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Evaluation of a Lower Tier Exposure Assessment Model for Veterinary Medicines

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Veterinary antibiotics are used in large quantities in the European Union, and one of the key environmental exposure routes is via the application of manure containing excreted antibiotics to arable land as fertilizer. It is a legal requirement to assess the environmental risk of veterinary medicines, and this is done in two stages. A key decision parameter in phase I of these assessments is the predicted environmental concentration (PEC) in soil, and if a trigger value of 100 μg/kg is exceeded, then further phase II studies on the fate, behavior, and effects are carried out. A widely used model to calculate manure and soil PECs is the Uniform Approach. This study evaluated the Uniform Approach in two ways: first, by reviewing existing data, addressing data gaps by performing degradation studies, and then calculating soil and manure PECs for the veterinary antibiotics sulfachloropyridazine, oxytetracycline, and tylosin applied to arable land via liquid pig manure and comparing these data with the results from two field-scale fate studies; second, by collating monitoring data and making a comparison with modeled data. The data comparisons indicated that the Uniform Approach model performed conservatively, with initial PECs being up to 2 orders of magnitude greater than measured environmental concentrations, providing confidence in the use of the model in the risk assessment process, although the assumption of first-order degradation kinetics in the model may underestimate the environmental persistence of veterinary antibiotics.

KEYWORDS: Exposure assessment; model evaluation; veterinary medicines; environmental risk assessment; uniform approach

INTRODUCTION

Antibiotics are used in large quantities for veterinary purposes [e.g., in the European Union (EU), >5000 tonnes was used in 1997 (*1*)] and can be excreted unchanged and thus may be released to the environment by grazing animals on pasture or by the spreading to land of manure as an organic fertilizer. There is growing awareness and concern over the occurrence of veterinary antibiotics in the environment and the potential effects these compounds may have (e.g., refs *2*−*4*). There are regulatory requirements for environmental risk assessments (ERA) to be carried out for veterinary medicinal products (VMPs), and the relevant legislative requirements in the EU, United States, and Canada have recently been summarized (*5*). The process of conducting ERAs in these areas, as well as in Japan and Australasia, has recently been harmonized with the assessments carried out in a phased manner (*6*). Phase I considers such factors as product dosage, treatment regimens, metabolism, manure production, and manure application to calculate predicted environmental concentrations (PEC) in soil. A PEC in soil of >100 μg/kg necessitates further phase II studies in which the environmental fate and effects of the VMP are considered in more detail through appropriate laboratory studies (tier A) such as ecotoxicity, sorption, and degradation tests and possibly semifield and field studies (tier B) such as field leaching and dissipation.

A key component of the ERA process is the determination of exposure concentrations. A number of approaches have been published to predict the concentrations of veterinary medicines in soil, groundwater, and surface waters. A scheme (Uniform Approach) has been developed by the European Federation for Animal Health (FEDESA) for harmonizing the calculation of PECs in soil of veterinary products (*7*). Using a database of information on cattle, pigs, and poultry and agricultural practice within the EU, the model provides standard equations to predict environmental concentrations of VMPs in manure and soils based on dose and treatment regimen. Another approach (ETox) has been developed using scenarios specific to agricultural

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practice in The Netherlands (*8*). The model predicts concentrations of VMPs in soil, groundwater, surface water, and biota. A third model has been published (VETPEC) that is a combination of existing models, including the Uniform Approach previously mentioned, two models used to predict pesticide transport to groundwater and river waters, and a model to estimate partitioning to soils. The model considers a number of animals such as cattle, pigs, poultry, and sheep and predicts concentrations of VMPs in soil, groundwater, and surface water (*9*).

A key limitation of these models is that they have not been validated. The authors of the Uniform Approach indicated that the model was created as a regulatory screening instrument and that model validation was not possible in the absence of available field data, but that evaluation of the model's validity would be useful in the future. The authors of the Uniform Approach also indicated that the calculated concentrations for manure and soil could be used as the input for further modeling of the environmental behavior of VMPs such as transport to aqueous environments via leaching and runoff. Data on the environmental concentrations in manure, soils, and surface waters of a number of veterinary medicines have become increasingly available over the past few years, and these data could now be used to provide confidence in the model predictions.

The aim of this study was to evaluate the use of the Uniform Approach, which is widely used in the EU for environmental risk assessments of VMPs and should be equally applicable in the United States, Canada, and Japan if animal husbandry data are available. In the interests of clarity, the other models discussed above were not evaluated. This was because the Etox model was specific to The Netherlands, and the VETPEC model was based on the Uniform Approach. Also, the Uniform Approach was published two and three years before the other models, respectively, and is specifically referenced in the VICH guidelines (*6*).

Two approaches were used: comparison of modeled data with the results of field-scale studies conducted into the fate of veterinary antibiotics applied to land in liquid pig manure at two sites in the United Kingdom; and comparison of modeled data with measured environmental concentrations of antibiotics published in recent monitoring studies. Initially, three specific antibiotics were selected for the fate studies: oxytetracycline (OTC), sulfachloropyridazine (SCP), and tylosin (TYL) from the tetracycline, sulfonamide, and macrolide groups of antibiotics, respectively. These represent model compounds from three of the most widely used classes of antibiotics (*1*) and have been identified as having high potential to reach the environment (*10*).

A review of existing published data on the usage, degradation, sorption, and treatment scenarios in pigs of the selected antibiotics, as well as relevant data on agricultural practices and pig husbandry, was carried out. The relative lack of degradation data for the study compounds in soils and liquid pig manure necessitated laboratory studies to help assess the persistence of these compounds. These data were then used to determine exposure concentrations of the selected antibiotics under typical U.K. usage scenarios in fattening pigs, and these concentrations were compared with the results of field-scale fate studies to provide an evaluation of the model. Finally, environmental concentration data were collated from monitoring studies of these classes of antibiotics. The measured environmental concentrations were compared with the modeled exposure concentrations to provide a further evaluation of the model.

MATERIALS AND METHODS

Review of Input Data Required for the Model. The calculation of PECs in manure, soil, and water using the Uniform Approach requires several input parameters. These are as follows: information on the treatment regimen of the animals (e.g., dosage rates of the antibiotics and treatment schedules); information on animal husbandry (e.g., stocking densities, manure production, and animal body weights); information on agricultural practice (e.g., soil and manure physical properties, manure application rates, and incorporation regimens such as plowing); information on metabolism and excretion of the VMP in animals; and sorption and persistence of the VMP (e.g., partition coefficients in soil, water, and manure and degradation rates in soil and manure). A search of the scientific literature was performed to obtain these data. This search highlighted the need to perform degradation studies to assess the persistence of the study compounds in soils and liquid pig manure.

