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Harmonization of environmental exposure assessment for veterinary pharmaceuticals and biocides: Ring test for validation of a draft test protocol for studies on transformation in manure

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Harmonization of environmental exposure assessment for veterinary pharmaceuticals and biocides: Ring test for validation of a draft test protocol for studies on transformation in manure

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Kurzbeschreibung

Die Ausbringung von Veterinärpharmaka und Bioziden mit Gülle auf landwirtschaftlich genutzte Flächen stellt einen sehr wichtigen Eintragspfad dieser Produktgruppen in die Umwelt dar. Aktuelle Bewertungsleitfäden (zum Beispiel: „Guideline on determining the fate of veterinary medicinal products in manure“ (EMA/CVMP/ERA/430327/2009) (EMA, 2011) sehen aus diesem Grund auch experimentelle Untersuchungen zur Transformation dieser Substanzen in Gülle vor. Allerdings beinhalten die Dokumente lediglich grundlegende regulatorische Vorgaben, eine experimentelle Prüfrichtlinie zur Durchführung von Studien zum Abbauverhalten von Veterinärpharmaka und Bioziden in Gülle liegt jedoch weder auf EU- noch auf OECD-Ebene vor. Um eine einheitliche Bewertung von Studien im Zulassungsverfahren zu gewährleisten wird jedoch ein harmonisiertes, international akzeptiertes und validiertes Testverfahren benötigt.

Vor diesem Hintergrund wurde im Rahmen des F+E-Vorhabens „Entwicklung einer Testvorschrift zum Abbauverhalten von Veterinärpharmaka und Bioziden in Gülle“ (Hennecke et al., 2015) ein Entwurf für eine experimentelle Richtlinie erarbeitet. Die experimentelle Methode wurde durch die Auswertung von Intralaborvergleichen sowie eines internationalen Interlaborvergleichs (prevalidation Ringversuch) mittels geeigneter statistischer Verfahren überprüft und überarbeitet.

Aufbauend auf diesen Vorarbeiten wurde im Vorhaben ein internationaler Ringversuch mit einem Tierarzneimittel (Florfenicol) in Schweinegülle sowie einem Biozid (Imidacloprid) in Rindergülle durchgeführt und ausgewertet. Darüber hinaus wurden zwei internationale Workshops organisiert; zu Beginn des Vorhabens in Zusammenhang mit dem Vorgängervorhaben, sowie am Ende des Projektes zur Auswertung des internationalen Ringversuchs. Basierend auf den experimentellen Ergebnissen des Ringversuchs sowie den Diskussionen und Schlussfolgerungen der beiden Workshops wurde ein überarbeiteter Prüfrichtlinienentwurf erstellt.

Abstract

The spread of veterinary medicinal products (VMP) and biocides onto agriculturally used areas represents a very important path of entry into the environment for these product groups. For this reason, current guidance (e.g. „Guideline on determining the fate of veterinary medicinal products in manure“ (EMA/CVMP/ERA/430327/2009) (EMA, 2011) stipulates experimental studies on transformation of VMPs and biocides in manure. Though, the documents only contain basic regulatory requirements, whereas an experimental test guideline is still missing, both on EU and OECD level. To allow for a consistent assessment of studies within the registration process, a harmonized internationally accepted and validated test method is needed.

A draft test guideline was developed within a previous R&D-Project “Development of test guidance for transformation of veterinary pharmaceuticals and biocides in liquid manure” (Hennecke et al., 2015). The experimental method was examined and revised by an intra-laboratory comparisons as well as an international inter-laboratory comparison (pre-validation ring test).

In the present project, an international ring test has been performed and evaluated testing a veterinary medicinal product (florfenicol) in pig manure and a biocide (imidacloprid) in cattle manure. Moreover, two international workshops were organized; one at the beginning in connection with preceding project (Hennecke et al., 2015) and one at the end of the project to discuss and evaluate the ring test. Based on the experimental results of the ring test as well as discussions and conclusions of both workshops, a revised draft test guideline was prepared.

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List of abbrevations

ACN	Acetonitrile
aR	applied radioactivity
ASE	accelerated solvent extraction
Bq	Bequerel (radioactive decay per second)
CAS	Chemical Abstracts Service
COV	coefficient of variation
CRO	Contract Research Organisation
cpm	counts per minute
d	day
DGGE	Denaturating Gradient Gel Electrophoresis
DMS	dimethylsulfide
DMSO	dimethylsulfoxide
DT_{50}	time [d] needed for the disappearance of 50 % of the parent compound, disappearance time
DT_{90}	time [d] needed for the disappearance of 90 % of the parent compound, disappearance time
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ER	extractable residues
ESI	electrospray Ionization
FKZ	Project No. (german: Forschungskennzahl)
FOCUS	Forum of the Co-ordination of pesticide fate models and their Use
GC	Gas chromatography
HLB	Hydrophilic-Lipophilic-Balanced
HPLC	high performance liquid chromatography
ISO	International Organization for Standardization
K_{oc}	soil adsorption coefficient
K_{ow}	octanol-water partition coefficient
LC	liquid chromatography
LSC	liquid scintillation counting
Max	Maximum
Min	Minimum
MRM	Multiple reaction monitoring
MS	mass spectromety

NaOAc	Sodium acetate
NaOH	Sodium hydroxide
NER	non-extractable residues
NH_4-N	ammonia nitrogen
NMR	Nuclear Magnetic Resonance
N_{tot}	total nitrogen
OECD	Organisation for Economic Co-operation and Development
OM	organic matter
PET	polyethylenterephthalat (polyester)
PIS	product ion scan
PTFE	polytetrafluorethylene
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
Rf	retardation factor (also known as retention factor)
SAX	strong anion exchange
SD	standard deviation
SETAC	Society of Environmental Toxicology and Chemistry
SFO	single first order kinetics
SPE	solid phase extraction
TFA	trifluoroacetic acid
TLC	thin layer chromatography
TP	transformation product
VDI	The Association of German Engineers (german: Verein Deutscher Ingenieure)
VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products
VMP	veterinary medicinal products

Zusammenfassung

Zielsetzung

Ziel des Projekts waren die Weiterentwicklung einer Testvorschrift zur experimentellen Untersuchung der Transformation von Veterinärpharmaka und Bioziden in Schweine- und Rindergülle, sowie die Validierung der Methode im Rahmen eines Ringtests.

Hintergrund

Die Ausbringung von Gülle stellt einen bedeutenden Eintragspfad für Veterinärpharmaka (VMP) und Biozide in die Umwelt dar. Aus diesem Grund sehen aktuelle Leit- und Richtlinien die experimentelle Untersuchung dieser Substanzen in Gülle vor (z.B. „Guideline on determining the fate of veterinary medicinal products in manure (EMA, 2011)). Allerdings ist zurzeit noch keine standardisierte experimentelle Methode zur Untersuchung der Transformation von Veterinärpharmaka und Bioziden in flüssiger Gülle verfügbar. Die EMA-Richtlinie zur Transformation in Gülle (EMA, 2011) beinhaltet zwar grundlegende regulatorische Anforderungen, um eine einheitliche Bewertung von Studien innerhalb regulatorischer Rahmenbedingungen zu ermöglichen, es wird jedoch eine harmonisierte, international akzeptierte und validierte Testmethode benötigt. In einem vorhergehenden Forschungsprojekt wurde daher in mehreren Schritten eine Testvorschrift entwickelt. Hierbei wurden Erfahrungen von Laboratorien bei der Durchführung von Simulationsstudien zur Transformation von Chemikalien in Boden und Wasser-Sediment Systemen genutzt und das Testdesign an die speziellen Anforderungen der Güllematrix angepasst.

Der Testvorschrift beschreibt Methoden zur Untersuchung der Transformation von Chemikalien in Schweine- und Rindergülle (90-95% Wassergehalt) unter anaeroben Bedingungen. Die Experimente werden durchgeführt, um die Transformationsrate der Testsubstanz, Identität, Bildungs- und Abnahmeraten von Transformationsprodukten, den Anteil der Substanz, welcher zu CO_2 oder CH_4 mineralisiert oder zu anderen volatilen Stoffen umgeformt wird, sowie die Menge an nicht-extrahierbaren Rückständen (NER) zu bestimmen.

Die Testsubstanz wird in die flüssige Gülleprobe appliziert und im Dunkeln unter kontrollierten Laborbedingungen inkubiert. Nach geeigneten Zeitintervallen werden Gülleproben entnommen, extrahiert und auf die Muttersubstanz sowie Transformationsprodukte analysiert. Volatile Transformationsprodukte werden zur Analyse in geeigneten Apparaturen aufgefangen um die Bildung von CO_2 und CH_4 zu quantifizieren. Durch die Verwendung von ^{14}C-radioaktiv markierter Testsubstanz können Mineralisierungsraten der Testsubstanz gemessen und eine Massenbilanz einschließlich der Bildung von NER erstellt werden. Die Ergebnisse ermöglichen die Berechnung von DT_{50}-Werten, und gegebenenfalls DT_{90}-Werten.

Die experimentelle Methode wurde entwickelt, indem drei unterschiedliche Wirkstoffe (Veterinärpharmaka und Biodzide) getestet wurden. Die Methode wurde aufgrund der Ergebnisse aus Diskussionen mit Interessenvertretern bei vier internationalen Konferenzen (2012 Berlin, 2013 Glasgow, 2014 Basel, 2015 Barcelona) verfeinert. In 2012/2013 wurde ein internationaler pre-validation Ringversuch durchgeführt, um einen ersten Eindruck über die Anwendbarkeit der Testmethode in anderen Laboratorien zu gewinnen. Die Erfahrungen der Teilnehmer wurden gesammelt und 2013 im Rahmen eines zweitägigen Workshops in Flörsheim diskutiert. Basierend auf den Ergebnissen und Empfehlungen des Workshops wurde die Testvorschrift überarbeitet. Der resultierende Richtlinienentwurf stellte die Grundlage für einen zweiten Ringversuch in 2013/2014 zur Validierung der experimentellen Methode dar. Darüber hinaus sollten Kriterien zur Qualität und zur Reproduzierbarkeit der Testergebnisse abgeleitet werden. Im Anschluss an diesen Ringversuch diente ein zweitägiger

internationaler Workshop in Gießen 2014 dazu, die Erfahrungen der Teilnehmer zusammen zu tragen und den Richtlinienentwurf weiter zu verfeinern.

Ringversuch zur Validierung eines Richtlinienentwurfs für Studien zur Transformation in Gülle

Um die Anwendbarkeit des überarbeiteten Richtlinienentwurfs zu prüfen, wurde ein internationaler Ringversuch organisiert. Im März 2013 wurden zu diesem Zweck 35 Institute aus Europa, 4 Institute aus Nordamerika und 2 Institute aus Asien eingeladen, am Ringversuch teilzunehmen. Zudem wurde eine Informationsveranstaltung in Glasgow im Rahmen der 23. SETAC Europe Jahrestagung im Mai 2013 organisiert.

Die folgenden sechs Institute (in alphabetischer Reihenfolge) nahmen schließlich am Ringversuch teil:

- Agriculture and Agrifood Canada (AAFC), London, Kanada
- Noack Laboratorien GmbH, Sarstedt, Deutschland
- ECT Oekotoxikologie GmbH, Flörsheim, Deutschland
- Fraunhofer Institut für Molekularbiologie und Angewandte Ökologie (IME), Schmallenberg, Deutschland
- IBACON GmbH, Rossdorf, Deutschland
- Universität Trier, Abteilung Bodenkunde, Trier, Deutschland

Der Ringversuch wurde basierend auf den Erfahrungen aus dem pre-validation Ringversuch sowie der aktuellen Version des Richtlinienentwurfs durchgeführt, welcher vor Beginn der Versuche an alle Teilnehmer verteilt wurde. Darüber hinaus wurden den Teilnehmern vorab substanz-spezifische Verfahren zur Durchführung des Ringversuchs zur Verfügung gestellt (z.B. Testdauer, Probenahmezeitpunkte, Testkonzentrationen, zu applizierende Radioaktivität je Testgefäß, Herstellung von Sterilkontrollen, empfohlene Extraktionsmethoden und analytische Verfahren, potentielle Transformationsprodukte).

Die anaerobe Transformation von zwei ^{14}C-markierten Substanzen - ein Tierarzneimittelwirkstoff in Schweinegülle (^{14}C-Florfenicol) und ein Biozid in Rindergülle (^{14}C-Imidacloprid) - wurde bei einer Temperatur von 20 ± 2°C getestet. Die Substanzen wurden aufgrund mehrerer Kriterien ausgewählt: die Substanzen werden nicht zu schnell abgebaut und erlauben somit eine durchführbare und reproduzierbare Probenahme; die Substanzen sind nicht persistent, woraus eine verlängerte Testdauer resultieren würde; der Abbau basiert hauptsächlich auf biologischen Prozessen; die Anforderungen für eine chemische Analytik sind nicht zu hoch; die Position der radioaktiven Markierung im Molekül erlaubt die Ermittlung der wesentlichen Transformationsprodukte; die Kosten für die Beschaffung der ^{14}C-markierten Substanzen sind vertretbar.

Darüber hinaus wurde die Transformation von Florfenicol in Schweinegülle bei einer Testtemperatur von 10 ± 2°C unter der Verwendung von radioaktiv markierter sowie unmarkierter Testsubstanz untersucht, um eine Anleitung für die Untersuchung von auftretenden Transformationsprodukten zu entwickeln. Die chemische Analytik wurde mittels radio-HPLC (^{14}C-markiert) und LC-ESI-MS/MS (unmarkiert) durchgeführt. Neue Extraktions- und Aufreinigungsmethoden wurden entwickelt.

Die Experimente des Ringversuchs wurden zwischen September 2013 und September 2014 durchgeführt.

Auswertung der Ergebnisse

Für jeden Probenahmezeitpunkt wurde eine ^{14}C-Massenbilanz durch Aufsummieren der Mengen an Radioaktivität [% der applizierten Radioaktivität; % aR] in den Extrakten (extrahierbare Rückstände, ER), den nicht-extrahierbaren Rückständen (NER) und der Mineralisierung ($^{14}CO_2$ und $^{14}CH_4$) erstellt. Da die Massenbilanzen zu Beginn der Experimente (Tag 0) Unterschiede zwischen den teilnehmenden Instituten zeigten, werden alle Resultate bezogen auf die Massenbilanz [% aR] zusätzlich in normalisierter Form präsentiert (Massenbilanz bei Tag 0 wird für jedes Institut auf 100% gesetzt).

Die Ergebnisse eines Instituts für Florfenicol in Schweinegülle konnten nicht für die weitere Auswertung herangezogen werden, da die verwendete Extraktionsmethode wesentliche Abweichungen von der im Vorfeld an die Teilnehmer verteilten empfohlenen Methode aufwies. Für die Extraktion wurden unpolare Lösemittel verwendet, welche zu einer geringeren Massenbilanz für diese Experimente geführt haben könnten.

DT_{50}-Werte wurden für die Muttersubstanz und für Transformationsprodukte (TP; nur falls eine Abnahme beobachtet wurde) ermittelt, um die Kinetik des Verschwindens der Testsubstanz zu beschreiben. DT_{50}-Werte wurden unter Verwendung einer Kinetik erster Ordnung (single first order, SFO) mit der Software KinGUI ermittelt und für die Berechnung von Mittelwerten und Standardabweichungen ln-transformiert. Als Maß für die Anpassung (goodness of fit) des gewählten Kinetikmodells wurde der chi^2-Fehler berechnet und eine visuelle Kontrolle der Transformationskurve vorgenommen.

Ergebnisse

Parameter zur Charakterisierung der Güllematrix

Die folgenden Parameter zur Charakterisierung der Güllematrix wurden von den Teilnehmern innerhalb der Testdauer bestimmt: Temperatur, Trockensubstanzgehalt, organischer Gehalt, Stickstoffgehalt, Redoxpotential, pH-Wert. Die in Schweine-und Rindergülle gemessenen Temperaturen lagen innerhalb des geforderten Bereichs von 20 ± 2°C. Der pH-Wert lag zwischen 7.4 und 9.3 für Schweinegülle und zwischen 6.1 und 8.8 für Rindergülle. Es konnte kein zeitabhängiger Verlauf während der Testperiode beobachtet werden. Alle Teilnehmer konnten im Test ein Redoxpotential von ≤ -100 mV in Schweine- und Rindergülle etablieren. Somit konnten zu jeder Zeit während der Experimente stabile anaerobe Bedingungen sichergestellt werden.

Verschwinden der Muttersubstanz

DT_{50}-Werte bei 20 ± 2°C für die Testsubstanzen (Muttersubstanz) wurden mittels SFO-Kinetik unter Verwendung der Software KinGUI ermittelt. Für Florfenicol in Schweinegülle bei 20± 2°C lag der beobachtete Mittelwert der DT_{50}-Werte zwischen 0.17 Tagen und 0.41 Tagen. Für Imidacloprid in Rindergülle lagen die mittleren DT_{50}-Werte im Bereich 17.4 Tage bis 40.0 Tage. Der Mittelwert der DT_{50}-Werte für Florfenicol in Schweinegülle bei einer Temperatur von 10 ± 2°C lag bei 1.4 Tagen (unmarkierte Testsubstanz) bzw. 2.3 Tagen (^{14}C-markierte Testsubstanz).

Der durchschnittliche chi^2-Fehler der Anpassungen lag bei 21.3 ± 6.3% für Florfenicol und bei 13.4 ± 4.4% für Imidacloprid. Obwohl für den chi^2-Fehler für Florfenicol und Imidacloprid in mehreren Fällen Werte > 15% berechnet wurden, lassen die Residuendiagramme (residual plots) keinen systematischen Fehler des SFO-Modells erkennen.

Mineralisierung

Weder für Florfenicol (maximale gemessene Mineralisierung von 6% aR) noch für Imidacloprid (maximale gemessene Mineralisierung von < 1% aR) konnte eine nennenswerte Mineralisierung gemessen werden.

Extrahierbare und nicht-extrahierbare Rückstände

Ein Rückgang der extrahierbaren Rückstände (ER) im Testverlauf konnte für Florfenicol und Imidacloprid beobachtet werden. Dementgegen zeigte sich ein Anstieg der nicht-extrahierbaren Rückstände (NER) für beide Testsubstanzen. Bei Testende lag der Anteil der NER nach Normalisierung (Massenbilanz bei Tag 0 wurde auf 100% aR gesetzt) zwischen 30.8% aR und 61.0% aR für Florfenicol in Schweinegülle und zwischen 11.3% aR und 65.3 % aR für Imidacloprid in Rindergülle. Die Variabilität der extrahierbaren und nicht-extrahierbaren Rückstände bei Testende könnte durch die Verwendung von leicht unterschiedlichen Extraktionsmethoden durch die teilnehmenden Institute verursacht worden sein.

Massenbilanz

Bei Testbeginn lag die mittlere Massenbilanz der einzelnen Institute im Bereich zwischen 92.8% aR und 100.3% aR für Florfenicol in Schweinegülle und zwischen 86.8% aR und 111.8% aR für Imidacloprid in Rindergülle. Nach Normalisierung auf 100% aR bei Testbeginn (Tag 0) lag die über alle Institute im Testverlauf gemittelte Massenbilanz zwischen 95.1% aR und 103.0% aR für Florfenicol und zwischen 92.3% aR und 103.7% aR für Imidacloprid.

Die Massenbilanzen aller Teilnehmer des Ringversuchs wurden ausgewertet. Als Empfehlung konnte ein Bereich von 100 ± 15% aR abgeleitet werden.

Betrachtung spezieller Methoden zur Identifikation von Transformationsprodukten

Die Transformation von Florfenicol in Schweinegülle bei 10 ± 2°C wurde unter Verwendung von ^{14}C-radioaktiv markierter sowie unmarkierter Testsubstanz untersucht. Chemische Analytik wurde mittels radio-HPLC (^{14}C-markiert) sowie LC-ESI-MS/MS (unmarkiert) durchgeführt. Für letztere wurde eine Methode zur Extraktion und Aufreinigung entwickelt.

Die Ergebnisse zeigen, dass Florfenicol und seine Haupt-Transformationsprodukte unter Verwendung einer dreifachen Extraktion mit 0.02 molarer KH_2PO_4/ACN 1:1 (v:v) ausreichend in Gülle nachgewiesen werden können. Für die LC-MS/MS-Analytik wird eine sorgfältige Aufreinigung des Extrakts durch Zentrifugation bei hoher Beschleunigung und mehrere Aufreinigungsschritte mit Festphasenextraktion und Filtration benötigt. Jedoch führt die Aufreinigung der Proben zu Verlusten der Analyten; Wiederfindungsraten um 60% wurden erreicht. Eine zusätzliche beschleunigte Lösemittelextraktion der Probe erwies sich als nicht zweckdienlich.

Nach Einmischen von Florfenicol in Schweinegülle zeigt sich ein schneller Rückgang der Extrahierbarkeit. Gleichzeitig werden nicht-extrahierbare Rückstände gebildet, welche anhand von ^{14}C-markiertem Florfenicol quantifiziert werden konnten.

Florfenicol wird in Transformationsprodukte umgewandelt. Nach Inkubation wurden vorübergehend Florfenicolamin, Florfenicoloxaminsäure sowie ein unbekanntes Transformationsprodukt in geringen Mengen gebildet. Mit der Kombination von radio-HPLC und LC-ESI-MS/MS unter Verwendung von Produkt-Ionen-Scan (PIS) und Multiple Reaction Monitoring (MRM) war es nicht möglich, weitere Transformationsprodukte zu erfassen.

Basierend auf den Ergebnissen liegt der folgende Transformationspfad nahe: Transformation zu Florfenicolamin durch Hydrolyse der Amid-Bindung und anschließende Bildung von Florfenicoloxaminsäure über Substitution von Fluor durch eine Hydroxylgruppe. Da keine Massenbestimmung und somit keine Molekülcharakterisierung des unbekannten Transformationsprodukts möglich war, kann für diese Substanz kein Transformationspfad vorgeschlagen werden.

Entwicklung der experimentellen Methode und des Prüfrichtlinienentwurfs

Die erste Version des Richtlinienentwurfs wurde im Rahmen des Vorgängerprojektes „Entwicklung einer Testvorschrift zum Abbauverhalten von Veterinärpharmaka und Bioziden in Gülle“ (Hennecke et al., 2015) erstellt. Die experimentelle Testmethode wurde zunächst durch Versuche innerhalb eines Labors und einem Ringversuch (pre-validation ringtest) überprüft. Basierend auf den Ergebnissen und den Diskussionen während eines Workshops im April 2013 in Flörsheim, Deutschland, wurde der Text des Richtlinienentwurfs überarbeitet und kritische Schritte bei der Testdurchführung wurden präzisiert (z.B. Applikation der Testsubstanz; Verlängerung der Testdauer auf bis zu 90 Tage; Details zur Anpassung des Trockensubstanzgehaltes).

Der überarbeitete Richtlinienentwurf wurde dann im internationalen Ringversuch 2013/2014 angewendet. Die Anwendbarkeit und Durchführbarkeit der Testmethode wurden während eines zweitägigen internationalen Workshops im September 2014 in Giessen, Deutschland, diskutiert. Vertreter aller Teilnehmer des Ringversuchs und internationale Experten nahmen an dem Workshop teil. Der Schwerpunkt des Workshops lag auf der Präsentation und Diskussion der Ergebnisse und den Erfahrungen der Teilnehmer mit der Testmethode. Darauf basierend wurde die aktuelle Version des Richtlinienentwurfs im Detail diskutiert und überarbeitet. Dem Richtlinienentwurf wurden Anhänge hinzugefügt, um genauere Anleitungen für wichtige Schritte der Methode zur Verfügung zu stellen.

Basierend auf den Ergebnissen des Ringversuchs und den Diskussionen bei den Workshops können die folgenden Schlussfolgerungen gezogen werden:

- Die im Richtlinienentwurf beschriebene Testmethode kann für die Routinemessung der Transformation von Veterinärpharmaka und Bioziden in flüssiger Schweine- und Rindergülle verwendet werden.
- Ein halbstatisches (semi-static) Testdesign sollte verwendet werden, wohingegen ein strikt statisches Testdesign nicht empfohlen wird. Ein Durchfluss-Testdesign kann darüber hinaus für Substanzen verwendet werden, welche keinerlei oder nur eine geringe Mineralisierung zeigen. Weitere Informationen können in Herrchen et al. (2016) nachgeschlagen werden.
- Redoxpotentiale im typischen Bereich für flüssige Gülle in Lagertanks oder Faulteichen (von -230 mV bis -400 mV (Weinfurtner, 2011)) können bei Verwendung der im Richtlinienentwurf beschriebenen Testmethode sichergestellt werden.
- Parallel zur Testsubstanz sollte eine Referenzsubstanz untersucht werden, um vergleichbare Bedingungen zwischen unterschiedlichen Experimenten mit unterschiedlichen Güllen sicherstellen zu können. Salizylsäure (z.B. als Natriumsalicylat, CAS: 54-21-7 oder als Salizylsäure, CAS: 69-72-7) wird als Referenzsubstanz vorgeschlagen, da die Transformation von Salizylsäure unter anaeroben Testbedingungen bereits in Schweine- und Rindergülle untersucht wurde und eine hohe Mineralisierung zu CO_2 und CH_4 nachgewiesen werden konnte (Hennecke et al., 2015; Herrchen et al., 2016). Sofern eine Referenzsubstanz getestet wird, besteht keine Notwendigkeit zur weiteren Testung auf mikrobielle Aktivität der Gülle (z.B. Mineralisierung von ^{14}C-Glukose).
- Die Testung einer Gülle wird als ausreichend angesehen, sofern diese den vorgegebenen Matrixparametern entspricht, zeitgleich die Mineralisierung der Referenzsubstanz in der Gülle untersucht wird.
- Autoklavieren wird zur Herstellung von Sterilproben empfohlen. Das Autoklavieren sollte mindestens zweimal erfolgen und die Proben sollten zuvor erhitzt werden (100°C für mindestens 12 Stunden).
- Die Zeitspanne zum Austreiben von potentiell gelöstem $^{14}CO_2$ nach Ansäuern der Gülleprobe sollte mindestens 24 Stunden betragen. Falls ein halbstatisches Testdesign verwendet wird, könnte eine zusätzliche Durchlüftung der Gülle notwendig sein. Hierzu Stickstoff sollte verwendet werden, um anaerobe Bedingungen sicherzustellen.

- Falls möglich sollte auch entstandenes $^{14}CH_4$ bestimmt werden, um Verluste und unvollständige Massenbilanzen zu vermeiden. Weiterhin kann die Messung von $^{14}CH_4$ zum Nachweis von methanogenen Mikroorganismen in der Gülle verwendet werden.
- Unter Berücksichtigung von Häufigkeitsverteilungen und Boxplot-Diagrammen kann ein Qualitätskriterium von 100 ± 15% aR für die Massenbilanz bei Testbeginn, sowie für die mittlere Massenbilanz für alle Probenahmezeitpunkte im Testverlauf empfohlen werden.
- Basierend auf chi^2-Fehlern und Residuendiagrammen wird das SFO-Modell wird als geeignet angesehen, um DT_{50}-Werte zu bestimmen.
- Die Mengen an extrahierbaren Rückständen (ER) und nicht-extrahierbaren Rückständen (NER) sind abhängig von der verwendeten Extraktionsmethode. Aus diesem Grund müssen die analytischen Methoden (inklusive Extraktions- und Aufreinigungsmethoden) sorgfältig entwickelt, validiert und berichtet werden.
- Transformationsprodukte müssen mit einbezogen und bei der Methodenentwicklung berücksichtigt werden. Für LC-MS/MS-Analytik ist eine gründliche Aufreinigung der Gülleextrakte erforderlich. Darüber hinaus wird die Verwendung eines internen Standards dringend empfohlen.
- Im Ringversuch konnte eine geringe Variabilität und Streuung der Ergebnisse für extrahierbare Rückstände (Variationskoeffizient (COV) bei Testende: 31.0-40.7%), nicht-extrahierbare Rückstände (COV bei Testende: 32.1-54.1%) und DT_{50}-Werte (Gesamt-COV: 37.2-52.6%), sowie insbesondere für die Massenbilanz (COV bei Testende: 9.8-10.0%) erreicht werden.

Schlussfolgerungen

Der Entwurf der Testvorschrift erwies sich in verschiedenen Laboratorien mit unterschiedlich großer Erfahrung bei der Arbeit mit Gülle, sowie bei der Verwendung unterschiedlicher Versuchsaufbauten als anwendbar. Die Testvorschrift erscheint durchaus robust im Hinblick auf Änderungen bezüglich Testequipment und Versuchsaufbau.

Die beobachtete Variabilität zwischen den teilnehmenden Laboratorien erlaubt die Gewinnung verlässlicher und reproduzierbarer Ergebnisse, welche für die Charakterisierung des Verbleibs und der Transformation von Chemikalien in Gülle geeignet sind.

Zusammenfassend kann somit festgestellt werden, dass die in der aktuellen Version des Richtlinienentwurfs beschriebene experimentelle Methode (siehe Annex 1) gut geeignet ist, um die Transformation organischer Substanzen, inklusive Veterinärpharmaka und Biozide, unter anaeroben Bedingungen in flüssiger Gülle zu untersuchen.

Summary

Objective

The aim of the project was to further develop a draft test protocol for an experimental method to study the transformation of veterinary pharmaceuticals and biocides in cattle and pig liquid manure and to start the validation of the method by conducting a ring test.

Background

Spreading of manure constitutes an important pathway by which veterinary medicinal products (VMP) and biocides enter the environment. For this reason, current guidance (e.g. „Guideline on determining the fate of veterinary medicinal products in manure“ (EMA, 2011) take transformation of VMPs and biocides in manure into account. However, currently, there is no standardized experimental test protocol available to examine the transformation of veterinary medicinal products (VMP) and biocides in liquid manure. The EMA guideline on transformation in manure (EMA, 2011) contains basic regulatory requirements. To allow for a consistent assessment of studies within regulatory frameworks, a harmonized internationally accepted and validated test method is needed. In a previous research project, a test protocol was developed in multiple steps taking into account experiences from labs performing simulation type studies like transformation of chemicals in soil and in water-sediment systems and adapting their test design to the specific requirements of the matrix manure.

The test protocol describes methods to examine the transformation of chemicals in pig and cattle manure (90-95% water content) under anaerobic conditions. The experiments are performed to determine the rate of transformation of the test substance, the identity and rates of formation and decline of transformation products, the amount of test substance that is mineralized to CO_2 or CH_4 or other volatiles, and the amount of non-extractable residues (NER).

Liquid manure samples are treated with the test substance and incubated in the dark under controlled laboratory conditions. After appropriate time intervals, manure samples are removed, extracted and analyzed for the parent substance and for transformation products. Volatile products are collected for analysis using appropriate trapping devices to quantify formation of CO_2 and CH_4. Using ^{14}C-radiolabelled material, mineralization rates of the test substance can be measured and a mass balance, including the formation of NER, can be established. Results enable the calculation of DT_{50}-values, and, if appropriate, DT_{90}-values.

The experimental method was developed by testing three different VMP and biocide active ingredients. The method was refined by results from discussions with stakeholders at four international meetings (2012 Berlin, 2013 Glasgow, 2014 Basel, 2015 Barcelona). In 2012/13 an international pre-validation ring test was conducted to get a first impression on how the protocol performs when transferred to other labs. The experiences of the participants were collected and discussed in detail in a technical two-day workshop held in Flörsheim in 2013. The developed test protocol was changed according to the recommendations from this workshop. This test protocol formed the basis for a ring test that was conducted 2013/14 with the purpose to validate the test protocol and derive criteria for quality and reproducibility of the test results. Following this validation ring test a two day international workshop held in Giessen in 2014 served to collect the experiences from the participants and to increase clarity and unambiguity of the draft test protocol.

Ring test for validation of a draft test protocol for studies on transformation in manure

To test the applicability of the revised draft test method, an international inter-laboratory comparison (ring test) was organized. For that purpose, 35 institutes from Europe, 4 institutes from Northern America and 2 institutes from Asia have been invited in March 2013 to participate in the ring test. In addition, an informative meeting has been organized in Glasgow in the framework of the 23rd SETAC Europe Annual Meeting in May 2013.

The following six institutes finally took part in the ring test (listed in alphabetical order):

- Agriculture and Agri-Food Canada (AAFC), London, Canada
- Noack Laboratorien GmbH, Sarstedt, Germany
- ECT Oekotoxikologie GmbH, Flörsheim, Germany
- Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany
- IBACON GmbH, Rossdorf, Germany
- University Trier, Soil Science Department, Trier, Germany

The ring test was performed based on the experiences gained from a previous pre-validation ring test and the current version of the draft test guideline, which was handed out to all participants prior to the start of the experiments. Furthermore, substance-specific procedures for the performance of the ring test (e.g. test duration, sampling time points, test concentrations, radioactivity to be applied per test vessel, sterile controls, recommended extraction methods and analytical procedures, potential transformation products) were provided to all participants in advance.

The anaerobic transformation of two ^{14}C-labeled compounds was tested at a temperature of 20 ± 2°C: one veterinary pharmaceutical in pig manure (^{14}C-florfenicol) and one biocide in cattle manure (^{14}C-imidacloprid). The compounds were chosen based on several selection criteria: the compounds do not degrade too fast and thus allow for a feasible and reproducible sampling; the substances are not persistent, which would result in a prolonged test period; degradation is mainly based on biological processes; requirements for chemical analyses are not extremely high; the position of the ^{14}C-label enables the detection of main transformation products and the costs for synthesis of ^{14}C-labeled compounds are acceptable.

In addition, the transformation of florfenicol in pig manure was investigated at 10 ± 2°C, using radio-labeled and unlabeled test compound to develop guidance on studying transformation products that arise during transformation in manure. Chemical analysis was performed by radio-HPLC (labeled) and LC-ESI-MS/MS (unlabeled). New extraction and clean-up procedures were developed.

The ring test experiments were performed between September 2013 and September 2014.

Evaluation of results

A ^{14}C-mass balance was determined and calculated for each sampling time point by summing-up the amount of radioactivity [% of applied radioactivity; % aR] in the extracts (extractable residues, ER), non-extractable residues (NER) and mineralization ($^{14}CO_2$ and $^{14}CH_4$). Since mass balances at the start of the experiments (day 0) showed differences between the participants, all results relating to the mass balance [% aR] are also presented in normalized from (mass balance at day 0 is set to 100% for each participant).

Results from one participant for florfenicol in pig manure had to be excluded from further evaluations as the applied extraction method deviated considerably from the recommended procedure in the ring test description handed out to all participants. Nonpolar extraction solvents were used which might have led to lower mass balances observed for these experiments.

DT_{50}-values were determined for the parent compound and for transformation products (TP; only if a decrease is observed) to describe the kinetics of the disappearance of the test substance. DT_{50}-values were calculated using "single first order" (SFO) kinetics by means of the KinGUI-software tool and were ln-transformed for calculation of mean and standard deviations. As a measure of the goodness of fit of the kinetic model selected, chi^2-error values were determined and a visual check of the transformation plots was performed.

Results

Manure Matrix characterization parameters

The manure matrix parameters temperature, dry matter content, organic matter content, nitrogen content, as well as redox potential and pH value were determined by the participants throughout the test period. Temperatures measured in pig manure and cattle manure were within the required range of 20 ± 2°C. The pH-value was in the range between 7.4 and 9.3 for pig manure and between 6.1 and 8.8 for cattle manure. No time-dependent trend could be observed throughout the test period. All participants managed to establish redox potentials ≤ -100 mV in pig manure and cattle manure Thus, stable anaerobic conditions could be proved at any time during the experiments.

Disappearance of the parent compound

DT_{50}-values for the test substances (parent compounds) were determined by means of the SFO-kinetic using the Software KinGUI. For imidacloprid in cattle manure mean DT_{50}-values (20 ± 2°C) were in the range of 17.4 days to 40.0 days. For florfenicol in pig manure the range observed for mean DT_{50}-values (20 ± 2°C) was between 0.17 days and 0.41 days. Mean DT_{50}-vlaues determined for florfenicol in pig manure at a temperature of 10 ± 2°C were 1.4 days (unlabeled florfenicol) and 2.3 days (^{14}C-labeled florfenicol).

Mean chi^2-error of the fits was 21.3 ± 6.3% for florfenicol and 13.4 ± 4.4% for imidacloprid. Although chi^2-error values are above 15% in most cases for florfenicol and imidacloprid, residual plots indicate no systematic error of the SFO model.

Mineralization

No noteworthy mineralization (neither $^{14}CO_2$ nor $^{14}CH_4$) could be measured in the experiments with florfenicol (maximum mineralization of 6% aR) and imidacloprid (maximum mineralization < 1% aR).

Extractable and non-extractable residues

A decrease in extractable residues (ER) for florfenicol and imidacloprid was observed over time. An increase of non-extractable residues (NER) was observed throughout the test period for both test compounds. At the end of the test the amount of NER after normalization (mass balance at day 0 was set to 100% aR) was between 30.8% aR and 61.0% aR for florfenicol in pig manure and between 11.3% aR and 65.3% aR for imidacloprid in cattle manure. The variation in extractable residues and non-extractable residues between the institutes at the end of the study might be caused by different extraction methods used by the participants.

Mass balance

At the beginning of the test, the mean mass balances for individual institutes were in the range between 92.8% aR and 100.3% aR for florfenicol in pig manure and between 86.8% aR and 111.8% aR for imidacloprid in cattle manure. After normalization to 100% aR at day 0, mass balances averaged across all participating institutes throughout the test period were within 95.1% aR and 103.0% aR for florfenicol and within 92.3% aR and 103.7% aR for imidacloprid.

The mass balances of all ring test participants were evaluated. As a recommendation, a mass balance range of 100 ± 15% aR was derived.

Special method considerations for identification of transformation products

The transformation of florfenicol in pig manure at 10 ± 2°C was investigated using ^{14}C-radiolabelled florfenicol and unlabeled test compound. Chemical analysis was performed by radio-HPLC (labeled) and LC-ESI-MS/MS (unlabeled). For the latter, an extraction and clean-up procedure was developed.

The results showed that florfenicol and its major transformation products are sufficiently extracted from manure using a threefold extraction with 0.02 M KH_2PO_4/ACN 1:1 (v:v). For LC-MS/MS-analysis, a thorough clean-up of the extract is required by using centrifugation at high acceleration and several clean-up steps with solid phase extraction materials as well as filtration. Yet, sample clean-up leads to losses of the analytes; recovery rates of about 60% were reached. Additional accelerated solvent extraction of the samples is not expedient.

Mixing florfenicol with manure is followed by a fast decline in florfenicol extractability. This is paralleled by the formation of non-extractable residues that were quantified from ^{14}C-labeled florfenicol.

Florfenicol is transformed into transformation products. Upon incubation, florfenicol amine and florfenicol oxamic acid as well as an unknown transformation product were formed in minor amounts followed by dissipation. With the combination of radio-HPLC and LC-ESI-MS/MS using product ion scan (PIS) and multiple reaction monitoring (MRM) experiments, it was not possible to discover further transformation products.

Based on the results the suggested transformation pathway for florfenicol is transformation to florfenicol amine by amide bond hydrolysis and to florfenicol oxamic acid through a substitution of fluorine with a hydroxyl group. Since no mass identification and thus molecular characterization of the unknown transformation product was possible, no transformation pathway can be suggested for this compound.

Development of the experimental method and the draft test guideline

The first version of the draft test guideline was compiled in the framework of the preceding project „Development of test guidance for transformation of veterinary pharmaceuticals and biocides in liquid manure“(Hennecke et al., 2015). The experimental test method was preliminary validated by intra-laboratory comparisons and an international inter-laboratory comparison (pre-validation ring test). Based on the results and the discussions during a workshop held in Flörsheim, Germany, in April 2013, the text of the draft test guideline has been revised and critical steps of the test performance have been specified more in detail (e.g. application of the test substance, a prolongation of the test duration up to 90 days, details on adjustment of the dry matter content).

The revised draft guideline was then used in an international ring test in 2013/2014. The applicability and feasibility of the test method were discussed at a two-day international technical workshop in Gießen, Germany in September 2014. Representatives of all ring test participants and international experts took part in the workshop. The main focus was the presentation and discussion of results and experiences of the participants with the test method. The current version of the draft test guideline was discussed in detail and revised based on the experiences of the participants. Annex documents were included to give more specific guidance on important steps of the procedure.

Based on the results of the ring test and the discussions at the workshop the following conclusions can be drawn:

- The test method described in the draft test guideline is applicable for routine measurements of the transformation of veterinary pharmaceuticals and biocides in pig and cattle manure.
- A semi-static test design should be used, whereas a strictly static test design is not recommended. A flow-through design might also be used for substances which show no or only low mineralization. More information can be found in Herrchen et al. (2016).
- Redox potentials in the typical range for liquid manure storage tanks or lagoons (from -230 mV to -400 mV (Weinfurtner, 2011)) can be ensured when using the test method described in the draft test guideline.
- A reference substance should be tested in parallel to the test compound to be able to ensure comparable conditions for different tests with different manures. Salicylic acid (e.g. as sodium salicylate CAS: 54-21-7 or as salicylic acid CAS: 69-72-7) is proposed as reference substance since it has been tested for transformation under anaerobic conditions in pig and cattle manure and found to be mineralized to a high extend to CO_2 and CH_4 (Hennecke et al., 2015; Herrchen et al., 2016). If a reference substance is tested, there is no need to test for microbial activity (e.g. mineralization of ^{14}C-glucose).
- One manure is considered to be sufficient if it conforms to the matrix parameters specified and the same manure is concurrently tested with the reference substance.
- Autoclaving is recommended to prepare sterile samples. Autoclaving should be performed at least twice and the samples should be preheated (100°C for at least 12 hours) in advance.
- The time period to strip out potentially dissolved $^{14}CO_2$ after acidification of the manure should be at least 24 hours. If a semi-static test system is used, bubbling manure with nitrogen might be necessary in addition. Nitrogen should be used to ensure anaerobic conditions.
- If feasible, $^{14}CH_4$ should be determined to avoid losses and an incomplete mass balance. Furthermore, measurement of $^{14}CH_4$ can be used to prove that methanogenic microorganisms are present in the manure.
- In consideration of frequency distributions and box-plot diagrams, a quality criterion of 100 ± 15% aR for mass balance at the beginning of the test, as well as for mean mass balances for all sampling time points throughout the test is recommended.
- The SFO model proved to be appropriate for determination of DT_{50}-values based on Chi^2error values and residual plots.
- The amounts of extractable residues (ER) and non-extractable residues (NER) depend on the extraction method used. Therefore, analytical methods (including extraction methods and clean-up methods) have to be carefully developed, validated and reported for each specific test substance.
- Transformation products have to be considered and to be included into the method development. For LC-MS/MS-analysis a thorough clean-up of manure extracts is required and the use of an internal standard is highly recommended.
- Low variability and spread in the results could be achieved in experiments in the ring test for extractable residues (COV at the end of the test: 31.0-40.7%), non-extractable residues (COV at the end of the test: 32.1-54.1%) and DT_{50} values (overall COV: 37.2-52.6%) and particularly for ^{14}C-mass balances (COV at the end of the test: 9.8-10.0%).

Conclusions

The draft test protocol proved to be applicable in different laboratories with different levels of experience in working with manure and using different experimental set-ups. The test protocol seems to be quite robust concerning variations in equipment and test setup. The observed variability in between different participating laboratories allows to obtain reliable and reproducible results suitable for the purpose of characterizing the fate and transformation of chemicals in manure.

In conclusion, the experimental method described in the current draft version of the test guideline (see Annex 1) is considered well-suited to examine the transformation of organic compounds, including veterinary pharmaceuticals and biocides, under anaerobic conditions in liquid manure.

1 Introduction

Spreading of manure constitutes an important pathway by which veterinary medicinal products (VMP) and biocides enter the environment. For this reason, current guidance (e.g. „Guideline on determining the fate of veterinary medicinal products in manure“ (EMA, 2011) take transformation of VMPs and biocides in manure into account. However, currently, there is no standardized experimental test protocol available to examine the transformation of veterinary medicinal products (VMP) and biocides in liquid manure. The EMA guideline on transformation in manure (EMA, 2011) contains basic regulatory requirements. To allow for a consistent assessment of studies within regulatory frameworks, a harmonized internationally accepted and validated test method is needed. In a previous research project a test protocol was developed in multiple steps taking into account experiences from labs performing simulation type studies like transformation of chemicals in soil and in water-sediment systems and adapting their test design to the specific requirements of the matrix manure.

The test protocol describes methods to examine the transformation of chemicals in pig and cattle manure under anaerobic conditions. The experiments are performed to determine the rate of transformation of the test substance, the identity and rates of formation and decline of transformation products, the amount of test substance that is mineralized to CO_2 or CH_4 or other volatiles, and the amount of non-extractable residues (NER).

Liquid manure samples are spiked with the test substance and incubated in the dark under controlled laboratory conditions. After appropriate time intervals, manure samples are removed, extracted and analyzed for the parent substance and for transformation products. Volatile products are collected for analysis using appropriate trapping devices to quantify formation of CO_2 and CH_4. Using ^{14}C-radiolabelled material, mineralization rates of the test substance can be measured and a mass balance, including the formation of NER, can be established. Results enable the calculation of DT_{50}-values, and, if appropriate, DT_{90}-values.

The experimental method was developed by testing three different VMP and biocide active ingredients. Subsequently the method was refined by results from discussions with stakeholders at four international meetings (2012 Berlin, 2013 Glasgow, 2014 Basel, 2015 Barcelona). In 2012/2013 an international pre-validation ring test was conducted to get a first impression on how the protocol performs when transferred to another lab. The experiences of the participants were collected and discussed in detail in a technical two-day workshop held in Flörsheim in 2013. The developed test protocol was changed according to the recommendations from this workshop. This guideline formed the basis for a ring test that was conducted 2013/2014 with the purpose to validate the test protocol and derive criteria for validity and acceptability of the test results. Following this validation ring test a two-day international workshop held in Giessen in 2014 served to collect the experiences from the participants and to increase clarity and unambiguity of the draft test protocol.

2 Material and methods

In order to test the applicability of the developed draft test method, an international inter-laboratory comparison (ring test) was organized.

For that purpose, thirty-five institutes from Europe, four institutes from Northern America and two institutes from Asia have been invited in March 2013 to take part in the ring test (see Annex 2 for the invitation, including outline of the ring test and registration form). In addition, an informative meeting has been organized in Glasgow in the framework of the 23rd SETAC Europe Annual Meeting in May 2013. Until September 2013 seven institutes (6 from Europe, 1 from Northern America) registered for the ring-test. Unfortunately, two institutes had to cancel their participation short-term.

The following five institutes finally took part in the ring test (listed in alphabetical order):

- Agriculture and Agri-Food Canada (AAFC), London, Canada
- Noack Laboratorien GmbH, Sarstedt, Germany
- ECT Oekotoxikologie GmbH, Flörsheim, Germany
- Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany
- IBACON GmbH, Rossdorf, Germany
- University Trier, Soil Science Department, Faculty VI, Trier, Germany

In addition, to include the identification of transformation products in more detail, the transformation of one test compound (florfenicol in pig manure) was investigated by one institute (Institute 6), using radiolabeled and unlabeled test compound. Chemical analysis was performed by radio-HPLC (labeled) and LC-ESI-MS/MS (unlabeled). For the latter, a new extraction and clean-up procedure was developed.

2.1 Test compounds

Two ^{14}C-labeled compounds were tested: one veterinary pharmaceutical (^{14}C-florfenicol, Batch-No. CFQ41813) in pig manure and one biocide (^{14}C-imidacloprid, Batch-No. CFQ41814) in cattle manure.

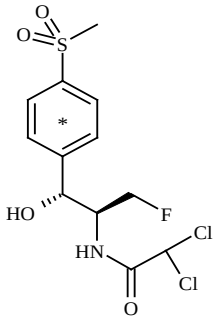
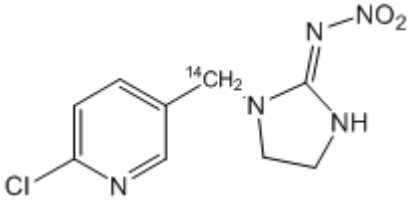
The compounds were selected based on regulatory relevance, i.e. the compounds had already been examined in respective marketing authorization applications. The substances were chosen to reflect a different range of physical-chemical properties and different behavior in manure: florfenicol is a rather polar antibiotic compound. It is expected to be rapidly transformed in manure while forming high amounts of non-extractable residues. Imidacloprid in contrast is an insecticide belonging to the neonicotinoids. It is less polar than florfenicol and expected to exhibit a longer half-life in manure than florfenicol. The participating labs could either test the two compounds in parallel or run consecutive experiments (e.g. due to limited lab space).

Moreover, the compounds fulfil the following requirements:

- The compounds do not degrade extremely fast and thus allow for a feasible and reproducible sampling.
- The substances are not persistent, which would result in a prolonged test period.
- Degradation is mainly based on biological processes.
- Requirements for chemical analyses are not extremely high.
- The position of the ^{14}C-label enables the detection of main transformation products.
- The costs for synthesis of ^{14}C-labeled compounds are acceptable.

The compounds have been shipped to all participants by the manufacturer Quotient Bioresearch Ltd in July 2013 (see Table 1 for information on test substances).

Table 1: Information on test substances

Name	[ring-U-^{14}C]Florfenicol	[methylene-^{14}C]Imidacloprid
CAS-Number	73231-34-2	138261-41-3
Chemical structure	 * ^{14}C-ring-label	
Molecular formula	$C_{12}H_{14}Cl_2FNO_4S$	$C_9H_{10}ClN_5O_2$
Molecular weight	360.2 g/mol [a]	257.5 g/mol [c]
Water solubility	1320 mg/L [b]	610 mg/L [d]
Log P_{ow}	0.37 [b]	0.57 [d]
Batch-No.	CFQ41813 [a]	CFQ41814 [c]
Purity	98.2% [a]	98.8% [c]
Appearance	Solid [a]	Solid [c]
Specific activity	2.29 GBq/mmol [a]	2.15 GBq/mmol [c]
Origin	Quotient Bioresearch Ltd.	Quotient Bioresearch Ltd.

[a] Product Specification Sheet, [ring-U-^{14}C]Florfenicol, Quotient Bioreserach (Radiochemicals) Ltd.;
[b] AQUAFLOR™, Technical monograph, Schering-Plough Animal Health, http://aqua.merck-animal-health.com/binaries/PDF_tech_monograph_tcm56-34642.pdf;
[c] Product Specification Sheet, [methylene-^{14}C]Imidacloprid, Quotient Bioreserach (Radiochemicals) Ltd.;
[d] Gervais et al. (2010)

2.2 Test design

As basis for the performance of the ring test all participating institutes were provided with the then current version of the draft test guideline (see Annex 1 for current version) and an evaluation sheet (Microsoft Excel) for documentation of the results. The draft test guideline gives a detailed description of the method to examine the transformation of chemicals in pig and cattle manure under anaerobic conditions. The main paragraphs of the draft guideline with regard to the test performance are described in the following subsections 2.2.1 to 2.2.4.

2.2.1 Collection, handling and storage of manure

Sampling of both manures (cattle and pig) was performed individually by each participant in accordance with the recommendations described in the following.

Prior to collection the liquid manure should be homogenized by mixing in the respective manure tank. Pig manure should be stirred immediately before sampling as separation into liquid and solid

phase easily occurs. Cattle manure should be stirred no more than one day before sampling. For mixing devices installed in the tank or external devices may be used. Mixing for one hour proved sufficient for homogenization of manure in the tanks independent from tank volume.

Liquid manure is collected from the tank by appropriate equipment (e.g. a ladle with a large beaker), and filled into containers. Filling up to approximately ¾ of maximum container volume might by appropriate. Containers are closed tightly but must allow gas, which is generated by continuous microbial activity, to expand. This can be achieved by connecting a tube with a fermentation air lock to an outlet in the container. This also prevents odors from escaping from the containers (see Figure 1).

The sampling site, the sampling procedure (time and duration of mixing), and the type and size of manure tank (above/below ground, covered/open) should be recorded in detail.

Prior to further processing, manure might by stored at 4°C to 20°C (preferably at the test temperature) for up to two months. Storage should ensure anaerobic conditions. Care has to be taken to allow gas, generated by biological activity during storage, to expand to avoid explosion of the container.

Figure 1: Container with fermentation air lock during storage of cattle manure

2.2.2 Manure characterization

Key parameters that have to be measured and reported (with reference to the method used) and the stage of the test at which those parameters have to be determined are summarized in the table hereafter. As far as possible, standardized methods should be used to determine the matrix parameters (see footnotes for examples).

Table 2: Measurement of matrix parameters for characterization of liquid manure

Parameter[1]	Stage of test procedure				
	Sampling (on site)	Start of acclimation	Start of test	During test	End of test
pH[2]	X	X	X	X	X
microbial activity[3]	(X)[4]		X		X
organic matter content [%OM][5]	X	X			
nitrogen content [N_{total}; mg/kg][6]	X		X		
nitrogen content [NH_4-N; mg/kg][7]	X		X		
redox potential [mV][8]	X	X	X	X	X
dry matter content [%][9]	X	X	X		X
temperature [°C]	X	X	X	X	X

2.2.3 Acclimation

Prior to the start of the acclimation period, the dry matter content of the manure has to be determined. To get comparable conditions it has to be adjusted to standardized values. The recommended dry matter content in cattle and pig manure is 10% ± 1% and 5% ± 1%, respectively (EMA (2011), Weinfurtner (2011). If the dry matter content is below the recommended value, it can be concentrated by careful centrifugation (e.g. for 10 minutes at 740 × g). However, the initial dry matter content should not be below 8% (cattle) or 3% (pig). If dry matter content is too high, water (deionized water, bubbled with nitrogen for 30 min) should be added as needed.

Thereafter, cattle manure should be homogenized by mixing. No additional measures to prevent introduction of oxygen are used. Subsamples of 50 – 100 g (wet weight) each should be directly filled into the incubation vessels which are used for the acclimation and transformation study.

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1 For all matrix parameters it has to be specified in the report whether they relate to dry or to wet mass of the sample.

2 e.g. ISO 10390 „Soil quality -- Determination of pH“ (ISO, 2005)

3 Optional. For testing the microbial activity several suggestions exist.
a) Reduction of DMSO to DMS (EMA (2011), Greibler and Slezak (2001)).
b) Mineralization of a readily degradable ^{14}C-labeled substance (e.g., ^{14}C-glucose) under anaerobic conditions (see Annex 4 of the draft test guideline in Annex 1). This method was not recommended for the ring test due to low predictivity in pre-validation ringtest.
c) Determination of gas volume (e.g. VDI-Richtlinie 4630 „Fermentation of organic materials“, http://www.vdi.de/uploads/tx_vdirili/pdf/9703240.pdf).
It is recommended to use a reference substance concurrently with each test (see section 3.12.1.2).

4 In brackets are optional determinations

5 e.g. DIN 12879 „Charakterisierung von Schlämmen - Bestimmung des Glühverlustes der Trockenmasse” (DIN, 2001a)

6 e.g. ISO 11261 “Soil quality - Determination of total nitrogen - Modified Kjeldahl method” (ISO, 1995). For conversion of mass based units in volume based units a density of 0.001 kg/m^3 is used

7 e.g. ISO 5664 “Water quality - Determination of ammonium - Distillation and titration method” (ISO, 1984).

8 e.g. ISO 11271 “Soil quality - Determination of redox potential - Field method” (ISO, 2002) and/or DIN 38404-6 “Determination of the oxidation reduction (redox) potential” (DIN,1984)

9 e.g. DIN 12880 “Characterization of sludges - Determination of dry residue and water content” (DIN, 2001b)

Pig manure should be homogenized under anaerobic conditions in order to obtain a fairly stable phase. This can be achieved, e.g. by filling the manure into a beaker, putting a mixer/homogenizer (e.g. hand blender) into the manure, and gently passing a nitrogen stream over the manure while mixing. Thereafter, the dry matter content should be adjusted. After a repeated homogenization under anaerobic conditions by thoroughly mixing (set up as above) subsamples of 50 – 100 g (fresh weight) are filled into the incubation vessels.

If a flow through apparatus is used, the incubation apparatus has to be closed and a constant, water saturated stream of nitrogen is passed over the manure at a rate in the range of approximately 50 - 200 mL/min. If a semi-static apparatus is used, the incubation system is flushed with moistened nitrogen for one hour to maintain anaerobic conditions. Subsequently, the incubation system is closed by valves. At regular intervals, the incubation system should be connected to the flow-through apparatus and purged with moistened nitrogen for pressure compensation.

The acclimation should be carried out for 21 ±1 days at test temperature.

2.2.4 Test conditions

2.2.4.1 Test temperature and light conditions

During the whole test period the manure samples should be incubated in the dark at the appropriate test temperature. Typical environmental temperatures observed in manure tanks are 10°C for Central European climate conditions (EFSA, 2007, Hennecke et al., 2015, Weinfurtner, 2011). In other regions, different temperatures might be considered typical. To facilitate laboratory studies, studies may be conducted at 20 °C (range of ± 2 °C) and resulting DT_x values converted to environmentally relevant temperatures (FOCUS, 2006). To determine the pathway of transformation, environmentally relevant temperatures might have to be used.

2.2.4.2 Anaerobic incubation conditions

Transformation studies in cattle and pig manure should be performed under anaerobic conditions. Anaerobic conditions should be demonstrated by Eh ≤ -100 mV (OECD, 2002b). Redox potentials measured in pig and cattle manure have been found to range from -230 mV to -400 mV (Weinfurtner, 2011) and experimental conditions should be comparable. Redox potential should be measured and recorded at least as often that stable anaerobic conditions can be assured throughout the experiment.

2.2.4.3 Abiotic controls

For information on the abiotic transformation of the test substance it is recommended to include sterile controls. For substances undergoing rapid abiotic transformation otherwise no meaningful results might be deduced from the study. Manure may be sterilized, treated with sterile test substance and flasks kept closed carefully. Sampling of sterile controls should be according to the sampling schedule but sampling can be reduced to fewer time points. Sterile controls should be sampled at the end of the test. Sterilization can be achieved by autoclaving twice (e.g. 15 min, 121°C, 100 bar). It might be helpful to pre-heat the manure to be autoclaved at 100°C overnight if severe foaming is otherwise observed.

Figure 2: Sterile control sample

2.2.4.4 Treatment and application of test substance

The test substance should be dosed into the manure at a concentration that reflects the maximum expected manure concentration. If this concentration is not sufficient for detection and identification of transformation products, the test may be conducted at increased substance start concentrations. However, excessively high concentrations should be avoided.

The test substance should be dissolved in an appropriate solvent and should be added into the acclimated manure in the respective incubation vessels followed by thoroughly mixing while maintaining anaerobic conditions. This can be achieved, e.g., as follows: during application passing the nitrogen stream over the samples has to be maintained. The required volume of stock solution should be pipetted into the manure under simultaneous stirring using the pipette tip. As soon as the solution is evenly distributed in the manure the pipette tip remains in the manure. The total volume of a water miscible organic solvent used for application should not exceed 1% by volume.

2.2.4.5 Test duration and sampling

Test duration will depend on the rate of transformation of the parent compound and transformation products. The maximum study duration is 90 days. This time was derived from a survey on typical manure storage times (VICH, 2008). In certain cases it might be reasonable to prolong the study. Ideally, the test substance and transformation products should each be present in amounts below 10% of the applied amount at the end of the study. If the study is further prolonged, e.g. because increasing amounts of transformation products have been observed, a test for microbial activity may be conducted at the beginning and end of the prolongation period. It might therefore be useful to have a further spare incubation vessel for this purpose.

At least duplicate incubation flasks are sacrificed per sampling. Sampling intervals should be selected in a way that the pattern of decline of the test substance, and the pattern both of formation and decline of transformation products can be established (e.g. 0, 1, 3, 7 days; 2, 3 weeks; 1, 2, 3 months, etc.). Besides sampling directly after application at least 9 additional sampling points should be included. More sampling time points may be necessary for kinetic modeling according to FOCUS recommendations (FOCUS, 2006) and to include transformation products. An experiment prior to the test start might give valuable indications for the behavior of the test substance and transformation

products. In some cases rapid dissipation of the test substance may be observed and sampling time points have to be adjusted accordingly.

Traps to measure mineralization are removed at the same time intervals and analyzed for trapped $^{14}CO_2$ and other evolved gases, respectively. Sampling procedures for the flow-through system and the static system are described in Annex 2 and Annex 4 of the draft guideline (see Annex 1 of this report).

2.2.4.6 Measurements and analysis

Manure samples are cleaned-up directly after sampling. The samples are extracted with appropriate solvents of different polarity. A sequential extraction approach should be followed for optimal recovery of parent substance and transformation products of different polarity. Aqueous solvent mixtures and acid and base systems should be used as solvents to ensure extraction of more polar transformation products. In case non-extractable residues are observed, exhaustive extraction methods should be applied additionally. These methods comprise e.g. pressurized liquid extraction (e.g. ASE®), reflux, soxhlet etc. with appropriate solvents. Extracts are quantified by liquid scintillation counting (LSC). When using ^{14}C-labeled test substance, the residues remaining after the last extraction step (non-extractable residues, NER) will be quantified by combustion and a mass balance will be calculated for each sampling interval. Analytes should not be altered by the respective extraction method. This can be demonstrated by appropriate controls for the known substances.

Figure 3: Measurement of radioactivity in extracts and in non-extractable residues

left: liquid scintillation counter (LSC) to determine radioactivity in extracts (extractable residues, ER); right: sample oxidizer for combustion of dried manure residues after extraction (non-extractable residues, NER)

Concentration of the test substance and the transformation products at every sampling time should be determined and reported. In general, transformation products detected at ≥ 10% of the applied radioactivity at any sampling time should be identified. Transformation products for which concentrations are continuously increasing during the study should also be identified, even if their concentrations do not exceed the limit given above, as this may indicate persistence.

Typically, identification is accomplished either by co-chromatography of the transformation product with known standards using two dissimilar systems or by techniques capable of positive structural identification such as MS, NMR, etc. In the case of co-chromatography, chromatographic techniques utilizing the same stationary phase with two different solvent systems are not adequate for the verification of the transformation product identity, since the methods are not independent. Identification by co-chromatography should be obtained using two dissimilar, analytically independent systems, such as reverse and normal phase thin layer chromatography (TLC) or TLC and high performance liquid chromatography (HPLC). Provided that the chromatographic separation is of suitable quality, then additional confirmation by spectroscopy is not required. Unambiguous identification can also

be obtained using methods providing structural information such as gas chromatography/mass spectrometry (GC-MS), liquid chromatography/mass spectrometry (LC-MS), liquid chromatography/tandem mass spectrometry (LC-MS/MS), and NMR.

The stereochemistry of transformation products generally does not need to be determined unless a differing behavior is observed.

New extraction and analysis techniques may be substituted for the techniques mentioned above. State of the art technology should be used, as appropriate, to fully elucidate the transformation pathway.

2.3 Substance-specific procedures for the ringtest

In addition to the general descriptions in the previous subsections 2.2.1 to 2.2.4, substance-specific procedures for the performance of the ring test (e.g. test duration, sampling time points, test concentrations, radioactivity to be applied per test vessel, sterile controls, extraction procedures, potential transformation products) were provided to all participants prior to the start of the experiments August 2013 and September 2013.

A summary of the test method parameters is shown in Figure 7.

2.3.1 ^{14}C-florfenicol in pig manure

10 sampling points:

- 0, 0.5, 1, 2, 4, 7, 24 h, 7±1 d, 28±1 d, 60±2 d, 90±2 d
  (until 24 h no mineralization is expected; i.e. CO_2-traps needed from day 7 on)

Sterile controls: 1 d, 28 d, 90 d

2.3.1.1 Test substance concentration

Based on previous transformation studies with manure the following test substance concentration was recommended:

Table 3: Florfenicol concentration in pig manure

Test compound	molecular weight [mg/mmol]	Specific activity [GBq/mmol]	recommended test concentration [mg/kg manure wet weight]
^{14}C-florfenicol	360.2	2.29	3.0

The amount of radioactivity applied per test vessel (containing 50 g manure fresh weight) should be 50 kBq, resulting in 1 MBq per kg manure fresh weight. In consideration of molecular weight and specific activity of the test substance, this corresponds to a concentration of 0.157 mg ^{14}C-florfenicol/kg manure fresh weight. The remaining 2.843 mg florfenicol/kg manure fresh weight had to be added using unlabeled florfenicol.

Table 4: Putative transformation products (TP)/suggested reference compounds for florfenicol

Substance	CAS-No:	Remark	Producer/supplier
florfenicol	73231-34-2	available	Sigma-Aldrich
florfenicol alcohol		presumably not available commercially	
monochloroflorfenicol		presumably not available commercially, probably first TP to be formed	
florfenicol oxamic acid		presumably not available commercially	
florfenicol amine	76639-93-5	available	Sigma-Aldrich
N-[2-Hydroxy-2-(4-methanesulfonyl-phenyl)-1-methyl-ethyl]-acetamide		presumably not available commercially	
N-[1-Fluoromethyl-2-hydroxy-2-(4-methanesulfonyl-phenyl)-ethyl]-acetamide		presumably not available commercially	

2.3.1.2 Extraction method and analytical procedures

The following two extraction procedures were recommended and provided to the participants (all amounts given refer to 50 g manure wet weight per test vessel):

Method proposal 1

The incubated manure sample is extracted with 50 mL of 0.02 M KH_2PO_4 solution: Acetonitrile (ACN) 1:1 (v:v) by shaking for 20 min followed by centrifugation at 2600 g for 10 min. This procedure is repeated 2 additional times and the (supernatant) extracts are combined and aliquots are submitted to LSC analysis. Before performing HPLC-RAM or LC-MS/MS analysis, the samples are filtered appropriately (e.g. 0.2 µm PTFE filters).

HPLC on a RP18 column is performed using the following eluents: 20 mM ammonium acetate, pH 4 (Solvent A) and acetonitrile (ACN) (Solvent B). The program is described below

Time in min	% Solvent A	%Solvent B
0	95	5
2	95	5
38	5	95
42	5	95
43	95	5
47	95	5

For LC-MS/MS quantification and identification further dilution/clean-up of samples is recommended and different eluents/gradient programs might be appropriate (see chapters 2.3.1.3 and 3.11).

Method proposal 2

At the sampling times the glass-flasks containing the respective spiked manure samples (50 g) 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 50 mL methanol and once by 50 mL methanol + 1.5 mL TFA. For extraction the samples are shaken for 30 minutes on a horizontal shaker and centrifuged for 15 minutes at 739 x g. After centrifugation the supernatant is decanted, and the pellet is subjected to the next extraction step. After the last extraction step the pellet is air dried and aliquots are subjected to combustion and liquid scintillation counting (LSC) to give the information on the amount of non-extractable residues (NER).

Extracts are quantified by liquid scintillation counting (LSC) and analyzed for the test item by TLC-analysis. The extracted manure is analyzed for non-extractable residues by combustion with subsequent LSC of the formed $^{14}CO_2$. Thereafter, the extracted manure is additionally extracted by "accelerated solvent extraction, ASE®" using methanol as solvent. ASE® is an extraction method which extracts under high pressure and temperature. Extraction was at 100°C and 12000 kPa. Heat up was for 5 minutes, followed by a static time (10 minutes). 2 cycles were run for each sample.

The volume of the absorption solutions is measured and radioactivity in each solution is determined by LSC at various time intervals (preferably: 7-day intervals during the first month and after one month in 14-day intervals) during and at the end of incubation of each manure sample and analyzed for evolved CO_2 and methane. Evolved $^{14}CO_2$ and $^{14}CH_4$ can be quantified and the rate of mineralization can be determined.

The following TLC-system is recommended. Ranges of Rf-values are rather broad. Allocation of peaks to florfenicol is enabled by co-chromatography of the VMP standard.

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

Characterization	*Rf-values*	
Florfenicol	Rf =	0.39 – 0.55
NIR 1_TLCstart	Rf =	0.01 – 0.10
NIR 2	Rf =	0.11 – 0.15
NIR 3	Rf =	0.20 – 0.35
NIR 4	Rf =	0.36 – 0.45
NIR 5	Rf =	0.47 – 0.56
NIR 6	Rf =	0.80 – 0.85
NIR 7	Rf =	0.89 – 0.92
NIR 8	Rf =	0.95 – 0.98

2.3.1.3 Analysis of florfenicol from incubation at 10°C

To be able to give guidance on methods for sample handling, clean-up and measurements for transformation products, the transformation of florfenicol in pig manure was studied in more detail at 10°C ± 2°C using radiolabeled and unlabeled test compound. Each test flask was filled with 25 g manure (wet weight). Non-radioactive samples were prepared in duplicate, radioactive samples in single determination. Five additional samples were prepared in case of an accidental loss of a sample or to have triplicate samples, if necessary. Chemical analysis was performed by radio-HPLC (labeled) and LC-ESI-MS/MS (unlabeled). Samples were taken at the following timepoints: 0, 0.5, 4, 24 h, 3±1, 7±1, 14±1, 21±1, 28±1, 49±2, 70±2, 90±2 d after application of the test substance.

Analysis of non-radiolabeled florfenicol was done using a Shimadzu LC-20 HPLC (Shimadzu, Duisburg, Germany) coupled to an API 3200 LC-ESI-MS/MS (Applied Biosystems/MDS Sciex Instruments, Toronto, Canada). The HPLC consisted of two LC-20 AD pumps, an autosampler SIL- 20 AC, a column oven CTO-10ASvp, and a system controller CBM-20A Lite. A Sunfire C18, 3.5 µm, 3.0×20 mm guard column and a Sunfire C18, 3.5 µm, 3.0×100 mm (Waters, Eschborn, Germany) were used for separation of florfenicol and its transformation products from other matrix components. The eluent consisted of 20 mM HCOOH (formic acid) in water + 5% ACN (solvent A) and 20 mM HCOOH in ACN (solvent B) which were delivered in a gradient program.

Each sample was analyzed in product ion scan (PIS) and multiple reaction monitoring (MRM) experiments using positive and negative ionization mode. The sample injection volume was 10 µL. To reduce the number of analytes and masses investigated at a single time, three methods were defined with three individual sets of masses detected. Consequently, every single sample was analyzed with six different LC-MS/MS methods for PIS. The software Analyst 1.4.2 (Applied Biosystems/MDS Sciex Instruments) was used for analysis of the data obtained. Quantification of the transformation products listed in Table 50 was done relatively to florfenicol. For florfenicol amine, though, a standard substance was commercially available and externally calibrated. Chloramphenicol was used as internal standard. All three chemicals were obtained from Sigma Aldrich.

Analysis of ^{14}C-radiolabelled florfenicol was done using an Agilent 1200 S system equipped with a Berthold LB 507 b radioactivity detector with yttrium-glass scintillation cell. Exactly similar mobile and stationary phases as well as further conditions were used as for the analysis of non-radiolabeled florfenicol using LC-MS/MS. This ensured unbiased comparison of retention times. It was only necessary to respect the different dead time of the two chromatographic systems, which was done by subtracting a value of 2 minutes. To detect the total radioactivity in extracts a MicroBeta LSC-Plate Counter and LSC Cocktail Optiphase Supermix (Perkin Elmer) were used.

2.3.2 ^{14}C-imidacloprid in cattle manure

10 sampling points: 0 h, 3±1 d, 7±1 d, 10±1 d, 14±1 d, 21±1 d, 28±1 d, 42±2 d, 56±2 d, 72±2 d, and 90±2 d.

Sterile controls: 7 d, 56 d, 90 d.

Note: The aqueous photolysis half-life of imidacloprid is very short (3.98 x 10^{-2} days (24°C, pH 7)). Therefore, please ensure that during incubation exposure to light is avoided (e.g. by wrapping the test vessels with aluminum foil).

2.3.2.1 Test substance concentrations

Based on previous transformation studies with manure the following test substance concentration was recommended:

Table 5: Imidacloprid concentration in cattle manure

Test compound	molecular weight [mg/mmol]	Specific activity [GBq/mmol]	recommended test concentration [mg/kg manure wet weight]
^{14}C-imidacloprid	257.5	2.15	1.0

The amount of radioactivity applied per test vessel (containing 50 g manure fresh weight) should be 50 kBq, resulting in 1 MBq per kg manure fresh weight. In consideration of molecular weight and specific activity of the test substance, this corresponds to a concentration of 0.120 mg ^{14}C-

imidacloprid/kg manure fresh weight. The remaining 0.88 mg/kg manure fresh weight had to be added using unlabeled imidacloprid.

Table 6: Putative transformation products (TP)/suggested reference compounds for imidacloprid

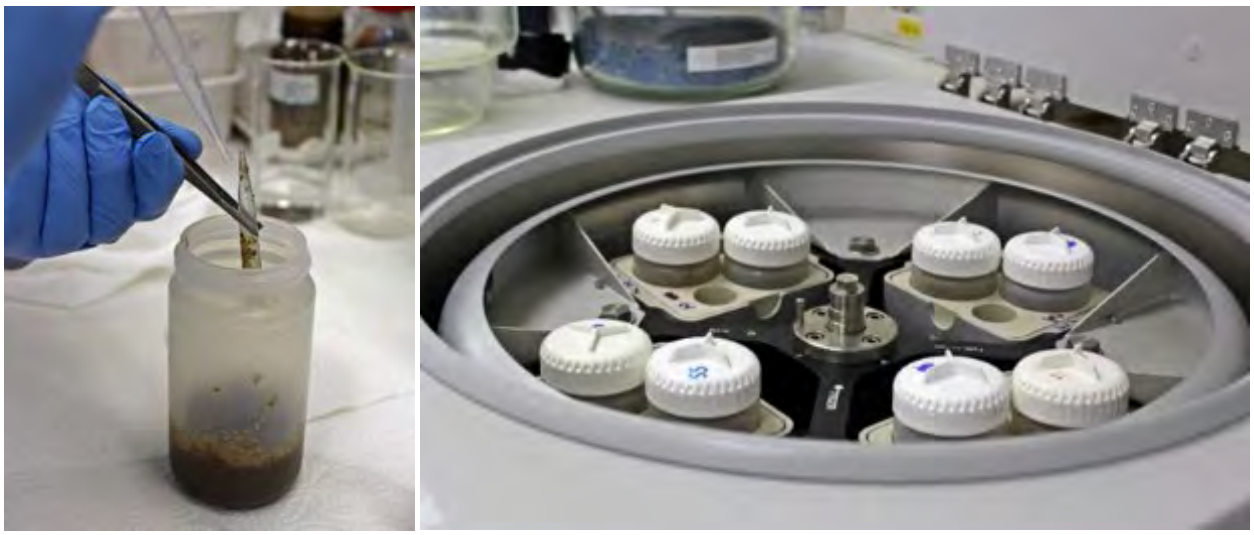
Substance	CAS-No:	Remark	Producer/supplier
Imidacloprid	138261-41-31	available	Sigma-Aldrich
N-(2-Hydroxyphenyl)-N-[(4-methylphenyl)sulfonyl]acetamide	71463-41-7	not available	
N-nitrosoguanidine	674-81-7	available	Select Lab
1-nitroguanidine	556-88-7	available	Sigma-Aldrich
6-chloronicotinic acid	5326-23-6	available	Sigma-Aldrich

2.3.2.2 Extraction method and analytical procedures

An extraction procedure was recommended (all amounts given refer to 50 g manure wet weight per test vessel) and provided to the participants:

50 g manure sample are extracted once by 80 mL acetonitrile, and thereafter twice by 50 mL acetonitrile. For extraction the samples are shaken for 30 minutes on a horizontal shaker and centrifuged for 15 minutes at 739 x g. After centrifugation the supernatant is decanted, and the pellet is subjected to the next extraction step. After the last extraction step the pellet is air dried and aliquots are subjected to combustion and radioassaying to give the information on the amount of non-extractable residues (NER).

Figure 4: Extraction of cattle manure during the ringtest (left: transfer of manure into centrifugation vessel; right: manure samples in centrifuge)



In addition to the described extraction a further extraction step using ASE® can be performed. The accelerated solvent extraction (ASE®), i.e. extraction under high pressure and temperature (100°C, 12000 kPa, heat up for 5 minutes, followed by a static time of 10 minutes) uses the same solvent mixture as for the first extraction steps (acetonitrile). Extraction is performed twice but extracts are not combined.

As the matrix manure influences HPLC, radio-TLC is preferred over HPLC. The following TLC-system is recommended:

- stationary phase: silica gel KG60
- mobile phase: ethylacetate / 2-propanol / water; 65/23/12 (v/v/v)

The radioactive peaks after the development of the TLC-plates are characterized by their Rf-values and allocation to the peaks of co-chromatographed imidacloprid and possible transformation products.

Characterization	*Rf-values*
Imidacloprid:	Rf = 0.58 – 0.64
Nitroguanidine:	Rf = 0.70 – 0.74
N-Nitrosoguanidine:	Rf = 0.65 – 0.70
6-Chloronicotinic acid:	Rf = 0.35 – 0.39

In addition, peaks might be observed, which cannot be allocated to any of the used reference substances. They are described by their Rf-values and named as transformation product T1 and T2. Respective Rf-values are:

T1: Rf = 0.00
T2: Rf = 0.03 – 0.1

Table 7: Summary of test method parameters for the ring test

Parameter		
Test compounds (radiolabeled) and test matrix	- Veterinary pharmaceutical: ^{14}C-florfenicol (CAS No. 73231-34-2) anaerobic pig manure (liquid manure sampled from a tank or lagoon adjusted to 5% dry matter content, provided by coordinator) - Biocide: ^{14}C-imidacloprid (CAS No. 138261-41-3); anaerobic cattle manure (liquid manure sampled from a manure tank or lagoon adjusted to 10% dry matter content, provided by coordinator)	
Pre-treatment	Homogenization (anaerobic)	
Storage of manure	maximum storage period: 2 months at 20°C (anaerobic)	
Manure matrix characterization (minimum, ref. to EMA, 2011)	- pH - temperature - organic matter [%] - redox potential	- dry matter content - nitrogen content (NH_4-N and N_{tot}) - microbial activity [1)]
Amount of manure	50 - 100 g wet weight per incubation vessel	
Pre-incubation	21 days at 20°C, anaerobic	
Test duration	90 d	
Redox conditions	Anaerobic: redox potential always below -100 mV (typical range -230-‹-400 mV)	
Temperature	20 ± 2°C	
Lighting	complete darkness	
Number of sampling time points	10 [2)]	
Number of test concentrations	1 (to be defined; e.g. maximum expected manure concentration)	
Number of replicates	3 (per sampling)	
Number of sterile controls	6 (without gas trapping; sampling at max. three time points incl. termination of incubation)	
Endpoints / parameters	- mineralization (CO_2 + CH_4), - formation of non-extractable residues (NER) - screening for and identification of transformation products - DT_{50} parent and transformation products - mass balance	
Evaluation of ring test results	by the Coordinator (excel file for data reporting will be provided to each participant)	

[1] optional; [2] For the test with florfenicol it is important to know that at 20°C sampling should be frequent during the first day. After one week, the sampling intervals can be increased. The dissipation half-life for the parent compound is expected to be in the rage of a few hours at 20°C.

2.4 Evaluation of ring test results

2.4.1 Manure matrix parameters

The results of the measurements of matrix parameters for characterization of liquid manure (e.g. pH, temperature, organic matter content, redox potential, dry matter content, nitrogen) are reported for each sampling point (see Table 2).

2.4.2 Degradation kinetics

By use of the KinGUI-software tool (Mikolasch et al., 2006), DT_{50}-values were calculated for the parent compound and for transformation products (TP) using "single first order" (SFO) kinetics. SFO-kinetics proved to be most suitable for the evaluation of transformation studies in manure within the preceding project (Hennecke et al., 2015).

Consequently, SFO-kinetics were also used within this project for reasons of comparability. In addition, chi^2-values were determined and a visual check of the graphs was performed. Chi^2-values are a measure of the goodness of fit of the kinetic model selected and indicate the robustness of the calculation. FOCUS (FOCUS, 2006) states that chi^2-values < 15% indicate that the resulting fit is of good quality.

2.4.3 ^{14}C-mass balance

A ^{14}C-mass balance was determined and calculated for each sampling time point. This was done by summing-up the amount of radioactivity [% of applied radioactivity; % aR] in the extracts (extractable residues, ER) + non-extractable residues (NER) + mineralization ($^{14}CO_2$ and $^{14}CH_4$). Results for mass balances and radioactivity distribution are presented as figures and tables. Mean values, standard deviations and coefficients of variation for mass balances were determined.

Since mass balances at the start of the experiments (day 0) show differences between the participants all results relating to the mass balance [% aR] are also presented in normalized from (mass balance at day 0 is set to 100% for each participant).

2.4.4 Statistical evaluation

DT_{50}-values were determined to describe the kinetics of the disappearance of the test substance. DT_X-values were ln-transformed for calculation of mean and standard deviations.

For DT_X values the following parameters were calculated:

- Re-transformed arithmetic mean of the ln-transformed data
- Standard deviation of the ln-transformed data
- Coefficient of variation as $\sqrt{e^{\sigma^2} - 1}$ with σ^2 = variance of the ln-transformed data

Furthermore, mean values, standard deviations and coefficients of variation for extractable residues (ER) and for non-extractable residues (NER) were determined.

DT_{50}-values have to be normalized to reference conditions in order to compare results determined under different test conditions. For example, the transformation rate of chemicals is dependent on temperature. This dependency can be described by the Arrhenius equation which specifies the degradation rate constant and accordingly the DT_{50}-value as a function of the temperature and the activation energy E_a.

$$DT_{50,ref} = DT_{50,act} \times exp\left(\frac{E_a}{R}\left[\frac{1}{T_{act}} - \frac{1}{T_{ref}}\right]\right)$$

Where: $DT_{50,ref}$ = DT_{50} at reference temperature
$DT_{50,act}$ = DT_{50} at actual temperature
E_a = activation energy (kJ mol^{-1})
R = gas constant (0.008314 kJ K^{-1} mol^{-1})
T_{act} = actual temperature (K)
T_{ref} = reference temperature (K)

The correction factor for the ratio between the degradation rates an actual temperature T_{act} that is 10°C lower than the reference Temperature T_{ref} is defined as the Q_{10} value.

$$DT_{50,ref} = DT_{50,act} \times Q_{10}^{\frac{(T_{act}-T_{ref})}{\Delta T}}$$

Here, a default E_a value of 65.4 kJ mol^{-1} is used, corresponding to a Q_{10} value of 2.58 (EFSA, 2008). The resulting equation can be written as

$$DT_{50,20°C} = DT_{50,10°C} \times Q_{10}^{\frac{(10-20)}{10}} = \frac{DT_{50,10°C}}{2.58}$$

3 Results and discussion

3.1 Overview

In the following the results of all ring test participants are presented. All results have been anonymized (Institute 1 to 6). The ring test experiments were performed between September 2013 and September 2014.

The following chapters present the applied incubation systems and results obtained for manure matrix parameters, DT_{50}-values, mineralization, extractable residues, non-extractable residues and ^{14}C-mass balances. Table 8 gives an overview of the collected data (see Annex 3 for the filled evaluation sheets (Microsoft Excel) from all participants).

Table 8: Parameters and endpoints determined by the participants during the ring test

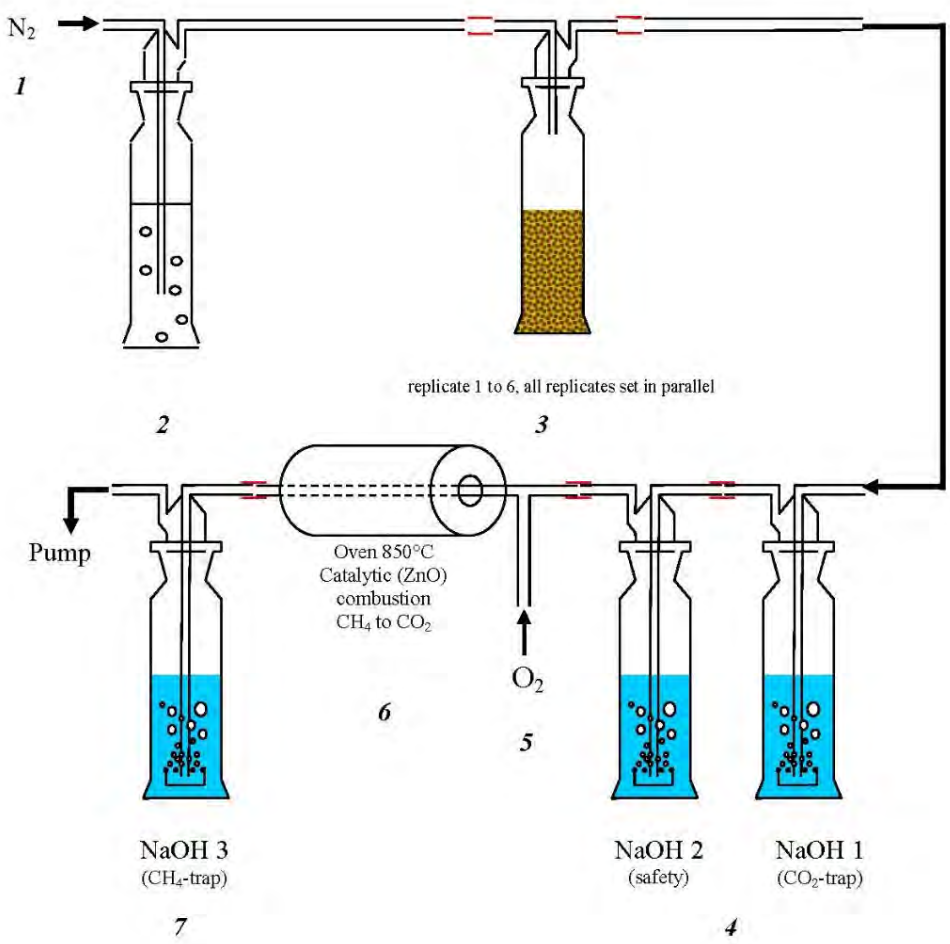
Institute	Imidacloprid / cattle manure					
	$^{14}CO_2$	$^{14}CH_4$	extracts	NER	chemical analyses	microbial activity
1	Yes	Yes	Yes	Yes	Yes	No
2	Yes	Yes	Yes	Yes	Yes	Yes [1)]
3	Yes	Yes	Yes	Yes	Yes	Yes [2)]
4	Yes	Yes	Yes	Yes	Yes	No
5	Yes	No	Yes	Yes	No	Yes [2)]
Institute	**Florfenicol / pig manure**					
	$^{14}CO_2$	$^{14}CH_4$	extracts	NER	chemical analyses	microbial activity
1	Yes	Yes	Yes	Yes	Yes	No
2	Yes	Yes	Yes	Yes	Yes	Yes [1)]
3	Yes	Yes	Yes	Yes	Yes	Yes [2)]
4	Yes	Yes	Yes	Yes	Yes	No
5	Yes	No	Yes	Yes	No	Yes [2)]
6	No	No	Yes	Yes	Yes	No

[1)] Microbial activity was assessed by measuring the gas production of the respective manure;
[2)] Microbial activity was assessed by measuring the mineralization of ^{14}C-glucose of the respective manure

3.2 Incubation systems

A flow-through test system (see schematic example in Figure 5 and pictures in Figure 6) was used by Institute 1, whereas all other participants used (semi-)static test systems (see schematic examples and pictures in Figure 7 to Figure 13). For a detailed description of the semi-static test system and the flow-through test system refer to Annex 3 and Annex 4 of the final draft test guideline in Annex 1 of this report.

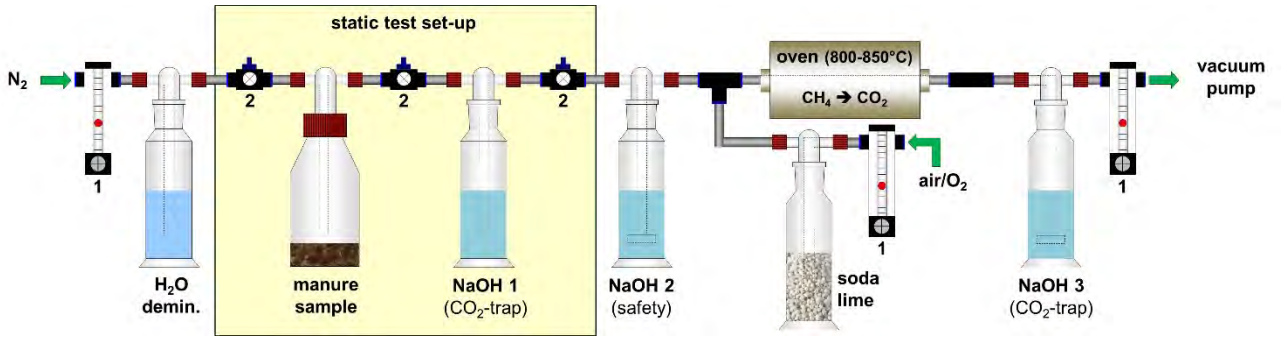
Figure 5: Schematic diagram of the flow-through test system used in the ring test by Institute 1



1: nitrogen is gently passed over the manure samples
2: gas washing bottle containing water
3: manure transformation flasks filled with 50 g manure (fresh weight)
4: for anaerobic transformation two NaOH-filled 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-filled trap for CO_2 formed from CH_4

Figure 6: Pictures of the flow-through test system used in the ring test by Institute 1

Figure 7: Schematic diagram of the semi-static test apparatus used in the ring test by Institute 2



1: variable area flowmeter
2: valve

Figure 8: Pictures semi-static test apparatus used in the ring test by Institute 2

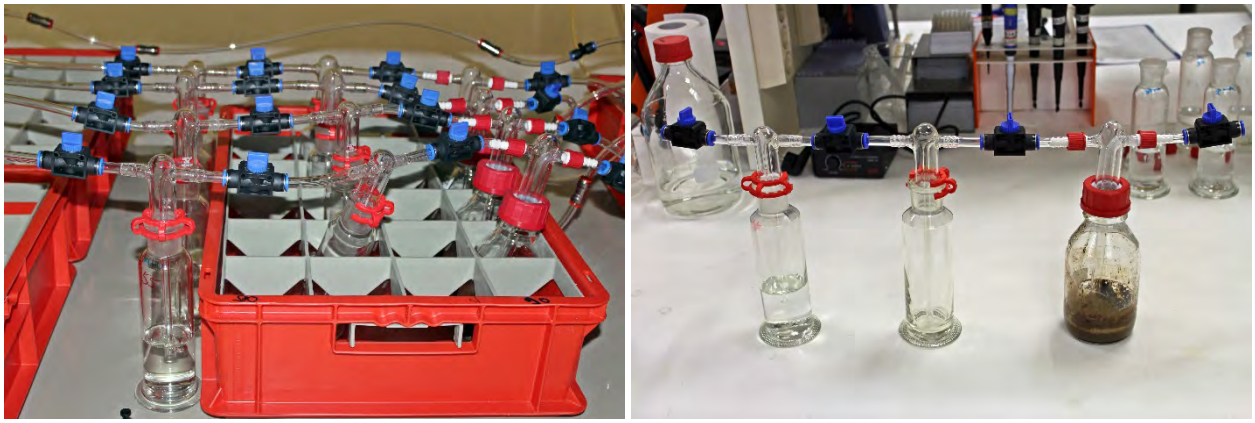
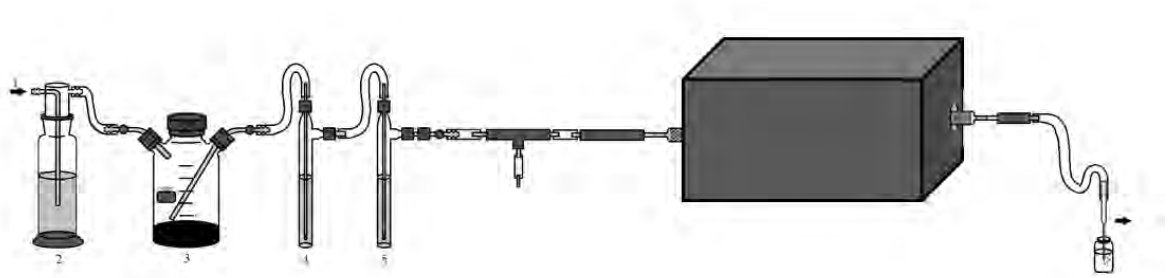


Figure 9: Schematic diagram of the semi-static test apparatus used in the ring test by Institute 3



1: nitrogen inlet
2: gas washing bottle containing deionised water
3: incubation flask containing manure
4: CO_2-trap (e.g. containing 2 M NaOH)
5: CO_2-trap (e.g. containing 2 M NaOH)
6: tube as bypass for further air/oxygen inlet containing silica gel or soda lime pellets
7: oven with quarz glass tube (filled with CuO as catalysator) at 800°C - 850°C
8: CO_2-trap (e.g. containing 2 M NaOH)

Figure 10: Pictures semi-static test apparatus used in the ring test by Institute 3

Figure 11: Pictures semi-static test apparatus used in the ring test by Institute 4

Figure 12: Pictures semi-static test apparatus used in the ring test by Institute 5

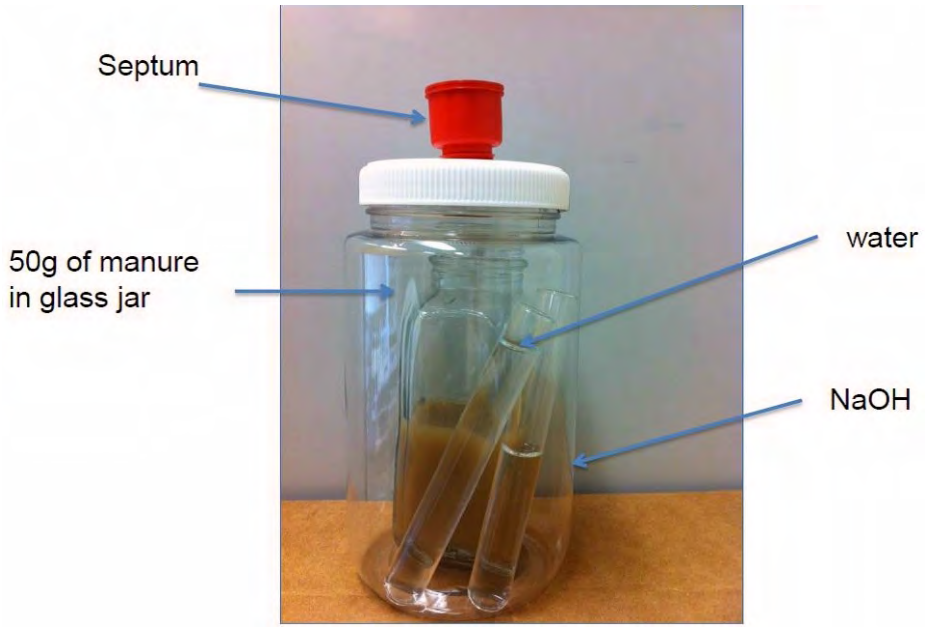


Figure 13: Picture semi-static test apparatus used in the ring test by Institute 6

3.3 Manure sampling

Pig and cattle manure was sampled individually by each participant. After measurement of physico-chemical parameters according to Table 2, manure was stored at test temperature until start of the acclimation period. For details on sampling and origin of manure see Table 9 (cattle manure) and Table 10 (pig manure).

Figure 14: Sampling of cattle manure for the ring test with imidacloprid

top left: cattle manure in tank during mixing; bottom left: mixing device; top right: measurement of manure temperature directly after sampling; bottom right: transfer of manure into storage container using a beaker

Table 9: Cattle manure origin and sampling

Cattle manure	Institute 1	Institute 2	Institute 3	Institute 4	Institute 5
Sampling date:	August 14, 2013	October 08, 2013	February 06, 2014	January 27, 2014	May 01, 2014
Sampling device/ sampling method:	beaker	beaker with a telescope bar	dipper with stirring	ladle	vacuum truck
Type of manure tank:	below ground, 100 m^3, stirring device	open, below ground, stirring device	below ground, 120 m^3	above ground	pit, ~ 345 m^3
Livestock/ type of animals:	80 cows 35 young cattle	290 dairy cows (age › 23 months), 20 female offspring (age ‹ 23 months)	50 feeder cattle	25	180 milking cows and heifers
Feed:	pasture, silage (pasture), mash	maize (silage), gras (silage), draff, winter wheat, winter barley, soybean, rape feed fat, straw	silage	n.a.	haylage, corn silage
Veterinary medicines/ biocides used:	n.a.	1-2 out of 385 cattles in the respective barn treated with ursolcyclin, prostaglandin, albiotic, procain penicillin	none	none	lincomycin, penicillin, $CaCO_3$
Storage time in the laboratory [d]:	55	7	34	14	26
Storage temperature in the laboratory [°C]:	20	20	n.a.	20	20
Remarks:	stirring device used one day before sampling	horse manure is added at 2 tons per 30 tons cattle manure per day.	-	manure in tank was frozen; sampling of fresh manure from smaller interim storage	-

n.a. = information not available

Table 10: Pig manure origin and sampling

Pig manure	Institute 1	Institute 2	Institute 3	Institute 4	Institute 5	Institute 6
Sampling date:	August 21, 2013	December 23, 2013	September 09, 2013	January 27, 2014	May 01, 2014	May 05, 2014
Sampling device/ sampling method:	beaker	n.a.	dipper with stirring	ladle	vacuum truck	n.a.
Type of manure tank:	below ground	n.a.	below ground, 20 m^3	above ground	pit, ~ 53 m^3	below ground
Livestock/ type of animals:	n.a.	1600 farrows, 600 fattened pigs	pigs, sows, fatteners	n.a.	farrowing barn	420 breeding sows, ~11000 fattening pigs (per year)
Feed:	n.a.	cereals, soy extract grit, rape extract grid, mineral nutrients	mash	n.a.	milled corn, premix hog feed	70% cereal mix (wheat, triticale, barley); 30% supplementary feed (soy flour, rapeseed meal, sunflower extract, minerals, etc.)
Veterinary medicines/ biocides used:	n.a.	lysovet (disinfection), alzogur (fly control), no regular use of antibiotics	none	none	chlortetracycline, penicillin, sulfamethazine	1 application of anthelmintic Panacur (Fenbendazole); individual pigs with Duphamox (Amoxycillin)
Storage time in the laboratory [d]:	33	14-29	36	29	26	14
Storage temperature in the laboratory [°C]:	20	20	20	18	20	8
Remarks:	sampled by the farmer	sampled by the farmer	-	tank mixed with ladle prior to sampling	-	Manure stirred before sampling

n.a. = information not available

3.4 Manure matrix characterization parameters

The following subsections present the measured data obtained for manure matrix parameters temperature (3.4.1), dry matter content (3.4.2), organic matter content (3.4.3), nitrogen content (3.4.4), as well as redox potential and pH values (3.4.5).

3.4.1 Temperature

Temperatures measured in pig manure and cattle manure throughout the test period were within the required range of 20 ± 2°C (Institutes 1-5) and 10 ± 2°C (Institute 6). The temperature was in the range between 19.5°C and 21.8°C for pig manure (Table 11; except Institute 6) and between 18.4°C and 21.7°C for cattle manure (Table 12).

Table 11: Temperature [°C] of pig manure at the given sampling points

Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
Institute 1	20.5	20.5	20.5	20.0	20.5	20.0	19.5	20.0	20.5	20.5	20.0
Institute 2	21.0	n.d.	n.d.	n.d.	21.5	n.d.	n.d.	20.8	21.1	20.2	21.8
Institute 3	20.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20.0	n.d.	21.0
Institute 4	20.7	n.d.	n.d.	n.d.	20.7	n.d.	20.7	21.2	20.7	20.9	20.6
Institute 5	n.d.	n.d.	n.d.	n.d.	n.d.	20.0	20.0	20.0	20.0	n.d.	20.0
Institute 6	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0[1)]	10.0

n.d. = not determined; [1)] measurements at day 49 and day 70

Table 12: Temperature [°C] of cattle manure at the given sampling points

Time [d]	0	3	7	10	14	21	28	42	56	72	90
Institute 1	20.5	20.0	19.5	20.0	20.5	20.0	19.5	19.5	20.0	20.5	20.0
Institute 2	18.4	21.6	20.4	n.d.	20.5	20.4	21.7	n.d.	n.d.	20.3	21.3
Institute 3	20.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20.0	n.d.	n.d.	20.0
Institute 4	20.8	20.4	20.7	21.3	19.7	21.1	20.9	20.7	20.8	20.4	20.9
Institute 5	20.0	20.0	20.0	n.d.	n.d.	n.d.	20.0	20.0	n.d.	n.d.	20.0

n.d. = not determined

3.4.2 Dry matter content

Prior to the start of the acclimation period, the dry matter content of the pig manure was adjusted to 5% ± 1% to get comparable conditions as described in section 2.2.1.

Table 13: Dry matter content of pig manure [%]

	Pig manure			
	Sampling	Start of acclimation	Start of test	End of test
Institute 1	4.1	4.1	n.d.	n.d.
Institute 2	5.6	5.0	4.7	4.9
Institute 3	5.5	5.0	n.d.	4.5
Institute 4	6.2	5.7	n.d.	5.3
Institute 5	3.5	n.d.	n.d.	n.d.
Institute 6	14.7	5.0	n.d.	4.1

n.d. = not determined

Prior to the start of the acclimation period, the dry matter content of the cattle manure was adjusted to 10% ± 1% to get comparable conditions as described in section 2.2.1.

Table 14: Dry matter content of cattle manure [%]

	Pig manure			
	Sampling	Start of acclimation	Start of test	End of test
Institute 1	9.5	9.5	n.d.	n.d.
Institute 2	9.6	9.7	8.9	7.5
Institute 3	11.7	9.1	n.d.	7.8
Institute 4	10.4	10.2	n.d.	9.0
Institute 5	6.4	n.d.	n.d.	n.d.

n.d. = not determined

3.4.3 Organic matter content

Table 15: Organic matter content of pig and cattle manure [% relating to wet weight]

	Pig manure		Cattle manure	
	Sampling	Start of acclimation	Sampling	Start of acclimation
Institute 1	4.3	4.3	7.9	7.9
Institute 2	n.d.	3.8	n.d.	7.9
Institute 3	4.5	n.d.	n.d.	n.d.
Institute 4	3.0*	2.6*	3.9*	2.8*
Institute 5	n.d.	n.d.	n.d.	n.d.
Institute 6	6.1*	2.1*	not tested	not tested

n.d. = not determined; * = values refer to organic carbon content instead of organic matter content

3.4.4 Nitrogen content

3.4.4.1 Total nitrogen

Table 16: Total nitrogen content of pig and cattle manure [N_{total}; mg/kg]

	Pig manure		Cattle manure	
	Sampling	Start of test	Sampling	Start of test
Institute 1	3418	n.d.	4194	n.d.
Institute 2	n.d.	3661	n.d.	4432
Institute 3	3000	n.d.	4000	n.d.
Institute 4	7120	n.d.	32900	3720
Institute 5	n.d.	n.d.	n.d.	n.d.
Institute 6	11190	3800 [1)]	not tested	not tested

n.d. = not determined; [1)] start of acclimation period

3.4.4.2 Ammonia nitrogen

Table 17: Ammonia nitrogen content of pig and cattle manure [NH_4-N; mg/kg]

	Pig manure		Cattle manure	
	Sampling	Start of test	Sampling	Start of test
Institute 1	2296	n.d.	2034	n.d.
Institute 2	n.d.	1843	n.d.	3952
Institute 3	1600	n.d.	n.d.	n.d.
Institute 4	5200	n.d.	15100	1670
Institute 5	3220	n.d.	1504	n.d.
Institute 6	7330	2490[1)]	not tested	not tested

n.d. = not determined; [1)] start of acclimation period

3.4.5 Redox potential and pH values

The redox-potential and pH-value are two important parameters, which reflect the test conditions. Thus, they might also serve to interpret the results. All measurements of physical-chemical parameters are documented in the respective evaluation sheets in Annex 3.

The pH-value was in the range between 7.4 and 9.3 for pig manure (Table 18) and between 6.1 and 8.8 for cattle manure (Table 19). No time-dependent trend could be observed throughout the test period.

During the experiments, the measured redox potentials in pig manure (Table 20) and cattle manure (Table 21) were in the range typically observed for liquid manure storage tanks or lagoons of -230 mV to -400 mV (Weinfurtner, 2011) and always below ≤ -100 mV. Thus, stable anaerobic conditions prevailed throughout the test period.

Table 18: pH-values of pig manure at the given sampling points

Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
Institute 1	8.9	8.7	8.2	8.6	9.1	9.3	8.4	7.9	8.7	9.3	8.4
Institute 2	7.8	n.a.	n.a.	n.a.	8.1	n.a.	n.a.	8.1	8.1	8.1	8.0
Institute 3	7.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	8.1	n.a.	7.7
Institute 4	8.2	n.a.	n.a.	n.a.	8.3	n.a.	8.3	8.4	8.4	8.5	8.7
Institute 5	n.a.	n.a.	n.a.	n.a.	n.a.	7.4	7.4	7.4	7.4	n.a.	7.4
Institute 6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	7.4
Minimum	7.4										
Maximum	9.3										
Mean	8.2										
SD	0.5										
COV%	6.6										

n.a. = not analyzed

Table 19: pH-values of cattle manure at the given sampling points

Time [d]	0	3	7	10	14	21	28	42	56	72	90
Institute 1	8.1	8.1	8.2	7.9	8.4	8.6	8.1	8.8	8.3	8.3	8.5
Institute 2	7.4	7.2	7.7	n.a.	7.6	8.0	7.6	n.a.	n.a.	7.6	7.8
Institute 3	7.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	8.0	n.a.	n.a.	8.1
Institute 4	6.3	6.2	6.2	6.3	6.1	6.4	6.6	6.5	7.6	7.4	7.5
Institute 5	6.9	6.9	6.9	n.a.	n.a.	n.a.	6.9	6.9	n.a.	n.a.	6.9
Minimum	6.1										
Maximum	8.8										
Mean	7.4										
SD	0.8										
COV%	10.3										

n.a. = not analyzed

Table 20: Redox-potential [mV] of pig manure at the given sampling points

Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
Institute 1	-421	-413	-421	-404	-415	-424	-410	-423	-408	-419	-427
Institute 2	-373	n.a.	n.a.	n.a.	-380	n.a.	n.a.	-325	-371	-375	-381
Institute 3	-373	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-251	n.a.	-328
Institute 4	-450	n.a.	n.a.	n.a.	-448	n.a.	-450	-328	-328	-266	-307
Institute 5	n.a.	n.a.	n.a.	n.a.	n.a.	-366	-362	-360	-370	n.a.	-370
Institute 6	-371	n.a.	n.a.	n.a.	n.a.	n.a.	-336	-374	-409	-378[1)]	-207
Minimum	-450										
Maximum	-207										
Mean	-374.3										
SD	54.2										
COV%	14.5										

n.a. = not analyzed; [1)] measurements at day 70

Table 21: Redox-potential [mV] of cattle manure at the given sampling points

Time [d]	0	3	7	10	14	21	28	42	56	72	90
Institute 1	-398	-399	-405	-420	-413	-432	-422	-431	-410	-416	-464
Institute 2	-261	-436	-453	n.a.	-420	-378	-410	n.a.	n.a.	-342	-325
Institute 3	-394	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-348	n.a.	n.a.	-369
Institute 4	-230	-165	-192	-143	-161	-162	-162	-143	-130	-114	-353
Institute 5	-270	-268	-250	n.a.	n.a.	n.a.	-270	-272	n.a.	n.a.	-300
Minimum	-464										
Maximum	-114										
Mean	-316.2										
SD	109.7										
COV%	34.7										

n.a. = not analyzed

3.5 Dissipation of the parent compound

DT_{50}-vlaues were determined for the experiments performed by Institutes 1, 2, 3 and 4 on both the transformation of florfenicol in pig manure and imidacloprid in cattle manure. For florfenicol at 20°C in pig manure the range observed for mean DT_{50}-values was between 0.17 d and 0.41 d. For imidacloprid in cattle manure, mean DT_{50}-values were in the range of 17.4 d to 40.0 d.

DT_{50}-vlaues were also determined in separate experiments on the transformation of florfenicol in pig manure at a reduced temperature of 10°C (^{14}C-labeled and unlabeled). The aim of this experiment was to get more insight into the transformation pathway of florfenicol. At 20°C transformation proceeds rapidly. However, the typical temperature in a manure tank is closer to 10°C (Weinfurtner, 2011). Therefore, special attention was put on the identification of transformation products at a lower transformation rate condition at 10°C in this experiment. A detailed description of the experiments is presented in section 3.11.

DT_{50} values determined at 10°C were normalized to 20°C for comparison with the results from the other institutes by using the Q_{10} value of 2.58 as described in section 2.4. The results are presented in Table 22.

Table 22: DT_{50}- values [d] determined in the ring test for the test substance florfenicol (parent) at a temperature of 10°C and normalized to 20°C, fitting of SFO model

Test substance	Florfenicol (10°C)		Florfenicol (normalized to 20°C)	
	unlabeled	^{14}C-labeled	unlabeled	^{14}C-labeled
Replicate 1	1.27	2.335	0.492	0.905
Replicate 2	1.52	n.a.	0.589	n.a.
N	2	1	2	1
Mean	1.39	2.335	0.538	0.905

n.a. = not analyzed

The results for all Institutes are presented in Table 23 (DT_{50}-values), Figure 15 (plots for florfenicol at 10°C), Figure 16 (plots for florfenicol at 20°C) and Figure 17 (plots for imidacloprid at 20°C). Results from Institute 4 were not considered for the evaluation due to deviations from the proposed extraction method (use of very unpolar solvents only, see chapter 3.9.1). DT_{50}-values could not be calculated for institute 5 since no chemical analyses have been performed.

The acceptability of the fits was judged on the basis of the chi^2-error and visual assessment. Mean chi^2-error was 21.3 ± 6.3% for florfenicol and 13.4 ± 4.4% for imidacloprid. Although chi^2-error values are above 15% in most cases for florfenicol and imidacloprid, residual plots indicate no systematic error of the SFO model.

The resulting plots for dissipation of parent compounds (overall mean and standard deviation) based on predicted values (SFO kinetics) are presented in Figure 18 (florfenicol at 20°C), Figure 19 (florfenicol at 10°C) and Figure 20 (imidacloprid at 20°C).

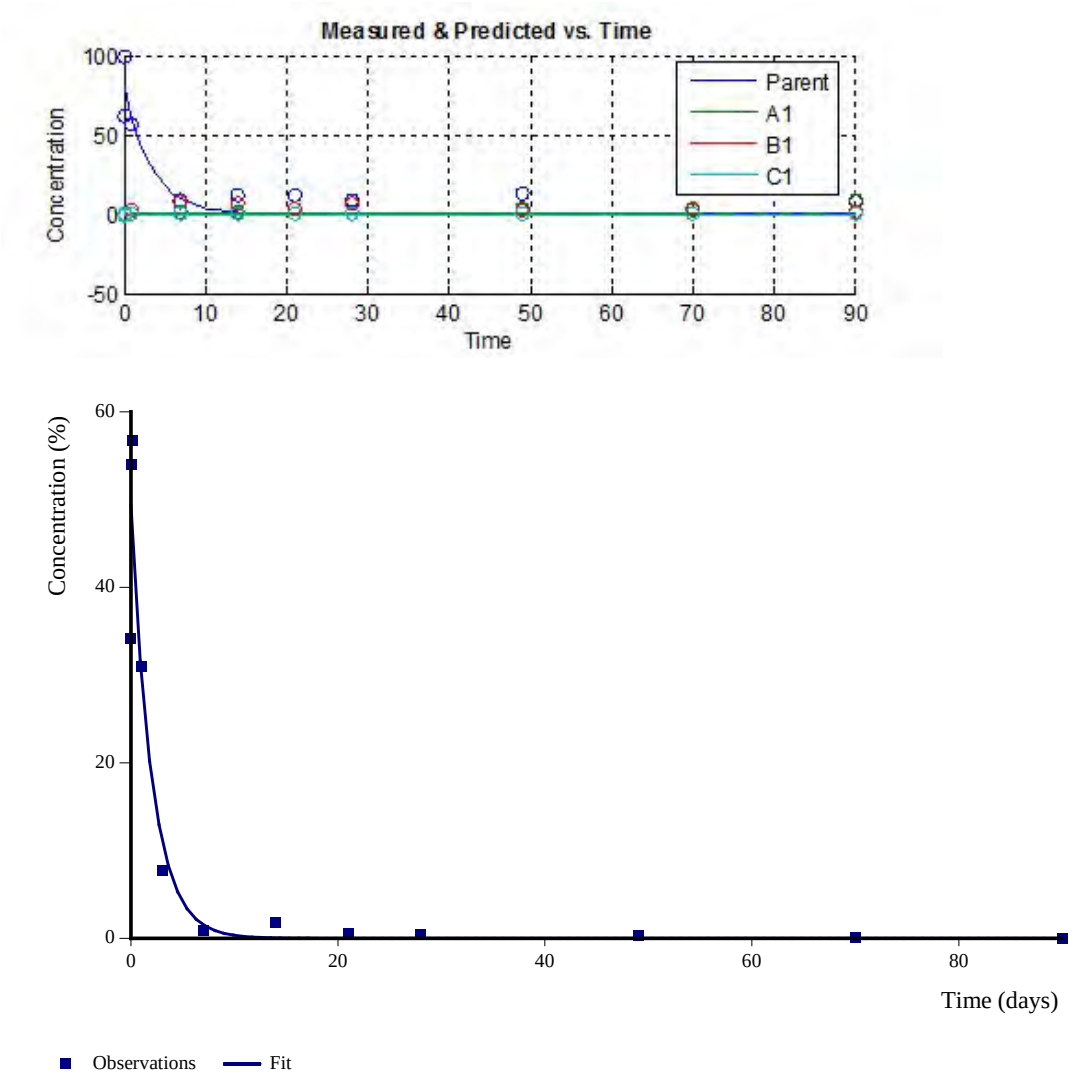
Table 23: DT_{50}- values [d] determined in the ring test for the test substances florfenicol (parent) and imidacloprid (parent) at 20°C, fitting of SFO model.

Test substance	Florfenicol					Imidacloprid			
Institute	1	2	3	6[1)]	4	1	2	3	4
Replicate 1	0.430	0.332	0.185	0.492	0.008	21.98	17.03	21.59	43.04
Replicate 2	0.394	0.490	0.155	0.589	0.008	21.58	17.02	21.00	37.16
Replicate 3	0.296	0.329	n.a.	n.a.	0.008	21.16	17.51	n.a.	40.12
Replicate 4	0.373	0.357	n.a.	n.a.	n.a.	21.53	16.82	n.a.	n.a.
Replicate 5	0.428	0.344	n.a.	n.a.	n.a.	22.18	18.24	n.a.	n.a.
Replicate 6	0.558	0.353	n.a.	n.a.	n.a.	21.75	17.86	n.a.	n.a.
N	6	6	2	2	3	6	6	2	3
Mean	0.406	0.364	0.169	0.538	0.008	21.69	17.41	21.29	40.03
SD	0.207	0.149	0.125	0.127	0.007	0.017	0.032	0.020	0.073
COV (%)	20.97	15.02	12.56	12.78	0.726	1.66	3.16	1.95	7.36
Minimum	0.169 [2)]					17.41			
Maximum	0.538 [2)]					40.03			
Overall mean	0.341 [2)]					23.82			
Overall SD	0.494 [2)]					0.360			
Overall COV (%)	52.61 [2)]					37.23			

n.a. = not analyzed; [1)] Values for unlabeled florfenicol; [2)] Results from Institute 4 are not considered for evaluation

The calculation of DT_{50}-values for detected transformation products is documented in Annex 4.

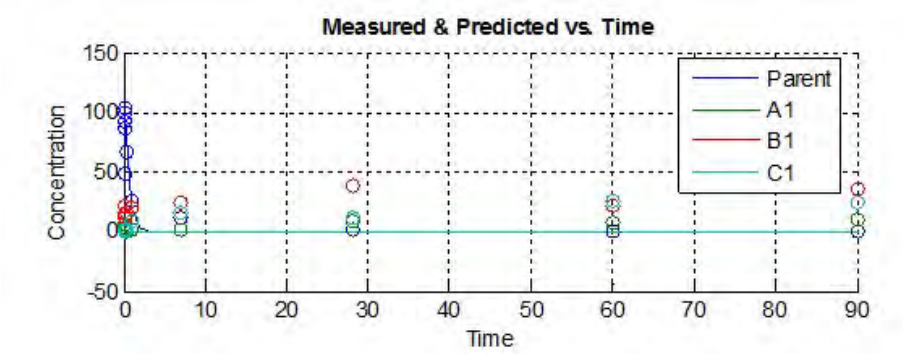
Figure 15: Plots for transformation of ^{14}C-labeled and unlabeled florfenicol in pig manure at a temperature of 10°C. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool).



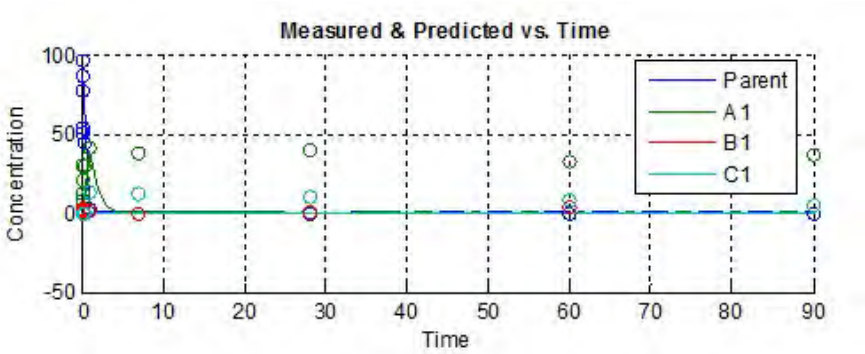
top: plot for ^{14}C-labeled florfenicol (only one replicate, KinGUI software tool); bottom: plot for unlabeled florfenicol (mean of two replicates, CAKE software tool, version 3.1)

Figure 16: Plots for transformation of florfenicol in pig manure for all institutes based on arithmetic mean of all replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen.

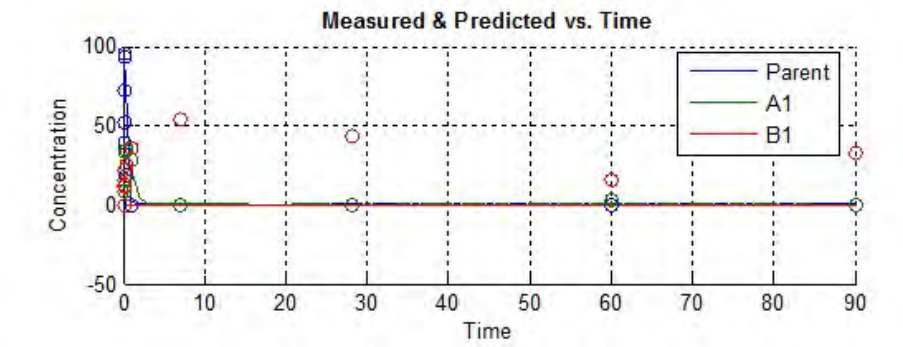
Institute 1



Institute 2



Institute 3



Institute 4

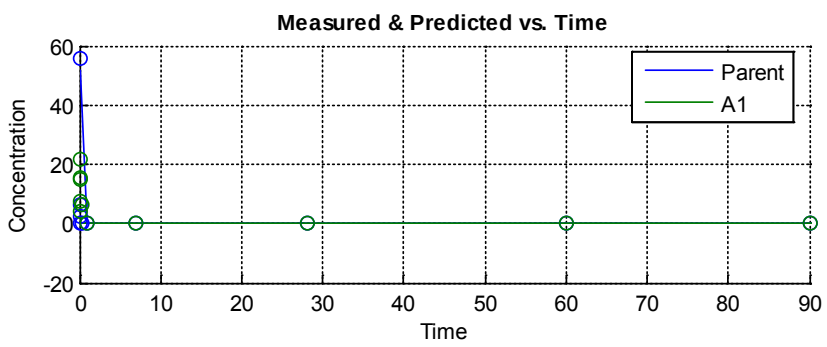
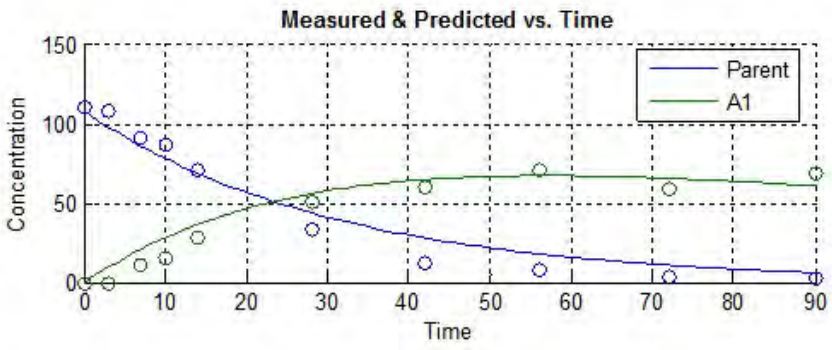
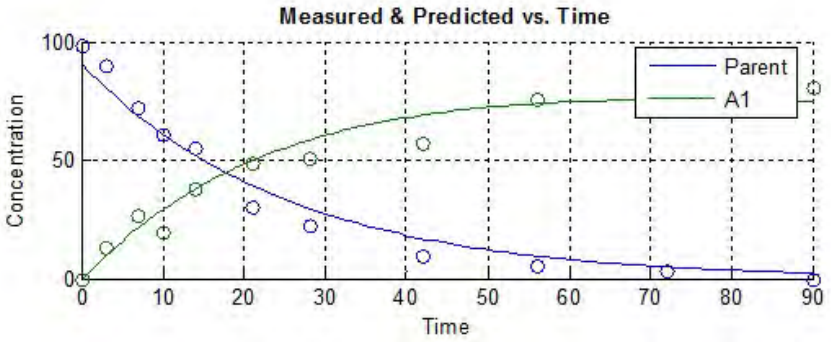


Figure 17: Plots for transformation of imidacloprid in cattle manure for all institutes based on arithmetic mean of all replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation products TP1 and TP2 (named as A1 and B1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of transformation products.

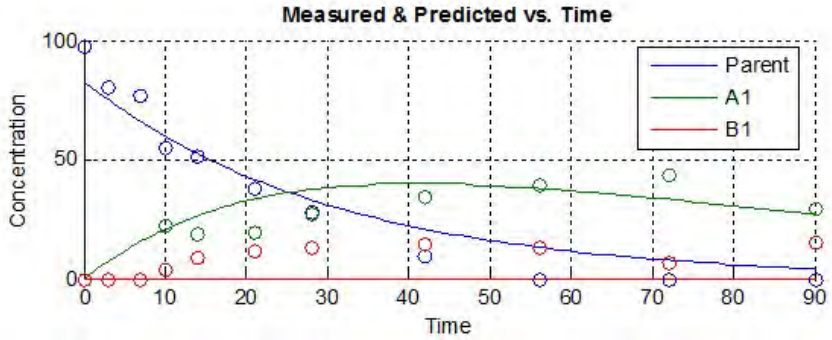
Institute 1



Institute 2



Institute 3



Institute 4

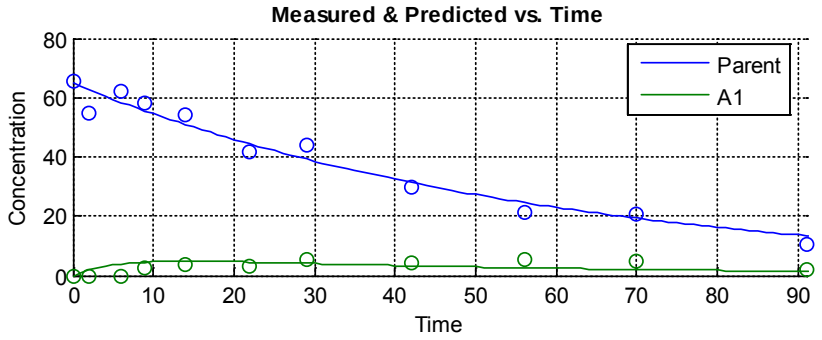


Figure 18: Dissipation plot for florfenicol in pig manure at 20°C based on overall mean of mean values for institutes (3 institutes, predicted values, SFO kinetics) and standard deviation (shaded area).

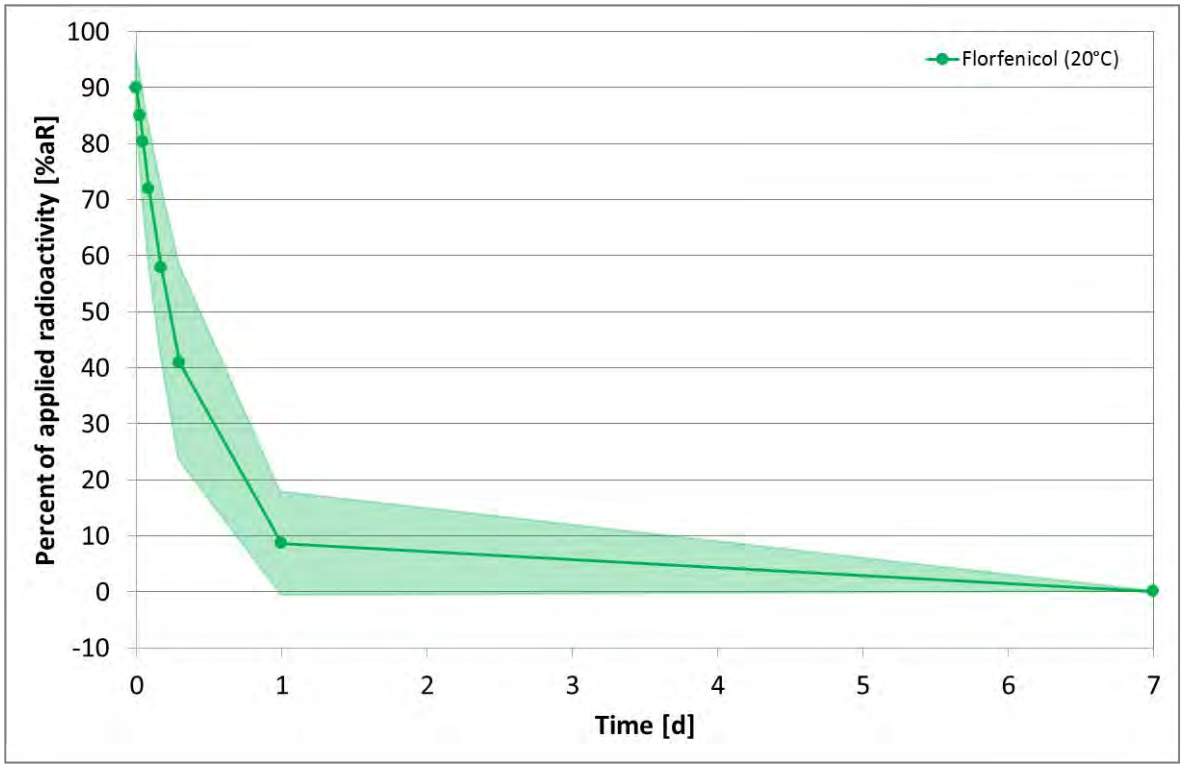


Figure 19: Dissipation plot for florfenicol in pig manure at 10°C based on mean of two replicates (institute 6, predicted values, SFO kinetics) and standard deviation (shaded area).

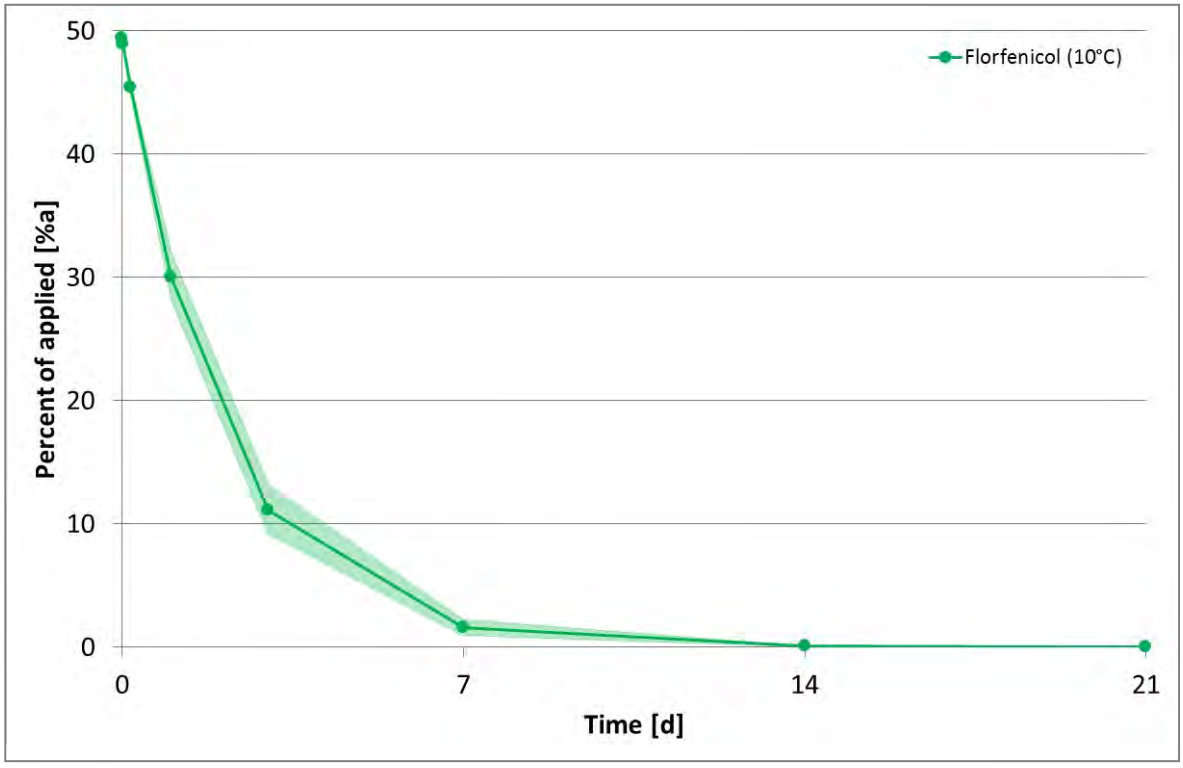
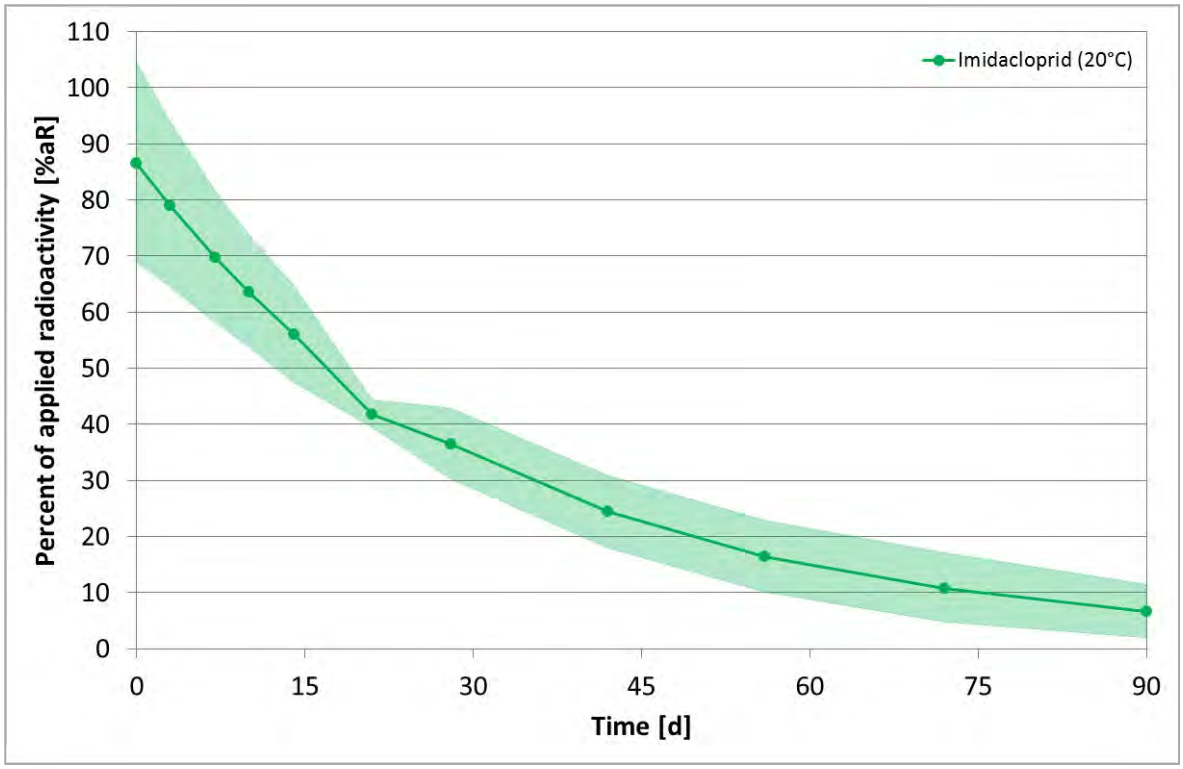


Figure 20: Dissipation plot for imidacloprid in cattle manure at 20°C based on overall mean of mean values for institutes (4 institutes, predicted values, SFO kinetics) and standard deviation (shaded area).



3.6 Mineralization

No relevant mineralization (neither $^{14}CO_2$ nor $^{14}CH_4$) was observed in the experiments with florfenicol and imidacloprid. Therefore, results are only presented in tabular form (see Table 24 to Table 27).

The highest amount of $^{14}CO_2$ in the experiments with florfenicol was measured by Institute 4 at the end of the incubation period (6.0% aR). In the experiment with imidacloprid, a high amount of $^{14}CH_4$ (33.2% aR) was measured by Institute 3 at day 7 of the incubation period. However, this value can be regarded as measuring error since the mass balance for that replicate is clearly too high and the other replicate shows no mineralization. For that reason the values were excluded from further analysis.

Total mineralization at the end of the incubation period was in the range between 0% aR and 6% aR for florfenicol and below 1% aR for imidacloprid in all experiments.

Earlier experiments during method development with different test substances however underline the importance of determining mineralization as a parameter in studies on transformation in manure. Using e.g. salicylic acid, a VMP, as test substance up to 67.9% aR of CO_2 and up to 4.8% aR of CH_4 formation were observed (Hennecke et al., 2015), the determination is warranted for every experiment, as otherwise no complete mass balances can be obtained and it is thus impossible to assess the quality of the study.

Table 24: mean-values and standard deviation (SD) of mineralization to $^{14}CO_2$ [% aR] measured for the transformation of ^{14}C-florfenicol in pig manure, 5 institutes.

Institute	Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
1	Mean	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.01	0.03	0.05	0.08
	SD	0.00	0.01	0.23	0.01	0.00	0.00	0.00	0.01	0.01	0.02	0.02
2	Mean	0.06	0.03	0.02	0.02	0.03	0.04	0.07	0.11	0.22	0.69	1.44
	SD	0.02	0.00	0.00	0.01	0.00	0.01	0.01	0.03	0.01	0.13	0.88
3	Mean	0.00	0.14	0.10	0.11	0.10	0.10	0.13	0.07	0.65	0.70	0.69
	SD	0.00	0.06	0.02	0.02	0.01	0.01	0.04	0.01	0.37	0.46	0.14
4	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.88	1.64	5.95
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.16	0.11	1.09
5	Mean	0.00	n.a.	0.00	0.00	n.a.	0.00	0.00	n.a.	0.00	n.a.	0.00
	SD	0.00	n.a.	0.00	0.00	n.a.	0.00	0.00	n.a.	0.00	n.a.	0.00

n.a. = not analyzed

Table 25: mean-values and standard deviation (SD) of mineralization to $^{14}CH_4$ [% aR] measured for the transformation of ^{14}C-florfenicol in pig manure, 5 institutes.

Institute	Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
1	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.03
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01
3	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. = not analyzed

Table 26: mean-values and standard deviation (SD) of mineralization to $^{14}CO_2$ [% aR] measured for the transformation of ^{14}C-imidacloprid in cattle manure, 5 institutes.

Institute	Time [d]	0	3	7	10	14	21	28	42	56	72	90
1	Mean	0.00	0.00	0.17	0.00	0.00	n.a.	0.00	0.03	0.01	0.03	0.05
	SD	0.00	0.00	0.23	0.01	0.00	n.a.	0.00	0.03	0.01	0.01	0.04
2	Mean	0.00	0.03	0.04	0.05	0.13	0.03	0.05	0.04	0.11	0.08	0.06
	SD	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01
3	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	Mean	0.00	0.00	0.00	n.a.	n.a.	n.a.	0.00	0.00	n.a.	n.a.	0.00
	SD	0.00	0.00	0.00	n.a.	n.a.	n.a.	0.00	0.00	n.a.	n.a.	0.00

n.a. = not analyzed

Table 27: mean-values and standard deviation (SD) of mineralization to $^{14}CH_4$ [% aR] measured for the transformation of ^{14}C-imidacloprid in cattle manure, 5 institutes.

Institute	Time [d]	0	3	7	10	14	21	28	42	56	72	90
1	Mean	0.00	0.00	0.00	0.00	0.00	n.a.	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	n.a.	0.00	0.00	0.00	0.00	0.00
2	Mean	0.00	0.01	0.00	0.01	0.03	0.00	0.01	0.01	0.02	0.02	0.01
	SD	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01
3	Mean	0.00	0.00	16.58	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
	SD	0.00	0.00	23.45	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
4	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. = not analyzed

3.7 Extractable residues

A typical time dependent behavior of extractable residues (ER) could be observed in both experiments with florfenicol in pig manure (Table 28) and with imidacloprid in cattle manure (Table 29) showing a decrease over time. For florfenicol one laboratory (Institute 4) used extraction methods differing considerably in solvent polarity from the recommended methods (for details see Annex 3). Therefore, these data had to be excluded from further evaluation but are nevertheless displayed here for information purposes.

At the end of the study with florfenicol in pig manure, after normalization, extractable residues ranged from 32.7% aR (Institute 3) to 70.9% aR (Institute 1). At the end of the study with imidacloprid in cattle manure, after normalization, extractable residues ranged from 26.4% aR (Institute 4) to 84.2% aR (Institute 5). The variability between the results of the different laboratories (COV of 31.0% for florfenicol and COV of 40.7% for imidacloprid) can be explained by differences in extraction procedures used by the participating institutes.

Table 28: mean-values, standard deviation (SD) and coefficient of variation (COV%) of extractable residues [% aR] measured for ^{14}C-florfenicol in pig manure, 5 institutes.

Institute	Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
1	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	99.5	103.6	104.5	105.0	79.3	92.4	62.6	58.9	68.6	61.7	71.2
	SD	2.7	3.2	2.8	2.4	5.0	2.3	3.1	4.5	7.8	1.2	0.9
	COV	2.7	3.1	2.7	2.3	6.3	2.5	5.0	7.6	11.4	1.9	1.3
2	n	6	6	6	6	6	6	6	6	6	4	4
	Mean	96.5	95.3	90.6	79.9	80.6	73.1	60.9	50.0	51.4	44.2	46.5
	SD	3.7	0.6	1.4	3.1	2.0	1.1	1.3	0.7	1.2	1.0	3.4
	COV	3.8	0.6	1.5	3.9	2.5	1.5	2.1	1.4	2.3	2.3	7.3
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	93.3	94.7	90.6	89.0	84.5	77.2	63.3	53.4	42.8	45.2	32.4
	SD	2.0	0.5	1.2	0.7	2.1	0.3	5.3	1.9	1.2	5.6	6.8
	COV	2.1	0.5	1.3	0.8	2.5	0.4	8.4	3.6	2.8	12.4	21.0
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	62.3	32.8	23.4	20.9	14.6	11.5	4.0	6.3	6.3	2.9	3.1
	SD	11.0	9.1	6.0	0.7	4.7	2.9	0.1	1.5	1.6	0.5	0.5
	COV	17.7	27.7	25.6	3.3	32.2	25.2	2.5	23.8	25.4	17.2	16.1
5	n	3	n.d.	3	3	n.d.	3	3	3	3	n.d.	3
	Mean	91.7	n.d.	95.6	83.2	n.d.	81.5	81.5	76.6	71.1	n.d.	48.5
	SD	2.5	n.d.	4.4	3.7	n.d.	4.9	5.0	20.6	11.0	n.d.	2.2
	COV	2.7	n.d.	4.6	4.4	n.d.	6.0	6.1	26.9	15.5	n.d.	4.5
Total [1)]	**n**	**4**	**3**	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**3**	**4**
	Min	**91.7**	**94.7**	**90.6**	**79.9**	**79.3**	**73.1**	**60.9**	**50.0**	**42.8**	**44.2**	**32.4**
	Max	**99.5**	**103.6**	**104.5**	**105.0**	**84.5**	**92.4**	**81.5**	**76.6**	**71.1**	**61.7**	**71.2**
	Mean	**95.2**	**97.9**	**95.3**	**89.3**	**81.5**	**81.1**	**67.1**	**59.7**	**58.5**	**50.4**	**49.6**
	SD	**3.5**	**5.0**	**6.6**	**11.1**	**2.7**	**8.3**	**9.7**	**11.8**	**13.6**	**9.8**	**16.1**
	COV%	**3.6**	**5.1**	**6.9**	**12.5**	**3.3**	**10.3**	**14.5**	**19.8**	**23.3**	**19.6**	**32.3**

n.d. = not determined; [1)] Results from Institute 4 are not considered for evaluation

Table 29: mean-values, standard deviation (SD) and coefficient of variation (COV%) of extractable residues [% aR] measured for ^{14}C-imidacloprid in cattle manure, 5 institutes.

Institute	Time [d]	0	3	7	10	14	21	28	42	56	72	90
1	n	6	6	6	6	6	n.d.	6	6	6	6	6
	Mean	109.9	107.6	102.8	104.1	100.2	n.d.	87.2	74.9	80.0	63.5	71.9
	SD	2.2	3.2	1.4	2.9	3.7	n.d.	3.6	1.8	6.4	1.0	1.7
	COV	2.0	3.0	1.4	2.8	3.7	n.d.	4.1	2.4	8.0	1.6	2.4
2	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	100.4	102.8	98.4	86.3	92.8	78.6	82.5	79.1	80.6	79.8	81.0
	SD	1.0	0.3	1.0	1.1	1.2	6.5	1.4	5.7	3.0	2.9	2.3
	COV	1.0	0.3	1.0	1.3	1.3	8.3	1.7	7.2	3.7	3.6	2.8
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	97.2	80.4	76.8	81.3	79.5	69.0	68.2	58.6	55.0	50.2	44.2
	SD	2.1	6.4	8.8	2.8	3.2	1.0	1.8	2.7	0.9	3.1	7.2
	COV	2.2	8.0	11.5	3.4	4.0	1.4	2.6	4.6	1.6	6.2	16.3
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	70.9	78.3	71.1	74.1	65.5	53.2	64.6	48.6	38.9	40.1	22.9
	SD	9.4	3.0	1.9	2.4	1.9	3.4	0.8	3.0	5.2	1.7	1.9
	COV	13.3	3.8	2.7	3.2	2.9	6.4	1.2	6.2	13.4	4.2	8.3
5	n	3	3	3	n.d.	n.d.	n.d.	3	3	n.d.	n.d.	3
	Mean	86.2	79.1	82.2	n.d.	n.d.	n.d.	71.1	72.3	n.d.	n.d.	73.6
	SD	8.4	7.1	10.2	n.d.	n.d.	n.d.	7.7	4.8	n.d.	n.d.	9.0
	COV	9.7	9.0	12.4	n.d.	n.d.	n.d.	10.8	6.6	n.d.	n.d.	12.2
Total	**n**	**5**	**5**	**5**	**4**	**4**	**3**	**5**	**5**	**4**	**4**	**5**
	Min	**70.9**	**78.3**	**71.1**	**74.1**	**65.5**	**53.2**	**64.6**	**48.6**	**38.9**	**40.1**	**22.9**
	Max	**109.9**	**107.6**	**102.8**	**104.1**	**100.2**	**78.6**	**87.2**	**79.1**	**80.6**	**79.8**	**81.0**
	Mean	**92.9**	**89.6**	**86.3**	**86.5**	**84.5**	**66.9**	**74.7**	**66.7**	**63.6**	**58.4**	**58.7**
	SD	**14.9**	**14.3**	**13.8**	**12.8**	**15.3**	**12.8**	**9.7**	**12.7**	**20.4**	**17.2**	**24.4**
	COV%	**16.1**	**16.0**	**16.0**	**14.8**	**18.1**	**19.1**	**13.0**	**19.0**	**32.0**	**29.4**	**41.6**

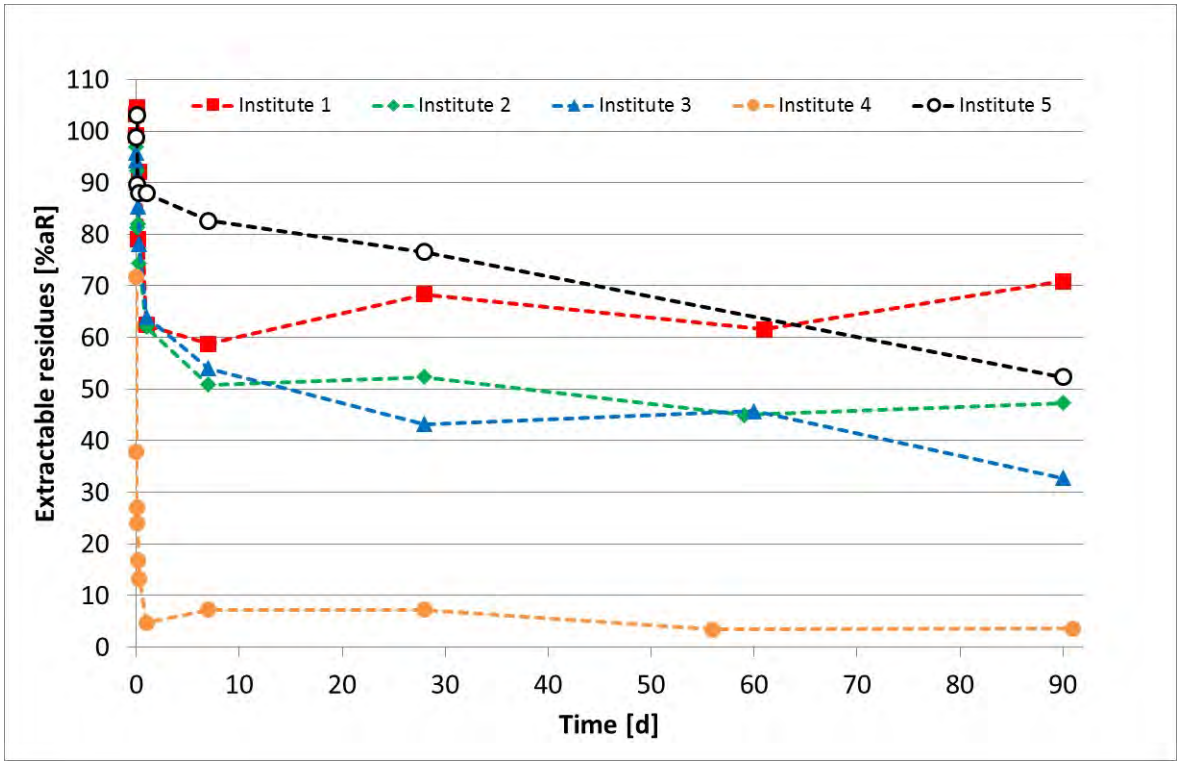
n.d. = not determined

Table 30: mean-values, standard deviation (SD)) and coefficient of variation (COV%) of extractable residues [normalized, %aR] measured for ^{14}C-florfenicol in pig manure, 5 institutes.

Institute	Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
1	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	99.1	103.3	104.2	104.7	79.1	92.1	62.4	58.7	68.4	61.5	70.9
	SD	2.7	3.2	2.8	2.4	5.0	2.3	3.0	4.5	7.7	1.2	0.9
	COV	2.7	3.1	2.7	2.3	6.3	2.5	4.9	7.6	11.3	2.0	1.3
2	n	6	6	6	6	6	6	6	6	6	4	4
	Mean	98.3	96.9	92.2	81.4	82.0	74.4	62.0	50.9	52.4	45.0	47.3
	SD	3.7	0.6	1.4	3.1	2.0	1.1	1.3	0.7	1.2	1.0	3.4
	COV	3.8	0.7	1.5	3.8	2.5	1.5	2.2	1.4	2.3	2.2	7.3
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	94.3	95.8	91.6	90.0	85.4	78.0	64.0	54.0	43.2	45.7	32.7
	SD	2.0	0.5	1.2	0.8	2.1	0.3	5.4	1.9	1.2	5.6	6.9
	COV	2.1	0.5	1.3	0.8	2.5	0.3	8.4	3.5	2.9	12.3	21.0
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	71.8	37.8	27.0	24.1	16.9	13.2	4.7	7.2	7.3	3.4	3.5
	SD	12.6	10.5	7.0	0.8	5.5	3.4	0.1	1.8	1.8	0.6	0.6
	COV	17.6	27.8	25.8	3.5	32.4	25.4	3.0	24.5	25.3	17.9	17.1
5	n	3	n.d.	3	3	n.d.	3	3	3	3	n.d.	3
	Mean	98.8	n.d.	103.1	89.7	n.d.	87.9	87.9	82.6	76.6	n.d.	52.3
	SD	2.7	n.d.	4.8	4.0	n.d.	5.3	5.4	22.2	11.8	n.d.	2.4
	COV	2.7	n.d.	4.6	4.5	n.d.	6.0	6.2	26.9	15.4	n.d.	4.6
Total [1)]	**n**	**4**	**3**	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**3**	**4**
	Min	**94.3**	**95.8**	**91.6**	**81.4**	**79.1**	**74.4**	**62.0**	**50.9**	**43.2**	**45.0**	**32.7**
	Max	**99.1**	**103.3**	**104.2**	**104.7**	**85.4**	**92.1**	**87.9**	**82.6**	**76.6**	**61.5**	**70.9**
	Mean	**97.6**	**98.7**	**97.8**	**91.4**	**82.2**	**83.1**	**69.1**	**61.6**	**60.1**	**50.7**	**50.8**
	SD	**2.2**	**4.0**	**6.8**	**9.7**	**3.2**	**8.3**	**12.6**	**14.4**	**15.1**	**9.4**	**15.8**
	COV%	**2.3**	**4.1**	**7.0**	**10.6**	**3.9**	**10.0**	**18.2**	**23.4**	**25.1**	**18.4**	**31.0**

n.d. = not determined; [1)] Results from Institute 4 are not considered for evaluation

Figure 21: Comparison of extractable residues [normalized, %aR] obtained from transformation of ^{14}C-florfenicol in pig manure for all participants of the ring test.



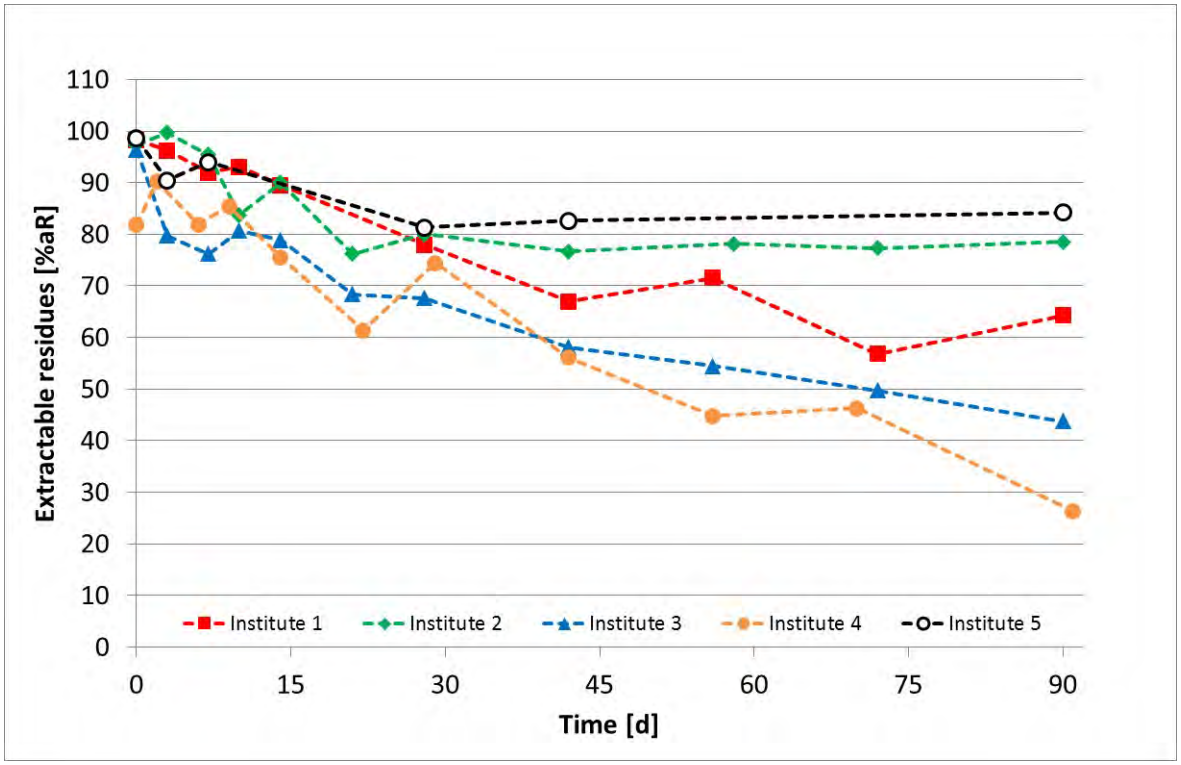
Results from Institute 4 were not considered for further evaluation because of deviations from the proposed extraction scheme.

Table 31: mean-values and standard deviation (SD) of extractable residues [normalized, %aR] measured for ^{14}C- imidacloprid in cattle manure, 5 institutes.

Institute	Time [d]	0	3	7	10	14	21	28	42	56	72	90
1	n	6	6	6	6	6	n.d.	6	6	6	6	6
	Mean	98.3	96.2	92.0	93.1	89.5	n.d.	77.9	66.9	71.6	56.8	64.3
	SD	1.9	2.9	1.3	2.6	3.3	n.d.	3.2	1.6	5.7	0.9	1.5
	COV	2.0	3.0	1.4	2.8	3.7	n.d.	4.1	2.4	7.9	1.5	2.4
2	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	97.3	99.7	95.4	83.7	90.0	76.2	80.0	76.7	78.2	77.3	78.5
	SD	0.9	0.3	1.0	1.1	1.2	6.3	1.3	5.5	3.0	2.8	2.3
	COV	1.0	0.3	1.1	1.3	1.3	8.2	1.6	7.2	3.8	3.7	2.9
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	96.4	79.7	76.2	80.7	78.9	68.4	67.6	58.1	54.5	49.8	43.8
	SD	2.1	6.3	8.7	2.8	3.1	1.0	1.8	2.7	0.9	3.1	7.2
	COV	2.2	7.9	11.4	3.5	4.0	1.5	2.6	4.6	1.6	6.2	16.4
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	81.8	90.2	81.9	85.4	75.5	61.3	74.4	56.0	44.8	46.2	26.4
	SD	10.8	3.5	2.2	2.8	2.2	3.9	1.0	3.5	6.0	2.0	2.1
	COV	13.2	3.9	2.6	3.3	2.9	6.4	1.3	6.2	13.4	4.2	8.1
5	n	3	3	3	n.d.	n.d.	n.d.	3	3	n.d.	n.d.	3
	Mean	98.5	90.4	94.0	n.d.	n.d.	n.d.	81.3	82.6	n.d.	n.d.	84.2
	SD	9.6	8.1	11.6	n.d.	n.d.	n.d.	8.8	5.5	n.d.	n.d.	10.3
	COV	9.7	9.0	12.4	n.d.	n.d.	n.d.	10.8	6.7	n.d.	n.d.	12.2
Total	**n**	**5**	**5**	**5**	**4**	**4**	**3**	**5**	**5**	**4**	**4**	**5**
	Min	**81.8**	**79.7**	**76.2**	**80.7**	**75.5**	**61.3**	**67.6**	**56.0**	**44.8**	**46.2**	**26.4**
	Max	**98.5**	**99.7**	**95.4**	**93.1**	**90.0**	**76.2**	**81.3**	**82.6**	**78.2**	**77.3**	**84.2**
	Mean	**94.5**	**91.3**	**87.9**	**85.7**	**83.5**	**68.6**	**76.2**	**68.1**	**62.3**	**57.5**	**59.4**
	SD	**7.2**	**7.6**	**8.4**	**5.3**	**7.4**	**7.4**	**5.5**	**11.5**	**15.3**	**13.9**	**24.2**
	COV%	**7.6**	**8.3**	**9.6**	**6.2**	**8.9**	**10.8**	**7.2**	**16.9**	**24.6**	**24.2**	**40.7**

n.d. = not determined

Figure 22: Comparison of extractable residues [normalized, %aR] obtained from transformation of ^{14}C-imidacloprid in cattle manure for all participants of the ring test.



3.8 Non-extractable residues

For pig manure an increase of NER until day 7 was observed by Institutes 1, 2 and 3. Afterwards, amounts of NER remain more or less constant until the end of the study. Different curve progressions were obtained by Institute 4 and Institute 5. Whereas NER slightly increase until day 60 at Institute 5, a rapid increase followed by a rapid decline was measured in the experiment by Institute 4. However, results from Institute 4 are not considered for evaluation since extraction methods differing considerably in solvent polarity from the recommended methods have been used (see chapter 3.9.1). At the end of the incubation period, after normalization, the amount of NER was between 30.8% aR (Institute 5) and 61.0% aR (Institute 3) (see Table 32, Table 33 and Figure 23).

A time dependent behavior of non-extractable residues (NER) can be observed in cattle manure showing an increase throughout the test period (Table 34 and Table 35). The amount of NER at the end of the study, after normalization, was between 11.3% aR (Institute 2) and 65.3% aR (Institute 4) (Figure 24). The variability between the results of the different laboratories (COV of 32.1% for florfenicol and COV of 54.1% for imidacloprid) can be explained by differences in extraction procedures used by the participating institutes.

