

Uptake of Galaxolide, Tonalide, and Triclosan by Carrot, Barley, and Meadow Fescue Plants

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ABSTRACT: Many xenobiotics entering wastewater treatment plants are known to be persistent during wastewater treatment and tend to adsorb to sewage sludge. The application of sewage sludge as fertilizer in agriculture may pose the risk of an incorporation of xenobiotics in the cultivated plants and, finally, an inclusion into the food chain. This study was performed to investigate the uptake of common sewage sludge contaminants, galaxolide, tonalide, and triclosan, by plants used for human consumption and livestock feeding. Barley, meadow fescue, and four carrot cultivars were sown and grown in spiked soils under greenhouse conditions. After harvesting the plants, roots and leaves were analyzed separately, and the respective bioconcentration factors were calculated. In carrots, a concentration gradient of the xenobiotics became evident that decreased from the root peel via root core to the leaves. A significant influence of the differing root lipid contents on the uptake rates cannot be supported by our data, but the crucial influence of soil organic carbon content was confirmed. Barley and meadow fescue roots incorporated higher amounts of the target substances than carrots, but translocation into the leaves was negligible. The results indicated that an introduction of persistent semi- and nonpolar xenobiotics into the food chain via edible plants like carrots could be of certain relevance when sludge is applied as fertilizer. Due to low rates found for the translocation of the xenobiotics into the aerial plant parts, the entrance pathway into food products via feeding livestock is less probable.

KEYWORDS: uptake, food plants, xenobiotics, polycyclic musk, triclosan, GC–MS

■ INTRODUCTION

Xenobiotics may enter the food chain via several pathways, e.g., via the use of antibiotics and other veterinary pharmaceuticals in livestock breeding, pesticides in crop farming, or contamination during manufacturing food. Potential transfer of xenobiotics from sewage sludge and manure amended soils into plants used for food and feed production has been less considered so far although the use of sewage sludge and manure as fertilizer in agriculture has been practiced in Europe for a long time. Currently, the European Commission even encourages the reutilization of sludge under observance of corresponding rules regarding toxic relevant sludge pollutants (Council Directive 86/278/EEC¹ or the German Sewage Sludge Act²). Annually, more than 11 million metric tons d.w. (dry weight) of sewage sludge are produced in the countries of the European Union,^{3,4} and about 40% are applied as fertilizer in agriculture.

Particularly, persistent organic substances with semipolar and lipophilic properties tend to accumulate in sewage sludge.^{5,6} For instance, galaxolide (HHCB) and tonalide (AHTN), polycyclic musk compounds frequently applied as ingredients of cosmetic and household products, have been determined in sewage sludge of Norwegian wastewater treatment plants at concentrations of 0.3–22.4 mg kg⁻¹ d.w. (HHCB) and at 0.1–3.5 mg kg⁻¹ (AHTN), or up to 14 mg kg⁻¹ and 2 mg kg⁻¹, respectively, in Austrian WWTPs.^{7,8} The bactericide triclosan

(TCS) released from personal care products such as toothpaste and cosmetics, detergents, or impregnated textiles is accumulated in sludge at concentrations between 0.04 and 7 mg kg⁻¹ d.w.^{9,10} Regular application of the contaminated sludge on fields results in soil concentration of about 1 μg kg⁻¹ as reported previously¹¹ which corresponds well with soil concentrations predicted.¹²

Among adverse effects of polycyclic musks and triclosan to aquatic organisms, there are also potential risks to organisms living in soils and sediments that have been revealed long time ago.^{13–15} Furthermore, triclosan has been proven to support antibiotic resistance of microorganisms.¹⁶ Possible chronic effects of these compounds on human health are still under discussion, but potential risks for human health have been assumed particularly, when they are present in food.¹⁷

Taking into account that plants form an essential basis of the animal and human diet, an evaluation of the uptake and accumulation of potential harmful organic contaminants in plants is of importance for risk assessment. Current studies on xenobiotic uptake by food and feed plants focus predominately on pesticides or veterinary drugs.¹⁸ Root uptake, translocation,




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Table 1. Selected Features of the HHCB, AHTN, and Triclosan Used for the Plant Exposure and Parameter of the Analytical Protocol, Limit of Quantification (LOQ), Recovery and Mean Relative Standard Deviation (RSD), Maximum Concentrations in European Sludges, and the Resulting Maximum Soil Concentrations

Trade name, abbreviation, structure	CAS-No.	log K _{OW}	Water solubility mg L ⁻¹	Henry's law constant Pa m ³ mol ⁻¹	LOQ in µg g ⁻¹ d.w.	Recovery in % (n=3-4)	% RSD (n=3-4)	Target ions (m/z) quantifier, qualifier	Exemplary sludge Concentration mg kg ⁻¹ d.w.	Calculated max. Soil Concentration mg kg ⁻¹ d.w.
Galaxolide (HHCB) 	1222-05-5	5.9	1.75 ^a	11.3 ^a	0.001 (soil) 0.02 (carrot root) 0.02 (meadow leaf)	115 102 63	7 8 1	243, 258	81 ^c	2.02
Tonalide (AHTN) 	1506-02-1	5.7	1.25 ^a	12.5 ^a	0.001 (soil) 0.05 (carrot root) 0.02 (meadow leaf)	110 97 65	7 10 2	243, 258	16 ^c	0.40
Triclosan (TCS) 	3380-34-5	4.8	10 ²⁵	1.5 × 10 ^{-2b}	0.005 (soil) 0.05 (carrot root) 0.06 (meadow leaf)	106 86 65	9 12 2	288, 290, 252	8.8 ^d	0.22

^aReference 13. ^bReference 23. ^cReference 24. ^dReference 25.

