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GUIDANCE DOCUMENT ON THE ASSESSMENT OF THE RELEVANCE OF METABOLITES IN GROUNDWATER OF SUBSTANCES REGULATED UNDER REGULATION (EC) No 1107/2009

This document has been conceived as a working document of the Commission Services, which was elaborated in co-operation with the Member States. It does not intend to produce legally binding effects and by its nature does not prejudice any measure taken by a Member State within the implementation prerogatives or Regulation (EC) No 1107/2009, nor any case law developed with regard to this provision. This document also does not preclude the possibility that the European Court of Justice may give one or another provision direct effect in Member States.

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Version history

Version	Applicable from	What
10	25 February 2003	Original version
11	Applies to dossiers submitted from 1 May 2022 2022 but can be applied earlier by applicants. To note that in any case Rev 10 requires the exclusion of genotoxicity at stage 1 of Step 3 – while a battery of 3 *in vitro* tests is listed that is not in line with the current state of scientific knowledge it must be recalled that these are the minimum requirements.	Stage 2 of Step 3 on the screening for genotoxicity - amended to provide clarification in view of ensuring that the relevant end-points (gene mutation, clastogenicity and aneugenicity) are fully considered. Version 10 of the guidance stated that all metabolites that passed step 1, step 2 and stage 1 of step 3 should be screened for their genotoxic activity and provided the minimum requirements in terms of tests to be submitted. In Version 11 the requirements to fully address genotoxicity were updated. The reference list was updated accordingly. References to classification have been updated to reflect Regulation (EC) No 1272/2008. References to Directive 91/414/EEC have been replaced by the relevant references to Regulation (EC) No 1107/2009 and associated implementing regulations. Obsolete information has been amended or removed. Reference to FOCUS groundwater exposure guidance has been updated. Clarification of how metabolites formed by active substances that are micro-organisms need to be assessed has been added. Corrections to footnotes and formatting have been made.

Contents

1. Introduction

This document, on the assessment of the relevance of metabolites in groundwater, is intended to provide guidance for applicants and Member States in the context of the assessment of active substances under Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market[1]. The document intends to identify a consensus approach in regulatory decision-making concerning the approval of active substances and the authorisation of plant protection products. It does not prejudice the authority of Member States in national authorisations, nor does it prejudice the application of other EU legislation in force. Nonetheless, the document still provides some recommendations, which might be helpful in maintaining harmonised assessment schemes and decision making in Member States.

Metabolites and breakdown products of active substances may occur in many environmental compartments (in particular in soil, surface waters, groundwater and air), in animal feed or in food for human consumers. It is the intention of the Commission Services to cover all of these aspects in guidance documents, which are to be continuously revised to keep the guidance in line with scientific and technical progress as well as regulatory changes. This guidance document focuses on groundwater, though the general approach may also be applicable for the regional management of surface water resources intended for the abstraction of drinking water in Member States.

Separate guidance documents on terrestrial and aquatic ecotoxicology have been produced and will be continuously adapted to scientific and regulatory progress to address the protection of non-target organisms in these environmental compartments These guidance documents address aspects of the ecotoxicology assessment not only for the active substances but also for metabolites or breakdown products, which are formed after their application of the active substance.

Regulation (EC) No 1107/2009 refers to metabolites that are relevant in Article 3(32) and point 9.2.4 in Part A of Regulation (EU) No 284/2013 refers to the need to assess the relevance of metabolites which occur in concentrations above 0,1 µg/L in groundwater.

Points 2.5.1.2 of Part I and 2.7.3 of Part II of the Uniform Principles (Regulation (EU) No 546/2011[2]) set out the conditions for authorisation of plant protection products with regards to contamination of groundwater, in which relevant metabolites are referred to in the former.
The term "*relevant metabolites*" is also used in the Drinking Water[3] and Groundwater[4] Directives, where it is provided that concentrations of pesticides and their relevant metabolites in drinking water and groundwater must not exceed 0.1 µg/L.
Article 3(32) of Regulation (EC) No 1107/2009 provides a definition of when a metabolite should be considered as relevant , however, it is the goal of this document to lay down the operational means of determining relevance insofar as this is necessary for the assessment of active substances under Regulation (EC) No 1107/2009 and to identify a consensus approach towards its application in regulatory decision-making concerning the approval of active substances and authorisation of plant protection products.

