Ecotoxicology and Environmental Safety 126 (2016) 228–237

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Phytotoxicity of veterinary antibiotics to seed germination and root elongation of crops

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ARTICLE INFO

Article history:
Received 19 June 2015
Received in revised form
16 December 2015
Accepted 22 December 2015
Available online 11 January 2016

Keywords:
Veterinary antibiotics
Phytotoxicity
Seed germination
Root elongation
Edible crops

ABSTRACT

Large quantities of veterinary antibiotics (VAs) are being used worldwide in agricultural fields through wastewater irrigation and manure application. They cause damages to the ecosystem when discharged into the environment, but there is a lack of information on their toxicity to plants and animals. This study evaluated the phytotoxic effects of five major VAs, namely tetracycline (TC), sulfamethazine (SMZ), norfloxacin (NOR), erythromycin (ERY) and chloramphenicol (CAP), on seed germination and root elongation in lettuce, tomato, carrot and cucumber, and investigated the relationship between their physicochemical properties and phytotoxicities. Results show that these compounds significantly inhibited root elongation ($p < 0.05$), the most sensitive endpoint for the phytotoxicity test. TC was associated with the highest level of toxicity, followed by NOR, ERY, SMZ and CAP. Regarding crop species, lettuce was found to be sensitive to most of the VAs. The median effect concentration (EC50) of TC, SMZ, NOR, ERY and CAP to lettuce was 14.4, 157, 49.4, 68.8 and 204 mg/L, respectively. A quantitative structure-activity relationship (QSAR) model has been established based on the measured data. It is evident that hydrophobicity was the most important factor governing the phytotoxicity of these compounds to seeds, which could be explained by the polar narcosis mechanism. Lettuce is considered a good biomarker for VAs in the environment. According to the derived equation, phytotoxicities of selected VA compounds on different crops can be calculated, which could be applicable to other VAs. Environmental risks of VAs were summarized based on the phytotoxicity results and other persistent factors.

1. Introduction

Veterinary antibiotics (VAs) are widely used to prevent infections in and promote the growth of farm animals. Because of their broad usage and persistence in the environment, there has been a mounting concern over VAs and their environmental impacts in recent years. Owing to their limited absorption in animals, antibiotics in parent and metabolite forms are excreted into the environment through manure and urine. As a result, a variety of VAs have been detected in relatively high concentrations in soil, surface water, groundwater and sediment (Tamtam et al., 2011; Tong et al., 2009). Their concentrations range from 0.01 to 1420 mg/kg in manure and soil (Chen et al., 2012; Kim et al., 2010; Luo et al., 2011). The growing discharge of VAs and their metabolites result in adverse effects on terrestrial and aquatic organisms (Chari and Halden, 2012; Pan et al., 2014). They include tetracyclines, sulfonamides, macroclides, fluoroquinolones and others (USEPA, 2012). Five VAs were studied as representatives of the commonly used antibiotic type, which are widely used in animal medicine, and are frequently detected with relatively high concentrations in the environment (Sarmah et al., 2006; Yan et al., 2013). Crops are an important component of the terrestrial environment and serve as a potential pathway for VA transport (Ma et al., 2010). Lettuce, carrot, cucumber and tomato were selected as the target crops since they have different edible parts that can used as salad, as well as significant economic and ecological values. Evaluation of the biological responses of terrestrial crops to antibiotics is important because such plants are often exposed to VAs by the routes of wastewater irrigation and/or animal manure fertilization. Seed germination and root elongation tests are simple, sensitive and inexpensive environmental bioassays (Reynolds, 1989; Salvatore et al., 2008), which are commonly used for the evaluation of phytotoxicity of organics and/or inorganics to plants (Liu et al., 2007; Wang and Zhou, 2005). Besides, the quantitative structure-activity relationship (QSAR) model is another common means to estimate the ecotoxicity of organic compounds with different physicochemical properties (Tropsha, 2010). Information about phytotoxicity is essential to ecological risk assessments of VAs, and crops should be taken into the test battery in order to develop a comprehensive toxicity profile for these pollutants. Few studies