Table 2. Plants Used for Exposure Experiments

plant species	analyzed plant parts	reason for selection
barley <i>Hordeum vulgare</i> , cv Edel	root, leaf	representative for cereal, mainly for animal feed
meadow fescue <i>Festuca pratense</i> , cv Fure	root, leaves	dominating forage in Norway
carrot <i>Daucus carota</i> ssp. <i>sativus</i> , cvs Napoli <i>Daucus carota</i> ssp. <i>sativus</i> , cvs Amager <i>Daucus carota</i> ssp. <i>sativus</i> , cvs Rothild <i>Daucus carota</i> ssp. <i>sativus</i> , cvs Nutri-Red	root, leaves	representative of root crop; common in Norway, an industrial cultivar
	root	representative of root crop an old cultivar, common
	root	representative of root crop red, high carotene
	root	representative of root crop deep red, high carotene and lycopene; commonly grown in kitchen gardens

and accumulation of semipolar sewage sludge contaminants have been less considered, although their inclusion into the food chain via plants should be evaluated to improve the assessment of the daily dose of xenobiotics the consumers encounter. Furthermore, a science-based decision whether the application of sewage sludge in agriculture poses any risks requires more data on the environmental fate of xenobiotics including the uptake by plants.¹⁹

In our study we investigated the uptake of triclosan, HHCB, and AHTN from spiked soil by four carrot cultivars, barley, and meadow fescue which are ranked among most essential food and feed plants in middle and northern European countries.²⁰ Furthermore, carrots are considered as worst case scenario for uptake studies due to their oil rich tap root, which facilitates the transfer of compounds into plants.²¹ Moreover, as a root vegetable especially the edible parts of the carrot are in steady and direct contact to contaminated soils. Barley and meadow fescue were included in this study as important crop and fodder plants of which the aerial compartments become part of the food chain.

In general, the probability of possible human exposure by contaminants arising from biosolids application in agriculture is addressed increasingly in food safety research.²²

MATERIALS AND METHODS

Test Substances. Both polycyclic musk compounds 1,3,4,6,7,8-hexahydro-4,6,6,7,8-hexamethylcyclopenta-[g]-2-benzopyran (galaxo-

lide, HHCB) and 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydro-naphthalene (tonalide, AHTN) were received from Dr. Ehrenstorfer (Augsburg, Germany), and the antibacterial compound triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) was from Fluka (Budes, Germany). All substances were of ≥95% purity and were used for spiking the soils and served as reference compounds for the analytical measurements.

Soil. The sandy soil used in the experiments (0.9% TOC, 0.21% total N and 1130 mg kg⁻¹ total P-content, pH 5.5) was air-dried and sieved to 4 mm before batches of the soil were mixed with a commercially available, slow-release fertilizer (3 g kg⁻¹ soil). The exact mineral composition was not characterized.

Spiking Procedure. The test substances from a stock solution made in acetone were spiked into 50 mL of acetone which was finally added to the soil of each pot (175 mm i.d., 210 mm high) filled with 4 kg d.w. of soil. All was mixed thoroughly manually in order to adjust concentrations of 10 mg kg⁻¹ d.w. for HHCB, AHTN, and triclosan. After spiking, all pots were stored at ambient temperature for three days to allow the residual acetone to evaporate from the soil. The applied xenobiotic amounts were chosen on the basis of calculated worst case concentrations using eq 1.¹² The prediction considered (i) the maximum concentrations reported for the target substances in sewage sludge (Table 1), (ii) a single sludge application to soil, and (iii) the disregarding of leaching and biodegradation processes of the xenobiotics.

$$c_{0, \text{Soil}} = \frac{c_{\text{Sludge}} \cdot \text{APPL}_{\text{Sludge}} \cdot 10}{\text{DEPTH}_{\text{Soil}} \cdot \text{RHO}_{\text{Soil}}} \quad (1)$$

$c_{0,\text{soil}}$ is the concentration of the contaminant in soil after one application of sewage sludge in mg kg^{-1} at $t = 0$, c_{sludge} is the concentration of the contaminant in the applied dry sewage sludge, $\text{APPL}_{\text{sludge}}$ is the application rate (0.6 kg m^{-2}) d.w., $\text{DEPTH}_{\text{soil}}$ is the mixing depth of the soil (0.2 m), and RHO_{soil} is the bulk density of soil (1200 kg m^{-3}), respectively.¹² As shown in Table 1, the expected concentrations of the target compounds in soil are in the low mg kg^{-1} range.

