25 July 2025
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Committee for Veterinary Medicinal Products (CVMP)

Overview of comments received on the guideline for the demonstration of efficacy for veterinary medicinal products containing anticoccidial substances (EMA/CVMP/EWP/755916/2016) – Revision 1 (second version)

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

Stakeholder no.	Name of organisation or individual
1	Cruelty Free Europe

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

1. General comments – overview

Stakeholder no.	General comment	Outcome
1	Cruelty free Europe welcomes the opportunity to provide comments on the revised draft 'Guideline for the demonstration of efficacy for veterinary medicinal products containing anticoccidial substances'. While we welcome the references to the 3R principles within the draft guideline, we believe further improvements could be made to reduce and replace the use of animals for efficacy testing of anticoccidial substances wherever possible. It is crucial that this guideline is future-proofed to allow flexibility for the development of in vitro and other non-animal methods as they become available. This is especially important given the European Commission's roadmap to phase out animal testing in chemical safety assessments, including medicines, which is expected to be released in 2026. We strongly encourage the Committee for Medicinal Products for Veterinary Use (CVMP) to consider holding a workshop to evaluate available non-animal approaches for the testing of anticoccidial drugs and to identify areas that could benefit from the development of novel methods to replace the use of animals. The workshop should specifically cover the following areas: • The use of in vitro assays or other non-animal methods for screening and efficacy testing • Opportunities to reduce the number of studies (such as dose determination) through combined studies and identifying where tests on animals can be waived. We suggest that the guideline is expanded to include guidance on using in vitro and/or other non-animal methods to reduce and, where possible, replace the use of animals required to demonstrate the efficacy of veterinary medicinal products (VMPs) for the prevention/reduction of coccidiosis. At a	Thank you for your comments. Please note that the text in lines 125-127 has been changed to: '*All animal experiments should be conducted taking into account section I.1.7 of Annex II of Regulation (EU) 2019/6 and the 3Rs principles (replacement, reduction and refinement), notwithstanding the place of conduct of the experiments*." Furthermore, the following sentence has been added to the guideline to give a clear message that the option of using non-animal methods is possible: '*Alternatives to in vivo test methods should be employed whenever possible*.' At present it is not considered possible to expand the guideline text to include specific and detailed guidance on using *in vitro* and other non-animal methods; these can be proposed by applicants and considered on a case-by-case basis. If an applicant considers waiving of a particular test requiring the use of animals, or using a 3Rs-compliant methodology, advice on the acceptability of the proposed approach can be requested through the EMA's scientific advice procedure. These further improvements ensure flexibility for the development and use of *in vitro* and other non-animal

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	minimum, we recommend that the guideline reference the option to use non-animal methods where possible, and as they become available. The World Association for the Advancement of Veterinary Parasitology (WAAVP)'s 2018 'guideline for evaluating the efficacy of anticoccidials in mammals' emphasizes the potential of in vitro methods to improve pre-clinical drug screening and efficacy testing. It highlights that "*there is a growing body of new methods of in vitro cultivation systems for mammalian coccidia which can be used for pre-clinical drug screening and efficacy testing in cases of suspected resistance*" (Joachim et al., 2018). The guideline also points out the significant drawbacks of the current animal tests, including high costs, the large number of animals required, ethical concerns and contamination risks, stressing the need for alternative approaches, particularly for the propagation and amplification of coccidia. The guideline also describes the use of several in vitro systems including the combination of cell culture with quantitative PCR (cc-qPCR) for assessing the efficacy of drugs against Cryptosporidium Parvum, cultivation systems like porcine intestinal cells and embryonated egg inoculation for propagating Cystoisospora suis and the use of in foetal primary cells to complete the whole life cycle of certain bovine Eimeria species in vitro. These systems offer promising alternatives to traditional animal testing. A 2021 review further underscores the value of in vitro methods, stating, "*in vivo studies can only provide an overview of the general benefits of treatment against coccidiosis, but the key processes that make it effective should be studied in vitro, too. In vitro research allows a step-by-step analysis of the mode of action of a treatment, which is essential for pathogens like coccidia, characterised by a multiphasic life cycle, in order to understand the actual target of the molecule*" (Felici, et al., 2021). While in vivo studies may still be required, the review advocates for the implementation of "fast and relatively cheap in vitro technologies" to	methods as they become available. The current wording also allows waiving of animal studies, thus reduction of the number of animals used in studies.

