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Concept development for an extended plant test in the environmental risk assessment of veterinary medicinal products

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Concept development for an extended plant test in the environmental risk assessment of veterinary medicinal products

by

Dr. Markus Simon, Dr. Monika Herrchen
Fraunhofer-Institute for Molecular Biology and Applied Ecology ,
Schmallenberg, Germany

Nadin Graf , Dr. Bernhard Förster, Dr. Jörg Römbke
ECT Oekotoxikologie GmbH, Flörsheim, Germany

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Umweltbundesamt
Wörlitzer Platz 1
06844 Dessau-Roßlau
Tel: +49 340-2103-0
Fax: +49 340-2103-2285
info@umweltbundesamt.de
Internet: www.umweltbundesamt.de

/umweltbundesamt.de
/umweltbundesamt

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57392 Schmallenberg

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Abstract

In the frame of this research project, an extended terrestrial plant test for veterinary medicinal products – especially antibiotics – with a more realistic exposure scenario via manure application was developed. This research project comprehends: i) development of methods of preparation, acclimation, incubation, and application of manure in a plant test, ii) tests investigating necessary technical background (e.g. suitable plant species, suitable manure concentration), iii) tests according to the OECD 208 standard test design and modified test designs, considering an application of the test substance via manure with and without incubation.

Main tests were conducted with six plant species, pig and cattle manure, and two representative veterinary antibiotics (florfenicol and tylosin tartrate). The test design considered additional effects of manure to the test substance (e.g. adsorption) as well as transformation/metabolization of the test substance in manure and formation of NER.

This report describes the results of the tests which were carried out by Fraunhofer IME and ECT Oekotoxikologie GmbH and a manual for an extended plant test with an exposure scenario via manure application after an anaerobic incubation period for the use in environmental risk assessment of veterinary medicinal products is presented.

Kurzbeschreibung

Im Rahmen dieses Projektes wurde ein weiterführender Pflanzentest für Tierarzneimittel – insbesondere für Antibiotika – mit einem realistischeren Expositionsszenario über Gülleapplikation entwickelt. Das Forschungsvorhaben beinhaltet: i) Entwicklung von Methoden zur Aufarbeitung, Akklimatisierung, Inkubation und Applikation von Gülle in einem Pflanzentest, ii) Tests zur Klärung notwendiger technischer Hintergründe (z.B. geeignete Pflanzenarten, geeignete Güllemenge), iii) Tests nach dem OECD 208 Standardtestdesign und nach modifizierten Designs, die eine Testsubstanzapplikation über Gülle – mit oder ohne Inkubation – berücksichtigen.

Die Haupttests wurden mit sechs Pflanzenarten, Schweine- und Rindergülle und zwei repräsentativen Veterinärantibiotika (Florfenicol und Tylosintartrat) durchgeführt. Das Testdesign berücksichtigte zusätzliche Effekte der Gülle auf die Testsubstanz (z.B. Adsorption) wie auch Transformation/Metabolisierung der Testsubstanz in der Gülle und Bildung von NER.

Dieser Bericht beschreibt die Ergebnisse der am Fraunhofer IME und bei ECT Oekotoxikologie GmbH durchgeführten Tests und stellt einen Leitfaden für einen weiterführenden Pflanzentest mit einem Expositionsszenario über Gülleapplikation nach anaerober Inkubation für die Verwendung in der Umweltrisikobewertung von Tierarzneitmitteln vor.

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List of Abbreviations

aR	Applied radioactivity
CAS #	CAS registry number
CH_4	Methane
CO2	Carbon dioxide
CL	Confidence limits
CV	Coefficient of variance
CVMP	Committee for Medicinal Products for Veterinary Use
D	Day
DM	Dry mass
Fw	Fresh weight
ECx	Effect concentration where x percent effect occurs
e.g.	For example
EMA	European Medicines Agency
ERA	Environmental Risk Assessment
FM	Fresh mass
GL	Guideline
HPLC	High performance liquid chromatography
kBq	Kilo Becquerel; Becquerel = is the SI-derived unit of radioactivity. One Bq is defined as the activity of a quantity of radioactive material in which one nucleus decays per second.
L: D	Light: Dark ratio
LSC	Liquid scintillation counting
LOD	Limit of detection
LOQ	Limit of quantification
MeOH	Methanol
NER	Non-extractable residue
NOEC	No effect concentration
n.s.s.	No seedlings survived
OECD	Organisation for Economic Co-operation and Development
Rf	Retention factor or retardation factor (R) is defined as the fraction of an analyte in the mobile phase of a chromatographic system
TFA	Trifluoro acetic acid
TI	Test item

TLC	Thin layer chromatography
TP	Transformation product
UV/VIS	Ultra violet / visible
VICH	VICH is a trilateral (EU-Japan-USA) programme aimed at harmonising technical requirements for veterinary product registration
Z.B.	Zum Beispiel

1 Summary

Effects on the environment caused by veterinary medicinal products are assessed according to the guidelines CVMP/VICH/592/98-FINAL [1] and VICH GL 38 (ECOTOXICITY PHASE II) [3] of the EMA and VICH. In Phase II of the environmental risk assessment effects on terrestrial plants are tested since residues of active substances can reach agricultural areas because manure and slurry from treated animals are used as fertilizer. Effects on terrestrial plants are examined according to the OECD guideline 208 “Seedling Emergence and Seedling Growth Test” [7].

The current risk assessment uses the initial predicted environmental concentrations. Degradation of active substances in manure is not considered. However, this approach often results in an unacceptable risk to terrestrial plants. One possibility to refine the risk is to conduct a modified approach considering a more realistic application form (manure enriched soil).

So far no harmonized concept exists for performance and assessment of such modified test design. A guidance paper for i) the kind of application via dung and/or manure in the plant test itself and ii) the treatment of the “carrier” manure, e.g. duration of pre-incubation (storage of the manure and application of the test substance before use in the plant test) and characterization of the matrix manure (e.g. according to EMA/CVMP/ERA/430327/2009 [4]), as well as a possible adaption of the test design (e.g. increase of number of replicates, identification and exclusion of unsuitable plant species) has to be defined.

On the European scale, since March 2011 a guideline of the European Medicines Agency (EMA) EMA/CVMP/ERA/430327/2009: Guideline on determining the fate of veterinary products in manure (adopted 10.03.2011) [4] exists for veterinary medicinal products. However, the EMA guideline does not include experimental specifications and details. It only describes some experimental parameters and mainly considers the interpretation of the results of studies on transformation in manure.

Based on the need for a standardized test design to test the effect of veterinary medicinal products on plants in a more realistic way, the following objectives of this research project arise:

- Development of a test design for a modified OECD 208 seedling emergence and growth plant test with a more realistic exposure in manured soil;
- Experimental verification of the practicability of the test design by means of two veterinary antibiotics and two kinds of manures (pig and cattle) in tests with six plant species;
- Preparation of a manual for the performance, evaluation and reporting of extended plant tests with an exposure scenario via manure application for the use in environmental risk assessment of veterinary medicinal products;

The realization was divided in three sections. The first section comprised the theoretical preparation of the project, the second section comprised the experimental part, and the third section comprised the preparation of a preliminary manual based on the experimental results, its discussion within an international workshop, and the publication of a finalized manual.

In the scope of a kick-off meeting in December 2011, the founder figured out the regulatory background for the research project. In extended plant tests submitted by applicants so far

problems regarding i) growth inhibition due to manure, ii) increase of coefficient of variation due to manure, and iii) partly lack of clear dose-response relationship occurred. Additionally, the lack of a higher tier approach for Phase II, Tier B was identified. The actual research project should have an important input in preparation of a manual for plant tests with an adapted test design concerning test substance application via manure to close this gap.

For this, within the project the following issues should be investigated:

- Development of methods for preparation, acclimatization, incubation, and application of manure in a plant test;
- Necessary technical background (e.g. suitable plant species, suitable manure concentration);
- Comparison of Tests according to the OECD 208 standard test design and modified test designs, considering an application of the test substance via manure;

Main tests were conducted with six plant species. The studies were conducted with pig and cattle manure. The veterinary antibiotics florfenicol and tylosin tartrate were chosen as representative test substances by the German Federal Environment Agency.

The test design considers additional effects of manure to the test substance (e.g. adsorption) as well as transformation/metabolization of the test substance in manure. To ensure significant results and to investigate the variance within replicates, in treatments with manure eight replicates were applied. The standard tests without manure application and the standard controls (without manure and test item) in the tests considering the manure effect were conducted with at least 4 replicates according to the OECD guideline 208. Test item concentration in tests with tylosin tartrate was verified in manure by chemical analysis just before incorporation into the soil. In contrast, for florfenicol the degradation kinetic over the incubation phase was monitored by recording non-extractable residues (NER), CO2, CH4, and transformation products in representative test concentrations.

Test performance was planned at two test facilities (Fraunhofer Institute for molecular biology and Applied Ecology (IME), Schmallenberg, Germany; ECT Oekotoxikologie GmbH (ECT), Flörsheim, Germany) using the same soil, manure, and seeds (as far as the same plant species were applied). The nomination of the individual test phases follows the new EMA-guideline on determining the fate of veterinary medicinal products in manure EMA/CVMP/ERA/430327/2009 (adopted March 10, 2011) [4] and the first draft for an upcoming OECD Guideline for the testing of chemicals – Anaerobic Transformation in Liquid Manure (status 19.03.2012) [10].

Pig and cattle manure applied in the project stem from animals that were reared under well controlled conditions. A contamination with VMPs, biocides and other material that might impair plant growth or survival can be neglected as far as possible. Prior to further processing manure was stored for 3 – 10 weeks at 8° C in the dark. Storage ensured anaerobic conditions. Prior to the start of the acclimation period, the dry matter content of the manure was determined and adjusted – if necessary - to standardized values of 5 % ± 1 % and 10 % ± 1 % for pig and cattle manure, respectively [12], [8]. Thereafter, manure was processed using a mixer in order to obtain a fairly stable phase. The acclimation was carried out for 21 – 26 days at 20°C in the dark. Processing and acclimation ensured anaerobic conditions. After acclimation, the manure was spiked with the antibiotics and incubation period started. Depending on test design, i) no incubation, ii) incubation for half-maximum (27 and 45 days for pig and cattle manure, respectively) and iii) maximum allowed storage duration (53 and 90 days for pig and cattle manure, respectively) at 20°C in the dark under anaerobic conditions took place. To monitor if the applied methods are suitable to maintain anaerobic conditions during the whole manure processing, redox potential and pH was measured in the manure for the main tests at the sampling site, before acclimation, after acclimation, and after anaerobic incubation. Additionally, individual tests regarding deviations in sequential arrangement of application or incubation conditions were conducted.

The objective of the studies was the assessment of the effects of the test item on emergence and early stages of growth of terrestrial plant seedlings. Seeds of different terrestrial plants were sown in a natural soil substrate containing the test item in different concentrations without or with manure. A single application of test item was made. The kind of introduction of the test item to the soil depended on the respective test design. Test item was either applied directly to soil via an aqueous application solution (tylosin tartrate, standard test), via quartz sand (florfenicol, standard test), or via spiked manure. In any case, the carrier was distributed on the surface of the spreaded soil and mixed thoroughly. The test was generally performed following the "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test" Guideline OECD 208 [7].

Plant species applied in the tests were *Allium cepa* (onion; Liliaceae) and *Triticum aestivum* (wheat; Poaceae), *Avena sativa* (oat; Poaceae), *Solanum lycopersicum* (tomato; Solanaceae), *Brassica napus* (oil seed rape; Brassicaceae), *Sinapis alba* (mustard; Brassicaceae), *Cucumis sativus* (cucumber; Cucurbitaceae), *Trifolium pratense* (red clover; Fabaceae), and *Phaseolus vulgaris* (common bean; Fabaceae), which are representatives for monocotyledonous, dicotyledonous, and legumes, respectively. Pre-tests for the evaluation of suitable plant species and manure concentration were applied. The following test series regarding the effect of veterinary antibiotics on plants were performed with a manure concentration of approximately 85 kg N/ha (22 g manure/kg soil).

However, since the dry matter content of manure is expected to have more influence on the effect of veterinary medicinal products in plant tests than the nitrogen content, it was decided that the test series should be performed with constant manure originated dry matter concentration instead of nitrogen concentration per kg soil. Consequently, all further tests were performed with 22 g manure (fresh mass)/kg soil (dry mass), independent from total nitrogen concentration in the manure applied.

The influence of manure on seedling emergence and growth

It was demonstrated, that manure, actually pig manure, potentially can impair germination, emergence and post-emergence survival of plant seedlings within a concentration corresponding to 170 kg N/ha, representing the maximum amount of manure allowed to be deployed per year in Europe. A maximum manure concentration representing 85 kg N/ha (1/2 maximum amount allowed per year in Europe) should not be exceeded in a plant test to ensure an unimpaired germination, emergence and post-emergence survival. At least the seven plant species (*A. cepa* (onion), *A. sativa* (oat), *S. lycopersicum* (tomato), *B. napus* (rape), *P. vulgaris* (common bean), *S. alba* (mustard), *and T. pratense* (red clover)), representing monocots, dicots, and legumes, were grown successfully at this manure concentration.

The influence of the exposure way on effects of antibiotics in plant tests

Neither the physical presence of pig or cattle manure, nor the sequential arrangement of application influenced the effect of the two antibiotics on shoot fresh mass (most sensitive parameter) of the plant species investigated. The NOEC and EC values are comparable to those from the standard test design.

The influence of the incubation conditions (aerobic/anaerobic) on effects of antibiotics in plant tests

Incubation conditions seriously influenced the effect of the two antibiotics on shoot fresh mass (most sensitive parameter) of the plant species investigated. An aerobic incubation of the spiked manure/soil did not influence the effect of the two antibiotics on shoot fresh mass of the plant species investigated, when compared with a standard test design. In contrast, an anaerobic incubation of spiked pig or cattle manure resulted in a significant decreased effect of both antibiotics on shoot fresh mass.

The influence of the incubation duration on effects of antibiotics in plant tests

Incubation duration seriously influenced the effect of the two antibiotics on shoot fresh mass of all plant species investigated. Freshly spiked manure did not influence the effect of the two antibiotics on shoot fresh mass (most sensitive parameter) of the plant species investigated, when compared with a standard test design. In contrast, an anaerobic incubation of spiked pig or cattle manure resulted in a significant decreased effect of both antibiotics on shoot fresh mass. In most cases the maximum decrease was already reached after incubation for the half-maximum storage duration.

Do manure application increase variability of the results generally?

Manure application did not generally result in increased variances. This was true for pig and cattle manure. An increase of replicates in our tests did not reduce variances in the most cases.

Does replicate number influence NOEC or EC_{10} in manure approaches?

The results of the tests do not allow a definite conclusion. However, to ensure representative and reproducible results, it is recommended to increase the number of replicates in a NOEC test design and the number of treatment concentrations in an EC_X test design.

Methods of preparation, acclimation, incubation, and application of manure in a plant test

Methods for storage, acclimation, and incubation of manure following the new EMA-Guideline on determining the fate of veterinary medicinal products in manure [4] combined with the methods for processing, were shown to be suitable to maintain anaerobic conditions during the whole manure processing. The methods enable comparable seedling emergence and growth to a standard OECD test and result in robust biological endpoints. The variance of the replicate results - and therefore the strictness of the calculated NOEC or EC values – in the modified test design was comparable to that in the standard test design according to OECD 208.

Regarding the objectives concerning a standardized test design to test the effect of veterinary medicinal products on plants in a more realistic way, formulated at the start of the project, it can be summed up that

- a test design for a modified OECD 208 seedling emergence and growth plant test with a more realistic exposure in manured soil was developed successfully and
- the practicability of the test design was verified experimentally by means of two veterinary antibiotics (florfenicol and tylosin tartrate) and two kinds of manures (pig and cattle) for six plant species.
- A draft manual with recommendations for the performance, evaluation and reporting of an extended plant test with an exposure scenario via manure application for the use in environmental risk assessment of veterinary medicinal products is presented.

2 Zusammenfassung

Durch Tierarzneimittel verursachte Effekte auf die Umwelt werden nach den EMA und VICH Guidelines CVMP/VICH/592/98-FINAL [1] and VICH GL 38 (ECOTOXICITY PHASE II) [3] bewertet. In Phase II der Umweltrisikobewertung werden Effekte auf terrestrische Pflanzen untersucht, da Rückstände der Wirkstoffe über als Dünger eingesetzter Gülle von behandelten Tieren auf landwirtschaftliche Flächen gelangen können. Effekte auf terrestrische Pflanzen werden nach der OECD Richtlinie 208 “Seedling Emergence and Seedling Growth Test” [7] untersucht. Bei der Risikobewertung wurde bisher die geschätzte initiale Umweltkonzentration verwendet. Ein Abbau der Wirkstoffe in Gülle wurde nicht berücksichtigt. Diese Vorgehensweise führt häufig zu einem unakzeptablen Risiko für terrestrische Pflanzen. Eine Möglichkeit das Risiko realistischer zu bewerten ist die Anwendung eines modifizierten Ansatzes, der eine realistischere Applikationsform (mit Gülle angereicherter Boden) berücksichtigt.

Bisher existiert kein einheitliches Konzept zur Durchführung und Bewertung eines solchen modifizierten Testdesigns. Es muss eine Handlungsanweisung entwickelt werden, welche i) die Art der Applikation über Dung und/oder Gülle im eigentlichen Pflanzentest, ii) die Behandlung/Aufarbeitung des Trägermaterials Gülle, z.B. die Dauer der Vorinkubation (Lagerung der Gülle und Applikation der Testsubstanz vor dem Einsatz im Pflanzentest) und Charakterisierung der Güllematrix (z.B. nach EMA/CVMP/ERA/430327/2009 [4]), sowie iii) eine mögliche Adaptation des Testdesigns (z.B. Erhöhung der Replikatzahl, Identifizierung und Ausschluss ungeeigneter Pflanzenarten) definiert.

Auf europäischer Ebene existiert seit März 2011 mit der Richtlinie EMA/CVMP/ERA/430327/2009: Guideline on determining the fate of veterinary products in manure (verabschiedet am 10.03.2011) [4] eine Bewertungsrichtlinie der European Medicines Agency (EMA) für Tierarzneimittel. Diese EMA Bewertungsrichtlinie beinhaltet aber keine experimentellen Spezifikationen und Details. Sie beschreibt nur einige experimentelle Parameter und geht hauptsächlich auf die Interpretation der Ergebnisse von Studien zur Transformation in Gülle ein.

Basierend auf der Notwendigkeit für ein standardisiertes Testdesign zur realistischeren Überprüfung der Effekte von Tierarzneimitteln auf Pflanzen, ergeben sich folgende Ziele für dieses Forschungsvorhaben:

- Entwicklung eines Testdesigns für einen modifizierten OECD 208 “Seedling emergence and growth” Pflanzentests mit einer realistischeren Exposition in mit Gülle versetztem Boden;
- Experimentelle Bestätigung der Praktikabilität des Testdesigns durch zwei Antibiotika und zwei Güllearten (Schwein und Rind) in Tests mit sechs Pflanzenarten;
- Erstellung einer Handlungsanweisung zur Durchführung, Auswertung und Berichterstellung von erweiterten Pflanzentests mit einem Expositionsszenario über Gülleapplikation für den Einsatz in der Umweltrisikobewertung von Tierarzneimitteln;

Die Umsetzung war in drei Sektionen gegliedert. Die erste Sektion umfasste die theoretische Vorbereitung des Projektes, die zweite Sektion den experimentellen Teil und die dritte Sektion beinhaltete die Erstellung einer vorläufigen Handlungsanweisung, basierend auf den experimentellen Ergebnissen, seiner Diskussion innerhalb eines internationalen Workshops sowie die Veröffentlichung einer finalisierten Handlungsanweisung.

Im Rahmen eines Kick-Off Meetings im Dezember 2011 wurde durch den Auftraggeber der regulatorische Hintergrund des Forschungsvorhabens dargestellt. In erweiterten Pflanzentests mit Gülle, die bisher von Antragstellern vorgelegt wurden, traten Probleme bezüglich i) Wachstumshemmung durch Güllezugabe, ii) Vergrößerung des Variationskoeffizienten durch Güllezugabe und iii) teilweisem Fehlen klarer Dosis-Wirkungsbeziehungen auf. Auch wurde das Fehlen eines Higher Tier Ansatzes für Phase II, Tier B aufgeführt. Dieses Forschungsvorhaben soll mit der Erstellung einer Handlungsanweisung für einen Pflanzentest mit adaptiertem Testdesign - bezüglich der Berücksichtigung einer Testsubstanzapplikation über Gülle - einen wichtigen Beitrag zum Schließen dieser Lücke bilden.

Dazu sollen in diesem Projekt die folgenden Punkte untersucht werden:

- Entwicklung von Methoden zur Aufarbeitung, Akklimatisierung, Inkubation und Applikation von Gülle in einem Pflanzentest;
- Notwendiger technischer Hintergrund (z.B. geeignete Pflanzenarten, geeignete Güllekonzentration;
- Vergleich von Tests nach dem OECD 208 Standardtestdesign und modifizierten Testdesigns, die eine Applikation der Testsubstanz über Gülle berücksichtigen;

Die Haupttests wurden mit sechs Pflanzenarten durchgeführt. Die Studien wurden mit Schweine- und Rindergülle durchgeführt. Das deutsche Umweltbundesamt wählte die Veterinärantibiotika Florfenicol und Tylosintartrate als repräsentative Testsubstanzen aus.

Das Testdesign berücksichtigt zusätzliche Effekte der Gülle auf die Testsubstanz (z.B. Adsorption), sowie Transformation beziehungsweise Metabolisierung der Testsubstanz in Gülle. Um aussagekräftige Ergebnisse sicherzustellen und um die Varianz zwischen den Replikaten zu untersuchen, wurden in Ansätzen mit Gülle acht Replikate eingesetzt. Die Standardtests ohne Gülleapplikation und die Standardkontrollen (ohne Gülle und Testsubstanz) in den Tests bezüglich der Gülleeffekte wurden entsprechend der OECD Richtlinie 208 mit mindestens 4 Replikaten durchgeführt. Die Testsubstanzkonzentration in Tests mit Tylosintartrat wurde in der Gülle durch chemische Analyse direkt vor der Einarbeitung in den Boden überprüft. Im Gegensatz dazu wurde bei Florfenicol die Abbaukinetik über die Inkubationsphase durch Erfassung nichtextrahierbarer Rückstände (NER), CO_2, CH_4 sowie von Transformationsprodukten in repräsentativen Testkonzentrationen verfolgt.

Die Testdurchführung fand an zwei Prüfeinrichtungen (Fraunhofer Institut für Molekularbiologie und Angewandte Oekologie (IME), Schmallenberg, Deutschland; ECT Oekotoxikologie GmbH (ECT), Flörsheim, Deutschland) mit dem Einsatz desgleichen Bodens, dergleichen Gülle sowie dergleichen Samencharge (soweit die gleichen Pflanzenarten eingesetzt wurden) statt. Die Bezeichnung der einzelnen Testphasen folgt der neuen EMA-Richtinie “Guideline on determining the fate of veterinary medicinal products in manure EMA/CVMP/ERA/430327/2009 (verabschiedet am 10.03.2011)” [4] und dem ersten Entwurf einer neuen OECD Richtlinie zur Testung von Chemikalien, “Anaerobic Transformation in Liquid Manure (Status 19.03.2012)” [10].

Die Schweine- und Rindergülle, die in diesem Projekt eingesetzt wurde, stammt von Tieren, die unter gut kontrollierten Bedingungen gehalten wurden. Eine Kontamination mit Tierarzneimitteln, Bioziden und anderen Materialien, die das Pflanzenwachstum oder das Überleben von Pflanzen beeinträchtigen könnten, kann weitestgehend ausgeschossen werden. Vor einer weiteren Behandlung wurde die Gülle für 3 – 10 Wochen bei 8 °C im Dunkeln gelagert. Vor dem Start der Akklimatisationsphase wurde der Trockenmasseanteil der Gülle bestimmt und wenn nötig auf Standardwerte von 5 % ± 1 % und 10 % ± 1 % für Schweine beziehungsweise Rindergülle eingestellt [12], [8]. Danach wurde die Gülle mit einem Mixer aufbereitet um eine ausreichend stabile Phase zu erhalten. Die Akklimatisation wurde für 21 – 26 Tage bei 20 °C im Dunkeln durchgeführt. Bei der e Gülleaufbereitung und Akklimatisation wurden anaerobe Bedingungen gewährleistet. Nach der Akklimatisation wurde die Gülle mit den Antibiotika versetzt und die Inkubationsperiode begann. Abhängig vom Testdesign wurde i) keine Inkubation, ii) Inkubation für ½maximal (27 beziehungsweise 45 Tage für Schweine- und Rindergülle) und iii) maximal erlaubte Lagerungsdauer (53 beziehungsweise 90 Tage für Schweine- und Rindergülle) bei 20 °C im Dunkeln unter anaeroben Bedingungen angewandt. Das Redoxpotential und der pH der Gülle wurden in den Haupttests bei Probename, vor der Akklimatisation, nach der Akklimatisation und nach anaerober Inkubation gemessen, um zu verfolgen, ob die angewandten Methoden geeignet sind anaerobe Bedingungen während des gesamten Aufbereitungsprozesses der Gülle zu gewährleisten. Zusätzlich wurden eigenständige Tests durchgeführt, die den Einfluss von Unterschieden in der Reihenfolge der Applikation oder von Unterschieden bei den Inkubationsbedingungen untersuchten.

Das Ziel der Studien war die Bewertung der Testsubstanzeffekte auf Auflauf und frühe Stadien des Wachstums bei terrestrischen Pflanzen. Samen verschiedener terrestrischer Pflanzenarten wurden in natürlichem Boden – mit oder ohne Gülle - ausgesät, der die Testsubstanz in verschiedenen Konzentrationen enthielt. Die Testsubstanz wurde einmalig appliziert. Die Art der Einarbeitung der Testsubstanz hing dabei vom jeweiligen Testdesign ab. Die Testsubstanz wurde entweder direkt über eine wässrige Applikationslösung (Tylosintartrat, Standardtest), über Quarzsand (Florfenicol, Standardtest), oder über gespikte Gülle auf den Boden aufgebracht. In jedem Fall wurde das Trägermaterial gleichmäßig auf der Oberfläche des ausgebreiteten Bodens verteilt und sorgfältig untergemischt. Die Tests wurden generell nach der OECD Richtlinie 208 "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test" durchgeführt [7].

In den Tests wurden die Pflanzenarten *Allium cepa* (Zwiebel; Liliaceae), *Triticum aestivum* (Weizen; Poaceae), *Avena sativa* (Hafer; Poaceae), *Solanum lycopersicum* (Tomate; Solanaceae), *Brassica napus* (Raps; Brassicaceae), *Sinapis alba* (Senf; Brassicaceae), *Cucumis sativus* (Gurke; Cucurbitaceae), *Trifolium pratense* (Rotklee; Fabaceae) und *Phaseolus vulgaris* (Gartenbohne; Fabaceae) eingesetzt. Diese Arten repräsentieren einkeimblättrige und zweikeimblättrige Arten, sowie Leguminosen. Es wurden Vortests zur Überprüfung geeigneter Pflanzenarten und Güllekonzentrationen durchgeführt. Die nachfolgenden Testserien bezüglich der Effekte von Veterinärantibiotika auf Pflanzen wurden bei einem Güllekonzentration von ungefähr 85 kg N/ha (22 g Gülle/kg Boden) durchgeführt.

Es kann davon ausgegangen werden, dass der Trockenmasseanteil der Gülle mehr Einfluss auf den Effekt von Tierarzneimitteln in Pflanzentests hat als der Stickstoffgehalt. Daher wurde beschlossen, dass die Testserien mit einer konstanten, güllebezogenen

Trockenmassekonzentration anstelle einer konstanten, güllebezogenen Stickstoffkonzentration pro kg Boden durchgeführt werden sollten. Alle weiteren Tests wurden mit 22 g Gülle (Frischmasse) pro kg Boden (Trockenmasse), unabhängig von der Gesamtstickstoffkonzentration der Gülle, durchgeführt.

Einfluss von Gülle auf Auflauf und Wachstum

Es konnte gezeigt werden, dass Gülle – insbesondere Schweinegülle – potentiell Keimung, Auflauf und Überlebensrate der Pflanzen nach Auflauf bei einer Konzentration entsprechend 170 kg N/ha – dies entspricht der maximalen Güllemenge, die in Europa pro Jahr ausgebracht werden darf - negativ beeinträchtigen kann. Eine maximale Güllekonzentration entsprechend 85 kg N/ha – dies entspricht der ½maximalen Güllemenge, die in Europa pro Jahr ausgebracht werden darf – sollte deshalb in einem Pflanzentest nicht überschritten werden um Keimung, Auflauf und Überlebensrate der Pflanzen nach Auflauf nicht negativ durch Gülleapplikation zu beeinträchtigen. Sieben Pflanzenarten (*A. cepa* (Zwiebel), *A. sativa* (Hafer), *S. lycopersicum* (Tomate), *B. napus* (Raps), *P. vulgaris* (Gartenbohne), *S. alba* (Senf) und *T. pratense* (Rotklee)) sind bei dieser Güllekonzentration erfolgreich gekeimt und gewachsen.

Einfluss des Expositionsweges auf Antibiotikaeffekte in Pflanzentests

Weder die physikalische Anwesenheit von Schweine- oder Rindergülle, noch die Reihenfolge der Applikation hatte einen Einfluss auf die Effekte der beiden Antibiotika auf die Sprossbiomasse (empfindlichster Parameter) der untersuchten Pflanzenarten. Die NOEC und EC Werte sind vergleichbar mit denen aus Tests nach dem Standarddesign.

Einfluss der Inkubationsbedingungen (aerob/anaerob) auf Antibiotikaeffekte in Pflanzentests

Die Inkubationsbedingungen hatten einen deutlichen Einfluss auf die Effekte der beiden Antibiotika auf die Sprossbiomasse (empfindlichster Parameter) der untersuchten Pflanzenarten. Eine aerobe Inkubation der/des gespikten Gülle/Boden hatte keinen Einfluss auf die Effekte der beiden Antibiotika auf die Sprossbiomasse, verglichen mit Tests nach dem Standarddesign. Im Gegensatz dazu führte eine anaerobe Inkubation von gespikter Schweine- oder Rindergülle zu signifikant reduzierten Effekten beider Antibiotika auf die Sprossbiomasse.

Einfluss der Inkubationsdauer auf Antibiotikaeffekte in Pflanzentests

Die Inkubationsdauer hatte einen deutlichen Einfluss auf die Effekte der beiden Antibiotika auf die Sprossbiomasse der untersuchten Pflanzenarten. Frisch gespikte Gülle hatte keinen Einfluss auf die Effekte der beiden Antibiotika auf die Sprossbiomasse (empfindlichster Parameter) der untersuchten Pflanzenarten, verglichen mit Tests nach dem Standarddesign. Im Gegensatz dazu führte eine anaerobe Inkubation von gespikter Schweine- oder Rindergülle zu signifikant reduzierten Effekten beider Antibiotika auf die Sprossbiomasse. In den meisten Fällen wurde die maximale Abnahme der Effekte schon nach halbmaximaler Lagerungsdauer erreicht.

Führt Gülleapplikation generell zu einer erhöhten Variabilität der Ergebnisse?

Gülleapplikation führte nicht generell zu einer Erhöhung der Varianzen. Dies gilt für Schweine- und Rindergülle. Eine Erhöhung der Replikatzahl führte aber in den meisten Fällen auch nicht zu einer Verminderung der Varianzen.

Beeinflusst die Replikatzahl NOEC oder EC_{10} Werte in Gülleansätzen?

Die untersuchten Tests sind nicht ausreichend, um eine generelle Schlussfolgerung abzuleiten. Es wird empfohlen die Anzahl an Replikaten in einem NOEC Testdesign und die Anzahl der Testkonzentrationen in einem EC_X Testdesign zu erhöhen, um repräsentative und reproduzierbare Ergebnisse zu gewährleisten.

Methoden zur Aufbereitung, Akklimatisierung, Inkubation und Applikation von Gülle in einem Pflanzentest.

Es wurde gezeigt, dass die Methoden zur Lagerung, Akklimatisierung und Inkubation von Gülle nach der neuen EMA Richtlinie “Guideline on determining the fate of veterinary medicinal products in manure” [4] kombiniert mit den Methoden für Aufbereitung dazu geeignet sind, während des gesamten Gülleaufbereitungsprozesses anaerobe Bedingungen zu gewährleisten. Die Methoden gewährleisten zum OECD 208 Standardtestdesign vergleichbare Werte für Pflanzenauflauf und Wachstum und führen zu robusten biologischen Endpunkten. Die Varianz der Ergebnisse der Replikate, und somit die Aussagekraft der berechneten NOEC oder EC Werte, waren im modifizierten Testdesign vergleichbar zu denen aus Tests nach dem OECD 208 Standardtestdesign.

Bezüglich der Ziele für ein standardisiertes Testdesign zur realistischeren Testung der Effekte von Tierarzneimitteln auf Pflanzen, die am Anfang des Projektes formuliert wurden, kann zusammenfassend gesagt werden:

- Ein Testdesign für einen modifizierten OECD 208 “Seedling emergence and growth plant test” mit einer realistischeren Exposition in mit Gülle versetztem Boden wurde erfolgreich entwickelt.
- Die Praktikabilität des Testdesigns wurde experimentell anhand von zwei Veterinärantibiotika (Florfenicol und Tylosintartrat) und zwei Güllearten (Schwein und Rind) für sechs Pflanzenarten belegt.
- Ein Entwurf für eine Handlungsanleitung für einen erweiterten Pflanzentests mit einem Expositionsszenario über Gülleapplikation für die Umweltrisikobewertung von Tierarzneimitteln mit Empfehlungen zur Durchführung, Auswertung und Berichtserstellung ist erstellt.

3 Background

Effects on the environment caused by veterinary medicinal products are assessed according to the manuals CVMP/VICH/592/98-FINAL [1] and VICH GL 38 (ECOTOXICITY PHASE II) [3] of the EMA and VICH. In Phase II of the environmental risk assessment effects on terrestrial plants are tested since residues of active substances can reach agricultural areas because manure and slurry from treated animals are used as fertilizer. Effects on terrestrial plants are examined according to the OECD guideline 208 “Seedling Emergence and Seedling Growth Test” [7].

However, this approach often results in strong effects, leading to an unacceptable risk to terrestrial plants. One possibility to refine the risk is to conduct a modified approach considering a more realistic application form (manure enriched soil).

So far no equal harmonized concept exists for performance and assessment of such modified test design. Thereby not only the kind of application via manure in the plant test itself has to be defined. Additionally, the treatment of the “carrier” manure, e.g. duration of pre-incubation (storage of the manure and application of the test substance before use in the plant test) and characterization of the matrix manure (e.g. according to EMA/CVMP/ERA/430327/2009 [4], as well as a possible adaption of the test design (e.g. increase of number of replicates, identification and exclusion of unsuitable plant species) have to be determined.

On the European scale, since March 2011 a guideline by the European Medicines Agency (EMA) EMA/CVMP/ERA/430327/2009: Guideline on determining the fate of veterinary products in manure (adopted 10.03.2011) [4] exists for veterinary medicinal products.

However, the EMA assessment guideline does not include experimental specifications and details. It only describes some experimental parameters and mainly considers the interpretation of the results of studies on transformation in manure.

4 Objectives

Based on the need for a standardized test design to test the effect of veterinary medicinal products on plants in a more realistic way, the following objectives of this research project arise:

- Development of a test design for a modified OECD 208 seedling emergence and growth plant test with a more realistic exposure in manured soil
- Experimental verification of the practicability of the test design by means of two veterinary antibiotics and two kind of manures (pig and cattle) in tests with six plant species
- Discussion of the informative value of the results in the risk assessment
- Preparation of a manual for the performance, evaluation and reporting of extended plant tests with an exposure scenario via manure application for the use in environmental risk assessment of veterinary medicinal products

5 Project realization

The realization was divided in three sections. The first section comprised the theoretical preparation of the project like the summary of the state of knowledge regarding plant tests with manure application in a kick-off meeting, the choice of the two veterinary antibiotics, and the preparation of the study plans. The second section comprised the experimental part and hence the performance, documentation, and evaluation of the plant tests (range finding and main tests) and representative fate studies in both manures and manure/soil mixtures, respectively. The third section comprised the preparation of a preliminary manual based on the experimental results, its discussion within an international workshop, and the publication of a finalized manual.

6 Section 1: Theoretical preparation

In the scope of a kick-off meeting in December 2011 at the German Federal Environment Agency (UBA) in Dessau, the founder figured out the regulatory background for the research project. In plant tests with manure performed according to own methods and submitted by applicants to the UBA so far following problems are revealed: i) growth in controls with manure application was partially significantly lower than in controls without manure, ii) in contrast, the coefficient of variation in these controls was significantly higher, and iii) for some plant species no clear dose-response relationship occurred.

In this research project these issues should have been investigated and a manual for an extended test design for plant tests with test substance application via manure considering possible properties of manure on a plant test should be developed.

<u>Development of methods for preparation, acclimatization, incubation, and application of manure in a plant test</u>

Storage, acclimation, and incubation of manure followed the new EMA-Guideline on determining the fate of veterinary medicinal products in manure [4]. To ensure a homogenous application, the manure was shredded and homogenized before acclimation. According to the guideline, characterization of the manure matrix was applied at the end of the acclimation phase. All operations were carried out in a way that the manure had minimal contact with oxygen.

<u>Tests investigating necessary technical background (e.g. suitable plant species, suitable manure concentration)</u>

Range finder tests to verify a suitable manure concentration in plant tests without impairment of seedling emergence and growth due to the manure itself were conducted before any tests with veterinary medicinal products were performed. For this pre-tests two Brassica species (known to be sensitive for antibiotics), four additional dicots and two monocots were chosen. For the following tests concerning the effects of veterinary antibiotics, the most suitable seven species (six for each test substance) out of these eight species were chosen after discussion with the German Federal Environment Agency.

<u>Tests according to the OECD 208 standard test design and modified test designs, considering an application of the test substance via manure</u>

To ensure a significant evidence of the studies regarding universal validity, main tests were conducted with six plant species. The studies were conducted with pig and cattle manure. The veterinary antibiotics florfenicol and tylosin tartrate were chosen as representative test substances by the German Federal Environment Agency.

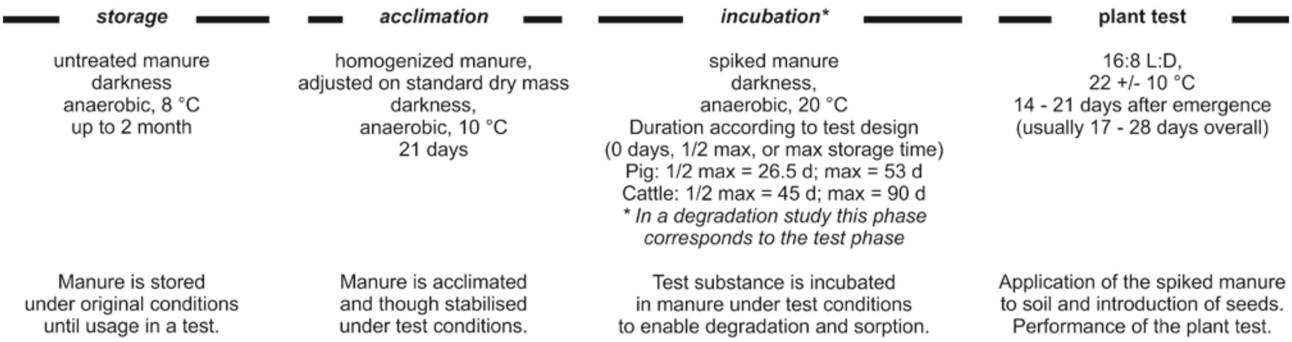
The test design considers additional effects of manure to the test substance (e.g. adsorption) as well as transformation/metabolization of the test substance in manure. For this, standard tests according to OECD 208 without manure application were performed as a benchmark. Subsequently tests with manure application investigating the influence of i) the duration of anaerobic incubation in manure, ii) the way of application, and iii) the incubation conditions (anaerobic/aerobic) on the effects of the antibiotics in plant tests were performed.

To ensure significant results and to investigate the variance within replicates, in treatments with manure eight replicates with 4 – 5 seeds per replicate were applied, ensuring 32 – 40 seeds per treatment and manure control. The standard tests without manure application and the standard controls (without manure and test item) in the tests considering the manure effect were conducted with at least 4 replicates and overall 20 seeds according to the OECD guideline 208.

Test item concentration in tests with tylosin tartrate was verified in manure by chemical analysis just before incorporation into the soil. In contrast to tylosin tartrate, for florfenicol the degradation kinetic over the incubation phase was monitored by recording non-extractable residues (NER), CO_2, CH_4, and transformation products. For this, ^{14}C-florfenicol was applied in additional fate assays. Since this issue was not the main question of this project, only one test substance was applied ^{14}C-labelled representatively. ^{14}C-florfenicol was assumed to be better suited than tylosin tartrate due to a usually faster transformation rate.

Test performance was planned at two test facilities (Fraunhofer Institute for molecular biology and Applied Ecology (IME), Schmallenberg, Germany; ECT Oekotoxikologie GmbH (ECT), Flörsheim, Germany) using the same soil, manure, and seeds (as far as the same plant species were applied). The nomination of the individual test phases follows the new EMA-guideline on determining the fate of veterinary medicinal products in manure EMA/CVMP/ERA/430327/2009 (adopted March 10, 2011) [4] and the first draft for an upcoming OECD Guideline for the testing of chemicals – Anaerobic Transformation in Liquid Manure (status 19.03.2012) [10] (Figure 1).

Figure 1: Nomination of the individual test phases follows the EMA-guideline on determining the fate of veterinary medicinal products in manure and the first draft for an upcoming OECD Guideline for the testing of chemicals – Anaerobic Transformation in Liquid Manure (status 19.03.2012)



Terms in italic are taken from the "**First Draft for An upcoming OECD Guideline for the testing of chemicals - Anaerobic Transformation in Liquid Manure**" (status 19.03.2012).

7 Section 2: Experimental work

7.1 Manure

Pig and cattle manure applied in the project stem from animals that were reared under well controlled conditions. A contamination with VMPs, biocides and other material that might impair plant growth or survival can be neglected as far as possible. The pig manure applied originated from a pig plant. The cattle manure originated from the milk cow plant. Both plants have below ground, covered manure storage tanks. Manure sampling took place on 11.01.2011 for the pre-tests (pig and cattle manure) and on 25.07.2012 (pig manure) and 17.10.2012 (cattle manure) for the main tests, respectively. Manure sampling, storage, preparation, acclimation, and characterization were performed at Fraunhofer IME.

7.1.1 Sampling

Prior to collection the liquid manure was homogenized by mixing in the respective manure tank. Stirring took place immediately before sampling. For mixing a device installed in the tank was used. Mixing was performed for one hour which is proved sufficient for homogenization of manure in tanks independent from tank volume. Liquid manure was collected from the tank by a ladle with a large beaker, and filled to 45 L into 60 L barrels. The barrels were sealed but allowed gas, which was generated by continuous microbial activity, to expand.

7.1.2 Storage

Prior to further processing manure was stored in the 60 L barrels at 8° C in the dark (Figure 2). Storage ensured anaerobic conditions. Care was taken to allow gas, generated by biological activity during storage, to expand and therefore avoid explosion of the container. Storage duration for the range finder tests regarding suitable manure concentrations for plant tests were 3 - 4 weeks. Storage duration for the range finder tests regarding the effects of florfenicol and tylosin tartrate were 10 weeks.

Figure 2: Storage of unprocessed manure. (A) Overview; (B) detail view on the barrels.

A B

7.1.3 Processing and acclimation

Prior to the start of the acclimation period, the dry matter content of the manure was determined. To get comparable conditions it has to be adjusted – if necessary - to standardized values. The recommended dry matter content in pig and cattle manure is 5 % ± 1 % and 10 % ± 1 %, respectively [12], [8]. Dry matter content of the original manure was 5.0 % (pre-tests) and 11.8 % (main tests) for pig manure and 10.0 % (pre-tests) and 9.8 % (main tests) for cattle manure. Since the dry matter content of the pig manure for the main tests was too high, water (deionized water, bubbled with nitrogen for 30 min) was added as needed.

Figure 3: Manure processing. (A) Shredding with a food processor; (B) processed pig manure.

A B

Thereafter, manure was processed using a mixer (a food processor) in order to obtain a fairly stable phase (Figure 3). To prevent introduction of oxygen, mixing was conducted in an extensively sealed beaker flooded with nitrogen. Thereafter, the dry matter content was checked. Subsequently, the processed manure was directly filled into the acclimation container which was used for the acclimation. The acclimation containers were filled up to approximately 3/4 - 4/5 of maximum container volume. After flooding the container with nitrogen, it was closed but allowed gas to expand. The acclimation was carried out for 21 – 26 days at 20°C in the dark (Figure 4).

Figure 4: Manure acclimation.

The manure needed for the tests performed at ECT was delivered in buckets within a maximum of three hours by car at the end of the third acclimation week, ensuring that temperature did not deviate too much from 20 °C and that anaerobic conditions maintained stable. After delivery, acclimation was continued under anaerobic conditions at 20 °C in the dark until use.

7.1.4 Manure characterization

Key parameters measured of the manure applied are summarized in the table hereafter.

Table 1: Schedule and key parameter for manure characterization

Parameter	stage of test procedure		
	Sampling (on site)	End of storage/start of acclimation	end of acclimation
pH	X	X	X
Temperature [°C]	X	X	X
Redox potential [mV]	X	X	X
Dry matter content [%]		X	X
Microbial activity			X
NH_4-N content [mg/kg]			X
Total N content [mg/kg]			X
Organic C content [mg/kg]			X
Total P content [mg/kg]			X
Total Cu content [mg/kg]			X

Table 2: Methods applied for manure characterization

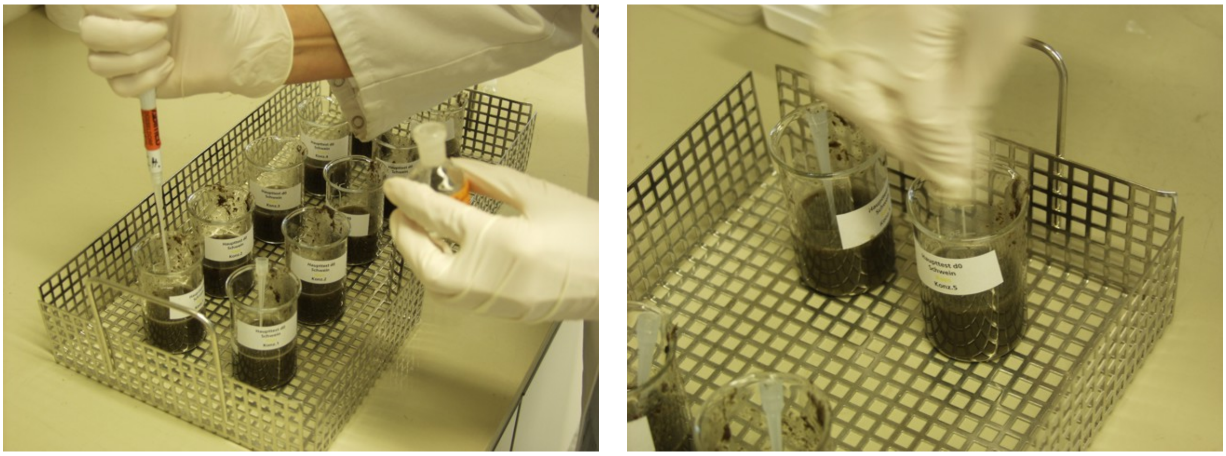
pH	Direct measurement in liquid manure.
Temperature	Direct measurement in liquid manure.
Redox potential	Direct measurement in liquid manure.
Dry matter content	Drying of subsamples of 10 g fresh mass at 105 °C over-night in a drying chamber. Quantification by weighting.
Microbial activity	For testing the microbial activity several suggestions exist. In this case it was mineralization of ^{14}C-labeled glucose under anaerobic conditions.
NH_4-N content	VDLUFA, Methodenbuch Band II.1, Die Untersuchung von Düngemitteln, 3.2.3 Ammonium-N, Ausblasemethode. Quantification by titration.
Nonvolatile N content	Analysis of dried manure by determination with element analyzer after combustion.
Total N content	Calculation by addition of NH4-N content and nonvolatile N content.
Organic C content	Analysis of dried manure by determination with element analyzer after combustion.
Total P content	ISO 11466:1995: Soil quality -- Extraction of trace elements soluble in aqua regia and DIN EN 13346: Charakterisierung von Schlämmen - Bestimmung von Spurenelementen und Phosphor - Extraktionsverfahren mit Königswasser; German version EN 13346:2000. Phosphor quantification by ICP-OES with a matrix adjusted calibration.
Total Cu content	ISO 11466: Soil quality -- Extraction of trace elements soluble in aqua regia and DIN EN 13346: Charakterisierung von Schlämmen - Bestimmung von Spurenelementen und Phosphor - Extraktionsverfahren mit Königswasser; German version EN 13346:2000. Copper quantification by ICP-OES with a matrix adjusted calibration.

7.1.5 Spiking and incubation of manure

<u>Tests with florfenicol at IME</u>

Due to the low water solubility of florfenicol, the test item had to be applied to the manure via an acetonic stock solution. The volume of stock solution required to obtain the desired concentrations was filled up to a fix total volume (< 600 µL, actual volume depending on test) with acetone and mixed with a portion of manure (44 g fresh mass manure per 2 kg test substrate) in 100 – 150 mL glass beakers (Figure 5). The solvent was evaporated by depressing three times on ca. 500 mbar for 1 minute in an anaerobic cabinet (Figure 7 A).

Figure 5: Manure spiking with florfenicol. (A) Application via pipette; (B) stirring.



A B

If the effect of duration of anaerobic incubation in manure was investigated, the spiked manure was incubated for half-maximum (26.5 and 45 days for pig and cattle manure, respectively) and maximum storage duration (53 and 90 days for pig and cattle manure, respectively) under anaerobic conditions in the dark at 20 °C. The incubation was conducted in an anaerobic cabinet (Figure 7 A).

If the effect of aerobic incubation was investigated, the samples of spiked manure were added to a portion of test soil in a ratio of 44 g fresh mass manure to 400 g dry mass soil, and mixed thoroughly. The pre-mixture was added to the rest of test soil (1600 g dry mass) and mixed thoroughly. The final mixture was placed into containers of nonporous plastic and the respective amount of water needed to adjust the test substrate to 40 % water holding capacity was added. The mixture was incubated for half-maximum storage duration (26.5 and 45 days for pig and cattle manure, respectively) under aerobic conditions in the dark at 20 °C.

<u>Tests with tylosin tartrate at ECT</u>

Due to the good water solubility of tylosin tartrate, the test item was applied to the manure on a weight basis. The amount of solid test item required to obtain the desired concentrations was added to 50 mL beakers (Figure 6 A). Subsequently, the portion of manure (22 g fresh mass manure per kg soil) was added on a weight basis (Figure 6 B) and mixed thoroughly. When the substance was incubated in the manure, test substance and manure were weighed into screw cap bottles of brown glass and mixed thoroughly (Figure 7 B). The bottles were flushed with nitrogen and sealed.

Figure 6: Manure spiking with tylosintartrate. (A) Adding test substance on a weight basis; (B) adding manure.

A B

If the effect of duration of anaerobic incubation in manure was investigated, the spiked manure was incubated for half-maximum (26.5 and 45 days for pig and cattle manure, respectively) and maximum storage duration (53 and 90 days for pig and cattle manure, respectively) under anaerobic conditions in the dark at 20 °C. The incubation was conducted in screw cap bottles (Figure 7 B).

Figure 7: Incubation of spiked manure. (A) Anaerobic cabinet at IME; (B) screw cap bottles at ECT.

A B

If the effect of aerobic incubation was investigated, the samples of spiked manure were added to a portion of test soil in a ratio of 88 g fresh mass manure to approximately 800 g dry mass soil, and mixed thoroughly. The pre-mixture was added to the rest of test soil (3200 g dry mass) and mixed thoroughly. The final mixture was placed into containers of nonporous plastic and the respective amount of water needed to adjust the test substrate to approximately 40 % water holding capacity was added. The mixture was incubated for half-maximum storage duration (26.5 and 45 days for pig and cattle manure, respectively) under aerobic conditions in the dark at 20 °C.

7.1.6 Suitability to maintain anaerobic conditions during manure processing

To monitor if the applied methods are suitable to maintain anaerobic conditions during the whole manure processing, redox potential and pH was measured in the manure for the main tests at the sampling site, before acclimation, after acclimation, and after anaerobic incubation.

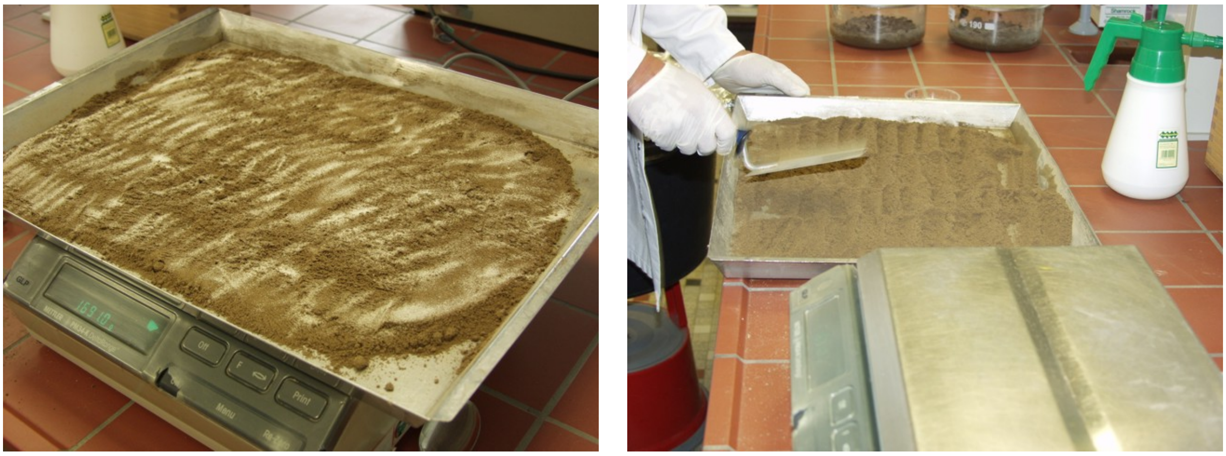
Table 3: Redox potential and pH during manure processing for main tests

Pig manure		**Cattle manure**	
Values at sampling site		**Values at sampling site**	
Redox potential:	-411 mV	Redox potential:	-345 mV
pH:	7.7	pH:	6.9
Before acclimation		Before acclimation	
Redox potential:	-388 mV	Redox potential:	-295 mV
pH:	7.6	pH:	6.9
After acclimation		After acclimation	
Redox potential:	-367 mV	Redox potential:	-327 mV
pH:	7.4	pH:	6.8
		After 45 days of anaerobic incubation in an anaerobic cabinet	
		Redox potential:	-397 mV
		pH:	7.8
After 53 days of anaerobic incubation in an anaerobic cabinet		After 90 days of anaerobic incubation in an anaerobic cabinet	
Redox potential:	-395 mV	Redox potential:	-238 mV
pH:	8.9	pH:	8.8

7.2 General test conditions for plant tests

The objective of the studies was the assessment of the effects of the test item on emergence and early stages of growth of terrestrial plant seedlings. Seeds of different terrestrial plants were sown in a natural soil substrate containing the test item in different concentrations without or with manure. A single application of test item was made. The kind of introduction of the test item to the soil depended on the respective test design. Test item was either applied directly to soil via an aqueous application solution (tylosin tartrate, standard test), via quartz sand (florfenicol, standard test), or via spiked manure. In any case, the carrier was distributed on the surface of the spreaded soil and mixed thoroughly. The way of introduction is shown exemplarily in Figure 8 and Figure 9.