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[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*

[2] Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products

[3] Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast) [Formerly Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption]

[4] Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. *OJ L 372, 27.12.2006, p. 19*

In explicitly referring to the term relevant metabolites in the legislation the legislator acknowledges that there may be metabolites that are not relevant. Therefore, the provisions of both the Drinking Water and Groundwater Directives are intended to regulate or place limits on a "relevant" subset of breakdown products in the same way as is done for active substances. Different provisions should apply for "non-relevant"" substances.

Although their relevance in the sense of the present guidance document cannot be excluded a priori, metabolites produced by micro-organisms in general fall outside the scope of the present document unless they are identified as metabolites of concern according to the applicable specific guidance[5], where the risks to the environment, including considerations for groundwater exposure is duly addressed.

This document describes a stepwise scheme, of increasing complexity, to identify "relevant metabolites" for which the limit value of the Groundwater and Drinking Water directives should apply. The document further describes a scheme for the assessment of those metabolites, which are not identified as relevant, but which have to be evaluated previous to a decision on (renewal of) approval of an active substance or authorisation of plant protection products in accordance with Regulation (EC) No 1107/2009.

2. Context and general approach

This guidance document focuses exclusively on the assessment, under Regulation (EC) No 1107/2009, of metabolites and breakdown products of active substances in groundwater, because the term "relevant metabolites" is used for this compartment in a unique legislative context. Issues related to the ecotoxicological aspects of metabolites and breakdown products present in groundwater (relevant since groundwater becomes surface water and thus an environment for aquatic organisms) and all ecotoxicological questions related to other environmental compartments such as soil, surface water and air, are treated in separate guidance documents for terrestrial and aquatic ecotoxicology.
Following the precautionary principle laid down in the Water Framework Directive[6], Groundwater must be regarded as a natural resource, which should be protected in its own right. In consideration of this principle, the limit value of 0.1 µg/L provided in Regulation 284/2013 (and in the Groundwater and Drinking water Directives) for active substances and their relevant metabolites is not solely based on toxicological criteria. A risk assessment on human toxicology therefore cannot be the exclusive basis for a decision as to whether a metabolite has to be considered relevant or not. Furthermore, also for non-relevant metabolites it is considered appropriate that an adequate level of protection is established for groundwater, which takes into account the unique properties of this environmental compartment.

Consequently, this document describes a scheme to determine whether a metabolite is relevant (and thus subject to the 0.1 µg/L limit) or not relevant using criteria of biological activity, genotoxicity and toxicological hazard but also other, pragmatic administrative criteria to allow efficient and transparent regulatory decision-making. If a metabolite is not relevant, it is still subject to other limits, as outlined in detail in this document, and to further case-by-case assessments in-line with the above principle of precaution.

As noted above, this document is intended to provide guidance for decisions concerning (renewal of)

[5] Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances - SANCO/2020/12258

[6] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy - OJ L 327, 22. December 2000, p.1

approval of active substances and authorisation of plant protection products in accordance with Regulation (EC) No 1107/2009.

According to Article 4(2)(a) of that Regulation "t*he residues of the plant protection products, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use, shall not have any harmful effects on……..groundwater"* . Furthermore Annex II point 3.10 states *"An active substance shall only be approved where it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6)."*

This possibility of potential groundwater - or drinking water - contamination is investigated generally on the basis of the convention that a soil layer of approximately 1 m is used to represent the "groundwater" aquifer. Such an assumption is far from representative for all regions of Europe but it is considered to provide a realistic worst case on the European scale. Should, at a future stage, more realistic assessment schemes and models become available for refined assessments at the European scale (e.g. probabilistic assessments based on real groundwater distribution data), this Guidance document will be revised to reflect such a progress.

This document does not prejudice the authority of Member States to grant national authorisations. It is recommended that Member States develop their own national and regional scenarios for the assessment of groundwater contamination to ensure that the limit values provided by EU legislation are respected at the points of abstraction of groundwater. It may also be considered that Member States use the guidance herein in conjunction with monitoring data where available as an orientation where regional or local schemes of resource management are developed to protect surface waters used for the abstraction of drinking water.

