Chemosphere 312 (2023) 137210

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Validation of a method to determine transformation of chemicals in anaerobic liquid pig and cattle manure for the OECD test guideline programme

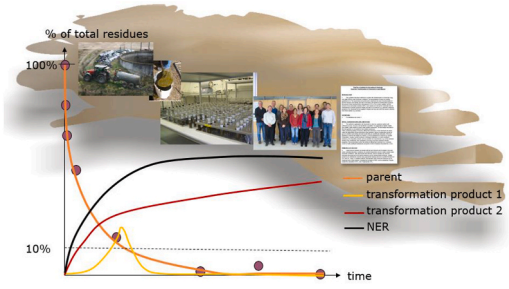
Silvia Berkner [a,*], Julia Margaretha Anke [a], Rolf-Alexander Düring [b], Silke Fiebig [d], Thomas Junker [e], Dieter Hennecke [c], Monika Herrchen [c], Maria Meinerling [f], Jörg Römbke [e], Sören Thiele-Bruhn [g], Edward Topp [h,i], Wolfgang Völkel [j], Susanne Walter-Rohde [a]

[a] German Environment Agency, Dessau-Rosslau, Germany
[b] Justus Liebig University, Institute of Soil Science and Soil Conservation, Giessen, Germany
[c] Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany
[d] Noack-Laboratorien GmbH, Sarstedt, Germany
[e] ECT Oekotoxikologie GmbH, Flörsheim, Germany
[f] Ibacon GmbH, Rossdorf, Germany
[g] University of Trier, Soil Science, Faculty VI, Trier, Germany
[h] Agriculture and Agri-Food Canada, London, ON, Canada
[i] Department of Biology, University of Western Ontario, London, ON, Canada
[j] Innovative Environmental Sciences (IES), Witterswil, Switzerland

HIGHLIGHTS

- A new OECD Test Guideline (TG 320 on) on anaerobic transformation of chemicals in liquid manure has been validated by an inter-laboratory ring test.
- This is the first report of the outcome of validation results for a simulation type OECD fate study design.
- a robust, reliable, repeatable and reproducible, standardized method is now available for the regulatory environmental risk assessment of veterinary pharmaceuticals, biocides and other chemicals.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
OECD Test guideline programme
Pharmaceuticals
Biocides
Transformation
Manure
Environmental risk assessment

ABSTRACT

Manure is widely used as a fertilizer and applied to agricultural land. It may contain highly active chemicals like veterinary medicinal products or biocides, which enter into the environment by this pathway. This is recognized by several regulatory frameworks, however, a detailed method for examining the transformation of chemicals in manure was lacking. This article describes the validation of a method for studying the anaerobic transformation of chemicals in pig and cattle liquid manure. Different steps are covered with an emphasis on the validation ring test and the OECD (Organisation for Economic Cooperation and Development) process that led to the recent adoption of the method as OECD Test Guideline (TG) 320.

* Corresponding author.
E-mail address: silvia.berkner@uba.de (S. Berkner).

https://doi.org/10.1016/j.chemosphere.2022.137210
Received 29 September 2022; Received in revised form 7 November 2022; Accepted 8 November 2022
Available online 8 November 2022

1. Introduction

Manure is used as a fertilizer and is widely applied to agricultural land. It is a recognized environmental concern that highly active chemicals (e.g. veterinary medicinal products (VMP) and biocides) with distinct modes of action enter the environment via manure (Wohde et al., 2016). Emissions of chemicals to manure (i.e. antibiotics, disinfectants) are also of interest for addressing the global challenge of antimicrobial resistance (AMR) within a one health approach that takes into account environmental spreading of AMR (Ghirardini et al., 2020). Therefore, information on the fate of chemicals in manure is crucial for the environmental risk and hazard assessments of chemicals.

These concerns are taken into consideration in regulatory frameworks. For instance, the internationally harmonized VICH (Veterinary International Conference on Harmonization) guidelines GL6 and GL38 (VICH, 2022) provide a common basis for the Environmental Impact Assessment (EIA) of VMPs in the European Union (EU), Japan, the United States, Canada and Australia/New Zealand. Both guidelines refer to transformation of VMP in manure as part of an EIA. The European Medicines Agency (EMA) adopted the VICH guidelines as CVMP/VICH/592/98-FINAL (EMEA, 2000) and CVMP/VICH/790/03-FINAL (EMEA, 2004). A further "Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH guidelines GL6 and GL38" (EMA, 2016) provides more detailed guidance for an environmental assessment, including transformation in manure. Furthermore, in 2011, EMA published the „Guideline on determining the fate of veterinary medicinal products in manure" (EMA, 2011). This guideline deals with regulatory guidance and defines some test conditions for studies on transformation in manure, however does not specify a test method or give experimental guidance on how to conduct such studies. There are also data requirements for biocides under the European Biocidal Products Regulation (EU, 2012) regarding transformation during manure storage for disinfectants used for veterinary hygiene (product type 3) and insecticides used in stables and manure storage systems (product type 18), also without specifying a test method.

