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Fate of Chlortetracycline in Korean Arable Soil

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Abstract The widespread use of the pharmaceutical chlortetracycline to treat human and animal infectious diseases is raising concern in Korea regarding the potential development of antibiotic-resistant bacteria. This study investigated the fate of chlortetracycline in Korean arable soils, to assess its environmental impact. The estimated half-life of chlortetracycline was 26.70–27.17 days indoors and 18.63–30.16 days outdoors. The chlortetracycline epimers and metabolites, 4-epichlortetracycline and 4-epi-anydrochlotetracycline were determined using liquid chromatography tandem mass spectroscopy. The respective concentrations of 4-epi-anydrochlotetracycline and 4-epichlortetracycline were 1.91%–9.71% and 11.12%–23.92% of the total chlortetracycline residual concentration indoors and 2.96%–11.44% and 17.61%–40.73% outdoors.

Keywords Chlortetracycline · Dissipation · Korean arable soil · Epimers

The occurrence of pharmaceuticals in the environment has become an important issue (Halling-Sørens et al. 1998; Sarmah et al. 2006). Animal manure and sewage sludge are dispersed on fields and the antibiotics they contain may contaminate the soil and eventually the groundwater (Geiger et al. 2003; Göbel et al. 2004). After fertilising fields with manure or sewage sludge that contains antibiotics, these compounds may undergo several processes resulting in the complete or partial elimination of the parent compound (Kümmerer 2001). The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) published guidelines on assessing the environmental impact of veterinary medicine products (VICH 2001; VICH 2005). The VICH adopted the Organisation for Economic Cooperation and Development (OECD) guideline 307 for assessing soil biodegradation. Most veterinary drugs are biologically active compounds designed to kill microbes or inhibit their growth. These basic characteristics are an obstacle to assessing biodegradation using standardised tests such as closed bottle, Zahn-Wellens, and CO_2-evolution tests, which proved ineffective, with limited applicability and questionable validity (Alexy et al. 2004; Kümmerer et al. 2004). A number of recent studies have explored the fate of veterinary drugs in soils and surface and ground waters. Field-plot and large-scale field studies using lysimeters have explored the transport and dissipation of drugs for environmental risk assessment, which is connected to the food chain and food safety (Kay et al. 2004; Blackwell et al. 2007). Animal wastes or manure composts are the largest source of antibiotic contamination in soil. To understand their environmental impact, it is important to investigate the fate of antibiotics under more realistic environmental conditions, including regional hydrological, climatic, and land-use characteristics. Tetracyclines are the antibiotics used most widely in humans and animals and are the highest antibiotic resistance-inducing agents among animal drugs in Korea. This study investigated the fate of chlortetracycline (CTC) in Korean arable soils, reflecting the Korean agricultural environment, to assess the environmental impact of CTC.

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Materials and Methods

Chlortetracycline and its epimers 4-epi-chlortetracycline (ECTC) and 4-epi-anhydro-chlotetracycline (EACTC) (VETRANAL®, analytical standard) were purchased from Fluka (Buchs, Switzerland). All solvents were of ULTRA RESI-ANALYZED™ grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical grade and commercially available, unless otherwise stated.

The study was performed on arable soil from the Pesticide Dissipation Test Fields of ChungBuk National University. These fields have been free from the application of veterinary drug and manure compost for more than 10 years. They are located in central Korea, and the soil properties are typical of soils in Korea. Studies were performed under indoor and outdoor conditions. The soil was a silty loam with the following characteristics: organic carbon 0.6%, cation exchange capacity (cmol(+) kg^{-1} soil) 8.4, and pH (H_2O, 1:5) 6.1–6.3. Solid manure compost was collected from an organic farm accredited by the government as animal-drug free. For the outdoor experiments, before spraying CTC, antibiotic-free compost was applied to the soil, according to the Standard Fertiliser Application (Rural Development Administration 2007) guidelines for Chinese cabbage culture (pre- and post-application; 32.0 N kg/10 acres, ~3.2 kg m^{-2}). A standard aqueous solution of CTC was sprayed to a 1 m^2 × 0.1 m soil depth at concentration of 1 mg kg^{-1} in soil using a garden sprayer. For the indoor experiment, the same levels of manure compost and CTC were applied to 100 g soil in test tubes, which were incubated in the dark at room temperature for 60 days, maintaining at least 40% of the field moisture capacity by adding distilled water.

