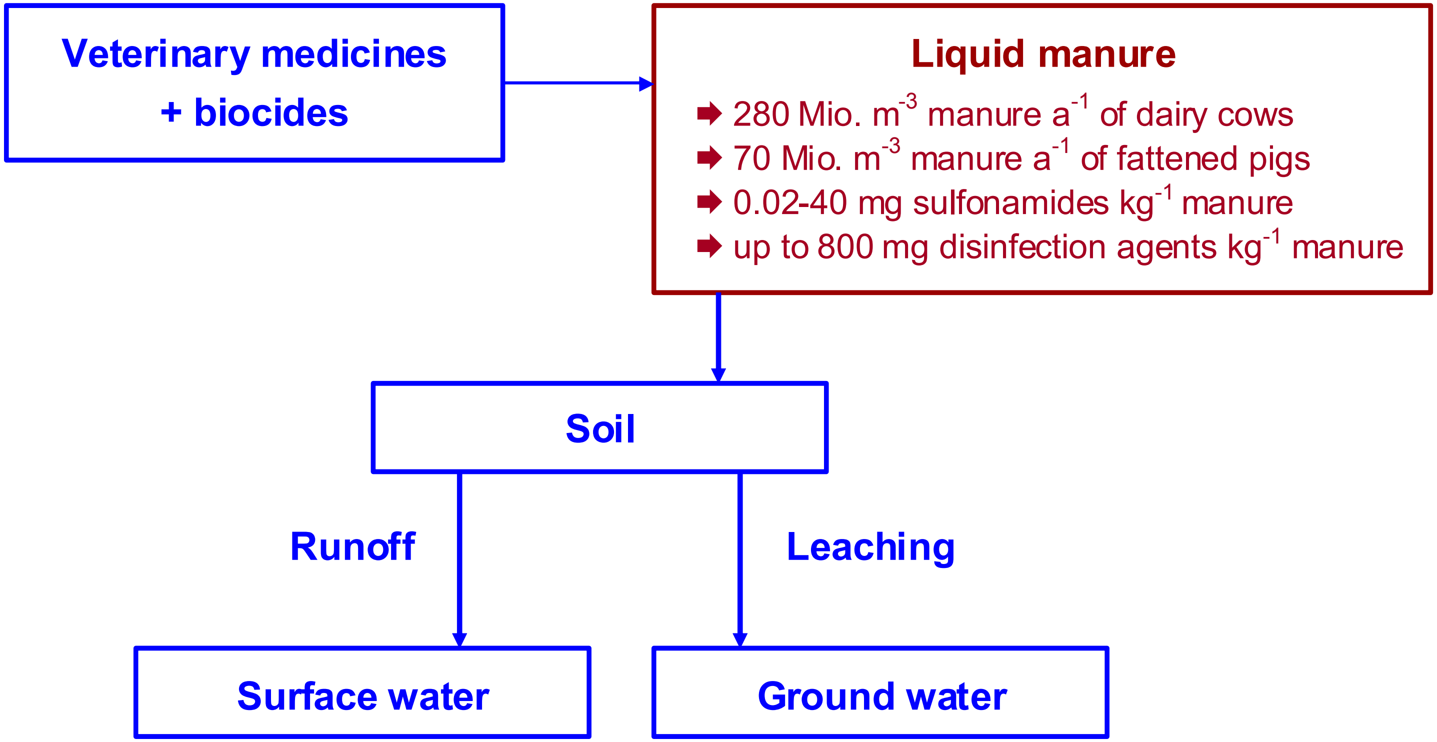
Technische Universität Braunschweig

Institute of Environmental and Sustainable Chemistry

Robert Kreuzig

Fate of Veterinary Medicines and Biocides in Manures and Soils: Laboratory Tests for Prospective Evaluation Concepts



Veterinary medicines + biocides

Liquid manure

- ➡ 280 Mio. m^{-3} manure a^{-1} of dairy cows
- ➡ 70 Mio. m^{-3} manure a^{-1} of fattened pigs
- ➡ 0.02-40 mg sulfonamides kg^{-1} manure
- ➡ up to 800 mg disinfection agents kg^{-1} manure

Soil

Runoff

Leaching

Surface water

Ground water

Manure Project (2004-2007; UBA-FKZ 20467455); Biocide Project (2007-2009; UBA-FKZ 370767403)

Environmental risk assessment for pesticides in soil (EU, 2006; BBA, 1998; Fichter & Holden, 1992)

Tier 1: Laboratory tests and fate modeling

Transformation: $DT_{50} > 120$ d
$MIN < 5$ %
$NER > 70$ % in 100 d

Sorption: $K_d < 5$ L kg^{-1}
$K_{OC} < 300$ L kg^{-1}

➡ ^{14}C-pesticide
➡ standard solution
➡ different soils
➡ controlled lab conditions

Tier 2: Lysimeter and field studies

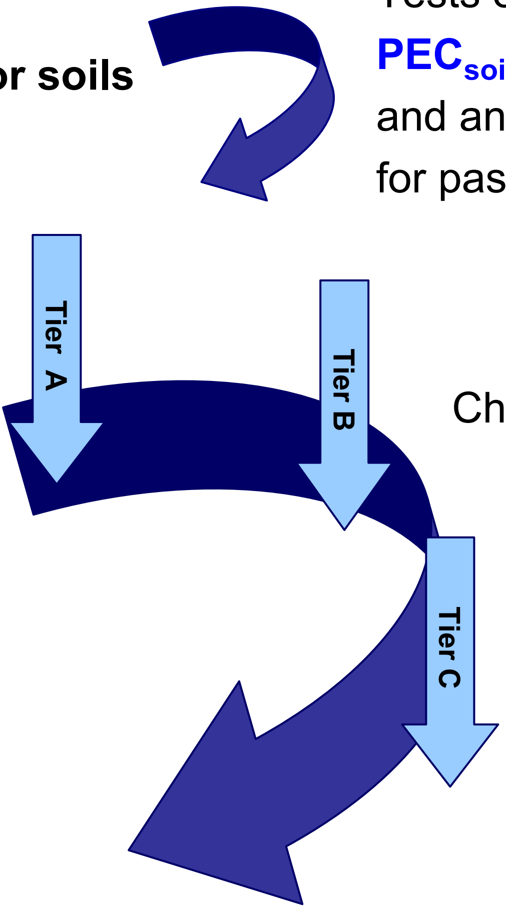
Lysimeter: 0.1 µg L^{-1}

Transformation: $DT_{90} > 360$ d

➡ ^{14}C-pesticide

➡ plant protection product
➡ field conditions
➡ good agricultural practice

Tier 3: Soil accumulation study and refined risk assessment for soil organisms



Phase I

Exposure assessment for soils

Tests on VMP with

PEC_{soil} > 100 µg kg^{-1}

and antiparasitics

for pasture animals

Phase II

Acute effects,

fate in soil and

surface water

Chronical effects, bioaccumulation

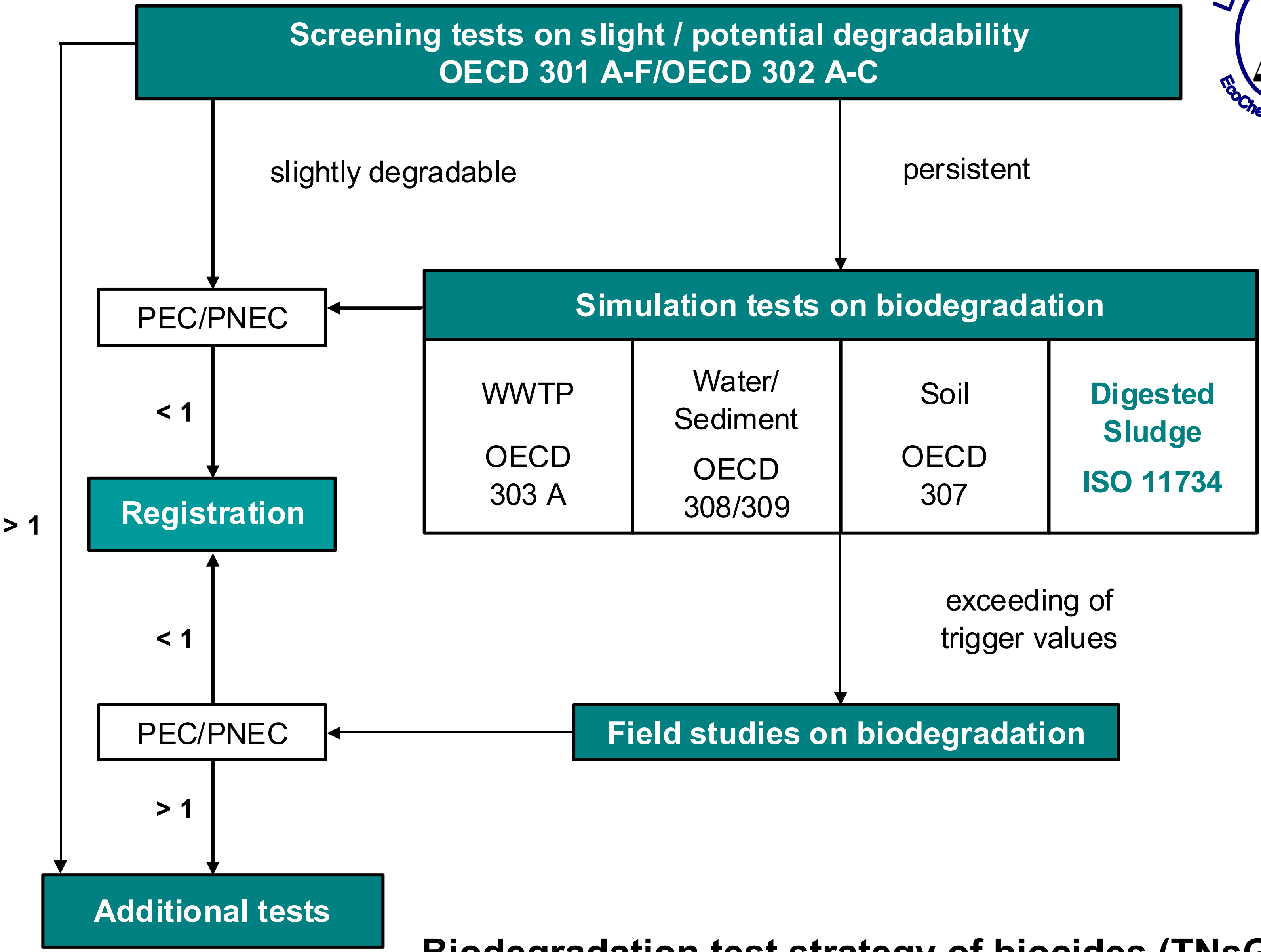
Extended tests

PEC/PNEC > 1

→ **Requirements for**

environmental protection

Environmental risk assessment for veterinary medicines (VICH, 2000, 2003)



Screening tests on slight / potential degradability
OECD 301 A-F/OECD 302 A-C

slightly degradable

persistent

PEC/PNEC

Simulation tests on biodegradation

WWTP OECD 303 A	Water/ Sediment OECD 308/309	Soil OECD 307	**Digested Sludge** **ISO 11734**

< 1

Registration

> 1

< 1

exceeding of trigger values

PEC/PNEC

Field studies on biodegradation

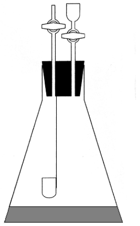
> 1

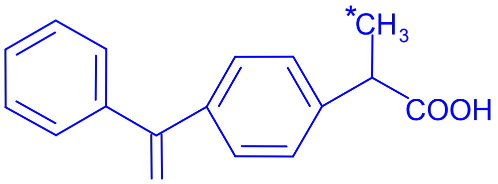
Additional tests

Biodegradation test strategy of biocides (TNsG, 2000)

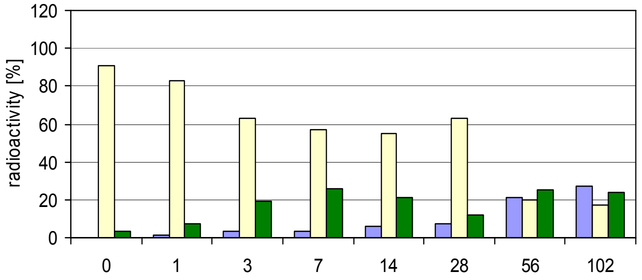
Anaerobic transformation tests of biocides

Digested-sludge test **ISO 11 734**	**Reference-manure test** **Technical Guidance (Draft)**
➡ screening test	➡ simulation test
➡ ultimate biodegradability	➡ metabolic fate
➡ digested sludge of WWTP	➡ reference manures (cattle, pig)
➡ fermentative bacteria	➡ heterogenic microorganisms
➡ unlabelled test substance	➡ ^{14}C-labelled test substance
➡ biogas production (CH_4, CO_2)	➡ radiotracer analysis
➡ disappearance of test substance	➡ balances: MIN, ER, NER
➡ test temperature: 35 °C	➡ test temperature: 20 °C
➡ test duration: 60 days	➡ test duration: 100 (180) days





ketoprofen



Transformation in digested sludge

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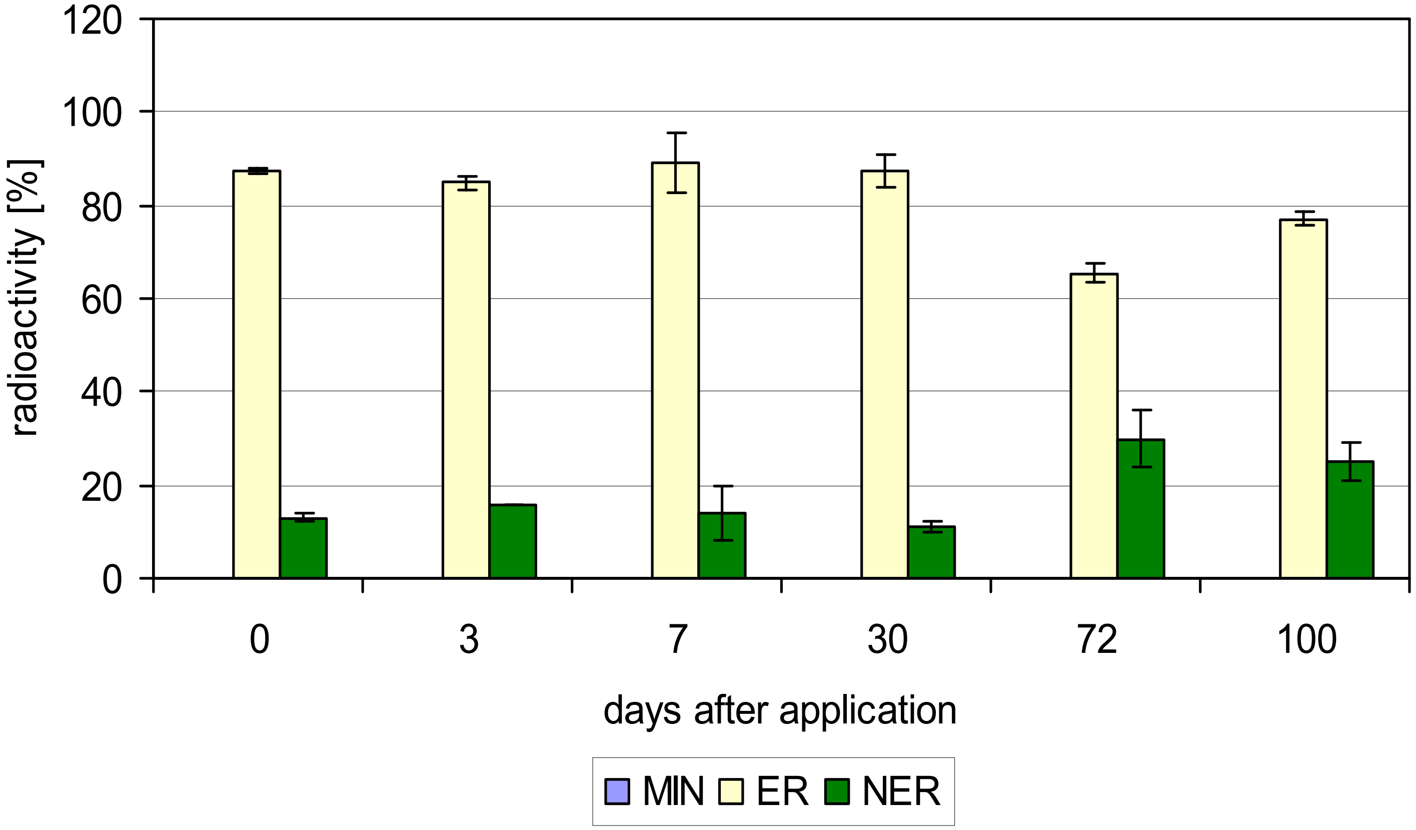
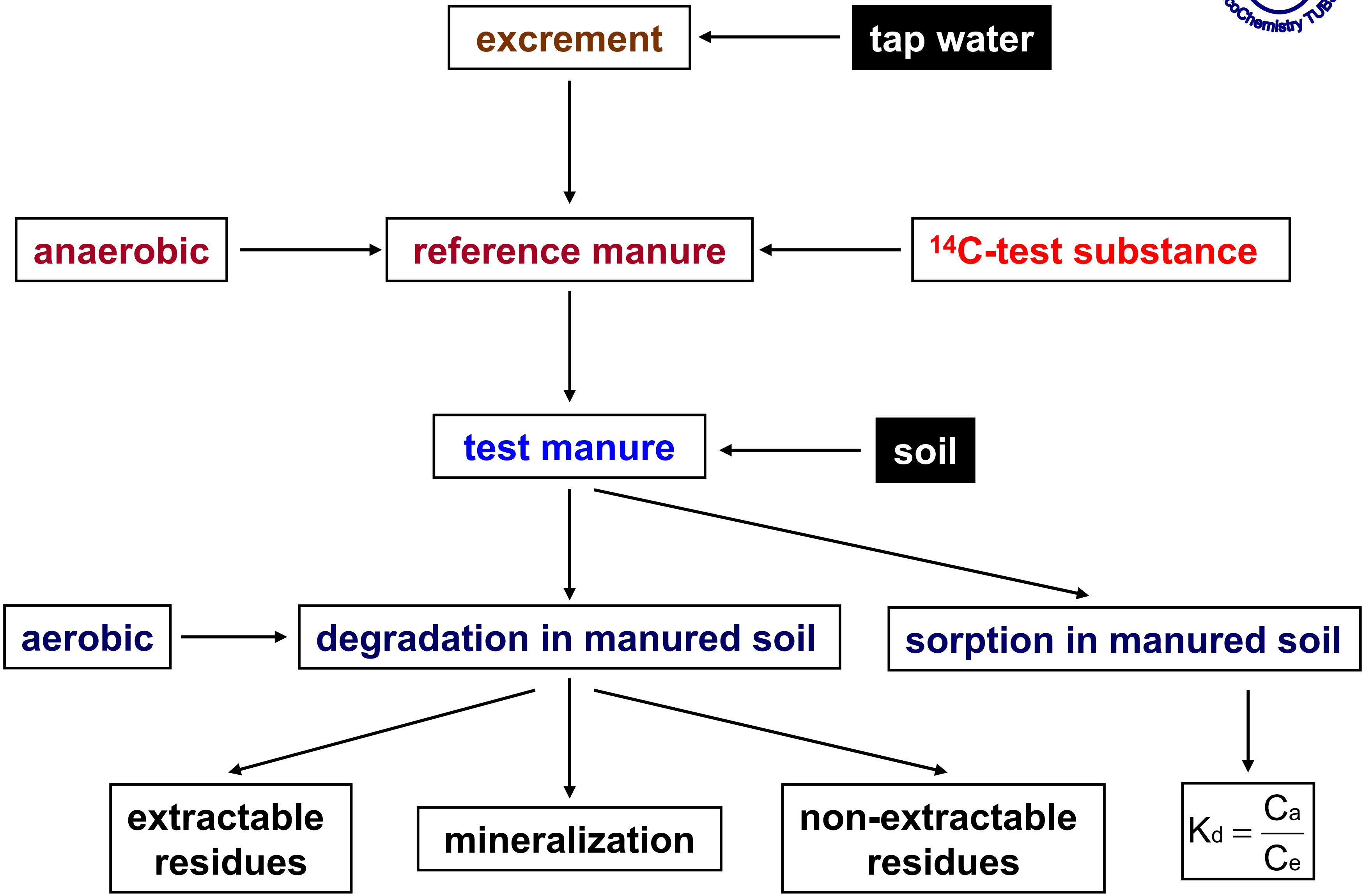


