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Uptake of Veterinary Medicines from Soils into Plants

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Medicines play an important role in the treatment and prevention of disease. Whereas the side effects on human and animal health resulting directly from treatment have been widely documented, only recently have the occurrence and fate of medicines in the environment and the potential consequences for human health been recognized as an issue warranting consideration. Medicines have been shown to be released to soils and to persist in the environment. This study was performed to investigate the potential for a range of veterinary medicines to be taken up from soil by plants used for human consumption and to assess the potential significance of this exposure route in terms of human health. Soil analyses indicated that, for selected substances, measurable residues of these are likely to occur in soils for at least 5 months following application of manure containing these compounds. Experimental studies on the uptake of veterinary medicines into carrot roots (tubers) and lettuce leaves showed that only florfenicol, levamisole, and trimethoprim were taken up by lettuces, whereas diazinon, enrofloxacin, florfenicol, and trimethoprim were detected in carrot roots. Measured concentrations in plant material were used to model potential adult human exposure to these compounds. Although exposure concentrations were appreciable in a few instances, accounting for ~10% of the acceptable daily intake values (ADI), all were lower than the ADI values, indicating that, at least for compounds with properties similar to those considered here, there is little evidence of an appreciable risk. This exposure route may, however, be important when veterinary medicines have a very low ADI, at which they elicit subtle effects over prolonged periods, or when exposure is occurring via a number of routes at once. Although degradation products (produced in the soil or the plant) were not measured, it is possible for some substances that these could increase the risks to consumers.

KEYWORDS: Pharmaceuticals; veterinary; plant uptake; indirect exposure; soil; environment

INTRODUCTION

Medicines play an important role in the treatment and prevention of disease in humans and animals. Whereas the side effects on human and animal health have been widely documented, only recently have the occurrence and fate and effects of such medicines in the environment been considered (see, e.g., refs *1*−*3*).

Veterinary medicines are widely used in livestock treatment and will be released to land either directly in feces or urine or indirectly through the application of manure as a fertilizer (*4*). A range of veterinary medicines, including hormones, antibiotics, and parasiticides, have been detected in soils, surface waters, and groundwaters (see, e.g., refs *5*−*8*). Although the reported concentrations are generally low, the substances have been observed throughout the year across a variety of hydrological, climatic, and land-use settings. Some substances (e.g.. oxytetracycline) may also persist in the environment for over a year (see, e.g., ref *9*). As a result, questions have been raised over the potential impacts of veterinary medicines in the environment on human and animal health, such as the promotion of the spread of antibiotic resistance or the triggering of adverse immunological reactions (see, e.g., ref *10*).

Humans may be exposed to residues of veterinary medicines in the environment (i.e., soil, water, sediment) by a number of routes including the consumption of (1) crops that have accumulated substances from soils as a result of exposure to contaminated manure and slurry; (2) livestock that have accumulated veterinary medicines through the food chain; (3) fish exposed to treatments used in aquaculture; and (4) abstracted groundwater and surface waters containing veterinary medicines. Exposure may also occur via the inhalation of dust emitted from intensively reared livestock facilities (see, e.g., ref *11*) or as a

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Table 1. Characteristics of Test Soil

parameter	value	parameter	value
sand content (%)	90.14	cation exchange capacity	5.4
silt content (%)	6.26	organic carbon content (%)	0.4
clay content (%)	3.60	water holding capacity (% w/w)	35.0
pH	6.3	biomass (μg of C/g)	67.3

result of contact with contaminated fleece from treated sheep. Although veterinary medicines are routinely monitored in food materials from treated animals to ensure that concentrations are below the maximum residue limits, the magnitude of the exposure via many of the routes listed and the health impacts of such exposure have not been extensively quantified. Recent work has assessed the potential risks arising from exposure to veterinary medicines in fish and water. Studies have demonstrated the presence of medicines in water bodies (see, e.g., refs *5* and *6*) and the accumulation of veterinary medicines from surface waters by fish, shellfish, and crustacea (see, e.g., refs *12*−*15*). A risk assessment of human medicines in the United States indicates that consumption of medicines via surface waters and fish poses no appreciable risk to human health (*16*).

With the exception of a few studies (see, e.g., refs *17*−*21*) that have shown that fluoroquinolones and sulfonamides can be taken up by crops, exposure via plants has not been considered, previously. There is, therefore, a need to determine the significance of uptake into plants from soil as a potential exposure route for veterinary medicines. This study was therefore performed to investigate the potential for a representative range of veterinary medicines to be taken up from soil by plants and to assess the potential significance of this route of exposure in terms of risk to human health.

MATERIALS AND METHODS

Studies were performed using a leaf crop (lettuce) and a root crop (carrot). Work focused on representative veterinary medicines from a range of classes and with a range of physicochemical properties.

Test Soil. A light loamy sand soil was used in the study. The soil was selected because it was highly homogeneous, with a low organic carbon content, and probably, therefore, represented a "worst case" in terms of bioavailability. The soil was collected in the summer of 2004 from an arable farm located close to Church Warsop, Nottinghamshire, U.K. To ensure homogeneity, following collection the soil was air-dried and passed through a 2 mm screen and mixed, prior to use in the uptake studies. **Table 1** summarizes the properties and characteristics of the test soil.

