EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 February 2010
EMA/CVMP/ERA/430327/2009
Committee for medicinal products for veterinary use (CVMP)

Guideline on determining the fate of veterinary medicinal products in manure

Draft

Draft Agreed by Working Party on Environmental Risk Assessment	3 January 2010
Adoption by CVMP for release for consultation	10 February 2010
End of consultation (deadline for comments)	31 August 2010

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Keywords	*Veterinary medicinal products (VMP), degradation, fate, manure, DT50, non-extractable residues, environmental risk assessment*

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Introduction

1.1. Background

The guideline Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH guidelines GL6 and GL38 (EMEA/CVMP/418282/2005-Rev.1)[1] gives further technical support to the implementation of the VICH guidelines GL6 and GL38 on the environmental risk assessment (ERA) of veterinary medicinal products (VMPs). Besides algorithms, models and default values for the determination of the predicted environmental concentration (PEC) of VMPs in the environment, the guideline provides a number of options to refine the exposure assessment. One of these options, is the determination of the degradability of a VMP in manure of stabled target animal(s). This type of study is not compulsory but the information can be used in Phase I if complete degradation or mineralization can be demonstrated or in Phase II to refine the PEC in soil.

At present no validated or standardized method for assessing the fate of VMPs in manure has been developed. Consequently the CVMP guideline provides only limited advice on this aspect of the risk assessment with a focus on the use of degradation studies as part of the Phase I assessment. Since the release of the CVMP guideline it has become clear that there is a need for guidance on the design, execution and interpretation of studies on the transformation of VMPs in manure which can be used in the preparation of the ERA by the applicant and in the evaluation of the studies by the competent authorities.

This guidance document has been prepared with input from researchers, industry and the competent authorities at a workshop organised by the CVMP/EMEA (Fate of veterinary medicinal products in manure Focus Group Meeting, 23 June 2009, http://www.emea.europa.eu/meetings/conferences/23jun09.htm).

1.2. General considerations

Animal manures contains excreta, i.e. urine and faeces, of the housed animals together with other materials of farming practice, e.g. spilled feed, straw, litter, sand, water, down and carcasses. Manure may be either liquid (slurries) or solid (farm yard manure).

Manure is a highly variable substance and the parameters that influence the degradation rate of VMPs can differ to a large extent. The most striking difference is the variation in dry matter content in manure due to the species of animal or the way the animals are housed. At present the impact of all the different parameters on degradation rate is not well known which makes it difficult to select one set of conditions which mimic all conditions of animal housing. In the future it may be possible to define types of reference manure which meet certain conditions.

This guidance document attempts to standardise the degradation tests as much as possible, recognising that more research is needed to examine the influence of variations in test conditions. This guidance should therefore be considered as a living document which might need revision when more information on the variability of manure properties and the effects of these variables on VMP degradation becomes available.

1.3. Outline

This guidance is divided into two main sections dealing with study design and the evaluation and use of the test results. These sections are subdivided into the following topics:

[1] referred to subsequently as "the CVMP guideline"

Study design (§ 2)

- Sampling of excreta and preparation of test/reference manure (§ 2.1)
- Matrix characterisation of the manure (§ 2.2)
- Establishing test conditions (§ 2.3)
- Test substance (§ 2.4)
- Analytical methods (§ 2.5)

Evaluation and use of test results (§ 3)

- Extrapolation within and between manure types of different target species (§ 3.1)
- Use of test results to refine PECs (§ 3.2)

2. Study design

2.1. Sampling of excreta and preparation of test/reference manure

Degradation studies should preferably be performed in manure of each major species to which the VMP is administered. Relevant major species are those defined in the CVMP guideline, i.e. pigs, cattle, sheep and chickens.

It is recommended that the degradation study is carried out in manure from animals, that are reared under well controlled conditions. Manure contaminated with other VMPs, biocides and other material that can alter the degradation rate of the VMP under investigation should not be used. The feed type, feeding regime and the veterinary history of the animals from which the manure is collected should be recorded.