Transformation of ^{14}C-ketoprofen in bovine reference manure

Laboratory test strategies

Relative testing	**Realistic testing**
➡ standard test methods	➡ advanced test procedures
➡ simplified test conditions	➡ complex test conditions
➡ reproducibility	➡ transferability to field conditions
Sorption tests for VMP or biocides	
➡ batch equilibrium tests	➡ batch equilibrium tests
➡ dried and equilibrated soil samples	➡ field fresh soil samples
➡ standard application	➡ test-manure application

Transformation and sorption tests in manured soils



excrement

tap water

anaerobic

reference manure

^{14}C-test substance

test manure

soil

aerobic

degradation in manured soil

sorption in manured soil

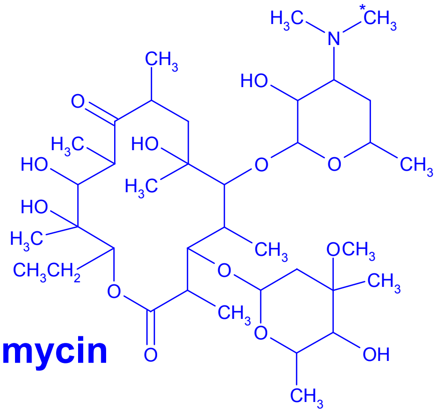
extractable residues

mineralization

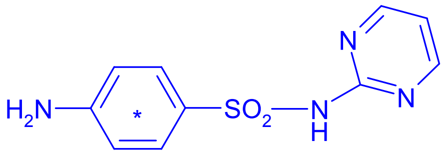
non-extractable residues

$$K_d = \frac{C_a}{C_e}$$

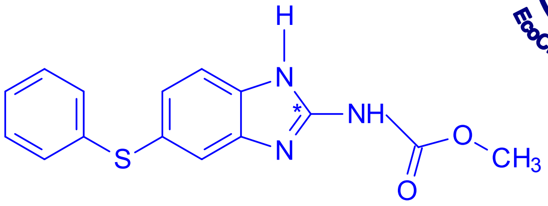
$$K_d = \frac{C_a}{C_e}$$



erythromycin



sulfadiazine

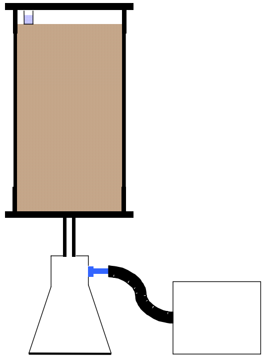


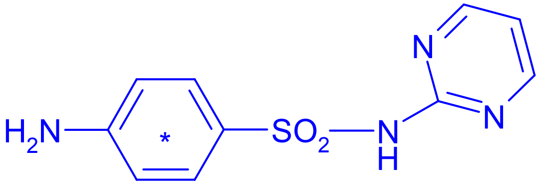
fenbendazole

Soil/water distribution coefficients [L kg^{-1}]

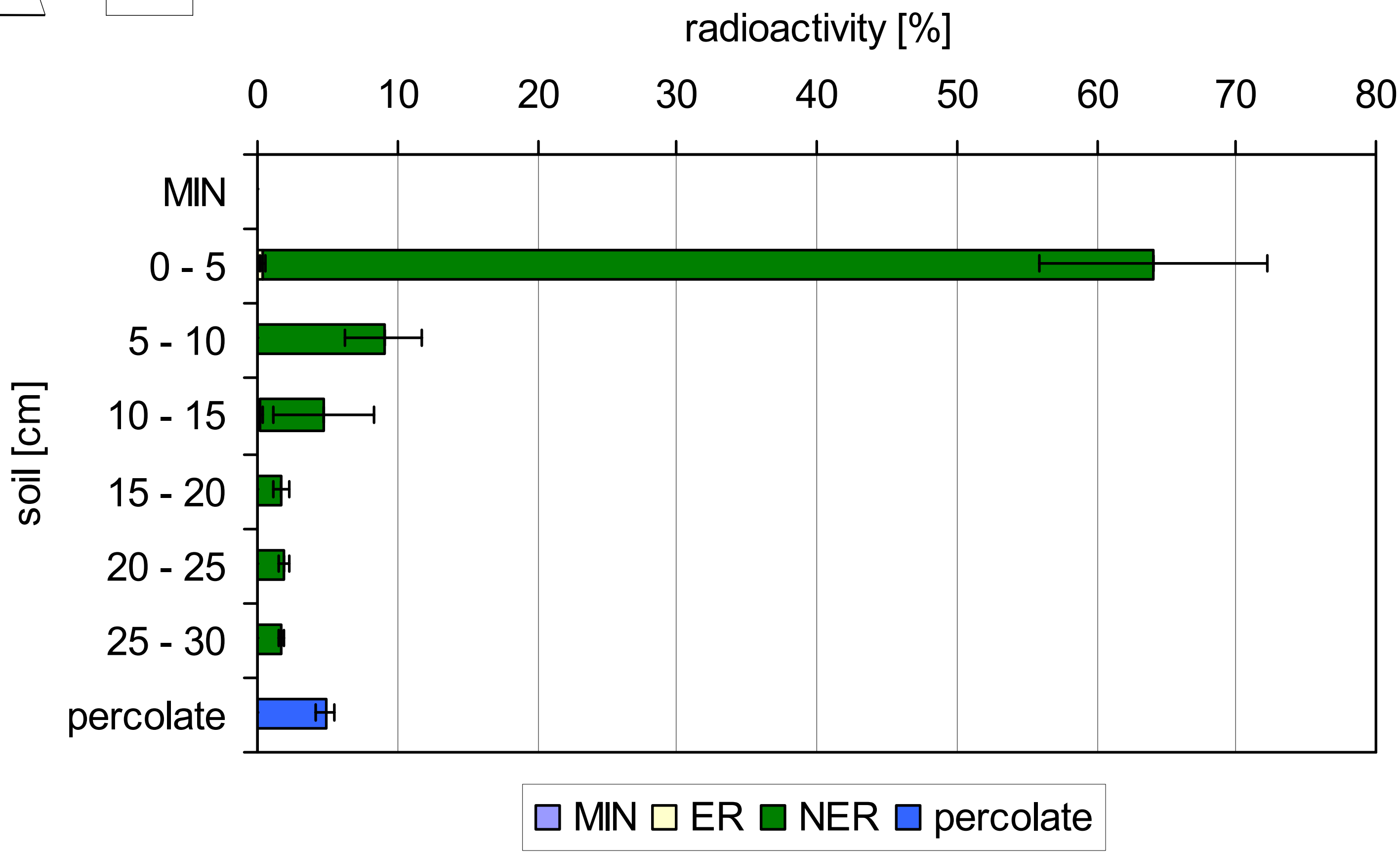
Soil texture	application	ERY	SDZ	FEN
Silty clay	**standard**	**24 ± 2**	**2 ± 0**	**63 ± 7**
	standard + PM	17 ± 2	---	---
Clay [%] : 39.0	**test manure (TM-P)**	**1 ± 0**	**---**	**42 ± 3**
OC [%] : 1.6	standard + BM	33 ± 4	3 ± 0	---
pH : 6.9	**test manure (TM-B)**	**3 ± 1**	**14 ± 1**	---
Silty sand	**standard**	**14 ± 3**	**2 ± 0**	**58 ± 3**
	standard + PM	7 ± 1	---	---
Clay [%] : 4.0	**test manure (TM-P)**	**0.1 ± 0**	**---**	**31 ± 1**
OC [%] : 0.8	standard + BM	7 ± 1	15 ± 3	---
pH : 5.4	**test manure (TM-B)**	**1 ± 0**	**84 ± 12**	---

K_d < 5: enhanced mobility (Fichter & Holden, 1992)

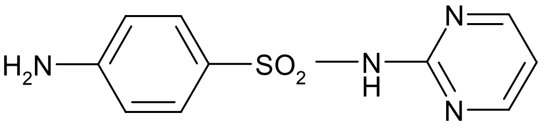




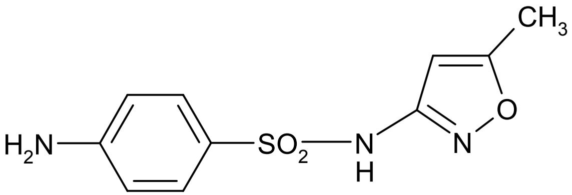
sulfadiazine



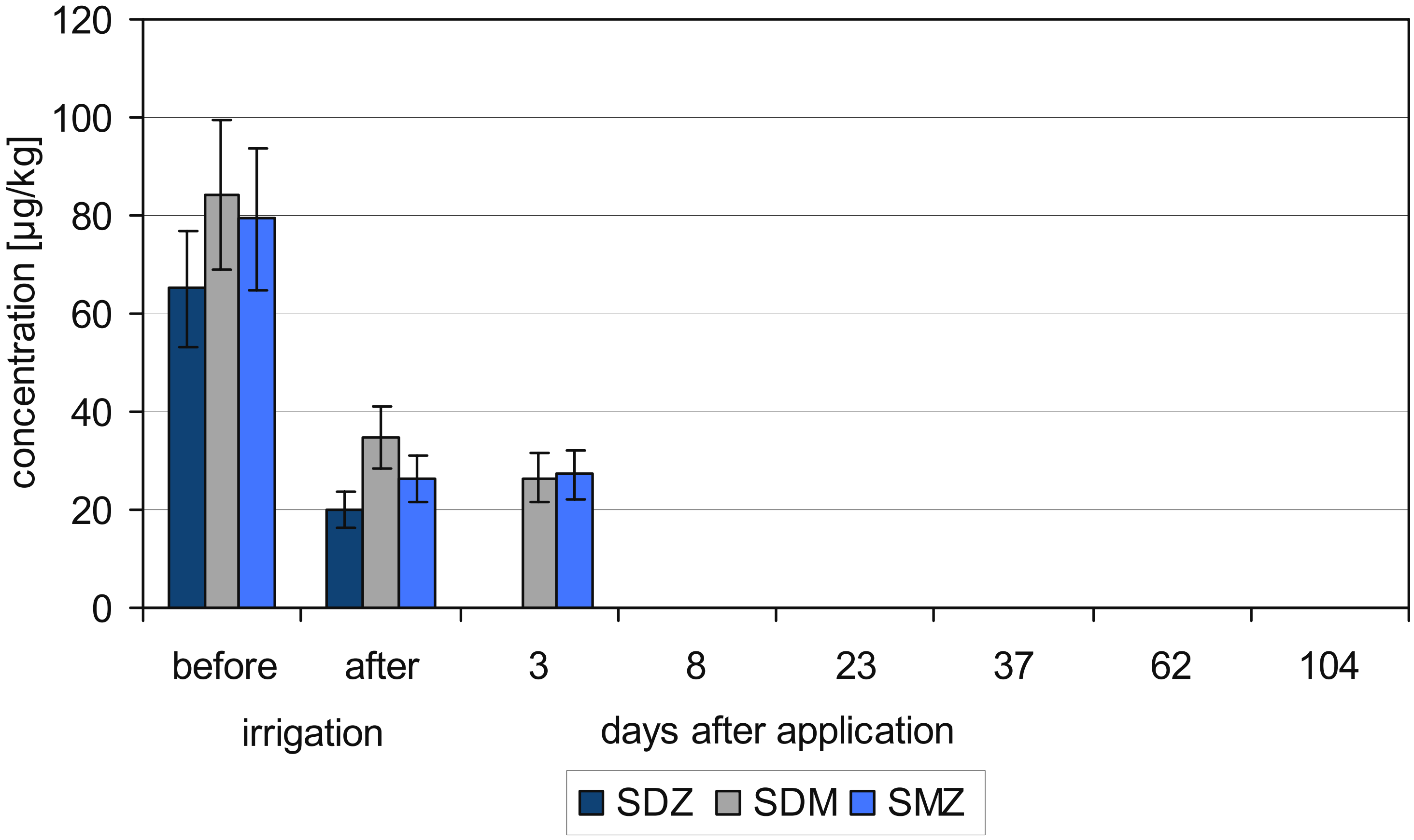
Laboratory lysimeter test of ^{14}C-sulfadiazine after test-manure application