Plant Cultivation and Exposure. The cultivation of the plants used for the experiments (Table 2) has been performed under controlled greenhouse conditions. Three days after spiking, the pots were sown with barley, carrots, and meadow fescue. All pots were kept at 14°C during germination. After germination, temperature was set to 20°C day and 14°C night with a 16 h day length. Lighting was given as $350 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photosynthetic photon flux (PPF) with SON-T lamps corresponding to $30 \text{ mol m}^{-2} \text{ day}^{-1}$. The pots were placed on individual trays and irrigated with water (pH 7.4, EC 1.5 mS cm^{-1}) to maintain a water content of about 70% of the soil's water holding capacity, which was approximately 11%. The number of plants per pot was 5–6 in the case of all carrot types, 10 for barley, and 20 for meadow fescue. Control plants of carrots (cultivar Napoli) and barley grown in nonspiked soil were placed in between the spiked pots to investigate whether significant amounts of the investigated xenobiotics are transferred directly from the soil to leaf tissue.

Harvesting. Plant materials were harvested during a period of two months, depending on the degree of ripeness of the seeds and the proper size of the carrots and meadow fescue, while all plants per pot were collected and pooled to one sample. Samples of roots and leaves were taken from the same pot. Roots were carefully washed with tap water. Carrots were peeled with a vegetable peeler (depth of 2 mm). All plant materials were dried in an oven for three days starting at 50°C (1 day) followed by 40°C . Control and exposed plant materials were dried separately to prevent cross-contamination. The dried plant samples were packed in paper bags and soil in glass vessels and stored about two weeks at ambient temperature until analysis.

Sample Preparation. After harvesting, the carrot plants were divided into root peel, root core, and leaves which were analyzed separately. Barley and meadow fescue plants were divided into roots and leaves. The dried samples were coarsely cut and ground with an ultracentrifuge mill (Retsch, Haan, Germany) to obtain fine-grained (0.2 mm), homogenized material. These samples were extracted by the QuEChERS (quick, easy, cheap, effective, rugged, and safe) methodology^{26,27} which was modified to enable GC–MS analysis. For this purpose, acetonitrile was replaced by a mixture of ethyl acetate/acetone (1:1 v/v) (10 mL). For the whole procedure, glass devices were applied to avoid sample contamination by plasticizers and loss of target compounds by adsorption. The applied MgSO_4 (Merck, Darmstadt, Germany) was heated at 300°C for 12 h in a muffle furnace for cleanup.

A 2 g sample of the oven-dried and ground plant material (for leaves 1 g) was processed with the modified QuEChERS method and analyzed by GC–MS. The cleanup of the carrot root and leaf extracts was performed using 60 mg and 100 mg Supelclean ENVI-Carb (Sigma-Aldrich, St. Louis), respectively, which were added to the PSA (Agilent Technologies, Waldbronn, Germany).

A 1 g amount of soil was extracted for 3 min with $2 \times 10 \text{ mL}$ acetone and $1 \times 10 \text{ mL}$ ethyl acetate using an ultrasonic probe [Sonoplus GM 200 (20 kHz), probe type MS 73 with microtip of $1/8$ in., Bandelin, Mörfelden-Walldorf, Germany]. After centrifugation the supernatants were combined and evaporated to a final volume of 1 mL and analyzed by GC–MS.

Recovery experiments were carried out using carrots purchased from a local supermarket. The carrots free of target analytes were lyophilized and ground as described above. For spiking, the reference compounds were dissolved in acetone and added to the respective sample to reach a concentration of $1\text{--}5 \mu\text{g g}^{-1}$. The solvent was allowed to evaporate, and the material was weighed into glass centrifuge tubes and processed with the QuEChERS procedure.

The recovery of the soil extraction method was determined by spiking 1 g of blank soil to a final concentration of $1 \mu\text{g g}^{-1}$ for each

analyte prior to ultrasound assisted extraction. The analyte recoveries and reproducibility of the protocols are given in Table 1.

In order to determine the soluble lipid content of the carrot roots, 2 g of the dried and homogenized root material was extracted by pressurized solvent extraction using a Dionex 200 accelerated solvent extractor (Dionex, Sunnyvale, CA). According to Dionex Application Note 321,²⁸ n-hexane was applied as solvent. The extracts were evaporated to dryness and the lipids determined gravimetrically.

Analysis. The GC–MS analyses of the extracts were performed on a 6890GC-5973MSD-system (Agilent Technologies, Waldbronn, Germany) by injecting $1 \mu\text{L}$ of each extract. The analytes were separated on a HP-5MS column of 30 m length, 0.25 mm i.d., and a film thickness of $0.25 \mu\text{m}$ (J&W by Agilent Technologies). The GC oven program started at an initial temperature of 50°C and was held for 1 min, increased at 10 K min^{-1} to 280°C , and held for 6 min. Helium was used as carrier gas at a constant flow of 1 mL min^{-1} . The directly coupled mass spectrometer analyzed the substances after electron impact ionization at 70 eV in selected ion monitoring (SIM) mode (target ions in Table 1). The results of duplicate analyses of 3 parallel extractions were averaged for quantification using external standards. Recoveries were generally corrected for the sample type (soil, plant species, and plant part) to consider possible matrix influence. The determined limits of quantification for the target substances are included in Table 1.