Figure 8: Application of test item to soil. (A) Application of spiked quartz sand for Florfenicol; (B) mixing of quartz sand and soil.



A B

Figure 9: Application of manure to soil. (A) Application of spiked manure to a subsample of soil; (B) mixing manure and subsample of soil.

A B

The test was generally performed following the "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test" Guideline OECD 208 [7].

Containers of nonporous plastic with a diameter of approximately 100 mm, a height of 80 mm, and holes at the bottom were used. The containers were filled up with an amount of ca. 530 g moist test substrate (Figure 10). Containers were placed in saucers (Figure 11). The saucers were filled with water daily. Holes at the bottom of the containers ensure consistent soil moisture. Fertilizer (e.g. COMPO, Floragard or Substral Grünpflanzendünger) was applied in two halves during the test on the surface of the soil. The amount applied corresponds to approximately 30 mg total nitrogen, 15 mg phosphate and 30 mg potassium oxide per kg soil.

Figure 10: Plant test start. (A) Transfer of the test soil into a test vessel; (B) test vessels with a spike stamp to create reproducible pots.

A B

Seeds were planted in the vessels as soon as possible but at least at the same day the test item was introduced in the manure/soil. All seeds of each species for each test were of the same size class. The seeds were not imbibed. After sowing, the test substrate was moistened to 60 % water holding capacity. Starting with the next day, the saucers were filled with water daily.

The effects on emergence and growth of the seedlings were determined at least 14 days, but not longer than 21 days after 50 % of the seedlings have emerged in the relevant control (standard control in tests without manure; manure control in tests with manure).

Tests at the IME were performed in a glasshouse (Figure 11 A); tests at ECT in a phytotron (Figure 11 B). Climatic conditions were 22 °C ± 10 °C, 70 % ± 25 % air humidity and a light duration of ≥ 16 h per day with a light intensity of approximately 15,000 – 25,000 lx or 250 – 350 µE $m^{-2}*s^{-1}$ (IME) and ≥ 200 µE $m^{-2}*s^{-1}$ (ECT), respectively.

Figure 11: Plant test run. (A) Glasshouse at IME; (B) phytotron at ECT.

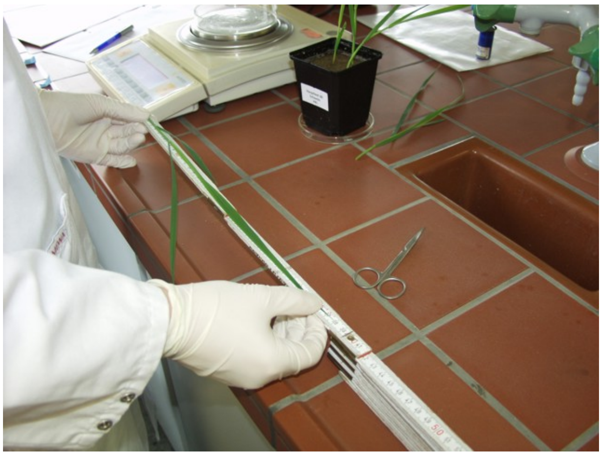
A

B

The 14 to 21-day-growth-phase started, when 50 % of the seedlings emerged in relevant control (standard control in tests without manure; manure control in tests with manure). This day was determined as "growth day 1". At harvest ("growth day” 14 – 21), all seedlings were counted and the shoot length and aboveground biomass were measured (Figure 12). For this, the shoot length and the fresh mass of each plant were measured individually immediately after harvesting. However, for evaluation, mean values per replicate were applied.

Figure 12: Harvesting at plant test end. (A) Cutting of shoots at soil surface; (B) shoot length measurement.

A



B

7.2.1 Test soil

The soil applied in all tests of the project was loamy sand from the UBA-founded RefeSol program [1], [13], [14]. The soil was sieved at 2 mm before application. The soil was not sterilized.

Table 4: Soil parameter of the test soil RefeSol 01-A.

Soil	Sand 2000 - 63 ↔m (%)	Silt 63 - 2 ↔m (%)	Clay < 2 ↔m (%)	Sand 2000 - 50 ↔m (%)	Silt 50 - 2 ↔m (%)	Clay < 2 ↔m (%)	Corg (%)	pH (0,01 M CaCl2)	CECeff (mmolc /kg)	WHCmax (g/kg)
	According to E DIN ISO 11277			According to USDA						
RefeSol 01-A	70	27	3	73	23	4	1.1	5.0	8.6	233

7.2.2 Plant seeds

The plant species used in the tests were *Allium cepa* (onion; Liliaceae) and *Triticum aestivum* (wheat; Poaceae), *Avena sativa* (oat; Poaceae), *Solanum lycopersicum* (tomato; Solanaceae), *Brassica napus* (oil seed rape; Brassicaceae), *Sinapis alba* (mustard; Brassicaceae), *Cucumis sativus* (cucumber; Cucurbitaceae), *Trifolium pratense* (red clover; Fabaceae), and *Phaseolus vulgaris* (common bean; Fabaceae), which are representatives for monocotyledonous, dicotyledonous, and legumes, respectively.

The species are recommended species according to the OECD 208 guideline.

Origin of the seeds:

Allium cepa: Amaryllidaceae	Enza Zaden Deutschland GmbH & Co. KG, Germany Cultivar Stuttgarter Riesen
Triticum aestivum: Poaceae	Nordsaat, Saatzuchtgesellschaft mbH, Granskevitz Germany.
Avena sativa: Poaceae	Raiffeisen Genossenschaft Nordwest eG, Germany
Solanum lycopersicum: Solanaceae	Enza Zaden Deutschland GmbH & Co. KG, Germany Cultivar Moneyberg
Brassica napus: Brassicaceae	KWS Saat AG, Germany Cultivar Kadore
Sinapis alba: Brassicaceae	Carl Sperling & Co. Lüneburg, Germany
Cucumis sativus: Cucurbitaceae	Enza Zaden Deutschland GmbH & Co. KG, Germany Cultivar Gurken Delikateß
Phaseolus vulgaris: Fabaceae	Enza Zaden Deutschland GmbH & Co. KG, Germany Cultivar Primel
Trifolium pratense: Fabaceae	Bruno Nebelung GmbH, Everswinkel, Germany.

7.3 Pre-tests for the evaluation of suitable plant species and manure concentration

Pre-tests with the eight plant species *Solanum lycopersicum* (tomato; Solanaceae), *Brassica napus* (oil seed rape; Brassicaceae), *Sinapis alba* (mustard; Brassicaceae), *Cucumis sativus* (cucumber; Cucurbitaceae), *Trifolium pratense* (red clover; Fabaceae), *Phaseolus vulgaris* (common bean; Fabaceae), *Allium cepa* (onion; Liliaceae) and *Triticum aestivum* (wheat; Poaceae) were conducted with five concentrations of manure applied to the soil.

The applied concentrations followed the maximum allowed amount of manure to be deployed in one year in Europe, corresponding to 170 kg N/ha. The five concentrations applied were 26, 42, 66, 106 und 170 kg N/ha, corresponding to approximately 7, 11, 18, 29 and 46 g manure (fresh mass) per kg soil (dry mass) based on the content of total nitrogen in the manure (Table 5) and assuming an incorporation depth of 0-5 cm and a soil density of 1.5 g/cm^3.

Table 5: Key parameter for manure characterization applied in pre-tests.

Pig manure			
Values at sampling site		Laboratory values	
pH:	7.79	Total nitrogen:	4.71 g/kg
Redox potential:	-374 mV	Dry mass content:	5 %
Temperature:	12.5 °C		
Cattle manure			
Values at sampling site		**Laboratory values**	
pH:	7.57	Total nitrogen:	5.11 g/kg
Redox potential:	-362 mV	Dry mass content:	10 %
Temperature:	5.8 °C		

For the pre-tests pH, redox potential and temperature at the sampling site, dry mass content at the start of acclimation, and total nitrogen content at the end of acclimation were determined. The results were in the expected range for liquid pig and cattle manure (Table 5).

The pre-tests verified that a maximum manure concentration representing 85 kg N/ha (1/2 maximum amount allowed per year in Europe) should not be exceeded to ensure an unimpaired germination, emergence and post-emergence survival. At least the seven plant species (*A. cepa* (onion), *A. sativa* (oat), *S. lycopersicum* (tomato), *B. napus* (rape), *P. vulgaris* (common bean), *S. alba* (mustard), and *T. pratense* (red clover)), representing monocots, dicots, and legumes, were grown successfully at this manure concentration.

All species tested (except of onion) showed significant impairment of emergence starting at pig manure concentrations of 106 kg N/ha (29 g manure/kg soil) or at the latest at 170 kg N/ha (46 g manure/kg soil). Oil seed rape, common bean, and red clover showed also increased post-emergence mortality at concentrations of 170 kg N/ha (46 g manure/kg soil). Shoot length and fresh mass were not impaired by pig manure up to and including a concentrations of 170 kg N/ha (46 g manure/kg soil). None Cattle manure had no negative effect on any of the species tested up to and including a concentrations of 170 kg N/ha (46 g manure/kg soil). In Figure 13 to Figure 20 exemplary results are presented for emergence rate and post-emergence survival for tomato, oil seed rape, mustard, and cucumber.

Figure 13: Emergence rate of tomato (A) and oil seed rape (B) depending on pig manure concentration.

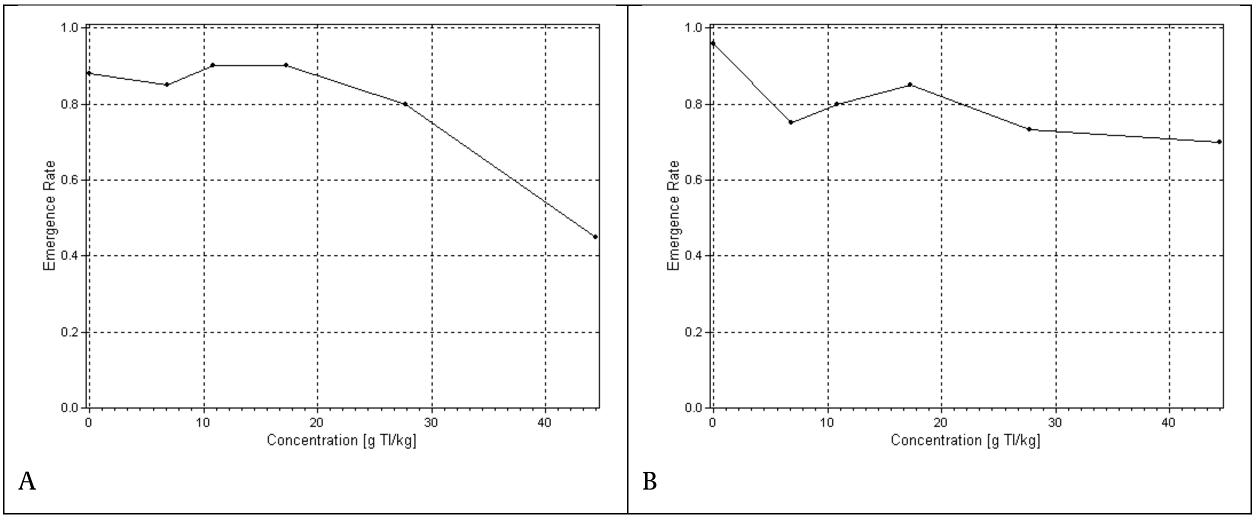


Figure 14: Emergence rate of mustard (A) and cucumber (B) depending on pig manure concentration.

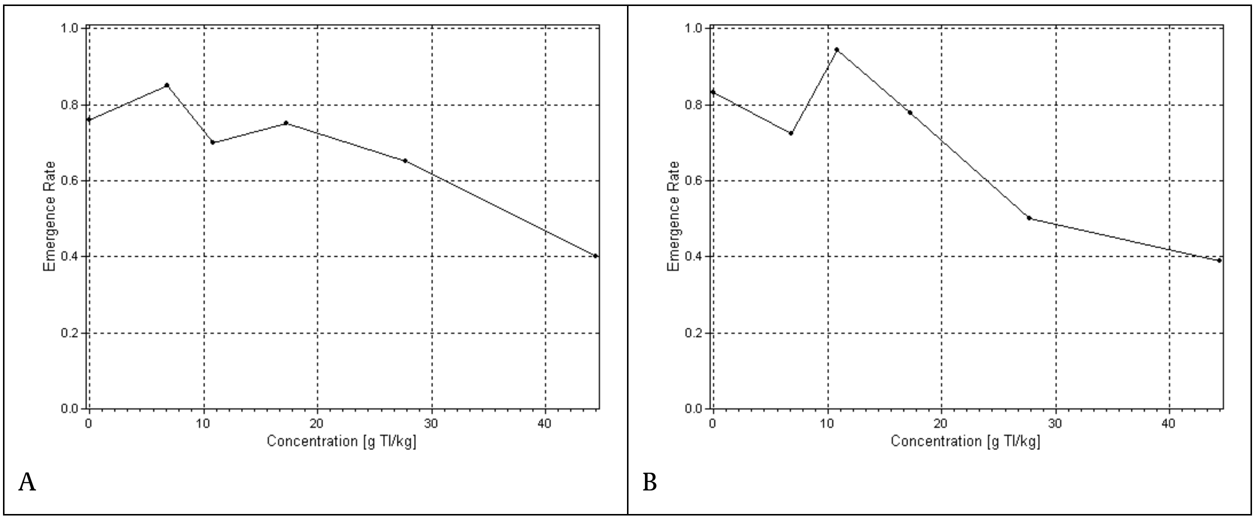


Figure 15: Post-emergence survival rate of tomato (A) and oil seed rape (B) depending on pig manure concentration.

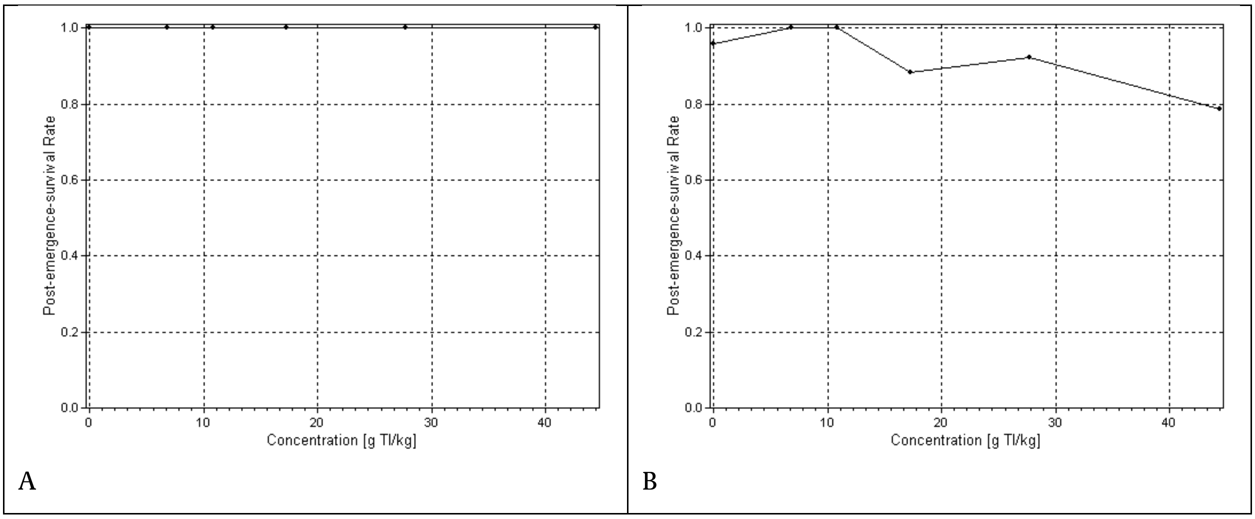


Figure 16: Post-emergence survival rate of mustard (A) and cucumber (B) depending on pig manure concentration.

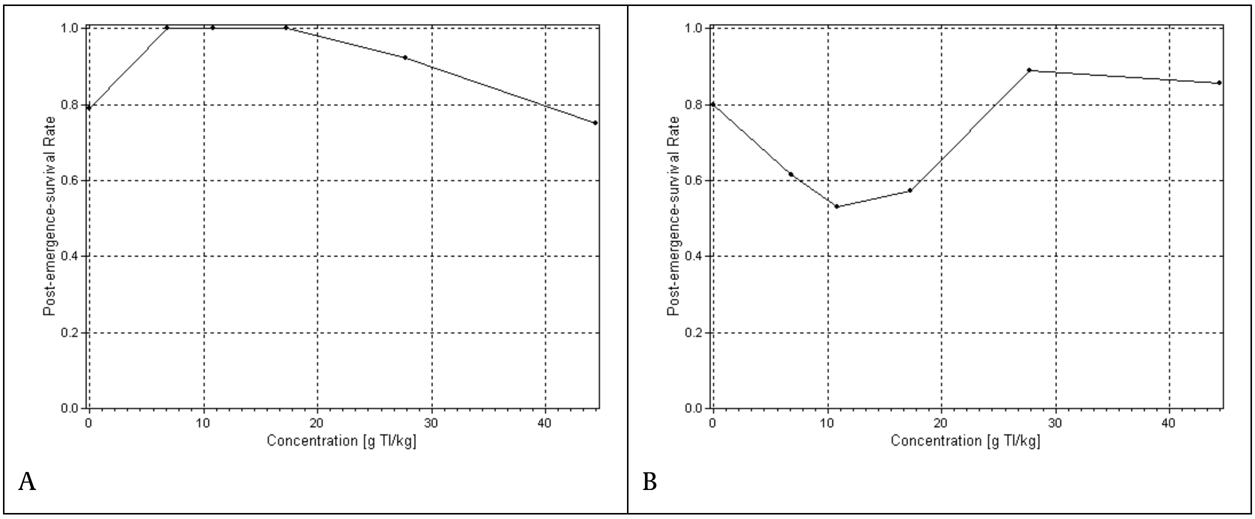


Figure 17: Emergence rate of tomato (A) and oil seed rape (B) depending on cattle manure concentration.

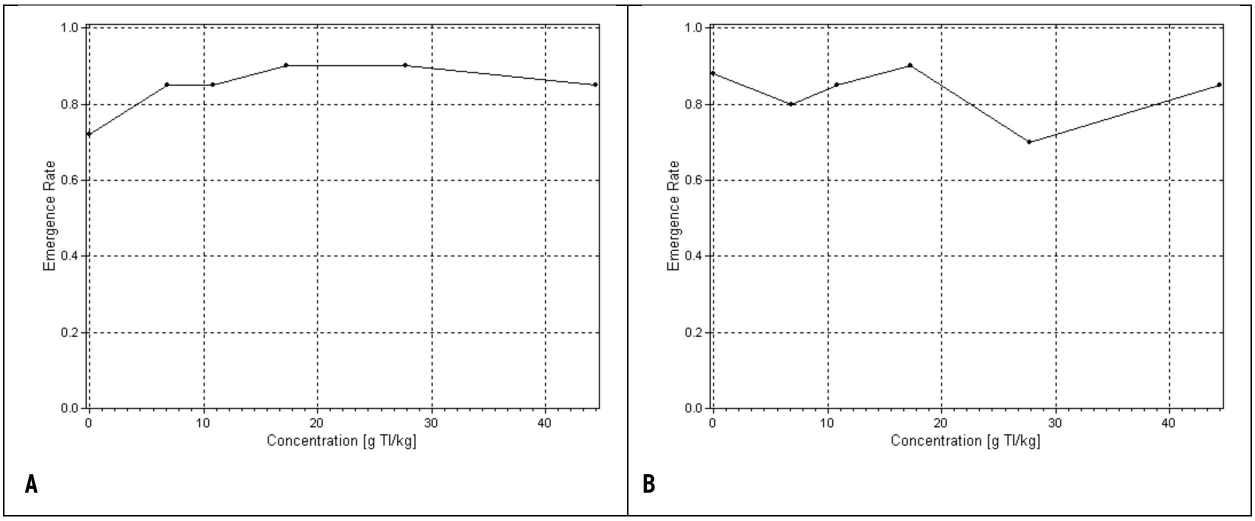


Figure 18: Emergence rate of mustard (A) and cucumber (B) depending on cattle manure concentration.

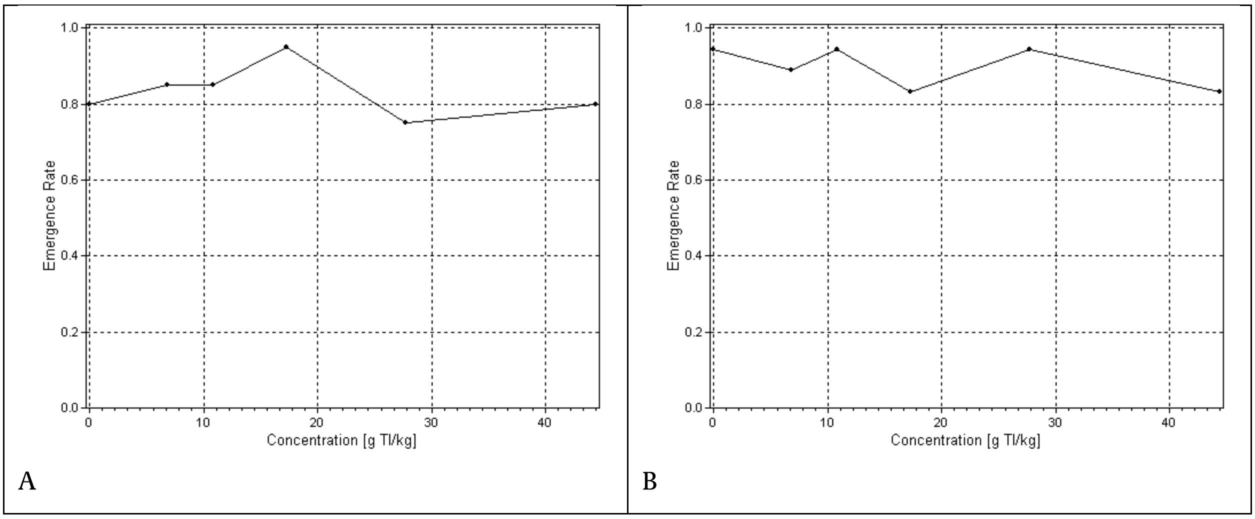


Figure 19: Post-emergence survival rate of tomato (A) and oil seed rape (B) depending on cattle manure concentration.

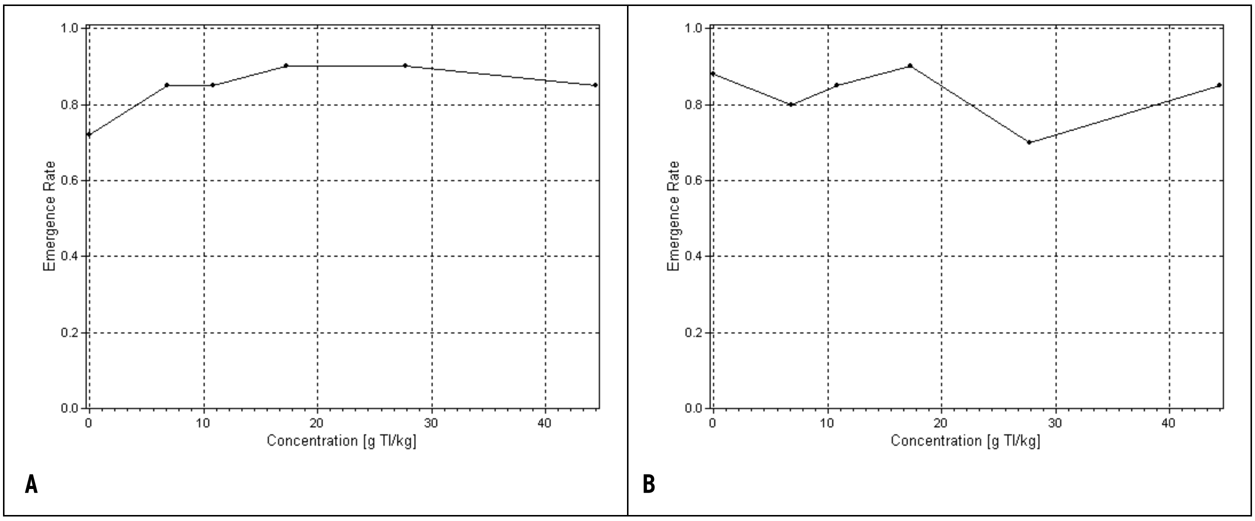
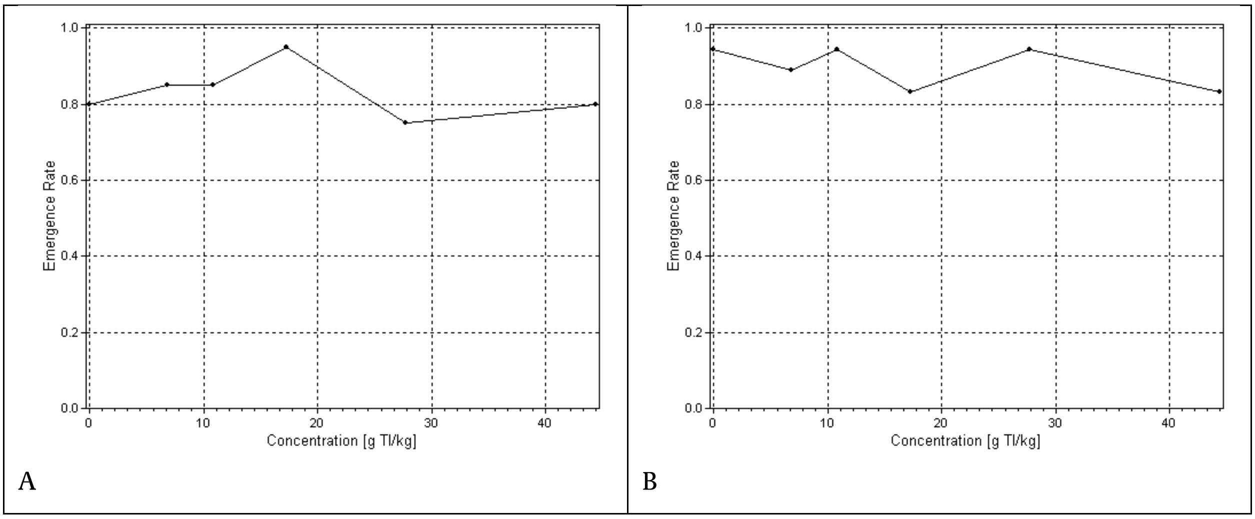


Figure 20: Post-emergence survival rate of mustard (A) and cucumber (B) depending on cattle manure concentration.



Under the chosen test conditions, cucumber (*Cucumis sativus*) showed generally a low survival rate – also in the control. Additionally, shoot length and fresh mass showed great variances within the replicates. Emergence rate of cucumber was impaired starting at pig manure concentrations of 106 kg N/ha (29 g manure/kg soil). Wheat (*Triticum aestivum*) generally showed a low emergence rate under the chosen test conditions – also in the control. Due to this, these species were not applied in further tests generally. To test a second monocot species, *Avena sativa* (oat; Poaceae) was used instead of wheat in the further tests without repetition of this pre-test. Oat is deemed to be an undemanding species in relation to soil and climatic requirements. Due to general problems regarding emergence of red clover (*Trifolium pratense*) at IME, this species was not applied in further tests with florfenicol (conducted at IME). Due to general problems regarding emergence of mustard (*Sinapis alba*) at ECT, this species was not applied in further tests with tylosin tartrate (conducted at ECT).

The respective six plant species applied in the further tests were:

- Tests with florfenicol (IME):

  Allium cepa (onion), *Avena sativa* (oat), *Solanum lycopersicum* (tomato), *Brassica napus* (oil seed rape), *Phaseolus vulgaris* (common bean), *Sinapis alba* (mustard)

- Tests with tylosin tartrate (ECT):

  Allium cepa (onion), *Avena sativa* (oat), *Solanum lycopersicum* (tomato), *Brassica napus* (oil seed rape), *Phaseolus vulgaris* (common bean), *Trifolium pratense* (red clover)

After discussion with the UBA, the following test series regarding the effect of veterinary antibiotics on plants were performed with a manure concentration of approximately 85 kg N/ha (22 g manure/kg soil) due to the impairment on plant emergence and growth starting at concentrations of 106 kg N/ha (29 g manure/kg soil). However, since the dry matter content of manure is expected to have more influence on the effect of veterinary medicinal products in plant tests than the nitrogen content, it was also decided that the test series should be performed with constant manure originated dry matter concentration instead of nitrogen concentration per kg soil. Consequently, all further tests were performed with 22 g manure (fresh mass)/kg soil (dry mass), independent from total nitrogen concentration in the manure applied.

7.4 Test items

The test items were provided by a commercial supplier (LKT Laboratories, Inc. and Sigma Aldrich, respectively) before the start of the study. Test item information was given by the supplier.

7.4.1 Test item 1

Test item name:	Florfenicol
Chemical name:	2,2-Dichlor-N-((1R,2S)-3-fluor-1-hydroxy-1-(4-(methylsulfonyl)-phenyl)-propan-2-yl)-ethanamid
CAS-Number:	76639-94-6
Lot/Batch Number:	23922307
Purity:	99.7 %
Water solubility:	Low soluble
State of matter and appearance:	White or almost white powder
Origin of test item:	LKT Laboratories, Inc.
Expiry date:	August 31, 2013 (expert judgement)
Storage conditions:	Container kept tightly closed in a cool, well-ventilated area.

7.4.2 Test item 2

Test item name:	Tylosin tartrate
Sigma Aldrich product number:	Sigma Aldrich product number
CAS-Number:	1405-69-0 (Tylosin)
Lot/Batch Number:	050M1205V
Units/mg (nominal):	≥800
Units/mg (nominal):	919
Water solubility:	Soluble in water
State of matter and appearance:	White to light yellow powder
Origin of test item:	Sigma-Aldrich
Expiry date:	May 2013
Storage conditions:	2 - 8°C

7.5 Main test manure, key parameter

In the main tests with modified test designs to investigate the influence of i) the duration of anaerobic incubation in manure, ii) the way of application, and iii) the incubation conditions (anaerobic/aerobic) on the effects of the two antibiotics pig and cattle manure was applied.

In the following, the key parameters at the sampling site, after storage, and after acclimation are listed.

7.5.1 Pig manure

7.5.1.1 Manure characterization on sampling site

Temperature:	18.0 °C
pH:	7.7
redox potential:	- 410 mV

7.5.1.2 Manure characterization after storage

Temperature:	8.0 °C
pH:	7.6
redox potential:	- 388 mV
Dry matter content (originally):	11.8 %
Dry matter content (after processing):	4.8 %

7.5.1.3 Manure characterization after acclimation

Temperature:	20.0 °C
pH:	7.4
redox potential:	- 367 mV
dry matter content:	4.8 %
NH_4-N:	1740 mg/kg fresh mass
Nonvolatile nitrogen:	1258 mg/kg fresh mass
Total nitrogen (calculated):	2998 mg/kg fresh mass
Organic carbon:	17380 mg/kg fresh mass
Total P content:	1320 mg/kg fresh mass
P_2O_5 equivalents:	3024 mg/kg fresh mass
Total Cu content:	14 mg/kg fresh mass
Microbial activity (in 7 days):	36 % glucose degradation (32 % CO_2, 4 % CH_4)

7.5.2 Cattle manure

7.5.2.1 Manure characterization on sampling site

Temperature:	11.0 °C
pH:	6.9
redox potential:	- 345 mV

7.5.2.2 Manure characterization after storage

Temperature:	8.0 °C
pH:	6.9
redox potential:	- 295 mV
Dry matter content (originally):	9.8 %
Dry matter content (after processing):	9.6 %

7.5.2.3 Manure characterization after acclimation

Temperature:	20.0 °C
pH:	6.8
redox potential:	- 327 mV
dry matter content:	9.6 %
NH_4-N:	1500 mg/kg fresh mass
Nonvolatile nitrogen:	2102 mg/kg fresh mass
Total nitrogen (calculated):	3602 mg/kg fresh mass
Organic carbon:	39725 mg/kg fresh mass
Total P content:	820 mg/kg fresh mass
P_2O_5 equivalents:	1877 mg/kg fresh mass
Total Cu content:	20 mg/kg fresh mass
Microbial activity (in 7 days):	59 % glucose degradation (50 % CO_2, 9 % CH_4)

7.6 Standard tests according to OECD 208

Tests according to the OECD 208 standard test design without manure application were performed as a benchmark.

7.6.1 Tests with florfenicol

7.6.1.1 Range Finding Tests

Range finding tests were performed. EC_{50} values of the most sensitive endpoint biomass were in the range of 0.20 – 1.83 mg/kg showing rape to be the most sensitive species.

Table 6: Florfenicol Standard Test Range Finder. Relevant EC_{50} values (biomass) according to the OECD 208 standard test design.

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Sinapis alba*
EC_{50}	1.16 mg/kg	1.36 mg/kg	0.20 mg/kg	0.59 mg/kg	0.25 mg/kg	1.83 mg/kg

7.6.1.2 Main tests

The nominal concentration in the test containers with test item was 0.06, 0.19, 0.56, 1.67, and 5.0 mg test item/kg dry mass soil. Five seeds (onion, oat, rape, mustard, and tomato) and four seeds (common bean), respectively, were planted in each replicate right after incorporation of the test item. For vessels with five and three seeds, four and five replicates were prepared, respectively. The total number of seeds applied per treatment was 20.

The quantity of test item required to obtain the desired concentrations was dissolved in acetone and mixed with a portion of quartz sand (10 g per kg test substrate). After evaporation of the solvent, the mixture and the respective amount of water needed to adjust the test substrate to 40 % water holding capacity was added to the test soil, placed into the test container and mixed thoroughly.

As the test item was applied to quartz sand using a solvent, a control reflecting solvent-mediated application followed by evaporation was conducted. Since it is known from experience that acetone is evaporated overnight completely and no adverse effect due to the solvent occur, no additional standard control was applied. Number of replicates and seeds per replicate were the same as for the treatments.

The general test conditions are listed in chapter 7.2.

With 16 – 32 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 280 – 400 µE/m2/s the permitted value of 200 – 400 µE/m2/s (OECD 208) was maintained. With 25 – 88 % the proposed range of 70 ± 25 % for air humidity (OECD 208) was under run sometimes. However, the growth of the control plants was proper and as expected.

To verify the test item concentration at the start of the plant test, samples of spiked soil at a representative test item concentration were measured. The recovery rate was 85 % florfenicol. Evaluation was done by using nominal concentrations.

The only obvious effect of the test item on the visual appearance regarding pathological symptoms of the seedlings was a partly discoloration of leafs in mustard, rape, and tomato.

There was no or only a slight concentration dependent effect on seedling emergence of *Allium cepa*, *Avena sativa*, and *Brassica napus* up to and including 5.00 mg florfenicol per kg dry soil, the highest concentration tested. Emergence of *Sinapis alba*, *Solanum lycopersicum*, and *Phaseolus vulgaris* was clearly affected by Florfenicol. The lowest NOEC with < 0.06 mg/kg dry soil was found for *S. lycopersicum*. The lowest EC_{10} with 0.01 mg/kg was calculated for B. napus, the lowest EC_{50} with 1.46 mg/kg for *S. alba*.

There was no or only a slight concentration dependent effect on post-emergence survival of *Avena sativa* and *Phaseolus vulgaris* up to and including 5.00 mg florfenicol per kg dry soil, the highest concentration tested. Post-emergence survival of *Allium cepa*, *Brassica napus*, *Sinapis alba*, and *Solanum lycopersicum* was clearly affected by florfenicol. The most sensitive species regarding post-emergence survival was *S. alba* with a NOEC of 0.56 mg/kg, an EC_{10} of 0.24 mg/kg, and an EC_{50} of 0.60 mg/kg dry soil.

There were clear concentration dependent effects on growth of all species tested due to florfenicol.

The most sensitive species regarding shoot length was *B. napus* with a NOEC of 0.06 mg/kg, an EC_{10} of 0.06 mg/kg, and an EC_{50} of 0.93 mg/kg dry soil. Also for fresh mass, *B. napus* was the most sensitive species with a NOEC of < 0.06 mg/kg, an EC_{10} of 0.05 mg/kg, and an EC_{50} of 0.25 mg/kg dry soil.

The NOEC, EC_{10} and EC_{50} values of florfenicol for emergence, growth, and post-emergence survival are presented in Table 7. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 63 to Table 70.

Table 7: Florfenicol Standard Test. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Sinapis alba*	*Solanum lycopersicum*	*Phaseolus vulgaris*
Emergence						
NOEC	✣ 5.00	✣ 5.00	✣ 5.00	0.56	< 0.06	1.67
EC_{10}	> 5.00	0.34	0.01	0.12	n.d.	0.57
EC_{50}	n.d.	> 5.00	> 5.00	1.46	> 5.00	> 5.00
Post-emergence survival						
NOEC	1.67	✣ 5.00	1.67	0.56	0.56*	✣ 5.00
EC_{10}	0.35	2.28	0.71	0.24	0.43	n.d.
EC_{50}	4.82	> 5.00	2.71	0.60	3.43	n.d.
Shoot length						
NOEC	0.19	✣ 5.00	0.06	0.19	0.56	0.56
EC_{10}	0.16	> 5.00	0.06	0.14	0.25	0.74
EC_{50}	2.57	n.d.	0.93	0.60	1.83	2.86
Fresh mass						
NOEC	0.06	5.00	< 0.06	0.19	0.56	0.56
EC_{10}	0.06	0.58	0.05	0.07	0.46	0.76
EC_{50}	0.75	> 5.00	0.25	0.32	0.76	2.59

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. * NOEC ✣ 5.00 mg/kg by ToxRat due to great variances in the highest treatment. Realistic NOEC is set on 0.56 mg/kg by the study director. Concentrations given as nominal values.

Conclusion:

Florfenicol has clear concentration depending effects on terrestrial plants under the chosen test conditions. The relevant NOEC for the most sensitive endpoint (biomass of *Brassica napus*) was found to be < 0.06 mg/kg dry soil. The EC_{10} and EC_{50} were calculated to be 0.05 mg/kg dry soil and 0.25 mg/kg dry soil, respectively.

7.6.2 Tests with tylosin tartrate

7.6.2.1 Range Finding Tests

Range finding tests were performed. EC_{50} values of the most sensitive endpoint biomass were in the range of 23.5 – 433.7 mg/kg showing red clover to be the most sensitive species.

Table 8: Tylosin tartrate Standard Test Range Finder. Relevant EC_{50} values (biomass) according to the OECD 208 standard test design.

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Trifolium pratense*
EC_{50}	n.d.	660.8 mg/kg	57.2 mg/kg	98.0 mg/kg	360.7 mg/kg	n.d. mg/kg

n.d. = not determined due to mathematical reasons.

7.6.2.2 Main tests

Five test item concentrations per species were tested against an untreated control (Table 9). Test concentrations had been specified in a previous range-finding test. The number of replicates was eight per treatment and species.

A stock solution (SL1) was prepared by dissolving 12.8017 g of the test item in one litre of deionised water. A second stock solution (SL2) was prepared by a 1:10 dilution of SL1. Test solutions (TL) were obtained by diluting defined volumes of the respective stock solution (SL1 or SL2) with deionised water (Table 10).

A defined volume of the appropriate test solution was mixed into the bulk soil for the respective treatment.

The untreated control soil was mixed with the same volume of deionised water.

After mixing the test solution into the bulk soil for the treatment, the soil was distributed to the plant pots and seeds were sown.

The general test conditions are listed in chapter 7.2.

The test plants were cultivated in air conditioned rooms equipped with high pressure metal halide lamps (Master HPI-T PLUS, Philips GmbH, Hamburg, Germany).

Air temperature ranged from 22 to 24 °C and was within the required range of 22 °C ± 10 °C (OECD 208). The light conditions were ≥200 µE/m^2/s; relative air humidity ranged from 38 – 50%, hence it was sometimes below the required range of 70 ± 25 % (OECD 208). However, the control plants grew healthy.

No verification of test item concentration was applied. Evaluation was done by using nominal concentrations.

Table 9: Treatment code, test item concentration in soil [mg/kg soil dry weight] and species in the definitive test.

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	X	X	X	X	X	X
T1	9.3	---	---	---	---	---	X
T2	16.0	---	---	X	---	---	X
T3	27.8	X	---	X	---	---	X
T4	36.1	---	---	---	X	---	---
T5	48.1	X	---	X	---	---	X
T6	50.0	---	X	---	---	X	---
T7	51.0	---	---	---	X	---	---
T8	72.2	---	---	---	X	---	---
T9	83.3	X	---	X	---	---	X
T10	100.0	---	X	---	---	X	---
T11	102.1	---	---	---	X	---	---
T12	144.3	X	---	X	X	---	---
T13	200.0	---	X	---	---	X	---
T14	250.0	X	---	---	---	---	---
T15	400.0	---	X	---	---	X	---
T16	800.0	---	X	---	---	X	---

Respective test item concentration tested (X) or not tested (---).

The only obvious effect of the test item on the visual appearance regarding pathological symptoms of the seedlings was a partly discoloration (chlorosis) of leafs in all species.

The test item Tylosin tartrate had no adverse effect on seedling emergence at concentrations up to and including 83.3 mg/kg soil dry weight (*T. pratense*), 144 mg/kg soil dry weight (*B. napus, S. lycopersicum*), 250 mg/kg soil dry weight (*A. cepa*) and 800 mg/kg soil dry weight (*A. sativa* and *P. vulgaris*).

The lowest observed concentration to increase seedling mortality was 83.3 mg/kg soil dry weight (*T. pratense*). For *A. cepa* and *B. napus* it was 144 mg/kg soil dry weight

The overall lowest EC_{50} in this study was 23.5 mg/kg soil dry weight and was observed with *T. pratense* for the end point shoot fresh weight.

The overall lowest NOEC and EC_{10} in this the study was 16.0 mg/kg soil dry weight and 7.7 mg/kg soil dw and was observed with *T. pratense* for shoot fresh weight.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate for emergence, growth, and post-emergence survival are presented in Table 11. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 71 to Table 78.

Table 10: Preparation of test solutions through dilutions of stock solutions to adjust test item concentrations in soil [mg/kg soil dry weight] for the different treatments.

Code	Test item [mg/kg soil]	Type of SL used to prepare TL	Volume of SL to prepare the TL [mL]	Volume of TL after completion with deionised water [mL]	Soil per treatment [kg dw]	Soil per treatment [kg fw]
C	0.0	n.a.	n.a.	n.a.	12.00	12.492
T1	9.3	SL2	14.5	200	2.00	2.082
T2	16.0	SL2	50	250	4.00	4.164
T3	27.8	SL2	130.3	500	6.00	6.246
T4	36.1	SL2	56.4	200	2.00	2.082
T5	48.1	SL2	225.5	500	6.00	6.246
T6	50.0	SL2	156.3	250	4.00	4.164
T7	51.0	SL1	8.0	200	2.00	2.082
T8	72.2	SL1	11.3	200	2.00	2.082
T9	83.3	SL1	39.0	500	6.00	6.246
T10	100.0	SL1	31.3	250	4.00	4.164
T11	102.1	SL1	16.0	200	2.00	2.082
T12	144.3	SL1	67.6	500	6.00	6.246
T13	200.0	SL1	62.5	250	4.00	4.164
T14	250.0	SL1	39.1	200	2.00	2.082
T15	400.0	SL1	125.0	250	4.00	4.164
T16	800.0	SL1	250.0	250	4.00	4.164

n.a. = not applicable; dw = dry weight; fw = fresh weight; TL = test solution; SL = stock solution.

Table 11: Tylosin tartrate Standard Test. NOEC, EC_{10} and EC_{50} values of tylosin tartrate for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Trifolium pratense*
Emergence						
NOEC	≥250	≥800	≥144	≥144	≥800	≥83.3
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Post-emergence survival						
NOEC	83.3	≥800	83.3	≥144	≥800	48.1
EC_{10}	71.2	n.d.	13.7	103	n.d.	28.1
EC_{50}	135	n.d.	82.6	n.d.	n.d.	57.6
Shoot length						
NOEC	27.8	≥800	48.1	102	≥800	16.0
EC_{10}	n.d.	565	54.9	63.2	32.8	11.7
EC_{50}	82.9	n.d.	96.0	127	434	49.8
Fresh mass						
NOEC	27.8	200	48.1	≥144	≥800	16.0
EC_{10}	n.d.	113	35.2	45.4	n.d.	7.7
EC_{50}	41.3	603	61.9	74.9	107	23.5

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

Conclusion:

Tylosin tartrate has clear concentration depending effects on terrestrial plants under the chosen test conditions. The relevant NOEC for the most sensitive endpoint (biomass of *Trifolium pratense*) was found to be 16 mg/kg dry soil. The EC_{10} and EC_{50} were calculated to be 7.7 mg/kg dry soil and 23.5 mg/kg dry soil, respectively.

7.7 Modified plant tests with fresh spiked manure

To mimic the realistic exposure way of veterinary antibiotics, the test item was applied via manure to the soil. To investigate if the sequential arrangement of application has an influence on possible effects on plant growth, tests were conducted with fresh spiked manure and additional assays with the two most sensitive plant species where the test item was applied directly to the soil with a subsequent manure application.

Test item application to processed manure was done according to 7.1. For test item application directly to the soil see 7.2. Subsequent manure application followed 7.1. After mixing of test item, manure, and soil, an OECD 208 test was applied (7.2). Seeds were planted in each replicate as soon as possible but at least at the same day the test item was introduced in the manure/soil.

7.7.1 Tests with florfenicol

7.7.1.1 Range Finding Tests

Range finding limit-tests were performed indicating adverse effects up to 25 % and 70 % in pig and cattle manure, respectively, at a concentration of 2 mg/kg dry soil. Tests revealed onion and mustard to be the most sensitive species. However, due to experience and after discussion with the UBA, onion (*A. cepa*) and oil seed rape (*B. napus*) were chosen for the additional approaches regarding differing sequential arrangement of application.

Table 12: Florfenicol Fresh Spiked Manure Range Finder. Percentage effects (% inhibition) on the most sensitive endpoint biomass when compared with control in a test design with spiked manure.

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Sinapis alba*
Pig manure % effect at 2 mg TI/kg *(1 mg TI/kg)	-28.7	7.0	16.2*	25.2	8.5*	5.1
Cattle manure % effect at 2 mg TI/kg *(1 mg TI/kg)	72.4	26.9	3.0*	66.6	-20.8*	40.4

7.7.1.2 Main tests, general approach

The nominal concentrations in the test containers with test item were 0.2, 0.6, 1.9, 5.6, and 16.7 mg test item/kg dry mass soil. Five seeds (onion, oat, rape, mustard, and tomato) and four seeds (common bean), respectively, were planted in each replicate immediately after incorporation of the test item. For approaches with manure, eight replicates were prepared, independent from the number of seeds per replicate. The total number of seeds applied per treatment was 32 - 40. For the standard control without manure, for vessels with five and four seeds, four and five replicates were prepared, respectively. The total number of seeds applied per treatment was 20.

As the test item was applied via manure or quartz sand using a solvent, a control reflecting solvent-mediated application to manure followed by evaporation was conducted (7.1.5). Since it is known from experience that no adverse effect due to the solvent occurs (pre-tests, data not shown) and since the combination of solvent spiked manure is seen as the carrier of the test item, no additional manure control without solvent was applied. Number of replicates and seeds per replicate were the same as for the treatments.

The general test conditions are listed in chapter 7.2.

7.7.1.3 Main tests with pig manure

With 19 – 28 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 135 – 242 µE/m²/s the permitted value of 200 – 400 µE/m²/s (OECD 208) was under run sometimes. With 35 – 70 % the proposed range of 70 ± 25 % for air humidity (OECD 208) was under run sometimes. However, the growth of the control plants was proper and as expected.

Test item concentration was verified in the highest application solution. The recovery was found to be within 85 – 95 % of nominal. To verify the test item concentration at the start of the plant test, samples of spiked manure and spiked soil/manure mixture at a representative test item concentration were measured. The recovery rate for spiked manure and spiked soil/manure mixture was 93 % and 98 % florfenicol, respectively. Evaluation was done by using nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

The only obvious effect of the test item on the visual appearance regarding pathological symptoms of the seedlings was a partly discoloration of leafs in mustard, rape and tomato.

There was no or only a slight concentration dependent effect on seedling emergence of *Allium cepa, Avena sativa*, *Brassica napus,* and *Sinapis alba* up to and including 16.7 mg Florfenicol per kg dry soil, the highest concentration tested. Emergence of *Solanum lycopersicum*, and *Phaseolus vulgaris* was clearly affected by Florfenicol. The most sensitive species regarding emergence was *P. vulgaris* with a NOEC of 0.6 mg/kg, an EC_{10} of 0.18 mg/kg, and an EC_{50} of 15.18 mg/kg dry soil.

There were clear concentration dependent effects on post-emergence survival of all plant species tested due to Florfenicol. The most sensitive species regarding post-emergence survival was *A. cepa* with a NOEC of 0.6 mg/kg, an EC_{10} of 0.13 mg/kg, and an EC_{50} of 0.44 mg/kg dry soil.

There were clear concentration dependent effects on growth of all species tested due to Florfenicol. The most sensitive species regarding shoot length and fresh mass was *S. alba* with a fresh mass NOEC of < 0.2 mg/kg, an EC_{10} of 0.08 mg/kg and an EC_{50} of 0.22 mg/kg dry soil.

Table 13: Florfenicol Fresh Spiked Pig Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Sinapis alba*	*Solanum lycopersicum*	*Phaseolus vulgaris*
Emergence						
NOEC	✣ 16.7	✣ 16.7	✣ 16.7	✣ 16.7	5.6	0.6
EC_{10}	4.77	n.d.	0.69	3.53	3.54	0.18
EC_{50}	n.d.	n.d.	n.d.	n.d.	10.62	15.18
Post-emergence survival						
NOEC	0.6	5.6	0.2	1.9	0.2	1.9
EC_{10}	0.13	7.94	0.21	0.61	0.12	2.35
EC_{50}	0.44	n.d.	0.48	1.35	0.80	5.52
Shoot length						
NOEC	0.6	0.6	< 0.2	< 0.2	0.2	0.2
EC_{10}	0.48	0.60	0.16	0.09	0.17	0.38
EC_{50}	0.79	8.90	0.48	0.43	0.92	2.86
Fresh mass						
NOEC	✣ 1.9	0.6	< 0.2	< 0.2	0.2	< 0.2
EC_{10}	0.43	0.15	0.08	0.08	0.33	0.07
EC_{50}	0.73	1.37	0.21	0.22	0.47	1.00

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values.

The NOEC, EC_{10} and EC_{50} values of florfenicol when spiked to fresh manure with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 13. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 79 to Table 82 and Table 87 to Table 90.

The sequential arrangement of application has no influence on the effects on seedling emergence, growth, or post-emergence survival. The NOEC, EC_{10} and EC_{50} values for *A. cepa* and B. *napus* are comparable, independent if test item spiked manure is applied to soil or manure is applied to test item spiked soil.

The NOEC, EC_{10} and EC_{50} values of florfenicol when spiked to soil with subsequent application of fresh manure for emergence, growth, and post-emergence survival are presented in Table 13. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 83 to Table 86 and Table 91 Table 94

Table 14: Florfenicol Fresh Spiked Soil with Pig Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Brassica napus*	Species	*Allium cepa*	*Brassica napus*
Emergence			Shoot length		
NOEC	✣ 16.7	✣ 16.7	NOEC	✣ 1.9	< 0.20
EC_{10}	9.93	1.13	EC_{10}	1.31	n.d.
EC_{50}	n.d.	> 16.7	EC_{50}	2.29	0.78
Post-emergence survival			Fresh mass		
NOEC	0.6	0.6	NOEC	0.6	< 0.20
EC_{10}	0.14	0.33	EC_{10}	0.78	n.d.
EC_{50}	0.75	0.91	EC_{50}	1.52	0.20

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values.

Conclusion:

The pig manure applied had no adverse effects on seedling emergence, growth, or post-emergence survival. Florfenicol has clear concentration depending effects on terrestrial plants under the chosen test conditions. The sequential arrangement of application has no influence on the effects. The relevant NOEC for the most sensitive endpoint (biomass of *Sinapis alba*) was found to be < 0.2 mg/kg dry soil. The EC_{10} and EC_{50} were calculated to be 0.08 mg/kg dry soil and 0.22 mg/kg dry soil, respectively.

7.7.1.4 Main tests with cattle manure

With 19 – 28 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 135 – 242 μE/m^2/s the permitted value of 200 – 400 μE/m^2/s (OECD 208) was under run sometimes. With 35 – 70 % the proposed range of 70 ± 25 % for air humidity (OECD 208) was under run sometimes. However, the growth of the control plants was proper and as expected.

Test item concentration was verified in the highest application solution. The recovery was found to be within 94 – 110 % of nominal. To verify the test item concentration at the start of the plant test, samples of spiked manure and spiked soil/manure mixture at a representative test item concentration were measured. The recovery rate for spiked manure and spiked soil/manure mixture was 98 % and 88 % florfenicol, respectively. Evaluation was done by using the nominal concentrations of the application solution.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

The only obvious effect of the test item on the visual appearance regarding pathological symptoms of the seedlings was a partly discoloration of leafs in mustard, rape, tomato, and common bean.

There was no or only a slight concentration dependent effect on seedling emergence of all species tested up to and including 16.7 mg Florfenicol per kg dry soil, the highest concentration tested. The most sensitive species regarding emergence was *Phaseolus vulgaris* with a NOEC of ≥ 16.7 mg/kg, an EC_{10} of 1.20 mg/kg, and an EC_{50} of > 16.7 mg/kg dry soil.

There were clear concentration dependent effects on post-emergence survival of all plant species tested due to Florfenicol. The most sensitive species regarding post-emergence survival was *Brassica napus* with a NOEC of 0.2 mg/kg, an EC_{10} of 0.35 mg/kg, and an EC_{50} of 0.1.04 mg/kg dry soil.

There were clear concentration dependent effects on growth of all species tested due to Florfenicol. The most sensitive species regarding shoot length and fresh mass was *B. napus* with a fresh mass NOEC of < 0.2 mg/kg, an EC_{10} of 0.07 mg/kg and an EC_{50} of 0.20 mg/kg dry soil.

The NOEC, EC_{10} and EC_{50} values of Florfenicol when spiked to fresh manure with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 15. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 95 to Table 98 and Table 103 to Table 106.

