24 February 2017
EMA/CHMP/CVMP/JEG-3Rs/25975/2015
Committee for Medicinal Products for Human Use (CHMP)
Committee for Medicinal Products for Veterinary Use (CVMP)

Overview of comments received on ' Guideline on regulatory acceptance of 3R (replacement, reduction, refinement) testing approaches' (EMA/CHMP/CVMP/JEG-3Rs/450091/2012)

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

Stakeholder no.	Name of organisation or individual
1	Apotex Inc.
2	Cell Therapy Catapult
3	European Coalition to End Animal Experiments (ECEAE)
4	EFPIA – European Federation of Pharmaceutical Industries and Associations
5	EGAN - Patient Network for Medical Research and Health
6	Medicines Evaluation Board, The Netherlands
7	AESGP - Association of the European Self-Medication Industry
8	Sanofi

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1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)
1	No comments	-
2	The Cell Therapy Catapult both welcomes and supports the generation of these guidelines detailing the submission and evaluation process for testing approaches in line with the 3Rs. There are no specific comments on the document at this time.	Noted
3	The concept paper suggesting revision of the Position paper on Replacement of Animal Studies by in vitro Methods (CPMP/SWP/728/95) was first published for comment in March 2011. We are extremely disappointed that it has taken three and a half years to draft this guidance, particularly as it has little substance and adds nothing to understanding of how the EMA will access and accept alternative approaches in a practical sense. The draft currently fails to give clear guidance on the process for submission of alternative test methods and how these will be evaluated by the Agency. Under Scope it states, "This guideline describes the process for submission and evaluation of a proposal for regulatory acceptance of 3R testing approaches for use in the development and quality control during production of human and veterinary medicinal products."- it does no such thing. The crux of the practical detail appears to be in the EMA guideline; Qualification of novel methodologies for drug development: guidance to applicants. However it is not obvious if this process is entirely appropriate for the submission of alternative methods and whether any adaptations are needed such as waiving of fees and revision of	The practical process resides indeed within the CHMP SAWP procedure for qualification of novel methodologies for drug development. The process is considered entirely appropriate for this purpose. The discussion related to fees is not the subject of this

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	EMA guidelines. Nor is it clear how this relates to submission of methods that have been validated formally via ECVAM or group consortia (see specific comments). The entirety of section 6. Regulatory acceptance of 3R testing approaches –the key part of the document-needs re writing with a particular focus on section 6.3. Particular revision of section 6.3 Criteria for regulatory acceptance of 3R testing approaches is required. It confuses validation with regulatory acceptance, it fails to define validation, the requirements for validation or how the regulatory acceptance process will proceed. A large part of section 6.3.2 Regulatory acceptance following formal validation actually discusses the process of validation (the subject of section 6.3.1) and fails to properly address how regulatory acceptance is achieved. The text under section 6.3 uses three points to describe the same thing, validation. We attach our recent publication called ADAPT which seeks to highlight to regulatory bodies that these processes are in fact different and need to be adequately addressed. We do not feel the document currently does this.	Guideline. Section 6.3 (5.3. & 5.4. revised GL) has been restructured in order to adequately (and separately) address the requirements for validation (5.4.) and the criteria for regulatory acceptance (5.3.).
	We are extremely concerned about the use of the 'safe harbour concept'. We feel the concept undermines the established concept of validation and is at odds to how validation is currently done in the chemical and cosmetics sectors. Appropriate documents are already referred to in the guideline. None refer to the safe harbour concept. We are aware that the concept has been recently employed in the revision of the ICH S1 guidance on Carcinogenicity testing. We opposed the approach there because there was already considerable retrospective 'validation' of the redundancy of the rodent cancer bioassay. Whilst we understand the nervousness towards new	With regards to the safe harbour process the term "safe harbour" has been deleted but the concept of voluntary submission of data obtained by using a new 3Rs testing approach in parallel with data generated using existing methods has been kept (5.4.3.). In addition, it has been clarified that this process is not meant as a routine add-on to standard validation but may be considered on a case-by-case basis in certain situations only.

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	methods and the importance of ensuring that they are fit for purpose, particularly for human pharmaceuticals, we feel the safe harbour concept is excessive in most situations. The document does not explain where the concept has come from nor explain why it is necessary. The only justification appears to be the need to establish validity in 'real life'. This misunderstands the validation process (described below). The most commonly employed methods of validation are retrospective and prospective validation. Safe harbour is a kind of prospective validation. Most common forms of prospective validation do not require side by side evaluation but consist of a single study (or more if the single study is inadequate) where the performance of the new method is assessed using substances that have been tested previously. In this sense there is an element of retrospection to the validation but this does not affect its robustness. The data is collected from the literature or in house data files. This is the most common method for validation of in vitro test methods in the agricultural, chemical and cosmetics sectors. Validation studies performed this way include the reconstituted human skin and eye irritation methods validated by ECVAM. This type of validation avoids the new use of animals as the data is already there. There is no obvious reason why this kind of validation cannot apply to the pharmaceutical sector. Retrospective validation can also be used. This is usually a desk based analysis of whether certain tests (or parameters within tests) were in retrospect useful. This involves looking at published, or in house data from the established test for a large number of substances. The test result is then compared usually with the outcome in real life, whether this was a regulatory decision, a	

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	classification, effects in seen in humans or in house company decision on the progress of the substance through their pipeline. Obvious examples in the pharmaceuticals sector include the removal of the single dose study and the redundancy to the rodent cancer bioassay. The safe harbour concept is of concern because it appears to be an additional validation to these methods described, certainly as far as the current version of the document appears. It is extremely unlikely that test method developers will not start the 'dual submission' phase without at least some validation already. The safe harbour validation process could therefore seek to further extend the time during which the new method is not in common use. Assuming the new method is better than the existing method in terms of predicting human outcomes then extending time before this method is in common use is of public health concern. We feel that it is likely that a safe harbour validation is likely to take much longer than a proper validation as typically performed, as the numbers of drug being submitted to the EMA remain relatively low and the test developers will already have done some validation, which could just be extended if considered inadequate by current standards. We are also concerned that by its very nature the safe harbour rule will lead to increased animal testing. Normal validations do not require additional use of animals due to the availability of existing data on similar substances. Assuming that the use of this concept will also delay the validation and regulatory acceptance process then further animals will be used unnecessarily as other pharmaceutical companies wait for regulatory acceptance. Furthermore, it is really not clear how a safe harbour approach could be used in practice in terms of refinement or reduction methods- do	