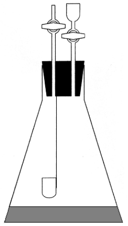
sulfadiazine, K_d: 14 L kg^{-1}

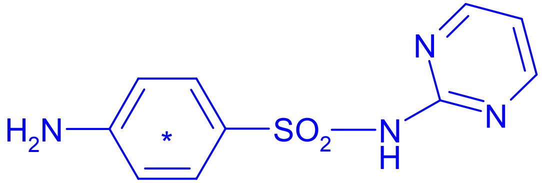


sulfamethoxazole , K_d: 11 L kg^{-1}

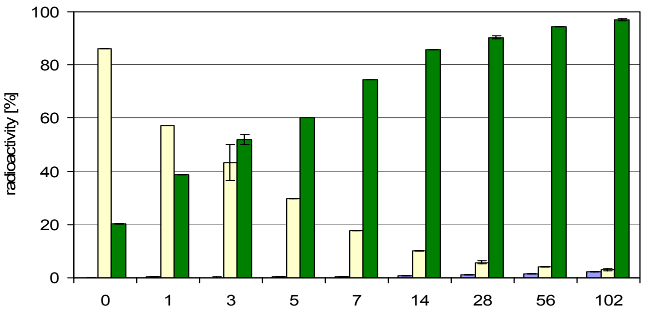


Sulfonamides in clay soil (0-15 cm) after bovine test-manure application, soil cultivation and sprinkler irrigation





sulfadiazine



Transformation after standard application

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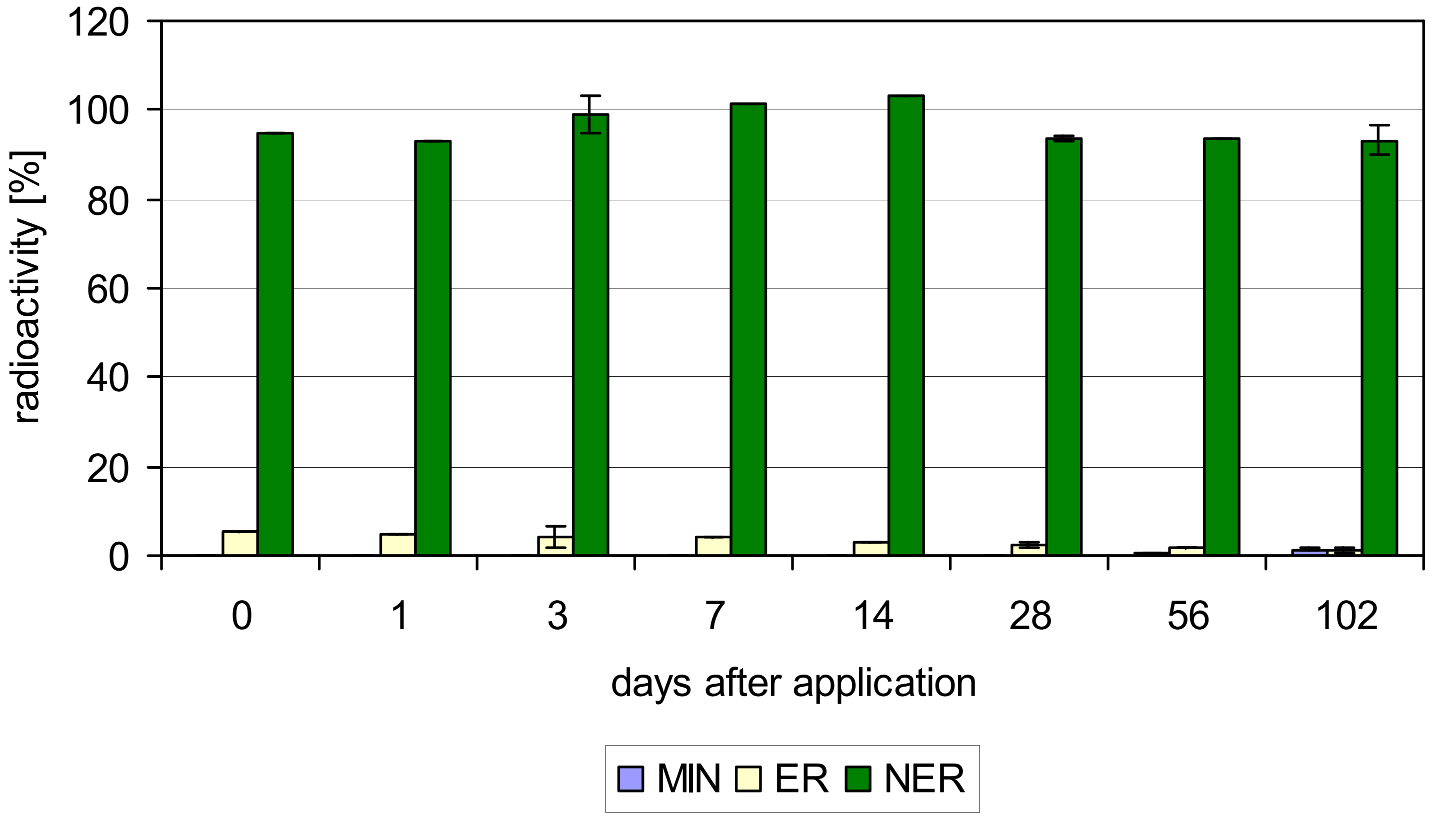
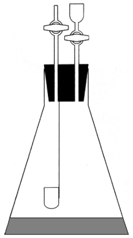
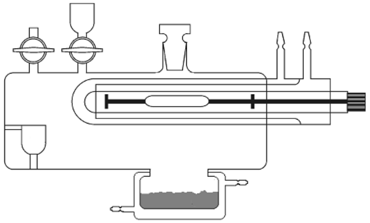
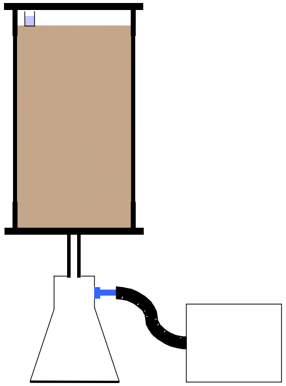


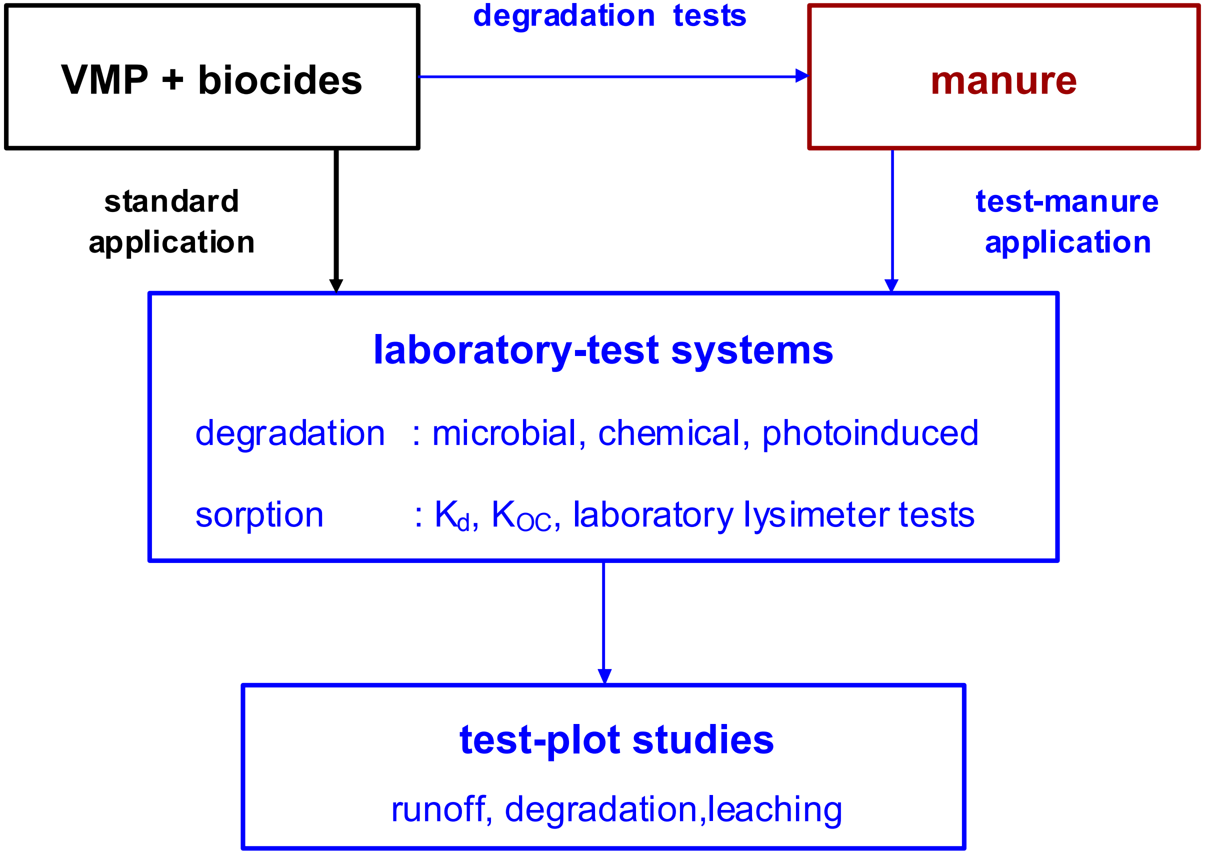
Transformation of ^{14}C-sulfadiazine in clay soil after test-manure application



Experimental design for fate monitoring







VMP + biocides

degradation tests

manure

standard application

test-manure application

laboratory-test systems

degradation : microbial, chemical, photoinduced

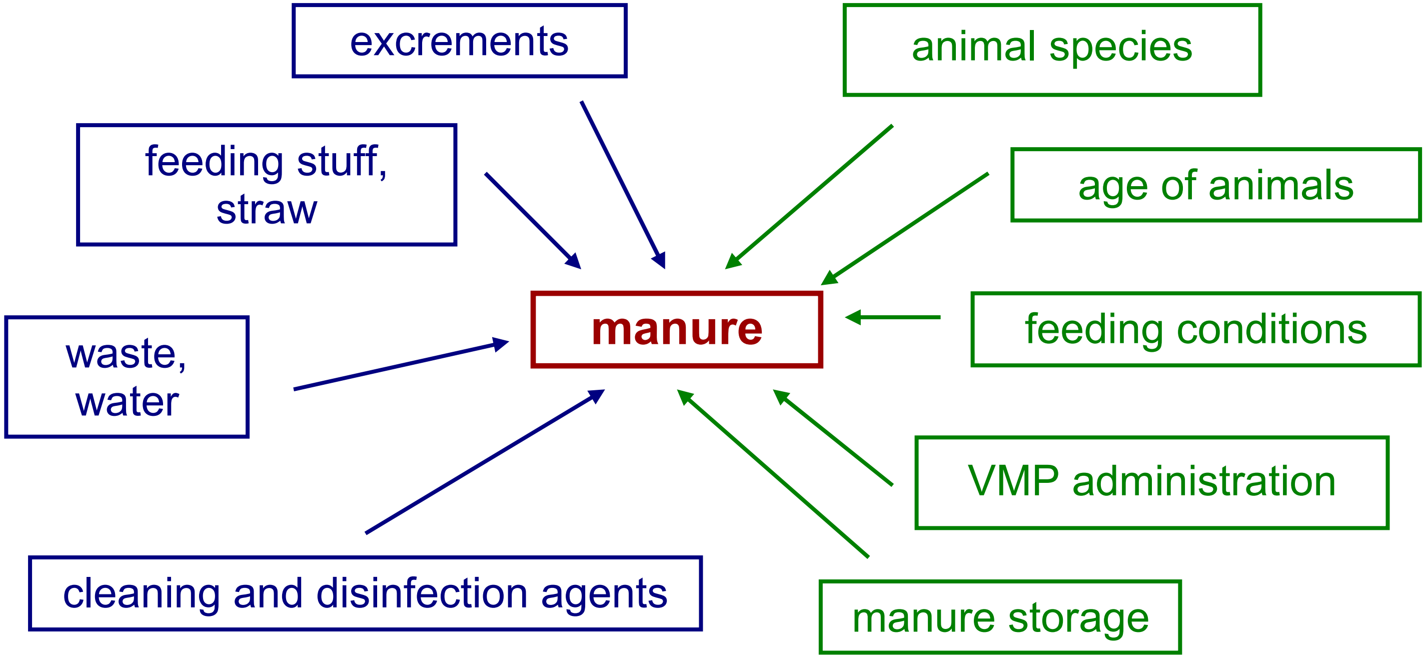
sorption : K_d, K_{OC}, laboratory lysimeter tests

test-plot studies

runoff, degradation,leaching

Runoff Project (UBA-FKZ20267435, 2002-2004)

Manure: Complexity, heterogeneity, variability


excrements

feeding stuff, straw

waste, water

cleaning and disinfection agents

manure

animal species

age of animals

feeding conditions

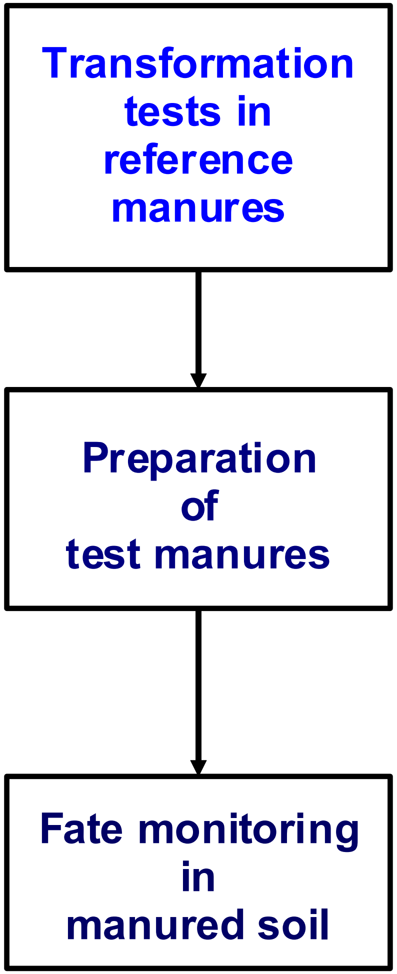
VMP administration

manure storage

Parameter	ds [%]	P_2O_5 [g kg^{-1}]	Cu [mg kg^{-1}]	NH_4-N [g kg^{-1}]	N_{total} [g kg^{-1}]
Bovine manure					
minimum	0.4	0.05	0.08	0.01	0.43
median	8.7	1.7	3.9	1.7	4.0
maximum	12.3	2.7	12.1	2.9	5.7
Pig manure					
minimum	0.4	0.03	0.22	0.27	0.60
median	4.9	2.3	16.1	2.7	4.6
maximum	11.6	6.3	53.1	4.9	8.3

Composition of bovine and pig manures (Merkel, 2005)

Development of a technical guidance for fate monitoring of VMP in liquid manures and manured soils



Transformation tests in reference manures

Preparation of test manures

Fate monitoring in manured soil

Reality:
→ composition of manures
→ storage conditions
→ entry route via manuring

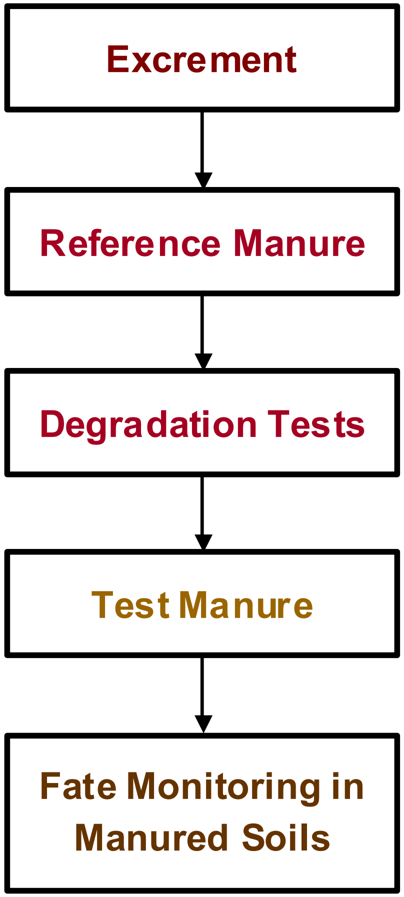
Practicability:
→ feasibility
→ time and costs

Quality assurance:
→ "repeatability"
→ "reproducibility"

Manure Project (UBA-FKZ 204 67 455; 2004-2007)

The technical guidance (Draft version)

Transformation of veterinary medicines and biocides in liquid bovine and pig manures and transformation and sorption in manured soils



Excrement

Reference Manure

Degradation Tests

Test Manure

Fate Monitoring in Manured Soils

A tiered experimental design in 5 parts:

I. **Sampling of excrements and preparation of reference manures.**

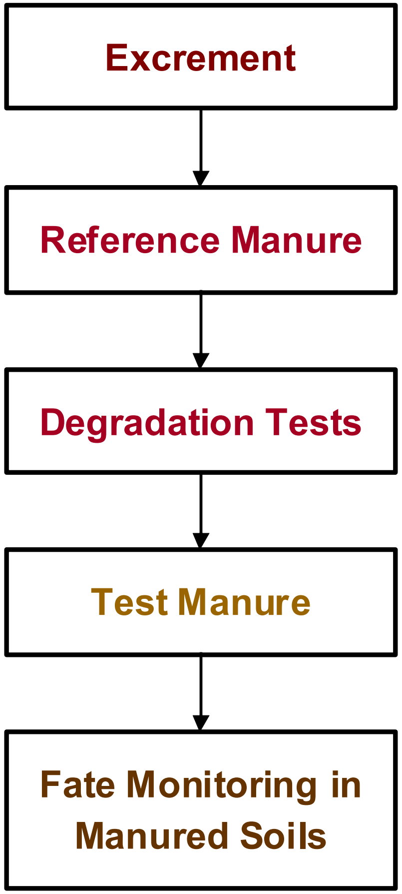
II. **Anaerobic degradation tests in reference manures.**

III. Preparation of test manures.

IV. Aerobic degradation in manured soils.

V. Sorption tests in manured soils.

Preparation of reference manures



Excrement

Reference Manure

Degradation Tests

Test Manure

Fate Monitoring in Manured Soils

- **Sampling of excrements from individual production animals**

• animals´ age:	cattle	:	8 – 60 months
	pigs	:	4 – 12 months
• nutrition:	cattle	:	silage (maize, grass), pellets
	pig	:	barley, wheat, potatoes, soya