Test Chemicals and Soil Spiking. Ten test substances were selected (**Table 2**), to cover a range of veterinary classes (including sulfonamides, tetracyclines, organophosphates, fluoroquinolones, macrolides, and β-lactams) and environmental properties (such as hydrophobicity, sorption potential, and persistence). The study compounds had previously been identified, in prioritization studies, to have a high potential to be released to the environment (*22*, *23*). Four of the substances, oxytetracycline, trimethoprim, sulfadiazine, and amoxicillin, are also used as human medicines. The sources and purities of the test chemicals were as follows: tylosin (>90%; Fluka, Buchs, Switzerland); diazinon (98.4%; Riedel-de Haen, Seelze, Germany); florfenicol (analytical grade; Sigma, Germany); sulfadiazine (99%; Sigma, Poole, U.K.); phenylbutazone (analytical grade; Sigma); oxytetracycline (>98%; Fluka); levamisole (99%; Sigma); amoxicillin (>97%; Fluka); trimethoprim (98.5%; Sigma, Switzerland); and enrofloxacin (>98%; Fluka).

Individual stock solutions of 32 mg L^{-1} of test substance were prepared in distilled water for each compound. For each substance, stock solutions (*11*) were added to 32 kg of air-dried soil to give a nominal substance concentration of 1 mg kg^{-1} of dry weight. This concentration was selected as it was toward the upper end of the concentration range of veterinary medicines measured in soils (see, e.g., ref *3*). The stock solution/soil mixture was stirred in an electric concrete mixer for 20 min, after which time additional distilled water was added to give a moisture content of 20% w/w. Samples of spiked and control soil were then taken at this stage for chemical analysis.

Uptake Studies. Aliquots (1.5 kg) of spiked soil were placed into nonporous plastic plant pots (15 cm diameter × 14 cm deep) to give a total of 16 pots for each substance. Lettuce seeds (All Year Round Variety, B&Q, U.K.) were planted into 6 of the 16 pots, and carrot seeds (Autumn King 2 Variety, B&Q) were planted in the remaining 10. This gave 4 replicates per compound for assessing uptake by lettuce; 4 replicates per compound for assessing total uptake by carrots; 4 replicates per compound for assessing uptake into carrot peel; and 2 soil analysis replicates per plant species. Each pot received six seeds as preliminary studies indicated that this provided enough biomass for chemical analysis.

The plants were grown under controlled conditions: light intensity, 10000 lx with a 16/8 h light/dark cycle; humidity, 70%; and temperature, 20 °C during the light cycle and 15 °C during the dark cycle. To maintain healthy plants and minimize disturbance of the soil, pots were bottom watered twice a week with 50 mL of distilled water. Plants were grown until they had reached maturity. Lettuces were grown for 103 days and carrots for 152 days. After these times, samples of leaf material (lettuce) or carrot were removed from each pot, weighed, and placed in glass jars prior to analysis. Half of the carrot replicates were peeled manually using a peeler. Samples of soil were obtained from the two soil analysis replicates. Samples were transported, within 8 h of collection, to the Health and Safety Laboratory (HSL) for analysis. Prior to chemical analysis, all soil samples were stored in freezers at −18 °C and all plant material was stored in refrigerators at 2−8 °C. Samples were extracted within 7 days of collection.

Chemical Analysis. Concentrations of the study compounds in soil and plant material were determined after extraction by either gas chromatography (GC) with mass spectrometry (MS), liquid chromatography (LC) with ultraviolet (UV) detection, or LC-MS/MS. The methods are summarized below and are described in detail elsewhere (*24*).

Extraction. *Diazinon.* Soil samples were extracted into a mixture of dichloromethane (DCM) and acetone. Plant samples were extracted into acetone. The extracts were cleaned up by gel permeation chromatography (GPC) prior to analysis by GC-MSD (*25*).

Phenylbutazone. Soil and plant samples were extracted using a citrate buffer and a trisolvent mixture of diethyl ether, dichloromethane, and hexane. The combined extracts were derivatized with *n*-trimethylsulfonium hydroxide in methanol prior to analysis by GC-MS.

Oxytetracycline and Florfenicol. Soil and plant samples were extracted into a solution of citric acid and methanol. The extract was loaded onto Oasis HLB SPE cartridges and eluted with methanol. The sample eluates were dried, and 1 mL of internal standard solution was added prior to analysis by LC-MS/MS.

Enrofloxacin. Soil samples were extracted using acetic acid and acetone. Extracts were applied to an Oasis HLB SPE cartridge. Elution was with ammonium hydroxide (2%) in methanol. The eluate was dried and reconstituted in 1 mL of internal standard solution prior to analysis by LC-MS/MS. Plant material was extracted using the same methodology used for extraction of oxytetracyline and florfenicol from soil and plants.

Trimethoprim, Sulfadiazine, Tylosin, and Levamisole. Soil samples were Soxhlet extracted into methanol. The extract was cleaned up using Oasis HLB SPE cartridges. Sulfadiazine, tylosin, and levamisole were extracted from plant material using a mixture of DCM and acetonitrile. The extract was cleaned up on Oasis HLB SPE cartridges with methanol elution. For trimethoprim, plant material was extracted using the same methodology used for the extraction of oxytetracyline and florfenicol from soil and plants. The sample eluates were dried, and 1 mL of internal standard solution was added prior to analysis by LC-MS/MS.

