case studies

KEY/guide to the tables:

Italics- metabolites are found in plants (or livestock) and are not in the 'mammalian' metabolism (laboratory animal metabolism, usually rat)

Cramer Class III – no shading

Cramer Class II

Cramer Class I

First step comparison: Classification intervals -Intake values are in µg/kg bw/day:

'Int 1' <0.0025

'Int 2' 0.0025 up to 0.3

'Int 3' 0.3 up to 1.5

'Int 4' 1.5 up to 9

'Int 5' 9 up to 30

'Int 6' ≥ 30

Second step comparison: Classification intervals -Intake values are in $\mu\text{g/kg bw/day}$:

Chronic comparisons

'Int C1' <0.0025

'Int C2' 0.0025 up to 0.3

'Int C3' 0.3 up to 1.5

'Int C4' 1.5 up to 30

'Int C5' >30

Acute comparisons

'Int A1' <0.0025

'Int A2' 0.0025 up to 0.3

'Int A3' 0.3 up to 5.1

'Int A4' 5.1 up to 30

'Int A5' >30

Primary crops: Missing values may be present in the results tables depending on the circumstances. For example: for azoxystrobin M30, M40 and M42 were not found in all of the crops studied in the metabolism data; for napropamide and bitertanol, only the DAR representative uses could be considered as data to support wider uses in the context of MRL setting are not yet available; for prohexadione calcium the DAR uses considered uses were for a limited number of crops and the specific metabolites were found in different species in the metabolism studies. The overall extent of uses considered at the time of MRL setting for prohexadione calcium were not that extensive (cereals and pome fruit).

In practice for the rotational crop situation (azoxystrobin) 'limited crop uses' were not considered since a range of crops need to be considered for the rotational crop situation. Therefore the results for option A and B are the same and those for C and D are the same.

For livestock metabolite assessment (boscalid) the exposure options A and C were not calculated, although this should be possible, since a more definitive assessment of animal product residues was available for the wider uses (options B and D).

Table 3: First step comparison: Primary crop metabolites/critical consumer intakes

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|------------------------------|------------------|-------|-------|-------|------|-------|------|-------|----------------|-------|------|-------|------|-------|-----|-------|
| Exposure option | A | | B | | C | | D | | A | | B | | C | | D | |
| azoxystrobin Primary Crop | | | | | | | | | | | | | | | | |
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| M02 | 0.74 | 3 | 0.74 | 3 | 0.77 | 3 | 18.4 | 5 | 2.0 | 4 | 2.0 | 4 | 19.5 | 5 | 233 | 6 |
| M09 | 0.21 | 2 | 0.21 | 2 | 0.42 | 3 | 10.1 | 5 | 4.9 | 4 | 4.9 | 4 | 10.7 | 5 | 128 | 6 |
| M13 | 0.57 | 3 | 0.57 | 3 | 0.61 | 3 | 14.7 | 5 | 15.6 | 5 | 15.6 | 5 | 15.6 | 5 | 187 | 6 |
| M19 | 0.08 | 2 | 0.08 | 2 | 0.11 | 2 | 2.8 | 4 | 2.0 | 4 | 2.0 | 4 | 2.9 | 4 | 35 | 6 |
| M23 | 0.011 | 2 | 0.011 | 2 | 0.15 | 2 | 3.7 | 4 | 0.2 | 2 | 0.2 | 2 | 3.9 | 4 | 47 | 6 |
| M24 | 0.405 | 3 | 0.405 | 3 | 0.42 | 3 | 10.1 | 5 | 10.7 | 5 | 10.7 | 5 | 10.7 | 5 | 128 | 6 |
| M28 | 0.58 | 3 | 0.58 | 3 | 0.69 | 3 | 16.5 | 5 | 14.7 | 5 | 14.7 | 5 | 17.6 | 5 | 210 | 6 |
| M30 | - | | - | | 0.23 | 2 | 5.5 | 4 | - | | - | | 5.9 | 4 | 70 | 6 |
| M35 | 0.27 | 2 | 0.27 | 2 | 0.34 | 3 | 8.3 | 4 | 6.8 | 4 | 6.8 | 4 | 8.8 | 4 | 105 | 6 |
| M40 | - | | - | | 0.46 | 3 | 11.0 | 5 | - | | - | | 11.7 | 5 | 140 | 6 |
| M42 | - | | - | | 0.57 | 3 | 13.8 | 5 | - | | - | | 14.7 | 5 | 175 | 6 |
| MU5 | 0.076 | 2 | 0.076 | 2 | 0.08 | 2 | 1.8 | 4 | 2.0 | 4 | 2.0 | 4 | 2.0 | 4 | 23 | 5 |
| MU6 | 0.008 | 2 | 0.008 | 2 | 0.11 | 2 | 2.8 | 4 | 0.15 | 2 | 0.15 | 2 | 2.9 | 4 | 35 | 6 |
| MU13 | 0.008 | 2 | 0.008 | 2 | 0.11 | 2 | 2.8 | 4 | 0.15 | 2 | 0.15 | 2 | 2.9 | 4 | 35 | 6 |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|------------------------------------|------------------|-------|---|-------|------|-------|---|-------|----------------|-------|---|-------|------|-------|---|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Bitertanol Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| BM1 Bitertanol ketone (BUE 1662) | 0.125 | 2 | | | 0.21 | 2 | | | 1.33 | 3 | | | 4.6 | 4 | | |
| BM2 4-hydroxybiphenyl | 0.125 | 2 | | | 0.21 | 2 | | | 1.33 | 3 | | | 4.6 | 4 | | |
| BM3 Triazolyl acetic acid | 0.54 | 3 | | | 1.5 | 4 | | | 0.88 | 3 | | | 5.98 | 4 | | |
| BM4 Triazolyl alanine | 1.24 | 3 | | | 3.4 | 4 | | | 2.02 | 4 | | | 13.7 | 5 | | |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|------------------------------|------------------|-------|-------|-------|-------|-------|-------|-------|----------------|-------|-------|-------|-------|-------|------|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Boscalid Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 chloronicotinic acid | 0.038 | 2 | 0.038 | 2 | 0.420 | 3 | 1.093 | 3 | 0.175 | 2 | 0.175 | 2 | 4.453 | 4 | 20.7 | 5 |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|-----------------------------|------------------|-------|---|-------|------|-------|---|-------|----------------|-------|---|-------|------|-------|---|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Napropamide Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 | 0.28 | 2 | | | 0.75 | 3 | | | 3.185 | 4 | | | 4.29 | 4 | | |
| M2 | 0.013 | 2 | | | 0.05 | 2 | | | 0.24 | 2 | | | 0.29 | 2 | | |
| M3 | 0.003 | 2 | | | 0.02 | 2 | | | 0.086 | 2 | | | 0.12 | 2 | | |
| M4 | 0.15 | 2 | | | 0.41 | 3 | | | 1.72 | 4 | | | 2.32 | 4 | | |
| M5 | 0.34 | 3 | | | 1.15 | 3 | | | 5.45 | 4 | | | 6.55 | 4 | | |
| M6 | 0.040 | 2 | | | 0.15 | 2 | | | 0.72 | 3 | | | 0.87 | 3 | | |
| M7 | 0.42 | 3 | | | 1.56 | 4 | | | 7.4 | 4 | | | 8.87 | 4 | | |
| M8 | 0.092 | 2 | | | 0.55 | 3 | | | 2.3 | 4 | | | 3.13 | 4 | | |
| M9 | 0.155 | 2 | | | 0.39 | 3 | | | 1.64 | 4 | | | 2.20 | 4 | | |
| M10 | 0.31 | 3 | | | 1.07 | 3 | | | 5.1 | 4 | | | 6.09 | 4 | | |
| M11 | 0.079 | 2 | | | 0.31 | 3 | | | 1.44 | 3 | | | 1.74 | 4 | | |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|---|------------------|-------|-----|-------|------|-------|------|-------|----------------|-------|------|-------|-----|-------|------|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Prohexadione calcium Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 | - | | 2.2 | 4 | 1.4 | 3 | 3.5 | 4 | - | | 15.3 | 5 | 3.0 | 4 | 15.3 | 5 |
| M2 | - | | 3.7 | 4 | 2.3 | 4 | 5.9 | 4 | - | | 25.5 | 5 | 5.1 | 4 | 25.5 | 5 |
| M3 | - | | 2.1 | 4 | 1.4 | 3 | 3.4 | 4 | - | | 14.8 | 5 | 2.9 | 4 | 14.8 | 5 |
| M4 | - | | 1.0 | 3 | 0.64 | 3 | 1.7 | 4 | - | | 7.1 | 4 | 1.4 | 3 | 7.1 | 4 |
| M5 | - | | 4.7 | 4 | 2.9 | 4 | 7.6 | 4 | - | | 32.6 | 6 | 6.5 | 4 | 32.6 | 6 |
| M6 | - | | 3.1 | 4 | 1.9 | 4 | 5.0 | 4 | - | | 21.4 | 5 | 4.2 | 4 | 21.4 | 5 |
| M7 | - | | - | | 0.23 | 2 | 0.59 | 3 | - | | - | | 0.5 | 3 | 2.5 | 4 |
| M8 | - | | - | | 0.32 | 3 | 0.83 | 3 | - | | - | | 0.7 | 3 | 3.6 | 4 |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|----------------------------|------------------|-------|---|-------|------|-------|------|-------|----------------|-------|---|-------|------|-------|------|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Dimethoate Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 | 0.053 | 2 | - | | 0.72 | 3 | 0.74 | 3 | 0.14 | 2 | - | | 1.5 | 4 | 1.33 | 3 |
| M2 | 1.3 | 3 | - | | 9.7 | 5 | 11.1 | 5 | 2.2 | 4 | - | | 11.7 | 5 | 19.9 | 5 |
| M3 | 17.7 | 5 | - | | 129 | 6 | 147 | 6 | 28.8 | 5 | - | | 155 | 6 | 264 | 6 |
| M4 | 0.089 | 2 | - | | 0.65 | 3 | 0.74 | 3 | 0.14 | 2 | - | | 0.78 | 3 | 1.33 | 3 |
| M5 | 2.6 | 4 | - | | 18.8 | 5 | 21.5 | 5 | 4.2 | 4 | - | | 22.6 | 5 | 38.5 | 6 |
| M6 | 2.6 | 4 | - | | 18.8 | 5 | 21.5 | 5 | 4.2 | 4 | - | | 22.6 | 5 | 38.5 | 6 |
| M7 | 0.089 | 2 | - | | 0.65 | 3 | 0.74 | 3 | 0.14 | 2 | - | | 0.91 | 3 | 1.33 | 3 |
| M8 | 0.089 | 2 | - | | 0.65 | 3 | 0.74 | 3 | 0.14 | 2 | - | | 0.91 | 3 | 1.33 | 3 |
| M9 | 0.089 | 2 | - | | 0.65 | 3 | 0.74 | 3 | 0.14 | 2 | - | | 0.78 | 3 | 1.33 | 3 |

Table 4: First step comparison: Primary crop metabolites/adult intakes

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|------------------------------|---------------|-------|-------|-------|------|-------|------|-------|-------------|-------|------|-------|-----|-------|----|-------|
| Exposure option | A | | B | | C | | D | | A | | B | | C | | D | |
| azoxystrobin Primary Crop | | | | | | | | | | | | | | | | |
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| M02 | 0.21 | 2 | 0.21 | 2 | 0.22 | 2 | 5.1 | 4 | 0.63 | 3 | 0.63 | 3 | 6.3 | 4 | 40 | 6 |
| M09 | 0.062 | 2 | 0.062 | 2 | 0.12 | 2 | 2.8 | 4 | 1.6 | 4 | 1.6 | 4 | 3.5 | 4 | 22 | 5 |
| M13 | 0.16 | 2 | 0.16 | 2 | 0.18 | 2 | 4.1 | 4 | 5.1 | 4 | 5.1 | 4 | 5.1 | 4 | 32 | 6 |
| M19 | 0.023 | 2 | 0.023 | 2 | 0.03 | 2 | 0.77 | 3 | 0.6 | 3 | 0.6 | 3 | 0.9 | 3 | 6 | 4 |
| M23 | 0.004 | 2 | 0.004 | 2 | 0.04 | 2 | 1.02 | 3 | 0.08 | 2 | 0.08 | 2 | 1.3 | 3 | 8 | 4 |
| M24 | 0.12 | 2 | 0.12 | 2 | 0.12 | 2 | 2.8 | 4 | 3.5 | 4 | 3.5 | 4 | 3.5 | 4 | 22 | 5 |
| M28 | 0.17 | 2 | 0.17 | 2 | 0.20 | 2 | 4.6 | 4 | 4.7 | 4 | 4.7 | 4 | 5.7 | 4 | 36 | 6 |
| M30 | - | | - | | 0.07 | 2 | 1.5 | 4 | - | | - | | 1.9 | 4 | 12 | 5 |
| M35 | 0.08 | 2 | 0.08 | 2 | 0.10 | 2 | 2.3 | 4 | 2.1 | 4 | 2.1 | 4 | 2.8 | 4 | 18 | 5 |
| M40 | - | | - | | 0.13 | 2 | 3.1 | 4 | - | | - | | 3.8 | 4 | 24 | 5 |
| M42 | - | | - | | 0.17 | 2 | 3.9 | 4 | - | | - | | 4.7 | 4 | 30 | 6 |
| MU5 | 0.02 | 2 | 0.02 | 2 | 0.02 | 2 | 0.51 | 3 | 0.6 | 3 | 0.6 | 3 | 0.6 | 3 | 4 | 4 |
| MU6 | 0.003 | 2 | 0.003 | 2 | 0.03 | 2 | 0.77 | 3 | 0.06 | 2 | 0.06 | 2 | 0.9 | 3 | 6 | 4 |
| MU13 | 0.003 | 2 | 0.003 | 2 | 0.03 | 2 | 0.77 | 3 | 0.06 | 2 | 0.06 | 2 | 0.9 | 3 | 6 | 4 |

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|------------------------------------|---------------|-------|---|-------|---|-------|---|-------|-------------|-------|---|-------|---|-------|---|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Bitertanol Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| BM1 Bitertanol ketone (BUE 1662) | 0.0225 | 2 | | 0.067 | 2 | | | 0.2 | 2 | | | 1.0 | 3 | | | |
| BM2 4-hydroxybiphenyl | 0.0225 | 2 | | 0.067 | 2 | | | 0.2 | 2 | | | 1.0 | 3 | | | |
| BM3 Triazolyl acetic acid | 0.22 | 2 | | 0.42 | 3 | | | 0.37 | 3 | | | 0.91 | 3 | | | |
| BM4 Triazolyl alanine | 0.51 | 3 | | 0.96 | 3 | | | 0.85 | 3 | | | 2.1 | 4 | | | |

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|------------------------------|---------------|-------|--------|-------|-------|-------|-------|-------|-------------|-------|-------|-------|-------|-------|-------|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Boscalid Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 chloronicotinic acid | 0.0105 | 2 | 0.0105 | 2 | 0.118 | 2 | 1.023 | 3 | 0.082 | 2 | 0.082 | 2 | 3.057 | 4 | 12.02 | 5 |

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|-------------------------------------|---------------|-------|---|-------|---|-------|---|-------|-------------|-------|---|-------|---|-------|---|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Napropamide Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 | 0.12 | 2 | | 0.36 | 3 | | | 0.90 | 3 | | | 1.11 | 3 | | | |
| M2 | 0.007 | 2 | | 0.02 | 2 | | | 0.052 | 2 | | | 0.08 | 2 | | | |
| M3 | 0.0011 | 1 | | 0.01 | 2 | | | 0.024 | 2 | | | 0.03 | 2 | | | |
| M4 | 0.069 | 2 | | 0.19 | 2 | | | 0.485 | 3 | | | 0.60 | 3 | | | |
| M5 | 0.17 | 2 | | 0.54 | 3 | | | 1.18 | 3 | | | 1.70 | 4 | | | |
| M6 | 0.021 | 2 | | 0.07 | 2 | | | 0.16 | 2 | | | 0.23 | 2 | | | |
| M7 | 0.22 | 2 | | 0.73 | 3 | | | 1.6 | 4 | | | 2.30 | 4 | | | |
| M8 | 0.029 | 2 | | 0.26 | 2 | | | 0.66 | 3 | | | 0.81 | 3 | | | |
| M9 | 0.070 | 2 | | 0.18 | 2 | | | 0.46 | 3 | | | 0.57 | 3 | | | |
| M10 | 0.16 | 2 | | 0.50 | 3 | | | 1.10 | 3 | | | 1.58 | 4 | | | |
| M11 | 0.041 | 2 | | 0.14 | 2 | | | 0.31 | 3 | | | 0.45 | 3 | | | |