Table 32: minimum (Min), maximum (Max) and mean-values, standard deviation (SD) and coefficient of variation (COV%) of NER [% aR] for the transformation of ^{14}C-florfenicol in pig manure, 5 institutes

Institute	Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
1	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	0.9	1.7	2.9	3.5	15.0	15.4	29.4	41.3	38.3	39.5	34.5
	SD	0.1	0.2	0.1	1.0	1.7	1.7	3.5	4.5	6.2	1.6	1.7
	COV	8.8	12.9	4.1	27.1	11.7	11.8	12.3	11.1	16.3	4.1	5.0
2	n	6	6	6	6	6	6	6	6	6	4	4
	Mean	1.7	4.2	5.7	13.2	15.9	23.6	35.2	47.8	43.7	48.1	50.8
	SD	0.2	0.3	1.1	3.7	1.4	1.4	3.7	2.1	4.0	4.3	3.5
	COV	13.3	7.5	19.8	28.1	8.5	5.8	10.6	4.5	9.1	8.9	7.0
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	5.6	6.8	8.6	8.4	14.2	20.5	30.8	43.7	45.7	44.5	60.3
	SD	2.6	6.0	0.1	3.3	1.1	0.5	0.5	3.5	2.3	12.2	6.5
	COV	45.5	87.4	1.6	39.7	7.8	2.4	1.7	8.0	5.1	27.3	10.8
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	24.4	47.9	54.9	61.5	77.0	65.5	64.2	18.9	15.8	16.8	9.7
	SD	1.0	2.1	1.6	3.0	10.0	3.3	2.6	1.3	2.0	2.4	1.5
	COV	4.0	4.4	3.0	4.9	13.0	5.1	4.0	7.1	12.8	14.1	15.6
5	n	3	n.d.	3	3	n.d.	3	3	3	3	n.d.	3
	Mean	1.1	n.a.	2.1	2.5	n.a.	3.8	7.2	18.3	12.8	n.a.	28.6
	SD	0.4	n.a.	0.4	1.0	n.a.	3.2	0.4	0.7	1.0	n.a.	2.6
	COV	33.7	n.a.	17.6	39.3	n.a.	84.5	5.8	4.1	8.0	n.a.	8.9
Total [1)]	**n**	**4**	**3**	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**3**	**4**
	Min	**0.9**	**1.7**	**2.1**	**2.5**	**14.2**	**3.8**	**7.2**	**18.3**	**12.8**	**38.8**	**28.6**
	Max	**5.6**	**6.8**	**8.6**	**13.2**	**15.9**	**23.6**	**35.2**	**47.8**	**45.7**	**48.1**	**60.3**
	Mean	**2.3**	**4.2**	**4.8**	**6.9**	**15.1**	**15.8**	**25.6**	**37.8**	**35.1**	**44.0**	**43.6**
	SD	**2.2**	**2.6**	**2.9**	**4.9**	**0.8**	**8.7**	**12.6**	**13.3**	**15.2**	**4.3**	**14.6**
	COV%	**96.5**	**60.9**	**60.8**	**71.2**	**5.6**	**54.8**	**49.0**	**35.1**	**43.3**	**9.8**	**33.5**

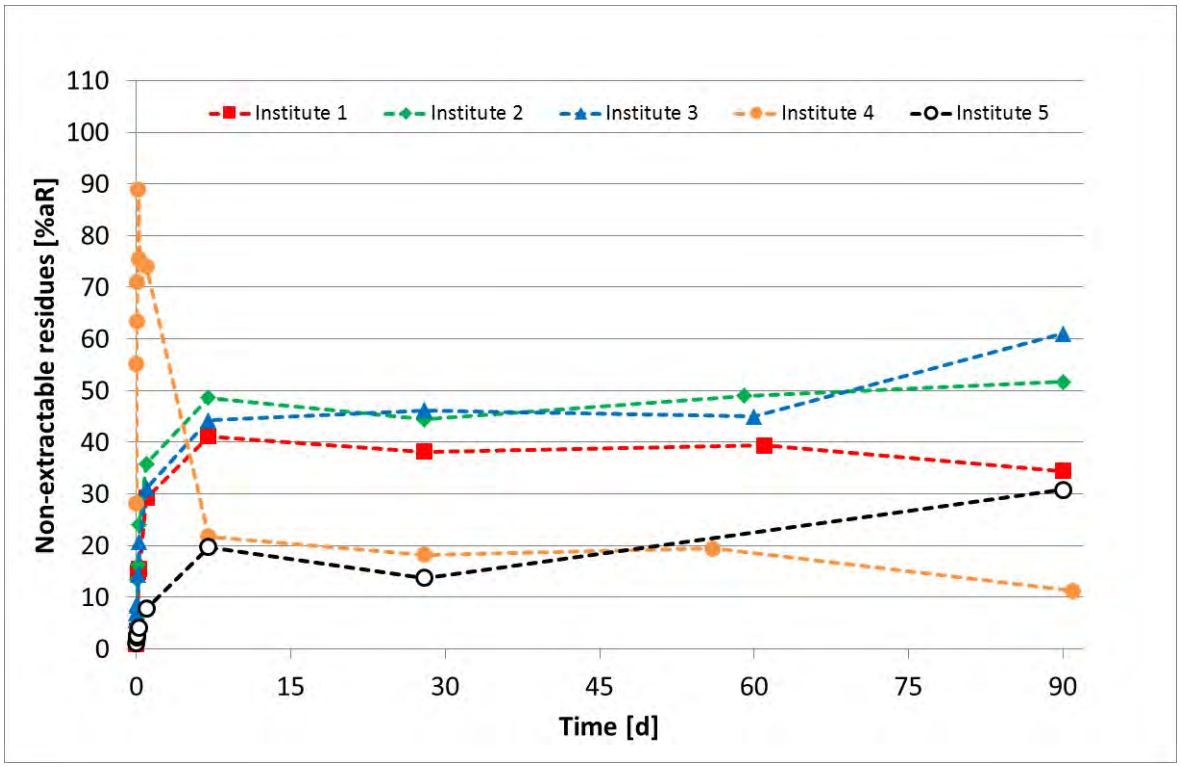
n.a. = not analyzed; [1)] Results from Institute 4 are not considered for evaluation

Table 33: minimum (Min), maximum (Max) and mean-values, standard deviation (SD) and coefficient of variation (COV%) of NER [normalized, % aR] for the transformation of ^{14}C-florfenicol in pig manure, 5 institutes.

Institute	Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
1	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	0.9	1.7	2.9	3.5	15.0	15.4	29.3	41.1	38.2	39.4	34.4
	SD	0.1	0.2	0.1	1.0	1.8	1.8	2.5	5.3	6.2	2.2	2.0
	COV	8.8	12.9	4.1	27.1	11.7	11.8	8.4	13.0	16.1	5.6	5.8
2	n	6	6	6	6	6	6	6	6	6	4	4
	Mean	1.7	4.3	5.8	13.5	16.2	24.0	35.9	48.6	44.4	48.9	51.7
	SD	0.2	0.3	1.2	3.8	1.4	1.4	3.8	2.2	4.1	4.4	3.6
	COV	13.3	7.5	19.8	28.1	8.5	5.8	10.6	4.5	9.1	8.9	7.0
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	5.7	6.9	8.7	8.5	14.4	20.7	31.1	44.2	46.2	45.0	61.0
	SD	2.6	6.0	0.1	3.4	1.1	0.5	0.5	3.5	2.4	12.3	6.6
	COV	45.5	87.4	1.6	39.7	7.8	2.4	1.7	8.0	5.1	27.3	10.8
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	28.2	55.2	63.3	70.9	88.9	75.5	74.1	21.8	18.3	19.4	11.2
	SD	1.1	2.5	1.9	3.5	11.6	3.9	2.9	1.5	2.3	2.7	1.8
	COV	4.0	4.4	3.0	4.9	13.0	5.1	4.0	7.1	12.8	14.1	15.6
5	n	3	n.d.	3	3	n.d.	3	3	3	3	n.d.	3
	Mean	1.2	n.d.	2.3	2.7	n.d.	4.1	7.8	19.7	13.8	n.d.	30.8
	SD	0.4	n.d.	0.4	1.1	n.d.	3.5	0.4	0.8	1.1	n.d.	2.8
	COV	33.7	n.d.	17.6	39.3	n.d.	84.5	5.8	4.1	8.0	n.d.	8.9
Total [1)]	**n**	**4**	**3**	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**3**	**4**
	Min	**0.9**	**1.7**	**2.3**	**2.7**	**14.4**	**4.1**	**7.8**	**19.7**	**13.8**	**39.4**	**30.8**
	Max	**5.7**	**6.9**	**8.7**	**13.5**	**16.2**	**24.0**	**35.9**	**48.6**	**46.2**	**48.9**	**61.0**
	Mean	**2.3**	**4.3**	**4.9**	**7.0**	**15.2**	**16.1**	**26.0**	**38.4**	**35.6**	**44.4**	**44.5**
	SD	**2.2**	**2.6**	**2.9**	**5.0**	**0.9**	**8.7**	**12.5**	**12.8**	**15.0**	**4.8**	**14.3**
	COV%	**95.3**	**61.2**	**59.7**	**70.7**	**6.1**	**54.2**	**48.0**	**33.4**	**42.0**	**10.8**	**32.1**

n.a. = not analyzed; [1)] Results from Institute 4 are not considered for evaluation

Figure 23: Comparison of non-extractable residues obtained from transformation of ^{14}C-florfenicol in pig manure for five participants of the ring test. The amount of radioactivity [% applied radioactivity, % aR] per sampling interval is given as mean. Values were normalized to 100% aR at day 0.



Results from Institute 4 were not considered for further evaluation because of deviations from the proposed extraction scheme.

Table 34: minimum (Min), maximum (Max) and mean-values, standard deviation (SD) and coefficient of variation (COV%) of NER [% aR] for the transformation of ^{14}C-imidacloprid in cattle manure, 5 institutes.

Institute	Time	0	3	7	10	14	21	28	42	58	72	90
1	n	6	6	6	6	6	n.d.	6	6	6	6	6
	Mean	2.0	5.1	8.4	10.4	14.8	n.a.	24.1	34.7	33.7	42.9	40.3
	SD	0.2	0.2	1.1	0.5	1.4	n.a.	0.8	2.9	2.2	4.7	2.6
	COV	11.7	3.8	13.0	4.6	9.2		3.3	8.2	6.4	11.0	6.5
2	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	2.8	5.4	5.8	7.6	7.2	8.5	9.3	12.6	10.2	10.5	11.7
	SD	0.3	0.3	0.3	0.5	0.6	0.9	1.0	6.4	0.6	0.2	0.7
	COV	12.6	6.0	5.8	7.0	7.6	11.2	10.3	50.8	5.8	1.9	6.1
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	3.6	10.2	15.4	19.7	24.4	28.7	31.9	39.4	51.0	51.5	57.8
	SD	0.0	2.0	1.4	0.3	0.2	0.6	0.7	4.6	5.6	1.0	2.7
	COV	1.2	19.9	8.9	1.7	0.9	2.1	2.1	11.7	11.1	1.9	4.6
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	15.8	18.7	20.1	24.9	27.4	29.7	29.0	40.2	47.6	46.1	56.6
	SD	9.2	1.1	2.2	0.9	1.1	1.4	1.4	4.9	4.0	3.1	2.7
	COV	86.7	5.7	11.0	3.8	4.0	4.8	4.7	12.3	8.4	6.8	4.8
5	n	3	3	3	n.d.	n.d.	n.d.	3	3	n.d.	n.d.	3
	Mean	1.3	10.0	9.3	n.a.	n.a.	n.a.	13.9	20.1	n.a.	n.a.	26.4
	SD	0.5	1.2	1.1	n.a.	n.a.	n.a.	1.9	2.4	n.a.	n.a.	4.9
	COV	41.1	12.1	11.8	n.a.	n.a.	n.a.	13.6	11.7	n.a.	n.a.	18.4
Total	**n**	**5**	**5**	**5**	**4**	**4**	**3**	**5**	**5**	**4**	**4**	**5**
	Min	**1.3**	**5.1**	**5.8**	**7.6**	**7.2**	**8.5**	**9.3**	**12.6**	**10.2**	**10.5**	**11.7**
	Max	**15.8**	**18.7**	**20.1**	**24.9**	**27.4**	**29.7**	**31.9**	**40.2**	**51.0**	**51.5**	**57.8**
	Mean	**5.1**	**9.9**	**11.8**	**15.7**	**18.5**	**22.3**	**21.7**	**29.4**	**35.6**	**37.7**	**38.6**
	SD	**6.1**	**5.5**	**5.8**	**8.0**	**9.2**	**12.0**	**9.7**	**12.4**	**18.5**	**18.5**	**19.8**
	COV%	**119.5**	**55.7**	**49.5**	**51.3**	**49.9**	**53.7**	**45.0**	**42.2**	**52.0**	**49.1**	**51.4**

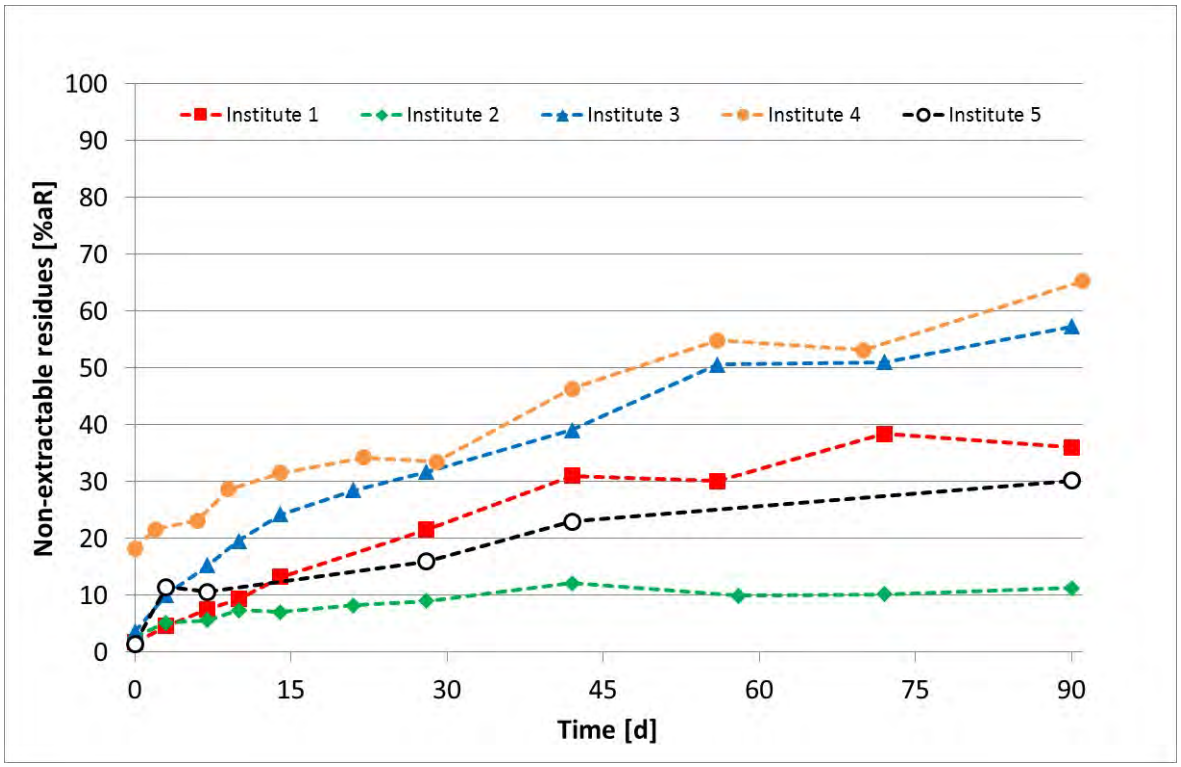
n.a. = not analyzed

Table 35: minimum (Min), maximum (Max) and mean-values, standard deviation (SD) and coefficient of variation (COV%) of NER [normalized, % aR] for the transformation of ^{14}C-imidacloprid in cattle manure, all institutes.

Institute	Time	0	3	7	10	14	21	28	42	58	72	90
1	n	6	6	6	6	6	n.d.	6	6	6	6	6
	Mean	1.7	4.6	7.5	9.3	13.2	n.d.	21.5	31.0	30.1	38.4	36.0
	SD	0.2	0.2	1.0	0.4	1.2	n.d.	0.7	2.5	1.9	4.2	2.3
	COV	11.7	3.8	13.0	4.6	9.2	n.d.	3.3	8.2	6.4	11.0	6.5
2	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	2.7	5.2	5.6	7.4	7.0	8.2	9.0	12.2	9.9	10.2	11.3
	SD	0.3	0.3	0.3	0.5	0.5	0.9	0.9	6.2	0.6	0.2	0.7
	COV	12.6	6.0	5.8	7.0	7.6	11.2	10.3	50.8	5.8	1.9	6.1
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	3.6	10.1	15.3	19.6	24.2	28.5	31.7	39.1	50.6	51.0	57.3
	SD	0.0	2.0	1.4	0.3	0.2	0.6	0.7	4.6	5.6	0.9	2.7
	COV	1.2	19.9	8.9	1.7	0.9	2.1	2.1	11.7	11.1	1.9	4.6
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	18.2	21.6	23.2	28.7	31.6	34.2	33.5	46.3	54.8	53.2	65.3
	SD	0.5	1.2	2.5	1.1	1.3	1.7	1.6	5.7	4.6	3.6	3.1
	COV	2.8	5.7	11.0	3.8	4.0	4.8	4.7	12.3	8.4	6.8	4.8
5	n	3	3	3	n.d.	n.d.	n.d.	3	3	n.d.	n.d.	3
	Mean	1.4	11.5	10.6	n.d.	n.d.	n.d.	15.9	23.0	n.d.	n.d.	30.2
	SD	0.6	1.4	1.3	n.d.	n.d.	n.d.	2.2	2.7	n.d.	n.d.	5.5
	COV	41.1	12.1	11.8	n.d.	n.d.	n.d.	13.6	11.7	n.d.	n.d.	18.4
Total	**n**	**5**	**5**	**5**	**4**	**4**	**3**	**5**	**5**	**4**	**4**	**5**
	Min	**1.4**	**4.6**	**5.6**	**7.4**	**7.0**	**8.2**	**9.0**	**12.2**	**9.9**	**10.2**	**11.3**
	Max	**18.2**	**21.6**	**23.2**	**28.7**	**31.6**	**34.2**	**33.5**	**46.3**	**54.8**	**53.2**	**65.3**
	Mean	**5.5**	**10.6**	**12.4**	**16.2**	**19.0**	**23.6**	**22.3**	**30.3**	**36.3**	**38.2**	**40.0**
	SD	**7.2**	**6.8**	**7.0**	**9.9**	**11.0**	**13.7**	**10.4**	**13.4**	**20.7**	**19.8**	**21.6**
	COV%	**129.2**	**64.5**	**56.5**	**60.7**	**57.8**	**57.8**	**46.5**	**44.1**	**56.9**	**51.8**	**54.1**

n.a. = not analyzed

Figure 24: Comparison of non-extractable residues obtained from transformation of ^{14}C-imidacloprid in cattle manure for all participants of the ring test. The amount of radioactivity [% applied radioactivity, % aR] per sampling interval is given as mean. Values were normalized to 100 % aR at day 0.



3.9 Mass balances

A ^{14}C-mass balance was determined for each sampling time point by summing-up the amount of radioactivity [% aR] in extracts, in non-extractable residues and for mineralization ($^{14}CO_2$ and $^{14}CH_4$).

According to established test guidelines on aerobic and anaerobic transformation (e.g. in soil (OECD, 2002a) and aquatic sediment systems (OECD, 2002b)) the mass balance throughout the study should be within a defined range (e.g. 100 ± 10% aR) to guarantee the quality of a study. However, since higher deviations are acceptable for initial measured mass balance values, it is crucial to normalize all results that are reported in % of the applied radioactivity to 100% mass balance at time point 0 days as losses due to sorption and dosing errors thereby are compensated. In the following, results of the mass balances (uncorrected and normalized data) are presented for ^{14}C-florfenicol in pig manure and ^{14}C-imidacloprid in cattle manure.

3.9.1 Florfenicol in pig manure

A summary of mass balances for the transformation of ^{14}C-florfenicol in pig manure from all participants is presented in Table 36 (uncorrected data), Table 37 (normalized data) and Figure 25 (normalized data).

Based on the results, data from Institute 4 were not considered for further evaluations (calculation of mean, standard deviation and coefficient of variation) since mass balances clearly decreased throughout the incubation period down to 21.6% aR at the end of the study. A potential reason might be the application of a different extraction method using a very unpolar solvent. With this method,

the polar test compound florfenicol and its even more polar transformation products were probably not extracted completely.

Mean mass balances are in the range between 92.0% aR and 102.1% aR (results from Institute 4 were not considered because of deviations from the proposed extraction scheme).

Table 36: Summarizing presentation of all minimum (Min), maximum (Max) and mean-values, standard deviation (SD) and coefficient of variation (COV%) of mass balances [% aR] for the transformation of ^{14}C-florfenicol in pig manure, 5 institutes

Institute	Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
1	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	100.3	105.3	107.6	108.6	94.4	107.9	91.9	100.2	106.9	101.3	105.8
	SD	2.7	3.0	2.7	2.4	5.3	1.9	2.0	1.4	2.1	2.5	2.3
	COV	2.7	2.9	2.5	2.2	5.6	1.8	2.2	1.4	2.0	2.5	2.2
	Min	94.8	101.3	104.5	106.0	88.0	105.8	88.6	98.7	103.5	96.7	102.2
	Max	101.6	109.2	112.0	112.7	103.2	110.3	94.7	101.7	109.3	103.5	108.5
2	n	6	6	6	6	6	6	6	6	6	4	4
	Mean	98.3	99.5	96.3	93.2	96.6	96.8	96.2	97.9	95.4	93.0	98.8
	SD	3.8	0.4	1.9	4.7	1.7	1.4	4.2	2.5	3.6	4.5	5.5
	COV	3.9	0.4	2.0	5.1	1.7	1.4	4.4	2.6	3.8	4.8	5.5
	Min	91.7	98.9	93.4	88.5	94.9	95.2	90.8	96.9	92.0	89.7	94.0
	Max	103.3	100.0	98.3	101.2	99.5	98.9	101.3	101.1	101.9	99.2	105.8
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	98.9	101.7	99.3	97.5	98.8	97.8	94.2	97.2	89.1	90.4	93.3
	SD	0.6	5.6	1.0	2.6	1.0	0.2	5.9	5.3	3.9	6.1	0.1
	COV	0.6	5.5	1.0	2.7	1.0	0.2	6.3	5.5	4.4	6.8	0.1
	Min	98.5	97.8	98.5	95.7	98.1	97.6	90.0	93.5	86.3	86.0	93.2
	Max	99.3	105.6	100.0	99.4	99.5	97.9	98.4	101.0	91.9	94.7	93.4
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	86.6	80.6	78.3	82.4	91.6	76.9	68.2	25.7	23.0	21.4	18.7
	SD	11.2	10.9	6.4	2.4	10.6	0.5	2.5	2.7	3.4	2.0	1.3
	COV	12.9	13.6	8.2	2.9	11.6	0.6	3.6	10.5	14.7	9.3	7.0
	Min	73.7	68.6	73.9	80.6	82.2	76.6	66.7	23.9	19.1	20.1	17.8
	Max	93.3	90.0	85.7	85.1	103.1	77.5	71.1	28.8	25.2	23.7	20.2
5	n	3	n.a.	3	3	n.a.	3	3	3	3	n.a.	3
	Mean	92.8	n.a.	97.7	85.7	n.a.	85.4	86.3	94.9	87.9	n.a.	77.2
	SD	2.8	n.a.	4.3	3.7	n.a.	5.7	7.0	21.3	10.8	n.a.	3.9
	COV	3.1	n.a.	4.4	4.3	n.a.	6.7	8.1	22.4	12.3	n.a.	5.1
	Min	90.2	n.a.	93.8	83.1	n.a.	81.6	81.1	71.6	80.3	n.a.	74.8

	Max	95.8	n.a.	102.4	89.9	n.a.	92.0	94.3	113.4	95.6	n.a.	81.7
Total, based on lab means [1)]	**n**	**4**	**3**	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**3**	**4**
	Min	**92.8**	**99.5**	**96.3**	**85.7**	**94.4**	**85.4**	**86.3**	**94.9**	**87.9**	**90.4**	**77.2**
	Max	**100.3**	**105.3**	**107.5**	**108.6**	**98.8**	**107.9**	**96.2**	**100.2**	**106.9**	**101.3**	**105.8**
	Mean	**97.6**	**102.1**	**100.2**	**96.2**	**96.6**	**96.9**	**92.2**	**97.6**	**94.8**	**94.9**	**93.8**
	SD	**3.3**	**2.9**	**5.1**	**9.6**	**2.2**	**9.2**	**4.3**	**2.2**	**8.7**	**5.7**	**12.2**
	COV%	**3.4**	**2.9**	**5.1**	**9.9**	**2.3**	**9.5**	**4.6**	**2.2**	**9.2**	**6.0**	**13.0**

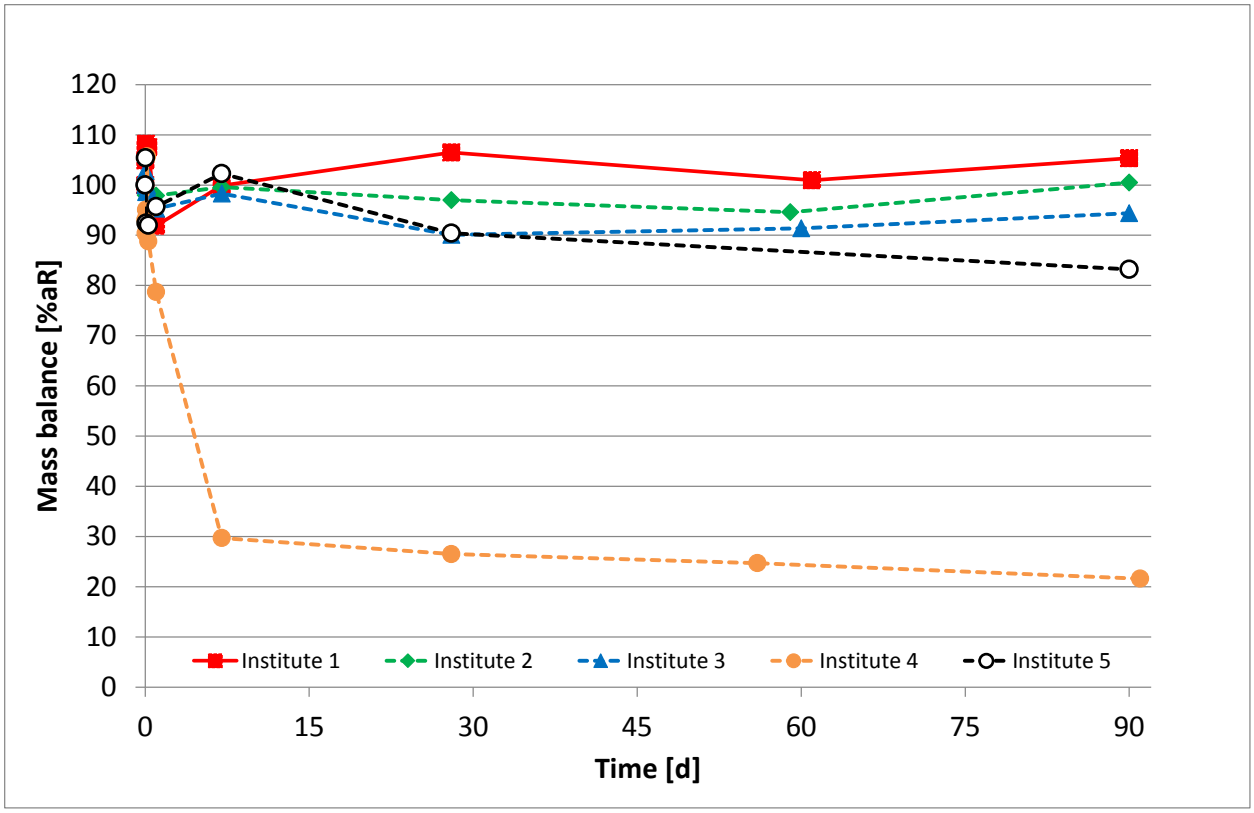
n.a. = not analyzed; [1)] Results from Institute 4 are not considered for evaluation due to a deviation from the proposed extraction scheme

Table 37: Summarizing presentation of all minimum (Min), maximum (Max) and mean-values, standard deviation (SD) and coefficient of variation (COV%) of normalized mass balances [% aR] for the transformation of ^{14}C-florfenicol in pig manure, participant: all.

Institute	Time	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
1	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	100.0	104.9	107.3	108.2	94.1	107.5	91.6	99.9	106.6	101.0	105.4
	SD	2.7	3.0	2.7	2.4	5.3	1.9	2.0	1.4	2.1	2.5	2.3
	COV	2.7	2.9	2.5	2.2	5.6	1.8	2.2	1.4	2.0	2.5	2.2
	Min	94.5	100.9	104.1	105.7	87.7	105.4	88.3	98.4	103.1	96.4	101.8
	Max	101.3	108.9	111.7	112.3	102.8	110.0	94.4	101.3	108.9	103.2	108.2
2	n	6	6	6	6	6	6	6	6	6	4	4
	Mean	100.0	101.2	98.0	94.8	98.3	98.5	97.9	99.6	97.0	94.6	100.5
	SD	3.9	0.4	2.0	4.8	1.7	1.4	4.3	2.5	3.7	4.6	5.6
	COV	3.9	0.4	2.0	5.1	1.7	1.4	4.4	2.6	3.8	4.8	5.5
	Min	93.3	100.6	95.0	90.1	96.6	96.9	92.4	96.8	93.6	91.3	95.7
	Max	105.1	101.8	100.0	103.0	101.3	100.7	103.1	102.9	103.8	101.0	107.7
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	100.0	102.8	100.4	98.6	99.9	98.8	95.3	98.3	90.1	91.4	94.4
	SD	0.6	5.6	1.0	2.6	1.0	0.2	6.0	5.4	4.0	6.2	0.1
	COV	0.6	5.5	1.0	2.7	1.0	0.2	6.3	5.5	4.4	6.8	0.1
	Min	99.6	98.9	99.6	96.7	99.2	98.7	91.0	94.5	87.3	95.8	94.3
	Max	100.4	106.8	101.0	100.5	100.7	99.0	99.5	102.1	92.2	87.0	94.5
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	100.0	93.1	90.4	95.1	105.7	88.8	78.7	29.7	26.5	24.7	21.6
	SD	12.9	12.6	7.4	2.8	12.3	0.5	2.9	3.1	3.9	2.3	1.5
	COV	12.9	13.6	8.2	2.9	11.6	0.6	3.6	10.5	14.7	9.3	7.0
	Min	85.1	79.2	85.3	93.1	94.9	88.4	77.0	27.6	22.0	23.2	20.5
	Max	107.2	103.9	98.9	98.2	119.0	89.4	82.0	33.3	29.1	27.3	23.4
5	n	3	n.a.	3	3	n.a.	3	3	3	3	n.a.	3
	Mean	100.0	n.a.	105.4	92.4	n.a.	92.0	95.7	102.3	90.4	n.a.	83.2
	SD	3.1	n.a.	4.7	4.0	n.a.	6.2	5.7	22.9	11.2	n.a.	4.2
	COV	3.1	n.a.	4.4	4.3	n.a.	6.7	6.0	22.4	12.4	n.a.	5.1
	Min	97.2	n.a.	101.1	89.6	n.a.	88.0	90.2	77.2	81.6	n.a.	80.6
	Max	103.3	n.a.	110.3	96.9	n.a.	99.2	101.6	122.2	103.0	n.a.	88.1
Total, based on lab means [1)]	**n**	**4**	**3**	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**3**	**4**
	Min	**100.0**	**101.2**	**98.0**	**92.4**	**94.1**	**92.0**	**91.6**	**98.3**	**90.1**	**91.4**	**83.2**
	Max	**100.0**	**104.9**	**107.3**	**108.2**	**99.9**	**107.5**	**97.9**	**102.3**	**106.6**	**101.0**	**105.4**
	Mean	**100.0**	**103.0**	**102.8**	**98.5**	**97.4**	**99.2**	**95.1**	**100.0**	**96.0**	**95.7**	**95.9**
	SD	**0.0**	**1.9**	**4.3**	**7.0**	**3.0**	**6.3**	**2.6**	**1.7**	**7.7**	**4.9**	**9.6**
	COV%	**0.0**	**1.8**	**4.2**	**7.1**	**3.1**	**6.4**	**2.7**	**1.7**	**8.1**	**5.1**	**10.0**

n.a. = not analyzed; [1)] Results from Institute 4 are not considered for evaluation due to a deviation from the proposed extraction scheme

Figure 25: Mass balances for transformation of ^{14}C-florfenicol in pig manure for all participants of the ring test. The amount of radioactivity [normalized, %aR] per sampling interval is given as mean.



Results from Institute 4 were not considered for further evaluation because of deviations from the proposed extraction scheme.

3.9.2 Imidacloprid in cattle manure

A summary of mass balances for the transformation of ^{14}C-imidacloprid in cattle manure from all participants is presented in Table 38 (uncorrected data), Table 39 (normalized data) and Figure 26 (normalized data). Mean mass balances of all Institutes were in the range between 89.2% aR and 103.1% aR.

Table 38: Summarizing presentation of all minimum (Min), maximum (Max) and mean-values, standard deviation (SD) and coefficient of variation (COV%) of mass balances for the transformation of ^{14}C-imidacloprid in cattle manure, participant: all.

Institute	Time [d]	0	3	7	10	14	21	28	42	56	72	90
1	n	6	6	6	6	6	n.a.	6	6	6	6	6
	Mean	111.8	112.7	111.4	114.5	115.3	n.a.	111.5	109.8	113.9	106.7	112.5
	SD	2.3	3.2	1.1	2.7	3.4	n.a.	3.7	2.1	5.6	4.4	2.3
	COV	2.1	2.8	1.0	2.4	2.9	n.a.	3.3	1.9	5.0	4.1	2.1
	Min	108.6	109.8	110.1	112.0	112.4	n.a.	107.8	107.3	109.9	102.7	109.4
	Max	114.5	118.1	113.0	119.0	119.0	n.a.	118.2	113.0	125.2	114.9	114.4
2	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	103.1	108.2	104.2	94.0	100.2	87.1	91.9	91.7	91.0	90.3	92.7
	SD	1.2	0.7	1.3	0.9	1.2	6.4	1.5	3.1	2.8	2.9	2.6
	COV	1.1	0.6	1.2	0.9	1.1	7.4	1.6	3.4	3.0	3.3	2.9
	Min	101.5	107.6	102.7	92.8	99.0	79.1	89.2	85.8	85.4	85.6	87.8
	Max	104.3	109.5	106.3	95.1	102.1	94.3	93.1	94.6	92.8	92.8	95.3
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	100.8	90.6	108.8	101.1	104.0	97.7	100.1	98.0	106.0	101.6	102.0
	SD	2.2	8.4	13.3	3.1	2.9	0.4	1.1	7.3	4.8	2.2	4.6
	COV	2.2	9.3	12.2	3.1	2.8	0.4	1.1	7.4	4.5	2.1	4.5
	Min	99.3	84.7	99.4	98.9	101.9	97.4	99.3	92.8	102.6	100.1	98.7
	Max	102.4	96.5	118.3	103.3	106.1	98.0	100.9	103.1	109.3	103.2	105.2
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	86.8	97.0	91.2	99.0	92.9	82.9	93.6	88.8	86.5	86.3	79.5
	SD	9.1	2.3	1.8	2.9	2.7	3.0	2.2	5.7	1.5	1.4	3.1
	COV	10.5	2.4	2.0	2.9	2.9	3.6	2.3	6.4	1.7	1.7	3.8
	Min	77.3	95.2	89.3	96.7	90.1	79.5	91.5	83.8	85.6	85.1	76.5
	Max	95.6	99.6	92.9	102.2	95.4	85.3	95.9	95.0	88.2	87.9	82.6
5	n	3	3	3	n.a.	n.a.	n.a.	3	3	n.a.	n.a.	3
	Mean	87.4	89.1	91.5	n.a.	n.a.	n.a.	85.0	92.4	n.a.	n.a.	100.0
	SD	8.1	7.4	11.2	n.a.	n.a.	n.a.	8.0	2.8	n.a.	n.a.	8.0
	COV	9.3	8.3	12.2	n.a.	n.a.	n.a.	9.4	3.0	n.a.	n.a.	8.0
	Min	82.0	81.9	81.9	n.a.	n.a.	n.a.	78.7	90.3	n.a.	n.a.	91.1
	Max	96.8	96.7	103.9	n.a.	n.a.	n.a.	94.0	95.5	n.a.	n.a.	106.7
Total, based on lab means	**n**	**5**	**5**	**5**	**4**	**4**	**3**	**5**	**5**	**4**	**4**	**5**
	Min	**86.8**	**89.1**	**91.2**	**94.0**	**92.9**	**82.9**	**85.0**	**88.8**	**86.5**	**86.3**	**79.5**
	Max	**111.8**	**112.7**	**111.4**	**114.5**	**115.3**	**97.7**	**111.5**	**109.8**	**113.9**	**106.7**	**112.5**
	Mean	**98.0**	**99.5**	**101.4**	**102.2**	**103.1**	**89.2**	**96.4**	**96.1**	**99.3**	**96.2**	**97.3**
	SD	**10.8**	**10.6**	**9.6**	**8.8**	**9.4**	**7.6**	**10.0**	**8.3**	**12.8**	**9.5**	**12.2**
	COV%	**11.0**	**10.6**	**9.4**	**8.6**	**9.1**	**8.5**	**10.3**	**8.7**	**12.9**	**9.9**	**12.6**

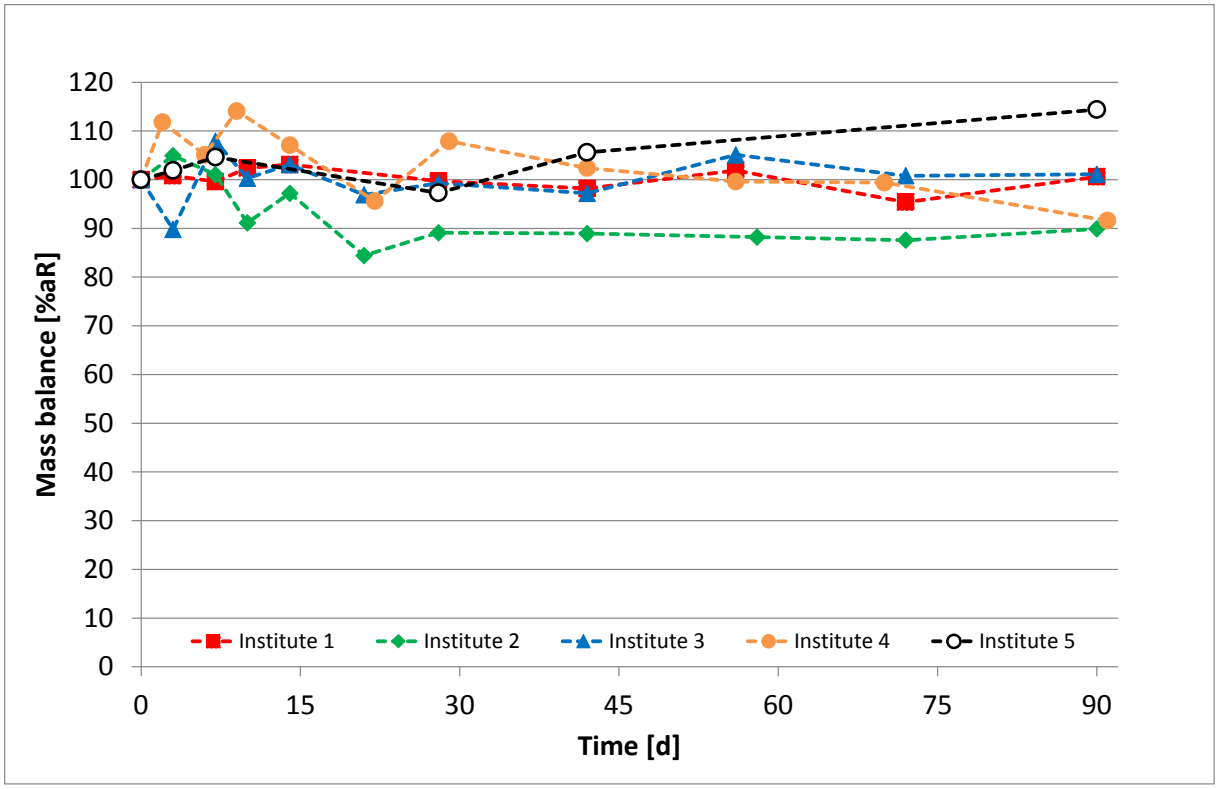
n.a. = not analyzed

Table 39: Summarizing presentation of all minimum (Min), maximum (Max) and mean-values, standard deviation (SD) and coefficient of variation (COV%) of normalized mass balances for the transformation of ^{14}C-imidacloprid in cattle manure, participant: all.

Institute	Time [d]	0	3	7	10	14	21	28	42	56	72	90
1	n	6	6	6	6	6	n.a.	6	6	6	6	6
	Mean	100.0	100.8	99.6	102.4	103.1	n.a.	99.7	98.2	101.9	95.4	100.6
	SD	2.1	2.8	1.0	2.4	3.0	n.a.	3.3	1.9	5.0	3.9	2.1
	COV	2.1	2.8	1.0	2.4	2.9	n.a.	3.3	1.9	5.0	4.1	2.1
	Min	97.1	98.1	98.5	100.2	100.5	n.a.	96.4	95.9	98.3	91.8	97.8
	Max	102.4	105.6	101.0	106.4	108.3	n.a.	105.7	101.1	112.0	102.7	102.2
2	n	6	6	6	6	6	6	6	6	6	6	6
	Mean	100.0	104.9	101.0	91.1	97.2	84.4	89.1	88.9	88.2	87.6	89.9
	SD	1.1	0.6	1.2	0.8	1.1	6.2	1.5	3.1	2.7	2.9	2.6
	COV	1.1	0.6	1.2	0.9	1.1	7.4	1.6	3.4	3.0	3.3	2.9
	Min	98.4	104.3	99.5	90.0	96.0	76.7	86.4	83.1	82.9	83.0	85.1
	Max	101.1	106.1	103.1	92.2	99.0	91.5	90.3	91.7	90.0	90.0	92.4
3	n	2	2	2	2	2	2	2	2	2	2	2
	Mean	100.0	89.8	107.9	100.2	103.1	96.9	99.3	97.2	105.1	100.8	101.1
	SD	2.2	8.3	13.2	3.1	2.9	0.4	1.1	7.2	4.7	2.2	4.5
	COV	2.2	9.3	12.2	3.1	2.8	0.4	1.1	7.4	4.5	2.1	4.5
	Min	98.5	84.0	98.6	98.0	101.1	96.6	98.5	92.1	101.7	99.3	97.9
	Max	101.5	95.7	117.3	102.5	105.2	97.1	100.0	102.3	108.4	102.3	104.3
4	n	3	3	3	3	3	3	3	3	3	3	3
	Mean	100.0	111.8	105.1	114.1	107.1	95.6	107.9	102.4	99.6	99.4	91.6
	SD	10.5	2.6	2.1	3.3	3.1	3.5	2.5	6.6	1.7	1.7	3.5
	COV	10.5	2.4	2.0	2.9	2.9	3.6	2.3	6.4	1.7	1.7	3.8
	Min	89.1	109.7	102.9	111.4	103.9	91.7	105.5	96.6	98.6	98.1	88.2
	Max	110.1	114.8	107.1	117.8	110.0	98.3	110.5	109.5	101.6	101.3	95.2
5	n	3	3	3	n.a.	n.a.	n.a.	3	3	n.a.	n.a.	3
	Mean	100.0	101.9	104.6	n.a.	n.a.	n.a.	97.3	105.6	n.a.	n.a.	114.4
	SD	9.3	8.4	12.8	n.a.	n.a.	n.a.	9.1	3.2	n.a.	n.a.	9.2
	COV	9.3	8.3	12.3	n.a.	n.a.	n.a.	9.4	3.0	n.a.	n.a.	8.0
	Min	93.8	93.7	93.7	n.a.	n.a.	n.a.	90.0	103.2	n.a.	n.a.	104.2
	Max	110.7	110.5	118.8	n.a.	n.a.	n.a.	107.5	109.2	n.a.	n.a.	122.0
Total, based on lab means	**n**	**5**	**5**	**5**	**4**	**4**	**3**	**5**	**5**	**4**	**4**	**5**
	Min	**100.0**	**89.8**	**99.6**	**91.1**	**97.2**	**84.4**	**89.1**	**88.9**	**88.2**	**87.6**	**89.9**
	Max	**100.0**	**111.8**	**107.9**	**114.1**	**107.1**	**96.9**	**107.9**	**105.6**	**105.1**	**100.8**	**114.4**
	Mean	**100.0**	**101.9**	**103.7**	**102.0**	**102.6**	**92.3**	**98.6**	**98.5**	**98.7**	**95.8**	**99.5**
	SD	**0.0**	**8.0**	**3.3**	**9.5**	**4.1**	**6.8**	**6.7**	**6.3**	**7.3**	**5.9**	**9.7**
	COV%	**0.0**	**7.8**	**3.2**	**9.3**	**4.0**	**7.4**	**6.8**	**6.4**	**7.4**	**6.2**	**9.8**

n.a. = not analyzed

Figure 26: Mass balances for transformation of ^{14}C-imidacloprid in cattle manure for all participants of the ring test. The amount of radioactivity [normalized, %aR] per sampling interval is given as mean.



3.9.3 Derivation of quality criteria

Mass balances can be taken as a quality criterion of a transformation study performed with radiolabeled test substance. The guidelines on aerobic and anaerobic transformation in soil (OECD, 2002b) and aerobic and anaerobic transformation in aquatic sediment systems (OECD, 2002a) assume a mass balance in between 90 %aR and 110 %aR as a criterion for a valid study. Table 40 shows an evaluation of test results from all participants with regard to obtained mass balances.

Mean mass balances of all Institutes were in the range between 92.2% aR and 102.1% aR for florfenicol in pig manure (Table 36; without Institute 4) and between 89.2% aR and 103.1% aR for imidacloprid in cattle manure (Table 38). However, the range of 90% aR to 110% aR was not met in many cases. To find a more adapted range, taking into account the complexity of the matrix manure, the mass balances of all ring test participants were evaluated in order to recommend a suitable range.

Figure 27 and Figure 28 show the frequency distribution of mass balances [% aR] for florfenicol in pig manure and for imidacloprid in cattle manure, respectively.

When looking at mass balance results between 85% aR and 115% aR for florfenicol in pig manure (highlighted in yellow in Figure 27), 162 out of 174 measured data are within that range, corresponding to 93% of the entire data set. However, only 86% of data (149 out of 174 mass balances) are in between 90% aR and 110% aR (highlighted in orange in Figure 27).

Referring to mass balance results between 85% aR and 115% aR for imidacloprid in cattle manure (highlighted in yellow in Figure 28), 175 out of 199 measured data are within that range, corresponding to 88% of the data, whereas only 60% of data (119 out of 199 mass balances) are between 90% aR and 110% aR (highlighted in orange in Figure 28).

Based on the frequency distributions of mass balances data (Figure 27 and Figure 28), we suggest to use the following quality criteria with regard to the mass balance:

"For radiolabeled compounds the mass balance at the beginning of the study should be within 85% to 115%. During the study average total mass balance for all samples within a treatment should fall within that range. For unlabeled test substances the analytical recovery of the test compound has to be at least 70%."

Only three criteria are slightly out of the range of 85% aR – 115% aR: the range of mean mass balances for ^{14}C-florfenicol in pig manure during the study for Institute 5 (lowest mass balance 77.2% aR), the range of mean mass balances for ^{14}C-imidacloprid in cattle manure during the study for institute 1 (highest mass balance 115.3% aR) and the range of mean mass balances for ^{14}C-imidacloprid in cattle manure during the study for institute 4 (lowest mass balance 79.5% aR) (Table 41).

Table 40: Comparison of obtained mass balances to the range of 90%-110% for all ring test experiments

Institute	Mass balance					
	Mean at test start		Mean all sampling time points		Overall mean	
	Value	Within 90%-110%?	Range	Within 90%-110%?	Value	Within 90%-110%?
Florfeniol in pig manure						
1	100.3	YES	91.2 - 108.6	YES	102.6	YES
2	98.3	YES	93.0 - 99.5	YES	96.5	YES
3	98.9	YES	89.1 - 101.7	**NO**	96.2	YES
4 [1)]	86.6	**NO**	18.7 - 91.6	**NO**	59.4	**NO**
5	92.8	YES	77.2 - 97.7	**NO**	88.5	**NO**
Imidacloprid in cattle manure						
1	111.8	**NO**	106.7 - 115.3	**NO**	112.0	**NO**
2	103.1	YES	87.1 - 108.2	**NO**	95.9	YES
3	100.8	YES	90.6 - 108.8	YES	101.0	YES
4	86.8	**NO**	79.5 - 99.0	**NO**	89.0	**NO**
5	87.4	**NO**	85.0 - 100.0	YES	90.9	YES

[1)] Results from Institute 4 are not considered for evaluation due to a deviation from the proposed extraction scheme

Figure 27: Frequency distribution of mass balances [% aR] for ^{14}C-florfenicol in pig manure for all participants of the ring test (without Institute 4); n=174

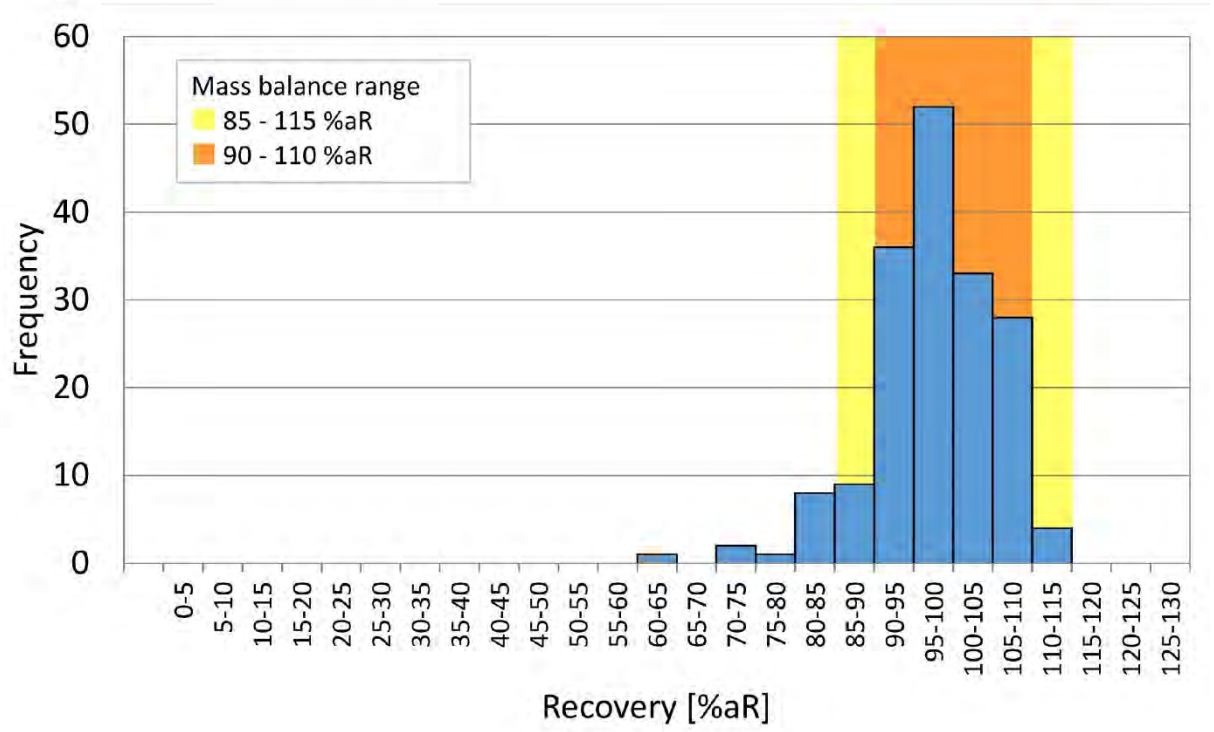


Figure 28: Frequency distribution of mass balances [% aR] for ^{14}C-imidacloprid in cattle manure for all participants of the ring test; n=199

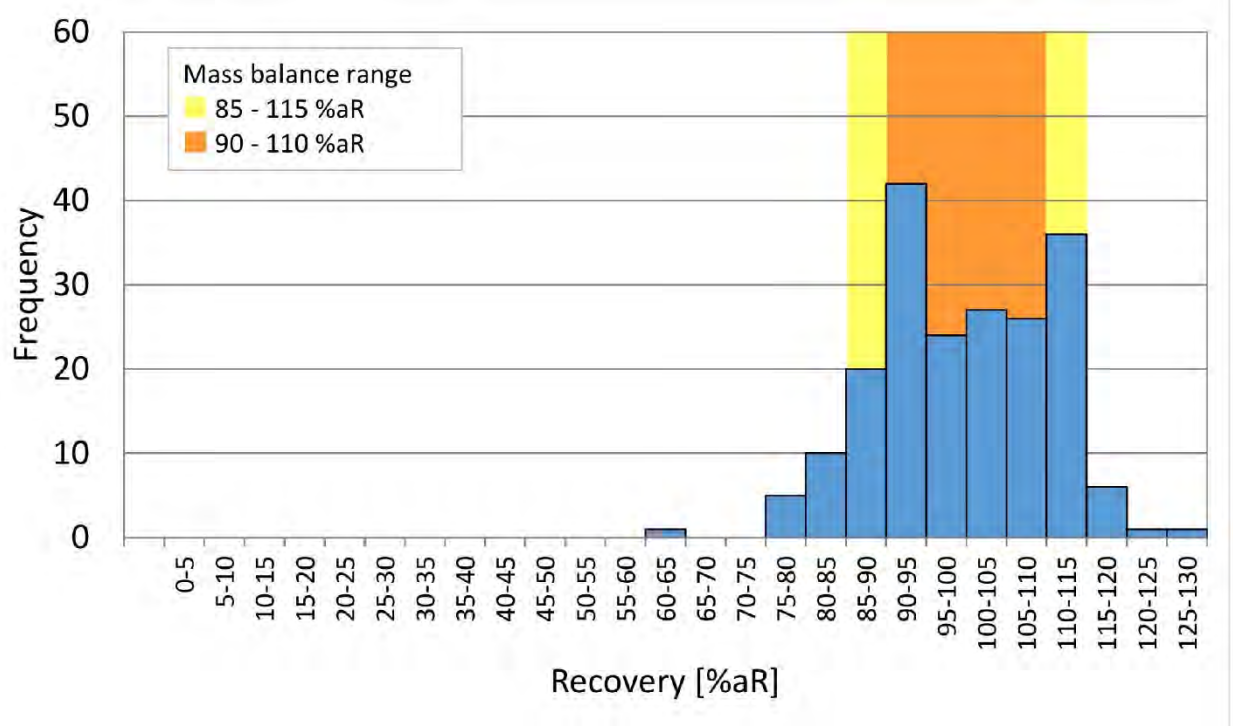


Table 41: Comparison of obtained mass balances to the range of 85%-115% for all ring test experiments

Institute	Mass balance					
	Mean at test start		Mean all sampling time points		Overall mean	
	Value	Within 85%-115%?	Range	Within 85%-115%?	Value	Within 85%-115%?
Florfeniol in pig manure						
1	100.3	YES	91.2 - 108.6	YES	102.6	YES
2	98.3	YES	93.0 - 99.5	YES	96.5	YES
3	98.9	YES	89.1 - 101.7	YES	96.2	YES
4 [1)]	86.6	YES	18.7 - 91.6	**NO**	59.4	**NO**
5	92.8	YES	77.2 - 97.7	**NO**	88.5	YES
Imidacloprid in cattle manure						
1	111.8	YES	106.7 - 115.3	**NO**	112.0	YES
2	103.1	YES	87.1 - 108.2	YES	95.9	YES
3	100.8	YES	90.6 - 108.8	YES	101.0	YES
4	86.8	YES	79.5 - 99.0	**NO**	89.0	YES
5	87.4	YES	85.0 - 100.0	YES	90.9	YES

[1)] Results from Institute 4 are not considered for evaluation due to a deviation from the proposed extraction scheme

3.10 Overview on the variability of the results obtained in the ring test in different laboratories with different manures

The variability of the results obtained in the ring test was investigated by comparing mean values, standard deviations and coefficients of variation for mineralization, extractable residues, NER and mass balance as described in detail in sections 3.6 to 3.9. The results are summarized in Table 42 and Table 43 for florfenicol in pig manure and in Table 44 and Table 45 for imidacloprid in cattle manure. For the parameters mass balance, extractables and NER, single data refer to mean values across all institutes for each sampling time point.

Graphs of the results are presented in Figure 29 (mean values and standard deviations for florfenicol) and Figure 30 (mean values and standard deviations for imidacloprid). Variability of results related to coefficients of variation for mass balances, extractable residues and non-extractable residues is shown in Figure 31.

It becomes apparent that variability between the results of the different laboratories is low regarding mass balance (florfenicol (90 d): 95.9 ± 9.6% aR, COV = 10.0%; imidacloprid (90 d): 99.5 ± 9.7% aR, COV = 9.8%), but higher with respect to extractable residues (florfenicol (90 d): 50.8 ± 15.8% aR, COV = 31.0%; imidacloprid (90 d): 59.4 ± 24.2% aR, COV = 40.7%) and non-extractable residues (e.g. florfenicol (90 d): 44.5 ± 14.3% aR, COV = 32.1%; imidacloprid (90 d): 40.0 ± 21.6% aR, COV = 54.1%). This can be explained by differences in extraction procedures used by the participating institutes.

No mineralization (neither to $^{14}CO_2$ nor $^{14}CH_4$) was observed in the experiments with florfenicol and imidacloprid. The peak in mineralization for imidacloprid after 7 days (3.33 ± 4.69 % aR) is caused by a high amount of $^{14}CH_4$ (33.2 % aR) measured in one replicate by Institute 3. However, this value can be regarded as a measuring error since the mass balance for that replicate is clearly too high and the other replicate shows no mineralization.

The results are further displayed in the box-plot diagram (Figure 32) and in Table 47. For florfenicol in pig manure only 2 out of 41 values are outside the upper and lower whiskers (comprising 95% of the data), for imidacloprid in cattle manure 4 out of 49 values are not within this range.

Results regarding dissipation of the parent compound (DT_{50}-values; see section 3.5) are summarized in Table 46. Single data refer to mean DT_{50} values across replicates for each institute. The mean coefficients of variation of 52.6% for florfenicol in pig manure and 37.2% for imidacloprid in cattle manure are acceptable, e.g. considering an estimated coefficient of variation for DT_{50}-values in different soils of about 100% (FOCUS, 2000). Clearly higher variation of DT_{50}-values in different soils were also reported by Howard (1993).

Table 42: Summary of ring test results for ^{14}C-florfenicol in pig manure [% aR]; mean, standard deviation and coefficient of variation for mineralization, extractables, NER and mass balance; 4 institutes[1)]; mean across institutes for each sampling time point

Time [d]	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
Mineralization											
Mean	0.02	0.06	0.07	0.04	0.04	0.04	0.05	0.07	0.23	0.48	0.56
SD	0.00	0.02	0.06	0.01	0.00	0.01	0.01	0.02	0.10	0.20	0.26
COV%	29.6	40.8	87.7	24.7	5.8	16.7	24.7	26.6	43.6	42.4	46.3
Extractables											
Mean	95.2	97.9	95.3	89.3	81.5	81.1	67.1	59.7	58.5	50.4	49.6
SD	3.5	5.0	6.6	11.1	2.7	8.3	9.7	11.8	13.6	9.8	16.1
COV%	3.6	5.1	6.9	12.5	3.3	10.3	14.5	19.8	23.3	19.6	32.3
NER											
Mean	2.3	4.2	4.8	6.9	15.1	15.8	25.6	37.8	35.1	44.0	43.6
SD	2.2	2.6	2.9	4.9	0.8	8.7	12.6	13.3	15.2	4.3	14.6
COV%	96.5	60.9	60.8	71.2	5.6	54.8	49.0	35.1	43.3	9.8	33.5
Mass balance											
Mean	97.6	102.1	100.2	96.2	96.6	96.9	92.2	97.6	94.8	94.9	93.8
SD	3.3	2.9	5.1	9.6	2.2	9.2	4.3	2.2	8.7	5.7	12.2
COV%	3.4	2.9	5.0	9.9	2.4	9.4	4.7	2.2	9.2	6.1	13.1

For florfenicol in pig manure, data from institute 4 were not considered for evaluation.

Table 43: Summary of ring test results for ^{14}C-florfenicol in pig manure [normalized, % aR]; mean, standard deviation and coefficient of variation for mineralization, extractables, NER and mass balance; 4 institutes[1)]; mean across institutes for each sampling time point

Time [d]	0h	0.5h	1h	2h	4h	7h	24h	7d	28d	60d	90d
Mineralization											
Mean	0.02	0.06	0.07	0.04	0.04	0.04	0.05	0.07	0.23	0.49	0.57
SD	0.00	0.02	0.06	0.01	0.00	0.01	0.01	0.02	0.10	0.21	0.26
COV%	29.6	40.7	87.3	24.7	5.8	16.7	24.7	26.5	43.6	42.4	46.4
Extractables											
Mean	97.6	98.7	97.8	91.4	82.2	83.1	69.1	61.6	60.1	50.7	50.8
SD	2.2	4.0	6.8	9.7	3.2	8.3	12.6	14.4	15.1	9.4	15.8
COV%	2.3	4.1	7.0	10.6	3.9	10.0	18.2	23.4	25.1	18.4	31.0
NER											
Mean	2.3	4.3	4.9	7.0	15.2	16.1	26.0	38.4	35.6	44.4	44.5
SD	2.2	2.6	2.9	5.0	0.9	8.7	12.5	12.8	15.0	4.8	14.3
COV%	95.3	61.2	59.7	70.7	6.1	54.2	48.0	33.4	42.0	10.8	32.1
Mass balance											
Mean	100.0	103.0	102.8	98.5	97.4	99.2	95.1	100.0	96.0	95.7	95.9
SD	0.0	1.9	4.3	7.0	3.0	6.3	2.6	1.7	7.7	4.9	9.6
COV%	0.0	1.8	4.2	7.1	3.1	6.4	2.7	1.7	8.1	5.1	10.0

For florfenicol in pig manure, data from institute 4 were not considered for evaluation.

Figure 29: Mean for mass balance, extractables, NER and mineralization data [normalized; % aR] and standard deviation (shaded area) within the ring test for ^{14}C-florfenicol in pig manure; 4 Institutes

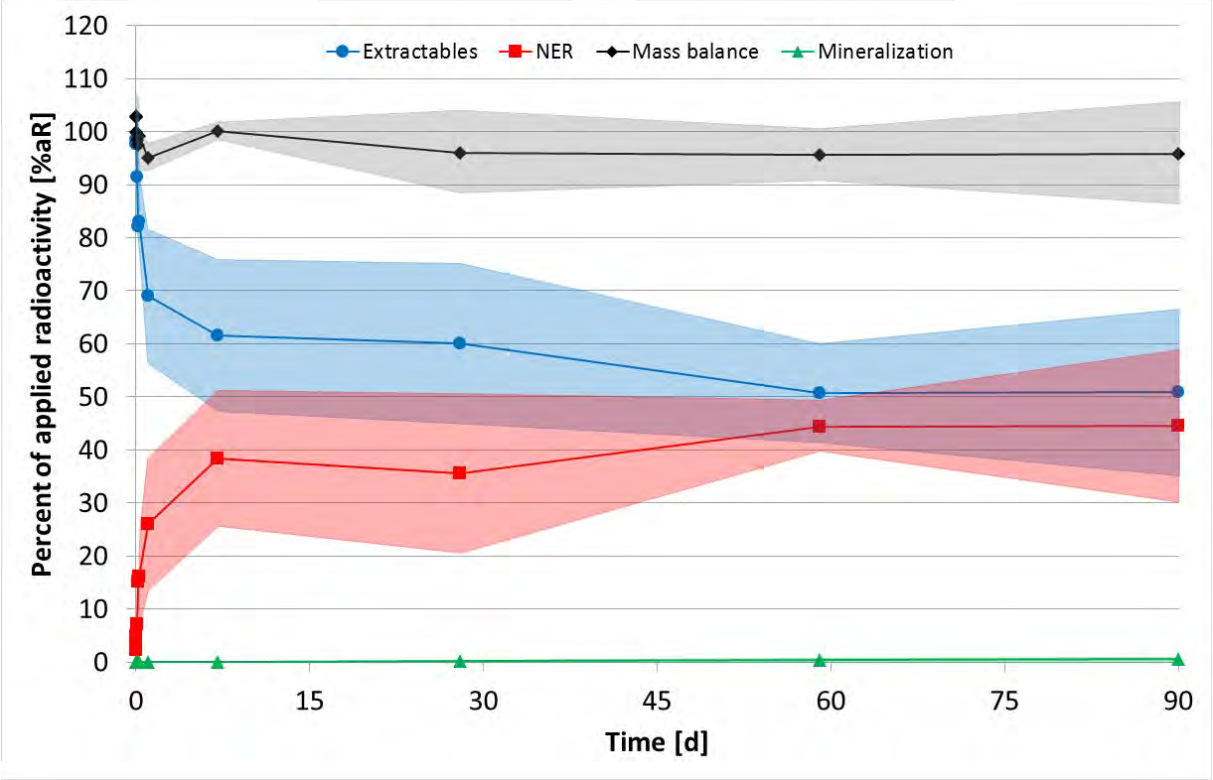


Figure 30: Mean for mass balance, extractables, NER and mineralization data [normalized; % aR] and standard deviation (shaded area) within the ring test for ^{14}C-imidacloprid in cattle manure; 4 Institutes;

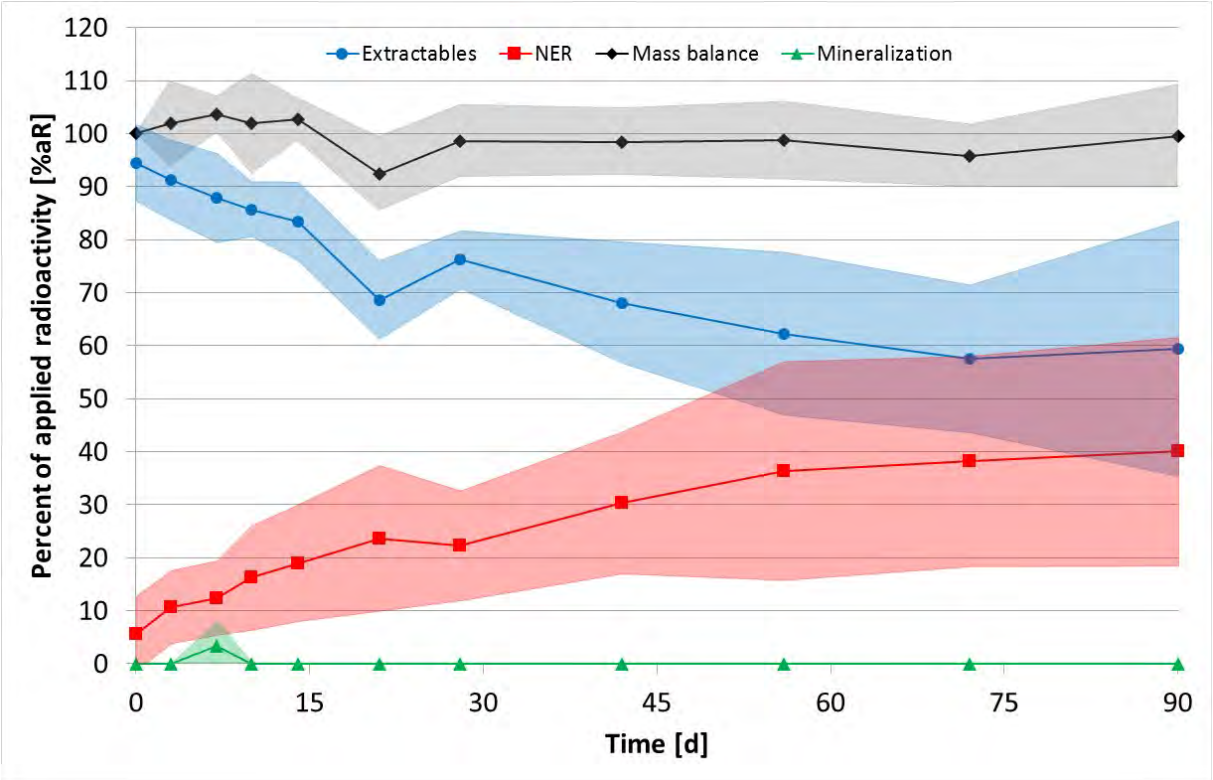


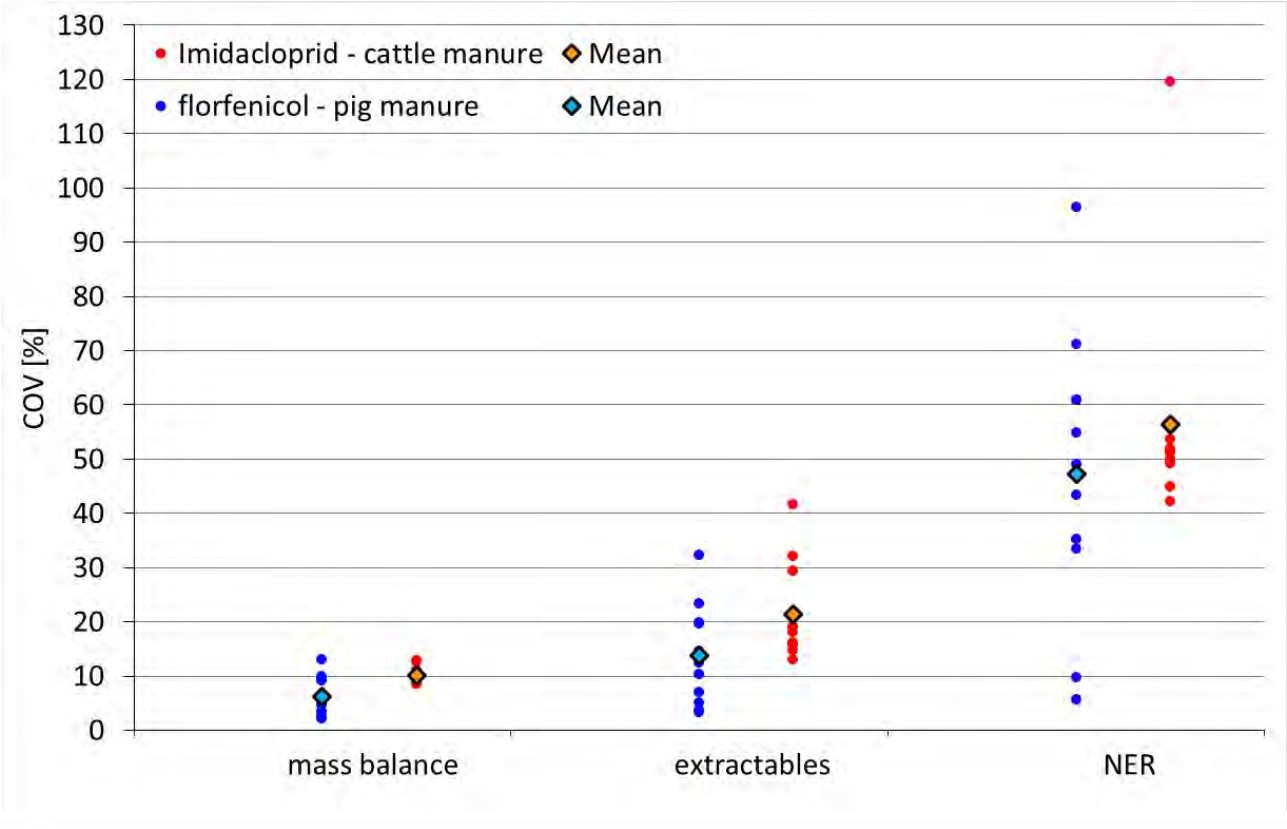
Table 44: Summary of ring test results for ^{14}C-imidacloprid in cattle manure [% aR]; mean, standard deviation and coefficient of variation for mineralization, extractables, NER and mass balance; 5 institutes; mean across institutes for each sampling time point

Time [d]	0	3	7	10	14	21	28	42	56	72	90
Mineralization											
Mean	0.00	0.01	3.36	0.01	0.04	0.01	0.01	0.02	0.03	0.03	0.02
SD	0.00	0.00	4.74	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01
COV%	0.00	35.0	141.1	31.5	4.4	26.7	15.2	46.1	26.7	35.4	46.9
Extractables											
Mean	92.9	89.6	86.3	86.5	84.5	66.9	74.7	66.7	63.6	58.4	58.7
SD	14.9	14.3	13.8	12.8	15.3	12.8	9.7	12.7	20.4	17.2	24.4
COV%	16.1	16.0	16.0	14.8	18.1	19.1	13.0	19.0	32.0	29.4	41.6
NER											
Mean	5.1	9.9	11.8	15.7	18.5	22.3	21.7	29.4	35.6	37.7	38.6
SD	6.1	5.5	5.8	8.0	9.2	12.0	9.7	12.4	18.5	18.5	19.8
COV%	119.5	55.7	49.5	51.3	49.9	53.7	45.0	42.2	52.0	49.1	51.4
Mass balance											
Mean	98.0	99.5	101.4	102.2	103.1	89.2	96.4	96.1	99.3	96.2	97.3
SD	10.8	10.6	9.6	8.8	9.4	7.6	10.0	8.3	12.8	9.5	12.2
COV%	12.7	10.6	9.4	8.6	9.1	8.5	10.3	8.7	12.9	9.9	12.6

Table 45: Summary of ring test results for ^{14}C-imidacloprid in cattle manure [normalized, % aR]; mean, standard deviation and coefficient of variation for mineralization, extractables, NER and mass balance; 5 institutes; mean across institutes for each sampling time point

Time [d]	0	3	7	10	14	21	28	42	56	72	90
Mineralization											
Mean	0.00	0.01	3.33	0.01	0.04	0.01	0.01	0.02	0.03	0.03	0.02
SD	0.00	0.00	4.69	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01
COV%	0.00	33.9	141.1	30.5	4.4	26.9	15.2	45.1	26.1	35.5	45.6
Extractables											
Mean	94.5	91.3	87.9	85.7	83.5	68.6	76.2	68.1	62.3	57.5	59.4
SD	7.2	7.6	8.4	5.3	7.4	7.4	5.5	11.5	15.3	13.9	24.2
COV%	7.6	8.3	9.6	6.2	8.9	10.8	7.2	16.9	24.6	24.2	40.7
NER											
Mean	5.5	10.6	12.4	16.2	19.0	23.6	22.3	30.3	36.3	38.2	40.0
SD	7.2	6.8	7.0	9.9	11.0	13.7	10.4	13.4	20.7	19.8	21.6
COV%	129.2	64.5	56.5	60.7	57.8	57.8	46.5	44.1	56.9	51.8	54.1
Mass balance											
Mean	100.0	101.9	103.7	102.0	102.6	92.3	98.6	98.5	98.7	95.8	99.5
SD	0.0	8.0	3.3	9.5	4.1	6.8	6.7	6.3	7.3	5.9	9.7
COV%	0.0	7.8	3.2	9.3	4.0	7.4	6.8	6.4	7.4	6.2	9.8

Figure 31: Coefficients of variation [%]: mean across institutes (dots) for mass balance, extractables and non-extractable residues (NER) and total mean (open square) during the transformation studies of ring test.



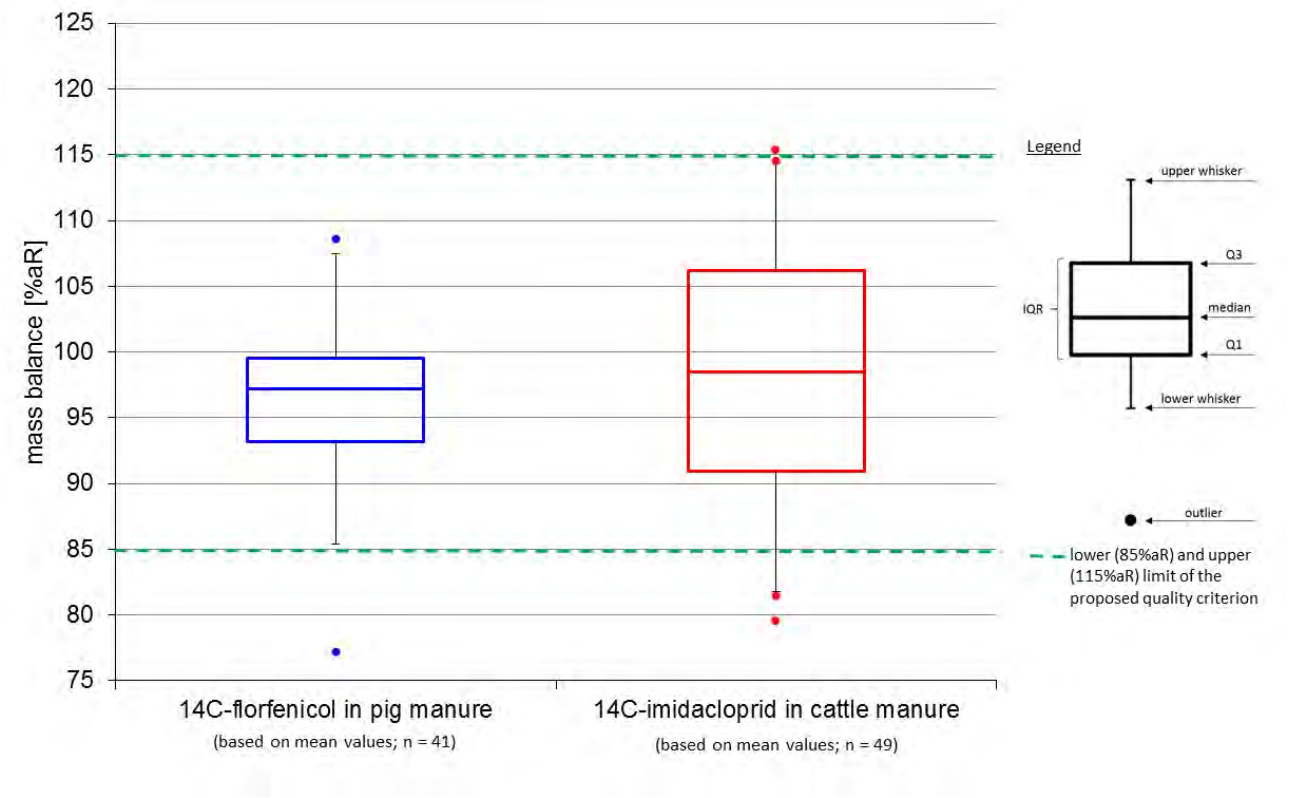
Note: For florfenicol in pig manure, data from institute 4 were not considered for evaluation.