Amoxicillin. Soil and plant samples were extracted into a mixed phosphate buffer solution consisting of 50% potassium dihydrogen phosphate (10 mM) and monobasic potassium phosphate (10 mM). The extract was centrifuged and filtered prior to analysis by LC.

Analysis. The GC system consisted of a Hewlett-Packard (HP) 5890 series II gas chromatograph fitted with a HP-5 MS column (30 m × 2.5 mm × 2.5 μm film thickness) and a HP 5972 series MS with HP

Table 2. Structures and Properties of the Study Compounds (Sources of Data Are Provided in the Supporting Information)

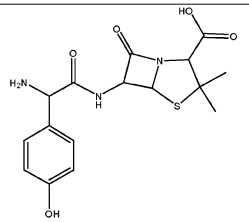
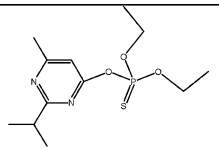
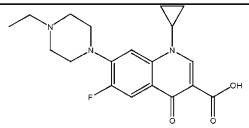
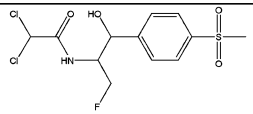
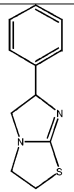
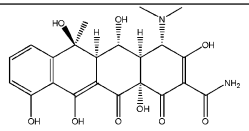
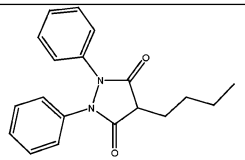
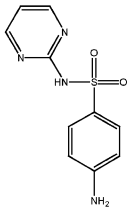
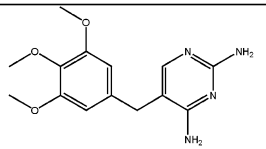
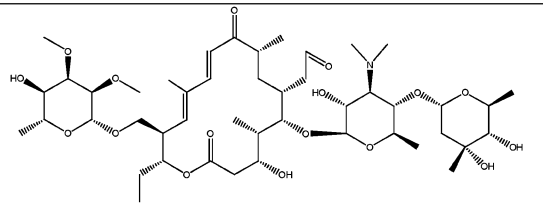
Compound	CAS	Molecular weight	Log K_{ow}	pKa	Log K_{oc} (ℓ/kg)	Soil DT_{50} (days)	Structure
Amoxicillin (antibacterial)	26787-78-0	365.4	0.87	2.8, 7.2	865.5	0.16–0.29	
Diazinon (ectoparasiticide)	333-41-5	304.35	3.81	<2.5[g]	229–1549	1.7–112	
Enrofloxacin (antibacterial)	93106-60-6	359.4	0.7	6.0, 8.8	15800	-	
Florfenicol (antibacterial)	73241-34-2	358.21	-0.04	-	24-52	4-27	
Levamisole (endoparasiticide)	14769-73-4	204.3	1.84	-	8652	-	
Oxytetracycline (antibacterial)	79-57-2	460.44	-0.9	3.3, 7.3, 9. j	2872–56060000	16–18	
Phenylbutazone (antinflammatory)	50-33-9	308.4	3.16	-	15800	-	

Table 2 (Continued)

Compound	CAS	Molecular weight	Log K_{ow}	pKa	Log K_{oc} (ℓ/kg)	Soil DT_{50} (days)	Structure
Sulfadiazine (antibacterial)	68-35-9	250.3	-0.09	6.48	confidential data		
Trimethoprim (antibacterial)	738-70-5	290.32	0.91	7.2	confidential data		
Tylosin (antibacterial)	1401-69-0	916.12	1.63	7.1	200–7988	3.3–105	

G1034C MS ChemStation software. The oven temperature program was 60 °C for 1 min, ramping at 10 °C/min to 300 °C and holding for 5 min, total run time of 30 min. Selected ion monitoring (SIM) data were collected between 8.5 and 25.0 min.

The LC system used to analyze amoxicillin consisted of a Waters 600 controller, a Waters 510 HPLC pump, a Waters 717 plus autosampler, and a Waters 996 photodiode array. The column fitted was a Waters Spherisorb ODS2 5 μm, 4.6 × 300 mm column. The injection volume was 20 μL. The mobile phase was 95% phosphate buffer and 5% acetonitrile at pH 4.8 and was run isocratically at 1 mL min^{-1} at ambient temperature. The monitoring wavelength was 229 nm.

The LC system used for all other analytes consisted of an Agilent 1100 and an Applied Biosystems API 2000 LC-MS/MS. The column fitted was a Restek Allure C18 5 μm, 2.1 × 250 mm, and the injection volume was 5 μL. Mobile phase A was 20 mM ammonium acetate with 0.1% acetic acid, and mobile phase B was acetonitrile with 0.1% acetic acid. The mobile phases were run as a gradient, and a multireaction monitoring (MRM) analysis was used.