RESULTS AND DISCUSSION

Analyte Concentration in the Soil. The concentration of the target substances in the soils was examined before seeding (day 0) and after 49 and 119 days of plant cultivation. The detected concentrations differed from the calculated nominal concentration (Figure 1). In the case of AHTN, the

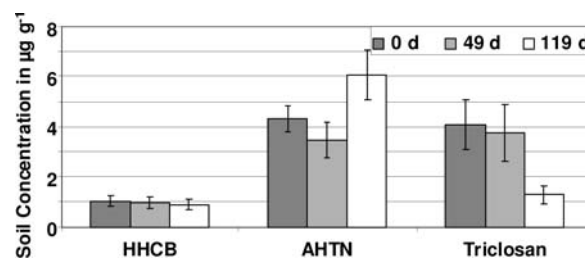


Figure 1. Concentration of HHCB, AHTN, and triclosan determined in soil after 0, 49, and, 119 days.

concentration detected at day 0 was smaller than after 119 days which was probably due to an inhomogeneous distribution of AHTN within the soil. However, in general, the concentration of both polycyclic musk compounds HHCB and AHTN were found to be fairly stable over the entire cultivation period of 119 days; dissipation under the exposure conditions was negligible. The resistance against abiotic degradation and low volatilization of AHTN and HHCB from soils goes along with literature data where half-lives between 10 and 17 months for HHCB and even 2–24 years for AHTN were reported.²¹

For the calculation of uptake factors, the concentrations of the target substances measured in initial soil, after 49 days, and after 119 days were averaged and used as “concentration in dry soil” (eq 2).

Plant Uptake Results. Most of the exposed plants were delayed in their development as compared to nonspiked controls, but they compensated for this lag during the cultivation period. This is in agreement with previous studies, in which plant exposure to 0.44 mg L^{-1} of triclosan caused plant death after germination under hydroponic conditions²⁹ or

Table 3. Average Concentration ($n = 3$) of the HHCB, AHTN, and Triclosan in the Carrot Napoli Peels, Cores, and Leaves and the Corresponding Bioconcentration Factors (Relative Standard Deviations in % Given in Parentheses)

	carrot roots			BCF	carrot leaves		BCF (total plant)
	c (peel) $\mu\text{g g}^{-1}$ d.w.	c (core) $\mu\text{g g}^{-1}$ d.w.	c (total root) $\mu\text{g g}^{-1}$ d.w.		c $\mu\text{g g}^{-1}$ d.w.	BCF	
HHCB	4.35 (11)	0.22 (16)	0.86 (12)	0.89	0.74 (16)	0.76	0.86
AHTN	13.41 (2)	0.30 (15)	2.32 (10)	0.50	0.82 (12)	0.18	0.44
triclosan	2.82 (7)	0.14 (4)	0.55 (7)	0.18	0.23 (18)	0.07	0.16

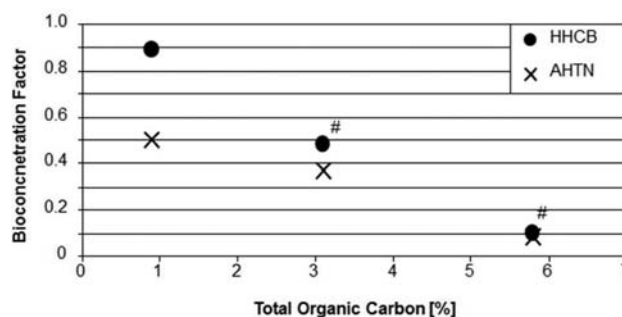
a significant reduced growth of carrot plants exposed to a mixture of veterinary medicines at 1 mg kg^{-1} soil concentration.¹⁸ Also, wheat seedlings have been shown to be sensitive to low exposure concentration of triclosan and HHCB ($0.2\text{--}0.3 \text{ mg L}^{-1}$ as reported by An et al.³⁰).

Uptake of the Target Substances by Carrots. *Uptake and Translocation in Carrot Type Napoli.* Exemplarily, the concentration of the target substances in different parts of the carrot cultivar “Napoli” is shown in Table 3. The studied xenobiotics HHCB, AHTN, and triclosan were detected in all compartments of the carrot plants. The highest concentrations were detected in the root peel and decreased gradually toward the inner parts of the root and to the leaves. The total root concentrations of the xenobiotics were calculated as sum of the concentration found in root peel and root core. The corresponding bioconcentration factors (BCF) included in Table 3 were calculated in accordance with eq 2 on the basis of the dry weight of the material analyzed.

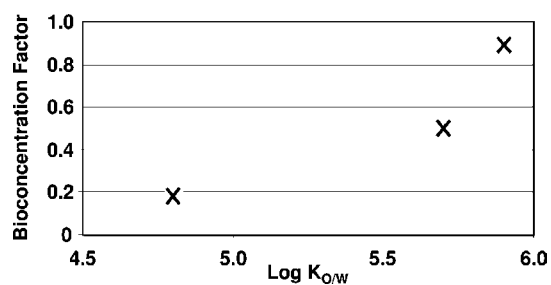
$$\text{BCF} = \frac{\text{concentration in dry plant tissue}(\mu\text{g g}^{-1})}{\text{concentration in dry soil}(\mu\text{g g}^{-1})} \quad (2)$$

With a BCF of 0.18, triclosan shows the lowest enrichment. The BCFs for AHTN and HHCB were up to 5 times higher at 0.50 and 0.89, respectively. Despite the very similar structural properties and $\log K_{\text{OW}}$ values of AHTN (5.7) and HHCB (5.9), their BCFs in the entire root tissue differed significantly. Relating to the entire root, the BCF of HHCB was calculated as almost twice as high as the BCF of AHTN. As the separate analysis of the root peel and core revealed, the concentrations of AHTN and HHCB in the peel reflected the different soil concentrations. The amounts of both substances in the core variations were less distinct due to similar translocation rates assumed for both compounds. For triclosan, about 20 times higher concentrations were found in the peel than in the core which is a comparable ratio as determined for the distribution of HHCB.