Table 46: Mean, standard deviation and coefficient of variation [%] for dissipation of the parent compound (DT_{50}-values) during the transformation studies of the ring test (mean across replicates for each institute at 20°C)

Institute	1	2	3	4	5	6	Mean
Florfenicol							
Mean	0.406	0.364	0.169	0.008	n.d.	0.538	0.341
SD	0.207	0.149	0.125	0.007	n.d.	0.127	0.494
COV%	20.97	15.02	12.56	0.73	n.d.	12.78	52.61
Imidacloprid							
Mean	21.69	17.41	21.29	40.03	n.d.	n.d.	23.819
SD	0.017	0.032	0.020	0.073	n.d.	n.d.	0.360
COV%	1.66	3.16	1.95	7.36	n.d.	n.d.	37.23

Note: For florfenicol in pig manure, data from institute 4 were not considered for evaluation. n.d. = not determined

Figure 32: Boxplot diagram of mass balances (based on mean values) [% aR] within the ring test (^{14}C-florfenicol in pig manure and ^{14}C-imidacloprid in cattle manure)



Note: The ends of the error bars (whiskers) are set at the 97.5%-percentile (upper whisker) and at the 2.5%-percentile (lower whisker). Thus, the whiskers comprise 95% of the data. Values outside this range, are shown as dots. For florfenicol in pig manure, data from institute 4 were not considered for evaluation.

Table 47: Maxima, minima and percentiles of mass balances (%aR) within the ring test

	^{14}C-florfenicol in pig manure	^{14}C-imidacloprid in cattle manure
Maximum	108.6	115.3
3rd quartile	99.5	106.0
median	97.2	98.0
1st quartile	93.2	91.0
Minimum	77.2	79.5
97.5 percentile	107.5	114.4
2.5 percentile	85.4	83.3

3.11 Transformation of florfenicol in pig manure at 10°C - special method considerations for identification of transformation products

3.11.1 Extraction and clean-up method

The identification of degradation products required the determination of transformation products using LC-MS/MS and non-radiolabeled florfenicol. Hence, it was mandatory to have a proper extraction, clean-up and concentrating of florfenicol and transformation products because the ionization in the ESI interface of an LC-MS/MS system, and thus the result of the analysis vastly depends on the sample matrix.

To deliver extracts suitable for LC-MS/MS analysis, a modified and advanced method was developed. This encompassed especially centrifugation at much higher acceleration, additional clean-up and concentration steps.

Original method (see 2.3.1.2 method 1)

a) Batch extraction

- Manure sample in 50 mL Falcon tube
- Add same volume of 0.02 M KH_2PO_4/ACN 1:1 (v:v)
- Shake end-over-end for 20 minutes
- Centrifuge at 2600 g for 10 minutes
- Repeat this twice and combine extracts.
- Filtration of extract using e.g. 0.2 mm PTFE filters.

b) Additional ASE

c) HPLC using RPC18 column and gradient elution using 20 mM ammonium acetate and ACN.

New method

d) Batch extraction

- Manure sample in 50 mL Falcon tube
- Add same volume of 0.02 M KH_2PO_4/ACN 1:1 (v:v)
- Shake end-over-end for 20 minutes.
- Centrifuge at 15 000 g for 15 minutes
- Repeat this two more times with 1 minute vortexing; do not combine extracts.

e) Two-step clean-up using solid phase extraction (SPE) (according to Salvia et al. (2012), modified)

First clean-up using strong anion exchanger (SAX) cartridge (e.g. SB, Chromabond, Macherey-Nagel, Düren, Germany)

- Conditioning: 5 mL ACN followed by 5 mL conditioning buffer (0.04 M citric acid)
- Pass extract No. 1 through one and extracts No. 2 and 3 through a second SAX cartridge
- Elute into and combine in a 100-mL pear-shaped flask
- Wash SAX with extraction solution and collect
- Remove ACN on a rotary evaporator; water remains

Second clean-up using HLB cartridge (e.g. Strata-X, Phenomenex, Aschaffenburg, Germany)

- Conditioning: 5 mL ACN followed by 5 mL conditioning buffer (0.04 M citric acid).
- Pass extract and sequentially wash cartridge with 5 mL 0.04 M citric acid and 5 mL 0.1 M sodium acetate (NaOAc).
- Dry cartridge using vacuum (min. 10 minutes).
- Elute analytes with 10 mL ACN into 25 mL pear-shaped flask.
- Evaporate the eluate on a rotary evaporator until dryness (40°C, ~100 mbar). To avoid foaming/boiling and to further diminish proteins etc. add ~0.5 g SAX (QuEChERS[10] technique).
- Re-dissolve in 1 mL of H_2O/ACN 50:50 (v:v).
- Add internal standard: 10 µL chloramphenicol 50 µg/mL; final concentration 500 ng/mL.
- Eventually filtrate using fiberglass filter (e.g. Chromafil GF-100/15MS).
- Store extracts at 8°C in the dark until measurement.

3.11.1.1 Test of additional accelerated solvent extraction (ASE) of centrifugation pellet

Because not all florfenicol and ^{14}C-radioactivity from radiolabeled florfenicol could be recovered by the aforementioned method, the following ASE procedure was tested. To this end centrifugation pellets remaining after manure extraction with 0.02 M KH_2PO_4/ACN 1:1 (v:v) were store the at -18°C in the dark until ASE.

ASE method

- Freeze dry the centrifugation pellet.
- Weigh 1 g into extraction cartridge (Dionex 350).
  ASE conditions: 100°C; 5 minutes heating, 10 minutes static extraction (12.000 kPa) using 100% ACN.
- Eventually further clean-up: Transfer extract into glass-centrifuge tube.
  QuEChERS: Conditioning of SAX powder in ACN. Add ~0.5 g to the extract (to remove additional water add ~0.5 g anhydrous Na_2SO_4).
  Centrifuge and reduce extract in a rotary evaporator to ~1 mL.
- Add internal standard, eventually filtrate using fiberglass filter. Store extracts at 8°C in the dark until measurement.

The LC-MS/MS analysis of florfenicol and major transformation products in ASE extracts from the spiked soil samples using non-radioactive florfenicol revealed that additional ASE after preceding mild solvent extraction using 0.02 M KH_2PO_4/ACN 1:1 (v:v) did not yield sufficiently measurable compounds. Only small amounts (< 1.8% of residing compounds) were extracted. Such detectable concentrations were limited to the freshly spiked soil samples (incubation time ≤ 1 day) and the sterilized samples from day 1 and 28. In all other samples, signals for all analytes (florfenicol and transformation products) were below the limit of detection. This result cannot be due to strong matrix interference with the ionization in the ESI source of the LC-MS/MS. This became evident from the good recovery of the spiked internal standard chloramphenicol. Instead it is concluded that the com-

[10] QuEChERS stands for Quick, Easy, Cheap, Effective, Rugged, Safe, and is the acronym for a simplified solid phase extraction (SPE) method adding bulk SPE material to the liquid sample or extract for removal of impurities and interfering substances instead of passing the sample through an SPE-cartridge. The method was first published by Anastassiades et al. (2003) and later adopted to a broader spectrum of analytes and matrices. The method has been collaboratively studied on a large number of commodity/pesticide combinations and is described in detail in the European Standard EN 15662:2008 (CEN, 2008).

pounds resisting threefold extraction with 0.02 M KH_2PO_4/ACN 1:1 (v:v) are strongly immobilized on manure organic matter. Thus, additional ASE is not recommended.

3.11.1.2 Recovery rate of the method

Analysis of florfenicol in freshly spiked manure samples (0.5 hours of incubation) showed that the recovery rate of the method was below 100%. For non-radioactive florfenicol spiked at 3 mg/kg to manure, the recovery rate was 59.8% (± 1.17%; standard deviation). For rapidly transforming substances it is thus advisable to determine analytical recovery immediately after spiking. It was determined that each of the three sequential extraction steps contributed to the recovery of florfenicol:

Step 1: 45.3% (± 1.60%)

Step 2: 10.0% (± 0.35%)

Step 3: 4.5% (± 0.08%)

This proved that the threefold extraction using 0.02 M KH_2PO_4/ACN 1:1 (v:v) is appropriate.

Recovery rates showed the same trend but were quantitatively somewhat different when measuring ^{14}C-radioactivity from radiolabeled florfenicol (see Table 48).

To further identify the work steps most relevant for losses of the analyte, the extraction method was followed up step-by-step using ^{14}C-radiolabelled florfenicol. Results for the recovery from spiked manure that was incubated for 24 hours are shown in Table 48. It can be seen that the recovery of the applied radioactivity (100 kBq = 100%) was quite high with 114 % recovery after the three-stage extraction, 111 % after clean-up by SPE with SAX sorbent and 114 % after the second SPE using HRX sorbent. Recoveries > 100% are probably due to inhomogeneous distribution of suspended and/or dissolved organic matter from manure, containing adsorbed florfenicol. Considerable losses (recovery of the applied radioactivity =74.3%) occurred upon rotary evaporation, which was combined with a third sample clean-up step. For the latter SAX sorbent was used and applied with the QuEChERS technique, to avoid foaming and to further diminish proteins etc. A further reduction in recovery down to 44% was obtained after filtration of the concentrated sample using a fiberglass filter (Table 48).

Table 48: Recovery of radioactivity from ^{14}C-florfenicol from spiked manure over the different work steps of the extraction and clean-up procedure.

Work step	Procedure	Activity [kBq/mL]	Volume [mL]	Activity [kBq]	Total Activity [kBq]
1	1st extraction	2.7595	32	88.3	114.0
2	2nd extraction	0.7795	28	21.8	
3	3rd extraction	0.1395	28	3.91	
4	1st extract after SAX	2.7305	32	87.4	111.7
5	2nd & 3rd extract after SAX	0.4345	56	24.3	
6.1	Loss from HRX	0.1035	47	4.86	
6.2	Eluate from HRX	11.4765	10	114.8	
7	1 mL concentrate from rotary evaporation	74.3115	1	74.3	
8	1 mL concentrate fiberglass filtrated	44.0435	1	44.0	

Initial radioactivity was 100 kBq so that activity [kBq] can be directly read as recovery rate [%]. Extraction was done 24 hours after spiking.

Further tests revealed that the losses in radioactivity were not due to volatilization, sorption of florfenicol to the fiberglass filter or incomplete extraction. Non-extractable residues in freshly spiked manure (0.5 hours of incubation) amounted to 2.3% of the spiked radioactivity from ^{14}C-florfenicol (data not shown). Furthermore, the fiberglass filter was shown to produce minor losses of florfenicol when pure standard was filtrated (Table 49).

Together with the polyester (PET) filter, fiberglass is most recommended to be used for filtration of florfenicol extracts. Instead, it is strongly assumed that the losses of florfenicol/radioactivity during rotary evaporation combined with QuEChERS clean-up and upon filtration are due to the intended removal of suspended organic matter from the analysis solution. The observed losses show that substantial parts of florfenicol are sorbed to this suspended organic matter, and thus get lost upon QuEChERS clean-up and filtration.

Table 49: Recovery of florfenicol in H_2O/ACN 50:50 (v:v) without (control) and with filtration using four different syringe filters (Macherey-Nagel, Düren, Germany).

Filter types		Recovery		Recovery rate
		[ng/mL]	SD	[%]
	Control without filter	860.67	3.74	100.00
1	Fiberglass - Chromafil GF 100/15MS	812.33	5.94	94.38
2	Regenerated cellulose - Chromafil RC 45/15	780.67	1.12	90.70
3	PET - Chromafil Xtra PET 45/25	818.00	2.12	95.04
4	PTFE - Chromafil 0-45/15MS	719.00	2.19	83.54

SD = standard deviation.

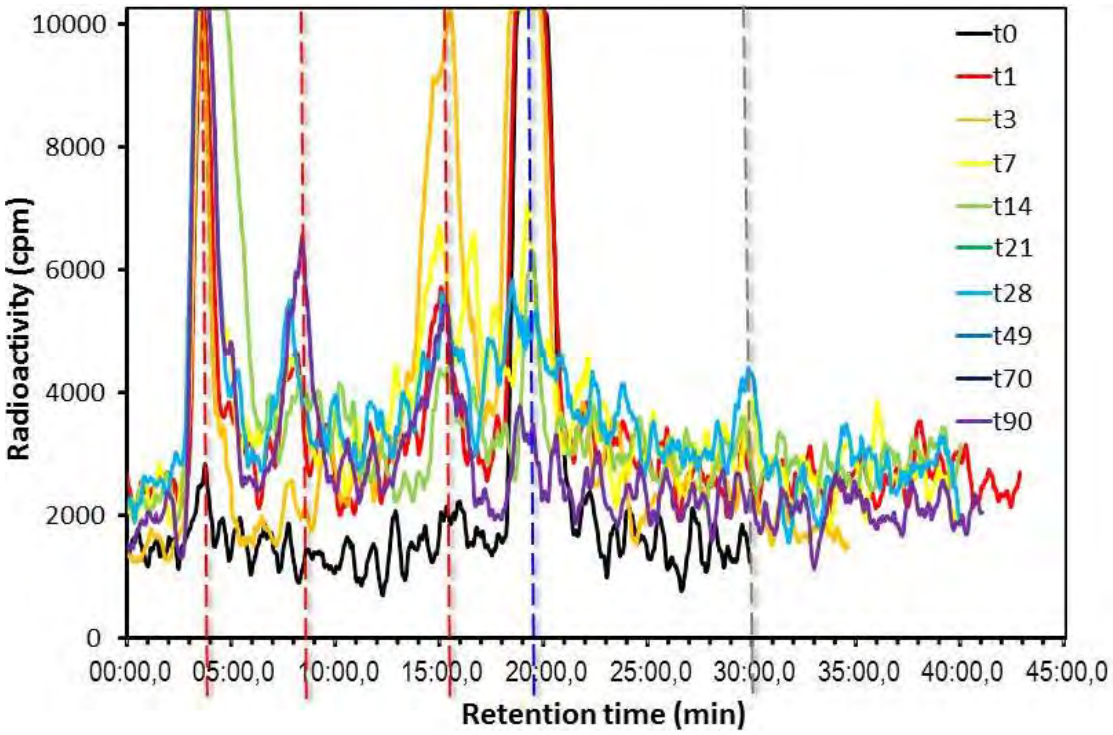
Calibration

Calibration of LC-MS/MS was done using external standards of florfenicol (50, 100, 200, 300, 400, 500, 750, 1000 ng/mL) and florfenicol amine (10, 20, 50, 100, 200, 500, 750, 1000 ng/mL). For radioactivity measurements the calibration curves were done using ^{14}C-florfenicol standard solutions with 0.1, 0.5, 1, 5, 10, 20, 30, 40 and 50 kBq/mL. All calibrations were linear within the tested range.

3.11.2 Assignment of transformation products

From radio-HPLC analyses it became clear that florfenicol not only dissipated over time due to immobilization. At least part of the decline in the signal from ^{14}C-florfenicol was due to transformation into transformation products. Three additional signals from radioactivity developed over time with retention times of 4.0, 8.3 and 15.0 minutes for radio-HPLC. Transferred to the retention times in LC-MS/MS (with shorter dead time) these were 2.0, 6.3 and 13.0 minutes. Due to the broadness of the new signals at 4.0 and 15.0 minutes each could represent one or two transformation products (Figure 33). The signal at 30.0 min only occurred in a few samples (day 7, day 28) without meaningful kinetics. All reported and/or suggested transformation products of florfenicol are more polar (smaller K_{ow} and/or higher electronegativity due to the type of functional groups; see Table 50). Hence, no transformation product is expected to occur in reversed phase chromatography at higher retention times compared to florfenicol. Thus, the signal at 30.0 minutes was not further considered.

Figure 33: Chromatograms from Radio-HPLC; data smoothing by adjacent averaging 100. Dashed lines show retention times of radioactivity signals that significantly deviate from background noise.



The assignment of transformation products was done using non-radiolabeled florfenicol and florfenicol amine. Compounds were analyzed using product ion scan (PIS) and multiple reaction monitoring (MRM) experiments in LC-MS/MS. The masses analyzed are listed in Table 50.

The results from LC-MS/MS showed that the retention time of florfenicol amine (2.0 minutes) corresponded to the retention time of one of the three new signals in radio-HPLC (4.0 minutes). Additionally the parent mass of florfenicol amine (m/z 247) and of the major fragments (products) matched with the retention time.

Focusing on the other two retention times, PIS experiments were done in ESI^+ and ESI^- ionization mode, using the parent and fragment masses of the proposed transformation products (Table 50). Based on this, the radioactivity and LC-MS/MS signals at 13.0 and 15.0 minutes, respectively, were assigned to florfenicol oxamic acid. The signal at 6.3 and 8.3 minutes, respectively, remained unidentified. No signal with this retention time was obtained in LC-MS/MS with neither of the applied measuring conditions and parent and fragment masses.

Using the external standard calibration, florfenicol and florfenicol amine were quantified. Clear kinetics of the dissipation and formation/dissipation of the two compounds were observed (see Annex 3 for results of chemical analyses and Annex 4 for transformation plots). Florfenicol oxamic acid and the unknown transformation product were only (semi-)quantitatively determined because no standard compounds were available for these compounds. Florfenicol oxamic acid was present in higher contents from the start of the experiment. Florfenicol amine as well as the unknown transformation product were formed during the incubation before dissipating again towards the end of the experiment.

It is obvious that the incline in the three transformation products could not quantitatively explain the decline in florfenicol. It was suspected that further transformation products occur especially at very short incubation times. However, this was not confirmed; no additional signals were determined in extracts from samples incubated for 0.5 and 4 hours.

Furthermore, additional transformation products were screened for. This is an acetyl-conjugate as well as a methyl-conjugate of florfenicol and a fragment [4-(methylsulfonyl)phenyl]methanol ($C_8H_{10}O_3S$) that has been reported to evolve in MS analysis (Alechaga et al., 2013). It was hypothesized that this typical fragmentation product of the molecule may also be a typical product of chemical and/or biological degradation. It was found that the conjugates did not occur during the degradation process. The same was found for [4-(methylsulfonyl)phenyl]methanol, although fragments with the typical m/z-ratio of [4-(methylsulfonyl)phenyl]methanol clearly occurred with high intensity already in the first samples. Yet, the concentrations of [4-(methylsulfonyl)phenyl]methanol did not follow meaningful kinetics but arbitrarily changed from sampling time to sampling time. Further analyses revealed that a different compound with similar m/z-ratio must be contained in manure.

In summary, the following transformation products have been assigned:

1. Florfenicol amine
2. unknown transformation product
3. florfenicol oxamic acid

We propose that florfenicol is first transformed to florfenicol oxamic acid by substitution of chloride with a carboxyl group. Most likely, this intermediate product is further transformed to florfenicol amine by amide bond hydrolysis. It is assumed that florfenicol amine is not the major parent compound for the formation of florfenicol amine because the formation of florfenicol amine goes along with the dissipation of florfenicol oxamic acid. The formation and subsequent dissipation of the unknown transformation product parallels that of florfenicol amine. Since no mass identification and thus molecular characterization of the unknown transformation product was possible, no transformation pathway can be suggested for this compound. From the retention time in reversed phase chromatography it is concluded that the polarity of the unknown transformation product is in between of florfenicol amine and florfenicol oxamic acid. All transformation products disappeared again during the incubation time, showing that no dead-end transformation products could be identified.

DT_{50}-values for the parent compound, calculated by means of the KinGUI-software tool using “single first order” (SFO) kinetics, are presented in section 3.5. The corresponding transformation plots can be looked up in Annex 4, chemical analysis raw data are summarized in Annex 3.

Table 50: Putative and identified transformation products of florfenicol and parameters for LC-MS/MS analysis

	Name	Formula	CAS	Molar mass	Fragments for LC-MS	Esi Mode[a] for LC-MS	logKow	Koc	S[b] [mg/L]	H-Donor/ Acceptor	Identified
1	**Florfenicol**	$C_{12}H_{14}Cl_2FNO_4S$	73231-34-2	357	185/336	negative	-0.04	205.2	5936	5/2	Yes
2	**Florfenicol amine**	$C_{10}H_{14}FNO_3S$	76639-93-5	247	130/230	positive	-1.27	79.8	1.00E+6	4/3	Yes
3	**Florfenicolalcohol** N-[1-(fluoromethyl)-2-hydroxy-2-(4-methylsulfonylphenyl) ethyl]-2-hydroxy-acetamide	$C_{12}H_{16}FNO_5S$	-	305	185c	negative	< -0.04	-	-	-	No
4	**Florfenicol oxamic acid** 2-[[1-(fluoro-methyl)-2-hydroxy-2-(4-methylsulfonyl phenyl)ethyl] amino]-2-oxo-acetic acid	$C_{12}H_{14}FNO_6S$	-	319	185	negative	< -0.04	-	-	-	Yes
5	**Monochloroflorfenicol** 2-chloro-N-[1-(fluoromethyl)-2-hydroxy-2-(4-methyl-sufonylphenyl)ethyl]acetamide	$C_{12}H_{15}ClFNO_4S$	-	323	185	negative	-	-	-	-	No
6	N-2-hydroxy-2-4-methansulfonylphenyl-1-methyl-ethyl-acetamide	$C_{12}H_{17}NO_4S$	-	271	185	negative	-1.05	34.8	1.41E+5	?	No
7	N-1-fluoromethyl-2-hydroxy-2-4-methane-sulfonylphenylethylacetamide	$C_{12}H_{16}FNO_4S$	-	289	185	negative	-1.11	67.3	1.25E+5	?	No
8	**Thiamphenicol**	$C_{12}H_{15}Cl_2NO_5S$	15318-45-3	355	185	negative	-0.27	10.0	9660	6/3	No
9	**Chloramphenicol**	$C_{11}H_{12}Cl_2N_2O_5$	56-75-7	323	152	negative	1.14	10.0	2500	7/3	No

a) ESI = Electrospray inonization used in LC-MS/MS; b) S = water solubility at 25°C; c) italized fragment masses were estimated from molecular structure; chemical data on logKow, Koc, S- and H-donor/acceptor properties were taken from ChemSpider (http://www.chemspider.com/Search.aspx) and calculated with EPI-Suite (© by US EPA, 2000), respectively

3.11.3 Conclusions on identification of transformation products

- For LC-MS/MS-analysis a thorough clean-up of manure extracts is required as many interfering substances are present in manure extracts without further clean-up.
- The following methods and techniques were found to be useful for extraction.
  - A three times repeated extraction using a mixture of an aqueous salt solution with a polar solvent. For florfenicol this was 0.02 M KH_2PO_4 / ACN 1:1 (v:v).
  - Centrifugation at high acceleration to enable maximum settling of manure particulate and colloidal matter. For florfenicol this was centrifugation at 15 000 g für 15 min.
  - Multi-step solid phase extraction using 1. SAX cartridge, 2. HLB cartridge and 3. additional SAX applied with the QuEChERS technique upon rotary evaporation of the solvent.
- The use of an internal standard is mandatory for chromatographic analysis. For florfenicol this was chloramphenicol.
- Further filtration of the sample might be required with best recovery obtained with PET filter and fiberglass filter.
- Additional extraction using pressurized liquid extraction did not yield sufficiently measurable contents of florfenicol.
- Analysis using LC-MS/MS is best suited for the detection of polar compounds such as pharmaceuticals.
- For the determination of florfenicol and its major transformation products analyses using the negative and the positive ionization mode are required. With the combination of radio-HPLC and LC-ESI-MS/MS using product ion scan (PIS) and multiple reaction monitoring (MRM) experiments, each performed with a suite of different modes and parameter settings proved useful to identify transformation products in manure
- The identification of transformation products and pathways is often hindered due to the unavailability of standard substances. This was the case for the majority of the suggested transformation products of florfenicol. This problem might be circumvented by the use of high resolution mass spectrometry.

3.12 Modification, discussion and further development of the draft test guideline

The first version of the draft test guideline was compiled in the framework of the preceding project „Development of test guidance for transformation of veterinary pharmaceuticals and biocides in liquid manure“ (Hennecke et al., 2015). The guideline had been updated several times based on experiences from intra-laboratory comparisons and an international inter-laboratory comparison (prevalidation ring test). Based on the results and the discussions during a workshop held in Flörsheim, Germany, in April 2013, the text of the draft test guideline has been revised and critical steps of the test performance have been specified.

The revised draft guideline was then used as basis in the international ring test in 2013/2014, which is described in the present report. After conducting the ring test the applicants presented their experiences at an international technical workshop in Gießen, Germany, in September 2014 (see section 3.12.1), resulting in an updated draft test guideline. Besides the revision of the existing guideline text (e.g. paragraphs on reference substance, preparation of sterile samples, stripping out potentially dissolved CO_2), several annexes were added to the guideline, describing e.g. extractions and clean-up methods and incubation systems.

In addition, the current state of the method development, the planning for ring testing and experimental results were discussed at four international meetings within the program of SETAC (Society of Environmental Toxicology and Chemistry) conferences (2012 Berlin, 2013 Glasgow, 2014 Basel, 2015 Barcelona). In total, 50 particpants from industry and Contract Research Organisations (CRO) as well as participants with regulatory background and academic researchers from 10 countries (Canada, Denmark, Finland, Germany, Japan, The Netherlands, South Korea, Switzerland, United Kingdom, United States) took part in the meetings.

For a complete list of all participants at the technical workshops and the meetings at SETAC conferences see Annex 5. For the current version of the draft test guideline see Annex 1.

3.12.1 Technical workshops

Two technical workshops were held on the pre-validation ring test at ECT Oekotoxiokologie GmbH, Flörsheim, Germany on April 18th/19th, 2013 and on the ring test at the University of Giessen, Germany on September 25th/26nd, 2014. The main focus of the workshops was the presentation and discussion of results and experiences of the participants with the ring test experiments together with international experts. Furthermore, the current version of the draft test guideline was discussed in detail and revised based on the experiences of the participants. In the following, the presentations and discussions during both workshops are summarized. The participants of both technical workshops are listed in Table 51.

Table 51: List of participants of the technical workshops in 2013 and 2014

Name of participant	Affiliation	Country	Workshop	
			2013	2014
Aikens, Peter	Huntingdon Life Sciences	UK	-	X
Atorf, Cornelia	Fraunhofer IME	Germany	X	-
Berkner, Silvia	Umweltbundesamt	Germany	X	X
Bicker, Su	Ibacon GmbH	Germany	X	X
Düring, Rolf-Alexander	University Giessen	Germany	X	X
Fiebig, Silke	Noack Laboratorien GmbH	Germany	-	X
Gilberg, Daniel	ECT Oekotoxikologie GmbH	Germany	X	-
Hennecke, Dieter	Fraunhofer IME	Germany	X	X
Herrchen, Monika	Fraunhofer IME	Germany	X	X
Heusner, Elena	ECT Oekotoxikologie GmbH	Germany	X	X
Junker, Thomas	ECT Oekotoxikologie GmbH	Germany	X	X
Konradi, Sabine	Umweltbundesamt	Germany	-	X
Meinerling, Maria	Ibacon GmbH	Germany	X	X
Römbke, Jörg	ECT Oekotoxikologie GmbH	Germany	X	-
Schwarz, Lisa	University Giessen	Germany	X	-
Thiele-Bruhn, Sören	University Trier	Germany	X	X
Topp, Ed	Agriculture and Agri-Food Canada	Canada	X	X
van Vlaardingen, Peter	RIVM	The Netherlands	X	-
Wehrhan, Anne	Harlan Laboratories Ltd.	Switzerland	X	-
Wohde, Manuel	University Giessen	Germany	X	X

Figure 34: Participants of the technical workshops in Flörsheim 2013 (left) and in Giessen 2014 (right)


3.12.1.1 Presentations and discussions at the workshop 2013

After a presentation on the background and the aim of the preceding project (Hennecke et al., 2015), the development of the test method and the draft guideline was presented. In addition, the results of the inter-laboratory comparison were presented and discussed. Based on these experiences, the current version of the draft test guideline as well as necessary changes or problems were discussed at the workshop.

Incubation conditions

Flow-through as well as static test systems have to maintain anaerobic/methanogenic conditions. It was questioned whether the flow-through conditions are realistic, because tanks or lagoons are more or less static systems. Furthermore, problems may occur, if H_2 is removed from the system with the gas flow since hydrogen is then missing for other processes, e.g. formation of CH_4. No final conclusion could be drawn on the suitability of the test design (static/flow-through) but, the option for using a static test system was added to the description of the test method. In addition, it was decided to compare static test systems and flow-through test system in further tests.

Dry matter content

Although the dry matter content might vary in the tank or lagoon due to different layers, the adjustment of the dry matter content to a uniform, representative value for testing purposes seems to be very important.

An extensive increase of the dry matter content by centrifugation was criticized because microorganisms and dissolved organic carbon are removed together with supernatant. Therefore, a minimum initial dry matter content was added to the description of the test method: If the dry matter content is below the recommended value, it can be concentrated by careful centrifugation (e.g. 10 minutes at 740 x g). However, the initial dry matter content should not be below 8% (cattle) or 3% (pig). If dry matter content is too high, water (deionized water, bubbled with nitrogen for 30 minutes) should be added as needed." It was further decided to conduct additional experiments to investigate the influence of the dry matter content of the manure on the parameters mineralization, DT_{50} and NER.

Microbial activity

The outcome of the ^{14}C-glucose mineralization tests as a measure of microbial activity of the manure, seemed to be not predictive for the results of the test substance. Further testing is required to check the suitability of the glucose test for measuring microbial activity of the manure. However, no other methods are available so far that are better suited.

Test item concentration/application

The test item concentration should always be mentioned when presenting the results. Different test results might be obtained due to different application techniques (locally high test concentrations might be toxic to the microorganisms). Therefore, the application of the test item into the manure was simulated in a practical demonstration. It became apparent that comparable application techniques have been used by all participants of the pre-validation ring test. Thus, differences in the results cannot be explained by different application techniques.

Measurements and Analysis

Problems may occur with regard to chemical analysis if transformation products are detected at very low but continuously increasing concentrations during the study (e.g. 1% aR continuously increasing up to 1.5% aR at the end of the study).

Beyond that, the required test item purity of ≥95% means that up to 5% impurities might be present. Therefore, it would not make sense to identify transformation products below 5% aR. Thus, a modified wording was suggested: "Transformation products once detected at ≥ 5% for which concentrations are continuously increasing during the study afterwards should also be identified, even if their concentrations do not exceed the limit given above, as this may indicate persistence."

Quality Criteria

The redox conditions throughout the study have to guarantee methanogenic conditions as is observed in a manure tank. Care has to be taken to fulfil these conditions. It was recommended to prevent air entering the system during removal of single replicates by using valves or a special set-up design (e.g. replicates for one sampling time point in series instead of in parallel).

A high mass balance is important to be able to use the results from the study. In other simulation type studies (e.g. OECD 307 (OECD, 2002a) and OECD 308 (OECD, 2002b)) the criterion is 90-110% mass balance for radiolabeled compounds. This range was not met by all participants, thus less stringent criteria might be needed. This point was addressed in the following ring test.

Variability of results

Large differences were observed in this first comparison exercise between the results of the participants for transformation of ^{14}C-salicylic acid in cattle and pig manure. The variability seemed to be mainly caused by differences in the test design and in test procedures at the different laboratories. This can be explained by the fact that many of the participants were unexperienced in performing transformation studies with manure and many of the test parameters had not been fixed sufficiently in advance (e.g. minimum and maximum dry matter content). For that reason, a precise description of manure handling, particularly for critical steps, had been added to the draft test guideline in view of the upcoming ring test.

3.12.1.2 Presentations and discussions at the workshop 2014

After a presentation on the background and the aim of this project the results of the preceding project and the first inter-laboratory comparison were summarized (see above). In the following, all ring test participants presented details on the test setup, sampling and extraction procedures as well as results of the experiments and observed problems. The presentations were followed by discussions on different topics regarding performance and evaluation of the ring test, which are summarized in the subsequent section.

Quality criteria and microbial activity of manure

Several methods are suggested for testing the microbial activity of manure (e.g. EMA, 2011).

The mineralization of a readily biodegradable compound (e.g. ^{14}C-glucose) was measured within the pre-validation ring test. However, results showed high variation and did not correlate with the results for the mineralization of the test compound ^{14}C-salicylic acid. Although, the variation might have been caused by insufficient time to strip out $^{14}CO_2$ after acidification of the manure, further testing would be required to check the suitability of the test method for measuring microbial activity of the manure. Additionally the suitability of glucose as a representative compound to conclude on the transformation ability of the manure for VMP and biocides was questioned. Reduction of DMSO to DMS can be used as measurement of anaerobic microbial activity without interference (Griebler and Slezak, 2001). The determination of gas production (e.g. according to VDI (2006)) of manure without addition of a test compound at several time points throughout acclimation and incubation was investigated as a method to check microbial activity of the manures. The gas production was similar for both cattle and pig manure at the beginning of the acclimation period, but gas production decreased inconsistently afterwards. Thus, further testing (e.g. using several different manures in parallel) would be needed as well to check if this method is suitable for measuring microbial activity of the manure.

Another method to determine the microbial biomass (not the microbial activity) is the fumigation method according to ISO 14240–2 (ISO, 1999). The active and the inactive biomass were determined, but false positive or false negative results were often obtained. The fumigation method can – in principle – be used to determine the microbial biomass under anaerobic conditions. However, preliminary tests showed that they are not applicable to the matrix manure due to very high background values (Hennecke et al., 2015).

Based on the experiences with ^{14}C glucose method in the pre-validation ring test, this method was not recommended for the ring test. As no other suitable methods were available, testing the microbial activity within the ring test (e.g. according to one of the methods mentioned above) was optional.

As an alternative, the participants agreed that the mineralization of ^{14}C-labeled salicylic acid as reference compound should be used to get information on microbial activity. The transformation behavior of salicylic acid was already examined previously by Hennecke et al. (2015). Salicylic acid (e.g. as sodium salicylate (CAS-No. 54-21-7) or as salicylic acid (CAS-No. 69-72-7) has been tested for transformation under anaerobic conditions in pig and cattle manure and found to be mineralized to a high extent to CO_2 and CH_4 (> 50% aR to 80% aR within 85 to 91 days). DT_{50}-values (SFO, dissipation of parent) observed in tests with 6 different pig and 3 different cattle manures ranged from 3 d to 30 d (Hennecke et al., 2015). $DT_{50,MIN}$ values (SFO, mineralization to CO_2 and CH_4) observed in tests with two pig and two cattle manures ranged from 15 d to 49 d (Herrchen et al., 2016). It is suggested to use the reference substance at a concentration of 24 mg/kg wet manure (corresponding to 0.02 mg ^{14}C salicylic acid (75 kBq) plus 1.18 mg unlabeled salicylic acid applied to 50 g manure fresh weight. For the reference compound, the same results as for the test substance have to be reported.

A new section for testing the reference substance salicylic acid was added to the final draft guideline (see Annex 1) to be able to ensure comparable conditions for different tests with different manures. Consequently, no other measurement of microbial activity is needed.

Anaerobic test conditions and redox potential

The redox potential does not directly measure the presence of anaerobic or methanogenic conditions but it is the most feasible measurement and is commonly used as an indicator. The threshold value of -100 mV was adopted from OECD Guideline 308 (OECD, 2002b). The intention is to get conditions in the transformation experiments that mimic closely the conditions observed in reality in storage facilities for liquid manure, where redox potentials have been found to be typically in the range of -230 mV to -400 mV (Weinfurtner, 2011). If the guideline is followed, it should be no problem to meet the redox criteria.

Measurement of methane (CH_4)

In the preceding project mineralisation to CH_4 was measured for salicylic acid (up to 6% aR) and glucose (up to 23% aR). If feasible, CH_4 should be determined to prevent losses of volatile transformation products and an incomplete mass balance. Furthermore, measurement of CH_4 can be used to prove the presence of methanogenic microorganisms, which is an indication for similar microbial communities as present in manure storage tanks (Barret et al., 2013).

Possible adaptation of microorganisms

Adaptation of microorganisms in manure pre-exposed to test substances cannot be excluded when sampling manure directly from a tank. However, it was agreed to use manure directly sampled from a tank or storage device and only to adjust the dry matter content instead of trying to set up a manure matrix from individual components, as this produces more problems in the long run. Mixtures obtained from mixing excrements (faces and urine) from animals were found to have a completely different microbial community structure based on DGGE (Denaturating Gradient Gel Electrophoresis) experiments (unpublished results, personal communication Kornelia Smalla, Julius-Kühn-Institut, Braunschweig, Germany). All ring test participants had been able to obtain suitable manure. It was stressed that if manure is sampled from a tank and has to be used directly in the experiment, there is a low risk of adaptation occurring compared to preparing some kind of “standardized” manure by mixing urine and faeces collected separately or rearing specialized animals for the purpose of collecting their manure. It was agreed, that transformation should be studied under conditions as close as possible to the situation in a real world manure tank, in which VMPs, biocides as well as other substances might be present. The inclusion of a positive control or reference substance into the method is a way of dealing with uncertainties stemming from this fact. To further take this into account, the medication that the animals received and used disinfection products in the stable should be recorded for the last 6 months prior to sampling and this information should be part of the study report. It is in the responsibility of the laboratory conducting the study to choose a manure suitable for testing purposes based on this information.

Number of manures

Transformation in manure is a complex study. For reasons of feasibility and practicability it was concluded that presently, one manure was considered to be sufficient if selected carefully and the same manure is concurrently tested with the reference compound.

Preparation of sterile manure samples

For laboratories that have no knowledge on working with the matrix manure, sterilization of samples might pose difficulties. Therefore detailed information on procedures that have been proven effective for the ring test laboratories have been included into the guideline, like e.g. autoclaving at least twice and preheating the samples (100°C for at least 12 hours) prior to autoclaving.

Test substance concentration in manure

Since the guideline should be applicable for several exposure scenarios with different calculations for maximum expected manure concentration it is not be possible to include detailed guidance how to calculate this value. It was added to the guideline that the calculation method and a rationale for its choice have to be reported. Substance specific regulatory frameworks (e.g. EMA, 2011) contain information on exposure scenarios.

Determination of mineralization

Some participants of the ring test observed that three hours of incubation after acidification were insufficient to strip out the entire dissolved CO_2. The time period should therefore be prolonged to at least 24 hours. If a (semi-)static test system is used, bubbling the manure might be necessary in addition. Nitrogen should be used to ensure anaerobic conditions.

Which extraction methods to use?

The text in the draft guideline was adopted from the EMA guideline (EMA, 2011). Accordingly, it is recommended that a sequential extraction method is followed and the remaining residues are combusted. The fraction of radiolabel therein is termed NER. The extraction method(s) have to be tailored to the specific test substance (and transformation products). Analytical methods (including extraction methods and clean-up methods) have to be validated and reported in detail for each test compound by the laboratory conducting the study.

Suitability of different test setups

According to the outcome of the inter-laboratory comparison, a semi-static as well as a flow-through test setup are suitable. Further experiments revealed that the parameter mineralization might show differences in between both systems (Herrchen et al., 2016). Therefore, a semi-static test system is recommended. Periodic purging with nitrogen is needed to avoid losses due to overpressure in the test vessels.

4 Conclusions

Based on the results and the experiences in the ring test, the discussions at the international workshops in 2013 and 2014 as well as the discussions with stakeholders at four international mettings at SETAC conferences (2012 Berlin, 2013 Glasgow, 2014 Basel, 2015 Barcelona), the following conclusions can be drawn:

- The test method described in the draft test guideline proved to be applicable for routine measurements of the transformation of veterinary pharmaceuticals and biocides in pig and cattle manure.
- A semi-static test design should be used, whereas a strictly static test design is not recommended due to the potential loss of volatile transformation products and an incomplete mass balance that might result from overpressure in the closed system without periodic flushing.
- Anaerobic conditions can be ensured when using the test method described in the draft test guideline as demonstrated by measured redox potentials below -100 mV throughout the entire test period. The observed redox potentials are in the range typically found in liquid manure storage tanks of -230 mV to -400 mV (Weinfurtner, 2011).
- A reference substance should be tested in parallel to the test compound to be able to ensure comparable conditions for different tests with different manures. Salicylic acid (e.g. as sodium salicylate CAS: 54-21-7 or as salicylic acid CAS: 69-72-7) is proposed as reference substance since it has been tested for transformation under anaerobic conditions in pig and cattle manure and found to be mineralized to a high extend to CO_2 and CH_4. If a reference substance is tested, there is no need to test for microbial activity (e.g. mineralization of ^{14}C-glucose).
- One manure is considered to be sufficient if selected carefully and the same manure is concurrently tested with the reference substance.
- Autoclaving is recommended to prepare sterile samples. Autoclaving should be performed at least twice and the samples should be heated (100°C for at least 12 hours) in advance.
- The time period to strip out potentially dissolved $^{14}CO_2$ after acidification of the manure should be at least 24 hours. If a semi-static test system is used, bubbling the manure might be necessary in addition. Nitrogen should be used to ensure anaerobic conditions.
- If feasible, $^{14}CH_4$ should be determined to avoid losses and an incomplete mass balance. Furthermore, measurement of $^{14}CH_4$ can be used to prove that methanogenic microorganisms are present in the manure.
- Mean ^{14}C mass balances averaged across all participants throughout the test period were in the range between 92.2% aR and 102.1% aR for florfenicol in pig manure and between 89.2% aR and 103.1% aR for imidacloprid in cattle manure. In consideration of frequency distributions of mass balances and other existing test guidelines on transformation of chemicals in different compartments, a quality criterion of 100 ± 15% aR for mass balance is recommended.
- For the identification of transformation products analysis using LC-MS/MS is suited best. For LC-MS/MS-analysis a thorough clean-up of manure extracts is required and further filtration of the sample might be needed. The use of an internal standard is recommended for chromatographic analysis.
- The SFO model is considered to be appropriate for determination of DT_{50}-values for transformation in manure. Mean DT_{50}-values were in the range between 17.4 d and 40.1 d for imidacloprid in cattle manure and between 0.17 d and 0.41 d for florfenicol in pig manure.
- The amounts of extractable residues (ER) and non-extractable residues (NER) depend on the extraction method used. Therefore, analytical procedures (including extraction and clean-up methods) have to be validated and reported test substance specific.

- Low variability and spread in the results were observed in the ring test for extractable residues (COV at the end of the test: 31.0-40.7%), non-extractable residues (COV at the end of the test: 32.1-54.1%) and DT_{50} values (overall COV: 37.2-52.6%) and particularly for ^{14}C-mass balances (COV at the end of the test: 9.8-10.0%).

In summary, the draft test protocol prove to be applicable in different laboratories with different levels of experience with manure and different experimental set-ups using individually sampled manure from storage tanks. The observed variability in between different participating laboratories allows to obtain reproducible results suitable for the purpose of characterizing the fate and transformation of chemicals in manure.

In conclusion, the experimental method described in the final draft version of the test guideline (see Annex 1) is considered well-suited to examine the anaerobic transformation of organic compounds, including veterinary pharmaceuticals and biocides, in liquid manure.

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Annex 1

Draft Guideline

DRAFT FOR A GUIDELINE FOR THE TESTING OF CHEMICALS

Anaerobic Transformation in Liquid Manure

INTRODUCTION

1. This guideline describes methods to examine the transformation of chemicals in pig and cattle manure under anaerobic conditions. The test guideline is based on existing guidelines [1,2]. The experiments are performed to determine the rate of transformation of the test substance, the identity and rates of formation and decline of transformation products, the amount of test substance that is mineralized to CO_2 or CH_4 or other volatiles, and the amount of non extractable residues (NER). Such studies are relevant for chemicals that are administered to animals housed in stables and later on excreted as e.g. veterinary medicinal products or for chemicals that are applied in stables and may also enter the manure collected from these stables (e.g. biocides).

PRINCIPLE OF THE TEST

4. Liquid manure samples are treated with the test substance and incubated in the dark under controlled laboratory conditions (at constant temperature and dry matter content under anaerobic conditions). After appropriate time intervals, manure samples are removed, extracted and analyzed for the parent substance and for transformation products. Volatile products are collected for analysis using appropriate trapping devices to quantify formation of CO_2 and CH_4. Using ^{14}C-labelled material, mineralization rates of the test substance can be measured and a mass balance, including the formation of NER, can be established. Results enable the calculation of DT_{50}, and, if appropriate, DT_{90}.

5. Transformation studies should be performed in manure of a relevant species. Liquid manure that is the predominant type of manure in Europe and North America [3] is a mixture of urine, faces and water used to clean the stables and may also contain bedding material. Typical dry matter contents for pig and cattle manure have been found to be 5% and 10%, respectively [3].

APPLICABILITY OF THE TEST

6. The method is applicable to all chemical substances for which an analytical method with sufficient accuracy and sensitivity is available. It is applicable to slightly volatile, non-volatile, water-soluble or poorly water-soluble compounds. The test should not be applied to chemicals which are volatile from water (e.g. fumigants, organic solvents).

INFORMATION ON THE TEST SUBSTANCE

7. Non-labeled or labeled test substance can be used to measure the rate of dissipation of the parent compound. ^{14}C-radio-labelled material is required for studying the pathway of transformation, for quantifying CO_2- and CH_4-formation, formation of NER, screening for and quantification of transformation products and for establishing a mass balance. The label(s) should be positioned in the most stable part(s) of the molecule. For complex molecules (e.g. containing more than one aromatic ring system) or for extensively substituted molecules, labeling in different positions might be necessary. The active ingredient should be labeled so that the transformation pathway can be traced as far as possible and transformation products can be tracked. If multiple ring structures or significant side chains are present, separate studies reflecting labeling of each ring or side chain will normally be required if it is anticipated that cleavage between these moieties may occur. A scientifically based rationale may be submitted in lieu of conducting studies with multiple radiolabels if no cleavage is anticipated. However, if cleavage of the molecule is evident, it may be necessary to

conduct an additional study with a radiolabel that tracks the portion of the molecule that is cleaved. In choosing the position to be labeled, assurance is needed that a stable position is selected. The choice of the labeling position(s) should be justified and illustrated in a structural formula of the test substance.

The use of stable isotopes such as ^{13}C, ^{15}N, or ^{2}D (nonexchangeable) together with the radio-labelled isotope is encouraged to aid in identification of metabolites by various spectroscopic methods (mass spectrometry (MS) or nuclear magnetic resonance (NMR)).

8. The purity of the test substance should be at least 95%. Deviations should be justified.

9. Before carrying out the test on transformation in manure, the following information on the test substance should be available and should be taken into account:

(a) solubility in water (TG 105; [4]),

(b) solubility in organic solvents,

(c) vapour pressure (TG 104; [5]) and Henry's law constant,

(d) n-octanol/water partition coefficient (TG 107; [6]),

(e) chemical stability in water (hydrolysis) (TG 111; [7]),

(f) pK_a if a molecule is liable to protonation or deprotonation (TG 112; [8]).

Other useful information may include data on toxicity of the test substance or transformation products to microorganisms, e.g. according to TG 209 [9] or TG 216 [10].

10. Analytical methods (including extraction and clean-up methods) for quantification and identification of the test substance and its transformation products should be available. If available, standard substances should be used for the characterization and/or identification of transformation products by spectroscopic and chromatographic methods.

If standard substances are unavailable identification based on spectrometric techniques (mass spectrometry, nuclear magnetic resonance spectrometry) may be attempted.

REFERENCE SUBSTANCE

11. A reference substance should be included to be able to ensure comparable conditions for different tests with different manures. Salicylic acid (e.g. as sodium salicylate CAS: 54-21-7 or as salicylic acid CAS: 69-72-7) has been tested for transformation under anaerobic conditions in pig and cattle manure and found to be mineralized to a high extent to CO_2 and CH_4 (> 50% to 80% within 85 to 91 d). DT_{50} values (SFO, dissipation of parent) observed in tests with 6 different pig and 3 different cattle manures ranged from 3 d to 30 d [11]. $DT_{50\ MIN}$ values (SFO, mineralization to CO_2 and CH_4) observed in tests with two pig and two cattle manures ranged from 15 d to 49 d [12]. It is suggested to use the reference substance at a concentration of 24 mg/kg wet manure (corresponding to 0.02 mg ^{14}C salicylic acid (75 kBq) plus 1.18 mg unlabeled salicylic acid applied to 50 g manure fresh weight. For the reference compound, the same results as for the test substance have to be reported. A description of an extraction scheme for salicylic acid can be found in Annex 1.

DEFINITIONS

12. See Annex 2.

Mass balance, recovery, repeatability and sensitivity of analytical method

13. Extraction and analysis of, at least, duplicate manure samples immediately after the addition of the test substance and at the end of incubation gives an indication of the recovery of the analytical method and of the uniformity of the application procedure for the test substance. Recoveries concerning radiolabeled material are given by the respective mass balances. At the beginning of the test, mass balance should range from 85% to 115% for labeled chemicals and 70% to 110% for non-labeled chemicals. In addition, the mean mass balance for all time points for labeled chemicals should be within 85% and 115%. Results given as % of applied radioactivity have to be normalized to 100% at time point 0. Repeatability of the analytical method (excluding the initial extraction efficiency) to quantify test substance and transformation products can be checked by duplicate extraction and analysis of the same extract of the manure, incubated long enough for formation of transformation products.

14. The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be at least 1% of the applied dose or at least 0.01 mg/kg, whichever is lower. The limit of quantification (LOQ) should also be specified.

Kinetic evaluation of the test data

15. The quality of the fit of an appropriate kinetic model to the test data should be evaluated according to the recommendations of the FOCUS group [13]. More sampling time points might be required if transformation products are observed to be able to derive DT_{50} and DT_{90}.

DESCRIPTION OF THE METHOD

Equipment and chemical reagents

16. The incubation is conducted in a suitable system. An example of a semi-static and a flow-through incubation apparatus are shown in Annex 3 and Annex 4, respectively. Other incubation systems are described in references [1] and [2].

17. To ensure anaerobic conditions, humidified nitrogen is passed over the samples in the beginning and intermittently (for the semi-static system) or continuously (for the flow-through system). NaOH filled traps (or another appropriate trapping solution) are used to trap evolving CO_2. Potentially formed methane (CH_4) accumulates in the incubation flask (no excess pressure to be expected, semi-static system) or passes through the CO_2-traps (flow-through-system) and is subsequently combusted in an oven (temperature details in the annexes) to form CO_2, and finally trapped in a CO_2-trap. To completely capture formed CO_2, acidification of the sample is recommended. A detailed description is given in Annex 3 (semi-static system) and Annex 4 (flow-through system). To verify that the radioactivity trapped in the CO_2-traps is $^{14}CO_2$ and not from potentially also formed volatile fatty acids (VFA), $Ba(OH)_2$ precipitation of the radioactivity can be conducted.

18. Standard laboratory equipment is required and especially the following:
- Analytical instruments such as GC or HPLC, including the appropriate detection systems for analyzing radiolabeled or non-labeled substances,
- Instruments for identification purposes (e.g. RAM, MS, HRMS, NMR, etc.),
- Liquid scintillation counter,
- Oxidiser for combustion of radioactive material,
- Centrifuge,
- Extraction apparatus (for example, centrifuge tubes for cold extraction, Soxhlet apparatus for continuous extraction under reflux, apparatus for accelerated solvent extraction, i.e. extraction under high pressure and temperature),
- Instrumentation for concentrating solutions and extracts (e.g. rotating evaporator),
- Water bath,
- Mechanical mixing device (e.g. kneading machine, rotating mixer, hand blender).

19. Chemical reagents used include, for example:
- NaOH, analytical grade (2 M), or other appropriate base (e.g. KOH, ethanolamine)
- $Ba(OH)_2$, analytical grade (0.25 M)
- H_2SO_4, analytical grade (0.05 M)
- HCl (10%), analytical grade,
- Organic solvents, analytical grade, such as acetone, methanol, etc.,
- Inorganic salts, analytical grade, such as KH_2PO_4 (for extraction solvents),
- Scintillation liquid.

Manure

Manure selection

20. Manure for testing purposes should be sampled from manure storage or pre-storage tanks or manure lagoons. Storage facilities may be above ground or below ground. Manure should stem from animals that are reared under well controlled conditions. The manure should not have been exposed to the test substance or compounds from the same substance class within the last six months. This should be demonstrated by obtaining information on the medication of the animals producing the manure in the respective time period. The laboratory conducting the studies is responsible for selecting an appropriate manure and providing a statement in the report, if farmers are not willing to consent that specific information is revealed in the study report. The number, type and age of animals should be known as well as their feed. Studies on transformation in manure should be conducted in manure of the relevant species (e.g. pigs, cattle). See also the paragraph 26 on manure characterization. At least one manure per species has to be used. If several manures were sampled and turned out to be not suitable e.g. due to matrix parameters not conforming to the specifications, then the results should nevertheless be reported.

Collection, handling and storage of manure

Sampling of pigs and cattle liquid manure

21. Prior to collection the liquid manure should be homogenized by mixing in the respective manure tank. Pig manure should be stirred immediately before sampling as separation into liquid and solid phase easily occurs. Cattle manure should be stirred no more than one day before sampling. For mixing devices installed in the tank or external devices may be used. Mixing for one hour proved sufficient for homogenization of manure in the tanks independent from tank volume.

22. Liquid manure is collected from the tank by appropriate equipment (e.g. a ladle with a large beaker), and filled into containers. Filling up to approximately ¾ of maximum container volume might be appropriate. Containers are closed tightly but must allow gas, which is generated by continuous microbial activity, to expand. This can be achieved by connecting a tube with a fermentation air lock to an outlet in the container. This also prevents odors from escaping from the containers.

23. The sampling site, the sampling procedure (time and duration of mixing), and the type and size of manure tank (above/below ground, covered/open) should be recorded in detail. A template can be found in Annex 5.

Storage of liquid manure

25. Prior to further processing manure might by stored at 4°C to 20°C (preferably at the test temperature) for up to two months. Storage should ensure anaerobic conditions. Care has to be taken to allow gas, generated by biological activity during storage, to expand to avoid explosion of the container. This can be achieved by connecting a tube with a fermentation air lock to an outlet of the container. This also prevents odors from escaping from the containers.

Manure characterization

26. Key parameters that have to be measured and reported (with reference to the method used) and the stage of the test at which those parameters have to be determined are summarized in the table hereafter.

Measurement of parameters for characterization of liquid manure

Parameter[11]	Stage of test procedure				
	Sampling	Start of acclimation	Start of test	During test	End of test
pH[12]	X	X	X	X	X
organic matter content [%][13]		X			
nitrogen content [N_{total}; mg/kg][14]		X			
nitrogen content [NH_4-N; mg/kg][15]		X			
redox potential [mV][16]	X	X	X	X[17]	X
dry matter content [%][18]	X	X	X		X
ash content [%][19]					
temperature [°C]	X	X	X	X	X

Data after sampling (start of acclimation, start of test, during test and at the end of the test) have to be reported based on the adjusted dry matter content and wet mass of the manure.

If non-labeled test substance is used, a background control or blank sample has to be analyzed, to exclude the presence of the test substance or to quantify a possible background concentration of the test substance in the manure.

Establishing of test conditions

Acclimation

27. Prior to the start of the acclimation period, the dry matter content of the manure has to be determined. To get comparable conditions it has to be adjusted to standardized values. The recommended dry matter content in cattle and pig manure is 10 ± 1% (m/m) and 5 ± 1% (m/m), respectively [1,3]. If the dry matter content is below the recommended value, it can be concentrated by careful centrifugation (e.g. for 10 minutes at 740 x g). However, the initial dry matter content has to be ≥ 8% (cattle) or ≥ 3% (pig). If these minimum values are not met, fresh manure has to be collected. If the dry matter content is too high, water (de-ionized water, bubbled with nitrogen for 30 minutes) should be added as needed.

28. Thereafter, cattle manure should be homogenized by mixing. No additional measures to prevent introduction of oxygen are used. Subsamples of 50 – 100 g (wet weight) each should be directly filled into the incubation vessels which are used for the acclimation and transformation study.

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[11] For all matrix parameters it has to be specified in the report whether they relate to dry or to wet mass of the sample.

[12] e.g. ISO 10390 „Soil quality -- Determination of pH“ [14]

[13] e.g. DIN 12879 „Charakterisierung von Schlämmen - Bestimmung des Glühverlustes der Trockenmasse” [15]

[14] e.g. ISO 11261 “Soil quality - Determination of total nitrogen - Modified Kjeldahl method” [16]. For conversion of mass based units in volume based units a density of 0.001 kg/m^3 is used

[15] e.g. ISO 5664 “Water quality - Determination of ammonium - Distillation and titration method” [17].

[16] e.g. ISO 11271 “Soil quality - Determination of redox potential - Field method” [18] and/or DIN 38404-6 “Determination of the oxidation reduction (redox) potential” [19]

[17] It has to be assured that the given specifications for the redox potential are met throughout the study. Therefore, at least one measurement in the middle of the test period is recommended.

[18] e.g. DIN 12880 “Characterization of sludges - Determination of dry residue and water content” [20]

[19] According to European Standard EN 15935 [21]

29. Pig manure should be homogenized under anaerobic conditions in order to obtain a fairly stable phase. This can be achieved, e.g. by filling the manure into a beaker, putting a mixer/homogenizer (e.g. hand blender) into the manure, and gently passing a nitrogen stream over the manure while mixing. Thereafter, the dry matter content should be adjusted. After a repeated homogenization under anaerobic conditions by thoroughly mixing (set up as above) subsamples of 50 g (fresh weight) are filled into the incubation vessels.

30. If a semi-static apparatus (see Annex 3) is used, the incubation system is flushed with nitrogen for 1 hour to maintain anaerobic conditions. Thereafter, the incubation system is closed by valves. If a flow-through apparatus (see Annex 4) is used, the incubation apparatus has to be closed and a constant, water saturated stream of nitrogen is passed over the manure at a rate in the range of approximately 50 - 200 mL/min.

31. The acclimation should be carried out for 21 (± 1 d) days at test temperature.

PERFORMANCE OF THE TEST

Test conditions

Test temperature and light conditions

32. During the whole test period the manure samples should be incubated in the dark at the appropriate test temperature. Typical environmental temperatures observed in manure tanks are 10°C for Central European climate conditions [3]. In other regions, different temperatures might be considered typical. To facilitate laboratory studies, studies may be conducted at 20°C (range of ± 2°C) and resulting DT_x values converted to environmentally relevant temperatures [22]. To determine the pathway of transformation, environmentally relevant temperatures might have to be used.

Anaerobic incubation conditions

33. Transformation studies in cattle and pig manure should be performed under anaerobic conditions similar to the conditions observed in a manure tank. Anaerobic conditions should be demonstrated by Eh ≤ -100 mV [2]. Redox potentials measured in pig and cattle manure have been found to range from -230 mV to -400 mV [3]. Redox potential should be measured and reported regularly to ensure stable anaerobic conditions throughout the experiment.

Abiotic controls

34. For information on the abiotic transformation of the test substance it is recommended to include sterile controls. For substances undergoing rapid abiotic transformation otherwise no meaningful results might be deduced from the study. Manure is sterilized, treated with sterile test substance and flasks kept closed carefully. Sampling of sterile controls should be according to the sampling schedule but sampling can be restricted to fewer time points. Sterile controls should be sampled at the end of the test. Sterilization can be achieved by autoclaving at least twice following this protocol: Preheat the manure in the test vessels overnight (at least 12 h) to 100°C. Let the vessels cool to room temperature during the day. Start first autoclaving cycle (15 min, 121°C, 100 bar) and let the test vessels again cool down to room temperature overnight to enable germination of bacterial spores. Then start the second autoclaving cycle (15 min, 121°C, 100 bar). This procedure helps to inactivate bacterial spores and prevents foaming. Other methods to stop the biological activity can be used, if appropriate (e.g. adding a toxicant or gamma irradiation).

Treatment and application of test substance

35. The test substance should be dosed into the manure at a concentration that reflects the maximum expected manure concentration, which depends on substance specific exposure scenarios. Concentrations in the mg/kg range are commonly observed for e.g. veterinary pharmaceuticals in manure [23]. The rationale for using a certain test substance concentration should be reported. If this concentration is not sufficient for detection and identification of transformation products, the test may be conducted at increased substance start concentrations. However, excessively high concentrations potentially toxic to microorganisms should be avoided.

36. The test substance should be dissolved in an appropriate solvent and should be added into the acclimated manure in the respective incubation vessels followed by thoroughly mixing while maintaining anaerobic conditions. This can be achieved, e.g., as follows: during application passing the nitrogen stream over the samples has to be maintained. The required volume of stock solution should be pipetted into the manure under simultaneous stirring using the pipette tip. As soon as the solution is evenly distributed in the manure the pipette tip remains in the manure. The total volume of a water miscible organic solvent used for application should not exceed 1% by volume.

Test duration and sampling

37. Test duration will depend on the rate of transformation of the parent compound and transformation products. The maximum study duration is 90 days. This time was derived from a survey on typical manure storage times [24] and during method development. Furthermore, validation studies were run up to 90 days. In certain cases it might be reasonable to prolong the study. Ideally, the test substance and transformation products should each be present in amounts below 10% of the applied amount at the end of the study. If the study is further prolonged, e.g. because increasing amounts of transformation products have been observed a test for microbial activity may be conducted at the beginning and end of the prolongation period. It might therefore be useful to have a further spare incubation vessel for this purpose.

38. At least duplicate incubation flasks are sacrificed per sampling. Sampling intervals should be selected in a way that the pattern of decline of the test substance, and the pattern both of formation and decline of transformation products can be established (e.g. 0, 1, 3, 7 days; 2, 3 weeks; 1, 2, 3 months, etc.). Besides sampling directly after application at least 9 additional sampling points should be included. More sampling time points may be necessary for kinetic modeling according to FOCUS recommendations [13] and to include transformation products. An experiment prior to the test start might give valuable indications for the behavior of the test substance and transformation products. In some cases rapid dissipation of the test substance may be observed and sampling time points have to be adjusted accordingly.

39. CO_2 and CH_4 are major volatile final transformation products which are expected from transformation under anaerobic conditions. Beside these compounds, volatile fatty acids (VFA) also might be formed. The experimental set-up has to be such, that the following requirements are fulfilled:

- quantitative capturing to avoid any losses of volatiles and enable establishment of a mass balance,
- differentiation between formed CO_2, CH_4 and VFAs.

For this purpose, traps to measure mineralization are removed at the same time intervals and analyzed for trapped $^{14}CO_2$ and other evolved gases, respectively. At first, adsorption traps are removed, replaced by freshly filled traps, and analyzed for radioactivity content. Thereafter, the manure incubation flasks to be removed at that particular sampling point are treated by addition of 10 mL 10% HCl in order to strip potentially dissolved CO_2 (or HCO_3^- / CO_3^{2-}). After addition of 10 mL 10% HCl, the incubation flasks are closed again and nitrogen is passed through for at least 24 hours. Thereafter, manure incubation flasks are removed and the samples are subjected to clean-up, extraction procedures and analyses. CO_2-traps are removed and radio-counted for additionally trapped $^{14}CO_2$.

For detailed descriptions of the sampling procedure see Annex 3 (semi-static system) and Annex 4 (flow-through system).

Measurements and analysis

40. Manure samples are cleaned-up directly after sampling. The samples are extracted with appropriate solvents of different polarity. A sequential extraction approach should be followed for optimal recovery of parent substance and transformation products of different polarity. Aqueous solvent mixtures and acid and base systems should be used as solvents to ensure extraction of more polar transformation products. In case non-extractable residues are observed, exhaustive extraction methods should be applied additionally. These methods comprise e.g. pressurized liquid extraction (e.g. ASE®), reflux, soxhlet etc. with appropriate solvents. When using ^{14}C-labeled test substance, the residues remaining after the last extraction step (non-extractable residues, NER) will be quantified by combustion and a mass balance will be calculated for each sampling interval. Analytes should not be altered by the respective extraction method. This can be demonstrated by appropriate controls for the known substances.

41. Concentration of the test substance and the transformation products at every sampling time should be determined and reported (see also paragraph 42). In general, transformation products detected at ≥ 10% of the applied radioactivity at any sampling time should be identified. Transformation products once detected at ≥ 5% of the applied radioactivity for which concentrations are continuously increasing during the study should also be identified, even if their concentrations do not exceed the limit given above, as this may indicate persistence.

42. Typically, identification is accomplished either by co-chromatography of the transformation product with known standards using two dissimilar systems or by techniques capable of positive structural identification such as MS, NMR, etc. In the case of co-chromatography, chromatographic techniques utilizing the same stationary phase with two different solvent systems are not adequate for the verification of the transformation product identity, since the methods are not independent.

Identification by co-chromatography should be obtained using two dissimilar, analytically independent systems, such as reverse and normal phase thin layer chromatography (TLC) or TLC and high performance liquid chromatography (HPLC). Provided that the chromatographic separation is of suitable quality, then additional confirmation by spectroscopy is not required. Unambiguous identification can also be obtained using methods providing structural information such as gas chromatography/mass spectrometry (GC-MS), liquid chromatography/mass spectrometry (LC-MS), liquid chromatography/tandem mass spectrometry (LC-MS/MS), and NMR.

The stereochemistry of transformation products generally does not need to be determined unless a differing behavior is observed.

New extraction and analysis techniques may be substituted for the techniques mentioned above. State of the art technology should be used, as appropriate, to fully elucidate the transformation pathway.

DATA AND REPORTING

Treatment of results and calculations

43. The results of the manure matrix parameters should be reported for each sampling point (based on wet weight, if applicable).

44. The amounts of test substance, transformation products, volatile substances, CO_2 and CH_4 and non-extractable residues should be given as % of applied initial amount and, where appropriate, as mg/kg manure (based on wet weight) for each sampling interval. A mass balance should be given in percentage of the applied initial amount for each sampling interval. Data should be reported separately for each replicate and as arithmetic mean of all replicates. Additionally, all data (see paragraph 48) should be given normalized to 100% radioactive mass balance at test start, separately for each replicate and as arithmetic mean of all replicates. A graphical presentation of the test substance and transformation product concentration against time on a non-logarithmic scale should be included. Major transformation products should be identified and their concentrations should also be plotted against time to show their rates of formation and decline. A major transformation product is any product representing ≥ 10% of the applied dose at any time during the study. Additionally transformation products should be identified if they show an increasing behavior towards the end of the study (see under 41).

45. Accurate determinations of DT_{50} and DT_{90}-values should be obtained by applying appropriate kinetic model calculations. The DT_x -values should be reported together with the description of the model used, and a measure for the goodness of fit. Details for calculations can be found in reference [13]. If appropriate, the calculations should also be applied to the major transformation products.

46. DT_x-values can be corrected to the relevant environmental temperature.

Validity of the test

47. The test has to be conducted under anaerobic/ methanogenic conditions typically found in manure tanks. As an indicator parameter the redox potential (reported as E_h) should be in a typical range for manure (-230 to -400 mV (or lower)) and never above -100 mV.

For radio-labeled compounds the mass balance in the beginning of the study should be within 85% to 115%. During the study, the arithmetic mean of the mass balances for all sampling time points should fall within that range. For unlabeled test substances the analytical recovery of the test compound should be at least 70% to 110%.

Test report

48. The report must include:

Test substance (and reference substance salicylic acid):

- common name, chemical name, CAS number, structural formula (indicating position of label(s) when radiolabeled material is used) and relevant physical-chemical properties,
- purity (impurities) of test substance,
- radiochemical purity of labeled chemical and specific activity (where appropriate);

Standard substance(s) for identification of transformation products:

- chemical name and structure of reference substances used for the characterization and/or identification of transformation products;

Analytical determinations

- methods for determination of manure matrix parameters
- methods for quantification and identification of the test substance, transformation products and reference substance
- recovery, repeatability, LOD and LOQ (expressed as % of applied amount and in mg/kg) of the analytical methods used,
- mass balance
- detailed description of sequential extraction procedure

Test manure:

- details of sampling site (date, location, type and number of animals, feed, type and size of manure tank, information of use of chemicals, e.g. biocides or veterinary medicinal products, in the last six months),
- date and procedure of manure sampling,
- matrix parameters of manure (pH, organic matter content, nitrogen content, redox potential, dry matter content, temperature),
- duration of manure storage and storage conditions (if stored in the lab);

Test conditions:

- dates of the performance of the studies,
- amount of test substance applied,
- calculation of maximum expected manure concentration (incl. rationale)
- solvents used (if appropriate) and method of application for the test substance,
- weight of manure treated (fresh weight),
- description of the incubation system used,
- nitrogen flow rates (for flow-through systems and semi-static systems only);
- temperature,

- matrix parameters of manure (pH, , organic matter content, nitrogen content, redox potential, dry matter content, temperature, as specified, method(s) of extraction, chemical analytical method;
- number of replicates and number of controls including sterile controls;

Results:

- result of manure matrix characterization for each time point,
- tables of results expressed as % of applied initial dose and, where appropriate, as mg/kg manure (on a wet weight basis) given for each replicate and as mean of all replicates for the following parameters and results expressed as % of applied initial dose, normalized to 100% radioactive mass balance at time point 0 for the following parameters,
- characterization of non-extractable radioactivity,
- quantification of released CO_2 and CH_4, and other volatile compounds,
- mass balance for each sampling point,
- substance specific quantification of test substance and transformation products
- plots (non-logarithmic) of concentrations versus time for the test substance and, where appropriate, for major transformation products,
- DT_{50}, and DT_{90} for the test substance and, where appropriate, for major transformation products including kinetic model used an procedure used for fitting,
- abiotic transformation under sterile conditions,
- substance storage stability, when samples are frozen prior to chemical analysis (for exceptional cases only);
- an assessment of transformation kinetics for the test substance and, where appropriate, for transformation products,
- a proposed pathway for transformation including structural formula and consistent names throughout the report for transformation products
- discussion and interpretation of results,
- raw data (e.g.. sample chromatograms, calculations of transformation rates and methods used to identify transformation products).

- The same results have to be supplied for the reference compound salicylic acid, except for the information on transformation products and transformation pathway.

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ANNEX 1: Sampling and analytical determination for the reference compound salicylic acid

50 g wet manure sample are extracted once by 80 mL methanol + 1% trifluoroacetic acid (TFA), and thereafter twice by 50 mL methanol + 1 % TFA. For extraction, the samples are shaken for 30 minutes on a horizontal shaker and centrifuged for 10 minutes at 739 x g. After centrifugation the supernatant extract is collected and the pellet is subjected to the next extraction step. Further extraction solvent is added to the pellet. The whole process is repeated twice. Extracts are combined, and further analyzed by radio TLC. After the last extraction step the pellet is air dried and aliquots are subjected to combustion and radioassaying to give the information on the amount of non-extractable residues (NER).

In addition to the described extraction a further extraction step using ASE® can be performed. The accelerated solvent extraction (ASE®), i.e. extraction under high pressure and temperature (100°C, 12000 kPa, heat up for 5 minutes, followed by a static time of 10 minutes) uses the same solvent mixture as for the first extraction steps (methanol + 1 % TFA). Extraction is performed twice but extracts are not combined.

As the extracts without further cleanup might influence HPLC resulting in broad peaks, radio-TLC is preferred over HPLC. The following TLC-system is suggested:

- stationary phase: silica gel KG60
- mobile phase: methanol / toluene / ethylacetate / acetic acid; 10/44/43 /3 (v/v/v/v)

The radioactive peaks obtained after the development of the TLC-plates are characterized by their R_f-values and allocation to the peaks of co-chromatographed Salicylic acid and possible transformation products.

Typical R_f-values are:

Salicylic acid: R_f = 0.50 – 0.55
Salicyluric acid: R_f = 0.31 – 0.36
Gentisinic acid: R_f = 0.42 – 0.47

If, in addition, peaks are observed which cannot be allocated to any of the used reference substances, they should be described by their Rf-values and named as transformation product T1, T2, etc.

To determine mineralization to CO_2 and CH_4 please refer to Annex 3.

A mass balance can be established by adding the amounts of radioactivity given in [% of applied radioactivity; % aR] in the aqueous/organic extracts, carbon dioxide ($^{14}CO_2$), methane ($^{14}CH_4$) and non-extractable residues (NER):

Mass balance [% aR] = extractables [% aR] + $^{14}CO_2$ [% aR] + $^{14}CH_4$ [% aR] + NER [% aR].

ANNEX 2: Definitions

Manure: mixture of urine, feces and water collected in a storage tank, high liquid content, may contain residual bedding material

Test substance: substance used to conduct the study

Transformation products: all substances resulting from biotic or abiotic transformation reactions of the test substance including CO_2, CH_4 and non-extractable residues.

Non-extractable residues (NER): represent compounds in manure, which persist in the matrix after extraction.

Mineralization is the complete transformation of an organic compound to CO_2 and H_2O and CH_4 (under anaerobic conditions).

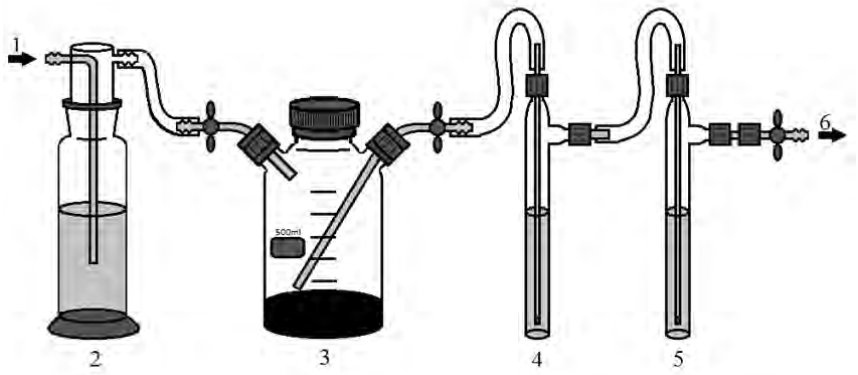
DT_{50} (Disappearance or Dissipation Time 50) is the time after which 50% of the initial amount of the compound has dissipated.

DT_{90} (Disappearance or Dissipation Time 90) is the time within which the concentration of the test substance is reduced by 90%.

ANNEX 3: Incubation in a semi-static system

Example of a semi-static apparatus

Incubation



[1] nitrogen inlet

[2] gas washing bottle containing deionised water

[3] incubation flask containing manure

[4] CO_2-trap (e.g. containing 2 *M* NaOH)

[5] CO_2-trap (e.g. containing 2 *M* NaOH)

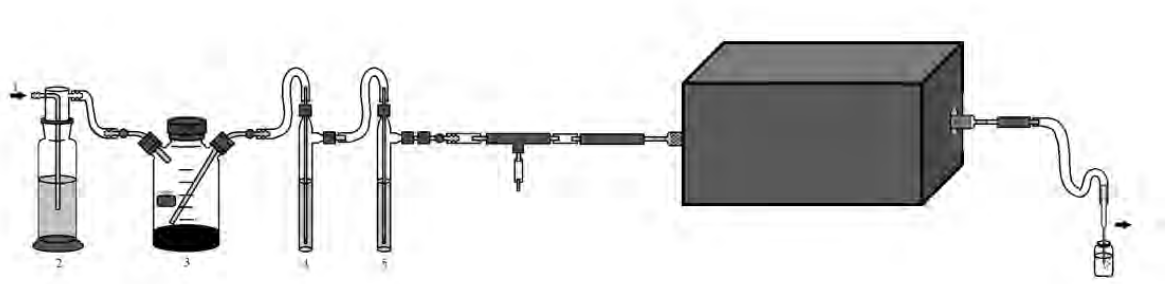
Manure samples are filled into the incubation flask [3]. The flask is connected to a semi-static apparatus. For a period of 1 hour a gentle stream of moistened nitrogen is passed over the manure to exclude air from the system and to ensure anaerobic conditions. After flushing with moistened nitrogen the system is closed by closing the two valves directly at the incubation flasks and the valve at the outlet of the second NaOH trap [5].

Note: The valve at the outlet of the incubation flask might be left open to increase the headspace of the semi-static system and to enable absorption of evolved CO_2 in the first CO_2-trap [4] during incubation. In that case, a safety trap (empty washing bottle) should be inserted in between the incubation flask [3] and CO_2-trap [4] to prevent a backflow of absorbing solution into the incubation flask in case of pressure drop in the test system.

At the end of the incubation period the respective incubation flasks are connected to the flow-through setup outlined below to detect formed CO_2, CH_4 and VFAs.

As a result of gas formation of the manure the pressure in the closed test vessel increases during the incubation period. To avoid losses of volatiles resulting in an incomplete mass balance, the test vessels should be connected to the flow-through apparatus and purged with moistened nitrogen at regular intervals (e.g. once a week).

Detection of CO_2, CH_4 and VFAs



[1] nitrogen inlet

[2] gas washing bottle containing deionised water

[3] incubation flask containing manure

[4] CO_2-trap (e.g. containing 2 *M* NaOH)

[5] CO_2-trap (e.g. containing 2 *M* NaOH)

[6] tube as bypass for further air/oxygen inlet containing silica gel or soda lime pellets

[7] oven with quarz glass tube (filled with CuO as catalysator) at 800°C - 850°C

[8] CO_2-trap (e.g. containing 2 *M* NaOH)

Differentiation between CO_2, CH_4 and VFAs

Humidified nitrogen is bubbled through the manure samples at a rate in the range of approximately 50 – 200 mL/min for at least 1 hour. Evolved $^{14}CO_2$ is purged from the manure samples, transported and captured in traps ([4] and [5]) containing a CO_2-absorber (e.g. 2 *M* NaOH). Potentially formed $^{14}CH_4$ passes the CO_2-traps ([4] and [5]). After the addition of oxygen or ambient air [6] it is catalytically (= CuO) oxidized in an oven [7] at 800°C - 850°C to form $^{14}CO_2$. The formed $^{14}CO_2$ is trapped in the CO_2-trap situated at the outlet of the oven [8].

Such a set-up enables the differentiation between evolved $^{14}CO_2$ (captured in traps [4] and [5]) and $^{14}CH_4$ (captured in trap [10]).

To verify that the radioactivity captured in the CO_2-traps [4] and [5] is $^{14}CO_2$ and not from potentially also formed volatile fatty acids (VFA), $BaCl_2$ precipitation of the radioactivity can be conducted. The radioactivity in the trapping solutions [4] and [5] is counted. Thereafter, 20 mL 0.25 *M* $BaCl_2$ is added to 10 mL aliquots of trapping solution [4] and [5] each. Precipitation of $Ba^{14}CO_2$ occurs. The supernatant is to be radio-counted again. The radioactive content in the supernatant after precipitation can be attributed to VFAs whereas the difference of radioactive content before precipitation minus radioactive content after precipitation can be attributed to evolved $^{14}CO_2$.

Quantification of volatiles

Quantification of trapped volatiles is by radio-counting (liquid scintillation counting, LSC) of aliquots of the trapping solutions.

When using a semi-static setup it is especially important to acidify the samples to release trapped CO_2 by addition of 10 mL 10% HCl, further incubation and trapping of further released CO_2.

This can be achieved as follows: after humidified nitrogen was bubbled through the manure samples for at least 1 hour, CO_2-traps ([4] and [5]) are removed and analyzed for trapped $^{14}CO_2$ and other evolved gases, respectively, as described above. The removed traps are replaced by freshly filled ones.

Thereafter, the manure incubation flasks to be removed at that particular sampling point are treated by addition of 10 mL 10% HCl in order to strip potentially dissolved CO_2 (or HCO_3^- / CO_3^{2-}). After adding of 10 mL 10% HCl the incubation flasks are closed again and moistened nitrogen is bubbled through the manure for at least 24 hours[20]. Samples are not stirred in order to avoid foaming. If foaming is nevertheless observed, the acid should be added slowly (e.g. dropwise) over the incubation period. Thereafter, manure incubation flasks are removed and manure is cleaned-up and extracted. CO_2-traps are also removed and radio-counted for additionally trapped CO_2.

Note:

Prior to the addition of 10% HCl to the manure it has to be checked whether the test substance and transformation products are stable under acidic conditions[21]. If this is not the case further replicates have to be incubated for that purpose.

