Incorporation of veterinary antibiotics into crops from manured soil[1]

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Abstract

Under farming conditions, winter wheat was cultivated on manure-fertilised soil. The liquid manure originated from pigs medicated under control with chlortetracycline (CTC), sulfadiazine (SFD) and trimethoprim (TMP). During 8 months of storage the initial concentration of CTC and SFD in the manure decreased by 50 - 60 %. This manure was used as a fertiliser, thereby remarkable amounts of antibiotics were administered to soil. Antibiotic recovery rates from soil peaked after the 2nd slurry administration (SFD: 90 µg/kg dw, CTC-compounds: 240 µg/kg dw) in the plough layer (0 - 25 cm) and dropped to a low level within 4 to 5 months. Wheat was grown on this soil. CTC and SFD were readily taken up by the roots, translocated within the plants into stems and leaves; CTC even into the wheat grain (~44 µg/kg fw) after twofold slurry application in spring. Experiments with non-labelled and ^{3}H-labelled antibiotics using hydroponically grown wheat, lamb's lettuce and carrots corroborate the result that intact plants can take up substantial amounts of antibiotics by the roots. Our study has revealed a novel way of human exposure to veterinary antibiotics by plant derived food.

Keywords: antibiotics in plants, liquid manure, chlortetracycline, sulfadiazine, trimethoprim, soil, winter wheat, consumer health protection

Zusammenfassung

Transfer von Antibiotika aus Wirtschaftsdüngern in Nahrungspflanzen

Unter praxisnahen landwirtschaftlichen Bedingungen wurde Winterweizen auf Versuchsparzellen angebaut, die mit Schweinegülle beaufschlagt waren. Zur Gewinnung der Antibiotika-belasteten Gülle waren Ferkel mit Chlortetracyclin (CTC), Sulfadiazin (SFD) und Trimethoprim (TMP) kontrolliert medikamentiert worden. Während der 8-monatigen Lagerung wurden in der Gülle CTC und SFD nur bis zu 50 - 60 % abgebaut, so dass durch die Düngung erhebliche Antibiotikafrachten auf die Felder gelangten. Nach zweimaliger organischer Düngung traten im Pflugbereich (0 - 25 cm) die höchsten (extrahierbaren) Bodengehalte an SFD (90 µg/kg TM) und CTC (Summenparameter: 240 µg/kg TM) auf, die sich nach 4 bis 5 Monaten erheblich verringert hatten. Auf diesem Boden wurde Weizen angebaut. SFD und CTC wurden vom Winterweizen aus dem Boden aufgenommen und in Stängel und Blätter transportiert, CTC bei zweifacher Gülledüngung im Frühjahr sogar bis ins Korn (~44 µg/kg FG). Die in Hydrokultur nachgewiesene Aufnahme und Verteilung unmarkierter und ^{3}H-markierter Antibiotika in Weizen, Feldsalat und Karotten bestätigt und ergänzt die Befunde der Feldversuche, dass intakte Pflanzen beträchtliche Mengen an Antibiotika über die Wurzel aufnehmen können. Die Ergebnisse unserer Studie können als deutlicher Hinweis auf einen Eintragspfad von Antibiotika aus der landwirtschaftlichen Tierhaltung in pflanzliche Lebensmittel gewertet werden.

Schlüsselworte: Antibiotika in Pflanzen, Gülle, Chlortetracyclin, Sulfadiazin, Trimethoprim, Boden, Winterweizen, gesundheitlicher Verbraucherschutz

