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Harmonization of environmental exposure assessment for veterinary pharmaceuticals and biocides: Literature review of studies on occurrence and transformation of veterinary pharmaceuticals and biocides in manure

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Harmonization of environmental exposure assessment for veterinary pharmaceuticals and biocides: Literature review of studies on occurrence and transformation of veterinary pharmaceuticals and biocides in manure

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Kurzbeschreibung

Die Ausbringung von Veterinärpharmaka und Bioziden mit Gülle auf landwirtschaftlich genutzte Flächen stellt einen sehr wichtigen Eintragspfad dieser Stoffgruppen in die Umwelt dar. In dieser Literaturstudie werden die öffentlich zugänglichen Transformationsstudien von Tierarzneimitteln und Bioziden mit Gülle zusammengefasst. Die 34 gefundenen Studien wurden bezüglich des Transformationsverhaltens der getesteten Substanzen, bezüglich der Herkunft und der Spezifikationen der verwendeten Gülle, bezüglich des experimentellen Setups und bezüglich der gefundenen Ergebnisse ausgewertet. Die in den Studien beschriebene Testdauer lag zwischen 2 und 374 Tagen, die Testtemperatur zwischen 5 °C und 55 °C. Als Grundaussage aus den 34 Studien kann die sehr große Abhängigkeit des Transformationsverhaltens von der Temperatur, dem Redoxpotential, der Trockenmasse und vieler weiterer Parameter festgehalten werden. Darüber hinaus wurden fehlende Informationen und Parameter in den gesammelten Studien kritisch herausgearbeitet. Leider wurde zum Beispiel nur in sechs Studien ein Redoxpotential der Gülle bestimmt. Zusätzlich fehlt häufig eine grundsätzliche Charakterisierung der Matrix Gülle über die Parameter Trockensubstanzgehalt, pH-Wert und total organic carbon (TOC).

In einem weiteren Teil der Literaturarbeit wurden öffentlich zugängliche Monitoring-Daten gesammelt und ausgewertet. Es wurden folgende Parameter tabellarisch zusammengefasst: Die Herkunft der Gülle, minimal und maximal gefundene Konzentrationen von Tierarzneimittel- und Biozidwirkstoffen, der Anteil an Positivfunden und der Trockensubstanzgehalt der untersuchten Gülle. Insgesamt wurden 39 verschiedene aktive Substanzen und 11 Metabolite und Transformationsprodukte gefunden. Es wurden insgesamt 27 Publikationen ausgewertet, innerhalb derer zusammengenommen 1568 Gülle-Proben analysiert wurden. Innerhalb der einzelnen Publikationen wurde hauptsächlich nach den Wirkstoffklassen der Sulfonamide, der Tetrazykline und der Fluorchinolone gesucht. Einen non-target Ansatz gab es in keiner der Publikationen. Einzelne Wirkstoffe, dieser Substanzklassen wurden in einzelnen Studien mit mehr als 100 untersuchten Proben in mehr als 50 % der Fälle gefunden. Dies gilt für Sulfadimidin, Chlortetracyclin, Oxytetracyclin und Tetracyclin.

Es kann festgehalten werden, dass eine standardisierte Herangehensweise sowohl für Transformations- als auch für Monitoring-Studien von Vorteil wäre. Auf diese Weise könnte die Vergleichbarkeit und der wissenschaftliche sowie regulatorische Wert solcher Studien enorm gesteigert werden.

Abstract

The spread of veterinary medicinal products (VMPs) and biocides onto agriculturally used areas represents a very important path of entry into the environment for these product groups. Within this literature study public available transformation studies with liquid manure are summarized. Transformation studies were evaluated considering the transformation fate of tested substances, the origin and characteristics of used manure, the experimental setup, the measured parameters and the main outcome of the studies. Test duration throughout the studies ranges from 2 to 374 days and study temperature ranges from 5 °C to 55 °C. As main topics within the 34 found transformation studies the high dependency of transformation on temperature, redox potential, dry matter content and many other parameters is reported. It was further critically analyzed which basic information and parameters were neglected. Unfortunately only six publications give information on the redox potential of the manure. Further, the characterization of the matrix in many cases is inadequate due to missing parameters such as dry matter content, pH, and TOC.

Additionally, public available monitoring data of VMPs in manure were collected and evaluated regarding the origin and characteristics of the manure, the minimum and maximum found concentrations, and percentage of identified compounds. Within the 27 found publications, 1568 manure samples were analyzed and 39 different active substances for VMPs and 11 metabolites and transformation products of VMPs could be found in manure. Mainly, the samples were analyzed for sulfonamides, tetracyclines and fluorquinolones. In no case a non-target approach was used. Single active substances were found in some studies with more than 100 analyzed samples in more than 50 % of the analyzed manure samples. This is the case for sulfadimidine, chlortetracycline, oxytetracycline, and tetracycline.

It can be concluded that for both transformation studies and monitoring studies a standardized guidance would be beneficial for their applicability in regulatory contexts and also enhance the scientific outcome of these studies.

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List of abbreviations

ASBR	anaerobic sequencing batch reactor
BOD	biological oxygen demand
CAFO	Concentrated Animal Feeding Operation
COD	chemical oxygen demand
CTC	chlortetracycline
CYN	cyanamide
DAD	diode array detector
DIF	difloxacin
DIN	german institute for standardization (german: Deutsches Institut für Normung e.V.)
DT50	time needed for disappearance of 50 % of the parent compound, disappearance time
DT90	time needed for disappearance of 90 % of the parent compound, disappearance time
dw	dry weight
ECTC	4-epi-chlortetracycline
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ERY	erythromycin
EU	European Union
F+E	research and development (german: Forschung und Entwicklung)
FEN	fenbendazole
FKZ	project no. (german: Forschungskennzahl)
FLU	flubendazole
GC	gas chromatography
HPLC	high performance liquid chromatography
HR-MS	high resolution - mass spectrometry
ICTC	iso-chlortetracycline
IMZ	imazalil
ISO	International Organization for Standardization
LC	liquid chromatography
LIN	lincomycin
LOD	limit of detection
LSC	liquid scintillation counting
MON	monensin
MS	mass spectrometry
n.d.	not determined or not defined

NER	non-extractable residues
ns	not specified
OECD	Organisation for Economic Cooperation and Development
OTC	oxytetracycline
RTLC	radio thin layer chromatography
SCP	sulfachloropyridazine
SDZ	sulfadiazine
SMZ	sulfamethoxazole
SPN	spectinomycin
TMP	trimethoprim
TOC	total organic carbon
TP	transformation product
TS	total solids
TYL	tylosin
UV	ultraviolet
VDI	Association of German Engineers (german: Verein Deutscher Ingenieure)
VFA	volatile fatty acid
VICH	Veterinary International Conference on Harmonization
VMP	veterinary medicinal product
VSS	volatile suspended solids
ww	wet weight

Zusammenfassung

Die Ausbringung von Veterinärpharmaka und Bioziden mit Gülle auf landwirtschaftlich genutzte Flächen stellt einen sehr wichtigen Eintragspfad dieser Stoffgruppen in die Umwelt dar. Aktuelle Bewertungsleitfäden (zum Beispiel: „Guideline on determining the fate of veterinary medicinal products in manure“ (EMA/CVMP/ERA/430327/2009) (EMA 2011) sehen aus diesem Grund auch experimentelle Untersuchungen zur Transformation dieser Substanzen in Gülle vor.

Allerdings beinhalten die Dokumente bisher lediglich nur grundlegende regulatorische Vorgaben. Eine experimentelle Prüfrichtlinie zur Durchführung von Studien zum Abbauverhalten von Veterinärpharmaka und Bioziden in Gülle liegt jedoch weder auf EU- noch auf OECD-Ebene vor. Um eine einheitliche Bewertung von Studien im Zulassungsverfahren zu gewährleisten, wird jedoch ein harmonisiertes, international akzeptiertes und validiertes Testverfahren benötigt. Vor diesem Hintergrund wurde im Rahmen des F+E-Vorhabens „Entwicklung einer Testvorschrift zum Abbauverhalten von Veterinärpharmaka und Bioziden in Gülle“ (FKZ 3710 67 422) (Hennecke et al. 2015) ein Entwurf für eine experimentelle Richtlinie erarbeitet.

In dieser Literaturstudie werden die öffentlich zugänglichen Transformationsstudien von Tierarzneimitteln und Bioziden mit Gülle zusammengefasst. Die 34 gefundenen Studien wurden bezüglich des Transformationsverhaltens der getesteten Substanzen, bezüglich der Herkunft und der Spezifikationen der verwendeten Gülle, bezüglich des experimentellen Setups und bezüglich der gefundenen Ergebnisse ausgewertet. Die in den Studien beschriebene Testdauer lag zwischen 2 und 374 Tagen, die Testtemperatur zwischen 5 °C und 55 °C. Als Grundaussage aus den 34 Studien kann die sehr große Abhängigkeit des Transformationsverhaltens von der Temperatur, dem Redoxpotential, der Trockenmasse und vieler weiterer Parameter festgehalten werden. Darüber hinaus wurden fehlende Informationen und Parameter in den gesammelten Studien kritisch herausgearbeitet. Leider wurde zum Beispiel nur in sechs Studien ein Redoxpotential der Gülle bestimmt. Zusätzlich fehlt häufig eine grundsätzliche Charakterisierung der Matrix Gülle über die Parameter Trockensubstanzgehalt, pH-Wert und total organic carbon (TOC).

In einem weiteren Teil der Literaturarbeit wurden öffentlich zugängliche Monitoring-Daten gesammelt und ausgewertet. Es wurden folgende Parameter tabellarisch zusammengefasst: Die Herkunft der Gülle, minimal und maximal gefundene Konzentrationen von Tierarzneimittel- und Biozidwirkstoffen, der Anteil an Positivfunden und der Trockensubstanzgehalt der untersuchten Gülle. Insgesamt wurden 39 verschiedene aktive Substanzen und 11 Metabolite und Transformationsprodukte gefunden. Es wurden insgesamt 27 Publikationen ausgewertet, innerhalb derer zusammengenommen 1568 Gülle-Proben analysiert wurden. Innerhalb der einzelnen Publikationen wurde hauptsächlich nach den Wirkstoffklassen der Sulfonamide, der Tetrazykline und der Fluorchinolone gesucht. Einen non-target Ansatz gab es in keiner der Publikationen. Einzelne Wirkstoffe, dieser Substanzklassen wurden in einzelnen Studien mit mehr als 100 untersuchten Proben in mehr als 50 % der Fälle gefunden. Dies gilt für Sulfadimidin, Chlortetracyclin, Oxytetracyclin und Tetracyclin.

Es kann festgehalten werden, dass eine standardisierte Herangehensweise sowohl für Transformations- als auch für Monitoring-Studien von Vorteil wäre. Auf diese Weise könnte die Vergleichbarkeit und der wissenschaftliche sowie regulatorische Wert solcher Studien enorm gesteigert werden.

Summary

The spread of veterinary medicinal products (VMPs) and biocides onto agriculturally used areas represents a very important path of entry into the environment for these product groups. For this reason, current guidance (e.g. „Guideline on determining the fate of veterinary medicinal products in manure“(EMA/CVMP/ERA/430327/2009) (EMA 2011) stipulates experimental studies on transformation of veterinary medicinal products (VMPs) and biocides in manure.

Though, the documents only contain basic regulatory requirements, whereas an experimental test guideline is still missing, both on EU and OECD level. To allow for a consistent assessment of studies within the registration process, a harmonized internationally accepted and validated test method is needed. A draft test guideline was developed within a previous R&D-Project “Development of test guidance for transformation of veterinary pharmaceuticals and biocides in liquid manure” (FKZ 3710 67 422) (Hennecke et al. 2015).

Within this literature study public available transformation studies with liquid manure are summarized. Transformation studies were evaluated considering the transformation fate of tested substances, the origin and characteristics of used manure, the experimental setup, the measured parameters and the main outcome of the studies. Test duration throughout the studies ranges from 2 to 374 days and study temperature ranges from 5 °C to 55 °C. As main topics within the 34 found transformation studies the high dependency of transformation on temperature, redox potential, dry matter content and many other parameters is reported. It was further critically analyzed which basic information and parameters were neglected. Unfortunately only six publications give information on the redox potential of the manure. Further, the characterization of the matrix in many cases is inadequate due to missing parameters such as dry matter content, pH, and TOC.

Additionally, public available monitoring data of VMPs in manure were collected and evaluated regarding the origin and characteristics of the manure, the minimum and maximum found concentrations, and percentage of identified compounds. Within the 27 found publications, 1568 manure samples were analyzed and 39 different active substances for VMPs and 11 metabolites and transformation products of VMPs could be found in manure. Mainly, the samples were analyzed for sulfonamides, tetracyclines and fluorquinolones. In no case a non-target approach was used. Single active substances were found in some studies with more than 100 analyzed samples in more than 50 % of the analyzed manure samples. This is the case for sulfadimidine, chlortetracycline, oxytetracycline, and tetracycline.

It can be concluded that for both transformation studies and monitoring studies a standardized guidance would be beneficial for their applicability in regulatory contexts and also enhance the scientific outcome of these studies.

1 Introduction: Literature research

Veterinary medicinal products (VMPs) are excreted by the treated animals as parent and metabolized compounds. The excrements from stabled animals in Europe and North America are collected and stored mainly as liquid or solid manure before they are used as fertilizers on arable land and grassland. Biocides, which are used for disinfection of the stable, end up in the stored animal excrements. Via manure application in agriculture, veterinary medicines and biocides are released to the environment and affect soil and water quality.