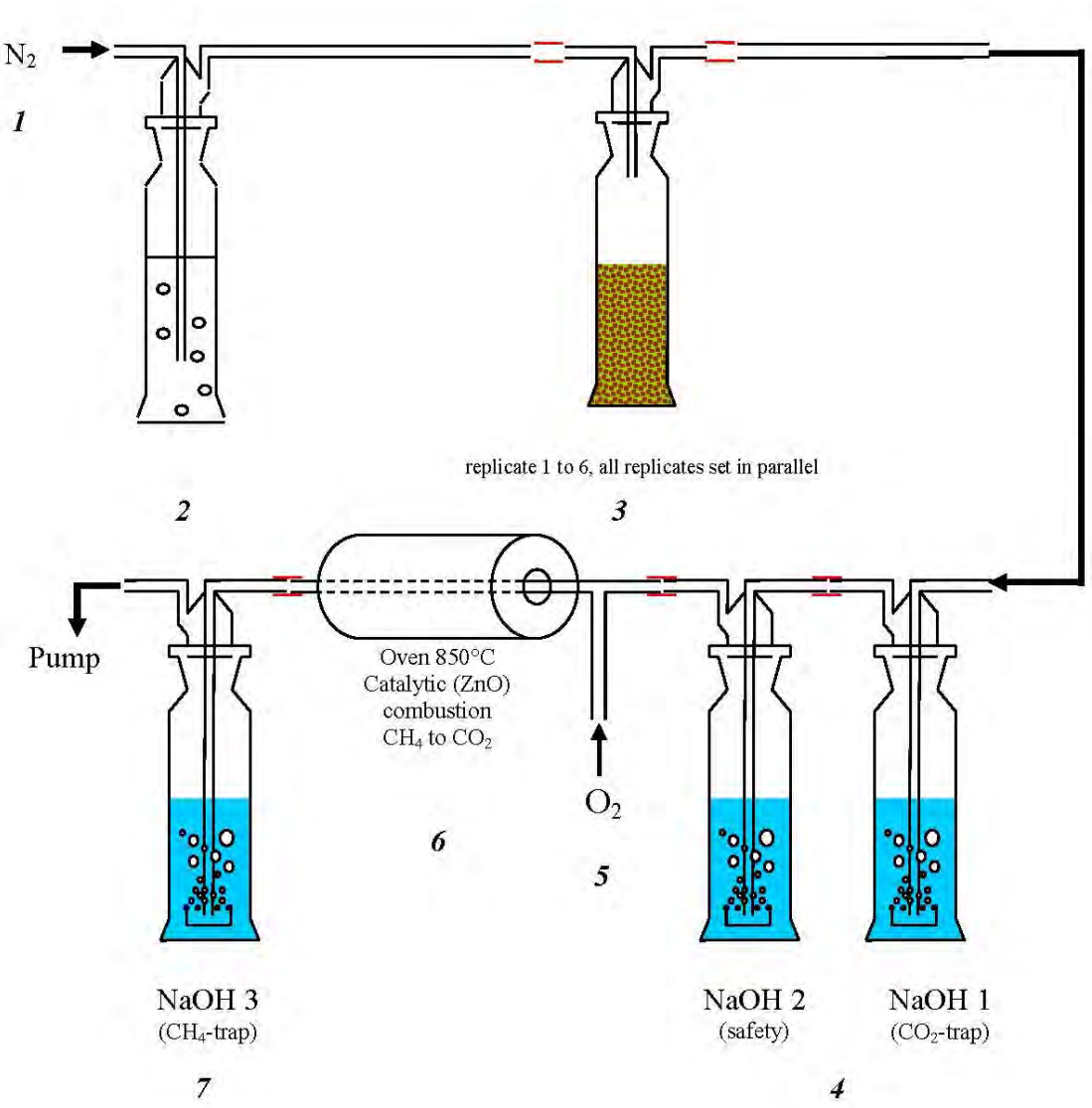
[20] The time needed for purging the $^{14}CO_2$ completely in the CO_2-traps, depends strongly on the amount of $^{14}CO_2$ formed and how fast this $^{14}CO_2$ formation is. For test substances with high mineralization purging for at least 24 h is recommended but time periods up to several days might be necessary to trap the $^{14}CO_2$ completely. If high mineralization is expected the optimal purging might be determined in preliminary investigations.

[21] In case of unknown transformation products, the stability check is not possible. A pre-test can be conducted to identify expected transformation products.

ANNEX 4: Incubation in a flow-through system

Example of a flow-through apparatus

Incubation



[1] nitrogen is gently passed over the manure samples

[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 or ambient air 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

Differentiation between CO_2, CH_4 and VFAs

Humidified nitrogen is passed over the manure sub-samples at a rate in the range of approximately 50 – 200 mL/min. By such a constant N_2-stream evolved $^{14}CO_2$ is purged from the manure samples, transported and captured in traps 1 and 2 (safety trap) containing a CO_2-absorber (e.g. 2 *M* NaOH). Potentially formed $^{14}CH_4$ passes the CO_2-traps. After the addition of oxygen it is catalytically (= CuO) oxidized in an oven at 800°C - 850°C to form $^{14}CO_2$. The formed $^{14}CO_2$ is trapped in the CO_2-trap situated at the outlet of the oven.

Such a set-up enables the differentiation between evolved $^{14}CO_2$ (captured in traps 1 and 2) and $^{14}CH_4$ (captured in trap 3).

To verify that the radioactivity captured in the CO_2-traps 1 and 2 is $^{14}CO_2$ and not from potentially also formed volatile fatty acids (VFA) $BaCl_2$ precipitation of the radioactivity can be conducted. The radioactivity in the trapping solutions 1 and 2 is counted. Thereafter, 20 mL 0.25 M $BaCl_2$ is added to 10 mL aliquots of trapping solution from traps 1 and 2 each. Precipitation of $Ba^{14}CO_2$ occurs. The supernatant is to be radio-counted again. The radioactive content in the supernatant after precipitation can be attributed to VFAs whereas the difference of radioactive content before precipitation minus radioactive content after precipitation can be attributed to evolved $^{14}CO_2$.

Quantification of volatiles

Quantification of trapped volatiles is by radio-counting (liquid scintillation counting, LSC) of aliquots of the trapping solutions.

Furthermore, it should be proven whether evolved $^{14}CO_2$ is purged quantitatively when passing the humidified nitrogen over the manure samples. This can be verified by addition of HCl to the manure sub-samples in order to strip CO_2 (or HCO_3^- / CO_3^{2-}) being potentially dissolved in the manure matrix. Purging by addition of HCl should be applied in case the amount of $^{14}CO_2$ exceeds the level of 10% of the total radioactivity (TRR).

This can be achieved as follows: CO_2-traps 1 and 2 are removed at the particular sampling point and analyzed for trapped $^{14}CO_2$ and other evolved gases, respectively, as described above. The removed traps are replaced by freshly filled ones.

Thereafter, the manure incubation flasks to be removed at that particular sampling point are treated by addition of 10 mL 10% HCl in order to strip potentially dissolved CO_2 (or HCO_3^- / CO_3^{2-}). After adding of 10 mL 10% HCl the incubation flasks are closed again and nitrogen is passed over for 3 hours. Samples are not stirred in order to avoid foaming. If foaming is nevertheless observed, the acid should be added slowly (e.g. dropwise) over the incubation period. Thereafter, manure incubation flasks are removed and manure is cleaned-up and extracted. CO_2-traps are also removed and radio-counted for additionally trapped CO_2. In order to avoid interferences and cross-contaminations by evolving gases sampling should start with samples being next to the outlet (e.g. samples 3 and 4 in figure, Annex 4).

Note:

Prior to the addition of 10% HCl to the manure sub-samples it has to be checked whether the test substance and transformation products is stable under acidic conditions[22]. If this is not the case further replicates have to be incubated.

[22] In case of unknown transformation products the stability check is not possible. A pre-test can be conducted to identify expected transformation products.

ANNEX 5: Template for documentation of manure sampling and storage information

Sampling & Storage

Please add as much information as possible

Participant:	
Manure sample:	
Sampling location:	
Sampling date:	
Storage time:	
Storage temperature:	
Sampling device/sampling method [1]**:**	
Type of manure tank [2]**:**	
Livestock/type of animals [3]**:**	
Feed:	
Veterinary medicines/biocides used:	
Remarks:	

[3]: How and at wchich time prior to sampling was the manure tank homogenized?
[2]: e.g. above ground, below ground, volume, with/w/o stirring device, covered, open, etc.
[3]: e.g. number of animals, feeder cattle, dairy cows, young cattle, etc.

Annex 2

Invitation Ring Test; Outline Ring Test; Registration Form

March 07, 2013

Validation Ring Test: Transformation of veterinary pharmaceuticals and biocides in manure

Dear colleagues,

on behalf of the German Federal Environmental Agency, I am pleased to invite you to take part in the international validation ring test on "Transformation of veterinary pharmaceuticals and biocides in liquid manure" in order to start the validation of a draft test method. The ring test is scheduled for September 2013 to April 2014. For details see the attached Outline of the Ring Test.

In general, participating institutes will be responsible for the funding of the laboratory work. A certificate for participating in the ring test will be provided to each participant at the end of the test.

I would be very grateful if you could indicate your interest to participate in the ring test by signing and returning the enclosed form by fax or by e-mail attachment until April 30th, 2013 at the latest. Regardless of whether you are able to participate or not, it would be helpful if you could name further colleagues who would be able and willing to take part. As soon as the participant list is completed you will receive further information.

I am convinced that your contribution will be significant for the achievement of the objectives of the ring test. If you have any technical questions relating to the ring test itself or in case you need further information, do not hesitate to contact us (addresses below).

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Boettgerstr. 2-14
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Yours sincerely

ECT Oekotoxikologie GmbH

Thomas Junker

Attachments:
- Outline of the Ring Test
- Registration form

Outline of the Ring Test

"Transformation of veterinary pharmaceuticals and biocides in (liquid) manure"

Introduction

Veterinary pharmaceuticals (VMPs) administered systemically to animals are excreted with urine and feces by treated animals. For animals housed in stables the resulting manure/slurry is collected and stored before being spread onto agricultural land. Disinfection products used to sanitize stables are also transferred into the manure. Therefore, the spreading of (liquid) manure/slurry is an important pathway of introducing veterinary pharmaceuticals, biocides and their metabolites and transformation products into the environment. As a consequence, the fate of VMPs/biocides in manure is taken into account in the environmental risk assessment for VMPs/biocides.

Although there is the need for guidance on the performance and evaluation of degradation studies with VMPs/biocides in manure, a standardized and validated method is currently lacking. At European level, the Committee for Medicinal Products for Veterinary Use (CVMP) of the European Medicines Agency (EMA) adopted a guidance document on determining the fate of veterinary medicinal products in manure in March 2011 (EMA, 2011). The document is intended to provide guidance on the general conditions of studies on the transformation of veterinary medicinal products in manure (e.g. matrix characterisation of manure, regulatory use of the results). However, the EMA guidance is not an experimental protocol and therefore further advice on experimental details is required to obtain reliable and sound results.

Therefore, a standardised experimental test method and a draft guideline are currently under development within a research project funded by the German Federal Environment Agency that should in the long run lead to a guideline on transformation of substances in (liquid) manure within the framework of the OECD test guideline program.

A pre-validation ring test has been performed in 2012/2013 and is currently evaluated. Based on the outcome of the pre-validation ring test and the currently planned validation test and taking into account existing guidelines, a draft guideline to be submitted to the OECD will be prepared.

Test Procedure

The performance and documentation should be done according to GLP-rules (OECD, 1998), but no formal certificate is required for the participating laboratories. Transformation tests will be run using ^{14}C-radioactively labelled substances. It is also possible and highly welcome to do LC-MS/MS-analysis including screening for and identification of transformation products. In this case working with ^{14}C-material is not required.

Bibliography

OECD (1998). The OECD Principles of Good Laboratory Practice (1998) (as revised in 1997). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring No.1. Organisation for Economic Co-Operation and Development, Paris.

Registration Form

Validation Ring Test on

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Name:
Institution:
Department :
Address:
Country:
Fax:
Phone:
E-Mail:

I will participate in the performance of the ring test:	☐ Yes	☐ No

Licence on handling ^{14}C-labelled material:	attached	☐
	will be delivered later	☐
	no licence available	☐

Comments:

....

Date Signature

If more than one person of your institute will participate, please use copies of this form.

Please return this form by fax or by e-mail to:
ECT Oekotoxikologie GmbH, Böttgerstr. 2-14, 65439 Flörsheim/Main, Germany
Thomas Junker, Fax: + 49 6145 9564 99, E-mail: th-junker@ect.de

Annex 3

Evaluation Sheets

Institute 1

Physico-chemical parameters

Figure A3_1: Physico-chemical parameters measured throughout the ring test experiments by Institute 1

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 1
Manure sample: Cattle
Test substance: Imidacloprid

Excel 2003

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) [mg/kg]	Nitrogen (N_{total}) [mg/kg]	Dry matter content [%]	Organic matter content (relating to wet weight) [%]	Other parameter(s)
Sampling	14.08.2013	7.1	-374	16.0	2034.000	4194.0	9.5	7.91	-
Start of acclimation	-	7.4	-417	20.0			9.5	7.91	-
Day 0	08.10.2013	8.1	-398	20.5					-
Day 3	-	8.1	-399	20.0					-
Day 7	-	8.2	-405	19.5					-
Day 10	-	7.9	-420	20.0					-
Day 14	-	8.4	-413	20.5					-
Day 21	-	8.6	-432	20.0					-
Day 28	-	8.1	-422	19.5					-
Day 42	-	8.8	-431	19.5					-
Day 56	-	8.3	-410	20.0					-
Day 72	-	8.3	-416	20.5					-
Day 90	-	8.5	-464	20.0			n.d.		-

Manure sample: Pig
Test substance: Florfenicol

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) [mg/kg]	Nitrogen (N_{total}) [mg/kg]	Dry matter content [%]	Organic matter content (relating to wet weight) [%]	Other parameter(s)
Sampling	21.08.2013	7.4	-395	13.5	2296.000	3418.0	4.1	4.28	-
Start of acclimation	-	7.9	-431	20.0			4.1	4.28	-
Day 0 - 0 h	23.09.2013	8.9	-421	20.5					-
Day 0 - 0.5 h	-	8.7	-413	20.5					-
Day 0 - 1 h	-	8.2	-421	20.5					-
Day 0 - 2 h	-	8.6	-404	20.0					-
Day 0 - 4 h	-	9.1	-415	20.5					-
Day 0 - 7 h	-	9.3	-424	20.0					-
Day 1 - 24 h	-	8.4	-410	19.5					-
Day 7	-	7.9	-423	20.0					-
Day 28	-	8.7	-408	20.5					-
Day 60	-	9.3	-419	20.5					-
Day 90	-	8.4	-427	20.0			n.d.		-

Mass Balance

Figure A3_2: Mass balance determined for ^{14}C-florfenicol in pig manure by Institute 1

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Test substance: Florfenicol
Participant: Institute 1
Manure sample: Pig
applied radioactivity(aR): 50.00 kBq
Specific radioactivity (sR): 0.01667 MBq/mg

Excel 2003

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Sample	activity in extracts (kBq)	amount in extracts % aR	extract (mean) (% aR)	NER/Activity in manure (combustion) (kBq)	NER/Manure combustion (% kBq/aR)	NER/Manure combustion (mean) (% kBq /aR)	activity in ASE (kBq)	amount ASE % aR	ASE (% aR) (mean)	Activity NaOH ($^{14}CO_2$) (kBq)	Activity NaOH ($^{14}CO_2$) (% kBq/aR)	Activity NaOH ($^{14}CH4$) (kBq)	Activity NaOH ($^{14}CH4$) (% kBq/aR)	total activity (kBq)	**recovery (% aR)**	**recovery mean (% aR)**
0 d - 1	50.120	100.2		0.440	0.9					-	-	-	-	50.560	**101.1**	
0 d - 2	50.210	100.4		0.440	0.9					-	-	-	-	50.650	**101.3**	
0 d - 3	50.308	100.6	99.0	0.512	1.0	0.9		-		-	-	-	-	50.820	**101.6**	100.3
0 d - 4	50.370	100.7		0.446	0.9					-	-	-	-	50.816	**101.6**	
0 d - 5	47.000	94.0		0.409	0.8					-	-	-	-	47.409	**94.8**	
0 d - 6	50.356	100.7		0.404	0.8					-	-	-	-	50.760	**101.5**	
0,5 h - 1	52.943	105.9		0.715	1.4					-	-	-	-	53.658	**107.3**	
0,5 h - 2	53.904	107.8		0.715	1.4					-	-	-	-	54.619	**109.2**	
0,5 h - 3	51.480	103.0	103.6	0.863	1.7	1.7		-		-	-	-	-	52.343	**104.7**	105.3
0,5 h - 4	52.449	104.9		0.912	1.8					-	-	-	-	53.361	**106.7**	
0,5 h - 5	49.650	99.3		0.986	2.0					-	-	-	-	50.636	**101.3**	
0,5 h - 6	50.386	100.8		0.843	1.7					-	-	-	-	51.229	**102.5**	
1 h - 1	52.815	105.6		1.415	2.8					-	-	-	-	54.230	**108.5**	
1 h - 2	51.938	103.9		1.417	2.8					-	-	-	-	53.355	**106.7**	
1 h - 3	54.540	109.1	104.5	1.398	2.8	2.9		-		-	-	-	-	55.938	**111.9**	107.5
1 h - 4	50.627	101.3		1.484	3.0					-	-	-	-	52.111	**104.2**	
1 h - 5	52.722	105.4		1.543	3.1					-	-	-	-	54.265	**108.5**	
1 h - 6	50.991	102.0		1.512	3.0					-	-	-	-	52.503	**105.0**	
2 h - 1	53.216	106.4		0.838	1.7					-	-	-	-	54.054	**108.1**	
2 h - 2	54.314	108.6		2.013	4.0					-	-	-	-	56.327	**112.7**	
2 h - 3	52.369	104.7	105.0	2.111	4.2	3.5		-		-	-	-	-	54.480	**109.0**	108.6
2 h - 4	50.956	101.9		2.057	4.1					-	-	-	-	53.013	**106.0**	
2 h - 5	52.606	105.2		1.926	3.9					-	-	-	-	54.532	**109.1**	
2 h - 6	51.570	103.1		1.699	3.4					-	-	-	-	53.269	**106.5**	
4 h - 1	39.381	78.8		6.443	12.9		0.726	1.5	1.5	-	-	-	-	46.550	**93.1**	
4 h - 2	35.528	71.1		7.726	15.5		0.768	1.5		-	-	-	-	43.254	**86.5**	
4 h - 3	38.749	77.5	79.3	6.479	13.0	14.5		-		-	-	-	-	45.228	**90.5**	94.1
4 h - 4	40.650	81.3		6.771	13.5					-	-	-	-	47.421	**94.8**	
4 h - 5	40.728	81.5		7.584	15.2					-	-	-	-	48.312	**96.6**	
4 h - 6	42.995	86.0		8.598	17.2					-	-	-	-	51.592	**103.2**	
7 h - 1	46.489	93.0		7.692	15.4		0.984	2.0	2.0	-	-	-	-	55.165	**110.3**	
7 h - 2	46.294	92.6		6.604	13.2		1.022	2.0		-	-	-	-	52.898	**105.8**	
7 h - 3	45.030	90.1	92.4	8.169	16.3	14.8		-		-	-	-	-	53.199	**106.4**	107.5
7 h - 4	47.310	94.6		6.062	12.1					-	-	-	-	53.372	**106.7**	
7 h - 5	44.670	89.3		8.228	16.5					-	-	-	-	52.898	**105.8**	
7 h - 6	47.454	94.9		7.544	15.1					-	-	-	-	54.998	**110.0**	
24 h - 1	33.723	67.4		12.904	25.8		0.730	1.5	2.3	-	-	-	-	46.627	**93.3**	
24 h - 2	31.005	62.0		11.739	23.5		1.579	3.2		-	-	-	-	42.744	**85.5**	
24 h - 3	30.292	60.6	62.6	15.261	30.5	28.6		-		-	-	-	-	45.553	**91.1**	91.2
24 h - 4	32.048	64.1		14.489	29.0					-	-	-	-	46.537	**93.1**	
24 h - 5	31.378	62.8		14.623	29.2					-	-	-	-	46.001	**92.0**	
24 h - 6	29.275	58.6		16.741	33.5					-	-	-	-	46.016	**92.0**	
7 d - 1	27.039	54.1		22.186	44.4		1.416	2.8	2.0	0.000				50.641	**101.3**	
7 d - 2	26.420	52.8		23.806	47.6		0.547	1.1		0.000				50.773	**101.5**	
7 d - 3	31.212	62.4	58.9	18.310	36.6	40.6		-		0.010	0.00	0.000	0.0	49.532	**99.1**	100.2
7 d - 4	29.486	59.0		20.019	40.0					0.010				49.515	**99.0**	
7 d - 5	31.427	62.9		19.417	38.8					0.000				50.844	**101.7**	
7 d - 6	31.190	62.4		18.165	36.3					0.010				49.365	**98.7**	
28 d - 1	34.532	69.1		18.454	36.9		0.972	1.9	1.1	0.020				53.993	**108.0**	
28 d - 2	35.407	70.8		18.557	37.1		0.094	0.2		0.020				54.093	**108.2**	
28 d - 3	26.712	53.4	68.6	25.012	50.0	37.9		-		0.010	0.04	0.015	0.0	51.749	**103.5**	107.0
28 d - 4	35.175	70.4		18.490	37.0					0.010				53.690	**107.4**	
28 d - 5	36.274	72.5		16.387	32.8					0.010				52.686	**105.4**	
28 d - 6	37.712	75.4		16.918	33.8					0.010				54.655	**109.3**	
61 d - 1	30.340	60.7		19.517	39.0		1.080	2.2	2.3	0.020				51.007	**102.0**	
61 d - 2	30.730	61.5		19.818	39.6		1.170	2.3		0.010				51.778	**103.6**	
61 d - 3	30.475	61.0	61.7	17.862	35.7	38.8		-		0.020	0.04	0.050	0.1	48.407	**96.8**	101.4
61 d - 4	30.446	60.9		20.014	40.0					0.020				50.530	**101.1**	
61 d - 5	31.853	63.7		19.872	39.7					0.030				51.805	**103.6**	
61 d - 6	31.332	62.7		19.271	38.5					0.040				50.693	**101.4**	
90 d - 1	36.248	72.5		16.743	33.5		0.888	1.8	1.8	0.050				53.979	**108.0**	
90 d - 2	35.973	71.9		17.314	34.6		0.938	1.9		0.050				54.325	**108.6**	
90 d - 3	35.570	71.1	71.2	15.485	31.0	33.9		-		0.040	0.10	0.050	0.1	51.145	**102.3**	105.9
90 d - 4	35.290	70.6		17.264	34.5					0.030				52.634	**105.3**	
90 d - 5	35.442	70.9		16.925	33.9					0.040				52.457	**104.9**	
90 d - 6	34.964	69.9		18.055	36.1					0.040				53.109	**106.2**	
1 d - 1 sterile	53.504	107.0	105.4	0.621	1.2	1.3				-	-	-	-	54.125	**108.2**	
1 d - 2 sterile	51.923	103.8		0.654	1.3			-		-	-	-	-	52.577	**105.2**	106.7
28 d - 1 sterile	57.107	114.2	112.4	2.521	5.0	5.7				-	-	-	-	59.628	**119.3**	
28 d - 2 sterile	55.326	110.7		3.184	6.4					-	-	-	-	58.510	**117.0**	118.1
90 d - 1 sterile	47.677	95.4	98.9		0.0	4.1				-	-	-	-	47.677	**95.4**	
90 d - 2 sterile	51.270	102.5		4.131	8.3					-	-	-	-	55.401	**110.8**	103.1
formula		100 / aR * B	mean of C		100 / aR *E	mean of F					100 / aR * K		100 / aR * I	B + D + F + H + J + L	**100 / aR * O**	mean of P

Figure A3_3: Mass balance determined for ^{14}C-imidacloprid in cattle manure by Institute 1

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Test substance: Imidacloprid
Participant: Institute 1
Manure sample: Cattle
applied radioactivity (aR): 50.00 kBq
Specific radioactivity (sR): MBq/mg

Excel 2003

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Sample	acticity in extracts (kBq)	amount in extracts % aR	extract (mean) (% aR)	NER/Activity in manure (combustion) (kBq)	NER/Manure combustion (% kBq/aR)	NER/Manure combustion (mean) (% kBq /aR)	activity in ASE (kBq)	amount ASE % aR	ASE (% aR) (mean)	Activity NaOH ($^{14}CO_2$) (kBq)	Activity NaOH ($^{14}CO_2$) (% kBq/aR)	Activity NaOH ($^{14}CH4$) (kBq)	Activity NaOH ($^{14}CH4$) (% kBq/aR)	total activity (kBq)	recovery (% aR)	recovery mean (% aR)
0 d - 1	54.774	109.5	109.9	0.871	1.7	2.0				-	-	-	-	55.645	111.3	111.8
0 d - 2	55.080	110.2		0.939	1.9					-	-	-	-	56.019	112.0	
0 d - 3	55.980	112.0		1.196	2.4					-	-	-	-	57.176	114.4	
0 d - 4	56.248	112.5		0.993	2.0					-	-	-	-	57.241	114.5	
0 d - 5	53.400	106.8		0.913	1.8					-	-	-	-	54.313	108.6	
0 d - 6	54.180	108.4		0.951	1.9					-	-	-	-	55.131	110.3	
3 d - 1	56.520	113.0	107.6	2.540	5.1	5.1				0.010	0.0	0.00	0.0	59.070	118.1	112.7
3 d - 2	53.214	106.4		2.695	5.4					0.001				55.910	111.8	
3 d - 3	52.272	104.5		2.641	5.3					0.000				54.913	109.8	
3 d - 4	52.448	104.9		2.432	4.9					0.001				54.882	109.8	
3 d - 5	54.782	109.6		2.492	5.0					0.000				57.275	114.5	
3 d - 6	53.640	107.3		2.554	5.1					0.000				56.194	112.4	
7 d - 1	50.864	101.7	102.8	4.197	8.4	8.4				0.000	0.0	0.00	0.0	55.061	110.1	111.4
7 d - 2	52.460	104.9		4.016	8.0					0.000				56.476	113.0	
7 d - 7	50.460	100.9		5.232	10.5					0.078				55.770	111.5	
7 d - 4	51.800	103.6		4.270	8.5					0.137				56.207	112.4	
7 d - 5	51.620	103.2		3.758	7.5					0.000				55.378	110.8	
7 d - 6	51.330	102.7		3.761	7.5					0.290				55.381	110.8	
10 d - 1	51.142	102.3	104.1	4.986	10.0	10.4				0.010	0.0	0.00	0.0	56.138	112.3	114.5
10 d - 2	52.128	104.3		5.013	10.0					0.000				57.141	114.3	
10 d - 3	51.223	102.4		5.382	10.8					0.000				56.605	113.2	
10 d - 4	50.414	100.8		5.602	11.2					0.000				56.016	112.0	
10 d - 5	53.100	106.2		5.094	10.2					0.000				58.194	116.4	
10 d - 6	54.280	108.6		5.243	10.5					0.000				59.523	119.0	
14 d - 1	53.100	106.2	100.2	6.930	13.9	14.8	0.531	1.1	1.2	0.000	0.0	0.00	0.0	60.561	121.1	115.3
14 d - 2	51.606	103.2		6.519	13.0		0.646	1.3		0.000				58.771	117.5	
14 d - 3	48.420	96.8		7.792	15.6		-	-	-	0.000				56.212	112.4	
14 d - 4	48.780	97.6		8.393	16.8		-	-	-	0.000				57.173	114.3	
14 d - 5	49.051	98.1		7.636	15.3		-	-	-	0.000				56.687	113.4	
14 d - 6	49.500	99.0		7.067	14.1		-	-	-	0.000				56.567	113.1	
28 d - 1	42.136	84.3	87.2	12.330	24.7	24.1	0.108	0.2	0.6	0.000	0.0	0.00	0.0	54.574	109.1	111.5
28 d - 2	46.872	93.7		11.707	23.4		0.538	1.1		0.000				59.117	118.2	
28 d - 3	44.100	88.2		11.621	23.2		-	-	-	0.000				55.721	111.4	
28 d - 4	42.173	84.3		11.746	23.5		-	-	-	0.000				53.919	107.8	
28 d - 5	43.560	87.1		12.531	25.1		-	-	-	0.000				56.091	112.2	
28 d - 6	42.639	85.3		12.329	24.7		-	-	-	0.000				54.968	109.9	
42 d - 1	38.130	76.3	74.9	16.417	32.8	34.7	0.470	0.9	0.8	0.020	0.0	0.00	0.0	55.041	110.1	109.8
42 d - 2	37.904	75.8		16.709	33.4		0.306	0.6		0.018				54.941	109.9	
42 d - 3	37.758	75.5		15.871	31.7		-	-	-	0.010				53.643	107.3	
42 d - 4	36.075	72.2		17.734	35.5		-	-	-	0.039				53.852	107.7	
42 d - 5	38.160	76.3		17.343	34.7		-	-	-	0.010				55.517	111.0	
42 d - 6	36.600	73.2		19.907	39.8		-	-	-	0.000				56.511	113.0	
56 d - 1	36.036	72.1	80.0	18.693	37.4	33.7	0.202	0.4	0.6	0.010	0.0	0.01	0.0	54.951	109.9	113.9
56 d - 2	38.824	77.6		17.061	34.1		0.380	0.8		0.010				56.285	112.6	
56 d - 3	38.850	77.7		16.578	33.2		-	-	-	0.000				55.438	110.9	
56 d - 4	45.632	91.3		16.974	33.9		-	-	-	0.000				62.616	125.2	
56 d - 5	40.176	80.4		16.137	32.3		-	-	-	0.000				56.323	112.6	
56 d - 6	40.608	81.2		15.521	31.0		-	-	-	0.000				56.139	112.3	
72 d - 1	30.960	61.9	63.5	21.773	43.5	42.9	0.580	1.2	0.9	0.020	0.0	0.00	0.0	53.336	106.7	106.7
72 d - 2	32.040	64.1		21.356	42.7		0.323	0.6		0.010				53.732	107.5	
72 d - 3	32.396	64.8		18.925	37.8		-	-	-	0.020				51.344	102.7	
72 d - 4	31.680	63.4		25.734	51.5		-	-	-	0.010				57.427	114.9	
72 d - 5	31.500	63.0		21.262	42.5		-	-	-	0.020				52.785	105.6	
72 d - 6	31.862	63.7		19.670	39.3		-	-	-	0.010				51.545	103.1	
90 d - 1	35.948	71.9	71.9	20.701	41.4	40.3	0.459	0.9	0.9	0.068	0.1	0.00	0.0	57.178	114.4	112.5
90 d - 2	35.948	71.9		20.221	40.4		0.412	0.8		0.020				56.603	113.2	
90 d - 3	34.713	69.4		22.375	44.7		-	-	-	0.030				57.120	114.2	
90 d - 4	36.040	72.1		18.635	37.3		-	-	-	0.010				54.687	109.4	
90 d - 5	35.616	71.2		19.252	38.5		-	-	-	0.020				54.890	109.8	
90 d - 6	37.368	74.7		19.762	39.5		-	-	-	0.010				57.142	114.3	
7 d - 1 sterile	53.792	107.6	108.2	1.902	3.8	3.6				-	-	-	-	55.694	111.4	111.8
7 d - 2 sterile	54.400	108.8		1.683	3.4					-	-	-	-	56.083	112.2	
56 d - 1 sterile	53.320	106.6	106.5	3.424	6.8	6.1				-	-	-	-	56.744	113.5	112.5
56 d - 2 sterile	53.148	106.3		2.629	5.3					-	-	-	-	55.777	111.6	
90 d - 1 sterile	50.464	100.9	103.1	2.816	5.6	5.7				-	-	-	-	53.280	106.6	108.8
90 d - 2 sterile	52.592	105.2		2.932	5.9					-	-	-	-	55.524	111.0	
formula		100 / aR * B	MW C		100 / aR * D	MW F		100 / aR * H	MW I	A 9 B	100 / aR * G	A 9 B 3	100 / aR * I	B + D + F + H + J + L	100 / aR * N	

Extraction methods

Figure A3_4: Extraction methods used within the ring test by Institute 1

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Extraction Methods

Please add as much information as possible

Excel 2003

Participant:	**Institute 1**	
Manure sample:	**Cattle**	**Pig**
Test substance	**Imidacloprid**	**Florfenicol**
Extraction Method:	The recommended extraction method has been used.	The recommended extraction method has been used (method proposal 2).

Chemical Analysis

Figure A3_5: Results of chemical analysis for ^{14}C-florfenicol in pig manure by Institute 1

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute
Manure sample: Pig
Test substance: Florfenicol

Replicate 1

Excel 2003

Sample	Radioactivity in combined extracts	florfenicol (Rf 0,56)		florfenicol-amine (Rf = 0,2)		tranformation product_1 (Rf-value = 0,0-0,1)		tranformation product_2 (Rf-value = 0,35)		tranformation product_3 (Rf-value = 0,65)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
Day 0 - 0 h_1	100.2	100	100.2	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 0.5 h_1	105.9	100	105.9	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 1 h_1	105.6	85.2	90.0	3.63	3.8	11.85	12.5	0	0.0	0	0.0
Day 0 - 2 h_1	106.4	92.48	98.4	1.2	1.3	3.35	3.6	0	0.0	0	0.0
Day 0 - 4 h_1	78.8	53.29	42.0	6.15	4.8	30.64	24.1	6.19	4.9	3.74	2.9
Day 0 - 7 h_1	93.0	76.97	71.6	0	0.0	17.01	15.8	0	0.0	6.02	5.6
Day 1 - 24 h_1	67.4	42.05	28.4	0	0.0	31.29	21.1	14.27	9.6	12.38	8.3
Day 7_1	54.1	8.76	4.7	0	0.0	51.08	27.6	30.03	16.2	10.13	5.5
Day 28_1	69.1	12.27	8.5	14.15	9.8	43.76	30.2	15.73	10.9	8.89	6.1
Day 60_1	60.7	0	0.0	17.17	10.4	32.06	19.5	29.9	18.1	20.86	12.7
Day 90_1	72.5	0	0.0	20.92	15.2	42.5	30.8	36.4	26.4	0	0.0

Replicate 2

Sample	Radioactivity in combined extracts	florfenicol (Rf 0,56)		florfenicol-amine (Rf = 0,2)		tranformation product_1 (Rf-value = 0,0-0,1)		tranformation product_2 (Rf-value = 0,35)		tranformation product_3 (Rf-value = 0,65)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
Day 0 - 0 h_2	100.4	100	100.4	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 0.5 h_2	107.8	100	107.8	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 1 h_2	103.9	78.51	81.6	1.67	1.7	19.82	20.6	0	0.0	0	0.0
Day 0 - 2 h_2	108.6	81.27	88.3	1.2	1.3	17.53	19.0	0	0.0	0	0.0
Day 0 - 4 h_2	71.1	53.84	38.3	6.07	4.3	35.16	25.0	0	0.0	4.52	3.2
Day 0 - 7 h_2	92.6	73.87	68.4	0	0.0	17.38	16.1	0	0.0	8.75	8.1
Day 1 - 24 h_2	62.0	44.65	27.7	0	0.0	31.8	19.7	12.68	7.9	10.8	6.7
Day 7_2	52.8	14.93	7.9	0	0.0	57.45	30.4	21.68	11.5	5.67	3.0
Day 28_2	70.8	8.77	6.2	14.03	9.9	59.7	42.3	9.79	6.9	0	0.0
Day 60_2	61.5	0	0.0	14.06	8.6	37.26	22.9	48.68	29.9	0	0.0
Day 90_2	71.9	0	0.0	11.39	8.2	49.52	35.6	39.09	28.1	0	0.0

Replicate 3

Sample	Radioactivity in combined extracts	florfenicol (Rf 0,56)		florfenicol-amine (Rf = 0,2)		tranformation product_1 (Rf-value = 0,0-0,1)		tranformation product_2 (Rf-value = 0,35)		tranformation product_3 (Rf-value = 0,65)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
Day 0 - 0 h_3	100.6	100	100.6	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 0.5 h_3	103.0	100	103.0	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 1 h_3	109.1	81.38	88.8	2.08	2.3	16.54	18.0	0	0.0	0	0.0
Day 0 - 2 h_3	104.7	84.3	88.3	1.13	1.2	14.57	15.3	0	0.0	0	0.0
Day 0 - 4 h_3	77.5	60.16	46.6	4.55	3.5	28.32	21.9	0	0.0	6.97	5.4
Day 0 - 7 h_3	90.1	64.46	58.1	0	0.0	18.68	16.8	5.05	4.5	11.81	10.6
Day 1 - 24 h_3	60.6	31.18	18.9	0	0.0	36.43	22.1	18.86	11.4	13.53	8.2
Day 7_3	62.4	17.76	11.1	0	0.0	44.71	27.9	27.99	17.5	9.54	6.0
Day 28_3	53.4	0	0.0	9.96	5.3	76.56	40.9	13.5	7.2	0	0.0
Day 60_3	61.0	0	0.0	18.85	11.5	31.46	19.2	40.15	24.5	0	0.0
Day 90_3	71.1	0	0.0	7.32	5.2	55.38	39.4	37.3	26.5	0	0.0

Replicate 4

Sample	Radioactivity in combined extracts	florfenicol (Rf 0,56)		florfenicol-amine (Rf = 0,2)		tranformation product_1 (Rf-value = 0,0-0,1)		tranformation product_2 (Rf-value = 0,35)		tranformation product_3 (Rf-value = 0,65)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
Day 0 - 0 h_4	100.7	100	100.7	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 0.5 h_4	104.9	100	104.9	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 1 h_4	101.3	88.28	89.4	2.88	2.9	8.94	9.1	0	0.0	0	0.0
Day 0 - 2 h_4	101.9	94.65	96.5	0	0.0	5.35	5.5	0	0.0	0	0.0
Day 0 - 4 h_4	81.3	63.62	51.7	6.32	5.1	30.07	24.4	0	0.0	0	0.0
Day 0 - 7 h_4	94.6	68.1	64.4	4.62	4.4	25.01	23.7	0	0.0	2.27	2.1
Day 1 - 24 h_4	64.1	34.98	22.4	8.27	5.3	38.66	24.8	13.55	8.7	4.55	2.9
Day 7_4	59.0	26.74	15.8	9.03	5.3	24.49	14.4	26.02	15.3	8.26	4.9
Day 28_4	70.4	0	0.0	17.85	12.6	53.3	37.5	28.74	20.2	0	0.0
Day 60_4	60.9	0	0.0	7.93	4.8	31.66	19.3	46.27	28.2	0	0.0
Day 90_4	70.6	0	0.0	8.51	6.0	53.99	38.1	37.5	26.5	0	0.0

Replicate 5

Sample	Radioactivity in combined extracts	florfenicol (Rf 0,56)		florfenicol-amine (Rf = 0,2)		tranformation product_1 (Rf-value = 0,0-0,1)		tranformation product_2 (Rf-value = 0,35)		tranformation product_3 (Rf-value = 0,65)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
Day 0 - 0 h_5	94.0	100	94.0	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 0.5 h_5	99.3	100	99.3	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 1 h_5	105.4	83.64	88.2	3.55	3.7	12.81	13.5	0	0.0	0	0.0
Day 0 - 2 h_5	105.2	91.55	96.3	0	0.0	6.94	7.3	1.51	1.6	0	0.0
Day 0 - 4 h_5	81.5	54.35	44.3	14.3	11.6	29.99	24.4	2.06	1.7	0	0.0
Day 0 - 7 h_5	89.3	68.96	61.6	0	0.0	16.24	14.5	5.32	4.8	9.5	8.5
Day 1 - 24 h_5	62.8	46.07	28.9	5.09	3.2	23.14	14.5	12.52	7.9	10.15	6.4
Day 7_5	62.9	24.64	15.5	9.55	6.0	37.86	23.8	24.64	15.5	15.41	9.7
Day 28_5	72.5	0	0.0	8.34	6.1	35.09	25.5	14.18	10.3	0	0.0
Day 60_5	63.7	0	0.0	14.59	9.3	38.77	24.7	46.64	29.7	0	0.0
Day 90_5	70.9	0	0.0	18.92	13.4	46.5	33.0	34.57	24.5	0	0.0

Replicate 6

Sample	Radioactivity in combined extracts	florfenicol (Rf 0,56)		florfenicol-amine (Rf = 0,2)		tranformation product_1 (Rf-value = 0,0-0,1)		tranformation product_2 (Rf-value = 0,35)		tranformation product_3 (Rf-value = 0,65)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
Day 0 - 0 h_6	100.7	100	100.7	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 0.5 h_6	100.8	100	100.8	0	0.0	0	0.0	0	0.0	0	0.0
Day 0 - 1 h_6	102.0	84.37	86.0	3.32	3.4	12.31	12.6	0	0.0	0	0.0
Day 0 - 2 h_6	103.1	85.23	87.9	0	0.0	14.77	15.2	0	0.0	0	0.0
Day 0 - 4 h_6	86.0	76.01	65.4	7.93	6.8	14.59	12.5	1.47	1.3	0	0.0
Day 0 - 7 h_6	94.9	82.03	77.9	0	0.0	13.03	12.4	0	0.0	4.93	4.7
Day 1 - 24 h_6	58.6	46.57	27.3	3.52	2.1	28.67	16.8	12.48	7.3	8.4	4.9
Day 7_6	62.4	17.71	11.0	0	0.0	33.93	21.2	37.67	23.5	10.7	6.7
Day 28_6	75.4	0	0.0	10.12	7.6	71.17	53.7	17.71	13.4	0	0.0
Day 60_6	62.7	0	0.0	0	0.0	43.4	27.2	35.57	22.3	0	0.0
Day 90_6	69.9	0	0.0	19.52	13.6	53.85	37.7	26.63	18.6	0	0.0

Figure A3_6: Results of chemical analysis for ^{14}C-imidacloprid in cattle manure by Institute 1

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 1
Manure sample: Cattle
Test substance: Imidacloprid

Excel 2003

Replicate 1

Sample	Radioactivity in combined extracts	imidacloprid Rf 0,65)		6-chloronictoic acid (Rf 0,35)		N-nitroso guanidine (Rf 0,67)		nitro-guanidine (Rf 0,75)		tranformation product_TP1 (Rf 0,1, starting radioactivity)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_1	109.5	100	109.5	0	0.0	0	0.0	0	0.0	0	0.0
3 d_1	113.0	100	113.0	0	0.0	0	0.0	0	0.0	0	0.0
7 d_1	101.7	89.61	91.2	0	0.0	0	0.0	0.68	0.7	9.71	9.9
10 d_1	102.3	83.96	85.9	0	0.0	0	0.0	0.86	0.9	15.16	15.5
14 d_1	106.2	71.11	75.5	0	0.0	0	0.0	1.96	2.1	26	27.6
28 d_1	84.3	38.1	32.1	0	0.0	0	0.0	1.92	1.6	57.69	48.6
42 d_1	76.3	16.13	12.3	0	0.0	0	0.0	1.99	1.5	81.01	61.8
56 d_1	72.1	9.72	7.0	0	0.0	0	0.0	0	0.0	90.28	65.1
72 d_1	61.9	4.42	2.7	0	0.0	0	0.0	0	0.0	95.58	59.2
90 d_1	71.9	2.64	1.9	0	0.0	0	0.0	0	0.0	97.36	70.0

Replicate 2

Sample	Radioactivity in combined extracts	imidacloprid		6-chloronictoic acid (Rf 0,35)		N-nitroso guanidine (Rf 0,67)		nitro-guanidine (Rf 0,75)		tranformation product_TP1 (Rf 0,1, starting radioactivity)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_2	110.2	100	110.2	0	0.0	0	0.0	0	0.0	0	0.0
3 d_2	106.4	100	106.4	0	0.0	0	0.0	0	0.0	0	0.0
7 d_2	104.9	89.09	93.5	0	0.0	0	0.0	0.76	0.8	9.72	10.2
10 d_2	104.3	82.35	85.9	0	0.0	0	0.0	1.05	1.1	15.97	16.6
14 d_2	103.2	71.22	73.5	0	0.0	0	0.0	1.56	1.6	26.23	27.1
28 d_2	93.7	38.43	36.0	0	0.0	0	0.0	1.86	1.7	58.18	54.5
42 d_2	75.8	14.05	10.7	0	0.0	0	0.0	1.83	1.4	82.73	62.7
56 d_2	77.6	9.8	7.6	0	0.0	0	0.0	0	0.0	90.2	70.0
72 d_2	64.1	6.52	4.2	0	0.0	0	0.0	1.9	1.2	91.58	58.7
90 d_2	71.9	3.6	2.6	0	0.0	0	0.0	0	0.0	96.4	69.3

Replicate 3

Sample	Radioactivity in combined extracts	imidacloprid		6-chloronictoic acid (Rf 0,35)		N-nitroso guanidine (Rf 0,67)		nitro-guanidine (Rf 0,75)		tranformation product_TP1 (Rf 0,1, starting radioactivity)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_3	112.0	100	112.0	0	0.0	0	0.0	0	0.0	0	0.0
3 d_3	104.5	100	104.5	0	0.0	0	0.0	0	0.0	0	0.0
7 d_3	100.9	88.92	89.7	0	0.0	0	0.0	0.74	0.7	10.34	10.4
10 d_3	102.4	83.42	85.5	0	0.0	0	0.0	1.14	1.2	15.45	15.8
14 d_3	96.8	70.09	67.9	0	0.0	0	0.0	1.04	1.0	28.02	27.1
28 d_3	88.2	36.74	32.4	0	0.0	0	0.0	1.39	1.2	60.25	53.1
42 d_3	75.5	16.08	12.1	0	0.0	0	0.0	1.47	1.1	83.91	63.4
56 d_3	77.7	11.83	9.2	0	0.0	0	0.0	1.63	1.3	86.53	67.2
72 d_3	64.8	5.83	3.8	0	0.0	0	0.0	0.97	0.6	91.33	59.2
90 d_3	69.4	4.13	2.9	0	0.0	0	0.0	1.66	1.2	94.2	65.4

Replicate 4

Sample	Radioactivity in combined extracts	imidacloprid		6-chloronictoic acid (Rf 0,35)		N-nitroso guanidine (Rf 0,67)		nitro-guanidine (Rf 0,75)		tranformation product_TP1 (Rf 0,1, starting radioactivity)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_4	112.5	100	112.5	0	0.0	0	0.0	0	0.0	0	0.0
3 d_4	104.9	100	104.9	0	0.0	0	0.0	0	0.0	0	0.0
7 d_4	103.6	88.36	91.5	0	0.0	0	0.0	0.72	0.7	10.28	10.7
10 d_4	100.8	83.05	83.7	0	0.0	0	0.0	1.34	1.4	14.88	15.0
14 d_4	97.6	71.21	69.5	0	0.0	0	0.0	1.07	1.0	27.72	27.0
28 d_4	84.3	39.23	33.1	0	0.0	0	0.0	2.57	2.2	58.2	49.1
42 d_4	72.2	14.14	10.2	0	0.0	0	0.0	2.06	1.5	82.28	59.4
56 d_4	91.3	9.25	8.4	0	0.0	0	0.0	0.79	0.7	87.27	79.6
72 d_4	63.4	6.67	4.2	0	0.0	0	0.0	1.64	1.0	91.72	58.1
90 d_4	72.1	2.43	1.8	0	0.0	0	0.0	1.95	1.4	95.62	68.9

Replicate 5

Sample	Radioactivity in combined extracts	imidacloprid		6-chloronictoic acid (Rf 0,35)		N-nitroso guanidine (Rf 0,67)		nitro-guanidine (Rf 0,75)		tranformation product_TP1 (Rf 0,1, starting radioactivity)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_5	106.8	100	106.8	0	0.0	0	0.0	0	0.0	0	0.0
3 d_5	109.6	100	109.6	0	0.0	0	0.0	0	0.0	0	0.0
7 d_5	103.2	88.32	91.2	0	0.0	0	0.0	0	0.0	11.68	12.1
10 d_5	106.2	81.92	87.0	0	0.0	0	0.0	1.46	1.6	16	17.0
14 d_5	98.1	68.6	67.3	0	0.0	0	0.0	0.95	0.9	30.45	29.9
28 d_5	87.1	42.97	37.4	0	0.0	0	0.0	1.58	1.4	55.46	48.3
42 d_5	76.3	20.32	15.5	0	0.0	0	0.0	3.63	2.8	74.12	56.6
56 d_5	80.4	9	7.2	0	0.0	0	0.0	1.49	1.2	88.04	70.7
72 d_5	63.0	5.06	3.2	0	0.0	0	0.0	1.87	1.2	93.06	58.6
90 d_5	71.2	4.04	2.9	0	0.0	0	0.0	0	0.0	95.96	68.4

Replicate 6

Sample	Radioactivity in combined extracts	imidacloprid		6-chloronictoic acid (Rf 0,35)		N-nitroso guanidine (Rf 0,67)		nitro-guanidine (Rf 0,75)		tranformation product_TP1 (Rf 0,1, starting radioactivity)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_6	108.4	100	108.4	0	0.0	0	0.0	0	0.0	0	0.0
3 d_6	107.3	100	107.3	0	0.0	0	0.0	0	0.0	0	0.0
7 d_6	102.7	88.09	90.4	0	0.0	0	0.0	0.43	0.4	11.48	11.8
10 d_6	108.6	84.02	91.2	0	0.0	0	0.0	1.06	1.2	14.47	15.7
14 d_6	99.0	69.68	69.0	0	0.0	0	0.0	0.99	1.0	29.33	29.0
28 d_6	85.3	36.28	30.9	0	0.0	0	0.0	2.24	1.9	61.48	52.4
42 d_6	73.2	18.99	13.9	0	0.0	0	0.0	2.87	2.1	78.12	57.2
56 d_6	81.2	10.77	8.7	0	0.0	0	0.0	1.27	1.0	85.88	69.7
72 d_6	63.7	4.52	2.9	0	0.0	0	0.0	2.33	1.5	93.15	59.4
90 d_6	74.7	3.26	2.4	0	0.0	0	0.0	1.91	1.4	92.64	69.2

Remark: at day 14 and later, ASE was performed; Result: approx. 1 % of aR / 100% imidacloprid in all samples / no correction of imidaclorpd values.

Institute 2

Physico-chemical parameters

Figure A3_7: Physico-chemical parameters measured throughout the ring test experiments by Institute 2

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 2
Manure sample: Cattle
Test substance: Imidacloprid

Excel 2003

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) [mg/kg]	Nitrogen (N_{total}) [mg/kg]	Dry matter content [%]	Organic matter content (relating to wet weight) [%]	Other parameter(s)
Sampling	08.10.2013	7.09	-10.3	16.4	n.d.	n.d.	9.6	n.d.	-
Start of acclimation	15.10.2013	7.25	-363.0	18.4			9.7	80.9	-
Day 0	05.11.2013	7.38	-261.0	n.d.	1842.6	3660.7	8.9		-
Day 3	08.11.2013	7.20	-436.0	21.6					-
Day 7	12.11.2013	7.70	-453.0	20.4					-
Day 10	n.d.	n.d.	n.d.	n.d.					-
Day 14	19.11.2013	7.60	-420.0	20.5					-
Day 21	26.11.2013	7.95	-378.0	20.4					-
Day 28	04.12.2013	7.63	-410.0	21.7					-
Day 42	n.d.	n.d.	n.d.	n.d.					-
Day 56	n.d.	n.d.	n.d.	n.d.					-
Day 72	21.01.2014	7.55	-342.0	20.3					-
Day 90	06.02.2014	7.82	-325.0	21.3			7.5		-

Manure sample: Pig
Test substance: Florfenicol

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) [mg/L]	Nitrogen (N_{total}) [mg/L]	Dry matter content [%]	Organic matter content (relating to wet weight) [%]	Other parameter(s)
Sampling	23.12.2014	n.d.	n.d.	n.d.	n.d.	n.d.	5.6	n.d.	-
Start of acclimation 1	07.01.2014	7.80	-320.0	20.0	4580.0	5104.0	5.0	76.1	-
Start of acclimation 2	21.01.2014	7.69	-325.0	19.3			4.7	n.d.	-
Day 0 - 0 h	28.01.2014	7.750	-373.0	21.0	3952.0	4432.0	4.7		-
Day 0 - 0.5 h	n.d.	n.d.	n.d.	n.d.					-
Day 0 - 1 h	n.d.	n.d.	n.d.	n.d.					-
Day 0 - 2 h	n.d.	n.d.	n.d.	n.d.					-
Day 0 - 4 h	12.02.2014	8.12	-380.0	21.5					-
Day 0 - 7 h	n.d.	n.d.	n.d.	n.d.					-
Day 1 - 24 h	n.d.	n.d.	n.d.	n.d.					-
Day 7	06.02.2014	8.10	-325.0	20.8					-
Day 28	28.02.2014	8.09	-371.0	21.1					-
Day 60	02.04.2014	8.10	-375.0	20.2					-
Day 90	30.04.2014	8.00	-381.0	21.8			4.9/2.9		-

Mass Balance

Figure A3_8: Mass balance determined for ^{14}C-florfenicol in pig manure by Institute 2

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 2
Manure sample: Pig
Test substance: Florfenicol
Applied radioactivity (aR): 50.00 kBq per test vessel

Excel 2003

A		B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Sample		Activity in extracts	Amount in extracts	Extract (mean)	NER/Activity in manure (combustion)	NER/Manure combustion	NER/Manure combustion (mean)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$) (mean)	Activity CO_2-stripping	Activity CO_2-stripping	Activity CO_2-stripping (mean)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$) (mean)	Total activity	Recovery	Recovery (mean)
		[kBq]	[% aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% aR]	[% aR]
0 d (0 h) - 1	1	49.215	98.4		0.959	1.9		0.014	0.0		0	0.0		0	0.0		50.189	100.4	
0 d (0 h) - 2	2	48.151	96.3		0.861	1.7		0.033	0.1		0	0.0		0	0.0		49.045	98.1	
0 d (0 h) - 3	3	50.699	101.4	96.5	0.923	1.8	1.7	0.034	0.1	0.063	0	0.0	0.0	0	0.0	0.0	51.656	103.3	98.3
0 d (0 h) - 4	4	45.108	90.2		0.690	1.4		0.039	0.1		0	0.0		0	0.0		45.837	91.7	
0 d (0 h) - 5	5	48.377	96.8		0.830	1.7		0.039	0.1		0	0.0		0	0.0		49.247	98.5	
0 d (0 h) - 6	6	48.093	96.2		0.707	1.4		0.029	0.1		0	0.0		0	0.0		48.830	97.7	
0 d (0.5 h) - 1	7	47.747	95.5		1.997	4.0		0.016	0.0		0	0.0		0	0.0		49.760	99.5	
0 d (0.5 h) - 2	8	47.725	95.5		2.091	4.2		0.015	0.0		0	0.0		0	0.0		49.831	99.7	
0 d (0.5 h) - 3	9	47.494	95.0	95.3	2.019	4.0	4.2	0.012	0.0	0.026	0	0.0	0.0000	0	0.0	0.0000	49.524	99.0	99.5
0 d (0.5 h) - 4	10	47.157	94.3		2.248	4.5		0.014	0.0		0	0.0		0	0.0		49.419	98.8	
0 d (0.5 h) - 5	11	48.112	96.2		1.883	3.8		0.012	0.0		0	0.0		0	0.0		50.006	100.0	
0 d (0.5 h) - 6	12	47.525	95.0		2.294	4.6		0.010	0.0		0	0.0		0	0.0		49.829	99.7	
0 d (1 h) - 1	13	46.447	92.9		2.516	5.0		0.011	0.0		0	0.0		0	0.0		48.973	97.9	
0 d (1 h) - 2	14	45.558	91.1		3.563	7.1		0.007	0.0		0	0.0		0	0.0		49.128	98.3	
0 d (1 h) - 3	15	45.130	90.3	90.6	3.606	7.2	5.7	0.010	0.0	0.017	0	0.0	0.000	0	0.0	0.0000	48.746	97.5	96.3
0 d (1 h) - 4	16	44.876	89.8		2.528	5.1		0.008	0.0		0	0.0		0	0.0		47.413	94.8	
0 d (1 h) - 5	17	44.388	88.8		2.297	4.6		0.006	0.0		0	0.0		0	0.0		46.691	93.4	
0 d (1 h) - 6	18	45.324	90.6		2.719	5.4		0.008	0.0		0	0.0		0	0.0		48.051	96.1	
0 d (2 h) - 1	19	40.382	80.8		6.942	13.9		0.018	0.0		0	0.0		0	0.0		47.342	94.7	
0 d (2 h) - 2	20	38.226	76.5		6.029	12.1		0.013	0.0		0	0.0		0	0.0		44.269	88.5	
0 d (2 h) - 3	21	39.946	79.9	79.9	6.978	14.0	13.2	0.007	0.0	0.024	0	0.0	0.000	0	0.0	0.000	46.931	93.9	93.2
0 d (2 h) - 4	22	38.228	76.5		5.989	12.0		0.013	0.0		0	0.0		0	0.0		44.230	88.5	
0 d (2 h) - 5	23	40.897	81.8		9.706	19.4		0.008	0.0		0	0.0		0	0.0		50.612	101.2	
0 d (2 h) - 6	24	42.125	84.2		4.019	8.0		0.013	0.0		0	0.0		0	0.0		46.156	92.3	
0 d (4 h) - 1	25	39.701	79.4		7.828	15.7		0.014	0.0		0	0.0		0	0.0		47.544	95.1	
0 d (4 h) - 2	26	40.827	81.7		7.198	14.4		0.013	0.0		0	0.0		0	0.0		48.039	96.1	
0 d (4 h) - 3	27	39.179	78.4	80.6	9.074	18.1	15.9	0.017	0.0	0.029	0	0.0	0.000	0	0.0	0.000	48.269	96.5	96.6
0 d (4 h) - 4	28	41.994	84.0		7.753	15.5		0.014	0.0		0	0.0		0	0.0		49.761	99.5	
0 d (4 h) - 5	29	39.968	79.9		7.477	15.0		0.014	0.0		0	0.0		0	0.0		47.459	94.9	
0 d (4 h) - 6	30	40.189	80.4		8.380	16.8		0.016	0.0		0	0.0		0	0.0		48.585	97.2	
0 d (7 h) - 1	31	36.771	73.5		12.053	24.1		0.034	0.1		0	0.0		0	0.0		48.858	97.7	
0 d (7 h) - 2	32	36.765	73.5		12.680	25.4		0.018	0.0		0	0.0		0	0.0		49.463	98.9	
0 d (7 h) - 3	33	37.209	74.4	73.1	10.615	21.2	23.6	0.021	0.0	0.041	0	0.0	0.000	0	0.0	0.000	47.845	95.7	96.8
0 d (7 h) - 4	34	35.786	71.6		11.785	23.6		0.018	0.0		0	0.0		0	0.0		47.588	95.2	
0 d (7 h) - 5	35	36.735	73.5		11.605	23.2		0.018	0.0		0	0.0		0	0.0		48.358	96.7	
0 d (7 h) - 6	36	36.028	72.1		12.113	24.2		0.016	0.0		0	0.0		0	0.0		48.157	96.3	
1 d - 1	37	29.405	58.8		16.185	32.4		0.025	0.1		0	0.0		0	0.0		45.618	91.2	
1 d - 2	38	30.521	61.0		20.066	40.1		0.042	0.1		0	0.0		0	0.0		50.629	101.3	
1 d - 3	39	31.171	62.3	60.9	18.069	36.1	35.2	0.033	0.1	0.073	0	0.0	0.000	0	0.0	0.000	49.273	98.5	96.2
1 d - 4	40	30.525	61.0		14.833	29.7		0.038	0.1		0	0.0		0	0.0		45.396	90.8	
1 d - 5	41	31.064	62.1		17.738	35.5		0.043	0.1		0	0.0		0	0.0		48.845	97.7	
1 d - 6	42	30.013	60.0		18.842	37.7		0.038	0.1		0	0.0		0	0.0		48.893	97.8	
7 d - 1	43	24.764	49.5		23.637	47.3		0.008	0.0		0.033	0.1		0.012	0.023		48.454	96.9	
7 d - 2	44	25.216	50.4		25.285	50.6		0.024	0.0		0.042	0.1		0.005	0.010		50.572	101.1	
7 d - 3	45	25.047	50.1	50.0	22.458	44.9	47.8	0.028	0.1	0.046	0.033	0.1	0.060	0.010	0.020	0.017	47.575	95.1	97.9
7 d - 4	46	24.433	48.9		23.203	46.4		0.024	0.0		0.033	0.1		0.008	0.017		47.701	95.4	
7 d - 5	47	25.381	50.8		23.733	47.5		0.025	0.1		0.005	0.0		0.003	0.007		49.147	98.3	
7 d - 6	48	25.143	50.3		24.942	49.9		0.028	0.1		0.037	0.1		0.012	0.023		50.162	100.3	
28 d - 1	49	25.043	50.1		21.748	43.5		0.018	0.0		0.081	0.2		0.012	0.023		46.901	93.8	
28 d - 2	50	25.534	51.1		25.327	50.7		0.033	0.1		0.072	0.1		0.010	0.020		50.975	101.9	
28 d - 3	51	25.982	52.0	51.4	19.887	39.8	43.7	0.034	0.1	0.061	0.083	0.2	0.157	0.003	0.007	0.017	45.989	92.0	95.4
28 d - 4	52	25.840	51.7		22.594	45.2		0.033	0.1		0.076	0.2		0.005	0.010		48.548	97.1	
28 d - 5	53	26.718	53.4		20.064	40.1		0.032	0.1		0.083	0.2		0.010	0.020		46.906	93.8	
28 d - 6	54	25.226	50.5		21.390	42.8		0.035	0.1		0.078	0.2		0.012	0.023		46.740	93.5	
59 d - 1	55	22.217	44.4		22.302	44.6		0.096	0.2		0.251	0.5		0.0	0.0		44.865	89.7	
59 d - 2	56	22.623	45.2	44.2	26.736	53.5	48.1	0.110	0.2	0.211	0.149	0.3	0.478	0.0	0.0	0.000	49.619	99.2	93.0
59 d - 3	57	21.439	42.9		24.813	49.6		0.099	0.2		0.263	0.5		0.0	0.0		46.613	93.2	
59 d - 4	58	22.128	44.3		22.339	44.7		0.118	0.2		0.293	0.6		0.0	0.0		44.877	89.8	
90 d - 1	61	23.405	46.8		23.435	46.9		0.227	0.5		0.373	0.7		0.015	0.030		47.455	94.9	
90 d - 2	62	25.410	50.8	46.5	27.313	54.6	50.8	0.121	0.2	0.532	0.052	0.1	0.912	0.009	0.018	0.031	52.905	105.8	98.8
90 d - 3	63	22.781	45.6		26.412	52.8		0.307	0.6		0.623	1.2		0.017	0.033		50.139	100.3	
90 d - 4	64	21.347	42.7		24.449	48.9		0.409	0.8		0.777	1.6		0.022	0.043		47.003	94.0	
1 d sterile - 1		43.495	87.0	89.3	0.623	1.2	1.2	-	-	-	-	-	-	-	-	-	44.118	88.2	90.5
1 d sterile - 2		45.786	91.6		0.581	1.2		-	-	-	-	-	-	-	-	-	46.367	92.7	
28 d sterile - 1		42.921	85.8	86.6	2.393	4.8	5.2	-	-	-	-	-	-	-	-	-	45.314	90.6	91.8
28 d sterile - 2		43.692	87.4		2.829	5.7		-	-	-	-	-	-	-	-	-	46.521	93.0	
90 d sterile - 1		35.596	71.2	70.2	8.339	16.7	19.1	-	-	-	-	-	-	-	-	-	43.935	87.9	89.3
90 d sterile - 2		34.607	69.2		10.805	21.6		-	-	-	-	-	-	-	-	-	45.412	90.8	
Formula			100 / aR * B	mean of C		100 / aR * E	mean of F		100 / aR * H	mean of I		100 / aR * K	mean of L		100 / aR * N	mean of O	B+E+H+K+N	100 / aR * Q	mean of R

Figure A3_9: Mass balance determined for ^{14}C-imidacloprid in cattle manure by Institute 2

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

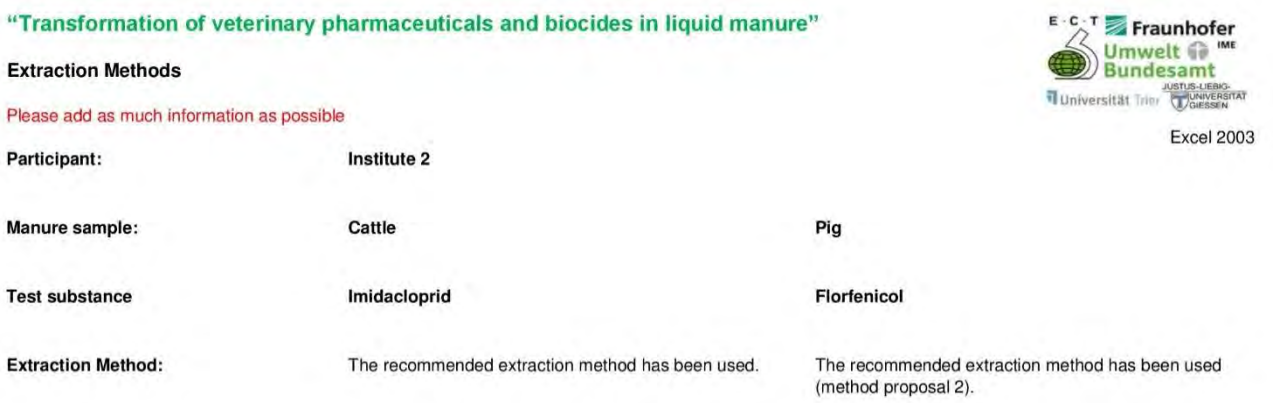
Participant: Institute 2
Manure sample: Cattle
Test substance: Imidacloprid
Applied radioactivity (aR): 50.00 kBq per test vessel

Excel 2003

A		B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Sample		Activity in extracts	Amount in extracts	Extract (mean)	NER/Activity in manure (combustion)	NER/Manure combustion	NER/Manure combustion (mean)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$) (mean)	Activity CO_2 stripping	Activity CO_2 stripping	Activity CO_2 stripping (mean)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$) (mean)	Total activity	Recovery	Recovery (mean)
		[kBq]	[% aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% aR]	[% aR]
0 d - 1	1	50.702	101.4		1.387	2.8		0	0.0		0	0.0		0	0.0		52.089	104.2	
0 d - 2	2	50.704	101.4		1.437	2.9		0	0.0		0	0.0		0	0.0		52.141	104.3	
0 d - 3	3	50.201	100.4	100.4	1.570	3.1	2.8	0	0.0	0.000	0	0.0	0.0	0	0.0	0.0	51.771	103.5	103.1
0 d - 4	4	50.169	100.3		1.524	3.0		0	0.0		0	0.0		0	0.0		51.693	103.4	
0 d - 5	5	49.869	99.7		1.087	2.2		0	0.0		0	0.0		0	0.0		50.956	101.9	
0 d - 6	6	49.445	98.9		1.298	2.6		0	0.0		0	0.0		0	0.0		50.744	101.5	
3 d - 1	7	51.436	102.9		2.660	5.3		0.005	0.0		0.007	0.0		0.003	0.0		54.110	108.2	
3 d - 2	8	51.415	102.8		2.676	5.4		0.013	0.0		0.011	0.0		0.001	0.0		54.116	108.2	
3 d - 3	9	51.722	103.4	102.8	2.990	6.0	5.4	0.010	0.0	0.018	0.007	0.0	0.0161	0.006	0.0	0.0078	54.735	109.5	108.2
3 d - 4	10	51.266	102.5		2.584	5.2		0.008	0.0		0.008	0.0		0.004	0.0		53.869	107.7	
3 d - 5	11	51.344	102.7		2.704	5.4		0.009	0.0		0.008	0.0		0.008	0.0		54.073	108.1	
3 d - 6	12	51.241	102.5		2.526	5.1		0.009	0.0		0.009	0.0		0.002	0.0		53.787	107.6	
7 d - 1	13	49.073	98.1		2.750	5.5		0.013	0.0		0.006	0.0		0.003	0.0		51.844	103.7	
7 d - 2	14	48.500	97.0		2.814	5.6		0.004	0.0		0.008	0.0		0.002	0.0		51.327	102.7	
7 d - 3	15	49.352	98.7	98.4	2.745	5.5	5.8	0.011	0.0	0.021	0.004	0.0	0.015	0.000	0.0	0.0033	52.112	104.2	104.2
7 d - 4	16	50.044	100.1		3.104	6.2		0.016	0.0		0.004	0.0		0.000	0.0		53.168	106.3	
7 d - 5	17	48.886	97.8		2.825	5.7		0.011	0.0		0.012	0.0		0.000	0.0		51.734	103.5	
7 d - 6	18	49.243	98.5		3.098	6.2		0.008	0.0		0.011	0.0		0.006	0.0		52.366	104.7	
10 d - 1	19	43.536	87.1		3.982	8.0		0.0183	0.0		0.005	0.0		0.0025	0.0		47.544	95.1	
10 d - 2	20	43.561	87.1		3.759	7.5		0.0092	0.0		0.005	0.0		0.0067	0.0		47.341	94.7	
10 d - 3	21	43.288	86.6	86.3	3.711	7.4	7.6	0.015	0.0	0.033	0.0100	0.0	0.013	0.005	0.0	0.008	47.029	94.1	94.0
10 d - 4	22	43.615	87.2		3.326	6.7		0.0225	0.0		0.005	0.0		0.0067	0.0		46.975	94.0	
10 d - 5	23	42.545	85.1		4.047	8.1		0.015	0.0		0.0058	0.0		0.0017	0.0		46.615	93.2	
10 d - 6	24	42.410	84.8		3.970	7.9		0.0192	0.0		0.0092	0.0		0.0000	0.0		46.408	92.8	
14 d - 1	25	46.706	93.4		3.352	6.7		0.044	0.1		0.023	0.0		0.014	0.0		50.140	100.3	
14 d - 2	26	47.193	94.4		3.791	7.6		0.041	0.1		0.026	0.1		0.015	0.0		51.066	102.1	
14 d - 3	27	46.126	92.3	92.8	3.302	6.6	7.2	0.038	0.1	0.079	0.029	0.1	0.054	0.016	0.0	0.031	49.511	99.0	100.2
14 d - 4	28	46.845	93.7		3.513	7.0		0.043	0.1		0.029	0.1		0.016	0.0		50.446	100.9	
14 d - 5	29	45.899	91.8		3.801	7.6		0.036	0.1		0.025	0.1		0.016	0.0		49.777	99.6	
14 d - 6	30	45.642	91.3		3.985	8.0		0.035	0.1		0.029	0.1		0.018	0.0		49.709	99.4	
21 d - 1	31	42.774	85.5		3.912	7.8		0.014	0.0		0.008	0.0		0.001	0.0		46.709	93.4	
21 d - 2	32	39.998	80.0		3.735	7.5		0.012	0.0		0.001	0.0		0.000	0.0		43.746	87.5	
21 d - 3	33	38.953	77.9	78.6	5.048	10.1	8.5	0.015	0.0	0.027	0.000	0.0	0.006	0.002	0.0	0.003	44.018	88.0	87.1
21 d - 4	34	42.771	85.5		4.370	8.7		0.013	0.0		0.005	0.0		0.002	0.0		47.161	94.3	
21 d - 5	35	35.162	70.3		4.395	8.8		0.012	0.0		0.000	0.0		0.003	0.0		39.572	79.1	
21 d - 6	36	36.066	72.1		3.975	8.0		0.016	0.0		0.003	0.0		0.000	0.0		40.060	80.1	
28 d - 1	37	40.172	80.3		4.379	8.8		0.020	0.0		0.004	0.0		0.003	0.0		44.578	89.2	
28 d - 2	38	42.224	84.4		4.164	8.3		0.019	0.0		0.003	0.0		0.005	0.0		46.415	92.8	
28 d - 3	39	41.142	82.3	82.5	5.102	10.2	9.3	0.018	0.0	0.039	0.003	0.0	0.009	0.005	0.0	0.008	46.270	92.5	91.9
28 d - 4	40	41.175	82.4		5.353	10.7		0.018	0.0		0.003	0.0		0.006	0.0		46.554	93.1	
28 d - 5	41	41.207	82.4		4.286	8.6		0.015	0.0		0.005	0.0		0.003	0.0		45.517	91.0	
28 d - 6	42	41.637	83.3		4.550	9.1		0.026	0.1		0.008	0.0		0.004	0.0		46.224	92.4	
42 d - 1	43	41.834	83.7		4.855	9.7		0.02	0.0		0.0067	0.0		0.005	0.0		46.721	93.4	
42 d - 2	44	40.891	81.8		5.439	10.9		0.0225	0.0		0.0033	0.0		0.0108	0.0		46.367	92.7	
42 d - 3	45	41.068	82.1	79.1	5.113	10.2	12.6	0.0217	0.0	0.034	0.005	0.0	0.010	0.0075	0.0	0.012	46.216	92.4	91.7
42 d - 4	46	41.161	82.3		4.525	9.1		0.0075	0.0		0.0067	0.0		0.0058	0.0		45.706	91.4	
42 d - 5	47	37.839	75.7		4.968	9.9		0.0166	0.0		0.0042	0.0		0.0025	0.0		42.831	85.7	
42 d - 6	48	34.519	69.0		12.756	25.5		0.0125	0.0		0.005	0.0		0.0042	0.0		47.297	94.6	
56 d - 1	49	41.261	82.5		4.898	9.8		0.0433	0.1		0.0083	0.0		0.0067	0.0		46.217	92.4	
56 d - 2	50	41.579	83.2		4.793	9.6		0.0325	0.1		0.0125	0.0		0.0075	0.0		46.425	92.8	
56 d - 3	51	37.336	74.7	80.6	5.316	10.6	10.2	0.0392	0.1	0.082	0.0175	0.0	0.024	0.015	0.0	0.015	42.724	85.4	91.0
56 d - 4	52	40.659	81.3		5.019	10.0		0.0442	0.1		0.015	0.0		0.005	0.0		45.743	91.5	
56 d - 5	53	40.589	81.2		4.979	10.0		0.0558	0.1		0.0092	0.0		0.0083	0.0		45.641	91.3	
56 d - 6	54	40.482	81.0		5.584	11.2		0.0308	0.1		0.0083	0.0		0.0033	0.0		46.108	92.2	
72 d - 1	55	37.612	75.2		5.143	10.3		0.0117	0.0		0.0217	0.0		0.0075	0.0		42.795	85.6	
72 d - 2	56	39.631	79.3		5.336	10.7		0.0175	0.0		0.0208	0.0		0.0133	0.0		45.019	90.0	
72 d - 3	57	40.927	81.9	79.8	5.240	10.5	10.5	0.0275	0.1	0.038	0.0217	0.0	0.039	0.0200	0.0	0.021	46.236	92.5	90.3
72 d - 4	58	38.835	77.7		5.295	10.6		0.0150	0.0		0.0167	0.0		0.0100	0.0		44.172	88.3	
72 d - 5	59	41.022	82.0		5.320	10.6		0.0317	0.1		0.0200	0.0		0.0100	0.0		46.404	92.8	
72 d - 6	60	41.266	82.5		5.098	10.2		0.0108	0.0		0.0167	0.0		0.0017	0.0		46.393	92.8	
90 d - 1	61	38.397	76.8		5.471	10.9		0.027	0.1		0.003	0.0		0.003	0.0		43.902	87.8	
90 d - 2	62	40.106	80.2		5.810	11.6		0.030	0.1		0.003	0.0		0.003	0.0		45.952	91.9	
90 d - 3	63	41.497	83.0	81.0	6.121	12.2	11.7	0.017	0.0	0.0	0.003	0.0	0.0	0.008	0.0	0.0	47.646	95.3	92.7
90 d - 4	64	40.459	80.9		6.375	12.8		0.028	0.1		0.008	0.0		0.002	0.0		46.872	93.7	
90 d - 5	65	40.909	81.8		5.707	11.4		0.026	0.1		0.003	0.0		0.003	0.0		46.647	93.3	
90 d - 6	66	41.563	83.1		5.500	11.0		0.016	0.0		0.008	0.0		0.000	0.0		47.086	94.2	
7 d sterile - 1		44.289	88.6	89.2	2.265	4.5	4.8	-	-	-	-	-	-	-	-	-	46.554	93.1	94.0
7 d sterile - 2		44.867	89.7		2.556	5.1		-	-	-	-	-	-	-	-	-	47.423	94.8	
58 d sterile - 1		44.863	89.7	89.8	2.073	4.1	4.5	-	-	-	-	-	-	-	-	-	46.936	93.9	94.2
58 d sterile - 2		44.887	89.8		2.411	4.8		-	-	-	-	-	-	-	-	-	47.298	94.6	
90 d sterile - 1		44.553	89.1	87.4	2.941	5.9	5.7	-	-	-	-	-	-	-	-	-	47.494	95.0	93.2
90 d sterile - 2		42.883	85.8		2.786	5.6		-	-	-	-	-	-	-	-	-	45.669	91.3	
Formula			100 / aR * B	mean of C		100 / aR * E	mean of F		100 / aR * H	mean of I		100 / aR * K	mean of L		100 / aR * N	mean of O	B+E+H+K+N	100 / aR * Q	mean of R

Extraction methods

Figure A3_10: Extraction methods used within the ring test by Institute 2



"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Extraction Methods

Please add as much information as possible

Excel 2003

Participant:	**Institute 2**	
Manure sample:	**Cattle**	**Pig**
Test substance	**Imidacloprid**	**Florfenicol**
Extraction Method:	The recommended extraction method has been used.	The recommended extraction method has been used (method proposal 2).

