Transfer of pharmaceutical residues in soils and plants from farmyard manure

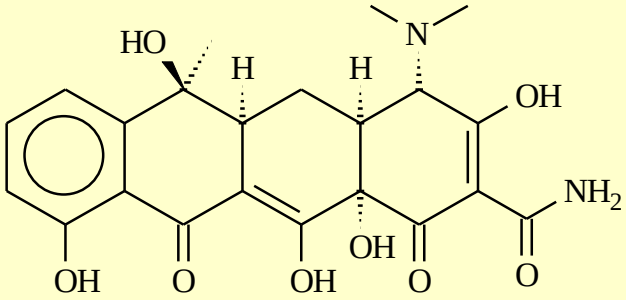
Anja Töpper
Center for Agricultural Technology *(LTZ) Augustenberg*
Karlsruhe, Germany

Uta Kraus
Chemical and Veterinarian Investigations Institute (CVUA)
Karlsruhe, Germany

Intention of the project

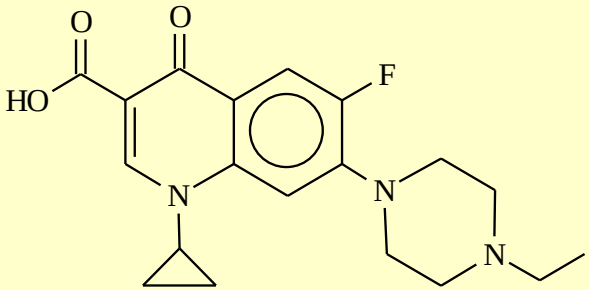
- pharmaceutical residues have become a noteworthy contamination factor in the environment during recent years
- many studies deal with pharmaceutical residues in water, such as surface, ground and drinking water
- more research is needed concerning pharmaceutical residues entering the feed and food chain through crop and vegetables as a result of plant fertilization with farmyard manure under practical conditions

Antibiotics of the trial



Tetracycline (TC)

- most frequently used antibiotic in veterinary medicine in Germany
- inhibition of the bacterial protein synthesis
- an entry of tetracycline into the food and feed chain from farmyard manure is most likely



Enrofloxacin (ENR)

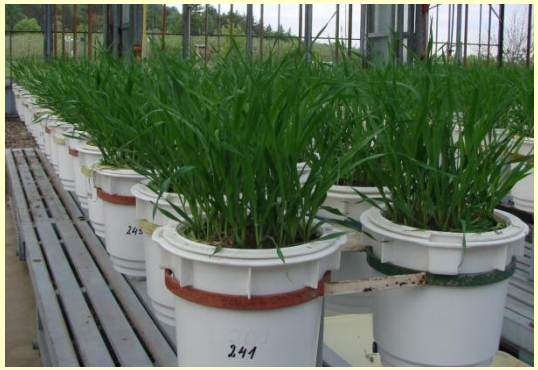
- belongs to the group of fluorqinolones
- gyrase inhibitor

- both antibiotics defined as persistent
- show high adsorption coefficient to soil
- accumulation in the environment

Experimental Setup

Pot experiments

- cultivation was performed in Kick-Brauckmann-pots under green house conditions
- 2 antibiotics in 3 concentrations to 5 or 3 harvest times and 2 replications (between 36 and 80 pots per culture)
- Kick-Brauckmann-pots are constructed to allow constant watering from below.
- additionally the plants were watered from the top once a week.



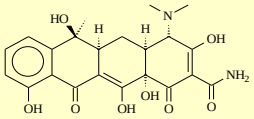
Field trials

- trial plots of 3 x 12 m (20 plots)
- randomised block design
- 5 variants in 4 replications

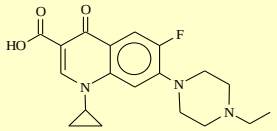
Estimation of added of antibiotics

realistic approach (initial situation)

Teracycline (TC):



Enrofloxacin (ENR):



- 6 month fattening (ca. 180 days)
- final fattening weight of 110-120 kg
- taking into account an average water consumption of 5,7 l/d
- single treatment over two days at the beginning
- dilution effects in slurry (caused by storage)

- daily intake of 20 mg/kg body weight

Tetracycline concentration under practical conditions

0,3 mg/l slurry

Estimation of added antibiotics

realistic approach (initial situation)