Depending on boundary conditions such as storage temperature, dry matter content, feeding of the animals and availability of electron acceptors, the pharmaceuticals and biocides can be further transformed in the liquid manure. Besides transformation other processes such as volatilization, sorption, and the formation of non-extractable residues (NER) can occur and contribute to the dissipation of the active agents.

Transformation products are also capable to persist in environmental matrices and can be ecotoxic. For tetracyclines the transformation products like epimers, isomers and anhydro-compounds have been detected. Metabolites of sulfadiazine showed transformation back to the parent compound. Transformation processes are influenced by the composition of matrix, temperature, pH value as well as aerobic or anaerobic conditions. Compounds could adsorb to the matrix depending on its sorption capacity. The higher the dry matter content in liquid manure, the higher is the number of sorption sites.

Generally, transformation under aerobic conditions occurs faster than transformation under anaerobic conditions. Also high temperatures promote the degradation of compounds in liquid manure. During manure storage in manure tanks, which is mostly applied in Europe, the storage conditions are anaerobic. In North America manure is stored in lagoons because of the large amounts of manure that accumulates in large-scale Concentrated Animal Feeding Operations (CAFOs). The outdoor lagoon storage is distinguished by more aerobic conditions on the large lagoon water surface but also by anaerobic conditions in deeper layers. Composting the separated manure under aerobic conditions is the favored treatment of manure in Asia. Concluding, the transformation process of compounds is affected intensely by the storage practice of manure.

There is increasing research activity regarding the transformation of single substances under laboratory conditions. Current guidance, e.g. „Guideline on determining the fate of veterinary medicinal products in manure“(EMA 2011), takes transformation of VMPs and biocides in manure into account. However, currently, there is no standardized experimental test protocol available to examine the transformation of veterinary medicinal products (VMPs) and biocides in liquid manure. The EMA guideline on transformation in manure (EMA 2011) contains basic regulatory requirements. To allow for a consistent assessment of studies within regulatory frameworks, a harmonized internationally accepted and validated test method is needed.

This literature study provides a survey on studies on (bio-) transformation processes in manure and on monitoring data of VMPs and biocides in manure. The objective of this review was to follow the questions which compounds are investigated, which methods and analytical techniques are used and which factors have been identified affecting the (bio-) transformation process in liquid manure. In addition, this report compiles recent monitoring data on VMP and biocide residues in manure samples.

2 Methodology

For this literature review the data bases “ISI Web of Knowledge” and “Google Scholar” were used. Categorized search items are given in Table 1. Boolean search for the most relevant two keywords, which were then combined with the following categories could limit the results to a manageable amount of citations.

Table 1: List of categorized keywords

1.	2.	3.	4.	5.	6.
manure	transformation	veterinary	medicine	biocide	florfenicol
slurry	metabolism		drug	pesticide	tetracycline
feces	catabolism		pharmaceutical	disinfectant	sulfonamide
faeces	anabolism				antibiotic
lagoon	degradation				antiparasitic
	decomposition				
	dissipation				
	fate				
	reaction				
	conversion				
	management				

International publications from the year 2000 to date were considered. In addition, cross references within the original publications were verified. Additionally, relevant authorities and organizations were asked for reports on related topics, see Table 2.

Furthermore, proven experts in this field were contacted in Canada and South Korea to assess specific local situations abroad:

Dr. Edward Topp
Agriculture and Agri-Food Canada
1391 Sandford Street
London, ON N5V 4T3
Canada

Dr. Jin-Wook Kwon
Ministry for Food, Agriculture
Forestry and Fisheries
620-2 Amnam-dong Seo-gu, Busan
Rep. of Korea

Table 2: List of requested relevant authorities and organizations

Ministerium für Energiewende, Umwelt, Landwirtschaft und ländliche Räume des Landes Schleswig-Holstein
Landesamt für Natur, Umwelt und Verbraucherschutz NRW (LANUV), Recklinghausen
Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten
Bayerische Landesanstalt für Landwirtschaft (LfL)
Bayerisches Staatsministerium für Umwelt und Gesundheit, München
Institut für Agrarökologie, Ökologischen Landbau und Bodenschutz (IAB), Freising
Thüringer Ministerium für Landwirtschaft, Forsten, Umwelt und Naturschutz; Abteilung Landwirtschaft, Markt, Ernährung
Thüringer Landesanstalt für Landwirtschaft (TLL), Jena, Abteilung Tierproduktion
Landwirtschaftliches Zentrum für Rinderhaltung, Grünlandwirtschaft, Milchwirtschaft, Wild und Fischerei Baden Württemberg (LAZBW); Aulendorf
Sächsische Landesanstalt für Landwirtschaft
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern
Hessisches Landesamt für Umwelt und Geologie
Biogas e.V.
Biogas Union

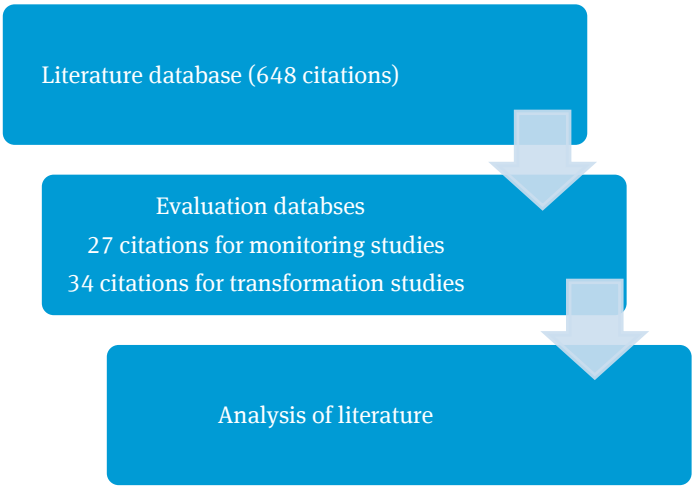
Citations were collected and entered into the software package “Citavi” (Swiss Academic Software, for Microsoft Windows). The established data base comprises literature which, in the broadest sense, deals with environmental burden due to active agents originated from husbandry. The results of this research were structured according to the following categories:

- Transformation of veterinary medicinal products and biocides in manure
- Transformation in soil and in soil/manure mixtures
- Sorption
- Fate in the aquatic system
- Stable Manure / Composting
- Treatment
- Antibiotic resistance genes
- Monitoring
- Biogas/ methane (antibiotics)
- Copper
- Metabolism in the animal

- Plant uptake
- Regulatory aspects
- International agricultural studies
- Miscellaneous

From 648 entered records, 27 were found to measure or monitor the occurrence of VMPs in liquid manure. Found substances, estimated concentrations, origin of the manure and further parameters were listed. From 648 records 34 publications were selected which deal explicitly with transformation of veterinary medicinal products and biocides in pig and cattle manure. These citations were systematically transferred to an Excel data base which considered specific parameters such as investigated compounds and substance amounts, characterization of matrices, aerobic/anaerobic conditions, transformation products, methodology and chemical analytics of the studies.

Figure 1: Literature selection process steps



3 Occurrence of veterinary medicines and biocides in manure

The monitoring data tables (Tables 3-6) summarize the results of 27 different publications measuring veterinary medicinal products in manure from 2001 until today. Manure was analyzed in North America (Canada), in Europe (Austria, Czech Republic, Denmark, Germany, Italy, Switzerland) and in Asia (China, Japan). The gathered results give a good general survey on the prevalent occurrence of pharmaceuticals in manure. Although it should be kept in mind, that the data do only cover certain locations, it can be assumed, that wherever veterinary medicinal products are used, portions of these will be found in the manure.

In some studies, a lot of samples were taken covering a large number of manures - up to 380 samples in Harms et al. (2005) - and in other studies only individual manures were sampled with the background knowledge of the previous medication. Within 19 publications only pig manure was analyzed, whereas 3 publications worked with cattle manure and 4 publications worked with pig, cattle or poultry manure. One publication did not specify the origin of the analyzed manure.

Manure and liquid manure samples with different dry matter content were considered in this literature study (range: 0.2 - 44.4 %). 16 of 27 Studies unfortunately did not specify dry matter content as a basic parameter. 12 of 27 studies reported concentrations of substances in manure in mg/kg dry weight (dw), 10 of 27 studies worked with mg/kg wet weight (ww) and 5 studies did not specify (ns) whether they calculated concentrations on the basis of dry or wet weight. Because of this, it is difficult to compare the found concentrations of the single active substances. The lowest values are found at the µg/kg order of magnitude - often restricted by the limit of detection (LOD) of the analytical method. For future studies it would be highly desirable to get at least all the information provided: Dry weight and wet weight concentrations of analyzed compound and dry matter content.

Among all reviewed literature, 39 different active substances of VMPs were found in manure. Moreover, 11 metabolites and transformation products of these active substances were also identified. For this, 1568 manure samples were analyzed within the 27 publications. Mainly, the samples were analyzed for sulfonamides, tetracyclines and fluorquinolones. None of the studies worked with a non-target approach. By far the most frequently found single active substances are sulfadimidine (599 positive), tetracycline (575 positive) and chlortetracycline (457 positive). There are 6 publications each of which analyzed more than 100 manure samples. All these come from Chinese or German institutes. Those active substances with the highest percentage of positive findings (> 50 %) within these 6 publications are chlortetracycline, oxytetracycline, tetracycline and sulfadimidine.

The 15 highest concentrations are found in pig manure from Germany or China. The highest concentration was 1420.76 mg/kg (dw) of enrofloxacin found in poultry manure from China, followed by 764.407 mg/kg (dw) chlortetracycline in pig manure from China and 330.7 mg/kg (ww) in pig manure from Germany. Further, very high values are found for other sulfonamides, tetracyclines and fluorchinolones.

Table 3 Sulfonamides and its metabolites found in manure (dw: dry weight, ww: wet weight, ns: not specified)

Substance	Reference	Matrix	Origin	Min	Max	Unit	Dry matter content, comments or quotation	n	n positive	% positive
Sulfachloropyridazine	Hu et al. 2010	pig and poultry manure	China	0.340	3.660	mg/kg (dw)	"liquid swine manure" (ns)	6	2	33
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.090	3.510	mg/kg (dw)	(ns)	143	7	5
Sulfadiazine	Engels 2004	pig manure	Germany (NI)	0.700	235.100	mg/kg (ww)	0.5 - 16.8 % (mean 5 %)	344	100	29
	Hamscher et al. 2005	pig manure	Germany	3.500	11.300	mg/kg (dw)	9.6 - 9.8 %	3	2	67
	Harms et al. 2005	pig manure	Germany (BY)	0.100	5.000	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	19	5
	Hu et al. 2010	pig and poultry manure	China	0.160	0.780	mg/kg (dw)	"liquid swine manure" (ns)	6	2	33
	Jacobsen and Halling Sørensen 2006	pig manure	Denmark	0.630	2.100	mg/kg (dw)	2.8 - 13.4 %	6	2	33
	Pfeifer et al. 2002	(ns)	Germany	0.011	0.080	mg/kg (ns)	"liquid manure" (ns)	4	2	50
	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	0.650	mg/kg (dw)	liquid and stable manure (ns)	34	5	15
	Winckler et al. 2004	pig manure	Germany	0.700	35.300	mg/kg (ww)	0.7 - 16.11 %	176	86	49
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.020	3.120	mg/kg (dw)	(ns)	143	14	10
4-Hydroxy-Sulfadiazine	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	9.050	mg/kg (dw)	liquid and stable manure (ns)	34	8	24
N4-Acetyl-Sulfadiazine	Harms et al. 2005	pig manure	Germany (BY)	-	-	not quantified	0.2 - 17.3 % (mean 3.7 %)	380	19	5
	Pfeifer et al. 2002	(ns)	Germany	0.010	0.270	mg/kg (ns)	"liquid manure" (ns)	4	2	50
	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	0.150	mg/kg (dw)	liquid and stable manure (ns)	34	6	18
Sulfadimethoxine	Harms et al. 2005	pig manure	Germany (BY)	0.050	0.600	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	5	1
	Pan et al. 2011	pig manure	China	0.120	1.255	mg/kg (dw)	(ns)	126	3	2
Sulfadimidine	Martìnez-Carballo et al. 2007	pig manure	Austria	-	< 20	mg/kg (dw)	"liquid manure" (ns)	30	18	60
	Aust et al. 2008	cattle manure	Canada	-	9.990	mg/kg (dw)	24.4 - 44.4 % (mean 37 %)	6	4	67
	Burkhardt et al. 2005	pig manure	Switzerland	-	14.400	mg/L (ww)	"in the supernatant" (water phase) (ns)	1	1	100
	Christian et al. 2003	pig manure	Germany	1.000	1.100	mg/kg (ww)	(ns)	2	2	100
	Christian et al. 2003	cattle manure	Germany	< 0.1	< 0.1	mg/kg (ww)	(ns)	2	2	100
	Engels 2004	pig manure	Germany (NI)	0.700	167.000	mg/kg (ww)	0.5 - 16.8 % (mean 5 %)	344	183	53
	Hamscher et al. 2005	pig manure	Germany	-	7.200	mg/kg (dw)	9.6 - 9.8 %	3	1	33
	Pan et al. 2011	pig manure	China	0.011	28.700	mg/kg (dw)	(ns)	126	65	52
	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	7.040	mg/kg (dw)	liquid and stable manure (ns)	34	6	18
	Sattelberger et al. 2005	pig manure	Germany	0.130	20.000	mg/kg (dw)	1.2 - 28 %	30	18	60
	Weiß 2008	pig manure	Germany (BY)	0.140	1.700	mg/L (ww)	3 - 2 %	8	8	100
	Winckler et al. 2004	pig manure	Germany	0.700	167.000	mg/kg (ww)	0.7 - 16.13 %	176	85	48
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.060	6.040	mg/kg (dw)	(ns)	143	17	12
	Haller et al. 2002	pig and cattle manure	Switzerland	0.130	8.700	mg/kg (ww)	1.1 - 3.7 %	6	6	100
	Harms et al. 2005	pig manure	Germany (BY)	0.050	38.000	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	181	48