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	improve understanding of coccidia pathogenesis and enhance anticoccidial drug screening. Recent advancements demonstrate the potential of in vitro methods to reduce animal use in evaluation of anticoccidial compounds. For example, a new screening model led to a 67% reduction in the number of animals needed for in vivo studies, and a miniaturised 96-well plate format further reduced animal use by 80% for the pre-screening of multiple compounds. (Arias-Maroto et al., 2024). Other models, such as using bovine colonic epithelial cells (BCEC) to assess anticoccidial efficacy in ruminants (Odden et al., 2019) and a reproduction inhibition assay (RIA) for evaluating *Eimeria tenella* sensitivity (Thabet et al., 2017), also support reducing animal testing in this area. Despite recent advancements in in vitro methods and a growing desire to move away from animal testing, the new draft guideline disappointingly fails to address opportunities to reduce the number of animal tests required. It currently recommends a disproportionate number of animal tests, such as the unclear need for two separate dose confirmation studies. To align with ethical considerations and scientific advancements, the guideline should explore opportunities to combine or waive certain tests based on the results of previous studies, in vitro or other non-animal methods (such as those outlined above) and/or existing data. Clear guidance on reducing animal testing should be incorporated throughout the guideline.	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Section 4, p.5	1	*Comment:* While the guideline explicitly states that single housing should be avoided for rabbits due to the stress it causes, it does not make the same recommendation for other species. Single housing is known to induce stress in social animals, which can confound experimental results. In fact, housing social species (e.g. dogs) on their own for prolonged periods is an example of a procedure that, under Annex VIII of the Directive 2010/63, can lead to severe suffering *Proposed change:* Possible additional point under section 4 – 'General considerations' **In line with Directive 2010/63 on the protection of animals used in scientific procedures, the use of group housing of animals should be implemented into study plans. The use of single housing can lead to stress, in particular in social species, which may confound experimental results.**	Partly accepted. The text in lines 125-127 has been changed to: '*All animal experiments should be conducted taking into account section I.1.7 of Annex II of Regulation (EU) 2019/6 and the 3Rs principles (replacement, reduction and refinement), notwithstanding the place of conduct of the experiments. Alternatives to in vivo test methods should be employed whenever possible*.' It is considered that "refinement" also implies that single housing of animals should be avoided when possible. In addition, the following sentence has been included under section 4 - General considerations: '*Housing of study animals should take account of animal welfare requirements whilst at the same time ensuring integrity of the study design*.'
Section 4: line 139-142. p.5	1	*Comment:* In line with the 3Rs principles, we urge the EMA to remove the use of mortality as an end point. At the very least, it should be explicitly stated that if mortality is to be used, proper scientific justification	Not accepted. Depending on the pathogen and the target animal species, coccidian infections can rapidly lead to mortality, without a significant time period during which clinical symptoms can be

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		must be provided, demonstrating that all other options have been fully considered and exhausted first. We recommend that this requirement be included throughout the document, including in the sections specific to each individual species. *Proposed change:* 3Rs principles should be followed through standardised methodology for infection and efficacy calculation, by avoiding mortality rate as ~~**primary**~~ **an** endpoint and by using other appropriate endpoints (e.g. lesions scores assessed in chickens euthanised prior to spontaneous death), where possible. **If mortality is to be used as an end point proper scientific justification should be provided.**	observed. Therefore, mortality may be quite high and thus may be the parameter with the highest relevance. In the guideline (section 5.5.6) it is stated that '*Animal welfare should be considered when establishing endpoints*.' Under general considerations (section 4) it is stated that: '*3Rs principles should be followed through standardised methodology for infection and efficacy calculation, by* ***avoiding mortality rate as primary endpoint*** *and by using other appropriate endpoints (e.g. lesions scores assessed in chickens euthanised prior to spontaneous death), where possible*.' The current wording included in the guideline is considered appropriate.
Section 4, p.5	1	*Comment:* We suggest an additional point is added under 'General considerations' to ensure that non-animal methods are used for screening and efficacy testing of anticoccidial substances wherever possible. *Proposed change:* Additional point – **Wherever possible non-animal methods should be used to replace the use of animals for the screening and efficacy testing of anticoccidial substances, in order to reduce overall animal use.**	Partly accepted. The following sentence has been added to the guideline (line 127): '*Alternatives to in vivo test methods should be employed whenever possible*.' It is considered that these further improvements ensure flexibility for the development and use of *in vitro* and other non-animal methods as they become available. The current wording also allows waiving of animal studies, thus reduction of the number of animals used in studies.