Ten-gram sub-samples of the soil samples were collected for simultaneous extraction of CTC and its epimers. In brief, 10 g of soil were weighed into a 100-mL plastic Erlenmeyer flask and 30 mL of extraction buffer [ethylenediaminetetraacetic acid (EDTA)-McIlvaine buffer/methanol (MeOH), 10:90 (v/v), pH 6.0] were added.

The flasks were shaken for 5 min, and placed in an ultrasonic bath for 20 min. The supernatant was decanted and the extraction procedure was repeated twice more. The combined supernatant was then filtered through GF/C with 1 g of Celite 545. The column was cleaned with a 1-g SPE C18 cartridge for activation and pre-treatment; 5 mL of MeOH and 5 mL of EDTA-McIlvaine buffer/MeOH (10:90, v/v, pH 4.0) were eluted, and then the extract was eluted from the column. After eluting the extract, 10 mL of water were eluted and discarded, and then 20 mL of methanolic 0.01 M oxalic acid were eluted and collected. The collected sample was concentrated under N_2 gas and 1 mL of MeCN:water (10:90, v/v) was added for analysis.

Table 1 MS/MS parameters for CTC and its epimers

Generic name	MRM transition precursor ion → production (m/z)	Cone voltage (V)	Collision energy (eV)
CTC	479 > 444, 479 > 462	36	18, 18
ECTC	479 > 444, 479 > 462	36	18, 18
EACTC	461 > 154, 461 > 444	36	18, 18

For the HPLC-MS-MS analysis, we used an ACQUITY UPLC system coupled to a TQD and XEVO triple quadrupole mass spectrometer from Waters (Milford, MA, USA) using an electrospray source. The HPLC mobile phase condition was a C18 ACQUITY UPLC BEH column (100 mm × 2.1 mm; particle diameter 1.7 µm) from Waters, programmable with 20 mM NH_4HCO_2/20% MeOH and 20 mM NH_4HCO_2/95% MeOH. Each multiple reaction monitoring (MRM) transition (m/z) of the precursor ion to the product ion of the target analyte is described Table 1. Data processing was performed using MassLynx 4.1 from Waters. The mean recovery ratio (%) of each analyte was 110.27% for CTC, 97.32% for ECTC, and 94.43% for EACTC. To avoid confusion arising from the various definitions of the limits of detection and quantification (LOD/LOQ), the practical quantification limits of CTC and its epimers were set at a level of 0.05 ng/g.

Results and Discussion

Figure 1 presents the dissipation patterns of CTC in Korean arable soil under the outdoor and indoor conditions. The estimated half-life (DT_{50}) of CTC was 18.63–30.16 days outdoors and 26.70–27.17 days indoors, by first-order kinetics. The outdoor and indoor experiments were performed in duplicate, i.e., two different 1 × 1 m plots outdoors and two different batches of the indoor test were set up and samples were collected on the same day. Sometimes a simple first-order kinetic equation cannot adequately describe the disappearance of chemicals, but its use is recommended in government regulations, to prevent the manipulation of DT_{50} and DT_{90} by second-order or other kinetic equations, when data are required for registering chemicals in Korea. The residual CTC concentrations differed in the two plots after 3 days, and the difference was greater after 26 days (Fig. 2). We postulate that the differences were due mainly to the non-homogeneity of the soil properties in the artificial plots. The differences were more pronounced with the prolonged precipitation in the Korean rainy season. However, no additional experiments were performed to determine whether this was caused by leaching or run-off, since this study sought to estimate

