

March 07, 2013

Validation Ring Test: Transformation of veterinary pharmaceuticals and biocides in manure

Dear colleagues,

on behalf of the German Federal Environmental Agency, I am pleased to invite you to take part in the international validation ring test on "Transformation of veterinary pharmaceuticals and biocides in liquid manure" in order to start the validation of a draft test method. The ring test is scheduled for September 2013 to April 2014. For details see the attached Outline of the Ring Test.

In general, participating institutes will be responsible for the funding of the laboratory work. A certificate for participating in the ring test will be provided to each participant at the end of the test.

I would be very grateful if you could indicate your interest to participate in the ring test by signing and returning the enclosed form by fax or by e-mail attachment until April 30th, 2013 at the latest. Regardless of whether you are able to participate or not, it would be helpful if you could name further colleagues who would be able and willing to take part. As soon as the participant list is completed you will receive further information.

I am convinced that your contribution will be significant for the achievement of the objectives of the ring test. If you have any technical questions relating to the ring test itself or in case you need further information, do not hesitate to contact us (addresses below).

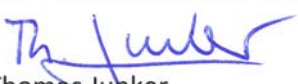
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Yours sincerely

ECT Oekotoxikologie GmbH


Thomas Junker

Attachments:

- Outline of the Ring Test
- Registration form

Outline of the Ring Test

“Transformation of veterinary pharmaceuticals and biocides in (liquid) manure”

Introduction

Veterinary pharmaceuticals (VMPs) administered systemically to animals are excreted with urine and feces by treated animals. For animals housed in stables the resulting manure/slurry is collected and stored before being spread onto agricultural land. Disinfection products used to sanitize stables are also transferred into the manure. Therefore, the spreading of (liquid) manure/slurry is an important pathway of introducing veterinary pharmaceuticals, biocides and their metabolites and transformation products into the environment. As a consequence, the fate of VMPs/biocides in manure is taken into account in the environmental risk assessment for VMPs/biocides.

Although there is the need for guidance on the performance and evaluation of degradation studies with VMPs/biocides in manure, a standardized and validated method is currently lacking. At European level, the Committee for Medicinal Products for Veterinary Use (CVMP) of the European Medicines Agency (EMA) adopted a guidance document on determining the fate of veterinary medicinal products in manure in March 2011 (EMA, 2011). The document is intended to provide guidance on the general conditions of studies on the transformation of veterinary medicinal products in manure (e.g. matrix characterisation of manure, regulatory use of the results). However, the EMA guidance is not an experimental protocol and therefore further advice on experimental details is required to obtain reliable and sound results.

Therefore, a standardised experimental test method and a draft guideline are currently under development within a research project funded by the German Federal Environment Agency that should in the long run lead to a guideline on transformation of substances in (liquid) manure within the framework of the OECD test guideline program.

A pre-validation ring test has been performed in 2012/2013 and is currently evaluated. Based on the outcome of the pre-validation ring test and the currently planned validation test and taking into account existing guidelines, a draft guideline to be submitted to the OECD will be prepared.

Test Procedure

The performance and documentation should be done according to GLP-rules (OECD, 1998), but no formal certificate is required for the participating laboratories. Transformation tests will be run using ^{14}C -radioactively labelled substances. It is also possible and highly welcome to do LC-MS/MS-analysis including screening for and identification of transformation products. In this case working with ^{14}C -material is not required.

Bibliography

OECD (1998). The OECD Principles of Good Laboratory Practice (1998) (as revised in 1997). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring No.1. Organisation for Economic Co-Operation and Development, Paris.

Technical Annex

Aim:	Validation of the test method described above.	
Co-ordination:	ECT Oekotoxikologie GmbH Boettgerstr. 2-14 65439 Floersheim/Main Germany	
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Scientific monitoring:	Dr. Dieter Hennecke Fraunhofer Institute for Molecular Biology and Applied Ecology 57392 Schmallenberg, Germany	
	Dr. Silvia Berkner Federal Environment Agency Section IV 2.2 Pharmaceuticals 06844 Dessau, Germany	
Participants:	As many as possible; at least 5 participants from different countries	
Chemicals:	Two substances will be selected and tested. Test substances will be provided by the Co-ordinator.	
Manure:	The minimum requirement is to test one substance in cattle manure and the other substance in pig manure. It is optional to test both substances in both pig and cattle manure. Laboratories that would like to do LC-MS/MS analysis including identification of transformation products are very welcome to participate. In this case working with ¹⁴ C-labelled compounds could be waived.	
Funding:	In general, participating institutes will be responsible for the funding of the laboratory work.	
Time Table:	03/2013 – 04/2013	Registration
	05/2013	Meeting at SETAC Glasgow (DOTTS group)
	06/2013	Distribution of draft test guideline to participants
	08/2013 – 09/2013	Distribution of chemicals, manure, documents
	09/2013 – 04/2014	Test performance
	01/2014 – 06/2014	Evaluation of results
	06/2014	Workshop: Discussion of ring test results with participants
	11/2014	planned submission of ring test results and Draft Guideline to OECD

Summary of the test method parameters (minimum test package)

Parameter	
Test compounds (radiolabelled) and Test matrix	- Veterinary pharmaceutical: ^{14}C florfenicol (CAS No. 73231-34-2); anaerobic pig manure (liquid manure sampled from a tank or lagoon adjusted to 5% dry matter content, sampled by each participant or provided by coordinator depending on evaluation of prevalidation results) - Biocide: ^{14}C imidacloprid (CAS No. 138261-41-3); anaerobic cattle manure (liquid manure sampled from a manure tank or lagoon adjusted to 10% dry matter content, sampled by each participant or provided by coordinator depending on evaluation of prevalidation results)
Pre-treatment	Homogenisation (anaerobic)
Storage of manure	maximum storage period: 2 months at 20°C (anaerobic)
Manure matrix characterisation (mimimum, ref. to EMA, 2011)	- pH - dry matter content - temperature - nitrogen content ($\text{NH}_4\text{-N}$ and N_{tot}) - organic matter [%] - microbial activity ¹ - redox potential
Amount of manure	50 - 100 g wet weight per incubation vessel
Pre-incubation	21 days at 20°C, anaerobic
Test duration	90 d
Test design	anaerobic
Temperature	20 ± 2°C
Lighting	complete darkness
Redox conditions	anaerobic (redox potential always below -100 mV); moistened nitrogen is passed above the manure
Number of sampling time points	10 ²
Number of test concentrations	1 (to be defined; e.g. maximum expected manure concentration)
Number of replicates	6 (per sampling)
Number of sterile controls	6 (without gas trapping; sampling at max. three time points incl. termination of incubation)
Endpoints / parameters	- mineralisation ($\text{CO}_2 + \text{CH}_4$), (may be waived if deailed LC-MS/MS analysis is done) - formation of non-extractable residues (NER) (may be waived if deailed LC-MS/MS analysis is done) - screening for and identification of transformation products - DT_{50} parent and transformation products - mass balance
Evaluation of ring test results	by the Co-ordinator (excel file for data reporting will be provided to each participant)

¹ e.g. mineralization of a readily degradable ^{14}C -labeled substance (e.g., ^{14}C -glucose) under anaerobic conditions (see Annex 3, draft guideline)

² For the test with florfenicol it is important to know that at 20°C sampling should be frequent during the first day. After one week, the sampling intervals can be increased. The dissipation half-life for the parent compound is in the rage of a few hours at 20°C and a few days at 10°C. Thus, frequent sampling during the first day can be avoided when testing at 10°C.

Optional experiments:

- ^{14}C -florfenicol, 20°C, cattle manure, 6 parallels, 10 sampling time points
- ^{14}C -imidacloprid, 20°C, pig manure, 6 parallels, 10 sampling time points
- florfenicol, unlabelled, 20°C, pig manure, 6 parallels, 10 sampling time points

Registration Form

Validation Ring Test on

“Transformation of veterinary pharmaceuticals and biocides in liquid manure”

Name:
Institution:
Department :
Address:
Country:
Fax:
Phone:
E-Mail:

I will participate in the performance of the ring test:	☐ Yes	☐ No
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Licence on handling ¹⁴ C-labelled material:	attached	☐
	will be delivered later	☐
	no licence available	☐

Comments:

.....
Date

.....
Signature

If more than one person of your institute will participate, please use copies of this form.

Please return this form by fax or by e-mail to:

ECT Oekotoxikologie GmbH, Böttgerstr. 2-14, 65439 Flörsheim/Main, Germany

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