	Pfeifer et al. 2002	(ns)	Germany	0.011	0.062	mg/kg (ns)	"liquid manure" (ns)	4	2	50
N4-Acetyl-Sulfadimidine	Haller et al. 2002	pig and cattle manure	Switzerland	‹ 0.1	2.600	mg/kg (ww)	1.1 - 3.7 %	6	5	83
	Harms et al. 2005	pig manure	Germany (BY)	0.050	27.000	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	117	31
	Weiß 2008	pig manure	Germany (BY)	0.120	1.000	mg/L (ww)	2 - 2 %	8	8	100
Sulfadoxine	Hu et al. 2010	pig and poultry manure	China	0.350	0.710	mg/kg (dw)	"liquid swine manure" (ns)	6	3	50
	Jacobsen and Halling Sørensen 2006	pig manure	Denmark	0.015	0.220	mg/kg (dw)	2.8 - 13.4 %	6	3	50
Sulfaguanidine	Zhao et al. 2010	pig, cattle and poultry manure	China	0.010	1.550	mg/kg (dw)	(ns)	143	27	19
Sulfamerazine	Harms et al. 2005	pig manure	Germany (BY)	0.700	0.900	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	7	2
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.090	0.660	mg/kg (dw)	(ns)	143	6	4
N4-Acetyl-Sulfamerazine	Harms et al. 2005	pig manure	Germany (BY)	-	-	not quantified	0.2 - 17.3 % (mean 3.7 %)	380	5	1
Sulfamethizole	Pan et al. 2011	pig manure	China	0.052	2.422	mg/kg (dw)	(ns)	126	35	28
Sulfamethoxazole	Harms et al. 2005	pig manure	Germany (BY)	0.050	0.050	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	3	1
	Hu et al. 2010	pig and poultry manure	China	0.340	1.290	mg/kg (dw)	"liquid swine manure" (ns)	6	2	33
	Motoyama et al. 2011	pig manure	Japan	0.002	0.035	mg/kg (ns)	(ns)	5	4	80
	Motoyama et al. 2011	cattle manure after fermentation	Japan	-	0.010	mg/kg (ns)	(ns)	8	1	13
	Pan et al. 2011	pig manure	China	0.137	0.639	mg/kg (dw)	(ns)	126	6	5
	Sattelberger et al. 2005	pig manure	Germany	‹ 0.1	2.400	mg/kg (dw)	1.2 - 28 %	30	2	7
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.120	2.800	mg/kg (dw)	(ns)	143	7	5
Sulfamethoxypyridazine	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	0.020	mg/kg (dw)	liquid and stable manure (ns)	34	4	12
Sulfamonomethoxine	Motoyama et al. 2011	pig manure	Japan	-	0.210	mg/kg (ns)	(ns)	5	1	20
	Motoyama et al. 2011	cattle manure after fermentation	Japan	-	0.022	mg/kg (ns)	(ns)	8	1	13
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.060	4.080	mg/kg (dw)	(ns)	143	39	27
Sulfanilamide	Zhao et al. 2010	pig, cattle and poultry manure	China	0.020	1.590	mg/kg (dw)	(ns)	143	5	3
Sulfaquinoxaline	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	0.670	mg/kg (dw)	liquid and stable manure (ns)	34	3	9
Sulfathiazole	Haller et al. 2002	pig and cattle manure	Switzerland	0.100	12.400	mg/kg (ww)	1.1 - 3.7 %	6	4	67
	Harms et al. 2005	pig manure	Germany (BY)	0.050	0.100	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	5	1
	Pan et al. 2011	pig manure	China	0.312		mg/kg (dw)	(ns)	126	1	1

Table 4 Tetracyclines and its metabolites found in manure (dw: dry weight, ww: wet weight, ns: not specified)

Substance	Reference	Matrix	Origin	Min	Max	Unit	Dry matter content, comments or quotation	n	n positive	% positive
Chlortetracycline	Martìnez-Carballo et al. 2007	pig manure	Austria	0.100	46.000	mg/kg (dw)	"liquid manure" (ns)	30	17	57
	Engels 2004	pig manure	Germany (NI)	1.100	330.700	mg/kg (ww)	0.5 - 16.8 % (mean 5 %)	344	44	13
	Hamscher et al. 2002	pig manure	Germany	0.090	0.100	mg/kg (ww)	(ns)	2	2	100
	Hamscher et al. 2005	pig manure	Germany	0.900	1.000	mg/kg (dw)	9.6 - 9.8 %	3	2	67
	Harms et al. 2005	pig manure	Germany (BY)	0.100	50.800	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	140	37
	Hu et al. 2010	pig and poultry manure	China	0.150	14.700	mg/kg (dw)	"liquid swine manure" (ns)	6	4	67
	Jacobsen and Halling Sørensen 2006	pig manure	Denmark	1.100	15.700	mg/kg (dw)	2.8 - 13.4 %	6	5	83
	Motoyama et al. 2011	pig manure	Japan	0.240	0.280	mg/kg (ns)	(ns)	5	2	40
	Motoyama et al. 2011	cattle manure after fermentation	Japan	-	0.001	mg/kg (ns)	(ns)	8	1	13
	Pan et al. 2011	pig manure	China	0.053	764.407	mg/kg (dw)	(ns)	126	122	97
	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	3.600	mg/kg (dw)	liquid and stable manure (ns)	34	7	21
	Sattelberger et al. 2005	pig manure	Germany	0.100	46.000	mg/kg (dw)	1.2 - 28 %	30	17	57
	Tylová et al. 2010	pig manure	Czech Republic	-	5.880	mg/kg (ns)	"Liquid hog manure" (ns)	5	1	20
	Weiß 2008	pig manure	Germany (BY)	0.600	2.000	mg/L (ww)	1 - 2 %	3	3	100
	Winckler et al. 2004	pig manure	Germany	1.100	25.700	mg/kg (ww)	0.7 - 16.1 %	176	18	10
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.160	27.590	mg/kg (dw)	(ns)	143	72	50
Epi-Chlortetracycline	Jacobsen and Halling Sørensen 2006	pig manure	Denmark	1.700	14.100	mg/kg (dw)	2.8 - 13.4 %	6	5	83
Doxycycline	Harms et al. 2005	pig manure	Germany (BY)	0.100	0.700	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	5	1
	Jacobsen and Halling Sørensen 2006	pig manure	Denmark	0.550	3.100	mg/kg (dw)	2.8 - 13.4 %	6	6	100
	Tylová et al. 2010	pig manure	Czech Republic	-	0.990	mg/kg (ns)	"Liquid hog manure" (ns)	5	1	20
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.230	13.500	mg/kg (dw)	(ns)	143	21	15
Metacycline	Zhao et al. 2010	pig, cattle and poultry manure	China	0.140	5.860	mg/kg (dw)	(ns)	143	50	35
Oxytetracycline	Martìnez-Carballo et al. 2007	pig manure	Austria	0.290	29.000	mg/kg (dw)	"liquid manure" (ns)	30	22	73
	De Liguoro et al. 2003	cattle manure	Italy	-	19.000	mg/kg (ns)	"heap" (ns)	1	1	100
	Engels 2004	pig manure	Germany (NI)	1.600	136.200	mg/kg (ww)	0.5 - 16.8 % (mean 5 %)	344	10	3
	Harms et al. 2005	pig manure	Germany (BY)	0.100	0.900	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	16	4
	Jacobsen and Halling Sørensen 2006	pig manure	Denmark	0.048	1.500	mg/kg (dw)	2.8 - 13.4 %	6	3	50

	Motoyama et al. 2011	pig manure	Japan	-	0.013	mg/kg (ns)	(ns)	5	1	20
	Motoyama et al. 2011	cattle manure after fermentation	Japan	-	0.001	mg/kg (ns)	(ns)	8	1	13
	Pan et al. 2011	pig manure	China	0.044	172.874	mg/kg (dw)	(ns)	126	114	90
	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	1.490	mg/kg (dw)	liquid and stable manure (ns)	34	5	15
	Sattelberger et al. 2005	pig manure	Germany	0.210	29.000	mg/kg (dw)	1.2 - 28 %	30	22	73
	Winckler et al. 2004	pig manure	Germany	1.600	136.200	mg/kg (ww)	0.7 - 16.9 %	176	9	5
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.150	59.590	mg/kg (dw)	(ns)	143	50	35
	Karcı and Balcıoğlu 2009	cattle manure	Turkey	-	0.060	mg/kg (ns)	(ns)	1	1	100
Epi-Oxytetracycline	Jacobsen and Halling Sørensen 2006	pig manure	Denmark	0.330	0.450	mg/kg (dw)	2.8 - 13.4 %	6	2	33
Tetracycline	Martìnez-Carballo et al. 2007	pig manure	Austria	0.360	23.000	mg/kg (dw)	"liquid manure" (ns)	30	22	73
	Hamscher et al. 2002	pig manure	Germany	3.200	4.000	mg/kg (ww)	(ns)	2	2	100
	Hamscher et al. 2005	pig manure	Germany	14.100	41.200	mg/kg (dw)	9.6 - 9.8 %	3	3	100
	Harms et al. 2005	pig manure	Germany (BY)	0.100	46.000	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	111	29
	Hu et al. 2010	pig and poultry manure	China	0.180	0.840	mg/kg (dw)	"liquid swine manure" (ns)	6	4	67
	Jacobsen and Halling Sørensen 2006	pig manure	Denmark	0.091	1.600	mg/kg (dw)	2.8 - 13.4 %	6	5	83
	Motoyama et al. 2011	pig manure	Japan	0.005	0.015	mg/kg (ns)	(ns)	5	3	60
	Motoyama et al. 2011	cattle manure after fermentation	Japan	-	0.001	mg/kg (ns)	(ns)	8	2	25
	Pan et al. 2011	pig manure	China	0.037	19.417	mg/kg (dw)	(ns)	126	107	85
	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	2.450	mg/kg (dw)	liquid and stable manure (ns)	34	12	35
	Sattelberger et al. 2005	pig manure	Germany	0.360	23.000	mg/kg (dw)	1.2 - 28 %	30	22	73
	Winckler and Grafe 2001	pig manure	Germany (NW)	0.600	66.000	mg/L (ww)	"pig slurry" (ns)	181	43	24
	Winckler et al. 2004	pig manure	Germany	0.900	43.100	mg/kg (ww)	0.7 - 16.8 %	176	87	49
	Engels 2004	pig manure	Germany (NI)	0.700	45.700	mg/kg (ww)	0.5 - 16.8 % (mean 5 %)	344	152	44
Epi-Tetracycline	Jacobsen and Halling Sørensen 2006	pig manure	Denmark	0.061	0.990	mg/kg (dw)	2.8 - 13.4 %	6	5	83

Table 5 Fluorchinolones found in manure (dw: dry weight, ww: wet weight, ns: not specified)

Substance	Reference	Matrix	Origin	Min	Max	Unit	Dry matter content, comments or quotation	n	n positive	% positive
Ciprofloxacin	Motoyama et al. 2011	pig manure	Japan	-	0.006	mg/kg (ns)	(ns)	5	1	20
	Motoyama et al. 2011	cattle manure after fermentation	Japan	0.002	0.012	mg/kg (ns)	(ns)	8	4	50
	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	0.070	mg/kg (dw)	liquid and stable manure (ns)	34	3	9
	Sattelberger et al. 2005	pig manure	Germany	0.180	0.620	mg/kg (dw)	1.2 - 28 %	30	4	13
	Weiß 2008	pig manure	Germany (BY)	0.005	0.028	mg/L (ww)	5 - 2 %	5	5	100
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.490	45.590	mg/kg (dw)	(ns)	143	44	31
Danofloxacin	Ratsak et al. 2013	pig and cattle manure	Germany (NW)		0.050	mg/kg (dw)	liquid and stable manure (ns)	34	1	3
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.080	3.060	mg/kg (dw)	(ns)	143	39	27
Difloxacin	Zhao et al. 2010	pig, cattle and poultry manure	China	0.410	12.380	mg/kg (dw)	(ns)	143	8	6
Enrofloxacin	Martìnez-Carballo et al. 2007	pig manure	Austria	0.130	0.750	mg/kg (dw)	"liquid manure" (ns)	-	-	-
	Ratsak et al. 2013	pig and cattle manure	Germany (NW)		0.550	mg/kg (dw)	liquid and stable manure (ns)	34	5	15
	Sattelberger et al. 2005	pig manure	Germany	0.130	0.750	mg/kg (dw)	1.2 - 28 %	30	5	17
	Weiß 2008	pig manure	Germany (BY)	0.050	0.116	mg/L (ww)	6 - 2 %	5	5	100
	Zhao et al. 2010	pig, cattle and poultry manure	China	0.330	1420.760	mg/kg (dw)	(ns)	143	67	47
Fleroxacin	Zhao et al. 2010	pig, cattle and poultry manure	China	0.760	99.430	mg/kg (dw)	(ns)	143	35	24
Levofloxacin	Motoyama et al. 2011	pig manure	Japan	-	0.003	mg/kg (ns)	(ns)	5	1	20
	Motoyama et al. 2011	cattle manure after fermentation	Japan	0.001	0.002	mg/kg (ns)	(ns)	8	2	25
Lomefloxacin	Zhao et al. 2010	pig, cattle and poultry manure	China	0.610	44.160	mg/kg (dw)	(ns)	143	45	31
Marbofloxacin	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	0.050	mg/kg (dw)	liquid and stable manure (ns)	34	3	9
Norfloxacin	Zhao et al. 2010	pig, cattle and poultry manure	China	0.560	225.450	mg/kg (dw)	(ns)	143	37	26
Ofloxacin	Hu et al. 2010	pig and poultry manure	China	0.450	3.870	mg/kg (dw)	"liquid swine manure" (ns)	6	2	33
Orbifloxacin	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	0.020	mg/kg (dw)	liquid and stable manure (ns)	34	1	3
Sarafloxacin	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	0.060	mg/kg (dw)	liquid and stable manure (ns)	34	1	3