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1 Introduction

Antibiotics are still used worldwide in large quantities as growth promoters and for therapeutic purposes in animal husbandry although legal restrictions have been set recently in the European Union (Sarmah et al., 2006). After application to pigs, up to 90 % of the drugs are excreted. The slurry obtained is used as liquid fertiliser in agricultural systems. Bacterial antibiotic resistance may be induced, either in animal guts, or after application to soil, in the environment (Smith 2005, Halling-Sørensen et al., 1998 and 2002). It has already been observed over forty years ago that naturally occuring antibiotics are taken up by roots from solution and are subsequently translocated within the plant (Krasilnikov 1958, Köhler 1960). The results of more recent experiments, e.g. under hydroponic and greenhouse conditions, foster the hypothesis that uptake of antibiotics by crops can occur (Batchelder 1982, Langhammer et al., 1990, Forni et al., 2002, Gujarathi et al., 2005, Boxall et al., 2006). Incorporation of CTC by maize and vegetables grown in pots was suggested to occur on the basis of analyses using ELISA (Kumar et al., 2005), a method prone to interferences. Moreover, the ELISA used cannot differentiate the various conversion products or metabolites of CTC (Table 1). The analysis of barley grown on manure fertilised soil gave no hints to the uptake of veterinary medicines (Jacobson et al., 2004).

It is important to emphasise that, until recently (Grote et al., 2005), no defined field experiments addressing the uptake of antibiotics from manured soil by marketable crops have been reported. This is why we set out to investigate, under controlled farming conditions, the uptake of antibiotics from manured soil and distribution of antibiotics within the plants using winter wheat (*Triticum aestivum*). In addition, the full potential for the uptake of antibiotics by vegetable plants was estimated by growing the plants under hydroponic conditions, where no sequestration by the soil can occur.

2 Materials and methods

2.1 Controlled medication of pigs and production of liquid manure

Five male castrated piglets, reared without antibiotic medication, were individually housed at the Bundesforschungsanstalt für Landwirtschaft FAL, Germany, and medicated twice for 10 days. Due to animal welfare regulations, medication was interrupted for 11 days in between. The antibiotics chlortetracycline (CTC) and sulfadiazine

Table 1:

Antibiotic residues in roots, stems, leaves and grains of wheat grown on soil, treated twice with liquid manure (plot 1)
(Results expressed as mg/kg of dry weight (roots, green parts) or fresh weight (grains as is); values are means ± SD from 4 samples, 3 measurements of each)

	Chlortetracycline (CTC)						Sulfadiazine
Sampling date/ Plant organ	CTC	enol-epi-	keto-epi-	iso-	epi-iso-	total	SFD
April 15, 2002							
Root	0.313 ± 0.035	0.174 ± 0.018	0.185 ± 0.020	0.115 ± 0.003	0.086 ± 0.007	0.874 ± 0.062	0.409 ± 0.076
April 29, 2002							
Root	0.396 ± 0.087	0.171 ± 0.038	0.114 ± 0.018	0.196 ± 0.028	0.251 0.030	1,104 0.176	0.487 ± 0.092
Stem/leaf	0.404 ± 0.120	0.083 ± 0.030	0.135 ± 0.050	0.126 ± 0.016	0.074 ± 0.004	0.822 ± 0.213	0.044 ± 0.005
August 8, 2002 (harvest)							
Root	0.043 ± 0.006	0.016 ± 0.003	0.012 ± 0.003	0.024 ± 0.001	0.018 ± 0.003	0.111 ± 0.010	n.d.
Straw	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Grain	0.043 ± 0.013	n.d.	n.d.	< LOD	< LOD	0.043 ± 0.013	n.d.

Abbreviations: enol-epi-CTC = enol-tautomer of epi-chlortetracycline; keto-epi-CTC = keto-tautomer of epi-chlortetracycline; epi-CTC = epimere of chlortetracycline, iso-CTC = isochlortetracycline; epi-iso-CTC = epimere of isochlortetracycline; LOD = Limit of Detection; n.d.= not detected, SD = Standard Deviation, dw = dry weight, fw = fresh weight

(SFD) combined with trimethoprim (TMP) were orally administered twice daily before each feeding. The total uptake of drugs for the first medication period was 45.0 g and for the second 56.1 g CTC (Chlortetracyclin 100®, Belapharm, 100 mg/kg lw), 112.6 and 161.4 g SFD as well as 22.5 and 32.3 g TMP (Antastmon®, Belapharm, 50 mg/kg lw), respectively.

Collected faeces and urine of each medication period were combined. The resulting two batches of slurry were stored in open plastic barrels from August 2001 until March 2002. Samples, monthly taken, were frozen at –30 °C before analyzing them by LC-MS/MS. Slurry batches were used as liquid manure for wheat fertilisation.