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|--|---------------|-------|---|-------|---|-------|---|-------|-------------|-------|-----|-------|-----|-------|---|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Prohexadione calcium Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 | - | 0.4 | 3 | 0.57 | 3 | 0.96 | 3 | - | 2.3 | 4 | 1.3 | 3 | 2.3 | 4 | | |
| M2 | - | 0.65 | 3 | 0.95 | 3 | 1.6 | 4 | - | 3.9 | 4 | 2.1 | 4 | 3.9 | 4 | | |
| M3 | - | 0.4 | 3 | 0.55 | 3 | 0.93 | 3 | - | 2.3 | 4 | 1.2 | 3 | 2.3 | 4 | | |
| M4 | - | 0.2 | 2 | 0.27 | 2 | 0.45 | 3 | - | 1.1 | 3 | 0.6 | 3 | 1.1 | 3 | | |
| M5 | - | 0.8 | 3 | 1.21 | 3 | 2.05 | 4 | - | 5.0 | 4 | 2.7 | 4 | 5.0 | 4 | | |
| M6 | - | 0.6 | 3 | 0.80 | 3 | 1.3 | 3 | - | 3.3 | 4 | 1.8 | 4 | 3.3 | 4 | | |
| M7 | - | - | | 0.095 | 2 | 0.16 | 2 | - | - | | 0.2 | 2 | 0.4 | 2 | | |
| M8 | - | - | | 0.133 | 2 | 0.22 | 2 | - | - | | 0.3 | 2 | 0.5 | 2 | | |

| Metabolite | Chronic adult | | | | Acute adult | | | |
|-----------------|---------------|---|---|---|-------------|---|---|---|
| Exposure option | A | B | C | D | A | B | C | D |

| Dimethoate Primary Crop | | | | | | | | | | | | | | | |
|-------------------------|-------|-------|---|-------|------|-------|------|-------|-------|-------|---|-------|------|-------|------|
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | |
| M1 | 0.022 | 2 | - | | 0.21 | 2 | 0.19 | 2 | 0.060 | 2 | - | | 0.37 | 3 | 0.23 |
| M2 | 0.54 | 3 | - | | 2.7 | 4 | 2.8 | 4 | 0.91 | 3 | - | | 3.9 | 4 | 3.4 |
| M3 | 7.2 | 4 | - | | 36 | 6 | 38 | 6 | 12.0 | 5 | - | | 51.6 | 6 | 45 |
| M4 | 0.036 | 2 | - | | 0.18 | 2 | 0.19 | 2 | 0.060 | 2 | - | | 0.26 | 2 | 0.23 |
| M5 | 1.0 | 3 | - | | 5.3 | 4 | 5.5 | 4 | 1.8 | 4 | - | | 7.5 | 4 | 6.6 |
| M6 | 1.0 | 3 | - | | 5.3 | 4 | 5.5 | 4 | 1.8 | 4 | - | | 7.5 | 4 | 6.6 |
| M7 | 0.036 | 2 | - | | 0.18 | 2 | 0.19 | 2 | 0.060 | 2 | - | | 0.50 | 3 | 0.23 |
| M8 | 0.036 | 2 | - | | 0.18 | 2 | 0.19 | 2 | 0.060 | 2 | - | | 0.50 | 3 | 0.23 |
| M9 | 0.036 | 2 | - | | 0.18 | 2 | 0.19 | 2 | 0.060 | 2 | - | | 0.26 | 2 | 0.23 |

Table 5: First step comparison: Rotational crop metabolites/critical consumer intakes

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|---------------------------------|------------------|-------|-------|-------|------|-------|------|-------|----------------|-------|------|-------|------|-------|------|-------|
| Exposure option | A | | B | | C | | D | | A | | B | | C | | D | |
| azoxystrobin Rotational Crop | | | | | | | | | | | | | | | | |
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| M03 | | | | | 0.98 | 3 | 0.98 | 3 | | | | | 2.0 | 4 | 2.0 | 4 |
| MN1(/MN2) | | | | | 1.1 | 3 | 1.1 | 3 | | | | | 2.6 | 4 | 2.6 | 4 |
| MN2 (/MN1) | | | | | 1.1 | 3 | 1.1 | 3 | | | | | 2.6 | 4 | 2.6 | 4 |
| MO1(/MO2/MO3) | 0.003 | 2 | 0.003 | 2 | 0.39 | 3 | 0.39 | 3 | 0.05 | 2 | 0.05 | 2 | 0.92 | 3 | 0.92 | 3 |
| MO2(/MO1/MO3) | 0.003 | 2 | 0.003 | 2 | 0.39 | 3 | 0.39 | 3 | 0.05 | 2 | 0.05 | 2 | 0.92 | 3 | 0.92 | 3 |
| MO3(/MO1/MO2) | 0.003 | 2 | 0.003 | 2 | 0.39 | 3 | 0.39 | 3 | 0.05 | 2 | 0.05 | 2 | 0.92 | 3 | 0.92 | 3 |
| M42* | 0.011 | 2 | 0.011 | 2 | 3.7 | 4 | 3.7 | 4 | 0.23 | 2 | 0.23 | 2 | 8.8 | 4 | 8.8 | 4 |
| MC | | | | | 1.1 | 3 | 1.1 | 3 | | | | | 2.6 | 4 | 2.6 | 4 |
| G 02 | | | | | 2.4 | 4 | 2.4 | 4 | | | | | 5.7 | 4 | 5.7 | 4 |
| MK2 | | | | | 0.43 | 3 | 0.43 | 3 | | | | | 1.0 | 3 | 1.0 | 3 |

*M42 was also a primary crop metabolite but was included here as it was a main rotational crop metabolite (other rotational crop metabolites also in the primary crop are not covered here)

Table 6: First step comparison: Rotational crop metabolites/adult intakes

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|-----------------|---------------|-------|-------|-------|------|-------|------|-------|-------------|-------|------|-------|------|-------|------|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| azoxystrobin | | | | | | | | | | | | | | | | |
| Rotational Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M03 | | | | | 0.29 | 2 | 0.29 | 2 | | | | | 0.31 | 3 | 0.31 | 3 |
| MN1(/MN2) | | | | | 0.33 | 3 | 0.33 | 3 | | | | | 0.41 | 3 | 0.41 | 3 |
| MN2 (/MN1) | | | | | 0.33 | 3 | 0.33 | 3 | | | | | 0.41 | 3 | 0.41 | 3 |
| MO1(/MO2/MO3) | 0.002 | 1 | 0.002 | 1 | 0.12 | 2 | 0.12 | 2 | 0.03 | 2 | 0.03 | 2 | 0.14 | 2 | 0.14 | 2 |
| MO2(/MO1/MO3) | 0.002 | 1 | 0.002 | 1 | 0.12 | 2 | 0.12 | 2 | 0.03 | 2 | 0.03 | 2 | 0.14 | 2 | 0.14 | 2 |
| MO3(/MO1/MO2) | 0.002 | 1 | 0.002 | 1 | 0.12 | 2 | 0.12 | 2 | 0.03 | 2 | 0.03 | 2 | 0.14 | 2 | 0.14 | 2 |
| M42 | 0.008 | 2 | 0.008 | 2 | 1.1 | 3 | 1.1 | 3 | 0.13 | 2 | 0.13 | 2 | 1.4 | 3 | 1.4 | 3 |
| MC | | | | | 0.33 | 3 | 0.33 | 3 | | | | | 0.41 | 3 | 0.41 | 3 |
| G 02 | | | | | 0.72 | 3 | 0.72 | 3 | | | | | 0.89 | 3 | 0.89 | 3 |
| MK2 | | | | | 0.13 | 2 | 0.13 | 2 | | | | | 0.16 | 2 | 0.16 | 2 |

*M42 was also a primary crop metabolite but was included here as it was a main rotational crop metabolite (other rotational crop metabolites also in the primary crop are not covered here)

Table 7: First step comparison: Animal product metabolites/critical consumer intakes

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|--------------------|------------------|-------|-------|-------|---|-------|------|-------|----------------|-------|-------|-------|---|-------|------|-------|
| Exposure option | A | | B | | C | | D | | A | | B | | C | | D | |
| Boscalid Livestock | | | | | | | | | | | | | | | | |
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| F1 | | | 5.8 | 4 | | | 8.4 | 4 | | | 11.2 | 5 | | | 16.1 | 5 |
| F2 | | | 5.3 | 4 | | | 5.1 | 6 | | | 17.4 | 5 | | | 94.4 | 6 |
| F54 | | | 0.008 | 2 | | | 0.12 | 2 | | | 0.022 | 2 | | | 0.37 | 3 |
| F49 | | | 2.6 | 4 | | | 5.2 | 4 | | | 5.0 | 4 | | | 9.9 | 5 |
| F51 | | | 4.2 | 4 | | | 4.2 | 4 | | | 8.2 | 4 | | | 8.7 | 4 |
| F52 | | | 0.49 | 3 | | | 16.7 | 5 | | | 6.8 | 4 | | | 29.8 | 5 |
| F53 | | | 3.1 | 4 | | | 3.1 | 4 | | | 6.3 | 4 | | | 6.2 | 4 |

Table 8: First step comparison: Animal product metabolites/adult consumer intakes

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|--------------------|---------------|-------|-------|-------|---|-------|------|-------|-------------|-------|-------|-------|---|-------|------|-------|
| Exposure option | A | | B | | C | | D | | A | | B | | C | | D | |
| Boscalid Livestock | | | | | | | | | | | | | | | | |
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| F1 | | | 0.56 | 3 | | | 0.91 | 3 | | | 1.1 | 3 | | | 1.7 | 4 |
| F2 | | | 0.68 | 3 | | | 5.6 | 4 | | | 7.9 | 4 | | | 9.8 | 5 |
| F54 | | | 0.002 | 1 | | | 0.02 | 2 | | | 0.005 | 2 | | | 0.04 | 2 |
| F49 | | | 0.23 | 2 | | | 0.57 | 3 | | | 0.52 | 3 | | | 1.0 | 3 |
| F51 | | | 0.37 | 3 | | | 0.46 | 3 | | | 0.85 | 3 | | | 0.91 | 3 |
| F52 | | | 0.10 | 2 | | | 1.8 | 4 | | | 2.3 | 4 | | | 3.1 | 4 |
| F53 | | | 0.27 | 2 | | | 0.35 | 3 | | | 0.66 | 3 | | | 0.65 | 3 |

Table 9: Second step comparison: Primary crop metabolites/critical consumer intakes

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|------------------------------|------------------|-------|-------|-------|------|-------|------|-------|----------------|-------|------|-------|------|-------|-----|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| azoxystrobin Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M02 | 0.74 | C3 | 0.74 | C3 | 0.77 | C3 | 18.4 | C4 | 2.0 | A3 | 2.0 | A3 | 19.5 | A4 | 233 | A5 |
| M09 | 0.21 | C2 | 0.21 | C2 | 0.42 | C3 | 10.1 | C4 | 4.9 | A3 | 4.9 | A3 | 10.7 | A4 | 128 | A5 |
| M13 | 0.57 | C3 | 0.57 | C3 | 0.61 | C3 | 14.7 | C4 | 15.6 | A4 | 15.6 | A4 | 15.6 | A4 | 187 | A5 |
| M19 | 0.08 | C2 | 0.08 | C2 | 0.11 | C2 | 2.8 | C4 | 2.0 | A3 | 2.0 | A3 | 2.9 | A3 | 35 | A5 |
| M23 | 0.011 | C2 | 0.011 | C2 | 0.15 | C2 | 3.7 | C4 | 0.2 | A2 | 0.2 | A2 | 3.9 | A3 | 47 | A5 |
| M24 | 0.405 | C3 | 0.405 | C3 | 0.42 | C3 | 10.1 | C4 | 10.7 | A4 | 10.7 | A4 | 10.7 | A4 | 128 | A5 |
| M28 | 0.58 | C3 | 0.58 | C3 | 0.69 | C3 | 16.5 | C4 | 14.7 | A4 | 14.7 | A4 | 17.6 | A4 | 210 | A5 |
| M30 | - | | - | | 0.23 | C2 | 5.5 | C4 | - | | - | | 5.9 | A4 | 70 | A5 |
| M35 | 0.27 | C2 | 0.27 | C2 | 0.34 | C3 | 8.3 | C4 | 6.8 | A4 | 6.8 | A4 | 8.8 | A4 | 105 | A5 |
| M40 | - | | - | | 0.46 | C3 | 11.0 | C4 | - | | - | | 11.7 | A4 | 140 | A5 |
| M42 | - | | - | | 0.57 | C3 | 13.8 | C4 | - | | - | | 14.7 | A4 | 175 | A5 |
| MU5 | 0.076 | C2 | 0.076 | C2 | 0.08 | C2 | 1.8 | C4 | 2.0 | A3 | 2.0 | A3 | 2.0 | A3 | 23 | A4 |
| MU6 | 0.008 | C2 | 0.008 | C2 | 0.11 | C2 | 2.8 | C4 | 0.15 | A2 | 0.15 | A2 | 2.9 | A3 | 35 | A5 |
| MU13 | 0.008 | C2 | 0.008 | C2 | 0.11 | C2 | 2.8 | C4 | 0.15 | A2 | 0.15 | A2 | 2.9 | A3 | 35 | A5 |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|------------------------------------|------------------|-------|---|-------|------|-------|---|-------|----------------|-------|---|-------|------|-------|---|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Bitertanol Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| BM1 Bitertanol ketone (BUE 1662) | 0.125 | C2 | | | 0.21 | C2 | | | 1.33 | A3 | | | 4.6 | A3 | | |
| BM2 4-hydroxybiphenyl | 0.125 | C2 | | | 0.21 | C2 | | | 1.33 | A3 | | | 4.6 | A3 | | |
| BM3 Triazolyl acetic acid | 0.54 | C3 | | | 1.5 | C4 | | | 0.88 | A3 | | | 5.98 | A4 | | |
| BM4 Triazolyl alanine | 1.24 | C3 | | | 3.4 | C4 | | | 2.02 | A3 | | | 13.7 | A4 | | |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|------------------------------|------------------|-------|-------|-------|-------|-------|-------|-------|----------------|-------|-------|-------|-------|-------|------|-------|
| Exposure option | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Boscalid Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 chloronicotinic acid | 0.038 | C2 | 0.038 | C2 | 0.420 | C3 | 1.093 | C3 | 0.175 | A2 | 0.175 | A2 | 4.453 | A3 | 20.7 | A4 |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|-----------------------------|------------------|-------|---|-------|------|-------|--|-------|----------------|-------|---|-------|------|-------|--|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| Napropamide Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 | 0.28 | C2 | | | 0.75 | C3 | | | 3.185 | A3 | | | 4.29 | A3 | | |
| M2 | 0.013 | C2 | | | 0.05 | C2 | | | 0.24 | A2 | | | 0.29 | A2 | | |
| M3 | 0.003 | C2 | | | 0.02 | C2 | | | 0.086 | A2 | | | 0.12 | A2 | | |
| M4 | 0.15 | C2 | | | 0.41 | C3 | | | 1.72 | A3 | | | 2.32 | A3 | | |
| M5 | 0.34 | C3 | | | 1.15 | C3 | | | 5.45 | A4 | | | 6.55 | A4 | | |
| M6 | 0.040 | C2 | | | 0.15 | C2 | | | 0.72 | A3 | | | 0.87 | A3 | | |
| M7 | 0.42 | C3 | | | 1.56 | C4 | | | 7.4 | A4 | | | 8.87 | A4 | | |
| M8 | 0.092 | C2 | | | 0.55 | C3 | | | 2.3 | A3 | | | 3.13 | A3 | | |
| M9 | 0.155 | C2 | | | 0.39 | C3 | | | 1.64 | A3 | | | 2.20 | A3 | | |
| M10 | 0.31 | C3 | | | 1.07 | C3 | | | 5.1 | A4 | | | 6.09 | A4 | | |
| M11 | 0.079 | C2 | | | 0.31 | C3 | | | 1.44 | A3 | | | 1.74 | A3 | | |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|---|------------------|-------|-----|-------|------|-------|------|-------|----------------|-------|------|-------|-----|-------|------|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| Prohexadione calcium Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 | - | | 2.2 | C4 | 1.4 | C3 | 3.5 | C4 | - | | 15.3 | A4 | 3.0 | A3 | 15.3 | A4 |
| M2 | - | | 3.7 | C4 | 2.3 | C4 | 5.9 | C4 | - | | 25.5 | A4 | 5.1 | A4 | 25.5 | A4 |
| M3 | - | | 2.1 | C4 | 1.4 | C3 | 3.4 | C4 | - | | 14.8 | A4 | 2.9 | A3 | 14.8 | A4 |
| M4 | - | | 1.0 | C3 | 0.64 | C3 | 1.7 | C4 | - | | 7.1 | A4 | 1.4 | A3 | 7.1 | A4 |
| M5 | - | | 4.7 | C4 | 2.9 | C4 | 7.6 | C4 | - | | 32.6 | A5 | 6.5 | A4 | 32.6 | A5 |
| M6 | - | | 3.1 | C4 | 1.9 | C4 | 5.0 | C4 | - | | 21.4 | A4 | 4.2 | A3 | 21.4 | A4 |
| M7 | - | | - | | 0.23 | C2 | 0.59 | C3 | - | | - | | 0.5 | A3 | 2.5 | A3 |
| M8 | - | | - | | 0.32 | C3 | 0.83 | C3 | - | | - | | 0.7 | A3 | 3.6 | A3 |