Concentration of tetracycline and chlortetracycline in slurry from conventional livestock production

	c tetracycline mg/kg FM	c chlortetracycline mg/kg FM	source
cattle slurry	n.d.	n.d.	own investigations
pig slurry	0,1	62	own investigations
pig slurry	0,5	n.d.	own investigations
pig slurry	-	100	CHRISTIAN ET AL., 2003
pig slurry	0,01-1,9	0,05-3,7	SATTELBERGER ET AL., 2005

n.d.= not detectable; FM = fresh matter

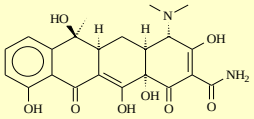
CHRISTIAN T, SCHNEIDER RJ, FÄRBER HA, SKUTLAREK D, MEYER MT, GOLDBACH HE (2003) DETERMINATION OF ANTIBIOTIC RESIDUES IN MANURE, SOIL AND SURFACE WATERS. ACTA HYDROCHIM. HYDROBIOL., 31:36-44

SATTELBERGER R, GANS O, MARTINEZ E (2005) VETERINÄRANTIBIOTIKA IN WIRTSCHAFTSDÜNGER UND BODEN. BUNDESUMWELTAMT GMBH WIEN, BERICHT BE-272

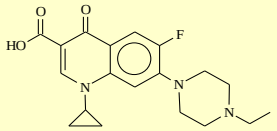
Estimation of added antibiotics

realistic approach (initial situation)

Teracycline (TC):



Enrofloxacin (ENR):



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- single treatment over two days at the beginning
- dilution effects in slurry (caused by storage)

- daily intake of 20 mg/kg body weight

Tetracycline concentration under practical conditions

0,3 mg/l slurry

- daily intake of 2,5 mg/kg body weight

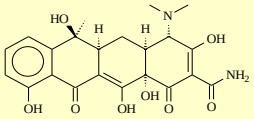
Enrofloxacin concentration under practical conditions

0,05 mg/l slurry

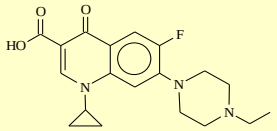
Estimation of added antibiotics

worst-case approach (initial situation)

Teracycline (TC):



Enrofloxacin (ENR):



- **6 month fattening (ca. 180 days)**
- **final fattening weight of 110-120 kg**
- **taking into account an average water consumption of 5,7 l/d**
- **single treatment over two days at the beginning**
- **in addition two treatments during fattening**
- **dilution effects in slurry (caused by storage)**

Tetracycline concentration under worst-case conditions

7 mg/l slurry

Enrofloxacin concentration under worst-case conditions

1 mg/l slurry

Application of antibiotics

Drug concentration of tetracycline (TC) and enrofloxacin (ENR) in slurry and calculated concentration in soil

trial	addition to slurry		theoretical drug concentration in soil			
			field trial		vessel experiments	
concentration c	c (TC) mg/l	c (ENR) mg/l	c (TC) µg/kg DM	c (ENR) µg/kg DM	c (TC) µg/kg DM	c (ENR) µg/kg DM
control	0	0	-	-	-	-
realistic approach	0,3	0,05	5	0,8	8	0,13
worst-case approach	7	1	116	16	187	26

DM = Dry Matter; Trial plots of 3x12 m; depth 20 cm

crops used for the trials: → **corn, wheat and barley**

Experimental Setup

crops used for the trials: → **corn, wheat and barley**

- addition of the calulated drug concentration to slurry
- taking into account even existing residues of the used antibiotics in the slurry
- spreading with a standard fertilizer application
- collection of plants and soil samples