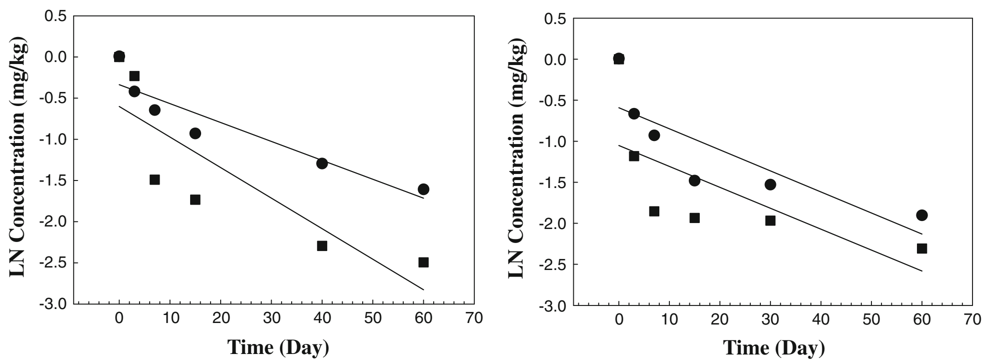

Fig. 1 Dissipation of chlortetracycline in soil outdoors and indoors with the corresponding 95% confidence limits. *Outdoors*: *filled square* $y = -0.0370971394x - 0.6009930908$ ($r^2 = 0.729$), $t_{1/2} = 18.68$ days, *filled circle* $y = -0.0229739462x - 0.3361020952$ ($r^2 = 0.876$), $t_{1/2} = 30.16$ days; *Indoors*: *filled square* $y = -0.025667433x - 0.5911865462$ ($r^2 = 0.703$), $t_{1/2} = 6.70$ days, *filled circle* $y = -0.025503356x - 1.0518641192$ ($r^2 = 0.676$), $t_{1/2} = 27.17$ days using first-order kinetics

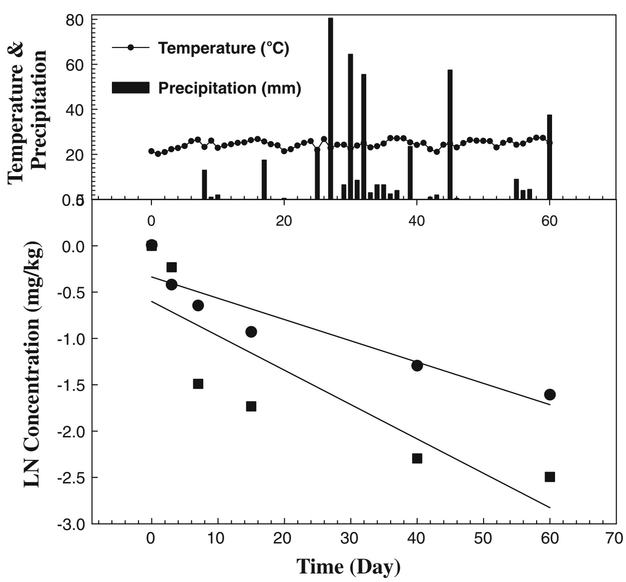

Fig. 2 Dissipation of chlortetracycline in soil outdoors

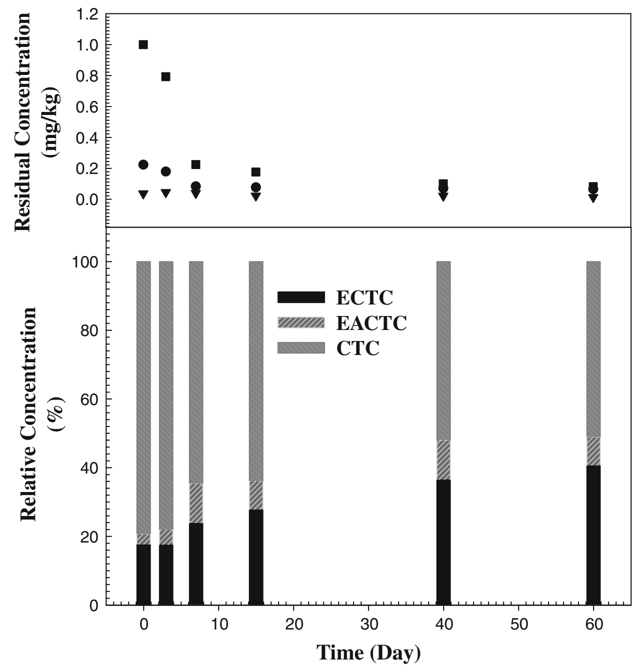

Fig. 3 Changes in CTC (*filled square*) and its epimers ECTC (*filled circle*) and EACTC (*filled inverted triangle*) in soil outdoors