Degradation Studies. *Chemicals.* Analytical grade OTC hydrochloride and SCP sodium were obtained from Vericore Ltd., Dundee, U.K., and Novartis Animal Health, Basle, Switzerland, respectively. Analytical grade TYL tartrate was purchased from Fluka (Gillingham, Dorset, U.K.). All other standards, reagents, and solvents were of analytical or HPLC grade (>99%).

Soil and Manure Sampling. The soils used in this study were top soils sampled from two different locations in the United Kingdom, where field-scale fate studies were undertaken with the study compounds: a clay loam from Osgathorpe, Leicestershire, and a sandy loam from Lockington, Leicestershire, in the East Midlands of the United Kingdom. The two soils were prepared by air-drying prior to being passed through a 5.6 mm sieve. Liquid pig manure was collected from slurry pits under fattening sheds at a pig farm also in Leicestershire. The manure was stirred prior to sample collection.

Soil and Manure Spiking Levels. The spiking levels used in the degradation studies were 1 mg/kg for SCP and 10 mg/kg for OTC and TYL in soil; and 26 mg/kg for SCP and 19 mg/kg for OTC in manure. The spiking levels were based on the calculated PECs assuming no degradation (see below).

Degradation in Soil Experimental Setup. The air-dried soils (of known moisture content) were weighed (10 ± 0.05 g) into 20 mL glass vials. Single-compound solutions of OTC, SCP, and TYL were prepared in distilled water at appropriate concentrations such that after spiking into the glass vials the moisture content of the soils was 50% of maximum water-holding capacity, the concentration of SCP was 1 mg/kg, and the concentrations of OTC and TYL were 10 mg/kg (w/w on a dry weight basis). Blanks were also prepared for each soil by adding the appropriate volume of distilled water into the soils. Sterile controls for OTC and TYL were prepared in soil that had been autoclaved twice and treated with the microbial inhibitor sodium azide. The vials were then stored in the dark at 20 ± 2 °C. Samples were taken after 0, 2, 4, 8, 16, 32, and 64 days with additional samples taken after 1 and 3 days for the soils spiked with SCP. All soils were frozen at −20 °C immediately after sampling and then defrosted prior to extraction. Three replicates of each compound for both soils per sampling time point were prepared, with the sterile controls having single preparations per time point. Moisture content was monitored over the course of the study and did not significantly alter.

Degradation in Manure Experimental Setup. Four amber glass bottles were each filled with 200 ± 1 mL of freshly collected liquid pig manure. Three of the bottles were inoculated with 2 mL of a mixed OTC and SCP solution such that the final concentrations were 19.2 mg/kg of OTC and 26.1 mg/kg of SCP, with the other bottle acting as a blank. The bottles were tightly capped and stored without agitation (closed-bottle test). Samples were taken by pipetting 2 mL of manure directly from each bottle, with the manure being homogenized by rapid stirring for 20 s prior to uncapping before sampling. Samples were taken immediately after inoculation and then after 6 h and 1, 2, 4, 8, 19, and 40 days. The study was conducted at 20 ± 2 °C.

Soil Extraction Method. The antibiotics were extracted from the soils using the same method for each compound. Briefly, aliquots of the moist soil (4.00 ± 0.05 g) were weighed into 10 mL centrifuge tubes, and 5 mL of extraction buffer (0.1 M McIlvaine buffer (pH 7)/0.1 M

EDTA/methanol 25:25:50 v/v) was added. The tubes were vortex mixed for 30 s, placed in an ultrasonic bath for 10 min, and centrifuged at 1160*g* for 15 min. The supernatant was decanted and the extraction procedure repeated twice more. The combined supernatant was then diluted to ~400 mL with distilled water and acidified to pH 2.9 with phosphoric acid and then cleaned up and preconcentrated by solid-phase extraction (SPE) using an Isolute SAX (IST, Hengoed, U.K.) sacrificial anion exchange cartridge to remove humic material and a Waters Oasis hydrophilic−lipophilic balance (HLB) (Waters, Watford, U.K.) polymer cartridge to retain the compounds. The HLB cartridges were washed and then eluted with 2 mL of methanol to provide the sample extract for HPLC analysis. The soil extraction method is more fully described elsewhere (*11*).

Manure Extraction Method. Exactly 2 mL of manure was accurately pipetted into a 15 mL centrifuge tube immediately after homogenization and 8 mL of extraction buffer (0.1 M EDTA/pH 7 McIlvaine buffer 50:50) added. The tubes were vortex mixed for 30 s and then placed into an ultrasonic bath for 10 min before being centrifuged at ~1200*g* for 15 min. Exactly 5 mL of the supernatant was then pipetted into a 15 mL centrifuge tube, and 50 μL of H_3PO_4 and 50 μL of MeCN were added to adjust the sample pH and help precipitate proteins in the extract. SAX-HLB SPE cartridges were set up in tandem, preconditioned with methanol and SPE conditioning buffer, and then the adjusted manure extract was passed through the cartridges at 10 mL/min. The SAX cartridges were then removed, and the HLB cartridge was washed sequentially with SPE washing buffer, 0.1 M NaOAc, distilled water, and 20% MeOH. The HLB cartridge was then air-dried for 10 min and then eluted with 2 × 1 mL of methanol to produce the extract for analysis. The manure extraction method is more fully described elsewhere (*11*).

HPLC Analysis. HPLC analysis was carried out using a Dionex summit system (Dionex, Camberley, U.K.) with a Genesis C_{18} column (4.6 × 150 mm, 4 μm, Jones Chromatography, Hengoed, U.K.). Briefly, a gradient elution was carried out over 25 min with THF, MeCN, and 0.05% TFA in water: THF remained at 5% throughout; MeCN was 2.5% from 0 to 4 min, rising to 75% from 4 to 18 min, then returning to 2.5% from 18 to 20 min and remaining at 2.5% 20−25 min; 0.05% TFA was 92.5% from 0 to 4 min, falling to 20% from 4 to 18 min, then returning to 92.5% from 18 to 20 min, and remaining at 92.5% from 20 to 25 min. The flow rate was 1 mL/min throughout, and simultaneous detection was performed at 285 nm for SCP and TYL and at 355 nm for OTC. The HPLC method is more fully described elsewhere (*12*).