Freshly collected manure from cattle and pigs should be conditioned at 20°C for 21 days to reach stable and strictly anaerobic conditions. As chicken manure is stored under aerobic conditions this does not need to be acclimatised. The manure samples have to be matrix characterised (see § 2.2). At present it is unclear whether storage of manure at low temperature (e.g. -20 °C) before testing could influence the degradability of VMPs. When stored at -20 °C, it is recommended that manure is reconditioned at ambient conditions for 3 days and the matrix characterised again. Tap water should be added to prepare reference-manure samples of defined dry substance contents (see § 2.3.3)

2.2. Matrix characterisation of the manure

The degradation of a substance can be a function of different characteristics of the manure. For this reason it is important that the following parameters are measured to characterise the manure:

- pH,
- microbial activity[2],
- organic matter,
- nitrogen content,
- redox potential,
- dry matter content,
- oxygen content.

2.3. Establishing test conditions

2.3.1. Treatment and application of test substance

The minimum amount of manure to use in the test is 50 to 100 g (fresh weight).

Preference is given to spiked manure although it may be possible to use manure from treated animals if a proper degradation rate and identification of the transformation products can be obtained.

The test substance should be dosed into manure at a concentration that reflects the expected manure concentration. If this concentration is not high enough to identify major transformation products, incubation of separate manure samples containing higher rates may be helpful, but excessive concentrations which may influence microbial functions should be avoided.

2.3.2. Oxygen content and redox potential

Cattle and pig studies should be performed under anaerobic conditions e.g. discontinuous nitrogen flow. Anaerobic conditions should be demonstrated by a negative redox potential or the absence of oxygen. For poultry and sheep manures, the conditions should be aerobic[3].

2.3.3. Dry matter content

The recommended dry matter content in bovine and pig manure is 10% ± 1% and 5% ± 1%, respectively. Poultry manure will normally contain a much higher dry matter content. Based on a study by Ellen (2000) a dry matter content of 60% ± 5% is recommended. If the test flasks are to be aerated, the air should be humidified to avoid loss of water from the poultry manure.

[2] The microbial activity of the manure samples could be proven by the measurement of the biological oxygen demand even though this method mainly determines the activity of aerobic microorganisms. The measurement of dehydrogenase activity, to determine the activities of aerobic and anaerobic microorganisms, may be limited by its final photometric measurement of triphenyl formazan at λ = 485 nm or λ = 546 nm because of the deeply coloured manure extracts. The application of a readily degradable standard substance, e.g., sodium benzoate, in parallel batch experiments causes other problems. Reduction of DMSO to DMS can be an alternative measurement of aerobic microbial activity without interference (Griebler & Slezak, 2001).

[3] In practice, strict aerobic conditions can be obtained just on the surface of the manure. Ventilation would be needed to keep aerobic conditions in the core of the manure pile. If the geometry of the pile is suitable, there will be an anaerobic, methane producing, thermopilic microbial community in the core with thick mainly fungal (actinomycetal) layer between aerobic and anaerobic world.

2.3.4. Test duration

The length of the study will depend on the rate of degradation of the compound and information on the average storage time for the manure under test (see CVMP guideline, Table 6).

2.3.5. Temperature and light condition

During the whole test period, the manure samples should be incubated in the dark at the relevant temperature. In the CVMP guideline relevant temperatures for testing are considered to be 20°C for pig manure, 10°C for cattle manure and 25°C for chicken manure. For the sake of standardisation, it is now recommended to test all manure types at a temperature of 20°C[4]. For the risk assessment the derived DT50 has to be corrected to the relevant environmental temperature (see § 3.2.5).

2.3.6. Sterile control

To obtain information on the relevance of abiotic transformation of a test substance, manure may be sterilized. This is most relevant when the study is conducted with non-radiolabelled test substance.

Further guidance on the test conditions for determining the degradation of VMPs in manure from pigs and cattle is given by Kreuzig et al., 2007 and 2009.

2.4. Test substance

Purity and/or radiochemical purity of the test substance should be reported.