- **Matrix characterisation of excrement samples**

- **Adjustment of excrement samples to definied water contents**
  - bovine manure : 2.5 %, 5 %, **10** % ds
  - pig manure : 2.5 %, **5** %, 10 % ds

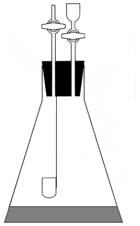
- **Matrix characterisation of reference manure samples**

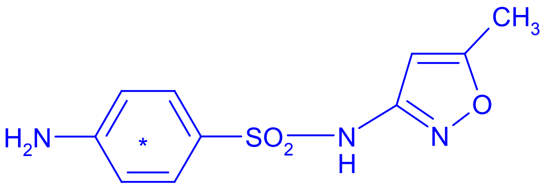
Composition of bovine manures (Merkel, 2005)

	ds [%]	P_2O_5 [g kg^{-1}]	Cu [mg kg^{-1}]	NH_4-N [g kg^{-1}]	N_{total} [g kg^{-1}]
Bovine manure					
minimum	0.4	0.05	0.08	0.01	0.43
median	8.7	1.7	3.9	1.7	4.0
maximum	12.3	2.7	12.1	2.9	5.7

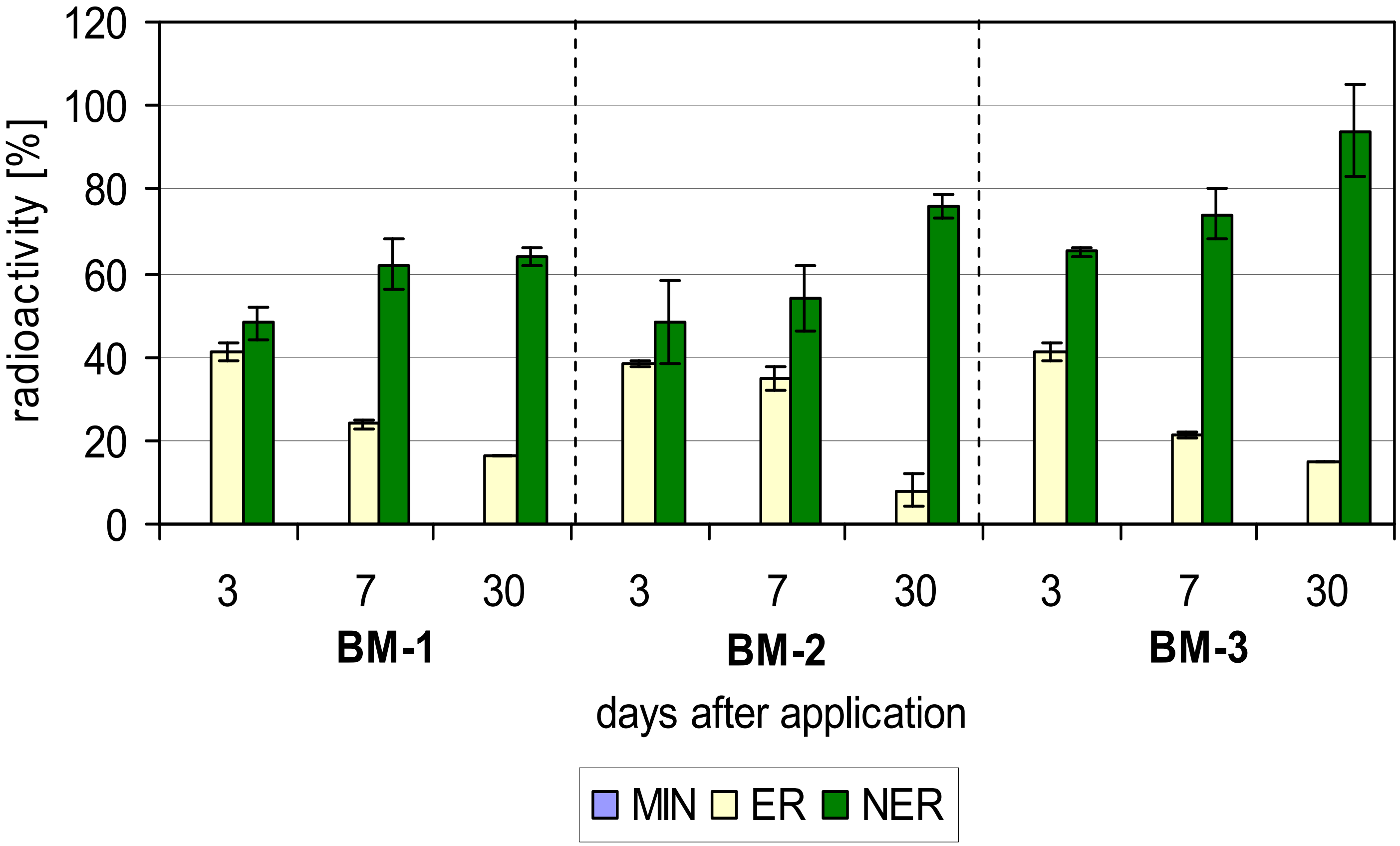
Composition of bovine excrements and reference manures

	Excrements			**Manures**		
Parameter	**BE-1**	**BE-2**	**BE-3**	**BM-1**	**BM-2**	**BM-3**
ds [%]	13	13	10	10	10	10
TOC [g kg^{-1}]	47	54	40	39	42	40
pH	6.9	8.4	8.0	7.0	8.1	8.0
Eh [mV]	40	10	-20	-40	-80	-20
O_2 [mg kg^{-1}]	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
NH_4-N [g kg^{-1}]	1.6	4.5	4.0	1.3	3.2	4.0
N_{total} [g kg^{-1}]	4.1	6.4	6.5	3.2	5.0	6.5
NH_4-N/N_{total}	0.4	0.7	0.6	0.4	0.6	0.6
BOD_5 [g kg^{-1}]	9.4	11	6.0	8.3	7.3	6.0
COD [g kg^{-1}]	76	70	65	71	60	65
BOD_5/COD	0.1	0.2	0.1	0.1	0.1	0.1

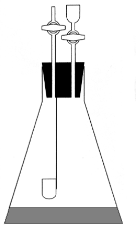


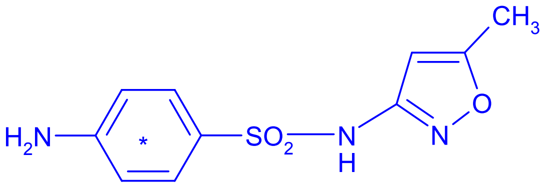


sulfamethoxazole



Transformation tests of ^{14}C-sulfamethoxazole in different bovine manures

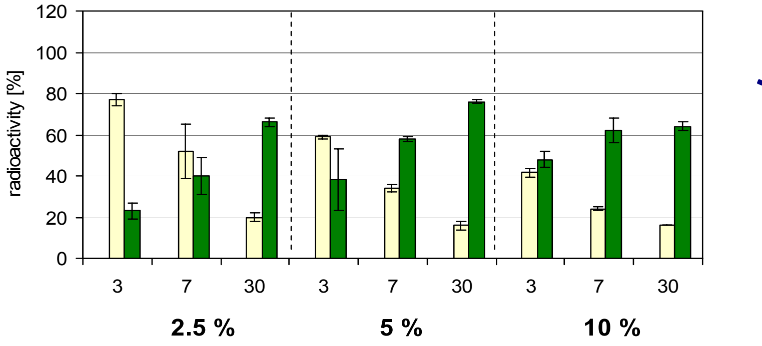




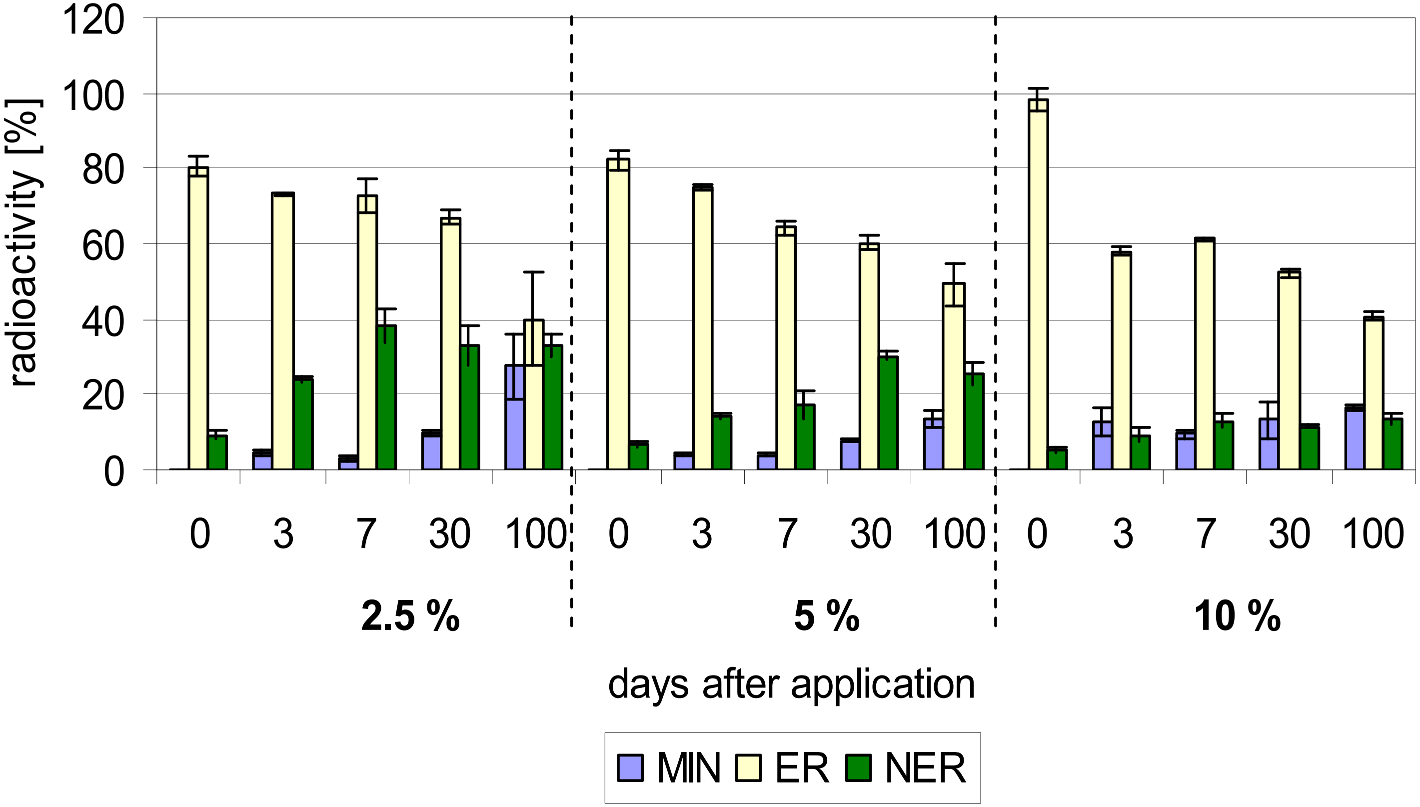
sulfamethoxazole

$N \equiv C^{*} - NH_2$

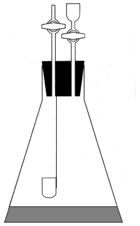
cyanamide

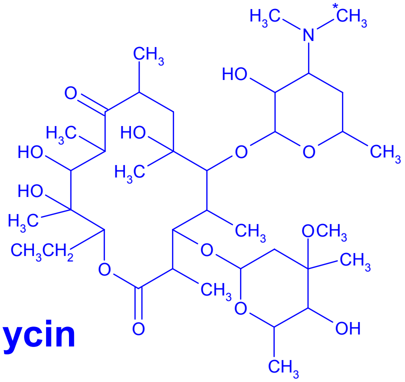


^{14}C-sulfamethoxazole in bovine reference manure



Transformation of ^{14}C-cyanamide in pig reference manure at different dry substance contents and at 20 ± 1 °C (balances: 92 ± 12%)





erythromycin

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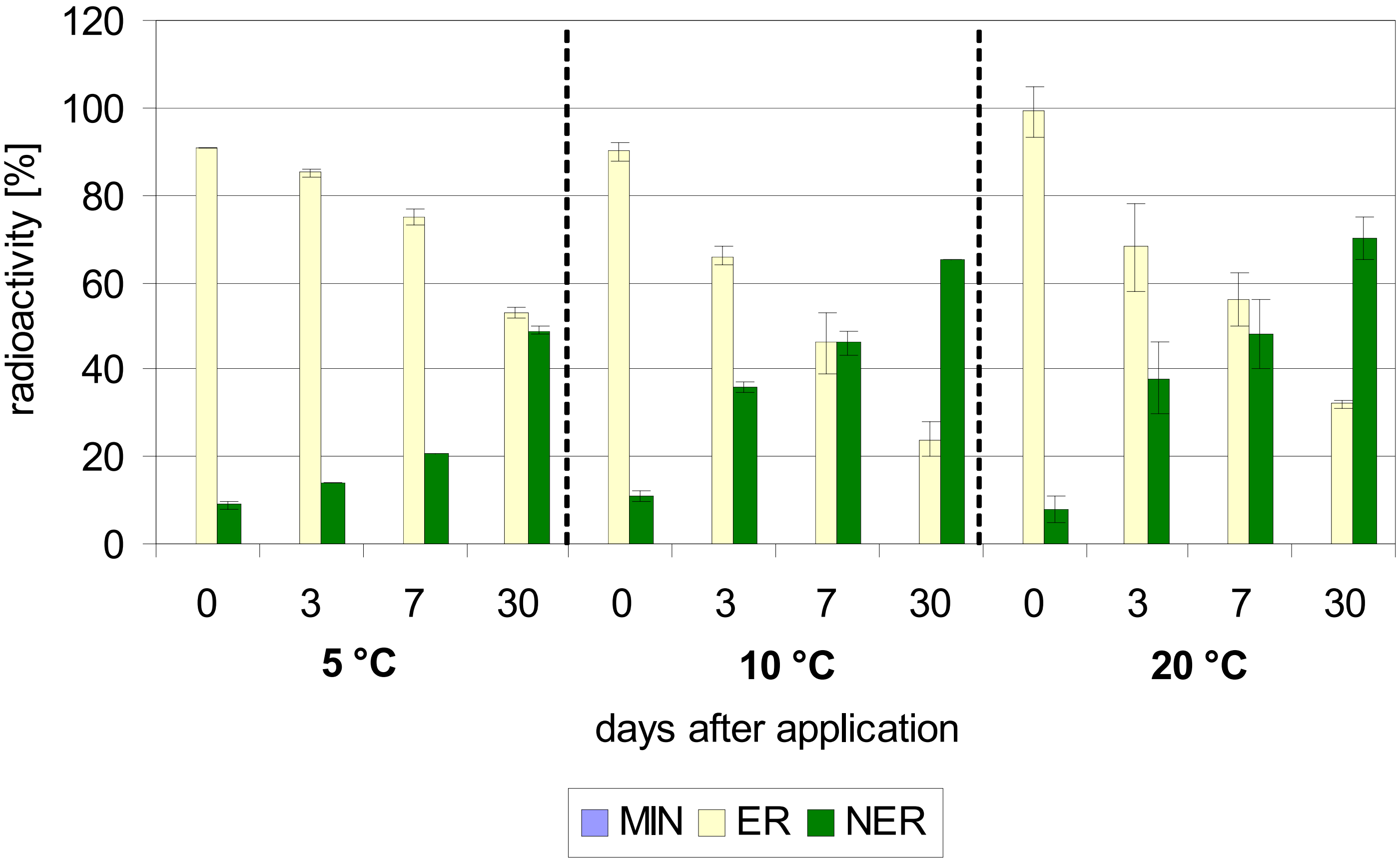
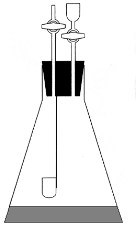
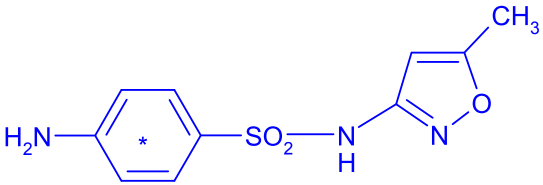
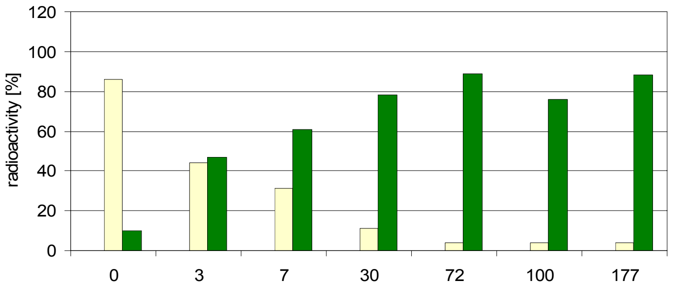