Calculation of Degradation Rates. Initially, first-order degradation kinetics were assumed for the compounds in manure and soil according to the first-order rate equation (eq 1)

$$\frac{dC}{dt} = -kC \qquad (1)$$

where C is the concentration in soil or manure (mg/kg) and k is the degradation constant (days^{-1}). The integrated form of this equation was fitted to the data using the least-squares method to give an exponential decay curve (eq 2)

$$C_t = C_0\, e^{(-kt)} \qquad (2)$$

where C_t is the concentration after a period of degradation (mg/kg), C_0 is the initial concentration (mg/kg), and t is the degradation time (days). However, where first-order kinetics did not adequately describe the disappearance of the compounds, biexponential curves were fitted to the data, which more closely followed the degradation (eq 3).

$$C_t = A\, e^{(-k_1 t)} + B\, e^{(-k_2 t)} \qquad (3)$$

The first-order rate equation is considered to be inadequate for describing the degradation kinetics if there is a poor fit to the data (determination coefficient $r^2 < 0.7$). This is often due to a decrease in the rate of degradation over time; that is, there is an initial period of rapid degradation followed by a slower rate of degradation. This method is described fully elsewhere (*13*) and is a practical and realistic method, which takes into account the fact that chemicals are often more persistent in soils than their initial disappearance rates would suggest.

Fate Studies. The fate studies were conducted at two field sites in the United Kingdom, which had contrasting soil types, a sandy loam and a clay loam, with soil properties given in **Table 3**. The results of the field studies are published in detail elsewhere (*14*−*16*). Liquid pig manure, sourced from fattening pigs that had been continuously fed with TYL and spiked with SCP and OTC, such that application rates were 1.15 kg/ha of SCP and 0.85 kg/ha of OTC, was applied to land at the two field sites. At the clay soil field site manure was applied, using a towed broadcast slurry spreader, to a 1.55 ha underdrained field, which had a single outflow point into a drainage ditch. At the sandy soil field site manure was manually applied to small-scale plots where soil water monitoring and sampling equipment had been previously installed. The manure application rates at the clay soil and sandy soil sites were 45000 and 33000 L/ha, respectively, but the compound application rates were the same at both sites. The manure was incorporated by plowing at the clay soil field site, but incorporation was not possible at the sandy soil field site. Replicate soil core samples were collected 1 day after application at both sites with subsequent further sampling over time. Cores were sectioned into 5 or 10 cm subsamples, and individual subsamples were analyzed from three cores at the clay soil site and four cores at the sandy soil site from each sampling time point. Additionally, soil water samples were taken at the sandy soil field site from depths of 40, 80, and 120 cm at various time points after application triggered by rainfall events, and drainflow water samples were taken from the clay soil field site drain outfall during periods of flow. Two sets of data were available at the clay soil field site as the application of OTC and SCP in manure was repeated the following year.

Modeling. Using the Uniform Approach (*7*), the PEC in manure was derived from information on the treatment scenario for the animal and information on the average body weight, average manure production, and stocking density of the animal as well as the metabolism of the compound in the animal (eq 4). The initial assumption was made that no degradation occurs during manure storage.

$$PEC_{MANURE} = \frac{(ID \times BW \times T \times N \times F_M)}{P_E} \qquad (4)$$

PEC_{MANURE} is the PEC in manure (mg/kg), ID is the dose rate (mg/kg of body weight), BW is the average body weight of the animal (kg), T is the number of treatments per animal, N is the number of animals raised each year per housing location (per year per place), F_M is the fraction of compound excreted to allow for absorption and metabolism in the animal, and P_E is the amount of manure produced per place in a year (kg per place per year). The PEC in soil was derived from a simple dilution of manure into the soil, using information on the soil bulk density, manure application rates, and plowing depth (eq 5). The assumption was made that the density of pig manure is close to unity.

$$PEC_{SOIL} = \frac{(M \times C_E)}{(100 \times D \times \rho) + M} \qquad (5)$$

PEC_{SOIL} is the PEC in soil after application of the manure (mg/kg), M is the amount of manure applied to a hectare of land in a year (kg/ha/year), C_E is the concentration of antibiotic in manure (mg/kg), D is the plow depth (cm), and ρ is the soil bulk density (kg/m^3). PECs in manure and soil may be refined by considering degradation of the antibiotics either during manure storage or after application to land (eq 6). It was assumed that the degradation of the compounds in soil and manure followed first-order kinetics.

$$PEC_S(t) = PEC_S(0) \times e^{(-\ln 2/DT_{50})t} \qquad (6)$$

$PEC_S(t)$ is the PEC in soil a specified period of time after application or in manure after a period of storage (mg/kg), $PEC_S(0)$ is the initial PEC in soil or manure calculated above (mg/kg), DT_{50} is the time taken for the concentration to fall to 50% of the initial value (half-life days), and t is the residence time during storage or after application (days).

Table 1. Relevant Data on Treatment Scenarios and Characteristics for Fattening Pigs

parameter	SCP	OTC	TYL
ID (mg/kg of BW)	20	20	2
T (days)	10	10	365
N (animals/year/place)	2.5	2.5	2.5
BW (kg)	95	95	95
P_E (kg/place/year)	1764	1764	1764
F_M	0.95	0.7	1.0

Although not covered in the Uniform Approach, PECs in water were also calculated for comparison with concentrations in water available from the field studies and in the literature. PECs in soil pore water were derived using a simple ratio between the PEC_{SOIL} and the sorption coefficients of the compounds in soil (eq 7)

$$PEC_{PW} = \frac{PEC_{SOIL}}{K_d} \quad (7)$$

where PEC_{PW} is the PEC in pore water in mg/L and K_d is the partition coefficient (L/kg).

Model Evaluation. Measured soil and water concentrations from the field-scale fate studies were collated. Normalized soil concentrations were calculated for each sampling time point by taking averages (and standard errors) of the results for the different depths and replicates. Soil and water PECs were calculated for the same time points using site-specific data on soil properties and mixing depths and compared with the measured data. A literature search indicated that a number of monitoring studies have been carried out over the past few years and that there is a growing amount of data in the public domain (*17*−*22*). Information from these studies on concentrations of antibiotics in manure, soil, and water, as well as any data on treatment regimens, animal body weights, and manure application rates was collated. If not stated, typical treatment scenarios for sulfonamides and tetracyclines (*23*) were assumed, and average body weight, stocking density, and manure production data were taken from the Uniform Approach. PECs in manure, soil, and water were calculated and compared with the results of the monitoring studies.