Table 6 Other veterinary medicines and its metabolites found in manure (dw: dry weight, ww: wet weight, ns: not specified)

Substance	Reference	Matrix	Origin	Min	Max	Unit	Dry matter content, comments or quotation	n	n positive	% positive
Flubendazole	Weiß 2008	pig manure	Germany (BY)	0.020	0.056	mg/L (ww)	8 - 2 %	7	7	100
Amino-Flubendazole	Weiß 2008	pig manure	Germany (BY)	0.032	0.110	mg/L (ww)	7 - 2 %	7	7	100
Hydroxy-Flubendazole	Weiß 2008	pig manure	Germany (BY)	0.018	0.075	mg/L (ww)	9 - 2 %	7	7	100
Lincomycin	Kuchta and Cessna 2009	pig manure	Canada	2.520	9.780	mg/L (ww)	mean 2.4 %	5	5	100
Salinomycin	Schlüsener et al. 2003	pig manure	Germany	-	0.011	mg/kg (ns)	5 %	4	1	25
Spectinomycin	Kuchta and Cessna 2009	pig manure	Canada	0.173	0.686	mg/L (ww)	mean 2.4 %	5	5	100
Tiamulin	Harms et al. 2005	pig manure	Germany (BY)	-	0.500	mg/kg (ww)	0.2 - 17.3 % (mean 3.7 %)	380	1	< 1
	Pan et al. 2011	pig manure	China	0.076	0.169	mg/kg (dw)	(ns)	126	6	5
	Schlüsener et al. 2003	pig manure	Germany	-	0.043	mg/kg (ns)	5 %	4	1	25
Toltrazuril	Olsen et al. 2012	pig manure	Denmark	-	0.114	mg/kg (dw)	"manure from a slurry storage tank" (ns)	1	1	100
Toltrazuril sulfone	Olsen et al. 2012	pig manure	Denmark	-	0.085	mg/kg (dw)	"manure from a slurry storage tank" (ns)	1	1	100
Toltrazuril sulfoxide	Olsen et al. 2012	pig manure	Denmark	-	0.007	mg/kg (dw)	"manure from a slurry storage tank" (ns)	1	1	100
Trimethoprim	Haller et al. 2002	pig and cattle manure	Switzerland	< 0.1	< 0.1	mg/kg (ww)	1.1 - 3.7 %	6	1	17
	Ratsak et al. 2013	pig and cattle manure	Germany (NW)	-	0.050	mg/kg (dw)	liquid and stable manure (ns)	34	1	3
Tylosin	De Liguoro et al. 2003	cattle manure	Italy	-	< 0.25	mg/kg (ns)	"heap" (ns)	1	1	100
	Solliec et al. 2014	pig manure	Canada	0.030	0.543	mg/kg (dw)	(ns)	-	-	-
	Weiß 2008	pig manure	Germany (BY)	0.130	0.320	mg/L (ww)	4 - 2 %	8	8	100

4 Transformation of veterinary pharmaceuticals and biocides in liquid manure

The focus of the literature search was on transformation studies with liquid manure and manure from lagoons. Liquid manure is the substrate found in a manure tank which consists of urine, feces, sometimes bedding material and water from cleaning the stables. It is important to note the difference to dung or excrements, which are distinguished from manure by being directly excreted and not collected and stored in some form for longer time periods under which anaerobic conditions develop (Weinfurtner 2011). In this review also studies using excrements and related matrices were included to get a comprehensive picture of available methods.

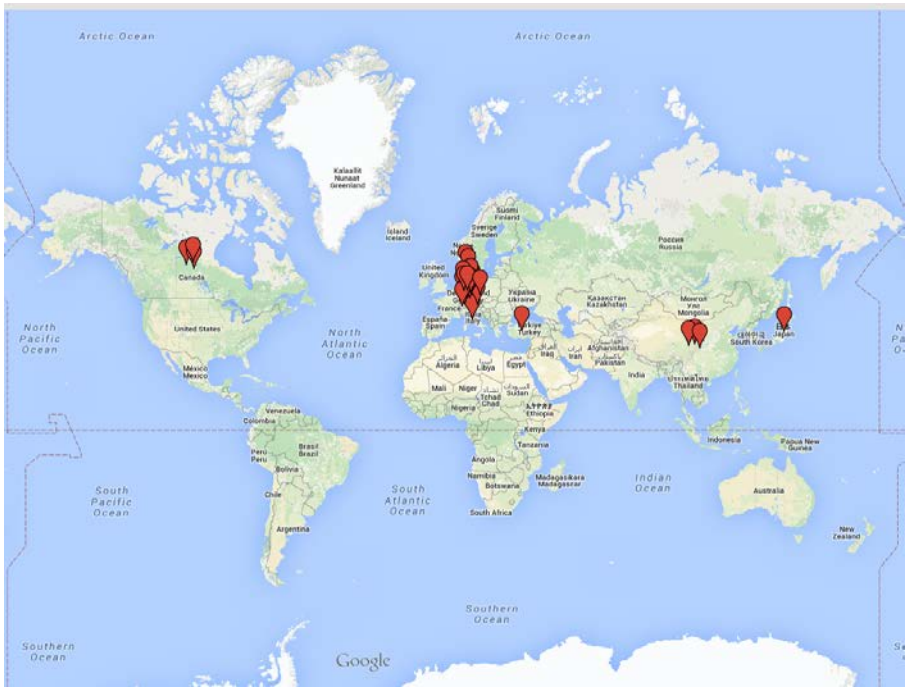
In literature many different studies using mixtures of soil and manure or test systems containing additionally plants can be found. These were not considered for the survey.

Studies on solid manure (mainly conducted at Asian institutions) are not considered in this review. The composition of this material is considerably more variable than the composition of liquid manure matrix which results in i.e. wide ranges of oxygen availability. Compared to solid manure, liquid manure exhibits a more homogeneous composition. This type of manure was considered primarily, as it has been found to be the predominant type of manure in the EU countries and North America (Weinfurtner 2011).

Out of a total of 648 publications a limited number of 34 relevant studies was selected. These studies together with information on their experimental design are compiled in Table 7 and Table 8. On the whole, there is only scarce data on transformation of veterinary medicinal products and especially on transformation of biocides.

Generally, the research on transformation of pharmaceuticals in manure is focused on North America, Europe, and Asia (Figure 2). However, there is an increasing publication rate worldwide which reflects the impact and necessity of this research field

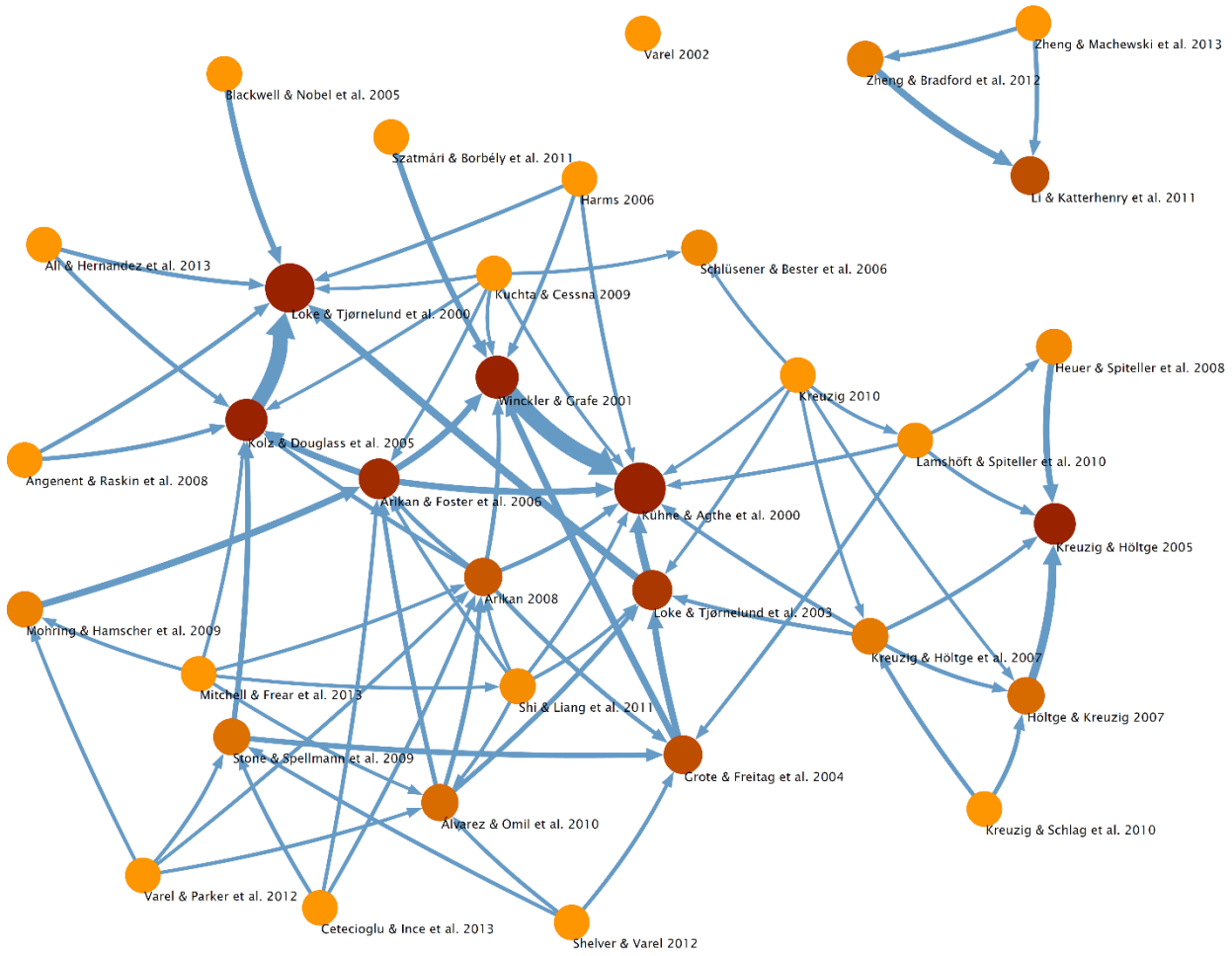
Figure 2 Origin of the 34 transformation studies



4.1 Citation map

The following citation map (Figure 3) provides an impression on the interconnection of the authors/working groups by generating a network and their respective impact in this field of research. Each node represents one publication. The darker and the bigger the node, the more often the publication is cited. The arrows show who cites whom, their thickness correlates with the citation flow indicating established thematic clusters. Only three publications are completely left out citing each other, owed to dealing with hormones and lagoon water. One isolated work of Varel (2002) considers deliberate application of (natural) biocides to manure. This was to stop microbial activity and prevent "odor emissions" during the storage of manure. This approach is contrary to all other considered publications. One cluster is implied on the right of this network, showing seven publications which used ^{14}C-labeled compounds, all originating from Germany (working groups Kreuzig and Spiteller). Most often cited papers are from Kühne et al. (2000) and Loke et al. (2000). This is partly explainable by the relatively early date of publication.

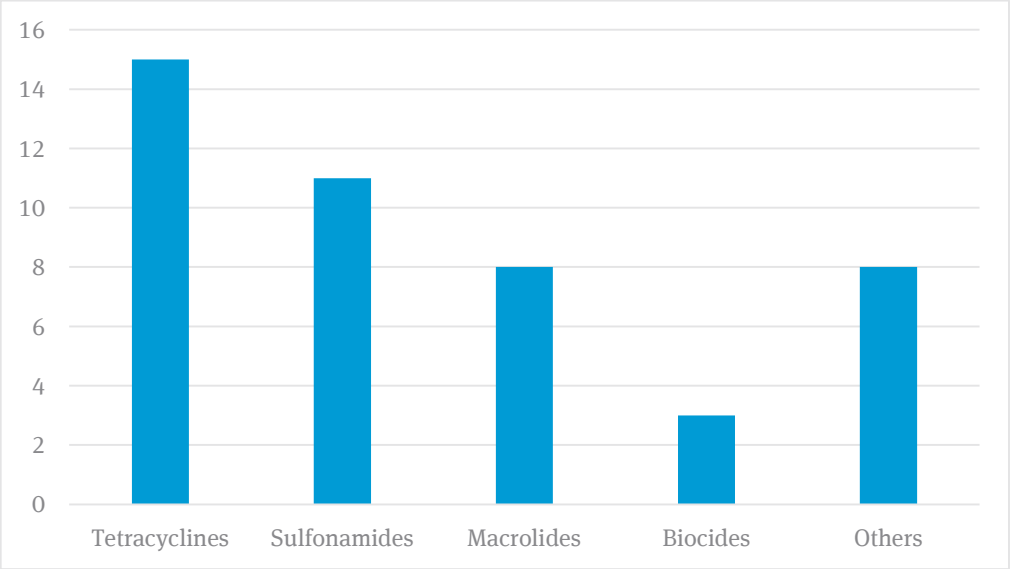
Figure 3 Citation Map (created via www.mapequation.org)



4.2 Studied substance classes

A compilation of the investigated active substances (on the basis of frequentness of substance classes) is depicted in Figure 4. Equivalent to the application practice in livestock breeding, mainly sulfonamide and tetracycline antibiotics were considered. There are only a few studies with parasiticides. For biocides only three publications were found (Kreuzig & Schlag et al. 2010, Kreuzig 2010, Varel 2002) Within 2 of 34 studies transformation of excreted hormones was investigated. Although they are not about VMPs, these publications were also considered because they are well documented (e.g. measured redox potential) and conducted similar to the rare transformation studies with VMPs.

Figure 4 Investigated substance classes in transformation studies



4.3 Aerobic vs. anaerobic conditions

It was tried to select studies that were conducted under primarily anaerobic conditions, as stated by the authors or deduced from the given information on the experimental set up. However, also studies are included in the table which used aerobic conditions (e.g. redox potentials above -100 mV, OECD 2002). Various authors assume anaerobic conditions without any further indication. Different terminology is used, such as “anaerobic digestion”, “anaerobic conditions”, “methanogenic conditions”, and "anaerobic tightly capped” vessels. Studies with this vague information were considered in this closer examination, otherwise the relevant publications would have been limited to a number of only six studies reporting a redox potential.

Besides using closed laboratory setups, many studies report the use of N_2 or He gas to purge the headspaces of the systems or to purge the used liquids and manures before starting the experiments. Others added reducing agents to the manures to guarantee reducing conditions (e.g. Na_2S by Álvarez & Omil et al. (2010) or titanium(III)citrate by Loke & Tjørnelund et al.(2003)). Loke & Tjørnelund et al. (2003)

further added resazurin as redox indicator. As the test bottles did not show a reddish coloring, they assumed anaerobic conditions. However, it might be difficult to interpret the coloring of this also pH-dependant indicator in deep brown liquid manure. For this, they also monitored methane gas production as a main indicator for methanogenic – and by this anaerobic - conditions. Varel & Parker et al. (2012) systematically studied methane production of their seed manure before starting transformation studies with this manure, to being able to work with stable methanogenic conditions.

Kuhne et al. (2000) used closed incubation systems to investigate the stability of tetracycline in pig manure. They determined that the DT50 (disappearance time 50 %) for tetracycline in their unventilated systems was 9 days, whereas it was 4.5 days when the slurry was ventilated.

Szatmári et al. (2011) compared an anaerobic laboratory study with a field study using manure composting. In the laboratory experiment more than 30 % and in the field study about 10 % of the initial doxycycline amount could be detected in manure samples after 16 and 12 weeks of manure ageing period, respectively. The half-life of doxycycline in manure was calculated to be 52.5 days under anaerobic conditions and 25.7 days under aerobic conditions.

Ali et al. (2013) were the only authors from all included studies who deliberately varied the redox potential. They established a set of microcosms with controlled redox potentials (Eh) (-100 mV, 0 mV, +250 mV, and +350 mV) and pH conditions (pH 5.5, 7.0, and 8.5). With increasing Eh - that is with increasing aerobic conditions - they found significantly increased dissipation rates for tylosin and could attribute this to microbial activity by comparison with sodium azide treated samples. Increasing pH resulted in increasing dissipation rates. With the addition of azide a decrease of Eh could be measured. Kolz et al. (2005) worked with redox potentials of slurry between -10 and -80 mV. The addition of azide resulted in a decrease of the redox potential to between -90 and -160 mV.

Although redox potential is not directly a proof of anaerobic conditions, it is a relatively easy to measure parameter to get insight into the system liquid manure. The internationally harmonized guideline concerned with transformation of chemicals in water sediment systems pragmatically sets a maximal upper limit of -100 mV (OECD 2002) for anaerobic conditions. Strictly considering this limit of -100 mV, only three studies meet the requirements for anaerobic conditions (Ali et al. (2013), Lamshöft et al. (2010), Zheng et al. (2013)). It should be considered, that redox potentials measured in real manure tanks are even much below -100 mV (Weinfurtner 2011). Generally, the transformation of VMPs in manure is faster and more complete under aerobic conditions than under anaerobic conditions.

4.4 Matrix characteristics and sorption to suspended solids

From the 34 studies, eight used cattle manure which showed dry matter contents from 1.1 up to 13 %. Two studies used both swine and cattle manure. One study relies on a synthetic matrix-water mixture to approximate properties of liquid manure. For the remaining 23 studies, swine manure was the matrix exhibiting dry matter contents from 2 up to 15.2%. Again, and similar to the point of varying oxygen availability, comparability is complicated due to differing dry matter contents. Kreuzig emphasizes substance specific interactions with the different pig or cattle manure matrices (Kreuzig 2010). He further mentioned that the dry substance content of manure is one of the most relevant factors affecting the transformation of VMPs and biocides. With a study on the stability of tylosin A in manure Loke et al. could not clarify whether the decrease in the concentration of this compound is caused by sorption, abiotic or biotic chemical degradation (Loke et al. 2000). Similarly, Shi et al. could not explain whether the rapid disappearance of the investigated antibiotics tetracycline and sulfamethoxydiazine could be due to their adsorption onto solid materials or degradation by microorganisms (Shi et al. 2011). In another study Loke et al. (2003) stated that very low free concentrations of oxytetracycline and metabolites in an anaerobic degradation experiment is due to high amounts of substances being bound to particles in the manure matrix rather than degradation to unknown compounds.

In 15 studies this dry matter content which is strongly influencing sorption of the test substances is not even mentioned, prohibiting a deeper interpretation of the results. As this is a key parameter which impacts the dissipation rate, could be shown by Álvarez et al. (2010), Arikan (2008), Kolz et al. (2005), Kreuzig (2010) and Kuchta and Cessna (2009). These authors investigated explicitly sorption onto solid matter which was already recognized as a crucial parameter by Winckler and Grafe (2001).

4.5 Biotic vs abiotic transformation

In some studies either sterilization with sodium azide or autoclaving allowed to differentiate between abiotic and biotic transformation. Generally, biotic transformation rates are substantially higher than pure abiotic transformation rates. However, the process of formation of non-extractable residues cannot be elucidated by this approach.

Loke & Tjørnelund et al. (2003) worked with autoclaved and non-autoclaved manure to study transformation of oxytetracycline (OTC) by measuring the free concentration of the VMP. By this, they did not find a difference between the sterile and the non-sterile setups, due to a fast sorption of OTC to the solid phase. They did not address the question, whether biotic transformation is inhibited by sorption of the substance to suspended solids.

Loke & Tjørnelund et al. (2000) studied transformation of tylosin A but were unfortunately not able to figure out whether the rapid decrease in the concentration of tylosin A is caused by sorption, abiotic or biotic chemical degradation. Ali & Hernandez et al. (2013) later reported among other conclusions, that under aerobic (Eh +350 mV) conditions, microbial degradation was much greater than under anaerobic conditions compared to abiotic transformation or sorption. Kolz & Douglass et al. (2005) concluded that both biodegradation and abiotic degradation occur during transformation of tylosin. However, strong sorption to slurry solids was probably the primary mechanism of tylosin disappearance.

Zheng & Bradford et al. (2012) found, that transformation of 17 αestradiol, 17 βestradiol, and estrone was mainly dominated by biodegradation than by physical or chemical transformation.

Li et al. found that the combined processes of hydrolysis and biodegradation were responsible for transformation of ceftiofur. The determined hydrolysis and total degradation rate constants in aqueous solutions varied with temperature (Li et al. 2011).

4.6 Metabolites and transformation products

With regard to VMPs it is important to distinguish between metabolites, which may be formed during metabolism in the treated animal, and transformation products, which may be formed from excreted parent and metabolites in the environment.

Transformation products were determined in 22 studies. This implies sophisticated methodology by liquid chromatography coupled to preferably tandem mass spectrometry or high resolution mass spectrometry (LC-MS/MS or LC-HR-MS). For specific applications, HPLC (high performance liquid chromatography) with UV (ultraviolet) detection may be sufficient (Winckler and Grafe 2001). Due to missing standard substances, transformation products are often determined only qualitatively.

Arikan (2008) studied the fate of chlortetracycline (CTC) during anaerobic digestion of manure from medicated calves. The CTC concentration decreased about 75 % and the concentration of the CTC epimer, 4-epi-chlortetracycline (ECTC), declined roughly 33 % during the 33 day experiment. The CTC metabolite, iso-chlortetracycline (ICTC), increased 2-fold in concentration. Referring to a higher water solubility, the authors concluded a possible occurrence of metabolites of CTC in water bodies.

Also Mitchell et al. stated that solid and liquid effluents from anaerobic digestion treatment with antibiotic transformation products could present an environmental concern (Mitchell et al. 2013).

In the study of Heuer et al. (2008) the concentration of Sulfadiazine (SDZ) increased by 42 % during storage of the manure due to deacetylation of the metabolite N-acetyl-SDZ. Basically the same was determined by Lamshöft et al. (2010) who alert environmental effects may be underestimated if the parent compound alone is considered for environmental risk assessment.

Table 7 Studies on transformation of VMPs and biocides in liquid manure and similar matrices (excrements, biosolids, etc, as specified in the second last column) under aerobic and anaerobic conditions. (n.d.: not determined or not defined)

Author	Year	Substances	Substance class	TP	Initial concentration	DT50	Mineralization	Manure (type and source)	Dry matter
Ali & Hernandez et al.	2013	Tylosin	Macrolide	-	160 mg/L	n.d. (highly pH and Eh dependant)	n.d.	cattle (spiked, mixed lagoon sediment)	2.7 %
Álvarez & Omil et al.	2010	Oxytetracycline (OTC), Chlortetracycline (CTC)	Tetracycline	+	10, 50, 100 mg/L	15.4 -12.0 (OTC), 4.1 - 3.2 (CTC) d	n.d.	pig (spiked, tank)	-
Angenent & Raskin et al.	2008	Tylosin-A	Macrolide	+	5.8 mg/L (measured)	2.49 h	n.d.	pig (spiked, tank/ASBR)	-
Arikan	2008	Chlortetracycline	Tetracycline	+	1.0 and 5.9 mg/L (EDTA-Buffer Extraction, pH 4)	18 d	n.d.	pig (medicated, mixed excrements)	5 %
Arikan & Foster et al.	2006	Oxytetracycline	Tetracycline	+	9.8 mg/L	56 d	n.d.	cattle (medicated, mixed excrements)	5 %
Blackwell & Noble et al.	2005	Oxytetracycline (OTC), Sulfachloropyridazine (SCP)	Tetracycline, Sulfonamide	-	19.2 (OTC), 26.1 (SCP) mg/L	79 (OTC), 127 (SCP) d	n.d.	pig (spiked, tank)	2 %
Cetecioglu & Ince et al.	2013	Tetracycline	Tetracycline	-	Gradient: 1.65, 5.7, 8.5 mg/L	n.d.	n.d.	synthetic (spiked, ASBR)	-
Grote & Freitag et al.	2004	Chlortetracycline (CTC), Sulfadiazine (SDZ), Trimethoprim (TMP)	Tetracycline, Sulfonamide	+	up to: 87.5 (CTC), 498.9 (SDZ), 15.8 (TMP) mg/kg	n.d.	n.d.	pig (medicated, 'barrels')	-
Harms	2006	20 different substances	Tetracycline, Sulfonamide, and others	-	numerous, many not given	n.d.	n.d.	pig (medicated & spiked, tank)	-
Heuer & Spiteller et al.	2008	Sulfadiazine (^{14}C)	Sulfonamide	+	› 80 mg/kg	n.d. (DT50 not reached)	‹ 1 %	pig (medicated, mixed excrements)	6 %