2.2 Application of liquid manure to fields and cultivation of winter wheat

Liquid manure of both medication periods was applied to the fields of the experimental farm „Versuchsgut Merklingsen“ of the University of Applied Sciences, FH Südwestfalen, Soest, Germany. No manure had been applied to these fields for the last 10 years. The composition of this soil (pseudogley para-brown earth: silt ~ 70 %, humic compounds ≤ 2 %, high water capacity) is typical for this area in Northrhine Westphalia. The water content determined before and after fertilisation was at maximum 25 % (soil depth 0 – 25 cm) and 15 % (25 – 50 cm), respectively (DIN ISO 11465). The pH-values ($CaCl_2$-extraction) were at 6.5 (0 – 5 cm) and 7.0 (20 – 25 cm).

Winter wheat (var. Drifter) was cultivated in a field divided into 6 plots, 24 m^2 each. Fertilisation with the liquid manure described was once (plots 3 to 6) or twice (plots 1 and 2) and complemented with mineral fertiliser (all plots). Winter wheat was sown on October 12, 2001. On March 12, 2002 liquid manure (2nd medication period) was applied to all plots at rates of 2 L/m^2 equivalent to 67 kg N/ha. The second dispersion of manure of the 1st medication period to plots 1 and 2 followed on April 15, 2002 (2.5 L/m^2 equivalent 46 kg N/ha) whereas plots 3 to 6 were fertilised with mineral fertiliser (46 kg N/ha). Finally, on May 25, 2002, all 6 plots were fertilised with mineral fertiliser (60 kg N/ha).

Soil was sampled before sowing of wheat and then monthly after the first fertilisation until harvest using a drilling machine (sleeves: Ø 50 mm x 255 mm). From plot 1 and 2 additional samples were taken immediately after the 2nd manure application. The sampled horizons were: 0 – 25 cm (plough layer) and deeper (25 – 50 cm), in some plots also segments of 0 - 10, 10 – 20, 20 – 30 and 30 – 60 cm. The segments of each horizon taken from the individual plots were combined, homogenised, and stored in polyethylene bags at - 30 °C.

Samples of whole wheat plants were taken from the fields before the 2nd manure dispersion on April 15, 2002 and once again on April 29. Grain harvest was timed such that the grain was ripe on every plot (August 2). Average yield was 97.1 dt/ha for all plots. This corresponds to the average yield of conventionally grown wheat in surrounding fields. Roots and straw were also taken. Nearly soil-free root material was recovered by thorough washing and rinsing with de-ionized water, and then separated from the stems. The plant organs were cut into small pieces (~0.5 cm length), washed, blotted dry and weighed. All plant materials were stored at -80 °C.

2.3 Uptake and distribution of antibiotics in hydroponically grown wheat

The experiments were carried out at the Federal Research Centre for Nutrition and Food, (BfEL) Detmold location, Germany. Winter wheat plants (and in addition lamb's lettuce and carrots) were grown in nutrient solution and exposed to selected representative antibiotics that we determined in the produced manure. These were SFD, CTC and iso-CTC, a non-active conversion product of CTC. Seeds were surface sterilised using sodium hypochlorite and hydrogen peroxide. After repeated extensive rinsing the seeds were transferred into sieves placed over nutrient solution (day 0). The seedlings were transferred to flower boxes filled with 4.5 L of Hoagland's nutrient solution (pH 6) which were placed in a climate-controlled growth cabinet (16 h photoperiod). The nutrient solution was protected from light by aluminium foil and was changed three times a week. After 13 days, the nutrient solution was spiked with either SFD or CTC combined with iso-CTC, so that concentrations of 2.5, 5, 10 or 20 µmol/L were reached for each compound. Non-spiked nutrient solution was used for the control wheat.

The plants, sampled 7, 14 and 21 days after spiking (or without), were intensively washed. Roots and green parts, stems, young and old leaves, were separated and stored at -80 °C.