The hazard and risk assessment of active substances under Regulation (EC) No 1007/2009 is highly developed and is supplemented by several guidance documents developed at EU level. The scientific assessment of metabolites and degradation products of these active substances should be of comparable transparency, scientific validity and degree of regulatory scrutiny as for the active substances themselves. The scheme described in this document for the evaluation of metabolites is aimed at achieving this goal and is aligned closely with that of the evaluation of active substances. The guiding principle of the assessment is that a metabolite or degradation product is considered relevant, if there is reason to assume that it has comparable intrinsic properties as the active substance in terms of its biological target activity, or that it has certain toxicological properties that are considered severe (i.e. genotoxic, toxic to reproduction, carcinogenic, toxic or very toxic), unless demonstrated to the contrary.

A stepwise procedure is used which aims in the first instance to select those cases which need further consideration (Steps 1 and 2). It then provides guidance on how metabolites should be treated in further, more complex, steps ranging from screening in a hazard assessment to (using further experimental data) a full risk assessment. A decision-tree to visualise the general approach is provided in Section 6.

Much of the data required for metabolites of individual active substances under the scheme may already be available. According to the data requirements for active substances[7], several studies have to be performed by the registrant on the metabolism of the active substance in the different environmental

[7] 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*

compartments where metabolites, degradation and reaction products may be formed. Data requirements concerning soil, which are of particular importance for the assessment of groundwater, are found in Section 7.1 of part A and section 7 of part B of the data requirements for active substances.

Consequently, for all compartments, at least some information on the metabolism, rate, route, and kinetics is available for use in the risk assessment of the active substance and its metabolites and breakdown products. Before performing additional tests, applicants should therefore examine existing studies and check whether metabolites or breakdown products under consideration have already been covered by studies required for the active substance or metabolites that reached levels triggering assessment, considering the use pattern and the fate of the compounds investigated. Based on existing knowledge on related compounds, some fate and effects characteristics of metabolites may also be anticipated and used to extrapolate the required information.

3. Definitions

The following definitions are used in this guidance document:

1. Metabolite: for the purpose of this document, the term is used for all reaction or breakdown products of an active substance of a plant protection product, which are formed in the environment after the application, be it by biotic (microbials, other taxa) or abiotic processes (hydrolysis, photolysis). The terms "metabolite", "breakdown product" and degradation product" are used interchangeably throughout this document.

2. Relevant metabolite: a metabolite for which there is reason to assume that it has comparable intrinsic properties as the active substance in terms of its biological target activity, or that it has certain toxicological properties that are considered severe and unacceptable with regard to the decision-making criteria described in the text. Such a metabolite is therefore treated like the parent active substance in the assessment according to point 9.2.4 of Part A of Regulation (EU) No 284/2013[8]. Where such a metabolite exceeds the maximum permissible concentration (0.1 µg/l) for groundwater for all representative use patterns assessed and for all geoclimatic situations for which the exposure assessment is available, non (renewal of) approval would be triggered at EU level for the active substance or a non-authorisation decision would be triggered at national level for specific uses of products containing that substance;
3. Metabolite of no concern: A metabolite which meets the criteria outlined in Step 1 of Part 4 below and is therefore deemed to be not relevant in the assessment according to point 9.2.4 of Part A of Regulation (EU) No 284/2013.
4. Non-relevant metabolite: a metabolite which does not meet the criteria provided for "relevant metabolites" and "metabolites of no concern". A non-relevant metabolite may be subject, on a case-by-case basis, to an individual groundwater limit concentration, as outlined in detail in this document.

4. Sequential assessment of the relevance of metabolites

As a general rule, all metabolites which are expected to occur in soil under normal use conditions on the

[8] Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, 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. 85–152*

basis of results from soil degradation studies should be subject to further assessments of their structure and environmental fate with the aim of quantitatively assessing their ability to contaminate groundwater. The same assessment should be done for all metabolites found in lysimeter studies, where such studies have been conducted. It is recognised that, for practical reasons and reasons of technical feasibility, it might not be possible to identify those minor metabolites (< 10% of total applied on a molar basis) which occur transiently in soil, or metabolites which are found in very low amounts (< 5 % of total applied on a molar basis) and with no tendency to accumulate. Similar limitations apply to lysimeter studies, where characterisation of metabolites found in small amounts in leachates is also not always feasible.

