Manure Degradation Studies Experiences

Intervet / Schering-Plough Animal Health

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Intervet

Manure degradation studies conducted

P = parent compound

M = metabolite

- Degradation studies conducted with two different parent compounds (P1 and P2)
- P1 in pig slurry and poultry manure (2003)
- P2 in pig slurry (2005) and cattle manure (2006)

➡ All studies conducted before EMEA/CVMP/ERA/418282/2005 became effective

➡ No regulatory guidance at that time

Study details - Bovine

	Bovine P2	EU TGD [1]	Tech. Protocol [2]
Media	Feces and urine	-	Feces and urine
Source	VMP-free cattle	-	VMP-free cattle
Conditions	Aerobic / anaerobic [3]	Anaerobic	Anaerobic
Label	^{14}C	-	^{14}C (recommended)
Introduction	Spiking	-	Spiking
System	Flow-through	-	Batch apparatus
Sterile samples	Yes	-	No
Temperature	10 ± 2° C	-	20 ± 2° C

[1] - EMEA/CVMP/ERA/418282/2005
[2] - Proposal for a Technical Protocol (Transformation of VMPs and Biocides in Liquid Bovine and Pig Manures)
[3] - Atmosphere aerobic, manure anaerobic

- No further characterization of manure (e.g. dry substance, TOC, etc.)

Study details - Porcine

	Porcine P1	Porcine P2	EU TGD [1]	Tech. Protocol [2]
Media	Feces, urine, cage wash	Feces and urine	-	Feces and urine
Source	Healthy pigs	VMP-free pigs	-	VMP-free pigs
Conditions	Aerobic [3]	Anaerobic	Anaerobic	Anaerobic
Label	^{14}C	^{14}C	-	^{14}C (recommended)
Introduction	Treatment of animals	Spiking	-	Spiking
System	Flow-through	Flow-through	-	Batch apparatus
Sterile samples	No	Yes	-	No
Temperature	~20° C	15 ± 2° C	-	20 ± 2° C

[1] - EMEA/CVMP/ERA/418282/2005

[2] - Proposal for a Technical Protocol (Transformation of VMPs and Biocides in Liquid Bovine and Pig Manures)

[3] - Bouwman GM and Reus JAWA. 1994. Persistence of Medicines in Manure. Centrum voor Landbouw en Milieu, Utrecht, The Netherlands

- No further characterization of manure (e.g. dry substance, TOC, etc.)

Study details – Poultry

	Porcine P1	EU TGD [1]	Tech. Protocol [2]
Media	Droppings	-	-
Source	Healthy chickens	-	-
Conditions	Aerobic [3]	Aerobic	-
Label	^{14}C	-	-
Introduction	Treatment of animals	-	-
System	Flow-through	-	-
Sterile samples	No	-	-
Temperature	~20° C	-	-

[1] - EMEA/CVMP/ERA/418282/2005

[2] - Proposal for a Technical Protocol (Transformation of VMPs and Biocides in Liquid Bovine and Pig Manures)

[3] - Bouwman GM and Reus JAWA. 1994. Persistence of Medicines in Manure. Centrum voor Landbouw en Milieu, Utrecht, The Netherlands

- No further characterization of manure (e.g. dry substance, TOC, etc.)

Extraction methods – Parent compound 1

Chicken manure and pig slurry

- Step 1: Centrifugation
- Step 2: Methanol

➡ relative mild methods

➡ recovery: 81% (chicken) (total)
91% (pig) (total)

Extraction methods – Parent compound 2

Porcine slurry

- Step 1: Centrifugation
- Step 2: Acetonitrile
- Step 3: Acetone / water (3:1)
- Step 4: Acetonitrile / 0.1M HCl (9:1)

➡ increasing extraction power
➡ continuous
➡ relative mild methods

➡ recovery: 93-108% (total)

Day 0 → Day 92
98% → 73% (Steps 1-4)
5% → 27% (bound)

Bovine manure

- Step 1: Centrifugation
- Step 2: Acetonitrile
- Step 3: Acetonitrile / 0.1M HCl (9:1)
- Step 4: 6M HCl reflux, overnight

➡ increasing extraction power
➡ continuous (Step 2 and 3) plus erratic (Step 4)
➡ relative mild (Step 2 and 3) and extremely harsh (Step 4)

➡ recovery: 95-107% (total)

Day 0 → Day 92
97% → 35% (Steps 1-3)
13% → 56 % (Step 4)
5% → 11% (bound)

Conduct of study – recommendations (1)

General study features

- Principally follows OECD 307 (Aerobic/anaerobic transformation soil)
  - Material balance (90-110%)
  - Rate and route of degradation (parent and metabolites)
- Duration: 90-120 days
  - including 9 sampling points
  - complimented with 3 sample points for sterile samples
  - Depending on degradation profile and typical manure storage time (e.g. as defined in EMEA/CVMP/ERA/418282/2005)
- ^{14}C-labelled compound, position of label important

Conduct of study – recommendations (2)

Manure

- Ideally from animals husbanded under controlled conditions (e.g. research stables)
- Fed on defined diet
- Collection procedure defined
  (e.g. feces and urine separately)
- Mixing of urine and feces at defined ratio
  - Pigs: feces + water + urine
  - Cattle: feces + urine

Set up

- Non-sterile and sterile samples (autoclavation or γ-irradiation)
- Acclimatization period (e.g. establishing of anaerobic conditions)
- Redox potential: to be measured for the proof of anaerobic conditions
- Microbial activity

Conduct of study – recommendations (3)

Extraction process

- Specific to substance and matrix
- Designed to extract analytes unaltered (influence of heat, pH, interaction with solvents)
- Increasing rigor
  - Weak (organic solvents at room temperature)
  - Moderate (organic solvents plus weak acid/base at room temperature)
  - Harsh (capable of breaking a covalent bond)

Chromatographic analysis

- Quantitative recovery during concentration phases
- Extracts measured via radiodetection
- Proportions of parent and metabolites in aqueous phase and extracts
- Rate of degradation for parent and metabolites (if possible)

ERA – Unclear issues
Old studies

- Before EMEA/CVMP/ERA/418282/2005 became effective at 01 Nov 2007, no guidance on how to conduct manure degradation studies
- Are old studies not in line with this guideline per se invalid?
- For example aerobic studies for bovine and porcine manure?