2.4 Uptake and distribution of radiolabelled antibiotics

These experiments were carried out at the Center for Environmental Research & Technology UFT, University of Bremen, Germany. Hoagland's solution (10 mL) was placed in a small plastic jar. After 13 days of germination and cultivation the nutrient solutions were spiked with 5 or 10 µmol/L of SFD or CTC and iso-CTC and tritium-labelled sulphonamide (740 KBq of (3,5)-^{3}H-sulfamethazine, Moravec Biochemicals, USA) or tritium-labelled tetracycline (740 KBq 7-^{3}H-tetracycline, Moravec Biochemicals, USA) was added to the corresponding spiked solution. Plantlets were taken after 7, 14 and 21 days, roots,

stems and leaves separated, cryofixated and lyophilised. Tissue solubiliser (Biolute S) was used to dissolve aliquots of the weighed samples. After several weeks a scintillation mixture (Quicksafe A) was added for liquid scintillation counting. Calculations to obtain the distribution of radioactivity in plant organs are based on quench corrected data and specific acitivities of the labelled compounds.

The experimental conditions used for the two sets of hydroponic experiments (applying labelled and exclusively non-labelled antibiotics) were similar but not identical. For example, the nutrient solutions containing radioactive tracers could not be exchanged every other day by contrast to the other experiments.

3 Analysis of antibiotic residues

Manure, soil and plant material were analysed at the University of Paderborn by means of HPLC-UV-MS^n.

3.1 Extraction and clean up

Liquid manure samples (1 - 5 g) were defrosted, homogenised and treated with 30 mL of aqueous McIllvain-buffer pH 4.1 (0.1 mol EDTA dissolved in 620 mL of 0.1 mol/L citric acid and 380 mL of 0.2 mol/L sodium dihydrogenphosphate).

Soil samples (5 g, air dried) were suspended in 10 mL of NH_3/NH_4Cl/EDTA buffer, pH 10 (20 mL of 0.05 mol/L NH_4Cl and 35 mL of ammonia solution 25 % (w/w) and 0.01 mol EDTA were added. The volume of the solution was adjusted to 100 mL with water.). After 30 min of shaking the suspension was centrifuged, the supernatant was removed and transferred into a beaker. The solid residue was treated a second time with fresh buffer solution. Both extracts were combined and adjusted to pH 4 by dropwise adding of 25 % (w/w) HCl under stirring. The ammoniacal buffer efficiently desorbs SFD, CTC and epi-CTC from the soil matrix. Under these conditions the chlortetracyclines were completely converted into iso-CTC and its epimer (epi-iso-CTC).

Plant materials, i.e. roots and green parts (stems and leaves), stored cool, were lyophilised (Christ alpha®) and the contents of water determined (roots: 46 – 56 %, green parts: 53 – 63 %). The dried materials were ground (swing mill Retsch MM 200®) and a weighed amount (1 - 2.5 g) of the resulting powder was transferred into 20 mL of McIllvain buffer pH 4.1. The extraction process, supported by sonication (5 min), was carried out two times and the resulting extracts were combined for further treatments. *Straw* was controlled crushed in a sieve-mill to a particle length of < 1 mm. These particles were treated with McIllvain buffer (ultrasonic bath). Lyophilised *wheat grains* were ground in a laboratory mill (Fritsch Pulverisette®) and sieved by an analytical sieving machine (Retsch). The mesh size fraction < 0.106 mm of the flour obtained was taken for repeated ultrasonic-supported extraction with McIllvain buffer. All extracts obtained were centrifuged (4000 rpm) prior to clean-up procedures.

3.2 Solid Phase Extraction (SPE)

Oasis HLB® cartridges (manure and plant materials: 3cc, 60 mg; soil: 6cc, 200 mg; Waters) were conditioned with 1 or 3 mL of methanol followed by an equal volume of water. The whole extract was passed slowly through the column (~35 - 40 drops per minute). After washing with 3 - 5 mL of methanol/dest. water 5/95 (v/v), elution was performed with 3 mL of methanol. The eluate was evaporated to dryness at 30 °C under a stream of nitrogen, except in the case of manure extracts.