Höltge & Kreuzig	2007	Sulfamethoxazole, Acetyl-Sulfamethoxazole (each ^{14}C)	Sulfonamide and metabolite	+	3 mg/kg	n.d.	≤ 1 %	cattle (spiked, mixed excrements)	13 %
Kolz & Douglass et al.	2005	Tylosin	Macrolide	+	20 and 195 mg/L	DT90: 40 - 500 h	n.d.	pig (spiked, lagoon water)	1.5, 3.6 %
Kreuzig	2010	Erythromycin (ERY), Sulfamethoxazole (SMZ), Cyanamide (CYN), Imazalil (IMZ), (each ^{14}C)	Macrolide, Sulfonamide, Biocide, Imidazole	-	only absolute radioactivity given; 0.1 to 0.2 MBq	n.d.	‹ 0.1 % (ERY, SMZ); 28 % (CYN); n.d. for (IMZ)	pig, cattle (spiked, mixed excrements)	2.5, 5, 10 %
Kreuzig & Höltge	2005	Sulfadiazine (^{14}C)	Sulfonamide	-	500 µg/kg	17 d	1 %	cattle (spiked, mixed excrements)	13 %
Kreuzig & Höltge et al.	2007	Fenbendazole (FEN), Flubendazole (FLU), (each ^{14}C)	Benzimidazole	+	200 (FEN), 2500 (FLU) µg/kg	n.d. (DT50 not reached)	‹ 0.6 %	pig (spiked, mixed excrements)	3 – 13 %
Kreuzig & Schlag et al.	2010	Imazalil (^{14}C)	Imidazole	+	4.3 and 4.5 mg/kg	› 177 d	0.1 %	pig, cattle (spiked, mixed excrements)	2.5, 5, 10 %
Kuchta & Cessna	2009	Lincomycin (LIN), Spectinomycin (SPN)	Antimicrobial	-	38.7 (LIN), 387 (SPN) µg/L	n.d.	n.d.	pig (spiked, lagoon water)	-
Kühne & Agthe et al.	2000	Tetracycline	Tetracycline	+	200 mg/L	9 d	n.d.	pig (spiked, tank)	-
Lamshöft & Spiteller et al.	2010	Difloxacin (DIF), Sulfadiazine (SDZ), (each ^{14}C)	Fluoroquinolone, Sulfonamide	+	17.1 ± 0.4 (DIF), 156.0 ± 4.2 (SDZ) mg/L	n.d. (DT50 not reached)	0.2 % (DIF), 0.5 % (SDZ)	pig (medicated, mixed excrements)	3.3 - 6 %
Li & Katterhenry et al.	2011	Ceftiofur	β-Lactam antibiotic	+	19.1 µmol/L	1.7 - 41 (highly dependant on T and dilution ratio with water)	n.d.	cattle (spiked, 'water from farm')	1.1 %
Loke & Tjørnelund et al.	2003	Oxytetracycline	Tetracycline	+	2 and 30 mg/L	n.d.	n.d.	pig (spiked, tank)	-
Loke & Tjørnelund et al.	2000	Tylosin A	Macrolide	+	5 mg/L	‹ 2 d	n.d.	pig (spiked, tank)	-

Mitchell & Frear et al.	2013	Ampicilin, Florfenicol, Sulfamethazine, Tylosin	β-Lactam antibiotic, Amphenicol, Sulfonamide, Macrolide	+	each 0.001 - 1.0 mM/L	n.d.	n.d.	cattle (spiked, mixed excrements)	3 - 6 %
Mohring & Hamscher et al.	2009	8 Sulfonamides	Sulfonamide	+	2 - 14 mg/kg	n.d.	n.d.	pig (spiked, biogas plant)	15.2 %
Schlüsener & Bester et al.	2006	Erythromycin, Roxithromycin, Salinomycin, Tiamulin	Macrolide, Ionophore, Pleuromutilin	+	2 mg/kg	6 d - ›180 d	n.d.	pig (spiked, tank)	-
Shelver & Varel	2012	Chlortetracycline	Tetracycline	+	›100 and ›300 ng/L (only given in figures)	› 21 d at 22 °C, ‹ 5 d at 38 °C and 55 °C	n.d.	pig (medicated, mixed excrements)	-
Shi & Liang et al.	2011	Tetracycline, Sulfamethoxydiazine	Tetracycline, Sulfonamide	-	each 25 and 50 mg/L	‹ 12 h	n.d.	pig (spiked, mixed excrements)	10 %
Stone & Spellmann et al.	2009	Chlortetracycline (CTC), Tylosin (TYL)	Tetracycline, Macrolide	+	28 (CTC), 1.1 (TYL) mg/L	n.d.	n.d.	pig (medicated, manure)	-
Szatmári & Borbély et al.	2011	Doxycycline	Tetracycline	-	61.57 ± 14.26 mg/kg	53 d	n.d.	pig (medicated, manure)	-
Varel	2002	Carvacrol, Thymol	Terpenoid	-	each 6.7 - 16.75 mmol/L	n.d.	n.d.	pig (spiked, mixed excrements)	-
Varel & Parker et al.	2012	Chlortetracycline (CTC), Monensin (MON)	Tetracycline, Ionophores	-	5.9 - 8.3 (CTC), 0.3 - 0.74 (MON) mg/L	n.d. (DT50 not reached for MON)	n.d.	pig, cattle (medicated, seed slurry and manure)	4 %
Winckler & Grafe	2001	Tetracycline	Tetracycline	-	20 and 100 mg/L	55 - 105 d	n.d.	pig (spiked, tank)	-
Zheng & Bradford et al.	2012	17-β-estradiol, 17-α-estradiol, estrone	Hormone	+	each 5 mg/L	n.d.	n.d.	cattle (spiked, lagoon water)	-
Zheng & Machewski et al.	2013	17 œstradiol-3-sulfate	Conjugate of a Hormone	+	5 mg/L	23 - 724 d	n.d.	cattle (spiked, lagoon water)	1.2 %

4.7 Chemical analysis

As already mentioned with regard to the citation map, seven studies used ^{14}C labeled test substances. By this, a mass balance of the experiment considering transformation, mineralization, volatilization, and the formation of non-extractable residues is possible. The methods used are radio thin layer chromatography (RTLC), oxidizers for solid samples and liquid scintillation counting (LSC). Only Heuer & Spiteller et al. (2008) and Lamshöft & Spiteller et al. (2010) further used LC-MS techniques in combination with radio techniques. An approach that will be inevitable in future studies to gain maximum information out of transformation studies in terms of transformation product identification and quantification.

Exemplary, Höltge and Kreuzig investigated the fate of ^{14}C-labeled sulfamethoxazole and acetyl-sulfamethoxazole in a laboratory experiment (Höltge and Kreuzig 2007). Within an incubation period of 72 d, 1 % at maximum of the initially applied radiotracers was released as $^{14}CO_2$ while more than 75 % was transferred to non-extractable residues that were operationally defined by an ethyl acetate extraction. In long-term degradability tests, the authors could determine an increase of non-extractable residues and 11 % mineralization at maximum.

However, by far most of the studies worked with unlabeled substances and used LC-MS or LC-MS/MS for detection and quantification of the VMPs and biocides and their transformation products (18 publications). Some of them combined UV-Vis / diode array detector (DAD) methods with MS-methods (3 publications). For example Schlüsener and Bester et al. (2006) used HR-MS (high resolution - mass spectrometry) for further salinomycin transformation product identification. Within 7 publications only UV-Vis/DAD-detection methods were used. The only GC method (gas chromatography) was applied by Varel (2002) for the detection of the terpenoids carvacrol and thymol. Varel (2012) applied an ELISA method (enzyme-linked immunosorbent assay) for the detection of chlortetracycline.

4.8 Methane production and microbial activity

The production of methane was considered as ongoing parameter in eight studies. In two studies with tetracyclines, Arikan et al. (2006) and Alvarez et al. (2010) found that methane production was reduced by 27 % during batch experiments and up to 62 % due to antibiotic dosage, respectively. Stone et al. (2009) found that generation of methane was inhibited by 27.8 % due to the presence of chlortetracycline. Dependent on the dosage, Cetecioglu et al. (2013) determined adverse impact of tetracycline with a total collapse of the microbial activity and metabolic functions at a concentration of 8.5 mg/L in a synthetic substrate mixture under anaerobic conditions. Shi et al. (2011) found a dosage dependent inhibition on CH_4 production and concluded antibiotics appear to inhibit bacterial activity resulting in a delay and overall decline in CH_4 production. Among these eight studies, two studies were explicitly concerned with microbiological issues (Stone et al. 2009; Heuer et al. 2008). Varel & Parker et al. (2012) mentioned that a 5 to 6 months adaption period was necessary for acclimatization of microorganisms to monensin and to reduce effects of antimicrobals on methane production. Against that background each future transformation study has to be analyzed critically. Composition of microbial community has a massive effect on transformation rates and routes. Without any further qualitative

and quantitative critical analysis of microbiology, it is not possible to produce reliable and reproducible transformation data of VMPs and biocides in liquid manure. From a regulatory point of view, this topic could enable a massive manipulation of transformation data.

4.9 Study temperature

Study temperatures ranged from 5 °C to 55 °C. Few publications study the temperature effect in detail by variation of study temperature.

Harms (2006) examined the stability of pharmaceuticals in manure during storage at 7 °C and found no degradation of chlortetracycline during six months. Sulfadiazine was reduced to 50 % after one week, but the remaining residuals were stable until the end of the trial (32 weeks). The author varied storage temperatures (-20°C, 7°C, room temperature) over a period of 16 weeks. Sulfamerazine, sulfamethoxypyrazine, sulfaguanidine and sulfisomedine persisted. Sulfamethoxazole was reduced up to 80 % at 7°C as well as at room temperature. The same degradation was reached more quickly with higher temperatures than under cooler conditions of storage. Enrofloxacin and tiamulin were reduced to 20 % at 7°C storage temperature. For tiamulin a reduction of 10 % at 7 °C and 25 % at room temperature was observed. Only sulfapyridazine and enrofloxacine showed a small decline at a storage temperature of -20 °C.

Li et al. (2011) studied the transformation of ceftiofur at temperatures between 15 °C and 45 °C and found increasing hydrolysis and biodegradation rates with temperature. Increasing study temperature from 35 °C to 45 °C resulted in an increasing relevance of hydrolysis on transformation of ceftiofur, whereas biodegradation remained static.

Similarly, Varel et al. (2012) found principally increasing dissipation rates with increasing temperature from 22°C to 55°C. They studied the effect of anaerobic digestion at different temperatures, among other parameters, on the fate of chlortetracycline (CTC) in swine manure and monensin (MON) in cattle manure. The authors concluded that anaerobic digestion at elevated temperatures may be an effective treatment to reduce CTC but not to reduce MON. Transformation of CTC mainly depends on abiotic transformation. This was also shown by Shelver et al. (2012), who also worked with CTC between 22°C and 55°C

Stone et al. (2009) worked with a temperature gradient to simulate field conditions commonly found in the northern mid-western United States of America. They started with 10°C (0-30 days) and increased temperature over time: 12°C (30-46 days), 15°C (46-56 days) and 20°C (56-216 days).

Approximately half of the studies (16 out of 34) took place at ambient temperatures (ranging from 20°C to 25°C). Some other experiments ran at elevated temperatures of 35°C to 40°C which enhances microbial activity (Arikan 2008). In general, transformation was found to be dependent on study temperature, with an increase in transformation rate with increasing temperature. Working with temperatures above the microbiological relevant limit of 35-40 °C results in an inhibition of microbial activity and biodegradation processes. In what way this effects transformation processes, mainly depends on the transformation routes for different substances.

Table 8 Studies on transformation of VMPs and biocides in liquid manure under aerobic and anaerobic conditions.

Author	Year	Focus and parameters	Setup	Amount manure	Preconditioning/acclimatization	Replicates	Study-T [°C]	Eh [mV]	Study duration [d]
Ali & Hernandez et al.	2013	pH and Eh	2.3 L erlenmeyer flask, continuously stirred and flushed with N_2/O_2 for different Eh (Fig. 5)	150 g wet lagoon sediment + 1.5 L 0.01 M $CaCl_2$	1 week for stabilization of pH and Eh	1	25	(-100), (0), (250), (350)	20
Álvarez & Omil et al.	2010	biogas compostion, pressure, sorption	500-mL glass flasks with coiled butyl rubber stoppers	385 mL + Inoculum (granular biomass from an anaerobic internal circulation digester)	Basal medium: cysteine (0.5 g/L), $NaHCO_3$ (5 g/L), pH 7.0–7.2; flushing with N_2, 1.2 mL Na_2S (20 g/L) (reducing agent)	2	35	-	21
Angenent & Raskin et al.	2008	antibiotic resistance, methane production, volatile solids removal, VFA	manure taken from ASBR effluent, 5 mL capped glass serum vials	1 mL	249 days of ASBR operation	1	25	-	2
Arikan	2008	sorption, pH, total solids, volatile solids, total alkalinity, NH_4-N, COD	1 L batch laboratory digester	800 mL manure + 200 mL inoculum from from a dairy manure digester	n.d.	3	35	-	33
Arikan & Foster et al.	2006	biogas production, total solids, total alkalinity, total N, total P	1.225 L batch laboratory digester	1 L manure + 225 mL inoculum from from a dairy manure digester	n.d.	3	35	-	64
Blackwell & Noble et al.	2005	exposure assessment, organic carbon, dry matter, available P and N	closed bottle test, tightly capped and stored without agitation	200 mL	n.d.	3	20	-	40
Cetecioglu & Ince et al.	2013	synthetic manure, COD, biogas production	ASBR, concentration influent and effluent, sludge	1 L	150 days of ASBR operation	1	35	-	155