To allow for a consistent assessment of studies within regulatory frameworks, a harmonized internationally accepted and validated test method was urgently needed, as there were no standardized methods available concerning the fate of chemicals in manure at the time. Consequently, a method to study transformation of chemicals in anaerobic liquid manure has been developed (Junker et al., 2020). In the present publication the validation of the developed method including the OECD process for international harmonization will be described.

2. Materials and methods

The OECD Guidance Document (GD) 34 on the validation and international acceptance of new or updated test methods for hazard assessment (OECD, 2005) describes specific requirements for method development and validation for the OECD TG Programme (TGP). A stepwise approach was followed and the draft test guideline was continuously developed, amended and improved throughout the whole process (Fig. 1).

The regulatory need as a prerequisite for starting the resource intensive work is outlined in the introduction. The first steps were to define the scope of the test method. The focus was set on pig and cattle anaerobic liquid manure (Weinfurtner, 2011, Wohde et al., 2016),

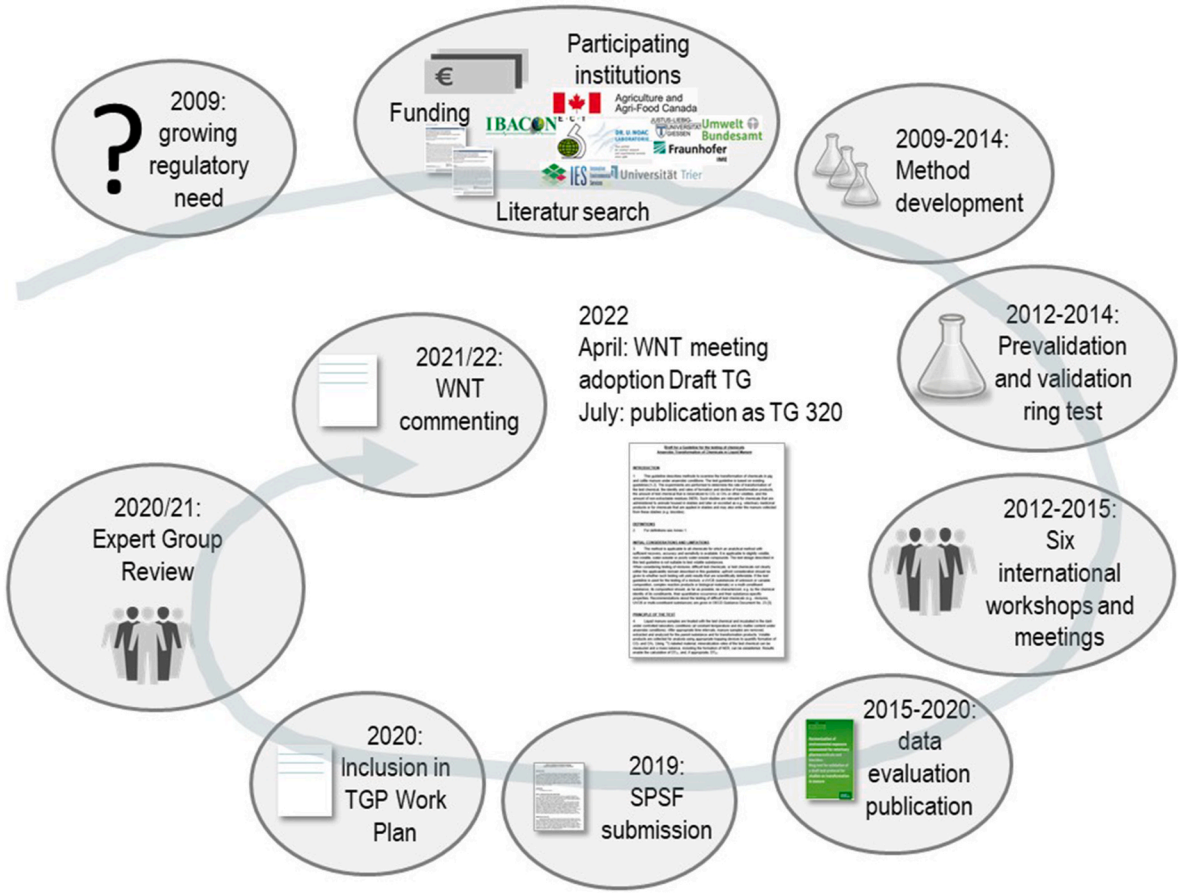


Fig. 1. The process overview details different steps in the development, validation and OECD approval for a new test guideline on transformation in aerobic liquid manure; SPSF: Standard Project Submission Form; TGP: Test Guidelines Programme; WNT: OECD Working Party of the National Coordinators for the Test Guidelines Programme.

relevant matrix parameters were identified based on existing guidance (EMA, 2011). Methods for reproducible sampling, homogenization and acclimation of manure were developed and test-setups to measure the transformation of a test chemical in manure were explored and used in a pre-validation ring test in 2012–2013 (Junker et al., 2020). Based on discussions with ring test participants and other experts from industry, regulatory agencies and academia, the developed draft test method was continuously adapted and used as basis for a subsequent validation ring test that was conducted in 2013–2014.