RESULTS AND DISCUSSION

Review of Input Data. Data on the average body weight, stocking density, and excreta production of fattening pigs have already been collated and published in the paper describing the Uniform Approach. The amount of manure that may be applied to land as fertilizer is limited in the United Kingdom by the amount of nitrogen that may be applied to land, with an application limit in the United Kingdom of 250 kg of N/ha/year (*24*). The liquid pig manure used in the field-scale fate studies was collected from under pig fattening sheds. The pigs were continuously treated with 100 g of TYL per tonne of feed, which was used to treat between 67 and 78 pigs per week. On the basis of the average fattening pig body weights given in the Uniform Approach, this equates to a daily dose of ∼2 mg/kg of body weight. This is lower than may be expected and is likely to be because the body weight of the pigs at the farm from which the manure was sourced was somewhat lower than the average. Typical treatment scenarios for SCP and OTC in fattening pigs are over a shorter time scale, typically a daily dose of 20 mg/kg of body weight over 5−20 days (*23*). The actual manuring rate at one of the field sites was ∼45000 L/ha, which is in line with agricultural practice. Following administration of the antibiotics the fraction of antibiotic excreted may also be estimated. Between 40 and 80% of OTC, between 50 and 100% of SCP, and between 94 and 100% of TYL can be excreted as parent compound with minimal metabolism (*15*, *25*−*27*). Sulfonamide metabolites may also revert to the parent compound during manure storage (*28*). Data on sorption and degradation of the study compounds in sediments, soils, water, and manure have been recently reported (*3*, *29*, *30*). Relatively few data were available on degradation of the compounds, hence the need for laboratory studies to be conducted. Generally, sorption coefficients had low values for sulfonamides, intermediate values for macrolides, and very high values for tetracyclines. The data indicate that SCP would be a highly mobile compound in the environment and that it was relatively nonpersistent in chicken faeces. OTC was highly sorbed to soils and was moderately to very persistent in marine sediments, although this may not necessarily be representative of persistence in agricultural soils. TYL has intermediate mobility and was

Table 2. Sorption and Degradation Data for the Study Compounds in Soil and Manure

compd	sorption and degradation data
SCP	K_d = 1.8 L/kg in clay loam; 0.9 L/kg in sandy loam[a]
	deg = 71% degraded after 3 months in laying hen feces; 65% degraded after 8 days in broiler feces[b]
OTC	K_d = 1500–3500 L/equiv in montmorillonite; 8500 L/equiv in sodium montmorillonite; 1070–1640 L/equiv in sodium kaolinite[c]
	K_d = 420–1030 L/kg in sandy/loamy soils[d]
	K_{oc} = 27800–93300 L/kg in sandy/loamy soils[d]
	K_d = 63–96 L/kg in pig manure[e]
	K_{oc} = 195 L/kg in pig manure[e]
TYL	K_d = 8.3–128 L/kg in sandy/loamy soils[d]
	K_{oc} = 550–7990 L/kg in sandy/loamy soils[d]
	K_d = 36–295 L/kg in pig manure[e]
	K_{oc} = 110 L/kg in pig manure[e]
	K_d = 3.3–8.1 L/kg in clay soil/mineral medium; 4.1–4.2 in sandy soil/mineral medium[f]
	deg = DT_{50} < 2 days in pig slurry[g]
	deg = 100% degraded after 30 days at 20 and 30 °C and 60% degraded at 4 °C in soil with 5% chicken manure[h]
	deg = DT_{50} = 6.2 days in cattle excreta; <7.6 days in chicken excreta; 7.6 days in swine excreta[i]

[a] Boxall et al. (*16*). [b] Van Dijk and Keukens (*31*). [c] Figuera et al. (*32*). [d] Rabølle and Spliid (*29*). [e] Loke et al. (*33*). [f] Ingerslev and Halling-Sørensen (*34*). [g] Loke et al. (*35*). [h] Gavalchin and Katz (*36*). [i] Teeter and Meyhoff (*37*).

Table 3. Properties of the Soils and Pig Manure Used in This Study

property	sandy loam	clay loam	liquid pig manure
sand (63 μm–2 mm), %	69.2	42.6	na
silt (2 μm–63 μm), %	20.5	32.3	na
clay (<2 μm), %	10.3	25.1	na
pH (1:2.5) extract in 0.01 M $CaCl_2$	6.6	6.8	na
CEC, mequiv/100 g	11.4	22.4	na
organic carbon, %	1.3	2.2	41.1 ± 5.0
bulk density, g/cm^3	1.68	1.30	na
maximum water-holding capacity, %	40.2	48.0	na
dry matter, %	na	na	2.00 ± 0.15
available P, mg/L	na	na	3260
available N, mg/L	na	na	6830

Table 4. Recoveries of the Study Compounds in Two Soils and Pig Manure

compd	recovery (%, mean ± SD)		
	sandy loam	clay loam	liquid pig manure
SCP	80 ± 1	68 ± 10	58–89
OTC	65 ± 7	38 ± 4	77–102
TYL	85 ± 2	47 ± 4	nd

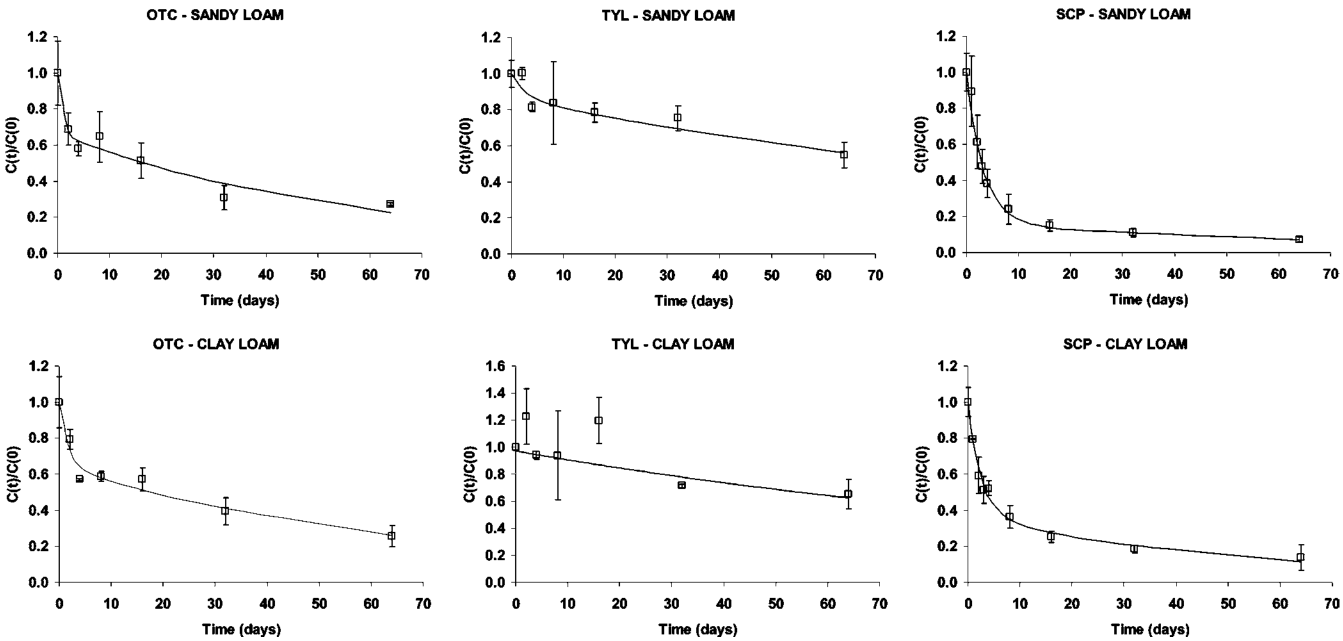

Figure 1. Degradation of the study compounds in soils.