Grote & Freitag et al.	2004	metabolism, transformation	outdoor realistic conditions with continuous influent of contaminated manure	“barrels”	n.d.	1	outdoor	-	240 + 210
Harms	2006	transformation	n.d.	n.d.	n.d.	n.d.	-20, 7, RT	-	112, 224
Heuer & Spiteller et al.	2008	bacterial community	n.d.	n.d.	n.d.	1	20	-	172
Höltge & Kreuzig	2007	transformation, NER	300-mL flasks, glass stoppers with inlet and outlet valves, $^{14}CO_2$-trap	50 g	7 days	3	20	-	72
Kolz & Douglass et al.	2005	aerobic vs anaerobic, sorption, pH, total solids, N, TOC, P	amber glass vials with teflon-lined caps, headspace filled with He	20 mL	‘homogenized stored in glass jars at 4 °C until use’	3	22	(-10) - (-160)	3
Kreuzig	2010	T,Eh, dry matter, O_2, N-total, NH_4-N, TOC, BOD	300-mL flasks, glass stoppers with inlet and outlet valves, $^{14}CO_2$-Trap	50 g	n.d.	2	5, 10, 20	(- 80)	30, 100, 177
Kreuzig & Höltge	2005	transformation, NER	300-mL flasks, glass stoppers with inlet and outlet valves, $^{14}CO_2$-Trap	50 g	n.d.	2	20	-	102
Kreuzig & Höltge et al.	2007	manure-soil mixtures, transformation, NER	300-mL flasks, glass stoppers with inlet and outlet valves, $^{14}CO_2$-Trap	50 g	n.d.	2	20	-	102
Kreuzig & Schlag et al.	2010	manure-soil mixtures, transformation, NER, biocides	300-mL flasks, glass stoppers with inlet and outlet valves, $^{14}CO_2$-Trap	50 g	n.d.	2	20	(< 120)	177
Kuchta & Cessna	2009	sorption, liquid-solid distribution after centrifugation	20-L stainless-steel storage container with clipdown cover	15.5 L	n.d.	2	20	-	160
Kühne & Agthe et al.	2000	transformation	vacuum desiccator (Fig. 6)	1 L	n.d.	2	RT	-	8
Lamshöft & Spiteller et al.	2010	T, Eh, dry mass, pH, BOD, COD, total carbon, conductivity	300-mL flasks with $^{14}CO_2$-Trap	50 g	‘the manure was allowed to attain room temperature’	3	10, 20	(- 280) - (- 329)	150

Li & Katterhenry et al.	2011	TOC, conductivity, pH, P,NH_4-N, Cl^-, Br^-, $NO3^-$, Na, K, Ca, Fe, Mg, Al, Si, Cu, Zn	Amber 250 mL bottles with teflon lined caps served as reactors	‹ 250 mL	n.d.	3	15, 25, 35, 45	-	72
Loke & Tjørnelund et al.	2003	pH, Eh via indicator, freely dissolved fraction	according to ISO 11734 (ISO 1998), 1 L bottles; titanium(III)citrate as reducing agent	525.0 ml mineral medium, 50.0 ml manure 100.0 ml stock solution	‹ 2 weeks storage at 4 °C	4	21	-	180
Loke & Tjørnelund et al.	2000	transformation, filtered vs non-filtered	according to ISO 11734 (ISO 1998), volumes x 50, 680 mL	680 mL (water with 6,4 % manure)	1 mm sieved, N_2 bubbled through manure, stored at 4 and -20 °C before usage	4	20	-	7
Mitchell & Frear et al.	2013	pH, CH_4, CO_2 inhibition,total solids (TS) and VSS	300 mL glass serum bottles fitted with rubber septum, headspace filled with N_2, inoculum used	200 mL	n.d.	3	37	-	40
Mohring & Hamscher et al.	2009	biogas production, pH	5-L fermentors (Bigatec, Rheinberg, Germany), German VDI 4630 guideline, DIN 38414 Part 8, control experiments in 500 mL flasks	1.89 kg manure, 1.89 L water, 0.42 kg inoculum	n.d.	2	37	-	34
Schlüsener & Bester et al.	2006	transformation	Erlenmeyer flasks closed with a fermenting tube	100 g	n.d.	1	20	-	180
Shelver & Varel	2012	pH, transformation	2 L digester flasks	n.d.	n.d.	3	22, 38, 55	-	28
Shi & Liang et al.	2011	methane production, pH, total solids	1 L digester with gas absorbing bottle and collector-bottle (Fig. 7)	1 L (including 100 g dry manure, 100 mL inoculum)	n.d.	3	25	-	20

Stone & Spellmann et al.	2009	CH_4, CO_2, volatile fatty acids, pH, Alkalinity, COD, VSS, VFA, hydrogentrophic methanogens, aceticlastic methanogens	120 mL batch reactors, butyl rubber stoppers, headspace N_2 purged	50 g	105 d at 4 °C	3	10-20 (gradient)	-	216
Szatmári & Borbély et al.	2011	transformation	300 ml BOD-bottles as used in closed bottle tests; referring to VICH/EMA (2010)	‹ 300 mL	n.d.	n.d.	20	-	112
Varel	2002	odor, total gas, VFA, L-lactate, pH	1 L Erlenmeyer flasks, N_2-gas, rubber stopper	500 mL (faeces, urine, distilled water; 50:35:15)	n.d.	2	25	-	62
Varel & Parker et al.	2012	odor, pH, VFA, aromatic fermentation products, methane, coliforms	2 L Erlenmeyer flasks with rubber stopper	600 mL (1:1 seed slurry and fresh manure)	establishing “seed slurry” over 2 - 5 months for stabilization of ph, methane and VFA-production	2	22, 38, 55	-	25, 28
Winckler & Grafe	2001	T, transformation	500 L tanks	295 L	not given	4	8	-	48
Zheng & Bradford et al.	2012	T, transformation	250 mL glass bottles with teflon-lined screw caps, glovebox, Na_2S, N_2	‹ 250 mL	1 d preconditioning	3	35	(- 277)	52
Zheng & Machewski et al.	2013	T, transformation	250 mL glass bottles with teflon-lined screw caps, glovebox, Na_2S, N_2	‹ 250 mL	1 d preconditioning	3	15, 25, 35, 45	-	65

4.10 Source of manure

Although all the publications work with liquid manure, there are different approaches on application of the test substance to manure for transformation studies. First of all with VMPs contaminated manure can be generated by taking manure from previously medicated animals. The advantage of this procedure is the occurrence of metabolites of the parent compound. By this a more realistic transformation scenario can be studied. The deacetylation of the metabolite N-acetyl-sulfadiazine in manure after excretion back to the parent compound sulfadiazine is a well-studied example. There might be many further not yet studied examples like this. (Heuer et al. 2008, Lamshöft et al. 2010) Further, VMPs influence intestinal microbiology and by this its own transformation fate in manure. Possibly intestinal built NER might also have an influence on VMP transformation in manure. This applies also to excipients of the medicinal products. Considering analytical method development, taking medicated manure makes it difficult to impossible to determine recovery rates of the analytes out of the excreted and then aged manure. At this point only radioactive methods can provide a valid survey on parent compound excretion and distribution. In literature only Heuer & Spiteller et al. (2008) and Lamshöft & Spiteller et al. (2010) worked with radioactive labeled VMPs and medicated manure (^{14}C-Sulfadiazine, ^{14}C-Difloxacin). Overall, 10 out of 34 studies were conducted with medicated manure. This approach seems to be more appropriate to study metabolism in the animal than transformation of compounds in manure under the regulatory perspective.

Spiking manure in laboratory scale is a much more reproducible way of generating contaminated manure and the only way to handle transformation studies of biocides. By this approach it is possible to determine recovery rates with unlabeled compounds and to study sorption processes.

Most of the studies considered manure from pigs (25 out of 34), the remaining studies investigated manure from cattle. Within one study a synthetic substrate mixture including volatile fatty acids, glucose and starch was used (Cetecioglu & Ince et al. 2013).

Generally liquid manure is characterized as an anaerobic liquid medium. Taking uncontaminated liquid manure directly from a tank at a farm is the most reliable source of liquid manure. By this an adapted and realistic anaerobic and methanogenic microbial community can be assumed. 9 out of 34 studies worked with liquid manure taken out of a bigger tank at a farm. In contrast to this 16 publications reported a procedure of mixing more or less fresh excrements with water and in some cases with an inoculum to produce liquid manure on laboratory scale. Out of these, only Varel & Parker et al. (2012) described a well-documented procedure of generating a “seed manure” over 2 to 5 months to mix it later with fresh manure in order to preserve a reproducible artificial liquid manure.

4 Studies worked with lagoon water, which mainly differs from liquid manure in its lower dry matter content of in these cases 1.2 - 3.6 %. Additionally, Li & Katterhenry et al. (2011) used “recycled water derived from a beef farm“. Within one publication lagoon sediment was mixed with water down to a dry matter content of 2.7 % (Ali and Hernandez 2013).

Cetecioglu & Ince et al. (2013) and Angenent & Raskin et al. (2008) took manure for transformation experiments out of a continuously running anaerobic sequencing batch reactor (ASBR), whereas Mohring & Hamscher et al. (2009) took manure directly out of a biogas plant.

4.11 Experimental setup

Considering the practical setup of transformation studies in literature, the whole variety of approaches becomes obvious. The amount of manure used for one replicate ranges from 1 mL (Angenent & Raskin et al. 2008) up to 295 L (Winckler & Grafe 2001). By far most of the studies were conducted with 50 - 500 mL manure. 8 studies do not report a clearly defined amount of manure used. Most studies seem to work without agitation of manure during experiments or they do not clearly report it. There are only a few studies who mention the periodically stirring of the test manure or at least directly before sampling the manure.

Some studies refer to several guidelines. Loke & Tjørnelund et al. (2000) and (2003) refer to ISO 11734 (ISO 1998). Mohring & Hamscher et al. (2009) refer to German VDI 4630 (VDI 2006) guideline and to DIN 38414 part 8 (DIN 1985). Szatmári & Borbély et al. (2011) refer to the former draft of the EMA-Guideline on determining the fate of veterinary medicinal products in manure (EMA 2011, VICH 2010).

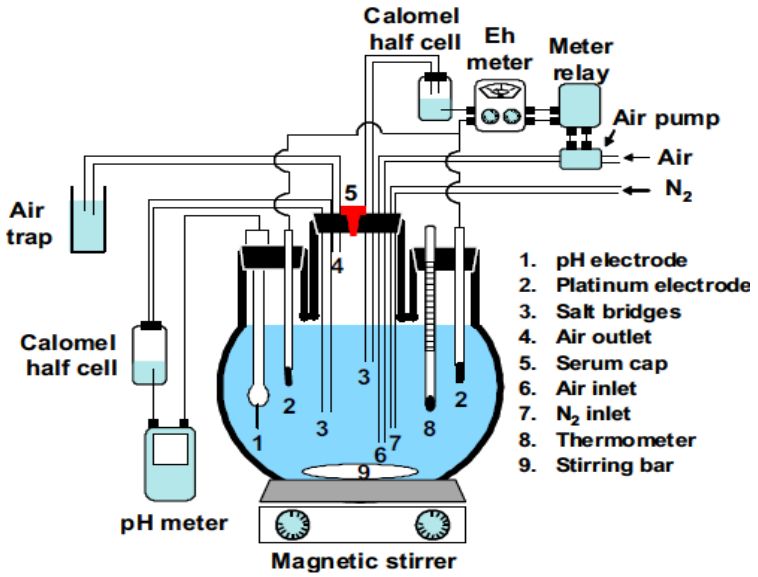
As already discussed under chapter 4.3 (Aerobic vs. anaerobic conditions) many of the studies try to establish anaerobic conditions by using an inert gas for flushing headspace or solutions of the experiments. In general it can be differenciated between flow-through systems and batch systems (also called static or semi static systems) on this topic. There is only one publication reporting a real flow-through system (Ali and Hernandez 2013). Especially for the reported batch systems it is often not well described on how exactly produced biogas was driven out of the system or how it was dealt with the generated biogas overpressure. This is important for studies monitoring biogas production or for those studies working with ^{14}C and monitoring mineralization.

Subsequently all the studies are shortly presented. There are two publications working with an anaerobic sequencing batch reactor (ASBR) and one study monitoring transformation under outdoor real farm conditions.

4.11.1 N_2 flow-through system

Ali and Hernandez (2013) worked with a continuous flow through of N_2 and O_2 in a defined ratio to reach a resulting Eh between -100 and +350 mV. With the addition of HCl or NaOH the pH was adjusted.

Figure 5 Schematic diagram of microcosm system used for controlling redox potential and pH in Ali and Hernandez (2013)



4.11.2 Batch assays (no N_2 flow-through)

Álvarez & Omil et al. (2010) worked with a batch assay approach. They used an inoculum to guarantee reproducible microbial starting conditions. Before starting the batch experiment N_2 was flushed through the 500 mL flasks. Further Na_2S was added at the beginning of the test to reach anaerobic conditions.

Arikan (2008) used a 1 L batch laboratory digester. It was filled with 800 mL fresh manure (diluted with water) from medicated pigs and 200 mL of an inoculum from the effluent of a dairy manure digester. After this the headspace of the digester was flushed with N_2. The digesters were stirred continuously during the experiments. Very similar to this Arikan & Foster et al. (2006) used the same setup. Additionally, biogas production was measured by using a water displacement technique.

Blackwell & Noble et al. (2005) worked with a closed glass bottle without agitation of the manure (200 mL). It is assumed that Heuer & Spiteller et al. (2008) also worked with a batch system. Manure was stored at 20 °C in the dark. Unfortunately the used amount and the storage setup is not given.

Höltge & Kreuzig (2007), Kreuzig & Höltge (2005), Kreuzig & Höltge et al. (2007) and Kreuzig (2010) used a stoppered 300 mL test setup with 50 g mixed excrements from untreated animals. ^{14}C-labelled biocides and pharmaceuticals were spiked to the manure and the whole setup was once flushed with nitrogen before starting the experiment. $^{14}CO_2$ was trapped within a KOH-solution. The manure was not stirred. Partially redox potentials and O_2-concentrations were measured to evaluate anaerobic conditions. Lamshöft & Spiteller et al. (2010) used a similar approach.

Kolz & Douglass et al. (2005) used amber glass vials with teflon-lined caps and filled the headspace with helium before storage. Kuchta & Cessna (2008) worked with the relatively high amount of 15.5 L manure for each replicate. It was stored in a 20-L stainless-steel container with clipdown cover to achieve similar storage conditions as they are given during storage in lagoons under a plastic cover.