dissipation only. There were only slight differences between the indoor tests. The chemical stability of tetracyclines under environmentally relevant conditions in terms of the matrix, pH, temperature, and presence of light, oxygen, and metal ions has been the subject of several papers (e.g., Søeborg et al. 2004). Although it is difficult to determine the cause of the differences in DT_{50} between the outdoor and indoor results, due to the large variation between the outdoor replicates, when considering the shorter half-life in the outdoors experiment, we postulated that temperature, light, and humidity affect the dissipation of CTC. The metabolites of CTC are bioactive and are included in the maximum residue limits (MRL) set for tetracyclines in food by the European Union (EU) (EMEA 1995). Eight potential degradation products of CTC have been suggested, depending on environmental conditions (Halling-Sørensen et al. 2002). CTC and CTC degradation products and epimers generally have shorter half-lives in soil interstitial water under environmentally relevant conditions and are more persistent in acidic environments at pH 3–4 than at pH above 5 (Søeborg et al. 2004). Figures 3 and 4 outline the formation of epimers of CTC in the outdoor and indoor soil dissipation experiments, respectively. To describe the degradation trend of CTC and formation of degradation products in soil simultaneously, the residual concentrations from the replicates were partially corrected; the CTC residue concentration was expressed as the sum of its epimers plus CTC from the mean of

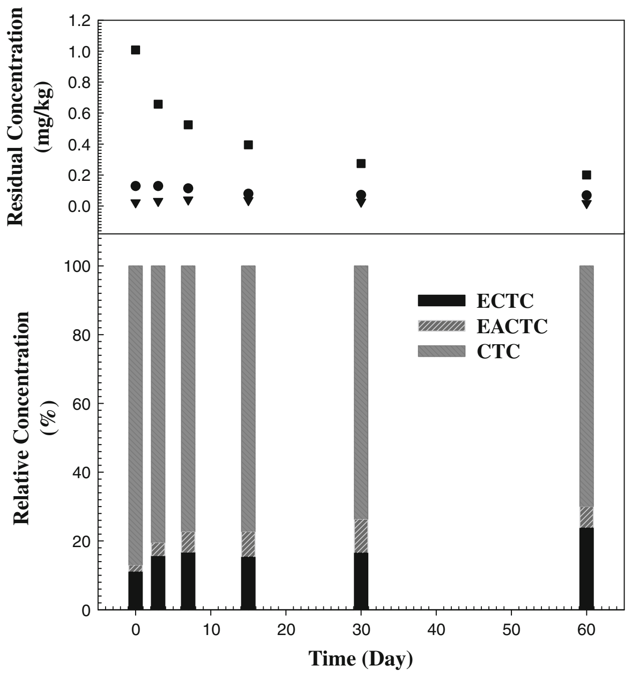

Fig. 4 Changes in CTC (*filled square*) and its epimers ECTC (*filled circle*) and EACTC (*filled inverted triangle*) in soil indoors

replicates, while the epimer concentrations were expressed as determined. Rapid degradation and the formation of degraded products were observed beginning on the first day of this study. We postulate that the temperature, soil pH, and application of manure compost affect the degradation and dissociation reaction in manure compost after spraying it with CTC solution. The formation of ECTC and EACTC supported this assumption, especially outdoors; CTC was readily degraded and the epimer concentrations were higher than those indoors. The respective concentrations of EACTC and ECTC were 1.91–9.71% and 11.12–23.92% indoors and 2.96–11.44% and 17.61–40.73% outdoors, relative to the total CTC residual concentration. In previous studies of CTC, the half-life was significantly shorter in the presence of light than without light; the disappearance rate is also temperature dependent (Søeborg et al. 2004). In summary, field and laboratory studies examined the fate of CTC in the soil environment. Overall, the results demonstrate that CTC has potential to persist in Korean arable soils, forming bioactive metabolites and epimers. The estimated half-life (DT_{50}) of CTC was 26.70–27.17 days indoors and 18.63–30.16 days outdoors.

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