Therefore, as a minimum, degradation products must be characterised and identified by the applicants to the extent that is technically feasible and their relevance must be assessed, if one of the following conditions applies:

a) Metabolites, which account for more than 10 % of the amount of active substance added in soil at any time during the studies; or
b) which account for more than 5 % of the amount of active substance added in soil in at least two sequential measurements during the studies; or
c) for which at the end of soil degradation studies the maximum of formation is not yet reached.

Moreover, all metabolites found in lysimeter studies at annual average concentrations exceeding 0.1 µg/l in the leachate should be identified and subject to further assessment.

Step 1: Exclusion of degradation products of no concern

This step applies to all metabolites. A degradation product which may be expected to occur in groundwater as a result of a soil degradation study or a lysimeter study will require further assessment unless one of the following conditions apply:

a) it is CO2 or an inorganic compound, not containing a heavy metal; or,
b) it is an organic compound of aliphatic structure, with a chain length of 4 or less, which consists only of C, H, N or O atoms and which has no "alerting structures" such as epoxide, nitrosamine, nitrile or other functional groups of known toxicological concern.
c) it is a substance, which is known to be of no toxicological or ecotoxicological concern, and which is naturally occurring at much higher concentrations in the respective compartment.

If condition a), b) or c) is met, the degradation product is considered to be a degradation product of no concern and no additional data are required.

Step 2: Quantification of potential groundwater contamination

All metabolites not excluded in Step 1 that are found in soil degradation and/or available lysimeter or field leaching studies should in principle be characterised and identified by the applicants to the extent that is technically feasible, as outlined above in the introductory remarks to this chapter. This is particularly the case for those metabolites which are predicted to be present in the leachate leaving the upper soil layer at an annual to triannual average flux (as defined by FOCUS[9]) concentration exceeding 0.1 µg/L. For these metabolites the predicted environmental concentration in groundwater needs to be estimated with the highest feasible accuracy and validity.

[9] European Commission (2014) "Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU" Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 3, 613 pp

To quantitatively assess the fate of these metabolites with the FOCUS groundwater models and scenarios, data on degradation and sorption are required as input. As the required input depends on the intended uses of the active substance under investigation, the FOCUS guidance document should be consulted for further information on extent and quality of input parameters needed. Also expert judgement may be used to estimate the necessary model input parameters on degradation and sorption in cases where experimental data cannot easily be provided. In these cases, a sensitivity analysis should be conducted to allow a judgement to be made on the level of confidence, which can be attributed to the calculations. However, experimental data should preferably be used.

For metabolites found in the leachate of lysimeter studies with annual average concentrations above 0.1 µg/L an attempt should be made to assess their leaching behaviour in other European regions with different soil and climatic conditions with the goal to extrapolate the experimental findings to other representative regions of European agriculture. All efforts should be made to quantitatively assess and identify individual compounds in the leachate fractions, as far as technically feasible. Since some Member States consider lysimeter studies as higher tier compared to model calculations, these provisions will not prejudice decision making on Member State level.

As far as valid and representative data are available for existing active substances, also monitoring data can be used to predict environmental concentrations of metabolites in groundwater. Monitoring data from regions with well-documented use of the active substance in question may provide a useful additional tool to supplement model calculations and lysimeter experiments to improve the accuracy and validity of estimates of potential groundwater contamination.
If, on the basis of these calculations and assessments, representative use-scenarios can be identified which predict no contamination of groundwater by the active substance or individual metabolites in excess of the limit values provided by the Drinking Water Directive or the Uniform Principles, then the active substance is eligible for further consideration for approval if at least one Member State indicates an interest in granting an authorisation.
Nonetheless, consideration should be given to the fact that groundwater is also an ecosystem containing non-target organisms and that surface water bodies may be supplied from groundwater resources and that there are substances which may give reason for ecotoxicological concern at levels even below the default limit value provided for drinking water. This aspect should be considered in the context of the ecotoxicological assessment of the active substance, which is outside the scope of this document.