2.1. Substance selection

Test substances were selected according to the following criteria: They should be of regulatory relevance, i.e. applications for the compounds in question should have been received by regulatory agencies. They should be widely used in important fields of application (i.e. veterinary medicine and biocides). The substances had to be available ^{14}C-radiolabeled with an acceptable label position. Hydrolyzing substances were excluded as well as highly persistent compounds (quantitative half-lives should be derivable from a 90 d-experiment). Suitable analytical methods had to be available. As practical considerations limited the maximal number of test substances for the ring test to two test substances, these were chosen to represent different behavior as follows: Widely varying transformation rates with one compound dissipating rapidly with a DT_{50} (time for half of the amount of parent compound to disappear) of less than 1 day for which sampling has to be done at frequent intervals to establish the kinetics, and a second substance with slower dissipation, but with kinetics such that a DT_{50} value with a maximal study duration of 90 d could be derived.

Accordingly, for method development, salicylic acid, paracetamol and biocide B (anonymized as the ^{14}C-labelled substance was a donation from a company conditionally on publishing data only anonymously) were chosen as test substances. For the validation ring test florfenicol and imidacloprid were used as test substances (respectively, as a mixture of ^{14}C-labelled and unlabeled compound). Table 1 gives an overview of characteristics for the test substances.

2.2. Matrices and test conditions

Liquid manure collected in pits and tanks has been found to be the relevant type for pig and cattle manure in Europe and North America (Weinfurtner, 2011). Therefore, the test conditions were chosen to be representative of conditions in manure storage tanks, namely anaerobic and with a rather low dry matter content (5% for pigs, 10% for cattle). Details on determining representative conditions as well as sampling, storage, acclimation and test setup are described in Junker et al. (2020) and in the recently published OECD TG 320 (OECD, 2022) and can also be found in the supporting information.

2.3. Endpoints

The proposed test protocol fits into the category of simulation type studies, i.e. studies on transformation in environmental compartments, or compartments of environmental relevance, as is the case for manure. The primary aim for such studies is to measure test substance concentration over time in the relevant matrix under defined test conditions. This is in order to derive kinetic parameters, expressed as the time by which half of the test substance has disappeared, the DT_{50}, for use in exposure assessment. This is the relevant endpoint used for validation assessments in the following. Other information derived from a simulation type study is the pathway of transformation including the identity and amount of transformation products (TP), gaseous products such as CO_2, CH_4 and other volatile TP such as volatile fatty acids, and so-called non-extractable residues (NER), which are operationally defined and may consist of parent compound and different types of further transformed products (Loeffler et al., 2020). It was not within the scope of the project to differentiate between different types of NER. NER were recorded in accordance with current procedures in other simulation type studies (e.g. transformation in soil, OECD TG 307, OECD, 2002a) as equivalent to radioactivity associated with the matrix after extraction of the manure sample. As radiolabeled test substances were used it was possible to establish a mass balance.

2.4. Repeatability

Repeatability is defined in OECD GD 34 as “the agreement among test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions”. Intra-laboratory repeatability was studied by conducting experiments with multiple replicates using the same manure at the same time in the same laboratory. For three test substances (salicylic acid, paracetamol and biocide B) tests were run in six replicates for three to six different cattle manures and three different pig manures. Repeatability was assessed by calculating the (geometric) mean DT_{50} value for all replicates, as well as standard deviation (SD) and coefficient of variation (COV) as a scale-independent measure of variability for each different manure and each test substance for a COV-analysis (OECD, 2005).

2.5. Reproducibility

Reproducibility is defined in OECD GD 34 as “the agreement among results obtained from testing the same substance using the same test protocol (see reliability)”. Reliability is then defined as “measures of the extent that a test method can be performed reproducibly within and

Table 1
Test substances for method development and validation studies; florfenicol and imidacloprid were used as test substances for the validation ring test; information is given for the respective ^{14}C-radiolabeled compound; n.a.: no information available.