The remaining residues were dissolved in 300 – 500 µL of mobile phase A (see below) and the resulting concentrates chromatographically analysed.

4 Analysis by HPLC-UV-MS^n (n = 2 or 3)

4.1 Stock and standard solutions

SFD and TMP were purchased from Sigma-Aldrich, CTC, epi-CTC and iso-CTC from Acros Chimica as the hydrochlorides. N4-SFD was synthesized by acylation of SFD according to common procedures. Usually, stock solutions (1.0 g/L) were prepared in methanol. Stock solutions of epi-iso-CTC were prepared by partial epimerisation (~ 50 %) of dissolved iso-CTC (100 mg/L, methanol/mobil phase A 1:1 v/v) at 60 °C (water bath, 90 min). The exact concentrations of both epimers were determined by difference based on the initial concentration of the iso-CTC standard (Vockel 2005, Vockel et al., 2005). The concentrations of the keto-enol tautomers of epi-CTC were analogously calculated. At -80 °C all stock solutions were stable for at least six months. To obtain the appropriate standard solutions for external calibration in the range of, e.g. 0.05 – 5 mg/L, aliquots of the defrosted stock solutions were diluted with the mobile phase A. Standard solutions were freshly prepared daily.

4.2 Chromatographic system

The concentrates were analysed using the following equipment:

HPLC-gradient pump SpectraSYSTEM P 4000, autosampler (10 °C), photodiode array-detector UV 6000 LP (190 – 800 nm) linked to the ESI-ion trap mass spectrometer LCQ *Advantage* (ThermoFinnigan/Thermo Electron). In a single chromatographic run SFD, N4-SFD, TMP and the

different chlortetracycline compounds were determined by using the following LC-systems.

Analytical column (manure and soil): Phenomenex, 250 x 2.0 mm, Synergi 4µ Hydro RP,

(plant materials): YMC-Pack ODS-AM, 150 x 3,0 mm i.d., S 5µm; guard column: YMC-Pack ODS-AM (19 x 3 mm, 5 µm).

The mobile phases A and B used for the gradient system were composed as follows: (A) acetonitrile/water/formic acid 10/89.9/0.1 (v/v/v); (B) 60/39.9/0.1 (v/v/v); flow: 0.4 mL/min.

The retention times are given for the chromatogram of a standard solution (5 mg/L of each compound):

YMC-column: SFD (5.14 min), epi-iso-CTC (7.29), keto-epi-CTC (7.84), iso-CTC (8.93), enol-epi-CTC (9.66), CTC (11.46); Phenomenex-column: SFD (4.59), N4-SFD/TMP (5.6), iso-CTC (7.83), CTC (10.17).

4.3 Mass spectrometry

Mass spectrometric analysis was carried out in the ESI positive ion mode by multiple reaction monitoring (MRM) of molecular ion adducts $[M+H]^+$ and confirming product ions.

Adjusted tune-page-parameter (LCQ Advantage): mass range: 80 – 2000 [m/z]; sheat gas flow rate: 47 [arb]; auxillary gas flow rate: 0 [arb]; ion spray voltage: 5 kV; capillary temperature: 250 ºC; capillary voltage: 9 V; tube lens offset: - 5.0 V; multipole 1 offset: - 1.5 V; lens voltage: - 32 V; multipole 2 offset: - 5.5 V.

For identification the following MS^1/MS^2 -transitions were utilised:

SFD: m/z 251 > 156, 251 > 174; N4-SFD: 293 > 136, 293 > 198, 293 > 227 and TMP: 291 > 123, 291 > 230, 291 > 258. In the case of CTC, epi-CTC and iso-CTC peaks of the following transitions were evaluated, showing different characteristic intensity ratios: 479 > 462, 479 > 444, 479 > 371, 479 > 154.