- Rationale to select aerobic conditions was to reflect agricultural practice (to the knowledge of the company) or to follow published information [1]
- EMEA/CVMP/ERA/418282/2005 considers anerobic conditions representative

➡ As long as old studies fulfill general quality criteria, they should be valid for ERA

➡ No request for new studies according to EMEA/CVMP/ERA/418282/2005

[1] - Bouwman GM and Reus JAWA. 1994. Persistence of Medicines in Manure. Centrum voor Landbouw en Milieu, Utrecht, The Netherlands

ERA – Unclear issues Metabolites (1)

- For P1, pig and poultry study were performed before Phase II studies were initiated
- P1 disappeared completely during storage, but M1 > 10% in pig study
- Pharmacologically active portion of the compound destroyed
- How to assess environmental risk adequately?
- PEC_{soil} > 100 µg/kg, for total residue approach and M1 exclusively
- Does it really make sense to conduct Phase II studies with P1?
- Is a complete Phase II study set necessary or can a reduced set be acceptable?

➡ Phase II studies should be conducted with M1 only, not with P1

ERA – Unclear issues Metabolites (2)

- For P2, pig and cattle study were performed after a complete Phase II dataset was generated for P2 (refinement PEC_{soil})
- P2 disappeared completely during storage, but M1, M2, and M3 > 10% each in cattle study
- M1-M3 are also metabolism products in target species and thus will be excreted
- Metabolites have a reduced pharmacological activity
- How to assess environmental risk adequately?
- PEC_{soil} > 100 µg/kg for total residue approach, but not for M1-M3 combined
- Does it really make sense to conduct Phase II studies with M1-M3?
- If so, is a complete Phase II study set necessary or can a reduced set be acceptable?

➡ Phase II studies conducted for P2 should be considered adequate to assess risk for M1-M3 (general rule: parent more toxic than metabolite)

➡ RQ based on PEC for M1-3 combined plus remainder P2

➡ PEC based on metabolites generated while storage and metabolites excreted

Open issues – ERA
Extractable and bound residues (1)

- Bovine manure degradation study with P2 raised question on residues released with extreme harsh extraction method (Step 4) only
- M1 and M2 likely covalently bound

	M1 [% appl. radioactivity]	**M2** [% appl. radioactivity]	**M3** [% appl. radioactivity]
Steps 1-3 [1] (mild)	2.3	4.4	9.9
Step 4 [2] (harsh)	13.1	7.3	1.0
Subtotal	15.3	11.7	10.9
Total (45 days)	**15.9**	**10.9**	**2.6**

[1] - Centrifugation, acetonitrile, and acetonitrile / 0.1M HCl (9:1)

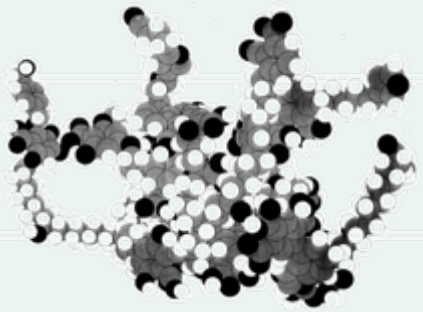
[2] - 6M HCl reflux, overnight

Open issues – ERA
Extractable and bound residues (2)

- As manure degradation studies are conducted once only, a maximum of information should be obtained
- Use of extremely harsh extraction methods is advised to allow maximum availability of compounds, e.g. for identification and analysis
- However, for the evaluation of environmental exposure, relevance of extracts gained with extreme methods, representing artificial laboratory conditions, need to be assessed carefully
- Residues which are bound in dung (and thus can be extracted with extreme methods only) will not be bioavailable after manure is applied to soil
- Binding in manure to its organic matrix, however in soil additionally to e.g. clay particles and humic substances

➡ Amount of bound residues should be subtracted for assessment

➡ How to define extremely harsh extraction methods? Compound specific!



Humic acid

Summary (1)

- ISPAH conducted degradation studies in cattle, pig, and chicken manure
- Two different parental compounds were involved
- Studies were conducted by two different CROs
- Principals of test protocols were different for P1 and P2
- Studies were accepted by Regulatory Authorities
- Clear answers with respect to the parent compounds
- New questions with respect to the metabolites

➡ Manure degradation studies do not necessarily solve all ERA-related issues immediately

Summary (2)

- Old studies not in line with EMEA/CVMP/ERA/418282/2005 should be accepted if fulfilling general quality criteria
- Metabolites should be dealt with case by case, however following a pragmatic and/or scientific reasonable approach
- Bound residues, extracted with harsh methods only, should not be considered
- Need for a standardized test protocol
- Although compound and matrix specific, general definition for rigor of extraction methods should be provided
  - Weak (organic solvents at room temperature)
  - Moderate (organic solvents plus weak acid/base at room temperature)
  - Harsh (capable of breaking a covalent bond)