Table 15: Florfenicol Fresh Spiked Cattle Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Sinapis alba*	*Solanum lycopersicum*	*Phaseolus vulgaris*
Emergence						
NOEC	✣ 16.7	✣ 16.7	✣ 16.7	✣ 16.7	✣ 16.7	✣ 16.7
EC_{10}	12.21	n.d.	3.60	n.d.	8.04	1.20
EC_{50}	> 16.7	n.d.	n.d.	n.d.	n.d.	> 16.7
Post-emergence survival						
NOEC	0.6	✣ 16.7	0.2	0.2	0.2	1.9
EC_{10}	0.41	16.25	0.35	0.30	0.22	1.24
EC_{50}	1.75	n.d.	1.04	1.13	4.66	> 16.7
Shoot length						
NOEC	0.2	0.2	< 0.2	< 0.2	0.2	0.6
EC_{10}	0.46	1.52	0.08	0.09	0.43	0.60
EC_{50}	1.31	> 16.7	0.61	0.16	2.79	3.31
Fresh mass						
NOEC	0.2	0.2	< 0.2	< 0.2	0.2	< 0.2
EC_{10}	0.30	0.56	0.07	0.06	0.24	0.08
EC_{50}	0.84	6.34	0.20	0.22	0.96	1.18

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values.

The sequential arrangement of application has no influence on the effects on seedling emergence, growth, or post-emergence survival. The NOEC, EC_{10} and EC_{50} values for *A. cepa* and B. *napus* are comparable, independent if test item spiked manure is applied to soil or manure is applied to test item spiked soil.

The NOEC, EC_{10} and EC_{50} values of florfenicol when spiked to soil with subsequent application of fresh manure for emergence, growth, and post-emergence survival are presented in Table 16 Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 99 to Table 102 and Table 107 to Table 110.

Table 16: Florfenicol Fresh Spiked Soil with Cattle Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Brassica napus*	Species	*Allium cepa*	*Brassica napus*
Emergence			Shoot length		
NOEC	✥ 16.7	✥ 16.7	NOEC	0.2	< 0.20
EC_{10}	2.68	n.d.	EC_{10}	0.34	0.07
EC_{50}	> 16.7	n.d.	EC_{50}	1.25	0.59
Post-emergence survival			Fresh mass		
NOEC	1.9	0.6	NOEC	< 0.20	< 0.20
EC_{10}	0.58	0.35	EC_{10}	0.18	0.06
EC_{50}	1.96	1.22	EC_{50}	0.73	0.17

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values.

Conclusion:

The cattle manure applied had no adverse effects on seedling emergence, growth, or post-emergence survival. Florfenicol has clear concentration depending effects on terrestrial plants under the chosen test conditions. The sequential arrangement of application has no influence on the effects. The relevant NOEC for the most sensitive endpoint (biomass of *Brassica napus*) was found to be < 0.2 mg/kg dry soil. The EC_{10} and EC_{50} were calculated to be 0.06 mg/kg dry soil and 0.17 mg/kg dry soil, respectively.

7.7.2 Tests with tylosin tartrate

7.7.2.1 Range Finding Tests

Range finding limit-tests were performed indicating adverse effects up to 15 % and 88.4 % in pig and cattle manure, respectively, at a concentration of 180 mg/kg dry soil (*A. cepa*), 800 mg/kg dw soil (*A. sativa*), 120 mg/kg dw soil (*B. napus* and *S. lycopersicum*), 600 mg/kg dw soil (*P. vulgaris*) and 50 mg/kg dw (*T. pratense*). Tests revealed onion (*A. cepa*) and red clover (*T. pratense*) to be the most sensitive species for the additional approaches regarding differing sequential arrangement of application.

Table 17: Tylosin tartrate Fresh Spiked Manure Range Finder. Percentage effects (% inhibition) on the most sensitive endpoint biomass when compared with control in a test design with spiked manure.

Species	*Allium cepa* 180 mg/kg	*Avena sativa* 800 mg/kg	*Brassica napus* 120 mg/kg	*Solanum lycopersicum* 120 mg/kg	*Phaseolus vulgaris* 600 mg/kg	*Trifolium pratense* 50 mg/kg
Pig manure	76	66.1	65.0	67.8	79.6	86.0
Cattle manure	15.2	63.2	77.8	83.7	82.0	88.4

7.7.2.2 Main tests, general approach

Five test item concentrations per species (Table 18) were tested against a standard control (soil only) and a manure control (soil amended with manure without the test item). The number of replicates was eight per treatment and species except for the standard control with four replicates per treatment and species. The number of seeds per pot was five for onion, oat, rape, red clover, and tomato) and three for common bean.

For approaches with manure, eight replicates were prepared, independent from the number of seeds per replicate. The total number of seeds per species treatment was 40 (24 for *P. vulgaris*). For the standard control without manure, four replicates were prepared. The total number of seeds per species and treatment was 20 (12 for *P. vulgaris*).

The general test conditions are listed in chapter 7.2.

Table 18: Treatment code, test item concentration in soil [mg/kg soil dry weight] and respective pot numbers per species and replicate.

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	X	X	X	X	X	X
MC	0.0	X	X	X	X	X	X
T1	5.0	--	--	X	X	--	X
T2	10.0	--	--	X	X	--	X
T3	15.0	X	--	--	--	--	--
T4	20.0	--	--	--	--	--	X
T5	25.0	--	--	X	X	--	--
T6	30.0	X	--	--	--	--	--
T7	40.0	--	--	--	--	X	--
T8	50.0	--	--	--	--	--	X
T9	60.0	X	--	--	--	--	--
T10	62.5	--	--	X	X	--	--
T11	88.0	--	--	--	--	X	--
T12	100	--	X	--	--	--	--
T13	125	--	--	--	--	--	X
T14	150	X	--	--	--	--	--
T15	156	--	--	X	X	--	--
T16	194	--	--	--	--	X	--
T17	200	--	X	--	--	--	--
T18	300	X	--	--	--	--	--
T19	391	--	--	X	X	--	--
T20	400	--	X	--	--	--	--
T21	426	--	--	--	--	X	--
T22	700	--	X	--	--	--	--
T23	937	--	--	--	--	X	--
T24	1000	--	X	--	--	--	--

C = untreated control; MC = manure control; T = test item amended manure; Respective test item concentration tested (X) or not tested (---).

7.7.2.3 Main tests with pig manure

The test plants were cultivated in air conditioned rooms equipped with high pressure metal halide lamps (Master HPI-T PLUS, Philips GmbH, Hamburg, Germany). Light intensity was >200 µE m-2s-1 for 16 h per day; 8 hours of darkness.

With 21 – 24 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 32 – 69 % the proposed range of 70 ± 25 % for air humidity (OECD 208) was under run sometimes. However, the growth of the control plants was proper and as expected.

To verify the test item concentration at the start of the plant test, samples of spiked manure and spiked soil/manure mixture at a representative test item concentration were measured. The recovery rate for spiked manure and spiked soil/manure mixture was 6.0 – 38.2 % tylosin tartrate, respectively. Evaluation was done by using the nominal concentrations of the application solution.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

The only obvious effect of the test item on the visual appearance regarding pathological symptoms of the seedlings was a partly discoloration of leafs in all species.

There was no or only a slight concentration dependent effect on seedling emergence of all species tested up to and including 1000 mg tylosin tartrate per kg dry soil, the highest concentration tested. The most sensitive species regarding emergence was *Brassica napus* with a NOEC of ≥ 391 mg/kg, an EC_{10} of 66.7 mg/kg. An EC_{50} could not be determined.

There were clear concentration dependent effects on post-emergence survival of all plant species tested due to tylosin tartrate. The most sensitive species regarding post-emergence survival was *Trifolium pratense* with a NOEC of 20.0 mg/kg, an EC_{10} of 17.9 mg/kg, and an EC_{50} of 36.8 mg/kg dry soil.

There were clear concentration dependent effects of tylosin tartrate on growth of all species tested. The most sensitive species regarding shoot length was *A. cepa* with a NOEC of 15.0 mg/kg, an EC_{10} of 14.7 mg/kg and an EC_{50} of 207mg/kg dry soil. Based on shoot fresh mass, the most sensitive species was *T. pratense* with a NOEC of 10.0 mg/kg, an EC_{10} of 8.5 mg/kg and an EC_{50} of 20.1 mg/kg dry soil.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when spiked to fresh manure with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 19. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 111 to Table 114 and Table 119 to Table 122.

Table 19: Tylosin tartrate Fresh Spiked Pig Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Trifolium pratense*
Emergence						
NOEC	✥300	✥1000	✥391	✥391	✥937	✥125
EC_{10}	n.d.	n.d.	66.7	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Post-emergence survival						
NOEC	60.0	700	62.5	156	✥937	20.0
EC_{10}	106	633	40.5	69.9	n.d.	17.9
EC_{50}	166	n.d.	165	212	n.d.	36.8
Shoot length						
NOEC	15.0	400	62.5	62.5	40.0	20.0
EC_{10}	14.7	548	109	57.0	26.3	15.6
EC_{50}	207	n.d.	164	171	432	40.7
Fresh mass						
NOEC	30.0	200	62.5	62.5	40.0	10.0
EC_{10}	18.8	226	101	53.0	n.d.	8.5
EC_{50}	62.7	499	131	88.1	142	20.1

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

The sequential arrangement of application has no influence on the effects on seedling emergence, growth, or post-emergence survival. The NOEC, EC_{10} and EC_{50} values for *A. cepa* and *T. pratense* are comparable, independent if test item spiked manure is applied to soil or manure is applied to test item spiked soil.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when spiked to soil with subsequent application of fresh manure for emergence, growth, and post-emergence survival are presented in Table 20. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 115 to Table 118 and Table 123 to Table 126.

Table 20: Tylosin tartrate Fresh Spiked Soil with Pig Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Trifolium pratense*	Species	*Allium cepa*	*Trifolium pratense*
Emergence			Shoot length		
NOEC	✥300	✥125	NOEC	30.0	✥125
EC_{10}	n.d.	n.d.	EC_{10}	19.3	14.7
EC_{50}	n.d.	n.d.	EC_{50}	182	39.3
Post-emergence survival			Fresh mass		
NOEC	60.0	20.0	NOEC	30.0	10.0
EC_{10}	69.6	33.3	EC_{10}	17.4	5.1
EC_{50}	190	39.6	EC_{50}	60.3	17.4

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

Conclusion:

The application of pig manure had no adverse effects on either seedling emergence, or growth, or post-emergence survival. Tylosin tartrate has clear concentration depending effects on terrestrial plants under the chosen test conditions. The relevant NOEC for the most sensitive endpoint (biomass of *Trifolium pratense*) was found to be 10 mg/kg dry soil. The EC_{10} and EC_{50} were calculated to be 5.1 mg/kg dry soil and 17.4 mg/kg dry soil, respectively.

7.7.2.4 Main tests with cattle manure

The test plants were cultivated in air conditioned rooms equipped with high pressure metal halide lamps (Master HPI-T PLUS, Philips GmbH, Hamburg, Germany). Light intensity was >200 µE m-2s-1 for 16 h per day; 8 hours of darkness.

With 17 – 28 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met With 23 – 62 % air humidity sometimes was below the proposed range of 70 ± 25 % (OECD 208). However, the growth of the control plants was proper and as expected.

To verify the test item concentration at the start of the plant test, samples of spiked manure and spiked soil/manure mixture at a representative test item concentration were measured. The recovery rate for spiked manure and spiked soil/manure mixture was 6.5 – 34.9 % tylosin tartrate, respectively. Therefore, evaluation was based on nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

The only obvious effect of the test item on the visual appearance regarding pathological symptoms of the seedlings was a partly discoloration of leafs in all species.

There was no concentration dependent effect on seedling emergence of all species tested up to and including 1000 mg tylosin tartrate per kg dry soil, the highest concentration tested. NOEC and EC_X could not be calculated.

There were clear concentration dependent effects of tylosin tartrate on post-emergence survival of all plant species (except *A. sativa* and *P. vulgaris*). The most sensitive species regarding post-emergence survival was *Trifolium pratense* with a NOEC of 20.0 mg/kg, an EC_{10} of 11.6 mg/kg, and an EC_{50} of 23.8 mg/kg dry soil.

There were clear concentration dependent effects of tylosin tartrate on growth of all species tested. The most sensitive species regarding fresh mass was *T. pratense* with a NOEC of < 5.0 mg/kg, an EC_{10} of 2.9 mg/kg and an EC_{50} of 10.7g/kg dry soil.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when spiked to fresh manure with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 21. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 127 to Table 130 and Table 135 to Table 138.

Table 21: Tylosin tartrate Fresh Spiked Cattle Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Trifolium pretense*
Emergence						
NOEC	✣300	✣1000	✣391	✣391	✣937	✣125
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Post-emergence survival						
NOEC	60.0	✣1000	156	156	n.d.	20.0
EC_{10}	52.4	n.d.	235	164	n.d.	11.6
EC_{50}	127	n.d.	n.d.	197	n.d.	23.8
Shoot length						
NOEC	15.0	200	25.0	62.5	40.0	5.0
EC_{10}	16.2	628	52.8	119	60.5	5.2
EC_{50}	113	n.d.	217	161	458	33.4
Fresh mass						
NOEC	15.0	200	25.0	n.d.	40.0	<5.0
EC_{10}	15.7	246	36.7	65.7	25.3	2.9
EC_{50}	46.3	775	78.7	100	177	10.7

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

The sequential arrangement of application has no influence on the effects on seedling emergence, growth, or post-emergence survival. The NOEC, EC_{10} and EC_{50} values for *A. cepa* and *T. pratense* are comparable, independent if test item spiked manure is applied to soil or manure is applied to test item spiked soil.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when spiked to soil with subsequent application of fresh manure for emergence, growth, and post-emergence survival are presented in Table 22. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 131 to Table 134 and Table 139 to Table 142.

Table 22: Tylosin tartrate Fresh Spiked Soil with Cattle Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Trifolium pratense*	Species	*Allium cepa*	*Trifolium pratense*
Emergence			Shoot length		
NOEC	✥300	✥125	NOEC	15.0	<5.0
EC_{10}	n.d.	n.d.	EC_{10}	15.3	5.6
EC_{50}	n.d.	n.d.	EC_{50}	137	23.7
Post-emergence survival			Fresh mass		
NOEC	60.0	10.0	NOEC	15.0	<5
EC_{10}	48.1	9.5	EC_{10}	16.7	2.4
EC_{50}	121	21.7	EC_{50}	53.0	9.2

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

Conclusion:

The cattle manure applied had no adverse effects on seedling emergence, growth, or post-emergence survival. Tylosin tartrate has clear concentration depending effects on terrestrial plants under the chosen test conditions. The sequential arrangement of application has no influence on the effects. The relevant NOEC for the most sensitive endpoint (biomass of *Trifolium pratense*) was found to be < 5.0mg/kg dry soil. The EC_{10} and EC_{50} were calculated to be 5.6 mg/kg dry soil and 23.7 mg/kg dry soil, respectively.

7.8 Modified plant tests with half-maximum incubated spiked manure

To simulate the realistic exposure way of veterinary antibiotics, the test item was applied via manure to the soil. The test item/manure mixture is aged for the half-maximum storage duration (27 and 45 days for pig and cattle manure, respectively) under anaerobic conditions. To investigate if the incubation condition has an influence on possible effects on plant growth, additional assays with the two most sensitive plant species are conducted where the test item/manure mixture is applied directly after spiking to the soil with a subsequent aerobic incubation.

Test item application was done according to 7.1. After the half-maximum incubation period, an OECD 208 test was applied (7.2). Seeds were planted in each replicate as soon as possible but at least at the same day the test item was introduced in the manure/soil.

7.8.1 Tests with florfenicol

7.8.1.1 Range Finding Tests

The concentrations in the main tests were chosen due to a limit test where the test item was applied via fresh manure indicating adverse effects up to 25 % and 70 % in pig and cattle manure, respectively, at a concentration of 2 mg/kg dry soil (Table 12), and a range finder test where the test item/manure mixture was aged for the maximum storage duration (53 and 90 days for pig and cattle manure, respectively) under anaerobic conditions indicating no significant effects up to 5 mg/kg dry soil (Table 32). Tests revealed onion and mustard to be the most sensitive species. However, due to experience and after discussion with the UBA, onion (*A. cepa*) and oil seed rape (*B. napus*) were chosen for the additional approaches regarding differing sequential arrangement of application.

7.8.1.2 Main tests, general approach

The nominal concentration in the test containers with test item was 0.62, 1.85, 5.56, 16.7, and 50 mg test item/kg dry mass soil. Five seeds (onion, oat, rape, mustard, and tomato) and four seeds (common bean), respectively, were planted in each replicate right after incorporation of the test item. For approaches with manure, eight replicates were prepared, independent from the number of seeds per replicate. The total number of seeds applied per treatment was 32 - 40. For the standard control without manure, for vessels with five and - four seeds, four and five replicates were prepared, respectively. The total number of seeds applied per treatment was 20.

As the test item was applied via manure using a solvent, a control reflecting solvent-mediated application to manure followed by evaporation was conducted (7.1.5). Since it is known from experience that no adverse effect due to the solvent occurs (pre-tests, data not shown) and since the combination of solvent spiked manure is seen as the carrier of the test item, no additional manure control without solvent was applied. Number of replicates and seeds per replicate were the same as for the treatments.

The general test conditions are listed in chapter 7.2.

7.8.1.3 Main tests with pig manure

With 16 – 28 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 212 – 445 µE/m^2/s the permitted value of 200 – 400 µE/m^2/s (OECD 208) was maintained. With 35 – 65 % air humidity sometimes was below the proposed range of 70 ± 25 % (OECD 208). However, the growth of the control plants was proper and as expected.

Test item concentration was verified in the highest application solution. The recovery was found to be within 94 – 100 % of nominal. To verify the test item concentration at the start of the plant test, samples of spiked manure and spiked soil/manure mixture at a representative test item concentration were measured. The recovery rate for spiked manure and spiked soil/manure mixture was 4 % and 81 % florfenicol, respectively. Evaluation was done by using nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

There was no effect of the test item on the visual appearance regarding pathological symptoms of the seedlings when the test item was incubated anaerobically. This was also true for the sensitive species rape. However, a partly discoloration of leafs due to the test item occurred in rape after aerobic incubation in the soil/manure mixture.

There was no or only a slight concentration dependent effect on seedling emergence of *Allium cepa, Avena sativa*, *Brassica napus, Sinapis alba*, and *Phaseolus vulgaris* up to and including 50 mg Florfenicol per kg dry soil, the highest concentration tested. Emergence of *Solanum lycopersicum* was clearly affected by Florfenicol. The most sensitive species regarding emergence was *S. lycopersicum* with a NOEC of 16.7 mg/kg, an EC_{10} of 5.50 mg/kg, and an EC_{50} of 29.56 mg/kg dry soil.

There were clear concentration dependent effects on post-emergence survival of all plant species tested due to Florfenicol. The most sensitive species regarding post-emergence survival was *B. napus* with a NOEC of 19.7 mg/kg, an EC_{10} of 3.33 mg/kg, and an EC_{50} of 30.16 mg/kg dry soil.

There were clear concentration dependent effects on growth of all species tested due to Florfenicol. The most sensitive species regarding shoot length and fresh mass were *S. alba* and *A. cepa* with a fresh mass NOEC of 1.9 and 5.6 mg/kg, respectively, and fresh mass EC_{10} and EC_{50} values of 5.43 – 10.18 and 17.53 - 19.97 mg/kg dry soil, respectively.

The NOEC, EC_{10} and EC_{50} values of florfenicol when incubated for half-maximum storage duration under anaerobic conditions with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 23 Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 143 to Table 146 and Table 151 to Table 154.

Table 23: Florfenicol Half-max Anaerobic Aged Pig Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Sinapis alba*	*Solanum lycopersicum*	*Phaseolus vulgaris*
Emergence						
NOEC	✣ 50	✣ 50	✣ 50	✣ 50	16.7	✣ 50
EC_{10}	6.34	n.d.	> 50	13.24	5.50	22.46
EC_{50}	n.d.	n.d.	n.d.	n.d.	29.56	> 50
Post-emergence survival						
NOEC	16.7	16.7	16.7	16.7	16.7	16.7
EC_{10}	7.93	31.92	3.33	11.93	16.90	8.62
EC_{50}	> 50	n.d.	30.16	32.25	42.02	> 50
Shoot length						
NOEC	5.6	16.7	5.6	5.6	16.7	5.6
EC_{10}	11.90	7.18	15.12	14.83	20.11	15.19
EC_{50}	29.87	40.42	29.15	27.48	31.39	32.99
Fresh mass						
NOEC	5.6	16.7	16.7	1.9	16.7	16.7
EC_{10}	5.43	18.89	14.08	10.18	12.67	11.82
EC_{50}	17.53	29.70	22.50	19.97	25.32	27.02

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values.

The kind of incubation has a serious influence on the effects on seedling emergence, growth, or post-emergence survival. The NOEC, EC_{10} and EC_{50} values for *A. cepa* and B. *napus* are significantly lower when the test item was incubated under aerobic conditions in the soil/manure mixture.

The NOEC, EC_{10} and EC_{50} values of florfenicol when applied to manure and mixed with soil with a subsequent half-maximum aerobic incubation of the mixture for emergence, growth, and post-emergence survival are presented in Table 24. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 147 to Table 150 and Table 155 to Table 158.

Table 24: Florfenicol Half-max Aerobic Aged Pig Manure/soil. NOEC, EC10 and EC50 values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Brassica napus*	Species	*Allium cepa*	*Brassica napus*
Emergence			Shoot length		
NOEC	≥ 50	≥ 50	NOEC	0.60	< 0.60
EC_{10}	n.d.	> 50	EC_{10}	0.63	n.d.
EC_{50}	n.d.	n.d.	EC_{50}	1.89	0.68
Post-emergence survival			Fresh mass		
NOEC	0.60	0.60	NOEC	0.60	< 0.60
EC_{10}	0.90	0.37	EC_{10}	0.54	n.d.
EC_{50}	2.05	3.78	EC_{50}	1.60	0.35

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values.

Conclusion:

The manure applied had no adverse effects on seedling emergence, growth, or post-emergence survival. Florfenicol has clear concentration depending effects on terrestrial plants under the chosen test conditions. The kind of incubation (anaerobic or aerobic) has a serious influence on the effects.

The relevant NOEC for the most sensitive endpoint (biomass of *Sinapis alba*) under anaerobic incubation conditions was found to be 1.9 mg/kg dry soil. The relevant EC_{10} and EC_{50} values (biomass *Allium cepa*) were calculated to be 5.43 mg/kg dry soil and 17.53 mg/kg dry soil, respectively.

The relevant NOEC and EC_{50} value for the most sensitive endpoint (biomass of *Sinapis alba*) under aerobic incubation conditions was found to be < 0.60 and 0.35 mg/kg dry soil, respectively. No EC_{10} could be calculated.

7.8.1.4 Main tests with cattle manure

With 18 – 24 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 110 – 293 µE/m²/s light intensity sometimes was below the permitted value of 200 – 400 µE/m²/s (OECD 208). With 28 – 55 % air humidity sometimes was below the proposed range of 70 ± 25 % (OECD 208). However, the growth of the control plants was proper and as expected.

Test item concentration was verified in the highest application solution. The recovery was found to be within 103 – 104 % of nominal. To verify the test item concentration at the start of the plant test, samples of spiked manure and spiked soil/manure mixture at a representative test item concentration were measured. The recovery rate for spiked manure and spiked soil/manure mixture was 3 % and 76 % florfenicol, respectively. Evaluation was done by using nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

There was no effect of the test item on the visual appearance regarding pathological symptoms of the seedlings when the test item was incubated anaerobically. This was also true for the sensitive species rape. However, a partly discoloration of leafs due to the test item occurred in rape after aerobic incubation in the soil/manure mixture.

There was no or only a slight concentration dependent effect on seedling emergence of *Allium cepa, Avena sativa*, *Brassica napus, Sinapis alba*, and *Phaseolus vulgaris* up to and including 50 mg Florfenicol per kg dry soil, the highest concentration tested. Emergence of *Solanum lycopersicum* was clearly affected by Florfenicol. The most sensitive species regarding emergence was *S. lycopersicum* with a NOEC of 16.7 mg/kg, an EC_{10} of 11.48 mg/kg, and an EC_{50} of 26.63 mg/kg dry soil.

Post-emergence survival of *A. cepa*, *B. napus*, *S. alba, and S. lycopersicum* was clearly affected by Florfenicol. The most sensitive species regarding post-emergence survival was *B. napus* with a NOEC of 19.7 mg/kg, an EC_{10} of 5.67 mg/kg, and an EC_{50} of 23.47 mg/kg dry soil.

There were clear concentration dependent effects on growth of all species tested due to Florfenicol. The most sensitive species regarding shoot length and fresh mass was *S. alba* with a fresh mass NOEC of 1.9 mg/kg and fresh mass EC_{10} and EC_{50} values of 8.10 and 21.14 mg/kg dry soil, respectively.

The NOEC, EC_{10} and EC_{50} values of florfenicol when incubated for half-maximum storage duration under anaerobic conditions with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 25. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 159 to Table 162 and Table 167 to Table 170.

Table 25: Florfenicol Half-max Anerobic Aged Cattle Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Sinapis alba*	*Solanum lycopersicum*	*Phaseolus vulgaris*
Emergence						
NOEC	✣ 50	✣ 50	✣ 50	✣ 50	16.7	✣ 50
EC_{10}	n.d.	n.d.	> 50	> 50	11.48	33.79
EC_{50}	n.d.	n.d.	n.d.	n.d.	26.63	> 50
Post-emergence survival						
NOEC	16.7	✣ 50	16.7	16.7	16.7*	✣ 50
EC_{10}	12.34	n.d.	5.67	22.16	20.63	39.62
EC_{50}	25.38	n.d.	23.47	32.57	> 50	> 50
Shoot length						
NOEC	✣ 16.7	16.7	5.6	1.9	5.6	16.7
EC_{10}	10.66	23.07	12.46	15.64	15.55	17.56
EC_{50}	37.81	42.35	33.85	27.59	26.69	34.78
Fresh mass						
NOEC	5.6	16.7	5.6	1.9	16.7	5.60
EC_{10}	7.71	14.24	12.05	8.10	16.68	11.83
EC_{50}	26.45	29.44	20.81	21.14	24.19	28.73

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. * NOEC ✣ 50 mg/kg by ToxRat due to great variances in the highest treatment. Realistic NOEC is set on 16.7 mg/kg by the study director. Concentrations given as nominal values.

The kind of incubation has a serious influence on the effects on seedling emergence, growth, or post-emergence survival. The NOEC, EC_{10} and EC_{50} values for *A. cepa* and B. *napus* are significantly lower when the test item was stored under aerobic conditions in the soil/manure mixture.

The NOEC, EC_{10} and EC_{50} values of florfenicol when applied to manure and mixed with soil with a subsequent half-maximum aerobic incubation of the mixture for emergence, growth, and post-emergence survival are presented in Table 26. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 163 to Table 166 and Table 171 to Table 174.

Table 26: Florfenicol Half-max Aerobic Aged Cattle Manure/soil. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Brassica napus*	Species	*Allium cepa*	*Brassica napus*
Emergence			Shoot length		
NOEC	✣ 50	16.7	NOEC	< 0.60	0.60
EC_{10}	8.20	7.92	EC_{10}	0.84	0.56
EC_{50}	> 50	> 50	EC_{50}	2.02	2.49
Post-emergence survival			Fresh mass		
NOEC	1.9	1.9	NOEC	< 0.60	0.60
EC_{10}	1.25	2.60	EC_{10}	0.35	0.55
EC_{50}	2.30	4.41	EC_{50}	1.18	1.10

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values.

Conclusion:

The manure applied had no adverse effects on seedling emergence, growth, or post-emergence survival. Florfenicol has clear concentration depending effects on terrestrial plants under the chosen test conditions. The kind of incubation (anaerobic or aerobic) has a serious influence on the effects.

The relevant NOEC for the most sensitive endpoint (biomass of *Sinapis alba*) under anaerobic incubation conditions was found to be 1.9 mg/kg dry soil. The relevant EC_{10} and EC_{50} values (biomass *Sinapis alba*) were calculated to be 8.10 mg/kg dry soil and 21.14 mg/kg dry soil, respectively.

The relevant NOEC, EC_{10} and EC_{50} values for the most sensitive endpoint (biomass of *Allium cepa*) under aerobic incubation conditions was found to be < 0.60, 0.35, and 1.18 mg/kg dry soil, respectively.

7.8.2 Tests with tylosin tartrate

7.8.2.1 Range Finding Tests

The concentrations in the main tests were chosen due to a limit test where the test item was applied via fresh manure indicating adverse effects up to 15 % and 88 % in pig and cattle manure, respectively, at concentrations between 50 and 800 mg/kg dry soil (Table 17), and a range finder test where the test item/manure mixture was aged for the maximum storage duration (53 and 90 days for pig and cattle manure, respectively) under anaerobic conditions indicating no effects up to 8000 mg/kg dry soil (Table 35). Tests revealed onion (*A. cepa*) and red clover (*T. pratense*) to be the most sensitive species for the additional approaches regarding differing sequential arrangement of application.

7.8.2.2 Main tests, general approach

Five test item concentrations per species (Table 27) were tested against a standard control (soil only) and a manure control (soil amended with manure without the test item). The number of replicates was eight per treatment and species except for the standard control with four replicates per treatment and species. The number of seeds per pot was five for onion, oat, rape, red clover, and tomato and three for common bean.

For approaches with manure, eight replicates per treatment and species were prepared. The total number of seeds per treatment and species was 40 (24 for *P. vulgaris*). For the standard control (without manure) the total number of seeds per treatment and species was 20 (12 for *P. vulgaris*).

The general test conditions are listed in chapter 7.2.

Table 27: Treatment code, test item concentration in soil [mg/kg soil dry weight] and respective pot numbers per species and replicate.

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	X	X	X	X	X	X
MC	0.0	X	X	X	X	X	X
T1	5.0	--	--	X	X	--	X
T2	10.0	--	--	X	X	--	X
T3	15.0	X	--	--	--	--	--
T4	20.0	--	--	--	--	--	X
T5	25.0	--	--	X	X	--	--
T6	30.0	X	--	--	--	--	--
T7	40.0	--	--	--	--	X	--
T8	50.0	--	--	--	--	--	X
T9	60.0	X	--	--	--	--	--
T10	62.5	--	--	X	X	--	--
T11	88.0	--	--	--	--	X	--
T12	100	--	X	--	--	--	--
T13	125	--	--	--	--	--	X
T14	150	X	--	--	--	--	--
T15	156	--	--	X	X	--	--
T16	194	--	--	--	--	X	--
T17	200	--	X	--	--	--	--
T18	300	X	--	--	--	--	--
T19	391	--	--	X	X	--	--
T20	400	--	X	--	--	--	--
T21	426	--	--	--	--	X	--
T22	700	--	X	--	--	--	--
T23	937	--	--	--	--	X	--
T24	1000	--	X	--	--	--	--

C = untreated control; MC = manure control; T = test item amended manure; Respective test item concentration tested (X) or not tested (---)

7.8.2.3 Main tests with pig manure

The test plants were cultivated in air conditioned rooms equipped with high pressure metal halide lamps (Master HPI-T PLUS, Philips GmbH, Hamburg, Germany). Light intensity was >200 µE m-2s-1 for 16 h per day; 8 hours of darkness.

With 21 – 26 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 35 – 70 % air humidity sometimes was below the proposed range of 70 ± 25 % (OECD 208). However, the growth of the control plants was proper and as expected.

To verify the test item concentration at the start of the plant test, samples of spiked manure and spiked soil/manure mixture at a representative test item concentration were measured. The recovery rate for spiked manure and spiked soil/manure mixture was 8.1 – 35.2 % tylosin tartrate, respectively. Evaluation was done by using nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

The only obvious effect of the test item on the visual appearance regarding pathological symptoms of the seedlings was a partly discoloration of leafs in *B. napus*, *S. lycopersicum*, *P. vulgaris* and *T. pratense.*

There was no concentration dependent effect tylosin tartrate on seedling emergence of any of the tested species at concentrations up to and including 1000 mg per kg dry soil, the highest concentration tested.

Post-emergence survival of all species was not affected by tylosin tartrate.

There were clear concentration dependent effects of tylosin tartrate on growth of all tested species except for *A. sativa* and *S. lycopersicum*. The most sensitive species regarding shoot length and fresh mass was T. pratense with a shoot length NOEC of 20.0 mg/kg and fresh mass EC_{10} value of 5.5 mg/kg dry soil, respectively.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when incubated for half-maximum storage duration under anaerobic conditions with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 28. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 175 to Table 178 and Table 183 to Table 186.

Table 28: Tylosin tartrate Half-max Anaerobic Aged Pig Manure. NOEC, EC10 and EC50 values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Trifolium pratense*
Emergence						
NOEC	✥300	✥1000	✥391	✥391	✥937	✥125
EC_{10}	n.d.	874.4	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Post-emergence survival						
NOEC	✥300	✥1000	✥391	✥391	n.d.	✥125
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Shoot length						
NOEC	150	✥1000	156	✥391	426	20.0
EC_{10}	n.d.	n.d.	n.d.	n.d.	534	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fresh mass						
NOEC	✥300	✥1000	156	✥391	426	✥125
EC_{10}	92.0	n.d.	n.d.	n.d.	581	5.5
EC50	n.d.	n.d.	n.d.	n.d.	837	n.d.

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

The kind of incubation has a serious influence on the effects on seedling emergence, growth, or post-emergence survival. The NOEC, EC_{10} and EC_{50} values for *A. cepa* and *T. pratense* are lower when the test item was stored under aerobic conditions in the soil/manure mixture.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when applied to manure and mixed with soil with a subsequent half-maximum aerobic incubation of the mixture for emergence, growth, and post-emergence survival are presented in Table 29. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 179 to Table 182 and Table 187 to Table 190.

Table 29: Tylosin tartrate Half-max Aerobic Aged Pig Manure/soil. NOEC, EC10 and EC50 values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Trifolium pratense*	Species	*Allium cepa*	*Trifolium pratense*
Emergence			Shoot length		
NOEC	✥300	✥125	NOEC	30.0	5.0
EC_{10}	n.d.	n.d.	EC_{10}	20.2	11.2
EC_{50}	n.d.	n.d.	EC_{50}	102	59.9
Post-emergence survival			Fresh mass		
NOEC	150	20.0	NOEC	30.0	10.0
EC_{10}	46.1	28.0	EC_{10}	20.3	7.7
EC_{50}	153	62.6	EC_{50}	65.4	29.8

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

Conclusion:

The manure applied had no adverse effects on either seedling emergence, or growth, or post-emergence survival. Tylosin tartrate had clear concentration depending effects on terrestrial plants under the chosen test conditions. The kind of incubation (anaerobic or aerobic) strongly influenced on the effects.

Under anaerobic incubation conditions the relevant NOEC for the most sensitive endpoint (shoot length of Trifolium pratense) was found to be 20 mg/kg dry soil. The relevant EC_{10} value (biomass *Trifolium pratense*) was calculated to be 5.5 mg/kg dry soil. An EC_{50} could not be determined

Under aerobic incubation conditions the relevant NOEC, EC_{10} and EC_{50} values for the most sensitive endpoint (of *Trifolium pratense*) was found to be 5.0 (shoot length), 7.7, and 29.8 mg/kg dry soil (biomass), respectively.

7.8.2.4 Main tests with cattle manure

With 16 – 26 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 26.7– 52.3 % air humidity sometimes was below the proposed range of 70 ± 25 % (OECD 208). However, the growth of the control plants was proper and as expected.

To verify the test item concentration at the start of the plant test, samples of spiked manure and spiked soil/manure mixture at a representative test item concentration were measured. The recovery rate for spiked manure and spiked soil/manure mixture was 16.7 – 27.1 % tylosin tartrate, respectively. Evaluation was done by using nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

The only obvious effect of the test item on the visual appearance regarding pathological symptoms of the seedlings was a partly discoloration of leafs in all species.

There was no or only a slight concentration dependent effect on seedling emergence of *Trifolium pratense*. Even for the sensitive species *T. pratense* a NOEC of ≥ 125 mg/kg, an EC_{10} or EC_{50} could not be calculated.

Post-emergence survival was not affected by tylosin tartrate. NOEC or EC values could not be determined.

There were clear concentration dependent effects of tylosin tartrate on growth of all species tested, except for *Avena sativa*. The most sensitive species regarding shoot length and fresh mass was *T. pratense* with a fresh mass NOEC of 50 mg/kg and fresh mass EC_{10} and EC_{50} values of 70.6 and 123 mg/kg dry soil, respectively.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when incubated for half-maximum storage duration under anaerobic conditions with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 30. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 191 to Table 194 and Table 199 to Table 202.

Table 30: Tylosin tartrate Half-max Anaerobic Aged Cattle Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Trifolium pratense*
Emergence						
NOEC	✣300	✣1000	✣391	✣391	✣937	✣125
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Post-emergence survival						
NOEC	✣300	n.d.	✣391	✣391	✣937	✣125
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Shoot length						
NOEC	150	✣1000	156	156	n.d.	50.0
EC_{10}	149	n.d.	95.5	79.6	188	49.3
EC_{50}	301	n.d.	957	578	753	n.d.
Fresh mass						
NOEC	150	✣1000	156	156	194	50.0
EC_{10}	156	n.d.	254	77.5	175	70.6
EC_{50}	231	n.d.	326	241	370	123

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

The kind of incubation has a serious influence on the effects on seedling emergence, growth, or post-emergence survival. The NOEC, EC_{10} and EC_{50} values for *A. cepa* and *T. pratense* are lower when the test item was stored under aerobic conditions in the soil/manure mixture.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when applied to manure and mixed with soil with a subsequent half-maximum aerobic incubation of the mixture for emergence, growth, and post-emergence survival are presented in Table 31. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 195 to Table 198 and Table 203 to Table 206.

Table 31: Tylosin tartrate Half-max Aerobic Aged Cattle Manure/soil. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Trifolium pratense*	Species	*Allium cepa*	*Trifolium pratense*
Emergence			Shoot length		
NOEC	✥300	✥125	NOEC	15.0	10.0
EC_{10}	n.d.	n.d.	EC_{10}	12.4	11.6
EC_{50}	n.d.	n.d.	EC_{50}	170	37.3
Post-emergence survival			Fresh mass		
NOEC	60.0	20.0	NOEC	15.0	5.0
EC_{10}	41.4	15.2	EC_{10}	10.7	5.3
EC_{50}	200	29.9	EC_{50}	46.0	16.9

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

Conclusion:

Application of manure had no adverse effects on either seedling emergence, or growth, or post-emergence survival. Tylosin tartrate had concentration depending effects on terrestrial plants under aerobic incubation conditions. The kind of incubation (anaerobic or aerobic) had a strong influence on the effects.

The relevant NOEC for the most sensitive endpoint (biomass and shoot length of *Trifolium pratense*) under anaerobic incubation conditions was found to be 50.0 mg/kg dry soil. The relevant EC_{10} and EC_{50} values were calculated to be 49.3 mg/kg dry soil (shoot length *T. pratense*) and 123 mg/kg dry soil (biomass *T. pratense*), respectively.

The relevant NOEC, EC_{10} and EC_{50} values for the most sensitive endpoint (biomass of *Trifolium pratense*) under aerobic incubation conditions was found to be 5.0, 5.3, and 16.9 mg/kg dry soil, respectively.

7.9 Modified plant tests with maximum incubated spiked manure

To simulate the realistic exposure way of veterinary antibiotics, the test item was applied manure to the soil. The test item/manure mixture is aged for the maximum storage duration (53 and 90 days for pig and cattle manure, respectively) under anaerobic conditions.

Test item application was done according to 7.1. After the maximum incubation period, an OECD 208 test was applied (7.2). Seeds were planted in each replicate as soon as possible but at least at the same day the test item was introduced in the soil.

7.9.1 Tests with florfenicol

7.9.1.1 Range Finding Tests

The concentrations in the main tests were chosen due to a range finding test where the test item/manure mixture was aged for the maximum storage duration (53 and 90 days for pig and cattle manure, respectively) under anaerobic conditions indicating no significant effects up to 5 mg/kg dry soil.

Table 32: Florfenicol Max Anaerobic Aged Manure Range Finder. NOEC, EC10 and EC50 values for the most sensitive endpoint fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Trifolium pratense*	*Solanum lycopersicum*	*Phaseolus vulgaris*
Pig manure						
NOEC	✣ 5.00	✣ 5.00	✣ 5.00	✣ 5.00	✣ 5.00	✣ 5.00
EC_{10}	n.d.	n.d.	n.d.	n.d.	1.75	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cattle manure						
NOEC	✣ 5.00	✣ 5.00	✣ 5.00	✣ 5.00	✣ 5.00	1.00
EC_{10}	n.d.	2.11	3.83	n.d.	n.d.	0.88
EC_{50}	n.d.	n.d.	> 5.00	n.d.	n.d.	> 5.00

TI = Test item; DM = Dry mass; Concentrations given as nominal values.

7.9.1.2 Main tests, general approach

The nominal concentration was 0.62, 1.85, 5.56, 16.7, and 50 mg test item/kg dry mass soil. Five seeds (onion, oat, rape, mustard, and tomato) and four seeds (common bean), respectively, were planted in each replicate right after incorporation of the test item. For approaches with manure, eight replicates were prepared, independent from the number of seeds per replicate. The total number of seeds applied per treatment was 32 - 40. For the standard control without manure, for vessels with five and four seeds, four and five replicates were prepared, respectively. The total number of seeds applied per treatment was 20.

As the test item was applied via manure using a solvent, a control reflecting solvent-mediated application to manure followed by evaporation was conducted (7.1.5). Since it is known from experience that no adverse effect due to the solvent occurs (pre-tests, data not shown) and since the combination of solvent spiked manure is seen as the carrier of the test item, no additional manure control without solvent was applied. Number of replicates and seeds per replicate were the same in the controls as for the treatments.

The general test conditions are listed in chapter 7.2.

7.9.1.3 Main tests with pig manure

With 16 – 28 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 212 – 445 $\mu E/m^2/s$ the permitted value of 200 – 400 $\mu E/m^2/s$ (OECD 208) was maintained. With 35 – 65 % air humidity sometimes was below the proposed range of 70 ± 25 % (OECD 208). However, the growth of the control plants was proper and as expected.

Test item concentration was verified in the highest application solution. The recovery was found to be within 94 – 100 % of nominal. To verify the test item concentration at the start of the plant test, samples of spiked manure at a representative test item concentration were measured. The recovery rate for spiked manure was 2 % florfenicol. Evaluation was done by using nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate, except of impaired seedling emergence in *A. cepa*.

There was no effect of the test item on the visual appearance regarding pathological symptoms of the seedlings when the test item was incubated anaerobically. This was also true for the sensitive species rape.

There was no or only a slight concentration dependent effect on seedling emergence of *Allium cepa*, *Avena sativa*, *Brassica napus*, and *Sinapis alba* up to and including 50 mg Florfenicol per kg dry soil, the highest concentration tested. Emergence of *Solanum lycopersicum* and *Phaseolus vulgaris* was clearly affected by Florfenicol. The most sensitive species regarding emergence was P. vulgaris with a NOEC of 16.7 mg/kg, and an EC_{10} of 1.33 mg/kg. An EC_{50} could not be calculated.

There was no concentration dependent effect on post-emergence survival of *Avena sativa* up to and including 50 mg florfenicol per kg dry soil, the highest concentration tested. Post-emergence survival was clearly affected by Florfenicol for the other five species tested. The most sensitive species regarding post-emergence survival was *S. lycopersicum* with a NOEC value of 1.9 mg/kg dry soil and an EC_{10} and EC_{50} values of 18.35 and 37.71 mg/kg dry soil, respectively. However, with EC_{10} and EC_{50} values of 2.08 – 5.77 and 18.47 – 22.32 mg/kg dry soil, respectively, sensitivity of *A. cepa* and *B. napus* is comparable.

There were clear concentration dependent effects on growth of all species tested due to Florfenicol.

The most sensitive species regarding shoot length and fresh mass were *S. lycopersicum* and *S. alba* with a NOEC of 1.9 and 5.6 mg/kg, respectively, and EC_{10} and EC_{50} values of 2.35 – 9.44 and 14.80 – 15. 66 mg/kg dry soil, respectively.

Table 33: Florfenicol Max Anaerobic Aged Pig Manure. NOEC, EC10 and EC50 values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Sinapis alba*	*Solanum lycopersicum*	*Phaseolus vulgaris*
Emergence						
NOEC	✣ 50	✣ 50	✣ 50	✣ 50	16.7	16.7
EC_{10}	6.34	n.d.	✣ 50	n.d.	18.34	1.33
EC_{50}	n.d.	n.d.	n.d.	n.d.	46.10	✣ 50
Post-emergence survival						
NOEC	16.7	✣ 50	16.7	16.7	1.9	16.7
EC_{10}	2.08	n.d.	5.77	6.87	18.35	16.95
EC_{50}	18.47	n.d.	22.32	> 50	37.71	✣ 50
Shoot length						
NOEC	16.7	16.7	✣ 16.7	5.6	1.9	16.7
EC_{10}	8.71	14.06	17.44	8.34	15.98	29.30
EC_{50}	33.74	> 50	n.d.	20.93	30.48	43.88
Fresh mass						
NOEC	16.7	16.7	✣ 16.7	5.6	1.9	16.7
EC_{10}	0.40	16.81	14.30	9.44	2.35	28.23
EC_{50}	19.76	37.20	27.78	15.66	14.80	41.18

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values.

The NOEC, EC_{10} and EC_{50} values of florfenicol when incubated for maximum storage duration under anaerobic conditions with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 33. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 207 to Table 214.

Conclusion:

The manure applied had no adverse effects on growth, or post-emergence survival. However, in *Allium cepa* emergence rate was significantly impaired due to manure. Florfenicol has clear concentration depending effects on terrestrial plants under the chosen test conditions.

The relevant NOEC for the most sensitive endpoint (biomass of *Solanum lycopersicum*) under anaerobic incubation conditions was found to be 1.9 mg/kg dry soil. The relevant EC_{10} and EC_{50} values (biomass *Sinapis alba*) were calculated to be 2.35 mg/kg dry soil and 14.80 mg/kg dry soil, respectively.

7.9.1.4 Main tests with cattle manure

With 18 – 27 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 183 – 319 µE/m^2/s light intensity sometimes was below the permitted value of 200 – 400 µE/m^2/s (OECD 208). With 25 – 50 % air humidity sometimes was below the proposed range of 70 ± 25 % (OECD 208). However, the growth of the control plants was proper and as expected.

Test item concentration was verified in the highest application solution. The recovery was found to be within 103 – 104 % of nominal. To verify the test item concentration at the start of the plant test, samples of spiked manure at a representative test item concentration were measured. The recovery rate for spiked manure was 3 % florfenicol. Evaluation was done by using nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

There was no effect of the test item on the visual appearance regarding pathological symptoms of the seedlings when the test item was incubated anaerobically. This was also true for the sensitive species rape.

There was no or only a slight concentration dependent effect on seedling emergence of the species tested. All species showed NOEC values ≥ 50 mg Florfenicol per kg dry soil. EC values could not be calculated.

Post-emergence survival was slightly affected by Florfenicol for *Solanum lycopersicum*. Post-emergence survival was not affected by Florfenicol for the other five species tested up to and including 50 mg Florfenicol per kg dry soil, the highest concentration tested. With ≥ 50 mg/kg dry soil, the NOEC was identical for all species tested. The most sensitive species *S. lycopersicum* showed EC_{10} and EC_{50} values of 24.19 and > 50 mg/kg dry soil mg/kg dry soil, respectively.

There were clear concentration dependent effects on growth all species tested due to Florfenicol. The most sensitive species regarding shoot length and fresh mass was *S. lycopersicum* with a shoot length NOEC of 5.6 mg/kg and fresh mass EC_{10} and EC_{50} values of 14.17 and 36.38 mg/kg dry soil, respectively. However, with a NOEC of 5.6 and 16.7 mg/kg dry soil and EC_{10} and EC_{50} values of 8.14 – 32.78 and 44.30 - > 50 mg/kg dry soil, respectively, sensitivity of *A. cepa*, *B. napus*, *S. alba*, and *P. vulgaris* is comparable.

The NOEC, EC_{10} and EC_{50} values of florfenicol when incubated for maximum storage duration under anaerobic conditions with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 34. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 215 to Table 222.

Table 34: Florfenicol Max Anaerobic Aged Cattle Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Sinapis alba*	*Solanum lycopersicum*	*Phaseolus vulgaris*
Emergence						
NOEC	✣ 50	✣ 50	✣ 50	✣ 50	✣ 50	✣ 50
EC_{10}	n.d.	n.d.	n.d.	> 50	0.04	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Post-emergence survival						
NOEC	✣ 50	✣ 50	✣ 50	✣ 50	✣ 50	✣ 50
EC_{10}	n.d.	n.d.	n.d.	n.d.	24.19	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	> 50	n.d.
Shoot length						
NOEC	16.7	16.7	16.7	16.7	5.6	✣ 50
EC_{10}	17.61	> 50	29.25	16.84	16.92	n.d.
EC_{50}	n.d.	n.d.	> 50	> 50	> 50	n.d.
Fresh mass						
NOEC	16.7	✣ 50	16.7	16.7	16.7	5.6
EC_{10}	32.78	> 50	27.02	10.12	14.17	8.14
EC_{50}	> 50	n.d.	> 50	44.30	36.83	> 50

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. * NOEC ✣ 50 mg/kg by ToxRat due to great variances in the highest treatment. Realistic NOEC is set on 16.7 mg/kg by the study director. Concentrations given as nominal values.

Conclusion:

The manure applied had no adverse effects on emergence rate, growth, or post-emergence survival. Florfenicol has clear concentration depending effects on terrestrial plants under the chosen test conditions.

The relevant NOEC for the most sensitive endpoint (biomass *Phaseolus vulgaris*) under anaerobic incubation conditions was found to be 5.6 mg/kg dry soil. The relevant EC_{10} and EC_{50} values (biomass *Phaseolus vulgaris*) were calculated to be 8.14 mg/kg dry soil and > 50 mg/kg dry soil, respectively.

7.9.2 Tests with tylosin tartrate

7.9.2.1 Range Finding Tests

Range finding limit-tests were performed indicating adverse effects up to 15 % and 88 % in pig and cattle manure, respectively, at a concentration of 180 mg/kg dry soil (*A. cepa*), 800 mg/kg dw soil (*A. sativa*), 120 mg/kg dw soil (*B. napus* and *S. lycopersicum*), 600 mg/kg dw soil (*P. vulgaris*) and 50 mg/kg dw (*T. pratense*). A range finder test where the test item/manure mixture was aged for the maximum storage duration (53 and 90 days for pig and cattle manure, respectively) under anaerobic conditions indicating no significant effects up to 5 mg/kg dry soil Table 35.

Table 35: Tylosin tartrate Max Anaerobic Aged Manure Range Finder. NOEC, EC_{10} and EC_{50} values for the most sensitive endpoint fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicon*	*Phaseolus vulgaris*	*Trifolium pratense*
Pig manure						
NOEC	✣ 250	✣ 800	✣ 144.3	✣ 144.3	✣ 800	✣ 83.3
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	56.2
EC_{50}	n.d.	n.d.	11.0	n.d.	n.d.	n.d.
Cattle manure						
NOEC	✣ 250	✣ 800	✣ 144.3	✣ 144.3	✣ 800	✣ 83.3
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d	n.d	n.d.	n.d.	6.6

TI = Test item; DM = Dry mass; Concentrations given as nominal values.

7.9.2.2 Main tests, general approach

Five test item concentrations per species (Table 36) were tested against a standard control (soil only) and a manure control (soil amended with manure without the test item). The number of replicates was eight per treatment and species except for the standard control with four replicates. Five seeds (onion, oat, rape, red clover, and tomato) and three seeds (common bean) were applied per replicate.

For approaches with manure, eight replicates were prepared, independent from the number of seeds per replicate. The total number of seeds per treatment and species was 40 (24 for *P. vulgaris*). For the standard control without manure the total number of seeds per treatment and species was 20 (12 for *P. vulgaris*).

The general test conditions are listed in chapter 7.2.

Table 36: Treatment code, test item concentration in soil [mg/kg soil dry weight] and respective pot numbers per species and replicate.

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	X	X	X	X	X	X
MC	0.0	X	X	X	X	X	X
T1	5.0	--	--	X	X	--	X
T2	10.0	--	--	X	X	--	X
T3	15.0	X	--	--	--	--	--
T4	20.0	--	--	--	--	--	X
T5	25.0	--	--	X	X	--	--
T6	30.0	X	--	--	--	--	--
T7	40.0	--	--	--	--	X	--
T8	50.0	--	--	--	--	--	X
T9	60.0	X	--	--	--	--	--
T10	62.5	--	--	X	X	--	--
T11	88.0	--	--	--	--	X	--
T12	100	--	X	--	--	--	--
T13	125	--	--	--	--	--	X
T14	150	X	--	--	--	--	--
T15	156	--	--	X	X	--	--
T16	194	--	--	--	--	X	--
T17	200	--	X	--	--	--	--
T18	300	X	--	--	--	--	--
T19	391	--	--	X	X	--	--
T20	400	--	X	--	--	--	--
T21	426	--	--	--	--	X	--
T22	700	--	X	--	--	--	--
T23	937	--	--	--	--	X	--
T24	1000	--	X	--	--	--	--

C = untreated control; MC = manure control; T = test item amended manure; Respective test item concentration tested (X) or not tested (---).

7.9.2.3 Main tests with pig manure

With 17 – 25 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 25 – 59 % air humidity sometimes was below the proposed range of 70 ± 25 % (OECD 208). However, the growth of the control plants was proper and as expected.

To verify the test item concentration at the start of the plant test, samples of spiked manure and spiked soil/manure mixture at a representative test item concentration were measured. The recovery rate for spiked manure and spiked soil/manure mixture was 11.4 – 31.9 % tylosin tartrate, respectively. Evaluation was done by using nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

There was no effect of the test item on the visual appearance regarding pathological symptoms of the seedlings when the test item was incubated anaerobically. This was not true for *P. vulgaris*.

There was no concentration dependent effect on seedling emergence of the species tested. All species showed NOEC values higher than the highest tested concentration.

Post-emergence survival was not affected by tylosin tartrate for all species tested up to and including 1000 mg tylosin tartrate per kg dry soil, the highest concentration tested. All species showed NOEC values higher than the highest tested concentration.

There were concentration dependent effects of tylosin tartrate on growth of *P. vulgaris* and *T. pratense* .The most sensitive species regarding shoot length was *P. vulgaris* with a NOEC of 426 mg/kg, and with an EC_{10} of 383 mg/kg. Also for fresh mass, *P. vulgaris* was the most sensitive species with a NOEC of 426 mg/kg, and *P. vulgaris* an EC_{50} of 553 mg/kg dry soil with and *T. pratense* with an EC_{10} of 66.8 mg/kg.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when incubated for maximum storage duration under anaerobic conditions with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 37. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 223 to Table 230.

Table 37: Tylosin tartrate Max Anaerobic Aged Pig Manure. NOEC, EC10 and EC50 values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Trifolium pratense*
Emergence						
NOEC	✥300	✥1000	✥391	✥391	✥937	✥125
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Post-emergence survival						
NOEC	✥300	✥1000	✥391	✥391	n.d.	n.d.
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Shoot length						
NOEC	✥300	✥1000	✥391	✥391	426	✥125
EC_{10}	n.d.	n.d.	n.d.	n.d.	383	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fresh mass						
NOEC	✥300	✥1000	✥391	✥391	426	✥125
EC_{10}	n.d.	n.d.	n.d.	n.d.	553	66.8
EC_{50}	n.d.	n.d.	n.d.	n.d.	885	n.d.

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

Conclusion:

The pig manure applied had no adverse effects on emergence rate, growth, or post-emergence survival. Tylosin tartrate has slight concentration depending effects on *P. vulgaris* and *T. pratense* under the chosen test conditions.

The relevant NOEC for the most sensitive endpoint (biomass and shoot length *Phaseolus vulgaris*) under anaerobic incubation conditions was found to be 426 mg/kg dry soil. The relevant EC_{10} (biomass *Trifolium pratense*) and EC50 values (biomass *Phaseolus vulgaris*) were calculated to be 66.8 mg/kg dry soil and 885 mg/kg dry soil, respectively.

7.9.2.4 Main tests with cattle manure

With 21 – 25 °C the permitted range of 22 °C ± 10 °C (OECD 208) was met. With 18 – 44 % air humidity sometimes was below the proposed range of 70 ± 25 % (OECD 208). However, the growth of the control plants was proper and as expected.

To verify the test item concentration at the start of the plant test, samples of spiked manure at a representative test item concentration were measured. The recovery rate for spiked manure was 14.9 – 30.4 % tylosin tartrate, respectively. Evaluation was done by using nominal concentrations.

There was no adverse effect of manure application noticeable on seedling emergence, growth, or post-emergence rate.

The only obvious effect of the test item on the visual appearance regarding pathological symptoms of the seedlings was a partly discoloration of leafs in *B. napus*, *P. vulgaris* and *T. pratense*.

There was no or only a slight concentration dependent effect on seedling emergence of the species tested. All species showed NOEC values ≥ then the highest concentration tested. An EC_{10} could be calculated for *Solanum lycopersicum*.

There was no concentration dependent effect on seedling emergence of the species tested. Hence, the NOEC was equal to or higher than the highest tested concentration. EC values could not be calculated

There were concentration dependent effects on growth all species (except *A. sativa* and *T. pratense* tested due to tylosin tartrate. The most sensitive species regarding shoot length was *B. napus* with a NOEC of 156 mg/kg, and *A. cepa* with an EC_{10} of 78.7 mg/kg. Also for fresh mass, *A. cepa* was the most sensitive species with a NOEC of 78.7 mg/kg, and *B. napus* with an EC_{10} of 78.7 mg/kg, and an EC50 of 391 mg/kg dry soil.

The NOEC, EC_{10} and EC_{50} values of tylosin tartrate when incubated for maximum storage duration under anaerobic conditions with subsequent application to soil for emergence, growth, and post-emergence survival are presented in Table 38. Effect concentrations were calculated based on nominal concentrations. The values for emergence and growth and the inhibition rates for the single treatments are presented as mean values in Annex 1, Table 231 to Table 238.

Table 38: Tylosin tartrate Max Anaerobic Aged Cattle Manure. NOEC, EC_{10} and EC_{50} values for emergence, post-emergence survival, shoot length, and fresh mass [mg TI/kg DM].

Species	*Allium cepa*	*Avena sativa*	*Brassica napus*	*Solanum lycopersicum*	*Phaseolus vulgaris*	*Trifolium pratense*
Emergence						
NOEC	✣300	✣1000	✣391	✣391	✣937	✣125
EC_{10}	n.d.	n.d.	n.d.	148	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Post-emergence survival						
NOEC	✣300	✣1000	n.d.	✣391	n.d.	✣125
EC_{10}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Shoot length						
NOEC	✣300	✣1000	156	✣391	194	✣125
EC_{10}	145	n.d.	119	n.d.	265	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fresh mass						
NOEC	150	✣1000	156	156	194	✣125
EC_{10}	104	n.d.	78.7	113	202	n.d.
EC_{50}	n.d.	n.d.	391	n.d.	525	n.d.

NOEC = No observed effect concentration; EC = Effect concentration; TI = Test item; DM = Dry mass; CL = Confidence level; n.d. = not determined due to mathematical reasons. Concentrations given as nominal values. n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

Conclusion:

The cattle manure applied had no adverse effects on emergence rate, growth, or post-emergence survival. Tylosin tartrate has clear concentration depending effects on terrestrial plants under the chosen test conditions.

The relevant NOEC for the most sensitive endpoint (biomass *Allium cepa*) under anaerobic incubation conditions was found to be 150 mg/kg dry soil. The relevant EC_{10} and EC_{50} values (biomass *Brassica napus*) were calculated to be 78.7 mg/kg dry soil and 391 mg/kg dry soil, respectively.

7.10 Chemical analyses

7.10.1 Materials and Methods

7.10.1.1 Information on test substances used in the project

Two substances (two veterinary pharmaceuticals) namely Florfenicol and Tylosin tartrate were used as test substances in the course of the research project.

Beside chemical analysis of the test substances in the plant growth tests, which aims at verification or falsification of the nominal test concentrations, it furthermore was an aim of the research project to follow the fate of one of the substances both in manure and soil. This was facilitated by the use of the radioactively labeled compound. As ^{14}C-florfenicol was easily available this substance was selected for the investigations of its fate in manure, soil, and manure applied soil.

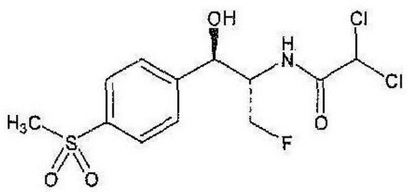
As the unlabeled substances are already described elsewhere in the report ^{14}C-Florfenicol only is characterized herein as follows:

Substance characterization

^{14}C-Florfenicol

Name:	[ring-U-^{14}C] Florfenicol
Chemical name:	^{14}C-2,2-Dichloro-N-[(1S,2R)-1-(fluoromethyl)-2-hydroxy-2-[4-(methylsulfonyl) phenyl] ethyl] acetamide
CAS-Number:	76639-94-6
Formula:	$C_{12}H_{14}Cl_2FNO_4S$

Figure 21: Structure of ^{14}C-Florfenicol.



Radiolabelling:	yes, [ring-U-14C]
Molecular weight:	362.1 g/mol (at that specific radioactivity)
Appearance	solid
Specific Radioactivity:	122 mCi/mmol; 4.51 GBq/mmol; 12.46 MBq/mg
Purity:	98.1% (date of analysis: 2 February 2012)
Code:	CFQ41389
Safety data sheet:	no

Receipt of test item: 13 August 2012

Waste disposal: hazardous waste

Expiry date: not given on the CoA

Origin: Quotient Bioresearch Ltd. (formerly: GE Healthcare)

7.10.1.2 Study design

Florfenicol

Arguments for the study design and sampling regime for 14C-Florfenicol fate investigations are given elsewhere in the report. Herein, the objective of study and the related sampling regime is briefly given:

Table 39: Study design and sampling regime for the investigations of the fate of ^{14}C-florfenicol in manure and manure applied soil.

Objective	Design	Sampling regime
Stability and transformation of Florfenicol in cattle manure	Anaerobic incubation of ^{14}C-Florfenicol in cattle manure 20 °C Flow-through apparatus Quantification of parent, possible transformation products (TP), evolving CO2 and CH4, and non-extractable residues Establishment of mass balance Determination of microbial activity of the manure at the start and the end of the incubation by testing an easily degradable substance (^{14}C-glucose)	0 d, 2 h, 4 h, 7 h, 24 h, 45 d (half of typical cattle manure storage period), 90 d (typical cattle manure storage period)
Stability and transformation of Florfenicol in pig manure		0 d, 2 h, 4 h, 7 h, 16 h, 24 h, 26 d (half of typical pig manure storage period), 53 d (typical pig manure storage period)
Stability and transformation of Florfenicol in manure applied soil (different modes of application9	Florfenicol applied onto quartz sand; quartz sand mixed into soil Florfenicol dissolved in an organic solvent and added to the soil Florfenicol added to pig and cattle manure, respectively; manure mixed into the soil; Subsequent fortification: aerobic incubation of the fortified soils	Soil sampling and clean-up after 0 d, 27 d, and 46 d

Besides these transformation experiments, concentrations of un-labeled Florfenicol in the application solutions which were used for the plant growth tests were measured.

Tylosin tartrate

Tylosin tartrate was analysed in various manure samples obtained from the partner ECT which were used for the plant growth test. It was the aim to check the nominal concentrations determined by weighing the test substance Tylosin tartrate. Selection of samples was such, that

- the Tylosin tartrate concentration range used in the plant growth tests was covered,
- plant species used in the growth tests were covered,
- representative samples of fresh manure, manure incubated for half of maximum storage duration, and manure incubated for maximum storage duration were analysed,
- pig and cattle manure was analysed.

Representative samples provided by ECT are listed in Table 40 and Table 41 in detail.

Table 40: Cattle manure samples provided by ECT for Tylosin tartrate analysis.

Nominal concentration	Sample
0.228 mg Tylosin tartrate/g cattle manure fw	Manure used for plant growth test with *T. pratense*; half of typical manure storage period (45 d)
	Manure used for plant growth test with *T. pratense*; typical manure storage period (90 d)
1.365 mg Tylosin tartrate/g cattle manure fw	Fresh cattle manure
	Manure used for plant growth test with *A. cepa*; half of typical manure storage period (45 d)
	Manure used for plant growth test with *A. cepa*; typical manure storage period (90 d)
4.0 mg Tylosin tartrate/g cattle manure fw	Fresh cattle manure
	Manure used for plant growth test with *P. vulgaris*; typical manure storage period (90 d)
7.1 mg Tylosin tartrate/g cattle manure fw	Manure used for plant growth test with *S. lycopersicum*; typical manure storage period (90 d)
17.75 mg Tylosin tartrate/g cattle manure fw	Manure used for plant growth test with *B. napus*; typical manure storage period (90 d)
45.45 mg Tylosin tartrate/g cattle manure fw	Fresh cattle manure
	Manure used for plant growth test with *A. sativa*; typical manure storage period (90 d)

Table 41: Pig manure samples provided by ECT for Tylosin tartrate analysis.

Nominal concentration	Sample
0.228 mg Tylosin tartrate/g pig manure fw	Fresh pig manure
	Manure used for plant growth test with *T. pratense*; half of typical manure storage period (26 d)
	Manure used for plant growth test with *T. pratense*; typical manure storage period (53 d)
1.365 mg Tylosin tartrate/g pig manure fw	Fresh pig manure
	Manure used for plant growth test with *A. cepa*; half of typical manure storage period (26 d)
	Manure used for plant growth test with *A. cepa*; typical manure storage period (53 d)
4.0 mg Tylosin tartrate/g pig manure fw	Fresh pig manure
	Manure used for plant growth test with *P. vulgaris*; half of typical manure storage period (26 d)
	Manure used for plant growth test with *P. vulgaris*; typical manure storage period (53 d)
7.1 mg Tylosin tartrate/g pig manure fw	Fresh pig manure
	Manure used for plant growth test with *S. lycopersicum*; half of typical manure storage period (26 d)
	Manure used for plant growth test with *S. lycopersicum* ; typical manure storage period (53 d)
17.75 mg Tylosin tartrate/g pig manure fw	Fresh pig manure
	Manure used for plant growth test with *B. napus*; half of typical manure storage period (26 d)
	Manure used for plant growth test with *B. napus*; typical manure storage period (53 d)
45.45 mg Tylosin tartrate/g pig manure fw	Fresh pig manure
	Manure used for plant growth test with *A. sativa*; half of typical manure storage period (26 d)
	Manure used for plant growth test with *A. sativa*; typical manure storage period (53 d)

7.10.1.3 Adjustment of dry matter content of manure

Before performing the transformation test with ^{14}C-Florfenicol, the manure was acclimatized to test conditions. Prior to the start of the acclimation period, the dry matter content of the manure was determined. To get comparable conditions it was adjusted to standardized values. The recommended dry matter content in cattle and pig manure is 10% ± 1% and 5% ± 1%, respectively (EMA/CVMP/ERA/430327/2009 (2011)). If the dry matter content of the original manure was below the recommended value, it was concentrated by careful centrifugation for 10 minutes at 740 x g. If the dry matter content was too high, water (de-ionized water, bubbled with nitrogen for 30 min) was added.

7.10.1.4 Acclimation of manure

After the adjustment of the dry matter content, cattle manure was homogenized by gently mixing using a glass bar. Subsamples of 50 g (wet weight) each were directly filled into the incubation vessels which are used for the acclimation and transformation study. No additional measures to prevent introduction of oxygen were used during both processes, homogenisation and filling of incubation vessels.

Pig manure was homogenized under anaerobic conditions by a knifetec mill (or similar apparatus) in order to obtain a fairly stable phase. This was achieved by filling the manure into a container or beaker, putting the knifetec mill into the manure, sealing with parafilm and gently passing a nitrogen stream over the manure while mixing for 1 minute. Thereafter, the dry matter content was adjusted. After a repeated homogenization under anaerobic conditions by thoroughly mixing (set up as above) subsamples of 50 g (fresh weight) were filled into the incubation vessels. Then the incubation apparatus was closed and a constant, water saturated stream of nitrogen was passed over the manure at a rate in the range of approximately 50 - 200 mL/min. As pre-tests have shown that an acclimation period of 21 days is appropriate the acclimation was routinely carried out for 21 days at 20°C.

7.10.1.5 Flow through apparatus (transformation in manure)

For the transformation studies with ^{14}C-Florfenicol a flow through apparatus was used. A schematic presentation of the system is shown on the following page.

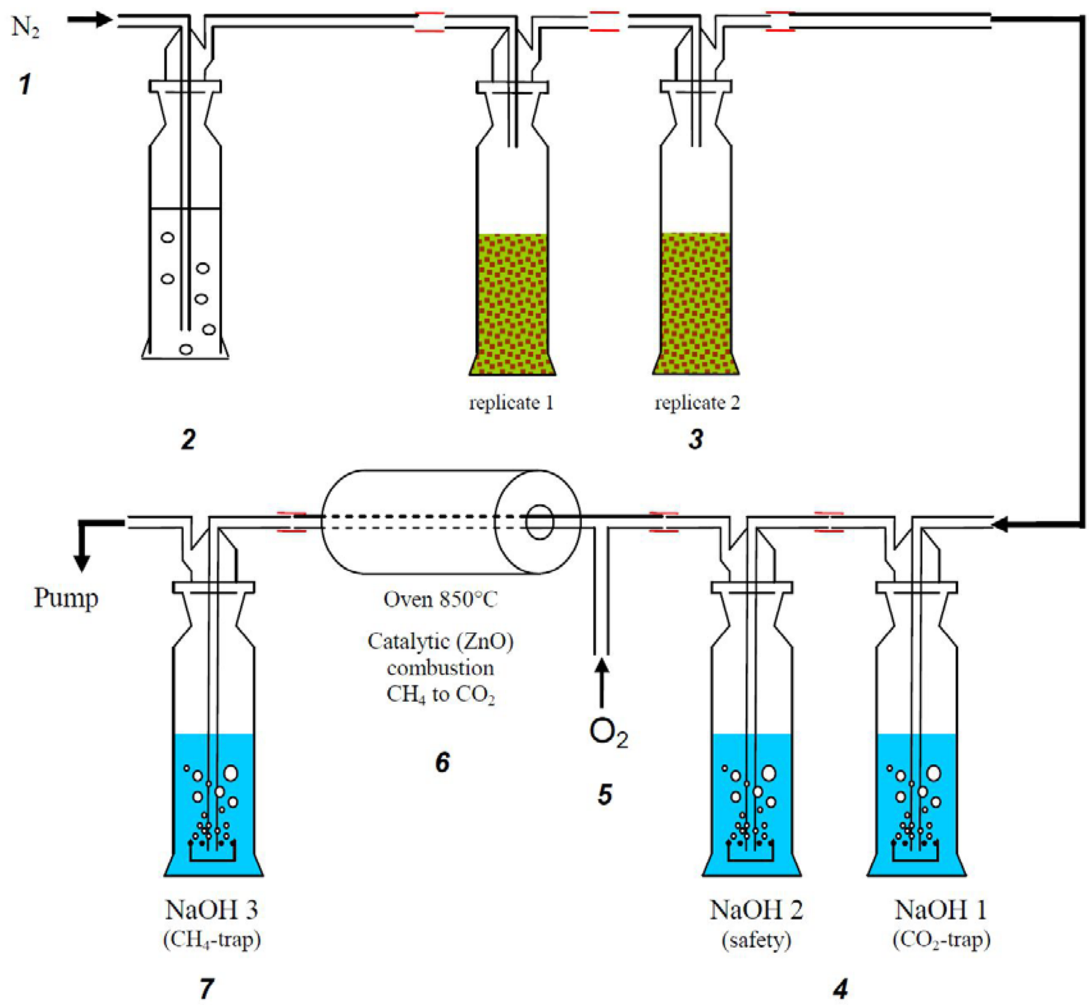
The flow through apparatus is a gas tight system of incubation vessels and traps set in sequence. Humidified nitrogen is gently passed over the liquid manure sub-samples. At the gas inlet nitrogen is given with a slight excess. By having a T-junction, excessive gas can escape via a washing flask, whereas the needed nitrogen is passed over the manure samples. By such a design back-flush can be avoided.

The nitrogen is passed over the samples at a flow rate of 50 – 200 mL/min. First, the nitrogen is bubbled through water in order to humidify the gas. Thereafter, the humidified gas is passed over the manure subsamples. Two replicates per sampling point are set in sequence. The vessels for the individual sampling points are set in parallel. Once the gas has passed over the second replicate it is bubbled through two adsorption traps in sequence containing 2 M NaOH. Traps are for sorbing the evolving $^{14}CO_2$ and other possibly occurring volatiles. Since the formation of $^{14}CH_4$ is expected in such an anaerobic system the gas is furthermore passed through an oven at 850°C. $^{14}CH_4$ is catalytically (CuO-catalyst + O_2 feeding to the tube) converted to $^{14}CO_2$ which again is trapped in a third NaOH trap.

During the whole test period the manure samples are incubated in the dark at 20°C.

The transformation studies in cattle and pig manure were performed under anaerobic conditions. Anaerobic conditions were demonstrated by Eh < -100 mV. Redox potentials were measured and recorded at start of the acclimation, at the start and at the end of the transformation studies.

Figure 22: Example of a flow-through apparatus to study transformation of chemicals in manure (2 replicates are shown).



1: nitrogen is gently passed over the manure samples (20 – 500 mL/h)
2: gas washing bottle containing water
3: manure transformation flasks filled with at least 50 – 100 g manure (fresh weight)
4: for anaerobic transformation two NaOH-traps in sequence are needed to trap evolving CO_2.
5: addition of oxygen for subsequent catalytic combustion of CH_4
6: oven for combustion of CH_4 to form CO_2
7: NaOH-trap for CO_2 formed from CH_4

7.10.1.6 Characteristics and preparation of soil

To study the stability of florfenicol in soil and in manure applied soil, respectively, the soil RefeSol 01-A was used. The soil was freshly sampled by Fh-IME. The properties of the soil are given in Table 42. Characterization has been performed by Fh-IME following GLP, study code: IME-010/7-85. Further information on the soil can be found under www.refesol.de.

Table 42: Soil parameter of the test soil RefeSol 01-A used in the fate approach.

Soil	Sand 2000 - 63 ↔m (%)	Silt 63 - 2 ↔m (%)	Clay < 2 ↔m (%)	Sand 2000 - 50 ↔m (%)	Silt 50 - 2 ↔m (%)	Clay < 2 ↔m (%)	Corg (%)	pH (0,01 M CaCl2)	CECeff (mmolc /kg)	WHCmax (g/kg)
	According to E DIN ISO 11277			According to USDA						
RefeSol 01-A	70.1	24.2	5.7	70.8	21.8	7.4	1.1	5.0	8.6	233

Prior to incubation the soil was sieved (2 mm sieve). The maximum water holding capacity (WHC) was determined again and the moisture content of the soil was adjusted to pF ~2 (approximately 50 % WHC).

After such preparation 50 g (dry weight) soil each were filled into incubation vessels and pre-incubated aerobically for 7 days at 20°C. In parallel, liquid manure was incubated anaerobically for 7 days at 20°C.

7.10.1.7 Substance application to manure

An existing ^{14}C-Florfenicol stock solution was diluted appropriately by using acetone. Unlabeled Florfenicol (LOT 23922307) was added to result in a final concentration in the stock solution of 25.458 g/L.

The required volume of stock solution (491 µL corresponding to 12.5 g ^{14}C-Florfenicol) was pipetted into 50 g manure fw under simultaneous stirring using the pipette tip. As soon as the solution was evenly distributed in the manure the pipette remained in the manure. Addition of the test substance was while maintaining anaerobic conditions. This was achieved by maintaining to pass the nitrogen stream over the samples during the application procedure.

The Florfenicol concentration was 12.5 g /50 g manure fw and 250 mg/kg manure fw. 100 kBq each were added to the manure subsamples. The specific radioactivity which was obtained after the addition of unlabeled Florfenicol was 0.008 MBq/mg.

7.10.1.8 Substance application to soil and manure applied soil

Florfenicol

Application to soil

Two ways of Florfenicol application were tested:

a) Application dissolved in an organic solvent

^{14}C-Florfenicol was dissolved in an as small as possible volume of actone. 12.5 mg ^{14}C-Florfenicol in 1 mL acetone were pipetted onto 50 g soil (dry weight) and evenly distributed by stirring. Thereafter, the fortified incubation dishes were put into the incubation chamber and left under aerobic conditions for 27 d and 46 d, respectively.

b) Application via quartz sand

Florfenicol was applied onto quartz sand. This was achieved by dissolving ^{14}C-Florfenicol in actone, and addition of an appropriate amount of quartz sand to that solution. Acetone was evaporated using a rotary evaporator. The fortified quartz sand (1 g) was added to 50 g soil dry weight. As for the transformation of ^{14}C-Florfenicol in manure, substance concentrations were 12.5 mg/50 g soil (dry weight).

Application to manure applied soil

1.1 g liquid manure (cattle manure: 10 % dm, pig manure: 5 % dm) was added to 50 g soil (dry weight). The water content of the soil had been adjusted accordingly under consideration of further water addition when adding liquid manure. Both, soil and manure previously had been acclimatized for 7 days at 20°C. After the addition of manure, the soil + manure mixture was stirred in order to obtain a homogenous distribution of both matrices.

Furthermore, Florfenicol was applied onto quartz sand. This was achieved by dissolving ^{14}C-Florfenicol in actone, and addition of an appropriate amount of quartz sand to that solution. Acetone was evaporated using a rotary evaporator. The fortified quartz sand was added to the manure applied soil. As for the transformation of ^{14}C-Florfenicol in manure, substance concentrations were equal to 12.5 mg/50 soil + manure.

Thereafter, the fortified samples were put into the incubation chamber and left under aerobic conditions for 27 d and 46 d, respectively.

7.10.1.9 Clean-up

Florfenicol in liquid manure

At the sampling times the glass-flasks containing the respective spiked manure samples and the corresponding absorption traps are removed from the incubation system. The content of each glass-flask is transferred to a glass centrifuge tube and extracted 3 times by 30 mL methanol and once by 30 mL methanol + 1.5 mL trifluoroacetic acid (TFA).

Extracts are quantified by liquid scintillation counting (LSC) and analysed for the test item by TLC-analysis. The extracted manure is analysed for non extractable residues by combustion with subsequent LSC of the formed $^{14}CO_2$.

The volume of the NaOH-adsorption solutions is measured and radioactivity in each solution is determined by LSC. Evolved $^{14}CO_2$ and $^{14}CH_4$ can be quantified and the rate of mineralization can be determined.

Florfenicol in soil

At the sampling times the glass-vessels containing the respective soil samples are removed from the incubation chamber. The content of each glass-dish is transferred into a glass centrifuge tube and extracted three times by 30 mL methanol. For extraction the centrifuge tube is shaken for 10 minutes on a horizontal shaker. After centrifugation for 10 min at 2000 rpm the liquid phase is separated from the soil. The methanol extracts are combined, and the volume of the combined extracts is determined. Extracts are concentrated if appropriate and subjected to radio-TLC.

The remaining soil is extracted once by addition of methanolic trifluoro acetic acid (TFA) and shaking overnight to additionally obtain polar compounds. The acid liquid phase is separated by centrifugation and subjected to radio-TLC for characterization of ^{14}C-Florfenicol and further polar metabolites if the amount of radioactivity exceeded 5 % aR.

An aliquot of the residual soil is combusted and radio-assayed.

The volume of the solutions in the absorption traps was measured and radioactivity in each solution was determined by LSC.

Tylosin tartrate in manure

50 g manure (fresh weight) is centrifuged for 30 minutes at 12500 x g. Thereafter, the supernatant is decanted and filled up to 100 mL using ultra pure water (solution 1).

25 mL methanol/acetonitrile/ascorbic acid (45/45/10; v/v/v) are added to the pellet, subjected to vortex for 1 min and to sonification for 30 minutes. Thereafter, the mixture is shaken for one hour on a horizontal shaker and centrifuged for 15 minutes at 12500 x g. The extraction is repeated and both extracts are combined. The combined extracts are transferred to a beaker. The centrifuge tube is rinsed with 40 mL ultra pure water and 2 mL methanol. Thereafter, the extracts are filled up to 200 mL using ultra pure water (solution 2).

SPE-columns (OASIS HLB6CC, 500 mg stationary phase) are conditioned by 10 mL methanol and 10 mL water. Solution 1 and 2 are each applied onto the columns, thereafter the columns are sucked to slight dryness. Elution of the analyte is by addition of 4 x 0.5 mL methanol + 1.25 % acetic acid. The eluates are combined and concentrated to dryness under nitrogen. The residuals are dissolved in methanol/water (70/30; v/v) and subjected to HPLC.

Recoveries for the described methodology were in the range of 65 – 70%. This is in agreement with literature data (e.g.: Kay P. et al. (2004): Fate of veterinary antibiotics in a macro-porous tile drained clay soil. Environmental Toxicology and Chemistry, Vol. 23 (5), 1136-1144).

7.10.1.10 Analytical methods

Liquid scintillation counting (LSC; for ^{14}C-Florfenicl)

After mixing an aliquot of the extract of interest with an aliquot of a suitable liquid scintillation cocktail (Pico-Fluor 40, Pico-Aqua or Pico-Fluor LLT for aqueous samples and Ultima Gold or

Pico-Fluor 40 for organic samples) LSC measurements are performed using a Packard Tri-Carb liquid scintillation analyzer. Each sample is measured for 5-10 minutes in duplicate in order to increase the sensitivity of the analytical method and to ensure reproducibility and the values reported are the mean values. Computer-constructed quench curves, derived from a commercially available series of sealed quenched standards (from Packard); automatically convert counts per minute to decays per minute.

Analysis by Thin Layer Chromatography with radio-detection (radio-TLC; for ^{14}C-Florfenicol)

As the matrix manure influences HPLC, radio-TLC is applied routinely to all extracts. The following TLC-system is used:

stationary phase:	silica gel KG60
mobile phase:	dichloromethane / methanol; 90/10 (v/v).

Florfenicol and transformation products (TP) were characterised by their Rf-values:

Florfenicol	Rf = 0.45 – 0.58
TP1	Rf = 0.0 – 0.1
TP2	Rf = 0.1 – 0.16
TP3	Rf = 0.32 – 0.36

The LOD is equal to 0.001 [% aR].

This can be derived as follows:

- 10 Bq in 100 µL are spotted onto the TLC-plate.
- A relative peak area of 5% can be integrated unequivocally, i.e. 0.05 * 10 Bq = 0.5 Bq can be integrated.
- 100 kBq Florfenicol are applied onto the manure or soil sample; 100 kBq = 100 [% aR]
- 0.5 Bq = 0.001 [% aR].

High performance liquid chromatography (for unlabeled Florfenicol)

Application solutions were analyzed for Florfenicol concentrations by HPLC-UV/VIS. Prior to HPLC-measurements the stock solutions were diluted; dilution factors are given in the results chapter. Measurement conditions and instrument settings are given as follows.

<u>HPLC conditions:</u>

HPLC pump:	gradient pump Ultimate 3000
UV detector:	Dionex Ultimate 3000
Radioactivity detector:	Raytest Ramona Star Beta
Column:	Luna C18 100 A (250 x 4.6 mm, 5µm)
Eluent A:	methanol
Eluent B:	UHQ water

Gradient:

Time (min)	Flow (mL/min)	% A	% B	Gradient
0	0.75	100	0	-
5	0.75	0	100	linear
7	0.75	0	100	-
15	0.75	100	0	linear

Thermostat: Lauda Ecoline RE 106

Temperature: 25°C

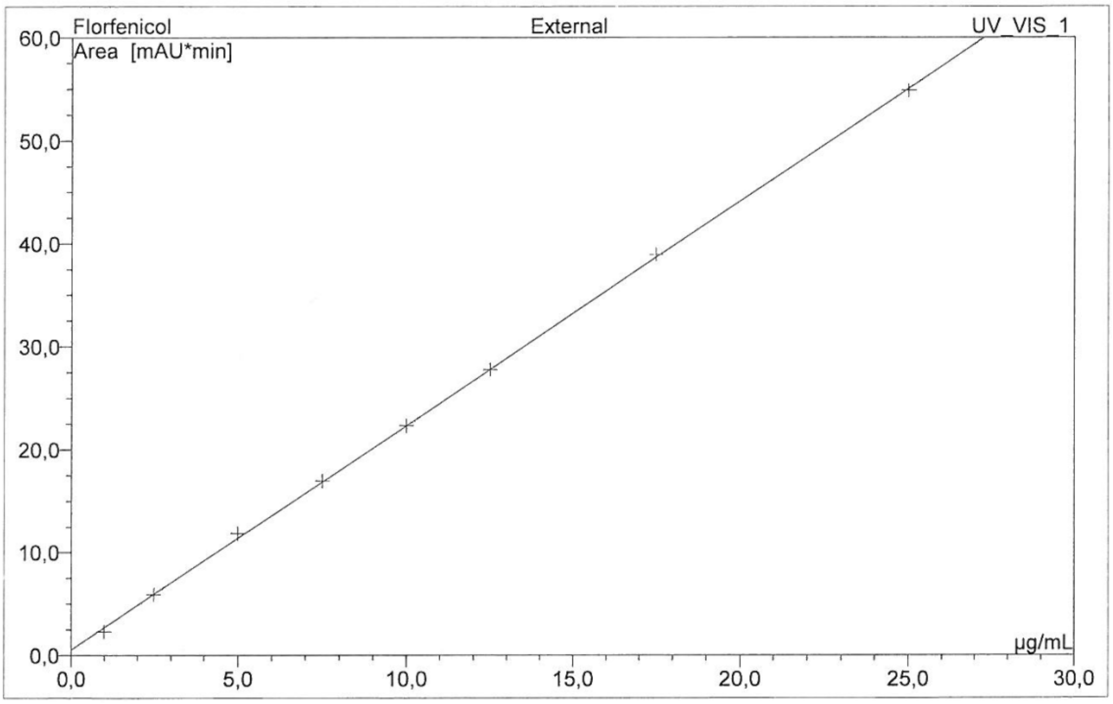
Retention time: 9.24 – 10.50 min

Wavelength: 225 nm

Calibration function: Peak area = 2.1819 * (cCal) +0.5256; r^2 = 0.9998

LOD: 1 mg/L (lowest concentration which can be determined by HPLC. The concentration was fixed pragmatically by defining the lowest concentration measured for the calibration function as LOD)

Figure 23: Calibration curve for unlabeled Florfenicol (x-axes = Florfenicol concentrations in the range of 1 mg/L – 25 mg/L; y-axes = Peak area in [mAU*min]).



High performance liquid chromatography (for Tylosin tartrate)

The samples were analyzed for Tylosin tartrate concentrations by HPLC-UV/VIS. Measurement conditions and instrument settings are given as follows.

HPLC conditions:

HPLC pump: gradient pump Dionex Ultimate 3000

DAD detector: Dionex Ultimate 3000

Column: 150 x 4.6 mm Stainless Steel; stationary phase: Luna C18 5µm, with pre column security guard cartridge C18, 4 x 3.0 mm ID; Phenomenex

Eluent A: 0.01 M ammonium acetate, pH=4.6

Eluent B: acetonitrile

Gradient:

Time (min)	Flow (mL/min)	% A	% B
0	0.5	90	10
1	0.5	90	10
15.0	0.5	0	100
17.0	0.5	0	100
19.0	0.5	90	10
25.0	0.5	90	10

Thermostat: Lauda Ecoline RE 106

Temperature: 40°C

Wavelength: 287 nm

Injection volume: 50 µL

Retention times: Tylosin A = 8.36 – 8.94 min

Tylosin B = 7.63 – 8.22 min

Tylosin C = 7.95 – 8.54 min

Tylosin D = 9.05 – 9.18 min

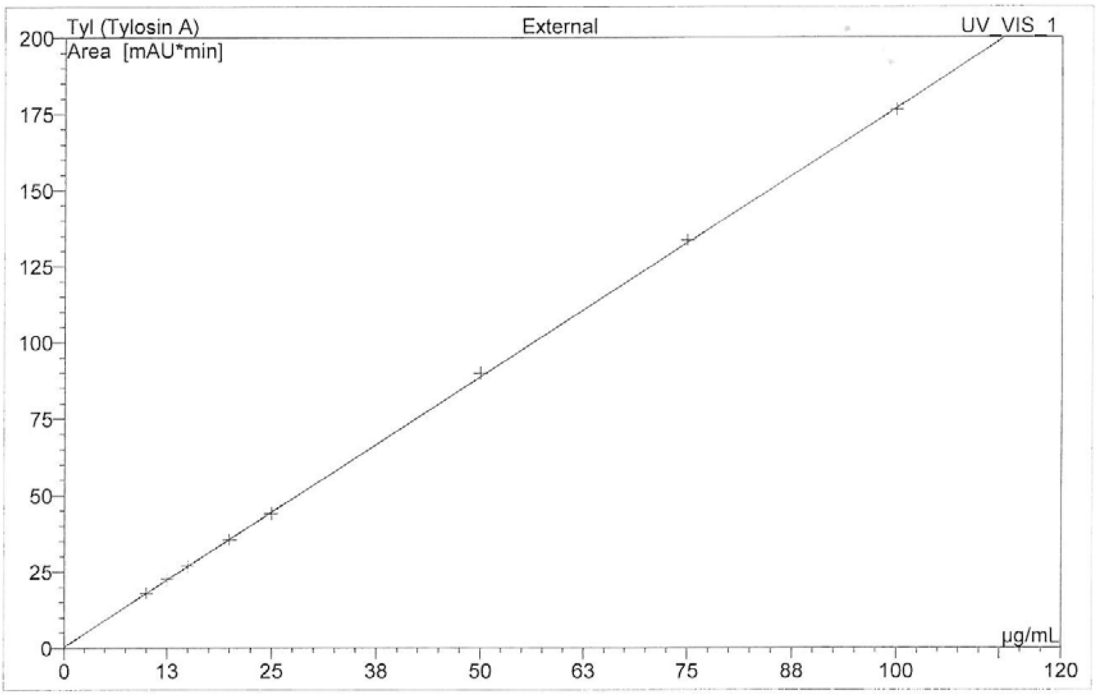
Calibration function: Peak area = 1.7674 * (cCal) + 0.4076; r^2 = 0.9898

LOD: 400 µg Tylosin tartrate/kg manure

This can be derived as follows:

- Lowest concentration which can be determined by HPLC: 10 mg/L (lowest measured concentration in the calibration curve
- Volume of solution obtained after clean-up and subjected to HPLC: 2 mL
- Total amount in 2 mL which can determined = 20 µg Tylosin tartrate
- Clean-up was for 50 g manure fresh weight: 20 µg Tylosin tartrate / 50 g manure = 400 µg/kg manure

Figure 24: Calibration curve for Tylosin tartrate in aqueous solution (x-axes = Tylosin tartrate concentrations in the working range of 10 mg/L – 100 mg/L; y-axes = Peak area in [mAU*min]).



7.10.2 Results

7.10.2.1 ^{14}C-Florfenicol in cattle and pig manure

^{14}C-Distribution and mass balances

The behavior of ^{14}C-florfenicol in cattle and pig manure was followed for 90 days (agreed maximum manure storage duration, cattle manure) and 53 days (agreed maximum manure storage duration, pig manure), respectively. As Florfenicol is known to rapidly dissipate in manure, the sampling regime was narrow within 24 hours after application. Furthermore, at half of maximum storage duration and maximum storage duration samples were taken.

Samples were analysed for radioactivity distribution, i.e. extractable residues, non-extractable residues, evolved CO_2 and CH_4. By addition of the amount of ^{14}C-radioactivity in these compartments a mass balance was established. The results are shown in the following tables and figure. Results in the figure are given as mean values as presented in the above table each.

In cattle manure, the amount of extractable residues decreases from 106.2 [% aR] at the test start to 36.8 [% aR] at termination of incubation after 90 days. In parallel, the amount of non-extractable residues (NER) increases from 7.7 [% aR] to 69.6 [% aR] after 45 days. Thereafter, no further increase is observed, but after 90 days the amount of NER (68.8 [% aR]) is comparable to that after 45 days. Mineralisation is modest, and the amount of formed $^{14}CO_2$ + $^{14}CH_4$ is 1.9 [% aR] after 90 days of incubation. The mass balance is above 100 [% aR] in all cases and ranges between 106.2 and 116.1 [% aR].

In pig manure, the amount of extractable residues decreases from 100.8 [% aR] at the beginning of incubation to 63.8 [% aR] after 26 days and 69.6 [% aR] after 53 days of incubation, respectively. The amount of NER increases from 1.6 [% aR] to 38.1 [% aR]. Formation of $^{14}CO_2$ + $^{14}CH_4$ is even less compared to cattle manure and is 0.2 [% aR] after 53 days of incubation. The mass balance is above 100 [% aR] in all cases and ranges between 102.4 and 108.0 [% aR].

Table 43: ^{14}C-Distribution and mass balance of Florfenicol in cattle manure. Applied amount: 250 mg/kg manure fw; 100 kBq.

Sample [incubation time _replicate]	Extr. [% aR]	Mean [% aR]	NE [% aR]	Mean [% aR]	$14CO_2$ [% aR]	$14CH_4$ [% aR]	Recovery [% aR]	Mean [% aR]
0 d_1	103.8	106.2	7.9	7.7	-	-	111.7	113.9
0 d_2	108.7		7.5		-	-	116.2	
2 h_1	106.8	107.0	8.2	9.2	0.0	0.0	115.0	116.1
2 h_2	107.1		10.1				117.1	
4 h_1	104.3	101.7	10.8	11.1	0.0	0.0	115.2	112.7
4 h_2	99.0		11.3				110.3	
7 h_1	100.1	102.0	12.4	11.8	0.0	0.0	112.5	113.8
7 h_2	104.0		11.2				115.2	
24 h_1	86.1	88.7	17.9	17.5	0.0	0.0	104.1	106.2
24 h_2	91.2		17.0				108.3	
45 d_1	36.3	39.5	70.5	69.6	0.1	1.6	108.5	110.8
45 d_2	42.6		68.8				113.1	
90 d_1	36.9	36.8	68.5	68.8	0.1	1.9	107.4	107.6
90 d_2	36.6		69.0				107.7	

Extr. = extractable; NE = non extractable.

Figure 25: Radioactivity distribution of ^{14}C-Florfenicol in cattle manure after different times of incubation. Data are given as mean values as presented in the above table.

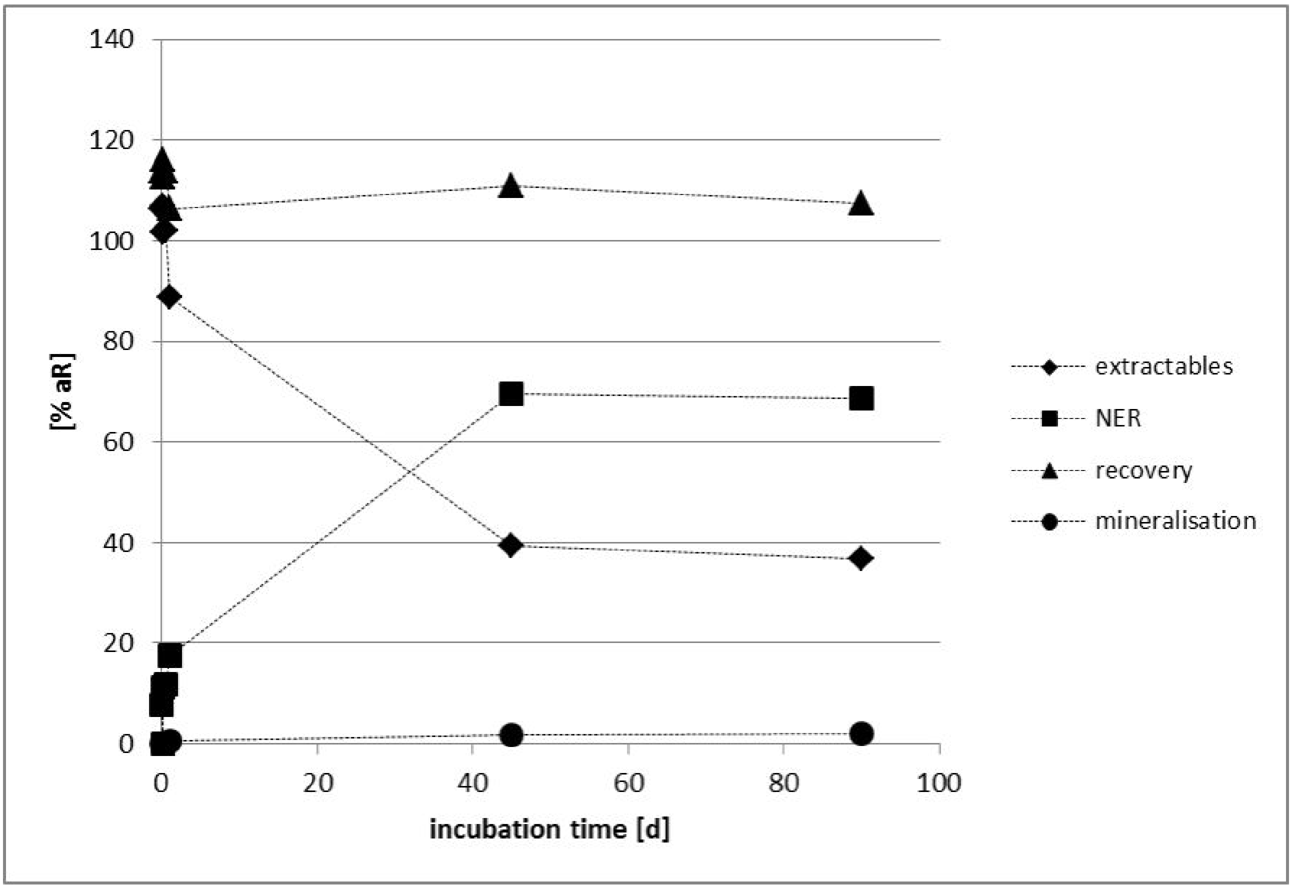
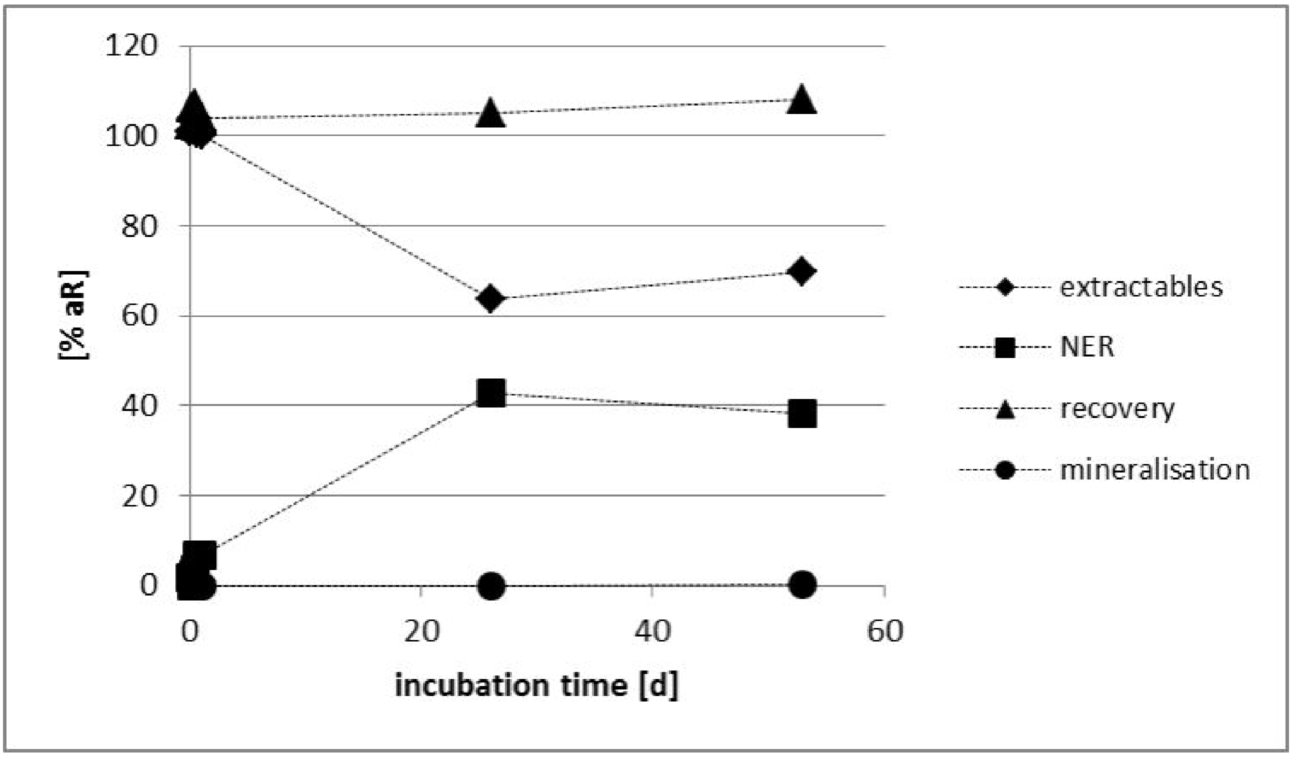


Table 44: ^{14}C-Distribution and mass balance of Florfenicol in pig manure. Applied amount: 250 mg/kg manure fw; 100 kBq.

Sample [incubation time _replicate]	Extr. [% aR]	Mean [% aR]	NE [% aR]	Mean [% aR]	$14CO_2$ [% aR]	$14CH_4$ [% aR]	Recovery [% aR]	Mean [% aR]
0 d_1	100.8	100.8	1.6	1.6	-	-	102.4	102.4
0 d_2*)	100.8		1.6		-	-	102.4	
2 h_1	105.5	103.7	2.4	2.8	0.0	0.0	107.8	106.4
2 h_2	101.9		3.1				105	
4 h_1	104	103.7	2.9	2.7	0.0	0.0	106.8	106.4
4 h_2	103.4		2.5				105.9	
7 h_1	101.5	103	3.7	3.6	0.0	0.0	105.2	106.7
7 h_2	104.5		3.5				108.1	
16 h_1	100.1	100.5	7.7	6.8	0.0	0.0	107.8	107.3
16 h_2	100.8		5.8				106.7	
24 h_1	97.9	100.2	9.1	6.7	0.0	0.0	106.9	106.9
24 h_2	102.5		4.4				106.9	
26 d_1	62.4	63.8	42.1	42.8	0.0	0.0	104.5	106.7
26 d_2	65.3		43.5				108.9	
53 d_1	64.6	69.6	38.9	38.1	0.2	0.0	103.7	108.0
53 d_2	74.6		37.3				112.2	

Extr. = extractable; NE = non extractable; *) sampled destroyed during clean-up.

Figure 26: Radioactivity distribution of ^{14}C-Florfenicol in pig manure after different times of incubation. Data are given as mean values as presented in the above table.



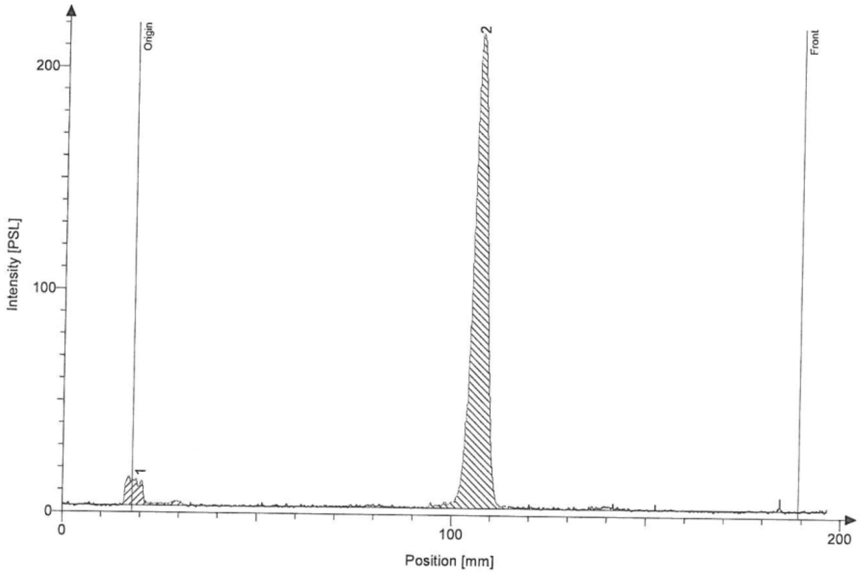
Behaviour of Florfenicol and transformation products in cattle and pig manure

Extracts were analysed for ^{14}C-Florfenicol and possible transformation products. Besides Florfenicol, three transformation products (TP1 to TP3) were observed. These compounds were not identified with respect to their chemical masses and possible structure, but characterized by their chromatographic behavior, i.e. by their Rf-values. These were for:

Florfenicol	Rf =
TP1	Rf = 0.0 – 0.1
TP2	Rf = 0.1 – 0.16
TP3	Rf = 0.32 – 0.36

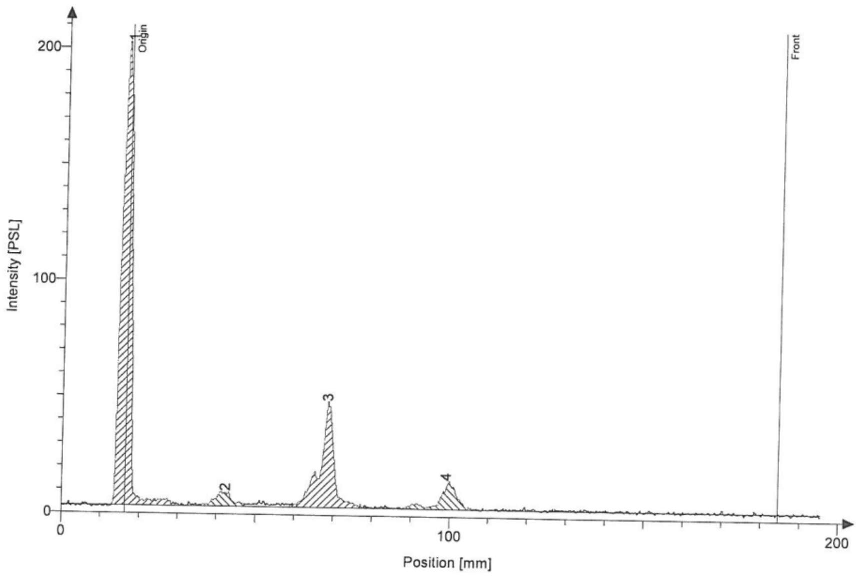
In cattle manure, the amount of Florfenicol descends from 97.8 [% aR] at the beginning of the incubation phase to 1.2 [% aR] at termination of incubation after 90 days. Within 24 hours of incubation, the Florfenicol amount decreases to 78.6 [% aR]. This is in contrast to other experiments where a much faster disappearance can be observed. The reduced transformation activity is supported by the observation of two transformation products, namely TP1 and TP3. From the chromatographic behaviour it can be concluded that TP1 might consist of one or even more small polar products; TP3 seems to be less polar but of a chromatographic behaviour comparable to that of Florfenicol. Such transformation products, in particular those with a behaviour similar to TP3, are not observed in transformation studies performed in manure at 20°C. An explanation for such behaviour might be the high Florfenicol concentration in liquid manure (applied amount: 250 mg/kg manure fw) and the bactericidal potency of Florfenicol. Exemplary chromatograms are shown in the following figures.

Figure 27: TLC-chromatogram of Florfenicol in cattle manure. Sample: 0 days of incubation.



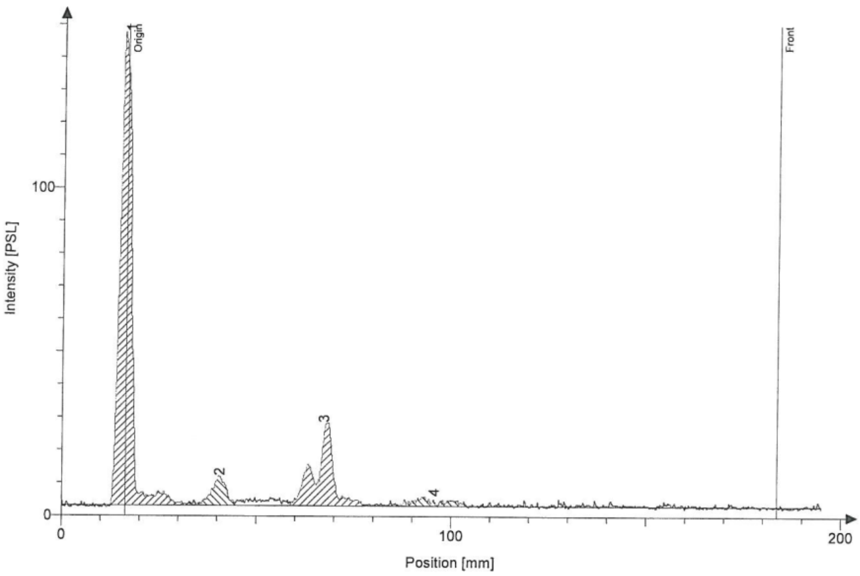
Stationary phase: silica gel KG60; mobile phase: dichloromethane / methanol; 90/10 (v/v); Florfenicol Rf = 0.45 – 0.58; TP1 Rf = 0.0 – 0.1; TP2 Rf = 0.1 – 0.16; TP3 Rf = 0.32 – 0.36.

Figure 28: TLC-chromatogram of Florfenicol in cattle manure. Sample: 45 days of incubation.



Stationary phase: silica gel KG60; mobile phase: dichloromethane / methanol; 90/10 (v/v); Florfenicol Rf = 0.45 – 0.58; TP1 Rf = 0.0 – 0.1; TP2 Rf = 0.1 – 0.16; TP3 Rf = 0.32 – 0.36.

Figure 29: TLC-chromatogram of Florfenicol in cattle manure. Sample: 90 days of incubation.



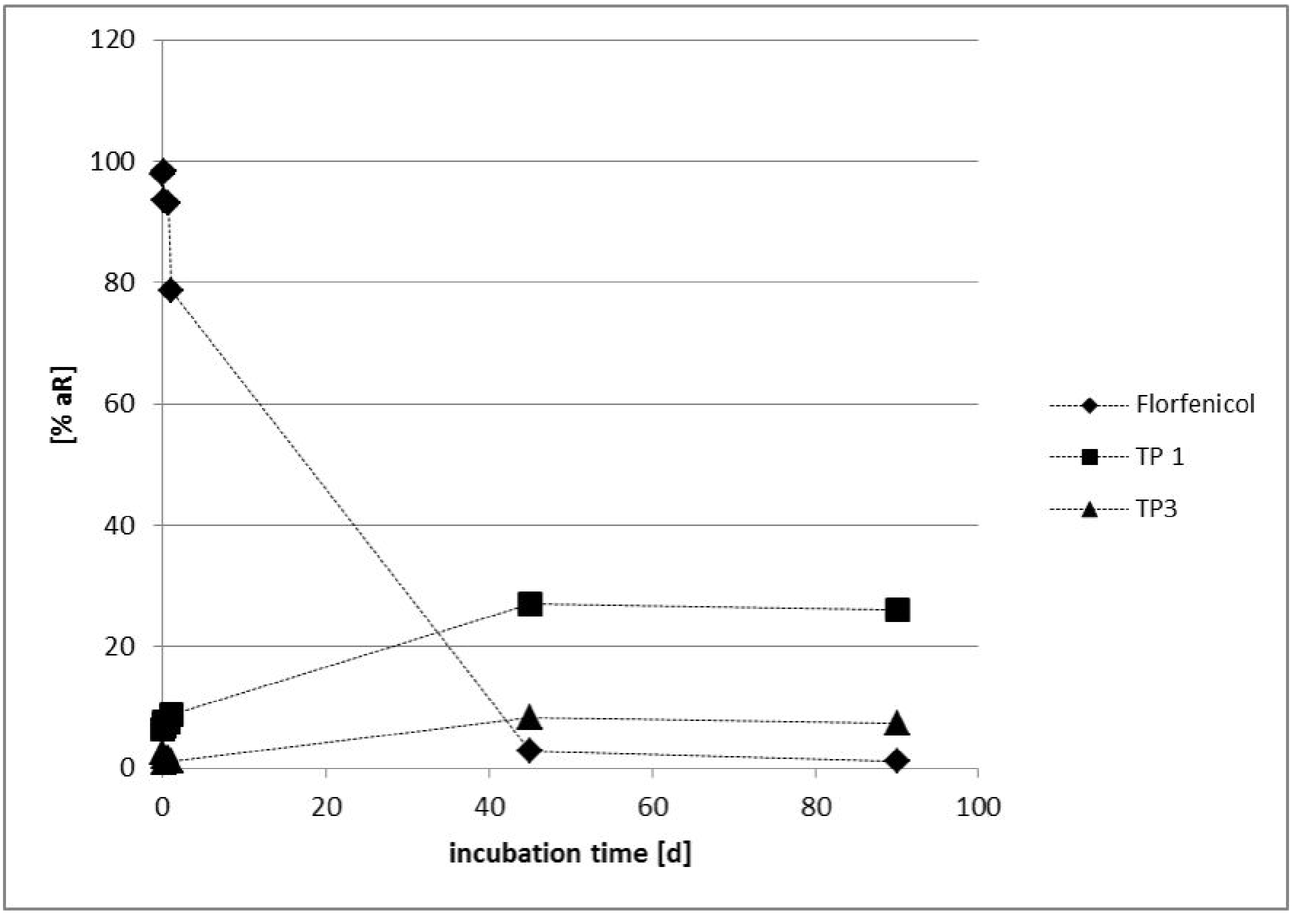
Stationary phase: silica gel KG60; mobile phase: dichloromethane / methanol; 90/10 (v/v); Florfenicol Rf = 0.45 – 0.58; TP1 Rf = 0.0 – 0.1; TP2 Rf = 0.1 – 0.16; TP3 Rf = 0.32 – 0.36.

Table 45: Concentration of Florfenicol and transformation products (TP) [% aR] in cattle manure.

Incubation time [d]	Florfenicol [%aR]	TP 1 [%aR]	TP2 [%aR]	TP3 [%aR]
0	97.8	6.5	n.d.	2.6
0.1	98.3	6.8	n.d.	1.8
0.2	93.4	7.5	n.d.	0.8
0.6	93	7.5	n.d.	1.6
1	78.6	8.8	n.d.	1.2
45	2.7	27.1	n.d.	8.3
90	1.2	26.1	n.d.	7.3

Applied amount: 250 mg/kg manure fw; 100 kBq. Values are mean values of two replicate measurements. n.d. = not detectable; LOD = 0.001[% aR].

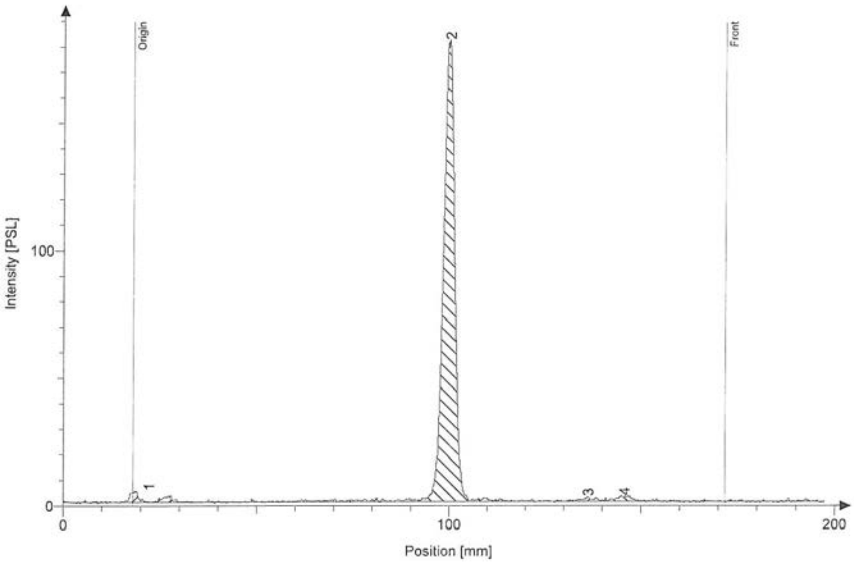
Figure 30: Behaviour of Florfenicol in cattle manure. Values are mean values of two replicate measurements.



In pig manure, the behaviour is similar. The amount of Florfenicol decreases from 92.5 [% aR] to 1.5 [% aR] after 53 days of incubation. Comparable to cattle manure, 85.1 [% aR] are still present after 24 hours of anaerobic incubation. The amount of TP1 increases from 7.5 [% aR] to 33.5 [% aR] after 53 days. Two further transformation products are observed, namely TP2 and TP3. TP2 occurs after 26 days and 53 days of incubation at concentrations of 3 [% aR] and 5.7 [% aR], respectively. TP3 is measured in an amount of 3.3 [% aR] one day after application and increases to 30.9 [% aR] and 26 [% aR] at day 26 and 53, respectively.

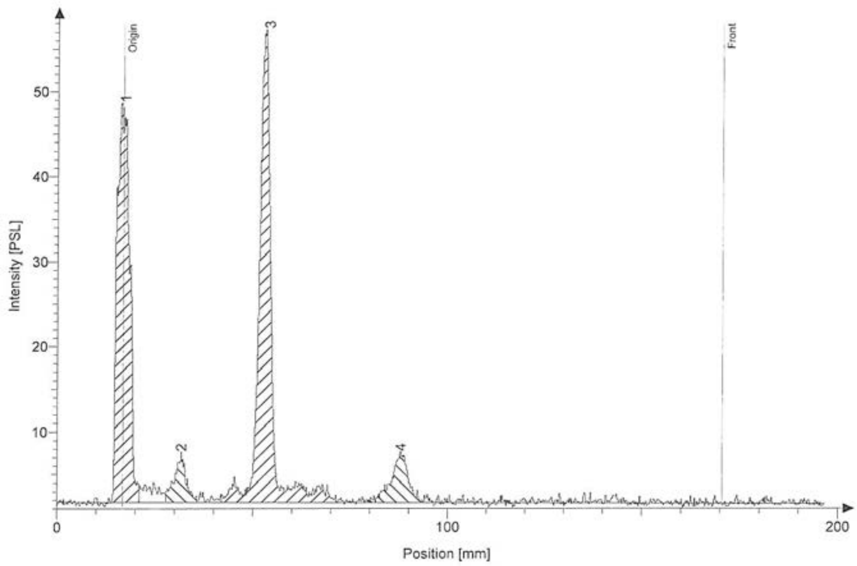
Again, the occurrence of transformation products is high compared to previous observations in manure transformation studies performed under anaerobic conditions at 20°C. Exemplary chromatograms are shown in the following figures.

Figure 31: TLC-chromatogram of Florfenicol in pig manure. Sample: 0 days of incubation.



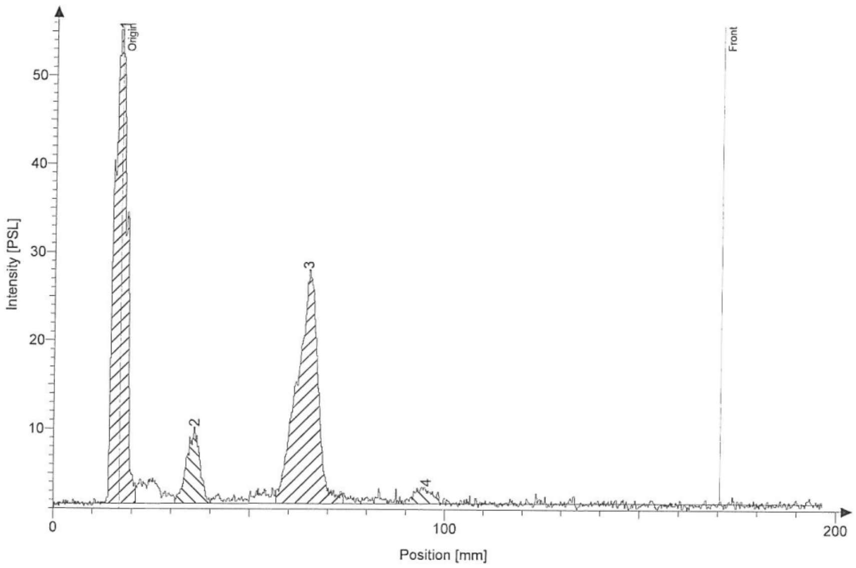
Stationary phase: silica gel KG60; mobile phase: dichloromethane / methanol; 90/10 (v/v); Florfenicol Rf = 0.45 – 0.58; TP1 Rf = 0.0 – 0.1; TP2 Rf = 0.1 – 0.16; TP3 Rf = 0.32 – 0.36.

Figure 32: TLC-chromatogram of Florfenicol in pig manure. Sample: 28 days of incubation.



Stationary phase: silica gel KG60; mobile phase: dichloromethane / methanol; 90/10 (v/v); Florfenicol Rf = 0.45 – 0.58; TP1 Rf = 0.0 – 0.1; TP2 Rf = 0.1 – 0.16; TP3 Rf = 0.32 – 0.36.

Figure 33: TLC-chromatogram of Florfenicol in cattle manure. Sample: 56 days of incubation.



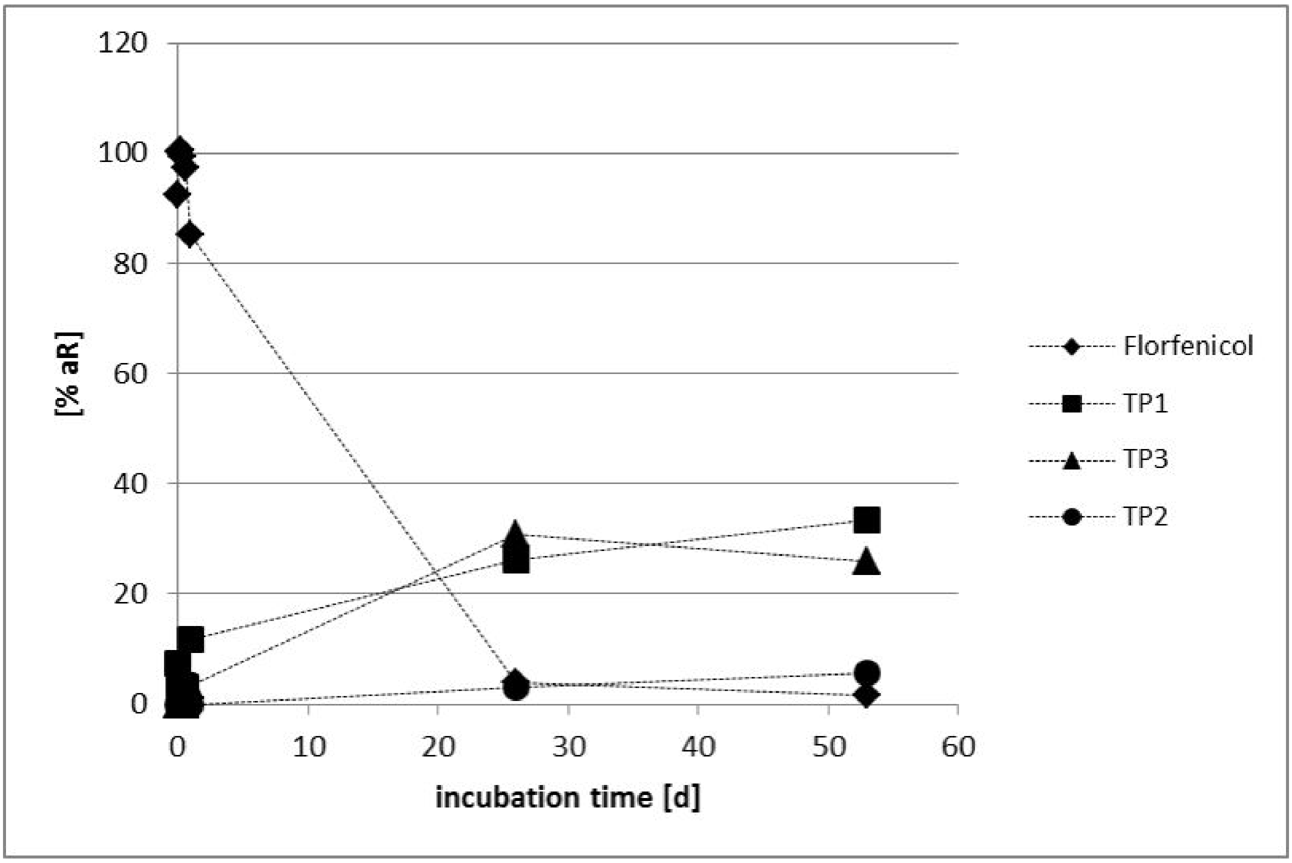
Stationary phase: silica gel KG60; mobile phase: dichloromethane / methanol; 90/10 (v/v); Florfenicol Rf = 0.45 – 0.58; TP1 Rf = 0.0 – 0.1; TP2 Rf = 0.1 – 0.16; TP3 Rf = 0.32 – 0.36.

Table 46: Concentration of Florfenicol and transformation products (TP) [% aR] in pig manure.

Incubation time [d]	Florfenicol [%aR]	TP 1 [%aR]	TP2 [%aR]	TP3 [%aR]
0	92.5	7.5	n.d.	n.d.
0.1	100.3	3.4	n.d.	n.d.
0.2	100.4	3.3	n.d.	n.d.
0.4	99.5	3.5	n.d.	n.d.
0.6	97.3	3.2	n.d.	n.d.
1	85.1	11.7	n.d.	3.3
26	3.8	26.2	3.0	30.9
53	1.5	33.5	5.7	26.0

Applied amount: 250 mg/kg manure fw; 100 kBq. Values are mean values of two replicate measurements. n.d. = not detectable; LOD = 0.001[% aR].

Figure 34: Behaviour of Florfenicol in pig manure. Values are mean values of two replicate measurements.



7.10.2.2 ^{14}C-Florfenicol in fortified soils

Besides the behavior of Florfenicol in liquid manure its behavior in soil and manure applied soil was investigated. Furthermore, two modes of application, namely application via quartz sand and via solvent, were tested. The ^{14}C-radioactivity distribution and mass balances as well as a substance characterization are shown in both tables on the following pages.

These preliminary tests lead to these observations:

- Transformation is faster in case the substance is applied via a solvent. This might be due to a reduced bioavailability when the substance is applied via a solid carrier such as quartz sand.
- Aerobic transformation in manure applied soil is much slower compared to the anaerobic transformation in manure. This might be attributed
  - to differences in redox-conditions (anaerobic versus aerobic),
  - to differences in the microorganisms populations,
  - and to differences in bioavailability in manure and manure applied soil.

Table 47: [14]C-Distribution and mass balance of Florfenicol in pig manure. Applied amount: 250 mg/kg manure fw; 100 kBq.

Sample	MeOH extr. [% aR]	Mean [% aR]	MeOH + 1%TFA-extr. [% aR]	Mean [% aR]	NER [% aR]	NER [% aR]	Minerali-sation [% aR]	Recovery [% aR]
0 d_1, soil	89.7	91.5	2.9	2.9	6.0	5.9	-	100.3
0 d_2, soil	93.2		3.0		5.8	-		
0 d_1, soil + cattle manure	95.9	95.4	4.0	3.9	6.8 7.3	7.0	-	106.4
0 d_2, soil + cattle manure	94.9		3.9					
0 d_1, soil + pig manure	106.7	105.9	3.1	3.1	5.7 6.1	5.9	-	114.9
0 d_1, soil + pig manure	105.2		3.1					
27 d_1, soil quartz sand applied	57.7	59.6	4.4	4.5	24.7 26.6	25.6	2.8	92.6
27 d_2, soil quartz sand applied	61.8		4.5					
27 d_1, soil solvent applied	29.6	29.8	3.8	3.8	38.1 33.8	35.9	2.8	72.3
27 d_2, soil solvent applied	29.9		3.8					
27 d_1, soil + pig manure	83.2	81.0	5.6	5.4	27.4 25.7	26.5	0.2	113.2
27 d_2, soil + pig manure	78.9		5.1					
46 d_1, soil quartz sand applied	54.6	53.3	4.6	4.5	34.4 35.7	35.1	3.9	96.7
46 d_2, soil quartz sand applied	52.1		4.4					
46 d_1, soil + cattle manure	70.8	78.3	4.8	5.1	26.2	28.1	0.2	111.6
46 d_2, soil + cattle manure	85.8		5.4					

Extr. = extract; NER = non extractable residue; *) sampled destroyed during clean-up.

Table 48: Florfenicol concentrations in various soil and manure samples.

samples	Duration	Florfenicol		TP1 (Rf =0.0-0.1)		TP2 (Rf =0.1-0.15)		TP3 (Rf =0.32-0.36)	
		% aR	mg/kg	% aR	mg/kg	% aR	mg/kg	% aR	mg/kg
Soil quartz sand applied	0 h	84.9	212.3	0.2	0.6	7.0	17.6	n.d.	n.d.
	27 d	57.5	143.8	0.8	2.0	4.6	11.5	n.d.	n.d.
	27 d (solvent applied!)	27.7	69.3	1.2	3.1	4.1	10.2	n.d.	n.d.
	46 d	50.4	126.0	1.0	2.5	5.1	12.7	n.d.	n.d.
Soil + pig manure	0 h	97.5	243.9	n.d.	n.d.	8.5	21.3	n.d.	n.d.
	27 d	80.7	201.7	0.5	1.4	5.2	12.9	n.d.	n.d.
Soil + cattle manure	0 h	87.5	218.7	0.2	0.6	7.5	18.8	n.d.	n.d.
	46 d	76.2	190.5	0.9	2.3	6.4	16.1	n.d.	n.d.
Pig manure	0 h	92.5	231.3	7.50	18.80	n.d.	n.d.	n.d.	n.d.
	27 d	3.8	9.5	26.20	65.40	3.00	7.49	30.90	77.15
Cattle manure	0 h	97.8	244.4	6.5	16.3	n.d.	n.d.	2.6	6.4
	46 d	2.7	6.1	27.1	68.0	1.3	4.0	8.3	20.0

Applied amount: 12.5 mg 14C-Florfenicol/50 g soil; /50 g soil + manure; /50 g manure = 250 mg/kg soil; /kg soil + manure; /kg manure. 100 kBq applied. n.d. = not detectable; LOD = 0.001 [% aR].

7.10.2.3 Concentration of Florfenicol in application solutions used for plant growth test

As routinely performed in plant growth tests the application solutions are subjected to chemical analysis in order to compare the analytically measured concentration and the concentration determined by weighing the test substance (nominal concentration). Quality of the test is sufficient in case the analytically measured concentration is in the range of 80 – 120 % of nominal.

Application solutions were chemically analysed by HPLC. For all measured application solutions the percent of nominal was in the range of 80 – 120 % as can be seen from the following table.

Table 49: Results of chemical analyses for Florfenicol of application solutions.

Test	Sample	measured concen-tration [mg/L]	dil. factor 1	Calc. conc. 1 [mg/L]	dil. factor 2	calc conc. 2 [mg/L]	nominal conc. [g/L]	percent of nominal [%]
PM_0d_1	Stock solution	10.82	8	86.56	2000	173120	204	84.9
PM_0d_2	Stock solution	11.07	40	442.8	400	177120	204	86.8
PM_0d_3	Stock solution	12.14	40	485.6	400	194240	204	95.2
PM_0d_3	Diluted solution	13.45	40	538	40	21520	23.5	91.6
PM_inc_1	Stock solution	11.74	40	469.6	400	187840	200.4	100.0
PM_inc_1	Diluted solution	12.77	40	510.8	40	20432	20.4	98.8
CM_0d_1	Stock solution	10.57	40	422.8	400	169120	174.1	103.3
CM_0d_1	Diluted solution	12.9	6.67	86	200	17200	17.4	109.7
CM_0d_2	Stock solution	11.24	40	449.6	400	179840	174.1	93.9
CM_0d_2	Diluted solution	14.33	6.67	95.5	200	19107	17.4	101.1
CM_0d_3 CM_inc_1	Stock solution	10.22	40	408.8	40	163520	174.1	102.9
CM_0d_3 CM_inc_1	Diluted solution	13.2	6.67	88	200	17600	17.4	104.2
CM_inc_2	Stock solution	11.2	40	448	40	179200	174.1	84.9
CM_inc_2	Diluted solution	13.61	6.67	90.7	200	18146.6	17.4	86.8

PM_ 0d = Tests with pig manure without incubation; PM_inc = tests with pig manure with incubation (27 or 53 days); CM_ 0d = Tests with cattle manure without incubation; CM_inc = tests with cattle manure with incubation (45 or 90 days); dil. factor = dilution factor; calc. Conc. = calculated concentration; nominal conc. = nominal concentration.

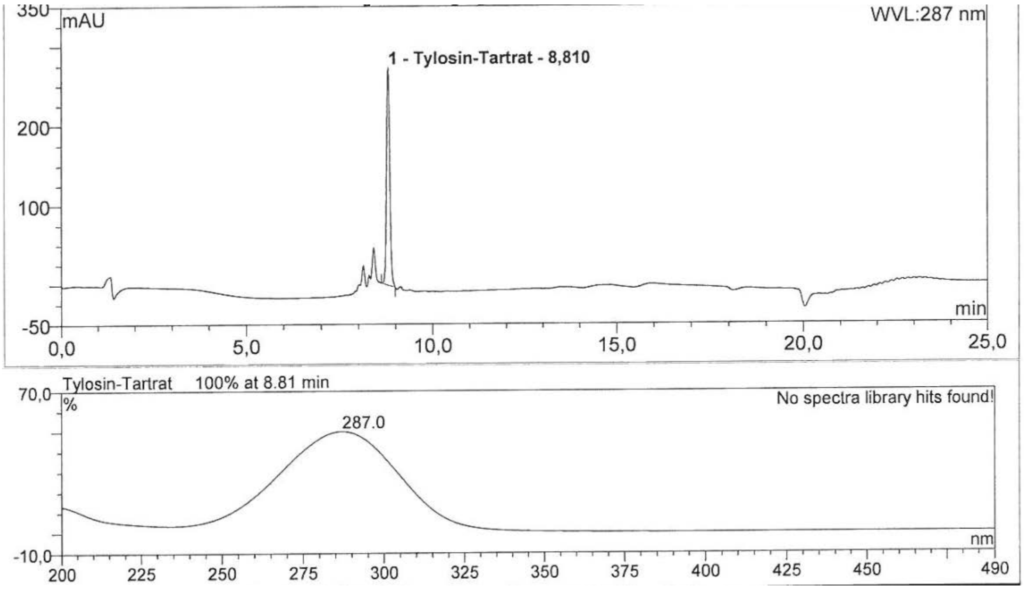
7.10.2.4 Tylosin tartrate in selected manure samples

Tylosin tartrate was analyzed in selected manure samples used for the plant growth tests. Selection of samples was such, that the Tylosin tartrate concentration range used in the plant growth tests was covered, plant species used in the growth tests were covered, representative samples of fresh manure, manure incubated for half of maximum storage duration, and manure incubated for maximum storage duration were analysed.

The clean-up and analytical procedure as described in the materials and methods chapter yielded a maximum recovery of 63% immediately after spiking manure with Tylosin tartrate. Such low recovery is known from literature.

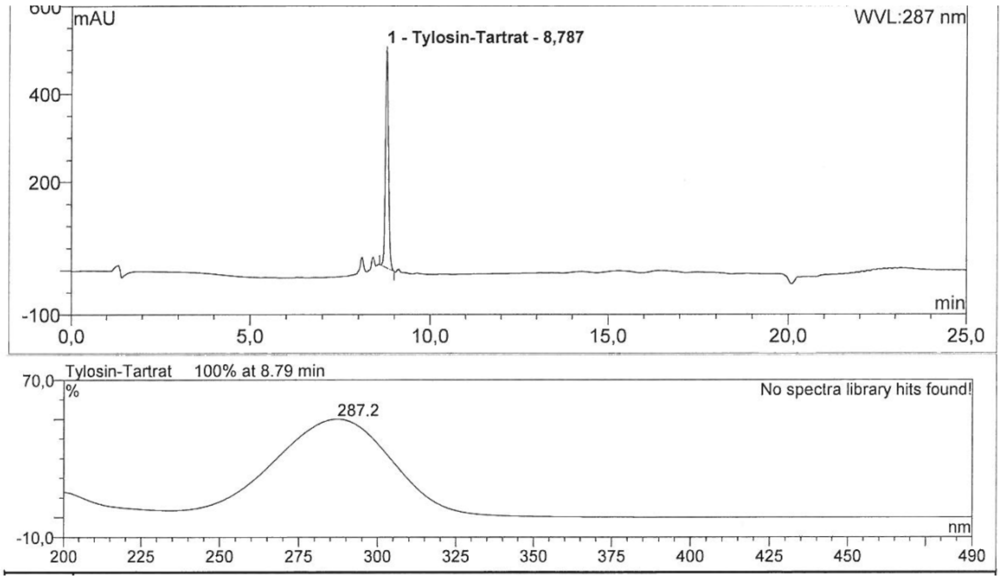
The following figures show two exemplary chromatograms of Tylosin tartrate in manure.

Figure 35: Representative chromatogram of Tylosin tartrate in pig manure (sample: *P. vulgaris*, half storage duration of pig manure, i.e., 26 d, nominal concentration = 4 mg/g manure fw).



Top: Chromatogram (wavelength: 287 nm); Bottom: UV spectrum of Tylosin tartrate

Figure 36: Representative chromatogram of Tylosin tartrate in fresh pig manure (nominal concentration =1.365 mg/kg manure fw).



Top: Chromatogram (wavelength: 287 nm); Bottom: UV spectrum of Tylosin tartrate

In detail, for the spiked manure samples the following Tylosin tartrate recoveries were measured.

Table 50: Results of chemical analyses for Tylosin tartrate of pig manure samples.

Nominal concentration	Sample	Recovery [% of nominal]
0.228 mg Tylosin tartrate/g pig manure fw	Fresh pig manure	11.9
	Manure used for plant growth test with *T. pratense*; half of typical manure storage period (26 d)	35.2
	Manure used for plant growth test with *T. pratense*; typical manure storage period (53 d)	29.8
1.365 mg Tylosin tartrate/g pig manure fw	Fresh pig manure	26.5
	Manure used for plant growth test with *A. cepa*; half of typical manure storage period (26 d)	15.9
	Manure used for plant growth test with *A. cepa*; typical manure storage period (53 d)	14.3
4.0 mg Tylosin tartrate/g pig manure fw	Fresh pig manure	33.8
	Manure used for plant growth test with *P. vulgaris*; half of typical manure storage period (26 d)	17.8
	Manure used for plant growth test with *P. vulgaris*; typical manure storage period (53 d)	11.6
7.1 mg Tylosin tartrate/g pig manure fw	Fresh pig manure	38.2
	Manure used for plant growth test with *S. lycopersicum*; half of typical manure storage period (26 d)	29.1
	Manure used for plant growth test with *S. lycopersicum*; typical manure storage period (53 d)	23.4
17.75 mg Tylosin tartrate/g pig manure fw	Fresh pig manure	15.2
	Manure used for plant growth test with *B. napus*; half of typical manure storage period (26 d)	27.5
	Manure used for plant growth test with *B. napus*; typical manure storage period (53 d)	31.9
45.45 mg Tylosin tartrate/g pig manure fw	Fresh pig manure	6.0
	Manure used for plant growth test with *A. sativa*; half of typical manure storage period (26 d)	8.1
	Manure used for plant growth test with *A. sativa*; typical manure storage period (53 d)	11.4

fw = fresh weight.

Table 51: Results of chemical analyses for Tylosin tartrate of cattle manure samples.

Nominal concentration	Sample	Recovery [% of nominal]
0.228 mg Tylosin tartrate/g cattle manure fw	Manure used for plant growth test with *T. pratense*; half of typical manure storage period (45 d)	27.1
	Manure used for plant growth test with *T. pratense*; typical manure storage period (90 d)	19.5
1.365 mg Tylosin tartrate/g cattle manure fw	Fresh cattle manure	18.9
	Manure used for plant growth test with *A. cepa*; half of typical manure storage period (45 d)	16.7
	Manure used for plant growth test with *A. cepa*; typical manure storage period (90 d)	14.9
4.0 mg Tylosin tartrate/g cattle manure fw	Fresh cattle manure	34.9
	Manure used for plant growth test with *P. vulgaris*; typical manure storage period (90 d)	30.4
7.1 mg Tylosin tartrate/g cattle manure fw	Manure used for plant growth test with *S. lycopersicum* ; typical manure storage period (90 d)	28.9
17.75 mg Tylosin tartrate/g cattle manure fw	Manure used for plant growth test with *B. napus*; typical manure storage period (90 d)	23.8
45.45 mg Tylosin tartrate/g cattle manure fw	Fresh cattle manure	6.5
	Manure used for plant growth test with *A. sativa*; typical manure storage period (90 d)	17.2

fw = fresh weight.

Results show low recoveries for all analysed manure samples. Even for the fresh manure samples, a recovery of 63 % as obtained when developing the method was not reached any more. Low recoveries might be attributed to

- Losses during clean-up, in particular when passing the extract through the SPE-columns,
- Matrix effects during HPLC, though calibration was done for extracts obtained from manure (matrix calibration).

Any clear conclusion cannot be drawn since no ^{14}C-radioactively labeled substance is used and thus, a mass balance for the clean-up and analytical procedure cannot be established.

It is recommended to use the nominal values obtained by weighing the substance. Any conclusion on a possible dissipation during the manure storage period cannot be drawn.

8 Discussion

8.1 The influence of manure on seedling emergence and growth

It was demonstrated, that pig manure potentially can impair germination, emergence and post-emergence survival of plant seedlings at concentration corresponding to 170 kg N/ha, representing the maximum amount of manure allowed to be deployed per year in Europe. A maximum manure concentration representing 85 kg N/ha (1/2 maximum amount allowed per year in Europe) should not be exceeded in a plant test to ensure an unimpaired germination, emergence and post-emergence survival. At least the seven plant species (*A. cepa* (onion), *A. sativa* (oat), *S. lycopersicum* (tomato), *B. napus* (rape), *P. vulgaris* (common bean), *S. alba* (mustard), and *T. pratense* (red clover)), representing monocots, dicots, and legumes, were grown successfully at this manure concentration.

8.2 The influence of the exposure way on effects of antibiotics in plant tests

To quantify the influence of manure on the effect of the test items due to its physical presence (e.g. sorption, interaction with compounds of the manure, etc.), additional tests with the two most sensitive species were performed. In these approaches, it was investigated if the sequential arrangement of application has an influence on possible effects on plant growth. The test item was applied directly to the soil with a subsequent manure application. The tests were performed at the same time as the tests with fresh spiked manure.

For both test items, fresh mass of the shoots was the most sensitive endpoint. Therefore, discussion of the influences is based on the results regarding fresh mass.

Neither the physical presence of pig or cattle manure, nor the sequential arrangement of application influenced the effect of the two antibiotics on shoot fresh mass of the plant species investigated. The NOEC and EC values (Table 52) are comparable to those from the standard test design (Table 7, Table 11). This can be explained by the chemical analysis of florfenicol. In all assays, recovery of extractable florfenicol is comparable (Table 48).

Table 52: NOEC and EC values for shoot fresh mass depending on the way of application.

Florfenicol				
Species	*Allium cepa*			
Spiked carrier	**Pig manure**	**Soil**	**Cattle manure**	**Soil**
NOEC	✥ 1.9	0.6	0.2	< 0.20
EC_{10}	0.43	0.78	0.30	0.18
EC_{50}	0.73	1.52	0.84	0.73
Species	*Brassica napus*			
Spiked carrier	**Pig manure**	**Soil**	**Cattle manure**	**Soil**
NOEC	< 0.2	< 0.20	< 0.2	< 0.20
EC_{10}	0.08	n.d.	0.07	0.06
EC_{50}	0.21	0.20	0.20	0.17
Tylosin tartrate				
Species	*Allium cepa*			
Spiked carrier	**Pig manure**	**Soil**	**Cattle manure**	**Soil**
NOEC	30.0	30.0	15.0	15.0
EC_{10}	18.8	17.4	15.7	16.7
EC_{50}	62.7	60.3	46.3	53.0
Species	*Trifolium pratense*			
Spiked carrier	**Pig manure**	**Soil**	**Cattle manure**	**Soil**
NOEC	10.0	10.0	✥125	<5
EC_{10}	8.5	5.1	n.d.	2.4
EC_{50}	20.1	17.4	n.d.	9.2

Values indicated as mg/kg DM; DM = Dry mass; n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

8.3 The influence of the incubation conditions (aerobic/anaerobic) on effects of antibiotics in plant tests

To quantify the influence of incubation conditions (anaerobic or aerobic) on the effect of the test items, additional tests with the two most sensitive species were applied. In these approaches, it was investigated if an aerobic incubation has an influence on possible effects on plant growth when compared to anaerobic incubation. The spiked manure was mixed directly after spiking into soil and was incubated aerobically for half-maximum storage duration. The tests were performed at the same time as the tests with anaerobically incubated manure.

For both test items, fresh mass of the shoots was the most sensitive endpoint. Therefore, discussion of the influences is based on the results regarding fresh mass.

Incubation conditions seriously influenced the effect of the two antibiotics on shoot fresh mass of the plant species investigated. An aerobic incubation of the spiked manure/soil did not influence the effect of the two antibiotics on shoot fresh mass of the plant species investigated, when compared with a standard test design. In contrast, an anaerobic incubation of spiked pig or cattle manure resulted in a significant decreased effect of both antibiotics on shoot fresh mass.

The NOEC and EC values for spiked manure/soil mixture incubated aerobically for half-maximum storage duration (Table 53) are comparable to those from the standard test design (Table 7, Table 11). The NOEC and EC values for spiked manure incubated anaerobically for half-maximum storage duration, decreased by a factor of 10 – 20 for florfenicol, independent from manure source. The same was true for tylosin tartrate with a decreasing factor of 5 – 10.

This can be explained by the chemical analysis of florfenicol. In all assays with aerobic incubation, recovery of extractable florfenicol is comparable with those of freshly spiked manure. In contrast, recovery of extractable florfenicol in anaerobically incubated spiked manure decreased clearly (Table 48).

Table 53: NOEC and EC values for shoot fresh mass depending on the way of application.

Florfenicol				
Species	***Allium cepa***			
Spiked carrier	**Pig manure**		**Cattle manure**	
Incubation condition	**Manure anaerobic**	**Manure/soil aerobic**	**Manure anaerobic**	**Manure/soil aerobic**
NOEC	5.6	0.60	5.60	<0.60
EC_{10}	5.43	0.54	7.71	0.35
EC_{50}	17.53	1.60	26.45	1.18
Species	***Brassica napus***			
Spiked carrier	**Pig manure**		**Cattle manure**	
Incubation condition	**Manure anaerobic**	**Manure/soil aerobic**	**Manure anaerobic**	**Manure/soil aerobic**
NOEC	16.7	< 0.60	5.60	0.60
EC_{10}	14.08	n.d.	12.05	0.55
EC_{50}	22.50	0.35	20.81	1.10
Tylosin tartrate				
Species	***Allium cepa***			
Spiked carrier	**Pig manure**		**Cattle manure**	
Incubation condition	**Manure anaerobic**	**Manure/soil aerobic**	**Manure anaerobic**	**Manure/soil aerobic**
NOEC	✥300	30.0	150	15.0
EC_{10}	92.0	20.3	156	10.7
EC_{50}	n.d.	65.4	156	46.0
Species	***Trifolium pratense***			
Spiked carrier	**Pig manure**		**Cattle manure**	
Incubation condition	**Manure anaerobic**	**Manure/soil aerobic**	**Manure anaerobic**	**Manure/soil aerobic**
NOEC	✥125	10.0	50.0	5.0
EC_{10}	5.5	7.7	70.6	5.3
EC_{50}	n.d.	29.8	123	16.9

Values indicated as mg/kg DM; DM = Dry mass; n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration.

8.4 The influence of the incubation duration on effects of antibiotics in plant tests

To quantify the influence of the duration of anaerobic incubation on the effect of the test items, tests with freshly spiked manure, and manure incubated for the half-maximum and maximum storage duration, respectively, with six species per test item were applied. In these approaches, it was investigated if the course of an anaerobic incubation has an influence on possible effects on plant growth.

For both test items, fresh mass of the shoots was the most sensitive endpoint. Therefore, discussion of the influences is based on the results regarding fresh mass.

Incubation duration seriously influenced the effect of the two antibiotics on shoot fresh mass of all plant species investigated. Freshly spiked manure did not influence the effect of the two antibiotics on shoot fresh mass of the plant species investigated, when compared with a standard test design. In contrast, an anaerobic incubation of spiked pig or cattle manure resulted in a significant decreased effect of both antibiotics on shoot fresh mass.

The NOEC and EC values for freshly spiked manure (Table 54) are comparable to those from the standard test design (Table 7, Table 11). The NOEC and EC values for spiked manure incubated anaerobically for half-maximum storage duration, decreased by a factor of 5 – 100 (10 – 40 in most cases) for florfenicol, independent from manure source. The same was true for tylosin tartrate with a decreasing factor of 5 – 10.

There was only a small ongoing decrease in effects prolonging the incubation period to maximum storage duration. In most cases the maximum decrease was already reached after incubation for the half-maximum storage duration. In a few cases, the NOEC was increased for one treatment concentration. For Florfenicol, it was shown that the EC_{50} values maintained stable for half-maximum and maximum incubation in pig manure. However, in cattle manure, EC_{50} values decreased by another factor of 2 when incubation was prolonged from half-maximum to maximum duration. The same was true for tylosin tartrate with an additional decreasing factor of 2.

This can be explained by the chemical analysis of florfenicol. In all assays with half-maximum incubated manure, recovery of extractable florfenicol decreased clearly when compared with freshly spiked manure. In contrast, recovery of extractable florfenicol in maximum incubated spiked manure decreased nearly maintain stable when compared with half-maximum incubated manure (Table 48).

Table 54: NOEC and EC values for shoot fresh mass depending on the incubation duration

Florfenicol												
Species	***Allium cepa***		***Avena sativa***		***Brassica napus***		***Sinapis alba***		***Solanum lycopersicum***		***Phaseolus vulgaris***	
Fresh manure	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**
NOEC	✢ 1.9	0.2	0.6	0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.2	0.2	< 0.2	< 0.2
EC_{10}	0.43	0.30	0.15	0.56	0.08	0.07	0.08	0.06	0.33	0.24	0.07	0.08
EC_{50}	0.73	0.84	1.37	6.34	0.21	0.20	0.22	0.22	0.47	0.96	1.00	1.18
Half-max incubated	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**
NOEC	5.6	5.6	16.7	16.7	16.7	5.6	1.9	1.9	16.7	16.7	16.7	5.6
EC_{10}	5.43	7.71	18. 9	14.2	14.1	12.1	10.2	8.10	12.7	16.7	11.8	11.8
EC_{50}	17.5	26.5	29.7	29.4	22.5	20.8	20.0	21.1	25.3	24.2	27.0	28.7
Max incubated	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**
NOEC	16.7	16.7	16.7	✢ 50	✢ 16.7	16.7	5.6	16.7	1.9	16.7	16.7	5.6
EC_{10}	0.40	32.8	16.8	> 50	14.3	27.0	9.4	10.1	2.4	14.2	28.2	8.14
EC_{50}	19.8	> 50	37.2	n.d.	27.8	> 50	15.7	44.3	14.80	36.8	41.2	> 50
Tylosin tartrate												
Species	***Allium cepa***		***Avena sativa***		***Brassica napus***		***Solanum lycopersicum***		***Phaseolus vulgaris***		***Trifolium pretense***	
Fresh manure	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**
NOEC	30.0	15.0	200	200	62.5	25.0	62.5	n.d.	40.0	40.0	10.0	<5.0
EC_{10}	18.8	15.7	226	246	101	36.7	53.0	65.7	n.d.	25.3	8.5	2.9
EC_{50}	62.7	46.3	499	775	131	78.7	88.1	100	142	177	20.1	10.7
Half-max incubated	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**
NOEC	✢300	150	✢1000	✢1000	156	156	✢391	156	426	194	✢125	50.0
EC_{10}	92.0	156	n.d.	n.d.	n.d.	254	n.d.	77.5	581	175	5.5	70.6
EC_{50}	n.d.	231	n.d.	n.d.	n.d.	326	n.d.	241	837	370	n.d.	123
Max incubated	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**	**PM**	**CM**
NOEC	✢300	150	✢1000	✢1000	✢391	156	✢391	156	426	194	✢125	✢125
EC_{10}	n.d.	104	n.d.	n.d.	n.d.	78.7	n.d.	113	553	202	66.8	n.d.
EC_{50}	n.d.	n.d.	n.d.	n.d.	n.d.	391	n.d.	n.d.	885	525	n.d.	n.d.

Values indicated as mg/kg DM; DM = Dry mass; PM = Pig manure; CM = Cattle manure; n.d. not determined due to the lacking of a meaningful concentration/response (ECx) or value above the highest tested concentration

8.5 Influence of manure and replicate number per treatment on the accuracy of the calculated effect values

8.5.1 Does manure application increase variability of the results generally?

To investigate if the physical presence of manure effected the variance of the biological endpoints in the plant tests, exemplary calculations were applied on the results of the main tests according to the standard test design without manure application and the modified test design with an application of florfenicol via spiked manure with or without subsequent anaerobic incubation.

The coefficient of variance (CV) of the standard controls was compared with the CV of the manure controls for the most sensitive endpoints shoot length and shoot fresh mass. Standard control data was available for four main tests (Standard test, standard controls from the tests with freshly spiked manure, half-maximum, and maximum incubation duration). Manure control data was available for three main tests per manure species (freshly spiked manure, half-maximum, and maximum incubation duration). To investigate the influence of manure only on the CV, replicate number must be the comparable for standard controls and manure controls. However, standard controls consisted of 4 – 5 replicates (depending on plant species) and manure controls consisted of 8 replicates. The number of replicates for the manure controls had to be adapted on the number available in standard controls. Artificial groups of 4 replicates were prepared. 50 groups were prepared randomly per Monte Carlo method per test. The mean CV value from the four standard control groups was calculated from the lowest and the highest CV per plant species. The mean CV value from the 150 artificial manure control groups was calculated per plant species and manure species.

The coefficient of variance in the standard controls was in the range of 3.5 – 38.1 %. Shoot fresh mass (10.5 – 38.1 %) generally showed higher variances than shoot length (3.5 – 20.2 %). The standard controls at Fraunhofer IME had CV values of 3.5 – 20.2 % and 10.5 – 31.8 % for shoot length and fresh mass, respectively. The values at ECT were in the range of 5.6 – 16.4 % and 12.4 – 38.1 % for shoot length and fresh mass, respectively. There was no difference in variance of the standard controls between the two test facilities.

In most cases (94 %, 179 out of 188 groups) the coefficient of variance was smaller than 25 %. To create a criterion for an influence due to manure on the coefficient of variance, 5 % deviation from the standard control CV was set as threshold value. E.g., if the standard control had a CV of 20 %, CV values of being ≤ 15 % or ≥ 25 % were assumed to be significantly decreased or increased due to manure, respectively.

Table 55: Coefficient of variance values (% CV) for shoot length and fresh mass in control sets at Fraunhofer IME (standard control and pig manure control)

Influence of pig manure			
Data set		**Shoot length**	**Fresh mass**
A. cepa	Standard control *	20.2	31.8
	Manure control 8 Replicates **	28.9 a,b	43.5
	Manure control 4 Replicates ***	20.2	32.6
B. napus	Standard control *	6.1	19.8
	Manure control 8 Replicates **	8.8	21.0
	Manure control 4 Replicates ***	7.3	16.7
A. sativa	Standard control *	3.8	14.3
	Manure control 8 Replicates **	6.2	13.1
	Manure control 4 Replicates ***	4.9	9.9
S. lycopersicum	Standard control *	3.5	13.8
	Manure control 8 Replicates **	9.1 a	24.9 a,b
	Manure control 4 Replicates ***	7.6	17.9
P. vulgaris	Standard control *	12.1	12.7
	Manure control 8 Replicates **	9.7	16.9
	Manure control 4 Replicates ***	8.6	13.0
S. alba	Standard control *	8.5	24.9
	Manure control 8 Replicates **	5.9	19.2 a
	Manure control 4 Replicates ***	4.6	14.4 a

Values indicated as percentage from mean length and mass, respectively. a = 5 % deviation from standard control; b = 5 % deviation from mean value of manure control with 4 replicates. * Arithmetic mean from lowest and highest CV out of four tests. ** Arithmetic mean from lowest and highest CV out of three tests. *** Arithmetic mean from 150 CV of randomly combined groups out of three tests (3 x 50 groups).

Comparing the variances of the standard control with the mean CV of the randomly prepared 150 data sets of four out of eight replicates, none of the plants showed an increased variance in shoot length or fresh mass due to manure application. However, 21 % (pig manure) and 29 % (cattle manure) of the calculated mean CV values showed decreased variances in the manure control.

Considering the original data sets with eight replicates, overall manure controls showed 13 % increased CV values, but also 15 % decreased CV values.

13 % of the original data sets of manure control showed increased variances when compared with the mean CV of the randomly prepared 150 data sets of four out of eight replicates. No decrease due to higher replicate number occurred.

Table 56: Coefficient of variance values (% CV) for shoot length and fresh mass in control sets at Fraunhofer IME (standard control and cattle manure control)

Influence of cattle manure			
Data set		**Shoot length**	**Fresh mass**
A. cepa	Standard control *	7.2	14.5
	Manure control 8 Replicates **	13.1 a	16.8
	Manure control 4 Replicates ***	10.6	14.5
B. napus	Standard control *	3.5	15.1
	Manure control 8 Replicates **	5.9	14.1
	Manure control 4 Replicates ***	4.7	11.7
A. sativa	Standard control *	5.7	10.5
	Manure control 8 Replicates **	3.9	12.0
	Manure control 4 Replicates ***	3.3	9.7
S. lycopersicum	Standard control *	8.5	16.5
	Manure control 8 Replicates **	6.8	15.7
	Manure control 4 Replicates ***	4.9	12.6
P. vulgaris	Standard control *	10.3	19.3
	Manure control 8 Replicates **	15.1	16.2
	Manure control 4 Replicates ***	11.2	13.5 a
S. alba	Standard control *	8.3	26.6
	Manure control 8 Replicates **	9.2	29.4 b
	Manure control 4 Replicates ***	6.6	20.9 a

Values indicated as percentage from mean length and mass, respectively. a = 5 % deviation from standard control; b = 5 % deviation from mean value of manure control with 4 replicates. * Arithmetic mean from lowest and highest CV out of four tests. ** Arithmetic mean from lowest and highest CV out of three tests. *** Arithmetic mean from 150 CV of randomly combined groups out of three tests (3 x 50 groups).

Generally, these tendencies were true for data sets of both test facilities. None of the plants showed an increased variance in shoot length or fresh mass due to manure application comparing only four replicates. However, 8 % (pig manure) and 17 % (cattle manure) at Fraunhofer IME and 33 % (pig manure) and 50 % (cattle manure) at ECT of the calculated mean CV values showed decreased variances in the manure control.

Considering the original data sets with eight replicates, overall manure controls showed 21 % increased CV values, but also 4 % decreased CV values at Fraunhofer IME. At ECT overall manure controls showed only 4 % increased CV values, but 25 % decreased CV values.

17 % of the original data sets of manure control showed increased variances when compared with the mean CV of the randomly prepared 150 data sets of four out of eight replicates at Fraunhofer IME. No decrease due to higher replicate number occurred. At ECT 8 % of the original data sets of manure control showed increased variances when compared with the mean CV of the randomly prepared data sets.

Table 57: Coefficient of variance values (% CV) for shoot length and fresh mass in control sets at ECT (standard control and pig manure control)

Influence of pig manure			
Data set		**Shoot length**	**Fresh mass**
A. cepa	Standard control *	10.7	17.3
	Manure control 8 Replicates **	9.9	20.2
	Manure control 4 Replicates ***	7.6	15.6
B. napus	Standard control *	7.9	16.9
	Manure control 8 Replicates **	6.8	14.9
	Manure control 4 Replicates ***	5.3	11.8 a
A. sativa	Standard control *	5.0	17.2
	Manure control 8 Replicates **	4.1	12.5
	Manure control 4 Replicates ***	3.2	9.2 a
S. lycopersicum	Standard control *	14.1	35.2
	Manure control 8 Replicates **	7.0 a	15.3 a
	Manure control 4 Replicates ***	5.5 a	12.7 a
P. vulgaris	Standard control *	9.0	12.4
	Manure control 8 Replicates **	17.5 a	16.9 b
	Manure control 4 Replicates ***	13.1	11.9
T. pratense	Standard control *	10.0	15.3
	Manure control 8 Replicates **	11.1	20.2
	Manure control 4 Replicates ***	8.2	15.2

Values indicated as percentage from mean length and mass, respectively. a = 5 % deviation from standard control; b = 5 % deviation from mean value of manure control with 4 replicates. * Arithmetic mean from lowest and highest CV out of four tests. ** Arithmetic mean from lowest and highest CV out of three tests. *** Arithmetic mean from 150 CV of randomly combined groups out of three tests (3 x 50 groups).

The kind of manure had no consistent influence on the variance of the results.

The lack of influence on the variance between the replicates was additionally shown by the comparability of the NOEC and EC_{10} values in the standard tests without manure application and the tests with freshly spiked manure (7.6.1, 7.6.2, 7.7.1, 7.7.2, 8.2).

To sum up, manure application did not generally result in increased variances. This was true for pig and cattle manure. An increase of replicates did not reduce variances in general.

Table 58: Coefficient of variance values (% CV) for shoot length and fresh mass in control sets at ECT (standard control and cattle manure control)

Influence of cattle manure			
Data set		***Shoot length***	***Fresh mass***
A. cepa	Standard control	12.6	23.4
	Manure control 8 Replicates	15.8	25.6 b
	Manure control 4 Replicates	11.2	18.5
B. napus	Standard control	10.2	21.9
	Manure control 8 Replicates	5.4	10.3 a
	Manure control 4 Replicates	3.6 a	7.6 a
A. sativa	Standard control	5.8	15.8
	Manure control 8 Replicates	6.9	11.8
	Manure control 4 Replicates	5.5	9.0 a
S. lycopersicum	Standard control	16.4	38.1
	Manure control 8 Replicates	3.9 a	7.8 a
	Manure control 4 Replicates	3.0 a	6.2 a
P. vulgaris	Standard control	12.2	20.8
	Manure control 8 Replicates	9.8	12.9 a
	Manure control 4 Replicates	7.4	9.6 a
T. pratense	Standard control	9.1	16.3
	Manure control 8 Replicates	9.4	19.5
	Manure control 4 Replicates	8.0	16.4

Values indicated as percentage from mean length and mass, respectively. a = 5 % deviation from standard control; b = 5 % deviation from mean value of manure control with 4 replicates.

8.5.2 Does replicate number influence NOEC or EC_{10} in manure approaches?

To investigate if the number of replicates has an influence on the accuracy of the results, especially the NOEC and EC_{10} values of the manure approaches, exemplary calculations were applied on the results of the main tests according to the modified test design with an application of florfenicol via spiked manure without subsequent anaerobic incubation.

The NOEC and EC_{10} values of the original data sets considering eight replicates were compared with the NOEC and EC_{10} values of two artificial data sets prepared by replicates 1 – 4 and 5 – 8, respectively, out of the eight replicates, for the most sensitive endpoints shoot length and shoot fresh mass.

Comparing the NOEC of the data sets reduced on 4 replicates with the original data sets of eight replicates, 15 % showed increased values. However, also 4 % showed decreased values. Changes in NOEC values are always accompanied by changes in %MDD (minimum detectable difference to control) - increased NOEC is based on increased %MDD and decreased NOEC is based on decreased %MDD (Table 61 and Table 62). However, changes in %MDD do not always result in changes of NOEC and it is not possible to set a threshold value for changes resulting in affected NOEC values.

EC_{10} values were nearly identical in all cases. EC_{10} never was increased due to a restricted number of replicates.

These results do not allow a definite conclusion. However, to ensure representative and reproducible results, it is recommended to increase the number of replicates in a NOEC test design and the number of treatment concentrations in an EC_X test design.

Table 59: NOEC and EC_{10} values (mg/kg) of Florfenicol for shoot length and fresh mass in freshly spiked pig manure

Pig manure				
A. cepa		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	< 0.20	> 0.60	0.60
	EC_{10}	0.0 (n.d.)	0.6 (0.6 – 0.6)	0.5 (n.d.)
Fresh mass	NOEC	< 0.20	> 0.60	> 0.60
	EC_{10}	n.d.	0.5 (0.5 – 0.5)	0.4 (0.4 – 0.4)
B. napus		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	0.60	> 0.60	< 0.20
	EC_{10}	0.2 (n.d.)	0.2 (n.d.)	0.2 (n.d.)
Fresh mass	NOEC	< 0.20	< 0.20	< 0.20
	EC_{10}	0.1 (0.1 – 0.1)	0.1 (0.1 – 0.1)	0.1 (0.1 – 0.1)
A. sativa		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	0.20	0.60	0.20
	EC_{10}	0.4 (0.0 – 0.9)	0.9 (0.1 – 2.0)	0.6 (0.1 – 1.5)
Fresh mass	NOEC	< 0.20	0.60	0.20
	EC_{10}	0.1 (0.0 – 0.1)	0.2 (0.0 – 0.6)	0.2 (0.0 – 0.4)
S. lycopersicum		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	0.20	0.20	0.20
	EC_{10}	0.2 (n.d.)	0.2 (n.d.)	0.2 (n.d.)
Fresh mass	NOEC	0.20	0.20	0.20
	EC_{10}	0.3 (n.d.)	0.2 (0.0 – 0.4)	0.3 (n.d.)
P. vulgaris		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	0.20	0.20	0.20
	EC_{10}	0.7 (0.3 – 1.1)	0.3 (0.0 – 0.7)	0.4 (0.0 – 0.9)
Fresh mass	NOEC	< 0.20	< 0.20	< 0.20
	EC_{10}	0.1 (0.0 – 0.2)	0.1 (0.0 – 0.2)	0.1 (0.0 – 0.3)
S. alba		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	< 0.20	< 0.20	< 0.20
	EC_{10}	0.1 (n.d.)	0.1 (n.d.)	0.1 (n.d.)
Fresh mass	NOEC	< 0.20	< 0.20	< 0.20
	EC_{10}	0.1 (n.d.)	0.1 (n.d.)	0.1 (n.d.)