Radiolabelled material is, in principle, preferred because detailed mass balances can be determined taking into account mineralisation, extractable and non-extractable residues. When non-radiolabelled material is used, only the disappearance of the parent compound initially applied can be followed unless specific methods exist for known transformation products. The study will need to be designed in such a way that it provides equivalent data to that which would be obtained in a radiolabelled study i.e. it will need to identify any major metabolites and characterise the formation of non-extractable residues. It is recommended that the manure is incubated in a flow-through system rinsed with nitrogen in stop-flow mode (OECD, 2002). In order to trap and analyse released 14C-carbon dioxide and 14C-volatile substances external traps filled with ethylene glycol, sulphuric acid and potassium hydroxide solution have to be linked to the incubation flask. For the determination of 14C-methane released out of the 14C-labelled test substance, the 14C-carbon dioxide free headspace of the incubation flask has to be transferred into a combustion apparatus where 14C-methane is oxidised to 14C-carbon dioxide. The latter is to be trapped again in an absorbing scintillation cocktail and then scintillation counted.

For addition to and distribution in manure, the test substance can be dissolved in water (deionised or distilled) or, when necessary, in minimum amounts of an organic solvent in which the test substance is sufficiently soluble and stable. However, the amount of solvent selected should not have a significant influence on manure microbial activity.

2.5. Analytical methods

The primary objective of the study is to determine the fate of the active substance in manure, to identify and if possible quantify all transformation products and remaining parent compound and to quantify the degree of mineralization. It is recommended that a sequential extraction method is

[4] If after consultation it is agreed that the incubation temperature for all manure types should be 20°C then the CVMP guidance will be amended to ensure consistency between the two guidance documents.

followed. An exhaustive extraction is necessary with various apolar and polar solvents and acid systems. The extraction efficiency should exceed 70%. The extraction of residues should not change the chemical nature of the active substance or the transformation products.

All transformation products formed at more than 10% of the applied dose should be identified and quantified.

The validation of the analytical methods should be the same for both the parent compound and the transformation products. Analytical methods (including extraction and clean-up methods) for identification and quantification of the test substance and its transformation products should be available. Further guidance on the validation of the analytical methods is given by De Knecht et al., 2001.

3. Evaluation and use of the test results

3.1. Extrapolation within and between manure types of different target species

In principle, extrapolation of degradation rates between manure types within the same species is possible provided that the composition of the manure and storage conditions are comparable. At present there is limited information available to provide more precise guidance for which species and manure types extrapolation is feasible. For pragmatic reasons, manure within the same animal type, i.e. pigs, cattle, sheep and poultry is considered to be comparable.

3.2. Use of test results to refine PECs

There are a number of potential outcomes from the study, namely:

- The parent compound is not degraded
- The parent compound is completely mineralised or degraded over 30 days
- The parent compound is partially mineralised
- The parent compound is primarily degraded into transformation products
- Non extractable residues (NER) are formed in the manure
- A combination of the above dissipation processes occurs

The outcome of studies in which there is complete degradation or mineralization can be used in Phase I. The approach for PEC refinement or use of the data in risk refinement is discussed in the following sections for each scenario.

3.2.1. Complete mineralization/degradation

In instances where degradation results in complete mineralization or complete degradation into transformation products all representing < 5% of the total dose within 30 days the exposure to the environment is considered negligible and the risk assessment can stop in Phase I.

3.2.2. Partial mineralisation

When partial mineralization is observed in the study, the PECsoil can be revised using the percentage mineralisation data and an appropriate kinetic model (selected based on the degradation pattern) and average storage times recommended in the CVMP guideline.

3.2.3. Degradation into transformation products

According to the VICH Phase II guideline, excreted metabolites representing 10% or more of the administered dose and which do not form part of biochemical pathways should be added to the active substance to allow the PEC to be recalculated. This rule can also be applied to degradation in manure. However, when NER remain, this rule should be applied with a certain restraint considering that these residues might still contain transformation products which when added to the extractable part will comprise a percentage > 10%.

In instances where major transformation products are formed, it is assumed that these compounds have a similar activity to the parent compound unless it can be shown that the ecotoxicity of these compounds is less than the parent compound. If the parent compound is completely degraded into a single transformation product, it is logical that the risk assessment should be focussed on this compound.