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|----------------------------|------------------|-------|---|-------|------|-------|------|-------|----------------|-------|---|-------|------|-------|------|-------|
| Exposure option | A | B | | C | | D | | A | B | | C | | D | | | |
| Dimethoate Primary Crop | | | | | | | | | | | | | | | | |
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| M1 | 0.053 | C2 | - | | 0.72 | C3 | 0.74 | C3 | 0.14 | A2 | - | | 1.5 | A3 | 1.33 | A3 |
| M2 | 1.3 | C3 | - | | 9.7 | C4 | 11.1 | C4 | 2.2 | A3 | - | | 11.7 | A4 | 19.9 | A4 |
| M3 | 17.7 | C4 | - | | 129 | C5 | 147 | C5 | 28.8 | A4 | - | | 155 | A5 | 264 | A5 |
| M4 | 0.089 | C2 | - | | 0.65 | C3 | 0.74 | C3 | 0.14 | A2 | - | | 0.78 | A3 | 1.33 | A3 |
| M5 | 2.6 | C4 | - | | 18.8 | C4 | 21.5 | C4 | 4.2 | A3 | - | | 22.6 | A4 | 38.5 | A5 |
| M6 | 2.6 | C4 | - | | 18.8 | C4 | 21.5 | C4 | 4.2 | A3 | - | | 22.6 | A4 | 38.5 | A5 |
| M7 | 0.089 | C2 | - | | 0.65 | C3 | 0.74 | C3 | 0.14 | A2 | - | | 0.91 | A3 | 1.33 | A3 |
| M8 | 0.089 | C2 | - | | 0.65 | C3 | 0.74 | C3 | 0.14 | A2 | - | | 0.91 | A3 | 1.33 | A3 |
| M9 | 0.089 | C2 | - | | 0.65 | C3 | 0.74 | C3 | 0.14 | A2 | - | | 0.78 | A3 | 1.33 | A3 |

Table 10: Second step comparison: Primary crop metabolites/adult intakes

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|------------------------------|---------------|-------|-------|-------|------|-------|------|-------|-------------|-------|------|-------|-----|-------|----|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| azoxystrobin Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M02 | 0.21 | C2 | 0.21 | C2 | 0.22 | C2 | 5.1 | C4 | 0.63 | A3 | 0.63 | A3 | 6.3 | A4 | 40 | A5 |
| M09 | 0.062 | C2 | 0.062 | C2 | 0.12 | C2 | 2.8 | C4 | 1.6 | A3 | 1.6 | A3 | 3.5 | A3 | 22 | A4 |
| M13 | 0.16 | C2 | 0.16 | C2 | 0.18 | C2 | 4.1 | C4 | 5.1 | A4 | 5.1 | A4 | 5.1 | A4 | 32 | A5 |
| M19 | 0.023 | C2 | 0.023 | C2 | 0.03 | C2 | 0.77 | C3 | 0.6 | A3 | 0.6 | A3 | 0.9 | A3 | 6 | A4 |
| M23 | 0.004 | C2 | 0.004 | C2 | 0.04 | C2 | 1.02 | C3 | 0.08 | A2 | 0.08 | A2 | 1.3 | A3 | 8 | A4 |
| M24 | 0.12 | C2 | 0.12 | C2 | 0.12 | C2 | 2.8 | C4 | 3.5 | A3 | 3.5 | A3 | 3.5 | A3 | 22 | A4 |
| M28 | 0.17 | C2 | 0.17 | C2 | 0.20 | C2 | 4.6 | C4 | 4.7 | A3 | 4.7 | A3 | 5.7 | A4 | 36 | A5 |
| M30 | - | | - | | 0.07 | C2 | 1.5 | C4 | - | | - | | 1.9 | A3 | 12 | A4 |
| M35 | 0.08 | C2 | 0.08 | C2 | 0.10 | C2 | 2.3 | C4 | 2.1 | A3 | 2.1 | A3 | 2.8 | A3 | 18 | A4 |
| M40 | - | | - | | 0.13 | C2 | 3.1 | C4 | - | | - | | 3.8 | A3 | 24 | A4 |
| M42 | - | | - | | 0.17 | C2 | 3.9 | C4 | - | | - | | 4.7 | A3 | 30 | A5 |
| MU5 | 0.02 | C2 | 0.02 | C2 | 0.02 | C2 | 0.51 | C3 | 0.6 | A3 | 0.6 | A3 | 0.6 | A3 | 4 | A3 |
| MU6 | 0.003 | C2 | 0.003 | C2 | 0.03 | C2 | 0.77 | C3 | 0.06 | A2 | 0.06 | A2 | 0.9 | A3 | 6 | A4 |
| MU13 | 0.003 | C2 | 0.003 | C2 | 0.03 | C2 | 0.77 | C3 | 0.06 | A2 | 0.06 | A2 | 0.9 | A3 | 6 | A4 |

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|------------------------------------|---------------|-------|---|-------|-------|-------|--|-------|-------------|-------|---|-------|------|-------|--|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| Bitertanol Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| BM1 Bitertanol ketone (BUE 1662) | 0.0225 | C2 | | | 0.067 | C2 | | | 0.2 | A2 | | | 1.0 | A3 | | |
| BM2 4-hydroxybiphenyl | 0.0225 | C2 | | | 0.067 | C2 | | | 0.2 | A2 | | | 1.0 | A3 | | |
| BM3 Triazolyl acetic acid | 0.22 | C2 | | | 0.42 | C3 | | | 0.37 | A3 | | | 0.91 | A3 | | |
| BM4 Triazolyl alanine | 0.51 | C3 | | | 0.96 | C3 | | | 0.85 | A3 | | | 2.1 | A3 | | |

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|------------------------------|---------------|-------|--------|-------|-------|-------|-------|-------|-------------|-------|-------|-------|-------|-------|-------|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| Boscalid Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 chloronicotinic acid | 0.0105 | C2 | 0.0105 | C2 | 0.118 | C2 | 1.023 | C3 | 0.082 | A2 | 0.082 | A2 | 3.057 | A3 | 12.02 | A4 |

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|-------------------------------------|---------------|-------|---|-------|----|-------|--|-------|-------------|-------|---|-------|----|-------|--|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| Napropamide Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 | 0.12 | C2 | | 0.36 | C3 | | | 0.90 | A3 | | | 1.11 | A3 | | | |
| M2 | 0.007 | C2 | | 0.02 | C2 | | | 0.052 | A2 | | | 0.08 | A2 | | | |
| M3 | 0.0011 | C1 | | 0.01 | C2 | | | 0.024 | A2 | | | 0.03 | A2 | | | |
| M4 | 0.069 | C2 | | 0.19 | C2 | | | 0.485 | A3 | | | 0.60 | A3 | | | |
| M5 | 0.17 | C2 | | 0.54 | C3 | | | 1.18 | A3 | | | 1.70 | A3 | | | |
| M6 | 0.021 | C2 | | 0.07 | C2 | | | 0.16 | A2 | | | 0.23 | A2 | | | |
| M7 | 0.22 | C2 | | 0.73 | C3 | | | 1.6 | A3 | | | 2.30 | A3 | | | |
| M8 | 0.029 | C2 | | 0.26 | C2 | | | 0.66 | A3 | | | 0.81 | A3 | | | |
| M9 | 0.070 | C2 | | 0.18 | C2 | | | 0.46 | A3 | | | 0.57 | A3 | | | |
| M10 | 0.16 | C2 | | 0.50 | C3 | | | 1.10 | A3 | | | 1.58 | A3 | | | |
| M11 | 0.041 | C2 | | 0.14 | C2 | | | 0.31 | A3 | | | 0.45 | A3 | | | |

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|--|---------------|-------|----|-------|----|-------|----|-------|-------------|-------|-----|-------|-----|-------|--|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| Prohexadione calcium Primary Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M1 | - | 0.4 | C3 | 0.57 | C3 | 0.96 | C3 | - | 2.3 | A3 | 1.3 | A3 | 2.3 | A3 | | |
| M2 | - | 0.65 | C3 | 0.95 | C3 | 1.6 | C4 | - | 3.9 | A3 | 2.1 | A3 | 3.9 | A3 | | |
| M3 | - | 0.4 | C3 | 0.55 | C3 | 0.93 | C3 | - | 2.3 | A3 | 1.2 | A3 | 2.3 | A3 | | |
| M4 | - | 0.2 | C2 | 0.27 | C2 | 0.45 | C3 | - | 1.1 | A3 | 0.6 | A3 | 1.1 | A3 | | |
| M5 | - | 0.8 | C3 | 1.21 | C3 | 2.05 | C4 | - | 5.0 | A3 | 2.7 | A3 | 5.0 | A3 | | |
| M6 | - | 0.6 | C3 | 0.80 | C3 | 1.3 | C3 | - | 3.3 | A3 | 1.8 | A3 | 3.3 | A3 | | |
| M7 | - | - | | 0.095 | C2 | 0.16 | C2 | - | - | | 0.2 | A2 | 0.4 | A2 | | |
| M8 | - | - | | 0.133 | C2 | 0.22 | C2 | - | - | | 0.3 | A2 | 0.5 | A2 | | |

| Metabolite | Chronic adult | | | | Acute adult | | | |
|-----------------|---------------|---|---|---|-------------|---|---|---|
| Exposure option | A | B | C | D | A | B | C | D |

| Dimethoate Primary Crop | | | | | | | | | | | | | | | | |
|----------------------------|-------|-------|---|-------|------|-------|------|-------|-------|-------|---|-------|------|-------|------|-------|
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| M1 | 0.022 | C2 | - | | 0.21 | C2 | 0.19 | C2 | 0.060 | A2 | - | | 0.37 | A3 | 0.23 | A2 |
| M2 | 0.54 | C3 | - | | 2.7 | C4 | 2.8 | C4 | 0.91 | A3 | - | | 3.9 | A3 | 3.4 | A3 |
| M3 | 7.2 | C4 | - | | 36 | C5 | 38 | C5 | 12.0 | A4 | - | | 51.6 | A5 | 45 | A5 |
| M4 | 0.036 | C2 | - | | 0.18 | C2 | 0.19 | C2 | 0.060 | A2 | - | | 0.26 | A3 | 0.23 | A2 |
| M5 | 1.0 | C3 | - | | 5.3 | C4 | 5.5 | C4 | 1.8 | A3 | - | | 7.5 | A4 | 6.6 | A4 |
| M6 | 1.0 | C3 | - | | 5.3 | C4 | 5.5 | C4 | 1.8 | A3 | - | | 7.5 | A4 | 6.6 | A4 |
| M7 | 0.036 | C2 | - | | 0.18 | C2 | 0.19 | C2 | 0.060 | A2 | - | | 0.50 | A3 | 0.23 | A2 |
| M8 | 0.036 | C2 | - | | 0.18 | C2 | 0.19 | C2 | 0.060 | A2 | - | | 0.50 | A3 | 0.23 | A2 |
| M9 | 0.036 | C2 | - | | 0.18 | C2 | 0.19 | C2 | 0.060 | A2 | - | | 0.26 | A2 | 0.23 | A2 |

Table 11: Second step comparison: Rotational crop metabolites/critical consumer intakes

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|---------------------------------|------------------|-------|-------|-------|------|-------|------|-------|----------------|-------|------|-------|------|-------|------|-------|
| Exposure option | A | | B | | C | | D | | A | | B | | C | | D | |
| azoxystrobin Rotational Crop | | | | | | | | | | | | | | | | |
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| M03 | | | | | 0.98 | C3 | 0.98 | C3 | | | | | 2.0 | A3 | 2.0 | A3 |
| MN1(/MN2) | | | | | 1.1 | C3 | 1.1 | C3 | | | | | 2.6 | A3 | 2.6 | A3 |
| MN2 (/MN1) | | | | | 1.1 | C3 | 1.1 | C3 | | | | | 2.6 | A3 | 2.6 | A3 |
| MO1(/MO2/MO3) | 0.003 | C2 | 0.003 | C2 | 0.39 | C3 | 0.39 | C3 | 0.05 | A2 | 0.05 | A2 | 0.92 | A3 | 0.92 | A3 |
| MO2(/MO1/MO3) | 0.003 | C2 | 0.003 | C2 | 0.39 | C3 | 0.39 | C3 | 0.05 | A2 | 0.05 | A2 | 0.92 | A3 | 0.92 | A3 |
| MO3(/MO1/MO2) | 0.003 | C2 | 0.003 | C2 | 0.39 | C3 | 0.39 | C3 | 0.05 | A2 | 0.05 | A2 | 0.92 | A3 | 0.92 | A3 |
| M42* | 0.011 | C2 | 0.011 | C2 | 3.7 | C4 | 3.7 | C4 | 0.23 | A2 | 0.23 | A2 | 8.8 | A4 | 8.8 | A4 |
| MC | | | | | 1.1 | C3 | 1.1 | C3 | | | | | 2.6 | A3 | 2.6 | A3 |
| G 02 | | | | | 2.4 | C4 | 2.4 | C4 | | | | | 5.7 | A4 | 5.7 | A4 |
| MK2 | | | | | 0.43 | C3 | 0.43 | C3 | | | | | 1.0 | A3 | 1.0 | A3 |

*M42 was also a primary crop metabolite but was included here as it was a main rotational crop metabolite (other rotational crop metabolites also in the primary crop are not covered here)

Table 12: Second step comparison: Rotational crop metabolites/adult intakes

| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|-----------------|---------------|-------|-------|-------|------|-------|------|-------|-------------|-------|------|-------|------|-------|------|-------|
| Exposure option | A | B | C | D | | | | | A | B | C | D | | | | |
| azoxystrobin | | | | | | | | | | | | | | | | |
| Rotational Crop | | | | | | | | | | | | | | | | |
| | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' | | 'Int' |
| M03 | | | | | 0.29 | C2 | 0.29 | C2 | | | | | 0.31 | A3 | 0.31 | A3 |
| MN1(/MN2) | | | | | 0.33 | C3 | 0.33 | C3 | | | | | 0.41 | A3 | 0.41 | A3 |
| MN2(/MN1) | | | | | 0.33 | C3 | 0.33 | C3 | | | | | 0.41 | A3 | 0.41 | A3 |
| MO1(/MO2/MO3) | 0.002 | C1 | 0.002 | C1 | 0.12 | C2 | 0.12 | C2 | 0.03 | A2 | 0.03 | A2 | 0.14 | A2 | 0.14 | A2 |
| MO2(/MO1/MO3) | 0.002 | C1 | 0.002 | C1 | 0.12 | C2 | 0.12 | C2 | 0.03 | A2 | 0.03 | A2 | 0.14 | A2 | 0.14 | A2 |
| MO3(/MO1/MO2) | 0.002 | C1 | 0.002 | C1 | 0.12 | C2 | 0.12 | C2 | 0.03 | A2 | 0.03 | A2 | 0.14 | A2 | 0.14 | A2 |
| M42 | 0.008 | C2 | 0.008 | C2 | 1.1 | C3 | 1.1 | C3 | 0.13 | A2 | 0.13 | A2 | 1.4 | A3 | 1.4 | A3 |
| MC | | | | | 0.33 | C3 | 0.33 | C3 | | | | | 0.41 | A3 | 0.41 | A3 |
| G 02 | | | | | 0.72 | C3 | 0.72 | C3 | | | | | 0.89 | A3 | 0.89 | A3 |
| MK2 | | | | | 0.13 | C2 | 0.13 | C2 | | | | | 0.16 | A2 | 0.16 | A2 |