Values indicated as mg florfenicol per kg dry mass soil. Values in brackets represent 95 % coincidence limits. A 4 replicates = replicate 1 – 4 out of the original data set; B 4 replicates = replicate 5 – 8 out of the original data set; 8 replicates = full original data set.

Table 60: NOEC and EC_{10} values (mg/kg) of Florfenicol for shoot length and fresh mass in freshly spiked cattle manure

Cattle manure				
A. cepa		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	0.60	0.20	0.20
	EC_{10}	0.6 (n.d.)	0.4 (0.0 – 0.7)	0.5 (n.d. – 0.9)
Fresh mass	NOEC	0.20	0.20	0.20
	EC_{10}	0.3 (0.1 – 0.5)	0.3 (0.2 – 0.4)	0.3 (0.2 – 0.4)
B. napus		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	< 0.20	< 0.20	< 0.20
	EC_{10}	0.1 (n.d.)	0.1 (n.d.)	0.1 (n.d.)
Fresh mass	NOEC	< 0.20	< 0.20	< 0.20
	EC_{10}	0.1 (n.d.)	0.1 (n.d.)	0.1 (n.d.)
A. sativa		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	0.20	0.20	0.20
	EC_{10}	1.4 (0.3 – 2.6)	1.7 (0.0 – 4.1)	1.5 (0.2 – 3.2)
Fresh mass	NOEC	0.20	0.20	0.20
	EC_{10}	0.6 (0.2 – 1.0)	0.6 (0.2 – 1.1)	0.6 (0.2 – 1.0)
S. lycopersicum		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	0.60	0.20	0.20
	EC_{10}	0.5 (0.1 – 0.9)	0.5 (0.3 – 0.8)	0.4 (0.2 – 0.7)
Fresh mass	NOEC	0.20	0.20	0.20
	EC_{10}	0.1 (0.1 – 0.2)	0.3 (0.2 – 0.4)	0.2 (0.2 – 0.3)
P. vulgaris		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	0.60	0.60	0.60
	EC_{10}	0.9 (0.0 – 1.8)	0.4 (0.0 – 1.0)	0.6 (0.0 – 1.3)
Fresh mass	NOEC	< 0.20	< 0.20	< 0.20
	EC_{10}	0.1 (0.0 – 0.3)	0.1 (0.0 – 0.2)	0.1 (0.0 – 0.2)
S. alba		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	NOEC	< 0.20	< 0.20	< 0.20
	EC_{10}	0.1 (0.0 – 0.3)	0.1 (n.d.)	0.1 (n.d.)
Fresh mass	NOEC	< 0.20	< 0.20	< 0.20
	EC_{10}	0.1 (0.1 – 0.1)	0.0 (0.0 – 0.1)	0.1 (0.0 – 0.1)

Values indicated as mg florfenicol per kg dry mass soil. Values in brackets represent 95 % coincidence limits. A 4 replicates = replicate 1 – 4 out of the original data set; B 4 replicates = replicate 5 – 8 out of the original data set; 8 replicates = full original data set.

Table 61: %MDD values in Florfenicol tests for shoot length and fresh mass in freshly spiked pig manure

Pig manure				
A. cepa		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	%MDD	< 23.1	> 165.2	92.5
Fresh mass	%MDD	< 39.5	> 298.6	> 159.4
B. napus		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	%MDD	19.9	>6.9	< 11.7
Fresh mass	%MDD	< 29.2	< 34.8	< 23.1
A. sativa		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	%MDD	10.7	15.8	- *
Fresh mass	%MDD	< 10.9	41.0	15.2
S. lycopersicum		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	%MDD	19.3	14.1	10.5
Fresh mass	%MDD	40.0	24.9	21.2
P. vulgaris		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	%MDD	8.4	9.2	7.7
Fresh mass	%MDD	< 12.8	< 12.1	< 9.0
S. alba		**A 4 replicates**	**B 4 replicates**	**8 replicates**
Shoot length	%MDD	< 11.9	< 8.9	< 8.5
Fresh mass	%MDD	< 29.4	< 32.5	- *

%MDD: minimum detectable difference to Control (in percent of Control). Indicated are %MDD values for the LOEC. A 4 replicates = replicate 1 – 4 out of the original data set; B 4 replicates = replicate 5 – 8 out of the original data set; 8 replicates = full original data set. * Test type does not indicate MDD% values.

Table 62: %MDD values in Florfenicol tests for shoot length and fresh mass in freshly spiked cattle manure

Pig manure				
A. cepa		A 4 replicates	B 4 replicates	8 replicates
Shoot length	%MDD	13.9	15.8	9.5
Fresh mass	%MDD	17.6	22.4	13.7
B. napus		A 4 replicates	B 4 replicates	8 replicates
Shoot length	%MDD	< 9.9	< 9.8	< 6.2
Fresh mass	%MDD	< 10.8	< 18.2	< 9.2
A. sativa		A 4 replicates	B 4 replicates	8 replicates
Shoot length	%MDD	4.1	4.1	4.0
Fresh mass	%MDD	11.7	14.7	9.0
S. lycopersicum		A 4 replicates	B 4 replicates	8 replicates
Shoot length	%MDD	12.4	10.6	7.2
Fresh mass	%MDD	24.9	19.8	21.3
P. vulgaris		A 4 replicates	B 4 replicates	8 replicates
Shoot length	%MDD	13.8	19.0	13.4
Fresh mass	%MDD	< 14.7	< 16.1	< 12.4
S. alba		A 4 replicates	B 4 replicates	8 replicates
Shoot length	%MDD	< 12.5	< 11.1	< 8.5
Fresh mass	%MDD	< 15.8	< 25.8	< 19.3

%MDD: minimum detectable difference to Control (in percent of Control). Indicated are %MDD values for the LOEC. A 4 replicates = replicate 1 – 4 out of the original data set; B 4 replicates = replicate 5 – 8 out of the original data set; 8 replicates = full original data set.

8.6 Methods of preparation, acclimatization, incubation, and application of manure in a plant test

Methods for storage, acclimation, and incubation of manure following the new EMA-Guideline on determining the fate of veterinary medicinal products in manure [4] combined with the methods for processing, were shown to be suitable to maintain anaerobic conditions during the whole manure processing. The methods enable comparable seedling emergence and growth to a standard OECD test and result in reliable biological endpoints. As discussed before (0), the variance of the replicate results - and therefore the accuracy of the calculated NOEC or EC values – in the modified test design was comparable to that in the standard test design according to OECD 208.

Sufficient growth conditions were verified for pig and cattle manure up to a maximum manure concentration representing 85 kg N/ha (1/2 maximum amount allowed per year in Europe), for at least the seven plant species *Allium cepa* (onion), *Avena sativa* (oat), *Solanum lycopersicum* (tomato), *Brassica napus* (rape), *Phaseolus vulgaris* (common bean), *Sinapis alba* (mustard), and *Trifolium pratense* (red clover), representing monocots, dicots, and legumes.

8.7 Conclusion

Regarding the objectives concerning a standardized test design to test the effect of veterinary medicinal products on plants in a more realistic way, formulated at the start of the project, it can be summed up that

- a test design for a modified OECD 208 seedling emergence and growth plant test with a more realistic exposure in manured soil was developed successfully and the
- practicability of the test design was verified experimentally by means of two veterinary antibiotics (florfenicol and tylosin tartrate) and two kind of manures (pig and cattle) for six plant species.
- A draft manual with recommendations for the performance, evaluation and reporting of an extended plant test with an exposure scenario via manure application for the use in environmental risk assessment of veterinary medicinal products is presented (see 11).

9 References

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10 Annex 1 — Raw data

10.1 Florfenicol - Standard Tests according to OECD 208 without manure

Table 63: Florfenicol Standard Test. Emergence rate at test end [%].

Species	Control	0.06 mg /kg	0.19 mg/kg	0.56 mg/kg	1.67 mg/kg	5.00 mg/kg
Allium cepa	85	90	80	85	55	80
Avena sativa	90	85	85	80	70	65
Brassica napus	90	70	90	85	70	75
Sinapis alba	85	80	70	65	40	20
Solanum lycopersicum	100	75	75	75	60	60
Phaseolus vulgaris	90	85	85	90	85	35

Concentrations given as nominal values.

Table 64: Florfenicol Standard Test. Length of the shoots. Mean values ± SD [cm].

Species	Control	0.06 mg /kg	0.19 mg/kg	0.56 mg/kg	1.67 mg/kg	5.00 mg/kg
Allium cepa	18.443 ± 1.530	17.634 ± 1.129	16.225 ± 1.861	14.098 ± 1.705	10.808 ± 2.494	6.867 ± 2.954
Avena sativa	38.995 ± 1.840	41.784 ± 1.506	38.030 ± 1.437	37.685 ± 5.055	38.283 ± 3.741	35.923 ± 1.760
Brassica napus	15.169 ± 0.613	13.798 ± 0.705	12.478 ± 2.129	8.400 ± 0.763	5.025 ± 1.880	4.400 ± 0.361
Sinapis alba	11.573 ± 1.099	10.961 ± 0.604	9.913 ± 1.100	6.021 ± 1.852	n.s.s.	n.s.s.
Solanum lycopersicum	14.489 ± 0.298	15.179 ± 2.617	15.192 ± 1.589	13.123 ± 3.116	3.700 ± 0.141	6.358 ± 2.590
Phaseolus vulgaris	23.210 ± 1.636	24.497 ± 1.353	23.327 ± 2.669	22.705 ± 0.922	15.482 ± 1.843	7.238 ± 0.810

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 65: Florfenicol Standard Test. Fresh weight of the shoots. Mean values ± SD [g].

Species	**Control**	**0.06 mg /kg**	**0.19 mg/kg**	**0.56 mg/kg**	**1.67 mg/kg**	**5.00 mg/kg**
Allium cepa	0.350 ± 0.067	0.322 ± 0.047	0.257 ± 0.046	0.199 ± 0.036	0.120 ± 0.038	0.056 ± 0.060
Avena sativa	1.530 ± 0.164	1.509 ± 0.118	1.328 ± 0.147	1.406 ± 0.206	1.332 ± 0.212	0.961 ± 0.119
Brassica napus	1.532 ± 0.188	1.249 ± 0.080	0.994 ± 0.497	0.360 ± 0.068	0.066 ± 0.053	0.033 ± 0.005
Sinapis alba	1.031 ± 0.345	0.880 ± 0.189	0.744 ± 0.188	0.307 ± 0.173	n.s.s.	n.s.s.
Solanum lycopersicum	1.858 ± 0.129	2.231 ± 1.006	1.955 ± 0.324	1.463 ± 0.938	0.038 ± 0.011	0.209 ± 0.158
Phaseolus vulgaris	4.539 ± 0.331	4.834 ± 0.438	4.582 ± 0.607	4.561 ± 0.468	2.867 ± 0.568	1.170 ± 0.431

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 66: Florfenicol Standard Test. Post-emergence survival rate at test end [%].

Species	**Control**	**0.06 mg /kg**	**0.19 mg/kg**	**0.56 mg/kg**	**1.67 mg/kg**	**5.00 mg/kg**
Allium cepa	100	94	94	94	82	38
Avena sativa	100	100	100	100	86	85
Brassica napus	92	93	94	94	57	27
Sinapis alba	94	94	86	62	0	0
Solanum lycopersicum	100	100	100	93	25	67
Phaseolus vulgaris	100	100	100	100	100	100

Concentrations given as nominal values.

Table 67: Florfenicol Standard Test. Emergence inhibition related to control [%].

Species	Control	0.06 mg /kg	0.19 mg/kg	0.56 mg/kg	1.67 mg/kg	5.00 mg/kg
Allium cepa		-6	6	0	35	6
Avena sativa		6	6	11	22	28
Brassica napus		22	0	6	22	17
Sinapis alba		6	18	24	53*	76*
Solanum lycopersicum		25	25	25	40	40
Phaseolus vulgaris		6	6	0	6	61*

Concentrations given as nominal values. *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 68: Florfenicol Standard Test. Shoot length inhibition related to control [%].

Species	Control	0.06 mg /kg	0.19 mg/kg	0.56 mg/kg	1.67 mg/kg	5.00 mg/kg
Allium cepa		4	12	24*	41*	63*
Avena sativa		-7	2	3	2	8
Brassica napus		10	19*	45*	67*	71*
Sinapis alba		5	14	48*	n.s.s.	n.s.s.
Solanum lycopersicum		-5	-5	9	74*	56*
Phaseolus vulgaris		-6	-1	2	33*	69*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 69: Florfenicol Standard Test. Fresh mass inhibition related to control [%].

Species	Control	0.06 mg /kg	0.19 mg/kg	0.56 mg/kg	1.67 mg/kg	5.00 mg/kg
Allium cepa		8	27*	43*	66*	84*
Avena sativa		1	13	8	13	37*
Brassica napus		23*	39*	78*	96*	98*
Sinapis alba		15	28	70*	n.s.s.	n.s.s.
Solanum lycopersicum		-20	-5	21	98*	89*
Phaseolus vulgaris		-9	-3	-2	36*	74*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 70: Florfenicol Standard Test. P.-em. survival inhibition related to control [%].

Species	Control	0.06 mg /kg	0.19 mg/kg	0.56 mg/kg	1.67 mg/kg	5.00 mg/kg
Allium cepa		6	6	6	18	63*
Avena sativa		0	0	0	14	15
Brassica napus		-1	-2	-2	38	71*
Sinapis alba		0	9	35	100*	100*
Solanum lycopersicum		0	0	7	75*	33
Phaseolus vulgaris		0	0	0	0	0

Concentrations given as nominal values. *: significant when compared to control (p = 0.05, one-sided smaller);
negative value = emergence stimulation.

10.2 Tylosin tartrate - Standard Tests according to OECD 208 without manure

Table 71: Tylosin tartrate Standard Test. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	85	85	60	60	58	90
T1	9.3	---	---	---	---	---	65
T2	16.0	---	---	80	---	---	65
T3	27.8	90	---	80	---	---	70
T4	36.1	---	---	---	60	---	---
T5	48.1	95	---	75	---	---	65
T6	50.0	---	90	---	---	75	---
T7	51.0	---	---	---	80	---	---
T8	72.2	---	---	---	80	---	---
T9	83.3	100	---	85	---	---	60
T10	100.0	---	95	---	---	100	---
T11	102.1	---	---	---	85	---	---
T12	144.3	100	---	95	90	---	---
T13	200.0	---	95	---	---	75	---
T14	250.0	100	---	---	---	---	---
T15	400.0	---	95	---	---	92	---
T16	800.0	---	95	---	---	100	---

Concentrations given as nominal values per kg dry mass soil. (C) untreated control; (T) treatments; (1) only 3 instead of 4 pots, i.e. 9 seeds instead of 12 seeds sown; (---) respective concentration not tested.

Table 72: Tylosin tartrate Standard Test. Length of the shoots. Mean values ± SD [cm].

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	165	386	164	125	343	89
T1	9.3	---	---	---	---	---	79
T2	16.0	---	---	194	---	---	77
T3	27.8	139	---	170	---	---	66
T4	36.1	---	---	---	140	---	---
T5	48.1	98	---	155	---	---	42
T6	50.0	---	384	---	---	283	---
T7	51.0	---	---	---	127	---	---
T8	72.2	---	---	---	102	---	---
T9	83.3	83	---	103	---	---	30
T10	100.0	---	375	---	---	292	---
T11	102.1	---	---	---	82	---	---
T12	144.3	60	---	n.s.s	52	---	---
T13	200.0	---	387	---	---	202	---
T14	250.0	n.s.s		---	---	---	---
T15	400.0	---	350	---	---	173	---
T16	800.0	---	335	---	---	138	---

n.s.s. = No seedlings survived; o.s.s. = One seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) control; (T) treatment;

Table 73: Tylosin tartrate Standard Test. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0.279	1.147	1.371	0.932	8.041	0.257
T1	9.3	---	---	---	---	---	0.186
T2	16.0	---	---	1.639	---	---	0.216
T3	27.8	0.174	---	1.311	---	---	0.118
T4	36.1	---	---	---	1.331	---	---
T5	48.1	0.096	---	0.973	---	---	0.040
T6	50.0	---	1.060	---	---	5.476	---
T7	51.0	---	---	---	0.632	---	---
T8	72.2	---	---	---	0.406	---	---
T9	83.3	0.071	---	0.341	---	---	0.012
T10	100.0	---	0.986	---	---	4.024	---
T11	102.1	---	---	---	0.147	---	---
T12	144.3	0.031	---	n.s.s	0.044	---	---
T13	200.0		0.998	---	---	2.754	---
T14	250.0	n.s.s	---	---	---	---	---
T15	400.0	---	0.726	---	---	2.016	---
T16	800.0	---	0.448	---	---	1.428	---

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) control; (T) treatment;

Table 74: Tylosin tartrate Standard Test. Post-emergence survival rate at test end [%].

Code	**Test item [mg/kg]**	***A cepa***	***A. sativa***	***B. napus***	***S. lycopersicum***	***P. vulgaris***	***T. pratense***
C	0.0	94	100	83	92	100	100
T1	9.3	---	---	---	---	---	100
T2	16.0	---	---	69	---	---	92
T3	27.8	94	---	75	---	---	100
T4	36.1	---	---	---	92	---	---
T5	48.1	100	---	100	---	---	69
T6	50.0	---	100	---	---	100	---
T7	51.0	---	---	---	100	---	---
T8	72.2	---	---	---	100	---	---
T9	83.3	95	---	83	---	---	17
T10	100.0	---	100	---	---	100	---
T11	102.1	---	---	---	100	---	---
T12	144.3	53	---	0	72	---	---
T13	200.0	---	100	---	---	100	---
T14	250.0	0	---	---	---	---	---
T15	400.0	---	100	---	---	100	---
T16	800.0	---	100	---	---	100	---

Concentrations given as nominal values per kg dry mass soil. (C) untreated control; (T) treatments; (dw) dry weight; (---) respective concentration not tested.

Table 75: Tylosin tartrate Standard Test. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0	0	0	0	0	0
T1	9.3	---	---	---	---	---	27.8
T2	16.0	---	---	-33.3	---	---	27.8
T3	27.8	-5.9	---	-33.3	---	---	22.2
T4	36.1	---	---	---	0	---	---
T5	48.1	-11.8	---	-25.0	---	---	27.8
T6	50.0	---	-5.9	---	---	-28.6	---
T7	51.0	---	---	---	-33.3	---	---
T8	72.2	---	---	---	-25.0	---	---
T9	83.3	-17.6	---	-41.7	---	---	33.3
T10	100.0	---	-11.8	---	---	-71.4	---
T11	102.1	---	---	---	-41.7	---	---
T12	144.3	-11.8	---	-58.3	-50.0	---	---
T13	200.0	---	-11.8	---	---	-28.6	---
T14	250.0	-17.6	---	---	---	---	---
T15	400.0	---	-11.8	---	---	-57.1	---
T16	800.0	---	-11.8	---	---	-71.4	---

Concentrations given as nominal values per kg dry mass soil. *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. C) untreated control; (T) treatments;

Table 76: Tylosin tartrate Standard Test. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0	0	0	0	0	0
T1	9.3	---	---	---	---	---	11
T2	16.0	---	---	-18	---	---	14
T3	27.8	16	---	-3	---	---	27 *
T4	36.1	---	---	---	-13	---	---
T5	48.1	41 *	---	6	---	---	53 *
T6	50.0	---	0.4	---	---	17	---
T7	51.0	---	---	---	-2.3	---	---
T8	72.2	---	---	---	18	---	---
T9	83.3	50 *	---	37 *	---	---	66 *
T10	100.0	---	3	---	---	15	---
T11	102.1	---	---	---	34	---	---
T12	144.3	64 *	---	n.s.s.	58 *	---	---
T13	200.0	---	-0.3	---	---	41	---
T14	250.0	n.s.s.	---	---	---	---	---
T15	400.0	---	9 *	---	---	50	---
T16	800.0	---	13	---	---	60	---

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 77: Tylosin tartrate Standard Test. Fresh mass inhibition related to control [%].

Code	**Test item [mg/kg]**	***A cepa***	***A. sativa***	***B. napus***	***S. lycopersicum***	***P. vulgaris***	***T. pratense***
C	0.0	0	0	0	0	0	0
T1	9.3	---	---	---	---	---	31
T2	16.0	---	---	-21	---	---	19
T3	27.8	32	---	3.5	---	---	56 *
T4	36.1	---	---	---	-74	---	---
T5	48.1	62 *	---	28	---	---	85 *
T6	50.0	---	8	---	---	32	---
T7	51.0	---	---	---	18	---	---
T8	72.2	---	---	---	47	---	---
T9	83.3	72 *	---	75 *	---	---	96 *
T10	100.0	---	14	---	---	50	---
T11	102.1	---	---	---	81	---	---
T12	144.3	88 *	---	n.s.s.	94	---	---
T13	200.0	---	13	---	---	66	---
T14	250.0	n.s.s.	---	---	---	---	---
T15	400.0	---	37 *	---	---	75	---
T16	800.0	---	61 *	---	---	80	---

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 78: Tylosin tartrate Standard Test. P.-em. survival inhibition related to control [%].

Code	**Test item [mg/kg]**	***A cepa***	***A. sativa***	***B. napus***	***S. lycopersicum***	***P. vulgaris***	***T. pratense***
C	0.0	0	0	0	0	0	0
T1	9.3	---	---	---	---	---	27.8
T2	16.0	---	---	-10.0	---	---	33.3
T3	27.8	-6.3	---	-20.0	---	---	22.2
T4	36.1	---	---	---	0	---	---
T5	48.1	-18.8	---	-30.0	---	---	50.0
T6	50.0	---	-5.9	---	---	-28.6	---
T7	51.0	---	---	---	-45.5	---	---
T8	72.2	---	---	---	-36.4	---	---
T9	83.3	-18.8	---	-30.0	---	---	88.9 *
T10	100.0	---	-11.8	---	---	-71.4	---
T11	102.1	---	---	---	-54.5	---	---
T12	144.3	37.5 *	---	100 *	-18.2	---	---
T13	200.0	---	-11.8	---	---	-28.6	---
T14	250.0	100 *	---	---	---	---	---
T15	400.0	---	-11.8	---	---	-57.1	---
T16	800.0	---	-11.8	---	---	-71.4	---

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = survival stimulation. Concentrations given as nominal values per kg dry mass soil. (C) untreated control; (T) treatments.

10.3 Florfenicol - Modified plant tests with fresh spiked pig manure

Table 79: Florfenicol Fresh Spiked Pig Manure. Emergence rate at test end [%].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	65.0	42.5	62.5	55.0	37.5	42.5	30.0
Avena sativa	90.0	85.0	82.5	87.5	92.5	85.0	80.0
Brassica napus	66.7	72.5	72.5	65.0	55.0	52.5	57.5
Sinapis alba	75.0	82.5	87.5	80.0	77.5	70.0	62.5
Solanum lycopersicum	75.0	55.0	60.0	57.5	57.5	40.0	17.5
Phaseolus vulgaris	80.0	81.3	87.5	71.9	28.1	56.3	50.0

Concentrations given as nominal values.

Table 80: Florfenicol Fresh Spiked Pig Manure. Length of the shoots. Mean values ± SD [cm].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	13.788 ± 2.699	9.407 ± 4.078	9.061 ± 5.818	7.200 ± 1.151	n.s.s.	n.s.s.	n.s.s.
Avena sativa	34.071 ± 1.606	37.366 ± 2.675	36.600 ± 2.741	32.300 ± 1.523	27.841 ± 1.160	24.685 ± 3.015	12.691 ± 5.576
Brassica napus	14.278 ± 1.367	15.398 ± 1.955	12.777 ± 2.512	6.442 ± 1.480	n.s.s.	n.s.s.	n.s.s.
Sinapis alba	14.548 ± 0.772	14.725 ± 1.594	11.285 ± 1.183	4.934 ± 0.711	2.393 ± 0.349	n.s.s.	n.s.s.
Solanum lycopersicum	14.417 ± 0.954	14.938 ± 1.865	14.421 ± 1.156	8.545 ± 2.336	4.171 ± 1.213	n.s.s.	3.200 ± -
Phaseolus vulgaris	23.678 ± 2.896	26.158 ± 3.816	25.310 ± 1.922	22.351 ± 1.089	16.338 ± 1.648	6.358 ± 0.803	5.900 ± -

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 81: Florfenicol Fresh Spiked Pig Manure. Fresh weight of the shoots. Mean values ± SD [g].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	0.184 ± 0.067	0.118 ± 0.079	0.121 ± 0.132	0.081 ± 0.026	n.s.s.	n.s.s.	n.s.s.
Avena sativa	0.982 ± 0.123	1.090 ± 0.184	1.015 ± 0.160	0.722 ± 0.102	0.391 ± 0.092	0.286 ± 0.058	0.103 ± 0.069
Brassica napus	2.226 ± 0.794	2.138 ± 0.622	1.148 ± 0.485	0.197 ± 0.093	n.s.s.	n.s.s.	n.s.s.
Sinapis alba	2.203 ± 0.532	2.026 ± 0.607	1.129 ± 0.358	0.225 ± 0.058	0.049 ± 0.024	n.s.s.	n.s.s.
Solanum lycopersicum	1.296 ± 0.360	1.535 ± 0.384	1.562 ± 0.541	0.300 ± 0.149	0.053 ± 0.050	n.s.s.	0.026 ± -
Phaseolus vulgaris	5.593 ± 0.860	6.688 ± 1.142	5.531 ± 0.731	3.993 ± 0.313	2.154 ± 0.464	1.038 ± 0.294	1.441 ± -

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 82: Florfenicol Fresh Spiked Pig Manure. Post-emergence survival rate at test end [%].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	61.5	64.7	48.0	31.8	0.0	0.0	0.0
Avena sativa	100.0	100.0	97.0	97.1	100.0	97.1	78.1
Brassica napus	60.0	69.0	62.1	26.9	0.0	0.0	0.0
Sinapis alba	93.3	93.9	97.1	84.4	29.0	0.0	0.0
Solanum lycopersicum	93.3	95.5	83.3	47.8	30.4	0.0	14.3
Phaseolus vulgaris	87.5	100.0	100.0	100.0	100.0	44.4	6.3

TI = Test item; DM = Dry mass. Concentrations given as nominal values.

Table 83: Florfenicol Fresh Spiked Soil with Pig Manure. Emergence rate at test end [%].

Species	**Standard control**	**Manure control**	**0.2 mg/kg**	**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**
Allium cepa	65.0	42.5	60.0	50.0	40.0	42.5	35.0
Brassica napus	66.7	72.5	97.5	80.0	57.5	37.5	62.5

TI = Test item; DM = Dry mass; Concentrations given as nominal values.

Table 84: Florfenicol Fresh Spiked Soil with Pig Manure. Length of the shoots. Mean values ± SD [cm].

Species	**Standard control**	**Manure control**	**0.2 mg/kg**	**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**
Allium cepa	13.788 ± 2.699	9.407 ± 4.078	10.708 ± 4.116	9.675 ± 1.413	6.267 ± 1.858	n.s.s.	n.s.s.
Brassica napus	14.278 ± 1.367	15.398 ± 1.955	12.035 ± 0.646	7.722 ± 2.385	5.600 ± -	2.300 ± -	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 85: Florfenicol Fresh Spiked Soil with Pig Manure. Fresh weight of the shoots. Mean values ± SD [g].

Species	**Standard control**	**Manure control**	**0.2 mg/kg**	**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**
Allium cepa	0.184 ± 0.067	0.118 ± 0.079	0.143 ± 0.073	0.113 ± 0.014	0.039 ± 0.021	n.s.s.	n.s.s.
Brassica napus	2.226 ± 0.794	2.138 ± 0.622	1.068 ± 0.116	0.357 ± 0.264	0.086 ± -	0.019 ± -	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 86: Florfenicol Fresh Spiked Soil with Pig Manure. Post-emergence survival rate at test end [%].

Species	**Standard control**	**Manure control**	**0.2 mg/kg**	**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**
Allium cepa	61.5	64.7	50.0	45.0	18.8	0.0	0.0
Brassica napus	60.0	69.0	69.2	46.9	4.3	6.7	0.0

Concentrations given as nominal values.

Table 87: Florfenicol Fresh Spiked Pig Manure. Emergence inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	-52.9		-47.1	-29.4	11.8	0.0	29.4
Avena sativa	-5.9		2.9	-2.9	-8.8	0.0	5.9
Brassica napus	8.0		0.0	10.3	24.1	27.6	20.7
Sinapis alba	9.1		-6.1	3.0	6.1	15.2	24.2
Solanum lycopersicum	-36.4		-9.1	-4.5	-4.5	27.3	68.2*
Phaseolus vulgaris	1.5		-7.7	11.5	65.4*	30.8*	38.5*

Concentrations given as nominal values. *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 88: Florfenicol Fresh Spiked Pig Manure. Shoot length inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	-46.6		3.7	23.5	n.s.s.*	n.s.s.	n.s.s.
Avena sativa	8.8*		2.0	13.6*	25.5*	33.9*	66.0*
Brassica napus	7.3		17.0*	58.2*	n.s.s.	n.s.s.	n.s.s.
Sinapis alba	1.2		23.4*	66.5*	83.7*	n.s.s.	n.s.s.
Solanum lycopersicum	3.5		3.5	42.8*	72.1*	n.s.s.	78.6
Phaseolus vulgaris	9.5		3.2	14.6*	37.5*	75.7*	77.4*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 89: Florfenicol Fresh Spiked Pig Manure. Fresh mass inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	-56.7		-2.6	31.6	n.s.s.	n.s.s.	n.s.s.
Avena sativa	9.9		6.8	33.7*	64.1*	73.7*	90.5*
Brassica napus	-4.1		46.3*	90.8*	n.s.s.	n.s.s.	n.s.s.
Sinapis alba	-8.7		44.3*	88.9*	97.6*	n.s.s.	n.s.s.
Solanum lycopersicum	15.6		-1.8	80.5*	96.5*	n.s.s.	98.3
Phaseolus vulgaris	16.4		17.3*	40.3*	67.8*	84.5*	78.5*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 90: Florfenicol Fresh Spiked Pig Manure. P.-em. survival inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	4.9		25.8	50.8	100.0*	100.0*	100.0*
Avena sativa	0.0		3.0	2.9	0.0	2.9	21.9*
Brassica napus	13.0		10.0	61.0*	100.0*	100.0*	100.0*
Sinapis alba	0.6		-3.4	10.2	69.1*	100.0*	100.0*
Solanum lycopersicum	2.2		12.7	49.9*	68.1*	100.0*	85.0*
Phaseolus vulgaris	12.5		0.0	0.0	0.0	55.6*	93.8*

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 91: Florfenicol Fresh Spiked Soil with Pig Manure. Emergence inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	-52.9		-41.2	-17.6	5.9	0.0	17.6
Brassica napus	8.0		-34.5	-10.3	20.7	48.3	13.8

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 92: Florfenicol Fresh Spiked Soil with Pig Manure. Shoot length inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	-46.6		-13.8	-2.8	33.4	n.s.s.	n.s.s.
Brassica napus	7.3		21.8*	49.9*	63.6*	85.1*	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 93: Florfenicol Fresh Spiked Soil with Pig Manure. Fresh mass inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	-56.7		-21.7	3.7	66.6*	n.s.s.	n.s.s.
Brassica napus	-4.1		50.0*	83.3*	96.0*	99.1*	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 94: Florfenicol Fresh Spiked Soil with Pig Manure. P.-em. survival inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	4.9		22.7	30.5	71.0*	100.0*	100.0*
Brassica napus	13.0		-0.4	32.0	93.7*	90.3*	100.0*

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

10.4 Florfenicol - Modified plant tests with fresh spiked cattle manure

Table 95: Florfenicol Fresh Spiked Cattle Manure. Emergence rate at test end [%].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	80.0	67.5	77.5	65.0	77.5	65.0	57.5
Avena sativa	85.0	87.5	80.0	95.0	95.0	90.0	80.0
Brassica napus	70.0	77.5	67.5	85.0	77.5	65.0	65.0
Sinapis alba	75.0	77.5	80.0	77.5	57.5	87.5	82.5
Solanum lycopersicum	85.0	70.0	72.5	82.5	70.0	67.5	47.5
Phaseolus vulgaris	80.0	68.8	75.0	71.9	53.1	40.6	43.8

Concentrations given as nominal values.

Table 96: Florfenicol Fresh Spiked Cattle Manure. Length of the shoots. Mean values ± SD [cm].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	12.825 ± 1.114	15.915 ± 2.102	16.191 ± 1.341	13.183 ± 1.938	5.174 ± 1.088	n.s.s.	1.500 ± -
Avena sativa	34.766 ± 1.712	40.006 ± 1.589	40.889 ± 1.550	36.204 ± 1.091	35.275 ± 2.260	32.178 ± 1.669	23.217 ± 2.591
Brassica napus	15.725 ± 1.257	18.158 ± 1.462	14.615 ± 1.197	7.820 ± 0.997	5.137 ± 1.696	n.s.s.	n.s.s.
Sinapis alba	14.732 ± 1.269	15.645 ± 1.392	12.374 ± 1.699	6.825 ± 1.753	2.260 ± 1.135	2.567 ± 0.981	n.s.s.
Solanum lycopersicum	13.914 ± 0.398	14.997 ± 1.156	14.653 ± 1.433	13.109 ± 1.312	8.969 ± 0.998	4.203 ± 1.012	2.442 ± 0.900
Phaseolus vulgaris	29.745 ± 3.386	32.600 ± 8.462	28.972 ± 3.633	30.067 ± 2.435	22.812 ± 3.973	9.220 ± 2.954	5.283 ± 2.715

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 97: Florfenicol Fresh Spiked Cattle Manure. Fresh weight of the shoots. Mean values ± SD [g].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	0.152 ± 0.024	0.252 ± 0.054	0.249 ± 0.040	0.164 ± 0.034	0.041 ±. 0.015	n.s.s.	0.007 ± -
Avena sativa	0.946 ± 0.109	1.222 ± 0.175	1.312 ± 0.111	1.031 ± 0.105	0.920 ± 0.123	0.665 ± 0.119	0.352 ± 0.094
Brassica napus	2.060 ± 0.596	2.495 ± 0.443	1.260 ± 0.235	0.241 ± 0.087	0.072 ± 0.046	n.s.s.	n.s.s.
Sinapis alba	1.378 ± 0.305	1.955 ± 0.492	1.055 ± 0.310	0.328 ± 0.148	0.031 ± 0.021	0.031 ± 0.015	n.s.s.
Solanum lycopersicum	1.336 ± 0.183	1.773 ± 0.352	1.627 ± 0.396	1.188 ± 0.318	0.469 ± 0.140	0.086 ± 0.056	0.020 ± 0.010
Phaseolus vulgaris	7.105 ± 2.676	7.648 ± 1.712	6.057 ± 1.096	5.066 ± 1.287	2.806 ± 0.672	1.765 ± 0.372	0.990 ± 0.539

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 98: Florfenicol Fresh Spiked Cattle Manure. Post-emergence survival rate at test end [%].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	93.8	85.2	77.4	80.8	48.4	0.0	4.3
Avena sativa	100.0	100.0	100.0	100.0	100.0	100.0	84.4
Brassica napus	100.0	87.1	85.2	61.8	25.8	0.0	0.0
Sinapis alba	100.0	83.9	90.6	54.8	21.7	8.6	0.0
Solanum lycopersicum	100.0	96.4	93.1	66.7	71.4	40.7	31.6
Phaseolus vulgaris	100.0	100.0	100.0	91.3	94.1	53.8	64.3

Concentrations given as nominal values.

Table 99: Florfenicol Fresh Spiked Soil with Cattle Manure. Emergence rate at test end [%].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	80.0	67.5	65.0	77.5	85.0	52.5	42.5
Brassica napus	70.0	77.5	65.0	50.0	65.0	45.0	65.0

Concentrations given as nominal values.

Table 100: Florfenicol Fresh Spiked Soil with Cattle Manure. Length of the shoots. Mean values ± SD [cm].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	12.825 ± 1.114	15.915 ± 2.102	15.292 ± 1.404	12.167 ± 1.776	5.404 ± 1.329	n.s.s.	n.s.s.
Brassica napus	15.725 ± 1.257	18.158 ± 1.462	14.672 ± 1.644	7.164 ± 1.272	5.590 ± 1.806	n.s.s.	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 101: Florfenicol Fresh Spiked Soil with Cattle Manure. Fresh weight of the shoots. Mean values ± SD [g].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	0.152 ± 0.024	0.252 ± 0.054	0.216 ± 0.033	0.151 ± 0.029	0.043 ± 0.020	n.s.s.	n.s.s.
Brassica napus	2.060 ± 0.596	2.495 ± 0.443	1.080 ± 0.294	0.176 ± 0.069	0.083 ± 0.076	n.s.s.	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 102: Florfenicol Fresh Spiked Soil with Cattle Manure. Post-emergence survival rate at test end [%].

Species	Standard control	Manure control	0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	93.8	85.2	84.6	67.7	61.8	0.0	0.0
Brassica napus	100.0	87.1	84.6	60.0	38.5	0.0	0.0

Concentrations given as nominal values.

Table 103: Florfenicol Fresh Spiked Cattle Manure. Emergence inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	-18.5		-14.8	3.7	-14.8	3.7	14.8
Avena sativa	2.9		8.6	-8.6	-8.6	-2.9	8.6
Brassica napus	9.7		12.9	-9.7	0.0	16.1	16.1
Sinapis alba	3.2		-3.2	0.0	25.8	-12.9	-6.5
Solanum lycopersicum	-21.4		-3.6	-17.9	0.0	3.6	32.1
Phaseolus vulgaris	-16.4		-9.1	-4.5	22.7	40.9	36.4

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 104: Florfenicol Fresh Spiked Cattle Manure. Shoot length inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	19.4*		-1.7	17.2*	67.5*	0.0*	90.6*
Avena sativa	13.1*		-2.2	9.5*	11.8*	19.6*	42.0*
Brassica napus	13.4*		19.5*	56.9*	71.7*	n.s.s.	n.s.s.
Sinapis alba	5.8		20.9*	56.4*	85.6*	83.6*	n.s.s.
Solanum lycopersicum	7.2*		2.3	12.6*	40.2*	72.0*	83.7*
Phaseolus vulgaris	8.8		11.1	7.8	30.0*	71.7*	83.8*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 105: Florfenicol Fresh Spiked Cattle Manure. Fresh mass inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	39.7*		1.4	34.9*	83.6*	n.s.s.	97.2*
Avena sativa	22.5*		-7.4	15.6*	24.7*	45.6*	71.2*
Brassica napus	17.4		49.5*	90.4*	97.1*	n.s.s.	n.s.s.
Sinapis alba	29.5*		46.0*	83.2*	98.4*	98.4*	n.s.s.
Solanum lycopersicum	24.6*		8.2	33.0*	73.5*	95.1*	98.9*
Phaseolus vulgaris	7.1		20.8*	33.8*	63.3*	76.9*	87.1*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 106: Florfenicol Fresh Spiked Cattle Manure. P.-em. survival inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	-10.1		9.1	5.2	43.2*	100.0*	94.9*
Avena sativa	0.0		0.0	0.0	0.0	0.0	15.6
Brassica napus	-14.8		2.2	29.1*	70.4*	100.0*	100.0*
Sinapis alba	-19.2		-8.1	34.6*	74.1*	89.8*	100.0*
Solanum lycopersicum	-3.7		3.4	30.9*	25.9*	57.8*	67.3*
Phaseolus vulgaris	0.0		0.0	8.7	5.9	46.2*	35.7*

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller);
negative value = emergence stimulation.

Table 107: Florfenicol Fresh Spiked Soil with Cattle Manure. Emergence inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	-18.5		3.7	-14.8	-25.9	22.2	37.0
Brassica napus	9.7		16.1	35.5*	16.1	41.9*	16.1

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 108: Florfenicol Fresh Spiked Soil with Cattle Manure. Shoot length inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	19.4*		3.9	23.6*	66.0*	n.s.s.	n.s.s.
Brassica napus	13.4*		19.2*	60.5*	69.2*	n.s.s.	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 109: Florfenicol Fresh Spiked Soil with Cattle Manure. Fresh mass inhibition related to control [%].

Species	Standard control		0.2 mg/kg	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg
Allium cepa	39.7*		14.6*	40.2*	83.1*	n.s.s.	n.s.s.
Brassica napus	15.3		56.7*	92.9*	96.7*	n.s.s.	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 110: Florfenicol Fresh Spiked Soil with Cattle Manure. P.-em. survival inhibition related to control [%].

Species	**Standard control**		**0.2 mg/kg**	**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**
Allium cepa	-10.1		0.7	20.5	27.5	100.0*	100.0*
Brassica napus	-14.8		2.8	31.1	55.8*	100.0*	100.0*

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller);
negative value = emergence stimulation.

10.5 Tylosin tartrate - Modified plant tests with fresh spiked pig manure

Table 111: Tylosin tartrate Fresh Spiked Pig Manure. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	95	90	90	80	83	75
MC	0.0	98	93	83	83	88	85
T1	5.0	--	--	--	--	--	85
T2	10.0	--	--	90	80	--	70
T3	15.0	90	--	--	--	--	--
T4	20.0	--	--	--	--	--	83
T5	25.0	--	--	93	95	--	--
T6	30.0	93	--	--	--	--	--
T7	40.0	--	--	--	--	79	--
T8	50.0	--	--	--	--	--	90
T9	60.0	100	--	--	--	--	--
T10	62.5	--	--	90	88	--	--
T11	88.0	--	--	--	--	92	--
T12	100	--	90	--	--	--	--
T13	125	--	--	--	--	--	80
T14	150	93	--	--	--	--	--
T15	156	--	--	93	90	--	--
T16	194	--	--	--	--	92	--
T17	200	--	85	--	--	--	--
T18	300	100	--	--	--	--	--
T19	391	--	--	85	95	--	--
T20	400	--	85	--	--	--	--
T21	426	--	--	--	--	92	--
T22	700	--	95	--	--	--	--
T23	937	--	--	--	--	100	--
T24	1000	--	100	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 112: Tylosin tartrate Fresh Spiked Pig Manure. Length of the shoots. Mean values ✢ SD [cm].

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested;

Code	Test item [mg/kg]	A cepa	A. sativa	B. napus	S. lycopersicum	P. vulgaris	T. pratense
C	0.0	168	377	145	129	289	106
MC	0.0	167	379	167	153	359	96
T1	5.0	--	--	--	--	--	99
T2	10.0	--	--	164	159	--	102
T3	15.0	161	--	--	--	--	--
T4	20.0	--	--	--	--	--	78
T5	25.0	--	--	171	155	--	--
T6	30.0	143	--	--	--	--	--
T7	40.0	--	--	--	--	347	--
T8	50.0	--	--	--	--	--	38
T9	60.0	104	--	--	--	--	--
T10	62.5	--	--	167	149	--	--
T11	88.0	--	--	--	--	300	--
T12	100	--	399	--	--	--	--
T13	125	--	--	--	--	--	n.s.s.
T14	150	93	--	--	--	--	--
T15	156	--	--	94	69	--	--
T16	194	--	--	--	--	209	--
T17	200	--	380	--	--	--	--
T18	300	78	--	--	--	--	--
T19	391	--	--	n.s.s.	38	--	--
T20	400	--	369	--	--	--	--
T21	426	--	--	--	--	180	--
T22	700	--	299	--	--	--	--
T23	937	--	--	--	--	136	--
T24	1000	--	211	--	--	--	--

Table 113: Tylosin tartrate Fresh Spiked Pig Manure. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	A cepa	A. sativa	B. napus	S. lycopersicum	P. vulgaris	T. pratense
C	0.0	0.276	1.050	1.002	0.872	6.080	0.365
MC	0.0	0.277	1.380	1.634	1.294	8.330	0.356
T1	5.0	--	--	--	--	--	0.381
T2	10.0	--	--	1.542	1.577	--	0.303
T3	15.0	0.269	--	--	--	--	--
T4	20.0	--	--	--	--	--	0.177
T5	25.0	--	--	1.521	1.331	--	--
T6	30.0	0.244	--	--	--	--	--
T7	40.0	--	--	--	--	8.110	--
T8	50.0	--	--	--	--	--	0.033
T9	60.0	0.107	--	--	--	--	--
T10	62.5	--	--	1.646	1.044	--	--
T11	88.0	--	--	--	--	4.964	--
T12	100	--	1.547	--	--	--	--
T13	125	--	--	--	--	--	n.s.s.
T14	150	0.059	--	--	--	--	--
T15	156	--	--	0.333	0.097	--	--
T16	194	--	--	--	--	2.830	--
T17	200	--	1.309	--	--	--	--
T18	300	0.043	--	--	--	--	--
T19	391	--	--	n.s.s.	0.023	--	--
T20	400	--	0.873	--	--	--	--
T21	426	--		--	--	2.320	--
T22	700	--	0.382	--	--	--	--
T23	937	--		--	--	1.453	--
T24	1000	--	0.212	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 114: Tylosin tartrate Fresh Spiked Pig Manure. Post-emergence survival rate at test end [%].

Code	Test item [mg/kg]	A cepa	A. sativa	B. napus	S. lycopersicum	P. vulgaris	T. pratense
C	0.0	100	100	100	100	100	100
MC	0.0	100	100	97	94	100	100
T1	5.0	--	--	--	--	--	97
T2	10.0	--	--	94	100	--	100
T3	15.0	100	--	--	--	--	--
T4	20.0	--	--	--	--	--	97
T5	25.0	--	--	95	95	--	--
T6	30.0	100	--	--	--	--	--
T7	40.0	--	--	--	--	95	--
T8	50.0	--	--	--	--	--	25
T9	60.0	97.5	--	--	--	--	--
T10	62.5	--	--	89	89	--	--
T11	88.0	--	--	--	--	96	--
T12	100	--	100	--	--	--	--
T13	125	--	--	--	--	--	0
T14	150	73	--	--	--	--	--
T15	156	--	--	76	97	--	--
T16	194	--	--	--	--	100	--
T17	200	--	97	--	--	--	--
T18	300	0	--	--	--	--	--
T19	391	--	--	0	5	--	--
T20	400	--	100	--	--	--	--
T21	426	--	--	--	--	96	--
T22	700	--	97	--	--	--	--
T23	937	--	--	--	--	92	--
T24	1000	--	65	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested; ([a]) values >100% represent emergence of additional seedlings after day 7.

Table 115: Tylosin tartrate Fresh Spiked Soil with Pig Manure. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	95	75
MC	0.0	98	85
T1	5.0	--	73
T2	10.0	--	80
T3	15.0	93	--
T4	20.0	--	80
T6	30.0	90	--
T8	50.0	--	60
T9	60.0	98	--
T13	125	--	68
T14	150	98	--
T18	300	98	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 116: Tylosin tartrate Fresh Spiked Soil with Pig Manure. Length of the shoots. Mean values ± SD [cm].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	168	106
MC	0.0	166	96
T1	5.0	--	94
T2	10.0	--	86
T3	15.0	165	--
T4	20.0	--	81
T6	30.0	150	--
T8	50.0	--	35
T9	60.0	103	--
T13	125	--	n.s.s.
T14	150	92	--
T18	300	69	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 117: Tylosin tartrate Fresh Spiked Soil with Pig Manure. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	0.276	0.365
MC	0.0	0.277	0.356
T1	5.0	--	0.301
T2	10.0	--	0.257
T3	15.0	0.282	--
T4	20.0	--	0.177
T6	30.0	0.225	--
T8	50.0	--	0.025
T9	60.0	0.107	--
T13	125	--	n.s.s.
T14	150	0.061	--
T18	300	0.038	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 118: Tylosin tartrate Fresh Spiked Soil with Pig Manure. Post-emergence survival rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	100	100
MC	0.0	100	100
T1	5.0	--	100
T2	10.0	--	100
T3	15.0	100	--
T4	20.0	--	100
T6	30.0	94	--
T8	50.0	--	4
T9	60.0	95	--
T13	125	--	0
T14	150	80	--
T18	300	15	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested

Table 119: Tylosin tartrate Fresh Spiked Pig Manure. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	3.1	3.2	-8.4	3.6	5.7	11.8
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	0
T2	10.0	--	--	-8.4	3.6	--	17.6
T3	15.0	8.2	--	--	--	--	--
T4	20.0	--	--	--	--	--	2.4
T5	25.0	--	--	-12.0	-14.5	--	--
T6	30.0	5.1	--	--	--	--	--
T7	40.0	--	--	--	--	10.2	--
T8	50.0	--	--	--	--	--	-5.9
T9	60.0	-2.0	--	--	--	--	--
T10	62.5	--	--	-8.4	-6.0	--	--
T11	88.0	--	--	--	--	-4.5	--
T12	100	--	3.2	--	--	--	--
T13	125	--	--	--	--	--	5.9
T14	150	5.1	--	--	--	--	--
T15	156	--	--	-12.0	-8.4	--	--
T16	194	--	--	--	--	-4.5	--
T17	200	--	8.6	--	--	--	--
T18	300	-2.0	--	--	--	--	--
T19	391	--	--	-2.4	-14.5	--	--
T20	400	--	8.6	--	--	--	--
T21	426	--	--	--	--	-4.5	--
T22	700	--	-2.2	--	--	--	--
T23	937	--	--	--	--	-13.6	--
T24	1000	--	-7.5	--	--	--	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 120: Tylosin tartrate Fresh Spiked Pig Manure. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	-1.1	0.7	13,3	-15.7	19.6	-10.3
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	-3.7
T2	10.0	--	--	2.2	-3,8	--	-6.5
T3	15.0	3.6	--	--	--	--	--
T4	20.0	--	--	--	--	--	18.5
T5	25.0	--	--	-1.9	-0.9	--	--
T6	30.0	14.0 *	--	--	--	--	--
T7	40.0	--	--	--	--	15.4	--
T8	50.0	--	--	--	--	--	60.4 *
T9	60.0	37.7 ,*	--	--	--	--	--
T10	62.5	--	--	0.11	2.9	--	--
T11	88.0	--	--	--	--	16.6 *	--
T12	100	--	-5.1	--	--	--	--
T13	125	--	--	--	--	--	n.s.s.
T14	150	44.1 *	--	--	--	--	--
T15	156	--	--	34.7 *	55.3 *	--	--
T16	194	--	--	--	--	41.9 *	--
T17	200	--	-0.2	--	--	--	--
T18	300	53.5 *	--	--	--	--	--
T19	391	--	--	n.s.s.	75.6 *	--	--
T20	400	--	2.5	--	--	--	--
T21	426	--	--	--	--	49.9 *	--
T22	700	--	21.0 *	--	--	--	--
T23	937	--	--	--	--	62.0 *	--
T24	1000	--	44.4 *	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 121: Tylosin tartrate Fresh Spiked Pig Manure. Fresh mass inhibition related to control [%].

Code	**Test item [mg/kg]**	***A cepa***	***A. sativa***	***B. napus***	***S. lycopersicum***	***P. vulgaris***	***T. pratense***
C	0.0	0.4	24.0	38.7	32.6	27.0	-2.2
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	-7.0
T2	10.0	--	--	5.6	-21.9	--	14.9
T3	15.0	2.9	--	--	--	--	--
T4	20.0	--	--	--	--	--	50.3 *
T5	25.0	--	--	7.0	-2.9	--	--
T6	30.0	11.9	--	--	--	--	--
T7	40.0	--	--	--	--	14.8	--
T8	50.0	--	--	--	--	--	90.8 *
T9	60.0	61.5 *	--	--	--	--	--
T10	62.5	--	--	-0.7	19.3	--	--
T11	88.0	--	--	--	--	40.4*	--
T12	100	--	-12.1	--	--	--	--
T13	125	--	--	--	--	--	n.s.s.
T14	150	78.7 *	--	--	--	--	--
T15	156	--	--	79.6 *	92.5 *	--	--
T16	194	--	--	--	--	66.0*	--
T17	200	--	5.2	--	--	--	--
T18	300	84.5 *	--	--	--	--	--
T19	391	--	--	n.s.s.	98.2 *	--	--
T20	400	--	36.7 *	--	--	--	--
T21	426	--	--	--	--	72.1 *	--
T22	700	--	72.3 *	--	--	--	--
T23	937	--	--	--	--	82.6 *	--
T24	1000	--	84.7 *	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 122: Tylosin tartrate Fresh Spiked Pig Manure. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0	0	-3.1	-6.4	0	0
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	2.9
T2	10.0	--	--	-3.1	-6.4	--	0
T3	15.0	0	--	--	--	--	--
T4	20.0	--	--	--	--	--	3.0
T5	25.0	--	--	-3.1	-1.1	--	--
T6	30.0	0	--	--	--	--	--
T7	40.0	--	--	--	--	5.3	--
T8	50.0	--	--	--	--	--	75.0 *
T9	60.0	2.5	--	--	--	--	--
T10	62.5	--	--	8.2	5.3	--	--
T11	88.0	--	--	--	--	4.5	--
T12	100	--	0	--	--	--	--
T13	125	--	--	--	--	--	100 *
T14	150	27.0 *	--	--	--	--	--
T15	156	--	--	21.6 *	-3.2	--	--
T16	194	--	--	--	--	0	--
T17	200	--	2.8	--	--	--	--
T18	300	100 *	--	--	--	--	--
T19	391	--	--	100 *	94.7 *	--	--
T20	400	--	0	--	--	--	--
T21	426	--	--	--	--	4.5	--
T22	700	--	2.6	--	--	--	--
T23	937	--	--	--	--	8.3	--
T24	1000	--	35.0 *	--	--	--	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 123: Tylosin tartrate Fresh Spiked Soil with Pig Manure. Emergence inhibition related to control [%].

Code	**Test item [mg/kg]**	***A. cepa***	***T. pratense***
C	0.0	3.1	11.8
MC	0.0	0	0
T1	5.0	--	14.1
T2	10.0	--	5.9
T3	15.0	5.1	--
T4	20.0	--	5.9
T6	30.0	8.2	--
T8	50.0	--	29.4
T9	60.0	0	--
T13	125	--	20.0
T14	150	0	--
T18	300	0	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 124: Tylosin tartrate Fresh Spiked Soil with Pig Manure. Shoot length inhibition related to control [%].