3.2.4. Non-extractable residues

The mechanisms for binding within the manure matrix can be numerous and complex though to date are not well understood. For a better understanding of the binding capacity of organic matter in various manure types further research is needed. It might also be necessary to highlight that, in contrast to soil and sediment, manure will be degraded to a large extent. In that respect it is important to rule out the possibility that the NER represents parent compound and transformation products that become available when the manure matrix degrades. In this context the general rule applied in the OECD guideline 307 and 308, that the extraction method must not substantially change the structure of the sample matrix under study, might not be appropriate for manure.

At present it is difficult to give guidance on which vigorous extraction method is sufficient to ensure that the NER will not cause any toxicity when manure degrades after having been spread on land. As a worst case estimate NER should be considered to represent parent compound, unless it can be shown, through appropriate experiments, that the toxicity of the NER is significantly lower than the parent compound. Initiatives on assessment of NER are currently under development.

3.2.5. Temperature correction

The CVMP guideline gives further guidance on how the test results can be used to refine the PEC based on the realistic storage times and environmental temperatures. As cattle and pig manure can be stored outdoor, 10 ºC is considered to be a realistic environmental temperature for both manure types (and not 20 ºC as recommended for pig manure in CVMP guideline[5]). For chicken manure 25 ºC is considered to be a realistic environmental temperature.

DT_{50} values can be normalized to the environmental temperature using the Arrhenius equation. The PPR Panel (EFSA, 2007) recently recommended that the median Ea value of 65.4 kJ/mol corresponding to a Q_{10} of 2.58 should be used instead of the previous recommended default Ea value of 68.9 kJ/mol, as mentioned in CVMP guideline. Based on a Q_{10} of 2.58 the following formula can be used when converting DT_{50} values determined at 20 ºC towards an environmental temperature T.

$$DT_{50,T} = DT_{50,20C} \cdot e^{0,095(20-T)}$$

in which:
$DT_{50,20C}$ = half-life at 20 °C

[5] If after consultation it is agreed that the storal temperature for pig manure is 10°C then the CVMP guidance will be amended to ensure consistency between the two guidance documents.

$DT_{50,T}$ = half-life at relevant temperature T [°C]

4. Interested parties

Industry, CROs and regulators

5. References to literature, guidelines etc

De Knecht JA, Janssen PAH and Van Vlaardingen PLA 2001. Guideline for evaluating studies to determine excretion of veterinary drugs. In R Luttik and MTM Van Raaij. Factsheets for the (eco)toxicological risk assessment strategy of the National Institute of Public Health and the Environment. RIVM report 601516 007.

EFSA. 2007. Opinion on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. The EFSA Journal 622: 1-32.

Ellen HH 2000. Overview of manure types and treatment. Praktijkonderzoek Pluimveehouderij 'Het Spelderhout' Beekbergen

Griebler C, Slezak D (2001): Microbial Activity in Aquatic Environments Measured by Dimethyl Sulfoxide Reduction and Intercomparison with Commonly Used Methods. Applied and Environmental Microbiology 67: 100–109

Kreuzig R, Höltge S, Heise J, Schmanteck I, Stein F, Batarseh M (2007): Veterinary Medicinal Products in Manures and Manured Soils: Development of a Technical Protocol for Laboratory Tests. UBA-Texte 45/07, ISSN 1862-4804. Umweltbundesamt, Dessau-Roßlau, 1-142. http://www.umweltdaten.de/publikationen/fpdf-l/3343.pdf

Kreuzig R, Schlag P, Teigeler J, Hartmann C, Cvetković B (2009): Technical protocol: Transformation of biocides in liquid manures. UBA-Texte 45/07, ISSN 1862-4804. Umweltbundesamt, Dessau-Roßlau, 1-116.

OECD, 2002. Guideline 307 for testing of chemicals. Aerobic and anaerobic transformation in soil.

OECD, 2006. Guideline 311 for testing of chemicals. Anaerobic biodegradability of organic compounds in digested sludge by measurement of gas production.

VICH Topic GL6 (Ecotoxicity Phase I) Guideline on environmental impact assessment (EIAS) for veterinary medicinal products – Phase I. CVMP/VICH/592/98-FINAL

VICH GL 38 (Environmental impact assessment for veterinary medicinal products - Phase II, CVMP/VICH/790/03-Final) Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL3