

**Compilation of comments from the Netherlands and IFAH-Europe  
on the second draft Guidance Document on the Determination of the Toxicity of a Chemical to Dung Beetles and responses from the lead  
(European Commission)**

Countries	Comments	Responses
	General comments	
<b>IFAH-Europe</b>	<p>The draft guideline recommends using <i>Aphodius constans</i> as the test organism. As indicated in the guideline itself, this species is available in nature only during a few months in early spring. It is very difficult to breed in the laboratory, as confirmed recently in email correspondence between an IFAH-Europe Member Company and dung organism experts K. Wardhaugh and K. Floate. The timeframe to perform the test therefore is restricted to about 4 months a year, which is a very narrow window, and makes the toxicity test cumbersome to apply within the structured regulatory timetables that exist within the EU for the authorisation of Veterinary Medicinal Products (VMPs), for which in fact this test was developed. We would therefore propose that the guideline should include the possibility to use other species of dung beetles (<i>Aphodius sp</i>, <i>Onthophagus Taurus</i>, <i>Euoniticellus intermedius</i>).</p>	<p>It is true that the testing of <i>Aphodius</i> sp. could be difficult due to the relatively small “time window” – and it is also true that the DOTTS group tried to find alternative and/or additional dung beetle test species. The obvious candidates are <i>O. taurus</i> and <i>E. intermedius</i>, since both have already been cultured in Australia and Europe (recently in Canada too). However, for about five years several European laboratories failed to set-up permanent cultures of <i>O. taurus</i>, starting with animals from colleagues in England and Australia (currently another attempt is planned but sending-out the beetles have again been delayed for months). The same happened in our own lab with <i>E. intermedius</i>, using beetles from Australia and South Africa. Actually, there are almost no test data with these species available – and those which are known have either not fully been published or did use different methods. More importantly, the British lab with the longest experience in <i>O. taurus</i> testing did not provide the DOTTS group with a text analogous to the one which is given in the current draft Guidance Document.</p> <p>The number of potential dung beetle test species can be increased in the future, but for the upcoming years the preconditions to include one of these species (broader experience in culturing and testing experience in more than 1 – 2 labs) are not fulfilled.</p>

<p><b>IFAH-Europe</b></p>	<p>The draft guideline recommends the possibility of a limit test at a maximum concentration of 1.000 mg per kg dry dung. The same recommendation is made in the toxicity test for dung flies. These concentrations and even increased ones are frequently observed in the dung of animals in the first days post treatment. It is our concern that restricting the test to that maximum concentration may preclude the solution of the Risk Quotient equations as prescribed in the VICH guidelines on Environmental Risk Assessments of VMPs. IFAH-Europe therefore recommends the addition of a maximum test at 10.000 mg per kg dry dung, or at any higher concentration selected by the applicant.</p> <p>Another possibility would be to express the maximum test concentration on a wet dung basis, for example at 1.000 mg per kg wet dung. Alternatively, it could be stated that the substance displaying no toxicity at 1.000 mg per kg dry dung is considered devoid of adverse impact on populations of dung organisms, comparable to statements made for example for the aquatic environment (where a substance with a toxicity over one hundred parts per million is regarded as practically non-toxic*).</p> <p>* OECD Series on Testing and Assessment - Number 27 - Guidance Document on the use of the Harmonised System for the Classification of Chemicals which are Hazardous for the Aquatic Environment.</p>	<p>Concentrations of 10.000 mg/kg dry dung for a veterinary pharmaceutical is unlikely. However, it might be possible. Therefore, Paragraph 33 was modified in line with the comment.</p>
<p><b>IFAH-Europe</b></p>	<p>The draft guideline recommends exclusively the use of cattle dung for the testing. We would like to re-emphasize our recommendation to include the statement that studies can also be conducted in sheep dung. Firstly, as already mentioned in our first comments, some VMPs are used in sheep only. Secondly, member companies of IFAH-Europe have evidence that some substances display higher toxicity in cattle dung than in sheep dung. Hence, in case a product is used in both, sheep and cattle, testing with sheep dung may eliminate an environmental risk, while limitation to cattle dung may suggest a risk for sheep as well which may not exist.</p>	<p>The reason why emphasis is given to cattle dung is that published experiences with other dung than cattle and this test method are rare (almost nothing, in fact). However, it is true that this test can be performed with sheep dung (I am not aware of tests with horse dung). Therefore, a sentence was added to address this comment. at the beginning of paragraph 16.</p>

<b>IFAH-Europe</b>	Finally, as it is stated in the introduction of the text “ <i>Preparation of test guidelines for dung beetles for formal submission to OECD currently is not possible. Partly this is due to unanswered questions concerning methods for O. taurus and A. constans, and partly because methods developed thus far have not been validated with a ring test</i> ”, it is our understanding that before finalization of this new test guideline, DOTTS will fully test the dung beetle method as it was done for the dung fly and also wait for the results from testing of other proposed test species, such as <i>Onthophagus taurus</i> and <i>Euoniticellus intermedius</i> . As indicated in Annex 6 (page 23), laboratory culture of <i>Onthophagus Taurus</i> has been achieved and preliminary results for <i>Euoniticellus intermedius</i> are promising. The definitive test results with these species need to be evaluated before they are excluded from the OECD test guideline. As indicated here again, this draft guideline is in fact not ready to be formally submitted to OECD for adoption.	The document currently developed is a Guidance Document, not a Test Guideline. As explained above when responding to Comment No. 1, despite considerable efforts it was not possible to set-up cultures of the additional species in European contract laboratories or to get test results from several laboratories with field-caught individuals with these species. Also it was already mentioned that no draft method description has been provided to DOTTS within the last years. Since there is clearly a need by EU authorities to get dung beetle data, it is strongly recommended to finalise this Guidance Document while at the same time working on improving the test species battery. This process could surely be facilitated if members of IFAH-Europe would actively support it.
	<b>Paragraph 3</b>	
<b>Netherlands</b>	Line 3: ‘Toxicity’ should read ‘Toxicology’  In addition, it seems the formulation of this and the next paragraph needs some further modification. It now reads as if the guidance document still is in development.	Please find a partly re-worded Paragraph 3 (no new information was included except one sentence explaining why only <i>A. constans</i> is described in detail).
	<b>Paragraph 23</b>	
<b>Netherlands</b>	Why is moisture content expressed on a wet weight basis? In general, use of dry weight is clearer and seems more consistent with other test guidelines.	Changed in the revised Paragraph 23
	<b>Annex 2</b>	
<b>Netherlands</b>	In first and second paragraph: please clarify whether weight of dung is expressed as dry or wet weight.	Changed according to the comment.
	<b>Annex 6</b>	
<b>Netherlands</b>	First paragraph, 5 <sup>th</sup> bullet: please mention which chemical has to be tested, I suppose it should be avermectin but that is not clear from the description.	The word “ <b>avermectin</b> ” was added in the first line of the fifth bullet point behind “mg” and before “/kg”

## **Name of Experts**

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