Degradation of ^{14}C-erythromycin in pig manure (PM-1) at different incubation temperatures (balances: 100 ± 5 %)

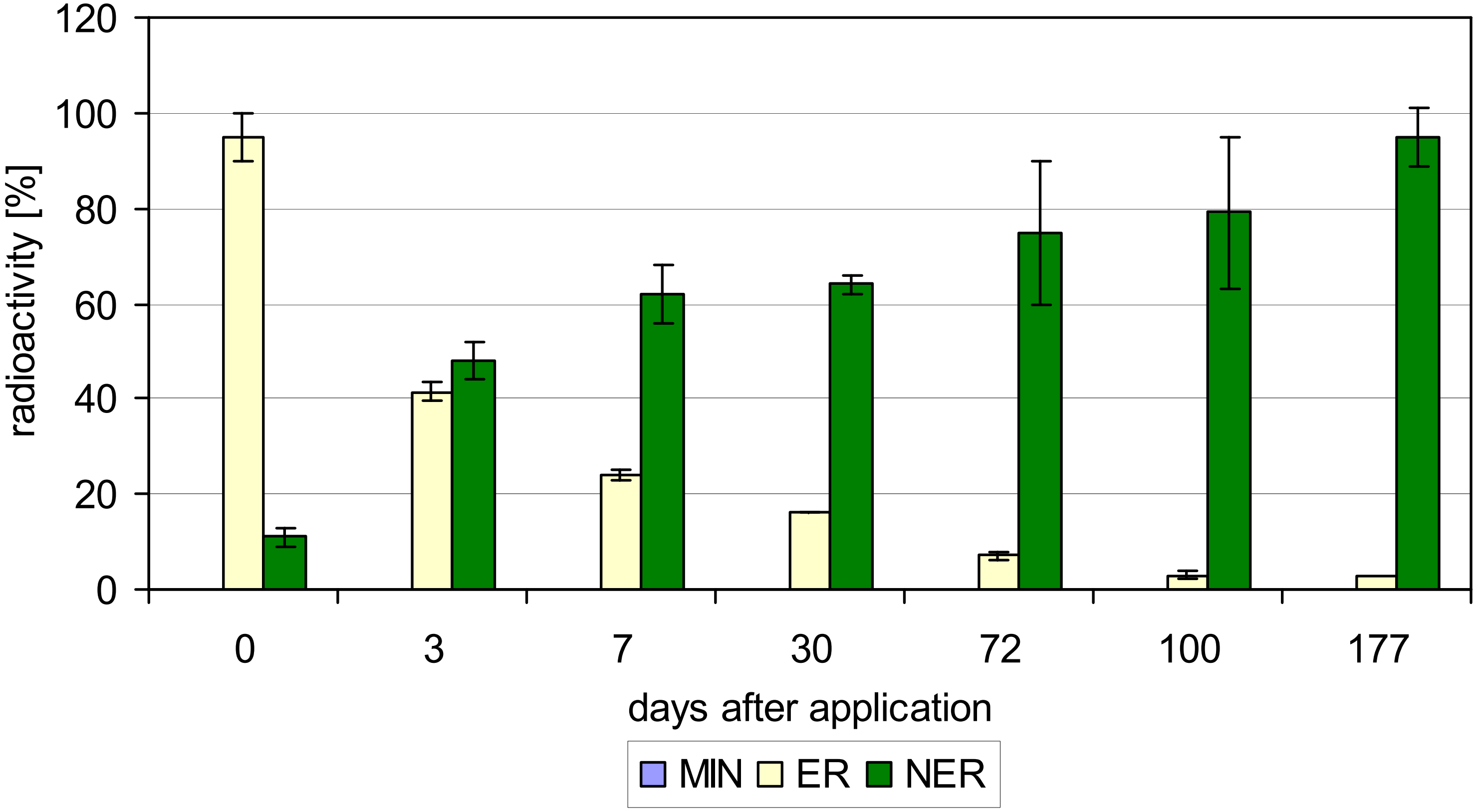




sulfamethoxazole



Transformation test in 2006 (1250 µg kg^{-1})



Transformation of ^{14}C-sulfamethoxazole in bovine manure in 2005 (560 µg kg^{-1})

2.5 Analytical methods

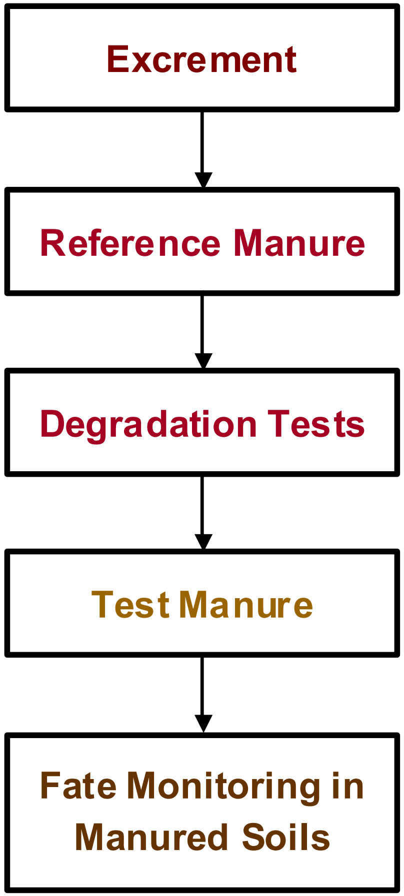
... **An exhaustive extraction is necessary with various apolar and polar solvents and acid systems.** The extraction efficiency should exceed 70%. The extraction of residues should not change the chemical nature of the active substance or the transformation products.

3.2.4 Non-extractable residues

The mechanisms for binding within the manure matrix can be numerous and complex though to date are not well understood. For a better understanding of the binding capacity of organic matter invarious manure types further research is needed. It might also be necessary to highlight that, in contrast to soil and sediment, manure will be degraded to a large extent. In that respect it is important to rule out the possibility that the NER represents parent compound and transformation products that become available when the manure matrix degrades. **In this context the general rule applied in the OECD guideline 307 and 308, that the extraction method must not substantially change the structure of the sample matrix under study, might not be appropriate for manure.**

The technical guidance (draft version)

Transformation of veterinary medicines and biocides in liquid bovine and pig manures and transformation and sorption in manured soils



Excrement

Reference Manure

Degradation Tests

Test Manure

Fate Monitoring in Manured Soils

A tiered experimental design in 5 parts:

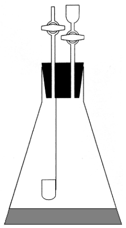
I. Sampling of excrements and preparation of reference manures.

II. Anaerobic degradation tests in reference manures.

III. Preparation of test manures.

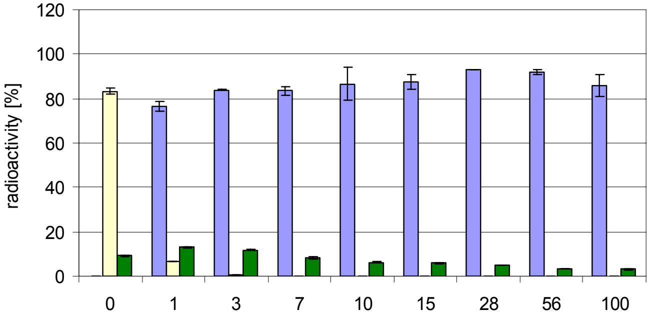
IV. Aerobic degradation in manured soils.

V. Sorption tests in manured soils.

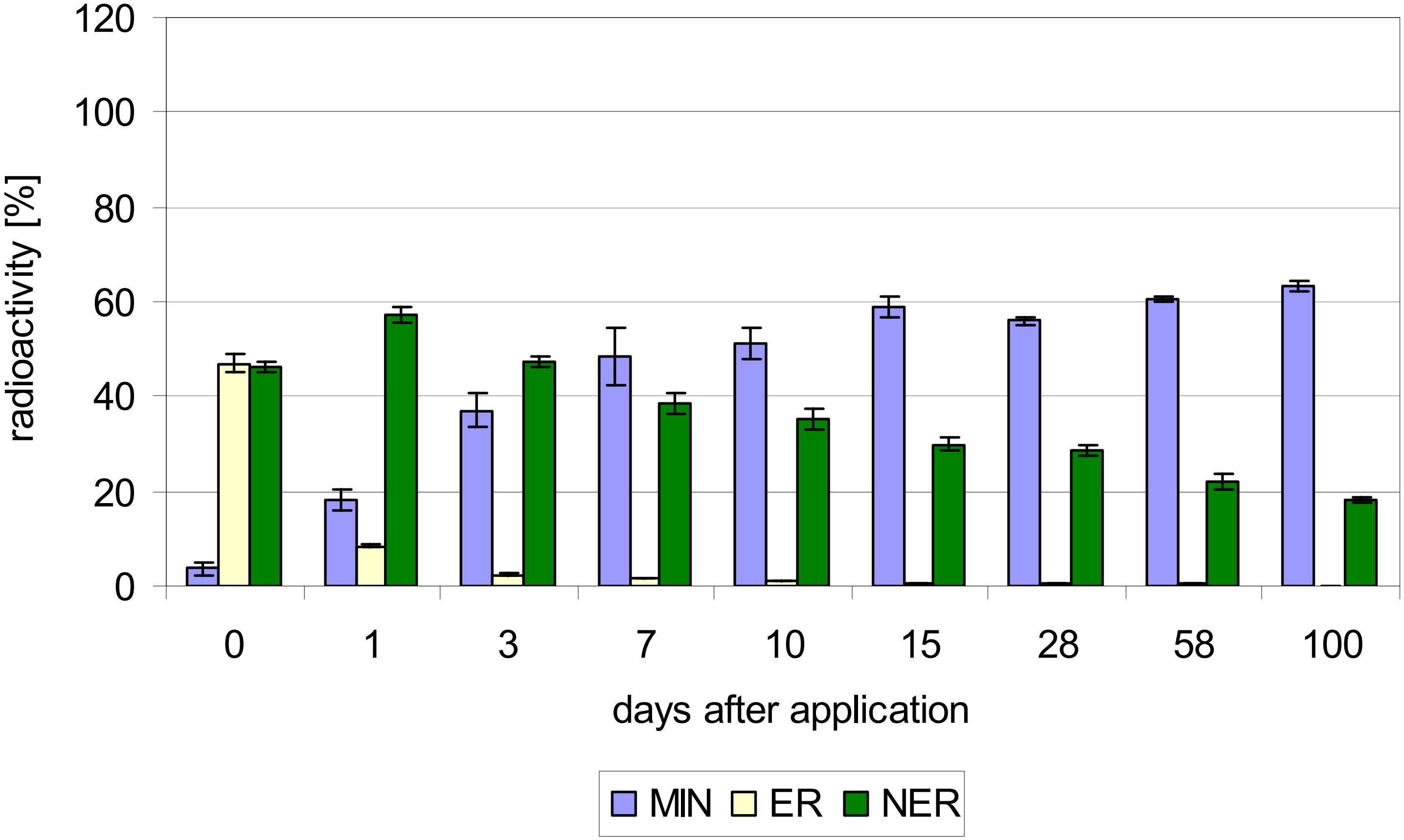


$N \equiv C^{*} — NH_2$

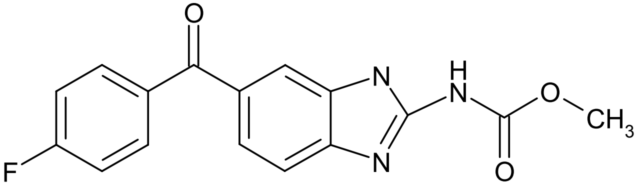
cyanamide



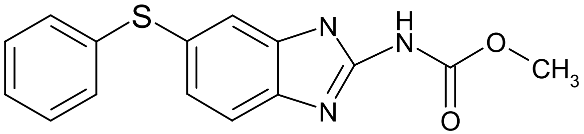
Transformation after standard application



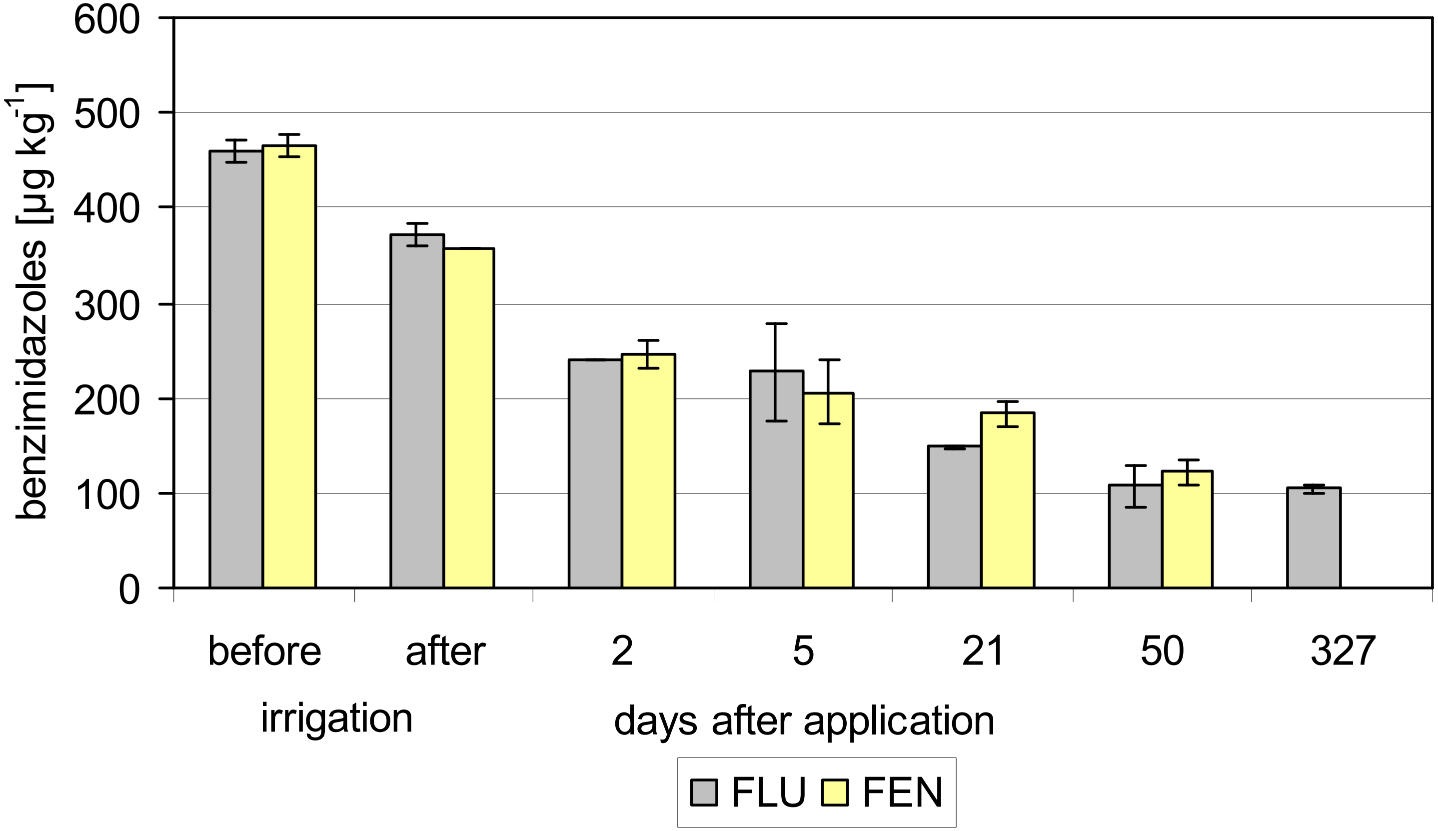
Transformation of ^{14}C-cyanamide in silty clay soil after pig test-manure application (balances: 87 ± 5 %)



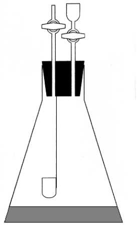
flubendazole, K_d: 141 L kg^{-1}

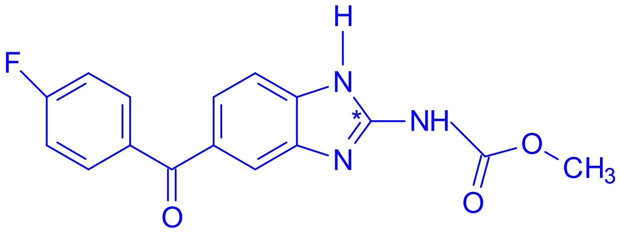


fenbendazole, K_d: 63 L kg^{-1}

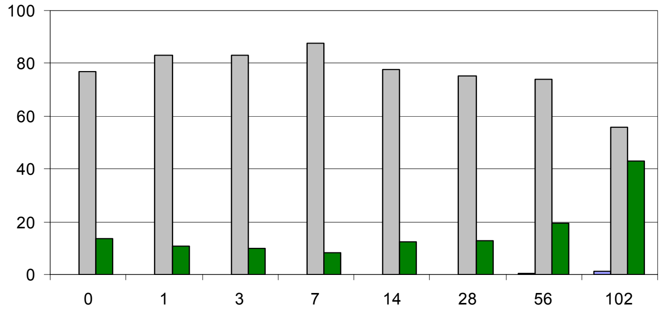


Benzimidazoles in clay soil (0-15 cm) after test-manure application (TM-P), soil cultivation and sprinkler irrigation