Table 5. Degradation Rates (DT_{50}, DT_{90}), Rate Constants (*k*), and Determination Coefficients (r^2) for the Study Compounds in Soils and Pig Manure

	sandy loam				clay loam				liquid pig manure		
compd	DT_{50} (days)	DT_{90} (days)	k (days^{-1})	r^2	DT_{50} (days)	DT_{90} (days)	k (days^{-1})	r^2	DT_{50} (days)	k (days^{-1})	r^2
SCP	2.8	38	$k_1 = 0.0135$; $k_2 = 0.3165$	0.99	3.5	71	$k_1 = 0.0184$; $k_2 = 0.4036$	0.99	127	0.0055	0.76
OTC	16	111	$k_1 = 1.1135$; $k_2 = 0.0170$	0.96	18	132	$k_1 = 0.5700$; $k_2 = 0.0147$	0.97	79	0.0088	0.88
TYL	97	427	$k_1 = 0.3627$; $k_2 = 0.0066$	0.91	95	316	0.0073	0.94	nd	nd	nd

nonpersistent in both manure and soil/mineral medium slurries. The input data used in the exposure calculations are summarized in **Table 1**. Sorption and degradation data for the compounds in soil and manure are summarized in **Table 2**.

Degradation Studies. *Soil and Manure Characterization.* The soil and manure properties were characterized using standard methodologies (*38*) and are summarized in **Table 3**. The soils represent typical sandy loam and clay loam soils in the United Kingdom.

Analytical Recoveries. Recoveries for the compounds in the two soils and pig manure are summarized in **Table 4**. Generally, recoveries for all three compounds were lower in the clay loam than in the sandy loam, especially for OTC and TYL, which have relatively high sorption coefficients in soils. This is likely to be explained by the greater clay content, that is, greater proportion of smaller-sized soil particles, giving a greater surface area for sorption in the clay loam. Recoveries were considered to be sufficiently high and reproducible for these studies.

Degradation in Soil. The results obtained for all three compounds and the fitted degradation curves are shown in **Figure 1**. Biexponential curves were fitted to all data except TYL in the clay loam for which it proved to be difficult to fit a curve. A number of approaches were attempted for TYL in clay, and ultimately a simple first-order curve was fitted to the data after the results from the 2 and 16 day time points were excluded. The calculated DT_{50} and DT_{90} values are summarized in **Table 5**. The results proved to be very similar for each compound regardless of the soil type. SCP rapidly degraded in both soils and would be classed as nonpersistent, OTC was found to be slightly persistent in both soils, and TYL was very persistent using standard definitions of persistence in soil (*39*). However, the DT_{90} values indicate that SCP has the potential to be moderately persistent in soils and that OTC has the potential to be very persistent in soils. Sterile controls were included for OTC and TYL because of the tendency of these compounds to strongly sorb to soils (*29*). Analysis of these controls over the first 16 days of the study indicates that degradation, rather than binding, is the major removal mechanism. Manure amendment of the soil was not considered as pig manure has been shown not to significantly alter the organic carbon content of soils (*40*), and other studies have shown that degradation rates of a number of veterinary antibiotics were not significantly affected by the addition of up to 10% manure (*34*). Excluding pig manure from the soil test system simplified both the experimental setup and the soil extraction and analysis and should not significantly affect the experimental results. Due to their specific modes of action, antibiotic compounds in soil could be toxic to microbes, and hence degradation rates could be affected at high concentrations. Research conducted in parallel with these studies and evaluation into the effects of the study compounds on soil microbes have indicated that although the spiking levels chosen may induce some effects in the most sensitive microbes in the soil community (*41*), overall degradation rates should not be significantly affected.

Degradation in Manure. The results obtained for SCP and OTC and the fitted degradation curves are shown in **Figure 2**, and the calculated DT_{50} values are summarized in **Table 5**. First-order curves were fitted to the data. Both compounds proved to be very persistent in pig manure with approximately 70% OTC and 85% SCP remaining after 40 days.

Model Evaluation. The Uniform Approach was selected for evaluation and for calculation of the spiking concentrations to

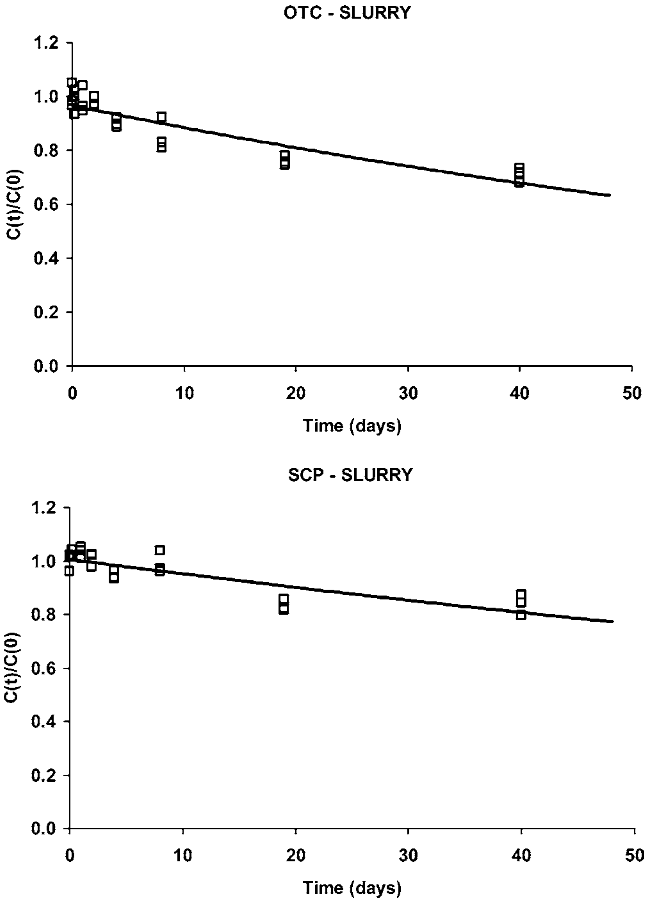

Figure 2. Degradation of OTC and SCP in pig manure.

be used in fate studies, as it is simple to use, has summary data available for a number of different animals, and is widely used for modeling exposure concentrations when risk assessments are conducted of VMPs in the EU being specifically referenced in the VICH guidelines (*6*), although the limitations of using a single simplified model have been identified; for instance, there are differences in agricultural practice and environmental conditions across the EU (*42*). The data from published studies in which environmental concentrations of tetracycline, sulfonamide, and macrolide antibiotics, related to agricultural use, have been determined in manures, soil, and water are summarized in **Table 6**.

