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Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers

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Abstract

In response to the request of the European Commission to EFSA, this document provides guidance on the information necessary to perform the risk assessment of plant protection active substances that contain stereoisomers in their composition as active components or impurities. The guidance should also be used for active substances that without containing any stereogenic element may generate transformation products or metabolites that do contain them. As a general principle, stereoisomers need to be treated as different chemical components for the risk assessment. Current data requirements in the EU regulatory framework (Regulation (EC) No 1107/2009 and Regulation (EC) No 396/2005) already establish that the substance tested should match the technical specifications (including its isomeric composition) and that formation and effects of metabolites, degradation and transformation products should be investigated (which certainly includes the case when transformation products are stereoisomers). Experience gained during the application of EU pesticides regulation has shown that guidance may be needed to provide applicants and evaluators advice on how to generate and assess the required data. Also, guidance is needed on how to make the best use of the available information to perform the risk assessment of these substances, particularly in situations when the information on individual isomers is not available or difficult to obtain, with a primary objective being to reduce the need for repeating vertebrate animal testing. This guidance does not aim to provide specific technical advice on analytical methods. In this guidance, the Regulation (EU) 283/2013 on the data requirements for the plant protection active substances is analysed and recommendations are given on how to best address and assess data requirements for active substances containing stereoisomers.

Keywords: Regulation (EC) No 1107/2009, Regulation (EC) No 396/2005, Regulation (EU) 283/2013, plant protection product, stereoisomers, enantiomers, diastereoisomers, risk assessment

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Summary

In October 2016, the European Commission sent a request to European Food Safety Authority (EFSA) to produce an EFSA guidance that addresses appropriate practice for completing risk assessments for active substances of plant protection products that have isomers and for transformation products of any active substances where these transformation products may have isomers. The users of the guidance will be applicants, consultants preparing applications, competent authorities of the Member States and EFSA staff. This guidance makes use of the considerations already made regarding stereoisomers in the EFSA Panel on plant protection products and their residues (PPR) scientific opinion on evaluation of the toxicological relevance of pesticide metabolites for dietary risk assessment.

A considerable number of active substances of plant protection products present stereogenic elements in their molecular structure (i.e. single atom, axis or a plane with an asymmetric configuration) that give rise to several potential stereoisomers. When this occurs, such substances are marketed either as a more or less purified stereoisomer or as a mixture of them. The isomeric constituents of the active substance should be taken into consideration when the risk assessment is performed. A number of cases may be found in the scientific literature where the differences on toxicological and environmental properties of stereoisomers have a potential impact on the outcome of the risk assessment. As a general principle, stereoisomers need to be treated as different chemical components for the risk assessment. Current data requirements in the EU regulatory framework (Regulation (EC) No 1107/2009 and Regulation (EC) No 396/2005) already establish that the substance tested should match the technical specifications (including its isomeric composition) and that formation and effects of metabolites, degradation and transformation products should be investigated (which certainly includes the case when transformation products are stereoisomers). Experience gained during the application of the EU pesticides regulation has shown that guidance may be needed to provide applicants and evaluators advice on how to generate and assess the required data. Also, guidance is needed on how to make the best use of the available information to perform the risk assessment of these substances, particularly in situations when the information on individual isomers is not available or difficult to obtain, with the primary objective to reduce the need to repeat vertebrate animal testing. In this guidance, Regulation (EU) 283/2013 on the data requirements for the plant protection active substances was analysed and recommendations are given on how to best address and assess the data requirements for *active substances containing stereoisomers*. One special situation is when active substances, that without containing any stereogenic element, may generate transformation products that do contain them. In these cases, it is appropriate to refer to *metabolites containing stereoisomers*. For these substances, the guidance should not be applied to the active substance but should be applied to assess metabolites that contain a stereogenic element. This guidance does not aim to provide specific technical advice on analytical methods.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Background

The current data requirements for active substances in plant protection products, establish that the active 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/transformation products are isomers of the active substance/are constituted by active substance components of a different isomer ratio to that defined by the technical specification. It is also specifically indicated that it is necessary to 'establish the isomeric composition and possible metabolic conversion of the isomers when relevant' and that for active substances 'the relative biological activity of each isomer, both in terms of efficacy and toxicity, shall be reported'. Thus, the information provided must be sufficient to permit an assessment of the nature and extent of the risks for man, and the identification of non-target species likely to be at risk from exposure to the active substance and/or its metabolites, where they are of toxicological or environmental significance taking account of their fate and behaviour in domesticated animals used in food production and the environment, including plants.

It has been estimated that around 25% of active substances possess at least one asymmetric centre or other stereogenic element in their molecular structure. EFSA in its conclusions on pesticide active substances and reasoned opinions on setting or modifying maximum residue levels (MRLs), currently identifies the concern that assessments are not finalised, where further information is considered necessary on the behaviour of stereoisomers in plants, animals, the environment or on effects to non-target organisms. This is concluded when this information is essential to better characterise the risk to humans and other non-target organisms. When making EU approval decisions on active substances, risk managers indicated that applicants must generate confirmatory studies which have to be submitted 2 years after the adoption of a specific guidance document on evaluation of the impact of isomers on the pertinent risk assessments. Therefore, there is the need for a guidance document to be prepared.

Terms of Reference

The Terms of Reference were discussed at the EFSA Pesticide Steering Network (PSN) meeting on 10th February 2015 taking into account the above reported background information.

The main focus of the guidance will be on *stereoisomers* because of the additional analytical challenges characterising this class of isomers. However, the guidance will also cover structural isomers.

The users of the guidance will be applicants, consultants preparing applications, competent authorities of the Member States and EFSA staff.

The guidance document developed should be consistent with the regulatory framework and data requirements for pesticides under Regulation (EC) No 1107/2009[1] and (EC) No 396/2005[2]. This framework has the aim of covering the risk to humans (in particular workers, bystanders, residents, consumers of treated produce, consumers of animal products derived from animals that have consumed treated produce, consumers of contaminated groundwater (primarily in the context of non relevant groundwater metabolites)) and other non target species (terrestrial and aquatic wildlife, wild birds and domestic animals used for food production). The risk assessment paradigms currently used (regarding indicator species selected, matrices in which exposure assessments are made and their temporal and spatial definitions) as set out in the legislation and existing noted guidance should not be changed by this guidance. The guidance needs to work with the risk characterisation approaches currently used. As far as is possible, the guidance will be developed with the aim of being compatible with risk assessment guidance that is being updated/is under development or may be developed in the future. This guidance will make use of the considerations already made regarding stereoisomers in the EFSA Panel on plant protection products and their residues (PPR) scientific opinion on evaluation of

[1] Regulation (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/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

[2] Regulation (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. OJ L 70, 16.3.2005, p. 1–16.

the toxicological relevance of pesticide metabolites for dietary risk assessment (EFSA PPR Panel, 2012). The aim of the guidance is to provide practical advice that uses available proven analytical and other technologies. The intended consequence of following the guidance, is that the uncertainty of a risk characterisation for a substance that contains isomers not resolved by non-chiral analytical techniques or one that transforms to substance/s with such isomers, should not be greater than for substances that do not have these properties. The guidance will aim to deliver the same level of confidence in the risk characterisation when there are isomer issues, as for a risk characterisation where there are no isomer issues.

Topics for which guidance needs to be developed are:

- Terminology used for describing different types of isomers with examples.
- Description of the capacities of different analytical techniques to discriminate and quantify different types of isomers.
- Guidance on uncertainty factors in the different risk assessments to be applied, without information on exposure and or effects from individual stereoisomers, being available, i.e. the factors needed when 'sum of isomers of unknown composition approaches' are followed.
- Guidance on the proportion of samples in the different radiolabelled metabolism study designs requiring stereoisomer separation, to provide sufficient information on exposure patterns of individual isomers (plants, animals, soil, sediment/water) when 'sum of isomers of unknown composition approaches' are insufficient for a robust risk assessment.
- Guidance on what change in stereoisomer ratio is considered too small to conclude that existing uncertainty factors used for risk assessment have not been eroded.
- Guidance on the need for non-radiolabelled residues (in plants, domestic animals, soil) studies to include some quantitative information on stereoisomer levels, guidance on interpretation of such studies and using this information in the exposure assessment where this information is available.
- Guidance on interpreting mammalian absorption, distribution, metabolism and excretion studies where information on stereoisomer ratios has been provided by the available analyses, guidance on interpretation of the studies where this information is available.
- Guidance on approaches, study designs and test organism selection, when risk characterisations indicate that toxicological or ecotoxicological reference values need to be determined for individual isomers or isomer compositions that differ from those in the technical substance originally tested.

It is envisaged a public consultation will be organised on a completed draft document. The target time to initiate the consultation is 2/3 of the way through the project. A stakeholder report will be prepared and input from the consultation considered, when finalising the guidance of EFSA.

1.2. Interpretation of the Terms of Reference

Following the agreement on the Terms of Reference by the PSN, the European Commission sent a mandate to European Food Safety Authority (EFSA). In this section, the approach followed by EFSA to address the mandate is presented.

Stereoisomerism is a frequent and well-known relationship among chemical substances at the molecular level. Stereoisomers are substances that share the same molecular formula, connectivity and bond multiplicity, and differ in the spatial arrangement of two or more atoms. A considerable number of active substances present stereogenic elements in their molecular structure (i.e. single atom, and axis or a plane with an asymmetric configuration) that give rise to several potential stereoisomers. When this occurs, such substances are marketed either as a more or less purified stereoisomer or as a mixture of them.

When the active substance is marketed as a mixture of stereoisomers, the purity is usually reported on basis to the sum of them.[3] This is done despite the fact that in many cases the biological activity, and hence the efficacy of the substance, will rely mostly on one or some of the stereoisomers in the mixture. Various pragmatic reasons have been given by industry and regulators to justify this practice:

[3] Examples of active substances that have been or are marketed as mixture of stereoisomers: cypermethrin (8 isomers), fenvalerate (4 isomers), dichlorprop (2 isomers), metalaxyl (2 isomers), diniconazole (2 isomers), metolachlor (4 isomers, generated by a chiral carbon and the impeded rotation: atropisomers), mecoprop (2 isomers), acetochlor (2 isomers, rotamers atropisomers), alachlor (2 isomers, rotamers atropisomers), fenamiphos (2 isomers), fonofos (2 isomers), malathion (2 isomers), imazapyr (2 isomers), imazaquin (2 isomers).

lack of exact knowledge of the relative biological activity of the stereoisomers, harmonisation with ISO, FAO and other international organisations etc.... Regulation (EU) 283/2013[4] establishes that those isomers that are not part of the ISO common name are considered impurities (Art 1.10).

In contrast to this, when the substance is marketed as enriched by one or containing a reduced number of the theoretically possible stereoisomers,[5] the purity is usually declared on the basis of the amount of the selected isomers. For some chemical structures, several different active substances are placed on the market for which the only difference among them is the proportion of the different isomers in the technical material. Usually these active substances share the same or a similar common name (e.g. cypermethrins).[2]

Independently of the consideration of whether the different isomers are included as active components or impurities of an active substance, they are applied to the crops and released to the environment as part of the plant protection products, resulting in exposure of humans, animals and non-target organisms. Therefore, the isomeric constituents of the active substance should be taken into consideration when the risk assessment is performed. Some stereoisomers, named **enantiomers** (IUPAC, 2013), have identical physical and chemical properties but will behave differently when confronted to an asymmetric environment as in metabolic processes, and will frequently show different biological activity. Enantiomers exist as pairs, corresponding to the two non-superimposable mirror images of otherwise identically connected molecular structures. The remaining stereoisomers, named **diastereomers** (IUPAC**,** 2013), may have similar but not identical physical and chemical properties and likely will present also significantly different biological activity. Diastereomers share the same atom connectivity at the level of molecular structure, but their structures cannot be related to each other through the reflection symmetry operation (e.g. RR isomer with respect to RS isomer in a molecule with two asymmetric centres, *cis–trans* isomerism, etc.).

A number of cases may be found in the scientific literature where the differences in toxicological and environmental properties of stereoisomers have a potential impact on the outcome of the risk assessment (Wong, 2006 and references there in).