*M42 was also a primary crop metabolite but was included here as it was a main rotational crop metabolite (other rotational crop metabolites also in the primary crop are not covered here)

Table 13: Second step comparison: Animal product metabolites/critical consumer intakes

| Metabolite | Chronic critical | | | | | | | | Acute critical | | | | | | | |
|--------------------|------------------|-------|-------|-------|---|-------|------|-------|----------------|-------|-------|-------|---|-------|------|-------|
| Exposure option | A | | B | | C | | D | | A | | B | | C | | D | |
| Boscalid Livestock | | | | | | | | | | | | | | | | |
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| F1 | | | 5.8 | C4 | | | 8.4 | C4 | | | 11.2 | A4 | | | 16.1 | A4 |
| F2 | | | 5.3 | C4 | | | 5.1 | C5 | | | 17.4 | A4 | | | 94.4 | A5 |
| F54 | | | 0.008 | C2 | | | 0.12 | C2 | | | 0.022 | A2 | | | 0.37 | A3 |
| F49 | | | 2.6 | C4 | | | 5.2 | C4 | | | 5.0 | A3 | | | 9.9 | A4 |
| F51 | | | 4.2 | C4 | | | 4.2 | C4 | | | 8.2 | A4 | | | 8.7 | A4 |
| F52 | | | 0.49 | C3 | | | 16.7 | C4 | | | 6.8 | A4 | | | 29.8 | A4 |
| F53 | | | 3.1 | C4 | | | 3.1 | C4 | | | 6.3 | A4 | | | 6.2 | A4 |

Table 14: Second step comparison: Animal product metabolites/adult consumer intakes

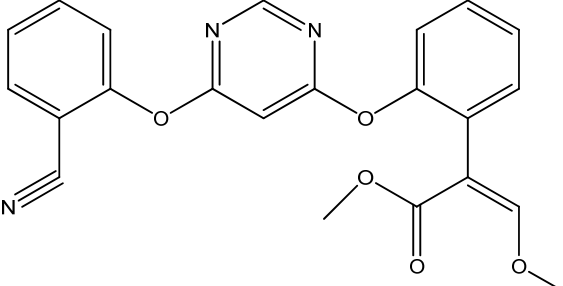
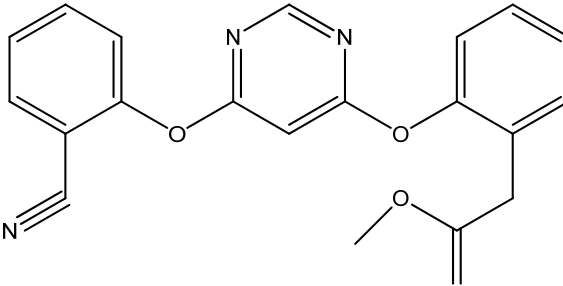
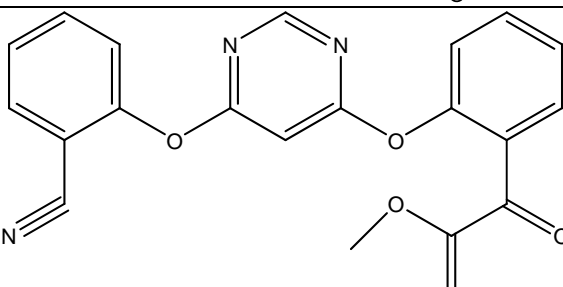
| Metabolite | Chronic adult | | | | | | | | Acute adult | | | | | | | |
|--------------------|---------------|-------|-------|-------|---|-------|------|-------|-------------|-------|-------|-------|---|-------|------|-------|
| Exposure option | A | | B | | C | | D | | A | | B | | C | | D | |
| Boscalid Livestock | | | | | | | | | | | | | | | | |
| | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ | | ‘Int’ |
| F1 | | | 0.56 | C3 | | | 0.91 | C3 | | | 1.1 | A3 | | | 1.7 | A3 |
| F2 | | | 0.68 | C3 | | | 5.6 | C4 | | | 7.9 | A4 | | | 9.8 | A4 |
| F54 | | | 0.002 | C1 | | | 0.02 | C2 | | | 0.005 | A2 | | | 0.04 | A2 |
| F49 | | | 0.23 | C2 | | | 0.57 | C3 | | | 0.52 | A3 | | | 1.0 | A3 |
| F51 | | | 0.37 | C3 | | | 0.46 | C3 | | | 0.85 | A3 | | | 0.91 | A3 |
| F52 | | | 0.10 | C2 | | | 1.8 | C4 | | | 2.3 | A4 | | | 3.1 | A3 |
| F53 | | | 0.27 | C2 | | | 0.35 | C3 | | | 0.66 | A3 | | | 0.65 | A3 |

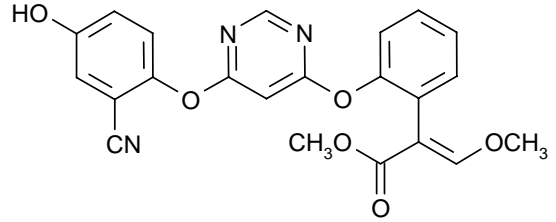
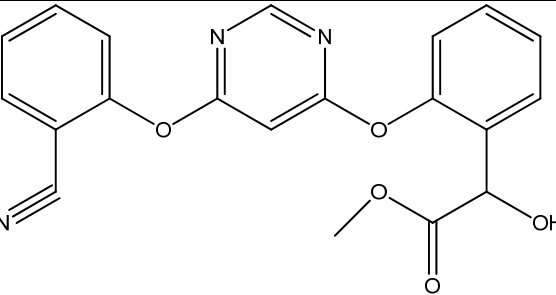
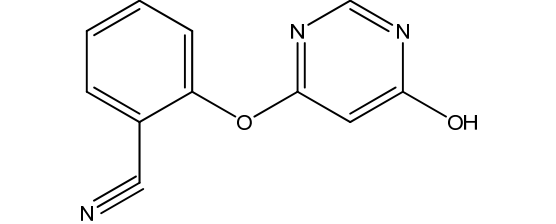
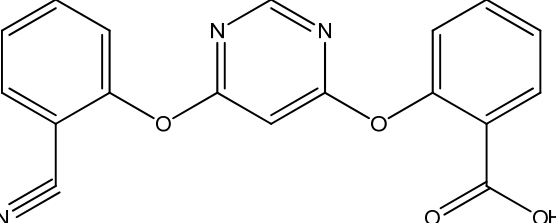
Tables 15 to 20 Metabolite structures, Toxtree assignments, and whether ‘mammalian’ metabolite (laboratory animal metabolite, usually rat) or plant/livestock specific metabolites.

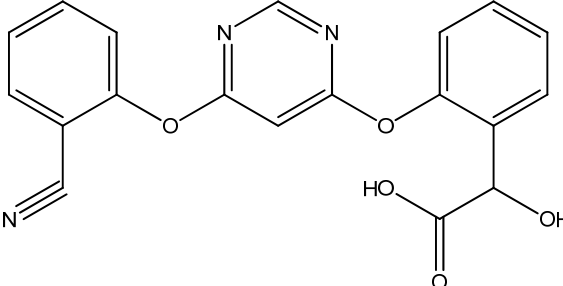
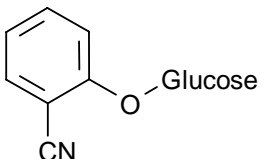
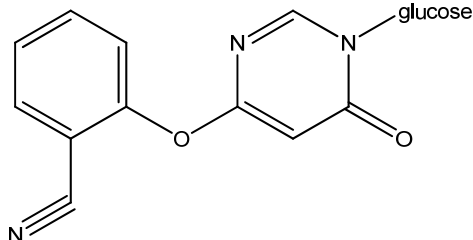
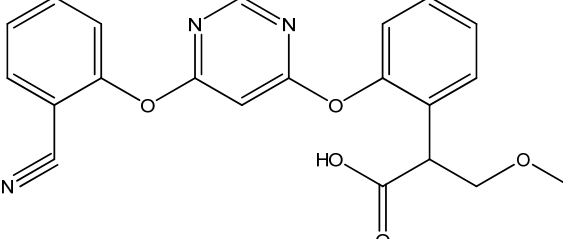
Table 15: azoxystrobin metabolites

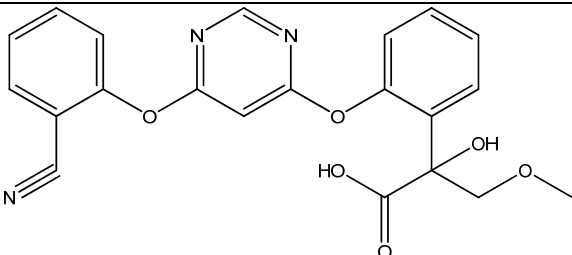
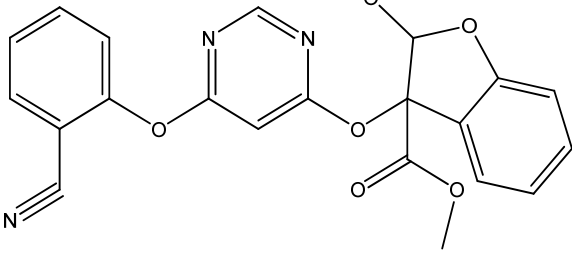
(A. primary crops)

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | ‘Mammalian’ (M) or Plant specific (P) |
|-----------------|---|-----------|--|---------------------------------------|
| | Azoxystrobin (parent pesticide) | | | |
| M2 (R234886) | (E)-2-(2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)phenyl)-3-methoxyacrylic acid | | III | M |

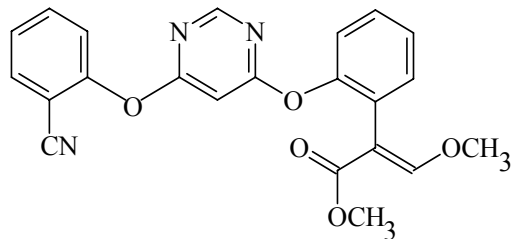
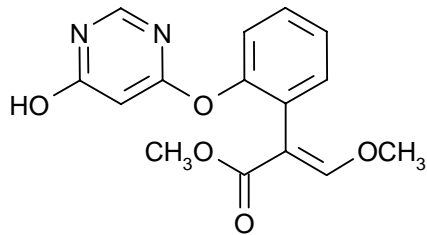
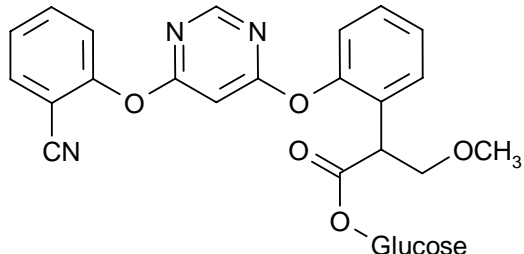
| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|--|--|---|--|---------------------------------------|
| M9 (R230310) Z isomer of azoxystrobin | (Z)-methyl 2-(2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)phenyl)-3-methoxyacrylate |  | III | M |
| M13 | methyl 2-(2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)phenyl)acetate |  | III | M |
| M19 | methyl 2-(2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)phenyl)-2-oxoacetate |  | III | P |

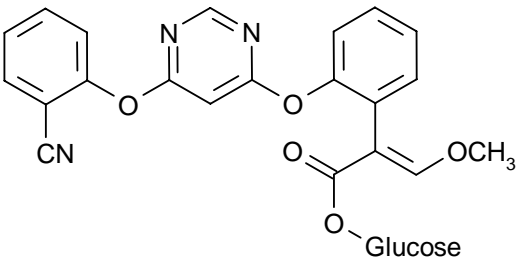
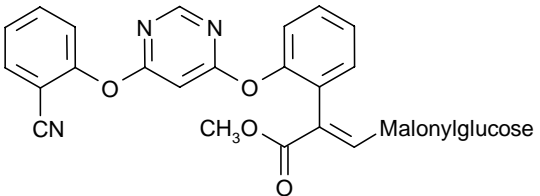
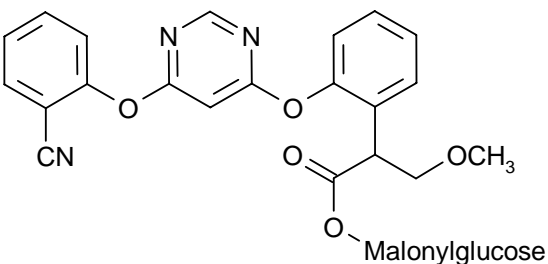
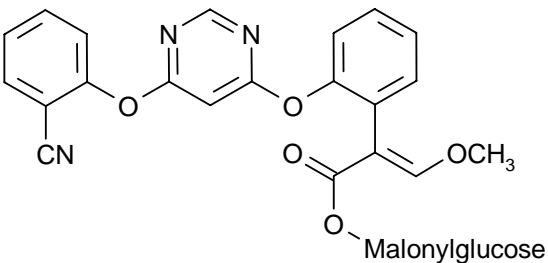
| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|---|--|--|---------------------------------------|
| M23 | Methyl (<i>E</i>)-2-{2-[6-(2-cyano-4-hydroxyphenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate |  | III | M |
| M24 | methyl 2-(2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)phenyl)-2-hydroxyacetate |  | III | P |
| M28 | 2-(6-hydroxypyrimidin-4-yloxy)benzonitrile |  | III | P |
| M30 | 2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)benzoic acid |  | III | P |

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|---|--|--|---------------------------------------|
| M35 | 2-(2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)phenyl)-2-hydroxyacetic acid |  | III | P |
| M40 | 2-glucosylbenzonitrile |  | III | P |
| M42 | 6-(2-cyanophenoxy)-3-glucosylpyrimidin-4-one |  | III | P |
| MU5 | 2-(2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)phenyl)-3-methoxypropanoic acid |  | III | P |

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|--|--|--|---------------------------------------|
| MU6 | 2-(2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)phenyl)-2-hydroxy-3-methoxypropanoic acid |  | III | P |
| MU13 | methyl 3-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)-2-methoxy-2,3-dihydrobenzofuran-3-carboxylate |  | III | P |

(B. rotational crops)

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|---|--|--|---------------------------------------|
| | Azoxystrobin (parent pesticide) |  | | |
| M03 | Methyl (E)-2-{2-[(6-hydroxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate |  | III | M |
| MN1 | glucosyl-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]-phenyl}-3-methoxypropionate |  | III | P |

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|--|--|--|---------------------------------------|
| MN2 | glucosyl2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]-phenyl}-3-methoxyacrylate |  | III | P |
| MO1 | Methyl(E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-(glucosylmalonyl)-acrylate |  | III | P |
| MO2 | glucosylmalonyl2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxypropionate |  | III | P |
| MO3 | glucosylmalonyl 2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate |  | III | P |