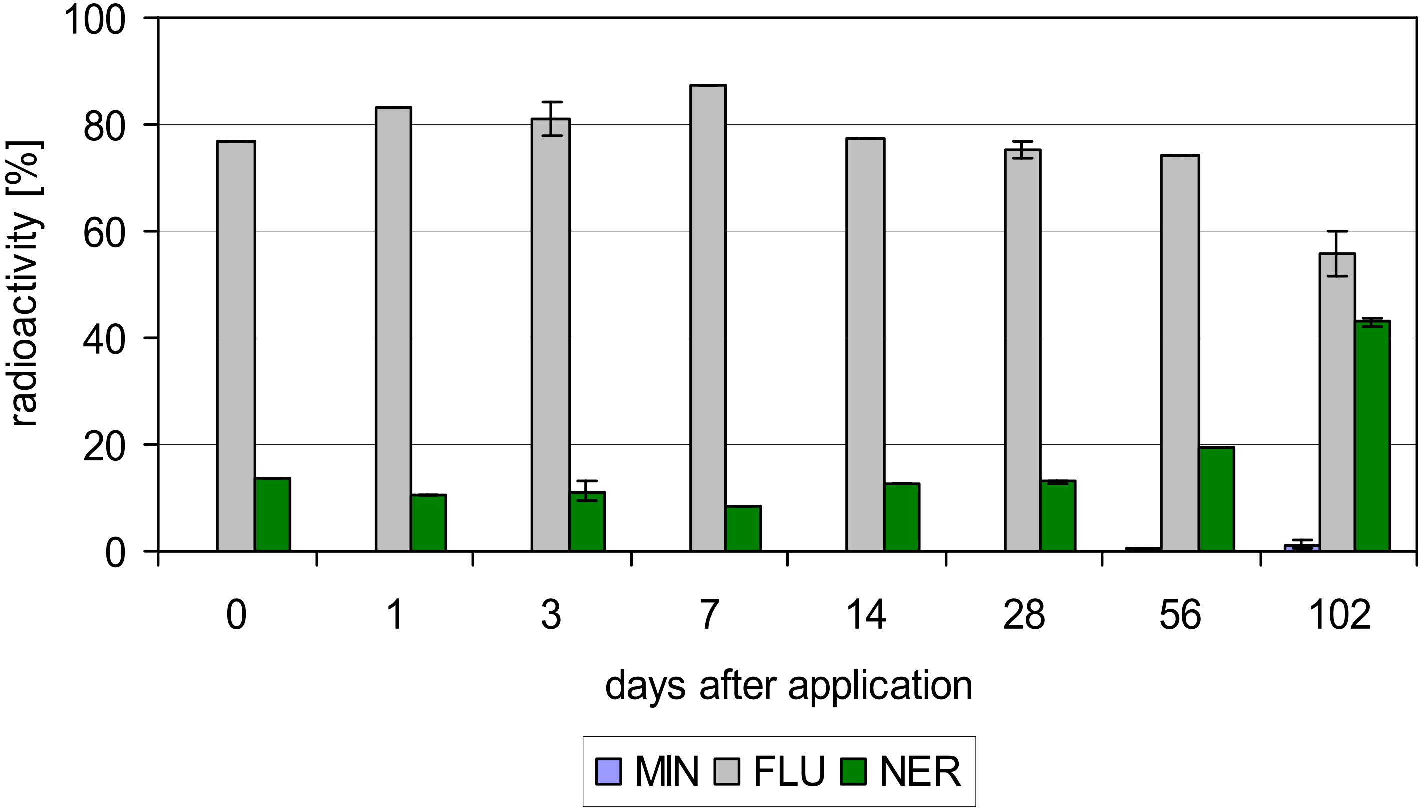




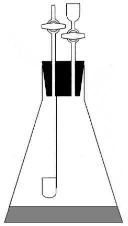
flubendazole

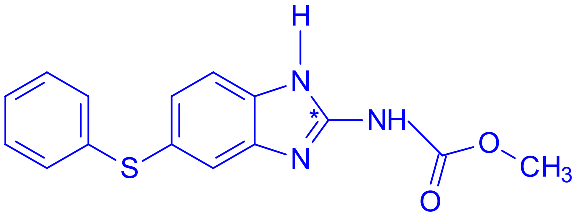


Transformation after standard application

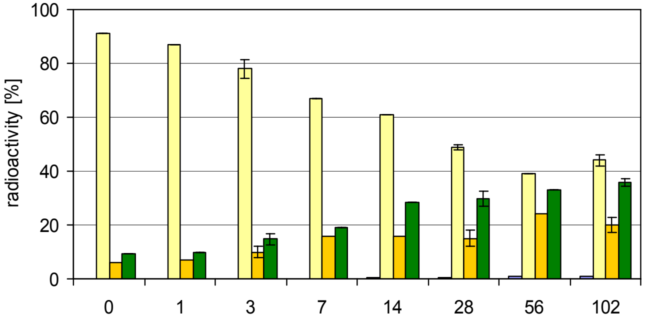


Degradation of ^{14}C-flubendazole in clay soil after test-manure application (TM-P, balances 93 ± 3 %)

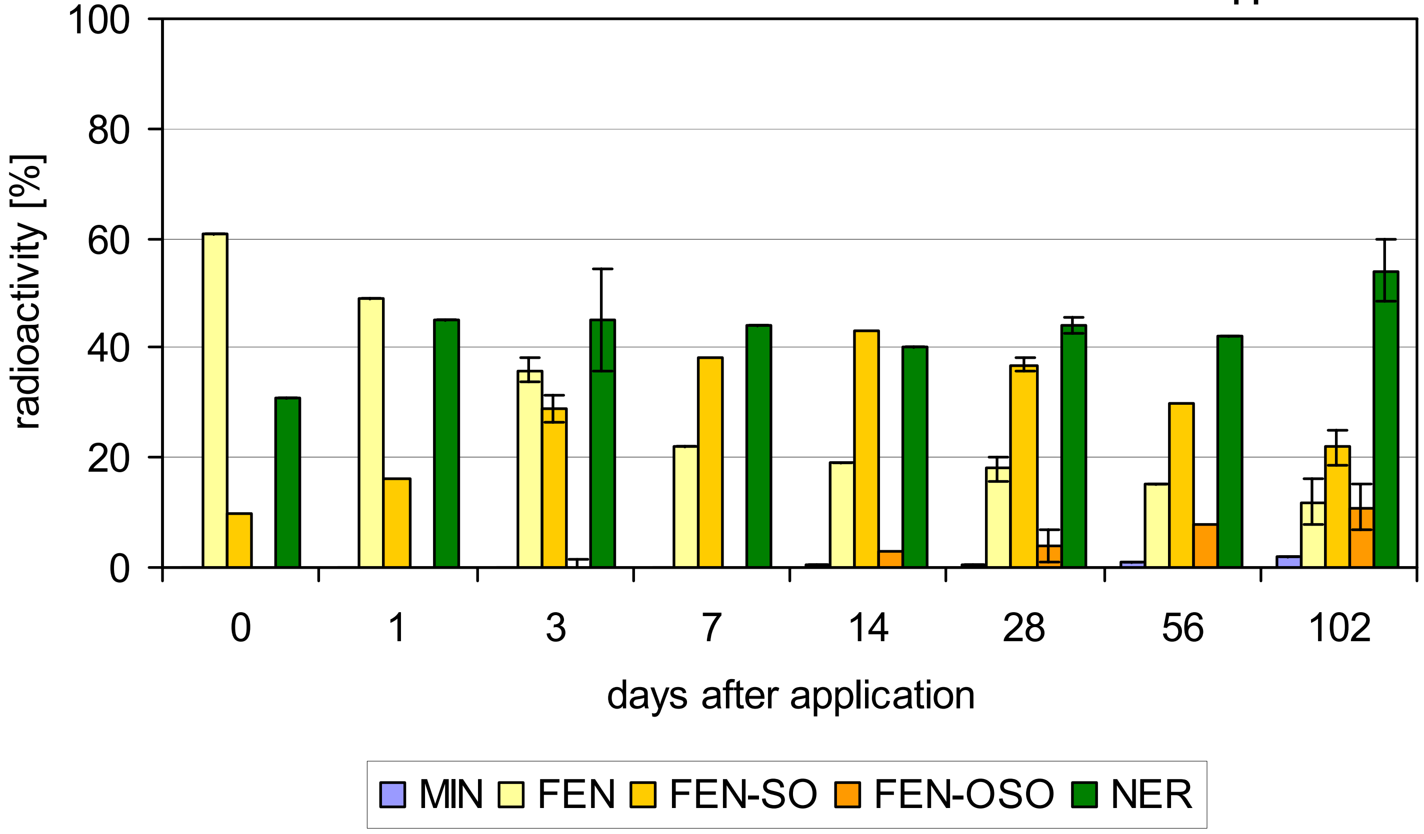




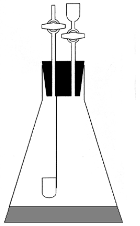
fenbendazole



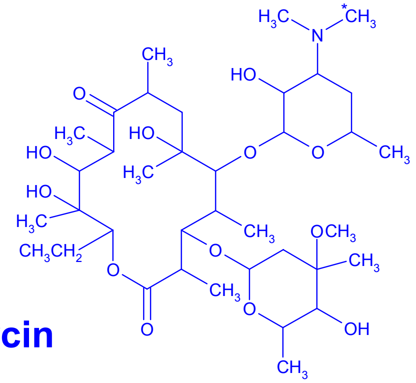
Transformation after standard application

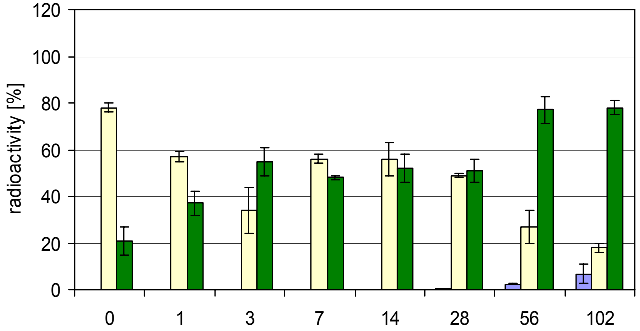


Transformation of ^{14}C-fenbendazole in clay soil after pig test-manure application (balances 104 ± 4 %)



erythromycin





Transformation after standard application

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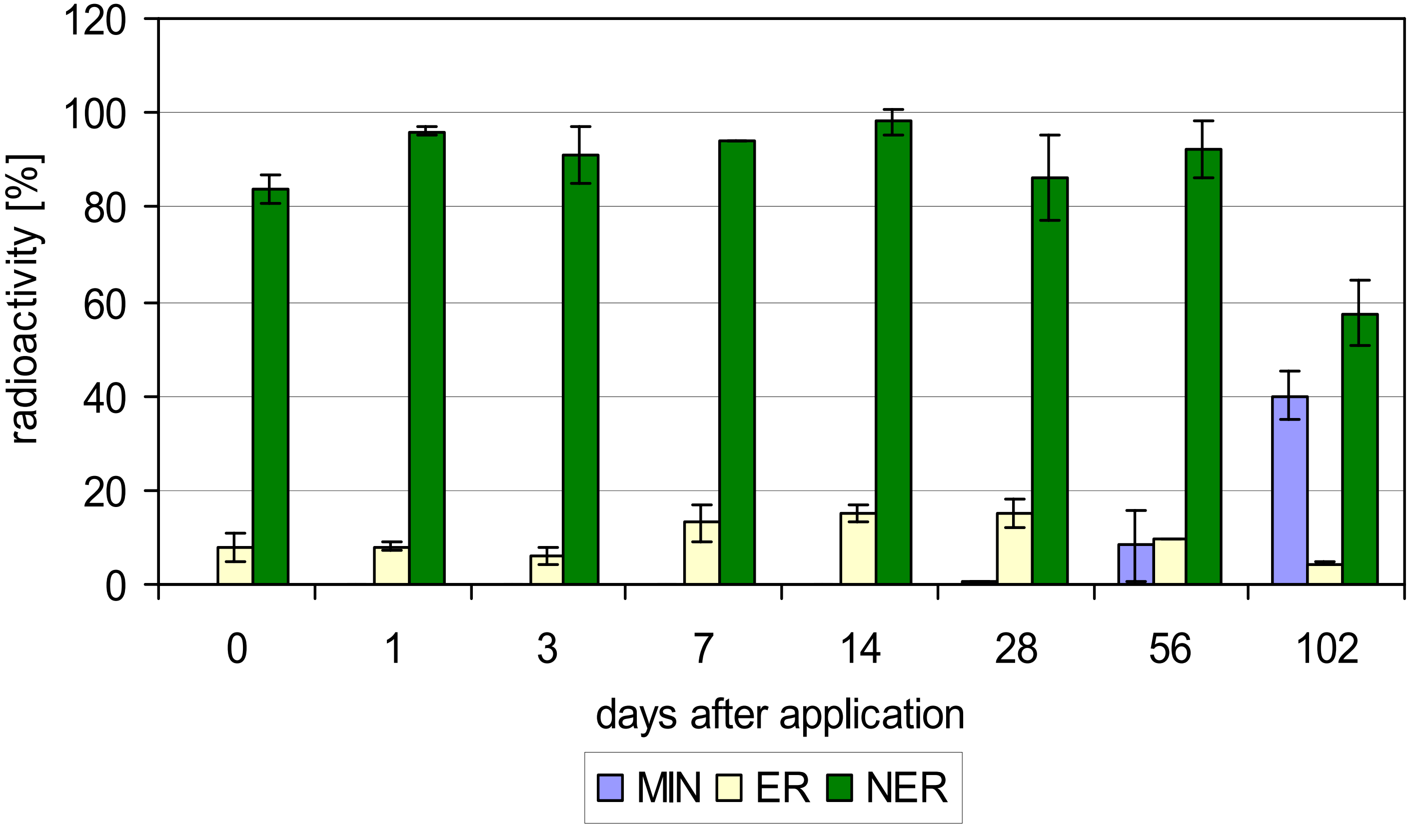
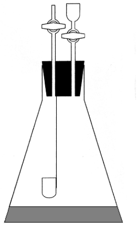
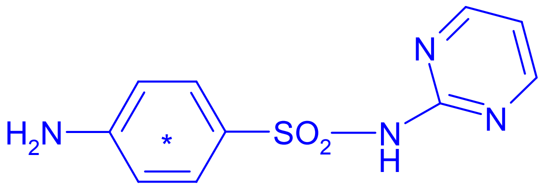
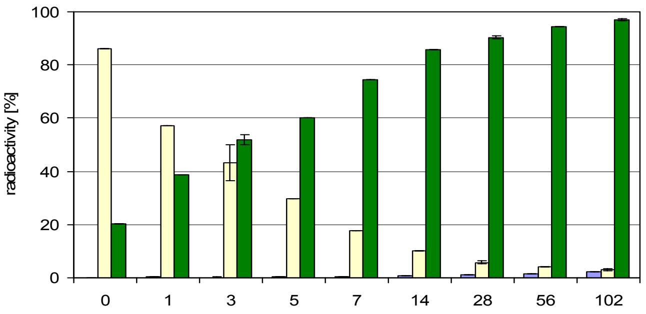


Transformation of ^{14}C-erythromycin in clay soil after pig test-manure application (balances 103 ± 7 %)