The observed transfer of the polycyclic musks from contaminated soils into carrot roots as well as the increased BCF of HHCB in comparison to AHTN goes along with the findings of Litz et al., from 2007.²¹ However, in contrast to their study, we found 1.9 and 1.6 times higher BCFs for both investigated musks. This deviance is probably caused by the total organic carbon content (TOC) of the soil which was with 0.9% quite low compared to the TOC of 3.1% in the study of Litz et al., in 2007.²¹ Data for HHCB and AHTN uptake obtained from our experiments and those provided by Litz et al., from 2007,²¹ are combined in Figure 2 indicating a strong relationship between the BCFs and the soils' TOC. The higher the TOC is, the lower the transferred amount of xenobiotics into the plants, which was also confirmed in a previous study,³¹ where in TOC-rich soil the mobility of several pharmaceutical compounds was reduced.

**Figure 2.** Dependence of bioconcentration factors of HHCB and AHTN in carrot roots on the soils' total organic carbon content (TOC), # marked points are taken from ref 21.

Due to the lack of comparable literature data, a relationship between the soils' TOC and the uptake of the more polar triclosan cannot be deduced. Compared to both polycyclic musk compounds, triclosan indicated a significantly lower bioconcentration factor which points to a direct correlation between uptake and lipophilic properties of the target substances (Figure 3). This is in agreement with the

**Figure 3.** Bioconcentration factors of HHCB, AHTN, and triclosan in carrot Napoli roots versus the $\log K_{\text{OW}}$ values of the compounds.

relationship published by Briggs et al., in 1982,³² concerning the uptake of xenobiotics by roots from a nutrient solution. Although the transfer of results obtained from hydroponic cultures to more complex plant–soil systems is problematic, our results fit the stated correlation: the higher the $\log K_{\text{OW}}$ of a compound the higher is its BCF. Since in our investigation the properties of the three target substances are quite similar ($4.8 < \log K_{\text{OW}} < 5.9$), more studies are required to confirm this relationship for plant–soil cultures and substances with different polarity.

As mentioned before, all compounds were quantified in the carrot leaves, as well. Triclosan was detected at mean concentration of $0.23 \mu\text{g g}^{-1} \pm 5 \text{ ng g}^{-1}$ and HHCB and AHTN at $0.74 \mu\text{g g}^{-1} \pm 7 \text{ ng g}^{-1}$ and $0.82 \mu\text{g g}^{-1} \pm 16 \text{ ng g}^{-1}$, respectively. The almost equal concentrations of the polycyclic musks are in contrast to the different soil and root peel concentrations and indicate that the transfer from the root peel, via the root core into the leaves, is rate limited. Transferring of

the target substances from soil to the leaf surface via transport by aerosols or bound to particles can be excluded. Neither the musk compounds nor triclosan were detected in leaves of the control plants grown among the exposed ones. Thus, the presence of the compounds in the leaves is the result of their translocation from the root compartments.

The gradual distribution of the xenobiotics, i.e., the decrease of their concentrations from the root peel via the root core to the leaves, is in agreement to the findings of Boxall et al., from 2006,¹⁸ although they reported lower BCFs for a set of veterinary pharmaceuticals. Also, the study of Litz et al., from 2007,²¹ supports our findings regarding a significant translocation of HHCB and AHTN from the carrot root into the aerial parts, but the translocated portions determined by Litz et al. were significantly smaller and the reported BCFs were lower by a factor of 10 in the leaves than in the roots. In our study the BCFs differ just by the factor of 2.7 for AHTN and were found to be comparable for HHCB. Thus, our results indicated an increased translocation of the incorporated substances into the leaves. These differences may arise from the time dependent passive distribution of the substances in the plant occurring via cascades of sorption/desorption and diffusion processes.³³ Furthermore, alterations in plant physiology like growth and aging may influence the substance concentrations in the specific plant tissues. Thus, uptake and translocation of the substances are dynamic, time dependent processes, and a state of equilibrium is not likely to be reached. The assumption of strong kinetic effects is supported by the fact that, despite the very different concentrations of HHCB and AHTN in the soil and the root peel, the amounts of both compounds in the leaf tissue were found to be similar. Thus, processes allowing the translocation of substances with comparable properties within plant tissue are dependent on time and the concentration gradients.