Time of sampling

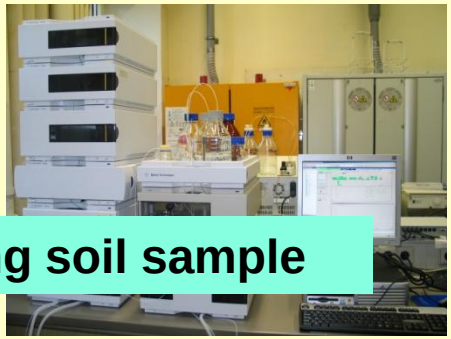
pot experiments

- up to tillering or 4-6 leaf stage
- at the beginning of ear emergence
- at grain harvest
- soil samples in 2 layers (0-5 cm and 6-20 cm) at harvest

field trials

Sample analytics

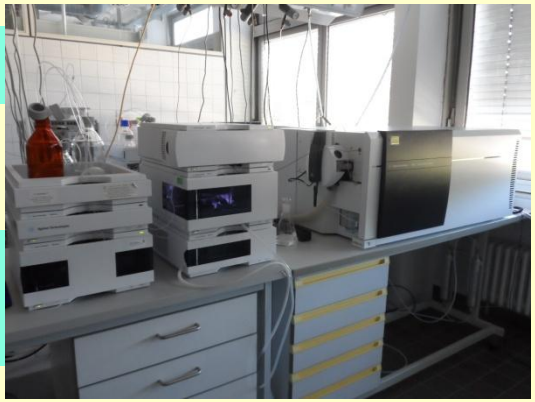
shearing and drying at 40 °C



homogenizing plant and grain

homogenizing soil sample

weights of 1 g sample material



Extraction with different extraction agents

measurement with HPLC and LC-MS/MS

Sample analytics

Limit of quantification and limit of detection of tetracycline and enroflofacin

matrix	limit of detection µg/kg DM	limit of quantification µg/kg DM
plant material	10	30
soil	10	30

Results – yield study

grain yield in t DM/ha

treatment	variant	corn	wheat	barley
control	without treatment	4,4	4,2	6,0
tetracycline	realistic worst-case	4,2 4,4	4,2 4,1	5,7 5,8
enrofloxacin	realistic worst-case	4,9 4,2	4,2 4,1	5,9 6,0

no significant differences between control and treatments

Results – residues in corn

pot experiments

analysis of 66 samples

- 10 samples at 4-6 leaf stage
- 10 variants at ear emergence
- 46 different harvest products

tetracycli… …s

NOT DETECTABLE

…acin residues

field trials

analysis of 60 samples

- 3 times of sampling
- 5 variants
- 4 replications

tetracycli… …es

NOT DETECTABLE

…xacin residues

Results – residues in wheat

pot experiments

analysis of 46 samples

- 10 samples at 4-6 leaf stage
- 10 variants at ear emergence
- 26 different harvest products

tetracycline

NOT DETECTABLE

acin residues

field trials

analysis of 60 samples

- 3 times of sampling
- 5 variants
- 4 replications

tetracycli

NOT DETECTABLE

xacin residues

Results – residues in barley

pot experiments

analysis of 46 samples

- 10 samples at 4-6 leaf stage
- 10 variants at ear emergence
- 26 different harvest products

tetracyclin[illegible]

[illegible]acin residues

NOT DETECTABLE

field trials

analysis of 60 samples

- 3 times of sampling
- 5 variants
- 4 replications

tetracyclin[illegible]

[illegible]xacin residues

NOT DETECTABLE

Results – residues in soil

tetracycline residues of the corn trials

	addition mg/l	addition µg/kg	4-6 leaf stage µg/kg	ear emergence µg/kg	harvest µg/kg
pot experiments					
realistic	0,3	8	-	-	n.d.
worst-case	7	187	-	-	<loq
field trials (0-20 cm)					
realistic	0,3	5	n.d.	n.d.	n.d.
worst-case	7	116	30	n.d.	n.d.
soil layer					
worst-case (0 - 5 cm)	7		-	-	30
worst-case (6-20 cm)	7		-	-	n.d.

- = no sampling; loq = limit of quantification n.d. = not detectable

no residues of tetracycline in any soil samples of the wheat and barley trials

no residues of enrofloxacin in any soil samples

Main findings

- **no entry of tetracycline and enrofloxacin into barley, wheat and corn under practical conditions**
- **soil: no detectable concentrations of these two antibiotics under realistic conditions**
- **no significant growth differences in various plants after administration of antibiotics**

Thank you:

- Ministry of Rural Affairs and Consumer Protection Baden-Württemberg for financing the project

- The Chemical and Veterinarian Investigation Institute (CVUA) Karlsruhe for the good cooperation

- All LTZ-colleagues involved
  (i.e. Klaus Mastel, Nicole Schneider-Götz, Markus Mokry, Mario Müller)

Thank you
for your attention