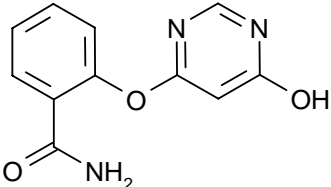
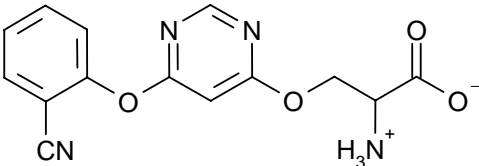
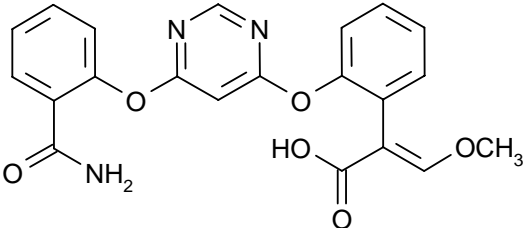
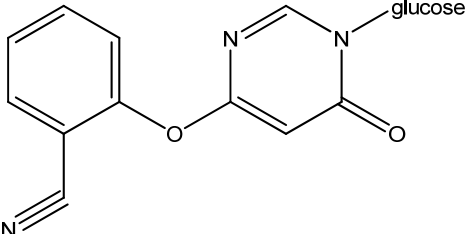
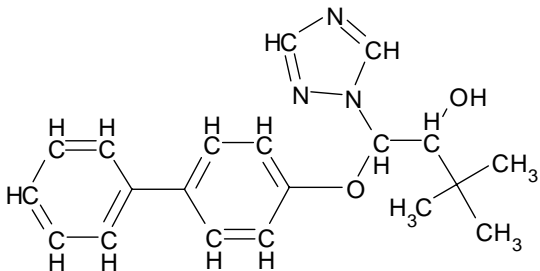
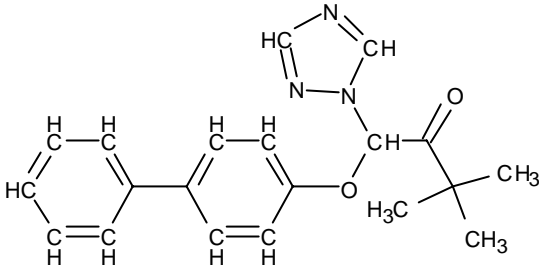
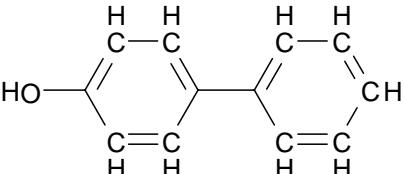
| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|--|--|--|---------------------------------------|
| MC | 2-[-(6-hydroxypyrimidin-4-yl-oxy)]benzamide |  | III | P |
| G02 | 2-ammonium-3-[6-(2-cyanophenoxy)pyrimidin-4-yl-oxy]-propionate |  | III | P |
| MK2 | (E)-2-{6-[2-(1-carboxy-2-methoxy-propyl)phenoxy]-pyrimidin-4-yl-oxy}-benzamide |  | III | P |
| M42 | 6-(2-cyanophenoxy)-3-glucosylpyrimidin-4-one |  | III | P |

Table 16: bitertanol metabolites

(A. primary crops)

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|-------------------------------|--|--|---------------------------------------|
| | Bitertanol (parent pesticide) |  | | |
| BM1 | Bitertanol ketone |  | III | P |
| BM2 | 4-hydroxybiphenyl |  | III | M |

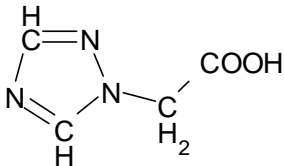
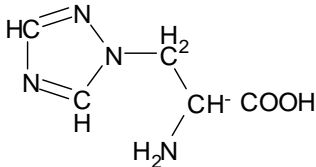
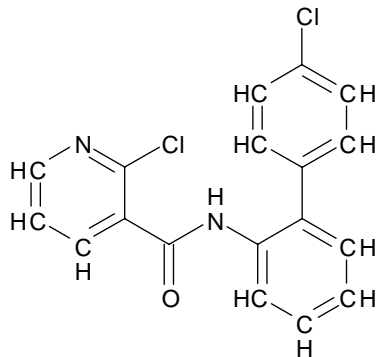
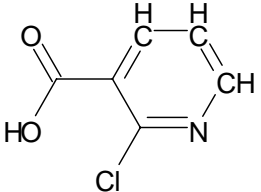
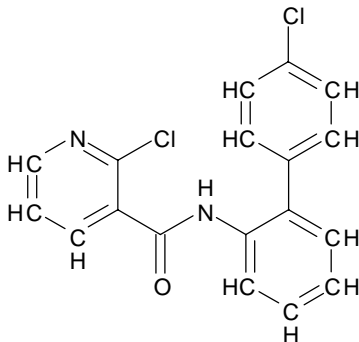
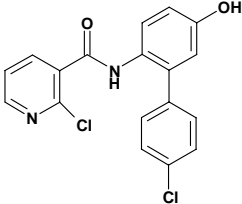
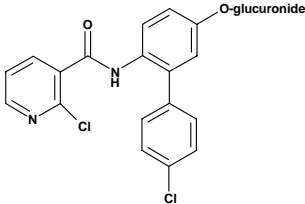
| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|-----------------------|--|--|--|
| | | | | |
| BM3 | Triazolyl acetic acid |  | III | P (no triazole label studies in the DAR for the rat) |
| BM4 | Triazolyl alanine |  | III | P (no triazole label studies in the DAR for the rat) |

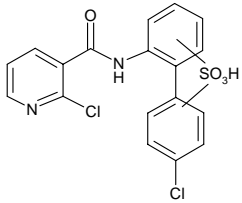
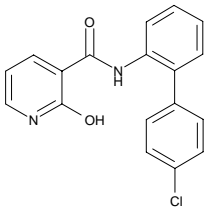
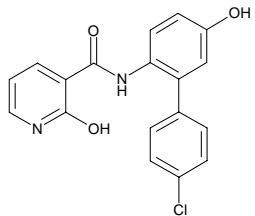
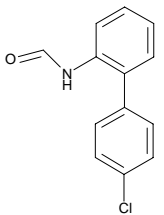
Table 17: boscalid metabolites

(A. primary crops)

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|--------------------------------------|---|--|---------------------------------------|
| | Boscalid (parent pesticide) |  | | |
| M1 | Chloronicotinic acid |  | III | M |

(B. livestock)

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Livestock specific (L) |
|------------|--|--|--|---|
| | Boscalid (parent pesticide) |  | | |
| F01 | 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)pyridine-3-carboxamide |  | III | M |
| F02 | 4'-chloro-6-[(2-chloropyridin-3-yl)carbonylamino]biphenyl-3-yl β-D-glucopyranosiduronic acid |  | III | M |

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Livestock specific (L) |
|------------|--|--|--|---|
| F54 | 4'-chloro-6-{{(2-chloropyridin-3-yl)carbonyl}amino}biphenyl-x-sulfonic acid or 4-chloro-2'-{{(2-chloropyridin-3-yl)carbonyl}amino}biphenyl-y-sulfonic acid |  | III | L |
| F49 | N-(4'-chlorobiphenyl-2-yl)-2-hydroxypyridine-3-carboxamide |  | III | L |
| F51 | N-(4'-chloro-5-hydroxybiphenyl-2-yl)-2-hydroxypyridine-3-carboxamide |  | III | L |
| F52 | N-(4'-chlorobiphenyl-2-yl)formamide |  | III | L |

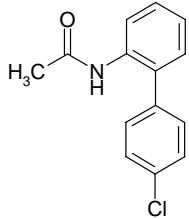
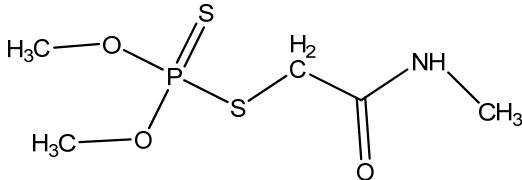
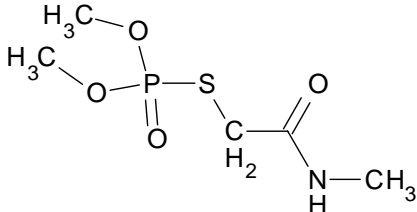
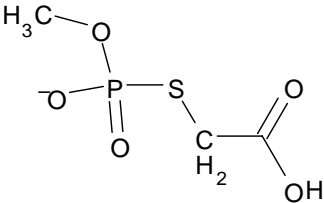
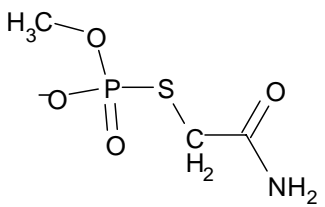
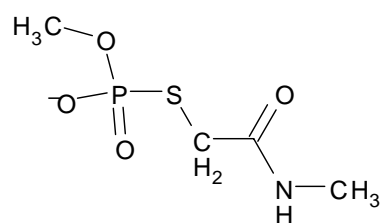
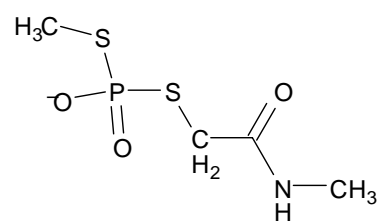
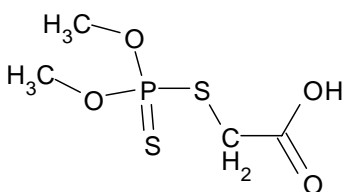
| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Livestock specific (L) |
|------------|-------------------------------------|--|--|---|
| F53 | N-(4'-chlorobiphenyl-2-yl)acetamide |  | III | L |

Table 18: dimethoate metabolites

(A. primary crops)

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|--|--|--|---------------------------------------|
| | Dimethoate (parent pesticide) |  | | |
| M1 | Omethoate |  | III | M |
| M2 | O-desmethyl carboxylic acid omethoate |  | III | P |
| M3 | O-desmethyl-N-desmethyl | | III | P |

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|----------------------------|--|--|---------------------------------------|
| | omethoate (total) |  | | |
| M4 | O-desmethyl omethoate |  | III | P |
| M5 | des-o-methyl isodimethoate |  | III | P |
| M6 | dimethoate carboxylic acid |  | III | M |

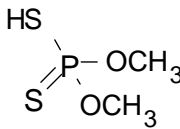
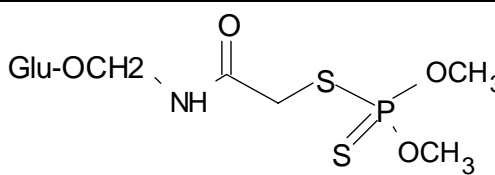
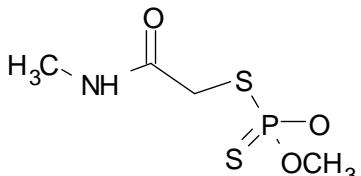
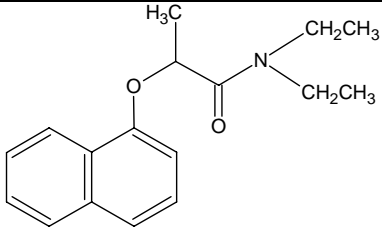
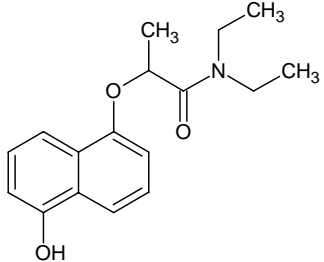
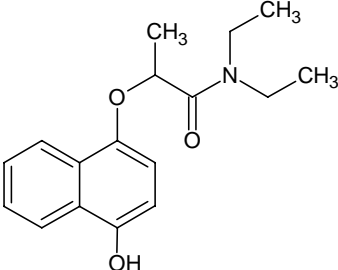
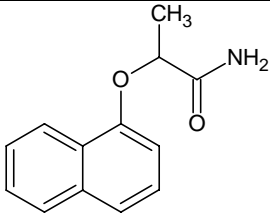
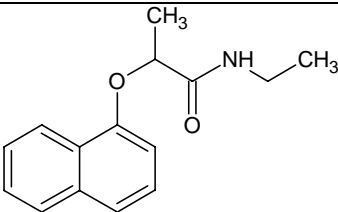
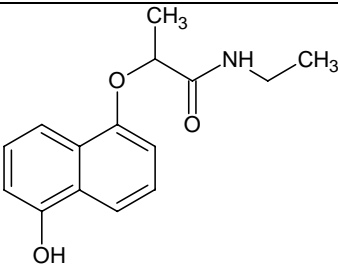
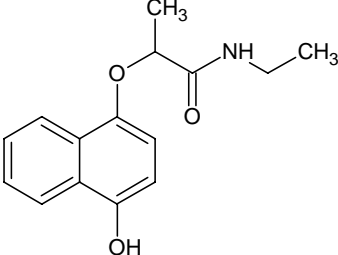
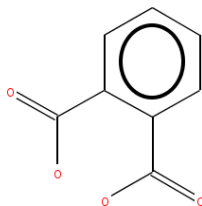
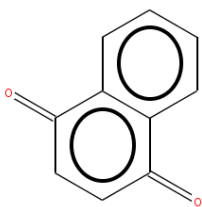
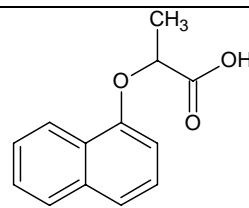
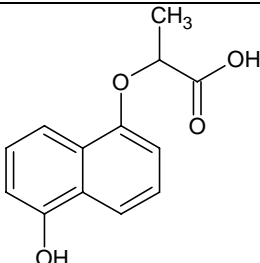
| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|--|---|--|---------------------------------------|
| | | | | |
| M7 | dimethyl dithiophosphate |  | III | M |
| M8 | glucose conjugate of hydroxydimethoate |  | III | P |
| M9 | desmethyl dimethoate |  | III | P |

Table 19: napropamide metabolites

(A. primary crops)

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|--------------------------------|--|--|---------------------------------------|
| | Napropamide (parent pesticide) |  | | |
| M1 | 5-hydroxynapropamide |  | III | M |
| M2 | 4-hydroxynapropamide |  | III | M |

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|-------------------------------|--|--|---------------------------------------|
| M3 | Naphthoxypropionamide |  | III | M |
| M4 | Desethylnapropamide |  | III | M |
| M5 | 5-hydroxy-desethylnapropamide |  | III | M |
| M6 | 4-hydroxy-desethylnapropamide |  | III | M |

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|-----------------------------------|--|--|---------------------------------------|
| M7 | O-phthalic acid |  | I | P |
| M8 | 1,4-Naphthoxyquinone |  | I | P |
| M9 | Naphthoxypropionic acid (NOPA) |  | III | M |
| M10 | 5-hydroxy-naphthoxypropionic acid |  | III | M |

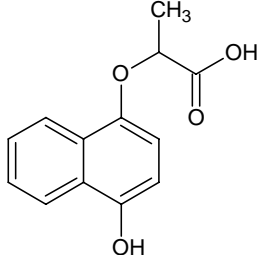
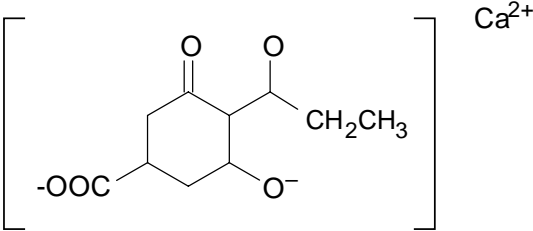
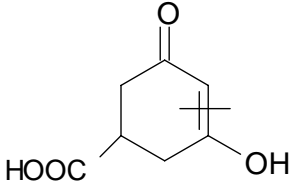
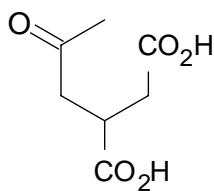
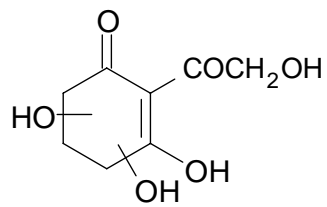
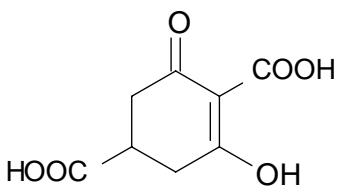
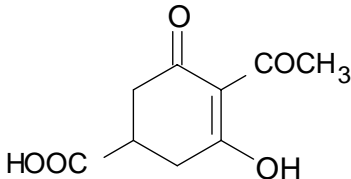
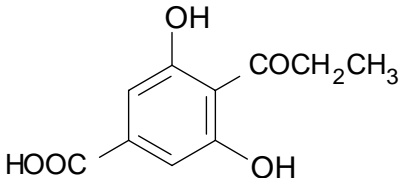
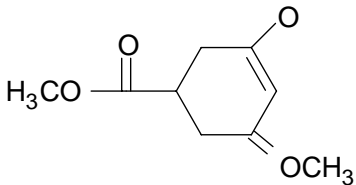
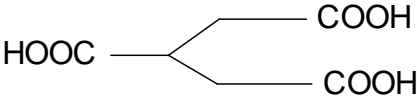
| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|------------|-----------------------------------|--|--|---------------------------------------|
| M11 | 4-hydroxy-naphthoxypropionic acid |  | III | M |

Table 20: prohexadione-calcium metabolites

(A. primary crops)

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|---------------------------|---|--|--|---------------------------------------|
| | Prohexadione calcium (parent pesticide) |  | | |
| Despropionyl (M1) KI 5376 | Despropionyl-prohexadione |  | II | M |