As a general principle, stereoisomers need to be treated as different chemical components for the risk assessment. The current data requirements in EU regulatory framework already establish that the substance tested should comply with the technical specifications of the substance on the market or to be marketed (including its isomeric composition) and that formation and effects of metabolites, degradation and transformation products should be investigated. This includes the case when transformation products are stereoisomers. In this sense, new specific data requirements for active substances or metabolites containing stereoisomers are not needed. Nevertheless, experience gained during the application of EU pesticides regulation has shown that guidance is needed for applicants and evaluators on how to generate and assess the required data. Also, guidance is needed on how to make the best use of the available information to perform the risk assessment of these substances, particularly in situations when the information on individual isomers is not available or difficult to obtain, with the primary objective of reducing repetition of vertebrate animal testing. For active substances containing stereoisomers or that may generate them, it is especially advisable that applicants consider the stereoisomeric nature of the active substance when data generation for a regulatory application is initiated. This will facilitate decisions on the best test design and the development of the methodologies needed to generate the required data for the risk assessment.

The guidance does not aim to provide specific technical advice on analytical methods. Such methodologies are not specific to pesticides; therefore, analytical techniques widely used for the separation of stereoisomers in organic chemistry are usually applicable to most of the pesticides. In addition, such advice may suffer from a rapid obsolescence due to the fast technological improvements. In such situations, restrictive reading of the guidance could actually be an obstacle to innovation.

In Section 2, Regulation (EU) 283/2013 on the data requirements for the plant protection active substances is considered and recommendations are given on how to best address these data

[4] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

[5] Examples of substances that have been or are marketed as purified or partly purified stereoisomers: zeta-cypermethrin (4 cypermethrin isomers), beta-cypermethrin (4 cypermethrin isomers, 2 of them also in zeta-cypermethrin), alpha-cypermethrin (2 cypermethrin isomers), esfenvalerate (one of the 4 isomers of fenvalerate purified up to 87%), dichlorprop-P (R isomer of dichlorprop), metalaxyl-M (R isomer of metalaxyl), diniconazole-M (R isomer of diniconazole), S-metolachlor (2 isomers, S isomers of metolachlor), mecoprop-P (R isomer of mecoprop).

requirements when active substances contain stereoisomers. Only those points of the Annex to the Regulation that need to be specifically addressed are referred to. Points not mentioned can either be addressed regardless of the isomeric composition of the active substance and its residues or can be trivially addressed by analogy to other points that have been specifically covered in this guidance. In addition, in case an active substance does not contain any stereogenic element, but it may generate transformation products that do, it is appropriate to refer to the advice that is provided for metabolites containing stereoisomers. For those active substances, the guidance does not need to be applied for the active substance or other non-stereogenic precursors. However, it still needs to be applied to address the pertinent metabolites that contain any stereogenic elements.

2. Requirements for active substances and metabolites containing stereoisomers and their assessment

2.1. General issues

Regulation (EU) 283/2013 on the data requirements for plant protection active substances (hereafter called 'the Regulation') requires that the information presented for the active substance shall be sufficient to *establish isomeric composition and possible conversion of isomers, when relevant* (Commission Regulation (EU) 283/2013 *ANNEX,* Introduction 1.11(n)).

The Regulation thus assumes that some active substances can consist of a mixture of isomers. In this guidance, this is only considered relevant with respect to stereoisomers, which is justified because stereoisomers either can share some physical, chemical and biological properties or they can be difficult to separate during the manufacturing process. Mixtures of structural isomers (constitutional isomers) are not expected to be commercialised as single active substances since in principle they are not expected to share physical, chemical or biological properties and can be easily purified or manufactured separately. Structural isomers can certainly occur as impurities during the manufacturing of the active substance, but do not deserve a specific treatment different to that given to other impurities.

For the purpose of this guidance, it will be considered that an active substance is an ***active substance containing stereoisomers*** when its three-dimensional chemical structure can give rise to stereoisomers by the exchange of two or more atoms, independently of the amount of those stereoisomers in the active substance as manufactured and their considerations as active substance components or impurities. Therefore, the term *active substance containing stereoisomers* applies to both

- active substances containing several components consisting of stereoisomers, or,
- active substances consisting of a single component with a chemical structure that has the potential of having stereoisomers (which may eventually be present in the active substance as impurities or formed by the active substance transformation through metabolic, chemical processes or transformation in the environmental compartments).

In a similar way, a transformation product will be considered a ***metabolite containing stereoisomers*** when its three-dimensional chemical structure can give rise to stereoisomers, independently of the amount of those stereoisomers formed and whether they originated from an active substance already containing stereoisomers or by asymmetric transformation of an active substance originally having a symmetric structure. In this sense, before taking a decision about whether further assessment is needed or not, all metabolites structurally related by stereoisomerism should be considered together and then confronted with the triggers used to decide whether further assessment is needed (e.g. 10% TRR (total radioactive residue) in food, 5% AR (applied radioactivity) in the environment).

The regulation requires that a *detailed description (specification) of the material shall be provided and test performed with material complying with these specifications (except where radiolabelled material or the purified active substance is used)* (Regulation (EU) 283/2013 *ANNEX* Introduction 4.1). This implies that stereoisomeric composition should be part of the description of the active substance and that stereoisomers need to be declared as impurities when appropriate. Hence, the stereoisomeric composition of the test substance used in all studies should be provided. In principle, tests should be performed with a material containing the same proportion of stereoisomers as specified (either as active components or impurities) to ensure that the risk assessment performed addresses the risk posed by the use of the active substance as manufactured. However, some tests may be required with

a different proportion of stereoisomers as given in the specification to complete the risk assessment (e.g. reflecting the ratio of isomers in the terminal residue on food or feed items – residue definition – rather than in the material prior to application, see also for example 2.6.1). When tests need to be performed with metabolites containing stereoisomers, the stereoisomeric composition should be representative of the likely composition to which the tested organism or environmental compartment is expected to be exposed. When stereoisomers need to be treated as individual components (including formed metabolites), studies have to use stereoselective analytical methods for the quantification of the active substance and metabolites.

2.2. Identity of the active substance

Identity of the active substance components should be given (IUPAC name, CAS, EC and CIPAC numbers etc.…) specifying the stereochemistry. The regulation also requires that the structural formula of each isomer present in the active substance shall be provided (Regulation (EU) 283/2013 *ANNEX,* Part A, Section 1, point 1.7). For enantiomers, in many instances, it is not possible to correlate a particular macroscopic form of an enantiomer (characterised by the optical rotation) with the corresponding structural formula (identified as *R* or *S*). In these situations, it is acceptable to provide an arbitrary assignation of each enantiomer to one of the two possible structural formulas, with the argumentation why it was not possible to determine the absolute configuration.

Isomers that are not part of the ISO common name are considered impurities (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 1, point 1.10). When isomers of the active component of the active substance are considered impurities, the provisions in the regulations in relation to impurities apply to them (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 1, point 1.8). This means that the applicant must provide:

- identity of the impurity isomers,
- origin of those impurity isomers (e.g. residues from starting material or formed by side reactions),
- toxicological, eco-toxicological and environmental relevance of impurities consisting of isomers of the active substance.

Whereas this information should be provided for any impurity, particular attention should be paid to those cases when the impurity is a stereoisomer of an active component, due to their structural similarity at molecular level. In this way, any stereoisomer that appears as impurity of the active substance or their components *exceeding 1 g/kg is to be considered by default a significant impurity* (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 1, point 1.10.2). In addition, those stereoisomers *considered particularly undesirable because of their toxicological, ecotoxicological or environmental properties, shall be considered relevant impurities* (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 1, point 1.10.3) and *shall be quantified separately in the batch analysis and the samples to generate toxicological data, even if present at quantities below 1 g/kg* (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 1, point 1.11).

When all stereoisomers related to a molecular structure are considered active components (i.e. are part of the ISO common name), the ratio or the ratio range of the content of the stereoisomers shall be provided. Isomer active components should also be quantified separately in the batch analysis and samples used to generate toxicological data to demonstrate equivalence with the active substance technical specifications (in agreement with Regulation (EU) 283/2013 *ANNEX,* Part A, Section 1, points 1.9 and 1.11). However, in case of manufacturing processes that yield racemic mixtures, it is also possible to demonstrate with a reasoned case that the synthetic steps do not promote the formation of a specific enantiomer; in such a case, the enantiomer-selective analysis is not requested.

In addition, the regulation requires that *the relative biological activity of each isomer, both in terms of efficacy and toxicity shall be reported* (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 1, point 1.9). This implies that when designing the corresponding studies, applicants need to obtain information not only on the active substance as mixture of stereoisomer components, but also on the individual stereoisomer components. Depending on the substance and the study under consideration, two possible general strategies may be considered in order to satisfy this requirement:

- During the course of the studies, chemical analysis able to identify and quantify the different stereoisomers is performed at various sampling times. This should enable to identify if conversion or preferential transformation of stereoisomers occurs and adequately relates the effects observed to the different stereoisomer compositions.

– For each effect data requirement, to perform repeated experiments with test materials containing proportions of stereoisomers different from those in the active substance. For example, if the active substance consists of two stereoisomers at 50% each, the tests could be performed with the active substance as manufactured (mixture 1:1) and, in additional experiments, with each of the pure stereoisomers.

In many situations, these strategies would not need to be applied exhaustively to all data requirements. Based on the results obtained in some tests, the relative behaviour/biological effect of the stereoisomers may be inferred for other similar conditions without the need of performing exhaustive analysis or repeated tests. In particular, special effort should be made in order to avoid or at least minimise the need of repeating tests on vertebrate animals.

When different active substances are or have been on the market, for which the only difference is the proportion of the corresponding stereoisomers in the active substance as manufactured, comparative assessment of the data available for all of them may be useful in determining the relative biological activity of each isomer, without the need of repeating the corresponding studies. Therefore, applicants should provide all available information on any active substance on the market that consists of stereoisomers or different stereoisomer combinations of the active substance under consideration.

2.3. Physical and chemical properties

In general, isomers, including most of the stereoisomers, are distinct chemical substances and have different physical and chemical properties. Enantiomers are an exception to this rule and present the same properties except when confronted to an asymmetric media or phenomenon (e.g. polarised light). Despite the fact that the separated enantiomers present the same physical properties, the racemic mixture may present different physical properties than each of the separated isomers (melting point, solubility etc…). Therefore, some physical properties measured for the racemic mixtures may not be assumed to be identical to those of the pure enantiomers. Then, for an active substance that is a pure enantiomer, physical properties (at least with respect to the solid-state properties) measured with its racemic mixture cannot be extrapolated to the active substance under evaluation.

In the case of *active substances which are optical active isomers, the optical purity shall be measured and reported* (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 2, point 2.4)). Whereas the regulation specifically refers to optical purity (optical rotation of polarised light), since this parameter is commonly equivalent to enantiomeric excess (*ee*), providing the *ee* of the active substance measured using another method (different than the measurement of optical rotations) will be acceptable (e.g. by chiral chromatography).

2.4. Further information on the active substance

When the active substance is a mixture of isomers, it should be clarified how this influences on the effects, based on the mode of action of the individual isomers (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 3, point 3.6). In some instances, experimental studies presented to cover other data requirements (e.g. metabolism studies) may be helpful to substantiate the assessment of the role of each isomer with respect to the mode of action of the mixture. Data on the biological activity of the separated isomers are also required (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 1, point 1.9) and are essential to determine their role on the mode of action of the active substance manufactured as a mixture of stereoisomers. This information should also allow risk managers to identify substances that can be considered candidates for substitution, since one of the conditions for considering a substance as candidate for substitution is that *it contains a significant proportion of non-active isomers* (Regulation (EU) 1107/2009 *ANNEX II*, point 4).

2.5. Analytical methods

2.5.1. Analytical methods for the generation of pre-approval data

2.5.1.1. Active substance as manufactured (technical material)

Analytical methods should be provided for the determination of the pure active ingredient in the active substance as manufactured.

If the active substance as manufactured contains all possible stereoisomers (sharing the same atom connectivity), the methods of analysis provided should allow the determination of the amount of each

isomer. However, for active substances consisting of racemic mixtures, a non-enantioselective method can be accepted in case the manufacturing process does not promote the formation of a specific enantiomer (European Commission, 2019).

If the active substance consists only of a single stereoisomer, or a purified mixture of some of the possible stereoisomers, analytical methods should allow to determine them separately and to separate them from other possible stereoisomers that may occur as impurities. Analytical methods should therefore be able to determine stereoisomers (including enantiomers) of the active ingredient components at levels above 1 g/kg if they are not considered impurities of toxicological concern. They need to be determined at lower levels if they cannot be excluded to be relevant impurities (i.e. it cannot be excluded they share the non-desirable effects attributed to the main components).