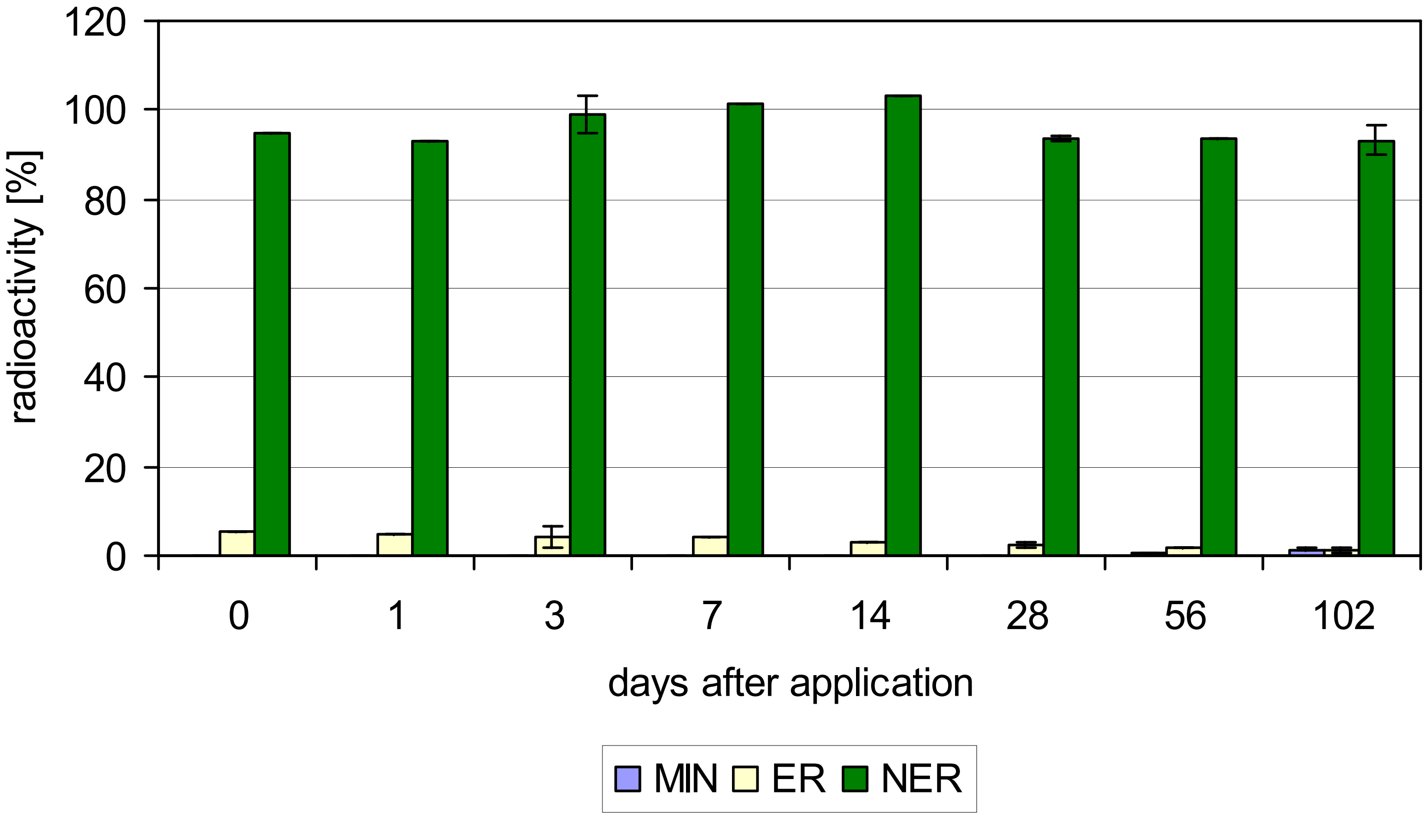




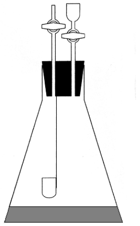
sulfadiazine

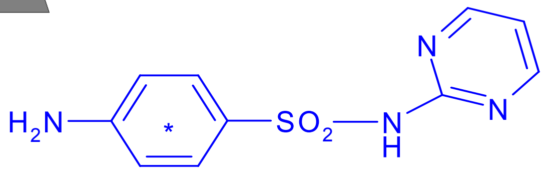


Transformation after standard application

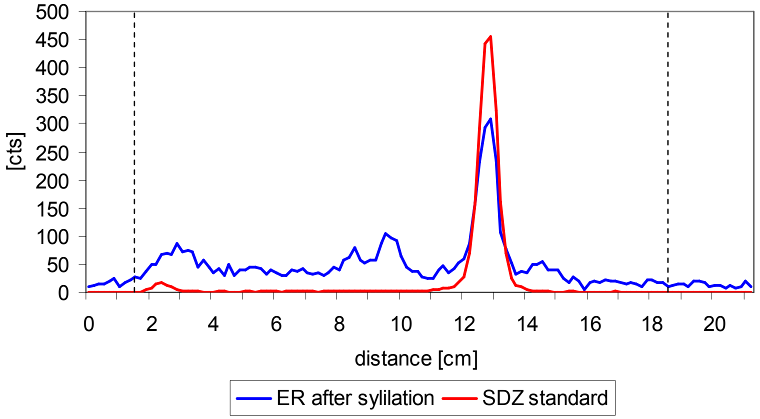


Transformation of ^{14}C-sulfadiazine in clay soil after test-manure application

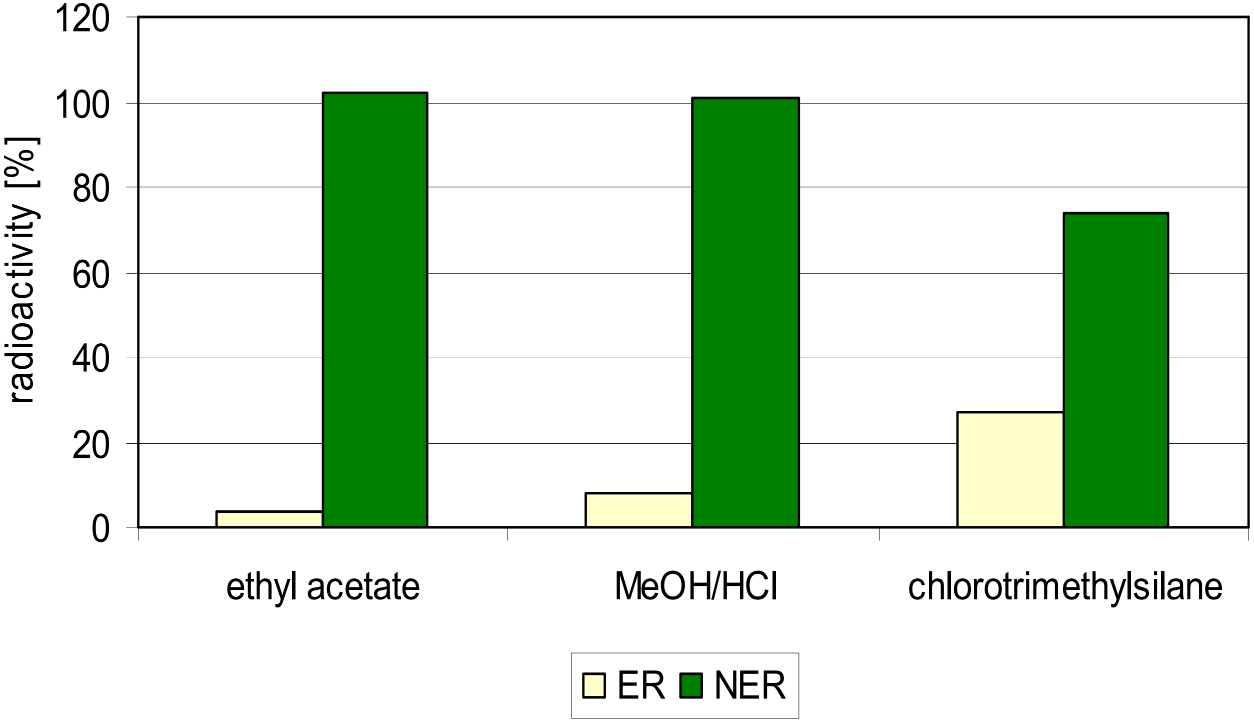




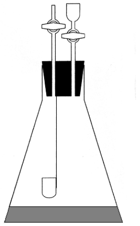
sulfadiazine

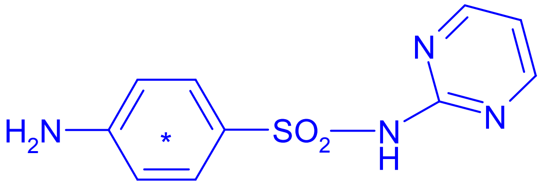


Release of SDZ after silylation



Chemical characterization of non-extractable ^{14}C-sulfadiazine residues in soil (balances 105 ± 3 %)





sulfadiazine

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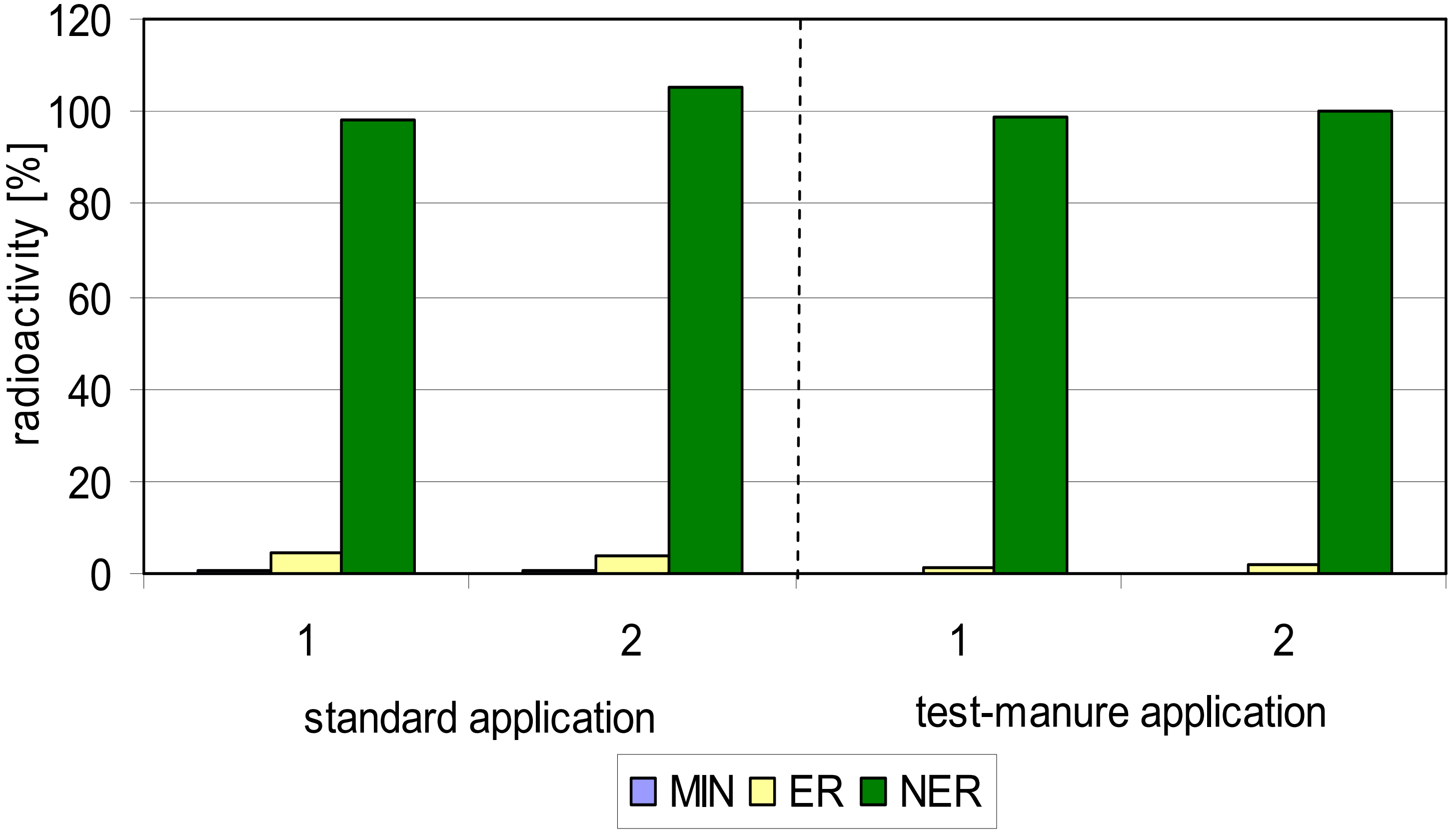
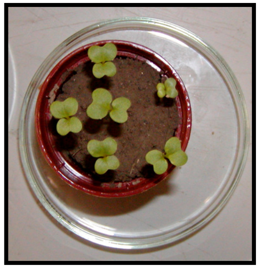
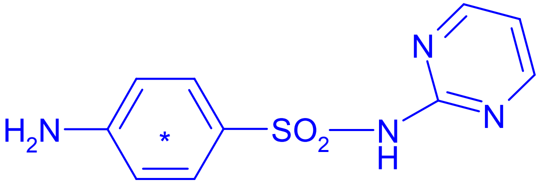


Activated-sludge test (28 d) on the remobilization of non-extractable ^{14}C-sulfadiazine residues in clay soil (balances 102 ± 2 %)





sulfadiazine

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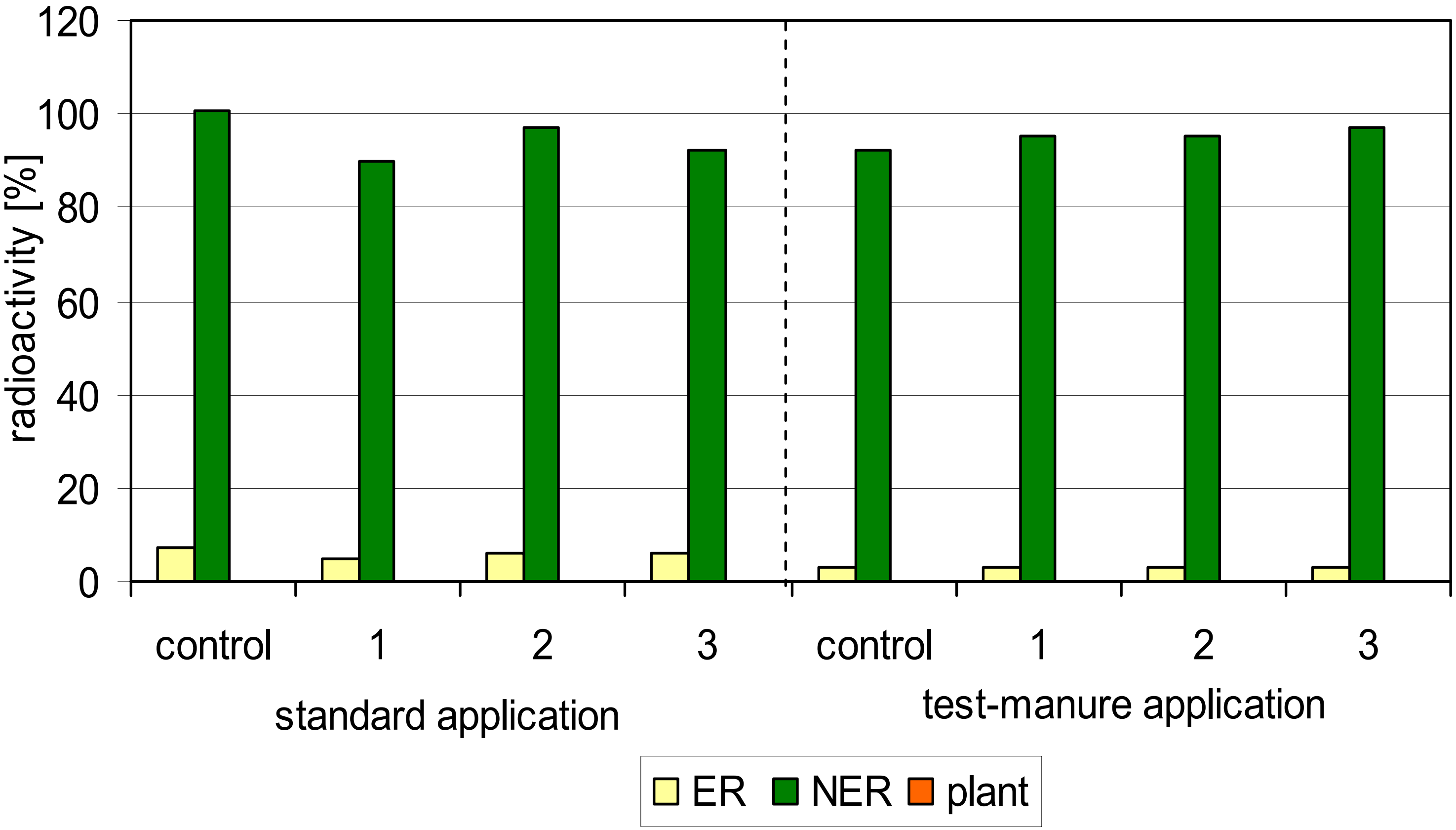
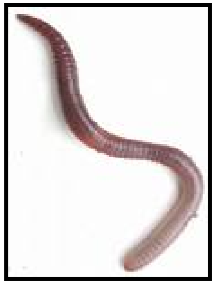
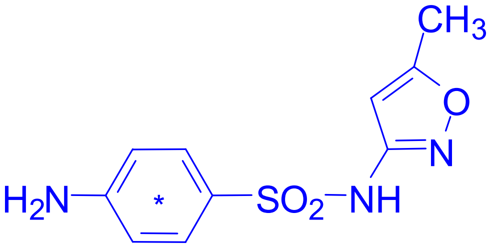
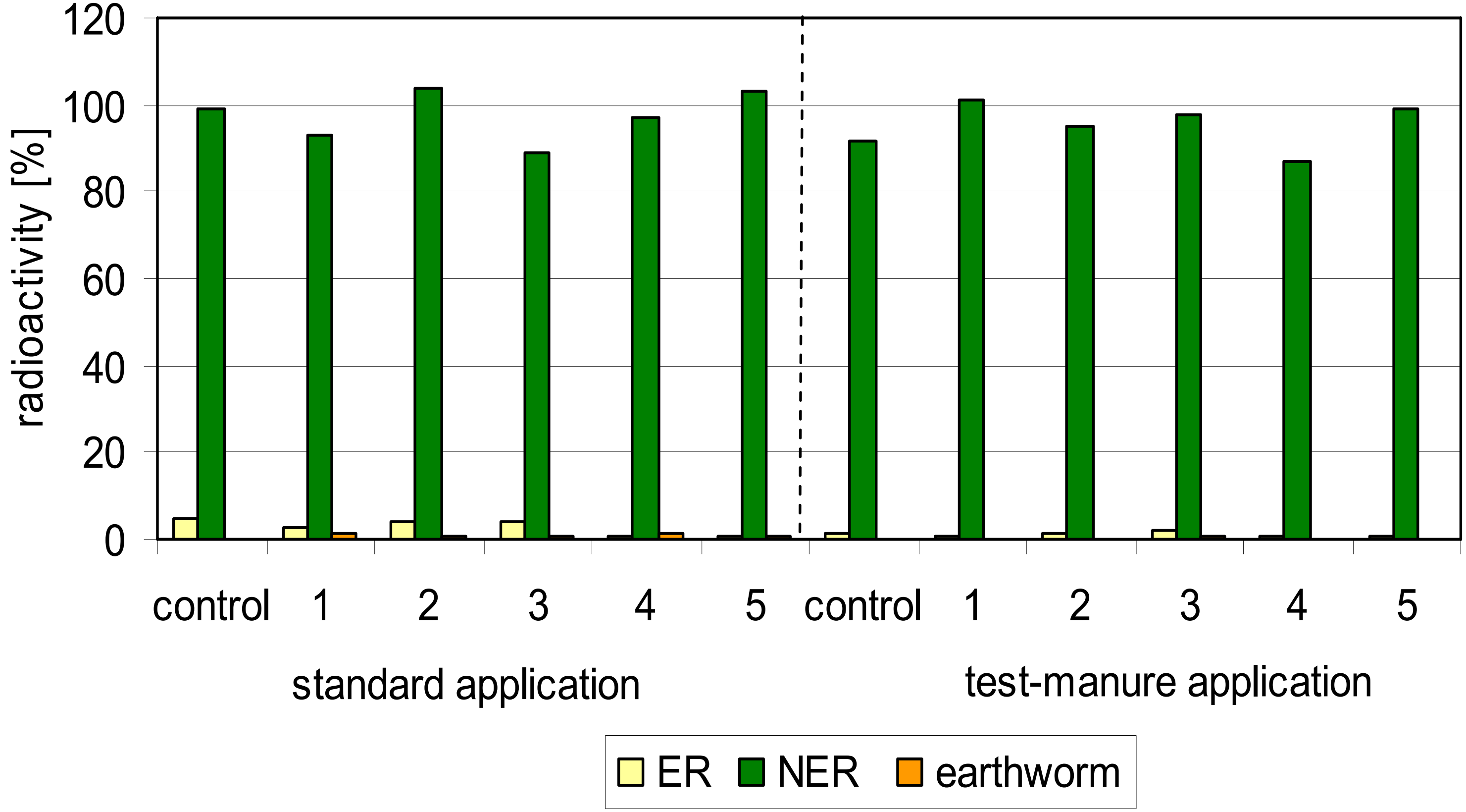