Active substances, for which not all uses and use conditions reviewed have resulted in acceptable predicted groundwater contamination need to be assessed further by Member States when granting national authorisations. The Review Reports for these substances will highlight this area of potential concern in such cases.

All metabolites, which might be expected to exceed the limits laid down in point 9.2.4 of Pat A of Regulation (EU) No 284/2013 should be further assessed in Step 3. Again, also in these cases the environmental concentration in groundwater needs to be estimated with the highest feasible accuracy and validity, following the principles outlined above.

Step 3: Hazard Assessment: Identification of relevant metabolites

Step 3 provides a pragmatic scheme for the regulatory decision-making concerning the "relevance" of a certain metabolite. It is a 3-stage assessment involving (i) biological activity screening, (ii) genotoxicity hazard screening, and (iii) toxicity hazard screening. Any metabolite that does not pass all three stages is considered as "relevant" under regulatory aspects and thus unacceptable at groundwater contamination levels exceeding 0.1 µg/L. Passing the three stages does not imply non-relevance – it simply means that further assessment in Step 4 is required.

a. Stage 1 of Step 3: Screening for biological activity:

Active substances of plant protection products are defined according to Art. 2 of the Directive on the basis of their biological activity against plants or harmful organisms (in the context of this document defined as the "biological activity"). The same criterion is used here to identify those breakdown products, which – from a regulatory perspective - should be treated in the same way as active substances with respect to groundwater protection.

The goal is to identify metabolites, which have a comparable target activity as the parent active ingredient, and to deal with cases where the parent molecule is a precursor of the active substance. Efficacy testing should be focused on this question of comparing the activity against the biological target. However, for parent compounds with a known range of activities, or for a compound belonging to a totally new group, it may be necessary to test a metabolite in a more extensive screening battery.

Structure-activity relationships may be considered on the basis of the mode of activity of the parent molecule (i.e. usually the active substance). In many cases for compounds belonging to a well defined group of active substances (e.g. sulfonyl thiourea herbicides) this may already provide useful and sufficient information for the assessment of this question in the absence of experimental data.

In cases where the above considerations do not lead to clear results, the metabolite should be evaluated in biological screening assays using standard methods comparing weight equivalents of the parent substance and the metabolites in question. As a possible refinement and if necessary, comparative testing may be done at the maximum application rate and based on a molar equivalent compared to the active substance. The maximum test rate then would be calculated by:

$$Rate_{metabolite} = M/A \bullet Rate_{a.s.}$$

in which:

$Rate_{metabolite}$ = application rate at which metabolite should be tested in screen (kg/ha)
$Rate_{a.s.}$ = use rate of active ingredient (kg/ha)
M = molar mass of metabolite
A = molar mass of active substance

On the basis of this maximum level, the effect of the metabolite against a range of target organisms should be compared to the activity of the parent compound. Metabolites of unknown structure (e.g. fractions from a lysimeter experiment) should be subject to a similar assessment, insofar as this is technically feasible. A case-by-case approach should be followed in this event, which should be developed in close collaboration between the applicant and the Rapporteur Member State. The metabolite(s) in question should be characterised as far as possible to allow an expert judgement on its activity.

In screening assays it will often not be possible to determine and compare the biological activity of a parent molecule and its metabolites with great precision, and this will also not be necessary in most cases. As a line of orientation, it should be sufficient to demonstrate that the biological activity of a metabolite is clearly less than 50% of the activity of the parent molecule. Otherwise the biological activity should be considered as "comparable".

From a regulatory perspective, metabolites with a comparable or higher biological activity than the parent are considered as relevant and must, therefore, not exceed a level of 0.1 µg/L in groundwater as determined according to Step 2.

All other products passing this stage should be further screened in Stage 2.

b. Stage 2 of Step 3: Screening for genotoxicity:

All metabolites that have passed step 1, step 2 and stage 1 of step 3 should be appropriately assessed in order to investigate their genotoxic potential.
For an adequate evaluation of the genotoxic potential of metabolites, the following end-points must be assessed:

- gene mutation (in both bacterial and mammalian cells)
- structural chromosomal alterations (clastogenicity)
- numerical chromosomal alterations (aneugenicity)

A combination of an Ames test, an i*n vitro* Mammalian Cell Gene Mutation Test (*tk* or *hprt* locus) and an *in vitro* micronucleus test fulfils the requirements to cover the genetic endpoints listed above. Any alternative study batteries must fully cover the endpoints listed above.