2.5.1.2. Methods for risk assessment

The methods for the risk assessment are the analytical methods used for the studies presented in the dossier that use non-radiolabelled material. The analytical methods of radioactive test material are considered within the respective sections where such tests are used.

As a general rule, methods employed should be able to separate the different stereoisomers related to the active substance, regardless of whether they are considered active substance components, impurities or metabolites/reaction/transformation products of the active substance components. When the fate of the active substance over time (degradation and residue decline studies) is investigated, the possibility of racemisation of asymmetric centres or preferential degradation of stereoisomers should be considered, and analytical methods should be able to identify and quantify these processes. In special situations, such as active substances that are constituted by only natural products, the analytical methods should allow to separate only those components known or expected to occur naturally. This is also the case for metabolites consisting of conjugates of a synthetic active substance to natural products (e.g. sugar conjugates), where the synthetic component does not contain a stereogenic element.

Depending on the test, enantiomers may not need to be separated in all test samples; therefore, it is possible to develop a method not discriminating enantiomers, used for routine analysis, and another method discriminating enantiomers for confirmation (to be used in a selected number of samples). In such cases, both methods should be fully validated. Quantification of stereoisomers (e.g. enantiomers) of non-radiolabelled compounds in complex matrices may present additional difficulties since the analytical response of the different stereoisomers is not always the same. This may be solved by using matrix-matched standards for the method validation.

2.5.2. Analytical methods for post approval control and monitoring purposes

The Regulation requires that monitoring methods, as far as practicable, shall employ the minimum cost, and require commonly available equipment. This implies that in situations when enantiomers may need to be separately monitored, the most cost-efficient method available should be provided. In general, this can be attained by using a non-enantioselective method for the bulk of the monitoring samples and a stereoselective method for confirmatory purposes and for cases where the proportion of enantiomers needs to be known. Quantification of stereoisomers (e.g. enantiomers) of non-radiolabelled compounds in complex matrices may present additional difficulties since the analytical response of the different stereoisomers is not always the same. This may be solved by using matrix-matched standards for the method validation.

Methods need to be provided to analyse the components in the residue definition to determine compliance with established MRLs (food and feed of plant or animal origin). In general, the stereoisomers specified in the residue definition will need to be separately quantified by the method. For the case of enantiomers, a method for routine use, not resolving the enantiomers, needs to be provided in addition to a confirmatory method able to resolve them if the enantiomers are listed separately in the corresponding residue definition (e.g. in situations when different active substances on the market, consisting of different proportions of the same enantiomers, need to be identified or for when isomers having different biological properties need to be monitored individually).

Methods need to be provided to analyse the components in the residue definition for soil and water. In general, the stereoisomers specified in the residue definition will need to be separately quantified by the method. For the case of enantiomers, a method for routine use, not resolving the enantiomers, needs to be provided in addition to a confirmatory method able to resolve them (e.g. in situations when different active substances on the market, consisting of different proportions of the same

enantiomers, need to be identified or for when isomers having different biological properties need to be monitored individually).

Methods for the analysis of the active substance and relevant breakdown products in air, formed during or after application, to monitor exposure of operators, workers, residents or bystanders need to be provided (field studies). Since these monitoring methods are generally used in circumstances when the nature of the applied substance is known (i.e. composition of the actual product applied is known by those performing the monitoring study), enantioselective methods are not usually needed, even when different mixtures of stereoisomers are or were to be authorised. A different approach should be followed in the case of volatile substances, for which air monitoring methods are needed for environmental reasons. In these situations, details on the application of the substance are not necessarily known by those performing the analysis, since monitoring is usually performed in locations far from the application place. In these cases, the stereoisomers specified in the air residue definition will always need to be separately quantified by the method.

The methods for the analysis of body fluid and tissue samples need to separate the stereoisomers specified as components identified in the residue definition for monitoring. For the case of enantiomers, a method for routine use, not resolving the enantiomers, needs to be provided in addition to a confirmatory method able to resolve them (e.g. in situations when different active substances on the market, consisting of different proportions of the same enantiomers, need to be identified or for when isomers having different biological properties need to be monitored individually).

2.6. Mammalian Toxicology and non-dietary exposure

The current data requirements for plant protection product active substances indicate that the information provided must be sufficient to assess the nature and extent of the risks for the different exposure groups from exposure to the active substance, its metabolites, degradation and reaction products, where they are of toxicological significance, and also that it is necessary to establish the isomeric composition and possible metabolic conversion of isomers when relevant. This also includes the case when metabolites are isomers of the active substance, e.g. when interconversion leads to the generation of stereoisomers of the active substance at levels that deviate from the technical specification of the active substance as manufactured (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 5, Introduction point 7).

The analytical methods to be used in toxicity studies shall be specific for the entity to be measured and shall be adequately validated (Regulation (EU) 283/2013 *ANNEX,* Part A, Section 5, Introduction point 6). Depending on the study and its particular conditions, this will imply the need to resolve enantiomeric mixtures.

For *active substances containing stereoisomers*, the fate of these stereoisomers in the plant, animal and the environmental metabolism studies must be considered, since the proportion of the isomers may change over time. In such cases, the toxicological impact of the actual residue needs to be assessed, following the residue definitions for risk assessment, established by the assessment of residues data in food and feed and fate and behaviour into the environment. The impact of stereochemistry on the toxicological relevance of pesticide metabolites for dietary risk assessments has previously been discussed in detail (EFSA PPR Panel, 2012). Since isomers may differ in their toxicological potency or profile, changes in stereoisomeric compositions need to be considered in the risk assessment. Therefore, the potential differences between the toxicologically tested isomeric mixture(s) and the stereoisomeric composition of the residues to which humans will be exposed needs to be addressed.

Regarding the dietary risk assessment, a stepwise approach was outlined in the PPR guidance document for residue definition for risk assessment (EFSA PPR Panel, 2016). The same principles are followed to consider stereoisomers. These principles are also applicable to non-dietary risk assessment.

The stepwise approach can be initiated with either considerations on the exposure profile (see Section 2.7 and 2.8) or on the hazard assessment of the different stereoisomers whatever is deemed most suitable and adequate for the specific situation.

The hazard assessment of stereoisomers or changed stereoisomeric composition includes a tiered approach, in order to minimise as much as possible *in vivo* vertebrate animal testing.

2.6.1. Studies on absorption, distribution, metabolism and excretion (ADME) in mammals[6]

Stereoisomers (including enantiomers) may be absorbed, distributed, transported and/or metabolised and/or excreted in a differentiated way. The separated stereoisomers may also behave differently in the laboratory studies on animals and in humans. *In vivo* absorption, distribution, metabolism and excretion (ADME) and *in vitro* metabolism studies (e.g. *in vitro* specific metabolism isoform) should address differential ADME properties of stereoisomers (including enantiomers). Frequently enantiomers exhibit only slight differences in metabolism; however, some notable examples are available where major differences in metabolism of enantiomers occurs (UK-DEFRA, 2003; Ye et al., 2015).

This information should allow the risk assessor to consider the biological relevance (EFSA Scientific Committee, 2017) of the ADME and toxicological data in the rat or other test species; also in comparison to the stereoisomeric mixture composition to which different groups will be likely exposed (e.g. same shift in enantiomer ratio in blood plasma in rats as the shift observed in plants).

2.6.2. Genotoxicity

The stereoisomeric mixture composition to which different groups will be likely exposed (see Section 2.7 and 2.8) should be compared with the specification of the material used in the genotoxicity studies conducted with the active substance or metabolites. If changes occur in the stereoisomeric composition with respect to what has been tested in the genotoxicity studies, these changes are not anticipated to alter the conclusion on genotoxicity hazard identification as long as all the stereoisomers present in the residue definition are adequately represented in the tested mixture used in the genotoxicity studies (EFSA PPR Panel, 2016).

However, if read-across (bridging) is proposed for one stereoisomer that has not been experimentally tested for genotoxicity alone or as a part of a stereoisomer mixture, bridging should be further supported considering all available information including:

- Structural alerts for genotoxicity,
- Potential differential metabolism,
- Whether differences in mutagenicity potency are likely (e.g. epoxide-7-8-oxide).

In case genotoxicity read-across for a particular stereoisomer is not supported by information available, it is recommended to address its genotoxic potential with an *in vitro* test battery following the EFSA Scientific Committee opinion on genotoxicity testing strategy (EFSA Scientific Committee, 2011).

2.6.3. General toxicity

The stereoisomeric mixture composition to which different groups will be exposed (see Section 2.7 and 2.8) should be compared with the composition of the material used in the toxicological studies conducted with the active substance or metabolites. If no significant change in composition is observed (e.g. changes in the expected stereoisomeric excess $< 10\%$), the toxicological data obtained by testing the active substance as manufactured can be used for hazard and risk assessment of the residues.

If changes in the stereoisomeric composition occur, with respect to what has been tested in the toxicological studies, any available information must be provided to characterise the hazard and inform the risk assessment. In order to substantiate read-across ('bridging') from available toxicological data to the incomplete data on the stereoisomeric composition to which exposure groups might be exposed, additional considerations are needed. The extent and type of data to substantiate read-across should be defined on a case-by-case basis and should consider all available information including:

- the toxicological profile of the active substance and its metabolites,
- the metabolism of the active substance and its metabolites (Ye et al., 2015),
- mechanistic data,
- the pesticide and toxicological mode of action (Ulrich et al., 2012),

[6] For a recent review on metabolism of chiral pesticides, please see Perez de Albuquerque et al., 2018.

- information from other active substances with the same stereoisomers in different proportions (e.g. cypermethrin and α-cypermethrin),
- whether quantitative differences (i.e. potency) are likely (e.g. organophosphates).

The purpose of the read-across is to decide whether there are qualitative (i.e. toxicological profile) and/or quantitative (i.e. toxicological potency) differences in toxicity of the stereoisomers. As for other situations where the use of different sources of incomplete information are used to fill information gaps, the bridging among different studies and sources of information may involve the need to consider those studies and data in a weight of evidence approach (EFSA Scientific Committee, 2017) taking into account the biological relevance and the uncertainty analysis (EFSA Scientific Committee, 2018) of the data available.

In case information to read across is not sufficient to characterise the hazard of the residues to which humans are exposed, then, before performing new vertebrate studies, a scientifically sound reasoned case could be made using the assumptions described in the non-dietary and consumer exposure sections (see Sections 2.6.4 and 2.7.4). Nevertheless, if such reasoned case is not conclusive (e.g. potential risk is identified), appropriate data and testing should be provided to refine the assessment.

2.6.4. Non-dietary exposure

The main non-dietary exposure groups that might be affected by changes in stereoisomeric composition after application are workers and residents.[7] The main routes of worker exposure during post-application activities are contact with foliage (here used to include fruits as well as leaves), soil and possible dust (EFSA, 2014). The main pathways of resident exposure after post-application are vapour, surface deposits and entry into treated crops (EFSA, 2014).

If no significant change in composition (e.g. changes in the expected stereoisomeric excess $< 10\%$, see Appendix A) is observed in residues in plants (e.g. foliage) and/or environmental fate studies (e.g. soil), the data from the toxicologically studies conducted with the active substance as manufactured can be directly used for risk assessment without need of any correction.

In case significant changes occur in the stereoisomeric composition and information available is not sufficient to characterise the hazard, further toxicological testing may be waived by deriving an uncertainty factor. This uncertainty factor is used to account for the potential change in the toxicological endpoints introduced by the change of ratio of the individual stereoisomers in the residue in plants and/or the environmental, with respect to the ratio initially tested in the toxicology studies with the active substance (e.g. active substance as manufactured) or the metabolites. This factor can be used in the human health risk assessment (see example below and Appendix B). The uncertainty added to the risk assessment by using such uncertainty factors is noted and should be described and considered in the risk assessment (EFSA Scientific Committee, 2018). The default assumption of this approach is that dose addition applies to toxicity potency and it is based on risk assessment, given that reference values are protective for any toxicological hazard.[8]

Example:

- Substance A is manufactured as a 50% mixture of two isomers: A1 and A2.
- Toxicological studies have been performed using the substance as manufactured and information on the contribution of the individual isomers to the toxicological effects is not available. For the substance as manufactured an acceptable operator exposure level (AOEL) of x mg/kg body weight per day has been established.
- Metabolism studies and studies determining the level of residues in crops were generated using methods that did not allow separate quantification of the isomers of A.
- Risk assessment based on measured residues of A will be done by applying an uncertainty factor of 2. This approach assumes that the target effect, as result of toxicodynamic and toxicokinetic/metabolism properties, is caused by only one of the isomers, assuming dose addition of toxicological effects (absence of synergism or antagonism between isomers).