Initial calculations for the study compounds in manure assumed no degradation during storage. The PEC_{MANURE} was 25.6 mg/kg for SCP, 18.9 mg/kg for OTC, and 98.5 mg/kg for TYL, which equated to application rates of the compounds of 1.15 kg/ha for SCP, 0.85 kg/ha for OTC, and 4.43 kg/ha for TYL. The higher PEC of TYL is to be expected as the treatment scenario was that of continual usage rather than over a discrete period. PEC_{MANURE} values of 2030 mg/kg of chlortetracycline (CTC) in pigs and 855 mg/kg for OTC and 533 mg/kg for TYL in cattle were calculated using the specific treatment regimens described in the monitoring studies to enable direct comparison with analytical data where the manure samples were analyzed immediately after excretion without mixing with uncontaminated manure. PEC_{MANURE} values were also calculated, based on typical treatment scenarios and assuming storage and mixing prior to application, to allow comparison with more general monitoring studies where grab samples of manure were taken. The range of values is derived from taking the upper and lower limits of typical treatment regimes. PEC_{MANURE} values for CTC and tetracycline (TC) were calculated to be in the range of 4.7−18.9 mg/kg in liquid manure from fattening pigs, and PEC_{MANURE} values for the sulfonamides sulfamethazine (SMZ) and sulfathiazole (STZ) were calculated to be in the ranges of 6.4−25.6 mg/kg for fattening pigs, 1.0−3.9 mg/kg for sows, and 6.5−26.1 mg/kg for calves. Additionally, a PEC_{MANURE} in the range of 17−51 mg/kg was calculated for sulfadimidine (SDM), where the treatment regimen was given but the manure had been mixed with uncontaminated manure.

Modeled PEC_{SOIL} values, based on the PEC_{MANURE} values above, were calculated for both of the fate studies for each sampling time point using measured site-specific soil bulk densities and the experimentally determined laboratory degradation half-lives. For the clay soil site, where the manure had been incorporated, weighted average concentrations in the top 30 cm of the soil profile were calculated for both SCP and OTC. For the sandy soil site, where the manure was not incorporated, the results indicated that SCP had rapidly moved to a depth of 30 cm after application but that OTC had remained almost exclusively within the top 10 cm of the soil; therefore, weighted average concentrations in the top 30 cm of the soil profile for SCP and in the top 10 cm of the soil profile were calculated for OTC. Depth-weighted average concentrations with standard errors and calculated PECs for SCP and OTC are shown in **Figure 3** for the clay soil site and in **Figure 4** for the sandy soil site. The modeled initial PEC_{SOIL} for TYL was 1120 μg/kg at the clay soil site and 872 μg/kg at the sandy soil site; however, TYL was not detected in any soil sample at either site. PEC_{SOIL} values were also calculated for the monitoring studies. Application rates and soil characteristics were assumed when not given in the monitoring studies. PEC_{SOIL} values were calculated to be in the range of 5.4−9.0 μg/kg for OTC, in the range of 31−208 μg/kg for CTC and TC, and in the range of 97−485 μg/kg for SDM.

PEC_{WATER} values for the clay soil field site 2 days after application were 109 μg/L for SCP, 0.48 μg/L for OTC, and 133 μg/L for TYL. These values compared with peak concentrations of 613 μg/L for SCP and 36.1 μg/L for OTC during the first flow event 2 days after manure application in the first year and peak concentrations of 6.1 μg/L for SCP and 0.8 μg/L for OTC during the first flow event, again 2 days after manure application, in the second year. The higher concentrations in the first year were believed to be caused by bypass flow of manure through field drains into surface water, which did not happen in the second year of the study as the ground was tilled prior to manure application. TYL was not detected in any drainflow samples. PEC_{WATER} values for the sandy soil field site 20 days after application were 2.3 μg/L for SCP, 0.22 μg/L for OTC, and 91 μg/L for TYL. These values were compared with a maximum concentration of 0.78 μg/L for SCP at a 40 cm depth 20 days after manure application, with neither OTC nor TYL detected in any samples ($<$0.35 μg/L). PEC_{WATER} values were also calculated for the monitoring studies. A PEC_{WATER} value in the range of 0.005−0.02 μg/L for OTC was compared with measured values of $<$1 μg/L. PEC_{WATER} values in the range from 0.03 to 0.50 μg/L for CTC and TC were compared with measured values of $<$0.05 μg/L. Additionally, antibiotics have been detected in surface waters in the United States, with concentrations ranging from 0.07 to 1.34 μg/L for tetracyclines and from 0.06 to 15 μg/L for sulfonamides (*20*).

Generally, the model outputs for initial soil and manure PECs were conservative by up to 2 orders of magnitude when compared with the results from monitoring studies (**Figure 5**). This suggested that the model output had a margin with respect to environmental safety. Comparison of the PEC_{MANURE} for