When choosing the tests to investigate the above endpoints, the latest scientific and technical knowledge should be taken into account[10,11,12].

If there are indications for the metabolite that specific metabolic pathways would be lacking in the standard *in vitro* systems, or it is known that the *in vitro* test system is inappropriate for that substance or for its mode of action, testing may require either appropriate modification of the *in vitro* tests or use of an *in vivo* test.

If all *in vitro* tests are clearly negative in adequately conducted tests, no *in vivo* testing is required.

Equivocal or inconclusive results from *in vitro* tests (or contradictory results if several *in vitro* studies of the same type are available) should be appropriately followed up according to the latest scientific knowledge on genotoxicity - it may be appropriate to conduct further testing *in vitro*, either by repetition of a test already conducted, perhaps under different conditions, or by conducting a different *in vitro* test, to try to obtain reliable and conclusive results for clear interpretation of the genotoxic potential. It may also be necessary to follow up with an appropriate *in-vivo* genotoxicity study.

Positive results of *in vitro* test(s*)* should be followed up with appropriate *in-vivo* test(s) to addresses the concerned endpoint(s). Evidence, either from the test itself or from other toxicokinetic or repeated-dose toxicological studies, that the target tissue(s) have been exposed to the test substance and/or its metabolites is essential for reliability and interpretation of results (EFSA, 2011, 2017).

Genotoxic metabolites are considered relevant based on their hazard.

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[10] EFSA Scientific Committee; Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379. [69 pp.] doi:10.2903/j.efsa.2011.2379. Available online: www.efsa.europa.eu/efsajournal

[11] EFSA Scientific Committee, Hardy, A, Benford, D, Halldorsson, T, Jeger, M, Knutsen, HK, More, S, Naegeli, H, Noteborn, H, Ockleford, C, Ricci, A, Rychen, G, Silano, V, Solecki, R, Turck, D, Younes, M, Aquilina, G, Crebelli, R, Gürtler, R, Hirsch-Ernst, KI, Mosesso, P, Nielsen, E, van Benthem, J, Carfì, M, Georgiadis, N, Maurici, D, Parra Morte, J and Schlatter, J, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. *EFSA Journal* 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113

[12] EFSA Scientific Committee, More SJ, Bampidis V, Bragard C, Halldorsson TI,Hernandez-Jerez AF, Hougaard Bennekou S, Koutsoumanis K, Lambre C, Machera K, Naegeli H, NielsenSS, Schlatter J, Schrenk D, Turck D, Younes M, Aquilina G, Bignami M, Bolognesi C, Crebelli R, G€urtlerR, Marcon F, Nielsen E, Vleminckx C, CarfiM, Martino C, Maurici D, Parra Morte J, Rossi A and BenfordD, 2021. Scientific Opinion on the guidance on aneugenicity assessment. EFSA Journal 2021;19(8):6770, 27 pp.https://doi.org/10.2903/j.efsa.2021.6770

c. Stage 3 of Step 3: Screening for toxicity

Stage 3 of Step 3 is aimed at the question of whether a metabolite has certain toxicological properties, which - from a regulatory perspective - qualify for considering it "relevant". A metabolite is considered "relevant" if its toxicological properties lead to certain classifications according to Regulation (EC) No 1272/2008.[13]

Reflecting the general concept of this document, the toxicity classification of the parent active substance as determined according to Regulation (EC) No 1272/2008 is used for pragmatic reasons as a starting point to focus the screening activity. The Step 3 screening is applied as follows:

For parent active substances, which are classified as Acute Tox. categories 1, 2 or 3, STOT SE1 or STOT RE1[14] , the acute or chronic toxicity of the metabolite must be determined.
Metabolites, which on the basis of an appropriate test qualify as Acute Tox. categories 1, 2 or 3, STOT SE1 or STOT RE1[15] according to Regulation (EC) No 1272/2008 are considered "relevant".