In case this approach (considering the uncertainty factor) results in exceedance of the reference value, further data either on the actual isomeric composition of the residues or on the specific toxicity

[7] No further considerations regarding operator and bystander exposure are needed since the ratio of stereoisomers must be stable in the technical product to comply with stability requirement.
[8] The aim is to identify potential risk and not the hazard.

of the isomers would need to be provided and used to refine the risk assessment. An extended consideration on how to apply this approach is presented in Appendix B. The same approach can be extended to a larger number of isomers by the appropriate uncertainty factor (e.g. in a substance containing four isomers at 25% each, an uncertainty factor of 4 will be used), but it should be taken into account that the higher the number of isomers the higher the uncertainty of the approach will be.

When toxicological data are available for all individual stereoisomers to which operators are exposed, the risk assessment can be conducted using one of the methods outlined in the EFSA opinion on methodologies to assess cumulative and synergistic risk from pesticides (EFSA PPR Panel, 2008). If the toxicological mode of action is the same and the isomers only differ in the potency of the effects, a relative potency factor among isomers can be established and used for the risk assessment.

2.7. Residues in food and feed

2.7.1. Storage stability studies

In the case of *active substances with stereoisomers,* studies on the effect of storage conditions on the stability of residues in frozen samples should also investigate stereoisomeric stability. This is especially relevant in case the toxicological profile of the isomers is assessed as different or the information available does not allow assessing whether the stereoisomers have a different toxicological profile. In these situations, stereoisomers need to be treated as individual components in the assessment process.

Although it may be sufficient to demonstrate isomeric stability for the longest storage period only, it is at the discretion of the applicant how many sampling points to address to come to a robust conclusion on the stereoisomeric stability of the residues during storage of samples and extracts prior to analysis.

2.7.2. Metabolism, distribution and expression of the residues

For *active substances containing stereoisomers*, metabolism studies that elucidate preferential metabolism, distribution of stereoisomers and stereoisomer interconversion are required. This information is essential to establish the residue definition for risk assessment. Also, this information would need to be considered in the design and interpretation of the toxicological studies to ensure that toxicological data generated cover the actual stereomeric composition to which consumers may be exposed. While diastereomers often can be quantified separately with conventional analytical methods, special 'chiral' analytical methods are needed to quantify individual enantiomers separately. If the active substance or metabolites contains enantiomers, a method capable to separate enantiomer pairs would need to be developed and used (e.g. using chiral chromatography).

It is acknowledged that, for many of the existing active substances, metabolism studies have not been conducted with stereoselective methods. Provided that enough information on the behaviour of individual stereoisomers in food and feed commodities can be demonstrated by field trials or animal feeding studies, these legacy metabolism data might be considered acceptable. In this case, field residue trials or animal feeding studies investigating stereoisomeric ratios could be used as surrogates for a new metabolism study, as long as all pertinent components of the risk assessment residue definition have been analysed. Nevertheless, these legacy metabolism studies should be fully reliable in aspects other than the lack of stereoselective analysis.

2.7.3. Magnitude of residue trials in plant and animal commodities

For *active substances containing stereoisomers*, depending on the results of the metabolism studies in relation to the transformation/interconversion of isomers, and the need to treat them as individual components in the assessment process, stereoselective methods may or may not be needed for the analysis of residues in the residue studies. Nevertheless, it is advisable that stereoisomers are individually followed and quantified in these studies, even when not strictly necessary, to increase the robustness of the data collected in the metabolism studies and to allow the use of data from legacy metabolism studies in which stereoisomers were not individually determined.

2.7.4. Risk assessment of the residues in food and feed

Two main possibilities need to be considered for *active substances containing stereoisomers*:

- Active substances containing only one stereoisomer among those possible on basis of the active substance molecular structure (if other isomers are present, they are considered impurities): in these cases, other stereoisomers formed should be treated as relevant metabolites with respect to the risk assessment, unless additional toxicological information shows them to be not relevant.
- Active substances defined as a mixture of stereoisomers, among those possible based on the active substance molecular structure: in these situations, preferential degradation or interconversion will trigger the need to assess the risk for the components separately.

For practical reasons, a trigger of 10% change in the stereoisomeric excess (e.g. 10% change of *ee* in case of enantiomers) in the residue, with respect to the substance as manufactured, is established for considering it to be potentially significant with respect to the dietary risk assessment. However, this trigger will need to be considered on a case-by-case basis in a weight of evidence approach, taking into account all the information already available for each individual stereoisomer. In this sense, it is advisable to quantify the individual stereoisomers separately in all relevant studies (metabolism and field residue trials), so a robust estimation of the specific behaviour of each isomer can be obtained. Nevertheless, the application of this trigger is limited to substances consisting of pairs of stereoisomers. For more complex mixtures of stereoisomers, it is recommended to use residue decline studies to investigate the fate of each individual stereoisomer in order to decide if the stereoisomers behave differently during metabolism and ageing of the residues (see Appendix A for further discussion and examples).

When the information available does not allow determining whether there is an enrichment of a specific stereoisomer and/or whether the toxicological properties of the stereoisomers are comparable, an uncertainty factor can be used to perform the consumer risk assessment, in order to account for the uncertainty associated to the missing information. For the calculation of the uncertainty factor, if neither the levels of the stereoisomers in the residue nor the relative toxicological potency of them is known, then it will be assumed that the toxicity of the original mixture can be all attributed to a single stereoisomer and that the whole residue is constituted by this same stereoisomer. In the same way, if the levels of the individual stereoisomers in the residue are not known, but the relative toxicological potency of them is known; it will be assumed that the residue is constituted by the most toxic stereoisomer. Finally, if the levels of the individual stereoisomers in the residue are known, but the relative toxicological potency of them is not known; it will be assumed that the toxicity of the original mixture can be attributed to the most abundant stereoisomer in the residue.

Example:

- Substance A is manufactured as a 50% mixture of two isomers: A1 and A2.
- Toxicological studies have been performed using the substance as manufactured and information on the separated contribution of the isomers to the toxicological effects is not available. For the substance as manufactured an acceptable daily intake (ADI) of x mg/kg body weight has been established.
- Metabolism studies and studies determining the level of residues in crops provided have been generated using methods that did not allow separate quantification of the isomers of A.
- Risk assessment based on measured residues of A will be done by applying an additional uncertainty factor of 2 in the risk assessment. This approach assumes that the target effect as result of toxicodynamic and toxicokinetic/metabolism properties is caused by only one of the stereoisomers, assuming additivity of toxicological effects (absence of synergies or antagonisms between isomers).

In case this approach (considering the uncertainty factor) results in exceedance of the reference value, further data either on the actual isomeric composition of the residues or on the specific toxicity of the individual stereoisomers will need to be provided and used to refine the risk assessment. A complete consideration with respect to the amount to information available on how to apply this approach is presented in Appendix B.

The same approach can be extended to a larger number of isomers by the appropriate factor (e.g. in a substance of four isomers at 25% each, an uncertainty factor of 4 will be used), but it should be taken into account that the higher the number of isomers considered the higher the uncertainty of the approach will be.

2.7.5. Definition of the residue in food and feed

The residue definition will be decided using the criteria and procedure given in the Guidance on the establishment of the residue definition for dietary risk assessment (EFSA PPR Panel, 2016).

It is acknowledged that for several existing active substances containing stereoisomers or with metabolites containing stereoisomers, legacy field residue trials or animal feeding studies, where the stereoisomers were not analysed separately, are frequently available. In order to be able to use these legacy residue trials or feeding studies in the risk assessment, for active substances containing stereoisomers that have a different toxicological profile, the residue definition for the risk assessment may need to be completed with information on the expected ranges/proportion of the individual stereoisomers in the relevant commodities. This information is not expected to be needed with new residue trials or feeding studies where the individual stereoisomers must be adequately quantified.

With respect to the residue definition for enforcement, it may be appropriate to consider the stereoisomeric composition of the residue if toxicological effects of isomers are different and/or if the risk managers need to know the origin of residues originated from a substance on the market containing the same stereoisomers but in different proportions.

2.8. Fate and behaviour into the environment

2.8.1. Degradation in soil

Route of degradation studies in soil should *permit the soil residue of concern to which non-target species are or may be exposed, to be defined* (Regulation (EU) 283/2013 *ANNEX* Part A, Section 7, point 7.1.1).

For *active substances containing stereoisomers*, this requirement implies that the composition of the stereoisomers to which the non-target organisms will be exposed has to be known. Changes in the proportion of stereoisomers compared to the active substance as manufactured should be considered a transformation process. Preferential transformation of isomers and isomer interconversion would need to be investigated in the route of degradation studies in soil. Analytical methods employed in the investigations of the degradation in soil should be capable of separating individual isomers. While diastereomers often can be quantified separately with conventional analytical methods, special 'chiral' analytical methods are needed to quantify individual enantiomers separately. If the active substance contains enantiomers, a method capable to separate enantiomer pairs would need to be developed and used (e.g. using chiral chromatography). The degradation and/or formation of individual isomers of the active substance or its metabolites should be characterised by the quantification of each of them individually at all sampling points taken for the route and rate studies in soil performed following current guidelines. If in the route of degradation studies, it is demonstrated that the proportion of enantiomers of a particular substance (active substance or metabolite) does not change in the course of the degradation, separate quantification of stereoisomers in the rest of the studies (rate of degradation, field dissipation studies) would be only required to the extent necessary to demonstrate that the case is generally applicable (e.g. by analysing them separately in a selected number of sampling times). In this respect, a trigger of 10% change in the isomeric excess (e.g. 10% change in the *ee*) in the residue (with respect to the substance as manufactured) is established for considering it to be potentially significant with respect to the environmental risk assessment. This trigger should be considered at the end of the study if at least 50% of the bulk substance (sum of stereoisomers) has been degraded or by extrapolation of the transformation pattern to consider what would be the situation when at least that amount of the bulk substance is degraded. However, such an extrapolation can only be done if sufficient degradation has occurred and a clear trend for each stereoisomer is observed. The same would apply to field dissipation studies. Nevertheless, the application of this trigger is limited to substances consisting of pairs of stereoisomers. For more complex mixtures of stereoisomers, it is recommended to investigate degradation of each individual stereoisomer and to compare kinetic degradation parameters in order to decide if the stereoisomers behave differently in soils (see Appendix A for further discussion and examples).[9] Based on these studies,

[9] In general, metabolites formed at level of 5% of the applied substance or higher need to be identified and considered in the risk assessment. In the case of a pair of stereoisomers, it is considered that a change of their relative amount is a transformation equivalent to the formation of a metabolite with respect to the risk assessment. Therefore, since a relative change of 5% of each isomer in the pair will result in a stereoisomeric excess of 10%, this trigger has been considered to be equivalent to 5% trigger generally considered for transformations giving rise to metabolites.

applicants should propose a degradation scheme in soil, including (if applicable) the interconversion of stereoisomers.

2.8.2. Adsorption desorption in soil and mobility in soil studies

Adsorption to soil is mostly a physical phenomenon associated with interactions of a solved substance with the solid soil matrix. Whereas components in the soil organic matter have a biological origin, it is not expected that the interactions between the soil organic matter and the solved leachate substances is selective and specific enough to discriminate between enantiomers.[10] However, some diastereomers may behave differently with respect to adsorption and desorption and mobility in soil. Information on the chromatographic behaviour of diastereomers may be helpful to assess the need of considering them separately with respect to soil mobility. OECD Test 121 (OECD, 2001) may be used as a preliminary test in the case of diastereomers. If based on the Koc calculated according to OECD Test 121 HPLC method, different diastereomers of the substance investigated fall in different mobility categories (FAO, 2000), then individual K_{Foc} would need to be calculated from the batch adsorption studies. Preliminary tests should be able not only to confirm the stability of the tested substance as a whole, but also its stereoisomeric stability. When there are indications that the diastereomers adsorption to soil may be different (e.g. after HPLC OECD Test 121), methods of analysis of the soil and aqueous phase should be able to quantify them separately in the batch soil adsorption/desorption studies. In this way, adsorption data and adsorption/desorption parameters may be obtained for the separated diastereomers without repeating the study with each of them.