Code	**Test item [mg/kg]**	***A. cepa***	***T. pratense***
C	0.0	-0.1	-10.3
MC	0.0	0	0
T1	5.0	--	2.2
T2	10.0	--	10.4
T3	15.0	1.1	--
T4	20.0	--	15.2
T6	30.0	9.7	--
T8	50.0	--	63.5
T9	60.0	37.9	--
T13	125	--	n.s.s.
T14	150	44.6	--
T18	300	58.3	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 125: Tylosin tartrate Fresh Spiked Soil with Pig Manure. Fresh mass inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	0.4	-2.5
MC	0.0	0	0
T1	5.0	--	15.3
T2	10.0	--	27.9
T3	15.0	-1.7	--
T4	20.0	--	50.2
T6	30.0	18.8	--
T8	50.0	--	93.0
T9	60.0	61.6	--
T13	125	--	n.s.s.
T14	150	77.8	--
T18	300	86.4	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 126: Tylosin tartrate Fresh Spiked Soil with Pig Manure. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	0	0
MC	0.0	0	0
T1	5.0	--	0
T2	10.0	--	0
T3	15.0	0	--
T4	20.0	--	0
T6	30.0	5.6	--
T8	50.0	--	95.8 *
T9	60.0	5.1	--
T13	125	--	100 *
T14	150	20.5 *	--
T18	300	84.6 *	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

10.6 Tylosin tartrate - Modified plant tests with fresh spiked cattle manure

Table 127: Tylosin tartrate Fresh Spiked Cattle Manure. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	75	100	100	100	100	80
MC	0.0	73	98	83	100	92	83
T1	5.0	--	--	--	--	--	75
T2	10.0	--	--	100	78	--	83
T3	15.0	80	--	--	--	--	--
T4	20.0	--	--	--	--	--	83
T5	25.0	--	--	88	93	--	--
T6	30.0	85	--	--	--	--	--
T7	40.0	--	--	--	--	96	--
T8	50.0	--	--	--	--	--	85
T9	60.0	88	--	--	--	--	--
T10	62.5	--	--	93	93	--	--
T11	88.0	--	--	--	--	96	--
T12	100	--	95	--	--	--	--
T13	125	--	--	--	--	--	68
T14	150	73	--	--	--	--	--
T15	156	--	--	95	98	--	--
T16	194	--	--	--	--	96	--
T17	200	--	100	--	--	--	--
T18	300	80	--	--	--	--	--
T19	391	--	--	93	93	--	--
T20	400	--	98	--	--	--	--
T21	426	--	--	--	--	100	--
T22	700	--	98	--	--	--	--
T23	937	--	--	--	--	88	--
T24	1000	--	95	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 128: Tylosin tartrate Fresh Spiked Cattle Manure. Length of the shoots. Mean values ± SD [cm].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	168	377	145	129	289	106
MC	0.0	167	379	167	153	359	96
T1	5.0	--	--	--	--	--	99
T2	10.0	--	--	164	159	--	102
T3	15.0	161	--	--	--	--	--
T4	20.0	--	--	--	--	--	78
T5	25.0	--	--	171	155	--	--
T6	30.0	143	--	--	--	--	--
T7	40.0	--	--	--	--	347	--
T8	50.0	--	--	--	--	--	38
T9	60.0	104	--	--	--	--	--
T10	62.5	--	--	167	149	--	--
T11	88.0	--	--	--	--	300	--
T12	100	--	399	--	--	--	--
T13	125	--	--	--	--	--	n.s.s.
T14	150	93	--	--	--	--	--
T15	156	--	--	94	69	--	--
T16	194	--	--	--	--	209	--
T17	200	--	380	--	--	--	--
T18	300	78	--	--	--	--	--
T19	391	--	--	n.s.s.	38	--	--
T20	400	--	369	--	--	--	--
T21	426	--	--	--	--	180	--
T22	700	--	299	--	--	--	--
T23	937	--	--	--	--	136	--
T24	1000	--	211	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 129: Tylosin tartrate Fresh Spiked Cattle Manure. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0.276	1.050	1.002	0.872	6.080	0.365
MC	0.0	0.277	1.380	1.634	1.294	8.330	0.356
T1	5.0	--	--	--	--	--	0.381
T2	10.0	--	--	1.542	1.577	--	0.303
T3	15.0	0.269	--	--	--	--	--
T4	20.0	--	--	--	--	--	0.177
T5	25.0	--	--	1.521	1.331	--	--
T6	30.0	0.244	--	--	--	--	--
T7	40.0	--	--	--	--	8.110	--
T8	50.0	--	--	--	--	--	0.033
T9	60.0	0.107	--	--	--	--	--
T10	62.5	--	--	1.646	1.044	--	--
T11	88.0	--	--	--	--	4.964	--
T12	100	--	1.547	--	--	--	--
T13	125	--	--	--	--	--	n.s.s.
T14	150	0.059	--	--	--	--	--
T15	156	--	--	0.333	0.097	--	--
T16	194	--	--	--	--	2.830	--
T17	200	--	1.309	--	--	--	--
T18	300	0.043	--	--	--	--	--
T19	391	--	--	n.s.s.	0.023	--	--
T20	400	--	0.873	--	--	--	--
T21	426	--	--	--	--	2.320	--
T22	700	--	0.382	--	--	--	--
T23	937	--	--	--	--	1.453	--
T24	1000	--	0.212	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 130: Tylosin tartrate Fresh Spiked Cattle Manure. Post-emergence survival rate at test end [%].

Code	**Test item [mg/kg]**	***A. cepa***	***A. sativa***	***B. napus***	***S. lycopersicum***	***P. vulgaris***	***T. pratense***
C	0.0	93	100	100	100	100	100
MC	0.0	97	100	100	100	100	97
T1	5.0	--	--	--	--	--	100
T2	10.0	--	--	100	100	--	85
T3	15.0	97	--	--	--	--	--
T4	20.0	--	--	--	--	--	82
T5	25.0	--	--	100	100	--	--
T6	30.0	97	--	--	--	--	--
T7	40.0	--	--	--	--	100	--
T8	50.0	--	--	--	--	--	3
T9	60.0	94	--	--	--	--	--
T10	62.5	--	--	100	100	--	--
T11	88.0	--	--	--	--	100	--
T12	100	--	100	--	--	--	--
T13	125	--	--	--	--	--	0
T14	150	50	--	--	--	--	--
T15	156	--	--	97	95	--	--
T16	194	--	--	--	--	100	--
T17	200	--	100	--	--	--	--
T18	300	0	--	--	--	--	--
T19	391	--	--	68	0	--	--
T20	400	--	100	--	--	--	--
T21	426	--	--	--	--	100	--
T22	700	--	100	--	--	--	--
T23	937	--	--	--	--	100	--
T24	1000	--	92	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 131: Tylosin tartrate Fresh Spiked Soil with Cattle Manure. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	75	80
MC	0.0	73	83
T1	5.0	--	88
T2	10.0	--	93
T3	15.0	75	--
T4	20.0	--	78
T6	30.0	78	--
T8	50.0	--	70
T9	60.0	68	--
T13	125	--	93
T14	150	70	--
T18	300	75	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 132: Tylosin tartrate Fresh Spiked Soil with Cattle Manure. Length of the shoots. Mean values ± SD [cm].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	162	105
MC	0.0	168	102
T1	5.0	--	91
T2	10.0	--	81
T3	15.0	158	--
T4	20.0	--	57
T6	30.0	139	--
T8	50.0	--	25
T9	60.0	105	--
T13	125	--	n.s.s.
T14	150	84	--
T18	300	55	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 133: Tylosin tartrate Fresh Spiked Soil with Cattle Manure. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	0.245	0.326
MC	0.0	0.267	0.333
T1	5.0	--	0.23
T2	10.0	--	0.172
T3	15.0	0.292	--
T4	20.0	--	0.072
T6	30.0	0.196	--
T8	50.0	--	0.007
T9	60.0	0.102	--
T13	125	--	n.s.s.
T14	150	0.046	--
T18	300	0.025	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 134: Tylosin tartrate Fresh Spiked Soil with Cattle Manure. Post-emergence survival rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	93	100
MC	0.0	97	100
T1	5.0	--	97
T2	10.0	--	89
T3	15.0	97	--
T4	20.0	--	61
T6	30.0	100	--
T8	50.0	--	7
T9	60.0	89	--
T13	125	--	0
T14	150	39	--
T18	300	7	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 135: Tylosin tartrate Fresh Spiked Cattle Manure. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	-2.7	-2.0	-20.5	0	-8.7	3.6
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	9.6
T2	10.0	--	--	-20.5 *	22.5 *	--	0
T3	15.0	-9.6	--	--	--	--	--
T4	20.0	--	--	--	--	--	0
T5	25.0	--	--	-6.0	7.5	--	--
T6	30.0	-16.4	--	--	--	--	--
T7	40.0	--	--	--	--	-4.3	--
T8	50.0	--	--	--	--	--	-2.4
T9	60.0	-20.5	--	--	--	--	--
T10	62.5	--	--	-12.0	7.5	--	--
T11	88.0	--	--	--	--	-4.3	--
T12	100	--	3.1	--	--	--	--
T13	125	--	--	--	--	--	18.1
T14	150	0	--	--	--	--	--
T15	156	--	--	-14.5	2.5	--	--
T16	194	--	--	--	--	-4.3	--
T17	200	--	-2	--	--	--	--
T18	300	-9.6	--	--	--	--	--
T19	391	--	--	-12.0	7.5	--	--
T20	400	--	-2.0	--	--	--	--
T21	426	--	--	--	--	-8.7	--
T22	700	--	0	--	--	--	--
T23	937	--	--	--	--	4.3	--
T24	1000	--	3.1	--	--	--	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 136: Tylosin tartrate Fresh Spiked Cattle Manure. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	3.4	-0.6	7.6	7.5	3.6	-3.4
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	8.7
T2	10.0	--	--	-0.6	-3.2	--	20.6 *
T3	15.0	-3.6	--	--	--	--	--
T4	20.0	--	--	--	--	--	36.5 *
T5	25.0	--	--	-4.5	0.7	--	--
T6	30.0	22.2 *	--	--	--	--	--
T7	40.0	--	--	--	--	-1.5	--
T8	50.0	--	--	--	--	--	60.6 *
T9	60.0	40.2 *	--	--	--	--	--
T10	62.5	--	--	8.7 *	2.7	--	--
T11	88.0	--	--	--	--	9.9 *	--
T12	100	--	4.3	--	--	--	--
T13	125	--	--	--	--	--	n.s.s.
T14	150	53.4 *	--	--	--	--	--
T15	156	--	--	44.1 *	44.7 *	--	--
T16	194	--	--	--	--	35.1 *	--
T17	200	--	2.0	--	--	--	--
T18	300	n.s.s.	--	--	--	--	--
T19	391	--	--	66.8 *	n.s.s.	--	--
T20	400	--	9.0 *	--	--	--	--
T21	426	--	--	--	--	54.2 *	--
T22	700	--	9.5 *	--	--	--	--
T23	937	--	--	--	--	60.5 *	--
T24	1000	--	13.9 *	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 137: Tylosin tartrate Fresh Spiked Cattle Manure. Fresh mass inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	8.2	5.4	20.0 *	21.5 *	24.7 *	2.1
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	23.7
T2	10.0	--	--	3.8	-27.0 *	--	46.5 *
T3	15.0	-9.4	--	--	--	--	--
T4	20.0	--	--	--	--	--	71.9 *
T5	25.0	--	--	-2.2	-11.3	--	--
T6	30.0	34.2 *	--	--	--	--	--
T7	40.0	--	--	--	--	7.19	--
T8	50.0	--	--	--	--	--	95.5 *
T9	60.0	65.7 *	--	--	--	--	--
T10	62.5	--	--	35.8 *	7.6	--	--
T11	88.0	--	--	--	--	5.40 *	--
T12	100	--	-0.7	--	--	--	--
T13	125	--	--	--	--	--	n.s.s.
T14	150	83.8 *	--	--	--	--	--
T15	156	--	--	86.8 *	90.9 *	--	--
T16	194	--	--	--	--	3.16 *	--
T17	200	--	3.6	--	--	--	--
T18	300	n.s.s.	--	--	--	--	--
T19	391	--	--	98.4 *	n.s.s.	--	--
T20	400	--	25.1 *	--	--	--	--
T21	426	--	--	--	--	2.09 *	--
T22	700	--	46.3 *	--	--	--	--
T23	937	--	--	--	--	1.82 *	--
T24	1000	--	59.8 *	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 138: Tylosin tartrate Fresh Spiked Cattle Manure. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	3.1	0	0	0	0	-3.1
MC	0.0	0	0	0	0	0	97
T1	5.0	--	--	--	--	--	-3.1
T2	10.0	--	--	0	0	--	12.4
T3	15.0	0	--	--	--	--	--
T4	20.0	--	--	--	--	--	15.5
T5	25.0	--	--	0	0	--	--
T6	30.0	0	--	--	--	--	--
T7	40.0	--	--	--	--	0	--
T8	50.0	--	--	--	--	--	96.9 *
T9	60.0	3.1	--	--	--	--	--
T10	62.5	--	--	0	0	--	--
T11	88.0	--	--	--	--	0	--
T12	100	--	0	--	--	--	--
T13	125	--	--	--	--	--	100 *
T14	150	48.5 *	--	--	--	--	--
T15	156	--	--	2.6	5.1	--	--
T16	194	--	--	--	--	0	--
T17	200	--	0	--	--	--	--
T18	300	100 *	--	--	--	--	--
T19	391	--	--	32.4 *	100 *	--	--
T20	400	--	0	--	--	--	--
T21	426	--	--	--	--	0	--
T22	700	--	0	--	--	--	--
T23	937	--	--	--	--	0	--
T24	1000	--	7.9	--	--	--	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 139: Tylosin tartrate Fresh Spiked Soil with Cattle Manure. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	-2.7	3.6
MC	0.0	0	0
T1	5.0	--	-6.0
T2	10.0	--	-12.0
T3	15.0	-2.7	--
T4	20.0	--	6.0
T6	30.0	-6.8	--
T8	50.0	--	15.7
T9	60.0	6.8	--
T13	125	--	-12.0
T14	150	4.1	--
T18	300	-2.7	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 140: Tylosin tartrate Fresh Spiked Soil with Cattle Manure. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	3.4	-3.4
MC	0.0	0	0
T1	5.0	--	10.6 *
T2	10.0	--	20.4 *
T3	15.0	6.1	--
T4	20.0	--	43.8 *
T6	30.0	17.5 *	--
T8	50.0	--	75.4 *
T9	60.0	37.4 *	--
T13	125	--	n.s.s.
T14	150	49.7 *	--
T18	300	67.3 *	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 141: Tylosin tartrate Fresh Spiked Soil with Cattle Manure. Fresh mass inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	8.2	2.1
MC	0.0	0	0
T1	5.0	--	31.1 *
T2	10.0	--	48.4 *
T3	15.0	-9.2	--
T4	20.0	--	78.4 *
T6	30.0	26.8 *	--
T8	50.0	--	97.9 *
T9	60.0	61.8 *	--
T13	125	--	n.s.s.
T14	150	82.6 *	--
T18	300	90.8 *	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 142: Tylosin tartrate Fresh Spiked Soil with Cattle Manure. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	4.1	0
MC	0.0	0	0
T1	5.0	--	2.9
T2	10.0	--	10.8
T3	15.0	0	--
T4	20.0	--	38.7 *
T6	30.0	-3.1	--
T8	50.0	--	12.9 *
T9	60.0	8.2	--
T13	125	--	100 *
T14	150	59.8 *	--
T18	300	12.8 *	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

10.7 Florfenicol - Modified plant tests with half-maximum incubated spiked pig manure

Table 143: Florfenicol Half-max Anaerobic Aged Pig Manure. Emergence rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	70.0	62.5	60.0	60.0	52.5	70.0	42.5
Avena sativa	80.0	85.0	90.0	95.0	92.5	100.0	77.5
Brassica napus	75.0	75.0	70.0	80.0	77.5	67.5	67.5
Sinapis alba	95.0	82.5	72.5	80.0	87.5	82.5	60.0
Solanum lycopersicum	95.0	87.5	90.0	85.0	70.0	82.5	17.5
Phaseolus vulgaris	80.0	59.4	78.1	75.0	59.4	62.5	34.4

Concentrations given as nominal values.

Table 144: Florfenicol Half-max Anaerobic Aged Pig Manure. Length of the shoots. Mean values ± SD [cm].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	11.238 ± 1.967	11.050 ± 2.132	10.840 ± 2.559	10.033 ± 1.917	10.792 ±. 2.555	8.771 ± 1.975	2.600 ± 1.414
Avena sativa	36.219 ± 1.963	40.596 ± 3.465	43.761 ± 2.917	42.456 ± 2.356	41.611 ± 4.151	39.608 ± 2.539	11.660 ± 3.567
Brassica napus	14.454 ± 1.148	15.802 ± 0.959	15.624 ± 1.103	16.310 ± 1.105	14.926 ± 0.682	13.632 ± 1.816	2.300 ± -
Sinapis alba	10.766 ± 1.532	11.788 ± 0.594	11.788 ± 0.679	12.384 ± 0.966	11.103 ± 0.881	10.026 ± 0.824	1.250 ± 0.212
Solanum lycopersicum	12.383 ± 0.174	13.874 ± 0.847	12.960 ± 0.883	13.421 ± 1.347	13.454 ± 0.978	13.394 ± 0.762	1.250 ± 0.212
Phaseolus vulgaris	21.870 ± 2.934	23.498 ± 3.135	23.280 ± 3.448	23.723 ± 2.272	23.004 ± 2.690	20.458 ± 3.002	5.775 ± 0.457

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 145: Florfenicol Half-max Anaerobic Aged Pig Manure. Fresh weight of the shoots. Mean values ± SD [g].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	0.134 ± 0.030	0.134 ± 0.036	0.134 ± 0.040	0.126 ± 0.047	0.110 ± 0.052	0.079 ± 0.041	0.007 ± 0.004
Avena sativa	1.197 ± 0.170	1.291 ± 0.156	1.470 ± 0.200	1.387 ± 0.150	1.383 ± 0.280	1.225 ± 0.144	0.091 ± 0.051
Brassica napus	1.527 ± 0.453	1.800 ± 0.285	1.999 ± 0.422	2.111 ± 0.412	1.914 ± 0.577	1.427 ± 0.532	0.026 ± -
Sinapis alba	0.971 ± 0.296	1.186 ± 0.186	1.119 ± 0.142	1.214 ± 0.205	1.012 ± 0.230	0.770 ± 0.118	0.008 ± 0.001
Solanum lycopersicum	0.911 ± 0.036	1.273 ± 0.229	1.085 ± 0.165	1.190 ± 0.281	1.147 ± 0.195	1.160 ± 0.144	0.006 ± 0.001
Phaseolus vulgaris	5.460 ± 1.155	7.633 ± 2.370	8.024 ± 4.622	6.268 ± 1.533	6.565 ± 1.559	6.031 ± 1.297	1.215 ± 0.302

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 146: Florfenicol Half-max Anaerobic Aged Pig Manure. Post-emergence survival rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	85.7	52.0	50.0	50.0	71.4	64.3	11.8
Avena sativa	100.0	100.0	100.0	100.0	100.0	100.0	80.6
Brassica napus	93.3	96.7	85.7	90.6	96.8	92.6	3.7
Sinapis alba	94.7	93.9	100.0	90.6	94.3	93.9	8.3
Solanum lycopersicum	94.7	88.6	97.2	94.1	89.3	81.8	28.6
Phaseolus vulgaris	100.0	100.0	96.0	100.0	94.7	100.0	36.4

Concentrations given as nominal values.

Table 147: Florfenicol Half-max Aerobic Aged Pig Manure/soil. Emergence rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	70.0	65.0	55.0	52.5	45.0	47.5	45.0
Brassica napus	75.0	80.0	87.5	77.5	72.5	70.0	77.5

Concentrations given as nominal values.

Table 148: Florfenicol Half-max Aerobic Aged Pig Manure/soil. Length of the shoots. Mean values ± SD [cm].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	11.238 ± 1.967	10.173 ± 2.222	8.679 ± 2.502	5.538 ± 2.873	0.500 ± -	n.s.s.	n.s.s.
Brassica napus	14.454 ± 1.148	14.634 ± 1.477	8.669 ± 1.294	3.211 ± 1.092	2.333 ± 0.569	2.075 ± 0.350	1.650 ± 0.420

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 149: Florfenicol Half-max Aerobic Aged Pig Manure/soil. Fresh weight of the shoots. Mean values ± SD [g].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	0.134 ± 0.030	0.106 ± 0.035	0.089 ± 0.034	0.049 ± 0.030	0.001 ± -	n.s.s.	n.s.s.
Brassica napus	1.527 ± 0.453	2.042 ± 0.427	0.510 ± 0.123	0.034 ± 0.024	0.011 ± 0.006	0.005 ± 0.001	0.006 ± 0.004

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 150: Florfenicol Half-max Aerobic Aged Pig Manure/soil. Post-emergence survival rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	100.0	61.5	77.3	28.6	5.6	0.0	0.0
Brassica napus	93.3	75.0	85.7	38.7	13.8	17.9	12.9

Concentrations given as nominal values.

Table 151: Florfenicol Half-max Anaerobic Aged Pig Manure. Emergence inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-12.0		4.0	4.0	16.0	-12.0	32.0
Avena sativa	5.9		-5.9	-11.8	-8.8	-17.6	8.8
Brassica napus	0.0		6.7	-6.7	-3.3	10.0	10.0
Sinapis alba	-15.2		12.1	3.0	-6.1	0.0	27.3
Solanum lycopersicum	-8.6		-2.9	2.9	20.0	5.7	80.0*
Phaseolus vulgaris	-34.7		-31.6	-26.3	0.0	-5.3	42.1

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 152: Florfenicol Half-max Anaerobic Aged Pig Manure. Shoot length inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-1.7		1.9	9.2	2.3	20.6*	76.5*
Avena sativa	10.8*		-7.8	-4.6	-2.5	2.4	71.3*
Brassica napus	7.7		1.1	-3.2	5.5	13.7*	85.4*
Sinapis alba	8.7		0.0	-5.1	5.8	14.9*	89.4*
Solanum lycopersicum	10.7*		6.6	3.3	3.0	3.5	91.0*
Phaseolus vulgaris	6.9		0.9	-1.0	2.1	12.9*	75.4*

Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 153: Florfenicol Half-max Anaerobic Aged Pig Manure. Fresh mass inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-0.3		0.0	6.0	17.6	40.6*	94.6*
Avena sativa	7.3		-13.8	-7.4	-7.1	5.2	93.0*
Brassica napus	15.1		-11.1	-17.3	-6.3	20.7	98.6*
Sinapis alba	18.2		5.7	-2.4	14.7*	35.1	99.4*
Solanum lycopersicum	28.5*		14.8	6.6	9.9	8.9	99.6*
Phaseolus vulgaris	28.5*		-5.1	17.9	14.0	21.0	84.1*

Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 154: Florfenicol Half-max Anaerobic Aged Pig Manure. P.-em. survival inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-64.8*		3.8	3.8	-37.4	-23.6	77.4*
Avena sativa	0.0		0.0	0.0	0.0	0.0	19.4*
Brassica napus	3.4		11.3	6.3	-0.1	4.2	96.2*
Sinapis alba	-0.8		-6.5	3.5	-0.4	0.0	91.1*
Solanum lycopersicum	-7.0		-9.8	-6.3	-0.8	7.6	67.7*
Phaseolus vulgaris	0.0		4.0	0.0	5.3	0.0	63.6*

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller);
negative value = emergence stimulation.

Table 155: Florfenicol Half-max Aerobic Aged Pig Manure/soil. Emergence inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-7.7		15.4	19.2	30.8	26.9	30.8
Brassica napus	6.3		-9.4	3.1	9.4	12.5	3.1

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 156: Florfenicol Half-max Aerobic Aged Pig Manure/soil. Shoot length inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-10.5		14.7	45.6*	95.1*	n.s.s.	n.s.s.
Brassica napus	0.3		40.8*	78.1*	84.1*	85.8*	88.7*

n.s.s. = No seedlings survived; Concentrations given as nominal values;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 157: Florfenicol Half-max Aerobic Aged Pig Manure/soil. Fresh mass inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-26.7		16.1	53.9*	99.1*	n.s.s.	n.s.s.
Brassica napus	25.2		75.0*	98.3*	99.5*	99.8*	99.7*

n.s.s. = No seedlings survived; Concentrations given as nominal values;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 158: Florfenicol Half-max Aerobic Aged Pig Manure/soil. P.-em. survival inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-62.5		-25.6	53.6*	91.0*	100.0*	100.0*
Brassica napus	-24.4		-14.3	48.4*	81.6*	76.2*	82.8*

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller);
negative value = emergence stimulation.

10.8 Florfenicol - Modified plant tests with half-maximum incubated spiked cattle manure

Table 159: Florfenicol Half-max Anaerobic Aged Cattle Manure. Emergence rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	85.0	72.5	55.0	87.5	67.5	65.0	57.5
Avena sativa	95.0	87.5	87.5	82.5	80.0	92.5	87.5
Brassica napus	85.0	80.0	75.0	80.0	77.5	77.5	72.5
Sinapis alba	55.0	75.0	90.0	67.5	77.5	65.0	70.0
Solanum lycopersicum	85.0	90.0	72.5	72.5	82.5	90.0	5.0
Phaseolus vulgaris	75.0	90.6	96.9	96.9	93.8	90.6	71.9

Concentrations given as nominal values.

Table 160: Florfenicol Half-max Anaerobic Aged Cattle Manure. Length of the shoots. Mean values ± SD [cm].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	8.799 ± 0.456	11.236 ± 0.775	12.477 ± 0.948	11.995 ± 1.151	10.937 ± 1.041	8.943 ± 2.230	n.s.s.
Avena sativa	38.253 ± 1.971	42.454 ± 1.513	40.388 ± 1.785	40.279 ± 2.134	41.400 ± 2.864	41.401 ± 1.931	15.418 ± 2.153
Brassica napus	14.506 ± 0.229	16.748 ± 1.053	16.660 ± 1.246	17.352 ± 1.322	16.572 ± 1.273	13.691 ± 2.152	n.s.s.
Sinapis alba	13.700 ± 1.435	16.236 ± 1.494	15.367 ± 1.924	16.221 ± 1.446	14.552 ± 1.076	14.161 ± 1.478	1.450 ± -
Solanum lycopersicum	12.656 ± 2.117	14.675 ± 0.997	15.581 ± 0.811	15.354 ± 1.321	14.263 ± 1.552	12.724 ± 1.676	1.000 ± -
Phaseolus vulgaris	21.020 ± 3.563	26.871 ± 4.228	27.586 ± 2.196	29.048 ± 2.608	27.546 ± 2.270	24.602 ± 2.409	6.667 ± 1.218

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 161: Florfenicol Half-max Anaerobic Aged Cattle Manure. Fresh weight of the shoots. Mean values ± SD [g].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	0.086 ± 0.009	0.142 ± 0.017	0.155 ± 0.012	0.144 ± 0.019	0.135 ± 0.017	0.097 ± 0.034	n.s.s.
Avena sativa	1.042 ± 0.098	1.383 ± 0.155	1.263 ± 0.180	1.201 ± 0.224	1.281 ± 0.182	1.352 ± 0.085	0.141 ± 0.043
Brassica napus	0.929 ± 0.098	1.238 ± 0.217	1.232 ± 0.211	1.276 ± 0.447	1.320 ± 0.245	0.863 ± 0.235	n.s.s.
Sinapis alba	1.476 ± 0.581	2.270 ± 0.918	1.863 ± 0.506	2.127 ± 0.376	1.683 ± 0.268	1.730 ± 0.458	0.009 ± -
Solanum lycopersicum	1.082 ± 0.341	1.458 ± 0.202	1.963 ± 0.412	1.972 ± 0.761	1.605 ± 0.445	1.311 ± 0.465	0.009 ± -
Phaseolus vulgaris	4.652 ± 1.007	5.904 ± 1.676	6.243 ± 0.816	6.280 ± 1.200	5.637 ± 0.461	4.661 ± 0.687	1.232 ± 0.213

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 162: Florfenicol Half-max Anaerobic Aged Cattle Manure. Post-emergence survival rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	100.0	89.7	90.9	100.0	88.9	84.6	0.0
Avena sativa	94.7	100.0	97.1	100.0	96.9	100.0	91.4
Brassica napus	100.0	96.9	93.3	96.9	83.9	93.5	0.0
Sinapis alba	100.0	96.7	86.1	92.6	96.8	100.0	7.1
Solanum lycopersicum	82.4	97.2	93.1	86.2	100.0	91.7	50.0
Phaseolus vulgaris	100.0	96.6	100.0	100.0	100.0	96.6	78.3

Concentrations given as nominal values.

Table 163: Florfenicol Half-max Aerobic Aged Cattle Manure/soil. Emergence rate at test end [%].

Species	**Standard control**	**Manure control**	**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**	**50 mg/kg**
Allium cepa	85.0	70.0	72.5	65.0	75.0	55.0	50.0
Brassica napus	85.0	82.5	87.5	77.5	80.0	67.5	55.0

Concentrations given as nominal values.

Table 164: Florfenicol Half-max Aerobic Aged Cattle Manure/soil. Length of the shoots. Mean values ± SD [cm].

Species	**Standard control**	**Manure control**	**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**	**50 mg/kg**
Allium cepa	8.799 ± 0.456	10.421 ± 1.477	9.102 ± 0.834	5.990 ± 1.211	n.s.s.	n.s.s.	n.s.s.
Brassica napus	14.506 ± 0.229	16.780 ± 0.812	16.367 ± 1.094	8.509 ± 0.740	4.967 ± 0.429	n.s.s.	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 165: Florfenicol Half-max Aerobic Aged Cattle Manure/soil. Fresh weight of the shoots. Mean values ± SD [g].

Species	**Standard control**	**Manure control**	**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**	**50 mg/kg**
Allium cepa	0.086 ± 0.009	0.117 ± 0.036	0.088 ± 0.013	0.039 ± 0.020	n.s.s.	n.s.s.	n.s.s.
Brassica napus	0.929 ± 0.098	1.441 ± 0.355	1.256 ± 0.136	0.222 ± 0.051	0.049 ± 0.017	n.s.s.	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 166: Florfenicol Half-max Aerobic Aged Cattle Manure/soil. Post-emergence survival rate at test end [%].

Species	**Standard control**	**Manure control**	**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**	**50 mg/kg**
Allium cepa	100.0	78.6	89.7	53.8	0.0	0.0	0.0
Brassica napus	100.0	93.9	100.0	93.5	25.0	0.0	0.0

TI = Test item; DM = Dry mass. Concentrations given as nominal values.

Table 167: Florfenicol Half-max Anaerobic Aged Cattle Manure. Emergence inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-17.2		24.1	-20.7	6.9	10.3	20.7
Avena sativa	-8.6		0.0	5.7	8.6	-5.7	0.0
Brassica napus	-6.3		6.3	0.0	3.1	3.1	9.4
Sinapis alba	26.7		-20.0	10.0	-3.3	13.3	6.7
Solanum lycopersicum	5.6		19.4	19.4	8.3	0.0	94.4*
Phaseolus vulgaris	17.2		-6.9	-6.9	-3.4	0.0	20.7

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 168: Florfenicol Half-max Anaerobic Aged Cattle Manure. Shoot length inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	21.7*		-11.0	-6.7	2.7	20.4	n.s.s.
Avena sativa	9.9*		4.9*	5.1	2.5	2.5	63.7*
Brassica napus	13.4*		0.5	-3.6	1.1	13.4*	n.s.s.
Sinapis alba	15.6*		5.4	0.1	10.4*	12.8*	91.1*
Solanum lycopersicum	13.8		-6.2	-4.6	2.8	13.3*	93.2*
Phaseolus vulgaris	21.8*		-2.7	-8.1	-2.5	8.4	75.2*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 169: Florfenicol Half-max Anaerobic Aged Cattle Manure. Fresh mass inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	39.8*		-8.9	-1.2	5.4	31.6*	n.s.s.
Avena sativa	24.6*		8.6	13.1	7.4	2.2	89.8*
Brassica napus	25.0*		0.4	-3.1	-6.7	25.0*	n.s.s.
Sinapis alba	35.0		17.9	6.3	25.9*	23.8*	99.6*
Solanum lycopersicum	25.8		-34.6	-35.2	-10.1	10.1	99.4*
Phaseolus vulgaris	21.2		-5.7	-6.4	4.5	21.1*	79.1*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 170: Florfenicol Half-max Anaerobic Aged Cattle Manure. P.-em. survival inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-11.5		-1.4	-11.5	0.9	5.6	100.0*
Avena sativa	5.3		2.9	0.0	3.1	0.0	8.6
Brassica napus	-3.2		3.7	0.0	13.4	3.4	100.0*
Sinapis alba	-3.4		10.9	4.2	-0.1	-3.4	92.6*
Solanum lycopersicum	15.3		4.2	11.3	-2.9	5.7	48.6*
Phaseolus vulgaris	-3.6		-3.6	-3.6	-3.6	0.0	18.9

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller);
negative value = emergence stimulation.

Table 171: Florfenicol Half-max Aerobic Aged Cattle Manure/soil. Emergence inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-21.4		-3.6	7.1	-7.1	21.4	28.6
Brassica napus	-3.0		-6.1	6.1	3.0	18.2	33.3*

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 172: Florfenicol Half-max Aerobic Aged Cattle Manure/soil. Shoot length inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	15.6*		12.7*	42.5*	n.s.s.	n.s.s.	n.s.s.
Brassica napus	13.6*		2.5	49.3*	70.4*	n.s.s.	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 173: Florfenicol Half-max Aerobic Aged Cattle Manure/soil. Fresh mass inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	27.0*		25.2*	66.9*	n.s.s.	n.s.s.	n.s.s.
Brassica napus	35.6*		12.9	84.6*	96.6*	n.s.s.	n.s.s.

n.s.s. = No seedlings survived; Concentrations given as nominal values;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 174: Florfenicol Half-max Aerobic Aged Cattle Manure/soil. P.-em. survival inhibition related to control [%].

Species	**Standard control**		**0.6 mg/kg**	**1.9 mg/kg**	**5.6 mg/kg**	**16.7 mg/kg**	**50 mg/kg**
Allium cepa	-27.3		-14.1	31.5	100.0*	100.0*	100.0*
Brassica napus	-6.5		-6.5	0.4	73.4*	100.0*	100.0*

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller);
negative value = emergence stimulation.

10.9 Tylosin tartrate - Modified plant tests with half-maximum incubated spiked pig manure

Table 175: Tylosin tartrate Half-max Anaerobic Aged Pig Manure. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	100	100	90	85	100	85
MC	0.0	95	100	93	95	96	85
T1	5.0	--	--	--	--	--	88
T2	10.0	--	--	100	98	--	90
T3	15.0	95	--	--	--	--	--
T4	20.0	--	--	--	--	--	90
T5	25.0	--	100	93	--	--	--
T6	30.0	90	--	--	--	--	--
T7	40.0	--	--	--	--	100	--
T8	50.0	--	--	--	--	--	88
T9	60.0	90	--	--	--	--	--
T10	62.5	--	--	80	93	--	--
T11	88.0	--	--	--	--	100	--
T12	100	--	98	--	--	--	--
T13	125	--	--	--	--	--	90
T14	150	90	--	--	--	--	--
T15	156	--	--	98	88	--	--
T16	194	--	--	--	--	100	--
T17	200	--	100	--	--	--	--
T18	300	95	--	--	--	--	--
T19	391	--	--	95	98	--	--
T20	400	--	100	--	--	--	--
T21	426	--	--	--	--	92	--
T22	700	--	88	--	--	--	--
T23	937	--	--	--	--	100	--
T24	1000	--	88	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 176: Tylosin tartrate Half-max Anaerobic Aged Pig Manure. Length of the shoots. Mean values ± SD [cm].

Code	**Test item [mg/kg]**	***A. cepa***	***A. sativa***	***B. napus***	***S. lycopersicum***	***P. vulgaris***	***T. pratense***
C	0.0	127	362	170	170	285	106
MC	0.0	175	360	197	187	306	123
T1	5.0	--	--	--	--	--	115
T2	10.0	--	--	188	191	--	117
T3	15.0	168	--	--	--	--	--
T4	20.0	--	--	--	--	--	116
T5	25.0	--	--	178	--	--	--
T6	30.0	193	--	--	--	--	--
T7	40.0	--	--	--	--	290	--
T8	50.0	--	--	--	--	--	113
T9	60.0	195	--	--	--	--	--
T10	62.5	--	--	192	178	--	--
T11	88.0	--	--	--	--	302	--
T12	100	--	388	--	--	--	--
T13	125	--	--	--	--	--	108
T14	150	191	--	--	--	--	--
T15	156	--	--	189	200	--	--
T16	194	--	--	--	--	282	--
T17	200	--	375	--	--	--	--
T18	300	148	--	--	--	--	--
T19	391	--	--	182	188	--	--
T20	400	--	367	--	--	--	--
T21	426	--	--	--	--	294	--
T22	700	--	359	--	--	--	--
T23	937	--	--	--	--	182	--
T24	1000	--	364	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 177: Tylosin tartrate Half-max Anaerobic Aged Pig Manure. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0.163	0.955	1.296	1.608	4.596	0.414
MC	0.0	0.304	1.099	1.856	2.333	6.688	0.649
T1	5.0	--	--	--	--	--	0.581
T2	10.0	--	--	1.637	2.282	--	0.595
T3	15.0	0.265	--	--	--	--	--
T4	20.0	--	--	--	--	--	0.544
T5	25.0	--	--	1.659	--	--	--
T6	30.0	0.336	--	--	--	--	--
T7	40.0	--	--	--	--	6.492	--
T8	50.0	--	--	--	--	--	0.529
T9	60.0	0.365	--	--	--	--	--
T10	62.5	--	--	2.045	2.11	--	--
T11	88.0	--	--	--	--	6.397	--
T12	100	--	1.363	--	--	--	--
T13	125	--	--	--	--	--	0.520
T14	150	0.367	--	--	--	--	--
T15	156	--	--	1.685	2.556	--	--
T16	194	--	--	--	--	6.296	--
T17	200	--	1.224	--	--	--	--
T18	300	0.219	--	--	--	--	--
T19	391	--	--	1.598	2.200	--	--
T20	400	--	1.138	--	--	--	--
T21	426	--	--	--	--	6.629	--
T22	700	--	0.952	--	--	--	--
T23	937	--	--	--	--	2.317	--
T24	1000	--	1.032	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 178: Tylosin tartrate Half-max Anaerobic Aged Pig Manure. Post-emergence survival rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	95	100	100	100	100	100
MC	0.0	92	100	100	97	100	94
T1	5.0	--	--	--	--	--	97
T2	10.0	--	--	98	100	--	100
T3	15.0	97	--	--	--	--	--
T4	20.0	--	--	--	--	--	100
T5	25.0	--	100	97	--	--	--
T6	30.0	100	--	--	--	--	--
T7	40.0	--	--	--	--	100	--
T8	50.0	--	--	--	--	--	94
T9	60.0	100	--	--	--	--	--
T10	62.5	--	--	100	97	--	--
T11	88.0	--	--	--	--	100	--
T12	100	--	100	--	--	--	--
T13	125	--	--	--	--	--	100
T14	150	94	--	--	--	--	--
T15	156	--	--	97	100	--	--
T16	194	--	--	--	--	100	--
T17	200	--	100	--	--	--	--
T18	300	90	--	--	--	--	--
T19	391	--	--	100	100	--	--
T20	400	--	100	--	--	--	--
T21	426	--	--	--	--	100	--
T22	700	--	97	--	--	--	--
T23	937	--	--	--	--	100	--
T24	1000	--	100	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested

Table 179: Tylosin tartrate Half-max Aerobic Aged Pig Manure/soil. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	100	85
MC	0.0	85	93
T1	5.0	--	75
T2	10.0	--	73
T3	15.0	90	--
T4	20.0	--	65
T6	30.0	90	--
T8	50.0	--	75
T9	60.0	88	--
T13	125	--	78
T14	150	93	--
T18	300	98	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 180: Tylosin tartrate Half-max Aerobic Aged Pig Manure/soil. Length of the shoots. Mean values ± SD [cm].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	163	67
MC	0.0	164	107
T1	5.0	--	96
T2	10.0	--	93
T3	15.0	160	--
T4	20.0	--	94
T6	30.0	147	--
T8	50.0	--	74
T9	60.0	95	--
T13	125	--	20
T14	150	60	--
T18	300	40	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 181: Tylosin tartrate Half-max Aerobic Aged Pig Manure/soil. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	0.298	0.206
MC	0.0	0.272	0.399
T1	5.0	--	0.378
T2	10.0	--	0.331
T3	15.0	0.278	--
T4	20.0	--	0.293
T6	30.0	0.230	--
T8	50.0	--	0.144
T9	60.0	0.124	--
T13	125	--	0.008
T14	150	0.061	--
T18	300	0.023	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 182: Tylosin tartrate Half-max Aerobic Aged Pig Manure/soil. Post-emergence survival rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	100	88
MC	0.0	91	97
T1	5.0	--	100
T2	10.0	--	100
T3	15.0	97	--
T4	20.0	--	92
T6	30.0	92	--
T8	50.0	--	73
T9	60.0	91	--
T13	125	--	9
T14	150	68	--
T18	300	10	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested

Table 183: Tylosin tartrate Half-max Anaerobic Aged Pig Manure. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	-5.3	0	3.2	10.5	-4.2	0
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	-3.5
T2	10.0	--	--	-7.5	-3.2	--	-5.9
T3	15.0	0	--	--	--	--	--
T4	20.0	--	--	--	--	--	-5.9
T5	25.0	--	0	0	--	--	--
T6	30.0	5.3	--	--	--	--	--
T7	40.0	--	--	--	--	-4.2	--
T8	50.0	--	--	--	--	--	-3.5
T9	60.0	5.3	--	--	--	--	--
T10	62.5	--	--	14.0	2.1	--	--
T11	88.0	--	--	--	--	-4.2	--
T12	100	--	2.5	--	--	--	--
T13	125	--	--	--	--	--	-5.9
T14	150	5.3	--	--	--	--	--
T15	156	--	--	-5.4	7.4	--	--
T16	194	--	--	--	--	-4.2	--
T17	200	--	0	--	--	--	--
T18	300	0	--	--	--	--	--
T19	391	--	--	-2.2	-3.2	--	--
T20	400	--	0	--	--	--	--
T21	426	--	--	--	--	4.2	--
T22	700	--	12.5	--	--	--	--
T23	937	--	--	--	--	-4.2	--
T24	1000	--	12.5	--	--	--	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 184: Tylosin tartrate Half-max Anaerobic Aged Pig Manure. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	27.3 *	-0.5	13.8 *	9.0 *	7.1	14.3 *
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	7.0
T2	10.0	--	--	4.7	-2.3	--	5.0
T3	15.0	4.2	--	--	--	--	--
T4	20.0	--	--	--	--	--	5.9
T5	25.0	--	0.1	9.4	--	--	--
T6	30.0	-10.1	--	--	--	--	--
T7	40.0	--	--	--	--	5.2	--
T8	50.0	--	--	--	--	--	8.3 *
T9	60.0	-11.2	--	--	--	--	--
T10	62.5	--	--	2.8	4.7	--	--
T11	88.0	--	--	--	--	1.5	--
T12	100	--	-7.8	--	--	--	--
T13	125	--	--	--	--	--	12.1 *
T14	150	-8.9	--	--	--	--	--
T15	156	--	--	4.1	-7.0	--	--
T16	194	--	--	--	--	7.9	--
T17	200	--	-4.1	--	--	--	--
T18	300	15.6 *	--	--	--	--	--
T19	391	--	--	7.5	-0.6	--	--
T20	400	--	-1.9	--	--	--	--
T21	426	--	--	--	--	4.0	--
T22	700	--	0.4	--	--	--	--
T23	937	--	--	--	--	40.5 *	--
T24	1000	--	-1.1	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 185: Tylosin tartrate Half-max Anaerobic Aged Pig Manure. Fresh mass inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	46.4 *	13.1	30.2 *	31.1 *	31.3 *	36.2 *
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	10.5
T2	10.0	--	--	11.8	2.2	--	8.4
T3	15.0	12.8	--	--	--	--	--
T4	20.0	--	--	--	--	--	16.1
T5	25.0	--	-2.5	10.6	--	--	--
T6	30.0	-10.7	--	--	--	--	--
T7	40.0	--	--	--	--	2.9	--
T8	50.0	--	--	--	--	--	18.6
T9	60.0	-20.0	--	--	--	--	--
T10	62.5	--	--	-10.2	9.5	--	--
T11	88.0	--	--	--	--	4.4	--
T12	100	--	-24.1	--	--	--	--
T13	125	--	--	--	--	--	20.0
T14	150	-20.8	--	--	--	--	--
T15	156	--	--	9.3	-9.6	--	--
T16	194	--	--	--	--	5.9	--
T17	200	--	-11.4	--	--	--	--
T18	300	-27.9	--	--	--	--	--
T19	391	--	--	13.9 *	5.7	--	--
T20	400	--	-3.6	--	--	--	--
T21	426	--	--	--	--	0.9	--
T22	700	--	13.3	--	--	--	--
T23	937	--	--	--	--	65.4 *	--
T24	1000	--	6.1	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 186: Tylosin tartrate Half-max Anaerobic Aged Pig Manure. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	-3.3	0	0	-3.1	0	-6.4
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	-3.2
T2	10.0	--	--	2.5	-3.1	--	-6.4
T3	15.0	-5.4	--	--	--	--	--
T4	20.0	--	--	--	--	--	-6.4
T5	25.0	--	0	2.7	--	--	--
T6	30.0	-8.7	--	--	--	--	--
T7	40.0	--	--	--	--	0	--
T8	50.0	--	--	--	--	--	0
T9	60.0	-8.7	--	--	--	--	--
T10	62.5	--	--	0	0	--	--
T11	88.0	--	--	--	--	0	--
T12	100	--	0	--	--	--	--
T13	125	--	--	--	--	--	-6.4
T14	150	-2.2	--	--	--	--	--
T15	156	--	--	2.6	-3.1	--	--
T16	194	--	--	--	--	0	--
T17	200	--	0	--	--	--	--
T18	300	2.2	--	--	--	--	--
T19	391	--	--	0	-3.1	--	--
T20	400	--	0	--	--	--	--
T21	426	--	--	--	--	0	--
T22	700	--	2.9	--	--	--	--
T23	937	--	--	--	--	0	--
T24	1000	--	0	--	--	--	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 187: Tylosin tartrate Half-max Aerobic Aged Pig Manure/soil. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	-17.6	8.6
MC	0.0	0	0
T1	5.0	--	19.4
T2	10.0	--	21.5
T3	15.0	-5.9	--
T4	20.0	--	30.1 *
T6	30.0	-5.9	--
T8	50.0	--	19.4
T9	60.0	-3.5	--
T13	125	--	16.1
T14	150	-9.4	--
T18	300	-15.3	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 188: Tylosin tartrate Half-max Aerobic Aged Pig Manure/soil. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	0.6	37.2 *
MC	0.0	0	0
T1	5.0	--	9.9
T2	10.0	--	13.4 *
T3	15.0	2.6	--
T4	20.0	--	23.3
T6	30.0	10.3	--
T8	50.0	--	30.5*
T9	60.0	42.0 *	--
T13	125	--	81.3 *
T14	150	63.3 *	--
T18	300	75.6 *	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 189: Tylosin tartrate Half-max Aerobic Aged Pig Manure/soil. Fresh mass inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	-9.6	48.4
MC	0.0	0	0
T1	5.0	--	5.2
T2	10.0	--	17.1
T3	15.0	-2.3	--
T4	20.0	--	35.7 *
T6	30.0	15.4	--
T8	50.0	--	63.8 *
T9	60.0	54.4 *	--
T13	125	--	98.0 *
T14	150	77.6 *	--
T18	300	91.5 *	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 190: Tylosin tartrate Half-max Aerobic Aged Pig Manure/soil. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	-9.9	9.3
MC	0.0	0	0
T1	5.0	--	-3.1
T2	10.0	--	-3.1
T3	15.0	-6.96	--
T4	20.0	--	5.2
T6	30.0	-1.1	--
T8	50.0	--	24.7 *
T9	60.0	0	--
T13	125	--	90.7 *
T14	150	25.3	--
T18	300	89.0 *	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller);negative value = survival stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

10.10 Tylosin tartrate - Modified plant tests with half-maximum incubated spiked cattle manure

Table 191: Tylosin tartrate Half-max Anaerobic Aged Cattle Manure. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	95	95	90	85	92	95
MC	0.0	90	95	88	95	100	70
T1	5.0	--	--	--	--	--	80
T2	10.0	--	--	95	88	--	85
T3	15.0	83	--	--	--	--	--
T4	20.0	--	--	--	--	--	73
T5	25.0	--	--	83	98	--	--
T6	30.0	75	--	--	--	--	--
T7	40.0	--	--	--	--	96	--
T8	50.0	--	--	--	--	--	70
T9	60.0	93	--	--	--	--	--
T10	62.5	--	--	88	98	--	--
T11	88.0	--	--	--	--	96	--
T12	100	--	100	--	--	--	--
T13	125	--	--	--	--	--	80
T14	150	93	--	--	--	--	--
T15	156	--	--	88	93	--	--
T16	194	--	--	--	--	96	--
T17	200	--	98	--	--	--	--
T18	300	93	--	--	--	--	--
T19	391	--	--	93	88	--	--
T20	400	--	95	--	--	--	--
T21	426	--	--	--	--	96	--
T22	700	--	100	--	--	--	--
T23	937	--	--	--	--	96	--
T24	1000	--	98	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 192: Tylosin tartrate Half-max Anaerobic Aged Cattle Manure. Length of the shoots. Mean values ± SD [cm].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	203	400	175	162	259	113
MC	0.0	198	412	180	170	274	107
T1	5.0	--	--	--	--	--	105
T2	10.0	--	--	183	174	--	111
T3	15.0	199	--	--	--	--	--
T4	20.0	--	--	--	--	--	117
T5	25.0	--	--	183	165	--	--
T6	30.0	191	--	--	--	--	--
T7	40.0	--	--	--	--	312	--
T8	50.0	--	--	--	--	--	106
T9	60.0	195	--	--	--	--	--
T10	62.5	--	--	181	176	--	--
T11	88.0	--	--	--	--	281	--
T12	100	--	397	--	--	--	--
T13	125	--	--	--	--	--	82
T14	150	178	--	--	--	--	--
T15	156	--	--	199	180	--	--
T16	194	--	--	--	--	284	--
T17	200	--	412	--	--	--	--
T18	300	100	--	--	--	--	--
T19	391	--	--	105	80	--	--
T20	400	--	376	--	--	--	--
T21	426	--	--	--	--	158	--
T22	700	--	396	--	--	--	--
T23	937	--	--	--	--	131	--
T24	1000	--	390	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 193: Tylosin tartrate Half-max Anaerobic Aged Cattle Manure. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0.335	1.368	1.860	1.679	6.335	0.371
MC	0.0	0.363	1.469	1.846	2.085	7.337	0.404
T1	5.0	--	--	--	--	--	0.368
T2	10.0	--	--	1.762	2.272	--	0.373
T3	15.0	0.400	--	--	--	--	--
T4	20.0	--	--	--	--	--	0.425
T5	25.0	--	--	1.961	2.131	--	--
T6	30.0	0.359	--	--	--	--	--
T7	40.0	--	--	--	--	7.750	--
T8	50.0	--	--	--	--	--	0.396
T9	60.0	0.380	--	--	--	--	--
T10	62.5	--	--	1.747	2.277	--	--
T11	88.0	--	--	--	--	7.643	--
T12	100	--	1.419	--	--	--	--
T13	125	--	--	--	--	--	0.196
T14	150	0.334	--	--	--	--	--
T15	156	--	--	1.902	2.307	--	--
T16	194	--	--	--	--	6.987	--
T17	200	--	1.503	--	--	--	--
T18	300	0.072	--	--	--	--	--
T19	391	--	--	0.326	0.166	--	--
T20	400	--	1.424	--	--	--	--
T21	426	--	--	--	--	2.249	--
T22	700	--	1.423	--	--	--	--
T23	937	--	--	--	--	1.647	--
T24	1000	--	1.278	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 194: Tylosin tartrate Half-max Anaerobic Aged Cattle Manure. Post-emergence survival rate at test end [%].