Brassica rapa **test (14 d) on the remobilization of non-extractable ^{14}C-sulfadiazine residues in clay soil (balances 99 ± 4 %)**



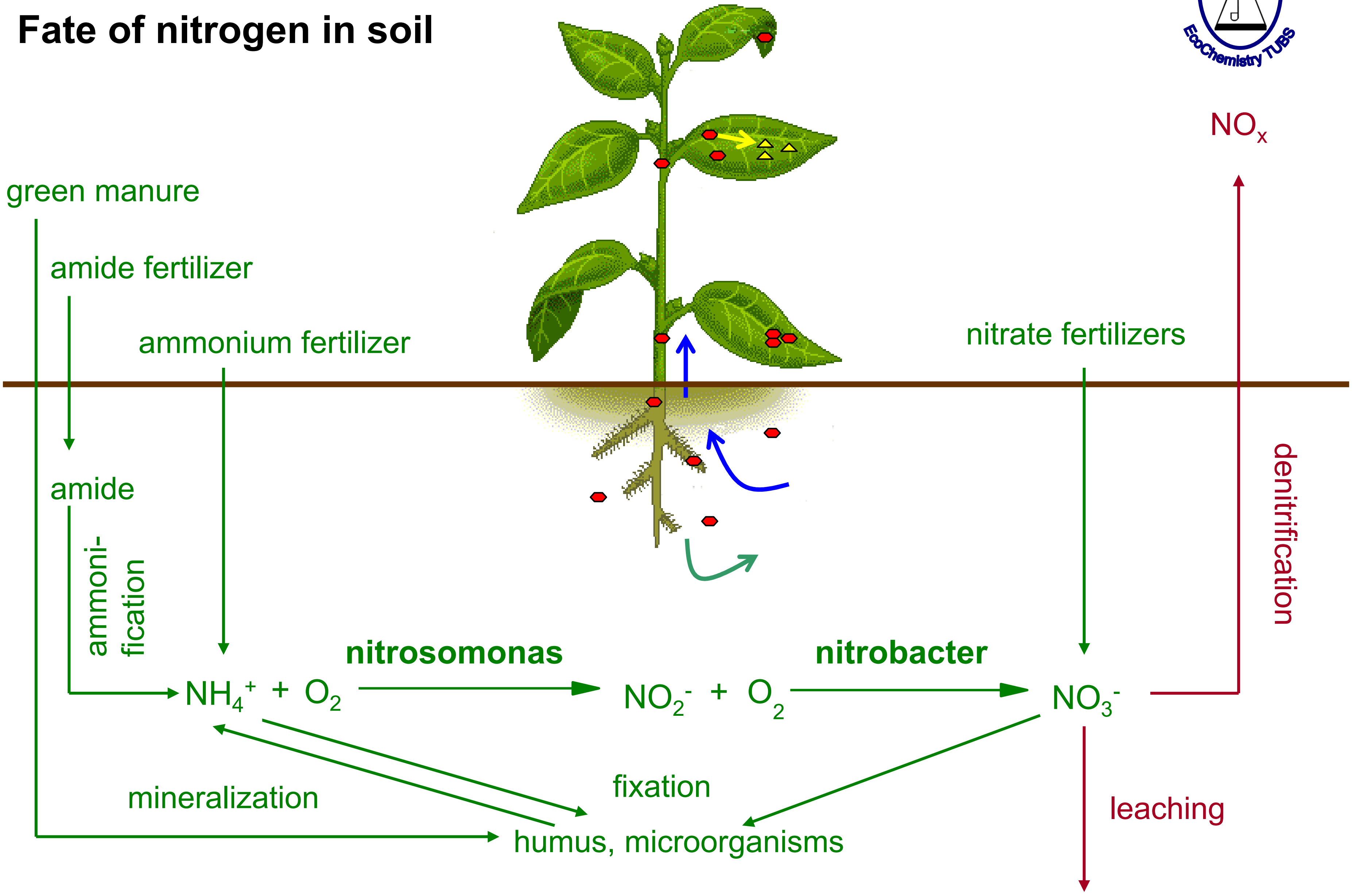


sulfamethoxazole



***Lumbricus terrestris* test A (14 d) on the remobilization of non-extractable ^{14}C-sulfamethoxazole residues in clay soil (balances 99 ± 5 %)**

Fate of nitrogen in soil



NOx

green manure

amide fertilizer

ammonium fertilizer

nitrate fertilizers

amide

ammoni-
fication

denitrification

nitrosomonas

nitrobacter

$NH_4^+ + O_2 \longrightarrow NO_2^- + O_2 \longrightarrow NO_3^-$

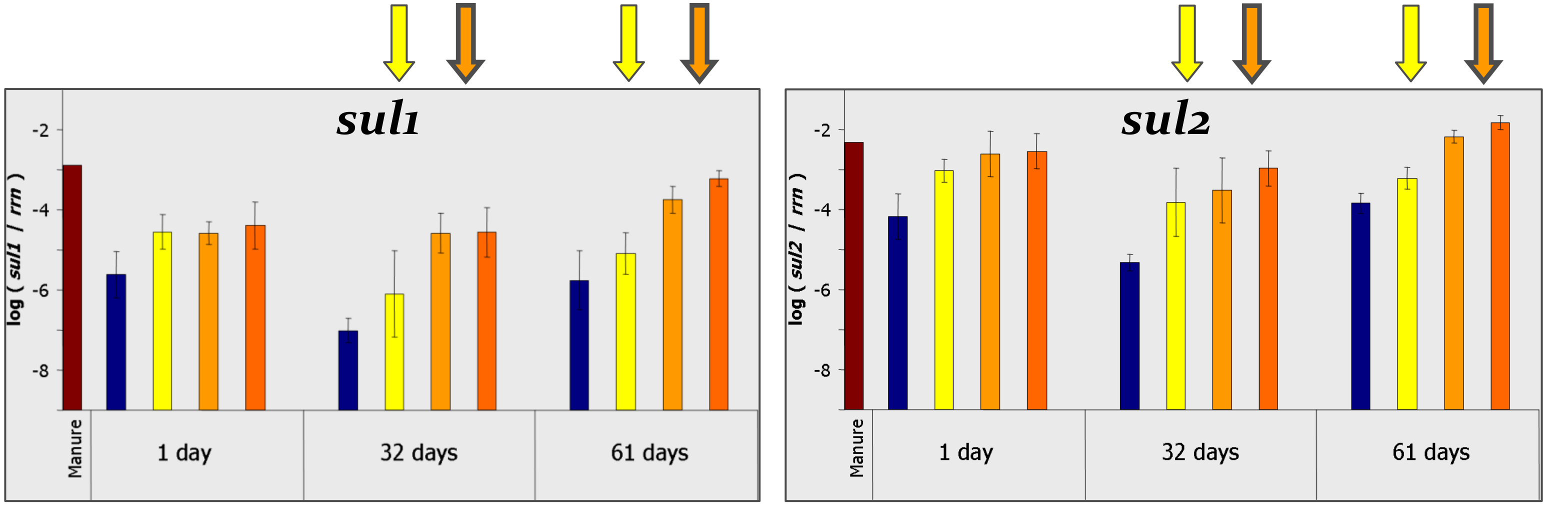
mineralization

fixation

leaching

humus, microorganisms

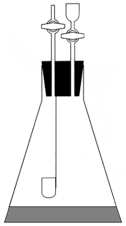
Dynamics of *sul* genes in total community DNA from soil followed by quantitative polymerase chain reaction (qPCR)



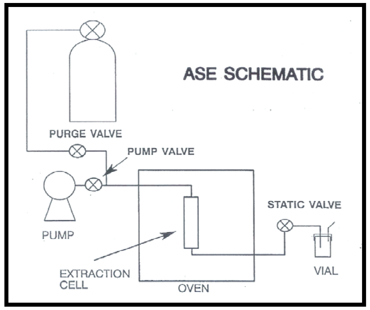
⇨ SDZ effect: ⇧ ⇧

🟦 Soil
🟨 Soil + Manure
🟧 Soil + Manure + SDZ^{10}
🟥 Soil + Manure + SDZ^{100}

Heuer, H. and K. Smalla. (2007): Synergistic effect of pig manure and sulfadiazine on the spread of bacterial antibiotic resistance in manured soil. Environ. Microbiol., 9, 657-666.

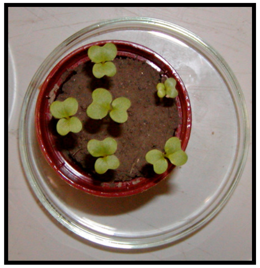


Chemical and biological characterization of non-extractable residues for environmental risk assessment



Open questions:

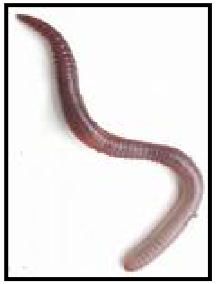
- parent compound or metabolites ?
- non-hazardous or hazardous substances ?
- physically entrapped or chemically bound ?
- reversible or irreversible processes ?
- benefit or environmental risk ?
- limits of soils´ buffer capacity ?

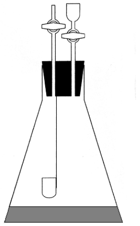


Possible measures:

- operational definition of non-extractability !
- definition of persistence criteria or NER triggers:

  MIN < 5 %; NER <10 %, 10-70 %, > 70 % in 100 days !





Transformation tests of veterinary medicines and biocides in liquid manures and transformation and sorption in manured soils

Excrement

Reference Manure

Degradation Tests

Test Manure

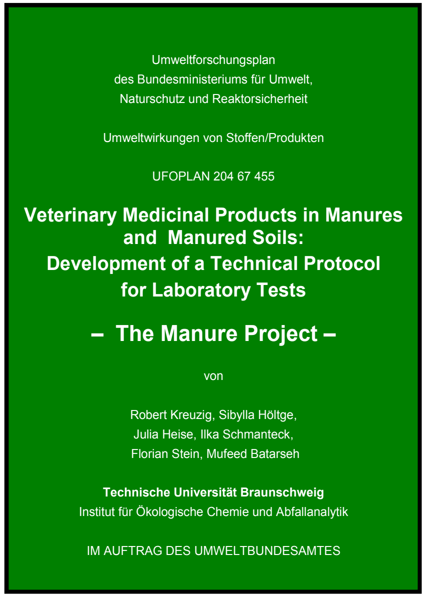
Fate Monitoring in Manured Soils

From excrements to reference manures:
The reference-manure concept facilitates reproducible laboratory testing at minimized experimental efforts !

Entry route and matrix effects define fate in soil:
The persistence of veterinary medicines and biocides in manures defines their entry into soil environments !

Transferability from laboratory to field:
Manure application to soil already under laboratory conditions improves understanding of VMP´s and biocides´ fate under field conditions !

Acknowledgments



Umweltforschungsplan
des Bundesministeriums für Umwelt,
Naturschutz und Reaktorsicherheit

Umweltwirkungen von Stoffen/Produkten

UFOPLAN 204 67 455

Veterinary Medicinal Products in Manures and Manured Soils:
Development of a Technical Protocol for Laboratory Tests

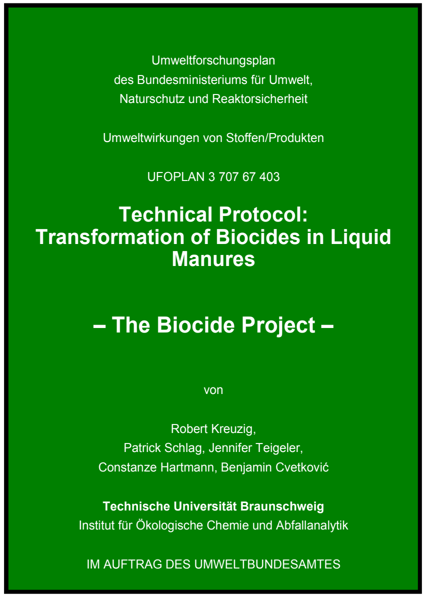
– The Manure Project –

von

Robert Kreuzig, Sibylla Höltge,
Julia Heise, Ilka Schmanteck,
Florian Stein, Mufeed Batarseh

Technische Universität Braunschweig
Institut für Ökologische Chemie und Abfallanalytik

IM AUFTRAG DES UMWELTBUNDESAMTES



Umweltforschungsplan
des Bundesministeriums für Umwelt,
Naturschutz und Reaktorsicherheit

Umweltwirkungen von Stoffen/Produkten

UFOPLAN 3 707 67 403

Technical Protocol:
Transformation of Biocides in Liquid Manures

– The Biocide Project –

von

Robert Kreuzig,
Patrick Schlag, Jennifer Teigeler,
Constanze Hartmann, Benjamin Cvetković

Technische Universität Braunschweig
Institut für Ökologische Chemie und Abfallanalytik

IM AUFTRAG DES UMWELTBUNDESAMTES

available at
www.umweltbundesamt.de

German Federal Environmental Agency:

Manure Project: Fate and Behaviour of Veterinary Medicinal Products in Liquid Manures and Manured Soils: Development of a Technical Protocol for Laboratory Tests (UBA-FKZ 204 67 455; 2004-2007)

Biocide Project: Technical Guidance: Transformation of Biocides in Manures (UBA-FKZ 3707 67 403; 2007-2009)

L. Van Leemput (Janssen Animal Health, Beerse, Belgium) for the supply of ^{14}C-imazalil and non-labelled reference standards of imazalil and metabolites as well as for fruitful discussions.

G. Nickel (SCC, Wendelsheim, Germany) for the supply of ^{14}C-cyanamide

LabTestSysTeam, EcoChemistry, TU Braunschweig:
P. Schlag, C. Hartmann, B. Cvetković, J. Teigeler and **the lab assistants**

... and many thanks for your attention !