Name	Salicylic acid	Paracetamol/Acetaminophen	Biocide B	Florfenicol	Imidacloprid
CAS-Number	69-72-2	103-90-2	n.a.	73231-34-2	138261-41-3
Chemical class	Aromatic acid	Acetic acid derivative, aminophenol	Neonicotinoid	Halogenated aromatic sulfone	Neonicotinoid
Product class	Chemical, natural compound, pharmaceutical active ingredient	Human and veterinary pharmaceutical active ingredient	Biocide	Veterinary pharmaceutical active ingredient	Biocide
Molecular formula	$C_7H_6O_3$	$C_8H_9NO_2$	n.a.	$C_{12}H_{14}Cl_2FNO_4S$	$C_9H_{10}ClN_5O_2$
Molecular weight [g/mol]	138.1	n.a.	n.a.	360.2	257.5
Lot-/Batch-No.	IO1112, ARC 0287	153-064-077-A-20080611-DR	n.a.	CFQ41813	CFQ41814
Radiochemical Purity [%]	99.0	99.2	>99.0	98.2	98.8
Appearance	white crystalline powder	crystalline solid	n.a.	solid	solid
Specific activity [MBq/mg]	4–5	18.8	4.44	6.4	8.3
Test concentration (labelled + unlabeled) [mg/kg manure fresh weight]	24	24	1.0	3.0	1.0

between laboratories over time, when performed using the same protocol. It is assessed by calculating intra- and inter-laboratory reproducibility and intra-laboratory repeatability."

Intra-laboratory reproducibility was assessed by comparing results from experiments conducted at two different timepoints one year apart with the same substance in the same laboratory. It should be kept in mind that the matrices in simulation type studies are sampled for each individual test and cannot be stored over a longer time period to repeat the test with the same batch of matrix later. Therefore, the parameter intra-laboratory reproducibility cannot be separated completely from variability introduced by different matrices. Comparative studies were conducted for the test compound salicylic acid in cattle and pig manure in the same laboratory with manure sampled at the same farm one year apart. The assessment was based on geometric mean values of three replicates (if only one manure was sampled per species (timepoint 2)) or on geometric mean values of means of six replicates for different manures (timepoint 1, six cattle manures, three pig manures).

Inter-laboratory reproducibility is an important parameter to assess the readiness of a protocol for application for regulatory purposes and was assessed based on a validation ring test (OECD, 2005). Such exercises consider all sources of variability encountered under real world conditions, when each laboratory uses only the test guideline as method description and different matrices, different equipment and different analytical methods. In the validation ring test six laboratories from the EU and Canada took part. Five participants used a mixture of ^{14}C-labelled and unlabeled test substance (i.e. florfenicol (5% w:w labelled) and imidacloprid (12% w:w labelled); for details see Table 1) and conducted incubations at 20 ± 2 °C. One laboratory performed experiments with florfenicol (unlabeled as well as mixture of labelled/unlabeled) at 10 ± 2 °C. Mean DT_{50}-values for florfenicol in pig manure at 10 °C were normalized to 20 °C for comparison with the results from the other institutes using the Arrhenius equation as recommended by EMA (2011). Laboratories received the test protocol consisting of the draft test guideline and information on the supplied test compounds. Test compounds were not distributed blind as stipulated in OECD GD 34, as this is not feasible for substances for which substance specific extraction and analytical methods have to be used. Protocols for extraction methods and analytical methods were also supplied (see SI1). The manures had to be sampled individually by each participant. Florfenicol was to be tested in pig manure and imidacloprid in cattle manure. Spreadsheets were sent to participants to document the experiments (manure sampling, matrix parameter determination, measured concentrations, see SI2). Kinetic evaluations were carried out by Fraunhofer Institute for Molecular Biology and Applied Ecology IME for all participants using the software KinGUI (version 1.1) or CAKE (version 3.1) to derive DT_{50} values from the concentration times series for interlaboratory comparison and COV-analysis (OECD, 2005). Single First Order (SFO) kinetics were most suitable for fitting and used for all experiments. The (geometric) mean of all replicates by one participant were calculated, as well as SD and COV. This procedure was then carried out for the mean values obtained for each participant to obtain the overall inter-laboratory reproducibility as COV in % obtained by dividing the mean by the SD.

2.6. OECD process

Based on the developed method and the results of the validation ring test, the draft method was submitted to the OECD Test Guideline Program (TGP) in 2019. A SPSF (Standard Project Submission Form) was prepared and revised in commenting rounds. A regulatory need was recognized and the proposal was included at the 2020 WNT (Working Party of the National Coordinators for the TGP) meeting into the OECD TGP Work Plan as project 3.18. The next step consisted of a series of OECD Expert Group meetings, comprising 19 experts from 11 different countries and organizations. The Expert Group reviewed all available information on the validation status and the potential availability and use of further information, and additional data from studies used in regulatory contexts was examined. A systematic search was conducted in the publicly available European Public Assessment Reports (EPAR) at the EMA website for reported results from studies on transformation in manure for the substances. For only one substance publicly available results could be identified and subsequently used for validation purposes, namely information from a referral procedure for eprinomectin (EMA, 2018). A series of specific questions was addressed in the Expert Group meetings, including validation and other detailed discussions on the topics reference substance, NER, number of manures to be tested and the mass balance as validity criterion. All issues could be resolved successfully and the draft test guideline was revised after each Expert Group meeting and commenting round, accordingly.