Table 6. Selected Monitoring Data for Tetracyclines, Sulfonamides, and Tylosin in Manures, Soil, and Waters

compd	treatment scenario	concentrations	additional information
chlortetracycline[a]	800 mg/kg in feed for 3 days for two 6-week-old pigs	112 mg/kg in manure immediately after treatment	no dilution or storage of manure
chlortetracycline[b]	no information given (typical: 10–20 mg/kg of body wt for 5–10 days)	0.09–0.1 mg/kg in liquid pig manure	manure sourced from fattening pigs
		4.6–7.1 μg/kg in soil shortly after application	manure application rate 30–50 m^3/ha
		5.0–6.0 μg/kg in soil 6 months after application	range of av concentrations given measured in top 0–30 cm soil
		<0.05 μg/L in soil water (80 cm depth)	no detections in soil below 30 cm depth
		<0.05 μg/L in groundwater (2.0–2.4 m depth)	
oxytetracycline[c]	60 mg/kg/day for 5 days in cattle	872 mg/kg in manure immediately after treatment	water sampled from drainage ditches after rainfall within 10 days of application
	(av body wt = 65 kg)	0.82 mg/kg in manure matured for 6 months	
		6–7 μg/kg in soil after application	
		<1 μg/L in water	
tetracycline[b]	no information given (typical: 10–20 mg/kg of body wt for 5–10 days)	3.2–4.0 mg/kg in liquid pig manure	manure sourced from fattening pigs
		56.4–198.7 μg/kg in soil shortly after application	manure application rate 30–50 m^3/ha
		43.4–94.2 μg/kg in soil 6 months after application	range of av concentrations given measured in top 0–30 cm soil
		<0.05 μg/L in soil water (80 cm depth)	no detections in soil below 30 cm depth
		<0.05 μg/L in groundwater (2.0–2.4 m depth)	
sulfadimidine[d]	16–24 mg/kg of body wt for 10 days in swine	1 mg/kg in swine manure	no details of manure storage time after treatment
		15 μg/kg in soil 7 months after application	manure and soil sampling simultaneous
sulfamethazine[e]	compound present in feed (typical: 10–20 mg/kg of body wt for 5–10 days)	0.13–0.23 mg/kg in fattening pig manure	no details of manure storage time after treatment
		3.3–8.7 mg/kg in sow manure	
		3.2 mg/kg in fattening calves manure	
sulfathiazole[e]	compound present in feed (typical: 10–20 mg/kg of body wt for 5–10 days)	0.10–0.17 mg/kg in fattening pig manure	no details of manure storage time after treatment
		<0.1–12.4 mg/kg in sow manure	
tylosin[c]	20 mg/kg/day for 5 days in cattle	116 mg/kg in manure immediately after treatment	water sampled from drainage ditches after rainfall within 10 days of application
	(av body wt = 85 kg)	<0.1 mg/kg in manure stored for 45 days	
		<10 μg/kg in soil after application	
		<10 μg/L in water	

[a] Hansen et al. (*17*). [b] Hamscher et al. (*18*). [c] De Liguoro et al. (*19*). [d] Christian et al. (*20*). [e] Haller et al. (*21*).

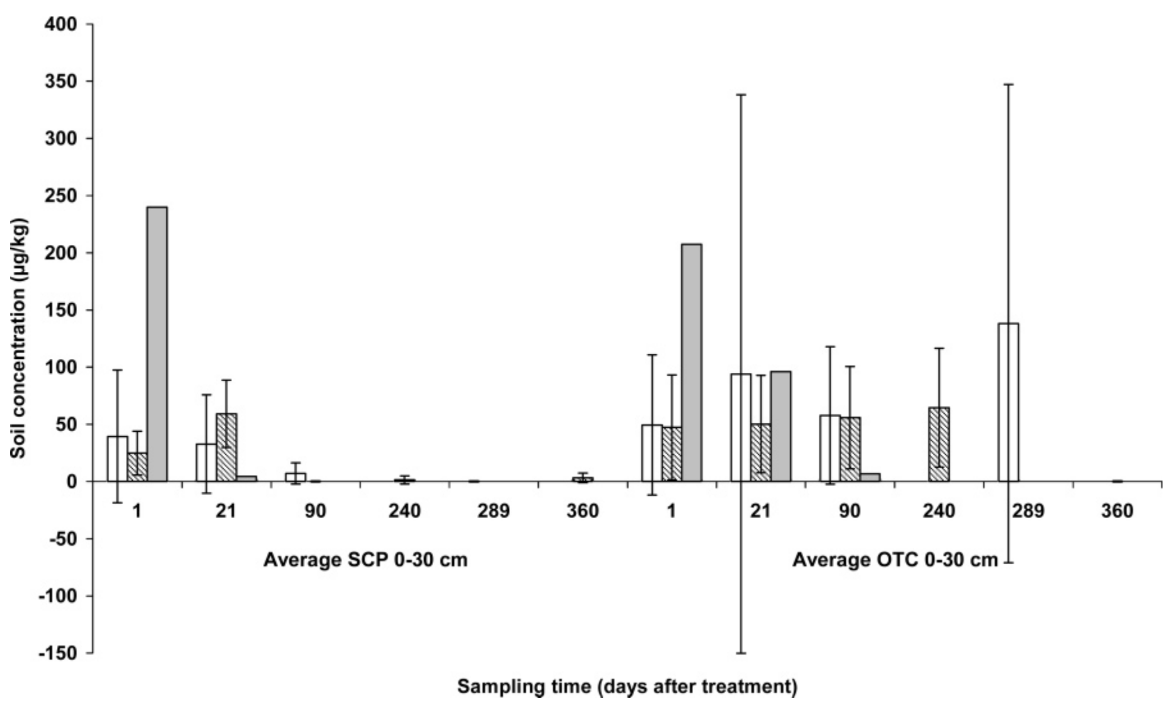

Figure 3. Average measured concentrations (with standard errors) and PECs for OTC and SCP at the clay soil field site versus time after manure application: (open columns) year 1; (hatched columns) year 2; (solid columns) PECs.

tetracyclines and sulfonamides with measured concentrations provided confidence that the manure used for the fate studies was spiked with realistic amounts of OTC and SCP and that the soil and water field results should be comparable with monitoring data. The initial PEC_{SOIL} values for the fate studies were generally within a factor of 10 of the average initial