Kühne & Agthe et al. (2000) used a closed vacuum desiccator to store 1 L liquid manure under unvented conditions. There is no mention of using N_2 or another inert gas to guarantee anaerobic conditions.

Figure 6 Setup for transformation studies presented by Kühne & Agthe et al. (2000)

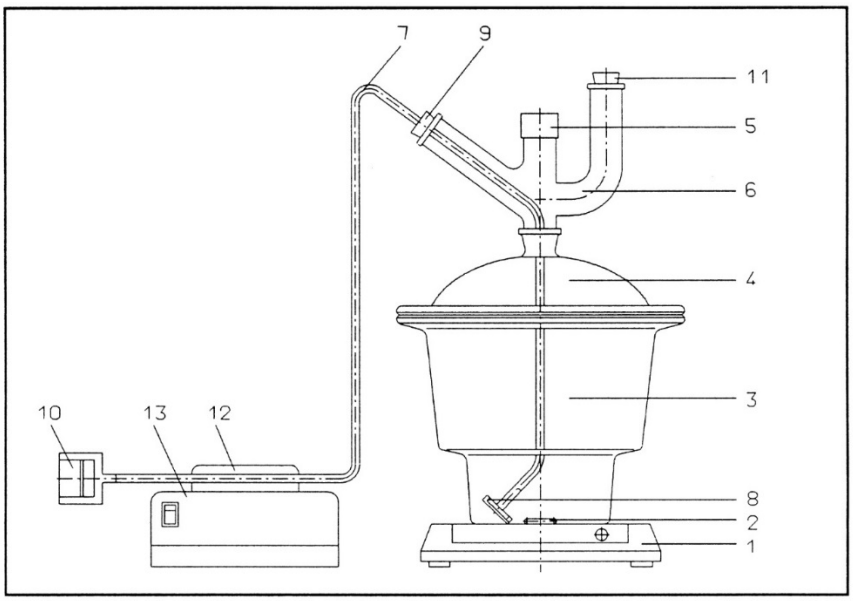


Fig. 1. Incubation system, ventilated. 1, magnetic stirrer; 2, magnetic stir stick; 3, desiccator base; 4, desiccator lid; 5, aluminium top piece; 6, three-headed top; 7, silicon tube; 8, gas distribution system; 9, rubber plug with drill hole; 10, filter holder; 11, rubber plug; 12, tube container; 13, pump.

Li & Katterhenry et al. (2011) used amber bottles (250 mL) with teflon lined caps as reactors. They did not report the usage of an inert gas.

Loke & Tjørnelund et al. (2000) and Loke & Tjørnelund et al. (2003) used a 1 L batch system according to ISO 11734 (1998). 50 g manure were mixed with 525.0 ml mineral medium and 100.0 ml stock solution. Titanium(III)citrate was added as reducing agent and resazurin (1.0 mg/L) was added as a redox indicator. Test conditions were assumed to be strictly anaerobic, because no reddish coloring from the resazurin occurred and methane gas was produced constantly. To further guarantee anaerobic conditions during the hole setup of the batch system and during sampling, it was worked under N_2-gas.

Mitchell & Frear et al. (2013) mixed manure with a total solid (TS) content of 20.1 % and an inoculum with a total solids content of 1.6 %. The inoculum of primary digested sludge was obtained from a wastewater treatment plant anaerobic digester. They mainly measured the inhibition of biogas-production out of a 200 mL batch system every day for the first 7 d and then every few days for 33 d using a syringe methodology.

Mohring & Hamscher et al. (2009) worked with a commercially available anaerobic 5L fermentor (Bigatec, Rheinberg, Germany). Based on the German VDI 4630 (VDI 2006) guideline and according to DIN 38414 part 8 (DIN 1985) they studied biogas production of a liquid manure taken from a biogas plant.

To achieve a dry matter content below 10 % they mixed the manure 1:1 with water. An inoculum of the biogas plant was added. The eight studied antibiotics were added at once to the two fermenters to realize similar degradation conditions for all of them.

Schlüsener & Bester et al. (2006) worked with 300 mL Erlenmeyer flasks closed with a fermenting tube "to maintain anaerobic conditions". Unfortunately no information is provided on how anaerobic conditions were established.

Shelver & Varel (2012) report the usage of incubators/ 2 L- digester flasks. It is not mentioned how they guaranteed anaerobic conditions.

Shi & Liang et al. (2011) took 100 g dried manure, mixed it with 100 mL inoculum obtained from an anaerobic digester on a farm and filled the mixture up to 1000 mL with water. Transformation experiments were carried out with the digester-setup shown in Figure 7. The produced biogas was led through a NaOH-collector to absorb carbon dioxide and the equivalent volume of the not absorbed amount of remaining biogas (methane) was pushed to the collector bottle. The system was flushed with N_2 before starting the transformation study and manure was stirred twice a day during the study

Figure 7 Setup for transformation studies presented by Shi and Liang et al. (2011)

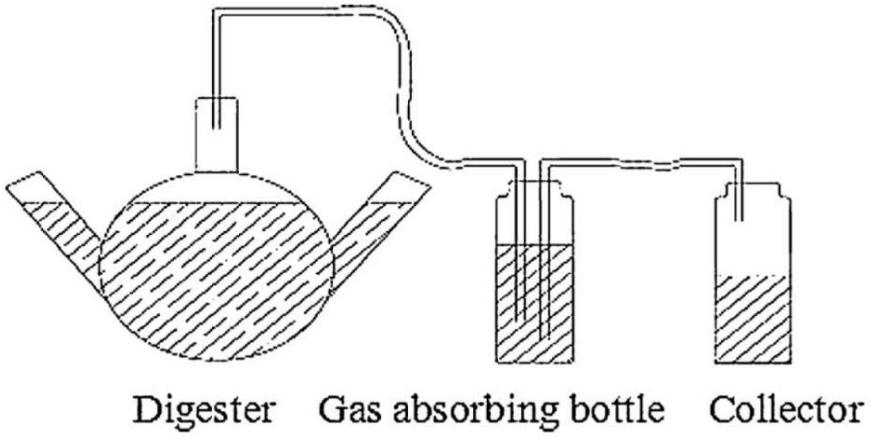


Fig. 1. Laboratory scale reactor for methane production in anaerobic digestion of pig manure.

Stone & Spellmann et al. (2009) filled 50 g manure into 120 mL batch reactors and purged the headspace with N_2 before starting the experiments. During the whole transformation studies, they continuously measured numerous parameters, such as: CH_4 and CO_2 production, volatile fatty acids (VFA), pH, alkalinity, chemical oxygen demand (COD), VSS, acetate, propionate, butyrate, isobutyrate, and the relative abundance of hydrogenotrophic methanogens and aceticlastic methanogens.

Szatmári & Borbély et al. (2011) conducted their studies referring to the former draft of the EMA-Guidline on determining the fate of veterinary medicinal products in manure (EMA 2011, VICH 2010). Nevertheless there is no mention of measured pH-values or working under N_2-gas within the 300 mL bottles as they are used in closed bottle tests. They compared transformation under anaerobic laboratory conditions with a simultaneously conducted field study under aerobic conditions.

Compared to all the other studies, Varel (2002) worked with a completely different approach and philosophy. He added the biocides Carvacrol or Thymol to the mixed excrements in order to reduce microbial activity and to reduce “offensive odor emissions”. In contrast to this, all the other studies were conducted in order to measure transformation under realistic conditions to assess potential environmental risks in the end. By intentionally adding biocides to manure, biotransformation of most VMPs will be reduced to a minimum.

Varel & Parker et al. (2012) spent a lot of efforts on establishing a well-defined seed-slurry over 2-5 months with a defined pH, methane and VFA-production. This seed slurry was later mixed with fresh manure from medicated animals. During their studies with a batch system under N_2-gas they monitored numerous concentrations of volatile fatty acids (VFA) and aromatic fermentation products (l-lactate, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, phenol, p-cresol, indole and skatole) besides the parameters pH and methane gas production and others. They further reported the acclimatization of the microbial activity to the presence of monensin.

Winckler & Grafe (2001) were one of the first to study transformation of pharmaceuticals in liquid manure. They worked with very large 500 l tanks under outdoor and under temperature controlled conditions.

Zheng & Bradford et al. (2012) studied the transformation of hormones - not a VMP - in lagoon water. Due to the limited amount of transformation studies in general and especially with lagoon water, this publication is considered within this literature review. They worked with 250 mL glass bottles and used Na_2S, N_2-gas and the glovebox technique to guarantee anaerobic conditions. Zheng & Machewski et al. (2013) used the same setup to study transformation of 17 œstradiol-3-sulfate.

4.11.3 Anaerobic sequencing batch reactor (ASBR)

Within the study of Angenent & Raskin et al. (2008) a 5 L ASBR was run by sequencing through a feed step (1 min), a react step (23.2 h), a settling step (45 min), and a decant step (2–5 min). Intermittent mixing was performed by biogas recycling (1 min of biogas recycling every hour at a flow rate of 26 L/h). Tylosin half-life experiments were conducted by taking manure from the ASBR, giving it into capped 5 mL glass serum vials (prepurged with N_2) and spiking it with tylosin. The vials were stored for 48 h at 25 °C in a water bath.

Cetecioglu & Ince et al. (2013) also used an ASBR with a 24-hour cycle to measure the impact of tetracycline on biogas production and biodegradation of a synthetic organic substrate. In contrast to Angenent & Raskin et al. (2008) they monitored the tetracycline mass balance between the influent and the effluent of the ASBR considering the sludge inside the ASBR. With this setup it was not possible to determine DT50-values.

4.11.4 Realistic outdoor conditions

Grote & Freitag et al. (2004) conducted the only transformation study with a realistic outdoor scenario with Chlortetracycline (CTC), Sulfadiazine (SDZ) and Trimethoprim (TMP). The medicated pigs excreted the pharmaceuticals over a long period of time. By this all the relevant metabolites were considered. Unless it is not easily possible to determine DT50-values with this approach, but it is possible to study the realistic transformation of a pharmaceutical.

4.12 Parameters

Within all the publications the following physical, chemical and biological parameters were measured or controlled during transformation studies:

Redoxpotential Eh [mV], dry matter content [%], pH, dissolved O_2-content [mg/kg], NH_4-N [g/kg], N_{total} [g/kg], total organic carbon (TOC) [g/kg], total carbon [g/kg], biological oxygen demand (BOD) [g/kg], chemical oxygen demand (COD) [g/kg], temperature [°C], volatile suspended solids (VSS), phosphorus [g/kg], conductivity [µs/cm], Cl^-, Br^-, NO_3^-, Na, K, Ca, Fe, Mg, Al, Si, Cu, Zn, relative abundance of hydrogenotrophic methanogens and aceticlastic methanogens. volatile fatty acids (VFA) and aromatic fermentation products (l-lactate, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, phenol, p-cresol, indole and skatole), methane/biogas production, mineralization [%], 50 and 90 % disappearance time (DT50, DT90), transformation products (TP), non-extractable residues (NER), mass balance/recovery [%], liquid-solid distribution.

4.13 Conclusions

The available studies on transformation of biocides and veterinary medicinal products in manure show large variations in the experimental set up and conditions such as temperature, redox potential, matrix effects, and physico-chemical properties.

Most frequently investigated VMPs are from the class of antibiotics, namely sulfonamides, tetracyclines, and macrolid antibiotics. In 10 out of the 34 studies, excrements or manure from medicated livestock was considered, all other studies are based on spiked manure. Most of the studies considered manure from pigs (25 out of 34), the remaining studies investigated manure from cattle or in one case used synthetic manure. Large differences are represented in terms of study duration and temperature, ranging from 2 to 374 days and 5 °C to 55 °C, respectively. Many studies suffer from insufficient control of basic parameters. Only six publications give information on the redox potential of the manure used for transformation. Further, the characterization of the matrix in many cases is inadequate due to missing parameters such as dry matter content, pH, and TOC. Information on dissipation rates or half-lives, transformation products, generation of methane and non-extractable residues (NER) are not available in the majority of the considered publications.

Overall, it can be stated that the majority of the studies describes at least one fundamental parameter of the experimental conditions poorly. Considering all the different approaches one can conclude that it is inevitable to give specific guidance for studies on transformation in manure in general and especially with regard to the applicability and acceptability of studies in regulatory contexts. Knowing that all the parameters - as they are studied particularly within single publications - do affect the basic outcome of a transformation study, it is necessary to standardize them or at least report them. Parameters as temperature, dry matter content, origin and preconditioning of the manure, pH and Eh do have massive effects on transformation rates or routes of VMPs and biocides in liquid manure. All these parameters are relatively easy to measure and should be monitored - or better standardized where possible- mandatorily. Another topic with knowledge gaps is related to the composition, the development or spread of resistance, or adaption and activity of the microbial community. For future studies this topic always needs to be addressed.

There are different tasks of academic interest within the research on monitoring data and transformation studies in manure, which are not directly related to the main question of how to standardize experiments on a laboratory scale to assess the environmental exposure by VMPs and biocides.

As an outcome of the summarized monitoring studies, a widespread distribution of VMPs in manure can be concluded. We found only one transformation study at real manure storage tanks. This is an important area for research to study transformation under realistic conditions. Comparing such realistic outdoor results with those produced on laboratory scale is important to validate the outcome of laboratory studies and to evaluate different experimental setups.

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