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	you seriously think companies will conduct two animal tests? And it is unnecessary in terms of in vitro methods or to demonstrate redundancy as these can be done using existing data. Assuming the validation is sufficient, e.g. a wide enough applicability domain and number of substances tested, we see no obvious reason why the safe harbour concept is needed. We understand that some side by side assessment may be required for biological batch tests as the batches vary so retrospective validation may not be possible. The document needs to be really clear about the sort of scenarios a safe harbour rule is likely to apply and it needs to justify this to the wider community. We did anticipate that the guideline would include a list of common alternative approaches for standard requirements. However we are comfortable with the idea that this sits separately to this document, particularly as it is hoped that the list would be a living document. However we do request that this list is provided as soon as possible. The tone of the guideline could be more positive and encouraging towards alternative approaches and actually seek to encourage submission of new approaches.	The annex referred to in the draft GL became two stand-alone reflection Papers for human and veterinary regulatory testing requirements to allow for updating in accordance with revision of the respective guidelines. Reflection Papers on regulatory testing requirements have been published for public consultation: • Draft Reflection paper providing an overview of the current regulatory testing requirements for veterinary medicinal & products and opportunities for implementation of the 3Rs • Draft Reflection paper providing an overview of the current regulatory testing requirements for human medicinal products and opportunities for implementation of the 3Rs
	We have requested before that the EMA considers a fee waiver for scientific advice (cf. The Guideline on Qualification of Novel	The request for fee waiver for scientific advice for qualification of 3R testing approaches is acknowledged but

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	Methodologies for Drug Development) and we encourage this to be considered again. There are some footnotes in the document, in the main text they need to be identified by the use of a super script.	not the aim of the current guideline. Accepted.
4	EFPIA welcomes the opportunity to comment on the draft "Guideline on regulatory acceptance of 3R (replacement, 5 reduction, refinement) testing approaches". We fully support this initiative to provide mechanisms for acceptance of alternative testing strategies. It will be beneficial both for stakeholders and animal welfare if delays in acceptance are minimised or eliminated. Therefore, EFPIA is generally supportive of the approaches outlined in the draft guideline. EFPIA would like to emphasize the importance of alignment of regulatory authorities globally (as referred to in Section 1 lines 54-57). Scenarios in which an improved testing paradigm is accepted in Europe and not accepted in another part of the world will lead to duplication of work and loss of any overall gain with respect to animal use considerations. EFPIA would like to emphasize that proper scientific validation should precede regulatory acceptance. Validation is a complex and long process that has to be anticipated by all interested parties. EFPIA also emphasizes that all three components (Replacement, Reduction, and Refinement) are critical aspects. With respect to method development and validation, there is at times an excessive focus on replacement and it is critical to consider all three aspects.	Noted.
5	The Patient's Network for Medical Research and Health (EGAN) is an alliance of both national Genetic Alliances and European disease specific patient organisations with a special interest in genetics,	Noted.

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	genomics and biotechnology. EGAN is a working voice in research and health policy and seeks a world in which genetic and other serious diseases are understood, effectively treated, prevented and the people affected supported. Patients are the direct beneficiaries of medical research; their voice and opinions should therefore be valued in discussions on this topic. The use of animals in medical research is a vital stage in the development of treatments for patients with unmet medical needs. When properly regulated animal research is necessary and EGAN supports this practice where it is essential for enabling much needed medical innovation. Investment into the 3Rs: Refinement, Reduction & Replacement, must be implemented and supported to limit the use of animals in research wherever this is practical. Implementation of the 3Rs also helps to stimulate the discovery of new methods, so that the use of animals in research can be replaced as alternative methods that have similar or more optimal capabilities are found. We support the development of a proportionate, appropriate and transparent regulatory framework for the use of animals in research that is designed with proper consideration of the reasons why such research is needed. This regulatory framework should not compromise the safety of products that may emerge, and not halt progress towards novel therapies. Therefore, EGAN supports the EMA's decision to put into place such a framework in the form of a regulatory testing guideline for the 3Rs, which includes the necessary flexibility in terms of accepting new, effective approaches that become available in the future.	
6	The MEB welcomes this draft guideline and supports the efforts that	It is expressed in the GL (e.g. 5.3.1.) that a new 3R

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	are made and hopefully will be made to Reduce, Refine and Replace the use of animals on the basis of this guidance. The emphasis of the guideline appears to be on validation of new approaches, which is considered an important step. However, rather little information is provided on criteria that should reveal whether a new 3R approach is as good as or better that existing methods. It may be possible that a new method has greater predictivity than an old (animal experiment) method. It should be made clear that in such cases a new approach will not be rejected because it compares poorly to the old method.	approach should be at least as useful as, and preferably better than existing methods (in the latter case concordance with the established standard would be necessarily imperfect but accepted as evidence of superiority).
7	In order to assure oversight as to which 3R updates in regulatory and VICH documents are in progress, a list of proposed changes across such documents would be helpful. This will ease oversight and allow visualising the consistency of 3R improvement implementation in such documents and across the regulatory document network. It will also show as to whether various (older) legal documents with 3R relevance have sufficient up-to-dateness to the demands of Dir 2010/63.	The annex referred to in the draft GL became two stand-alone reflection Papers for human and veterinary regulatory testing requirements to allow for updating in accordance with revision of the respective guidelines. Reflection Papers on regulatory testing requirements have been published for public consultation: • Draft Reflection paper providing an overview of the current regulatory testing requirements for veterinary medicinal & products and opportunities for implementation of the 3Rs • Draft Reflection paper providing an overview of the current regulatory testing requirements for human medicinal products and opportunities for implementation of the 3Rs
	Is not only the availability of a defined “formal” acceptance process that fosters the regulatory agreement to new 3R testing approaches, as e.g. the inclusion of 3Rs into scientific advice. But it is also the	The plea for involvement of regulators into collaborative approaches is acknowledged and certainly encouraged, however, not the aim of the current guideline.

