



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

17 March 2016
EMA/CVMP/ERA/603511/2015
Committee for Medicinal Products for Veterinary Use (CVMP)

Overview of comments received during the public consultation on 'Reflection paper on poorly extractable and/or non-radiolabelled substances' (EMA/CVMP/ERA/349254/2014)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	Blue Frog Scientific
2	Fraunhofer Institute for Molecular Biology and Applied Ecology
3	Federal Environment Agency of Germany (UBA)



1. General comments – overview

Comment	Stakeholder no.	General comment (if any)	Outcome (if applicable)
1	1	<p>Thank for very much for the efforts to prepare this reflection paper on poorly extractable substances in soil.</p> <p>The definitions from ECETOC (2013), i.e. BR and NER are not generally accepted in the scientific community and should not be used in this reflection paper as general discussion basis. In literature, BR and NER are used differently depending on the respective authors and the differentiation is also not relevant during the environmental assessment of VMPs at the moment. We suggest only differentiating between ER and NER, whereas NER stands for all un-extracted / not-extractable residues.</p>	<p>Thank you for your comment.</p> <p>We agree that in the scientific community, bound residue (BR) and non-extractable residue (NER) are used differently depending on the authors. Whichever definition would be chosen, there will always be part of the scientific community which would think it should be otherwise. However, for this paper a working definition is needed, and we decided to use the ECETOC definition. This definition was agreed on during a workshop where industry, scientists and regulators were present.</p> <p>We do need to distinguish between BR and NER, since it is very relevant to know what fraction is irreversibly bound (and can be assumed to be degraded), and what fraction may still be extracted.</p>
2	2	<p>The reflection paper very well summarise the problems which might occur if no 14C-radiolabelled substance is available for an OECD 307 test.</p> <p>A reliable answer how to deal with substances which show a recovery of e.g. <25% at t=0 and where no radiolabel is available, is missing. The last chapter should be more detailed with regard to that point. In addition, a discussion on trigger values for the recovery below which the performance of OECD 307 might not give meaningful and interpretable results should be stimulated.</p>	<p>Thank you for your comment.</p> <p>In principle, almost all compounds can be radiolabelled. Thus, when a test is performed without a radiolabelled substance and recovery is low, the test could be re-done with a radiolabelled compound. Even if a particular radiolabelled compound is not commercially available, they often can be synthesized in sufficient quantities for reasonable prices. (See comment below). Text in chapter 2 has been added in relation to this point.</p> <p>In relation to the minimum recovery of 70%, section 3 of the paper addresses this point.</p>

Comment	Stakeholder no.	General comment (if any)	Outcome (if applicable)
3	3	Thank you for the reflection paper, which gives relevant information how to deal with poorly extractable and non-radiolabelled substances.	Thank you for your comment.

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
23	1	Add "often" between "are" and "very".	Agreed
	1	Change 14C to radiolabelled. Comment: Even if compounds are not commercially available with 14C label, they often can be synthesized in sufficient quantities for less than 20,000 Euro.	Agreed. Sentence about costs is added to Chapter 2.
30 - Note box	1	Change "identification of metabolites" to "identification of metabolites and transformation products". Change "metabolic pathway" to "transformation pathway" since in OECD 307 rather the fate in soils (transformation) and not in bodies (animal metabolism) is investigated.	Please see answer to comment below. Metabolites is changed into 'transformation products', and metabolic pathway is changed into 'transformation pathway' in line with OECD 307.
30ff	3	Comment: The term 'metabolites' should be substituted to stay in line with the wording of the OECD 307 and to avoid confusion with veterinary metabolites from metabolisms/excretion studies. Proposed change (if any): Please replace 'metabolite' by 'transformation product' and 'metabolic' by 'transformation' (pathway).	Agreed. Metabolites is changed into 'transformation products'. Metabolic pathway is changed into 'transformation pathway'.

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39-40	2	<p>Comment: without radiolabel mass balances are impossible if bound residues occur.</p> <p>Proposed change (if any): <i>However, a radiolabelled analyte is strongly recommended as it makes several requirements easier to accomplish or is even necessary to enable requirements, such as a complete mass balance at all sampling points</i></p>	Agreed. Text is changed.
41	1	Change "bound residues" to "non-extractable residues".	Agreed. Text is changed.
41	3	<p>Comment: The term 'bound residues' is not clearly defined in the scientific literature therefore we propose to use the term 'non-extractable residues (NER)' which includes the total non-extractable fraction.</p> <p>Proposed change (if any): Please replace 'bound residues' by 'non-extractable residues (NER)'.</p>	Proposal not accepted. See the answer to the first comment at 'General comments' section.
43-44	2	<p>Comment: the recovery of 70%-110% for non-labelled substances cannot be required for <u>each sampling point!</u> This is a quality criteria for the initial sampling (0 day) only. If the amount of parent falls below 70% due to degradation over time, the recovery cannot be 70% if transformation products are unknown or no analytical standards are available.</p> <p>In line 89 of the reflection paper it is stated in the right way.</p>	Agreed. Text is changed.

