21 June 2018
EMA/CHMP/CVMP/3Rs/731924/2016
Committee for Medicinal Products for Veterinary Use (CVMP)

Overview of comments received on 'Reflection paper providing an overview of the current regulatory testing requirements for veterinary medicinal products and opportunities for implementation of the 3Rs' (EMA/CHMP/CVMP/3Rs/164002/2016)

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

Stakeholder no.	Name of organisation or individual
1	Zoetis
2	European Coalition to End Animal Experiments (ECEAE)
3	PETA International Science Consortium Ltd.

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1. General comments – overview

Stakeholder no. *(See cover page)*	General comment (if any)	Outcome (if applicable)
1	We are supportive of many of the proposed methods for 3Rs. Mechanisms for timely and iterative discussion with CVMP would be helpful to ensure that the recommendations proposed in the document are sufficient to underwrite the risk assessment. Without such discussion, all of the risk associated with the recommendations will be placed upon the pharmaceutical industry. Currently, there is insufficient communication with CVMP for sponsors to determine how the recommendations will be evaluated. We believe and strongly recommend increasing open communication between CVMP and industry as that would improve 3Rs implementation significantly.	There is no special communication channel foreseen between CVMP and the industry, but very early stage inquires could be at the J3Rs working group (J3RsWG), otherwise communication via the Scientific Advice Working Party (SAWPv) or Innovation Task Force (ITF) or through participation of J3RsWG in EU 3Rs initiatives e.g. questions raised by the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) Network for Preliminary Assessment of Regulatory Relevance (PARERE). In general, all 3Rs matters from J3RsWG are presented and discussed with CVMP by the 3RsWG secretariat and Chair, who are both in close contact with CVMP.
2	The European Coalition to End Animal Experiments (ECEAE) welcomes this reflection paper and thanks the CVMP for their efforts in its production. We support much of the content of the document and we are grateful that areas have been identified where further 3Rs steps can be made for example in the areas of pyrogens, abnormal toxicity, genotoxicity and carcinogenicity. We think the document is clear and should help provide guidance to pharmaceutical companies on the current requirements and further	Comment noted. There is the J3RWGs which also cares about monitoring of the different guidelines and considers innovations; however, an annual review is not feasible, although there is an ambition to consider reviewing the Reflection Paper in light of scientific updates in the area of 3Rs.

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	opportunities to replace, reduce and refine animal testing. We hope the CVMP have dedicated resources to follow through the recommendations in this document and will regularly (annually?) monitor progress with these. We have specific comments on some endpoints, which should not be considered exhaustive. We thank you for the opportunity to comment.	
3	Throughout the reflection paper, there are missed opportunities to identify additional non-animal approaches. We recommend the inclusion of the information in the following table in the final paper. Additionally, an overview of the opportunities for implementation of the 3Rs should encourage the development, validation, and use of non-standard non-animal methods, as described in the Organisation for Economic Co-Operation and Development (OECD) Series on testing and assessment no. 211 Guidance document for describing non-guideline in vitro test methods (2014) and the European Commission Joint Research Centre report Alternative methods for regulatory toxicology – a state-of-the-art review (2014).	The insertion of cross-references can be considered in a future update of the document. The J3RSWG has developed a new guideline which provides reflections on 3Rs methodologies (Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches. Ad The EMA/CVMP does require that justification be provided when additional tests are conducted: However, the legal responsibility for monitoring of the 3Rs is a national competence (and not EMA's), therefore the mandate of EMA is limited to encourage the adherence to the 3Rs in accordance with Directive 2010/63/EU. Also discussions already take place in the J3RsWG with respect to future optimization of the use of experimental animals e.g. optimal species, test principles, batch tests, precision, accuracy etc.