Chemical Analysis

Figure A3_11: Results of chemical analysis for ^{14}C-florfenicol in pig manure by Institute 2

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 2
Manure sample: Pig
Test substance: Florfenicol

Replicate 1

Excel 2003

Sample	Radioactivity in combined extracts	Florfenicol		TP1 Start		TP1 b		Florfenicol amine		TP2 a		TP2 b		TP1a+b		TP2a+b	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0	98.4	100	98.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0	0.0	0.0	0.0
0.021	95.5	92.05	87.9	7.95	7.6	0	0.0	0	0.0	0	0.0	0	0.0	8.0	7.6	0.0	0.0
0.042	92.9	86.23	80.1	13.77	12.8	0	0.0	0	0.0	0	0.0	0	0.0	13.8	12.8	0.0	0.0
0.083	80.8	67.59	54.6	25.63	20.7	2.64	2.1	4.15	3.4	0	0.0	0	0.0	28.3	22.8	0.0	0.0
0.167	79.4	58.73	46.6	41.27	32.8	0	0.0	0	0.0	0	0.0	0	0.0	41.3	32.8	0.0	0.0
0.292	73.5	58.54	43.1	41.46	30.5	0	0.0	0	0.0	0	0.0	0	0.0	41.5	30.5	0.0	0.0
1	58.8	0	0.0	68.66	40.4	0	0.0	3.73	2.2	27.62	16.2	0	0.0	68.7	40.4	27.6	16.2
7	49.5	0	0.0	80.02	39.6	0	0.0	0	0.0	12.25	6.1	7.73	3.8	80.0	39.6	20.0	9.9
28	50.1	0	0.0	77.9	39.0	3.03	1.5	0	0.0	15.02	7.5	4.05	2.0	80.9	40.5	19.1	9.6
60	44.4	0	0.0	70.55	31.3	9.57	4.3	0	0.0	16.8	7.5	3.08	1.4	80.1	35.6	19.9	8.8
90	46.8	0	0.0	79.81	37.4	11.82	5.5	0	0.0	8.38	3.9	0	0.0	91.6	42.9	8.4	3.9

Replicate 2

Sample	Radioactivity in combined extracts	Florfenicol		TP1 Start		TP1 b		Florfenicol amine		TP2 a		TP2 b		TP1a+b		TP2a+b	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0	96.3	100	96.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0	0.0	0.0	0.0
0.021	95.5	89.75	85.7	10.25	9.8	0	0.0	0	0.0	0	0.0	0	0.0	10.3	9.8	0.0	0.0
0.042	91.1	86.39	78.7	13.61	12.4	0	0.0	0	0.0	0	0.0	0	0.0	13.6	12.4	0.0	0.0
0.083	76.5	67.72	51.8	25.45	19.5	3.23	2.5	3.6	2.8	0	0.0	0	0.0	28.7	21.9	0.0	0.0
0.167	81.7	63.16	51.6	36.84	30.1	0	0.0	0	0.0	0	0.0	0	0.0	36.8	30.1	0.0	0.0
0.292	73.5	58.37	42.9	41.63	30.6	0	0.0	0	0.0	0	0.0	0	0.0	41.6	30.6	0.0	0.0
1	61.0	27.23	16.6	45.08	27.5	3.62	2.2	5.65	3.4	18.42	11.2	0	0.0	48.7	29.7	18.4	11.2
7	50.4	0	0.0	76.92	38.8	0	0.0	0	0.0	12.64	6.4	10.44	5.3	76.9	38.8	23.1	11.6
28	51.1	0	0.0	72.6	37.1	4.35	2.2	0	0.0	15.66	8.0	7.38	3.8	77.0	39.3	23.0	11.8
60	45.2	0	0.0	73.12	33.1	4.53	2.0	0	0.0	20.05	9.1	2.3	1.0	77.7	35.1	22.4	10.1
90	50.8	0	0.0	83.34	42.4	4.17	2.1	0	0.0	3.82	1.9	8.66	4.4	87.5	44.5	12.5	6.3

Replicate 3

Sample	Radioactivity in combined extracts	Florfenicol		TP1 Start		TP1 b		Florfenicol amine		TP2 a		TP2 b		TP1a+b		TP2a+b	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0	101.4	100.00	101.4	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
0.021	95.0	89.98	85.5	7.90	7.5	2.12	2.0	0.00	0.0	0.00	0.0	0.00	0.0	10.02	9.5	0.00	0.0
0.042	90.3	84.84	76.6	15.16	13.7	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	15.16	13.7	0.00	0.0
0.083	79.9	65.98	52.7	27.60	22.1	3.05	2.4	3.36	2.7	0.00	0.0	0.00	0.0	30.65	24.5	0.00	0.0
0.167	78.4	56.84	44.5	43.16	33.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	43.16	33.8	0.00	0.0
0.292	74.4	59.86	44.5	40.14	29.9	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	40.14	29.9	0.00	0.0
1	62.3	0.00	0.0	61.36	38.3	7.24	4.5	5.62	3.5	25.79	16.1	0.00	0.0	68.60	42.8	25.79	16.1
7	50.1	0.00	0.0	76.55	38.3	0.00	0.0	0.00	0.0	13.50	6.8	9.95	5.0	76.55	38.3	23.45	11.7
28	52.0	0.00	0.0	75.74	39.4	2.86	1.5	0.00	0.0	15.23	7.9	6.18	3.2	78.60	40.8	21.41	11.1
60	42.9	0.00	0.0	72.67	31.2	13.65	5.9	0.00	0.0	13.68	5.9	0.00	0.0	86.32	37.0	13.68	5.9
90	45.6	0.00	0.0	83.65	38.1	10.13	4.6	0.00	0.0	6.05	2.8	0.00	0.0	93.78	42.7	6.05	2.8

Replicate 4

Sample	Radioactivity in combined extracts	Florfenicol		TP1 Start		TP1 b		Florfenicol amine		TP2 a		TP2 b		TP1a+b		TP2a+b	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0	90.2	100.00	90.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
0.021	94.3	89.22	84.1	8.91	8.4	1.87	1.8	0.00	0.0	0.00	0.0	0.00	0.0	10.78	10.2	0.00	0.0
0.042	89.8	86.91	78.0	11.83	10.6	1.26	1.1	0.00	0.0	0.00	0.0	0.00	0.0	13.09	11.7	0.00	0.0
0.083	76.5	63.52	48.6	27.90	21.3	3.85	2.9	4.74	3.6	0.00	0.0	0.00	0.0	31.75	24.3	0.00	0.0
0.167	84.0	65.93	55.4	34.07	28.6	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	34.07	28.6	0.00	0.0
0.292	71.6	60.33	43.2	39.67	28.4	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	39.67	28.4	0.00	0.0
1	61.0	0.00	0.0	70.73	43.2	0.00	0.0	7.20	4.4	22.07	1.0	0.00	0.0	70.73	43.2	22.07	13.5
7	48.9	0.00	0.0	73.09	35.7	0.00	0.0	0.00	0.0	14.50	0.0	12.41	6.1	73.09	35.7	26.91	13.1
28	51.7	0.00	0.0	81.72	42.2	0.00	0.0	0.00	0.0	13.59	0.0	4.70	2.4	81.72	42.2	18.29	9.5
60	44.3	0.00	0.0	81.33	36.0	5.12	2.3	0.00	0.0	13.55	0.0	0.00	0.0	86.45	38.3	13.55	6.0
90	42.7	0.00	0.0	71.83	30.7	12.85	5.5	0.00	0.0	15.31	0.0	0.00	0.0	84.68	36.2	15.31	6.5

Replicate 5

Sample	Radioactivity in combined extracts	Florfenicol		TP1 Start		TP1 b		Florfenicol amine		TP2 a		TP2 b		TP1a+b		TP2a+b	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0	96.8	100.00	96.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
0.021	96.2	91.47	88.0	7.48	7.2	1.05	1.0	0.00	0.0	0.00	0.0	0.00	0.0	8.53	8.2	0.00	0.0
0.042	88.8	82.92	73.6	13.43	11.9	3.65	3.2	0.00	0.0	0.00	0.0	0.00	0.0	17.08	15.2	0.00	0.0
0.083	81.8	60.79	49.7	29.66	24.3	4.39	3.6	5.16	4.2	0.00	0.0	0.00	0.0	34.05	27.9	0.00	0.0
0.167	79.9	68.03	54.4	31.97	25.6	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	31.97	25.6	0.00	0.0
0.292	73.5	64.83	47.6	35.17	25.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	35.17	25.8	0.00	0.0
1	62.1	0.00	0.0	83.67	52.0	0.00	0.0	0.00	0.0	16.33	10.1	0.00	0.0	83.67	52.0	16.33	10.1
7	50.8	0.00	0.0	72.36	36.7	0.00	0.0	0.00	0.0	27.64	14.0	0.00	0.0	72.36	36.7	27.64	14.0
28	53.4	0.00	0.0	81.36	43.5	0.00	0.0	0.00	0.0	18.64	10.0	0.00	0.0	81.36	43.5	18.64	10.0
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Replicate 6

Sample	Radioactivity in combined extracts	Florfenicol		TP1 Start		TP1 b		Florfenicol amine		TP2 a		TP2 b		TP1a+b		TP2a+b	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0	96.2	100.00	96.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
0.021	95.0	91.05	86.5	8.36	7.9	0.59	0.6	0.00	0.0	0.00	0.0	0.00	0.0	8.95	8.5	0.00	0.0
0.042	90.6	83.31	75.5	13.20	12.0	3.48	3.2	0.00	0.0	0.00	0.0	0.00	0.0	16.68	15.1	0.00	0.0
0.083	84.2	76.86	64.8	16.95	14.3	6.19	5.2	0.00	0.0	0.00	0.0	0.00	0.0	23.14	19.5	0.00	0.0
0.167	80.4	61.10	49.1	38.90	31.3	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	38.90	31.3	0.00	0.0
0.292	72.1	60.19	43.4	39.81	28.7	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	39.81	28.7	0.00	0.0
1	60.0	0.00	0.0	70.52	42.3	0.00	0.0	6.98	4.2	22.50	13.5	0.00	0.0	70.52	42.3	22.50	13.5
7	50.3	0.00	0.0	78.68	39.6	0.00	0.0	0.00	0.0	9.11	4.6	12.21	6.1	78.68	39.6	21.32	10.7
28	50.5	0.00	0.0	77.82	39.3	0.00	0.0	0.00	0.0	17.00	8.6	5.19	2.6	77.82	39.3	22.19	11.2
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Sterile

Sample	Radioactivity in combined extracts	Florfenicol		TP1 Start		TP1 b		Florfenicol amine		TP2 a		TP2 b		TP1a+b		TP2a+b	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
1	87.0	95.65	83.2	4.35	3.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	4.35	3.8	0.00	0.0
1	91.6	95.01	87.0	4.99	4.6	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	4.99	4.6	0.00	0.0
28	85.8	92.78	79.6	7.22	6.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	7.22	6.2	0.00	0.0
28	87.4	91.37	79.8	8.63	7.5	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	8.63	7.5	0.00	0.0
91	71.2	64.81	46.1	29.37	20.9	0.00	0.0	0.00	0.0	5.82	4.1	0.00	0.0	29.37	20.9	5.82	4.1
91	69.2	61.84	42.8	32.39	22.4	0.00	0.0	0.00	0.0	5.77	4.0	0.00	0.0	32.39	22.4	5.77	4.0

Figure A3_12: Results of chemical analysis for ^{14}C-imidacloprid in cattle manure by Institute 2

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 2
Manure sample: Cattle
Test substance: Imidacloprid

Replicate 1

Excel 2003

Sample	Radioactivity in combined extracts	imidacloprid (Rf 0.53-0.67)		transformation product_1 (starting radioactivity; Rf 0.07-0.12)		tranformation product_2 (Rf 0.17-0.21)		Chloropiridin (Rf 0.25-0.26)		tranformation product_3 (Rf 0.38-0.40)		tranformation product_4 (Rf 0.48-0.58)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_1	101.4	96.68	98.0	0.00	0.0	0.00	0.0	0.00	0.0	3.32	3.4	0.00	0.0
3 d_1	102.9	87.81	90.3	12.19	12.5	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
7 d_1	98.1	73.80	72.4	26.20	25.7	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
10 d_1	87.1	72.61	63.2	22.42	19.5	4.97	4.3	0.00	0.0	0.00	0.0	0.00	0.0
14 d_1	93.4	59.40	55.5	40.60	37.9	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
21 d_1	85.5	39.09	33.4	60.91	52.1	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
28 d_1	80.3	29.58	23.8	62.01	49.8	0.00	0.0	0.00	0.0	0.00	0.0	8.41	6.8
42 d_1	83.7	14.49	12.1	85.51	71.5	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
56 d_1	82.5	4.98	4.1	95.02	78.4	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
72 d_1	75.2	2.83	2.1	97.17	73.1	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
90 d_1	76.8	0.00	0.0	100.00	76.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0

Replicate 2

Sample	Radioactivity in combined extracts	imidacloprid (Rf 0.53-0.67)		transformation product_1 (starting radioactivity; Rf 0.07-0.12)		tranformation product_2 (Rf 0.17-0.21)		Chloropiridin (Rf 0.25-0.26)		tranformation product_3 (Rf 0.38-0.40)		tranformation product_4 (Rf 0.48-0.58)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_2	101.4	98.51	99.9	0.00	0.0	0.00	0.0	0.00	0.0	1.49	1.5	0.00	0.0
3 d_2	102.8	88.53	91.0	11.47	11.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
7 d_2	97.0	73.20	71.0	26.84	26.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
10 d_2	87.1	75.69	65.9	18.37	16.0	5.94	5.2	0.00	0.0	0.00	0.0	0.00	0.0
14 d_2	94.4	56.40	53.2	43.60	41.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
21 d_2	80.0	36.78	29.4	63.22	50.6	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
28 d_2	84.4	28.59	24.1	61.33	51.8	0.00	0.0	0.00	0.0	0.00	0.0	10.08	8.5
42 d_2	81.8	14.09	11.5	85.91	70.3	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
56 d_2	83.2	7.53	6.3	92.47	76.9	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
72 d_2	79.3	2.87	2.3	97.13	77.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
90 d_2	80.2	0.00	0.0	100.00	80.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0

Replicate 3

Sample	Radioactivity in combined extracts	imidacloprid (Rf 0.53-0.67)		transformation product_1 (starting radioactivity; Rf 0.07-0.12)		tranformation product_2 (Rf 0.17-0.21)		Chloropiridin (Rf 0.25-0.26)		tranformation product_3 (Rf 0.38-0.40)		tranformation product_4 (Rf 0.48-0.58)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_3	100.4	97.19	97.6	0.00	0.0	0.00	0.0	0.00	0.0	2.81	2.8	0.00	0.0
3 d_3	103.4	88.04	91.1	11.96	12.4	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
7 d_3	98.7	73.45	72.5	26.55	26.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
10 d_3	86.6	68.43	59.2	25.52	22.1	6.05	5.2	0.00	0.0	0.00	0.0	0.00	0.0
14 d_3	92.3	59.76	55.1	40.24	37.1	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
21 d_3	77.9	41.59	32.4	58.41	45.5	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
28 d_3	82.3	29.47	24.2	65.59	54.0	0.00	0.0	0.00	0.0	0.00	0.0	4.93	4.1
42 d_3	82.1	17.16	14.1	82.84	68.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
56 d_3	74.7	5.39	4.0	94.61	70.6	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
72 d_3	81.9	4.64	3.8	90.65	74.2	0.00	0.0	1.72	1.4	0.00	0.0	2.99	2.4
90 d_3	83.0	0.00	0.0	100.00	83.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0

Replicate 4

Sample	Radioactivity in combined extracts	imidacloprid (Rf 0.53-0.67)		transformation product_1 (starting radioactivity; Rf 0.07-0.12)		tranformation product_2 (Rf 0.17-0.21)		Chloropiridin (Rf 0.25-0.26)		tranformation product_3 (Rf 0.38-0.40)		tranformation product_4 (Rf 0.48-0.58)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_4	100.3	98.26	98.6	0.00	0.0	0.00	0.0	0.00	0.0	1.74	1.7	0.00	0.0
3 d_4	102.5	88.70	90.9	11.30	11.6	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
7 d_4	100.1	74.93	75.0	25.07	25.1	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
10 d_4	87.2	72.75	63.5	21.18	18.5	6.07	5.3	0.00	0.0	0.00	0.0	0.00	0.0
14 d_4	93.7	60.04	56.3	39.96	37.4	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
21 d_4	85.5	36.14	30.9	63.68	54.5	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
28 d_4	82.4	27.60	22.7	68.33	56.3	0.00	0.0	0.00	0.0	0.00	0.0	4.08	3.4
42 d_4	82.3	15.26	12.6	84.74	69.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
56 d_4	81.3	6.13	5.0	93.87	76.3	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
72 d_4	77.7	3.26	2.5	94.89	73.7	0.00	0.0	1.86	1.4	0.00	0.0	0.00	0.0
90 d_4	80.9	0.00	0.0	97.78	79.1	0.00	0.0	0.00	0.0	0.00	0.0	2.22	1.8

Replicate 5

Sample	Radioactivity in combined extracts	imidacloprid (Rf 0.53-0.67)		transformation product_1 (starting radioactivity; Rf 0.07-0.12)		tranformation product_2 (Rf 0.17-0.21)		Chloropiridin (Rf 0.25-0.26)		tranformation product_3 (Rf 0.38-0.40)		tranformation product_4 (Rf 0.48-0.58)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_5	99.7	97.76	97.5	0.00	0.0	0.00	0.0	0.00	0.0	2.24	2.2	0.00	0.0
3 d_5	102.7	86.36	88.7	13.64	14.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
7 d_5	97.8	70.71	69.1	29.29	28.6	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
10 d_5	85.1	67.55	57.5	24.41	20.8	8.03	6.8	0.00	0.0	0.00	0.0	0.00	0.0
14 d_5	91.8	63.20	58.0	36.80	33.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
21 d_5	70.3	36.77	25.9	63.23	44.5	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
28 d_5	82.4	18.33	15.1	38.07	31.4	0.00	0.0	0.00	0.0	0.00	0.0	43.61	35.9
42 d_5	75.7	11.62	8.8	75.41	57.1	0.00	0.0	0.00	0.0	0.00	0.0	12.97	9.8
56 d_5	81.2	7.78	6.3	92.22	74.9	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
72 d_5	82.0	4.72	3.9	95.28	78.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
90 d_5	81.8	0.00	0.0	97.88	80.1	0.00	0.0	2.12	1.7	0.00	0.0	0.00	0.0

Replicate 6

Sample	Radioactivity in combined extracts	imidacloprid (Rf 0.53-0.67)		transformation product_1 (starting radioactivity; Rf 0.07-0.12)		tranformation product_2 (Rf 0.17-0.21)		Chloropiridin (Rf 0.25-0.26)		tranformation product_3 (Rf 0.38-0.40)		tranformation product_4 (Rf 0.49-0.58)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_6	98.9	98.32	97.2	0.00	0.0	0.00	0.0	0.00	0.0	1.68	1.7	0.00	0.0
3 d_6	102.5	85.18	87.3	14.82	15.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
7 d_6	98.5	73.07	72.0	26.93	26.5	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
10 d_6	84.8	66.33	56.3	25.29	21.5	8.37	7.1	0.00	0.0	0.00	0.0	0.00	0.0
14 d_6	91.3	55.21	50.4	44.79	40.9	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
21 d_6	72.1	37.28	26.9	62.72	45.2	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
28 d_6	83.3	29.05	24.2	70.95	59.1	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
42 d_6	69.0	0.00	0.0	18.72	12.9	0.00	0.0	0.00	0.0	0.00	0.0	81.28	56.1
56 d_6	81.0	7.39	6.0	92.61	75.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
72 d_6	82.5	4.04	3.3	94.76	78.2	0.00	0.0	1.20	1.0	0.00	0.0	0.00	0.0
90 d_6	83.1	0.00	0.0	100.00	83.1	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0

Sterile

Sample	Radioactivity in combined extracts	imidacloprid (Rf 0.53-0.67)	
	(% aR)	% peak-area	(% aR)
7 d_1	88.6	100	88.6
7 d_2	89.7	100	89.7
58 d_1	89.7	100	89.7
58 d_2	89.8	100	89.8
90 d_1	89.1	100	89.1
90 d_2	85.8	100	85.8

Institute 3

Physico-chemical parameters

Figure A3_13: Physico-chemical parameters measured throughout the ring test experiments by Institute 3

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 3
Manure sample: Cattle
Test substance: Imidacloprid

Excel 2003

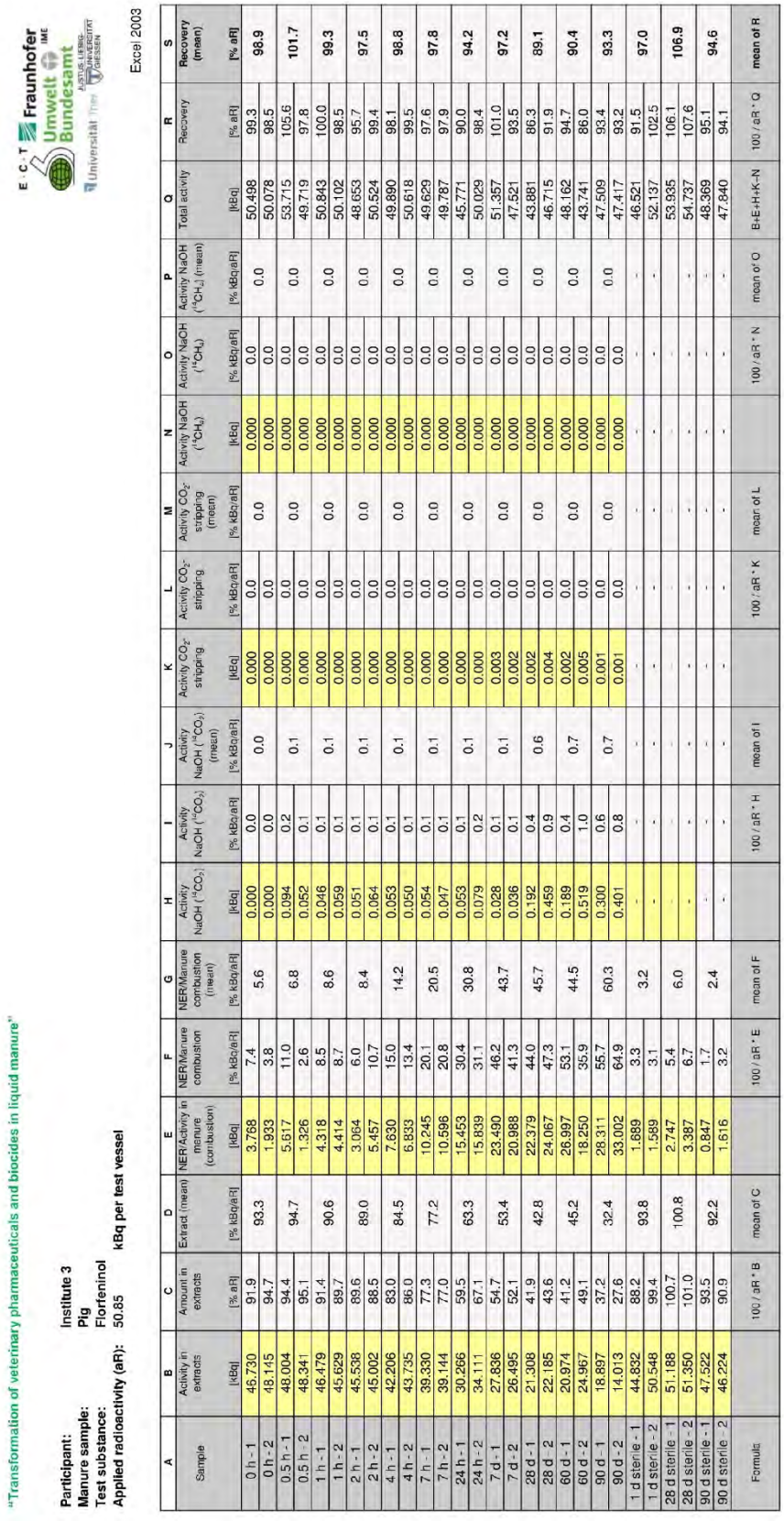
Time Point	Date	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) related to wet weight [mg/kg]	Nitrogen (N_{total}) related to wet weight [mg/kg]	Dry matter content [%]	Organic matter content (relating to wet weight) [%]	Other parameter(s)
Sampling	06.02.2014	7.4	-363.0	n.d.	n.d.	4000	11.7	n.d.	-
Start of acclimation	12.03.2014	7.4	<-200	20.0			9.1	n.d.	-
Day 0	02.04.2014	7.5	-394.0	20.0					-
Day 3	05.04.2014	n.d.	n.d.	n.d.					-
Day 7	09.04.2014	n.d.	n.d.	n.d.					-
Day 10	12.04.2014	n.d.	n.d.	n.d.					-
Day 14	16.04.2014	n.d.	n.d.	n.d.					-
Day 21	23.04.2014	n.d.	n.d.	n.d.					-
Day 28	20.04.2014	n.d.	n.d.	n.d.					-
Day 42	14.05.2014	8.0	-348.0	20.0					-
Day 56	28.05.2014	n.d.	n.d.	n.d.					-
Day 72	13.06.2014	n.d.	n.d.	n.d.					-
Day 90	01.07.2014	8.1	-369.0	20.0			7.8		-

Manure sample: Pig
Test substance: Florfenicol

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) related to wet weight [mg/kg]	Nitrogen (N_{total}) related to wet weight [mg/kg]	Dry matter content [%]	Organic matter content (relating to wet weight) [%]	Other parameter(s)
Sampling	09.09.2013	8.3	n.d.	n.d.	1600	3000	5.5	4.5	-
Start of acclimation	15.10.2013	n.d.	-420.0	21			5.0	n.d.	-
Day 0 - 0 h	07.11.2013	7.8	-373.0	20					-
Day 0 - 0.5 h	07.11.2013	n.d.	n.d.	n.d.					-
Day 0 - 1 h	07.11.2013	n.d.	n.d.	n.d.					-
Day 0 - 2 h	07.11.2013	n.d.	n.d.	n.d.					-
Day 0 - 4 h	07.11.2013	n.d.	n.d.	n.d.					-
Day 0 - 7 h	07.11.2013	n.d.	n.d.	n.d.					-
Day 1 - 24 h	08.11.2013	n.d.	n.d.	n.d.					-
Day 7	14.11.2013	n.d.	n.d.	n.d.					-
Day 28	16.12.2013	8.1	-251.0	20.0					-
Day 60	17.01.2014	n.d.	n.d.	n.d.					-
Day 90	05.02.2014	7.7	-328.0	21.0			4.5		-

Mass Balance

Figure A3_14: Mass balance determined for ^{14}C-florfenicol in pig manure by Institute 3



"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 3
Manure sample: Pig
Test substance: Florfenicol
Applied radioactivity (aR): 50.85 kBq per test vessel

Excel 2003

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Sample	Activity in extracts	Amount in extracts	Extract (mean)	NER/Activity in manure (combustion)	NER/Manure combustion	NER/Manure combustion (mean)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$) (mean)	Activity CO_2-stripping	Activity CO_2-stripping	Activity CO_2-stripping (mean)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$) (mean)	Total activity	Recovery	Recovery (mean)
	[kBq]	[% aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% aR]	[% aR]
0 h - 1	46.730	91.9	93.3	3.768	7.4	5.6	0.000	0.0	0.0	0.000	0.0	0.0	0.000	0.0	0.0	50.498	99.3	98.9
0 h - 2	48.145	94.7		1.993	3.8		0.000	0.0		0.000	0.0		0.000	0.0		50.078	98.5	
0.5 h - 1	48.004	94.4	94.7	5.617	11.0	6.8	0.094	0.2	0.1	0.000	0.0	0.0	0.000	0.0	0.0	53.715	105.6	101.7
0.5 h - 2	48.341	95.1		1.326	2.6		0.052	0.1		0.000	0.0		0.000	0.0		49.719	97.8	
1 h - 1	46.479	91.4	90.6	4.318	8.5	8.6	0.046	0.1	0.1	0.000	0.0	0.0	0.000	0.0	0.0	50.843	100.0	99.3
1 h - 2	45.629	89.7		4.414	8.7		0.059	0.1		0.000	0.0		0.000	0.0		50.102	98.5	
2 h - 1	45.538	89.6	89.0	3.064	6.0	8.4	0.051	0.1	0.1	0.000	0.0	0.0	0.000	0.0	0.0	48.653	95.7	97.5
2 h - 2	45.002	88.5		5.457	10.7		0.064	0.1		0.000	0.0		0.000	0.0		50.524	99.4	
4 h - 1	42.206	83.0	84.5	7.630	15.0	14.2	0.053	0.1	0.1	0.000	0.0	0.0	0.000	0.0	0.0	49.890	98.1	98.8
4 h - 2	43.735	86.0		6.833	13.4		0.050	0.1		0.000	0.0		0.000	0.0		50.618	99.5	
7 h - 1	39.330	77.3	77.2	10.245	20.1	20.5	0.054	0.1	0.1	0.000	0.0	0.0	0.000	0.0	0.0	49.629	97.6	97.8
7 h - 2	39.144	77.0		10.596	20.8		0.047	0.1		0.000	0.0		0.000	0.0		49.787	97.9	
24 h - 1	30.266	59.5	63.3	15.453	30.4	30.8	0.053	0.1	0.1	0.000	0.0	0.0	0.000	0.0	0.0	45.771	90.0	94.2
24 h - 2	34.111	67.1		15.839	31.1		0.079	0.2		0.000	0.0		0.000	0.0		50.029	98.4	
7 d - 1	27.836	54.7	53.4	23.490	46.2	43.7	0.028	0.1	0.1	0.003	0.0	0.0	0.000	0.0	0.0	51.357	101.0	97.2
7 d - 2	26.495	52.1		20.988	41.3		0.036	0.1		0.002	0.0		0.000	0.0		47.521	93.5	
28 d - 1	21.308	41.9	42.8	22.379	44.0	45.7	0.192	0.4	0.6	0.002	0.0	0.0	0.000	0.0	0.0	43.881	86.3	89.1
28 d - 2	22.185	43.6		24.067	47.3		0.459	0.9		0.004	0.0		0.000	0.0		46.715	91.9	
60 d - 1	20.974	41.2	45.2	26.997	53.1	44.5	0.189	0.4	0.7	0.002	0.0	0.0	0.000	0.0	0.0	48.162	94.7	90.4
60 d - 2	24.967	49.1		18.250	35.9		0.519	1.0		0.005	0.0		0.000	0.0		43.741	86.0	
90 d - 1	18.897	37.2	32.4	28.311	55.7	60.3	0.300	0.6	0.7	0.001	0.0	0.0	0.000	0.0	0.0	47.509	93.4	93.3
90 d - 2	14.013	27.6		33.002	64.9		0.401	0.8		0.001	0.0		0.000	0.0		47.417	93.2	
1 d sterile - 1	44.832	88.2	93.8	1.689	3.3	3.2	-	-	-	-	-	-	-	-	-	46.521	91.5	97.0
1 d sterile - 2	50.548	99.4		1.589	3.1		-	-		-	-		-	-	-	52.137	102.5	
28 d sterile - 1	51.188	100.7	100.8	2.747	5.4	6.0	-	-	-	-	-	-	-	-	-	53.935	106.1	106.9
28 d sterile - 2	51.350	101.0		3.387	6.7		-	-		-	-		-	-	-	54.737	107.6	
90 d sterile - 1	47.522	93.5	92.2	0.847	1.7	2.4	-	-	-	-	-	-	-	-	-	48.369	95.1	94.6
90 d sterile - 2	46.224	90.9		1.616	3.2		-	-		-	-		-	-	-	47.840	94.1	
Formula		100 / aR * B	mean of C		100 / aR * E	mean of F		100 / aR * H	mean of I		100 / aR * K	mean of L		100 / aR * N	mean of O	B+E+H+K+N	100 / aR * Q	mean of R

Figure A3_15: Mass balance determined for ^{14}C-imidacloprid in cattle manure by Institute 3

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 3
Manure sample: Cattle
Test substance: Imidacloprid
Applied radioactivity (aR): 54.29 kBq per test vessel

Excel 2003

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Sample	Activity in extracts	Amount in extracts	Extract (mean)	NER/Activity in manure (combustion)	NER/Manure combustion	NER/Manure combustion (mean)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$) (mean)	Activity CO_2-stripping	Activity CO_2-stripping	Activity CO_2-stripping (mean)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$) (mean)	Total activity	Recovery	Recovery (mean)
	[kBq]	[% aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% aR]	[% aR]
0 d - 1	53.6	98.8	97.2	2.0	3.6	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	55.583	102.4	**100.8**
0 d - 2	52.0	95.7		1.9	3.6		0.0	0.0		0.0	0.0		0.0	0.0		53.901	99.3	
3 d - 1	41.2	75.9	80.4	4.8	8.8	10.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	45.958	84.7	**90.6**
3 d - 2	46.1	84.9		6.3	11.7		0.0	0.0		0.0	0.0		0.0	0.0		52.401	96.5	
7 d - 1	45.1	83.0	76.8	8.9	16.4	15.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.6	53.971	99.4	**108.8**
7 d - 2	38.3	70.6		7.9	14.5		0.0	0.0		0.0	0.0		18.0	33.2		64.198	118.3	
10 d - 1	45.2	83.3	81.3	10.8	20.0	19.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	56.086	103.3	**101.1**
10 d - 2	43.1	79.4		10.6	19.5		0.0	0.0		0.0	0.0		0.0	0.0		53.668	98.9	
14 d - 1	42.0	77.3	79.5	13.4	24.6	24.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	55.323	101.9	**104.0**
14 d - 2	44.4	81.8		13.2	24.3		0.0	0.0		0.0	0.0		0.0	0.0		57.574	106.1	
21 d - 1	37.8	69.7	69.0	15.4	28.3	28.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	53.180	98.0	**97.7**
21 d - 2	37.1	68.3		15.8	29.2		0.0	0.0		0.0	0.0		0.0	0.0		52.882	97.4	
28 d - 1	36.3	66.9	68.2	17.6	32.4	31.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	53.923	99.3	**100.1**
28 d - 2	37.7	69.4		17.1	31.5		0.0	0.0		0.0	0.0		0.0	0.0		54.760	100.9	
42 d - 1	30.8	56.7	58.6	19.6	36.1	39.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.393	92.8	**98.0**
42 d - 2	32.8	60.5		23.2	42.7		0.0	0.0		0.0	0.0		0.0	0.0		55.994	103.1	
56 d - 1	30.2	55.6	55.0	25.5	47.0	51.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	55.693	102.6	**106.0**
56 d - 2	29.5	54.3		29.9	55.0		0.0	0.0		0.0	0.0		0.0	0.0		59.353	109.3	
72 d - 1	28.4	52.4	50.2	27.6	50.8	51.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	56.006	103.2	**101.6**
72 d - 2	26.0	48.0		28.3	52.1		0.0	0.0		0.0	0.0		0.0	0.0		54.341	100.1	
90 d - 1	21.2	39.0	44.2	32.4	59.7	57.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	53.601	98.7	**102.0**
90 d - 2	26.8	49.3		30.4	55.9		0.0	0.0		0.0	0.0		0.0	0.0		57.106	105.2	
7 d sterile - 1	49.002	90.3	89.9	1.4	2.6	2.9	-	-	-	-	-	-	-	-	-	50.425	92.9	**92.8**
7 d sterile - 2	48.596	89.5		1.8	3.2		-	-	-	-	-	-	-	-	-	50.354	92.8	
56 d sterile - 1	51.625	95.1	94.4	4.1	7.6	7.4	-	-	-	-	-	-	-	-	-	55.738	102.7	**101.9**
56 d sterile - 2	50.894	93.8		4.0	7.3		-	-	-	-	-	-	-	-	-	54.865	101.1	
90 d sterile - 1	48.277	88.9	88.6	4.1	7.6	7.7	-	-	-	-	-	-	-	-	-	52.389	96.5	**96.2**
90 d sterile - 2	47.866	88.2		4.2	7.8		-	-	-	-	-	-	-	-	-	52.082	95.9	
Formula		100 / aR * B	mean of C		100 / aR * E	mean of F		100 / aR * H	mean of I		100 / aR * K	mean of L		100 / aR * N	mean of O	B+E+H+K+N	100 / aR * Q	**mean of R**

Extraction methods

Figure A3_16: Extraction methods used within the ring test by Institute 3

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Extraction Methods

Please add as much information as possible

Excel 2003

Participant:	**Institute 3**	
Manure sample:	**Cattle**	**Pig**
Test substance	**Imidacloprid**	**Florfenicol**
Extraction Method:	Extraction of manure samples using acetonitrile: 1. An aliquot of about 10 g of each sample was diluted with 15 mL acetonitrile 2. 30 min vigorous shaking on a reciprocal (=overhead) shaker 3. 10 centrifugation (at 3500 rpm, 4°C) 4. Decantation of the supernatant in a measuring cylinder 5. Steps 1 – 4 were repeated twice using 10 mL acetonitrile 6. The supernatants were combined and the final volume was recorded	Extraction of manure samples using acetonitrile and 0.02 M buffer solution: 1. An aliquot of about 10 g of each sample was diluted with 10 ml acetonitrile/0,02 M KH_2PO_4 (50/50 v/v) 2. 10 min intensively mixed on a vortexer 3. 10 min centrifugation (at 3500 rpm, 4°C) 4. Decantation of the supernatant 5. Steps 1 – 4 were repeated twice using 2.5 mL acetonitrile/0,02 M KH_2PO_4 (50/50 v/v) 6. The supernatants were combined and filled up to 25 mL in a volumetric flask using acetonitrile

Chemical Analysis

Figure A3_17: Results of chemical analysis for ^{14}C-florfenicol in pig manure by Institute 3

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 3
Manure sample: Pig
Test substance: Florfenicol

Excel 2003

Replicate 1

Sample	Radioactivity in combined extracts	florfenicol		MCF		tranformation product_1 (t: 1.6 min)		tranformation product_2 (t:)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
Day 0 - 0 h_1	91.9	100.0	91.9	0.0	0.0	0.0	0.0	0.0	0.0
Day 0 - 0.5 h_1	94.4	100.0	94.4	0.0	0.0	0.0	0.0	0.0	0.0
Day 0 - 1 h_1	91.4	82.6	75.5	0.0	0.0	17.4	15.9	0.0	0.0
Day 0 - 2 h_1	89.6	66.4	59.5	15.1	13.5	18.5	16.6	0.0	0.0
Day 0 - 4 h_1	83.0	44.0	36.5	37.3	31.0	18.7	15.5	0.0	0.0
Day 0 - 7 h_1	77.3	22.5	17.4	42.0	32.5	35.5	27.5	0.0	0.0
Day 1 - 24 h_1	59.5	0.0	0.0	49.3	29.3	50.8	30.2	0.0	0.0
Day 7_1	54.7	0.0	0.0	0.0	0.0	100.0	54.7	0.0	0.0
Day 28_1	41.9	0.0	0.0	0.0	0.0	100.0	41.9	0.0	0.0
Day 60_1	41.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Day 90_1	37.2	0.0	0.0	0.0	0.0	100.0	37.2	0.0	0.0

Replicate 2

Sample	Radioactivity in combined extracts	florfenicol		MCF		tranformation product_1 (t: 1.6 min)		tranformation product_2 (t: 4.5 min)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
Day 0 - 0 h_2	94.7	100.0	94.7	0.0	0.0	0.0	0.0	0.0	0.0
Day 0 - 0.5 h_2	95.1	100.0	95.1	0.0	0.0	0.0	0.0	0.0	0.0
Day 0 - 1 h_2	89.7	75.0	67.3	17.6	15.8	7.4	6.6	0.0	0.0
Day 0 - 2 h_2	88.5	50.0	44.3	19.6	17.3	30.4	26.9	0.0	0.0
Day 0 - 4 h_2	86.0	47.0	40.4	42.5	36.6	10.2	8.8	0.0	0.0
Day 0 - 7 h_2	77.0	25.3	19.5	46.8	36.0	27.9	21.5	0.0	0.0
Day 1 - 24 h_2	67.1	0.0	0.0	39.8	26.7	60.2	40.4	0.0	0.0
Day 7_2	52.1	0.0	0.0	0.0	0.0	100.0	52.1	0.0	0.0
Day 28_2	43.6	0.0	0.0	0.0	0.0	100.0	43.6	0.0	0.0
Day 60_2	49.1	0.0	0.0	10.8	5.3	62.7	30.8	26.5	13.0
Day 90_2	27.6	0.0	0.0	0.0	0.0	100.0	27.6	0.0	0.0

% aR = % of applied radioactivity

Figure A3_18: Results of chemical analysis for ^{14}C-imidacloprid in cattle manure by Institute 3

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 3
Manure sample: Cattle
Test substance: Imidacloprid

Replicate 1

Excel 2003

Sample	Radioactivity in combined extracts	imidacloprid		transformation product_1 (please characterize by retention time 5.7 min)		tranformation product_2 (please characterize by retention time 2.1 min)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_1	98.8	100.0	98.8	0.0	0.0	0.0	0.0
3 d_1	75.9	100.0	75.9	0.0	0.0	0.0	0.0
7 d_1	83.0	100.0	83.0	0.0	0.0	0.0	0.0
10 d_1	83.3	62.0	51.6	29.0	24.2	9.0	7.5
14 d_1	77.3	66.0	51.0	22.0	17.0	12.0	9.3
21 d_1	69.7	55.0	38.3	31.0	21.6	14.0	9.8
28 d_1	66.9	40.0	26.8	40.0	26.8	21.0	14.0
42 d_1	56.7	33.0	18.7	51.0	28.9	16.0	9.1
56 d_1	55.6	0.0	0.0	73.0	40.6	27.0	15.0
72 d_1	52.4	0.0	0.0	75.0	39.3	25.0	13.1
90 d_1	39.0	0.0	0.0	66.0	25.7	34.0	13.3

Replicate 2

Sample	Radioactivity in combined extracts	imidacloprid		transformation product_1 (please characterize by retention time 5.7 min)		tranformation product_2 (please characterize by retention time 2.1 min)	
	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)	% peak-area	(% aR)
0 d_2	95.7	100.0	95.7	0.0	0.0	0.0	0.0
3 d_2	84.9	100.0	84.9	0.0	0.0	0.0	0.0
7 d_2	70.6	100.0	70.6	0.0	0.0	0.0	0.0
10 d_2	79.4	74.0	58.8	26.0	20.6	0.0	0.0
14 d_2	81.8	64.0	52.4	26.0	21.3	10.0	8.2
21 d_2	68.3	55.0	37.6	25.0	17.1	20.0	13.7
28 d_2	69.4	43.0	29.8	40.0	27.8	17.0	11.8
42 d_2	60.5	0.0	0.0	66.0	39.9	34.0	20.6
56 d_2	54.3	0.0	0.0	70.0	38.0	20.0	10.9
72 d_2	48.0	0.0	0.0	100.0	48.0	0.0	0.0
90 d_2	49.3	0.0	0.0	66.0	32.5	34.0	16.8

% aR = % of applied radioactivity

Institute 4

Physico-chemical parameters

Figure A3_19: Physico-chemical parameters measured throughout the ring test experiments by Institute 4

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

E·C·T Fraunhofer Umwelt IME Bundesamt Universität Trier JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Excel 2003

Participant: Institute 4
Manure sample: Cattle
Test substance: Imidacloprid

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) [mg/kg]	Nitrogen (N_{total}) [mg/kg]	Dry matter content [%]	Organic carbon content (relating to dry weight) [%]	Other parameter(s)
Sampling	27.01.2014	7.67	-227.0	15.2	1670	3720	10.4	3.90	-
Start of acclimation	10.02.2014	7.53	-304.0	19.7			10.2	2.83	-
Day 0	14.03.2014	6.30	-230.0	20.8					-
Day 3	17.03.2014	6.19	-165.2	20.4					-
Day 7	19.03.2014	6.18	-191.8	20.7					-
Day 10	21.03.2014	6.32	-142.7	21.3					-
Day 14	26.03.2014	6.11	-161.3	19.7					-
Day 22	03.04.2014	6.41	-162.3	21.1					-
Day 29	10.04.2014	6.56	-161.7	20.9					-
Day 42	23.04.2014	6.49	-143.2	20.7					-
Day 56	07.05.2014	7.58	-130.0	20.8					-
Day 70	22.05.2014	7.44	-114.2	20.4					-
Day 91	11.06.2014	7.49	-353.7	20.9			9.0		-

If you measure parameters that are not covered by the sheet, then please add columns as needed.

Manure sample: Pig
Test substance: Florfenicol

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) [mg/kg]	Nitrogen (N_{total}) [mg/kg]	Dry matter content [%]	Organic carbon content (relating to dry weight) [%]	Other parameter(s)
Sampling	27.01.2014	7.71	-408.3	11.7	5150	7120	6.2	2.95	-
Start of acclimation	25.02.2014	8.25	-448.2	18.4			5.7	2.61	-
Day 0 - 0 h	01.04.2014	8.24	-449.5	20.7					-
Day 0 - 0.5 h	n.d.	n.d.	n.d.	n.d.					-
Day 0 - 1 h	n.d.	n.d.	n.d.	n.d.					-
Day 0 - 2 h	n.d.	n.d.	n.d.	n.d.					-
Day 0 - 4 h	01.04.2014	8.26	-447.6	20.7					-
Day 0 - 7 h	n.d.	n.d.	n.d.	n.d.					-
Day 1 - 24 h	02.04.2014	8.34	-450.2	20.7					-
Day 7	09.04.2014	8.41	-328.9	21.2					-
Day 28	29.04.2014	8.44	-328.2	20.7					-
Day 56	27.05.2014	8,48	-266.2	20.9					-
Day 90	14.06.2014	8.67	-307.0	20.6			5.3		-

Mass Balance

Figure A3_20: Mass balance determined for ^{14}C-florfenicol in pig manure by Institute 4

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 4
Manure sample: Pig
Test substance: Florfeninol
Applied radioactivity (aR): 100.00 kBq per test vessel

Excel 2003

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Sample	Activity in extracts	Amount in extracts	Extract (mean)	NER/Activity in manure (combustion)	NER/Manure combustion	NER/Manure combustion (mean)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$) (mean)	Activity CO_2-stripping	Activity CO_2-stripping	Activity CO_2-stripping (mean)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$) (mean)	Total activity	Recovery	Recovery (mean)
	[kBq]	[% aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% aR]	[% aR]
0 h - 1	69.32	69.3	62.3	23.60	23.6	24.4		0.0	0.0		0.0	0.0		0.0	0.0	92.923	92.9	86.6
0 h - 2	67.80	67.8		25.48	25.5			0.0			0.0			0.0		93.286	93.3	
0 h - 3	49.63	49.6		24.10	24.1			0.0			0.0			0.0		73.732	73.7	
0.5 h - 1	36.08	36.1	32.8	47.20	47.2	47.9		0.0	0.0		0.0	0.0		0.0	0.0	83.279	83.3	80.6
0.5 h - 2	39.80	39.8		50.24	50.2			0.0			0.0			0.0		90.031	90.0	
0.5 h - 3	22.49	22.5		46.14	46.1			0.0			0.0			0.0		68.632	68.6	
1 h - 1	29.92	29.9	23.4	55.75	55.7	54.9		0.0	0.0		0.0	0.0		0.0	0.0	85.673	85.7	78.3
1 h - 2	17.99	18.0		55.88	55.9			0.0			0.0			0.0		73.874	73.9	
1 h - 3	22.37	22.4		52.98	53.0			0.0			0.0			0.0		75.349	75.3	
2 h - 1	20.20	20.2	20.9	64.91	64.9	61.5		0.0	0.0		0.0	0.0		0.0	0.0	85.108	85.1	82.4
2 h - 2	20.89	20.9		59.75	59.8			0.0			0.0			0.0		80.643	80.6	
2 h - 3	21.64	21.6		59.71	59.7			0.0			0.0			0.0		81.357	81.4	
4 h - 1	14.61	14.6	14.6	88.53	88.5	77.0		0.0	0.0		0.0	0.0		0.0	0.0	103.142	103.1	91.6
4 h - 2	9.88	9.9		72.36	72.4			0.0			0.0			0.0		82.237	82.2	
4 h - 3	19.34	19.3		70.14	70.1			0.0			0.0			0.0		89.477	89.5	
7 h - 1	8.60	8.6	11.5	68.88	68.9	65.5		0.0	0.0		0.0	0.0		0.0	0.0	77.473	77.5	76.9
7 h - 2	11.40	11.4		65.31	65.3			0.0			0.0			0.0		76.707	76.7	
7 h - 3	14.43	14.4		62.18	62.2			0.0			0.0			0.0		76.612	76.6	
24 h - 1	3.99	4.0	4.0	62.91	62.9	64.2		0.0	0.0		0.0	0.0		0.0	0.0	66.905	66.9	68.2
24 h - 2	3.97	4.0		67.12	67.1			0.0			0.0			0.0		71.084	71.1	
24 h - 3	4.19	4.2		62.51	62.5			0.0			0.0			0.0		66.701	66.7	
7 d - 1	7.98	8.0	6.3	20.31	20.3	18.9	0.07	0.1	0.0	0.48	0.5	0.5	0.00	0.0	0.0	28.848	28.8	25.7
7 d - 2	4.98	5.0		18.71	18.7		0.01	0.0		0.70	0.7		0.00	0.0		24.405	24.4	
7 d - 3	5.86	5.9		17.65	17.6		0.03	0.0		0.39	0.4		0.00	0.0		23.928	23.9	
28 d - 1	7.46	7.5	6.3	16.47	16.5	15.8	0.11	0.1	0.1	0.68	0.7	0.8	0.00	0.0	0.0	24.716	24.7	23.0
28 d - 2	4.47	4.5		13.55	13.6		0.13	0.1		0.93	0.9		0.00	0.0		19.092	19.1	
28 d - 3	6.92	6.9		17.46	17.5		0.11	0.1		0.68	0.7		0.00	0.0		25.172	25.2	
56 d - 1	2.59	2.6	2.9	19.51	19.5	16.8	0.16	0.2	0.2	1.40	1.4	1.5	0.00	0.0	0.0	23.659	23.7	21.4
56 d - 2	3.51	3.5		15.05	15.0		0.20	0.2		1.56	1.6		0.00	0.0		20.318	20.3	
56 d - 3	2.63	2.6		15.92	15.9		0.20	0.2		1.40	1.4		0.00	0.0		20.144	20.1	
91 d - 1	2.57	2.6	3.1	8.51	8.5	9.7	0.94	0.9	0.7	6.17	6.2	5.3	0.00	0.0	0.0	18.185	18.2	18.7
91 d - 2	3.62	3.6		9.22	9.2		0.50	0.5		4.46	4.5		0.00	0.0		17.795	17.8	
91 d - 3	3.02	3.0		11.42	11.4		0.65	0.6		5.14	5.1		0.00	0.0		20.235	20.2	
1 d sterile - 1	60.53	60.5	53.4	16.48	16.5	19.5	-	-	-	-	-	-	-	-	-	77.012	77.0	73.0
1 d sterile - 2	46.35	46.4		22.60	22.6		-	-	-	-	-	-	-	-	-	68.957	69.0	
28 d sterile - 1	58.89	58.9	63.2	35.06	35.1	39.6	-	-	-	-	-	-	-	-	-	93.955	94.0	102.8
28 d sterile - 2	67.52	67.5		44.17	44.2		-	-	-	-	-	-	-	-	-	111.691	111.7	
91 d sterile - 1	36.28	36.3	35.5	9.17	9.2	6.5	-	-	-	-	-	-	-	-	-	45.444	45.4	42.0
91 d sterile - 2	34.82	34.8		3.76	3.8		-	-	-	-	-	-	-	-	-	38.579	38.6	
Formula		100 / aR * B	mean of C		100 / aR * E	mean of F		100 / aR * H	mean of I		100 / aR * K	mean of L		100 / aR * N	mean of O	B+E+H+K+N	100 / aR * Q	mean of R

Figure A3_21: Mass balance determined for ^{14}C-imidacloprid in cattle manure by Institute 4

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 4
Manure sample: Cattle
Test substance: Imidacloprid
Applied radioactivity (aR): 83.00 kBq per test vessel

Excel 2003

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Sample	Activity in extracts	Amount in extracts	Extract (mean)	NER/Activity in manure (combustion)	NER/Manure combustion	NER/Manure combustion (mean)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$)	Activity NaOH ($^{14}CO_2$) (mean)	Activity CO_2-stripping	Activity CO_2-stripping	Activity CO_2-stripping (mean)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$) (mean)	Total activity	Recovery	Recovery (mean)
	[kBq]	[% aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% aR]	[% aR]
0 d - 1	51.02	61.5	70.9	0.000	0.0	15.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	51.016	61.5	81.5
0 d - 2	66.54	80.2		12.774	15.4		0.0	0.0		0.0	0.0		0.0	0.0		79.311	95.6	
0 d - 3	59.09	71.2		13.509	16.3		0.0	0.0		0.0	0.0		0.0	0.0		72.600	87.5	
2 d - 1	67.50	81.3	78.3	15.149	18.3	18.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	82.652	99.6	97.0
2 d - 2	65.00	78.3		14.926	18.0		0.0	0.0		0.0	0.0		0.0	0.0		79.927	96.3	
2 d - 3	62.46	75.3		16.550	19.9		0.0	0.0		0.0	0.0		0.0	0.0		79.011	95.2	
6 d - 1	59.30	71.4	71.1	14.800	17.8	20.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	74.096	89.3	91.2
6 d - 2	60.33	72.7		16.802	20.2		0.0	0.0		0.0	0.0		0.0	0.0		77.137	92.9	
6 d - 3	57.28	69.0		18.465	22.2		0.0	0.0		0.0	0.0		0.0	0.0		75.749	91.3	
9 d - 1	60.50	72.9	74.1	19.748	23.8	24.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	80.251	96.7	99.0
9 d - 2	63.85	76.9		20.970	25.3		0.0	0.0		0.0	0.0		0.0	0.0		84.825	102.2	
9 d - 3	60.24	72.6		21.209	25.6		0.0	0.0		0.0	0.0		0.0	0.0		81.452	98.1	
14 d - 1	52.56	63.3	65.5	22.259	26.8	27.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	74.821	90.1	92.9
14 d - 2	55.43	66.8		23.785	28.7		0.0	0.0		0.0	0.0		0.0	0.0		79.216	95.4	
14 d - 3	55.12	66.4		22.168	26.7		0.0	0.0		0.0	0.0		0.0	0.0		77.292	93.1	
22 d - 1	45.20	54.5	53.2	25.593	30.8	29.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	70.789	85.3	82.9
22 d - 2	40.96	49.4		25.058	30.2		0.0	0.0		0.0	0.0		0.0	0.0		66.022	79.5	
22 d - 3	46.34	55.8		23.319	28.1		0.0	0.0		0.0	0.0		0.0	0.0		69.662	83.9	
29 d - 1	54.25	65.4	64.6	25.321	30.5	29.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	79.570	95.9	93.6
29 d - 2	52.87	63.7		23.105	27.8		0.0	0.0		0.0	0.0		0.0	0.0		75.977	91.5	
29 d - 3	53.61	64.6		23.893	28.8		0.0	0.0		0.0	0.0		0.0	0.0		77.504	93.4	
42 d - 1	37.63	45.3	48.6	31.934	38.5	40.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	69.566	83.8	88.8
42 d - 2	42.56	51.3		30.185	36.4		0.0	0.0		0.0	0.0		0.0	0.0		72.748	87.6	
42 d - 3	40.91	49.3		37.981	45.8		0.0	0.0		0.0	0.0		0.0	0.0		78.887	95.0	
56 d - 1	32.30	38.9	38.9	38.726	46.7	47.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	71.028	85.6	86.5
56 d - 2	36.58	44.1		36.618	44.1		0.0	0.0		0.0	0.0		0.0	0.0		73.195	88.2	
56 d - 3	27.96	33.7		43.112	51.9		0.0	0.0		0.0	0.0		0.0	0.0		71.067	85.6	
70 d - 1	34.54	41.6	40.1	36.099	43.5	46.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	70.643	85.1	86.3
70 d - 2	33.61	40.5		37.586	45.3		0.0	0.0		0.0	0.0		0.0	0.0		71.195	85.8	
70 d - 3	31.76	38.3		41.168	49.6		0.0	0.0		0.0	0.0		0.0	0.0		72.930	87.9	
91 d - 1	20.49	24.7	22.9	48.091	57.9	56.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	68.578	82.6	79.5
91 d - 2	19.07	23.0		44.437	53.5		0.0	0.0		0.0	0.0		0.0	0.0		63.505	76.5	
91 d - 3	17.42	21.0		48.497	58.4		0.0	0.0		0.0	0.0		0.0	0.0		65.914	79.4	
6 d sterile - 1	63.60	76.6	84.8	12.051	14.5	14.6	-	-	-	-	-	-	-	-	-	75.651	91.1	99.3
6 d sterile - 2	77.08	92.9		12.180	14.7		-	-	-	-	-	-	-	-	-	89.264	107.5	
56 d sterile - 1	74.82	90.1	84.3	16.143	19.4	18.5	-	-	-	-	-	-	-	-	-	90.967	109.6	102.8
56 d sterile - 2	65.08	78.4		14.630	17.6		-	-	-	-	-	-	-	-	-	79.713	96.0	
90 d sterile - 1	63.88	77.0	79.0	17.971	21.7	21.5	-	-	-	-	-	-	-	-	-	81.855	98.6	100.5
90 d sterile - 2	67.23	81.0		17.752	21.4		-	-	-	-	-	-	-	-	-	84.978	102.4	
Formula		100 / aR * B	mean of C		100 / aR * E	mean of F		100 / aR * H	mean of I		100 / aR * K	mean of L		100 / aR * N	mean of O	B+E+H+K+N	100 / aR * Q	mean of R

Extraction methods

Figure A3_22: Extraction methods used within the ring test by Institute 4

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Extraction Methods

Please add as much information as possible

Excel 2003

Participant: **Institute 4**

Manure sample: **Pig**

Test substance **Florfenicol**

Extraction Method:

25 g manuresample
- Extraction with 20 mL methylene chloride by shaking for 30 min on overhead shaker and centrifugation at 3000 rpm for 5 min
- Methylene chloride phase withdrawn with a syringe
- Extraction of pellet repeated 2 times with15 mL methylene chloride and reduced time on overhead shaker (15 min)

Combined methylene chloride extracts evaporated to dryness and transferred with 2 x 2.5 mL Acetonitrile/Water 50/50 in a volumetric flask
- Filtered samples measured by HPLC-FSA and LSC
- Dried pellet combusted and NER determined via LSC

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Extraction Methods

Please add as much information as possible

Excel 2003

Participant: **Institute 4**

Manure sample: **Cattle**

Test substance **Imidacloprid**

Extraction Method:

1st extraction step
25 g manuresample
- Extraction with 20 mL diethylether by shaking for 30 min on overhead shaker and centrifugation at 3000 rpm for 5 min
- Ether phase separated
- Extraction of pellet repeated 2 times (reduced time on overhead shaker (15 min))

2nd extraction step
Pellet of extraction step 1
- Extraction with 2 mL NaOH (10 mol/L) and 15 mL methylene chloride by shakingfor 30 min on overhead shaker and centrifugation at 3000 rpm for 5 min
- Methylene chloride phase withdrawn with a syringe and given to the ether phase of extraction step 1
- Extraction of pellet repeated 2 times (reduced time on overhead shaker(15 min))

Combined extracts evaporated to dryness and transferred with 2 x 2.5 mL Acetonitrile/Water 50/50 in a volumetric flask
- Filtered samples measured by HPLC-FSA and LSC
- Dried pellet combusted and NER determined via LSC

Chemical Analysis

Figure A3_23: Results of chemical analysis for ^{14}C-florfenicol in pig manure by Institute 4

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

E·C·T Fraunhofer Umwelt IME Bundesamt Universität Trier JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Participant: Institute 4
Manure sample: Pig
Test substance: Florfenicol

Replicate 1 Excel 2003

Sample	Radioactivity in combined extracts		florfenicol (retention time 20,93)			tranformation product_1 (retention time 15,93)		
	kBq	(% aR)	kBq	(% aR Extract)	(%aR)	kBq	(% aR Extract)	(% aR)
Day 0 - 0 h_1	69.32	69.32	56.98	82.2	57.0	5.07	7.3	5.1
Day 0 - 0.5 h_1	36.08	36.08	6.97	19.3	7.0	24.27	67.3	24.3
Day 0 - 1 h_1	29.92	29.92	1.93	6.4	1.9	17.13	57.3	17.1
Day 0 - 2 h_1	20.20	20.20	0.59	2.9	0.6	14.22	70.4	14.2
Day 0 - 4 h_1	14.61	14.61	0.00	0.0	0.0	5.07	34.7	5.1
Day 0 - 7 h_1	8.60	8.60	0.00	0.0	0.0	3.30	38.4	3.3
Day 1 - 24 h_1	3.99	3.99	0.00	0.0	0.0	0.00	0.0	0.0
Day 7_1	7.98	7.98	0.00	0.0	0.0	0.00	0.0	0.0
Day 28_1	7.46	7.46	0.00	0.0	0.0	0.00	0.0	0.0
Day 56_1	2.59	2.59	0.00	0.0	0.0	0.00	0.0	0.0
Day 91_1	2.57	2.57	0.00	0.0	0.0	0.00	0.0	0.0

Replicate 2

Sample	Radioactivity in combined extracts		florfenicol (retention time 20,93)			tranformation product_1 (retention time 15,93)		
	kBq	(% aR)	kBq	(% aR Extract)	(%aR)	kBq	(% aR Extract)	(% aR)
Day 0 - 0 h_2	67.80	67.80	62.34	91.9	62.3	4.00	5.9	4.0
Day 0 - 0.5 h_2	39.80	39.80	7.67	19.3	7.7	26.79	67.3	26.8
Day 0 - 1 h_2	17.99	17.99	1.53	8.5	1.5	14.75	82.0	14.8
Day 0 - 2 h_2	20.89	20.89	0.00	0.0	0.0	16.98	81.3	17.0
Day 0 - 4 h_2	9.88	9.88	0.00	0.0	0.0	5.43	55.0	5.4
Day 0 - 7 h_2	11.40	11.40	0.00	0.0	0.0	6.57	57.6	6.6
Day 1 - 24 h_2	3.97	3.97	0.00	0.0	0.0	0.00	0.0	0.0
Day 7_2	4.98	4.98	0.00	0.0	0.0	0.00	0.0	0.0
Day 28_2	4.47	4.47	0.00	0.0	0.0	0.00	0.0	0.0
Day 56_2	3.51	3.51	0.00	0.0	0.0	0.00	0.0	0.0
Day 91_2	9.22	9.22	0.00	0.0	0.0	0.00	0.0	0.0

Replicate 3

Sample	Radioactivity in combined extracts		florfenicol (retention time 20,93)			tranformation product_1 (retention time 15,93)		
	kBq	(% aR)	kBq	(% aR Extract)	(%aR)	kBq	(% aR Extract)	(% aR)
Day 0 - 0 h_3	49.63	49.63	48.20	97.1	48.2	3.65	7.4	3.6
Day 0 - 0.5 h_3	22.49	22.49	4.75	21.1	4.8	13.90	61.8	13.9
Day 0 - 1 h_3	22.37	22.37	3.00	13.4	3.0	12.08	54.0	12.1
Day 0 - 2 h_3	21.64	21.64	0.00	0.0	0.0	15.49	71.6	15.5
Day 0 - 4 h_3	19.34	19.34	0.00	0.0	0.0	13.80	71.4	13.8
Day 0 - 7 h_3	14.43	14.43	0.00	0.0	0.0	10.44	72.3	10.4
Day 1 - 24 h_3	4.19	4.19	0.00	0.0	0.0	0.00	0.0	0.0
Day 7_3	5.86	5.86	0.00	0.0	0.0	0.00	0.0	0.0
Day 28_3	6.92	6.92	0.00	0.0	0.0	0.00	0.0	0.0
Day 56_3	2.63	2.63	0.00	0.0	0.0	0.00	0.0	0.0
Day 91_3	3.02	3.02	0.00	0.0	0.0	0.00	0.0	0.0

% aR = % of applied radioactivity

Figure A3_24: Results of chemical analysis for ^{14}C-imidacloprid in cattle manure by Institute 4

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

E·C·T Fraunhofer Umwelt IME Bundesamt Universität Trier JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Participant: Institute 4
Manure sample: Cattle
Test substance: Imidacloprid

Replicate 1 Excel 2003

Sample	Radioactivity in combined extracts		imidacloprid (retention time 21,60)			tranformation product_1 (retention time 13,20)		
	(kBq)	(% aR)	kBq	(% aR Extract)	(% aR)	kBq	(% aR Extract)	(% aR)
0 d_1	51.02	61.47	49.15	96.3	59.2	0.00	0.0	0.0
2 d_1	67.50	81.33	47.96	71.0	57.8	0.00	0.0	0.0
6 d_1	59.30	71.44	49.74	83.9	59.9	0.00	0.0	0.0
9 d_1	60.50	72.90	47.84	79.1	57.6	1.32	2.2	1.6
14 d_1	52.56	63.33	46.15	87.8	55.6	2.75	5.2	3.3
22 d_1	45.20	54.45	34.38	76.1	41.4	2.80	6.2	3.4
29 d_1	54.25	65.36	36.25	66.8	43.7	4.76	8.8	5.7
42 d_1	37.63	45.34	24.62	65.4	29.7	3.13	8.3	3.8
56 d_1	32.30	38.92	19.33	59.8	23.3	4.92	15.2	5.9
70 d_1	34.54	41.62	17.38	50.3	20.9	4.30	12.4	5.2
91 d_1	20.49	24.68	9.55	46.6	11.5	1.55	7.6	1.9

Replicate 2

Sample	Radioactivity in combined extracts		imidacloprid (retention time 21,60)			tranformation product_1 (retention time 13,20)		
	(kBq)	(% aR)	kBq	(% aR Extract)	(% aR)	kBq	(% aR Extract)	(% aR)
0 d_2	66.54	80.17	62.72	94.3	75.6	0.00	0.0	0.0
2 d_2	65.00	78.31	45.48	70.0	54.8	0.00	0.0	0.0
6 d_2	60.33	72.69	52.30	86.7	63.0	0.00	0.0	0.0
9 d_2	63.85	76.93	48.73	76.3	58.7	2.39	3.7	2.9
14 d_2	55.43	66.78	44.79	80.8	54.0	3.03	5.5	3.7
22 d_2	40.96	49.35	32.56	79.5	39.2	1.72	4.2	2.1
29 d_2	52.87	63.70	34.25	64.8	41.3	3.93	7.4	4.7
42 d_2	42.56	51.28	24.32	57.1	29.3	3.96	9.3	4.8
56 d_2	36.58	44.07	21.45	58.6	25.8	4.55	12.4	5.5
70 d_2	33.61	40.49	17.25	51.3	20.8	4.17	12.4	5.0
91 d_2	19.07	22.97	7.50	39.3	9.0	2.54	13.3	3.1

Replicate 3

Sample	Radioactivity in combined extracts		imidacloprid (retention time 21,60)			tranformation product_1 (retention time 13,20)		
	(kBq)	(% aR)	kBq	(% aR Extract)	(% aR)	kBq	(% aR Extract)	(% aR)
0 d_3	59.09	71.19	51.60	87.3	62.2	0.00	0.0	0.0
2 d_3	62.46	75.25	43.51	69.7	52.4	0.00	0.0	0.0
6 d_3	57.28	69.02	52.60	91.8	63.4	0.00	0.0	0.0
9 d_3	60.24	72.58	47.62	79.0	57.4	2.43	4.0	2.9
14 d_3	55.12	66.41	44.14	80.1	53.2	2.76	5.0	3.3
22 d_3	46.34	55.84	36.95	79.7	44.5	3.18	6.9	3.8
29 d_3	53.61	64.59	38.44	71.7	46.3	5.19	9.7	6.3
42 d_3	40.91	49.29	25.60	62.6	30.8	3.16	7.7	3.8
56 d_3	27.96	33.68	11.72	41.9	14.1	3.74	13.4	4.5
70 d_3	31.76	38.27	16.81	52.9	20.3	3.94	12.4	4.7
91 d_3	17.42	20.98	9.04	51.9	10.9	1.17	6.7	1.4

% aR = % of applied radioactivity

Institute 5

Physico-chemical parameters

Figure A3_25: Physico-chemical parameters measured throughout the ring test experiments by Institute 5

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 5
Manure sample: Cattle
Test substance: Imidacloprid

Excel 2003

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) [mg/kg]	Nitrogen (N_{total}) %	Dry matter content [%]	Organic matter content (relating to wet weight) [%]	Other parameter(s)
Sampling	01.05.2014	6.9	NA	NA	1504	n.d.	6.4		-
Start of acclimation	27.05.2014	6.9	-278.0	20.0			-	NA	-
Day 0	25.06.2014	6.9	-270.0	20.0					-
Day 3	28.06.2014	6.9	-268.0	20.0					-
Day 7	08.07.2014	6.9	-250.0	20.0					-
Day 28	28.07.2014	6.9	-270.0	20.0					-
Day 42	06.08.2014	6.9	-272.0	20.0					-
Day 90	22.09.2014	6.9	-300.0	20.0			-		-

If you measure parameters that are not covered by the sheet, then please add columns as needed.

Manure sample: Pig
Test substance: Florfenicol

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) [mg/kg]	Nitrogen (N_{total}) %	Dry matter content [%]	Organic matter content (relating to wet weight) [%]	Other parameter(s)
Sampling	01.05.2014	7.4	NA	NA	3220	n.d.	3.5	-	-
Start of acclimation	27.05.2014	7.4	-366.0	20.0			-	-	-
Day 0 - 0 h	24.06.2014	-	-	-					-
Day 0 - 1 h	24.06.2014	-	-	-					-
Day 0 - 2 h	24.06.2014	-	-	-					-
Day 0 - 7 h	24.06.2014	7.4	-366.0	20.0					-
Day 1 - 24 h	25.06.2014	7.4	-362.0	20.0					-
Day 7	07.07.2014	7.4	-360.0	20.0					-
Day 28	29.07.2014	7.4	-370.0	20.0					-
Day 90	23.09.2014	7.4	-370.0	20.0			-		-

Mass Balance

Figure A3_26: Mass balance determined for ^{14}C-florfenicol in pig manure by Institute 5

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 5
Manure sample: Pig
Test substance: Florfeninol
Applied radioactivity (aR): 101.80 kBq per test vessel

Excel 2003

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Sample	Activity in extracts	Amount in extracts	Extract (mean)	NER/Activity in manure (combustion)	NER/Manure combustion	NER/Manure combustion (mean)	Activity $NaOH$ ($^{14}CO_2$)	Activity $NaOH$ ($^{14}CO_2$)	Activity $NaOH$ ($^{14}CO_2$) (mean)	Activity CO_2-stripping	Activity CO_2-stripping	Activity CO_2-stripping (mean)	Activity $NaOH$ ($^{14}CH_4$)	Activity $NaOH$ ($^{14}CH_4$)	Activity $NaOH$ ($^{14}CH_4$) (mean)	Total activity	Recovery	Recovery (mean)
	[kBq]	[% aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% aR]	[% aR]
0 h - 1	93.000	91.4		1.000	1.0		0.000	0.0		0.000	0.0		-	-	-	94.000	92.3	
0 h - 2	91.000	89.4	91.7	0.780	0.8	1.1	0.000	0.0	0.0	0.000	0.0	0.0	-	-	-	91.780	90.2	92.8
0 h - 3	96.000	94.3		1.500	1.5		0.000	0.0		0.000	0.0		-	-	-	97.500	95.8	
1 h - 1	102.000	100.2		2.200	2.2		0.000	0.0		0.000	0.0		-	-	-	104.200	102.4	
1 h - 2	97.000	95.3	95.6	1.750	1.7	2.1	0.000	0.0	0.0	0.000	0.0	0.0	-	-	-	98.750	97.0	97.7
1 h - 3	93.000	91.4		2.500	2.5		0.000	0.0		0.000	0.0		-	-	-	95.500	93.8	
2 h - 1	83.000	81.5		1.570	1.5		-	-		-	-		-	-	-	84.570	83.1	
2 h - 2	82.000	80.6	83.2	3.570	3.5	2.5	-	-	-	-	-	-	-	-	-	85.570	84.1	85.7
2 h - 3	69.000	67.4		2.500	2.5		-	-		-	-		-	-	-	91.500	89.9	
7 h - 1	78.000	76.6		6.000	5.9		0.000	0.0		0.000	0.0		-	-	-	84.000	82.5	
7 h - 2	88.000	86.4	81.5	5.630	5.5	3.8	0.000	0.0	0.0	0.000	0.0	0.0	-	-	-	93.630	92.0	85.4
7 h - 3	83.000	81.5		0.100	0.1		0.000	0.0		0.000	0.0		-	-	-	83.100	81.6	
24 h - 1	78.130	70.0		7.020	6.9		0.000	0.0		0.000	0.0		-	-	-	85.150	83.6	
24 h - 2	88.350	76.0	73.7	7.020	7.5	7.2	0.000	0.0	0.0	0.000	0.0	0.0	-	-	-	95.970	94.3	86.3
24 h - 3	82.550	75.0		-	-		0.000	0.0		0.000	0.0		-	-	-	82.550	81.1	
7 d - 1	83.000	81.5		18.500	18.2		0.000	0.0		0.000	0.0		-	-	-	101.500	99.7	
7 d - 2	96.000	94.3	76.6	19.400	19.1	18.3	0.000	0.0	0.0	0.000	0.0	0.0	-	-	-	115.400	113.4	94.9
7 d - 3	55.000	54.0		17.900	17.6		0.000	0.0		0.000	0.0		-	-	-	72.900	71.6	
28 d - 1	68.000	66.8		13.750	13.5		0.000	0.0		0.000	0.0		-	-	-	81.750	80.3	
28 d - 2	85.000	-	64.8	12.280	12.1	12.8	0.000	0.0	0.0	0.000	0.0	0.0	-	-	-	97.280	95.6	79.6
28 d - 3	64.000	62.9		-	-		0.000	0.0		0.000	0.0		-	-	-	64.000	62.9	
90 d - 1	46.960	46.1		29.180	28.7		0.000	0.0		0.000	0.0		-	-	-	76.140	74.8	
90 d - 2	49.840	49.0	48.5	26.500	31.5	32.9	0.000	0.0	0.0	0.000	0.0	0.0	-	-	-	76.340	75.0	77.2
90 d - 3	51.470	50.6		31.700	38.4		0.000	0.0		0.000	0.0		-	-	-	83.170	81.7	
1 d sterile - 1	87.520	86.0		11.200	11.0		-	-		-	-		-	-	-	98.720	97.0	
1 d sterile - 2	88.540	87.0	86.5	9.800	9.6	10.3	-	-	-	-	-	-	-	-	-	98.340	96.6	96.8
28 d sterile - 1	77.290	75.9		7.000	6.9		-	-		-	-		-	-	-	84.290	82.8	
28 d sterile - 2	85.950	84.4	80.2	10.200	10.0	8.4	-	-	-	-	-	-	-	-	-	96.150	94.4	88.6
90 d sterile - 1	84.700	83.2		10.240	10.1		-	-		-	-		-	-	-	94.940	93.3	
90 d sterile - 2	86.420	84.9	84.0	7.600	7.5	8.8	-	-	-	-	-	-	-	-	-	94.020	92.4	92.8
Formula		100 / aR * B	mean of C		100 / aR * E	mean of F		100 / aR * H	mean of I		100 / aR * K	mean of L		100 / aR * N	mean of O	B + E + H + K + N	100 / aR * Q	mean of R

Figure A3_27: Mass balance determined for ^{14}C-imidacloprid in cattle manure by Institute 5

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 5
Manure sample: Cattle
Test substance: Imidacloprid
Applied radioactivity (aR): 15.00 kBq per test vessel

Excel 2003

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Sample	Activity in extracts	Amount in extracts	Extract (mean)	NER/Activity in manure (combustion)	NER/Manure combustion	NER/Manure combustion (mean)	Activity $NaOH (^{14}CO_2)$	Activity $NaOH (^{14}CO_2)$	Activity $NaOH (^{14}CO_2)$ (mean)	Activity CO_2-stripping	Activity CO_2-stripping	Activity CO_2-stripping (mean)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$)	Activity NaOH ($^{14}CH_4$) (mean)	Total activity	Recovery	Recovery (mean)
	[kBq]	[% aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	[% kBq/aR]	[% kBq/aR]	[kBq]	% kBq/aR]	[% kBq/aR]	[kBq]	[% aR]	[% aR]
0 d - 1	12.25	81.7		0.280	0.0		0.000	0.0		0.000	0.0		0.000	0.0		12.530	83.5	
0 d - 2	14.38	95.9	86.2	0.140	0.0	0.0	0.000	0.0	0.0	0.000	0.0	0.0	0.000	0.0	0.0	14.520	96.8	87.4
0 d - 3	12.15	81.0		0.150	0.0		0.000	0.0		0.000	0.0		0.000	0.0		12.300	82.0	
3 d - 1	12.850	85.7		1.650	11.0		0.000	0.0		0.000	0.0		0.000	0.0		14.500	96.7	
3 d - 2	12.000	80.0	79.1	1.300	8.7	10.0	0.000	0.0	0.0	0.000	0.0	0.0	0.000	0.0	0.0	13.300	88.7	89.1
3 d - 3	10.730	71.5		1.560	10.4		0.000	0.0		0.000	0.0		0.000	0.0		12.290	81.9	
7 d - 1	11.000	73.3		1.290	8.6		0.000	0.0		0.000	0.0		0.000	0.0		12.290	81.9	
7 d - 2	14.000	93.3	82.2	1.580	10.5	9.3	0.000	0.0	0.0	0.000	0.0	0.0	0.000	0.0	0.0	15.580	103.9	91.5
7 d - 3	12.000	80.0		1.300	8.7		0.000	0.0		0.000	0.0		0.000	0.0		13.300	88.7	
28 d - 1	10.000	66.7		2.370	15.8		0.000	0.0		0.000	0.0		0.000	0.0		12.370	82.5	
28 d - 2	10.000	66.7	71.1	1.800	12.0	13.9	0.000	0.0	0.0	0.000	0.0	0.0	0.000	0.0	0.0	11.800	78.7	85.0
28 d - 3	12.000	80.0		2.100	14.0		0.000	0.0		0.000	0.0		0.000	0.0		14.100	94.0	
42 d - 1	10.93	72.2		2.870	19.1		0.000	0.0		0.000	0.0		0.000	0.0		13.700	91.3	
42 d - 2	10.12	67.5	72.3	3.420	22.8	20.1	0.000	0.0	0.0	0.000	0.0	0.0	0.000	0.0	0.0	13.540	90.3	92.4
42 d - 3	11.57	77.1		2.760	18.4		0.000	0.0		0.000	0.0		0.000	0.0		14.330	95.5	
90 d - 1	10.550	70.3		4.790	31.9		0.000	0.0		0.000	0.0		0.000	0.0		15.340	102.3	
90 d - 2	10.010	66.7	73.6	3.660	24.4	28.4	0.000	0.0	0.0	0.000	0.0	0.0	0.000	0.0	0.0	13.670	91.1	100.0
90 d - 3	12.570	83.8		3.430	22.9		0.000	0.0		0.000	0.0		0.000	0.0		16.000	106.7	
7 d sterile - 1	14.130	94.2		0.600	4.0		-	-	-	-	-	-	-	-	-	14.730	98.2	
7 d sterile - 2	13.940	92.9	93.6	1.100	7.3	5.7	-	-	-	-	-	-	-	-	-	15.040	100.3	99.2
90 d sterile - 1	13.150	87.7		1.020	6.8		-	-		-	-		-	-		14.170	94.5	
90 d sterile - 2	14.250	95.0	91.3	1.250	8.3	7.6	-	-	-	-	-	-	-	-	-	15.500	103.3	98.9
Formula	14.28	100 / aR * B	mean of C		100 / aR * F	mean of F		100 / aR * H	mean of I		100 / aR * K	mean of L		100 / aR * N	mean of O	R+E+H+K+N	100 / aR * Q	mean of R

Extraction methods

Figure A3_28: Extraction methods used within the ring test by Institute 5

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Extraction Methods

Please add as much information as possible

Excel 2003

Participant:	Institute 5	
Manure sample:	Cattle	Pig
Test substance	Imidacloprid	Florfenicol
Extraction Method:	EXTRACTION IMADOCLOPRID 1. Transfer manure to a Teflon bottle 2. Extract once by 80 mL acetonitrile (shake 30 min) 3. Centrifuge at 2100rpm for 15 min. 4. Pour supernatant through paper filter into a beaker. 5. Extract twice more with 50 ml acetonitrile (shake for 30 min). 6. Count 2 x 1ml of pooled supernatant with LSC to measure extractable radiolabelled compound. 7. Leave manure to dry in hood overnight. 8. When dry, use oxidizer to quantify bound radiolabelled compound.	EXTRACTION FLORFENICOL 1. Transfer manure to a Teflon bottle 2. Extract 2x by 50 mL methanol (shake 30 min) 3. Centrifuge at 2100 rpm for 15 min. 4. Pour supernatant through paper filter into a beaker. 5. Extract 1x with 50 mL methanol + 1.5 ml TFA (shake for 30 min). 6. Count 2 x 1ml of pooled supernatant with LSC to measure extractable radiolabelled compound. 7. Leave manure to dry in hood overnight. 8. When dry, use oxidizer to quantify bound radiolabelled compound.