The revised draft test guideline was then sent out for commenting via the WNT. The comments received were addressed and the draft TG was further revised and adopted at the OECD WNT meeting in April 2022 and published in July 2022 as OECD TG 320 (OECD, 2022).

3. Results and discussion

3.1. Validation

3.1.1. Repeatability

Repeatability was determined by calculating the variability in between different replicates for tests conducted at the same laboratory, with the same manure, at the same time. Table 2 summarizes the outcome of experiments on repeatability (see Table 3).

Observed COV values range from 3% to 46%. More than 90% of COV values are below 25%, 75% are below 15%, the mean is 13%. The observed mean COV of 13% for six experimental replicates in 21 different experiments with up to nine different pig and cattle manures chosen to represent the most diverse manure origin possible (Junker et al., 2020) with three test chemicals indicates good repeatability of the test method.

3.1.2. Reproducibility

Intra-laboratory reproducibility was assessed by comparing the range of results from experiments with salicylic acid in the same laboratory at timepoint 1 and timepoint 2 (timepoint 1 plus 1 year).

The respective species-specific DT_{50}-values at timepoint 2 fell within the range observed at timepoint 1 (Table 3). This type of comparison is difficult for simulation type fate studies, as it is not possible to test the same matrix at two different time points, as it cannot be preserved without undergoing changes. Therefore, this comparison includes sources of variability outside of the intended scope. Nevertheless, this evaluation concluded, that comparable results are obtained in the same laboratory over time.

3.1.3. Inter-laboratory reproducibility

Results from the validation ring test are presented in Tables 4 and 5. Two participating laboratories' results had to be excluded, as one laboratory was not able to conduct substance specific analysis due to capacity limitations and another laboratory used a very unpolar extraction solvent for the polar test substance florfenicol, which precluded comparability of results. One laboratory took part only for florfenicol and not imidacloprid. Therefore, for the inter-laboratory comparison results from in total four participants could be evaluated for each manure and each test substance, respectively. Two of the laboratories used six replicates, whereas the remaining laboratories used 2–3 replicates, 2 replicates being the minimum requirement from the draft guideline.

For florfenicol in pig manure the range observed for mean DT_{50}-values (20 ± 2 °C) were between 0.1 days and 0.5 days. For imidacloprid in cattle manure mean DT_{50}-values (20 ± 2 °C) were in the range of 17.4 days–40.1 days. Inter-laboratory reproducibility was assessed by the overall COV calculated from the means of the replicates for each

Table 2
DT_{50} (d) given as mean, SD (d) and COV (%) for six replicates each, performed in the same laboratory with different manures (NW, BY: sampled in North Rhine-Westphalia or Bavaria; c: cattle manure, p: pig manure, s: manure sampled in summer, other manures sampled during winter).

test chemical manure	salicylic acid			paracetamol			biocide B		
	mean	SD	COV	mean	SD	COV	mean	SD	COV
NW1c	26.5	1.1	4	9.4	1.1	12	13.0	0.9	7
NW2c	15.8	2.5	16	16.3	1.4	9	16.5	0.5	3
BY1c	3.6	0.1	3	10.6	1.3	12	11.3	1.0	9
NW1cs	22.3	10.3	46	not determined for summer manure					
NW2cs	27.9	8.4	30						
BY1cs	19.3	4.1	21						
NW1p	7.2	0.8	11	7.2	1.6	22	31.5	2.5	8
NW2p	5.6	0.5	9	6.2	0.5	8	20.9	1.2	6
BY2p	3.9	0.3	8	4.8	0.5	10	13.0	2.5	19

Table 3
Range of DT_{50} values (d) derived in the same laboratory at two different time-points with manure sampled from the same manure tank one year apart.

species	cattle	pig
timepoint 1 (6 manures cattle, 3 manures pig, mean values)	3.6–27.9	3.9–7.2
timepoint 2 (1 manure each, replicate values)	24.1–27.6	6.1–6.6

Table 4
DT_{50} (d) obtained in the ring test for florfenicol; single DT_{50} values are reported with three decimal places as derived from kinetic modeling (for participant 6 measured at 10 °C and converted to 20 °C). Detailed results can be found in the supporting information. nd: not determined.

ring test participant replicate	1	2	3	6
1	0.430	0.332	0.185	0.492
2	0.394	0.490	0.155	0.589
3	0.296	0.329	nd	nd
4	0.373	0.357	nd	nd
5	0.428	0.344	nd	nd
6	0.558	0.353	nd	nd
mean (d)	0.4	0.4	0.2	0.5
SD (d)	0.1	0.1	0.02	0.1
COV (%)	21.3	16.8	12.5	12.7
mean all (d)	0.3			
SD all (d)	0.2			
COV all (%)	45			