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	early “informal” involvement of authorities to discuss 3R proposals and strategies to start testing such in collaborative approaches (e.g. like in EPAA, IMI) before they reach the ‘”formal” alignment and development of incentives (reduced fees, faster evaluation, etc.) for the industry. This should be encouraged more. When conceptual options for implementation of 3Rs solutions are discussed prior to the establishment of testing program (not only bilateral between authorities and company) and including all stakeholders (such in case of EPAA or industry initiatives as IMI) this will allow incremental approximation to what is considered to be acceptable, hence increase confidence of parties for acceptance, increasing the probability and reliability of investments for the specific alternative to be made. Mechanisms, incentives, leverages need to be incorporated into regulatory acceptance process by EMA (and into this document) that do not only take into consideration already established alternative methods, but also ask for efforts and proofs for 3R realisation, to get such marketing authorisation. This could include waving/reducing fees for marketing authorisation with certain 3R contribution.	The request for incentives and leverages for regulatory acceptance of 3R testing approaches is acknowledged but not the aim of the current guideline.
8	Thank you for the opportunity to submit our comments on this Regulatory guideline. Previous main comment on international acceptance has been partially included. Alignment between EU and the rest of the world is critical. Indeed, even if there are alternatives enforced in EU, we can still be obliged to ‘do testing outside Europe to support international development of release tests’. Sufficient time should be allowed for development and	

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	validation. A general comment on this guideline is that it is very focused on approved product testing and replacement, reduction, or refinement of animal tests with validated in vitro tests. It therefore does not address the product development phase where (in vitro alternate to animal) tests are not all validated (at least in the early phases of clinical trial development) and may be used for clinical trial applications, safety assessments, and quality control (potency). It would be beneficial to include a section or statement on the consistency approach to manufacture as an overall approach to replacement of animal-based assays. By implementing a battery of well-characterized in vitro assays, predictive of critical quality attributes, throughout the manufacture of the drug substances/drug products, this approach could provide much better overall control of the product than end-point animal based testing. So rather than viewing replacement as a one-to-one exercise, there should be a view to a holistic approach. There is just one sentence (line 56) describing cross sectorial regulatory acceptance. As many medicinal drug manufacturers are global in nature, there is a real need for more collaboration across regulatory jurisdictions to harmonize and streamline processes, evaluation and acceptance for regulatory testing. This is needed to further the 3Rs movement in a meaningful way, in order	The guidelines concerned provide recommendations for testing requirements for VMPs and HMPs in clinical trials as well as MAAs. Drug discovery is not part of regulatory testing and as such not covered. The revised GL now includes a paragraph on the value of the consistency approach (Section 1 ,line 85-91). Agreed.

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	to prevent duplication of testing/efforts for same products. The manufacturer of the product should build and lead an annual operational 3R program. This program should be discussed with authorities and/or experts during the early stages of the in vitro methods development in order to substitute in vivo test by appropriate methods.	Acknowledged.

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Lines 48-49	3	Comment: "Ethical and animal welfare considerations require that animal use is limited as much as possible." More positive language is required. The Directive 2010/63 requires that animal testing is avoided if there is an alternative method. Proposed change: Ethical and animal welfare considerations require that animal use is limited, <u>if not avoided</u>, as much as possible."	Accepted. Included in line 65 (animal use is limited, and preferably avoided).
Lines 54 and 59	4	Comment: Agency abbreviations (eg. EDQA, EPAA (V) ICH etc.) should be spelled out. Proposed change: Define acronyms	Accepted Acronyms defined throughout the document where considered necessary e.g. European Directorate for the Quality of Medicines and Healthcare (EDQM) line 75 & 76
Lines 54-57	1	Comment: Is there a reason to have a harmonised approach between these different initiatives and organizations?	Section removed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: Further clarification requested.	
Lines 54-57	3	Comment: "Various large scale international initiatives and organisations (e.g. EDQM, EPAA, EURL ECVAM, ICCVAM/NICEATM, JACVAM, OECD) are involved either directly or indirectly in the development, validation and dissemination of 3R testing approaches. In addition some initiatives attempt to foster cross-sectorial regulatory acceptance." This is a bit over general and could be a bit more helpful by explaining what these organisations do. Proposed change: "Various ~~large scale international initiatives and~~ organisations are involved in the validation of alternative methods (e.g. EDQM for quality control methods, ~~EPAA~~, EURL ECVAM in Europe, ICCVAM/NICEATM in the USA, JACVAM in Japan. The OECD is also ~~are~~ involved either directly or indirectly in the development, validation and dissemination of 3R testing approaches. In addition the EPAA in Europe has some initiatives to attempt to foster cross-sectorial regulatory acceptance."	Noted. Section removed from final GL as considered not particular relevant to the revised EU GL. The role of international initiatives and organisations is recognised and acknowledged but the GL focuses on the regulatory acceptance of 3Rs testing approaches for EU regulatory activities
Lines 56-57	7	Proposed change : "In addition some initiatives attempt to foster cross-	Section removed from final GL as considered not particular relevant to the revised EU GL. The role of international