Code	**Test item [mg/kg]**	***A. cepa***	***A. sativa***	***B. napus***	***S. lycopersicum***	***P. vulgaris***	***T. pratense***
C	0.0	95	100	100	100	100	100
MC	0.0	100	100	100	100	100	100
T1	5.0	--	--	--	--	--	97
T2	10.0	--	--	100	100	--	100
T3	15.0	100	--	--	--	--	--
T4	20.0	--	--	--	--	--	100
T5	25.0	--	100	100	100	--	--
T6	30.0	100	--	--	--	--	--
T7	40.0	--	--	--	--	100	--
T8	50.0	--	--	--	--	--	100
T9	60.0	100	--	--	--	--	--
T10	62.5	--	--	100	97	--	--
T11	88.0	--	--	--	--	100	--
T12	100	--	100	--	--	--	--
T13	125	--	--	--	--	--	100
T14	150	100	--	--	--	--	--
T15	156	--	--	100	100	--	--
T16	194	--	--	--	--	100	--
T17	200	--	100	--	--	--	--
T18	300	97	--	--	--	--	--
T19	391	--	--	95	97	--	--
T20	400	--	100	--	--	--	--
T21	426	--	--	--	--	96	--
T22	700	--	100	--	--	--	--
T23	937	--	--	--	--	96	--
T24	1000	--	100	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 195: Tylosin tartrate Half-max Aerobic Aged Cattle Manure/soil. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	95	95
MC	0.0	80	68
T1	5.0	--	73
T2	10.0	--	65
T3	15.0	78	--
T4	20.0	--	68
T6	30.0	83	--
T8	50.0	--	78
T9	60.0	60	--
T13	125	--	75
T14	150	85	--
T18	300	73	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 196: Tylosin tartrate Half-max Aerobic Aged Cattle Manure/soil. Length of the shoots. Mean values ± SD [cm].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	203	113
MC	0.0	170	110
T1	5.0	--	114
T2	10.0	--	103
T3	15.0	156	--
T4	20.0	--	81
T6	30.0	147	--
T8	50.0	--	42
T9	60.0	98	--
T13	125	--	n.s.s.
T14	150	94	--
T18	300	69	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 197: Tylosin tartrate Half-max Aerobic Aged Cattle Manure/soil. Fresh weight of shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	0.335	0.371
MC	0.0	0.276	0.453
T1	5.0	--	0.396
T2	10.0	--	0.357
T3	15.0	0.235	--
T4	20.0	--	0.162
T6	30.0	0.194	--
T8	50.0	--	0.074
T9	60.0	0.083	--
T13	125	--	n.s.s.
T14	150	0.053	--
T18	300	0.030	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil. Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 198: Tylosin tartrate Half-max Aerobic Aged Cattle Manure/soil. Post-emergence survival rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	100	100
MC	0.0	97	100
T1	5.0	--	100
T2	10.0	--	96
T3	15.0	90	--
T4	20.0	--	82
T6	30.0	97	--
T8	50.0	--	16
T9	60.0	100	--
T13	125	--	0
T14	150	65	--
T18	300	24	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 199: Tylosin tartrate Half-max Anaerobic Aged Cattle Manure. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	-5.6	0	-2.3	10.5	8.3	-35.7
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	-14.3
T2	10.0	--	--	-8.0	7.4	--	-21.4
T3	15.0	7.8	--	--	--	--	--
T4	20.0	--	--	--	--	--	-4.3
T5	25.0	--	--	5.7	-3.2	--	--
T6	30.0	16.4	--	--	--	--	--
T7	40.0	--	--	--	--	4.2	--
T8	50.0	--	--	--	--	--	0
T9	60.0	-3.3	--	--	--	--	--
T10	62.5	--	--	0	-3.2	--	--
T11	88.0	--	--	--	--	4.2	--
T12	100	--	-5.3	--	--	--	--
T13	125	--	--	--	--	--	-14.3
T14	150	-.3.3	--	--	--	--	--
T15	156	--	--	0	2.1	--	--
T16	194	--	--	--	--	4.2	--
T17	200	--	-3.2	--	--	--	--
T18	300	-3.3	--	--	--	--	--
T19	391	--	--	-5.7	7.4	--	--
T20	400	--	0	--	--	--	--
T21	426	--	--	--	--	4.2	--
T22	700	--	-5.3	--	--	--	--
T23	937	--	--	--	--	4.2	--
T24	1000	--	-3.2	--	--	--	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 200: Tylosin tartrate Half-max Anaerobic Aged Cattle Manure. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	-2.2	3.0	3.2	4.8	5.4	-5.5
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	2.1
T2	10.0	--	--	-1.4	-2.6	--	-4.2
T3	15.0	-0.4	--	--	--	--	--
T4	20.0	--	--	--	--	--	-9.3
T5	25.0	--	--	-1.6	3.3	--	--
T6	30.0	3.5	--	--	--	--	--
T7	40.0	--	--	--	--	-13.7 *	--
T8	50.0	--	--	--	--	--	0.6
T9	60.0	1.6	--	--	--	--	--
T10	62.5	--	--	-0.7	-3.6	--	--
T11	88.0	--	--	--	--	-2.3	--
T12	100	--	3.6	--	--	--	--
T13	125	--	--	--	--	--	23.5 *
T14	150	10.2	--	--	--	--	--
T15	156	--	--	-10.3	-5.7	--	--
T16	194	--	--	--	--	-3.7	--
T17	200	--	0	--	--	--	--
T18	300	49.8 *	--	--	--	--	--
T19	391	--	--	-41.5 *	53.1 *	--	--
T20	400	--	8.6	--	--	--	--
T21	426	--	--	--	--	42.3 *	--
T22	700	--	3.8	--	--	--	--
T23	937	--	--	--	--	52.3 *	--
T24	1000	--	5.4	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 201: Tylosin tartrate Half-max Anaerobic Aged Cattle Manure. Fresh mass inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	7.7	6.9	-0.8	19.5	13.6	8.2
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	8.8
T2	10.0	--	--	4.6	-9.0	--	7.7
T3	15.0	-9.9	--	--	--	--	--
T4	20.0	--	--	--	--	--	-5.3
T5	25.0	--	--	-6.2	-2.2	--	--
T6	30.0	1.2	--	--	--	--	--
T7	40.0	--	--	--	--	-5.6	--
T8	50.0	--	--	--	--	--	1.9
T9	60.0	-4.7	--	--	--	--	--
T10	62.5	--	--	5.4	-9.2	--	--
T11	88.0	--	--	--	--	-4.2	--
T12	100	--	3.4	--	--	--	--
T13	125	--	--	--	--	--	51.4 *
T14	150	8.0	--	--	--	--	--
T15	156	--	--	-3.0	-10.7	--	--
T16	194	--	--	--	--	4.8	--
T17	200	--	-2.3	--	--	--	--
T18	300	80.1 *	--	--	--	--	--
T19	391	--	--	82.3 *	92.1 *	--	--
T20	400	--	3.1	--	--	--	--
T21	426	--	--	--	--	69.3 *	--
T22	700	--	3.1	--	--	--	--
T23	937	--	--	--	--	77.6 *	--
T24	1000	--	13.0	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 202: Tylosin tartrate Half-max Anaerobic Aged Cattle Manure. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	5	0	0	0	0	0
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	3
T2	10.0	--	--	0	0	--	0
T3	15.0	0	--	--	--	--	--
T4	20.0	--	--	--	--	--	0
T5	25.0	--	0	0	0	--	--
T6	30.0	0	--	--	--	--	--
T7	40.0	--	--	--	--	0	--
T8	50.0	--	--	--	--	--	0
T9	60.0	0	--	--	--	--	--
T10	62.5	--	--	0	3	--	--
T11	88.0	--	--	--	--	0	--
T12	100	--	0	--	--	--	--
T13	125	--	--	--	--	--	0
T14	150	0	--	--	--	--	--
T15	156	--	--	0	0	--	--
T16	194	--	--	--	--	0	--
T17	200	--	0	--	--	--	--
T18	300	3	--	--	--	--	--
T19	391	--	--	5	3	--	--
T20	400	--	0	--	--	--	--
T21	426	--	--	--	--	4	--
T22	700	--	0	--	--	--	--
T23	937	--	--	--	--	4	--
T24	1000	--	0	--	--	--	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested

Table 203: Tylosin tartrate Half-max Aerobic Aged Cattle Manure/soil. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	-18.8	-39.7
MC	0.0	0	0
T1	5.0	--	-7.4
T2	10.0	--	4.4
T3	15.0	2.5	--
T4	20.0	--	0
T6	30.0	-3.8	--
T8	50.0	--	-14.7
T9	60.0	25	--
T13	125	--	-10.3
T14	150	-6.3	--
T18	300	8.7	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 204: Tylosin tartrate Half-max Aerobic Aged Cattle Manure/soil. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	-19.4 *	-2.6
MC	0.0	0	0
T1	5.0	--	-4,3
T2	10.0	--	6.2
T3	15.0	8.1	--
T4	20.0	--	26.2
T6	30.0	13.7	--
T8	50.0	--	62.0 *
T9	60.0	42.5	--
T13	125	--	n.s.s.
T14	150	44.9	--
T18	300	59.1	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 205: Tylosin tartrate Half-max Aerobic Aged Cattle Manure/soil. Fresh mass inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	-21.4	18.1
MC	0.0	0	0
T1	5.0	--	12.4
T2	10.0	--	21.1 *
T3	15.0	14.7	--
T4	20.0	--	64.2 *
T6	30.0	29.7 *	--
T8	50.0	--	83.7 *
T9	60.0	69.8 *	--
T13	125	--	n.s.s.
T14	150	80.7 *	--
T18	300	89.1 *	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;
*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation(C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 206: Tylosin tartrate Half-max Aerobic Aged Cattle Manure/soil. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*T. pratense*
C	0.0	0	0
MC	0.0	3	0
T1	5.0	--	0
T2	10.0	--	4
T3	15.0	10	--
T4	20.0	--	18
T6	30.0	3	--
T8	50.0	--	84
T9	60.0	0	--
T13	125	--	100
T14	150	35	--
T18	300	76	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

10.11 Florfenicol – Modified plant tests with maximum incubated spiked pig manure

Table 207: Florfenicol Max Anaerobic Aged Pig Manure. Emergence rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	80.0	42.5	20.0	57.5	35.0	22.5	30.0
Avena sativa	100.0	92.5	97.5	77.5	85.0	95.0	92.5
Brassica napus	80.0	75.0	52.5	77.5	57.5	57.5	55.0
Sinapis alba	90.0	95.0	82.5	85.0	90.0	90.0	85.0
Solanum lycopersicum	85.0	70.0	72.5	77.5	72.5	67.5	30.0
Phaseolus vulgaris	70.0	87.5	78.1	78.1	78.1	68.8	56.3

Concentrations given as nominal values.

Table 208: Florfenicol Max Anaerobic Aged Pig Manure. Length of the shoots. Mean values ± SD [cm].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	9.496 ± 3.343	12.547 ± 2.437	10.240 ± 2.626	10.976 ± 1.206	10.933 ±. 1.018	11.775 ± 2.348	3.200 ± -
Avena sativa	31.015 ± 0.693	33.694 ± 1.598	33.808 ± 1.039	34.176 ± 3.352	34.321 ± 3.052	32.709 ± 2.722	25.476 ± 2.713
Brassica napus	14.538 ± 1.511	14.332 ± 1.356	15.775 ± 0.779	15.044 ± 0.992	15.714 ± 2.212	12.864 ± 2.254	n.s.s.
Sinapis alba	10.407 ± 0.585	11.279 ± 0.206	12.360 ± 1.476	12.806 ± 1.255	11.825 ± 1.331	6.890 ± 0.678	1.407 ± 0.397
Solanum lycopersicum	11.404 ± 0.682	13.860 ± 1.858	13.573 ± 1.129	13.566 ± 0.772	12.375 ± 0.783	12.272 ± 0.986	2.250 ± 1.344
Phaseolus vulgaris	24.405 ± 2.721	23.203 ± 1.444	24.446 ± 1.810	23.933 ± 1.490	23.720 ± 1.591	23.495 ± 3.011	7.871 ± 0.786

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 209: Florfenicol Max Anaerobic Aged Pig Manure. Fresh weight of the shoots. Mean values ± SD [g].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	0.087 ± 0.044	0.134 ± 0.049	0.081 ± 0.047	0.109 ± 0.043	0.100 ± 0.013	0.116 ± 0.047	0.009 ± -
Avena sativa	0.713 ± 0.128	0.890 ± 0.098	0.911 ± 0.054	0.933 ± 0.141	0.898 ± 0.153	0.803 ± 0.136	0.282 ± 0.055
Brassica napus	1.696 ± 0.467	1.501 ± 0.336	1.852 ± 0.212	1.640 ± 0.208	2.224 ± 0.839	1.256 ± 0.481	n.s.s.
Sinapis alba	0.842 ± 0.231	0.962 ± 0.105	1.119 ± 0.176	1.300 ± 0.432	1.077 ± 0.315	0.418 ± 0.103	0.010 ± 0.008
Solanum lycopersicum	0.883 ± 0.052	1.369 ± 0.482	1.215 ± 0.219	1.147 ± 0.190	0.959 ± 0.137	0.920 ± 0.208	0.025 ± 0.012
Phaseolus vulgaris	5.060 ± 1.058	4.997 ± 0.249	6.086 ± 0.721	5.593 ± 0.685	5.523 ± 0.688	5.957 ± 1.779	1.274 ± 0.186

n.s.s. = No seedlings survived; Concentrations given as nominal values.

Table 210: Florfenicol Max Anaerobic Aged Pig Manure. Post-emergence survival rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	81.3	94.1	87.5	82.6	78.6	77.8	8.3
Avena sativa	100.0	100.0	100.0	100.0	97.1	100.0	94.6
Brassica napus	81.3	93.3	100.0	87.1	87.0	87.0	0.0
Sinapis alba	100.0	97.4	93.9	100.0	86.1	91.7	50.0
Solanum lycopersicum	100.0	78.6	89.7	96.8	100.0	96.3	16.7
Phaseolus vulgaris	92.9	100.0	100.0	100.0	96.0	100.0	55.6

Concentrations given as nominal values.

Table 211: Florfenicol Max Anaerobic Aged Pig Manure. Emergence inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-88.2*		52.9	-35.3	17.6	47.1	29.4
Avena sativa	-8.1		-5.4	16.2	8.1	-2.7	0.0
Brassica napus	-6.7		30.0	-3.3	23.3	23.3	26.7
Sinapis alba	5.3		13.2	10.5	5.3	5.3	10.5
Solanum lycopersicum	-21.4		-3.6	-10.7	-3.6	3.6	57.1*
Phaseolus vulgaris	20.0		10.7	10.7	10.7	21.4	35.7*

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 212: Florfenicol Max Anaerobic Aged Pig Manure. Shoot length inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	24.3		18.4	12.5	12.9	6.2	74.5*
Avena sativa	8.0*		-0.3	-1.4	-1.9	2.9	24.4*
Brassica napus	4.9		-10.1	-5.0	-9.6	10.2	n.s.s.
Sinapis alba	7.7*		-9.6	-13.5	-4.8	38.9*	87.5*
Solanum lycopersicum	17.7*		2.1	2.1	10.7*	11.5*	83.8*
Phaseolus vulgaris	-5.2		-5.4	-3.1	-2.2	-1.3	66.1*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 213: Florfenicol Max Anaerobic Aged Pig Manure. Fresh mass inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	35.3		39.5	18.8	25.6	14.0	93.3*
Avena sativa	20.0*		-2.4	-4.8	-0.9	9.8	68.3*
Brassica napus	1.8		-23.4	-9.3	-48.2	16.3	n.s.s.
Sinapis alba	12.4		-16.4	-35.2	-12.0	56.5*	98.9*
Solanum lycopersicum	35.5*		11.3	16.2	29.9*	32.8*	98.2*
Phaseolus vulgaris	-1.3		-21.8	-11.9	-10.5	-19.2	74.5*

n.s.s. = No seedlings survived; Concentrations given as nominal values;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 214: Florfenicol Max Anaerobic Aged Pig Manure. Post-em. survival inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	13.7		7.0	12.2	16.5	17.4	91.1*
Avena sativa	0.0		0.0	0.0	2.9	0.0	5.4
Brassica napus	12.9		-7.1	6.7	6.8	6.8	100.0*
Sinapis alba	-2.7		3.5	-2.7	11.6	5.9	48.6*
Solanum lycopersicum	-27.3		-14.1	-23.2	-27.3*	-22.6	78.8*
Phaseolus vulgaris	7.1		0.0	0.0	4.0	0.0	44.4*

*: significant when compared to control (p = 0.05, one-sided smaller);

negative value = emergence stimulation. Concentrations given as nominal values.

10.12 Florfenicol - Modified plant tests with maximum incubated spiked cattle manure

Table 215: Florfenicol Max Anaerobic Aged Cattle Manure. Emergence rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	90.0	65.0	75.0	72.5	82.5	75.0	82.5
Avena sativa	85.0	85.0	85.0	85.0	82.5	90.0	82.5
Brassica napus	70.0	72.5	62.5	75.0	82.5	80.0	77.5
Sinapis alba	60.0	67.5	82.5	85.0	72.5	90.0	62.5
Solanum lycopersicum	85.0	92.5	82.5	75.0	77.5	67.5	77.5
Phaseolus vulgaris	95.0	96.9	84.4	90.6	93.8	96.9	90.6

Concentrations given as nominal values.

Table 216: Florfenicol Max Anaerobic Aged Cattle Manure. Length of the shoots. Mean values ± SD [cm].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	11.608 ± 0.790	13.150 ± 2.762	13.333 ± 1.652	13.356 ± 1.771	12.824 ±. 1.667	12.686 ± 1.342	10.627 ± 1.062
Avena sativa	38.538 ± 2.601	41.877 ± 1.998	43.900 ± 2.423	43.741 ± 3.106	42.460 ± 2.286	41.657 ± 1.685	39.482 ± 1.300
Brassica napus	13.540 ± 0.297	15.636 ± 0.715	15.083 ± 1.006	15.275 ± 1.259	16.406 ± 1.424	15.550 ± 1.304	13.104 ± 0.926
Sinapis alba	14.013 ± 1.381	13.879 ± 1.500	13.721 ± 1.432	13.896 ± 1.792	14.348 ± 0.988	12.438 ± 1.168	8.954 ± 2.167
Solanum lycopersicum	12.358 ± 1.025	12.369 ± 0.887	12.697 ± 0.874	12.901 ± 1.222	12.751 ± 1.249	10.799 ± 0.995	8.018 ± 1.212
Phaseolus vulgaris	22.100 ± 1.327	23.122 ± 1.457	24.443 ± 2.376	24.690 ± 1.075	24.203 ± 3.046	22.835 ± 1.286	21.988 ± 1.924

Concentrations given as nominal values.

Table 217: Florfenicol Max Anaerobic Aged Cattle Manure. Fresh weight of the shoots. Mean values ± SD [g].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	0.124 ± 0.014	0.168 ± 0.041	0.165 ± 0.025	0.185 ± 0.034	0.156 ± 0.023	0.166 ± 0.036	0.127 ± 0.018
Avena sativa	1.130 ± 0.112	1.280 ± 0.165	1.399 ± 0.245	1.382 ± 0.170	1.436 ± 0.286	1.279 ± 0.123	1.162 ± 0.167
Brassica napus	1.129 ± 0.067	1.452 ± 0.179	1.368 ± 0.266	1.338 ± 0.277	1.568 ± 0.247	1.415 ± 0.195	0.966 ± 0.207
Sinapis alba	1.772 ± 0.620	1.541 ± 0.344	1.386 ± 0.303	1.514 ± 0.476	1.534 ± 0.202	1.209 ± 0.278	0.715 ± 0.353
Solanum lycopersicum	1.112 ± 0.299	1.188 ± 0.230	1.232 ± 0.204	1.337 ± 0.536	1.378 ± 0.313	1.016 ± 0.398	0.405 ± 0.147
Phaseolus vulgaris	5.474 ± 0.294	5.549 ± 0.342	5.908 ± 0.546	5.617 ± 0.582	5.219 ± 1.101	4.387 ± 0.618	3.239 ± 0.643

Concentrations given as nominal values.

Table 218: Florfenicol Max Anaerobic Aged Cattle Manure. Post-emergence survival rate at test end [%].

Species	Standard control	Manure control	0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	100.0	88.5	93.3	93.1	90.9	93.3	93.9
Avena sativa	100.0	100.0	100.0	100.0	100.0	97.2	100.0
Brassica napus	100.0	96.6	100.0	96.7	93.9	96.9	96.8
Sinapis alba	100.0	92.6	93.9	97.1	96.6	100.0	92.0
Solanum lycopersicum	82.4	97.3	97.0	100.0	90.3	88.9	83.9
Phaseolus vulgaris	89.5	100.0	100.0	100.0	96.7	100.0	100.0

Concentrations given as nominal values.

Table 219: Florfenicol Max Anaerobic Aged Cattle Manure. Emergence inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-38.5		-15.4	-11.5	-26.9	-15.4	-26.9
Avena sativa	0.0		0.0	0.0	2.9	-5.9	2.9
Brassica napus	3.4		13.8	-3.4	-13.8	-10.3	-6.9
Sinapis alba	11.1		-22.2	-25.9	-7.4	-33.3	7.4
Solanum lycopersicum	8.1		10.8	18.9	16.2	27.0	16.2
Phaseolus vulgaris	1.9		12.9	6.5	3.2	0.0	6.5

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation.

Table 220: Florfenicol Max Anaerobic Aged Cattle Manure. Shoot length inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	11.7		-1.4	-1.6	2.5	3.5	19.2*
Avena sativa	8.0*		-4.8	-4.5	-1.4	0.5	5.7*
Brassica napus	13.4*		3.5	2.3	-4.9	0.6	16.2*
Sinapis alba	-1.0		1.1	-0.1	-3.4	10.4*	35.5*
Solanum lycopersicum	0.1		-2.7	-4.3	-3.1	12.7	35.2
Phaseolus vulgaris	4.4		-5.7	-6.8	-4.7	1.2	4.9

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 221: Florfenicol Max Anaerobic Aged Cattle Manure. Fresh mass inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	25.9*		1.8	-10.5	6.8	1.2	24.0*
Avena sativa	11.7		-9.2	-8.0	-12.1	0.1	9.3
Brassica napus	22.3*		5.8	7.9	-8.0	2.6	33.5*
Sinapis alba	-15.0		10.0	1.7	0.5	21.5*	53.6*
Solanum lycopersicum	6.5		-3.7	-12.5	-16.0	14.5*	65.9*
Phaseolus vulgaris	1.4		-6.5	-1.2	5.9	20.9*	41.6*

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation.

Table 222: Florfenicol Max Anaerobic Aged Cattle Manure. P.-em. survival inhibition related to control [%].

Species	Standard control		0.6 mg/kg	1.9 mg/kg	5.6 mg/kg	16.7 mg/kg	50 mg/kg
Allium cepa	-13.0		-5.5	-5.2	-2.8	-5.5	-6.2
Avena sativa	0.0		0.0	0.0	0.0	2.8	0.0
Brassica napus	-3.6		-3.6	-0.1	2.7	-0.3	-0.2
Sinapis alba	-8.0		-1.5	-4.8	-4.3	-8.0	0.6
Solanum lycopersicum	15.4		0.3	-2.8	7.2	8.6	13.8*
Phaseolus vulgaris	10.5		0.0	0.0	3.3	0.0	0.0

Concentrations given as nominal values; *: significant when compared to control (p = 0.05, one-sided smaller);
negative value = emergence stimulation.

10.13 Tylosin tartrate - Modified plant tests with maximum incubated spiked pig manure

Table 223: Tylosin tartrate Max Anaerobic Aged Pig Manure. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	95	100	95	95	92	70
MC	0.0	95	100	98	98	96	90
T1	5.0	--	--	--	--	--	75
T2	10.0	--	--	93	95	--	85
T3	15.0	100	--	--	--	--	--
T4	20.0	--	--	--	--	--	85
T5	25.0	--	--	95	98	--	--
T6	30.0	88	--	--	--	--	--
T7	40.0	--	--	--	--	100	--
T8	50.0	--	--	--	--	--	80
T9	60.0	98	--	--	--	--	--
T10	62.5	--	--	100	85	--	--
T11	88.0	--	--	--	--	100	--
T12	100	--	93	--	--	--	--
T13	125	--	--	--	--	--	90
T14	150	98	--	--	--	--	--
T15	156	--	--	88	98	--	--
T16	194	--	--	--	--	96	--
T17	200	--	93	--	--	--	--
T18	300	90	--	--	--	--	--
T19	391	--	--	98	98	--	--
T20	400	--	98	--	--	--	--
T21	426	--	--	--	--	96	--
T22	700	--	98	--	--	--	--
T23	937	--	--	--	--	100	--
T24	1000	--	98	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 224: Tylosin tartrate Max Anaerobic Aged Pig Manure. Length of the shoots. Mean values ± SD [cm].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	143	404	190	144	292	104
MC	0.0	169	405	199	157	307	104
T1	5.0	--	--	--	--	--	98
T2	10.0	--	--	205	177	--	111
T3	15.0	170	--	--	--	--	--
T4	20.0	--	--	--	--	--	104
T5	25.0	--	--	208	157	--	--
T6	30.0	176	--	--	--	--	--
T7	40.0	--	--	--	--	295	--
T8	50.0	--	--	--	--	--	90
T9	60.0	175	--	--	--	--	--
T10	62.5	--	--	210	171	--	--
T11	88.0	--	--	--	--	323	--
T12	100	--	436	--	--	--	--
T13	125	--	--	--	--	--	98
T14	150	173	--	--	--	--	--
T15	156	--	--	205	163	--	--
T16	194	--	--	--	--	281	--
T17	200	--	423	--	--	--	--
T18	300	180	--	--	--	--	--
T19	391	--	--	209	158	--	--
T20	400	--	421	--	--	--	--
T21	426	--	--	--	--	284	--
T22	700	--	411	--	--	--	--
T23	937	--	--	--	--	210	--
T24	1000	--	394	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 225: Tylosin tartrate Max Anaerobic Aged Pig Manure. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0.216	1.390	1.736	1.329	6.615	0.353
MC	0.0	0.269	1.611	2.078	1.718	7.760	0.323
T1	5.0	--	--	--	--	--	0.311
T2	10.0	--	--	2.113	2.108	--	0.371
T3	15.0	0.279	--	--	--	--	--
T4	20.0	--	--	--	--	--	0.408
T5	25.0	--	--	2.25	1.779	--	--
T6	30.0	0.296	--	--	--	--	--
T7	40.0	--	--	--	--	7.818	--
T8	50.0	--	--	--	--	--	0.256
T9	60.0	0.298	--	--	--	--	--
T10	62.5	--	--	2.117	2.168	--	--
T11	88.0	--	--	--	--	7.811	--
T12	100	--	1.803	--	--	--	--
T13	125	--	--	--	--	--	0.296
T14	150	0.275	--	--	--	--	--
T15	156	--	--	2.127	1.702	--	--
T16	194	--	--	--	--	6.836	--
T17	200	--	1.744	--	--	--	--
T18	300	0.282	--	--	--	--	--
T19	391	--	--	2.178	1.734	--	--
T20	400	--	1.635	--	--	--	--
T21	426	--	--	--	--	7.582	--
T22	700	--	1.574	--	--	--	--
T23	937	--	--	--	--	3.399	--
T24	1000	--	1.425	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 226: Tylosin tartrate Max Anaerobic Aged Pig Manure. Post-emergence survival rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	95	100	100	100	100	100
MC	0.0	95	100	100	100	100	100
T1	5.0	--	--	--	--	--	100
T2	10.0	--	--	100	100	--	100
T3	15.0	93	--	--	--	--	--
T4	20.0	--	--	--	--	--	100
T5	25.0	--	--	100	--	--	--
T6	30.0	94	--	--	--	--	--
T7	40.0	--	--	--	--	100	--
T8	50.0	--	--	--	--	--	100
T9	60.0	97	--	--	--	--	--
T10	62.5	--	--	100	100	--	--
T11	88.0	--	--	--	--	100	--
T12	100	--	100	--	--	--	--
T13	125	--	--	--	--	--	100
T14	150	97	--	--	--	--	--
T15	156	--	--	94	100	--	--
T16	194	--	--	--	--	100	--
T17	200	--	100	--	--	--	--
T18	300	97	--	--	--	--	--
T19	391	--	--	100	100	--	--
T20	400	--	100	--	--	--	--
T21	426	--	--	--	--	100	--
T22	700	--	100	--	--	--	--
T23	937	--	--	--	--	100	--
T24	1000	--	100	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 227: Tylosin tartrate Max Anaerobic Aged Pig Manure. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0	0	3.1	3.1	4.2	22.2
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	16.7
T2	10.0	--	--	5.1	3.1	--	5.6
T3	15.0	-5.3	--	--	--	--	--
T4	20.0	--	--	--	--	--	11.1
T5	25.0	--	--	3.1	0	--	--
T6	30.0	7.4	--	--	--	--	--
T7	40.0	--	--	--	--	-4.2	--
T8	50.0	--	--	--	--	--	11.1
T9	60.0	3.2	--	--	--	--	--
T10	62.5	--	--	-2.0	13.3	--	--
T11	88.0	--	--	--	--	-4.2	--
T12	100	--	7.0	--	--	--	--
T13	125	--	--	--	--	--	0
T14	150	3.2	--	--	--	--	--
T15	156	--	--	10.2	0	--	--
T16	194	--	--	--	--	0	--
T17	200	--	7.0	--	--	--	--
T18	300	5.3	--	--	--	--	--
T19	391	--	--	0	0	--	--
T20	400	--	2.0	--	--	--	--
T21	426	--	--	--	--	0	--
T22	700	--	2.0	--	--	--	--
T23	937	--	--	--	--	-4.2	--
T24	1000	--	2.0	--	--	--	--

Concentrations given as nominal values per kg dry mass soil; *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 228: Tylosin tartrate Max Anaerobic Aged Pig Manure. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	15.6	0.1	4.6	8.6 *	5.0	-0.8
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	5.5
T2	10.0	--	--	-2.8	-12.3 *	--	-7.7
T3	15.0	-0.7	--	--	--	--	--
T4	20.0	--	--	--	--	--	-0.5
T5	25.0	--	--	-4.7	0.3	--	--
T6	30.0	-4.4	--	--	--	--	--
T7	40.0	--	--	--	--	4.1	--
T8	50.0	--	--	--	--	--	12.7
T9	60.0	-3.6	--	--	--	--	--
T10	62.5	--	--	-5.5	-8.7	--	--
T11	88.0	--	--	--	--	-5.0	--
T12	100	--	-7.7 *	--	--	--	--
T13	125	--	--	--	--	--	5.3
T14	150	-2.2	--	--	--	--	--
T15	156	--	--	-3.2	-3.4	--	--
T16	194	--	--	--	--	8.7	--
T17	200	--	-4.6	--	--	--	--
T18	300	-6.4	--	--	--	--	--
T19	391	--	--	-4.9	-0.3	--	--
T20	400	--	-4.0	--	--	--	--
T21	426	--	--	--	--	7.7	--
T22	700	--	-1.3	--	--	--	--
T23	937	--	--	--	--	31.7 *	--
T24	1000	--	2.6	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 229: Tylosin tartrate Max Anaerobic Aged Pig Manure. Fresh mass inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	19.7	13.7 *	16.5 *	22.6 *	14.8	-9.3
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	3.7
T2	10.0	--	--	-1.7	-22.7 *	--	-14.9
T3	15.0	-3.8	--	--	--	--	--
T4	20.0	--	--	--	--	--	-26.3 *
T5	25.0	--	--	-8.3	-3.5 *	--	--
T6	30.0	10.0	--	--	--	--	--
T7	40.0	--	--	--	--	-0.8	--
T8	50.0	--	--	--	--	--	20.7
T9	60.0	-10.6	--	--	--	--	--
T10	62.5	--	--	-1.9	-26.2	--	--
T11	88.0	--	--	--	--	-0.7	--
T12	100	--	-11.9 *	--	--	--	--
T13	125	--	--	--	--	--	8.3
T14	150	-2.1	--	--	--	--	--
T15	156	--	--	-2.4	1.0	--	--
T16	194	--	--	--	--	11.9	--
T17	200	--	-8.2	--	--	--	--
T18	300	-4.8	--	--	--	--	--
T19	391	--	--	-4.8	-0.9	--	--
T20	400	--	-1.5	--	--	--	--
T21	426	--	--	--	--	2.3	--
T22	700	--	3.2	--	--	--	--
T23	937	--	--	--	--	56.2 *	--
T24	1000	--	11.6 *	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil;

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 230: Tylosin tartrate Max Anaerobic Aged Pig Manure. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0	0	0	0	0	0
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	0
T2	10.0	--	--	0	0	--	0
T3	15.0	2.1	--	--	--	--	--
T4	20.0	--	--	--	--	--	0
T5	25.0	--	--	0	--	--	--
T6	30.0	1.1	--	--	--	--	--
T7	40.0	--	--	--	--	0	--
T8	50.0	--	--	--	--	--	0
T9	60.0	-2.1	--	--	--	--	--
T10	62.5	--	--	0	0	--	--
T11	88.0	--	--	--	--	0	--
T12	100	--	0	--	--	--	--
T13	125	--	--	--	--	--	0
T14	150	-2.1	--	--	--	--	--
T15	156	--	--	6	0	--	--
T16	194	--	--	--	--	0	--
T17	200	--	0	--	--	--	--
T18	300	-2.1	--	--	--	--	--
T19	391	--	--	0	0	--	--
T20	400	--	0	--	--	--	--
T21	426	--	--	--	--	0	--
T22	700	--	0	--	--	--	--
T23	937	--	--	--	--	0	--
T24	1000	--	0	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

10.14 Tylosin tartrate - Modified plant tests with maximum incubated spiked cattle manure

Table 231: Tylosin tartrate Max Anaerobic Aged Cattle Manure. Emergence rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	95	95	95	95	92	85
MC	0.0	80	95	93	95	96	80
T1	5.0	--	--	--	--	--	90
T2	10.0	--	--	80	100	--	80
T3	15.0	65	--	--	--	--	--
T4	20.0	--	--	--	--	--	83
T5	25.0	--	--	88	95	--	--
T6	30.0	87.5	--	--	--	--	--
T7	40.0	--	--	--	--	96	--
T8	50.0	--	--	--	--	--	83
T9	60.0	77.5	--	--	--	--	--
T10	62.5	--	--	95	93	--	--
T11	88.0	--	--	--	--	100	--
T12	100	--	90	--	--	--	--
T13	125	--	--	--	--	--	78
T14	150	72.5	--	--	--	--	--
T15	156	--	--	93	93	--	--
T16	194	--	--	--	--	88	--
T17	200	--	100	--	--	--	--
T18	300	67.5	--	--	--	--	--
T19	391	--	--	85	83	--	--
T20	400	--	98	--	--	--	--
T21	426	--	--	--	--	92	--
T22	700	--	95	--	--	--	--
T23	937	--	--	--	--	100	--
T24	1000	--	98	--	--	--	--

Concentrations given as nominal values per kg dry mass soil.. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 232: Tylosin tartrate Max Anaerobic Aged Cattle Manure. Length of the shoots. Mean values ± SD [cm].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	159	408	173	156	291	85
MC	0.0	157	413	187	162	277	90
T1	5.0	--	--	--	--	--	93
T2	10.0	--	--	191	168	--	88
T3	15.0	154	--	--	--	--	--
T4	20.0	--	--	--	--	--	94
T5	25.0	--	--	189	165	--	--
T6	30.0	162	--	--	--	--	--
T7	40.0	--	--	--	--	294	--
T8	50.0	--	--	--	--	--	96
T9	60.0	159	--	--	--	--	--
T10	62.5	--	--	190	169	--	--
T11	88.0	--	--	--	--	318	--
T12	100	--	403	--	--	--	--
T13	125	--	--	--	--	--	90
T14	150	155	--	--	--	--	--
T15	156	--	--	193	168	--	--
T16	194	--	--	--	--	263	--
T17	200	--	408	--	--	--	--
T18	300	123	--	--	--	--	--
T19	391	--	--	130	154	--	--
T20	400	--	391	--	--	--	--
T21	426	--	--	--	--	244	--
T22	700	--	401	--	--	--	--
T23	937	--	--	--	--	165	--
T24	1000	--	397	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 233: Tylosin tartrate Max Anaerobic Aged Cattle Manure. Fresh weight of the shoots. Mean values ± SD [g].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	0.243	1.651	1.521	1.692	7.445	0.252
MC	0.0	0.238	1.747	1.661	1.938	7.478	0.283
T1	5.0	--	--	--	--	--	0.283
T2	10.0	--	--	1.749	1.922	--	0.28
T3	15.0	0.252	--	--	--	--	--
T4	20.0	--	--	--	--	--	0.310
T5	25.0	--	--	1.645	1.977	--	--
T6	30.0	0.260	--	--	--	--	--
T7	40.0	--	--	--	--	7.623	--
T8	50.0	--	--	--	--	--	0.323
T9	60.0	0.257	--	--	--	--	--
T10	62.5	--	--	1.795	2.060	--	--
T11	88.0	--	--	--	--	7.771	--
T12	100	--	1.877	--	--	--	--
T13	125	--	--	--	--	--	0.273
T14	150	0.233	--	--	--	--	--
T15	156	--	--	1.747	2.071	--	--
T16	194	--	--	--	--	7.414	--
T17	200	--	1.772	--	--	--	--
T18	300	0.162	--	--	--	--	--
T19	391	--	--	0.514	1.321	--	--
T20	400	--	1.619	--	--	--	--
T21	426	--	--	--	--	4.027	--
T22	700	--	1.681	--	--	--	--
T23	937	--	--	--	--	1.989	--
T24	1000	--	1.648	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 234: Tylosin tartrate Max Anaerobic Aged Cattle Manure. Post-emergence survival rate at test end [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	95	100	100	100	100	100
MC	0.0	94	100	100	100	100	100
T1	5.0	--	--	--	--	--	97
T2	10.0	--	--	100	100	--	97
T3	15.0	100	--	--	--	--	--
T4	20.0	--	--	--	--	--	100
T5	25.0	--	--	100	97	--	--
T6	30.0	94	--	--	--	--	--
T7	40.0	--	--	--	--	100	--
T8	50.0	--	--	--	--	--	100
T9	60.0	100	--	--	--	--	--
T10	62.5	--	--	100	100	--	--
T11	88.0	--	--	--	--	100	--
T12	100	--	100	--	--	--	--
T13	125	--	--	--	--	--	100
T14	150	100	--	--	--	--	--
T15	156	--	--	100	95	--	--
T16	194	--	--	--	--	100	--
T17	200	--	98	--	--	--	--
T18	300	93	--	--	--	--	--
T19	391	--	--	100	100	--	--
T20	400	--	100	--	--	--	--
T21	426	--	--	--	--	100	--
T22	700	--	100	--	--	--	--
T23	937	--	--	--	--	100	--
T24	1000	--	100	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 235: Tylosin tartrate Max Anaerobic Aged Cattle Manure. Emergence inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	-18.8	0	-2.2	0	4.2	-6.3
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	-12.5
T2	10.0	--	--	14.0	-5.3	--	0
T3	15.0	8.7	--	--	--	--	--
T4	20.0	--	--	--	--	--	-3.8
T5	25.0	--	--	5.4	0	--	--
T6	30.0	9.4	--	--	--	--	--
T7	40.0	--	--	--	--	0	--
T8	50.0	--	--	--	--	--	3.8
T9	60.0	9.1	--	--	--	--	--
T10	62.5	--	--	-2.2	2.1	--	--
T11	88.0	--	--	--	--	-4.2	--
T12	100	--	5.3	--	--	--	--
T13	125	--	--	--	--	--	2.5
T14	150	9.4	--	--	--	--	--
T15	156	--	--	0	2.1	--	--
T16	194	--	--	--	--	8.3	--
T17	200	--	-5.3	--	--	--	--
T18	300	15.6	--	--	--	--	--
T19	391	--	--	8.6	12.6	--	--
T20	400	--	-3.2	--	--	--	--
T21	426	--	--	--	--	4.2	--
T22	700	--	0	--	--	--	--
T23	937	--	--	--	--	-4.2	--
T24	1000	--	-3.2	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. (C) standard control; (MC) manure control; (T) test item treated; the number of seeds sown was 20 (12 for *P.vulgaris*) in the case of (C) and 40 (24 for *P.vulgaris*) in the case of (MC) and (T); (---) respective concentration not tested.

Table 236: Tylosin tartrate Max Anaerobic Aged Cattle Manure. Shoot length inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	-1.4	1.3	7.8 *	3.8	-4.9	5.5
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	-3.2
T2	10.0	--	--	-2.0	-3.4	--	2.3
T3	15.0	2.2	--	--	--	--	--
T4	20.0	--	--	--	--	--	-3.9
T5	25.0	--	--	-0.7	-1.7	--	--
T6	30.0	-3.0	--	--	--	--	--
T7	40.0	--	--	--	--	-5.9	--
T8	50.0	--	--	--	--	--	-6.6
T9	60.0	-1.2	--	--	--	--	--
T10	62.5	--	--	-1.3	-3.8	--	--
T11	88.0	--	--	--	--	-14.5	--
T12	100	--	2.3	--	--	--	--
T13	125	--	--	--	--	--	0.3
T14	150	1.7	--	--	--	--	--
T15	156	--	--	-3.0	-3.5	--	--
T16	194	--	--	--	--	5.1	--
T17	200	--	1.2	--	--	--	--
T18	300	21.7	--	--	--	--	--
T19	391	--	--	30.7 *	5.3	--	--
T20	400	--	5.3	--	--	--	--
T21	426	--	--	--	--	11.9 *	--
T22	700	--	2.8	--	--	--	--
T23	937	--	--	--	--	40.6 *	--
T24	1000	--	3.9	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil.

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 237: Tylosin tartrate Max Anaerobic Aged Cattle Manure. Fresh mass inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	-2.1	5.5	8.4	12.7	0.4	11.0
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	0.1
T2	10.0	--	--	-5.3	0.8	--	1.1
T3	15.0	-5.5	--	--	--	--	--
T4	20.0	--	--	--	--	--	-9.7
T5	25.0	--	--	1.0	-2.0	--	--
T6	30.0	-9.0	--	--	--	--	--
T7	40.0	--	--	--	--	-1.9	--
T8	50.0	--	--	--	--	--	-14.3
T9	60.0	-7.9	--	--	--	--	--
T10	62.5	--	--	-8.1	-6.3	--	--
T11	88.0	--	--	--	--	-3.9	--
T12	100	--	-7.5	--	--	--	--
T13	125	--	--	--	--	--	3.5
T14	150	2.1	--	--	--	--	--
T15	156	--	--	-5.2	-6.9	--	--
T16	194	--	--	--	--	0.8	--
T17	200	--	-1.4	--	--	--	--
T18	300	32.3 *	--	--	--	--	--
T19	391	--	--	69.1 *	31.8 *	--	--
T20	400	--	7.3	--	--	--	--
T21	426	--	--	--	--	46.2 *	--
T22	700	--	3.7	--	--	--	--
T23	937	--	--	--	--	73.4 *	--
T24	1000	--	5.6	--	--	--	--

n.s.s. = No seedlings survived; Concentrations given as nominal values per kg dry mass soil.

*: significant when compared to control (p = 0.05, one-sided smaller); negative value = growth stimulation. (C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested.

Table 238: Tylosin tartrate Max Anaerobic Aged Cattle Manure. P.-em. survival inhibition related to control [%].

Code	Test item [mg/kg]	*A. cepa*	*A. sativa*	*B. napus*	*S. lycopersicum*	*P. vulgaris*	*T. pratense*
C	0.0	1.1	0	0	0	0	0
MC	0.0	0	0	0	0	0	0
T1	5.0	--	--	--	--	--	3
T2	10.0	--	--	0	0	--	3
T3	15.0	-5.3	--	--	--	--	--
T4	20.0	--	--	--	--	--	0
T5	25.0	--	--	0	3	--	--
T6	30.0	1.1	--	--	--	--	--
T7	40.0	--	--	--	--	0	--
T8	50.0	--	--	--	--	--	0
T9	60.0	5.3	--	--	--	--	--
T10	62.5	--	--	0	0	--	--
T11	88.0	--	--	--	--	0	--
T12	100	--	0	--	--	--	--
T13	125	--	--	--	--	--	0
T14	150	5.3	--	--	--	--	--
T15	156	--	--	0	5	--	--
T16	194	--	--	--	--	0	--
T17	200	--	2	--	--	--	--
T18	300	2.1	--	--	--	--	--
T19	391	--	--	0	0	--	--
T20	400	--	0	--	--	--	--
T21	426	--	--	--	--	0	--
T22	700	--	0	--	--	--	--
T23	937	--	--	--	--	0	--
T24	1000	--	0	--	--	--	--

Concentrations given as nominal values per kg dry mass soil. *: significant when compared to control (p = 0.05, one-sided smaller); negative value = emergence stimulation. C) standard control; (MC) manure control; (T) test item treated; (---) respective concentration not tested; (a) values >100% represent emergence of additional seedlings after day 7.

11 Annex 2 — DRAFT Recommendations for an extended plant test with a more realistic exposure scenario for Veterinary Medicinal Products (especially Antibiotics) via manure

Introduction

1. This recommendation is a guidance to assess the effects of veterinary medicinal products (VMPs), especially antibiotics, on terrestrial plants in an extended OECD 208 test considering a more realistic exposure scenario via pig and cattle manure application. However, all requirements and recommendations of the OECD 208 [1] guideline still apply to this extended approach.

2. In this recommendation the test substance is applied to manure and incubated under anaerobic conditions. The scenario of spiking manure is intended to simulate the fate and behaviour of VMPs in manure, which is stored in tanks before it is released to the environment e.g. by spreading to agricultural soil.

3. Veterinary medicinal products applied oral or by injection to the target animal usually reach the environment via manure. The modified exposure scenario of this approach takes into account degradation of the parent compound into transformation products and/or formation of NER (Non Extractable Residues).

4. The recommended test design was successfully verified with pig and cattle manure [6].

5. The techniques for manure storage and acclimation generally follow the EMA guideline on determining the fate of veterinary medicinal products in manure [2].

Definitions

6. Acclimation means storing of manure after processing (mixing, adjustment to standardized dry matter content), at incubation conditions to acclimate the micro-organisms before incubation. The acclimation period should be at least 7 days. Following the OECD 307 guideline for transformation in soil [3], an acclimation period of 28 days should not be exceeded.

7. Half-maximum storage duration is the half of the mean maximum storage time of manure in storage tanks at farms according to Table 6 of EMEA/CVMP/ERA/418282/2005-Rev.1 [2].

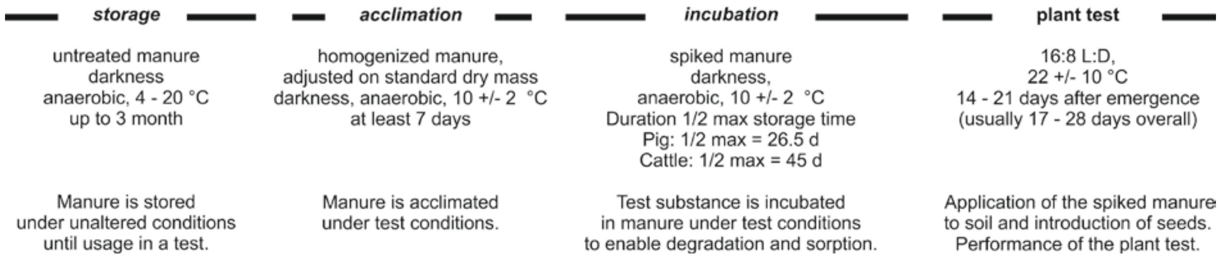
8. Incubation means storing of manure after acclimation and application of the test substance, at conditions which mimic the abidance of the manure in storage tanks at a farm under standardised conditions.

9. Manure, in this recommendation, means liquid manure (mixture of urine and dung).

10. Manure storage or pre-storage tank is the basin where the manure is stored at the farm.

11. Storage means storing of manure after sampling under unaltered conditions (unprocessed, at anaerobic conditions, 4 – 20 °C, in the dark), comparable with those of storage or pre-storage tanks at farms until use.

Figure 37: Schedule and definition of main phases in the extended test design.



Manure

12. The manure applied should come from animals that are reared under well controlled conditions. Use of manure contaminated with VMPs, biocides and other material that might impair plant growth or survival should be avoided. The feed type, feed regime and the veterinary history of the animals from which the manure will be collected should be recorded.

13. Manure should be sampled from manure storage or pre-storage tanks which may be above ground or below ground. Prior to collection the liquid manure should be thoroughly mixed in the respective manure tank. Pig manure should be stirred immediately before sampling as separation into liquid and solid phase easily occurs. Duration of mixing depends on the kind of storage tank. However, it should be ensured that at sampling the liquid manure is a representative mixture of the liquid and the solid phase. The sampling site, the sampling procedure, and the type and size of manure tank (above/below ground, covered/open) should be recorded.

14. Prior to further processing manure can be stored at 4° C to 20° C (preferably at acclimation and incubation temperature) for a maximum of three months [2]. Storage should ensure anaerobic conditions.

15. For acclimation, the dry matter content of the manure has to be adjusted to standardized values. The recommended dry matter content in pig manure is 5% ± 1%, in cattle manure 10% ± 1% [2], [4]. Manure should be processed using a mixer (e.g. a food processor or similar apparatus) in order to obtain a homogenised phase and to reduce test result variability. All operations should be carried out under anaerobic conditions; exposure to oxygen has to be kept to an absolute minimum if it cannot be avoided. The anaerobic acclimation should last for at least 7 days at 10 ± 2 °C in the dark.

16. Key parameters of the manure as mentioned in the EMA guideline on determining the fate of veterinary medicinal products in manure [2] listed in table 1 should be measured and reported.

Table 239: Schedule for manure key parameter measurements.

Parameter	stage of test procedure	
	Start of storage	during acclimation
pH	X	X
microbial activity[2]		X
organic carbon content [Corg mg/kg]		X
total nitrogen content [Ntotal; mg/kg]		X
ammonium content [NH_4-N; mg/kg]		X
phosphate content [mg/kg]		X
copper content [mg/kg] optional (for pig manure only)	X	
redox potential [mV]	X	X*
dry matter content [%]		X*
temperature [°C]	X	X

* Should be measured at the start and end of acclimation.

18. Anaerobic conditions should be ensured and demonstrated by measuring the manure redox potentials at the end of the acclimation period of Eh < -100 mV [3]. Redox potentials measured in pig and cattle manure have been found to range from -230 mV to -400 mV [4].

Application of the test substance

19. Manure can impair seedling emergence [6]. It is advised to check in a pre-test without test substance whether the intended manure concentration in soil has adverse effects on the test plants.

20. Based on nitrogen, the maximum amount of manure must not exceed 227 mg N/kg dry soil (= 170 kg N/ha per year assuming an incorporation depth of 5 cm and a soil density of 1.5 g/cm3). An amount of 20 g fresh manure per kg dry soil, corresponding to approximately 45 – 55 kg N/ha, was shown to be a suitable amount regarding handling and seedling tolerance [6].

21. The quantity of test substance required to obtain the theoretical test concentrations in soil assuming no transformation during incubation is mixed with a portion of manure (dry mass content of the manure: 5 ± 1 % for pig manure, 10 ± 1 % for cattle manure) e.g. in glass beakers. Example: If 20 g fresh manure should be applied to 1 kg dry soil and a theoretical test concentrations in soil assuming no transformation during incubation should be 100 mg/kg, 100 mg test substance have to be applied to 20 g fresh manure.

22. Substances which are water-soluble or suspended in water can be added directly to the manure, and then the spiked manure is mixed e.g. with a pipette tip. The volume of water added should be the same for each test concentration and should not result in a difference to the desired dry mass content of the manure (see paragraph 15). The water additionally provided by the stock solution, has to be taken into account when adjusting the manure for acclimation (i.e. the manure should thus be adjusted to an appropriate higher dry mass content for acclimation).

23. Substances with low water solubility should be dissolved in a suitable volatile solvent (e.g. acetone) and mixed either directly with the manure or via quartz sand. For direct application, the solvent concentration should not be greater than 0.1 mL/L manure and it should be the same concentration in all vessels. The solvent shall be removed from the manure e.g. using low-pressure followed by pressure compensation using oxygen free air or nitrogen. If the test substance will be applied using a solvent, a solvent control is necessary. For a direct application, a solvent control containing manure and solvent has to be applied. If the test substance is spiked on quartz sand (as little as possible), a solvent control containing manure and evaporated spiked quartz sand has to be applied. The quartz sand added is not considered for dry mass content of the manure. However, every effort should be made to keep the solvent concentration to a minimum.

24. For solid, insoluble test substances, the chemical can be applied either directly to the manure or via quartz sand. For the latter, the chemical and finely ground industrial quartz sand (as little as possible) is mixed in a suitable mixing device. Hereafter, the mixture is added to the manure and mixed thoroughly. The quartz sand added is not considered for dry mass content of the manure.

25. It should be kept in mind that all spiking and mixing operations should be carried out in a way that the manure has minimal contact with oxygen.

Incubation of the spiked manure

26. To reflect representative influences of storage in manure, the spiked manure is incubated under anaerobic conditions in the dark for a period representing half-maximum storage duration of the respective manure type (26.5 days for pig manure, 45 days for cattle manure) [2].
To reflect a realistic case scenario, incubation temperature should be 10 +/- 2 °C.

Incorporation of the spiked manure to the soil

27. It is recommended to mix the spiked manure in a two-step approach with the soil to ensure a homogenous distribution. The incubated spiked manure is added to a sub-portion of test soil and mixed thoroughly. Subsequently, the pre-mixture is added to the rest of test soil and mixed thoroughly.

Verification of test substance concentration

28. The concentrations/rates of application into the fresh manure must be confirmed by an appropriate chemical analysis, comparable to the requirements of the standard OECD 208 guideline.

29. Prior to the start of the plant test (i.e. incorporation of the manure into the soil), it is strongly recommended to measure the test substance concentration in the incubated manure. As a minimum, samples of the manure with the highest concentration and one lower concentration should be considered for analysis. These determinations of test substance concentration provide information about the degradation/adsorption of the tested chemical in the manure. (Depending on the question to be addressed by the test, determination of transformation products and non-extractable residues might be required.)

Plant test

30. In general, a standard test according to OECD 208 is applied and all requirements and recommendations still apply to this extended approach. However, modifications are listed below. Additional information from the guidance document has to be considered [7].

31. Seeds are planted in each replicate on the same day of incorporation of the test item/manure mixture to prevent aerobic transformation of the test item before contact with the seeds.

32. Control groups with manure are used to assure that effects observed are associated with or attributed only to the test substance exposure. This control (manure control) or solvent manure control is used for evaluation of the test item depending effects (see paragraph 23). The number of replicates and seeds depends on the chosen test design (see paragraph 35).

33. A standard control without manure has to be applied to detect possible adverse effects of the manure on seedling emergence or growth. At least four replicates (with an overall of at least 20 seeds) should be conducted as standard control, independent from the chosen test design (see paragraph 36). The standard control should not be used for test substance effect evaluation.

34. The start of the 14 – 21 day growth period is defined by a 50 % emergence in the manure control, not in the standard control.

35. Endpoints: The purpose of this approach is to achieve NOEC and/or ECx values.

36. In setting the range of concentrations, the following should be considered:

- Prior knowledge of the toxicity of the test substance should help in selecting appropriate test concentrations, for example from a standard test according to OECD 208. However, it is strongly recommended to apply a range finding test according to the extended test design. The magnitude of test substance depending effect decrease due to the incubation in manure often is not predictable.
- A combined approach allows for determination of both the NOEC and ECx is highly recommended. Eight treatment concentrations in a geometric series should be used. Four replicates for each treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 2.5.
- For determination of the NOEC, at least five concentrations in a geometric series should be tested. Eight replicates for each test concentration plus eight manure controls are recommended. The concentrations should be spaced by a factor not exceeding three.

Validity of the test

37. In general, all requirements according to OECD 208 still apply to this extended approach. Especially requirements regarding seedling emergence rate (70 %) and post-emergence survival rate (90 %) have to be fulfilled in all controls.

Treatment of the results

38. In general, all requirements according to OECD 208 still apply to this approach. Effect values expressed and based on dry mass soil, are calculated based on initial concentrations. For

this calculation, measured concentration of the application solution in case of soluble test substances or weights in case of insoluble test substances are applied (see paragraph 21).

Test report

39. In general, all requirements according to OECD 208 still apply to this extended approach. However, additional issues regarding the manure and its preparation, acclimation, incubation, and application are listed below and should be reported.

- Kind of manure (pig or cattle)
- Name and location of the farm, the manure originated from
- Feed type, feed regime and the veterinary history of the animals from which the manure originated (if data are available)
- Kind of manure tank from which the manure originated (e.g. above/below ground, open/covered, size) (if data are available). Was the manure mixed before sampling?
- Key parameters of the manure (at the respective time: date, temperature, pH, redox potential, dry matter content, Corg, N, P, etc.)
- Techniques for manure storage, preparation, acclimation, and incubation (e.g. cooling and/or incubation chamber, mixing device for manure homogenisation).
- Details on preparation of the spiked manure and verification of test concentrations as specified in paragraph 13 – 16, 19 and 20.

References

[1] OECD 208 (19 July 2006): OECD guideline for testing of chemicals – Terrestrial Plants Test: Seedling Emergence and Seedling Growth Test.

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[3] OECD (2002a). Guideline 307 for testing of chemicals. Aerobic and anaerobic transformation in soil.

[4] Weinfurtner K. (2010). Matrix parameters and storage conditions of manure. UBA-Texte 02/2011, ISSN 1862-4804. Umweltbundesamt, Dessau-Roßlau, 1- 54, http://www.uba.de/uba-info-medien-e/4054.html

[5] VICH (2008) Guideline on Environmental Impact Assessment for Veterinary Medicinal Products, EMEA/CVMP/ERA/418282/2005-Rev.1

[6] Simon M., Herrchen M., Förster B., Graf N., Römbke J., Kühnen U., Ebert I. (2013): Veterinary Antibiotics in Terrestrial Plant Tests – Effects of a more realistic exposure way via manure. Extended abstract of a platform presentation at SETAC Europe 23rd Annual Meeting, Glasgow, UK, 12 – 16. May 2013.

[7] EMA/CVMP/ERA/147844/2011 (9 December 2011): Reflection paper on testing strategy and risk assessment for plants. EMA.