Institute 6

Physico-chemical parameters

Figure A3_29: Physico-chemical parameters measured throughout the ring test experiments by Institute 6

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 6
Manure sample: Pig
Test substance: Florfenicol

Excel 2003

Time Point	Date (yy/mm/dd)	pH	Redox potential [mV]	Temperature [°C]	Ammonia (NH_4-N) [mg/kg]	Nitrogen (N_{total}) [mg/kg]	Dry matter content [%]	Organic matter content (relating to wet weight) [%]	Other parameter(s)	Redox potential sterile samples [mV]
Sampling	05.05.2014	7.1	-300.0	20.0	7330	11190	14.7	OC 6.1% [1)]	C/N 18,1	
Start of acclimation	19.05.2014	n.d.	n.d.	10.0	2490	3800	5.0	OC 2.1% [1)]	C/N 18,1	
Day 0 - 0 h	02.06.2014	n.d.	-371.0	10.0						
Day 0 - 0.5 h	n.d.	n.d.	n.d.	10.0						
Day 0 - 1 h	n.d.	n.d.	n.d.	10.0						
Day 0 - 2 h	n.d.	n.d.	n.d.	10.0						
Day 0 - 4 h	n.d.	n.d.	n.d.	10.0						
Day 0 - 7 h	n.d.	n.d.	n.d.	10.0						
Day 1 - 24 h	03.06.2014	n.d.	-336.0	10.0						
Day 3	27.06.2014*	n.d.	-330.4	10.0						
Day 7	01.07.2014*	n.d.	-374.0	10.0						
Day 14	08.07.2014*	n.d.	-258.1	10.0						
Day 21	15.07.2014*	n.d.	-347.4	10.0						
Day 28	30.06.2014	n.d.	-409.0	10.0						-151.2
Day 49	21.07.2014	n.d.	-325.0	10.0						
Day 70	11.08.2014	n.d.	-378.0	10.0						
Day 90	01.09.2014	7.4	-207.0	10.0			4.1			-138.5

* start of experiment for time points 3, 7, 14, 21 d on 24.06.2014 with repetition of sampling at t=0; [1)] The organic carbon content (OC) was determined instead of the organic matter content.

Mass Balance

Figure A3_30: Mass balance determined for ^{14}C-florfenicol in pig manure by Institute 6

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 6
Manure sample: Pig
Test substance: Florfenicol

Applied radioactivity (aR): 25.00 kBq per test vessel Excel 2003

Sample	Extract (mean)	NER/Manure combustion (mean)	**Recovery (mean)**
	[% kBq/aR]	[% kBq/aR]	**[% aR]**
0 h	100.0	3.2	**103.2**
0.5 h	63.3	2.3	**65.6**
24 h	60.0	8.8	**68.9**
3 d	35.2	40.7	**75.9**
7 d	19.5	58.8	**78.3**
14 d	20.8	9.7	**30.4**
21 d	17.6	49.2	**66.8**
28 d	23.3	70.8	**94.0**
49 d	20.0	52.2	**72.2**
70 d	23.6	54.4	**78.0**
90 d	18.9	59.6	**78.5**
1 d sterile	35.6	9.6	**45.3**
28 d sterile	28.9	23.6	**52.4**
90 d sterile	8.1	39.3	**47.5**

Chemical Analysis

Figure A3_31: Results of chemical analysis for ^{14}C-florfenicol in pig manure by Institute 6

"Transformation of veterinary pharmaceuticals and biocides in liquid manure"

Participant: Institute 6
Manure sample: Pig
Test substance: Florfenicol

Excel 2003

^{14}C-labeled Florfenicol (radio-HPLC)

Replicate 1

Sample	Radioactivity in combined extracts	NER/Manure combustion	florfenicol (in MSE)		florfenicol amine (in MSE)		florfenicol oxamic acid (in MSE)		unknown metabolite (M?) (in MSE)	
	MSE (% aR)	NER (% aR)	µg/kg	(% aR)	µg/kg	(% aR)	µg/kg	(% aR)	µg/kg	(% aR)
Day 0 - 0 h	100	3.2	1794	57.4	0	0.0	0	0.0	0	0.0
Day 0 - 0.5 h	63.3	2.3	1620	37.3	0.938	0.0	27.5405	0.6	0.079	0.0
Day 0 - 4 h	n.d.	n.d.	1702	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 1 - 24 h	60	8.8	804.399	70.8	1.132	0.1	37.2299	3.3	0.082	0.0
Day 3	35.2	40.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 7	19.5	58.8	19.465	11.4	4.230	2.5	17.5055	10.3	0.446	0.3
Day 14	20.8	9.7*	53.974	n.d.	7.160	n.d.	27.3471	n.d.	0.533	n.d.
Day 21	17.6	49.2	25.149	n.d.	1.628	n.d.	8.0712	n.d.	0.147	n.d.
Day 28	23.3	70.8	12.759	9.0	8.700	6.2	8.5881	6.1	1.178	0.8
Day 49	20	52.2	6.511	n.d.	1.794	n.d.	1.3329	n.d.	0.209	n.d.
Day 70	23.6	54.4	5.957	3.2	0.816	0.4	1.4339	0.8	0.085	0.0
Day 90	18.9	59.6	5.151	3.1	5.310	3.2	1.1317	0.7	0.059	0.0

n.d. = not determined; MSE = mild solvent extract; * = outlier

Sterile samples

Sample	Radioactivity in combined extracts	NER/Manure combustion	florfenicol (in MSE)		florfenicol amine (in MSE)		florfenicol oxamic acid (in MSE)		unknown metabolite (M?) (in MSE)	
	MSE (% aR)	NER (% aR)	µg/kg	(% aR)	µg/kg	(% aR)	µg/kg	(% aR)	µg/kg	(% aR)
Day 0 - 0 h	100	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 1 - 24 h	21.6	11.5	1053.451	121.1	24.640	2.8	0.0000	0.0	0	0.0
Day 28	49.6	7.8	55.182	4.3	3.032	0.2	9.3686	0.7	0.1635	0.0
Day 90	8.1	39.3	85.240	33.5	1.316	0.5	1.1079	0.4	0.0509	0.0

Unlabeled Florfenicol (LC-MS/MS)

Replicate 1

Sample	florfenicol (in MSE)		florfenicol (in MSE plus ASE)		florfenicol amine (in MSE)		florfenicol amine (in MSE plus ASE)	
	ng/ml	(% a)	ng/ml	(% aR)	ng/ml	(% aR)	ng/ml	(% aR)
Day 0 - 0 h	25900	34.5	25900	n.d.	34.7	n.d.	37.45	n.d.
Day 0 - 0.5 h	40500	54.0	40500	n.d.	n.d.	n.d.	n.d.	n.d.
Day 0 - 4 h	42550	56.7	42550	n.d.	n.d.	n.d.	n.d.	n.d.
Day 1 - 24 h	20950	27.9	20950	n.d.	26.8	n.d.	29.4	n.d.
Day 3	5344	7.1	5344	n.d.	37.0	n.d.	40.5	n.d.
Day 7	681	0.9	681	n.d.	126	n.d.	131	n.d.
Day 14	1520	2.0	1520	n.d.	141	n.d.	150	n.d.
Day 21	522	0.7	522	n.d.	38.0	n.d.	41.5	n.d.
Day 28	425	0.6	425	n.d.	239	n.d.	245	n.d.
Day 49	366	0.5	366	n.d.	50.8	n.d.	53.7	n.d.
Day 70	51.2	0.1	51	n.d.	21.3	n.d.	24.1	n.d.
Day 90	16.2	0.0	16	n.d.	20.5	n.d.	23.5	n.d.

Replicate 2

Sample	florfenicol (in MSE)		florfenicol (in MSE plus ASE)		florfenicol amine (in MSE)		florfenicol amine (in MSE plus ASE)	
	ng/ml	(% a)	ng/ml	(% aR)	ng/ml	(% aR)	ng/ml	(% aR)
Day 0 - 0 h	25279	33.7	25279	n.d.	12.2	n.d.	14.8	n.d.
Day 1 - 24 h	25600	34.1	25600	n.d.	29.8	n.d.	33.2	n.d.
Day 3	6170	8.2	6170	n.d.	61.7	n.d.	64.7	n.d.
Day 7	645	0.9	645	n.d.	85.5	n.d.	89	n.d.
Day 14	1246	1.7	1246	n.d.	217	n.d.	222	n.d.
Day 21	303	0.4	303	n.d.	43.4	n.d.	46.3	n.d.
Day 28	262	0.3	262	n.d.	196	n.d.	202	n.d.
Day 49	131	0.2	131	n.d.	38.9	n.d.	41.7	n.d.
Day 70	144	0.2	144	n.d.	19.5	n.d.	22.2	n.d.
Day 90	15.4	0.0	15.4	n.d.	24.5	n.d.	28.4	n.d.

Sterile samples

Sample	florfenicol (in MSE)		florfenicol (in MSE plus ASE)		florfenicol amine (in MSE)		florfenicol amine (in MSE plus ASE)	
	ng/ml	(% a)	ng/ml	(% aR)	ng/ml	(% aR)	ng/ml	(% aR)
Day 1 - 24 h	26340	n.d.	26340	n.d.	76.5	n.d.	79.0	n.d.
Day 28	2177	n.d.	2847	n.d.	86.2	n.d.	89.2	n.d.
Day 90	2266	n.d.	2266	n.d.	32.9	n.d.	36.9	n.d.

Annex 4

Degradation kinetics and statistical evaluation

Figure A5_1: Plots for transformation of florfenicol in pig manure, Institute 1, replicate 1. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by a modest increase of transformation products. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 19.5.

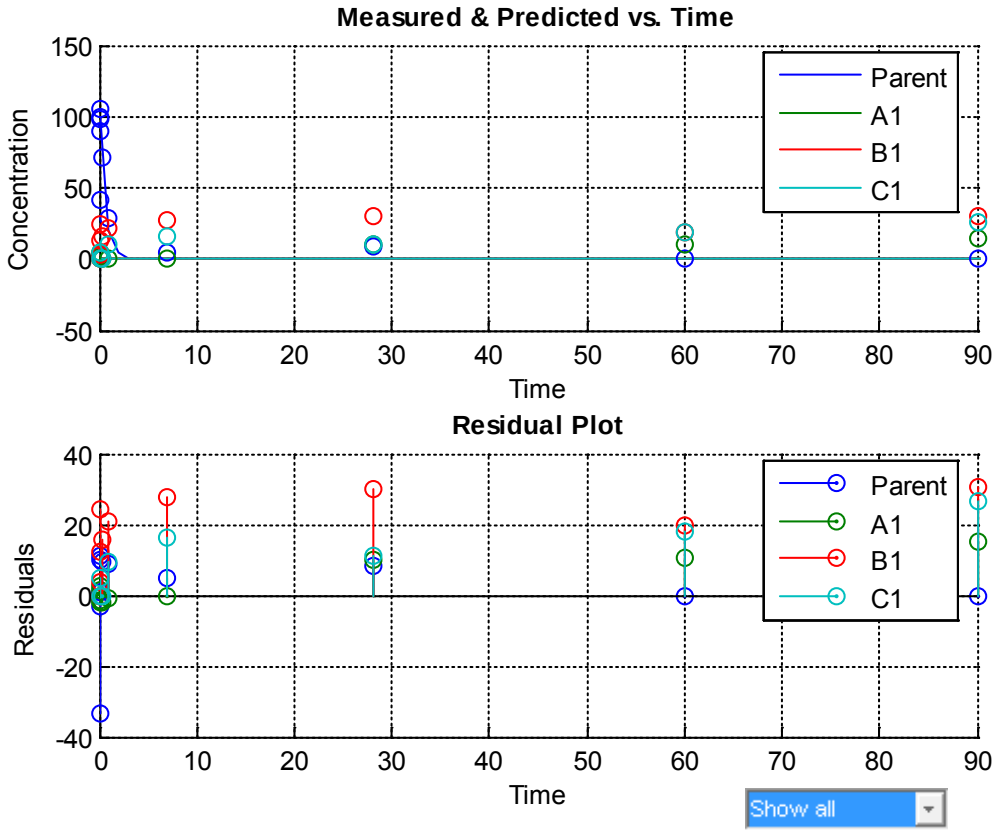


Figure A5_2: Plots for transformation of florfenicol in pig manure, Institute 1, replicate 2. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by a modest increase of transformation products. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 21.3.

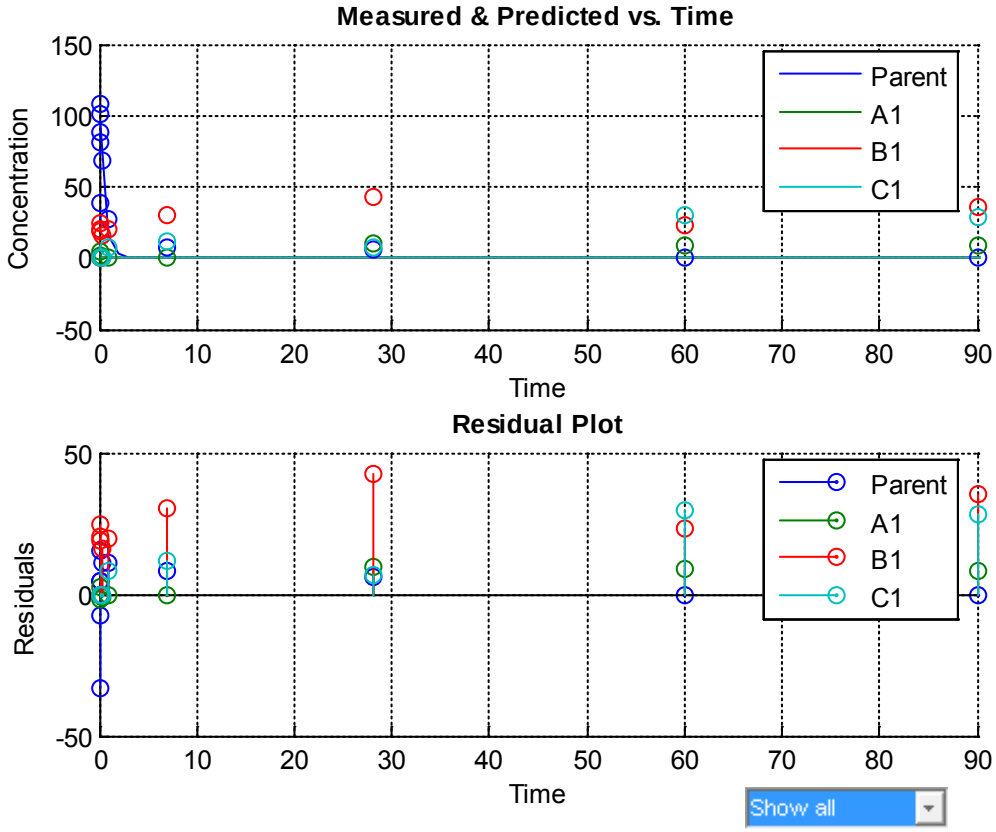


Figure A5_3: Plots for transformation of florfenicol in pig manure, Institute 1, replicate 3. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by a modest increase of transformation products. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 14.5.

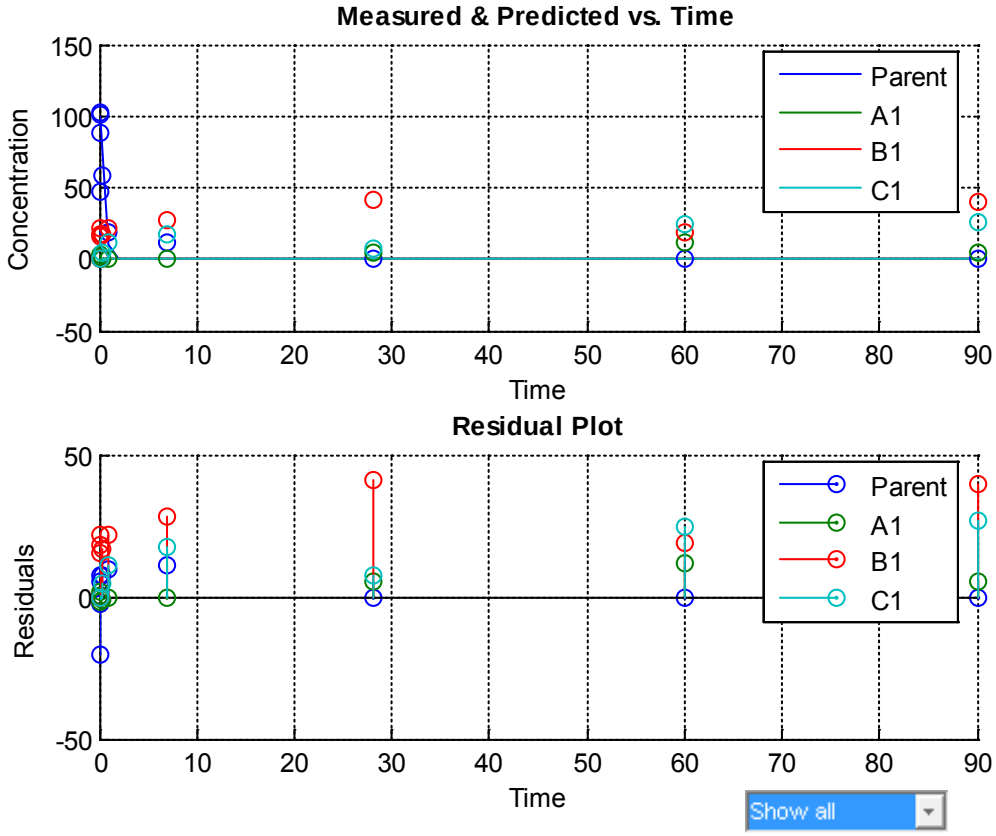


Figure A5_4: Plots for transformation of florfenicol in pig manure, Institute 1, replicate 4. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by a modest increase of transformation products. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 15.3.

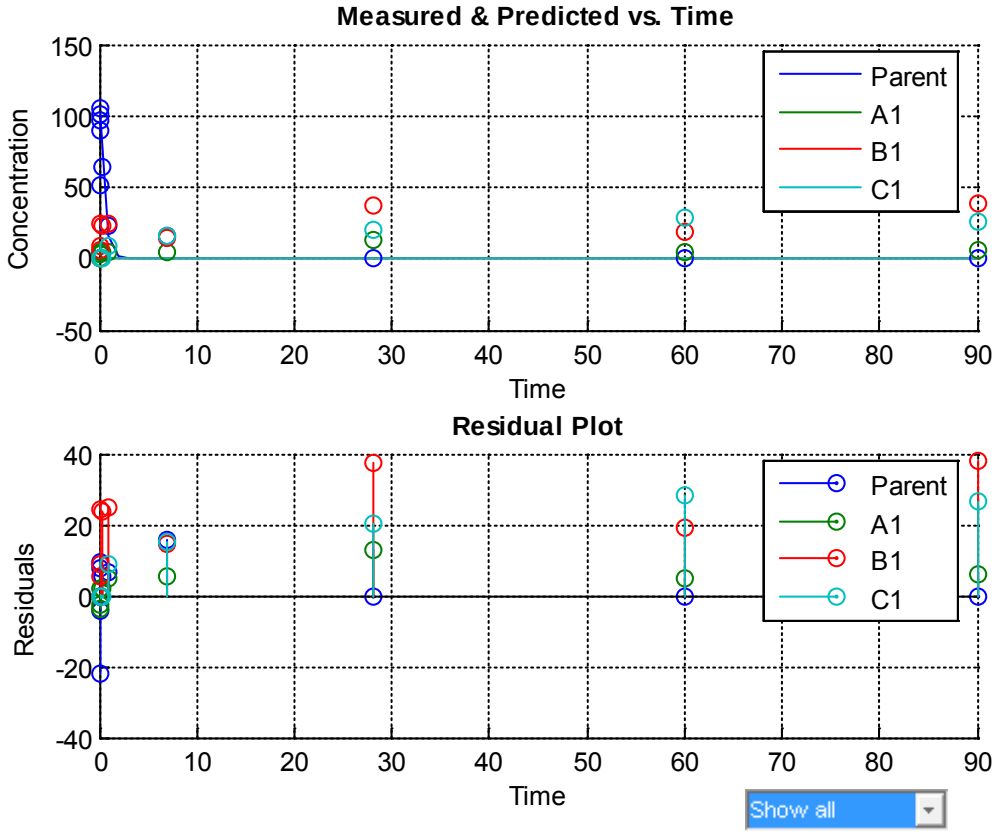


Figure A5_5: Plots for transformation of florfenicol in pig manure, Institute 1, replicate 5. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by a modest increase of transformation products. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 18.5.

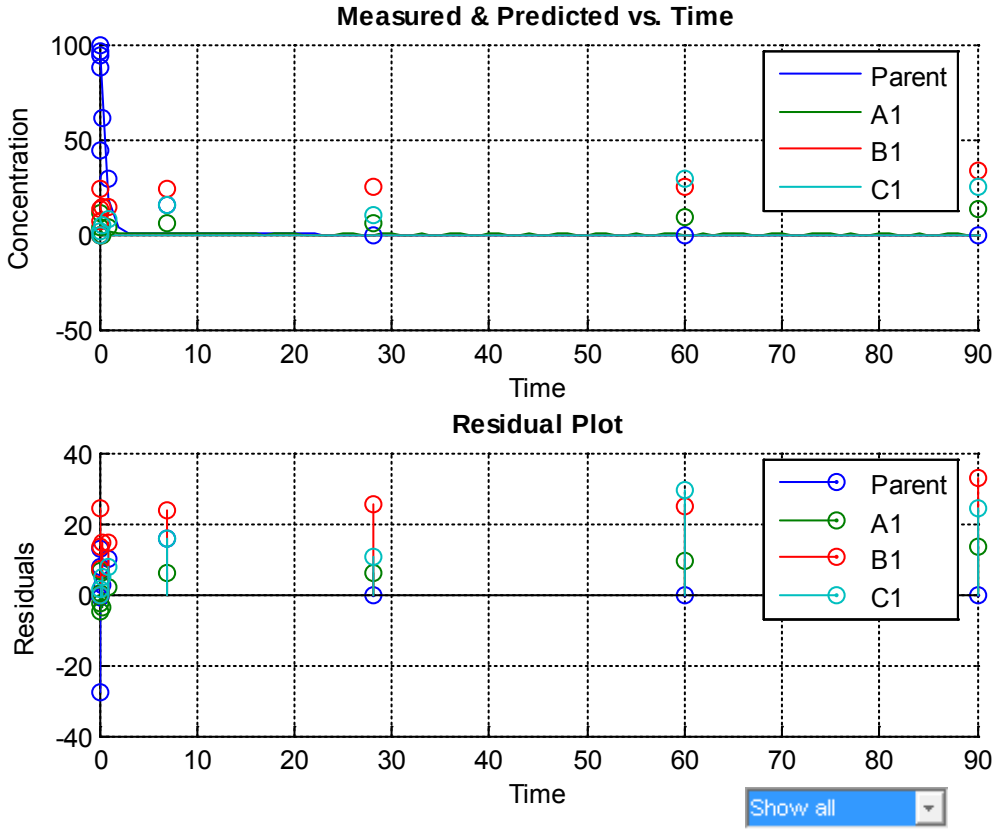


Figure A5_6: Plots for transformation of florfenicol in pig manure, Institute 1, replicate 6. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by a modest increase of transformation products. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 10.6.

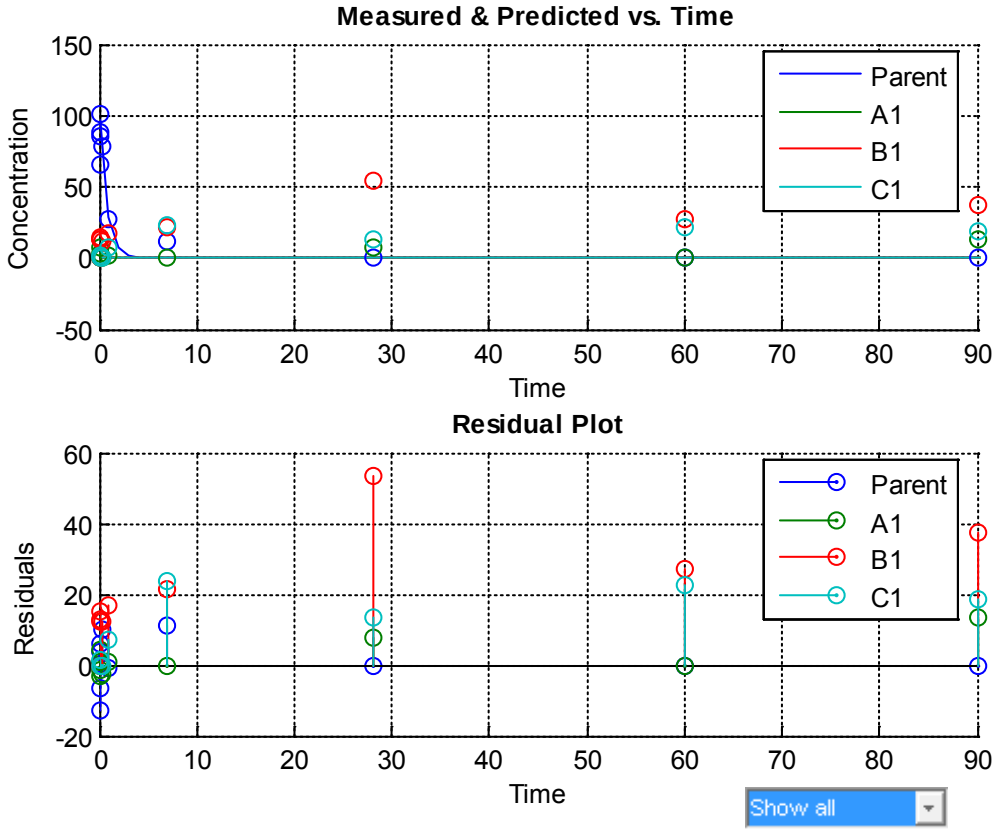


Figure A5_7: Plots for transformation of florfenicol in pig manure, Institute 1, mean of replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 16.6.

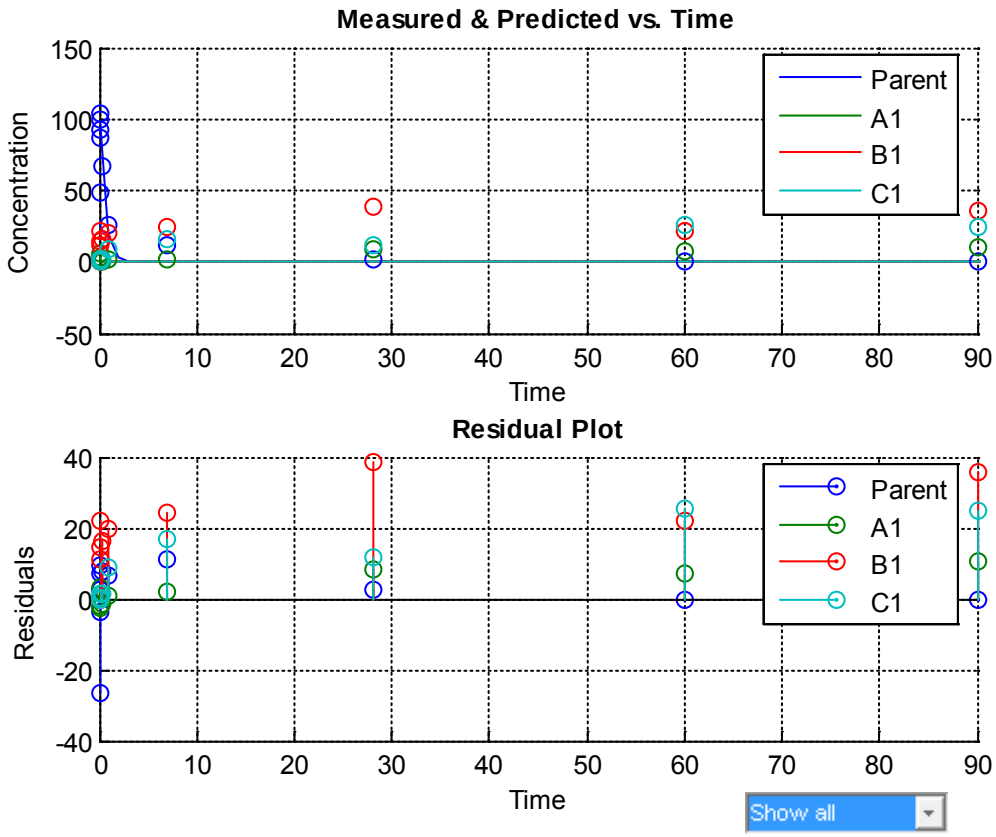


Table A5_1: Measured, predicted and residual values for transformation of the parent compound florfenicol in pig manure (Institute 1, mean of replicates, single first order kinetics)

Time	Florfenicol (parent)		
	Measured [%]	Predicted [%]	residual
0.0	99.5000	97.0908	2.4092
0.021	103.6000	93.9682	9.6318
0.042	87.3000	90.9460	-3.6460
0.083	92.6000	85.1902	7.4098
0.167	48.0000	74.7482	-26.7482
0.292	67.0000	59.4595	7.5405
1.0	25.6000	18.9375	6.6625
7.0	11.0000	0.0010	10.9990
28.0	2.4000	0.0000	2.4000

60.0	0.0000	0.0000	-0.0000
90.0	0.0000	0.0000	-0.0000

Figure A5_8: Plots for transformation of florfenicol in pig manure, Institute 2, replicate 1. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of TP1, followed by a modest decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 23.3; chi²-value (TP1) is 40.5.

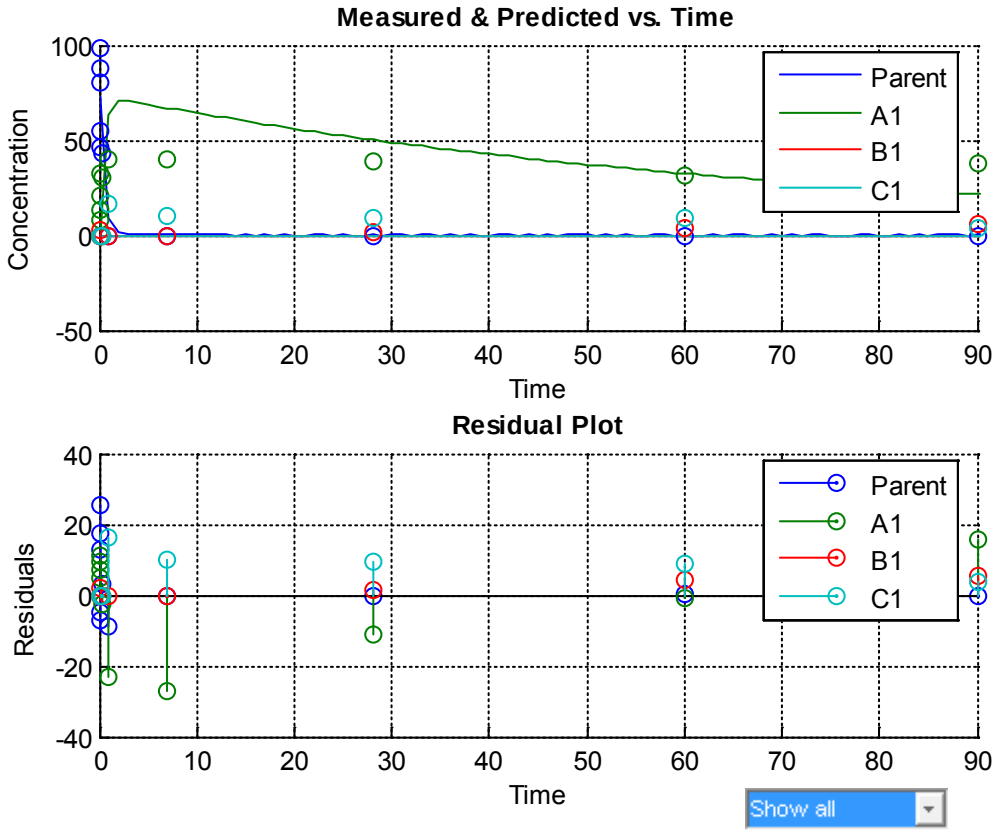


Figure A5_9: Plots for transformation of florfenicol in pig manure, Institute 2, replicate 2. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of TP1 followed by a modest decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 22.9; chi²-value (TP1) is 46.2.

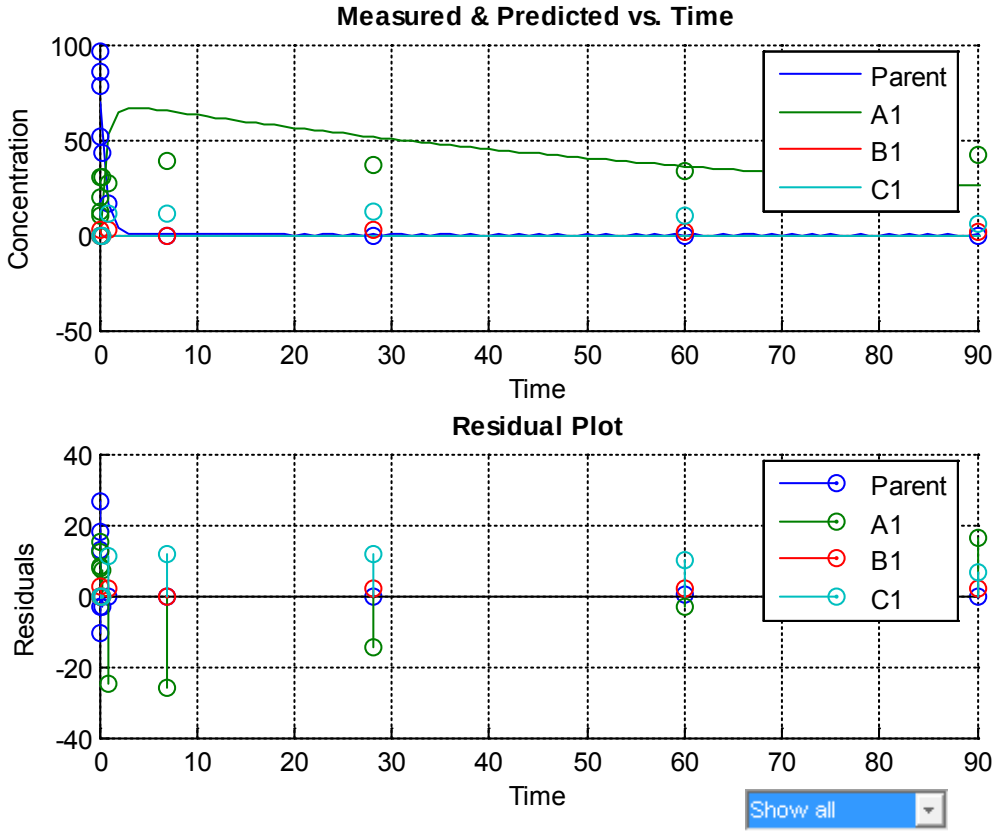


Figure A5_10: Plots for transformation of florfenicol in pig manure, Institute 2, replicate 3. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of TP1 followed by a modest decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 25.1; chi²-value (TP1) is 42.0.

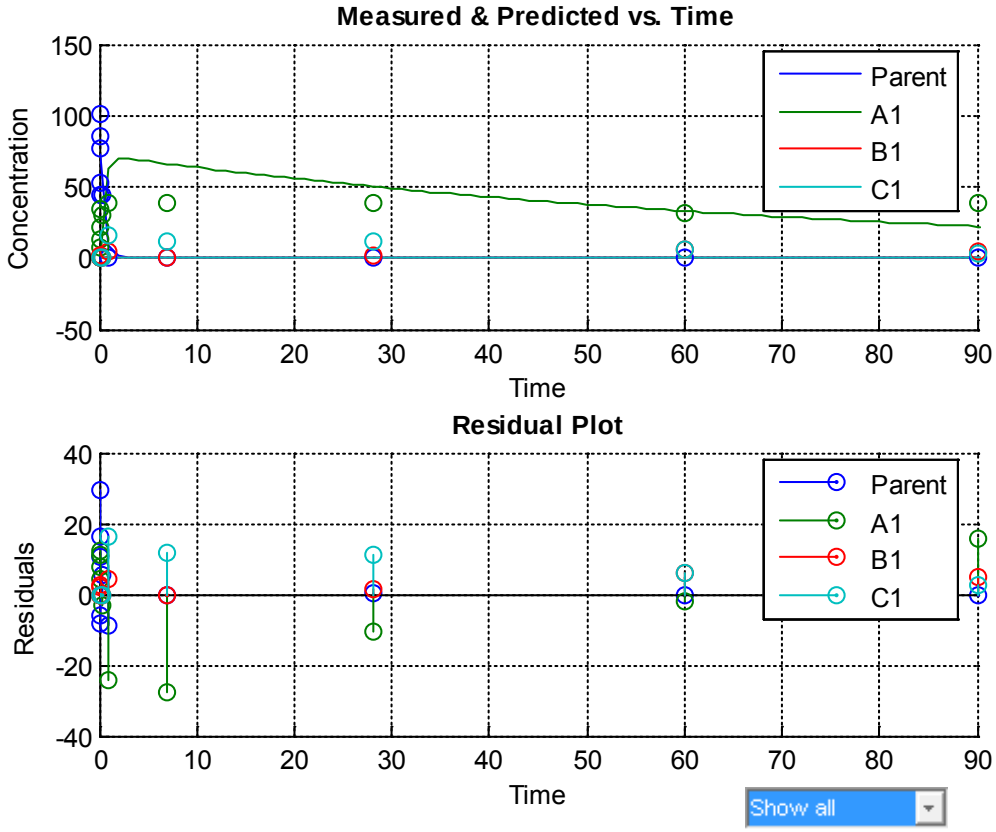


Figure A5_11: Plots for transformation of florfenicol in pig manure, Institute 2, replicate 4. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of TP1 followed by a modest decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 21.8; chi²-value (TP1) is 37.0.

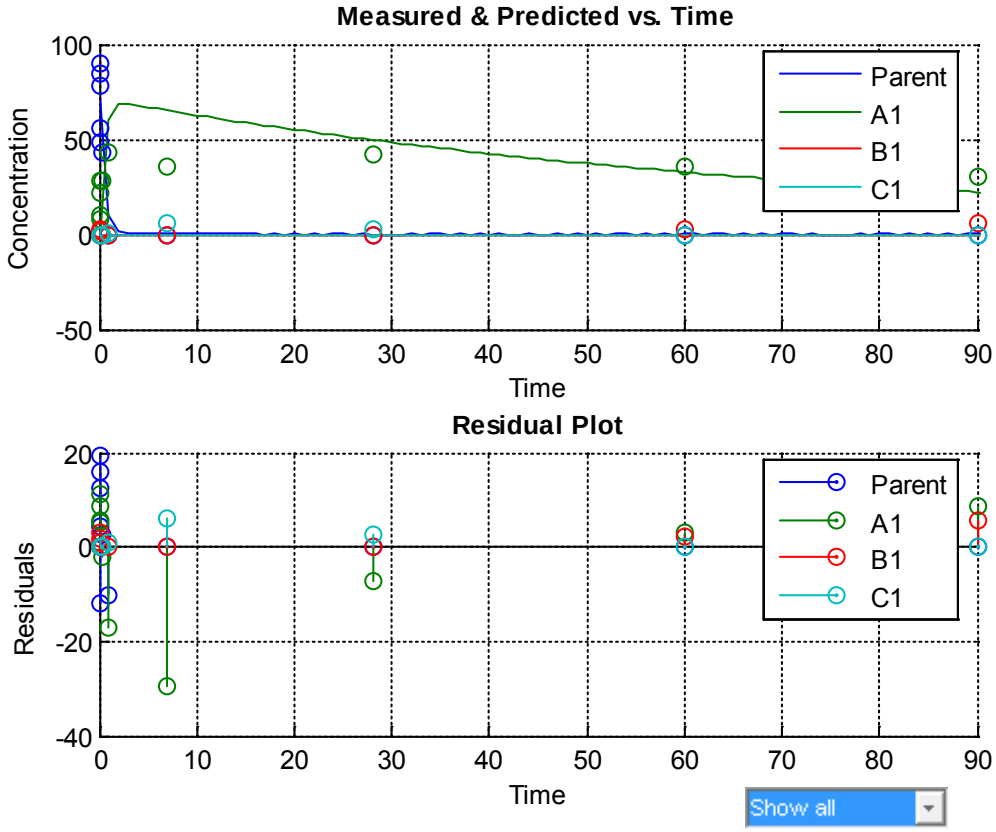


Figure A5_12: Plots for transformation of florfenicol in pig manure, Institute 2, replicate 5. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of TP1, followed by a modest decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 18.5; chi²-value (TP1) is 37.2.

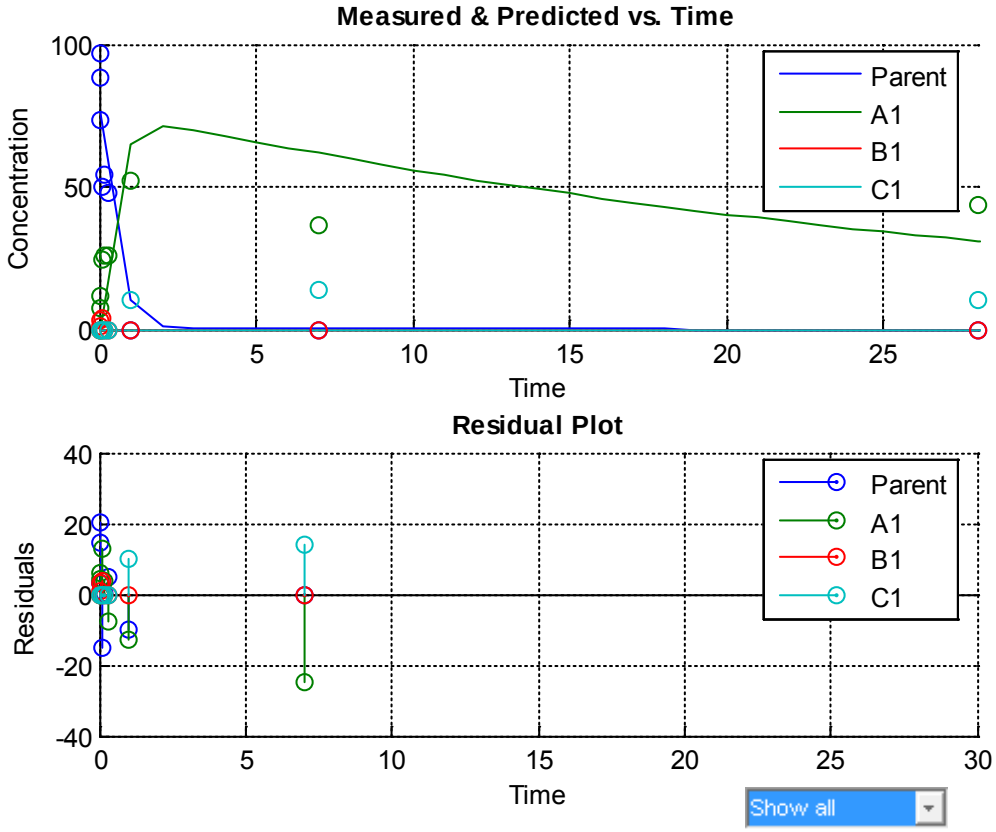


Figure A5_13: Plots for transformation of florfenicol in pig manure, Institute 2, replicate 6. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of A1, followed by a modest decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 18.5; chi²-value (TP1) is 37.2.

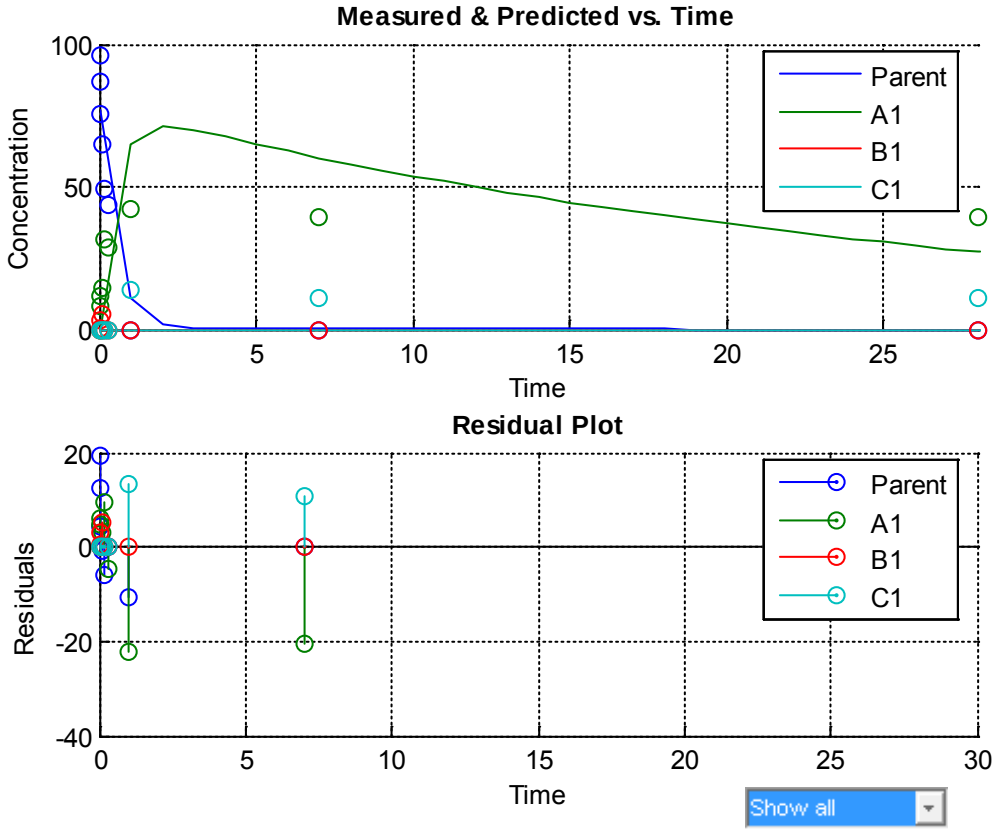


Figure A5_14: Plots for transformation of florfenicol in pig manure, Institute 2, mean of replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 13.1.

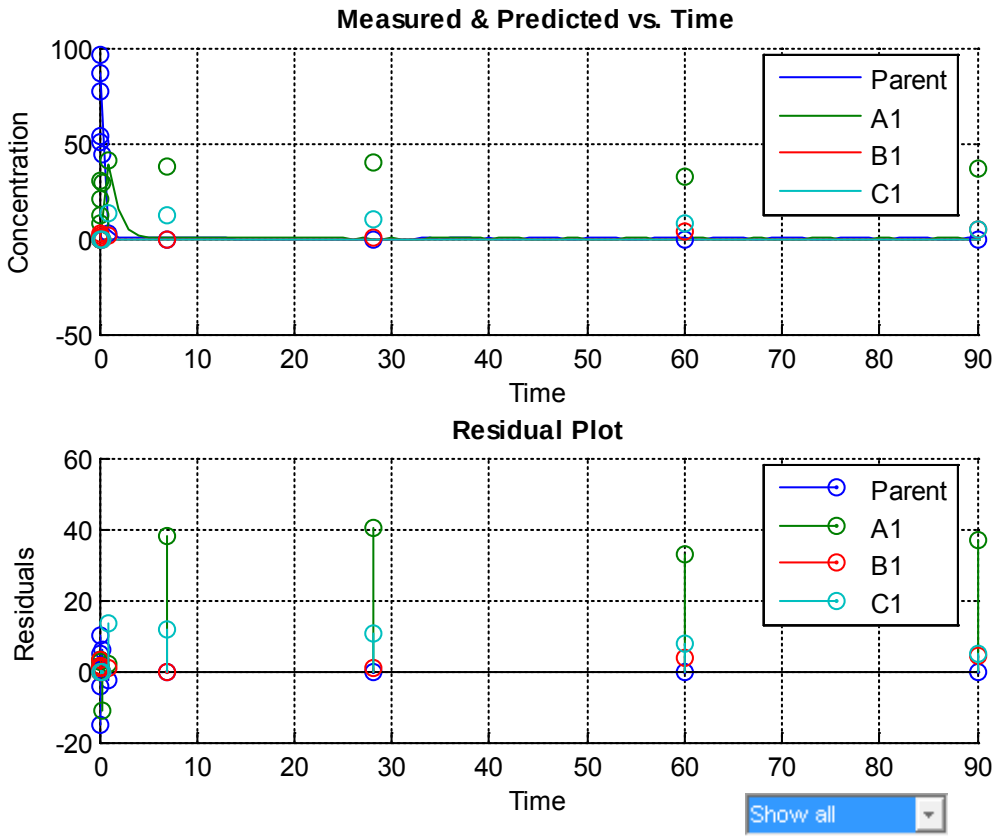


Table A5_2: Measured, predicted and residual values for transformation of the parent compound florfenicol in pig manure (Institute 2, mean of replicates, single first order kinetics)

Time	Florfenicol (parent)		
	Measured [%]	Predicted [%]	residual
0.0	96.5000	86.4695	10.0305
0.021	86.3000	81.5419	4.7581
0.042	77.1000	76.8952	0.2048
0.083	53.6000	68.5724	-14.9724
0.167	50.2000	54.2279	-4.0279
0.292	44.1000	38.2425	5.8575
1.0	2.8000	5.2899	-2.4899
7.0	0.0000	0.0000	-0.0000
28.0	0.0000	-0.0000	0.0000

60.0	0.0000	0.0000	-0.0000
90.0	0.0000	0.0000	-0.0000

Figure A5_15: Plots for transformation of florfenicol in pig manure, Institute 3, replicate 1. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1 and TP2 (named as A1 and B1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of TP1 and TP2. Whereas TP1 decreased rapidly afterwards, TP2 remains constant until the end of the study. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 15.7; chi²-value (TP1) is 48.2; chi²-value (TP2) is 103.4.

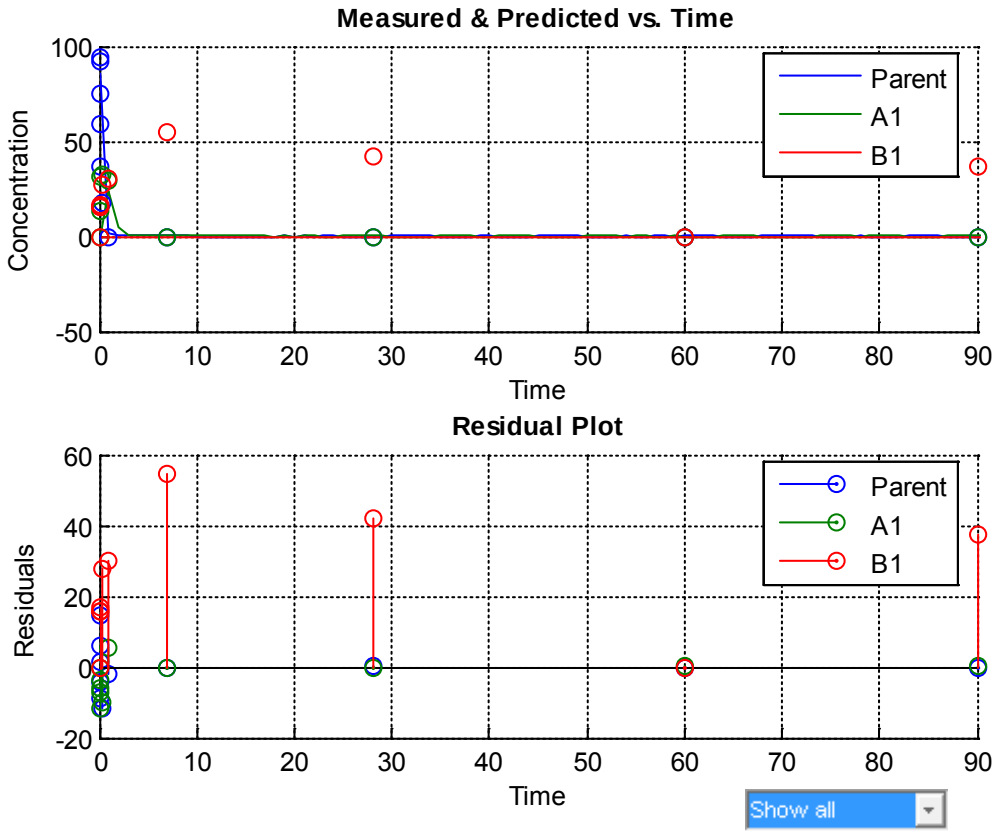


Figure A5_16: Plots for transformation of florfenicol in pig manure, Institute 3, replicate 2. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1 and TP2 (named as A1 and B1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of TP1 and TP2. Whereas TP1 decreased rapidly afterwards, TP2 shows a modest decrease until the end of the study Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 18.6; chi²-value (TP1) is 31.1; chi²-value (TP1) is 99.7.

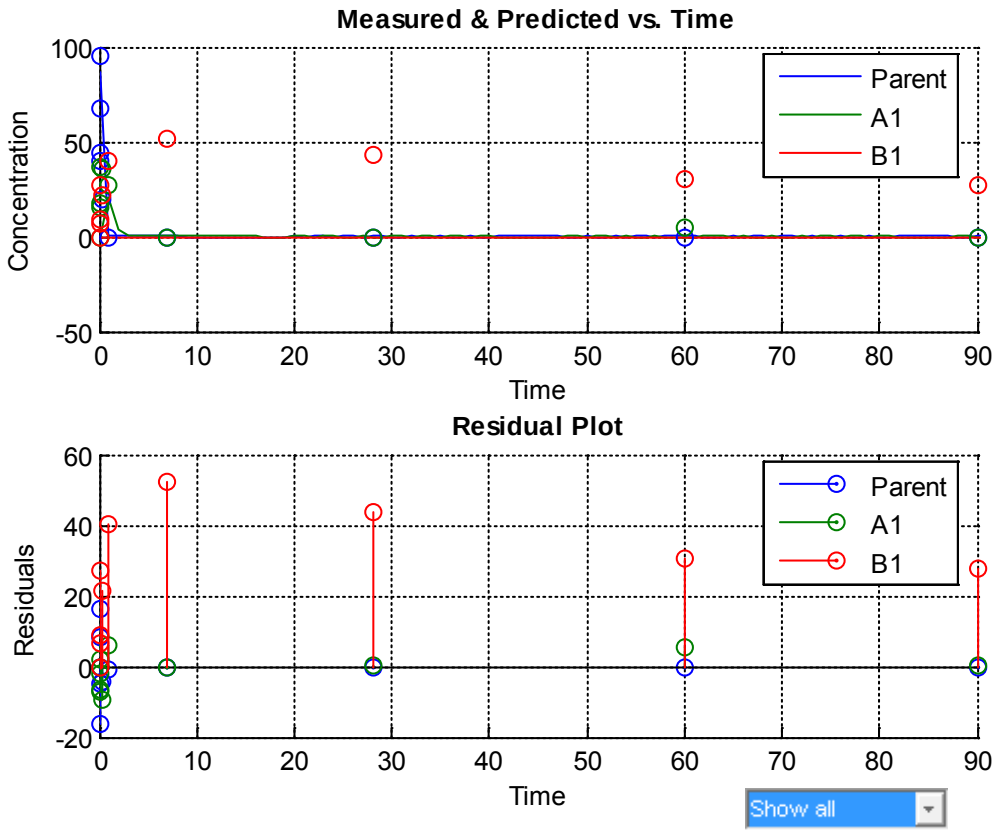


Figure A5_17: Plots for transformation of florfenicol in pig manure, Institute 3, mean of replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1 and TP2 (named as A1 and B1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 15.8.

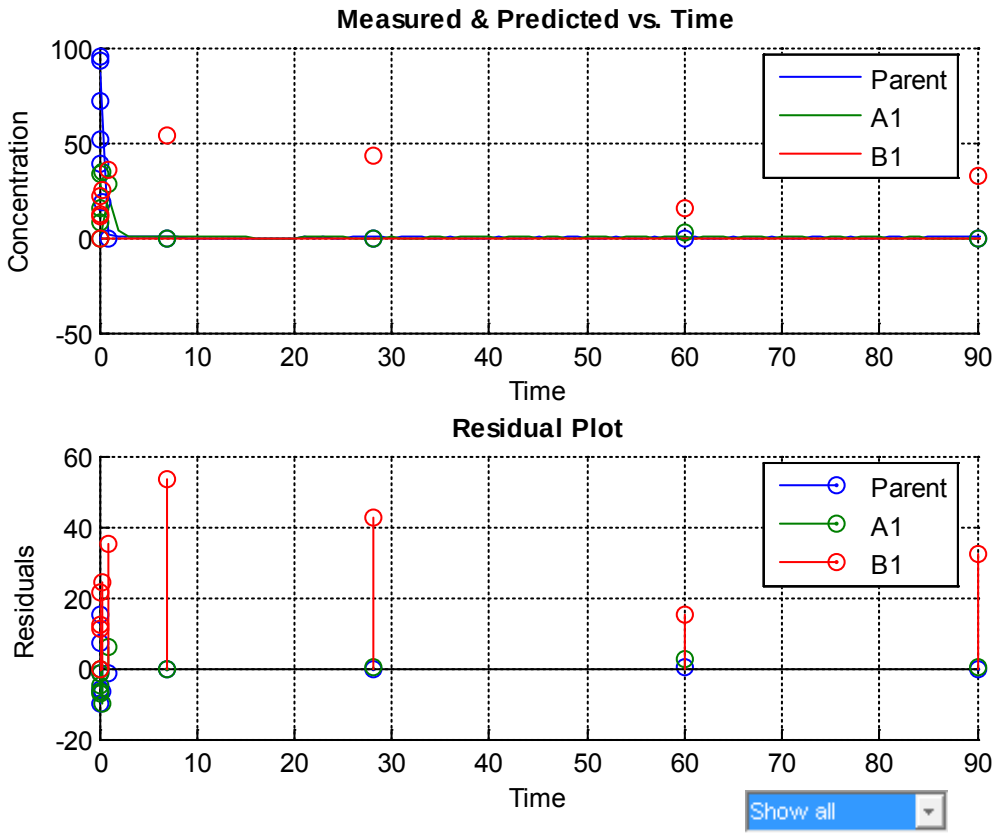


Table A5_3: Measured, predicted and residual values for transformation of the parent compound florfenicol in pig manure (Institute 3, mean of replicates, single first order kinetics)

Time	Florfenicol (parent)		
	Measured [%]	Predicted [%]	residual
0.0	93.3000	86.1812	7.1188
0.021	94.7000	79.3602	15.3398
0.042	71.4000	73.0791	-1.6791
0.083	51.9000	61.9689	-10.0689
0.167	38.5000	44.5589	-6.0589
0.292	18.4000	25.0188	-6.6188
1.0	0.0000	1.3961	-1.3961
7.0	0.0000	0.0000	-0.0000
28.0	0.0000	0.0000	-0.0000

60.0	0.0000	-0.0000	0.0000
90.0	0.0000	0.0000	-0.0000

Figure A5_18: Plots for transformation of florfenicol in pig manure, Institute 4, replicate 1. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of A1, followed by a rapid decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 15.1; chi²-value (TP1) is 59.2.

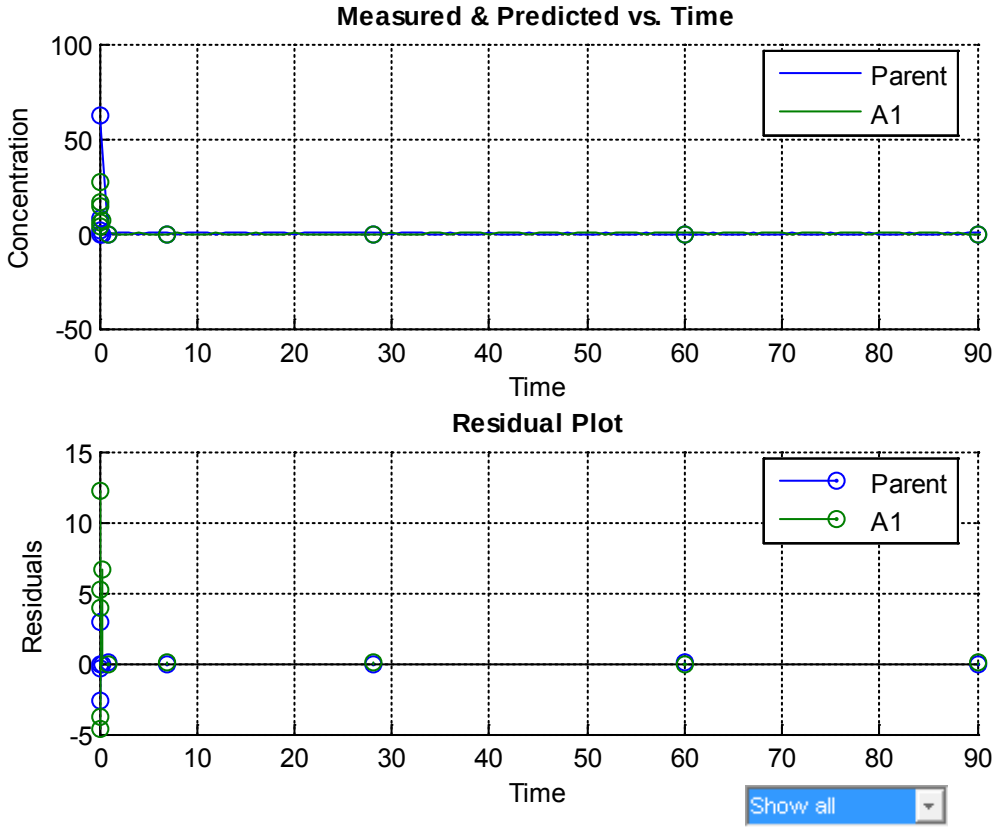


Figure A5_19: Plots for transformation of florfenicol in pig manure, Institute 4, replicate 2. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of A1, followed by a rapid decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 15.0; chi²-value (TP1) is 59.2.

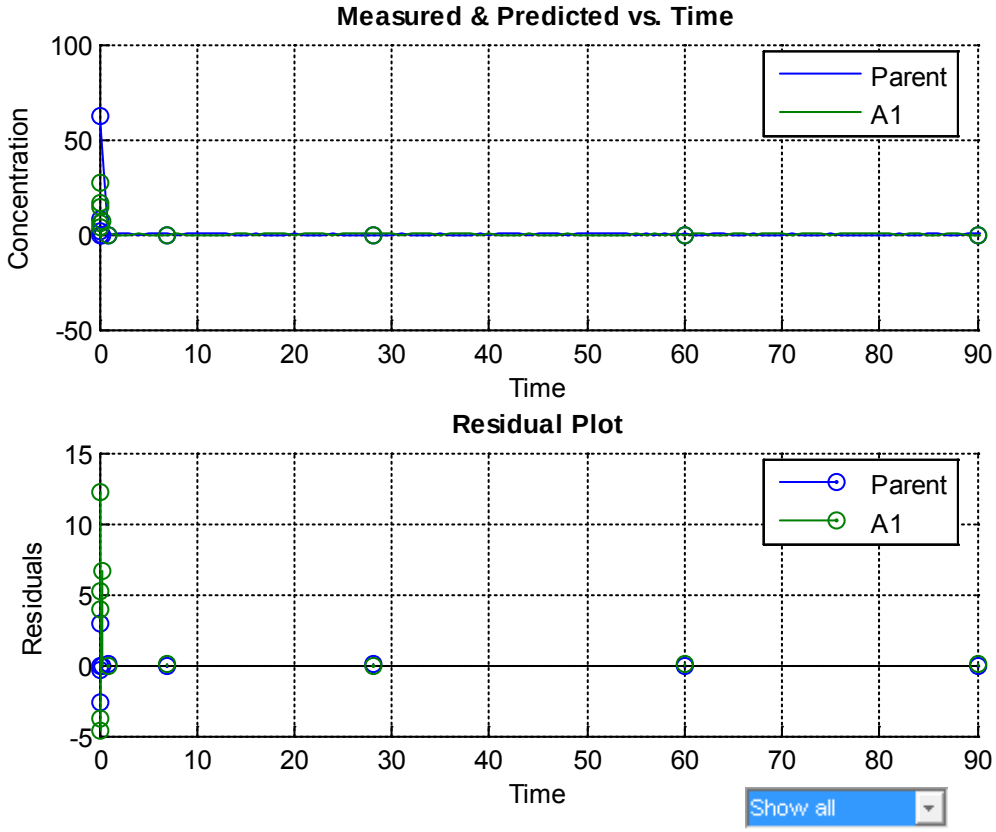


Figure A5_20: Plots for transformation of florfenicol in pig manure, Institute 4, replicate 3. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen accompanied by an increase of A1, followed by a rapid decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 15.0; chi²-value (TP1) is 59.2.

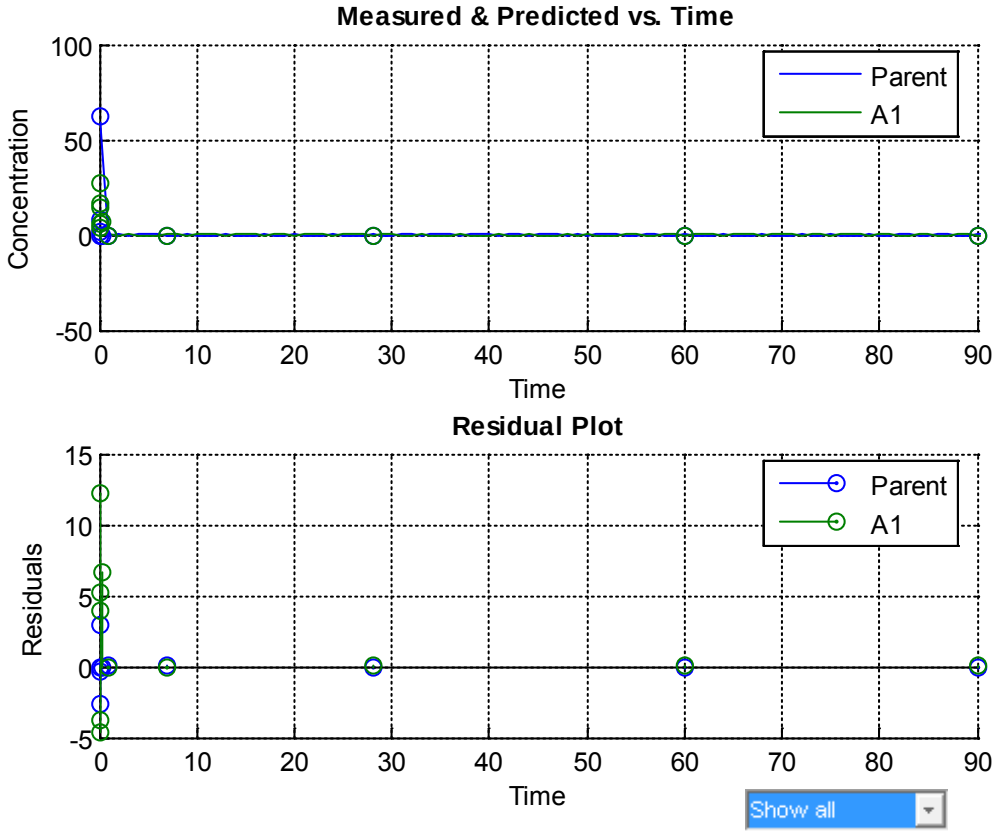


Figure A5_21: Plots for transformation of florfenicol in pig manure, Institute 4, mean of replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A rapid decrease of the parent compound can be seen. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 25.1.

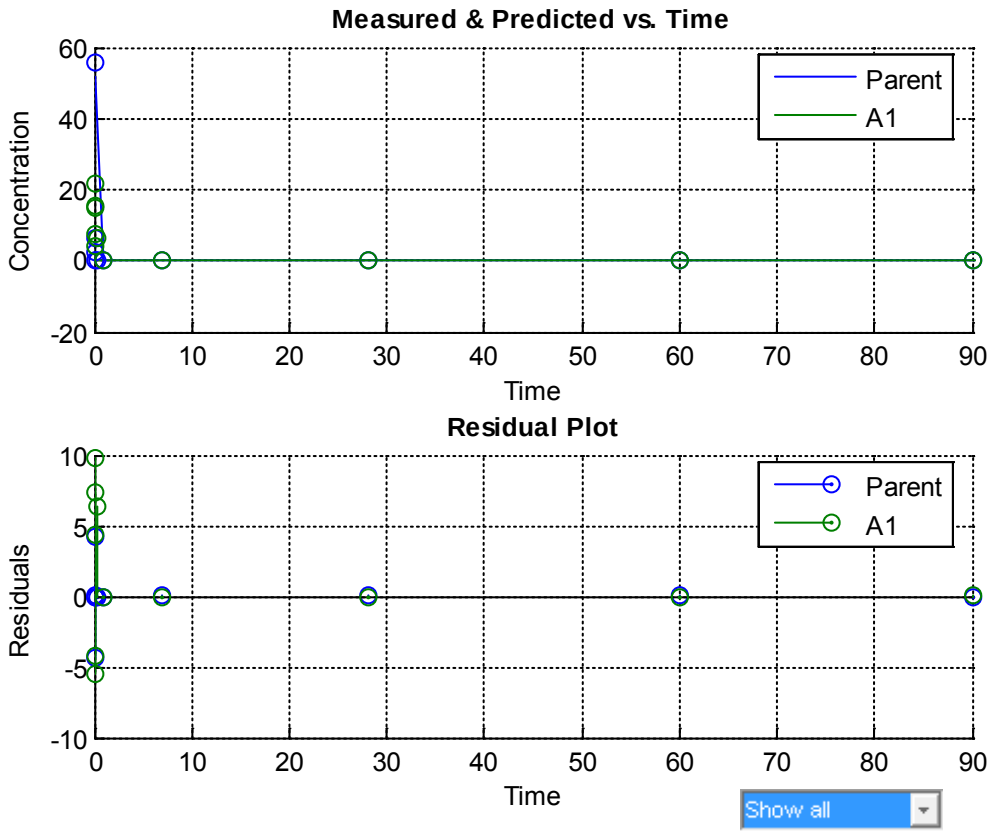


Table A5_4: Measured, predicted and residual values for transformation of the parent compound florfenicol in pig manure (Institute 4, mean of replicates, single first order kinetics)

Time	Florfenicol (parent)		
	Measured [%]	Predicted [%]	residual
0.0	55.8000	51.5930	4.2070
0.021	6.5000	10.8872	-4.3872
0.042	2.2000	2.2974	-0.0974
0.083	0.2000	0.1023	0.0977
0.167	0.0000	0.0002	-0.0002
0.292	0.0000	0.0000	-0.0000
1.0	0.0000	0.0000	-0.0000
7.0	0.0000	-0.0000	0.0000
28.0	0.0000	-0.0000	0.0000

60.0	0.0000	-0.0000	0.0000
90.0	0.0000	0.0000	-0.0000

Figure A5_22: Plots for transformation of florfenicol in pig manure at 10°C, Institute 6, replicate 1 (radiolabeled). The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol and the transformation products TP1, TP2 and TP3 (named as A1, B1 and C1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of transformation products, followed by a decrease. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 33.8

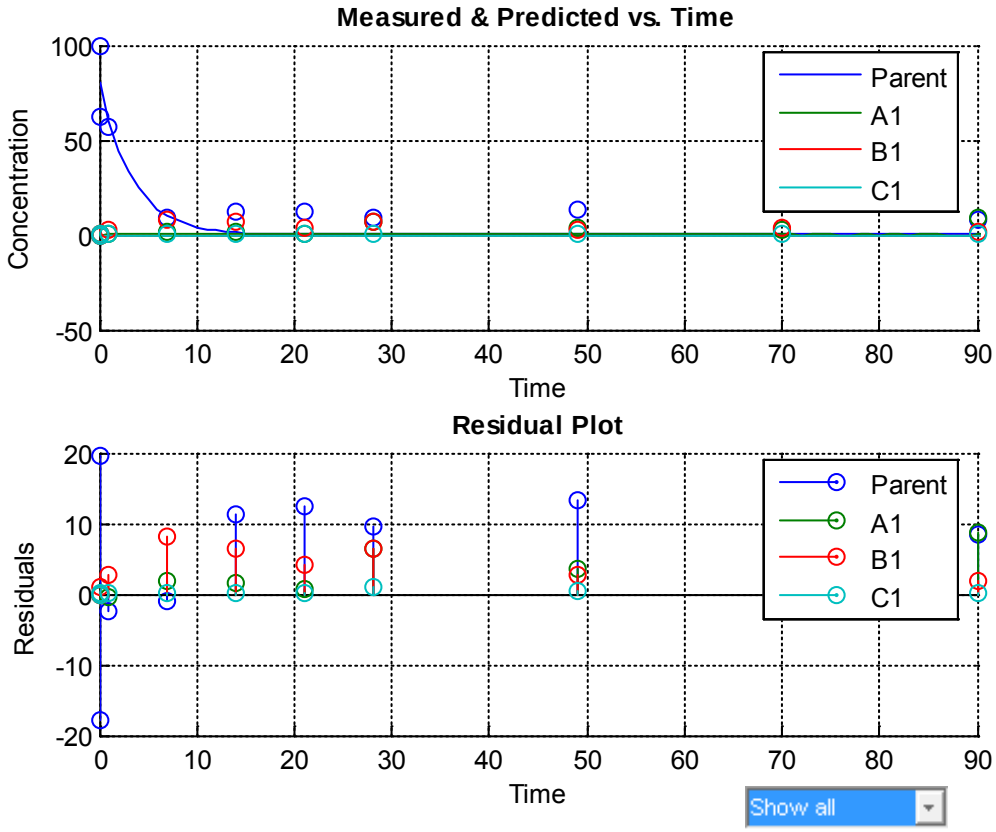


Figure A5_23: Plots for transformation of florfenicol in pig manure at 10°C, Institute 6, replicate 1 (unlabeled). The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol (using the CAKE software tool, version 3.1). A decrease of the parent compound can be seen. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 30.2

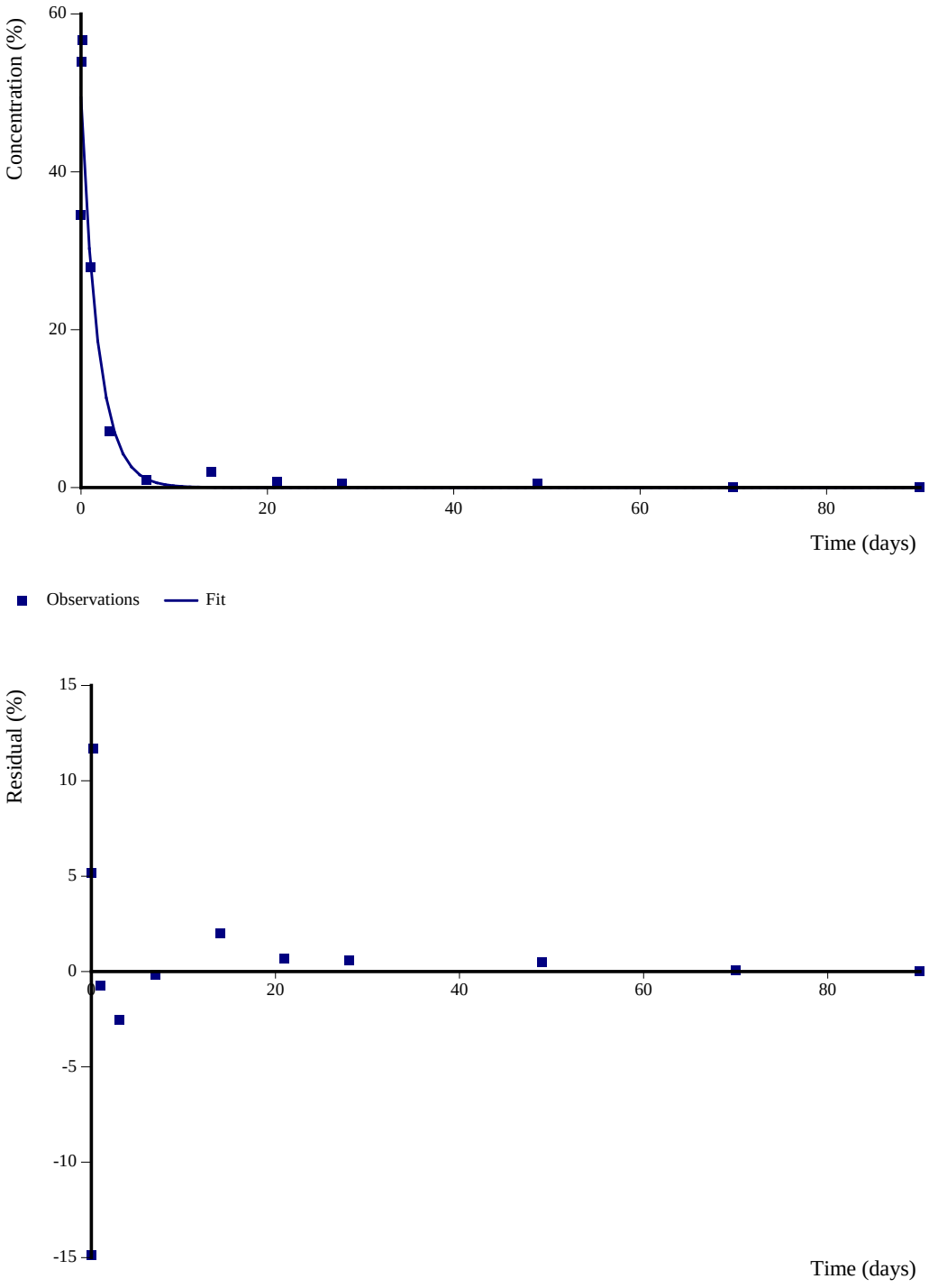


Figure A5_24: Plots for transformation of florfenicol in pig manure at 10°C, Institute 6, replicate 2 (unlabeled). The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol (using the CAKE software tool, version 3.1). A decrease of the parent compound can be seen. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 30.3

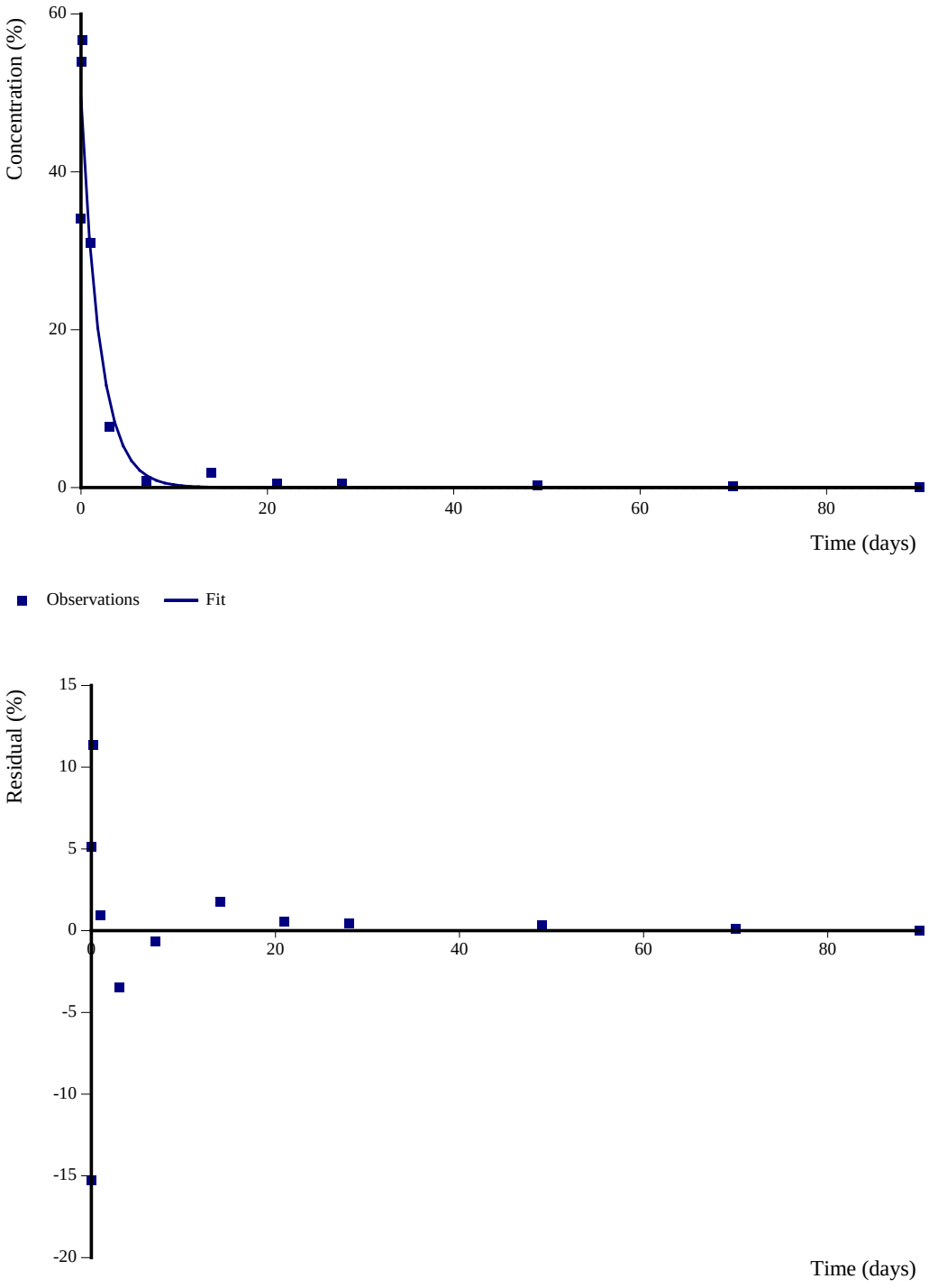


Figure A5_25: Plots for transformation of florfenicol in pig manure at 10°C, Institute 6, mean of replicates (unlabeled). The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound florfenicol (using the CAKE software tool, version 3.1). A decrease of the parent compound can be seen. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 30.1

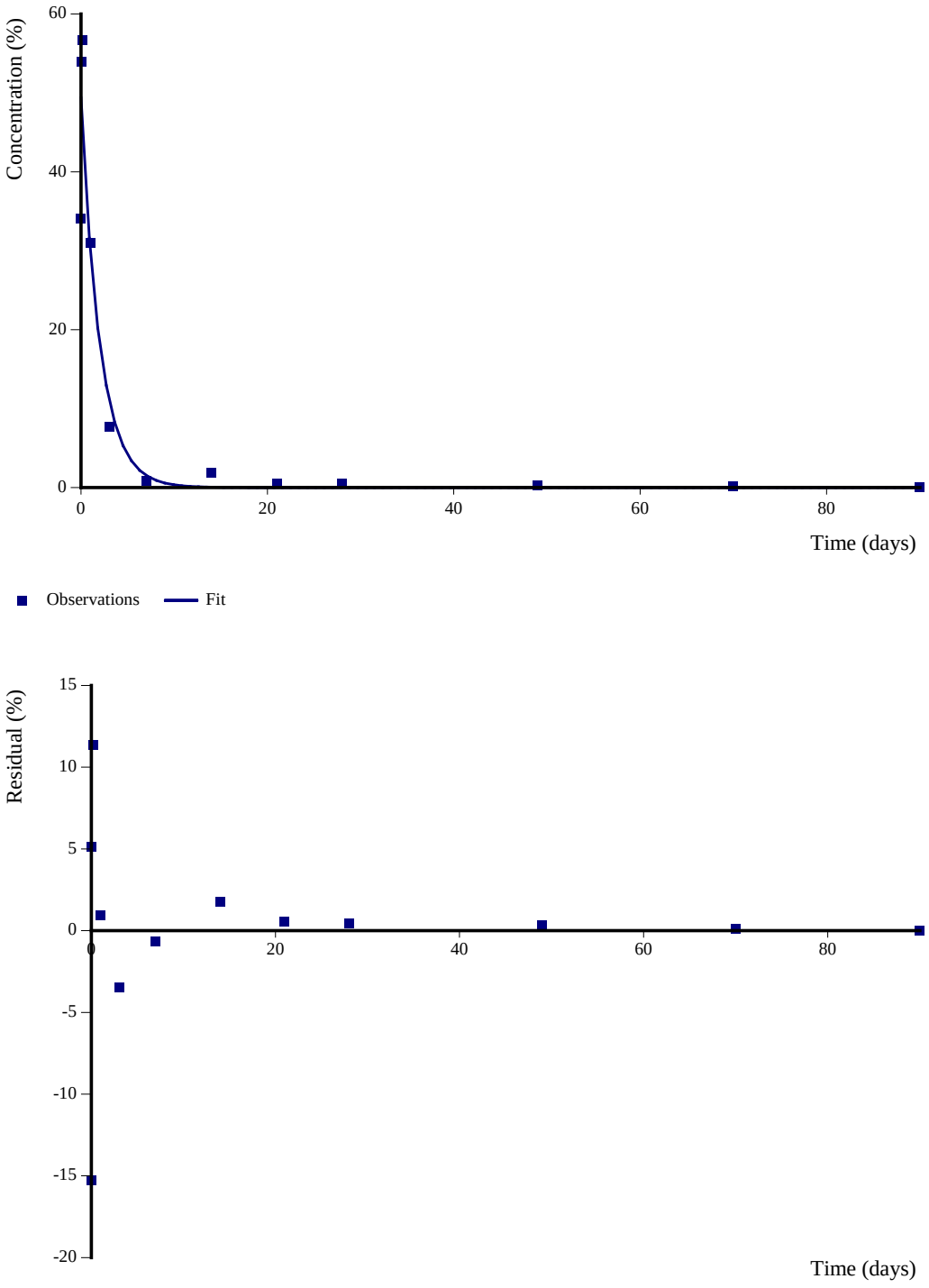


Table A5_5: Measured, predicted and residual values for transformation of the parent compound florfenicol at 10°C in pig manure (Institute 6, mean of replicates, single first order kinetics)

Time	Florfenicol (parent)		
	Measured [%]	Predicted [%]	residual
0	34.12	49.38	-15.26
0.02	54.00	48.90	5.10
0.17	56.73	45.39	11.34
1	31.03	30.07	0.96
3	7.68	11.15	-3.47
7	0.88	1.53	-0.65
14	1.84	0.05	1.79
21	0.55	0.00	0.55
28	0.46	0.00	0.46
49	0.33	0.00	0.33
70	0.13	0	0.13
90	0.02	0	0.02

Table A5_6: Overview on chi²-values of all replicates and measurements for the parent compound florfenicol (SFO-kinetics)

Test substance	Florfenicol (parent)				
Institute	1	2	3	4	6 [2)]
Replicate 1	19.503	23.250	15.710	15.066	30.200
Replicate 2	21.286	22.940	18.625	14.986	30.300
Replicate 3	14.476	25.090	n.a.	14.977	n.a.
Replicate 4	15.270	21.781	n.a.	n.a.	n.a.
Replicate 5	18.550	18.537	n.a.	n.a.	n.a.
Replicate 6	10.620	15.295	n.a.	n.a.	n.a.
N	6	6	2	3	2
Mean	16.618	21.149	17.168	15.010	30.250
SD	3.905	3.596	2.061	0.049	0.071
COV (%)	23.497	17.002	12.007	0.325	0.234
Minimum	16.618 [1)]				
Maximum	30.300 [1)]				
Overall mean	21.296 [1)]				
Overall SD	6.302 [1)]				
Overall COV (%)	29.590 [1)]				

n.a. = not analyzed; [1] Results from Institute 4 are not considered for evaluation; [2] results obtained for unlabeled florfenicol at 10 ± 2°C

Table A5_7: Dissipation of florfenicol at 20°C in pig manure (overall mean and standard deviation) based on predicted values (SFO kinetics)

Day	Institute 1 Mean	Institute 2 Mean	Institute 3 Mean	Overall Mean	Standard Deviation
0	97.09	86.47	86.18	89.9	6.2
0.021	93.97	81.54	79.36	85.0	7.9
0.042	90.95	76.90	73.08	80.3	9.4
0.083	85.19	68.57	61.97	71.9	12.0
0.167	74.75	54.23	44.56	57.8	15.4
0.292	59.46	38.24	25.02	40.9	17.4
1	18.94	5.29	1.40	8.5	9.2
7	0.00	0.00	0.00	0.0	0.0
28	0.00	0.00	0.00	0.0	0.0
59	0.00	0.00	0.00	0.0	0.0
90	0.00	0.00	0.00	0.0	0.0

Table A5_8: Dissipation of unlabeled florfenicol at 10°C in pig manure (mean and standard deviation) based on predicted values (SFO kinetics)

Day	Institute 6 Replicate 1	Institute 6 Replicate 2	Mean	Standard Deviation
0	49.38	49.45	49.42	0.05
0.02	48.85	49.00	48.93	0.11
0.17	45.02	45.76	45.39	0.52
1	28.66	31.33	30.00	1.89
3	9.65	12.57	11.11	2.06
7	1.10	2.03	1.56	0.66
14	0.02	0.08	0.05	0.04
21	0.00	0.00	0.00	0.00
28	0.00	0.00	0.00	0.00
49	0.00	0.00	0.00	0.00
70	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00

Figure A5_26: Plots for transformation of imidacloprid in cattle manure, Institute 1, replicate 1. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 14.9; chi²-value (TP1) is 17.7.