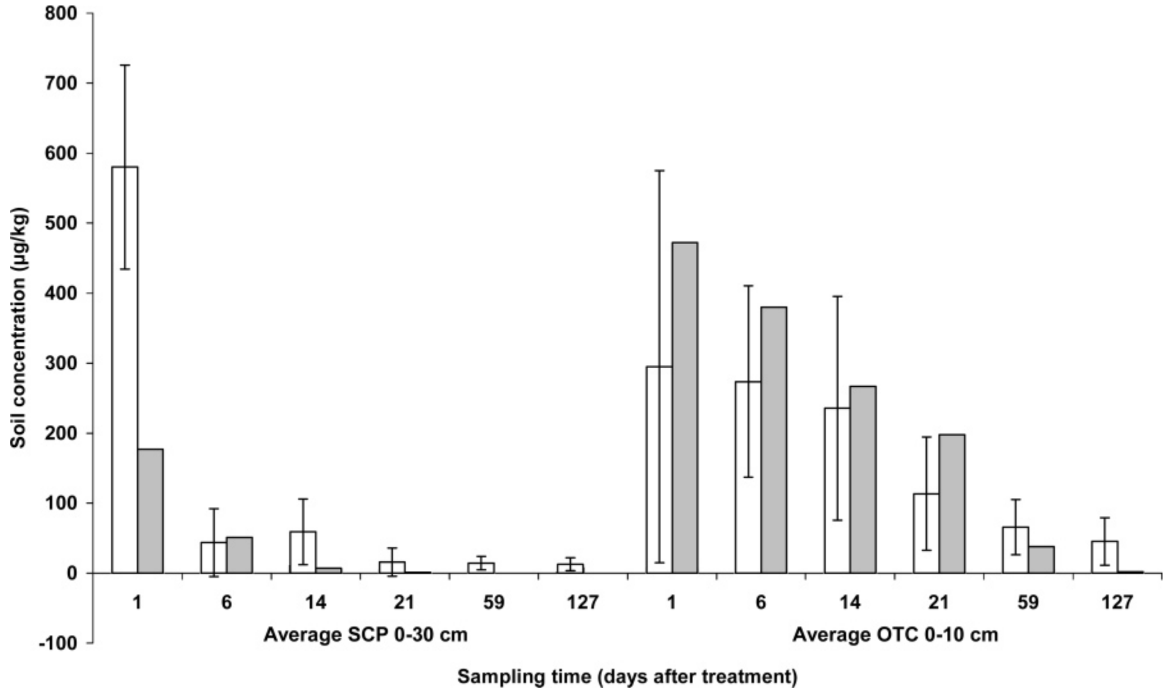

Figure 4. Average measured concentrations (with standard errors) and PECs for OTC and SCP at the sandy soil field site versus time after manure application: (open columns) measured concentrations; (solid columns) PECs.

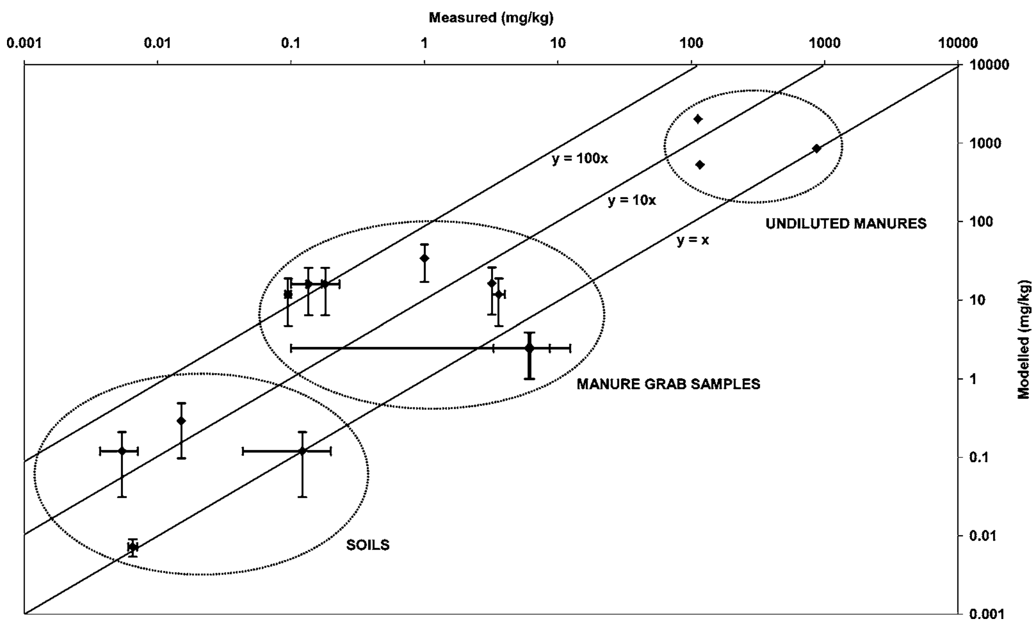

Figure 5. Comparison of measured and modeled data (in milligrams per kilogram) for antibiotics in manure and soil.

measured soil concentrations for OTC and SCP. This was to be expected given that the manure had been spiked with these compounds, The model also predicted soil concentrations reasonably accurately over the first few weeks of the studies but increasingly underestimated the soil concentrations of both OTC and SCP over time. This suggested that a key limitation of the Uniform Approach was the assumption of first-order degradation kinetics, and results from both the fate and monitoring studies indicated that the Uniform Approach may underestimate the long-term persistence of antibiotics in soil, especially for tetracyclines as the combination of their persistence and high sorption means there is high potential for these compounds to accumulate in the environment. Additionally, there appeared to be considerable variability in sorption and persistence data available for use in the model, possibly due to a wide range of experimental setups being employed, and given that the degradation half-life is an exponential term, the model is very sensitive to this parameter.

PEC_{WATER} values for OTC and SCP agreed reasonably well with measured concentrations in the fate studies, although the model appeared to significantly underestimate peak concentrations of OTC in the first year of the study. The high concentration of OTC was believed to be caused by bypass flow of manure through field drains into surface water, which did not happen in the second year of the study as the ground was tilled prior to manure application. PEC_{WATER} values for the monitoring studies agreed well with some surface water monitoring data from the United States. The lack of detections in the field studies may be explained by the sampling depth and the fact that tetracyclines were not observed to move to depth. The lack of

TYL detections in the fate studies is believed to be the result of its degradation in manure prior to application to land (*15*), which agreed well with the lack of detections in the monitoring studies and with PECs once degradation in manure prior to application to land was considered.

The harmonized guidelines for conducting Environmental Impact Assessments indicate that it is permissible to refine (i.e., reduce) the soil PEC by including results from degradation studies and that if the refined PEC is less than the trigger value, then assessment may stop at phase I. Results from the fate studies as well as monitoring data indicate that this approach is justified for compounds which degrade rapidly in manure such as TYL and that manure storage practices prior to application are therefore an important part of risk mitigation and are a key way of reducing environmental exposure.

Although there remain limited data available on environmental concentrations of veterinary antibiotics, a brief evaluation using these data and the results of two fate studies suggested that the model performed reasonably well at calculating initial PECs. Being generally conservative by up to 2 orders of magnitude, the model will therefore have a safety factor with respect to its use in the ERA process. Although longer term concentrations in soil (on the order of a few tens of micrograms per kilogram) may be underestimated for some persistent and highly sorbing compounds, the model will still generally overestimate initial environmental concentrations and hence overestimate the acute environmental risk. Refinement of the equations used, for instance, by considering biexponential degradation of antibiotics in soil, should enable the model to be used for estimating longer term environmental persistence.

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