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		sectorial regulatory acceptance (e.g. Innovative Medicines Initiative 2)."	initiatives and organisations is recognised and acknowledged but the GL focuses on the regulatory acceptance of 3Rs testing approaches for EU regulatory activities
Line 57	8	Comment: It would be interesting to mention examples of "cross sectorial regulatory acceptance".	Section removed from final GL as considered not particular relevant to the revised EU GL. The role of international initiatives and organisations is recognised and acknowledged but the GL focuses on the regulatory acceptance of 3Rs testing approaches for EU regulatory activities
Lines 58-59	3	Comment: "The application of all 3Rs is currently embedded in the drafting process of non-clinical regulatory 58 guidance both at the European and at (V)ICH level." This needs qualification (i.e. proof) with a reference perhaps to a public statement. Proposed change: Insert reference	Reference is made to tabulated overview of guidelines in Reflection Papers providing an overview of the current regulatory testing requirements for human and veterinary medicinal products and opportunities for implementation of the 3Rs: • Draft Reflection paper providing an overview of the current regulatory testing requirements for veterinary medicinal & products and opportunities for implementation of the 3Rs • Draft Reflection paper providing an overview of the current regulatory testing requirements for human medicinal products and opportunities for implementation of the 3Rs
Lines 63-65	3	Comment: "over the past few years, new in vitro methods have been accepted for regulatory use via multiple and flexible approaches" Include some examples? Proposed change:	Reference is made to tabulated overview of guidelines in Reflection Papers providing an overview of the current regulatory testing requirements for human and veterinary medicinal products and opportunities for implementation of the 3Rs: • Draft Reflection paper providing an overview of the

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		"over the past few years, new in vitro methods have been developed and accepted for regulatory use via multiple and flexible approaches such as…"	current regulatory testing requirements for veterinary medicinal & products and opportunities for implementation of the 3Rs • Draft Reflection paper providing an overview of the current regulatory testing requirements for human medicinal products and opportunities for implementation of the 3Rs
Lines 66-69	1	Comment: Does EMA consider working together with FDA to reduce/replace the animal studies? FDA already reduced requirements for conduct of studies in animals: for example non-clinical studies for biosimilar products. Proposed change: Further clarification requested.	Collaboration with FDA is not within scope of this Guideline but mentioned as a possibility in the frame of the SAWP qualification of novel methodologies and would occur in cases where implementation of 3R methods is done as part of the (V)ICH process.
Line 66	8	Comment: rewording Proposed change: "Whilst replacement of animal studies remains the ultimate goal" to be reworded as follows :"The ultimate goal is the suppression of animal studies which can be achieved by replacement by in vitro methods and in addition the use of broader and new testing approaches allowing for better characterization of medicinal products."	Not accepted. The revised wording (line 83-84) is in line with Directive 2010/63/EU and is acknowledging all 3Rs. Whilst replacement of animal studies remains the ultimate goal, approaches aiming at reducing or refining animal studies are routinely implemented in regulatory guidelines, where applicable.

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Line 68-69	4	Comment: Specific examples, rather than the general reference to the M3 and S2 would be more helpful to readers. Also as these are not necessarily recent, remove reference to “recent” Proposed changes: Include more detail of the specific examples instead of just citing the guidance (detail could be included in a footnote). Remove “recently” in line 68. Consider adding ICH S6 and S9 as they also aid in addressing appropriate animal use.	References to M3 and S2 have been removed. Reference is made to the Reflection Papers providing an overview of the current regulatory testing requirements for human and veterinary medicinal products and opportunities for implementation of the 3Rs: • Draft Reflection paper providing an overview of the current regulatory testing requirements for veterinary medicinal & products and opportunities for implementation of the 3Rs • Draft Reflection paper providing an overview of the current regulatory testing requirements for human medicinal products and opportunities for implementation of the 3Rs
Lines 73	3	Comment: 73- “foster the regulatory agreement to new 3R testing approaches” Poor English	Accepted partially. Revised wording line 65-69. “In this respect, Directive 2010/63/EU [5] on the protection of animals used for scientific purposes, which is fully applicable to regulatory testing of human and veterinary medicinal

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		Proposed change: "foster the regulatory ~~agreement to~~ acceptance of new 3R testing approaches"	products , unambiguously fosters the application of the principle of the 3Rs (Replacement, Reduction and Refinement) when considering the choice of methods to be used."
Line 77	8	Comment: Speaks about a path for regulatory acceptance of development work following the 3Rs but does not later differentiate the quality level at which this work should be (i.e.only discussed validation of assays which would not be required in all cases). Proposed change: Expand on this area of a regulatory path for replacement of animal use with in vitro tests for clinical trial applications or monitoring.	Section 2 "Scope" has been completely reworded. Regulatory paths are addressed later in the GL and therefore are not included within the scope.
Lines 92 and 102	8	Comment: the date of issuance of the directive 2010/63 is 22nd of Sept 2010. Not June.	Noted. Reference updated.
Lines 92 and 102	4	The date of issuance of the directive 2010/63 is 22nd of September 2010. Not June.	Noted. Reference updated.
Line 97	4	Comment: While the 3Rs were first defined by Russell and Burch (1959), their definitions, particularly for refinement have evolved considerably.	Original reference kept for historical perspective but definitions updated to include replacement, reduction and refinement in a modern context

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: Replace definitions and citation to a more modern interpretation (for example see http://www.nc3rs.org.uk/the-3rs)	
Line 100	4	Refinement should reflect a decrease in the incidence or severity of all procedures (uses), not just those that might be considered “inhumane” as stated. Proposed change: Delete the word “inhumane” or consider updating definitions of the 3Rs (see previous comment)	Original reference kept for historical perspective but definitions updated to include replacement, reduction and refinement in a modern context
Lines 104 to 110	8	Comment: The manufacturer of product and/or the authorities’ requirements should avoid, wherever possible, redundant in vivo testing in the analytical profile of the product.	Agreed but this section only refers to literal excerpts from the Directive 2010/63/EU and as such no change is proposed (line 129-135 in updated GL).
Line 124	8	Comment: rewording Proposed change: … laboratory animal regulatory studies are mainly used for 2 purposes: …	Section 5. Application of 3Rs during drug development deleted from final GL.
Lines 124-126	8	Comment: This paragraph mentions two main purposes of animal testing (non-clinical/safety testing and quality	Section 5. Application of 3Rs during drug development deleted from final GL Not agreed as quality batch control encompasses both safety and potency testing.