Parts of the incorporated triclosan have entered the carrot leaves as well. The distribution between roots and leaves is comparable to that found for AHTN; the BCF in leaves is about 3 times lower than that in the root tissue. The ability of triclosan to enter the aerial parts of a plant after its uptake via roots has previously been observed for soybean plants³⁴ where the concentrations in leaves exceed those in roots even multiply. In general, the observed distribution of the chemicals is in contrast to the assumption that plants with swollen storage roots as carrots and parsnips fail to translocate chemicals from the roots into the leaves.³²

Influence of the Lipid Content of Roots on the Uptake of the Target Substances. The pivotal feature considered in most uptake models is the lipophilic nature of the neutral organic compound expressed as $\log K_{ow}$ which indicates that the plant specific content on lipophilic root constituents might influence the uptake and accumulation of chemicals, as well. Therefore, four carrot cultivars Napoli, Rothild, Nutri Red, and Amagar were examined regarding their root contents of soluble lipids and a possible correlation with the incorporated quantities of HHCB, AHTN, and triclosan. The content of soluble lipids of the different carrot cultivars was determined in the peels and cores separately. In general, the lipid contents were below 0.2% of the fresh weight of the investigated carrot roots. As shown in Table 4, except for carrot Amagar, the lipid contents in the peels were slightly higher than in the cores. Even though the soluble lipid contents were 2 or 3 times higher in Nutri Red and Amagar than in Napoli roots, the incorporated xenobiotic amounts were fairly similar (Table 4). Furthermore, the portion

Table 4. Soluble Lipid Content of Root Peels and Root Cores of the Different Carrot Cultivars

	Napoli mg g ⁻¹ f.w.	Amagar mg g ⁻¹ f.w.	Rothild mg g ⁻¹ f.w.	Nutri Red mg g ⁻¹ f.w.
peel	0.87	1.49	1.39	1.61
core	0.59	1.85	0.86	1.27
total	0.65	1.77	0.95	1.31

of xenobiotics translocated from peel into the core was similar in all four carrot cultivars, namely 22%, 11%, and 19% for HHCB, AHTN, and triclosan (data not shown), respectively (see Table 5 for details for each compound).

Table 5. Average ($n = 3$) Total Concentration of the HHCB, AHTN, and Triclosan in the Roots of the Different Carrot Cultivars (Relative Standard Deviations in % Given in Parentheses)

	Napoli $\mu\text{g g}^{-1}$ d.w.	Amagar $\mu\text{g g}^{-1}$ d.w.	Rothild $\mu\text{g g}^{-1}$ d.w.	Nutri Red $\mu\text{g g}^{-1}$ d.w.
HHCB	0.86 (12)	0.64 (26)	0.42 (37)	0.73 (21)
AHTN	2.32 (10)	1.51 (12)	1.29 (30)	2.45 (20)
triclosan	0.55 (7)	0.47 (10)	0.31 (33)	0.50 (13)

A positive correlation between the lipid content of roots and the incorporation of lipophilic chemicals in the tissue has been stated in previous studies.^{35,36} For example, Gao et al., in 2006³⁶ investigated the uptake of selected PAHs into the roots of a set of plants covering a range of lipid contents from 0.1% to 1.0% and deduced a positive correlation between uptake rates and lipid content. In contrast to the findings of these previous studies, a correlation between the lipid content of the roots and the incorporated amount of target compounds is not supported by our data (Figure 4).

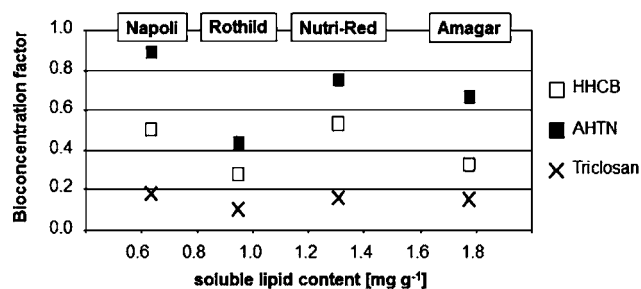


Figure 4. Fresh weight concentrations of HHCB, AHTN, and triclosan in the peels of different carrot types versus the lipid contents of the peel.

Uptake and Translocation of the Target Substances into Barley and Meadow Fescue. Barley and meadow fescue were examined to evaluate their uptake potential for the selected chemicals and to recognize possible entrance pathways into the animal or human food chain. All these substances, HHCB, AHTN, and triclosan, were detected in the expansive network of the fine roots of both plant species. Similar to carrots, the mean BCF of HHCB in barley roots was determined to be 0.83, but in contrast, the BCFs of AHTN (1.49) and triclosan (1.43) in barley roots exceeded those in carrots (Table 6). Both plants incorporated significantly different amounts of the target substances. While the BCFs of HHCB (1.82) and AHTN (2.74) were twice as high in

Table 6. Mean ($n = 3$) Total Concentrations and Bioconcentration Factors of HHCB, AHTN, and Triclosan Found in the Roots of Barley and Meadow Fescue Plants (Relative Standard Deviations in % Given in Parentheses)

	barley		meadow fescue	
	c in $\mu\text{g g}^{-1}$ d.w.	BCF	c in $\mu\text{g g}^{-1}$ d.w.	BCF
HHCB	0.81 (33)	0.83	1.77 (3)	1.82
AHTN	6.90 (31)	1.49	12.66 (36)	2.74
triclosan	4.36 (28)	1.43	1.11 (49)	0.36

meadow fescue roots as in barley roots, triclosan showed a significantly lower BCF (0.36) in meadow fescue. In general, the roots of the two grass plant species showed an increased incorporation of the target substances in comparison to the carrot roots. This is probably caused by the higher surface-to-volume ratio of the feathery barley and meadow fescue roots which facilitates the diffusive transfer of the substances into the roots. Their permanent root growth changes also the contact surfaces to soil particles, and exchange of substances is enhanced.