Table 5
Results on DT_{50} (d) at 20 ± 2 °C for imidacloprid; single DT_{50} values are reported with three decimal places as derived from kinetic modeling. Detailed results can be found in the supporting information. nd: not determined.

ring test participant replicate	1	2	3	4
1	21.980	17.034	21.588	43.038
2	21.580	17.020	21.000	37.158
3	21.160	17.506	nd	40.122
4	21.530	16.823	nd	nd
5	22.180	18.235	nd	nd
6	21.750	17.858	nd	nd
mean (d)	21.7	17.4	21.3	40.1
SD (d)	0.4	0.6	0.4	2.9
COV (%)	1.7	3.2	2.0	7.3
mean all (d)	23.8			
SD all (d)	10.1			
COV all (%)	43			

participant. For florfenicol in pig manure inter-laboratory reproducibility was found to be 45% and 43% for imidacloprid in cattle manure.

Values for comparison are not available, as for other simulation type OECD test guidelines no information on their validation is publicly available. For the most recent addition to the OECD test guideline program for simulation type fate tests, OECD TG 314, ring testing is not reported. Therefore, a literature search was conducted to try to identify suitable data. Information on inter-laboratory reproducibility was not available. To be able to put the obtained values into context, variabilities observed across environmental fate simulation type studies were used. Results were available for OECD TG 307 studies on transformation in soil from studies with plant protection products. A mean COV of 100% is reported by FOCUS (2000). A substance specific comparison was possible for eprinomectin, for which a COV of 109% was derived from values reported for four different soils and a COV of 59% derived from five different manures (EMA, 2018). Considering this additional information as a background for the ring test results, which used different manures and were conducted in different laboratories, it was concluded that inter-laboratory reproducibility is fulfilled by the test method.

3.2. *Further discussion points in workshops, commenting rounds and the OECD Expert Group*

3.2.1. *Reference substance*

Inclusion and purpose of a reference substance was extensively discussed. The term reference substance is understood to have different implications depending on the context. For screening studies like the OECD TG 301 test series on ready biodegradability, a reference substance is mandatory and used to determine validity of the test. For more complex simulation type studies, which use substance specific endpoints, reference substances are not used, e.g. OECD TG 307 and 308 on transformation in soil and water/sediment-systems (OECD, 2002a; OECD, 2002b), or they are included in the method but are not required for validity or any other outcome, as in OECD TG 309 on mineralization in surface water (OECD, 2004). The results from one reference substance would not yield any information on how a different compound requiring different biotransformation enzymes may behave in a test. The reason information on a reference substance was included is that the method is new to many laboratories and requires anaerobic conditions and thus different test setups from what many laboratories routinely running studies for regulatory purposes are used to. Therefore, the reference substance is meant to facilitate establishing the method at a laboratory and provides a means for testing the setup before starting the time-consuming definitive test. For that reason, salicylic acid was chosen as a reference substance that is easily obtainable at low cost and does show transformation in a suitable timeframe without needing additional specialized analytical techniques.

3.2.2. *Non-extractable residues (NER)*

As the topic on NER has advanced during the development and validation process, with different options being considered to be included into REACH guidance (Kästner et al., 2014; Loeffler et al., 2020), the topic was also addressed by the OECD Expert Group. It was noted that the topic is currently much discussed and it is important to consider, that all experimental information and development of methods for characterization of NER has been conducted for soil and no

information is available for the matrix under consideration, namely manure. There was consensus that the topic of the fate of compounds in the NER-fraction is certainly important especially for manure, as the manure matrix itself consists to a very high degree of organic matter and itself biodegrades after application to agricultural land. Therefore, any parent compound or TP in the NER are highly likely to be released to the environment. In the OECD Expert Group, it was agreed that manure has to be seen as a transitional compartment and the NER may consist of or contain parent compound (or TP). As different regulatory frameworks around the world have different approaches in dealing with NER (e.g. FDA-CVM considering NER as equivalent to mineralized, while EMA considers NER to be equivalent to parent compound (EMA; 2011), and this issue is under dynamic development, the topic was addressed in a way in the TG that is sufficiently flexible to guarantee applicability of the TG for all frameworks. A point that was agreed on is the importance of suitable and thorough extraction procedures as part of the analytical methods in the TG, also with regard to NER. Consequently, the TG states that exhaustive extraction methods like pressurized liquid extraction (PLE) should be used with appropriate solvents, as recommended for characterization of NER (Loeffler et al., 2020).

3.2.3. Number of manures

It was noted that in other simulation type studies in soil and water-sediment systems different test systems (e.g. four aerobic soils and two aerobic and anerobic water/sediment systems; OECD, 2002a; OECD, 2002b)) are used. For manure, during method development matrix parameters and study outcomes had been shown to differ between manures from different species, i.e. pigs and cattle (Junker et al., 2020). Within the same species, matrix parameters from method development and validation showed a narrow range, even when manures were chosen to be as diverse as possible (see Table 6). Additionally, by standardizing dry matter content as the TG requires, variability is further reduced.