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Section 4: lines 143-151, p.5	1	*Comment:* We appreciate that the document acknowledges the possibility of omitting certain recommended study types. However, the wording could be strengthened to more strongly encourage a reduction in animal use. Given the disproportionate number of animal studies recommended, we urge the EMA to provide more explicit guidance on when animal studies can be waived and to highlight opportunities for combining studies to further reduce the overall use of animals. *Proposed change:* - The omission of a type of study (laboratory or field conditions), **particularly those involving animals**, may be accepted, if appropriately justified and where the provided data are sufficiently robust to demonstrate efficacy. **Furthermore, where non-animal methods exist these should be used wherever possible.** - ***Add additional guidance on when studies can be combined and the criteria for waiving an animal study.***	Partly accepted. The following sentence has been added to the guideline (line 127): '*Alternatives to in vivo test methods should be employed whenever possible*.' It is considered that these further improvements ensure flexibility for the development and use of *in vitro* and other non-animal methods as they become available. The current wording also allows waiving of animal studies, thus reduction of the number of animals used in studies. The current wording allows for omission of a type of study where the provided data are sufficiently robust to demonstrate efficacy. These situations are considered on a case-by-case basis.
Section 5.5.1, paragraph 2, lines	1	*Comment:* We encourage the EMA to consider expanding the wording to more explicitly apply the 3Rs principles in dose determination studies. Animal use for studies	Not accepted. Please refer to responses above. The proposed amendment in lines 125-127 ensures flexibility for the development and use of *in vitro* and other non-animal methods as they

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200-201, p.7		should, at the least, be kept to a minimum and ideally replaced wherever possible. This will also allow for the inclusion of non-animal approaches as they become available. *Proposed change:* Infective dose, number of animals and endpoints should be considered carefully to take account of the 3Rs principles, whilst adhering to statistical principles and allowing for robust data. **The use of animals should be kept to a minimum and ideally replaced wherever possible.**	become available. The current wording also allows waiving of animal studies, thus reduction of the number of animals used in studies.
Section 5.5.6, paragraph2, lines 295-297, p. 9	1	*Comment:* Again, mortality should not be recommended as an endpoint, at the least, if mortality is to be used proper scientific justification should be provided. *Proposed change:* The endpoints may include clinical signs, ~~**mortality,**~~ macroscopical and histopathological changes, or body weight gain, depending on the target animal species (see below in the target species specific section). Animal welfare should be considered when establishing endpoints **and mortality should only be used with proper scientific justification**.	Not accepted. Depending on the pathogen and the target animal species, coccidian infections can rapidly lead to mortality, without a significant time period during which clinical symptoms can be observed. Therefore, mortality may be quite high and thus may be the parameter with the highest relevance. In the guideline (section 5.5.6) it is stated that '*Animal welfare should be considered when establishing endpoints*.' Under general considerations (section 4) it is stated that: '*3Rs principles should be followed through standardised methodology for infection and efficacy calculation, by* ***avoiding mortality rate as primary endpoint*** *and by using other appropriate endpoints (e.g. lesions scores assessed in chickens euthanised prior to spontaneous death), where possible*.'

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			The current wording included in the guideline is considered appropriate.
Section 5.5.6.2, paragraph 2, line 320, p.10	1	*Comment:* The unnecessary use of more invasive procedures should be avoided in line with the 3Rs principles. This draft version of the guideline states that individual faecal samples are 'necessary' whereas in the previous version it was stated that individual faecal samples are 'preferred'. We suggest that wherever possible pooled faecal samples are used. *Proposed change:* For dose determination and dose confirmation studies, ~~**individual**~~ **pooled** faecal samples ~~**(e.g. by rectal sampling)**~~ are generally ~~**necessary**~~ **preferred** for oocysts quantification. **Individual faecal samples (e.g. by rectal sampling) should only be used if properly justified.**	Not accepted. Pooled samples or litter samples may only be used for checking the presence of the parasite and are not relevant for correlation with the level of infection. Individual samples are necessary for quantification of oocysts and proper statistical analysis of the data. The current wording included in the guideline is considered acceptable.
	1	**References** Arias-Maroto et al. (2024). Reduction of chickens use to perform in vitro pre-screening of novel anticoccidials by miniaturisation and increased throughput of the current Eimeria tenella compound-screening model. *F1000Research*, 11:1135. Felici et al. (2021). In vitro assessment of anticoccidials: methods and molecules. *Animals*, 11(7): 1962.	Noted.

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		Joachim et al. (2018). WAAVP guideline for evaluating the efficacy of anticoccidials in mammals (pigs, dogs, cattle, sheep). *Veterinary Parasitology*, 253: 102-119. Odden, A. et al. (2019). Preliminary studies on in vitro methods for the evaluation of anticoccidial efficacy/resistance in ruminants. *Experimental Parasitology*, 201: 34-41. Thabet, A. et al. (2017). Anticoccidial efficacy testing: In vitro Eimeria tenella assays as replacement for animal experiments. *Veterinary Pathology*, 233: 86-96.	