In order to assure the MS^2-based identification of chlortetracyclines found in wheat grain by a complementary system, the more sensitive Linear-Ion-Trap-mass-spectrometer Finnigan LTQ (Thermo Electron, Dreieich, Germany) was employed in the MS^2 and MS^3-mode. Thus, epimers as well as keto- and enol-tautomers can be clearly distinguished by the following precursor and product ions (MS^2/MS^3-transitions):

keto-epi- CTC: 462.1 > 366.2 (30 % rel. intensity), 417.1 (50), 434.1 (20), 444.1 (100).

enol-epi- CTC: 462.1 > 444.1 (100); 444.1 > 355.1 (50), 381.0 (50), 399.1 (50), 426.1 (100).

iso-CTC: 462.1 > 373.1 (20), 416.1 (60), 434.7 (70), 444.1 (100).

epi-iso-CTC: 462.1 > 197.0 (40), 417.1 (100), 434.1 (50), 444.1 (60).

CTC: 462.1 > 444.1 (100); 444.1 > 154.0 (100), 371.1 (40), 399.1 (30), 426.1 (30).

Quantification was based on the summarised signal intensities of corresponding transitions (TIC). Limits of detection (LOD) were estimated according to the signal-to-noise ratios (S/N = 3:1). For SFD the LOD values lie between 10 µg/kg dw (soil) and 25 - 30 µg/kg dw/fw (wheat roots, green parts and grain), for CTC between 10 µg/kg dw/fw (soil, grain) and 25 µg/kg dw (roots, green parts). Limits of quantification (LOQ, S/N = 10:1) determined for CTC lie between 25 - 30 µg/kg dw/fw (soil, grain), for CTC and SFD about 50 µg/kg dw (roots, green parts). Further details of the analytical methods applied are described elsewhere (Grote et al., 2006).

Recoveries of chlortetracycline-derivatives determined for spiked grain samples (0.05 and 1µg per 2.5 g of grain) were about 44 - 66 % for iso-CTC, 100 - 120 % for epi-iso-CTC and 95 – 110 % for CTC, keto- and enol-epi-CTC. For SFD recoveries were between 43 and 115 %.

Lower recovery values were obtained for SFD (< 40 %) in the case of spiked green samples (stems, leaves). These values are probably caused by suppression effects in the ESI-source of the mass selective detector (Vockel et al., 2005, Vockel 2005). In such a case the standard addition procedure is to prefer to the external calibration method we used throughout for CTC-determination (Kloepfer et al., 2005). Furthermore, for the determination of SFD the addition of sulfamethoxypyridazine as an internal standard is recommended (Forni et al., 2002).

5 Results and discussion

5.1 Field Experiments

During storage of pig slurry for 8 months, excreted antibiotics were only partially degraded, so that considerable concentrations of SFD (~135 - 250 mg/kg fw), and CTC (~40 - 90 mg/kg fw) remained. Initial high concentrations of the main metabolite of SFD, N4-acetyl-SFD (~300 mg/kg fw), drastically declined during storage time whereas traces of TMP could be found only at the beginning of storage (Freitag et al., 2003, Grote et al., 2004 and 2005). Besides CTC its antibiotic active metabolites epi-CTC and anhydro-CTC and the non active iso-CTC and epi-iso-CTC were detected. In both batches the concentration of CTC and its active metabolites was higher than that of non active metabolites at the beginning of the storage period. Non active metabolites dominated at the end (Freitag et al., 2003, Grote et al., 2004, 2005 and 2006).

With the resulting liquid manure winter wheat was fertilised once or twice according to standard farming practice.

Until harvest, soil and whole plants were sampled in intervals and analysed for extractable antibiotics. After single manure application, overall antibiotic input to soil was 557 mg/m^2 SFD and 176 mg/m^2 CTC, whereas double application resulted in antibiotic input of 922 mg/m^2 SFD and 284 mg/m^2 CTC. In plots with double slurry fertilisation, peak concentrations of 240 µg/kg dw CTC and 90 µg/kg dw SFD were reached in the plough layer (0 – 25 cm depth). Underneath, no antibiotic residues were detected. During the vegetation period concentrations diminished to levels at LOD and LOQ (~10 to 30 µg/kg dw). These observations are in agreement with other studies (Hamscher et al. 2005).