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		Proposed change (if any): <i>is not considered to be problematic if 70-110% of the added substance can be accounted for at the start of the experiment.</i>	
55	1	Change "should be" to "has to be".	Agreed. Text is changed.
55-56 (box)	2	<p>Comment: Definitions are not in line with OECD 307! In 307 chapter 55, "results" it says "<i>characterisation of non-extractable (bound) radioactivity or residues in soil</i>".</p> <p>It does not clarify but lead to maximum confusion if the "non-extractable residue NER" represents a fraction which can be determined by (harsh) extraction. NER is a well-accepted term in pesticide regulation – where the OECD 307 is applied since many years. An alternative (and more reasonable) definition is given e.g. in the EFSA Journal 2015;13(7):4175, Figure 2 on page 18.</p> <p>Proposed change (if any): change definitions according to EFSA Journal Figure 2, page 18 http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4175.pdf to avoid confusion when working according to OECD 307</p>	<p>Proposed change not agreed. OECD 307 was specifically designed to test pesticides, in which framework NER is assumed to be equal to BR. Scientific evidence suggests that that is often not the case, as the amount of NER strongly depends on the extraction method used.</p> <p>See comment above at general comments about the use of the definitions.</p> <p>Some more explanation is added to the definitions box.</p>
55-56	2	Comment: ASE is a registered trademark of the company Dionex, today Thermo. It should be avoided to put registered trademarks in guidance. In e.g. ISO	Agreed. Text is changed.

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		<p>standards as an alternative this technique is termed "PLE pressurized liquid extraction".</p> <p>Proposed change (if any): change ASE accelerated solvent extraction to PLE pressurized liquid extraction (see line 96).</p>	
55-56 (Definitions)	3	<p>Comment: The definitions from ECETOC (2013) are not generally accepted and are used interchangeably as written below. They should not be part of this reflection paper that deals with poorly extractable and non-radiolabelled substances and not with establishing a scheme for differentiating the strength of association of different up to now undefined fractions with the matrix. Therefore the differentiation between NER and bound residues should be avoided and only the term non-extractable residues should be used, which means all not extracted/ not-extractable fractions. The differentiation between reversible and irreversible bound residues is not as clear as defined by ECETOC. The distinction is not technically feasible at the moment. Furthermore the differentiation into NER and BR is not relevant for the environmental risk assessment of VMPS according to the current guidelines.</p> <p>Proposed change (if any): Delete the definitions by ECETOC and avoid the term 'bound residues'.</p>	<p>Disagree. In this reflection paper, a working definition is needed to be able to distinguish between NER and BR, as NER reflects the analytical procedure and BR the chemical state of the compound. Currently, no method can distinguish between the two. This is especially important since it shows the importance of using the right extraction method, so the amount of NER is as close the actual amount of BR as possible. This information is needed to calculate the degradation half life. When the amount of NER is (too) high because of a bad extraction method, this influences the degradation half life and thus this is very relevant.</p>
59	1	Change "bound residue (BR) fraction" to " non-	Agreed. Text is changed.

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		extractable fraction”.	
59	3	Comment: see below Proposed change (if any): To determine the non-extractable residues the best available extraction techniques should be used.	Agreed. Text is changed.
64-66	2	Comment: does that mean that at testing with non-radiolabelled substances in the future the difference between initially applied and analytically recovered substance amount will be considered as BR in the assessment? Proposed change (if any): please clarify. Please also indicate how BR (considered as difference between initially applied and analytically recovered substance) will be evaluated in the context of risk assessment.	Thank you for your comment. The sentence is considered to confusing and thus has been deleted.
64-66	3	Comment: It cannot be assumed that the whole fraction not accounted for in a non-radiolabelled study is irreversibly bound residue. Proposed change (if any): The term BR should be replaced by NER.	Thank you for your comment. The sentence is considered to confusing and thus has been deleted.
66	1	Change “BR” to “NER”.	Thank you for your comment. The sentence is considered to confusing and thus has been deleted.
72	1	Change to “the compound is transformed to CO ₂ ...”. add “or volatile transformation products” after CO ₂ . In addition to CO ₂ also volatile transformation products	Agreed. Text is changed.

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		<p>which can be formed during the testing will not be observed.</p> <p>Delete "BR".</p>	
72	3	<p>Comment: see above</p> <p>Proposed change (if any): Delete BR</p>	Agreed.
73-76	2	<p>Comment: According to the given definition BR are often bound with covalent bonds to the matrix. In this case the parent substance does not exist anymore. Adding of e.g. an OH group to an organic molecule change its properties significantly. Formation of covalent bonds changes a substance – ethanol cannot be compared with ethene. Thus, it is not plausible why formation of BR leads to underestimation of persistency.</p> <p>Proposed change (if any): Delete the sentences. Information cannot be generalized and may lead to wrong conclusions.</p>	Agreed. The sentence has been re-written for clarification.
75	1	Change "BR" to "NER".	Not accepted. The reference to BR is correct in this sentence.
75-77	3	<p>Comment: The sentence 'If it is assumed that the BR fraction has been transformed, the persistency of the compound will be (highly) underestimated' is misunderstanding.</p> <p>Proposed change (if any): Please delete this sentence.</p>	The sentence has been re-written for clarification.
76ff	1	This is not true! Often transformation products (TPs) of VMP are known and hence they could also be traced and	Partially agreed. The word 'often' is added.