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		batch control as part of the manufacturing process) but does not specifically refer to potency testing. Proposed change: Suggest to include a third “purpose” item – (3) Potency testing for release of final product.	
Lines 124-127	1	Comment: Would EMA consider reducing or eliminating requirements for conduction of a non-clinical study for biosimilar products since biosimilars are not a ‘new human’ drug? Proposed change: Further clarification requested.	Section 5. Application of 3Rs during drug development deleted from final GL For biosimilars an in vivo study is not the default choice. For further information see Van Aerts et al. (2014) MAbs;6(5):1155-62. Biosimilars entering the clinic without animal studies. A paradigm shift in the European Union.
Line 124	4	Comment: Clarify that scope if for those studies subject to regulatory review. Proposed change: “… regulatory studies conducted in laboratory animals …	Section 5. Application of 3Rs during drug development deleted from final GL
Lines 124-127	3	Comment: A huge number of animals are used in efficacy, proof of concept tests prior to human clinical trials. Why are these not considered both here and in the guideline	Not within scope of this guideline.

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		generally? Proposed change: Please address	
Line 126	8	Comment: To add at the end of the second purpose, "the cases of use in vivo testing for product troubleshooting investigations".	Not agreed.
Lines 128-134	3	Comment: The use of proportions serves to minimise the impact of the pharmaceutical sector on animal numbers. We are actually not sure if the figures (percentages) are correct- we cannot find them in the official statistics. In addition the numbers of animals used within the sector in general (mostly in efficacy studies) is not presented. Proposed change: "The number of animals used for experimental and other scientific purposes in the EU Member States is reported by the European Commission on a 3 yearly basis4. The latest report (European Commission, 2013) provides an overview of the number of animals used in the Member States for experimental purposes for 2011. The use of animals for research and development of human and veterinary medicines is currently over 2 million annually. ~~As such,~~ In addition, a further 1 million animals are used for regulatory	Section 5. Application of 3Rs during drug development deleted from final GL.

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		safety studies of which 40% is for human and veterinary medicinal products. ~~account for approximately 4.4% of the total number of experimental animals used~~. In addition ~~Animal use for~~ production and quality batch control testing of human and veterinary medicinal products accounts, respectively for 1,260,011 and 337,798 ~~10.9% and 4%~~ of experimental animals."	
Line 129	7	"The latest report (European Commission 2013) provides an overview of the number of animals used in the Member States for experimental purposes for 2011." Comment: A link of animal use statistics with information to the frequency of alternatives use should be implemented to drive decisions by both industry and authorities towards more use of alternatives and/or if not used sufficiently to further clarify "restraining factors" to remove such and further foster the use.	No specific comment. Noted. The reference to the latest report of the EC on the number of animals used has been removed from the document.
Lines 129-131	7	Comment: Advance in innovation and use intake can only be reached with specific monitoring and steering instruments, which generate transparency. Proposed change: The latest report (European Commission 2013) provides an overview of the number of animals used in	No specific comment. Noted. No specific comment. Noted. The reference to the latest report of the EC on the number of animals used has been removed from the document.

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		the Member States for experimental purposes for 2011. **This shall also include naming of the implemented alternatives (particularly in the case of replacements) to the animal use in each of the categories of animal use reporting and the different sub-categories of procedures therein.**	
Lines 135-137	7	Comments: The proposed tabulation should very much help to have a complete overview of areas in which alternatives are already available and used. It should also present in which "maturity" or planning phase not yet fully established alternatives are (research, pre-validation, validation). The reason is that planning perspectives must be clear at the start of R&D programs for medicines. Linking animal numbers of a reporting category (e.g. toxicology) together with the degree of alternatives already used in this category of marketing authorisations will provide transparency. It can be an effective driver for the further implementation of 3R (here replacement), because it will become clear if the 3R system is effective and attractive in order to pursue it. Proposed change: A tabulated overview of the current regulatory testing requirements for human and veterinary medicinal products and opportunities for implementation of the 3Rs is under development and will be published	Reference is made to the Reflection paper Papers providing an overview of the current regulatory testing requirements for human and veterinary medicinal products and opportunities for implementation of the 3Rs: • Draft Reflection paper providing an overview of the current regulatory testing requirements for veterinary medicinal & products and opportunities for implementation of the 3Rs • Draft Reflection paper providing an overview of the current regulatory testing requirements for human medicinal products and opportunities for implementation of the 3Rs

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		separately. **This tabulation will link the animal use in subcategories of procedures (acc. to EU Commission Implementation decision C(2012) 8064)) with available alternatives or such to come/expected/fostered in the next 5-10 years.**	
Line 138	7	Regulatory acceptance of 3R testing approaches Comment: The whole section on regulatory acceptance refers to alternatives that reach certain quality criteria to become acceptable. Nevertheless this section should also include a passage laying out as to how institutions/companies will be required (as questioned by marketing authorisation authorities) to invest into own 3R (replacement) efforts as part of their research programs to reach such marketing authorisation. It should be outlined that it is hence not only the role of the (local national) animal welfare authorities to request diligent consideration of 3Rs on study level (mostly reduction and refinement), but it should now also be part of the role of the marketing authorisation authority (on regional level) to request and foster such efforts as an integral part of R&D programs to achieve marketing authorisation. EMAs central role will have an immense leverage effect on 3R fostering, if this is considered on this level. In other words mechanisms, incentives, leverages need to be incorporated into regulatory acceptance process	Although the comment is acknowledged and supported to a certain extent it is not considered within the scope of this Guideline. Consequently no specific wording is proposed in the text.