Data Analysis and Estimation of Exposure Concentrations. Analytical results for each of the veterinary medicines in lettuce and whole carrot were used to derive soil-based and soil pore water-based uptake factors (UFs) at harvest. The UFs were derived from the K_{oc} value for each compound and the soil and plant measured concentrations using eqs 1 and 2

$$\mathrm{UF_{soil}} = \frac{C_p}{C_s} \tag{1}$$

$$\mathrm{UF_{pw}} = \frac{C_p}{C_s \frac{1}{K_{oc} f_{oc}}} \tag{2}$$

where $\mathrm{UF_{soil}}$ is the soil-based UF, $\mathrm{UF_{pw}}$ is the soil pore water-based UF, C_p is the concentration in plant material, C_s is the concentration in soil, f_{oc} is the fraction of organic carbon in the test soil (0.4%), and K_{oc} is the organic carbon normalized soil sorption coefficient. When a substance was not detected, a theoretical maximum uptake factor was obtained using the analytical detection limit for either lettuce or carrot.

Predicted Exposure Concentrations in Plant Material. Uptake studies were performed using a standard spiking concentration of 1 mg kg^{-1}. Environmental exposure modeling approaches were therefore employed to assess likely exposure concentrations following typical usage patterns.

Predictions of concentrations of each of the veterinary medicines in soils were initially obtained using the exposure modeling approach of Spaepen et al. (*26*). The equations and scenarios used are described below. Initially, the amount of substance excreted by an animal was calculated, on the basis of typical treatment scenarios for each of the study compounds, using eq 3

$$A_{\mathrm{excreted}} = MDT_{\mathrm{treatment}} \tag{3}$$

where A_{excreted} is the amount excreted (mg/animal), M is the mass of the animal [kg (cattle = 600; pigs = 70; poultry = 2)], D is the dosage (mg/kg), and $T_{\mathrm{treatment}}$ is the treatment duration (days; a maximum of 70 days was set). Animal masses were those proposed by Spaepen et al (*26*).

The concentration of each study substance in slurry [C_{slurry} (mg kg^{-1})] was then determined from the amount excreted, the slurry storage time (T_{storage}), and the slurry production for individual animals [P_{slurry} (kg animal^{-1} day^{-1} (cattle = 78.5; pigs = 3.8; poultry = 0.072] (*26*) using eq 4. A storage time of 70 days was used.

$$C_{\mathrm{slurry}} = \frac{A_{\mathrm{excreted}}}{P_{\mathrm{slurry}} T_{\mathrm{storage}}} \tag{4}$$

The maximum recommended amount of manure or slurry that could be applied to land was then calculated using eq 5 and data on the concentration of nitrogen in different manure/slurry types [P_N (kg/place/year)] and on recommended nitrogen limits for the United Kingdom [A_N (kg/ha/year; 170 kg ha^{-1} year^{-1})] and the typical manure/slurry output for an animal holding (P_E).

$$S = \frac{A_N}{P_N} \times P_E \tag{5}$$

The amount of each veterinary medicine applied to land [A_{applied} (mg/ha)] was calculated, using eq 6, from the predicted manure/slurry concentration and the amount of manure that can be applied.

$$A_{\mathrm{applied}} = SC_{\mathrm{slurry}} \tag{6}$$

The concentration in soil (C_{sp}) was calculated using eq 7. It was assumed that all of the manure/slurry is applied to a field on one occasion each year and that the mixing depth (P_{depth}) is 20 cm. A soil bulk density (B_{density}) of 1500 kg/m^3 was used.

$$C_{sp} = \frac{A_{\mathrm{applied}}}{S + [((P_{\mathrm{depth}}//100) \times 100 \times 100) \times B_{\mathrm{density}}]} \tag{7}$$

Concentrations of each of the veterinary medicines in plant material were then estimated from the predicted soil concentrations and soil-based UFs obtained from the experimental studies using eq 8

$$C_p = \left(C_{sp} \frac{1}{K_{oc} f_{oc}} \mathrm{UF_{soil}} \right) \mathrm{e}^{-\mathrm{RC_{soil}} t} \tag{8}$$

where C_p is the concentration of the substance in the edible part of the plant, C_{sp} is the predicted soil concentration, K_{oc} is the organic carbon normalized sorption coefficient, f_{oc} is the soil organic carbon content (assumed to be 0.4%), UF is the uptake factor for soil, $\mathrm{RC_{soil}}$ is the degradation rate constant for soil (derived using eq 9), and t is the time of harvest (100 days for lettuce and 150 days for carrot). To provide a conservative estimate, the longest DT_{50} value from **Table 2** was used in the predictions.

$$\mathrm{RC_{soil}} = \frac{\ln 2}{\mathrm{DT_{50,soil}}} \tag{9}$$

RESULTS

Analytical Performance. Limits of detection ranged from 0.3 (diazinon) to 22 (enrofloxacin) μg kg^{-1} in soil, from 0.7 (trimethoprim) to 17 (sulfadiazine) μg kg^{-1} in lettuce, and from 0.3 (diazinon) to 23 (oxytetracycline) μg kg^{-1} in carrot. Although recoveries for most determinands were good, low but reproducible recoveries were obtained for selected substances in soil and/or plant material, so all measured values were recovery corrected. These low recoveries were observed for the highly sorptive study substances.

Soil Analyses. With the exception of tylosin, none of the study compounds were detected in control soils. Tylosin was detected at 8.7 μg kg^{-1}. Amoxicillin was not detected in any of the spiked soils. Initial measured concentrations of the other test chemicals were lower than the nominal spiking concentration (1 mg kg^{-1}) and ranged from 130 (phenylbutazone) to 900 μg kg^{-1} (oxytetracycline).