HHCB was found in just one barley sample ($0.04 \mu\text{g g}^{-1}$) above the analytical limit of quantification. AHTN was detected in the leaves of both barley ($0.17 \mu\text{g g}^{-1}$) and meadow fescue ($0.04 \mu\text{g g}^{-1}$), but in this case a foliar uptake of AHTN cannot be excluded. Although previous studies pointed to its low tendency for volatilization,²¹ the corresponding control leaves of barley contained $0.05 \mu\text{g g}^{-1}$ of AHTN. This represents about one-third of the detected AHTN concentration in exposed barley plants and is even equal to those detected in meadow fescue. Also, triclosan could not be detected in any of the leaf samples which is in contrast to the findings for carrot plants. None of the target compounds were detected in barley seeds above the LOQs (data not shown). In contrast to carrot plants, in barley and meadow fescue the chemicals were hardly translocated from the roots into the leaves; thus, their bioaccumulation in aerial parts is of low probability.

Our data imply that the uptake and translocation from contaminated soils in plants is not restricted to polar compounds as, for instance, proved for some veterinary drugs which are spread by manure application. For the more lipophilic compounds HHCB, AHTN, and triclosan, plant specific absorption and translocation were observed. Thus, an introduction of semi- and nonpolar xenobiotics into the food chain via the application of sewage sludge can be of certain relevance particularly for root vegetables. Soil parameters like the TOC were revealed to have a crucial influence on uptake rates which have to be investigated more in detail for a larger set of xenobiotics covering a broad range of structural and physicochemical properties. Furthermore, the influence of the pore water (volume, pH, and accompanying water-soluble matrix) on the uptake of chemicals by plants is another important aspect that was not considered within our investigations but needs further studies.

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Notes

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ABBREVIATIONS

AHTN, tonalide
 HHCB, galaxolide
 BCF, bioconcentration factor
 TOC, total organic carbon
 PAH, polycyclic aromatic hydrocarbon
 WWTP, wastewater treatment plant

REFERENCES

- (1) Council of the European Communities. Council Directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (86/278/EEC). *Off. J. Eur. Communities* **1986** L 181, 6–12.
- (2) Federal Republic of Germany—Federal Ministry of Justice. Sewage Sludge Act (Klärschlammverordnung (AbfKlärV)) *Bundesgesetzblatt* **1992** (last update 02-29-2012) 21, 912–935.
- (3) Milieu Ltd., Wrc, Risk and Policy Analysts Ltd. (RPA) for the European Commission. Environmental, economic and social impacts of the use of sewage sludge on land. *Final Report—Part 1: Overview Report*; 2010; pp 1–16.
- (4) European Commission, Consortium ESWI. *Study on Waste Related Issues of Newly Listed POPs and Candidate POPs, No ENV.G.4/FRA/2007/0066, Final Report*; Update13 April 2011; p 242.
- (5) Böhm, L.; Düring, R.-A. Partitioning of polycyclic musk compounds in soil and aquatic environment—experimental determination of K_{DOC} . *J. Soils Sediments* **2010**, 10, 708–713.
- (6) Butler, E.; Whelan, M. J.; Sakrabani, R.; van Egmond, R. Fate of triclosan in field soils receiving sewage sludge. *Environ. Pollut.* **2012**, 167, 101–109.
- (7) Mogensen, B. B.; Pritzl, G.; Rastogi, S.; Glesne, O.; Hedlund, B.; Hirvi, J.-P.; Lundgren, A.; Sigurdsson, A. Musk Compounds in the Nordic environment. *TemaNord—Nordic Council of Ministers, Copenhagen* **2004**, 503, 1–68.
- (8) Clara, M.; Gans, O.; Windhofer, G.; Krenn, U.; Hartl, W.; Braun, K.; Scharf, S.; Scheffknecht, S. Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. *Chemosphere* **2011**, 82, 1116–1123.
- (9) Gatidou, G.; Thomaidis, N. S.; Stasinakis, A. S.; Lekkas, T. D. Simultaneous determination of the endocrine disrupting compounds nonylphenol, nonylphenol ethoxylates, triclosan and bisphenol A in wastewater and sewage sludge by gas chromatography-mass spectrometry. *J. Chromatogr., A* **2007**, 1138, 32–41.
- (10) Kuch, C.; Schneider, C.; Metzger, J. W. Monitoring of triclosan, triclocarban and hexachlorphen in surface water, sediments, sludge, influents and effluents of sewage works (title translated). *FZKA-BWPLUS (BWB 21009)*; University of Stuttgart: Stuttgart, Germany, 2003; pp 1–15.
- (11) Cha, J.; Cupples, A. M. Detection of the antimicrobials triclocarban and triclosan in agricultural soils following land application of municipal biosolids. *Water Res.* **2009**, 43, 2522–2530.
- (12) Eriksen, G.; Amundsen, C.; Berndorf, A.; Eggen, T.; Grave, K.; Halling-Sørensen, B.; Källqvist, T.; Sogn, T.; Sverdrup, L. Risk assessment of contaminants in sewage sludge applied on Norwegian soils. *Norwegian Scientific Committee for Food Safety* **2009**, 1–244.
- (13) Balk, F.; Ford, R. A. Environmental risk assessment for the polycyclic musks, AHTN and HHCB. II. Effect assessment and risk characterisation. *Toxicol. Lett.* **1999**, 111, 81–94.