2.8.3. Fate and behaviour in water and sediment

In the case of active substances or metabolites constituted as racemates, the separation of enantiomers is considered not necessary in the analysis of OECD guideline sterile hydrolysis and sterile aqueous photochemical degradation studies because water and buffers are not 'an asymmetric environment'. In the case of active substances or metabolites consisting of pure or enriched enantiomers, a method allowing to separate enantiomers should be used in the analysis of the sterile hydrolysis and sterile aqueous photochemical degradation studies, because some chemical reactions can induce racemisation of pure enantiomers.

In the case of active substances or metabolites consisting of mixtures of diastereomers, different hydrolysis and photolysis degradation behaviour may be expected. Provided the analytical method used resolves the diastereomers, the fate of these components can be investigated together in a single study and separate hydrolysis or photolysis studies with each diastereomer will not be needed.

In the aerobic mineralisation in surface water study, individual transformation products need to be identified (Regulation (EU) 283/2013 *ANNEX,* Part A, Section 7, point 7.2.2.2.f). Changes in the proportion of stereoisomers with respect to the active substance as manufactured should be considered a transformation process and be adequately characterised. Differentiated transformation of stereoisomers and stereoisomer interconversion would need to be investigated in the aerobic mineralisation studies (including separated enantiomers), because 'an asymmetric environment' is likely to be present through biological transformation processes.

The water/sediment study *shall be sufficient to define the sediment residue of concern* (Regulation (EU) 283/2013 *ANNEX,* Part A, Section 7, point 7.2.2.3). For *active substances containing stereoisomers*, this requirement implies that the composition of the stereoisomers to which the non-target organisms will be exposed has to be determined. Changes in the proportion of stereoisomers with respect to the active substance as manufactured should be considered a transformation process. Differentiated transformation of stereoisomers and stereoisomer interconversion would need to be investigated in the water sediment studies (including separated enantiomers), because 'an asymmetric environment' is likely to be present through the biologically mediated transformation processes. Analytical methods employed in the investigations of the degradation in water-sediment studies should be capable to separate individual stereoisomers. While diastereomers often can be quantified separately with conventional analytical methods, special analytical methods are needed to quantify individual enantiomers separately (e.g. using chiral chromatography). If the active substance contains

[10] A published study (Gámiz et al., 2016) gives indications of possible enantioselective adsorption of soil. However, in the lack of further confirmation and understanding of the underlying phenomena and taking into account the observed magnitude of the selectivity in that study, this guidance assumes that adsorption to soil is a phenomenon insensitive to the chirality of the adsorbed molecule.

enantiomers, a method capable to separate enantiomer pairs would need to be developed and used (e.g. using chiral chromatography). The degradation and/or formation of individual stereoisomers of the active substance or its metabolites should be characterised by the quantification of each of them individually at all sampling points taken for the water sediment study. Exceptionally, if it is demonstrated that the proportion of enantiomers of a particular substance (active substance or metabolite) does not change in the course of the degradation, separation of them would only be required to the extent necessary to demonstrate that the case is generally applicable (e.g. by analysing them separately in a selected number of sampling times). In this respect, a trigger of 10% change in the isomeric excess (e.g. 10% change in the enantiomeric composition) in the residue (with respect to the active substance as manufactured) is established for considering it to be potentially significant with respect to the environmental risk assessment. As for the soil degradation studies, this trigger should be considered at the end of the study if at least 50% of the bulk substance (sum of stereoisomers) has been degraded or by extrapolation of the transformation pattern to consider what would be the situation when at least that amount of the bulk substance is degraded. However, in such extrapolation can only be done if sufficient degradation has occurred and a clear trend for each stereoisomer is observed. Nevertheless, the application of this trigger is limited to substances consisting of pairs of stereoisomers. For more complex mixtures of stereoisomers, it is recommended to investigate degradation of each stereoisomer separately and to compare kinetic degradation parameters in order to decide if the diastereomers behave differently in water sediment systems (see Appendix A for further discussion and examples).

The same provisions provided for the water/sediment study in the dark, apply to the irradiated water/sediment study.

2.8.4. Fate and behaviour in air

For *active substances containing stereoisomers,* the vapour pressure may need to be reported separately for the individual diastereomers of the active substance. Enantiomers are expected to have the same vapour pressure. Stereoisomerism is not considered to influence the calculated half-lives in the upper atmosphere. Also, stereoisomerism is not to be considered with respect to global warming potential, ozone depletion potential, photochemical ozone creation potential, accumulation in the troposphere, acidification potential and eutrophication potential.

2.8.5. Definition of the residue

2.8.5.1. Definition of the residue for risk assessment

The residue definition relevant for risk assessment for each compartment shall be defined to include all components (active substance, metabolites, breakdown and reaction products) that were identified in accordance with the criteria referred to in this Section – fate and behaviour into the environment– *The chemical composition of residues occurring in soil, groundwater, surface water (freshwater, estuarine and marine), sediment and air, resulting from use, or proposed use, of a plant protection product containing the active substance, shall be taken into account* (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 7, point 7.4.1).

For *active substances containing stereoisomers*, the residue definition for the risk assessment needs to be specified taking into account the composition of the chemical residues in the different environmental compartments, considering the identity and relative proportion of the actual stereoisomers in the residue to which non-target organisms are expected to be exposed.

When the rate of transformation of each stereoisomer composing the active substance is different, or stereoisomers are interconverted in the environment, and their proportion in the final residue changes compared to the composition of the active substance as manufactured, they are considered separately for the residue definition for the risk assessment into the environment. A trigger of 10% change in the stereoisomeric excess (e.g. 10% change in the *ee*) in the residue, compared to the substance as manufactured, is established for considering it to be potentially significant with respect to the environmental risk assessment (see Appendix A for further discussion and examples).

2.8.5.2. Definition of the residue for monitoring

Considering the results of toxicological and ecotoxicological testing, the residue for monitoring shall be defined to include those components from the definition of the residue for risk assessment, which

are considered relevant when assessing the results in those tests (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 7, point 7.4.2).

For *active substances containing stereoisomers*, all stereoisomers that are part of the residue definition for enforcement will need to be monitored separately.

2.8.6. Monitoring data

Available monitoring data concerning fate and behaviour of the active substance and relevant metabolites, breakdown and reaction products in soil, groundwater, surface water, sediment and air shall be reported (Regulation (EU) 283/2013 *ANNEX,* Part A, Section 7, point 7.5).

For *active substances containing stereoisomers*, monitoring data corresponding to all related active substances, containing different proportion of the stereoisomers, should be presented if available (e.g. for different active substances when both the pure enantiomer and the racemic substance have been commercialised).

2.9. Ecotoxicology

2.9.1. General issues

The ecotoxicological assessment shall be based on the risk that the proposed active substance used in a plant protection product poses to non-target organisms. In carrying out a risk assessment, toxicity shall be compared with exposure. The general term for the output from such a comparison is 'risk quotient' or RQ. It shall be noted that RQ can be expressed in several ways, for example, toxicity: exposure ratio (TER) and as a hazard quotient (HQ). The applicant shall take into account the information from Sections 2, 5, 6, 7 and 8 of the regulation (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 8, Introduction 2).

For *active substances containing stereoisomers*, separate risk quotients may need to be established for each of the isomers when either their fate into the environment is different (e.g. preferential degradation, stereoisomers interconversion) or when the toxicological and/or ecotoxicological properties of each isomer is different.

For the ecotoxicological assessment, it is considered that the toxicological/ecotoxicological properties of two stereoisomers are different when a difference of a factor of 3 is observed in relation to the toxicological/ecotoxicological endpoints (European Commission, 2012).[11]

It may be necessary to conduct separate studies for metabolites, breakdown or reaction products derived from the active substance where non-target organisms may be exposed and where their effects cannot be evaluated by the available results relating to the active substance. Before such studies are performed, the applicant shall take into account the information from Sections 5, 6 and 7. Studies undertaken shall permit characterisation of metabolites, breakdown or reaction products as being significant or not, and reflect the nature and extent of the effects judged likely to arise (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 8, Introduction 3).

In this sense, changes on the proportion of isomers with respect to the active substance as manufactured should be considered a transformation process. Differentiated transformation of stereoisomers and stereoisomer interconversion would need to be considered when designing the ecotoxicological studies. Residue definition for the risk assessment established on the basis of studies in fate and behaviour in the environment and residues in plants should be considered before initiating the ecotoxicological studies.

For *active substances containing stereoisomers*, it is necessary to determine the relative toxicity of the different stereoisomers present in the substance or in the residue of the active substance, as defined for the risk assessment. These will be the compounds to which the non-target organism will be exposed in the corresponding environmental compartment. Tests on invertebrate organisms, algae and plants can be performed using the active substance as manufactured and the purified stereoisomer(s) (of the active substance and/or of the relevant metabolites) listed in the corresponding residue

[11] See SANCO/10597/2003-rev. 10.1: *Where experimental or reliable modelled data are available for the new source, the ecotoxicological profile will be considered equivalent to that of the reference profile where the ecotoxicological data provided on the technical a.s. do not differ by more than a factor of 3 compared to the reference (or by the appropriate spacing factor of the respective test system, if greater than 3), when determined using the same species. (Note: this factor is meant to account for the variability of ecotoxicological test results and must not be interpreted as if an actual difference in ecotoxicity with a factor < 3 was in principle irrelevant with regard to the risk assessment).*

definitions for risk assessment. This should allow identifying differences in the relative biological activity of the isomers.

In order to minimise ecotoxicological tests in vertebrate organisms, all available information on related active substances constituted by different proportion of the same stereoisomer components of the active substance under assessment must be provided and assessed (e.g. when assessing a specific cypermethrin information available should be provided on other cypermethrins with different stereoisomeric compositions). This may allow to characterise toxicity of the different stereoisomers without needing to repeat the studies. In addition, information from metabolisms studies in mammals and poultry (originally presented in residues and/or human health sections) may also provide valuable information on preferential metabolism of the stereoisomers. As regards aquatic organisms, before deciding the need to perform additional studies, the toxicity values derived from tests with no stereoselective chemical analysis may be compared. When, based on this comparison (and considering other relevant data available), the most sensitive group of species can clearly be identified (i.e. a factor of 10 between the most sensitive and the second most sensitive group of species considering both the acute and long-them risk if applicable), the additional testing (i.e. with stereoselective analysis, tests for individual isomers) may focus only on that group of species.

If information allowing the relative toxicity of the stereoisomer components to be determined is not available, a risk assessment using an uncertainty factor for the relevant endpoints should be performed before deciding if additional vertebrate studies are needed (see Section 2.9.7 and Appendix B).

Higher tier studies shall be designed and data analysed using suitable statistical methods. Full details of the statistical methods shall be reported. Where appropriate and necessary, higher tier studies shall be supported by chemical analysis to verify exposure has occurred at an appropriate level (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 8, Introduction 9).

For *active substances containing stereoisomers*, the chemical analysis of the test system during the course of the higher tier tests is highly recommended. Since the potential interconversion and/or preferential degradation of the isomers in the environment needs to be taken into account for the risk assessment, any variation of those during the higher tier studies should be taken into account. The chemical analysis applied should be able to discriminate among the different stereoisomers in the active substance or the stereoisomeric components in of the residue definition for the risk assessment. In situations where selective transformation of isomers was not checked (i.e. higher tier legacy studies for which stereoselective chemical analysis was not performed during the test), all available information on transformation of stereoisomers in the relevant media must be provided and assessed. This may allow to estimate the characteristic of the stereoisomeric composition throughout the study and this assessment could be used in a weight of evidence approach for deciding how to consider that study for the risk assessment.

For those guidelines which allow for the study to be designed to determine an effective concentration (ECx), the study shall be conducted to determine an EC10, EC20 and EC50, when required, along with corresponding 95% confidence intervals. If an ECx approach is used, a no observed effect concentration (NOEC) shall still be determined. Existing acceptable studies that have been designed to generate a NOEC shall not be repeated. An assessment of the statistical power of the NOEC derived from those studies shall be carried out (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 8, Introduction 6).