As samples of soil were analyzed at the start of the experiment and following harvest of the crops, it was possible to generate information on the dissipation of each of the test substances in soils (**Figure 1**). Amoxicillin degrades very quickly in soil with half-life (DT_{50}) and time to 90% dissipation (DT_{90}) times of <1 day. With the exception of enrofloxacin, the DT_{50} values for all other substances were <103 days (**Table 3**). DT_{90} values for florfenicol, enrofloxacin, and oxytetracyline were >152 days.

Experimental Uptake by Plants and Prediction of Exposure Concentrations. Plant weight data indicated that, with the exception of phenylbutazone, oxytetracycline, and enrofloxacin, there was no effect of treatment with veterinary medicines on

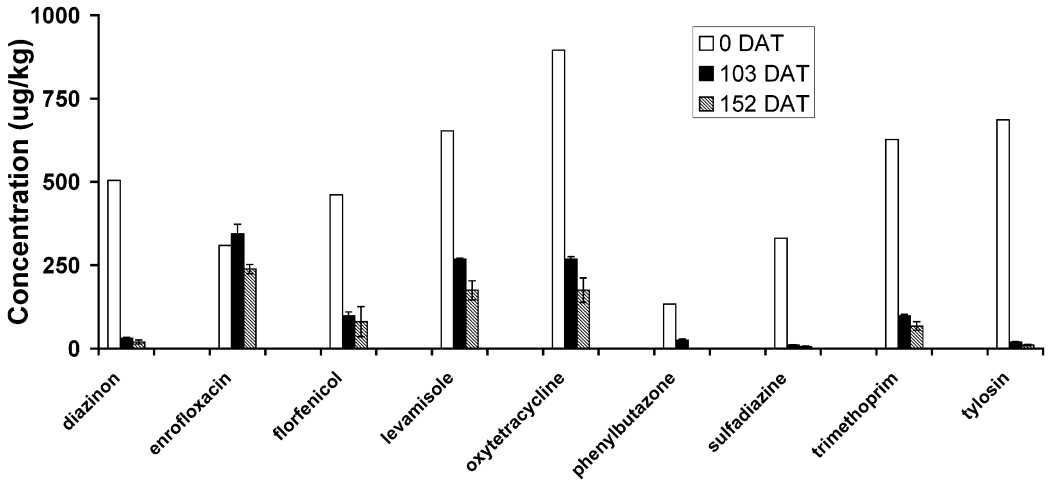


Figure 1. Mean concentrations (+ 1 SE) of study compounds in test soil over time (DAT, days after soil treatment).

Table 3. DT_{50} and DT_{90} Values, Mean Concentrations (of Four Replicates) and Uptake Factors in Plants for Test Substances in the Plant Uptake Studies (Standard Errors Are Given in Parentheses)

test substance	DT_{50} (days)	DT_{90} (days)	concn in lettuce (μg kg^{-1})	concn in whole carrot (μg kg^{-1})	concn in carrot peel (μg kg^{-1})	soil-based UF lettuce	soil-based UF carrot	pore water-based UF lettuce	pore water-based UF carrot
amoxicillin	<1	<1							
diazinon	<103	<152	<1	13 (1.7)	24 (0.65)	<0.03	0.64	0.11	2.3
enrofloxacin	>152	>152	<1.6	2.8 (0.3)	8.5 (2.2)	<0.004	0.01	<4.3	11
florfenicol	<103	>152	15 (8.3)	5 (1.1)	38 (6.4)	0.15	0.06	0.024	0.010
levamisole	<103	>152	170 (22)	<11	<11	1.4	<0.08	48	<2.7
oxytetracycline	<103	>152	<7.2	<23	<23	<0.03	<0.13	<5.7	<28
phenylbutazone	<103	<152	<1.1	<1.1	<1.1	<0.04	<0.79	<2.7	<50
sulfadiazine	<103	<103	<17	<6.1	<6.1	<1.6	<1.0	<0.55	<0.34
trimethoprim	<103	>152	6 (3)	5.3 (2.7)	1 (0.4)	0.06	0.08	0.68	0.86
tylosin	<103	<103	< 1.5	<0.5	<0.5	<0.08	<0.05	<0.1	<0.54

Table 4. Calculated Exposure Concentrations for the Veterinary Medicines in Soil, Carrot and Lettuce for Typical Treatment Scenarios

compound	ADI (μg kg day^{-1})	treatment group	treatment dose (mg kg day^{-1})	treatment duration (days)	soil concn (μg kg^{-1})		predicted concn (μg kg^{-1})	
					lettuce	carrot	lettuce	carrot
amoxicillin	0.5	pigs	15	15	0.0	0.0	0.0	0.0
diazinon	2	sheep	3000 animal^{-1}	1	35	25	<1.1	16
enrofloxacin	6.2	cattle	5	5	336	324	<1.3	3.2
florfenicol	3	cattle	20	2	28	8	4.2	0.48
levamisole	6	sheep	8	1	35	25	49	<2
oxytetracycline	30	pigs	20	15	75	11	<2.3	<1.4
phenylbutazone	NA	horses	4.2	10	52	37	<2.1	<29
sulfadiazine	20	poultry	22	10	2	0.0	<3.2	<0.0
trimethoprim	12.5	poultry	8	21	1372	1001	82	80
tylosin	6	pigs	25	3	26	18	<2.1	<0.9

plant growth; the growth of plants exposed to phenylbutazone, oxytetracycline, and enrofloxacin was significantly reduced. Plants were grown in discrete groups for each test chemical, so this finding may be an experimental artifact.