For parent active substances, which are classified for reproductive toxicity (any category: 1A, 1B or 2 according to Regulation (EC) No 1272/2008[16]), it must be shown by an appropriate test or convincing other evidence that the metabolite does not qualify for the same classification. Metabolites, which qualify for a classification of their reproductive toxicity (any category) are considered to be "relevant".

For parent active substances classified as category 1A or category 1B carcinogens according to Regulation (EC) No 1272/2008[17]) all metabolites are considered to be "relevant".

For parent active substances classified as category 2 carcinogens according to Regulation (EC) No 1272/2008[18]), convincing evidence must be provided that the metabolite will not lead to any risk of carcinogenicity. This may be done by appropriate carcinogenicity testing, by the provision of mechanistic evidence (e.g. absence of the likely mechanistic effect leading to carcinogenicity with the parent molecule, such as target organ pathology, peroxisome proliferation, cytochrome P450 induction or metabolism of thyroid hormones) or by a convincing toxicological assessment taking into consideration all available data.

However, independent of the classification of the parent active substance, if there is reason to expect that a certain degradation product may have toxicological hazards of concern, a targeted testing may be necessary.

All metabolites passing stage 3 of step 3 and are not considered as "relevant" are subject to an exposure and/or risk assessment as outlined in the steps below.

Step 4: Exposure assessment - threshold of concern approach

Metabolites which have not been identified as being relevant according to the hazard screening outlined in

[13] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006
[14] Formerly acutely or chronically toxic or very toxic - T followed by R25, R24, R23 or R48, or T+ followed by R28, R27, R26 or R39 according to Directive 67/548/EEC
[15] Formerly toxic or very toxic (T or T+) according to Directive 67/548/EEC
[16] Formerly R60 R61, R62 or R63 under Directive 67/548/EEC
[17] Formerly Carc. Cat. 1; R 45 or Carc. Cat. 2; R 45, respectively under Directive 67/548/EEC
[18] Formerly Carc. Cat. 3; R 40 under Directive 67/548/EEC

Step 3, should be further tested in an exposure assessment to make sure that any contamination of groundwater will not lead to unacceptable exposure of consumers via their drinking water.

Such an assessment, if done in isolation, would require, in principle, a full set of toxicological data in accordance with Commission Regulation (EU) No 283/2013 to ultimately establish an Acceptable Daily Intake value for these substances and is not excluded in Step 5 below. However, as a pragmatic alternative in cases where a full quantitative risk assessment cannot be provided, an approach following a "threshold of concern" should be followed. The approach is based on a statistical evaluation of lifetime carcinogenicity studies for more than 500 substances, which were originally compiled by Gold *et al.* 1989[19] and later supplemented and refined by other authors. Both, the Scientific Committee on Plants[20] and the Scientific Committee on Food[21] have discussed this concept and found that the available scientific information base is sufficiently large to consider an application of a threshold of toxicological concern as a concept, which is rational, pragmatic and scientifically valid.

Following this concept, for substances of unknown structure the Scientific Committee on Plants proposed a toxicological threshold of concern of 1.5 µg/person/day or 0.02 µg/kg body weight/day[22], which is in line with the threshold developed by the US-FDA. Assuming a consumption of 2 liters of water per day[23], all of which comes from the upper soil layer, such an acceptable exposure level relates to an acceptable estimated upper limit for the concentration of a metabolite of 0.75 µg/L.

When carrying out this assessment, it must be checked whether there is potential exposure for consumers via other sources but drinking water, e.g. if the metabolite in question is also found among the residues on treated commodities. Such a potential exposure from other sources should be taken into account in order to ensure that total exposure of consumers to the metabolite will not exceed the acceptable overall threshold of concern of 0.02 µg/kg body weight/day.

Such a threshold can only be considered acceptable if the metabolite in question
- does not exceed 0.75 µg/L (or a lower level, if consumers are exposed also via other routes)

and has passed Step 3 i.e.
- has a lower biological activity than the parent,
- is not genotoxic and
- is not defined as toxic.

Substances for which all metabolites meet all these criteria can be further considered for approval or authorisation. Where there is insufficient information to do a satisfactory assessment at this Step, then a refinement is necessary and further data will be required in Step 5.