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|---|---|--|--|---------------------------------------|
| BX112-M10 (M2) | 2-(2-oxopropyl)butanedioic acid |  | I | P |
| 25F1-A (M3) | 3,4,5-trihydroxy-2-(hydroxyacetyl)cyclohex-2-en-1-one or 3,4,6-trihydroxy-2-(hydroxyacetyl)cyclohex-2-en-1-one or 3,5,6-trihydroxy-2-(hydroxyacetyl)cyclohex-2-en-1-one |  | III | P |
| 27F2-A and 27F1-A (identical products) (M4) | 3,5-dihydroxy-4-propanoylbenzoic acid |  | III | P |

| Metabolite | Chemical name | Structure | Toxtree evaluation for the metabolite (1.60) | 'Mammalian' (M) or Plant specific (P) |
|---|--|--|--|---------------------------------------|
| BX112-I5 (M5) | 3-oxido-4-acetyl-5-oxo-3-cyclohexene carboxylic acid |  | III | P |
| 27F2-B and 45F2-A (identical products) (M6) | |  | I | P |
| BX112-M8 deriv. KI 5376 (M7) | Methyl 3-methoxy-5-oxo-3-cyclohexene-1-carboxylate |  | III | M |
| Tri-COOH (M8) | Tricarballic acid |  | I | P |

F. CHECKLIST IN SUPPORT OF ASSESSMENT OF ADEQUACY OF (Q)SAR PREDICTIONS

Table 21: Checklist of questions to help establish the adequacy of a (Q)SAR prediction (corresponds to Table 2.4 of EC, 2011)

| No | Question | Interpretation |
|----|---|--|
| 1 | Is the predicted endpoint clearly defined? | If the endpoint is not clearly defined, the use of the prediction will be open to different interpretations, and thus of questionable value. |
| 2 | If the predicted endpoint is clearly defined (“yes” to Q1), does it represent a direct information requirement under the legislation of interest (e.g. PPP directive), or is it related to one of the information requirements? | If the predicted endpoint corresponds directly with an information requirement, it may be possible to use the prediction instead of experimental data. Alternatively, if the predicted endpoint is indirectly related to an information requirement, it may be useful as supporting information. |
| 3 | If the model is statistically based (as opposed to knowledge-based), is the model training set fully available? | If the model training set of a statistically-based model is not fully available (e.g. because the data are proprietary), it will be impossible for another practitioner to independently reproduce the model, which may reduce confidence in the model estimates. However, this may not be an issue if the model is coded into a software tool. This does not apply to knowledge-based models, which are based on human knowledge and do not have a clearly identified training set. |
| 4 | Is the method used to develop the model documented or referenced (e.g. in a scientific paper or QMRF)? | If the details of model development are not documented, it will be impossible for another practitioner to independently develop and confirm the model, which may reduce confidence in the model estimates. Even if the method is documented, it will require a QSAR specialist to determine whether the documentation is sufficiently detailed to reproduce the model. |
| 5 | Is information available (in terms of statistical properties) concerning the performance of the model, including its goodness-of-fit, predictivity, robustness and error of prediction (uncertainty)? | The statistical properties of a model can provide evidence of its usefulness in a given context (e.g. need to minimise false negatives) and can also be used to assess whether the model has been overfitted (see question 7). |

| No | Question | Interpretation |
|----|---|--|
| 6 | If the model is statistically based (as opposed to knowledge-based), does examination of the available statistics indicate that the model may have been overfitted? | The overfitting of statistically based models is undesirable because it can result in unpredictable errors. This consideration does not apply to knowledge-based models. Overfitted statistical models typically show worse predictivity (outside their training sets) than their internal validation statistics imply. Several simple diagnostics exist, for example: a) the model estimation error (uncertainty of prediction) should not be significantly less than the known experimental error. b) the ratio of datapoints (chemicals) to variables (descriptors) should be at least 5:1. |
| 7 | Does the model training set contain the chemical of interest? | If the model training set contains the chemical of interest, then a prediction is not needed because some experimental data is available for direct use. |
| 8 | Does the model make reliable predictions for analogues of the chemical structure of interest? | The generation of reliable predictions for analogues of the chemical of interest increases confidence in the prediction. In the case of a software tool, it should be indicated whether the software automatically identifies analogues and their associated data within the model training set. In the case of a literature model, it should be considered whether suitable analogues can be identified in the training set (if available). |
| 9 | Is the model prediction substantiated with argumentation based on the applicability domain of the model? | Confidence in a prediction is increased if information is available concerning the applicability domain of the model, and thus whether the model is applicable to the chemical of interest. The applicability domain can include physicochemical and structural space, as well as mechanistic and metabolic considerations. |

G. ACUTE EXPOSURE THRESHOLDS

Table 22: Pesticide active substances (non- organophosphate/carbamate) analysed for derivation of an acute exposure threshold

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|---------------------------------------|---------------------|-------------------|--------------------|------------------------------|--------------|---------------|------|
| 1,3-Dichloropropene | 20 | 0.2 | 100 | 2 wk dog | 3 | EFSA | 2009 |
| 1-Naphthylacetamide | 15 | 0.1 | 150 | Rat developmental | 3 | EFSA | 2011 |
| 1-Naphthylacetic acid | 15 | 0.1 | 150 | Rat developmental | 3 | EFSA | 2011 |
| 2-Naphthyloxyacetic acid | 60 | 0.6 | 100 | developmental | 3 | EFSA | 2011 |
| 8-Hydroxyquinoline incl. oxyquinoline | 5 | 0.05 | 100 | rabbit developmental | 3 | DAR | 2011 |
| Acetamiprid | 10 | 0.1 | 100 | acute rat neurotoxicity | 3 | COM | 2004 |
| Acetochlor | 150 | 1.5 | 100 | acute rat neurotoxicity | 3 | EFSA | 2008 |
| Acrinathrin | 1 | 0.01 | 100 | acute rat neurotoxicity | 3 | EFSA | 2011 |
| alpha-Cypermethrin | 4 | 0.04 | 100 | acute rat neurotoxicity | 3 | COM | 2004 |
| Aluminium phosphide | 3.2 | 0.032 | 100 | rat developmental inhalation | 3 | EFSA | 2008 |
| Aminopyralid | 26 | 0.26 | 100 | rabbit developmental | 3 | DAR | 2006 |
| Amisulbrom | 30 | 0.3 | 100 | rabbit developmental | 3 | DAR | 2008 |
| Amitraz | 1 | 0.01 | 100 | 90 d dog, 2 yr dog | 3 | ECCO | 2003 |
| Asulam | 100 | 1 | 100 | 12 mo dog | 3 | DAR | 2009 |
| Atrazine | 2.5 | 0.025 | 100 | rat developmental | 3 | ECCO | 2003 |
| Azocyclotin | 2 | 0.02 | 100 | rabbit developmental | 3 | JMPR | 2005 |
| Bentazone | 25 | 0.25 | 100 | 90 d rat | 3 | COM | 2000 |
| beta-Cyfluthrin | 2 | 0.02 | 100 | acute rat neurotoxicity | 3 | COM | 2002 |
| BifenoX | 50 | 0.5 | 100 | rabbit developmental | 3 | EFSA | 2007 |
| Bifenthrin | 3 | 0.03 | 100 | 90 d rat neurotoxicity | 3 | EFSA | 2008 |
| Bitertanol | 1 | 0.01 | 100 | 13 wk dog initial findings | 3 | DAR EFSA 2010 | 2003 |
| Bromoxynil | 4 | 0.04 | 100 | rat developmental | 3 | COM | 2004 |
| Bromuconazole | 10 | 0.1 | 100 | rat developmental | 3 | EFSA | 2008 |
| Buprofezin | 50 | 0.5 | 100 | rat developmental | 3 | EFSA | 2008 |
| Calcium phosphide | 5.1 | 0.051 | 100 | rat developmental inhalation | 3 | EFSA | 2008 |
| Captan | 30 | 0.3 | 100 | rabbit teratogenicity | 3 | EFSA | 2009 |
| Carbetamide | 30 | 0.3 | 100 | 1 yr and 90 d dog | 3 | EFSA | 2011 |
| Chloromequat | 9 | 0.09 | 100 | 28 d dog | 3 | EFSA | 2008 |
| Chloropicrin | 0.1 | 0.001 | 100 | 1 yr dog | 3 | DAR | 2010 |
| Chlorothalonil | 60 | 0.6 | 100 | rat mechanistic studies | 3 | COM | 2006 |

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|------------------------------|---------------------|-------------------|--------------------|---|--------------|-----------|------|
| Chlorpropham | 50 | 0.5 | 100 | 90 d dog, acute dog | 3 | COM | 2003 |
| Chlorthal-dimethyl | 50 | 0.5 | 100 | 90 d rat | 3 | DAR | 2006 |
| Clodinafop | 5 | 0.05 | 100 | rat multigeneration, rat developmental | 3 | EFSA | 2005 |
| Clothianidin | 10 | 0.1 | 100 | rat developmental, rabbit developmental | 3 | COM | 2005 |
| Cyanamide (H & Ca cyanamide) | 5 | 0.05 | 100 | rat developmental supported by human experience | 3 | EFSA | 2010 |
| Cyclanilide | 3 | 0.015 | 200 | rabbit developmental | 3 | COM | 2001 |
| Cycloxydim | 200 | 2 | 100 | rabbit and rat developmental | 3 | EFSA | 2010 |
| Cyflufenamid | 5 | 0.05 | 100 | rabbit developmental | 3 | EFSA | 2009 |
| Cyfluthrin | 2 | 0.02 | 100 | acute rat neurotoxicity | 3 | COM | 2002 |
| Cyhexatin | 2 | 0.02 | 100 | rabbit developmental | 3 | JMPR | 2005 |
| Cymoxanil | 8 | 0.08 | 100 | rat developmental | 3 | EFSA | 2008 |
| Cypermethrin | 20 | 0.2 | 100 | acute rat neurotoxicity | 3 | COM | 2005 |
| Cyproconazole | 2 | 0.02 | 100 | rat and rabbit developmental | 3 | DAR | 2010 |
| Cyromazine | 10 | 0.1 | 100 | rabbit developmental | 3 | EFSA | 2008 |
| Dazomet | 3 | 0.03 | 100 | rat developmental | 3 | EFSA | 2011 |
| Deltamethrin | 1 | 0.01 | 100 | 1 yr dog, 90 d dog | 3 | COM | 2002 |
| Desmedipham | 10 | 0.1 | 100 | 80 d dog, rat developmental | 3 | COM | 2004 |
| Dicamba | 30 | 0.3 | 100 | rabbit developmental | 3 | DAR | 2007 |
| Dichlorprop-P | 50 | 0.5 | 100 | rabbit developmental | 3 | EFSA | 2006 |
| Diclofop | 3 | 0.03 | 100 | Rabbit developmental | 3 | PRAPeR 73 | 2010 |
| Dicloran | 2.5 | 0.025 | 100 | 1 yr dog | 3 | DAR | 2009 |
| Dicofol | 15 | 0.15 | 100 | rat acute neurotoxicity | 3 | DAR | 2006 |
| Difenoconazole | 16 | 0.16 | 100 | rat developmental | 3 | PRAPeR 83 | 2010 |
| Dimethachlor | 50 | 0.5 | 100 | rat developmental | 3 | EFSA | 2008 |
| Dimethenamid | 25 | 0.25 | 100 | 4 d rat mechanistic | 3 | EFSA | 2005 |
| Dimethenamid – P | 25 | 0.25 | 100 | 4 d rat mechanistic | 3 | COM | 2003 |
| Dimethipin | 20 | 0.2 | 100 | rabbit developmental | 3 | JMPR | 2004 |
| Dimethomorph | 60 | 0.6 | 100 | rat developmental | 3 | EFSA | 2006 |
| Dimoxystrobin | 4 | 0.004 | 1000 | 1 wk rat | 3 | EFSA | 2005 |
| Diniconazole-M | 5 | 0.02 | 250 | rat developmental | 3 | DAR | 2006 |
| Dinocap | 0.4 | 0.004 | 100 | 2 yr dog | 3 | COM | 2006 |
| Dithianon | 12 | 0.12 | 100 | 7 d and 28 d rat oral toxicity | 3 | DAR | 2010 |
| Diuron | 1.6 | 0.016 | 100 | 28 d rat, 6 mo rat | 3 | EFSA | 2005 |

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|-----------------------------------|---------------------|-------------------|--------------------|---|--------------|-------------|------|
| Dodemorph | 33 | 0.33 | 100 | rabbit developmental | 3 | EFSA | 2008 |
| Dodine | 10 | 0.1 | 100 | Rat developmental | 3 | EFSA | 2010 |
| Endosulfan | 1.5 | 0.015 | 100 | rat acute neurotoxicity | 3 | ECCO | 2001 |
| Epoxiconazole | 2.3 | 0.023 | 100 | rat multigeneration | 3 | EFSA | 2008 |
| Esfenvalerate | 5 | 0.05 | 100 | acute rat, acute mouse, acute rat neurotoxicity | 3 | COM | 2005 |
| Ethephon | 5 | 0.05 | 100 | 28 d dog (AChE inhibition study), human data | 3 | EFSA | 2008 |
| Ethoxyquin | 50 | 0.5 | 100 | dog single dose | 3 | JMPR | 2005 |
| Etofenprox | 100 | 1 | 100 | developmental rabbit | 3 | EFSA | 2008 |
| Etridiazole | 15 | 0.15 | 100 | rabbit developmental | 3 | DAR | 2009 |
| ETU (Ethylenethiourea) | 5 | 0.05 | 100 | rat teratogenicity | 3 | COM | 2005 |
| Famoxadone | 20 | 0.2 | 100 | 14 d mouse | 3 | COM | 2002 |
| Fenarimol | 2 | 0.02 | 100 | rat multigeneration | 3 | COM | 2007 |
| Fenazaquin | 10 | 0.1 | 100 | Rat developmental | 3 | EFSA | 2011 |
| Fenbuconazole | 30 | 0.3 | 100 | rat developmental | 3 | EFSA | 2010 |
| Fenbutatin oxide | 10 | 0.1 | 100 | multigeneration rat study | 3 | EFSA | 2011 |
| Fenoxaprop-P | 10 | 0.1 | 100 | rat developmental | 3 | EFSA (2009) | 2007 |
| Fenoxycarb | 200 | 2 | 100 | rabbit development | 3 | EFSA | 2011 |
| Fenpropidin | 2 | 0.02 | 100 | 28 d dog | 3 | EFSA | 2007 |
| Fenpropimorph | 15 | 0.03 | 500 | rabbit developmental | 3 | EFSA | 2008 |
| Fenpyroximate | 2 | 0.02 | 100 | 1 d and 5 d dog mechanistic, rabbit developmental | 3 | EFSA | 2008 |
| Fentin acetate | 0.1 | 0.001 | 100 | rabbit developmental (maternal effects) | 3 | ECCO 61 | 2001 |
| Fentin hydroxide | 0.1 | 0.001 | 100 | rabbit developmental (maternal effects) | 3 | ECCO | 2001 |
| Fipronil | 0.9 | 0.009 | 100 | rat developmental | 3 | EFSA | 2006 |
| Flonicamid | 2.5 | 0.025 | 100 | rabbit developmental | 3 | EFSA | 2010 |
| Fluazifop-p | 2 | 0.017 | 100 | rat developmental | 3 | EFSA | 2010 |
| Fluazinam | 7 | 0.07 | 100 | rabbit developmental (1988) | 3 | EFSA | 2008 |
| Flubendiamide | 20 | 0.2 | 100 | rabbit developmental toxicity | 3 | DAR | 2008 |
| Flufenacet (formerly fluthiamide) | 1.7 | 0.017 | 100 | 90 d dog, 1 yr dog | 3 | COM | 2003 |
| Flumioxazin | 10 | 0.05 | 200 | rat developmental | 3 | COM | 2002 |
| Fluometuron | 0.8 | 0.008 | 100 | Rat multigeneration | 3 | EFSA | 2011 |