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	Lastly, companies may submit animal tests in addition to what is required for product approval. To decrease the use of animals in testing and adhere to the use of animals only as a last resort, the EMA should require that justification be provided when additional tests are conducted.	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
page 10, Reproductive toxicity including developmental toxicity (MRL)	1	Comment: The extension of the one generation study (i.e., addition of a neurotoxicity or immunotoxicity arm to the study) will complicate the study design and not reduce the number of animals required. For user safety assessments, standard developmental toxicity testing is required. For target animal safety, developmental toxicity testing in one species is required if the product is intended for use in female animals that may be used for breeding. Proposed change (if any): none	This is partly accepted. The current requirement is a multigeneration reproductive toxicity study in one species and a developmental toxicity testing (in 1-2 species). The possibility of replacing the standard two-generation study, VICH GL 22, Safety studies for veterinary drug residues in human food: Reproduction Testing(ongoing activity at VICH) by the extended one generation reproductive toxicity study (EOGRTS) is being investigated by VICH. A reduction of used animals by 40% may be expected, even including additional endpoints. If the EOGRTS is accepted then further consideration of the elements routinely included in the study may be appropriate. The ongoing update on the veterinary legislation also needs to include the possibility to use the one generation test and not as stated at present per default to require the two generation reproductive toxicity study.
page 10, Genotoxicity studies (MRL)	1	Comment: This is being discussed at VICH. In vivo genotoxicity studies are smaller and more economical than carcinogenicity studies Proposed change (if any): none	Comment noted. However, in vivo genotoxicity studies do not cover the whole information (e.g. non-genotoxic carcinogenicity).
page 16, Repeat dose toxicity (MA of VMPs	1	Comment: The sponsor has attempted to utilize this recommendation in the past but the approach was unsuccessful.	It is noted that the document mentions “The published Summary Report/European Public MRL Assessment Report may be submitted in place of data”. However we cannot comment on the specifics of this individual case. It also

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for use in food producing species)		Proposed change (if any): none	depends on the legal basis, e.g. in the case of a full application it is not acceptable.
Page 11 (MRL) and 19 (MA of VMPs for use in companion animals) Carcinogenicity	2	Comment: The requirements, implemented 3Rs opportunities and newly identified 3Rs opportunities do seem at odds for this endpoint. The newly identified opportunities seem to imply that testing in 2 species is a requirement, but the requirements and implemented 3rs opportunities suggest otherwise. Perhaps the intention is that specific deletion of carcinogenicity (in 2 species) should be sought from the legislation? We would agree with that approach. However we would also support efforts to completely remove the carcinogenicity study given its unreliability and the caveats already provided in this document. A comparable effort is underway for human medicines so the veterinary sector should follow this. Proposed change (if any):	Comment noted (small correction /consistency proof in the reflection paper). Activities on the human side are currently monitored.
Page 16 (MA for VMPs for use in food producing species) and 18 (MA for VMPs for use in	2	Comment: It appears that although not specified there are reasons why single dose studies may be conducted, even though they could be avoided. Animals ARE used in target animal safety tests. E.g. EMA/CVMP/EWP/117899/2004-Rev.1 We suggest that seeking clarity on the need/avoidance of single dose studies including target animal safety	There seems to be a misunderstanding, this section (safety) relates to studies in experimental animals and not to target animal species.

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companion animals) Single dose toxicity		tests should be placed as a newly identified 3rs opportunity for this endpoint in all tables. Proposed change (if any):	
Page 16 Repeated dose, etc	2	Comment: Reference to the requirements for MRLs is given here. Perhaps it should be make more explicit that the 3Rs opportunities apply equally here and refer the reader to the specific Table and rows above or better, provide the same information in an endpoint by endpoint basis as you have for the MRL requirements. Proposed change (if any):	These editorial comments are accepted, this can be clarified.
Page 16 and 20 Other tests for user RA	2	Comment: There are several opportunities to avoid animal testing for these other tests and it would be better to separate out these tests (as with the other areas) so that 3Rs opportunities can be identified. The skin sensitisation opportunities for example should be moved to a specific line for skin sensitisation. There are further opportunities to avoid skin and eye irritation tests using in vitro methods, if these test are warranted at all, for example. Proposed change (if any):	Comment accepted, skin sensitisation may be listed as separate point in the revised document.
Page 18 (MA for VMPs for	2	Comment: Why is the extended one generation test not included	This chapter relates to target animal safety where developmental toxicity testing in one species is only needed if

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use in companion animals) Reproductive toxicity		here as a newly identified 3Rs opportunity? Proposed change (if any):	the product is intended for use in female animals that may be used for breeding. There is no requirement for further testing including two generations.
Page 32 Bioconcentration factor in fish	2	Comment: This endpoint can be avoided if the log Kow of the substance is a certain size. Furthermore this endpoint can be predicted well using various QSAR models. Can investigation of this be added under newly identified opportunities please? Proposed change (if any):	Using the logKOW value as a trigger for a fish bioaccumulation study is correct. Reference to this will be added in the relevant section. Quantitative structure–activity relationship (QSAR) modelling is not recommended to replace a BCF study as for ionisable substances these models are not robust enough at this point in time. Two OECD test guidelines using S9 or cryopreserved hepatocytes from rainbow trout to determine *in vitro* intrinsic clearance have been approved by OECD WNT in April 2018. *In vitro* intrinsic clearance rate is used to inform in silico BCF prediction models, thus making them more predictive and in consequence avoid unnecessary animal tests.
73	3	In the table “Overview of animal testing requirements for active substances of synthetic, semi-synthetic, fermentation origin as well as medicinal products (Quality 74 Working Party - CHMP/CVMP)”, we recommend the following inclusion.	Accepted. The text in the Refelction paper has been revised accordingly. .