Depending on antibiotic input, roots of growing wheat contained up to 0.5 mg SFD and 1.1 mg/kg dw of total CTC, declining to 0.1 mg/kg dw at harvest (Table 1). Leaves and stems contained a maximum of 1.1 mg/kg dw of CTC and traces of SFD. No extractable amounts of antibiotics were detected in straw, but wheat grain contained an average of 0.043 mg/kg fw of CTC in twice fertilised plots (Table 1).

5.2 Experiments with hydroponic cultures

Wheat was grown hydroponically in order to determine the potential of intact wheat plantlets for the uptake of antibiotics. Information of the distribution of antibiotics was also gained. In a first set of experiments, unlabelled antibiotics were added to the nutrient solution so as to reach different concentrations.

SFD was readily taken up by the roots (Table 2). In only two weeks of incubation, considerable concentrations were reached that were four to five times higher than those observed in the field experiments. The results indicate an approximately linear relationship between SFD concentration in the roots and incubation time. Doubling the SFD-concentration in the nutrient solution gave a threefold increase of the SFD concentration in the roots.

Table 2:
SFD concentration in wheat plants grown hydroponically

Plant organ/ Incubation time	SFD concentration	
	Culture medium [µmol/L]	Plant organ [mg/kg fw]
Root		
2 weeks	10	2.27
3 weeks	10	3.17
2 weeks	20	6.58
Leaf		
3 weeks	10	0.05
3 weeks	20	0.15

SFD was translocated to the leaves although the concentrations were low in the leaves by comparison to those in the roots (1.6 %). Doubling the SFD-concentration in the nutrient solution gave exactly the same threefold increase of SFD in the leaves as found in the roots.

CTC in the roots was two orders of magnitude higher than in the field experiments if CTC combined with iso-CTC was present in the nutrient solution (Table 3). Doubling the CTC concentration in the solution resulted in only 50 % increase of CTC in the roots. Like SFD, both chlortetracyclines were translocated to the leaves. Leaves of different developmental stage were compared in the CTC experiments. At the same duration of incubation, CTC concentrations, (i.e. the sum of CTC and iso-CTC concentration) in

Table 3:
CTC and iso-CTC concentrations in culture medium and wheat plants grown hydroponically

Plant organ/Incubation time	Culture medium	Plant organ	Mass ratios of CTC-isomers
	CTC and iso-CTC [µmol/L]	*Sum of CTC and iso-CTC [mg/kg fw]	$\frac{\text{CTC}}{\text{iso-CTC}}$
Root			
1 week	5	48.30	4.1
1 week	10	82.70	3.9
Leaf, developing			
3 weeks	5	1.97	< 0.1
3 weeks	10	3.01	< 0.1
Leaf, developed			$\frac{\text{iso-CTC}}{\text{CTC}}$
3 weeks	5	9.45	77.8
3 weeks	10	15.80	51.9

*(sum of concentrations including the epimers)

fully developed leaves were five times higher than those in leaves under development. One explanation of these results is that antibiotics are transported with the transpiration stream. It is most probable that the fully developed leaves were photosynthetically more active and therefore transpired more water than the leaves under development. However, more than one physiological mechanism may be responsible for the translocation of xenobiotics within plants absorbed by roots (Korte et al., 2000, Trapp S. 2004).

In the roots the concentration of CTC was fourfold higher than the iso-CTC concentration. It is emphasised that more iso-CTC than CTC accumulated during development of leaves, so that the mass ratio iso-CTC/CTC reached a maximum value of 78 (Table 3).

On-going research in our laboratory addresses the metabolism of chlortetracyclines in wheat plants grown hydroponically and under field conditions.

These results taken together show that the potential of wheat for the uptake of CTC is much higher than estimated from the field experiments. In soil a great portion of the CTC is reportedly sequestered by the soil matrix. It is conceivable that this can markedly reduce the transfer of organic xenobiotics into plants (Trapp S. et al., 2001, Boxall et al., 2006).

Carrots and lamb's lettuce were also hydroponically grown in presence of non-labelled and radiolabelled antibiotics (lamb's lettuce). In both cases the uptake of SFD and CTC by the plants was confirmed. Details will be reported separately.