None of the study substances were detected in the control lettuce leaves, whole carrot roots, or carrot root peeling materials. Only florfenicol, levamisole, and trimethoprim were detected in lettuce leaves (**Table 3**), whereas diazinon, enrofloxacin, florfenicol, and trimethoprim were detected in carrot root material (**Table 3**). Concentrations in lettuce leaves ranged from 6 (trimethoprim) to 170 (levamisole) μg kg^{-1}, whereas concentrations in carrot root ranged from 2.8 (enrofloxacin) to 13 μg kg^{-1} (diazinon). Concentrations of diazinon, enrofloxacin, and florfenicol on the outer layer of the carrot root were higher than concentrations inside the body of the carrot root, whereas trimethoprim was found at higher concentrations inside the crop (**Table 3**). Pore water-based UFs for lettuce ranged from 0.024 to 48, whereas UFs for whole carrot ranged from 0.01 to 11 (**Table 3**).

By combining the results of the uptake experiments with exposure modeling approaches, predictions of concentrations of each of the study compounds in plant material, following typical veterinary use, were obtained (**Table 4**). With the exception of oxytetracycline, predicted concentrations of the test chemicals in soil, arising from typical usage scenarios, were lower than the spike concentration of 1 mg kg^{-1}. On the basis of these predicted concentrations, predicted concentrations in lettuce leaves ranged from ND (amoxicillin) to 82 (trimethoprim) μg kg^{-1}, whereas concentrations in carrot root ranged from ND (amoxicillin) to <29 (phenylbutazone) μg kg^{-1}.

DISCUSSION

Veterinary and human medicines are increasingly being monitored in slurry, soils, surface waters, and groundwaters (see,

e.g., refs *5*, *6*, and *27*). Concerns have therefore been raised over the impacts of environmental exposure routes on human and environmental health. In this study the potential for medicines to enter the food chain via uptake from soil into food plants was explored. The results demonstrate that following application of veterinary medicines to soils at environmentally realistic concentrations, selected substances are taken up at detectable levels.

The study design was such that it was possible to generate semiquantitative information on the dissipation of veterinary medicines in the soil environment. The results demonstrated that amoxicillin is rapidly dissipated and that, with the exception of enrofloxacin, all of the other test substances had DT_{50} values of <15 weeks. The results are in agreement with previous degradation studies on amoxicillin (*28*), diazinon (*29*), oxytetracycline (*30*), florfenicol (*31*), and enrofloxacin (*32*). DT_{90} values for florfenicol, enrofloxacin, and oxytetracycline were >150 days, indicating that for these compounds residues could persist in the soil environment for >6 months following application. These findings are supported by other work with oxytetracycline where the substance was measured in soil ∼1 year after application (*9*).

In the plant studies, a decline in plant growth was observed for the phenylbutazone, oxytetracycline, and enrofloxacin exposures. Although the results could be an artifact of the experimental design, the findings are supported by previous laboratory in vitro studies in which the growth and development of a range of plants (e.g., *Phaseolus valugaris*, *Glycine nax*, *Medicago saliva*, and *Zea mays*) were affected by veterinary medicines (reviewed in ref *33*) and in vivo experiments with enrofloxacin where effects on root and leaf growth were observed at 5 mg kg^{-1} (*18*). As veterinary medicines are applied in urine, manure, and slurry to land that will be used for crop production, further studies may be warranted to explore in more detail the potential toxicity of veterinary medicines to plants under realistic exposure conditions and the impacts of such exposures on crop productivity.

In the uptake studies, both carrot roots and lettuce leaves took up florfenicol and trimethoprim. Uptake of levamisole was also observed in lettuce leaves, and the carrot roots took up diazinon and enrofloxacin. The uptake of enrofloxacin into plants has been demonstrated previously (*18*). With the exception of trimethoprim, data for whole carrot root and carrot root peelings indicated that the majority of the veterinary medicines that were taken up were associated with the outer layer of the carrot. Peeling of carrots prior to consumption will therefore greatly reduce the potential for exposure.

None of the other study compounds were detected in plant material. The lack of uptake observed may be due to factors such as high limits of detection or significant degradation during the study. For example, results for amoxicillin, sulfadiazine, and tylosin could well be explained by their dissipation in soils, with >90% dissipation being observed by the time lettuces were harvested. The results for sulfadiazine contrast with previous studies into the uptake of sulfonamide antibiotics (sulfamethoxine), where uptake was observed by roots and stems of certain plant species (*17*, *34*). Exposure concentrations in these previous studies were, however, significantly higher (from 13 to >2000 mg kg^{-1}) than the concentration used in our study (1 mg kg^{-1}).