Therefore, it was concluded that manure from each different species has to be tested. Within one species, the TG requires at least one manure to be tested based on low variability in matrix parameters for manure of the same species.

3.2.4. Mass balance and recovery as validity criteria

It was unanimously decided, that it is important to specify a range for acceptable values for mass balance and recovery. Other simulation type studies like OECD TG 307 and TG 308 (OECD, 2002a; OECD, 2002b) include mass balance (90%–110%) and recovery (70%–110%) as a criterion for considering a test to be valid. Evaluation of the mass balance values obtained in the ring test however showed that only 60%–86% of all values conformed to the 90%–110% range, whereas 88%–93% of all mass balance values met the range of 85%–115% (Junker et al., 2020). Therefore, it was proposed to use the range of 85%–115% as validity criterion, to avoid discarding a relatively high percentage of studies, which are resource-, time-, and cost-intensive. Discussions in the end led to a more detailed criterion for mass balance. Directly after dosing at timepoint zero, the mass balance has to be in the range of 90%–110%. For the following timepoints, meeting the range of 85%–115% was considered to be acceptable and it was included into the TG that all values given in % of applied test substance have to be normalized to the initially dosed % test substance.

Table 6
Manure matrix parameters measured for manures sampled during method development and validation (ring test); values are reported as mean value and range.

species	matrix parameter	mean ± range	reference
cattle	dry matter content	9.95 ± 2.2%	Junker et al. (2020)
	organic carbon content	4.1% ± 0.9%	Junker et al. (2020)
	pH	7.1 ± 0.6	Hennecke, 2015
pig	dry matter content	5.2% ± 3.2%	Junker et al. (2020)
	organic carbon content	2.1% ± 1.3%	Junker et al. (2020)
	pH	7.3 ± 0.1	Hennecke, 2015

4. Conclusion and outlook

Following OECD GD 34, it could be demonstrated that the developed test method for anaerobic transformation of chemicals in liquid manure is reliable, repeatable and reproducible. The inclusion of different manures and different test setups demonstrates the test method to be robust. OECD GD 34 gives valuable guidance for method development and validation for test guidelines. It should be noted that some points mentioned in OECD GD 34 are only applicable for effect testing.

The approach to learn from actually submitted information from marketing authorization applications and use information from studies for method development and standardization is a valuable path to follow, however this is rendered difficult by problems in data availability and transparency (Oelkers and Floeter, 2019). A search for results from marketing authorization study results that should be reported by EMA in the respective EPAR on their website was unsuccessful for all of the active ingredients where studies on transformation in manure were known to be available. As the information is not published, it was not possible to use this information for validation. One exception are the results from a referral procedure on eprinomectin. These results were important for the validation and acceptance of the method in Expert Group discussions. This highlights the need for more transparency concerning reports on regulatory studies also for scientific purposes.

A step wise approach was very helpful in developing and improving the draft for the TG over time. Discussing the draft in different groups, for example. SETAC Special Interest Groups, project meetings, workshops for ring test participants, and the OECD Expert Group helped to get a multifaceted feedback from different perspectives. As OECD GD 34 mentions, practicability was considered to be an important criterion for TG development. The TG was drafted considering what would be helpful information and guidance for laboratories conducting the studies and regulatory scientists using the studies for assessment purposes. This led to the inclusion of detailed experimental information, consideration of multiple possible test set-ups and a reporting template. We trust that the work on which the newly adopted TG was build, will be helpful for further development of environmental fate testing and that OECD TG 320 will further contribute to assuring a harmonized environmental impact assessment for chemicals.

Credit authors statement

Silvia Berkner: Conceptualization; Data curation; Funding acquisition; Project administration; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing. Julia Margaretha Anke: Validation; Visualization; Writing - review & editing. Rolf-Alexander Düring: Funding acquisition; Investigation; Methodology; Supervision, Writing - review & editing. Silke Fiebig: Data curation; Investigation; Methodology; Validation. Thomas Junker: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Supervision; Validation; Writing - review & editing. Dieter Hennecke: Conceptualization; Funding acquisition; Investigation; Methodology; Supervision, Writing - review & editing. Monika Herrchen: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Supervision; Validation. Maria Meinerling: Investigation; Methodology. Jörg Römbke: Funding acquisition; Supervision. Sören Thiele-Bruhn: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Supervision; Writing - review & editing. Edward Topp: Investigation; Methodology; Validation, Writing - review & editing. Wolfgang Völkel: Investigation; Methodology. Susanne Walter-Rohde: Validation; Writing - review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: S. F., T.J., D.H., M.M., J.R., W.V. are employed by institutes or companies conducting commercial environmental fate studies including studies on transformation in manure.

Data availability

Data are supplied in supplementary information.