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		Bacterial Endotoxins (amoebocyte lysate from Limulus polyphemus or Tachypleus tridentatus) – The reflection paper notes that horseshoe crabs do not fall under the scope of Directive 2010/63/EU. European Pharmacopoeia general chapter 5.1.10, "Guidelines for Using the Test for Bacterial Endotoxins" explains that the BET nevertheless requires harvesting cells from live endangered horseshoe crab species in a procedure that has been documented as a likely contributor to the decline of those species' populations in the wild. Consequently, European Pharmacopoeia (PhEur) general chapter 5.1.10 makes reference to the use of recombinant Factor C (rFC) in place of harvested amoebocyte lysate as an approach that does not require the use of horseshoe crabs. This information should be included in the final document.	
76 (MRLs)	3	In the table "Overview of animal testing requirements for safety studies to be submitted in support of applications for maximum residue limits (Safety Working Party - CVMP)", regarding the endpoint reproductive toxicity including developmental toxicity, it should be pointed out that the basic extended one-generation reproductive toxicity study (EOGRTS) design without inclusion of extended F2 generation and cohorts for developmental neurotoxicity (DNT) and developmental immunotoxicity (DIT) is required here. DNT and DIT cohorts are mentioned in the sections on the respective endpoints.	This is misunderstood. A developmental toxicity testing in the rat is always required (covering teratogenicity endpoints). No 2nd study in a 2nd animal species is necessary if the VMP shows to be teratogenic. A 2nd study (mostly in rabbits) is needed if the results in the first species are negative. In addition to this requirement a multigeneration reproduction toxicity study is required (see VICH GL 22). A new approach currently discussed in VICH as a possible replacement for the Multigeneration study is to require instead an extended one generation reproductive toxicity study (EOGRTS). The design

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			of an EOGRTS covers also developmental neurotoxicity (DNT) and developmental immunotoxicity (DIT) endpoints and still uses 40% less animals.
82 (MA for VMPs for use in companion animals)	3	In the table "Overview of animal testing requirements for safety studies to be submitted in support of applications for marketing authorisations for veterinary medicinal products for use in companion animals (Safety Working Party - CVMP)", we recommend inclusion of the following: • Single Dose Toxicity o Oral: Waivers for oral testing should be granted when a test chemical is corrosive to the skin, when end-use product design prevents oral exposure, and for other reasons described in the OECD guidance document 237 on considerations for waiving or bridging of mammalian acute toxicity tests (OECD, 2016). The 3T3 neutral red uptake cytotoxicity test can be used in a weight-of-evidence approach to identify substances not requiring classification for acute oral toxicity (EURL ECVAM recommendation, 2013). When EMA requires *in vivo* oral testing, OECD guidance document 129 on using cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests should be followed (2010).	General comment: Single dose toxicity data is intended to characterise signs of overdose of VMPs in target animal species and for user safety assessment. These studies may provide information useful for setting doses to be used in repeated dose studies. The submitted data can be bibliographic. In addition, data from repeated dose studies may provide an alternative. Based on this, the suggested waivers are considered too specific; however the insertion of the following sentence is suggested: "Acute oral toxicity studies may be waived also considering the criteria as listed in the OECD guidance document No. 237 ("Guidance Document on Considerations for Waiving or Bridging of Mammalian Acute Toxicity Tests". This needs to be considered as case-by-case decision.