- (14) Steinberg, P.; Fischer, T.; Arand, M.; Park, E.; Elmadafa, I.; Rinkus, G.; Brunn, H.; Dienes, H.-P. Acute hepatotoxicity of the polycyclic musk 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydro-naphthalene (AHTN). *Toxicol. Lett.* **1999**, *111*, 151–160.
- (15) Lin, D.; Zhou, Q.; Xie, X.; Liu, Y. Potential biochemical and genetic toxicity of triclosan as an emerging pollutant on earthworms (*Eisenia fetida*). *Chemosphere* **2010**, *81*, 1328–1333.
- (16) Suller, M. T. E.; Russell, A. D. Triclosan and antibiotic resistance in *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **2000**, *46*, 11–18.
- (17) Kroes, R.; Renwick, A. G.; Cheeseman, M.; Kleiner, J.; Mangelsdorf, I.; Piersma, A.; Schilter, B.; Schlatter, J.; van Schothorst, F.; Vos, J. G.; Würtzen, G. Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet. *Food Chem. Toxicol.* **2004**, *42*, 65–83.
- (18) Boxall, A. B. A.; Johnson, P.; Smith, E. J.; Sinclair, C. J.; Stutt, E.; Levy, L. S. Uptake of veterinary medicines from soils into plants. *J. Agric. Food. Chem.* **2006**, *54*, 2288–2297.
- (19) Jones-Lepp, T. L.; Stevens, R. Pharmaceuticals and personal care products in biosolids/sewage sludge: The interface between analytical chemistry and regulation. *Anal. Bioanal. Chem.* **2007**, *387*, 1173–1183.
- (20) European Commission—Eurostat. Europe in figures 2011. *Eurostat Yearbook 2011*; 2011.
- (21) Litz, N. T.; Müller, J.; Böhmer, W. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants part 2: Investigation of polycyclic musks in soils and plants. *J. Soils Sediments* **2007**, *7*, 36–44.
- (22) Aryal, N.; Reinhold, D. M. Phytoaccumulation of antimicrobials from biosolids: Impacts on environmental fate and relevance to human exposure. *Water Res.* **2011**, *45*, 5545–5552.
- (23) Australian Government—Department of Health and Ageing (NICNAS). Priority existing chemical assessment report no. 30. *Industrial Chemicals Notification and Assessment*; Industrial Chemicals Notification and Assessment Scheme (NICNAS): Sydney, Australia, 2009; pp 1–537.
- (24) Stevens, J. L.; Northcott, G. L.; Stern, G. A.; Tomy, G. T.; Jones, K. C. PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks, and polychlorinated n-alkanes in U.K. Sewage sludge: Survey results and implications. *Environ. Sci. Technol.* **2003**, *37*, 462–467.
- (25) Bester, K. Triclosan in a sewage treatment process—balances and monitoring data. *Water Res.* **2003**, *37*, 3891–3896.
- (26) Anastassiades, M.; Lehotay, S.; Staynbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412–431.
- (27) Paya, P.; Anastassiades, M.; Mack, D.; Sigalova, I.; Tasdelen, B.; Oliva, J.; Barba, A. Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Anal. Bioanal. Chem.* **2007**, *389*, 1697–1714.
- (28) Dionex Corporation. *Application Note 321 Determination of Unbound Fat in Various Food Matrices Using Accelerated Solvent Extraction (ASE)*; 2004; pp 1–3.
- (29) Herklotz, P. A.; Gurung, P.; Vanden Heuvel, B.; Kinney, C. A. Uptake of human pharmaceuticals by plants grown under hydroponic conditions. *Chemosphere* **2010**, *78*, 1416–1421.
- (30) An, J.; Zhou, Q.; Sun, Y.; Xu, Z. Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (*Triticum aestivum* L.). *Chemosphere* **2009**, *76*, 1428–1434.
- (31) Chefetz, B.; Mualem, T.; Ben-Ari, J. Sorption and mobility of pharmaceutical compounds in soil irrigated with reclaimed wastewater. *Chemosphere* **2008**, *73*, 1335–1343.
- (32) Briggs, G. G.; Bromilow, R. H.; Evans, A. A. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pestic. Sci.* **1982**, *13*, 495–504.
- (33) Bromilow, R. H.; Chamberlain, K. Principles governing uptake and transport of chemicals. In *Plant Contamination: Modeling and Simulation of Organic Chemical Processes*; Trapp, S., McFarlane, C., Eds.; Lewis Publishers: Boca Raton, FL, 1995; pp 38–64.
- (34) Wu, C.; Spongberg, A. L.; Witter, J. D.; Fang, M.; Czajkowski, K. P. Uptake of Pharmaceutical and Personal Care Products by Soybean Plants from Soils Applied with Biosolids and Irrigated with Contaminated Water. *Environ. Sci. Technol.* **2010**, *44*, 6157–6161.
- (35) Schwab, A. P.; Al-Assi, A. A.; Banks, M. K. Adsorption of naphthalene onto plant roots. *J. Environ. Qual.* **1998**, *27*, 220–224.
- (36) Gao, Y.; Ling, L. Comparison for plant uptake of phenanthrene and pyrene from soil and water. *Biol. Fertil. Soils* **2006**, *42*, 387–394.