5.3 Radiolabelled antibiotics

In a second set of experiments radiolabelled antibiotics were employed. Nutrient solutions spiked with 5 or 10 µmol/L of SFD or CTC combined with iso-CTC, were additionally spiked with tritium-labelled sulphamethazine or tetracycline. Distribution of radioactivity in roots, stems and leaves sampled after 7, 14 and 21 days was measured by liquid scintillation counting. The results in Figure 1 demonstrate considerable translocation of tetracycline-compounds and to a lesser extent of sulphonamide from root to vegetative organs.

The antibiotic concentrations in the plantlets calculated on quench corrected data and specific activities were approximately ten times lower compared to the non-labelled hydroponics. However, the relative distribution of the tritium-labelled sulphonamide and tetracycline between roots and green parts correspond to that in hydroponically grown wheat exposed to non-labelled antibiotics, and to that in wheat plants grown under field conditions as well.

Current microautoradiographic investigations should give further information about the localization of the antibiotics in tissue and compartiments of plant cells (Korte et al., 2000).

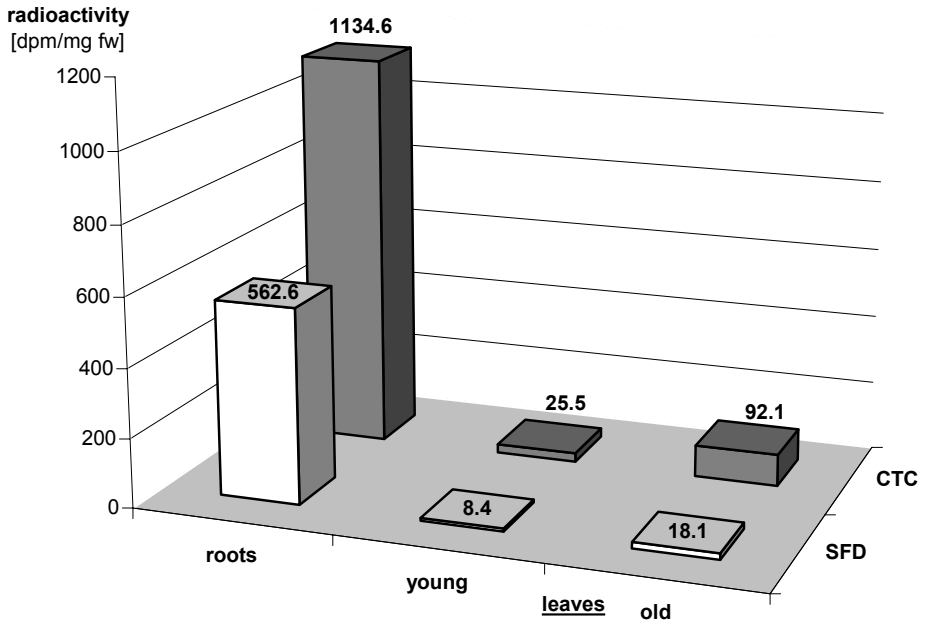

Figure 1:

Distribution of radioactivity in wheat plantlets after 7 days exposure to ^{3}H-labelled antibiotics in nutrient solution (10 µmol/L sulfadiazine and 740 KBq (3,5)-^{3}H-sulfamethazine or 10 µmol/L CTC, iso-CTC and 740 KBq 7-^{3}H-tetracycline; dpm: disintegrations per minute)

6 Conclusions

Our results demonstrate that humans and livestock can become exposed to antibiotics by the consumption of plants or food derived thereof. Wheat has a high potential for the uptake of antibiotics as demonstrated by our hydroponic culture experiments. The results of the field experiments show that chlortetracycline is also taken up from soil and is distributed in the plants.

It cannot be excluded to date that the observed antibiotic concentrations in the soil and wheat, although low in grains, can contribute to the risk of developing bacterial antibiotic resistance. It is clear, however, that a sound risk assessment with regard to food safety requires further investigations. Crops harvested from fields of common agricultural practise are under study.

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