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		Additionally, when an *in vivo* oral test is required, OECD guidance document 24 (2001) compares the three OECD test guidelines that are available. The oral fixed dose procedure (OECD test guideline (TG) 420) uses evident toxicity as its endpoint rather than mortality and, on average, uses the fewest animals. o Inhalation: Waivers for inhalation testing should be granted when there is little or no significant human inhalation exposure, when it is not technical feasible to perform a study, and for numerous other reasons described in OECD, 2016. When an *in vivo* inhalation test is required, prior knowledge of the oral toxicity class can be used to reduce the number of animals used (by starting testing in the most likely exposure band). OECD TG 436 provides a reduction in animal use compared to OECD TG 403. Draft OECD TG 433 provides a reduction and refinement to OECD TG 403, but is not yet an official OECD TG. o Dermal: Waivers for dermal testing should be granted if a test chemical is corrosive or severely irritating to the skin, if a test chemical shows no	

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		adverse effects in an acute oral toxicity test up to 2000 mg/kg, when there is low dermal penetration, and for numerous other reasons described in OECD, 2016. • Carcinogenicity – Cell transformation assays (CTAs), including those using SHE cells (Series on testing and assessment no. 214 *Guidance document on the* in vitro *Syrian Hamster Embryo (SHE) cell transformation assay*, 2015) or Bhas 42 cells (Series on testing and assessment no. 231 *Guidance document on the* in vitro *Bhas 42 cell transformation assay*, 2016) can be used in a weight-of-evidence approach to predict carcinogenic potential. An integrated approach to testing and assessment on non-genotoxic carcinogens was accepted into the OECD work plan this year which will illustrate how CTAs can be used in a predictive strategy (Work plan for test guideline programme, July 2016). Additionally, carcinogenicity testing can be combined with chronic toxicity testing as described in OECD TG 453. • Other Endpoints – The reflection paper combines "[o]ther tests required for the user risk assessment, possibly including skin and eye irritation, sensitisation and inhalation toxicity" and only mentions skin sensitisation tests in the area for "newly identified	Ad Carcinogenicity: These specific tests are not routinely required within the veterinary medicines sector and consequently there is no specific guidance available. Where testing is appropriate OECD Guidance Documents No. 214 (Guidance document on the in vitro Syrian hamster embryo (SHE) cell) and No. 231 (Guidance document on the in vitro Bhas 42 cell transformation assay) should be considered. With this respect the applicant is advised to seek Scientific Advice regarding e.g. an integrated approach without further long-term rat or mouse bioassays. Ad other endpoints: also these tests are not routinely required within the veterinary medicines sector. Regarding these endpoints the following sentence can be amended: "Where testing is appropriate consideration of the following alternatives should be considered, including OECD TG 492, OECD TG 491, OECD TG 460, OECD TG 438, and OECD TG 437 (serious eye damage and eye irritation); OECD TG 439, OECD TG 431, OECD TG 435, and OECD TG 430 (skin irritation and corrosion); and three-dimensional tissue models (such as MucilAir™ [Epithelix], SmallAir™ [Epithelix], and EpiAirway™ [MatTek Corp] (inhalation toxicity)).

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		opportunities for 3R implementation". However, companies continue to conduct testing on animals for other endpoints even though alternative test methods exist - including skin and eye irritation and inhalation toxicity. It would be beneficial to state available alternatives, including OECD TG 492, OECD TG 491, OECD TG 460, OECD TG 438, and OECD TG 437 (serious eye damage and eye irritation); OECD TG 439, OECD TG 431, OECD TG 435, and OECD TG 430 (skin irritation and corrosion); and three-dimensional tissue models (such as MucilAir™ [Epithelix], SmallAir™ [Epithelix], and EpiAirway™ [MatTek Corp] (inhalation toxicity)).	
85	3	In the table "Overview of animal testing requirements for immunological veterinary medicinal products - tests required during licensing (Immunologicals Working Party - 86 CVMP)", we recommend an emphasis on non-animal affinity reagents. The availability of modern non-animal affinity reagents, including recombinant antibodies (rAbs) and aptamers, makes the replacement of conventional animal-based antisera and antibody production methods feasible on a scale that should be recognized in the final document. Relative to animal-derived antibodies, the financial and time-saving benefits of rAbs and aptamers are clear. They can be sequenced	The current reflection paper provides a snapshot of the animal testing requirements and newly identified opportunities at the time of publication. It is to be expected that, over time, new testing approaches will become accepted and the tables should be considered accordingly. At the current time we are now aware of at the application or developments in non-animal affinity antibodies and aptamers for the testing of immunological veterinary medicinal products.

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		and reproduced as needed, selected for in controlled conditions, modified to fill a specific purpose, and bind a variety of targets with high affinity. (See Groff K, Brown J, Clippinger AJ. Modern affinity reagents: recombinant antibodies and aptamers. Biotechnol Adv. 2015;33(8):1787-1798.)	