Acknowledgements

Funding for experimental work was provided by the German Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection (grant no. 3710 67 422 and 3712 65 420). We would like to thank Stefanie Jäger and Sabine Konradi for their contributions to project management and René Lehmann for help with data evaluation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.137210.

References

EMA, 2000. VICH GL6 Environmental Impact Assessment (EIAS) for Veterinary Medicinal Products - Phase I. CVMP/VICH/790/03-FINAL.

EMA, 2004. VICH GL38 Environmental Impact Assessments for Veterinary Medicinal Products - Phase II. CVMP/VICH/790/03-FINAL.

EMA, 2011. Guideline on Determining the Fate of Veterinary Medicinal Products in Manure". EMA/CVMP/ERA/430327/2009.

EMA, 2016. Revised Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in Support of the VICH Guidelines GL6 and GL38. EMEA/CVMP/ERA/418282/2005-Rev.1-Corr.

EMA, 2018. Committee for medicinal products for veterinary use (CVMP) - final CVMP assessment report for LONGRANGE (EMEA/V/C/004291/0000). Available at: https://www.ema.europa.eu/documents/assessment-report/longrange-epar-refusal-public-assessment-report_en.pdf.

EU, 2012. Biocidal Products Regulation. Regulation (EU) 528/2012.

FOCUS, 2000. FOCUS groundwater scenarios in the EU review of active substances - the report of the work of the Groundwater Scenarios Workgroup of FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe). Version 1 of November 2000. EC Document Reference Sanco/321/2000 rev.2, 202pp. Available at: https://esdac.jrc.ec.europa.eu/projects/ground-water.

Ghirardini, A., Grillini, V., Verlicchi, P., 2020. A review of the occurrence of selected micropollutants and microorganisms in different raw and treated manure - environmental risk due to antibiotics after application to soil. Sci. Total Environ. 707 (10), 136118.

Hennecke, et al., 2015. Development of a test protocol to study the transformation of veterinary pharmaceuticals and biocides in liquid manure. UBA-Texte 78/2015. 148 pages. Umweltbundesamt, Dessau-Roßlau, Germany. Available from: https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_78_2015_development_of_a_test_protocol_to_study_the_transformation.pdf.

Junker, T., Atorf, C., Berkner, S., Düring, R.-A., Hennecke, D., Herrchen, M., Konradi, S., Merrettig-Bruns, U., Römbke, J., Wagner, J., Weinfurtner, K., 2020. Development of a test method for transformation of veterinary pharmaceuticals and biocides in anaerobic liquid manure. Environ. Sci. Eur. 32, 39.

Kästner, M., Nowak, Karolina M., Miltner, Anja, Trapp, Stefan, Schäffer, Andreas, 2014. Classification and modelling of nonextractable residue (NER) formation of xenobiotics in soil – a synthesis. Crit. Rev. Environ. Sci. Technol. 44 (19), 2107–2171.

Loeffler, D., Hatz, A., Albrecht, D., Fligg, M., Hogeback, J., Ternes, T.A., 2020. Determination of non-extractable residues in soils: towards a standardised approach. Environ. Pollut. 259, 113826.

OECD, 2002a. Test No. 307: Aerobic and Anaerobic Transformation in Soil, OECD Guidelines for the Testing of Chemicals, Section, vol. 3. OECD Publishing, Paris. https://doi.org/10.1787/9789264070509-en.

OECD, 2002b. Test No. 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, OECD Guidelines for the Testing of Chemicals, Section, vol. 3. OECD Publishing, Paris. https://doi.org/10.1787/9789264070523-en.

OECD, 2004. In: Test No. 309: Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test. OECD, Paris, France. https://doi.org/10.1787/9789264070547-en.

OECD, 2005. ENV/JM/MONO(2005). Guidance Document 34 on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment, vol. 14. https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2005)14&doclanguage=en.

OECD, 2022. Test No. 320. In: Determining Anaerobic Transformation of Chemicals in Liquid Manure, OECD Guidelines for the Testing of Chemicals, Section, vol. 3. OECD Publishing, Paris. https://doi.org/10.1787/62f5be61-en.

Oelkers, K., Floeter, C., 2019. The Accessibility of Data on Environmental Risk Assessment of Pharmaceuticals: Is the Marketing Authorisation Procedure in Conflict with the International Right of Access to Environmental Information?, vol. 31 Environmental Sciences, Europe, p. 1.

VICH, 2022. https://www.vichsec.org/guidelines/pharmaceuticals/pharma-safety/environmental-safety.html.

Weinfurtner, 2011. Matrix parameters and storage conditions of manure. UBA-Texte 02/2011. 54 pages. Umweltbundesamt. Dessau-Roßlau, Germany. Available from: https://www.umweltbundesamt.de/publikationen/matrix-parameters-storage-conditions-of-manure.

Wohde, et al., 2016. Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. Environ. Sci. Eur. 28, 23.