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|---|---------------------|-------------------|--------------------|---|--------------|-----------|------|
| Fluopicolide | 18 | 0.18 | 100 | 28 d rat, rabbit developmental | 3 | EFSA | 2009 |
| Fluoroglycofene (not existing in CIRCA) | 60 | 0.6 | 100 | 1 mo dog | 3 | UK | 2007 |
| Fluoxastrobin | 30 | 0.3 | 100 | 90 d dog, 1 yr dog | 3 | EFSA | 2005 |
| Fluquinconazole | 2 | 0.02 | 100 | rat developmental, rabbit developmental | 3 | EFSA | 2006 |
| Flurochloridone | 20 | 0.04 | 500 | rat developmental | 3 | EFSA | 2010 |
| Flurprimidol | 9 | 0.09 | 100 | rabbit developmental, rat developmental | 3 | EFSA | 2008 |
| Flusilazole | 0.5 | 0.005 | 100 | rat developmental | 3 | COM | 2007 |
| Flutriafol | 5 | 0.05 | 100 | | 3 | EFSA | 2011 |
| Folpet | 20 | 0.2 | 100 | rabbits teratogenicity | 3 | EFSA | 2009 |
| Forchlorfenuron | 100 | 1 | 100 | rabbit developmental | 3 | COM | 2005 |
| Fuberidazole | 8 | 0.08 | 100 | rat developmental | 3 | EFSA | 2007 |
| Gamma-cyhalothrin | 0.75 | 0.004 | 200 | 6 wk dog | 3 | DAR | 2005 |
| Glufosinate | 6.3 | 0.021 | 300 | rabbit developmental | 3 | EFSA | 2005 |
| Haloxypop | 7.5 | 0.075 | 100 | rabbit developmental | 3 | EFSA | 2006 |
| Haloxypop-R | 7.5 | 0.075 | 100 | rabbit developmental toxicity | 3 | EFSA | 2010 |
| Hymexazol | 50 | 0.5 | 100 | Rabbit developmental | 3 | PRAPeR 73 | 2010 |
| Imazalil | 5 | 0.05 | 100 | rabbit developmental | 3 | EFSA | 2010 |
| Imidacloprid | 8 | 0.08 | 100 | 90 d dog, rabbit developmental | 3 | EFSA | 2008 |
| Indoxacarb | 12.5 | 0.125 | 100 | acute rat neurotoxicity | 3 | COM | 2005 |
| Ioxynil | 4 | 0.04 | 100 | rat developmental | 3 | COM | 2004 |
| Ipconazole | 10 | 0.05 | 200 | rat developmental | 3 | DAR | 2008 |
| lambda-Cyhalothrin | 0.75 | 0.0075 | 100 | 42 d dog | 3 | COM | 2001 |
| Lindane | 5 | 0.01 | 500 | rabbit developmental | 3 | ECCO | 1999 |
| Linuron | 10 | 0.03 | 300 | rabbit developmental | 3 | COM | 2002 |
| Magnesium phosphide | 3.8 | 0.038 | 100 | rat developmental inhalation | 3 | EFSA | 2008 |
| Mancozeb | 60 | 0.6 | 100 | rat developmental | 3 | COM | 2005 |
| Maneb | 20 | 0.2 | 100 | rat developmental | 3 | COM | 2005 |
| MCPA | 15 | 0.15 | 100 | rabbit developmental | 3 | COM | 2008 |
| MCPB | 5 | 0.05 | 100 | rabbit developmental | 3 | COM | 2005 |
| Mepiquat chloride | 30 | 0.3 | 100 | rat neurotoxicity | 3 | EFSA | 2008 |
| Metaflumizone | 40 | 0.13 | 300 | rat developmental | 3 | DAR | 2008 |
| Metalaxyl-M | 50 | 0.5 | 100 | rat developmental | 3 | COM | 2002 |

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|---|---------------------|-------------------|--------------------|---|--------------|-----------|------|
| Metaldehyde | 30 | 0.3 | 100 | Dog 52 wk | 3 | EFSA | 2011 |
| Metam (incl. - potassium and - sodium) | 10 | 0.1 | 100 | rat developmental | 3 | PRAPeR 54 | 2008 |
| Metamitron | 10 | 0.1 | 100 | rat developmental | 3 | EFSA | 2008 |
| Metazachlor | 50 | 0.5 | 100 | rat developmental | 3 | EFSA | 2008 |
| Metconazole | 4 | 0.01 | 400 | rabbit developmental | 3 | EFSA | 2006 |
| Methoxyfenozide | 20 | 0.2 | 100 | 2 wk dog | 3 | COM | 2004 |
| Methyl bromide | 0.3 | 0.003 | 100 | 1 yr dog | 3 | EFSA | 2011 |
| Metosulam | 25 | 0.25 | 100 | 2 wk dog | 3 | EFSA | 2010 |
| Metribuzin | 2 | 0.02 | 100 | acute rat neurotoxicity | 3 | EFSA | 2006 |
| MITC | 3 | 0.03 | 100 | rat developmental | 3 | EFSA | 2008 |
| Molinate | 10 | 0.1 | 100 | mechanistic study | 3 | COM | 2003 |
| Myclobutanil | 31 | 0.31 | 100 | rat developmental | 3 | EFSA | 2011 |
| Nicotine | 0.8 | 0.0008 | 1000 | human | 3 | EFSA | 2008 |
| Oxyfluorfen | 30 | 0.3 | 100 | rabbit developmental | 3 | EFSA | 2011 |
| Paclobutrazol | 10 | 0.1 | 100 | rat developmental | 3 | EFSA | 2011 |
| Paraquat | 0.5 | 0.005 | 100 | 90 d dog | 3 | COM | 2003 |
| Penconazole | 50 | 0.5 | 100 | rabbit developmental (maternal NOAEL) | 3 | EFSA | 2008 |
| Pethoxamid | 8 | 0.08 | 100 | 90 d rat, 90 d dog, rat developmental | 3 | COM | 2005 |
| Picloram | 30 | 0.3 | 100 | rabbit developmental, supported by 1 yr dog | 3 | EFSA | 2009 |
| Picolinafen | 5 | 0.05 | 100 | rabbit developmental | 3 | COM | 2002 |
| Pinoxaden | 10 | 0.1 | 100 | rabbit developmental | 3 | DAR | 2005 |
| Potassium thiocyanate (values are based on SCN) | 24 | 0.24 | 100 | acute dog | 3 | DAR | 2007 |
| Prochloraz | 2.5 | 0.025 | 100 | 90 d dog, rat multigeneration, 14 d dog | 3 | EFSA | 2011 |
| Procymidone | 3.5 | 0.035 | 100 | rat developmental | 3 | COM | 2007 |
| Propachlor | 58 | 0.58 | 100 | rabbit developmental | 3 | DAR | 2007 |
| Propamocarb hydrochloride | 100 | 1 | 100 | 28 d rat | 3 | EFSA | 2006 |
| Propiconazole | 30 | 0.3 | 100 | rat developmental | 3 | COM | 2003 |
| Propineb (monomer) | 10 | 0.1 | 100 | rat developmental | 3 | COM | 2003 |
| Propisochlor | 5 | 0.05 | 100 | 1 y dog (acute effects) | 3 | EFSA | 2010 |
| Proquinazid | 20 | 0.2 | 100 | 90 d dog | 3 | EFSA | 2009 |
| Prosulfocarb | 10 | 0.1 | 100 | rat developmental | 3 | EFSA | 2007 |
| Prothioconazole | 20 | 0.2 | 100 | rat developmental | 3 | EFSA | 2007 |
| Prothioconazole-desthio | 1 | 0.01 | 100 | rat developmental | 3 | EFSA | |

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|-------------------------|---------------------|-------------------|--------------------|--|--------------|-----------------|------|
| (metabolite) | | | | | | | |
| PTU (Propylenethiourea) | 0.3 | 0.003 | 100 | Rat developmental | 3 | EFSA | 2003 |
| Pymetrozine | 10 | 0.1 | 100 | rabbit developmental study, 28 d rat | 3 | COM | 2002 |
| Pyraclostrobin | 3 | 0.03 | 100 | rabbit developmental | 3 | COM | 2004 |
| Pyraflufen-ethyl | 20 | 0.2 | 100 | 2 yr rat, 2 yr mouse, rabbit developmental | 3 | COM | 2002 |
| Pyridaben | 5 | 0.05 | 100 | rabbit and rat developmental study | 3 | EFSA | 2010 |
| Quinmerac | 30 | 0.3 | 100 | rabbit developmental toxicity | 3 | EFSA | 2010 |
| Quinoclamine | 5 | 0.05 | 100 | 28 d rat, rat developmental | 3 | EFSA | 2007 |
| Simazine | 2.5 | 0.025 | 100 | acute rat | 3 | DE ECCO | 2003 |
| Spiromesifen | 200 | 2 | 100 | acute rat neurotoxicity | 3 | EFSA | 2007 |
| Spirotetramat | 100 | 1 | 100 | acute rat neurotoxicity | 3 | DAR (EFSA) | 2008 |
| Spiroxamine | 10 | 0.1 | 100 | Acute neurotoxicity | 3 | COM | 2010 |
| Sulfuryl fluoride | 70 | 0.7 | 100 | acute rat neurotoxicity | 3 | EFSA | 2010 |
| tau-Fluvalinate | 5 | 0.05 | 100 | 28 d rat neurotoxicity, rabbit developmental | 3 | EFSA | 2011 |
| Tebufenpyrad | 2 | 0.02 | 100 | dog acute | 3 | EFSA | 2008 |
| Tefluthrin | 0.5 | 0.005 | 100 | 90 d dog | 3 | DAR (EFSA 2010) | 2006 |
| Tembotrione | 1 | 0.01 | 100 | rabbit developmental | 3 | PRAPeR 69 | 2009 |
| Tepraloxymid | 40 | 0.4 | 100 | rat developmental | 3 | COM | 2004 |
| Terbutylazine | 0.8 | 0.008 | 100 | rabbit developmental | 3 | EFSA | 2011 |
| Tetraconazole | 5 | 0.05 | 100 | rat developmental toxicity, maternal effects | 3 | EFSA | 2008 |
| Thiacloprid | 3 | 0.03 | 100 | rat acute neurotoxicity | 3 | COM | 2003 |
| Thiamethoxam | 50 | 0.5 | 100 | rabbit developmental | 3 | COM | 2006 |
| Thidiazuron | 25 | 0.25 | 100 | rabbit developmental | 3 | DAR | 2006 |
| Thiobencarb | 25 | 0.25 | 100 | rat developmental | 3 | DAR | 2005 |
| Thiodicarb | 1 | 0.01 | 100 | rat developmental, acute rat neurotoxicity | 3 | EFSA | 2005 |
| Thiram | 60 | 0.6 | 100 | acute rat neurotoxicity | 3 | COM | 2003 |
| Tolylfluanid | 25 | 0.25 | 100 | rabbit developmental | 3 | EFSA | 2005 |
| Topramezone BAS 670H | 0.5 | 0.001 | 500 | rabbit developmental | 3 | DAR | 2006 |
| Tralkoxydim | 1 | 0.01 | 100 | rat developmental | 3 | EFSA | 2008 |
| Triadimefon | 2 | 0.08 | 25 | acute rat neurotoxicity | 3 | JMPR | 2004 |

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|--------------------------------|---------------------|-------------------|--------------------|--|--------------|------------|------|
| Triadimenol | 5 | 0.05 | 100 | 2 yr rat, multigeneration rat, rat acute, rat subchronic | 3 | EFSA | 2008 |
| Triazamate | 1.5 | 0.015 | 100 | dog single dose | 3 | BE | 2002 |
| Triazoxide | 1.5 | 0.015 | 100 | 28 d rat | 3 | EFSA | 2011 |
| Tribenuron (aka metometuron) | 20 | 0.2 | 100 | rabbit developmental | 3 | EFSA | 2004 |
| Triclopyr | 30 | 0.3 | 100 | rabbit developmental | 3 | EFSA | 2005 |
| Triflumizole | 10 | 0.1 | 100 | rat developmental | 3 | EFSA | 2009 |
| Triflumuron | 0.5 | 0.005 | 100 | 6 d rat (For metabolite M07) | 3 | EFSA | 2011 |
| Triflusalufuron | 120 | 1.2 | 100 | rat developmental | 3 | EFSA | 2008 |
| Triticonazole | 5 | 0.05 | 100 | rabbit developmental | 3 | EFSA | 2005 |
| Vinclozolin | 6 | 0.06 | 100 | developmental toxicity rat | 3 | COM - LOEP | 2006 |
| zeta-Cypermethrin | 12.5 | 0.125 | 100 | rat developmental, rat acute neurotoxicity | 3 | EFSA | 2008 |
| Zinc phosphide incl. phosphine | 7.3 | 0.073 | 100 | rat developmental inhalation | 3 | EFSA | 2007 |
| Ziram | 8 | 0.08 | 100 | rat developmental | 3 | COM | 2004 |
| 1-Methyl-cyclopropene | 70 | 0.07 | 1000 | 21 d rat inhalation | 2 | EFSA | 2005 |
| Acequinocyl | 8 | 0.08 | 100 | rat single dose | 2 | DAR | 2006 |

| | | | | | | | |
|--|---------------|-----------------|--|--|--|--|--|
| 5th percentile of cumulative distribution of NOAELs | 0.5125 | mg/kg bw | | | | | |
|--|---------------|-----------------|--|--|--|--|--|

*Derived by removing the Uncertainty Factor (UF) from the ARfD

Table 23: Pesticide active substances (organophosphate/carbamates) analysed for an acute exposure threshold for neurotoxicity

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|------------------------|---------------------|-------------------|--------------------|--|--------------|--|------|
| Acephate | 1 | 0.1 | 10 | acute human | 3 | JMPR | 2005 |
| Azinphos-methyl | 1 | 0.01 | 100 | rat acute neurotoxicity | 3 | SCFCAH March 2006 (Draft review report 7587/VI/97) | 2005 |
| Cadusafos (aka ebufos) | 0.3 | 0.003 | 100 | rabbit developmental | 3 | EFSA | 2008 |
| Chlorpyrifos | 10 | 0.1 | 100 | acute rat neurotoxicity, delayed neurotoxicity | 3 | COM | 2005 |
| Chlorpyrifos-methyl | 10 | 0.1 | 100 | acute rat neurotoxicity, delayed neurotoxicity | 3 | COM | 2005 |
| Diazinon | 2.5 | 0.025 | 100 | acute rat, acute rat | 3 | EFSA | 2006 |

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|-----------------------------------|---------------------|-------------------|--------------------|--|--------------|----------|------|
| | | | | AChE inhibition, acute rat neurotoxicity | | | |
| Dimethoate | 1 | 0.01 | 100 | rat acute neurotoxicity | 3 | EFSA | 2006 |
| Disulfoton | 0.3 | 0.003 | 100 | acute rat neurotoxicity | 3 | JMPR | 1996 |
| Ethoprophos | 1 | 0.01 | 100 | rat cholinesterase inhibition | 3 | EFSA | 2006 |
| Fenamiphos (aka phenamiphos) | 0.25 | 0.0025 | 100 | acute dog neurotoxicity | 3 | EFSA | 2006 |
| Fenitrothion | 1.3 | 0.013 | 100 | 90 d rat neurotoxicity | 3 | EFSA | 2006 |
| Fenthion | 0.07 | 0.01 | 7 | 28 d human | 3 | ECCO | 2001 |
| Fosthiazate | 0.5 | 0.005 | 100 | 90 d dog, 1 yr dog | 3 | COM | 2003 |
| Malathion | 30 | 0.3 | 100 | rat developmental | 3 | EFSA | 2009 |
| Methamidophos | 0.3 | 0.003 | 100 | rat acute neurotoxicity | 3 | COM | 2008 |
| Methidathion | 0.1 | 0.01 | 10 | 6 wk human | 3 | JMPR | 1997 |
| Mevinphos | 0.03 | 0.003 | 10 | 28 d human | 3 | JMPR | 1996 |
| Monocrotophos | 0.02 | 0.002 | 10 | 10 d human (7 doses) | 3 | JMPR | 1995 |
| Naled | 0.2 | 0.002 | 100 | 90 d dog | 3 | DAR | 2004 |
| Omethoate | 0.2 | 0.002 | 100 | acute neurotoxicity | 3 | EFSA | 2006 |
| Oxydemeton-methyl | 0.15 | 0.0015 | 100 | 14 d rat | 3 | EFSA | 2006 |
| Parathion | 0.5 | 0.005 | 100 | acute rat neurotoxicity | 3 | ECCO 100 | 2001 |
| Parathion-methyl | 0.3 | 0.03 | 10 | human | 3 | ECCO 127 | 2002 |
| Phorate | 0.3 | 0.003 | 100 | rat single dose | 3 | JMPR | 2004 |
| Phosalone | 10 | 0.1 | 100 | rabbit developmental | 3 | EFSA | 2006 |
| Phosmet | 4.5 | 0.045 | 100 | acute rat neurotoxicity | 3 | EFSA | 2006 |
| Pirimiphos-methyl | 15 | 0.15 | 100 | acute rat neurotoxicity | 3 | EFSA | 2005 |
| Profenofos | 100 | 1 | 100 | acute rat neurotoxicity | 3 | JMPR | 2007 |
| Propanil | 7 | 0.07 | 100 | 30 d dog | 3 | PRAPeR | 2010 |
| Terbufos | 0.2 | 0.002 | 100 | acute rat neurotoxicity | 3 | JMPR | 2003 |
| Triazophos | 0.01 | 0.001 | 10 | 21 d human | 3 | JMPR | 2002 |
| Aldicarb | 0.03 | 0.003 | 10 | acute human | 3 | JMPR | 1995 |
| Benfuracarb | 2 | 0.02 | 100 | 28 d rat neurotoxicity | 3 | EFSA | 2009 |
| Carbaryl | 1 | 0.01 | 100 | 90 d rat neurotoxicity | 3 | EFSA | 2006 |
| Carbosulfan | 0.5 | 0.005 | 100 | rat acute neurotoxicity | 3 | EFSA | 2009 |
| Formetanate | 0.5 | 0.005 | 100 | acute rat cholinesterase kinetics | 3 | EFSA | 2006 |
| Methiocarb (aka mercaptodimethur) | 1.3 | 0.013 | 100 | 90 d dog | 3 | EFSA | 2006 |
| Methomyl | 0.25 | 0.0025 | 100 | rat acute neurotoxicity | 3 | EFSA | 2008 |
| Oxamyl | 0.1 | 0.001 | 100 | acute rat neurotoxicity | 3 | EFSA | 2005 |
| Pirimicarb | 10 | 0.1 | 100 | acute rat neurotoxicity | 3 | EFSA | 2006 |

| Compound Name | NOAEL (mg/kg bw/d)* | ARfD (mg/kg bw/d) | Uncertainty Factor | Study | Cramer Class | Source | Year |
|---|---------------------|-------------------|--------------------|-------|--------------|--------|------|
| 5th percentile of cumulative distribution of NOAELs | 0.0295 | mg/kg bw | | | | | |

*Derived by removing the Uncertainty Factor (UF) from the ARfD

GLOSSARY

Absolute configuration: The spatial arrangement of the atoms in a chiral molecule that distinguishes it from its mirror image, and its stereochemical description (*R* or *S*, *M* or *P*).