It is generally recognized that chemicals are taken up into plants via the soil pore water. Root uptake of organic chemicals from soil water is typically related to the octanol−water partition coefficient of the compound (*35*, *36*). Uptake of chemicals by roots is greatest for more lipophilic compounds, whereas polar compounds are accumulated to a lesser extent (*35*). Studies of translocation of pesticides into shoots indicate that uptake into shoots (and hence above ground plant material) is related to log K_{ow} by a Gaussian curve distribution (*35*, *36*). Maximum translocation is observed at a log K_{ow} of ∼1.8. More polar compounds are taken up less well by shoots, and uptake of highly lipophilic compounds (log K_{ow} > 4.5) is low. Comparison of measured pore water-based UFs and theoretical UFs (obtained from the detection limits for soil and plant material) for lettuce and carrot with log K_{ow} data (**Figure 2**) indicates that there is no relationship between log K_{ow} and pore water-based UFs for carrots for the veterinary medicines. This is not surprising as data for other environmental processes (e.g., sorption to soil) indicate that the behavior of veterinary medicines in the environment is poorly related to hydrophobicity but is determined by a range of factors including H-bonding potential, cation exchange, cation bridging at clay surfaces, and complexation (*37*).

In contrast, results for lettuce suggest that uptake of veterinary medicines may follow a Gaussian relationship similar to that observed previously, with the maximum measured UF being observed for levamisole with a log K_{ow} value of 1.84 (**Figure 2**). As we were able to detect only three of the study substances in lettuce, additional work is required to confirm this. We recommend that in the future a more thorough assessment of the routes of uptake of medicines in plants be undertaken. Experiments could include the use of hydroponics, sampling of sap rather than the whole plant, and assessment of uptake across a range of concentrations and across a range of soil types and climatic conditions. The effects of manure and slurry amendment on the behavior of the materials should also be assessed as studies have indicated that the manure matrix can alter the behavior and transport of medicines (see, e.g., refs *4*, *38*, and *39*).

Using data generated from the study with published fate and behavior data and environmental exposure models, concentrations of each of the veterinary medicines in crop material, following typical usage regimes, were estimated to be generally in the low micrograms per kilogram range. With the exception of phenylbutazone, acceptable daily intake (ADI) values are available for all of the study compounds. Using estimates of dietary intakes from the Global Environment Monitoring System/Food Contamination Monitoring and Assessment Program (GEMS/Food) dataset, predicted plant concentration data, and these ADIs, it was possible to estimate the potential exposure to veterinary medicines in plants and risk to adult human health. On a daily basis, an adult typically consumes 0.512 kg of plant material from crops grown above ground (including cereals, pulses, and green vegetables) and 0.333 kg of plant material from crops grown below ground (including potatoes and bulb vegetables). The potential daily intake of each veterinary medicine (micrograms per day) was estimated by using the lettuce data as a surrogate for concentrations in aboveground crops and the carrot data as a surrogate for vegetables grown below ground.

Comparison of the calculated daily intakes with the ADIs (**Figure 3**) suggests that for the study compounds exposure of consumers to veterinary medicines in soils via plants is likely to be considerably below the ADI and that the risk to human health is probably low. This simplistic risk assessment is very conservative, because it assumes that all plant material consumed in the diet is derived from crops grown with manure containing veterinary medicines. Nonetheless, exposure via the plant material consumed in the diet could potentially be a significant