When ecotoxicological endpoints for *active substances containing stereoisomers* are determined and reported, they should be clearly associated to the specific composition of stereoisomers tested and the stereoisomeric stability and consistency of the test material should be demonstrated. In order to demonstrate that, stereoselective analysis at the beginning and during the corresponding tests should be performed as necessary.

In situations where selective transformation of isomers occurs and no separate ecotoxicological endpoints for the stereoisomers are available, in order to minimise the need to perform additional tests, an uncertainty factor on the relevant endpoints can be applied for the individual isomers for the risk assessment. The approach exemplified in Section 2.9.7 can be adopted for the ecotoxicological risk assessment (See also Appendix B for an extended consideration of this approach). In case this approach results in a breach of the trigger value(s), ecotoxicological tests with the separate isomer(s), as considered in the residue definition for the risk assessment for the environment, will be needed.

In situations where selective transformation of isomers occurs during the test, the endpoint should be associated with the pattern of the exposure that includes a brief description of the change in stereoisomeric ratio (e.g. stereoisomeric ratio at day 0 was 50:50, but changed to 20:80 by day 4). In situations where selective transformation of isomers was not checked (i.e. stereoselective chemical

analysis was not performed during the test), the endpoint should be linked to the composition of the test item (i.e. the initial ratio of the isomers), but it should be clearly indicated that it is unknown whether selective transformation of isomers occurred during the test. In those situations, and when no separate ecotoxicological endpoints for the stereoisomers are available, in order to minimise the need to perform additional tests, an uncertainty factor on the relevant endpoints can be applied for the individual isomers for the risk assessment. The approach exemplified in Section 2.9.7 can be adopted for the ecotoxicological risk assessment (See also Appendix B for an extended consideration of this approach). In case this approach results in a breach of the trigger value(s), ecotoxicological tests with the separate isomer(s), as considered in the residue definition for the risk assessment for the environment, will be needed.

2.9.2. Effects on birds and other terrestrial vertebrates

For all avian and mammalian feeding studies, average achieved dose shall be reported, including where possible the dose in mg substance/kg body weight. Where dosing via the diet is utilised, the active substance shall be distributed uniformly in the diet (Regulation (EU) 283/2013 *ANNEX,* Part A, Section 8, point 8.1).

For *active substances containing stereoisomers*, the isomeric proportion in the tested substance needs to be checked and reported. Stereoisomeric stability over the course of the test in the diet matrix needs to be demonstrated unless freshly prepared for each feeding dose (i.e. with such a frequency that stereoisomeric excess changes $>$ 10% is not expected for any of the dosing periods considering relevant changes in the available information). This means that in case of feeding studies with short exposure (e.g. acute study with an exposure period $\leq$ 24 h and the spiked food is prepared only once for this period), the confirmation of the stereoisomeric stability is unlikely to be necessary. However, it may become necessary in cases when the stereoisomeric stability in this matrix is expected to be very low and a very rapid change in the isomeric proportion is expected. In case of long-term feeding studies, the need for demonstrating the stereoisomeric proportion in the food is likely to be necessary. Studies (usually acute studies) with other types of oral treatment(s) (i.e. *per os*) are not considered to be feeding studies; therefore, the above requirements are not applicable to these.

2.9.3. Effects on aquatic organisms

Reports of the tests referred to in points 8.2.1, 8.2.4 and 8.2.6 shall be submitted for every active substance and supported with analytical data on concentrations of the substance in the test media.

Toxicity endpoints (such as LC50, EC10, EC20, EC50 and NOEC) shall be calculated on the basis of nominal or mean/initial measured concentrations (Regulation (EU) 283/2013 *ANNEX,* Part A, Section 8, point 8.2)).

For *active substances containing stereoisomers*, the isomeric proportion in the tested substance needs to be checked and reported. However, it is noted that in case of flow through or semi-static studies, this may not be necessary provided that the renewal period of the test water is such that stereoisomeric excess change $>$ 10% is not expected. In situations where selective transformation of isomers occurs and no separate ecotoxicological endpoints for the stereoisomers are available, in order to minimise the need to perform additional tests with vertebrates, uncertainty factors can be applied to relevant endpoints for the individual isomers to perform the risk assessment. The approach exemplified in Section 2.9.7 can be adopted for the aquatic ecotoxicological risk assessment (See also Appendix B for an extended consideration of this approach).

2.9.4. Effects on bees

Effects on bees shall be assessed and the risk evaluated, including the risk deriving from residues of the active substance or its metabolites in nectar, pollen and water, including guttation. Reports of the tests referred to in points 8.3.1.1, 8.3.1.2 and 8.3.1.3 shall be submitted, except where plant protection products containing the active substance are for exclusive use in situations where bees are not likely to be exposed (Regulation (EU) 283/2013 *ANNEX,* Part A, Section 8, point 8.3.1).

For *active substances containing stereoisomers*, residues of the active substance in nectar, pollen and water (including guttation) should be investigated taking into account its isomeric variation. The exposure estimation for lower tier risk assessment is usually based on default worst-case values that do not consider transformation. However, isomer specific ecotoxicological endpoints on bees may be needed if isomers composition changes in/on the relevant matrices to which the bees are exposed to

is expected or cannot be excluded. Alternatively, uncertainty factors may be applied to relevant ecotoxicological endpoints (see Section 2.9.7 and Appendix A).

Changes on the isomer proportions in these matrices should be considered in the higher tier risk assessment. Isomer selective chemical analysis of the test system during the course of the higher tier tests is highly recommended.

2.9.5. Effects on non-target arthropods other than bees, effects on non-target soil meso- and macrofauna, effects on soil nitrogen transformation, effects on terrestrial non-target higher plants, effects on other terrestrial organisms (flora and fauna) and effects on biological methods for sewage treatment

The specific Annex points of Regulation (EU) 283/2013 on the data requirements for the plant protection active substances do not contain specific requirements to the situation of *active substances containing stereoisomers*. In the case of *active substances containing stereoisomers*, the risk assessments for these organisms and processes can be addressed by analogy to similar points specifically considered in this guidance. As a starting point, the general issues as described in Section 2.9.1 should be consulted.

2.9.6. Monitoring data

Available monitoring data concerning adverse effects of the active substance to non-target organisms shall be reported (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 8, point 8.9).

For *active substances containing stereoisomers*, monitoring data corresponding to different active substances containing different stereoisomeric composition should be presented if available (e.g. for different active substances when both a pure enantiomer and the racemic substance are or have been commercialised).

2.9.7. Ecotoxicological risk assessment

When performing the ecotoxicological risk assessment for non-target organisms, two main possibilities exist for *active substances containing stereoisomers*.

- Active substances containing only one stereoisomer among those possibly formed on the basis of the active substance molecular structure (if present, other isomers are only impurities). In those cases, the formation of other stereoisomers in the environment can be treated as any other metabolite with respect to its assessment.
- Active substances containing a mixture of stereoisomers among those possibly formed on the basis of the active substance molecular structure. In those situations, preferential degradation or interconversion in the environment will trigger the need to evaluate them as separate components.

When the rate of transformation of each stereoisomer is different, or they are interconverted during the fate and behaviour studies, and their proportion in the final residue changes with respect of the composition of the active substance as manufactured, they are considered separately for the residue definition for the risk assessment in the environment.

When the information available does not allow determining whether there is an enrichment of a specific stereoisomer and/or whether the ecotoxicological properties of the stereoisomers are comparable, an uncertainty factor can be used in the ecotoxicological risk assessment, in order to account for the uncertainty associated with the missing information. For the calculation of the uncertainty factor, if neither the levels of the stereoisomers nor the relative toxicological potency of them is known, then it will be assumed that the toxicity of the original mixture can be entirely attributed to a single stereoisomer and that the whole residue is constituted by this same stereoisomer. In the same way, if the levels of the individual stereoisomers in the residue are not known, but the relative toxicological potency of them is known; it will be assumed that the residue is constituted by the most toxic stereoisomer. Finally, if the levels of the individual stereoisomers in the residue are known, but the relative toxicological potency of them is not known, it will be assumed that the toxicity of the original mixture can be attributed to the most abundant stereoisomer in the residue. This approach is equally applicable for the risk assessment for vertebrates and for the risk assessment for invertebrates.

Example:

- Substance A is manufactured as a 50% mixture of two isomers: A1 and A2.
- Ecotoxicological studies have been performed using the substance as manufactured and information on the separated contribution of the isomers to the toxicological effects is not available. For the substance as manufactured, a NOEC of x mg/kg body weight has been established.
- Fate and behaviour in the environment studies and studies determining the amount of residues in animal food have been generated using methods that did not allow separate quantification of the isomers of A.
- Risk assessment based on measured or estimated residues of A will be done against the NOEC corrected by an uncertainty factor of 2 (NOEC [established for total A]/2). This approach assumes that the target effect as result of toxicodynamic and toxicokinetic/ metabolism properties is caused by only one of the stereoisomers.

When the application of this approach and the corrected ecotoxicological endpoints (considering the uncertainty factor) results in an exceedance of the risk quotient (RQ) reference value, further data either on the actual isomeric composition of the residues or on the specific toxicity of the isomers (or both) would need to be provided and used to refine the risk assessment.

The same approach can be extended to a larger number of isomers by the appropriate factor (e.g. in a substance of four isomers at 25% each, an uncertainty factor of 4 will be used), but it should be taken into account that the higher the number of isomers considered the higher the uncertainty of the approach will be. A complete consideration with respect to the amount of information available on how to apply this approach is presented in Appendix B.

When ecotoxicological data are available for all individual stereoisomers, the risk assessment can be performed using one of the methods outlined in the EFSA opinion on methodologies to assess cumulative and synergistic risk from pesticides (EFSA PPR Panel, 2008). In this case, the approach would be applied to ecotoxicological endpoints in the same way the opinion applies them to toxicological ones.

2.10. Literature data

A summary of all relevant data from the scientific peer reviewed open literature on the active substance, metabolites and breakdown or reaction products and plant protection products containing the active substance shall be submitted (Regulation (EU) 283/2013 *ANNEX*, Part A, Section 9).

For *active substances containing stereoisomers*, peer-reviewed scientific literature should be searched for all separated isomers as well on their mixtures known to be or to have been commercially available. In these cases, scientific literature may contain very valuable information for the characterisation of the different fate and behaviour and biological activity of the individual isomers and their mixtures allowing completing the respective data requirements.

The scientific literature review should conform to EFSA guidance on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA, 2011).

References

EFSA (European Food Safety Authority), 2011. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1–50). EFSA Journal 2011;9(2):2092, 49 pp. https://doi.org/10.2903/j.efsa.2011.2092

EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874, 55 pp. https://doi.org/10.2903/j.efsa.2014.3874

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2008. Opinion of the Scientific Panel on Plant Protection products and their Residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005. EFSA Journal 2008;6(4):705, 84 pp. https://doi.org/10.2903/j.efsa.2008.705

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2012. Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment. EFSA Journal 2012;10(7):2799, 187 pp. https://doi.org/10.2903/j.efsa.2012.2799