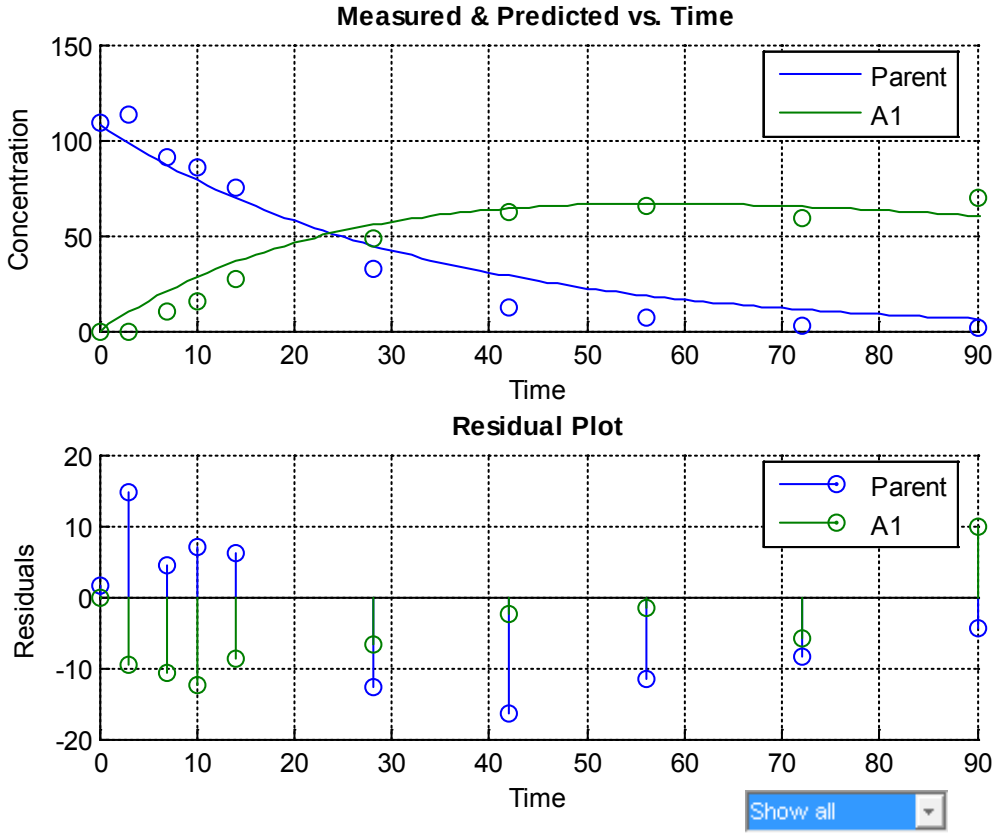


Figure A5_27: Plots for transformation of imidacloprid in cattle manure, Institute 1, replicate 2. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 12.8; chi²-value (TP1) is 16.8.

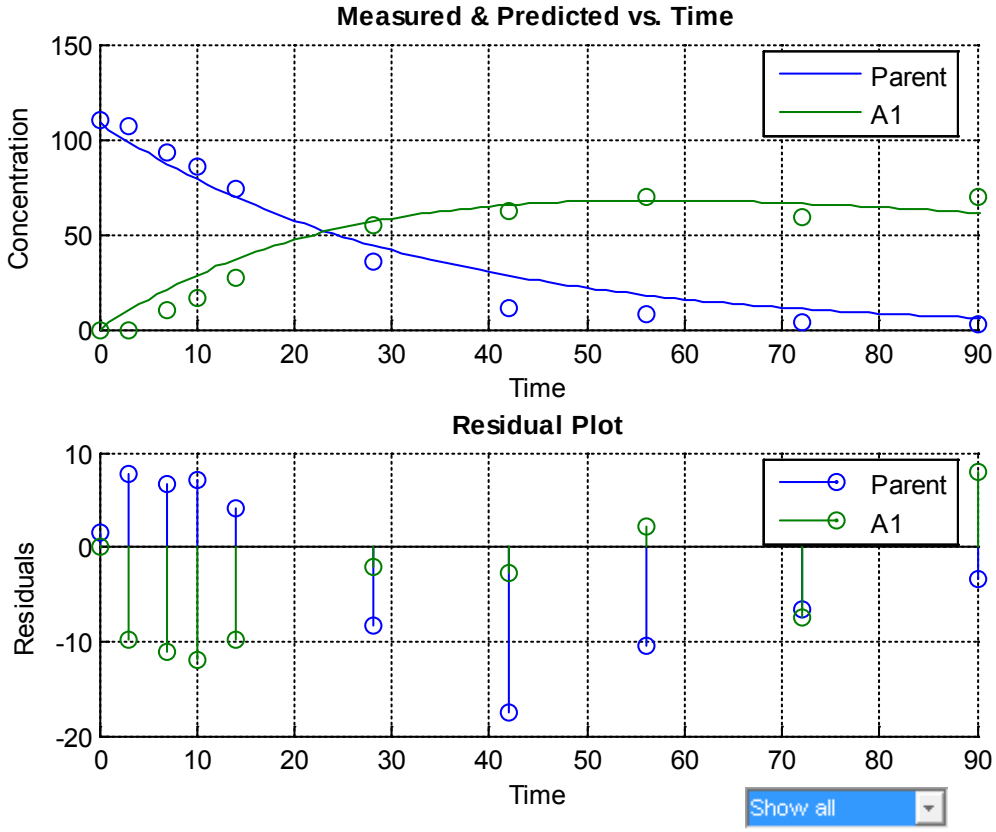


Figure A5_28: Plots for transformation of imidacloprid in cattle manure, Institute 1, replicate 3. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 12.0; chi²-value (TP1) is 16.5.

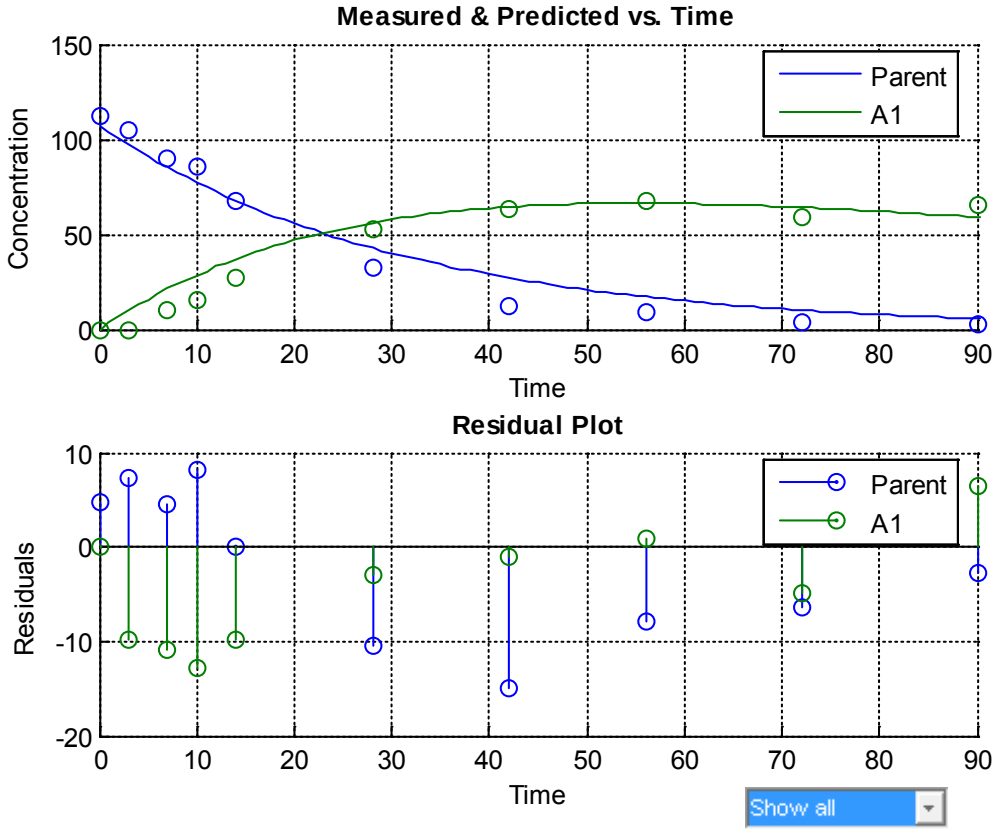


Figure A5_29: Plots for transformation of imidacloprid in cattle manure, Institute 1, replicate 4. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 13.3; chi²-value (TP1) is 19.4.

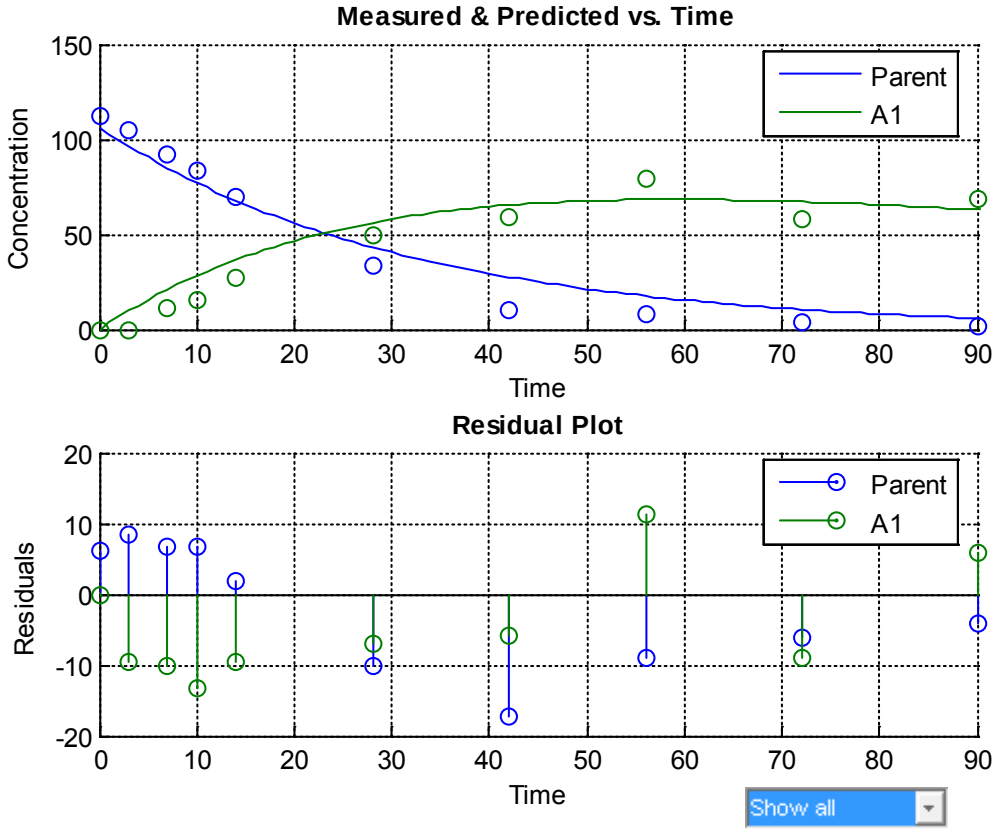


Figure A5_30: Plots for transformation of imidacloprid in cattle manure, Institute 1, replicate 5. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 12.7; chi²-value (TP1) is 15.8.

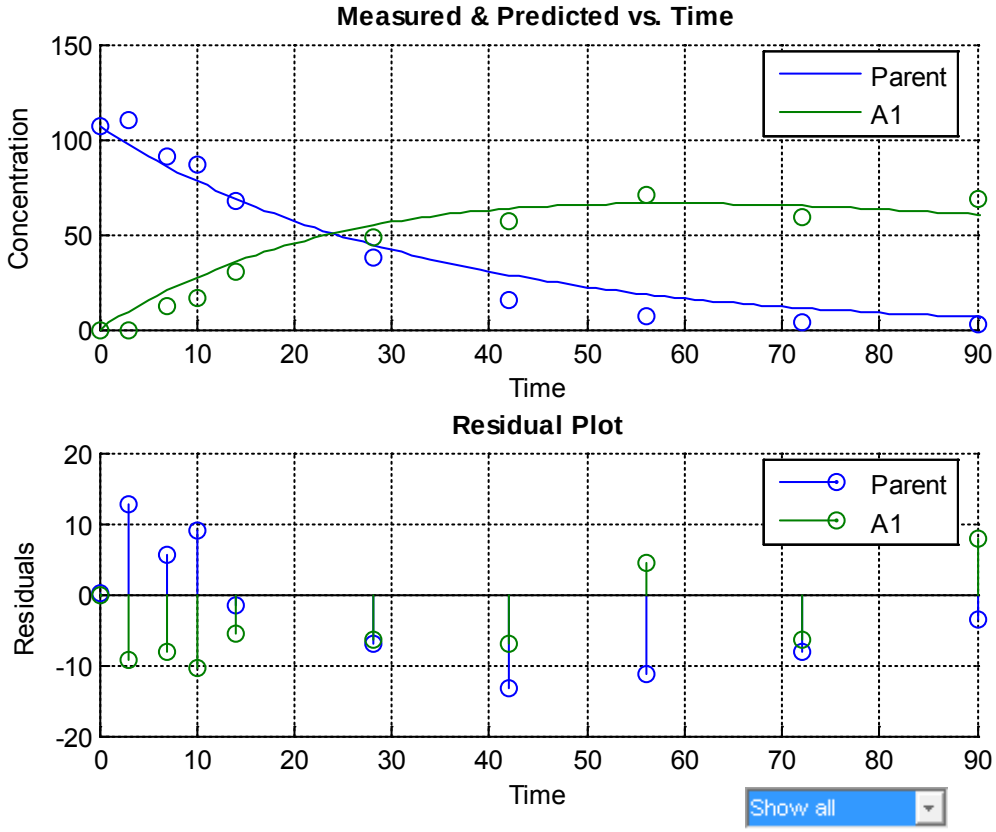


Figure A5_31: Plots for transformation of imidacloprid in cattle manure, Institute 1, replicate 6. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 14.0; chi²-value (TP1) is 16.4.

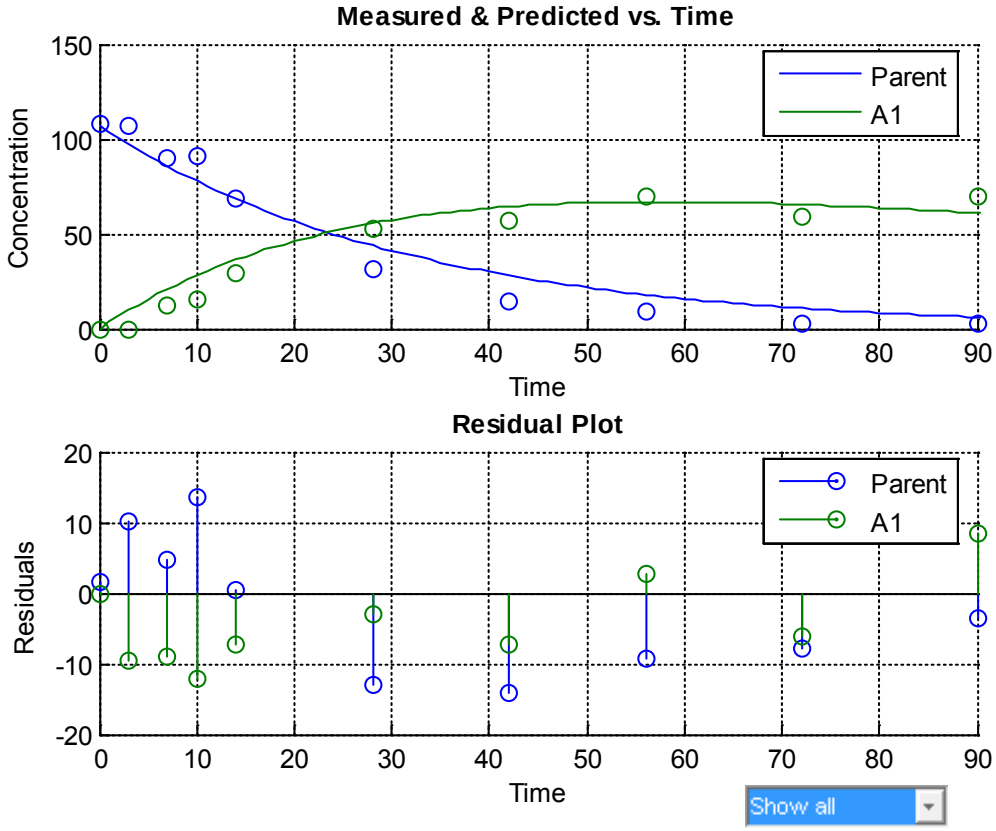


Figure A5_32: Plots for transformation of imidacloprid in cattle manure, Institute 1, mean of replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 12.9.

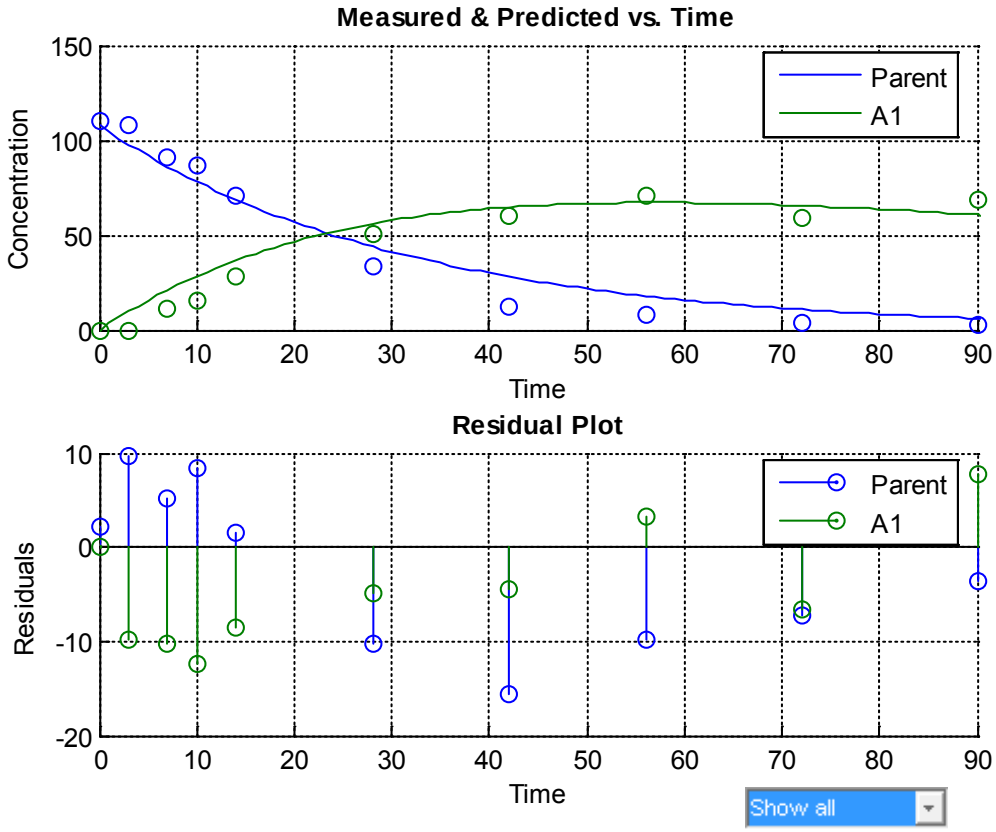


Table A5_9: Measured, predicted and residual values for transformation of the parent compound imidacloprid in cattle manure (Institute 1, mean of replicates, single first order kinetics)

Time	Florfenicol (parent)		
	Measured [%]	Predicted [%]	residual
0.0	109.9000	107.7284	2.1716
3.0	107.6000	97.8586	9.7414
7.0	91.3000	86.0909	5.2091
10.0	86.5000	78.2035	8.2965
14.0	70.4000	68.7994	1.6006
28.0	33.7000	43.9379	-10.2379
42.0	12.5000	28.0604	-15.5604
56.0	8.0000	17.9204	-9.9204
72.0	3.5000	10.7345	-7.2345

90.0	2.4000	6.0311	-3.6311

Figure A5_33: Plots for transformation of imidacloprid in cattle manure, Institute 2, replicate 1. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 9.0; chi²-value (TP1) is 9.1.

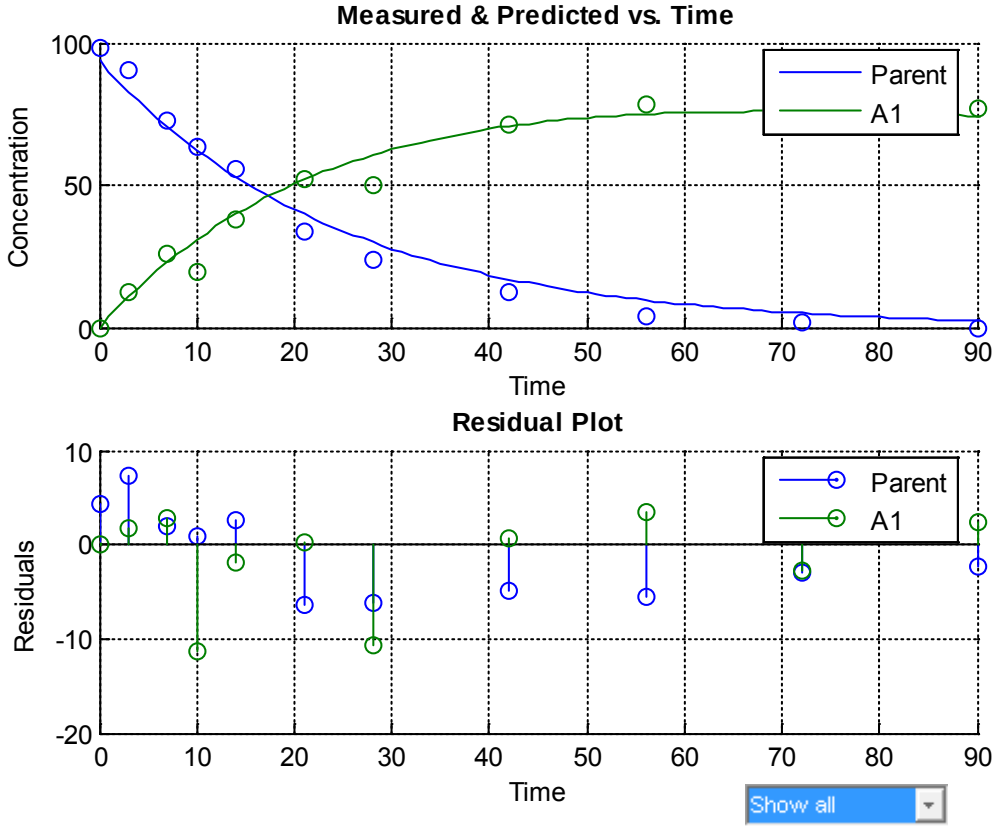


Figure A5_34: Plots for transformation of imidacloprid in cattle manure, Institute 2, replicate 2. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 10.5; chi²-value (TP1) is 9.7.

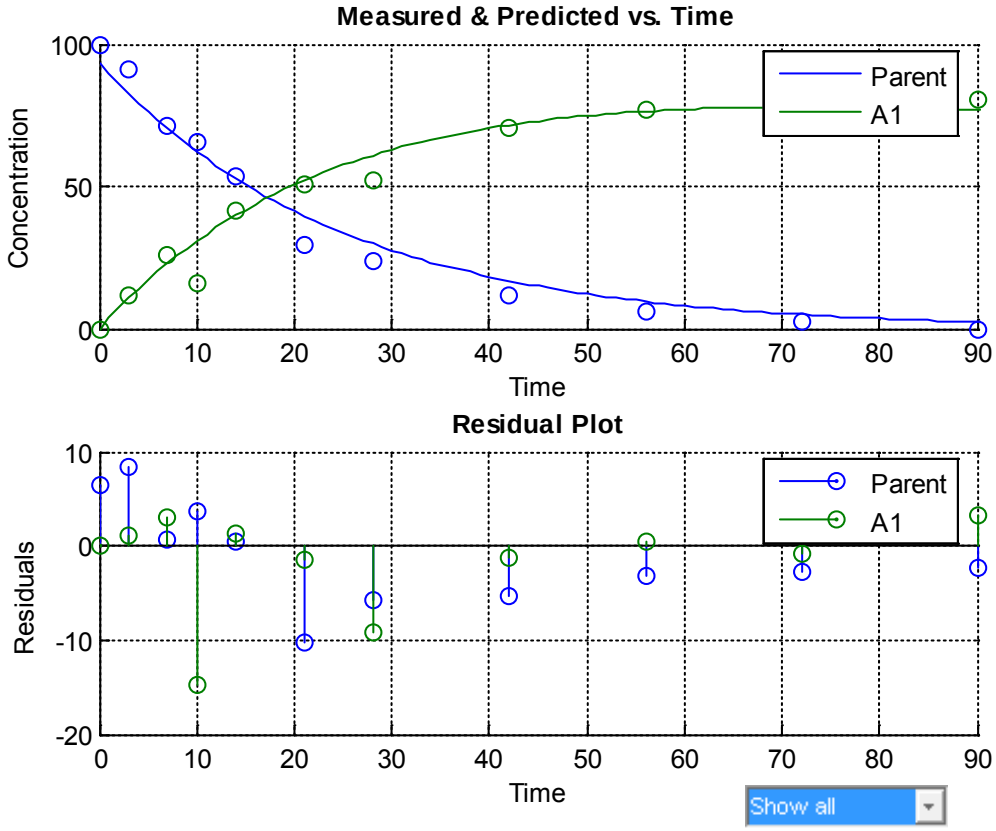


Figure A5_35: Plots for transformation of imidacloprid in cattle manure, Institute 2, replicate 3. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 10.2; chi²-value (TP1) is 7.7.

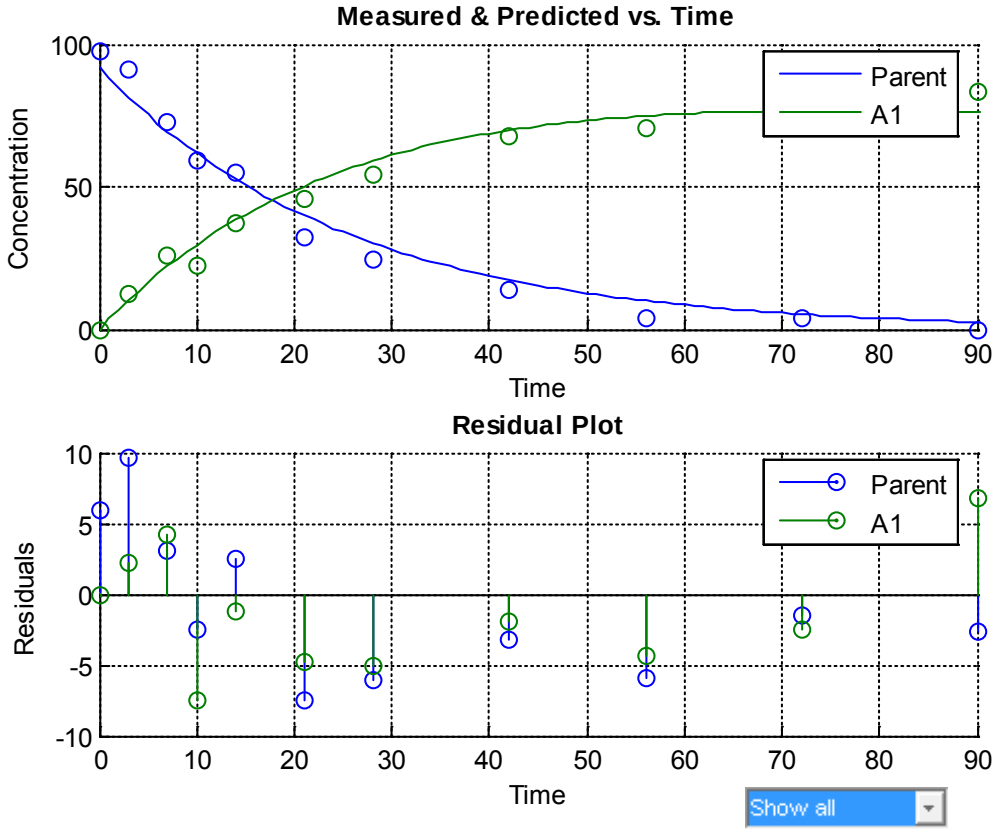


Figure A5_36: Plots for transformation of imidacloprid in cattle manure, Institute 2, replicate 4. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 9.7; chi²-value (TP1) is 8.2.

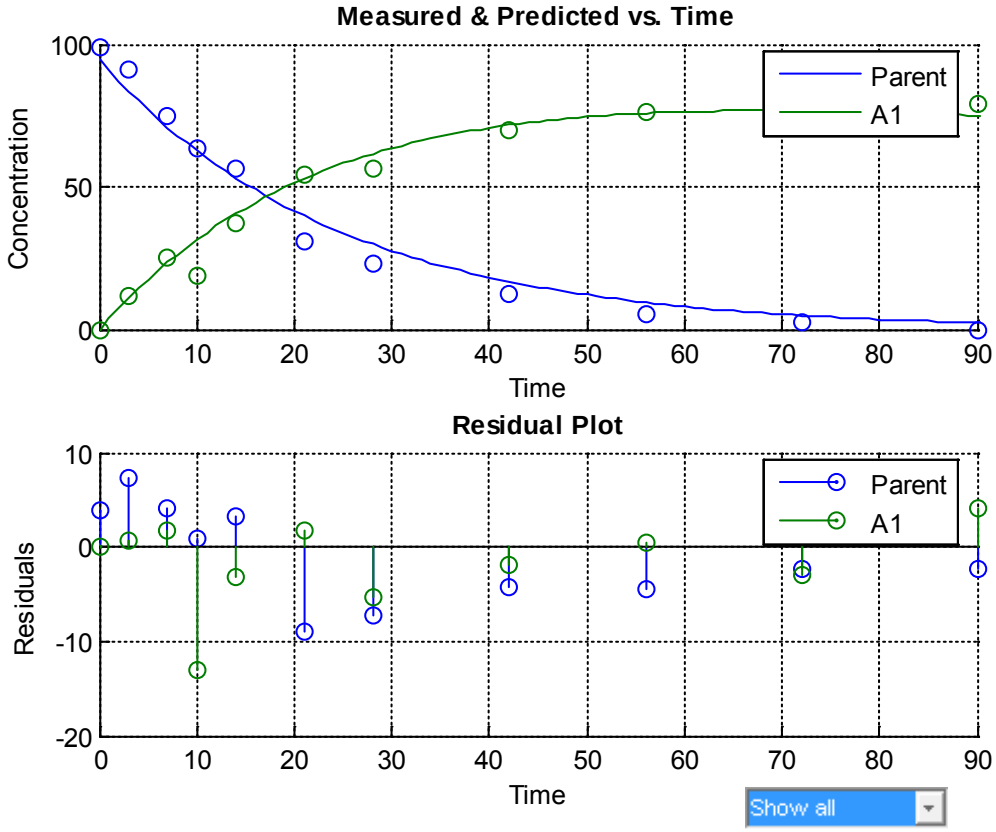


Figure A5_37: Plots for transformation of imidacloprid in cattle manure, Institute 2, replicate 5. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 17.9, chi²-value (TP1) is 16.4.

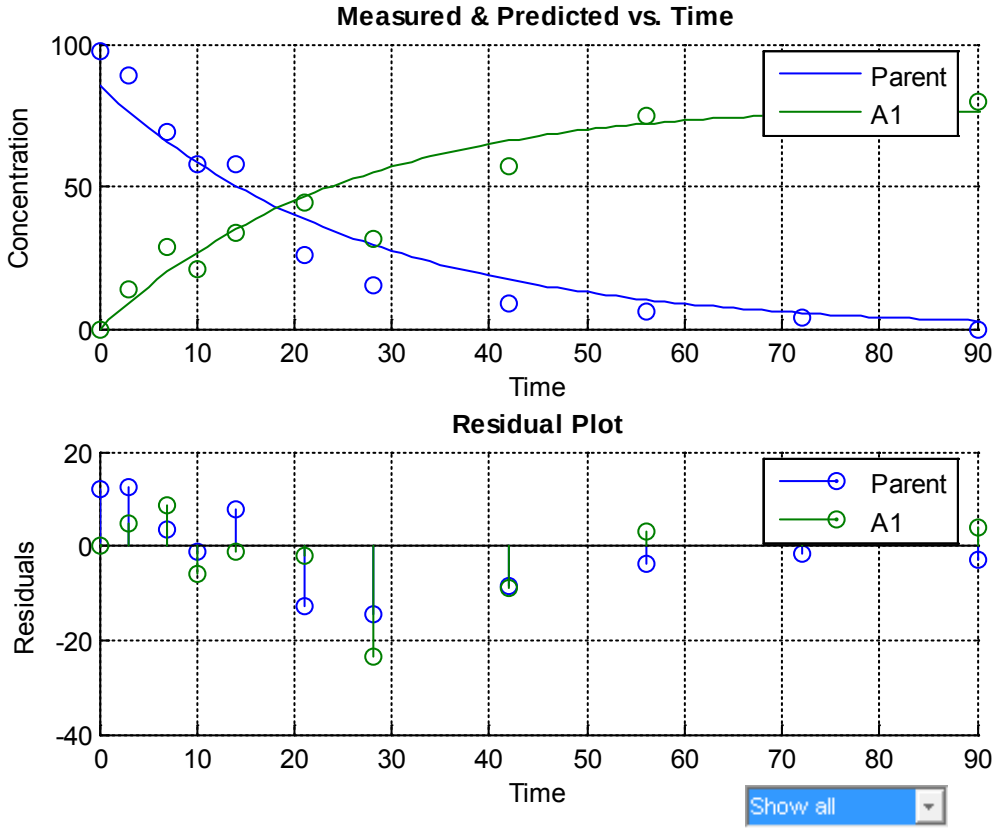


Figure A5_38: Plots for transformation of imidacloprid in cattle manure, Institute 2, replicate 6. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 17.8, chi²-value (TP1) is 32.1.

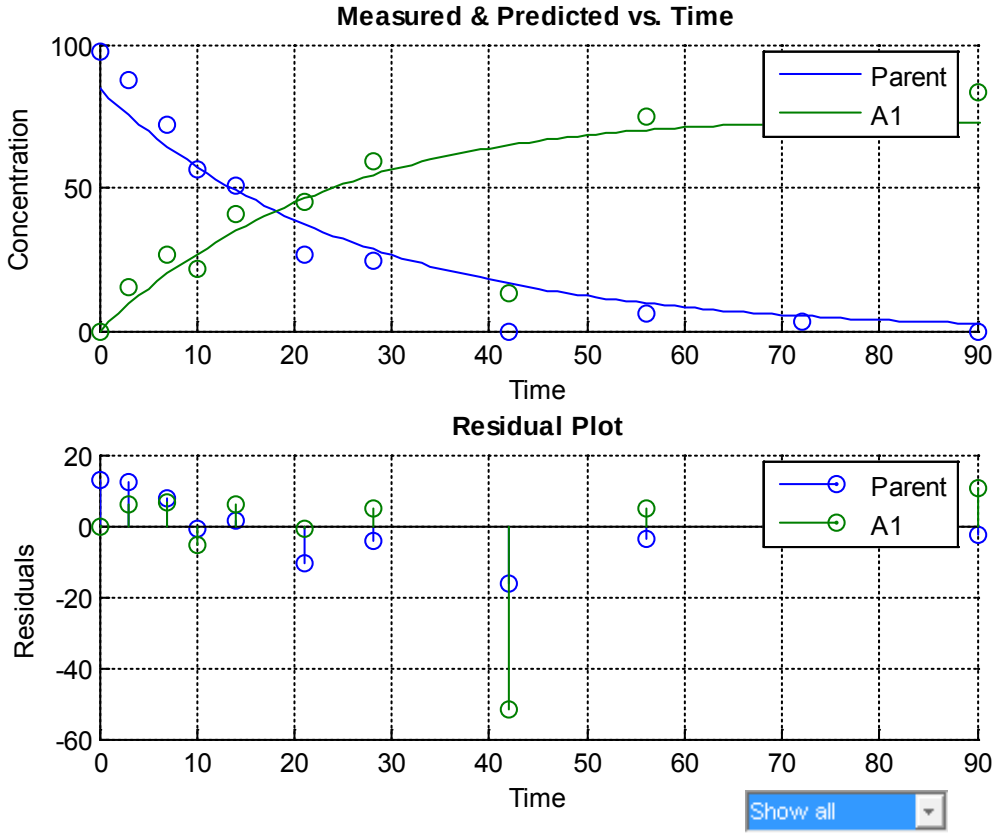


Figure A5_39: Plots for transformation of imidacloprid in cattle manure, Institute 2, mean of replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 11.9.

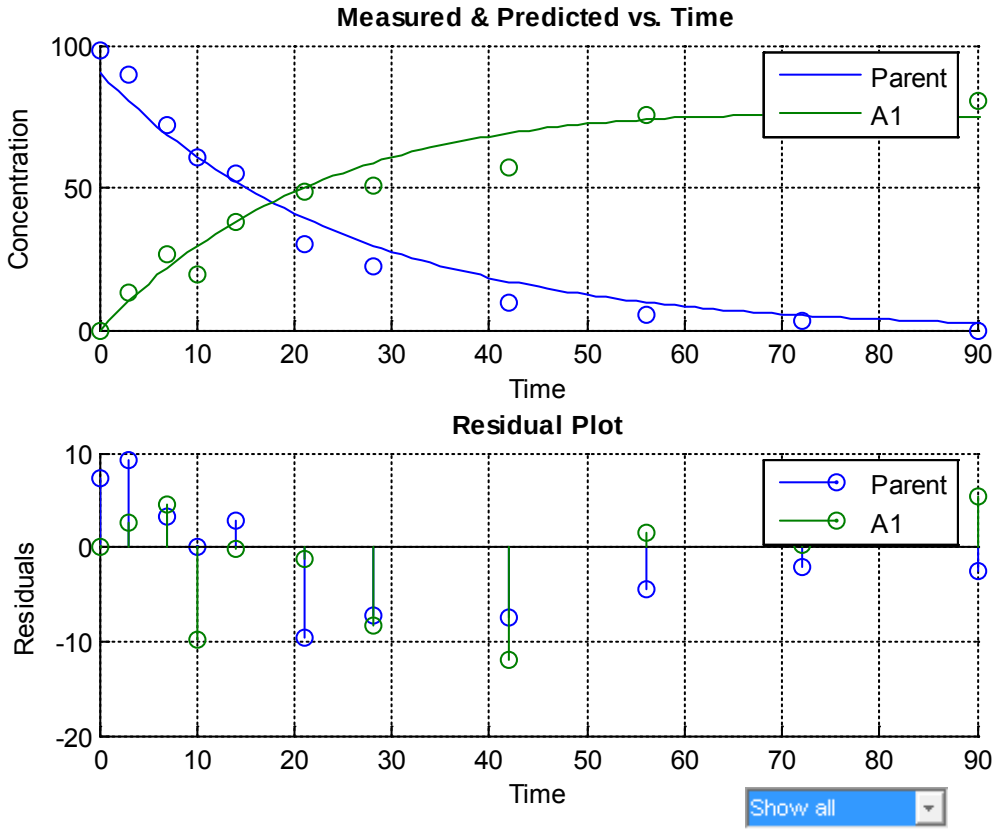


Table A5_10: Measured, predicted and residual values for transformation of the parent compound imidacloprid in cattle manure (Institute 2, mean of replicates, single first order kinetics)

Time	Florfenicol (parent)		
	Measured [%]	Predicted [%]	residual
0.0	98.1000	90.7744	7.3256
3.0	89.9000	80.5445	9.3555
7.0	72.0000	68.6751	3.3249
10.0	60.9000	60.9358	-0.0358
14.0	54.8000	51.9560	2.8440
21.0	29.8000	39.3072	-9.5072
28.0	22.4000	29.7378	-7.3378
42.0	9.6000	17.0209	-7.4209
56.0	5.3000	9.7421	-4.4421

72.0	3.0000	5.1488	-2.1488
90.0	0.0000	2.5127	-2.5127

Figure A5_40: Plots for transformation of imidacloprid in cattle manure, Institute 3, replicate 1. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation products TP1 and TP2 (named as A1 and B1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1 and TP2. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 18.7, chi²-value (TP1) is 33.4; chi²-value (TP2) is 97.0.

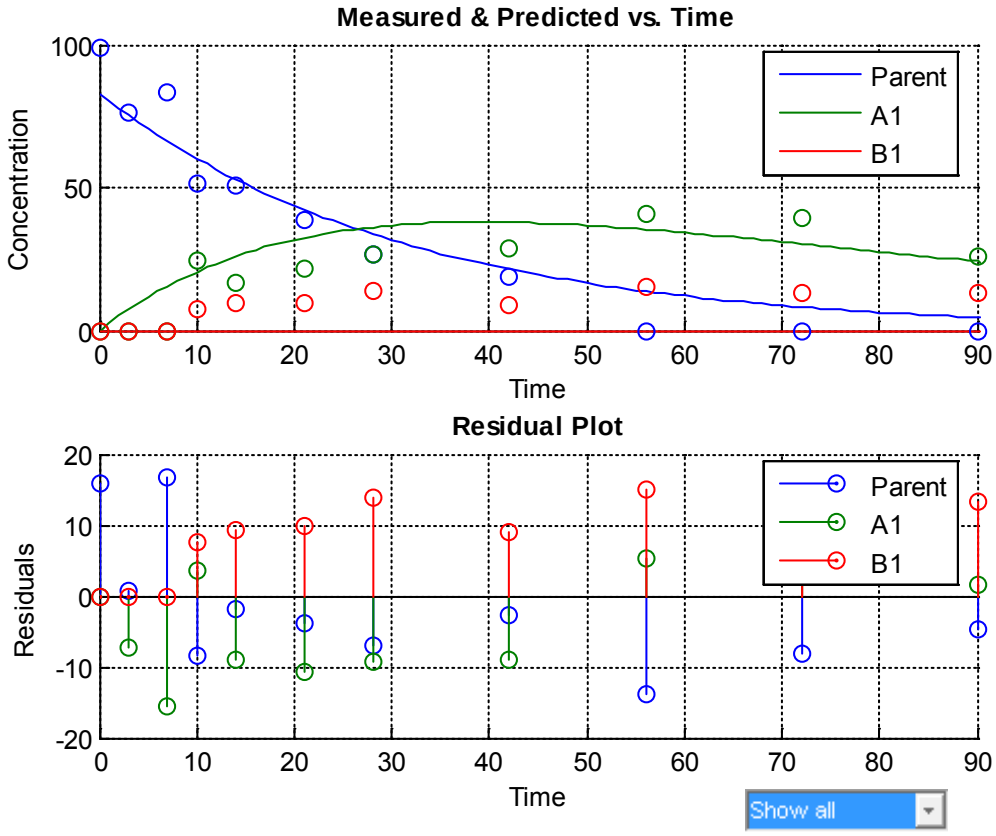


Figure A5_41: Plots for transformation of imidacloprid in cattle manure, Institute 3, replicate 2. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation products TP1 and TP2 (named as A1 and B1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1 and TP2. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 19.7, chi²-value (TP1) is 33.2; chi²-value (TP2) is 113.9.

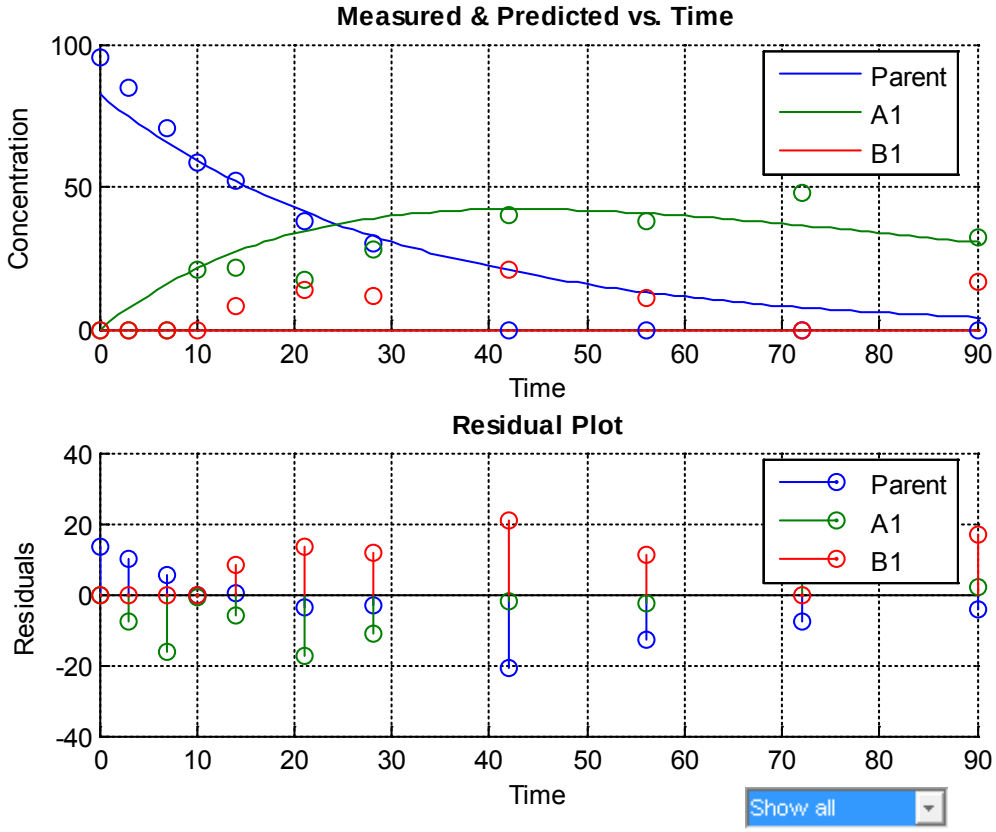


Figure A5_42: Plots for transformation of imidacloprid in cattle manure, Institute 3, mean of replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation products TP1 and TP2 (named as A1 and B1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1 and TP2. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 17.4.

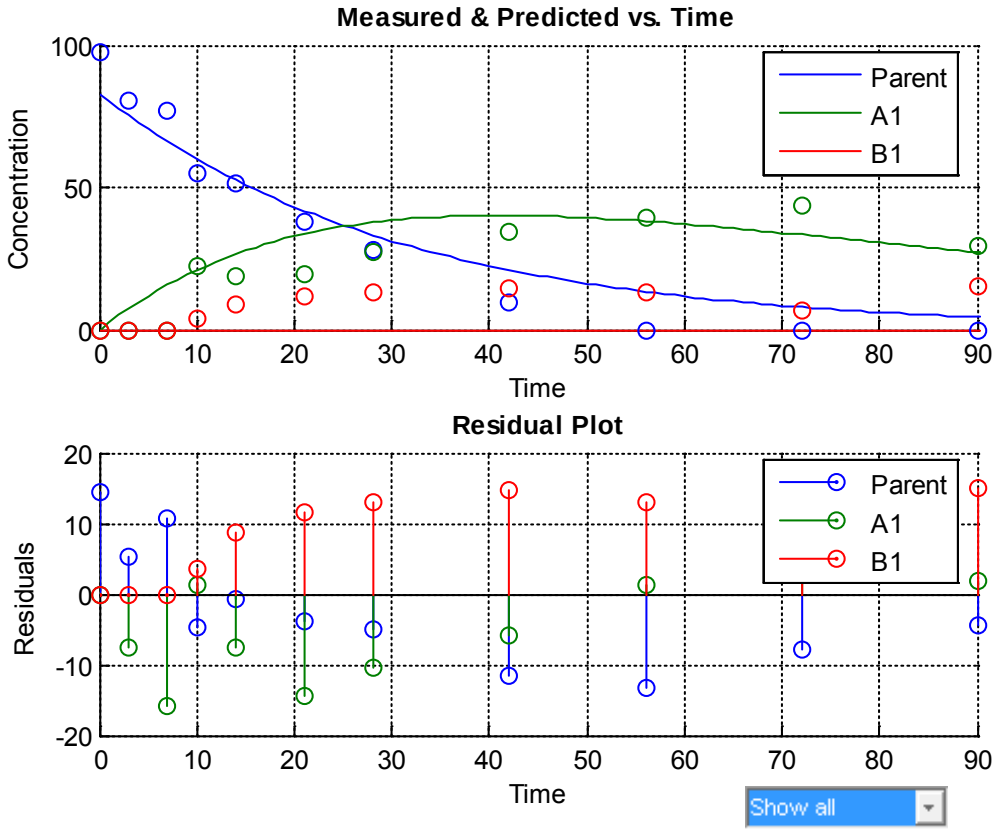


Table A5_11: Measured, predicted and residual values for transformation of the parent compound imidacloprid in cattle manure (Institute 3, mean of replicates, single first order kinetics)

Time	Florfenicol (parent)		
	Measured [%]	Predicted [%]	residual
0.0	97.3000	82.9211	14.3789
3.0	80.4000	75.1935	5.2065
7.0	76.8000	65.9985	10.8015
10.0	55.2000	59.8479	-4.6479
14.0	51.7000	52.5295	-0.8295
21.0	38.0000	41.8092	-3.8092
28.0	28.3000	33.2768	-4.9768
42.0	9.4000	21.0804	-11.6804
56.0	0.0000	13.3542	-13.3542

72.0	0.0000	7.9256	-7.9256
90.0	0.0000	4.4068	-4.4068

Figure A5_43: Plots for transformation of imidacloprid in cattle manure, Institute 4, replicate 1. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by a low increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 6.2, chi²-value (TP1) is 60.7.

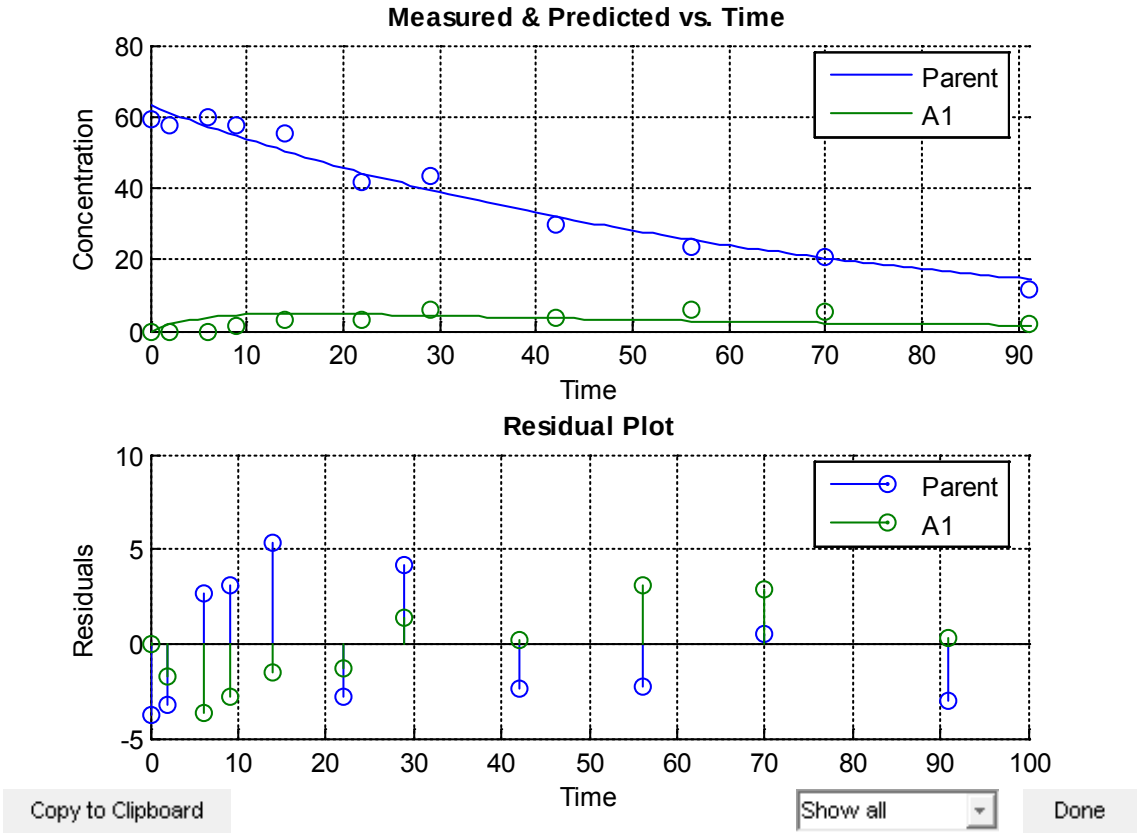


Figure A5_44: Plots for transformation of imidacloprid in cattle manure, Institute 4, replicate 2. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by a low increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 8.8, chi²-value (TP1) is 63.8.

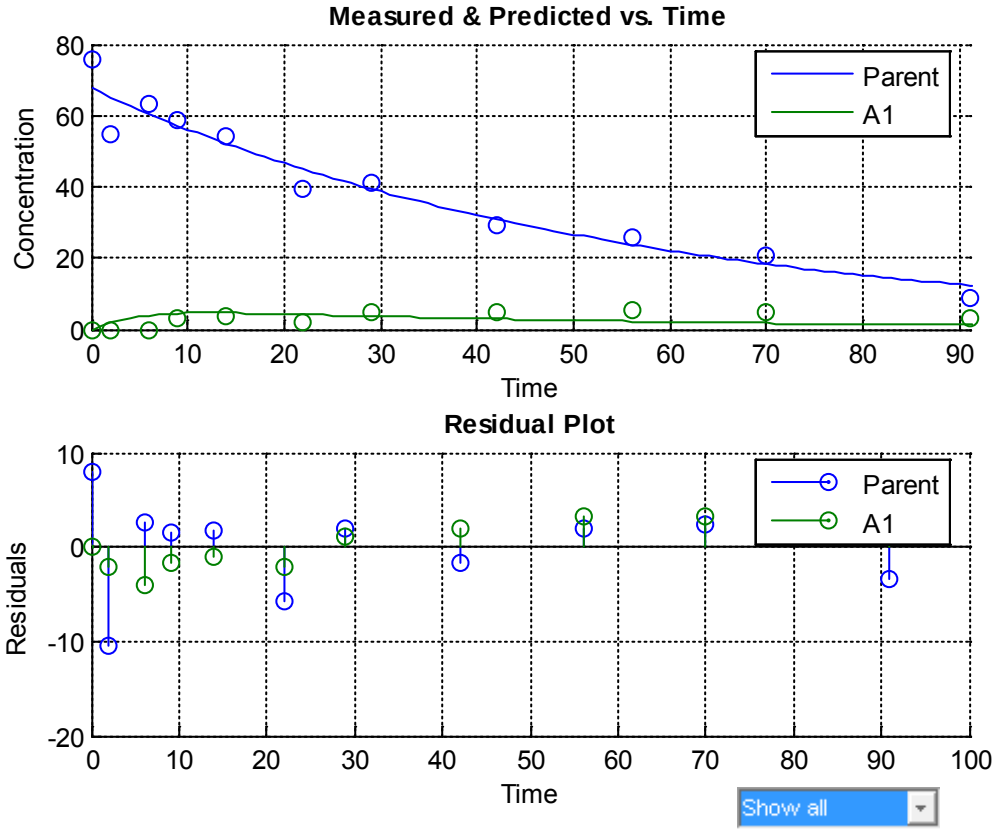


Figure A5_45: Plots for transformation of imidacloprid in cattle manure, Institute 4, replicate 3. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by a low increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 10.3, chi²-value (TP1) is 54.1.

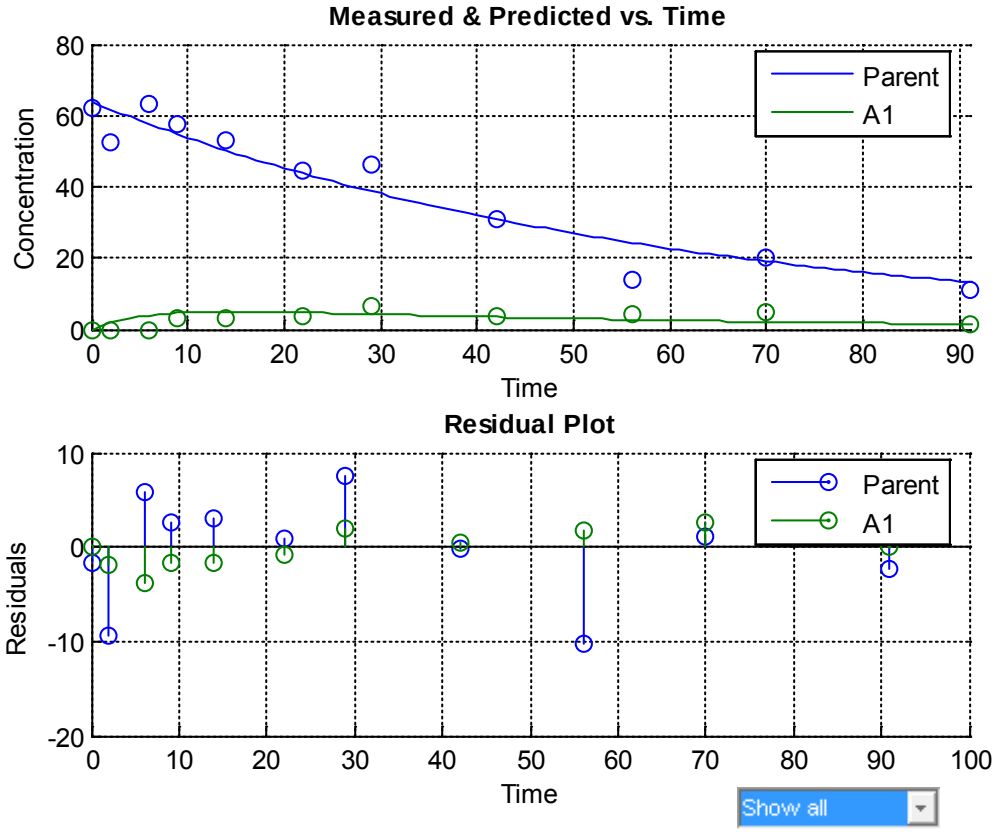


Figure A5_46: Plots for transformation of imidacloprid in cattle manure, Institute 4, mean of replicates. The measured values and the values calculated by means of applying the single first order kinetic model are shown for the parent compound imidacloprid and the transformation product TP1 (named as A1 in the nomenclature of the KinGUI software tool). A decrease of the parent compound can be seen accompanied by an increase of TP1. Furthermore, in the graph below, the difference between measured value and calculated value is given. Chi²-value (parent) is 6.9.

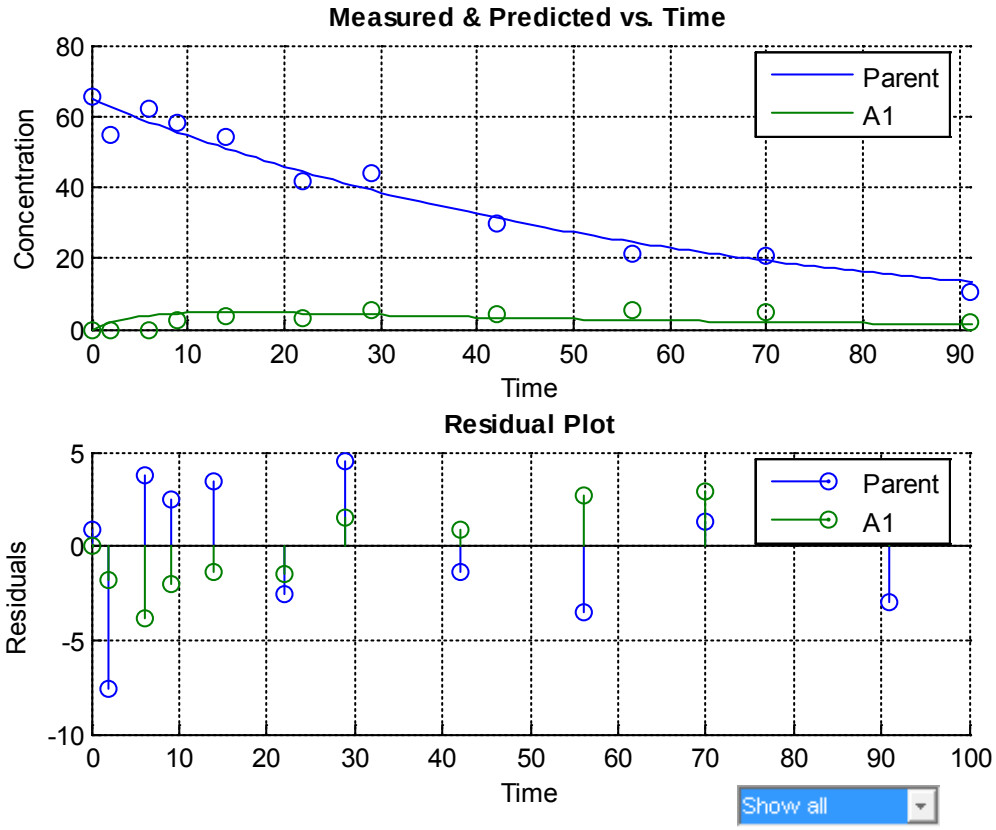


Table A5_12: Measured, predicted and residual values for transformation of the parent compound imidacloprid in cattle manure (Institute 4, mean of replicates, single first order kinetics)

Time	Florfenicol (parent)		
	Measured [%]	Predicted [%]	residual
0.0	65.6500	64.7628	0.8872
2.0	55.0000	62.5619	7.5619
6.0	62.1000	58.3821	3.7179
9.0	57.9100	55.4315	2.4785
14.0	54.2500	50.8415	3.4085
22.0	41.7200	44.2749	-2.5549
29.0	43.7500	39.2288	4.5212
42.0	29.9300	31.3333	-1.4033
56.0	21.0800	24.5980	-3.5180

70.0	20.6600	19.3105	1.3495
91.0	10.4800	13.4318	-2.9518

Table A5_13: Overview on chi²-values of all replicates and measurements for the parent compound imidacloprid (SFO-kinetics)

Test substance	Imidacloprid (parent)			
Institute	**1**	**2**	**3**	**4**
Replicate 1	14.942	8.954	18.678	6.245
Replicate 2	12.750	10.505	19.685	8.825
Replicate 3	12.040	10.182	n.a.	10.302
Replicate 4	13.270	9.683	n.a.	n.a.
Replicate 5	12.736	17.926	n.a.	n.a.
Replicate 6	13.984	17.796	n.a.	n.a.
N	6	6	2	3
Mean	13.287	12.508	19.182	8.457
SD	1.037	4.180	0.712	2.053
COV (%)	7.801	33.417	3.712	24.279
Minimum	**8.457**			
Maximum	**19.182**			
Overall mean	**13.358**			
Overall SD	**4.422**			
Overall COV (%)	**33.102**			

n.a. = not analyzed

Table A5_14: Dissipation of imidacloprid at 20°C in cattle manure (overall mean and standard deviation) based on predicted values (SFO kinetics)

Day	Institute 1 Mean	Institute 2 Mean	Institute 3 Mean	Institute 4 Mean	Overall Mean	Standard Deviation
0	107.73	90.77	82.92	64.76	86.5	17.8
3	97.86	80.54	75.19	62.56	79.0	14.6
7	86.09	68.68	66.00	58.38	69.8	11.7
10	78.20	60.94	59.85	55.43	63.6	10.0
14	68.80	51.96	52.53	50.84	56.0	8.5
21	n.d.	39.31	41.81	44.27	41.8	2.5
28	43.94	29.74	33.28	39.23	36.5	6.3
42	28.06	17.02	21.08	31.33	24.4	6.5
56	17.92	9.74	13.35	24.60	16.4	6.4
72	10.73	5.15	7.93	19.31	10.8	6.1
90	6.03	2.51	4.41	13.43	6.6	4.8

n.d. = not determined

Annex 5

List of Participants

Technical Workshops and SETAC-Meetings

Table A5: List of participants at the technical workshops and the SETAC-Meetings

Name	Institution	Country	Work-shops		SETAC-Meetings			
			2013 Flörsheim	2014 Giessen	2012 Berlin	2013 Glasgow	2014 Basel	2015 Barcelona
Adler, Nicole	Umweltbundesamt	Germany			X			
Aikens, Peter	Huntingdon Life Sciences	UK		X				
Atorf, Cornelia	Fraunhofer IME	Germany	X		X			
Berkner, Silvia	Umweltbundesamt	Germany	X	X	X	X	X	
Bicker, Sudaporn	Ibacon GmbH	Germany	X	X		X	X	
Blanckenhorn, Wolf	Univerity Zurich	Switzerland					X	
Düring, Rolf-Alexander	Universität Giessen	Germany	X	X			X	
Fiebig, Silke	Dr.U.Noack-Laboratorien	Germany		X		X	X	X
Floeter, Carolin	Hamburg Applied University	Germany						X
Förster, Bernhard	ECT Oekotoxikologie GmbH	Germany						X
Ganssmann, Matthias	Ibacon GmbH	Germany				X	X	
Gassen, Michael	Harlan Laboratories Ltd.	Switzerland				X		
Gilberg, Daniel	ECT Oekotoxikologie GmbH	Germany	X					
Hahn, Stefan	Fraunhofer ITEM	Germany					X	
Hellstern, Jutta	Eurofins Agroscience Services EcoChem GmbH	Germany			X			
Hennecke, Dieter	Fraunhofer IME	Germany	X	X	X		X	
Herrchen, Monika	Fraunhofer IME	Germany	X	X	X		X	
Heusner, Elena	ECT Oekotoxikologie GmbH	Germany	X	X		X		
Hickmann, Silke	Umweltbundesamt	Germany					X	

Table A5 (continued): List of participants at the technical workshops and the SETAC-Meetings

Name	Institution	Country	Work-shops		SETAC-Meetings			
			2013 Flörsheim	2014 Giessen	2012 Berlin	2013 Glasgow	2014 Basel	2015 Barcelona
Jenkins, Carole	Huntingdon Life Sciences	UK				X		
Jensen, John	Aarhus University	Denmark			X			
Junker, Thomas	ECT Oekotoxikologie GmbH	Germany	X	X	X		X	X
Kaiser, Sibylle	Dr. Knoell Consult	Germany				X		
Knäbe, Silvio	Eurofins Agroscience Services EcoChem GmbH	Germany					X	
Konradi, Sabine	Umweltbundesamt	Germany		X				X
Krogh, Kristine	University of Copenhagen	Denmark			X			
Kwon, Jin-Wook	QIA	South Korea			X			
Lahr, Joost	Alterra	The Netherlands			X		X	
Lichtenberger, Melanie	Ibacon GmbH	Germany						X
Meinerling, Maria	Ibacon GmbH	Germany	X	X	X			
Moermond, Caroline	RIVM	The Netherlands			X			
Muurinen, Johanna	University of Helsinki	Finland						X
Nishimura, Tetsuji	Teikyo Heisei University	Japan				X		
Reinhard, Dominik	Innovative Environmental Services (IES) Ltd.	Switzerland			X			
Römbke, Jörg	ECT Oekotoxikologie GmbH	Germany	X		X			
Scheffczyk, Adam	ECT Oekotoxikologie GmbH	Germany			X		X	
Schmidt, Thomas	Harlan Laboratories Ltd.	Switzerland			X			
Schwarz, Lisa	Universität Giessen	Germany	X					

Table A5 (continued): List of participants at the technical workshops and the SETAC-Meetings

Name	Institution	Country	Work-shops		SETAC-Meetings			
			2013 Flörsheim	2014 Giessen	2012 Berlin	2013 Glasgow	2014 Basel	2015 Barcelona
Schwonbeck, Susanne	Fraunhofer ITEM	Germany						X
Sekine, Tatsu-ya	Ibacon GmbH	Germany					X	
Sinclair, Chris	FERA	UK					X	
Smit, Els	RIVM	The Nether-lands					X	
Thiele-Bruhn, Sören	Universität Trier	Germany	X	X		X	X	X
Topp, Ed	Agriculture and Agri-Food Canada	Canada	X	X		X		
van Vlaarding-en, Peter	RIVM	The Nether-lands	X			X		
Vigon, Bruce	SETAC North America	United States			X			
Wagenhoff, Eiko	Eurofins Agroscience Ser-vices EcoChem GmbH	Germany					X	X
Wehrhan, Anne	Harlan Laboratories Ltd.	Switzerland	X		X			
Wess, Arno	Harlan Laboratories Ltd.	Switzerland			X		X	X
Wohde, Ma-nuel	Universität Giessen	Germany	X	X			X	X
Total			**18**	**13**	**21**	**12**	**21**	**12**

Annex 6

List of Publications

PUBLICATIONS

Schwarz, L. (2014). Masterarbeit Lisa Schwartz „Transformation von Tierarzneimitteln und Bioziden in Gülle – Eine Literaturstudie. UBA-Texte 54/2014. Umweltbundesamt, Dessau-Roßlau, Germany. Available at: https://www.umweltbundesamt.de/publikationen/transformation-von-tierarzneimitteln-bioziden-in.

Hennecke, D., Atorf, C., Bickert, C., Herrchen, M., Hommen, U., Klein, M., Weinfurtner, K., Heusner, E., Knacker, T., Junker, T., Römbke, J., Merrettig-Bruns, U. (2015): Development of a test protocol to study the transformation of veterinary pharmaceuticals and biocides in liquid manure. Available at: www.umweltbundesamt.de

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