Step 5: Refined risk assessments for non-relevant metabolites

Metabolites which have passed steps 1 to 3 and for which levels of estimated concentrations of metabolites in groundwater (as defined in Step 2) lie between 0.75 µg/L (from Step 4) and 10 µg/L[24] will require a

[19] Gold et al. 1989
[20] SCP 2000
[21] SCF, 1996;
[22] Munro *et al.*, 1996; Munro *et al.*, 1999
[23] Which is a conservative value also recommended by WHO (1994).
[24] This limit value of 10 µg/L is selected for pragmatic reasons. It is also the current limit value defined in the Drinking Water Directive for chlorinated aliphatic hydrocarbons such as trichlorethene. Some degradation products of pesticides may belong into this chemical category. Note that some other products may also belong to other defined categories in Drinking Water Directive and are, therefore, subject to a different limit.

refined assessment of their potential toxicological significance for consumers. All such metabolites, which are estimated to occur at levels exceeding the toxicological threshold for unknown substances, must be fully identified and also synthesised by the applicant, if necessary to allow their further testing.

The appropriate strategy for the assessment of these cases has to be developed on a case-by-case basis in collaboration between the applicant and the Rapporteur Member State.

As a general principle, it should be understood that data requirements raised in this context do not always have to be addressed by experimental studies. Applicants may, if possible, address open questions by using available information in support of a scientific and rational assessment. Valuable sources of information include, but are not limited to:

- consideration of molecular structure of the metabolite (active part intact?);
- the occurrence of metabolites in existing tests with the active substance;
- general knowledge on the relationship between the toxicity of metabolites and their parent substances;
- available knowledge on related compounds.

In such cases expert judgement may be used to determine the necessity of requiring additional information.

With regard to human toxicology it should be investigated in particular whether a metabolite was also identified in mammalian laboratory animals and, consequently, has been intrinsically subject to toxicity studies along with the active substance. The occurrence of metabolites, their quantification and the extrapolation of this information to humans should be based on expert judgement, taking into consideration known differences in phase one and two metabolism and assessments of biochemical plausibility. For example, a metabolite formed in soil, which may be present in the 1 m groundwater horizon may be a plausible metabolite in mammals, which is only transient and, therefore, not detectable in significant quantity in laboratory animals. Conjugates formed in biotic processes in the soil may be readily cleaved in the gastro-intestinal tract and may give rise to known metabolites in mammals.

If the metabolite is found in laboratory animals, the acceptable limit in groundwater for this compound may be defined on the basis of existing studies with the active substance.

If a metabolite is not likely to be formed in laboratory animals upon exposure to the active substance, stepwise testing should be conducted to determine the full toxicological profile of the metabolite or to generate enough information to allow a comparison with the toxicology profile of the active substance to be made. The extent of the toxicology testing should be determined by expert judgement on a case-by-case basis. The applicant should always be required to provide justification when a full toxicological profile is not produced. Possible reasons for avoiding unnecessary testing include the use of existing information on alerting structures (SAR's[25]), or toxicological information derived from structurally related chemicals.

For non-genotoxic substances, and in the absence of any particular alerting chemical structures in the metabolite, an extrapolation from subchronic to chronic study results will be possible in most cases. It is largely agreed in the scientific community[26] that an extra assessment factor of 10 will be adequate to cover the additional uncertainty when an acceptable daily intake for consumers is derived from a 90-day study result.

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[25] Structure-activity relationships.

[26] Lewis *et al.* 1990 A new approach to deriving community exposure guidelines from "no-observed-adverse-effect levels". Regul Toxicol Pharmacol11: 314-330.

As provided above for step 4, also in step 5 the question must be addressed whether there are other sources of exposure for consumers but groundwater. The permissible exposure of consumers via water is calculated on the basis of a daily consumption of 2 L/day and taking into account exposure from all other routes, if appropriate.

Where actual or predicted concentrations of a non-relevant metabolite in groundwater exceed 10 µg/L, no general guidance can be provided in the context of this document. As outlined in the introduction, regulatory decisions must maintain a high level of protection for groundwater. Therefore, it is necessary to carefully evaluate case by case, whether the requirements of Article 4 of Regulation (EC) No 1107/2009 are still fulfilled and the active substance can be approved or not. Such an assessment must consider the overall profile and use pattern of the substance and it must be based on strict precaution.

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6. Decision Tree