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2016. Guidance on the establishment of the residue definition for dietary risk assessment. EFSA Journal 2016;14(12):4549, 129 pp. https://doi.org/10.2903/j.efsa.2016.4549
EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 68 pp. https://doi.org/10.2903/j.efsa.2011.2379
EFSA Scientific Committee, 2017. Scientific Opinion on the guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. https://doi.org/10.2903/j.efsa.2017.4971
EFSA Scientific Committee, 2018. Guidance on Uncertainty Analysis in Scientific Assessments. EFSA Journal 2018;16(1):5123, 39 pp. https://doi.org/10.2903/j.efsa.2018.5123
European Commission, 2012. Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No 1107/2009, SANCO/10597/2003-rev. 10.1, 13 July 2012.
European Commission, 2019. Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre-and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013, SANCO/3030/99 rev. 5, 22 March 2019.
FAO (Food and Agriculture Organization), 2000. Appendix 2. Parameters of pesticides that influence processes in the soil. In FAO Information Division Editorial Group (Ed.), *Pesticide Disposal Series 8. Assessing Soil Contamination. A Reference Manual.* Rome: Food & Agriculture Organization of the United Nations (FAO). (Accessed June 4, 2019).
Gámiz B, Facenda G and Celis R, 2016. Evidence for the effect of sorption enantioselectivity on the availability of chiral pesticide enantiomers in soil. Environmental Pollution, 213, 966–973.
IUPAC, 2013. Henri A Favre and Warren H Powell, Nomenclature of Organic Chemistry IUPAC Recommendations and Preferred Names 2013, EDITION 1 1, Royal Society of Chemistry, (2013), ISBN:978-0-85404-182-4, PDF eISBN:978-1-84973-306-9
OECD, 2001. OECD Test No. 121: Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC), OECD Guidelines for the Testing of Chemicals, Section 1, OECD. Publishing, Paris. https://doi.org/10.1787/9789264069909-en
Perez de Albuquerque NC, Daniel Blascke Carrão D, Habenschus MD and Moraes de Oliveira AR, 2018. Metabolism studies of chiral pesticides: A critical review. Journal of Pharmaceutical and Biomedical Analysis, 147, 89–109.
UK-DEFRA (Department for Environment Food and Rural Affairs), 2003. Final Report: Differential Metabolism of Chiral Compounds – PS2501. Available online: http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=11787
Ulrich EM, Morrison CN, Goldsmith MR and Foreman WT, 2012. Chiral pesticides: identification, description, and environmental implications. Reviews of Environmental Contamination and Toxicology, 2012, 1–74. https://doi.org/10.1007/978-1-4614-2329-4_1
Wong CS, 2006. Environmental fate processes and biochemical transformations of chiral emerging organic pollutants. Analytical and bioanalytical chemistry, 386, 544–558.
Ye J, Zhao M, Niu L and Liu W, 2015. Enantioselective environmental toxicology of chiral pesticides. Chemical Research in Toxicology, 28, 325–338. https://doi.org/10.1021/tx500481n. Epub 2015 Feb

Abbreviations

ADI	Acceptable daily intake
ADME	Adsorption, distribution, metabolism and excretion
AOEL	Acceptable operator exposure level
AR	Applied radioactivity
a.s.	Active substance
bw	Body weight
CAS	Chemical American Society
DAT	Days after treatment
DEFRA	Department for Environmental, Food & Rural Affairs (UK)
ee	Enantiomeric excess
ECx	Effective concentration x (being x a percentile)
F_{A1}	Molar fraction of the metabolite A1
FAOHQ	Food and Agriculture Organization of the United NationsHazard quotient
ISO	International Standard Organization
IUPAC	International Union of Pure and Applied Chemistry
MRL	maximum residue level
NOEC	No observable effect concentration
per os	by mouth, orally (Latin)

PPR	EFSA panel on plant protection products and their residues
PSN	EFSA Pesticide Steering Network
RPF	Relative Potency Factor
RQ	Risk quotient
se	Stereoisomeric excess
TER	Toxicity exposure ratio
TRR	total radioactive residue

Appendix A – On the trigger of 10% stereoisomeric excess change, considered in the metabolism and transformation studies used as criteria to consider that stereoisomers in a pair behave differently

In different places of this guidance, the following situations are considered:

- when the rate of transformation of each stereoisomer is different,
- or they are interconverted during the studies,

resulting in a change in their proportion in the final residue with respect to the proportion in the active substance as manufactured. In these situations, the stereoisomers may need to be considered separately in the residue definitions for risk assessment and monitoring. A trigger of 10% change in the stereoisomeric excess (e.g. 10% change of *ee* in case of enantiomers) in the residue, with respect to the substance as manufactured, is used in the guidance for considering it to be a change potentially significant with respect to the dietary, non-dietary and environmental risk assessments.[12] The same trigger may be considered with respect to the metabolites. In this sense, if a given metabolite with stereoisomers shows during their life cycle changes in their relative levels representing a stereoisomeric excess of 10%, metabolite stereoisomers will need to be considered separately for the risk assessment. However, this trigger has to be considered on a case-by-case basis in a weight of evidence approach, taking into account all the information already available for each separated stereoisomer. Here, some additional definitions regarding this trigger and how to use it in the context of this guidance is provided.

The first issue to be considered is that the stereoisomeric excess is only defined for substances occurring as pair of stereoisomers. The concept of *ee* originated with enantiomeric pairs and has been extended for diastereomers when they occur also in pairs, but it remains an undefined concept when more than two components occur in the mixture of isomers. Therefore, the case of two stereoisomers will be analysed first.

In a pair of stereoisomers **A1** and **A2**, the stereoisomeric excess (either for enantiomers or diastereomers) is defined as the absolute difference between the mole fractions of each stereoisomer:

(1) $\boldsymbol{se} = |F_{\mathbf{A1}} - F_{\mathbf{A2}}|$, where $F_{\mathbf{A1}} + F_{\mathbf{A2}} = 1$

Most frequently (as in this guidance), the stereoisomeric excess is expressed as a percentage.

(2) $\boldsymbol{se}\ (\%) = (|F_{\mathbf{A1}} - F_{\mathbf{A2}}| \times 100)\%$

In an example with enantiomers, a sample containing 60% of R isomer and 40% of S isomer will have an *ee* of 20%. This can also be thought of as a mixture of 20% pure R with 40% of a racemic mixture (which contributes 40% R and 40% S to the overall composition).

In this same example, a transformation of 5% of S isomer to the R isomer will result on a mixture of 65% R and 35% S and the resulting sample will show an *ee* of 30%, which actually represents a 10% change in the *ee*.

In a particular transformation study, performed with radioactive material, observed percentile of applied radioactivity (% AR) will need to be transformed to the corresponding % molar fractions for each pair of stereoisomers in order to assess whether the trigger of 10% change in the stereoisomeric excess is met.

(3) $\boldsymbol{se}\ (\%) = ([(\mathbf{A1}\%\ \mathrm{AR} - \mathbf{A2}\%\ \mathrm{AR})/(\mathbf{A1}\%\ \mathrm{AR} + \mathbf{A2}\%\ \mathrm{AR})] \times 100)\%$

Below some examples are presented based on real data sets to clarify how the concept of stereoisomeric excess is used in the context of this guidance.

Example 1.

In a laboratory soil incubation study, the degradation of an active substance defined as one of two possible stereoisomers, which contains the other isomer as an impurity, is investigated. In a particular soil the following measurements are obtained for each of the isomers:

[12] In general, metabolites formed at level of 5% AR of the applied substance or higher need to be identified and considered in the environmental risk assessment. In the case of a pair of stereoisomers, it is considered that a change of their relative amount is a transformation equivalent to the formation of a metabolite with respect to the risk assessment. Therefore, since a relative change of 5% AR of each isomer in the pair will result on a stereoisomeric excess of 10%, this trigger has been considered to be equivalent to 5% trigger generally considered for transformations giving rise to metabolites needing further assessment.

t (days)	*stereoisomer A1 (%AR)*	*stereoisomer A2 (%AR)*	*se*
0	*94.0*	*2.8*	94.2
7	*90.1*	*2.7*	94.2
30	*80.4*	*2.2*	94.7
91	*70.0*	*2.1*	94.2
120	*68.8*	*2.2*	93.8
179	*58.3*	*1.9*	93.7
270	*51.0*	*2.2*	91.7
365	*49.3*	*2.2*	91.5

*In this case, the **se** calculated according to formula (3) ranges from 94.7% to 91.5%, which is a **se** change of 3.3%. Therefore, in this case, it is considered that this study shows neither stereoisomers interconversion nor that the potential differential transformation of the stereoisomers is relevant for the soil compartment in this soil.*

Normally, at least four studies, like the one illustrated in *Example 1*, are available in the dossier of a particular active substance. If the results are consistent between the different available studies, the calculation of the changes of the stereoisomeric excess in each experiment will be sufficient to take a decision on the need for considering isomers separately for the risk assessments associated with the soil compartment. However, in case discrepancies are observed between the studies (i.e. some show a change of ***se*** > 10% but others not), a weight of evidence approach will need to be applied in order to decide how much effort is needed for further investigation of the effects and the environmental fate and behaviour of separated isomers in the particular compartment. In these cases, applicants will need to justify their decision. In providing this justification, all available information on the substance may be used, in particular the available information on the potential for different biological effects of the separated stereoisomers can be useful to demonstrate the need or not for further stereoisomer specific investigations.

Three further issues need to be considered here:

- Selective transformation of stereoisomers cannot be presumed to occur to the same extent and in the same sense in all matrices investigated. For example, preferential degradation of one stereoisomer that may occur in a system (e.g. soil), may not occur in another one (e.g. in/on plants or between different plants) or even the *sense* of the preferential degradation may be reversed.[13] Therefore, comparison of the stereoisomers transformation in different media needs to be done with caution, and discrepant behaviour cannot be automatically interpreted as random variability.
- The initial proportion between isomers may influence the decision on the need for further investigations. In the *Example 1*, data indicated that the degradation rate is significantly different, as the stereoisomer impurity is significantly more persistent than the active substance stereoisomer. If the initial proportions were reversed or the active substance had consisted of a 50% mixture of these two stereoisomers, then the trigger of 10% *se* change would very probably be breached in these transformation experiments.
- Special care has to be taken to appropriately consider the errors in the analytical measurements of the separated stereoisomers. In particular, in the later data points of decline studies. When the amounts measured are close to the LOQ, quantification errors may be higher than 5% of the actual % AR value measured. Consequently, it would be impossible to determine if the trigger of 10% *se* is actually breached. Analysis of consistency in the changes observed among data points and among different analogous studies (performed in the same compartment; soil, plant etc.…) may be especially helpful in these situations to decide if the apparent change is real or an analytical artefact. Due to the limitations of analytical quantification, a change in the stereoisomeric excess of 10% is in general of low relevance when the total amount is already below 10% of AR.

[13] Whereas the formal definition of stereoisomeric excess is given as the absolute difference of the two stereoisomers molar fractions, when decline studies are analysed for stereoisomeric excess changes, the direction of the differences must be maintained in order to identify apparent changes in the sense of the transformation.

Example 2.

In a similar decline experiment to the one presented in Example 1, the degradation of an active substance composed of two stereoisomers in an initial 50% proportion is presented.

t (days)	***stereoisomer B1 (%AR)***	***stereoisomer B2 (%AR)***	***se***	***se change***
0	*42.7*	*42.4*	*0.3*	*0*
1	*35.8*	*36.2*	*0.6*	*−0.9*
3	*25.4*	*28.3*	*5.4*	*−5.7*
10	*13.3*	*17.2*	*12.8*	*−13.1*
14	*7.4*	*7.7*	*2*	*−2.3*
31	*7.9*	*9.3*	*8.1*	*−8.4*
63	*3.1*	*3.7*	*8.8*	*−9.1*
91	*1.6*	*1.9*	*8.6*	*−8.9*
122	*1.7*	*2.4*	*17.1*	*−17.4*

*In this case, the **se change** ranges from 0.9% at 1 DAT (days after treatment) to 17.4% at 122 DAT. In two sampling data points 10 DAT and 122 DAT, the trigger of 10% of stereoisomeric excess change is breached. However, 90% of degradation has already occurred at 63 DAT and the significant change observed at 10 DAT is not confirmed by subsequent data points indicating that this can be attributed to experimental error. A similar analysis of the other degradation studies would need to be performed to confirm the conclusion that the apparent faster degradation of isomer B1 with respect to B2 des not need to be considered further for the risk assessment.*

The definition of ***se*** in formula (1) is not applicable when mixtures with a higher number of isomers are considered. The analysis of stereoselective degradation and stereoisomer interconversions becomes necessary and more complex in such mixtures. Where it is eventually still possible to do an analysis by grouping the isomers in pairs, according the configuration around each stereogenic element, it is not a recommended option, due to the high number of analyses that would need to be done and the difficulty of reaching solid conclusions from these analyses. In such situations, the calculation of half-lives and comparison of them seems to be a more practical approach than the use of the calculation and comparison of stereoisomeric excess changes. In such situations, studies should be available to allow the kinetic analysis of the degradation (or the formation and degradation in the case of metabolites) of each individual stereoisomer. This will allow to perform a separate risk assessment for each stereoisomer or identify a set of worst-case kinetic parameters that can be used for the exposure assessment that covers all isomers.