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		by EMA (and into this document) that do not only take into consideration already established alternative methods, but also ask for efforts and proofs for 3R realisation, to get such marketing authorisation. This includes waving/reducing fees for marketing authorisation with certain 3R contribution. In addition this would make Dir 2010/63 of equal value to other regulations of marketing authorisations on a “system” level.	
Lines 147-149	3	Comment: Why are efficacy studies not included? Proposed change: Please address	The guideline applies only to regulatory guideline driven studies. As primary pharmacology studies for human pharmaceuticals are not guideline driven, no specific recommendations except general application of 3R principles in the design and selection of the type of studies to be carried out can be provided. With regards the veterinary medicinal products, regulatory guidelines related to clinical requirements are concerned by the guideline and thus included. Revised line 164-166 Regulatory guidelines concerned are those related to the quality or non-clinical (safety) requirements for human or veterinary medicinal products, residues requirements for veterinary medicinal products and safety and efficacy target species test requirements for veterinary medicinal products. Furthermore Reference is made to the Reflection paper Papers providing an overview of the current regulatory testing requirements for

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			human and veterinary medicinal products and opportunities for implementation of the 3Rs: • Draft Reflection paper providing an overview of the current regulatory testing requirements for veterinary medicinal & products and opportunities for implementation of the 3Rs. • Draft Reflection paper providing an overview of the current regulatory testing requirements for human medicinal products and opportunities for implementation of the 3Rs.
Line 147-149	4	Comment: The sentences are a little confusing to read. Proposed change: Combine the 2 sentences to give "…requirements for human or veterinary medicinal products and clinical requirements for veterinary medicinal products"	Partially accepted. Revised wording: Regulatory guidelines concerned are those related to the quality or non-clinical (safety) requirements for human or veterinary medicinal products, residues requirements for veterinary medicinal products and safety and efficacy target species test requirements for veterinary medicinal products.
Lines 148-149		"In addition, regulatory guidelines related to clinical requirements for veterinary medicinal products are concerned." Comment: This makes sense as also clinical veterinary trials are potentially matter at least for reduction and refinement. The situation however is that Dir 2010/63 in Art 1. Nr. 5 excludes clinical veterinary trials for MA	Noted.

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		from the scope and other (particularly older) regulations e.g. EU Directive 2001/82/EC do not sufficiently cover the 3Rs. This gap of coverage of 3Rs marketing authorisation will hence need to be addressed appropriately in some of the regulatory guidelines.	
Line 152	4	Proposed change: …at the same time increase predictive power and robustness of regulatory testing…	Accepted. Revised line 173-174.
Line 152	8	Comment: rewording Proposed change: "… at the same time increase predictive power and the robustness of regulatory testing …" To be added as mainly for QC purposes, lack of reproducibility and accuracy, with too large IC.	Accepted. Revised line 173-174.
Lines 153-156	4	Comment: Consider expanding a few of the examples listed that are recent and pragmatic. Proposed change: These levels range from discrete modifications of existing testing approaches. (eg. reduction of the top concentration used in in vitro genotoxicity testing in ICH s2R, consideration of smaller animal groups, the	Not accepted but revised wording section line 167-175.

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		need for both sexes or reversibility groups)	
Page 6, paragraph 6.3	8	Comment: Inclusion of consistency approach, as the WG of EPAA on vaccines, is a key area for progress. In addition, it is recognized that 1-to-1 validation for replacement is not always possible, nor desirable. The objective of the 3R testing approach is not to strictly establish a correlation between in vivo and in vitro methods but to select relevant and reliable testing methods. In the same section, consideration of waiving for testing assays which are not anymore scientifically valid is not included in 'replacement/reduction' approaches. This has been demonstrated as an effective way to make some progresses.	This comment is acknowledged. The guideline does not focus on 1 to 1 replacement. Waiving of testing assays that lost scientific validity is indeed considered a way forward however, is not stricto senso considered as regulatory acceptance of 3R testing approaches. The consistency approach is a good example of a methodology that can entail waiving of animal testing and the approach could be either submitted for qualification to the SAWP as a replacement/reduction approach or via a product-specific variation application once ready for implementation. The revised GL now includes a paragraph on the value of the consistency approach (Section 1 ,line 85-91).
Lines 157-165	3	Comment: This section doesn't add much and basically says the methods have to be validated. Points 1-3 all describe validation. Proposed change: Suggest delete and include more about the requirements for validation in section 6.3.1.	Partially agreed. Section 6.3. (5.3. in revised GL) has been reworded.
Lines 158-165	4	Comment: Inclusion of consistency approach, as the WG of EPAA on vaccines, is a key area for progress. In addition, it	This comment is acknowledged. The guideline does not focus on 1 to 1 replacement. Waiving of testing assays that lost scientific validity is indeed considered a way forward however, is not stricto senso

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		is recognized that 1-to1 validation for replacement is not always possible, nor desirable. In the same section, consideration of waiving for testing assays, which are not anymore scientifically valid is not included in “replacement/reduction” approaches. This has been demonstrated as an effective way to make some progress on animal use.	considered as regulatory acceptance of 3R testing approaches. The consistency approach is a good example of a methodology that can entail waiving of animal testing and the approach could be either submitted for qualification to the SAWP as a replacement/reduction approach or via a product-specific variation application once ready for implementation. The revised GL now includes a paragraph on the value of the consistency approach (Section 1, line 85-91).
Line 160	7	“Demonstration of method validation” Comment: Many historical methods were not validated or cannot be validated due to very high variability. The question remains whether the historic and the new method should be linked to each other. To increase predictive power of regulatory testing, potency results can sometimes not be compared to each other. Proposed change: In cases where the traditional method was not validated or cannot be validated due to very high variability, the validation of the 3R method can be done independently since it will increase predictive power of regulatory testing. Hence, no link between test results of the traditional method and the 3R method is possible nor needed. To compensate the gap between the methods, the validation should cover acceptable and several different non-acceptable	Noted. Proposed changed not included but revised wording in section 5.3. It is considered that method validation should be indeed be conducted on a case by case basis taking into account the existing methodology, the area of application and the degree of validity of the in vivo methods. These aspects are further elaborated under Section 5.