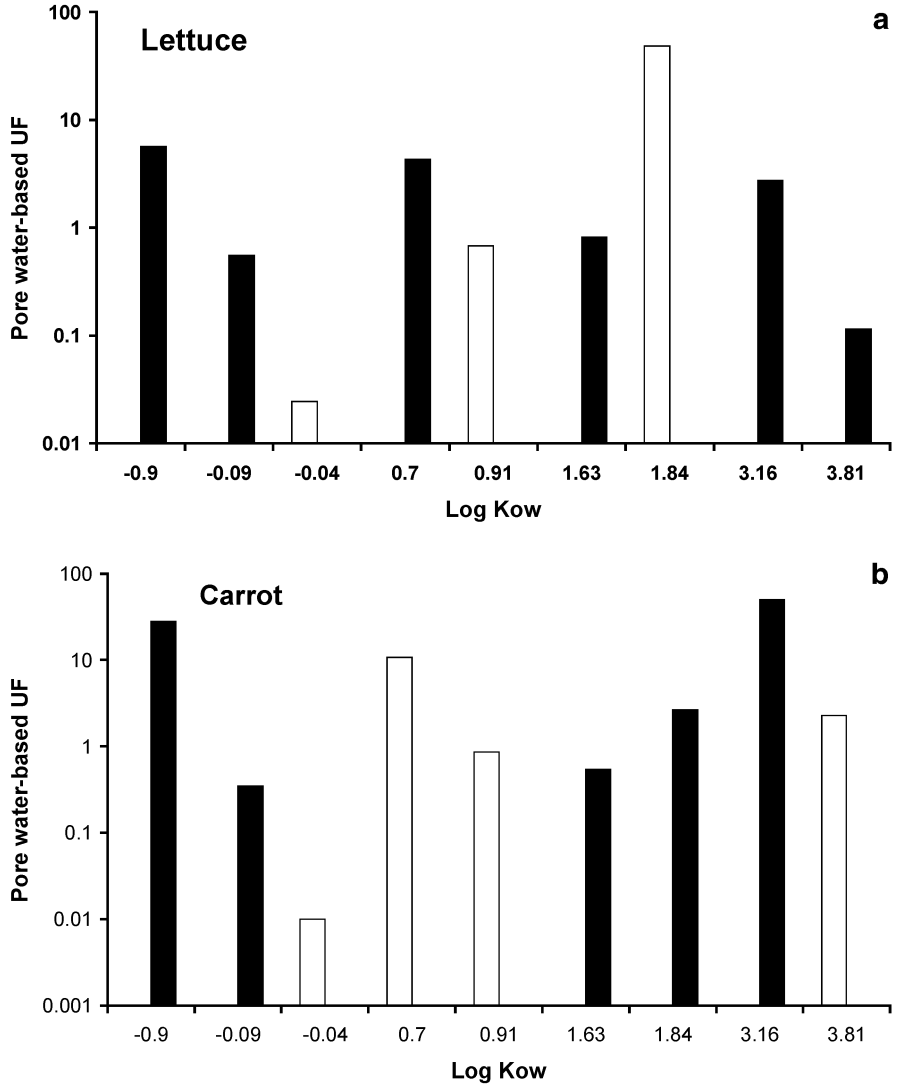


Figure 2. Relationship between log octanol–water partition coefficient and pore water-based uptake factors for veterinary medicines in (**a**) lettuce and (**b**) carrot. Open bars represent measured UF values, and solid bars represent theoretical maximum UF values derived from the limits of quantification for soil and plant material.

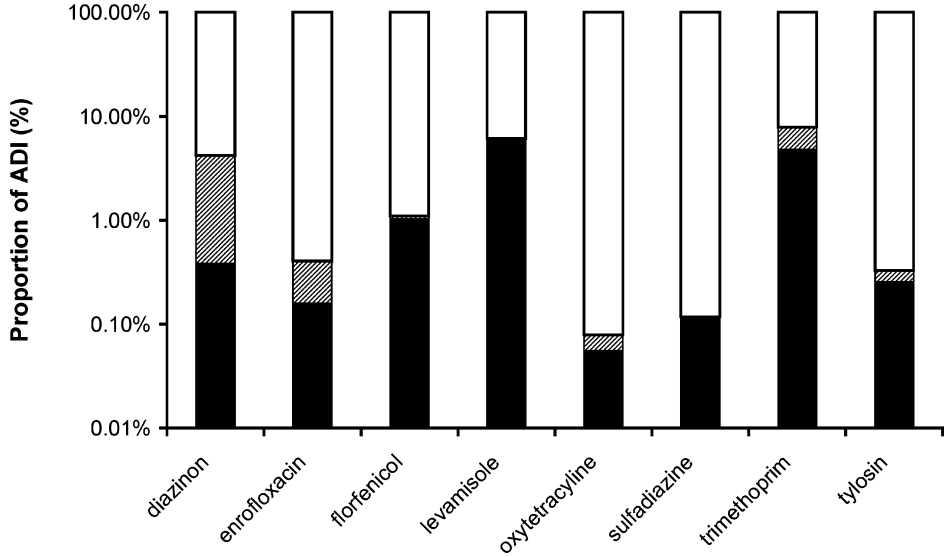


Figure 3. Potential contribution of veterinary medicines in vegetable material to the acceptable daily intake. Solid bars represent exposure via nongreen vegetables, and hatched bars represent exposure via green vegetables.

element in those instances when (1) the dose of a medicine taken up via other routes of exposure (e.g., consumption of meat) is close to the ADI, (2) a veterinary medicine has a low ADI, (3) a substance is banned for use in food-producing animals (e.g.,

phenylbutazone), or (4) there are concerns over subtle health effects resulting from long-term low-dose exposure (e.g., promotion of antibiotic resistance or endocrine disruption).

The current study has focused exclusively on parent medicines and on single-substance exposures. It is likely that many of the study compounds will have degraded in the soil or in the plant into transformation products. For example, for pesticides, it is known that many of these compounds are degraded in soils and plants into other organic compounds (see, e.g., refs *40* and *41*). These substances may be toxicologically active and could also be taken up. Although in most cases transformation products have lower toxicity than their parents, there are instances of transformation products having toxicity similar to or greater than that of the parent compound (*42*), so the potential risks of these substances should not be discounted. In the natural environment, veterinary medicines will also co-occur with other veterinary medicines and other synthetic organic compounds. For example, in a recent monitoring study of waters the antibacterial lincomycin was shown to co-occur with 27 other synthetic compounds (*6*). Assuming that similar mixtures occur in soils and that a number are taken up into plants, there is a possibility that humans will be exposed to mixtures of such contaminants via plant-derived foodstuffs.

In conclusion, veterinary medicines are increasingly being detected in environmental matrices. It is therefore appropriate to give consideration to the potential impact on humans from exposure to veterinary medicines in environmental matrices. This study has explored the potential for humans to be exposed to medicines in plant material. A number of the veterinary medicines were shown to be taken up by plants from soils. Predictions of the potential exposure of each of the study compounds following typical usage patterns indicate that exposure of humans via plant-derived foodstuffs is generally low and that effects on human health are unlikely. This route of exposure may, however, be more significant for the small number of highly toxic medicines or in situations when long-term low-level exposure could elicit subtler effects (e.g., promotion of antibacterial resistance or endocrine disruption). Further work to explore the mechanisms of uptake of medicines from soils into plants and the potential risks of transformation products and mixtures of substances that are likely to occur in soils is therefore warranted.

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Supporting Information Available: Full version of Table 2, including original sources of data. This material is available free of charge via the Internet at http://pubs.acs.org.

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