Appendix B – Examples of situations of incomplete exposure or hazard information on individual stereoisomers of active substances containing stereoisomers

For an active substance containing stereoisomers, in principle the information provided should be sufficient to characterise both the fate and the hazard posed by the separated stereoisomer in each of the different aspects for which a risk assessment is performed (human and animal health and environment). However, in many instances, this information is not complete and there are technical or ethical considerations that justify not generating additional data (e.g. to minimise toxicity tests with vertebrates). It is therefore appropriate to analyse the different cases when the risk assessment can be completed, and which assumptions are needed in the case of incomplete information with respect to individual stereoisomers.

It should be noted that the solutions proposed, based on additional risk assessment uncertainty factors, presume that toxicity of stereoisomers is additive and that there is no antagonism or synergism of the toxicological effects between stereoisomers.

In this appendix, the different situations with respect to limited information, either on the exposure part or the hazard part of the risk assessment, for active substances containing stereoisomers are presented together with the acceptable risk assessment approaches to be applied to overcome this lack of information (e.g. application of uncertainty factors).

In Table B.1, a matrix of the different cases is presented together with the corresponding risk assessment recommendations.

In Table B.2, the approaches proposed in Table B.1 are applied to theoretical examples for toxicology and ecotoxicology risk assessment.

Table B.1: Different cases and risk assessment recommendations

Exposure	**Hazard**	
	Hazard information is available only for the a.s. as manufactured	**Hazard Information is available for the a.s. as manufactured and for the individual isomers[(a)]**
It is demonstrated that changes in the stereoisomeric excess are < 10%	Compare the exposure estimation for the sum of the isomers with the available combined toxicity endpoint	Compare the exposure estimation for the sum of the isomers with the available combined toxicity endpoint. In this case, hazard information on individual metabolites would not be strictly needed for the risk assessment but may be useful to confirm that the additive toxicity hypothesis is applicable
It is not known whether changes in the stereo isomeric excess occur or the extent of such changes is not known	Compare the exposure estimation for the sum of the isomers with the available combined toxicity endpoint and adjust the risk assessment with the default worst-case uncertainty factor. This factor is calculated assuming that the toxicological effects of the mixture of isomers can be attributed to a single isomer and that the measured residue is also all constituted by this single isomer	Two situations are possible: • Individual isomers and the mixture have equivalent toxicity: Compare the exposure estimation for the sum of the isomers with the available combined toxicity endpoint • At least one of the individual isomers has higher toxicity than the others in the mixture: Consider the toxicity of the most toxic isomer in combination with the exposure estimation for the sum of the isomers[(b)]

Exposure	Hazard	
	Hazard information is available only for the a.s. as manufactured	**Hazard Information is available for the a.s. as manufactured and for the individual isomers[(a)]**
It is demonstrated that changes in the stereoisomeric excess are > 10% and those changes have been quantitatively determined	Compare the exposure estimation for the sum of the isomers with the available combined toxicity endpoint and adjust the risk assessment with the calculated uncertainty factor. As above, this factor is calculated assuming that the toxicological effects of the mixture of isomers can be attributed to a single isomer. However, since in this case, the composition of the isomers in the residue is known, it is assumed that only the major isomer is responsible for the toxicological properties observed and not the whole estimated residue	Two situations are possible: • Individual isomers and the mixture have equivalent toxicity: Compare the exposure estimation for the sum of the isomers with the available combined toxicity endpoint • At least one of the individual isomers has higher toxicity than another or of the mixture: Consider the exposure of all single isomers taking into account their occurrence at a relevant time point or relevant time frame (e.g. max occurrence, occurrence at harvest, twa) and compare these exposure figures with the respective toxicity endpoints. Then, it will be necessary to weight the contribution of each isomer to the overall risk following the methods outlined in EFSA PPR opinion on methodologies to assess cumulative and synergistic risk from pesticides (EFSA PPR Panel, 2008). In some instances, when the toxicity potency difference among isomers is high (> 1 order of magnitude) and the levels of the most toxic isomers are significant (> 10%), the risk assessment is driven by the most toxic isomer and the contribution of the less toxic ones to the risk is frequently negligible

(a): Situations where the toxicological endpoints are known for a mixture or different mixtures of isomers and at least one of the purified isomers may be frequent when different active substances with different isomer proportions are or have been placed on the market over time (e.g. racemic and pure enantiomer). Availability of complete information on the toxicity of all separated isomers and the mixture allows testing the validity of the additive toxicity hypothesis.
(b): Since the isomeric composition of the residue is not known, for the risk assessment it is assumed that the residue is constituted by the most toxic isomer.

Table B.2: Examples of the application of the criteria in Table B.1 for an a.s. consisting on a racemic mixture

Exposure	Hazard	
	Information on toxicity is available only for the a.s. as manufactured - Tox(A1 + A2)	**Information on toxicity is available for the a.s. as manufactured and for the individual isomers**
It is demonstrated that changes in the stereoisomeric excess are < 10%	*Example for ecotoxicology risk assessment* The necessary toxicity tests on fish are available only for the a.s. as manufactured which is a racemic mixture (50:50) of A1 and A2 stereoisomers. The risk assessment for fish will simply compare the PEC and the toxicity figure expressed for the racemic mixture: PECsw(A1 + A2) vs. Tox(A1 + A2)	*Example for ecotoxicology risk assessment* The long-term toxicity tests on daphnids is available for the a.s. as manufactured which is a racemic mixture (50:50) and for the separated isomers. It is found that isomer A1 is more toxic than A2. Since a change in the proportion of isomers is not expected to occur into the environment, the risk assessment can be performed based solely on the total PEC and the mixture toxicity endpoint. PECsw(A1 + A2) vs. Tox(A1 + A2) The risk quotient could also be calculated based on the individual isomers weighted for their respective toxicity. If the additive toxicity hypothesis is applicable (neither synergies nor antagonism between isomers occur), the resulting risk quotient should be nearly identical to the one calculated on basis of the mixture

Exposure	Hazard	
	Information on toxicity is available only for the a.s. as manufactured - Tox(A1 + A2)	**Information on toxicity is available for the a.s. as manufactured and for the individual isomers**
It is not known whether changes in the stereo isomeric excess occur or the extent of such changes is not known.	*Example for toxicological risk assessment* An ADI has been established for a mixture of two stereoisomers at 50% each. It is not known if there is any inter conversion of isomers or preferential degradation. The uncertainty factor that will be used for the risk assessment will be 2 in this case. This uncertainty factor is used either to divide the toxicological endpoint, or to multiply the risk quotient (which achieve equivalent outcomes). In the case, there was a mixture of four isomers (25% each) the factor used will be 4. *Example for ecotoxicology risk assessment* A long-term fish endpoint has been established for a mixture of two stereoisomers (A1 and A2) at 50% each. It is not known if there is any inter conversion of isomers or preferential degradation. The uncertainty factor that will be used for the risk assessment will be 2 in this case. This uncertainty factor can be used to divide the ecotoxicological endpoint; however, equivalent mathematical solutions might also be used (i.e. consider this factor for the PEC or for the resulting risk quotient): PECsw (A1 + A2) vs. Tox(A1 + A2) and uncertainty factor of 2 In the case there was a mixture of four isomers (25% each), the uncertainty factor used would be 4.	*Example for toxicological risk assessment* In a substance produced as a 50% mixture of two isomers A1 and A2, it has been established that ADI A1 = 0.1 mg/kg bw and ADI A2 = 1 mg/kg bw. However, it is only possible to estimate the total residue A1A2. Then, the risk assessment for the residue of the mixture will be done against the ADI endpoint of isomer A1 (the most toxic one). *Example for ecotoxicology risk assessment* The substance as manufactured is a racemic mixture (50:50) of A1 and A2 stereoisomers. The available long-term toxicity tests on daphnids indicate that A1 isomer is more toxic than A2 and the a.s. as manufactured. However, it is not known if there is any inter conversion of isomers or preferential degradation, therefore PEC calculations for the individual isomers are not available, but only for the a.s. as manufactured. Then, the risk assessment for this a.s. will be performed considering the endpoint of A1 (the more toxic one). PECsw(A1 + A2) vs. Tox(A1)

Exposure	Hazard	
	Information on toxicity is available only for the a.s. as manufactured - Tox(A1 + A2)	**Information on toxicity is available for the a.s. as manufactured and for the individual isomers**
It is demonstrated that changes in the stereoisomeric excess are > 10% and those changes have been quantitatively determined	*Example for toxicology risk assessment* An ADI has been established for a mixture of two stereoisomers A and B at 50% each. It has also been estimated that in the final residue, the isomeric composition is 80% A and 20% B. For the risk assessment, a factor of 1.6 (0.8 × 2) will be used instead of 2 (assuming the figure used to represent exposure corresponds to the sum of isomers). Alternatively, a factor of 2 can be used in a risk assessment where the figure used for exposure represents the major isomer in the residue. In a specific case where residue trials used were done without separation of isomers, but there is consistent evidence that the isomeric composition changes and the residue is 80% of one of the two isomers, then the dietary risk assessment would be conducted against an ADI corrected by a factor of 1.6. Risk assessment ADI = ADI (mixture)/1.6. *Example for ecotoxicology risk assessment* A long-term fish endpoint has been established for a mixture of two stereoisomers (A1 and A2) at 50% each. The ratio of the isomers changes with time in environmental matrices, and the ratio predicted for surface water becomes 80:20. It could not be excluded that the major isomer is more toxic than the other isomer or the mixture. The factor that will be used for the risk assessment will be in this case 1.6 (80/100 × 2). This factor can be used to divide the toxicological endpoint; however, equivalent mathematical solutions might also be used (i.e. consider this factor for the PEC or for the resulting risk quotient): PECsw (A1 + A2) vs. Tox (A1 + A2) and uncertainty factor of 1.6	*Example for toxicological risk assessment* For a substance manufactured as a 50% mixture of two isomers A1 and A2, it is known that the ADI A1 = 0.01 mg/kg bw and ADI A2 = 0.1 mg/kg bw. In this example, the case is considered in which the final residue is 95% A2 and 5% A1. In order to sum them up for the risk assessment, the residue of stereoisomer A1 would need to be weighted for its higher toxicity in a risk assessment considering both isomers. Using the method of the Relative Potency Factor (RPF) (EFSA PPR Panel, 2008): –The risk is estimated for the consumption of 1 kg of a commodity with an MRL = 1 mg/kg for the sum of the stereoisomers (established on basis of studies where isomers were not separated), knowing that the likely composition of the residue is 95% of isomer A2 and 5% of isomer A1. –One of the isomers is selected as index compound, e.g. isomer A1. –The RPF of isomer A2 is calculated: RPF A2 = ADI isomer A1/ADI isomer A2 = 0.1 –The combined exposure is calculated corrected by the RPF. Exposure isomer A2 × RPF A2 + Exposure isomer A1 = (1 kg commodity × 1 mg a.s./kg commodity × 0.95 mg isomer A2/mg a.s. × 0.1 (RPF A2)) + (1 kg commodity × 1 mg a.s./kg commodity × 0.05 mg isomer A1/mg a.s.) = 0.145 mg isomer A1 equivalents. –Risk assessment of the corrected exposure is performed against the endpoint of the isomer selected as the index compound, i.e. ADI isomer A1. e.g. Calculation of % ADI for an adult consuming 1 kg of this commodity: % ADI = [0.145 mg isomer A1 equivalents/(60 kg bw × 0.01 mg isomer A1/kg bw)] × 100 = 24% ADI The same result would be obtained in case isomer A2 is selected as the index compound. *Example for ecotoxicology risk assessment* The same method outlined in the toxicology example above can be applied to an ecotoxicological endpoint. A substance as manufactured is a racemic mixture (50:50) of A1 and A2 stereoisomers but degrade with different degradation rates. PEC values and toxicity figures are available for each individual isomer. The available long-term toxicity tests on earthworms indicate that NOEC A1 = 1 mg/kg soil and NOEC A2 = 10 mg/kg soil. The calculated PECsoil for A1 is 0.05 mg/kg soil and the PECsoil for A2 is 0.8 mg/kg soil. –Taking A1 as index compound, then RPF A2 = NOEC A1/NOEC A2 = 0.1. –The combined exposure is calculated by considering the RPF. PECsoil combined = PECsoil A1 + (PECsoil A2 × RPF) = 0.05 mg/kg soil + (0.8 mg/kg soil × 0.1) = 0.13 mg/kg soil. –Risk assessment is performed with the combined exposure against the endpoint of the index compound (A1). TER = NOEC A1/PEC soil combined = 1/0.13 = 7.7