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		batches to reflect possible failures in production, such as low antigen content. Conditions likely to affect the potency such as abnormal pH should also be tested. The demonstration of discriminatory power of the 3R potency test method should then be sufficient for regulatory acceptance. Data collection through the safe harbour concept is not affected by this.	
Lines 164-165	6	Comment: Does criterium 3 mean that in all cases first safe harbour data should be gathered before a 3R testing approach is accepted? Wouldn't it be possible to accept a method if it has been thoroughly validated and proven useful according to criteria 1 and 2? Proposed change: Make explicit that not all 3 criteria need to be met and/or indicate the level of flexibility.	Following criteria should be followed before consideration of a 3Rs testing approach for regulatory acceptance: • Availability of defined test methodology including standard protocols with clear defined/scientifically sound endpoints. • Relevance, where relevance describes the relationship of the test method to the effect of interest and whether it is meaningful and useful for a particular purpose (context of use). It is the extent to which the test correctly measures or predicts the biological effect of interest. Relevance incorporates consideration of the accuracy (e.g. concordance with comparable validated test method with established performance standards) of a test method [10]. • Context of use includes a description of the circumstances under which the 3Rs testing approach is applicable in the assessment of human or veterinary medicinal products and the limitations within which the available data adequately support use of the 3Rs testing approach. It should for instance be demonstrated that the new or substitute testing method or testing strategy provides

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			either new data that fill a recognised gap or data that are at least as useful as, and preferably better than those obtained using existing methods. • Reliability/robustness; a measure of the extent that a test method can be performed reproducibly over time when using the same protocol.
Line 166	4	Comment: Section 6.3.1 should retain the concepts of "robustness" and "sensitivity" under "reliability", as these are important parameters in testing, especially for regulatory purposes. These concepts are in the current version of the guideline (CPMP/SWP/728/95).	The terms are better explained using the definitions from OECD Guideline (2005) Glossary (see below). Partially accepted. Revised section 5.3 Relevance, where relevance describes the relationship of the test method to the effect of interest and whether it is meaningful and useful for a particular purpose (context of use). It is the extent to which the test correctly measures or predicts the biological effect of interest. Relevance incorporates consideration of the accuracy (e.g. concordance with comparable validated test method with established performance standards) of a test method [10]. • Context of use includes a description of the circumstances under which the 3Rs testing approach is applicable in the assessment of human or veterinary medicinal products and the limitations within which the available data adequately support use of the 3Rs testing approach. It should for instance be demonstrated that the new or substitute testing method or testing strategy provides either new data that fill a recognised gap or data that are at least as useful as, and preferably better than those obtained using existing methods. • Reliability/robustness; a measure of the extent that a test

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			method can be performed reproducibly over time when using the same protocol.
Lines 167-183	3	Comment: The requirements for a method to be considered valid and validated are key to this document. If the EMA is not able to be prescriptive then at least explain what is meant by each of these requirements in more detail. More text is needed to explain the principles of validation and the various steps as used by bodies such as ECVAM and OECD. A large section of 6.3.2 needs to be moved (and edited) into this section. Proposed change: Refer to the literature references 6-10 for definition of these terms. Include within and between laboratory variability as measures of reliability and explain what is meant by 'relevance'- we assume this means validated.	Guideline text has been modified accordingly. Revised section 5.3 • Relevance, where relevance describes the relationship of the test method to the effect of interest and whether it is meaningful and useful for a particular purpose (context of use). It is the extent to which the test correctly measures or predicts the biological effect of interest. Relevance incorporates consideration of the accuracy (e.g. concordance with comparable validated test method with established performance standards) of a test method [10]. • Context of use includes a description of the circumstances under which the 3Rs testing approach is applicable in the assessment of human or veterinary medicinal products and the limitations within which the available data adequately support use of the 3Rs testing approach. It should for instance be demonstrated that the new or substitute testing method or testing strategy provides either new data that fill a recognised gap or data that are at least as useful as, and preferably better than those obtained using existing methods. • Reliability/robustness; a measure of the extent that a test method can be performed reproducibly over time when using the same protocol.

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Line 172	6	Comment: Relevance for what? And how is this determined? Proposed change : Please elaborate	Guideline text has been modified accordingly. Revised section 5.3 • Relevance, where relevance describes the relationship of the test method to the effect of interest and whether it is meaningful and useful for a particular purpose (context of use). It is the extent to which the test correctly measures or predicts the biological effect of interest. Relevance incorporates consideration of the accuracy (e.g. concordance with comparable validated test method with established performance standards) of a test method [10]. • Context of use includes a description of the circumstances under which the 3Rs testing approach is applicable in the assessment of human or veterinary medicinal products and the limitations within which the available data adequately support use of the 3Rs testing approach. It should for instance be demonstrated that the new or substitute testing method or testing strategy provides either new data that fill a recognised gap or data that are at least as useful as, and preferably better than those obtained using existing methods. • Reliability/robustness; a measure of the extent that a test method can be performed reproducibly over time when using the same protocol.
Lines 181-183	6	Comment: Different routes are said to be acceptable, including VAMs and EDQM. However, subsequently only VAMs	Alternative ways of “non-formal” assay validation are described in revised section 5.4.2. and 5.4.3.

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		and EDQM routes are elaborated upon. Proposed change : Please elaborate	
Lines 184-200	3	Comment: Part of section 6.3.2 actually describes validation (not regulatory acceptance) and how other bodies do it. Are these acceptable to the EMA? Which process does the EMA endorse? This needs to be moved up to 6.3.1. See also Worth, A and Balls, M. 2002.Chapter 2: The Principles and Procedures of Validation ATLA 30, Supplement 1, 13-19. And Balls, M. and Fentem, J.H. 1999. The validation and acceptance of alternatives to animal testing. Toxicology in Vitro 13, 837-846. Proposed change: Please revise	The section 6.3.2. (Now section 5.4.1) (regulatory acceptance of 3R testing approaches) has been revised.
Line 196	6	Comment: 'predefined criteria'. It is very important which criteria these are. Using an animal experiment as golden standard may even lead to the rejection of a better predicting 3R approach. Comparing with human outcome parameters may not always be possible.	This section refers to criteria as described in 'Test Method Decision Criteria and Data Interpretation' and 'Data Analysis' as described in detail in the OECD validation guideline 2005, Reference 11) and is therefore not repeated in detail here. Reference has been added.