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		<p>quantified (if the transformation product is commercially available) during the OECD 307 study. In addition also using non-labelled compounds, the formation the transformation products and even the elucidation of the structure of previously unknown TPs can be achieved when using the appropriate techniques (Blank incubation, HRMS, MSⁿ ...).</p> <p>Please weaken this sentence and state that additional effort is needed to gather information about TPs when using non-labelled substances.</p>	
83ff	1	<p>This is not true: CO₂-formation (or at least the combined formation of volatile TPs and CO₂) can also be traced when using ³H labelled compounds if additional techniques are used. For example a cooling trap and a subsequent water trap can be feasible (e.g. used by Prasse et al., Chemosphere 77 (2009) 1321–1325). Please correct this statement.</p> <p>Nevertheless, we prefer ¹⁴C labelling!</p>	<p>As CO₂ does not contain any H-atoms, it can of course be traced but no radioactivity will be in the CO₂ trap. Sentence is not changed.</p>
95ff	1	<p>In our opinion ASE and PLE represent the same extraction technique, namely the extraction from the solid matrix under an elevated temperature and an elevated pressure. Hence, only one of the two terms should be mentioned.</p> <p>Please correct.</p>	<p>Agreed. Reference to the ASE method is removed.</p>

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96	2	<p>Comment: PLE and ASE is the same technique</p> <p>Proposed change (if any): delete ASE for the above mentioned reasons.</p>	Agreed. Reference to the ASE method is removed.
98ff	1	<p>Not only the sample preparation techniques, but also the detection methods have to be optimized. For example, when using MS as detector, e.g. the ionisation process has to be optimized. As sorptive compounds are often rather apolar, techniques such as APCI or APPI might be used preferably to the commonly used ESI-interface.</p> <p>Results of these tests for the optimization of the MS detection method should also be reported.</p>	Thanks for your comment. However, this is not considered a detection problem, but a recovery problem. Detection is normally calibrated to 100%. The text has not been changed.
101	2	<p>Comment: not clear if 10 µg/kg or 1% of the starting concentration. In some guidelines it is stated "<i>whichever is lower</i>" leading to unreasonable additional work if 1% differs in orders of magnitude from 10 µg/kg.</p> <p>Proposed change (if any): a clear guidance is needed! When 10µ/kg and when 1%? At which starting concentration range applies which value?</p>	Proposal not agreed, as it is considered that the text clearly states that it should be the lowest of these two values. The text has not been changed.
112	1	Change "if CO ₂ if formed" to "if CO ₂ is formed".	Agreed.
115ff	1	<p>We do not agree with the conclusion!</p> <p>What about transformation products? TPs can also account for the missing parts in the mass balance and</p>	Agreed. Text is changed.

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		not all has to be assigned to NER.	
116-117	2	<p>Comment: metabolites may require different extraction procedures / solvents compared to the parent. Thus, missing portions from the mass balance can mean transformation but must not be BR. Cannot be determined without radiolabel.</p> <p>Proposed change (if any): <i>not extracted, it is missing from the mass balance and it can be assumed to be bound or to be degraded to a product which cannot be extracted by the applied procedure.</i></p>	Agreed. Text is changed.
123ff	1	We again suggest not to differentiate between NER and BR.	Proposal not accepted; as explained above – General comments.
123-125	3	<p>Comment: We do not support the differentiation in NER and BR (see above).</p> <p>Proposed change (if any): The sentence 'Second, when it has been demonstrated that -apparently- the sorption of the analytes to the matrix (i.e. soil) is strong and/or irreversible, the non-extractable portion could be considered 'bound residue' should be deleted.</p>	Not agreed with the proposal (see above). However, the text has been changed to clarify this better. It is important to state that the non-extractable residue can only be assumed to be bound residue when the best available extraction method has been used.
130ff	1	Another point against is the difficulty to provide standardized manure for all testing. In contrast to soils (e.g. Eurosoil, Lufa), sampling, preservation and shipping of stable, standardized manure is not feasible. Therefore, adding "any manure" adds an extreme	Agreed. Text is changed.

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		<p>amount of uncertainty and variability to a necessarily standardized test system such as OECD 307.</p> <p>Please add this point.</p>	
130-136	2	<p>Comment: the degradation / NER formation in manure should be considered in the exposure assessment for soils then.</p> <p>The increase of microbial activity by manure application might lead to dramatic changes in degradation kinetics. Might also be lower degradation rate by reduced substance availability for microorganisms.</p> <p>Proposed change (if any): one soil could be tested additionally with both manure and no manure application to estimate the influence of the increased microbial activity / content of organic matter on the degradation kinetic of the substance in soils.</p>	Disagreed, the text already states that using manure is unrealistic for this test.
132	1	Delete "BR".	Agreed. BR is deleted.
132	3	<p>Comment: see below</p> <p>Proposed change (if any): Delete BR</p>	<p>Agreed. BR is deleted.</p> <p>(Note no additional comment was listed below, as indicated).</p>