Acute exposure: A contact between an agent and a target occurring over a short time, generally less than a day. Other terms, such as “short-term exposure” and “single dose” are also used.

Acute reference dose (ARfD): Estimate of the amount of a substance in food and/or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation.

Acute toxicity: Adverse effects of finite duration occurring within a short time (up to 14 d) after administration of a single dose (or exposure to a given concentration) of a test substance or after multiple doses (exposures), usually within 24 h of a starting point (which may be exposure to the toxicant, or loss of reserve capacity, or development change, etc.).

Amphoteric nature: A chemical species that behaves both as an acid and as a base (IUPAC, 2006).

Asymmetric carbon atom: A carbon atom with four different substituents, *C_{abde}*. The term, originally coined by van't Hoff, may also be applied to other tetrahedral atoms (e.g. *N_{abcd}*).

Axial chirality: Chirality stemming from the nonplanar arrangement of four groups about an axis, called a chiral axis; as, for example, in metolachlor.

Chiral: Not superposable (q.v.) with its mirror image, as applied to molecules, conformations, as well as macroscopic objects, such as crystals. The term has been extended to samples of substances whose molecules are chiral, even if the macroscopic assembly of such molecules is racemic (q.v.).

Chiral center: In a tetrahedral (*Xabcd*) or trigonal pyramidal (*Xabc*) structure, the atom (*X*) to which four (or three, respectively) different ligands *abc(d)* are attached and to which a CIP (q.v.) chirality descriptor *R* or *S* can be assigned. Reflection of the molecule reverses the sense of chirality (q.v.) and changes the descriptor.

Chronic exposure: A continuous or intermittent long-term contact between an agent and a target. (Other terms, such as “long-term exposure,” are also used.)

Chronic effect: Consequence that develops slowly and/or has a long lasting course: may be applied to an effect that develops rapidly and is long-lasting.

Chronic toxicity: Adverse effects following chronic exposure. Effects that persist over a long period of time whether or not they occur immediately upon exposure or are delayed.

CIP system: Abbreviation for the 'Cahn-Ingold-Prelog' system. A system of rules for the assignment of descriptors (*R*, *S*, *M*, *P*, *r*, *s*, *m*, *p*, *E*, *Z*) for stereoisomers.

cis (c): A stereochemical term for the relationship between ligands located on the same side of a double bond or of a ring structure in a conformation (real or hypothetical) devoid of reentrant angles. In the case of alkenes only, *E* or *Z* (q.v.) are preferred as descriptors in conjunction with a chemical name.

Configuration: The spatial arrangement of atoms that distinguishes stereoisomers (isomers of the same constitution) other than distinctions due to differences in conformation (q.v.). See also Sense of chirality, Absolute configuration, and Relative configuration.

Conversion factor: Multiplication factor used to when the residue definition for monitoring and risk assessment differ, to address the same toxicological end-point. Conversion factors are applied to monitoring data in order to take into account the exposure to metabolites that are not measured during monitoring.

Critical consumer: The group of consumers that gave the highest intake for a particular crop/residue combination and which have the highest exceeding of the toxicological relevant endpoint.

Degradates: See *metabolite*.

Diastereomers: (diastereoisomers). Stereoisomers not related as mirror images. They usually differ in physical and chemical properties.

E (entgegen), Z (zusammen): Stereochemical descriptors for alkenes or for cumulenes with an odd number of double bonds (and their hetero analogues, such as oximes, hydrazones, and azo compounds) with at least two nongeminal substituents (other than H) at the two ends of the double bonds. *E* (*entgegen*) denotes that the substituents of highest CIP priority at each end of the double bond are trans to each other, that is, on opposite sides. See also CIP system, *trans*. If the pertinent substituents are on the same side (cis to each other) the descriptor is *Z* (*zusammen*). The nomenclature may be used also with respect to partial double bonds such as the C-N bond in N-methylformamide, OHC-NHCH₃ • *E* and *Z* should *not* be used for substituted cycloalkanes.

Enantiomer: One of a pair of molecular species that are mirror images of each other and not superposable. Mirror-image stereoisomers.

Enantiomer composition: An expression of the proportion *R*: *S* of enantiomers *R*, *S* present in a sample of a chiral compound.

Enantiomeric purity: This term is not clearly defined. It may be used synonymously to enantiomer excess (q.v.) or it may (less commonly) refer to the percentage of the major isomer. In the latter case it is better to refer to enantiomer composition (q.v.) or to enantiomer ratio %*R*/% *S*. See Enantiomer excess and Optical purity.

Enantiomerically enriched (enantioenriched): Having an enantiomer excess of more than 0 but less than 100%.

Enantiomerically pure (enantiopure): Having 100% ee (within the limits of measurement).

Enantiomerization: Conversion of one enantiomer into the other. Usually not applied to racemisation (q. v.).

Epimers: Diastereomers differing in configuration at one of two or more stereogenic elements (q.v.). Originally, the term applied to aldoses of opposite configuration at C(2), such as glucose and mannose, but it has now been generalised.

Equivocal: Borderline biological or statistical significance. In (Q)SAR analysis a result is predicted as “equivocal” when the computed probability of a positive effect is between 0.7 and 0.3.

Exposure: Concentration or amount of a pesticide (or agent) that reaches a target organism, system, or (sub) population in a specific frequency for a detailed duration.

Exposure assessment: The process of estimating or measuring the magnitude, frequency and duration of exposure to an agent, along with the number and characteristics of the population exposed. Ideally, it describes the source, pathway, routes and the uncertainties in the assessment.

False negative: known positive compound that are incorrectly predicted as negative.

False positive: known negative compound that is incorrectly predicted as positive.

Genotoxic (or genotoxicity): is a broad term and refers to agents which interact with DNA and/or the cellular apparatus which regulates the fidelity of the genome, e.g. the spindle apparatus, and enzymes such as the topoisomerases, DNA repair systems and DNA polymerases and includes all the adverse effects on genetic information.

Good Agricultural Practice (GAP): In the use of pesticides includes the official recommended or nationally authorised uses of pesticides under actual conditions necessary for effective and reliable pest control. It encompasses a range of levels of pesticides applications up to the highest authorised use, applied in a manner which leaves a residue which is the smallest amount practicable.

Good Plant Protection Practice: means a practice whereby the treatments with plant protection products applied to given plants or plant products, in conformity with the conditions of their authorised uses (Regulation (EC) No 1107/2009).

Isomers: Chemical species that have the same number and kind of atoms but differ in physical and /or chemical properties because of a difference in structure [constitution and /or configuration and/or conformation (q.v.)]. The time scale of the experiment matters in the distinction of isomers from homomers (q.v.).

Metabolite: Any metabolite or a degradation product of an active substance, safener or synergist, formed either in the organism or in the environment.

Mutation (or mutagenicity): refers to a permanent change in the amount or structure of the genetic material of an organism, which may result in a heritable change in the characteristics of the organism. These alterations may involve: individual genes, blocks of genes, or whole chromosomes.

Nonracemic: A term describing a sample of a chiral substance in which molecules of one enantiomer are in excess over those of the other.

Negative predictivity: probability of a negative prediction being correct.

Positive predictivity: probability of a positive prediction being correct.

Optical rotation: The rotation of the plane of plane-polarised light, generally measured in a polarimeter, caused by the presence of either chiral molecules or achiral crystal in the light path. The angle of rotation α is positive, symbol (+), if the plane is turned clockwise as seen by an observer *towards whom* the light travels, negative, symbol (-), if the plane is turned counterclockwise.

Preharvest interval (PHI): The time interval between treatment and harvest.

Pre-registration: The time period where a data submitter is preparing a PPP dossier for designated authorities in support of proposed uses in agriculture.

Post-registration: The time after a certain PPP has been registered for use in agriculture.

Prochirality: A term referring to the existence of stereoheterotopic ligands or faces (q.v.) in a molecule, such that appropriate replacement of one such ligand or addition to one such face in an achiral precursor gives rise to chiral products. A more general term is "prostereoisomerism," since, in some cases, replacement of one or other of two heterotopic ligands or addition to one or other of two heterotopic faces gives rise to achiral diastereomers that contain stereogenic (but not chiral) elements (q.v.). The descriptors *pro-R* or *pro-S* are used for heterotopic ligands, depending on whether

replacement of a given ligand by one identical, but arbitrarily assumed to be of higher priority, gives rise to a chiral element with descriptor *R* or *S*, respectively. The descriptors *Re*, *Si* (q.v.) are used for heterotopic faces. See Chapter 8. If the elements or faces are prostereogenic but not prochiral, the descriptors are *pro-r*, *pro-s*, *re*, and *si*.

Racemate: A composite (solid, liquid, gaseous, or in solution) of equimolar quantities of two enantiomeric species.

Racemic compound: A racemate in which the two enantiomers form a crystalline compound (which can be recognised from the melting phase diagram or by X-ray structure analysis: The unit cell contains equal numbers of enantiomeric molecules). Formerly sometimes called "true racemate".

***R* (rectus), *S* (sinister):** Stereochemical descriptors in the CIP (Cahn-Ingold-Prelog) system. When the descriptors refer to axial chirality (q.v.), they may be modified to *aR*, as, if referring to planar chirality (q.v.) to *pR*, *pS*. If the chiral atom being described is other than carbon, its atomic symbol is sometimes indicated as a subscript, such as *R_p*, *R_s* for *R* chirality sense atphosphorus or sulfur, respectively. The symbols *R** and *S** may be used for relative configuration.

Read-across: Is a technique of filling the data gap. To read across is to apply data from a tested chemical for a particular property or effect to a similar untested chemical. The read-across technique is often applied within groups of similar chemicals assembled for assessment using either analog approach or category approach.

Relevant metabolite: A metabolite is deemed relevant if there is a reason to assume that it has intrinsic properties comparable to the parent substance in terms of its biological target activity, or that it poses a higher or comparable risk to organisms than the parent substance or that it has certain toxicological properties that are considered unacceptable (Regulation (EC) 1107/2009).

Sensitivity: Known positives compounds that are correctly predicted for a toxicological endpoint.

Specificity: Known negatives compounds that are correctly predicted for a toxicological endpoint.

Stereochemistry (adjective stereochemical): Chemistry in three dimensions, chemistry with consideration of its three-dimensional aspects, but also used in relation to chemical and physical properties of *cis-trans* isomers (q.v.) in alkenes.

Stereoisomers: Isomers of identical constitution but differing in the arrangement of their atoms in space. Subclasses are Enantiomers and Diastereomers (q.v.). Stereomutation. A general term for the conversion of one stereoisomer into another, such as Racemisation, Epimerisation, or Asymmetric transformation (q.v.).

Stereoselectivity: The preferential formation of one stereoisomer over another in a chemical reaction. If the stereoisomers are enantiomers, one speaks of enantioselectivity [quantified by enantiomer excess (q.v.)]; if they are diastereomers, one speaks of diastereoselectivity (q.v.). The term "enantioselective" may be applied to the ultimate outcome of a sequence of reactions, even if individual steps are diastereoselective.

Stereospecific: A reaction is termed stereospecific if, in such a reaction, starting materials differing only in their configuration are converted to stereoisomerically distinct products. According to this definition, a stereospecific process is necessarily stereoselective, but stereoselectivity does not necessarily imply stereospecificity. The term may be extended to a process involving a chiral catalyst (including an enzyme) or chiral reagent when the configuration of the product of the reaction depends uniquely on the configuration of the catalyst or reagent i.e. becomes reversed when a catalyst or reagent of opposite configuration is employed. The use of the term "stereospecific" merely to mean "highly stereoselective" is discouraged.

Supervised trial: Scientific studies for estimating maximum residue limits in which pesticides are applied to crops or animals according to specified conditions intended to reflect commercial practice after which harvested crops or tissues of slaughtered animals are analysed for pesticide residues. Usually specified conditions are those which approximate existing or proposed good agricultural practice.

Supervised trials median residue (STMR): The median of the residue value (one from each trial) from supervised trials conducted according to maximum good agricultural practice.

Transformation product: Chemical species resulting from environmental, chemical, or metabolic processes on a pesticide. See also *degradation product*, *metabolite*.

Toxicophore: A 'toxicophore' is a feature or group within a chemical structure that is thought to be responsible for the toxic properties, either directly or via metabolic activation.

ABBREVIATIONS

| | |
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| ADME | Absorption, Distribution, Metabolism and Excretion studies |
| AGES | Austrian Agency for Health and Food Safety (Österreichische Agentur für Gesundheit und Ernährungssicherheit) |
| ArfD | Acute Reference Dose |
| CAC | Codex Alimentarius Commission |
| CRD | Chemicals Regulation Directorate, UK |
| PCA | Principal Component Analysis |
| CCPR | Codex Committee on Pesticide Residues |
| CPDB | Carcinogenic Potency Database |
| DAR | Draft Assessment Report |
| FDA | Food and Drug Administration in USA |
| ECHA | European Chemicals Agency |
| EMA | European Medicine Agency |
| FAO | Food and Agriculture Organisation of the United Nations |
| GAP | Good Agricultural Practice |
| HR | Highest Residue |
| IESTI | International Estimated Short Term Intake |
| IEDI | International Estimate of Dietary Intake |
| JECFA | Joint FAO/WHO Meeting on Food Additives to evaluate flavouring substances |
| JMPR | Joint FAO/WHO Meeting on Pesticide Residues in Food and the Environment |
| JRC | Joint Research Center |
| MRL | Maximum Residue Limit |
| NOEL | No Observed Effect Level |
| OECD | The Organisation for Economic Co-operation and Development |
| PBPK | Physiologically based pharmacokinetic modeling |
| (Q)SAR | Quantitative Structure Activity Relationship |
| REACH | Registration, Evaluation, Authorisation and Restriction of Chemicals |
| SAR | Structure Activity Relationship |

| | |
|------|---|
| STMR | Supervised Trials Median Residue |
| TN | True negative |
| TP | True positive |
| TTC | Treshold of Toxicological Concern |
| WHO | World Health Organisation of the United Nations |
| WTO | World Trade Organisation |