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		Proposed change: Please elaborate on the type of criteria and the level expectancy.	
Lines 201-213	3	Comment: This part of the section does relate to regulatory acceptance but describes how the EDQM does it. Earlier in the document it states that the regulatory acceptance of quality control methods is not covered. We think it should be covered and we support this section remaining. However a reference for the BSP process is needed and examples of tests that have been validated and included (such as the LAL method for pyrogenicity). It is also not clear how this process fits into EMA process such as market authorisations- does the EMA not look at whether these quality control methods are good enough, does the EMA not play a role? Proposed change: Please revise	In the revised section 5.4.1 which now refers to EDQM's BSP references for more details on the BSP process are provided in a footnote.
Lines 214-224	3	Comment: This section does not explain how alternative routes are accessible and appears to describe standard routes, e.g. ICH processes. How does this relate to the "Qualification of novel methodologies for drug development: guidance to applicants" document?	The EU or ICH guideline development processes are considered as alternative routes to the 'standard' approach i.e., formal validation (e.g. through ECVAM) followed by transfer into OECD GL. While in this process new methods are selected and evaluated

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		Proposed change: Please revise	by regulatory experts involved in the development of regulatory guidelines the procedures for submission as described in section 5.5 are also open for external assay developers and other stakeholders.
Lines 225-234	3	Comment: See general comments on the safe harbour concept. We oppose the use of the concept in general and ask at the very least that it is explained more clearly in what situations it may be required. Who decides and who decides the point at which dual submission can stop and, crucially, who evaluates the outcome of the process and by what criteria? Proposed change: Please revise	With regards to the safe harbour process the term “safe harbour” has been deleted but the concept of voluntary submission of data obtained by using a new 3Rs testing approach in parallel with data generated using existing methods has been kept (5.4.3.). In addition, it has been clarified that this process is not meant as a routine add-on to standard validation but may be considered on a case-by-case basis in certain situations only.
Lines 226-234	4	Comment: We welcome the concept of the safe harbour collection of the data as outlined in Section 6.3.4;however, we welcome additional details around the type of data to be submitted, the format of the data, and an indication regarding the timelines for the evaluation of the alternative method.	With regards to the safe harbour process the term “safe harbour” has been deleted but the concept of voluntary submission of data obtained by using a new 3Rs testing approach in parallel with data generated using existing methods has been kept (5.4.3.). In addition, it has been clarified that this process is not meant as a routine add-on to standard validation but may be considered on a case-by-case basis in certain situations only.
Lines 226-234	8	Comment: It would helpful to have a more detailed guidance here on what the real-life data collection should include.	With regards to the safe harbour process the term “safe harbour” has been deleted but the concept of voluntary submission of data obtained by using a new 3Rs testing approach in parallel with data generated using existing

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		Proposed change: Suggest to include - 1) the approximate number of lots to be tested using both methods; 2) For potency tests, the mechanisms of action of some drug products are unknown. It would be helpful in these cases to include a general guidance on the requirements for replacing an in vivo method with an in vitro test.	methods has been kept (5.4.3.). In addition, it has been clarified that this process is not meant as a routine add-on to standard validation but may be considered on a case-by-case basis in certain situations only.
Lines 235-254	3	Comment: See general comments. The crux of this document appears to actually lie in another document and we are concerned that even in this document the process is not clear and may be inappropriate for 3Rs methods. Can the process as described in the Guideline on Qualification of Novel Methodologies for Drug be briefly outlined? Perhaps in the form of a timeline. For example see the Scheme for regulatory approval of methods under REACH http://tsar.jrc.ec.europa.eu/documents/Revised_Procedure_Adopted_by_CAs.pdf Can the process cover both submission by individual companies on a product specific basis as well as submission by a consortia or body such as ECVAM for consideration as entry into a new or revised guideline? Who is responsible for submission, evaluation and putting forward as a guideline? What will happen in terms of ICH? Can fees be waived if the method is a	Comments/proposal in general agreed. Further information in Section 5.5.and 5.6. included to better demonstrate how the EMA's data submission/evaluation process works and that it is appropriate for this purpose

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		3Rs method? Proposed change: Please revise	
Page 8 paragraph 6.4	8	Comment: It has been suggested by the European Commission to have change submission to the CTD free of charge in order to stimulate and accelerate 3Rs implementation. Could this be formally suggested/requested?	Not in the scope of this guideline.
Lines 239-242	7	Comment: The inclusion of 3R related aspects into the “scientific advice” procedure will provide (e.g. for replacement options) the possibility of alignment with authorities on questions of data generation, compilation and 3R validation requirements, so that chances are increased that own 3R efforts possibly become usable within the timelines of R&D program and marketing authorisation. This will allow consistent implementation of safe harbour concept early. However the weakness is that the scientific advice is only voluntary. Demonstration by institutions of their efforts related to all 3Rs in a marketing authorisation would draw more awareness on a dossier level and over a whole program. This would be particularly relevant as replacements do not appear in any of the EU statistical reporting requirements. It would also be important as local animal welfare authorities (in various countries	Not in the scope of this guideline.

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		and regions) do not have the oversight to be able to provide support on a 3R support on a marketing authorisation level and complete program as they are focused on single studies.	
Lines 252-254	4	Comment: The intent of this last sentence is unclear and does not follow the preceding text. Proposed change: Please clarify.	Sentence has been deleted.
Lines 235-251	4	Comment: It has been suggested by the European Commission to have change submission to the CTD free of charge in order to stimulate and accelerate 3Rs implementation. Could this be formally suggested/requested?	Not in the scope of this guideline.