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http://dx.doi.org/10.1016/j.ecoenv.2015.12.027
0147-6513/

evaluated the toxic effects of pharmaceutical compounds on crops. Migliore et al. (2003) found that enrofloxacin induced both toxic effects and hormesis in plants. Jin et al. (2009) investigated the ecotoxicological effects of paracetamol on wheat and found that a concentration between 1.4 and 22.4 mg/L adversely interfered with chlorophyll accumulation and protein synthesis. However, there is a paucity of information on the ecotoxicity of different types of VAs and the mechanisms of their phytotoxicity in crops. In addition, most of the phytotoxicity tests of organic or inorganic compounds focus on only one or two plant species (Moore et al., 1999; Zhang et al., 2012). Few data are available about the sensitivity of crop species to different types of VAs. Moreover, there is no study on the relationship between physicochemical properties of VAs and their phytotoxic effects on crops. In the present study, the phytotoxic effects of five selected VAs on four crops were investigated using the most simple and efficient method, and identified the most sensitive crops and the most phytotoxic VAs. The relationships were assessed between the phytotoxicity of VAs and their physicochemical properties by measuring root elongation. Furthermore, the inhibition concentration for root elongation of VA compounds were used to assess their phytotoxicity to different crops. This should be the first study to evaluate the relationship between the physicochemical properties of VAs and their phytotoxic effects on crops, as well as to derive an equation for their phytotoxicity calculations. The phytotoxicity of VAs was an important factor to summarize their terrestrial environmental risks. The objectives of the present study were to (1) evaluate the sensitivity of different tissues across the crop species, (2) identify the most sensitive crop to the VAs used, and (3) explore the relationship between the physicochemical properties and the phytotoxicity of these compounds. Results from this study would help facilitate understanding of the phytotoxicity of VAs to different crop species and provide more information about the toxicity mechanisms of organic pollutants in terrestrial organisms.

2. Materials and methods

2.1. Standards

Tetracycline (TC), sulfamethazine (SMZ), norfloxaxin (NOR), erythromycin (ERY) and chloramphenicol (CAP) ($>98\%$) were selected in the present study to represent the various types of VAs. They were obtained from Sigma-Aldrich (St. Louis, MO, USA). Their major physicochemical properties are shown in Table S1.

2.2. Crop species used and pretreatment

Seeds of lettuce (*Lactuca sativa*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*) and tomato (*Lycopersicon esculentum*) which are recommended by USEPA (1996), OECD (1984) and USFDA (1987) as model crops were used for seed germination and root elongation tests. With various edible parts, these crops have significant economic and ecological values. The seeds used were purchased from a local seed supplier (Choi Hing Lee International Ltd, Hong Kong). Prior to germination, all seeds were surface-sterilized in sodium hypochlorite solution (0.1%) for 10 min, and rinsed with deionized water afterwards.

2.3. Seed germination test

Aqueous solutions of the five VAs were prepared using distilled water at seven concentrations: 0.00, 0.01, 0.10, 1.00, 10.0, 100 and 300 mg/L. Five milliliters of each solution was added to a 10 cm petri dish, in which 20 seeds of each crop species had been placed on a piece of filter paper in accordance with the ASTM standard germination protocol (ATSM, 2003). Each treatment was replicated five times. Determination of selected VA concentrations in petri dish was carried out according to the procedures described in our previous study (Pan et al., 2014). The concentrations of target VAs were analyzed by HPLC-MS/MS (Agilent 1100 series HPLC System coupled to an Agilent 6410 Triple Quadrupole MS) equipped with an electrospray ionization (ESI) source (Agilent, USA) in multiple-reaction monitoring (MRM) mode. Ten concentrations (0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100 and 500 mg/L) of individual VAs were used to calculate the calibration curves ($R^2 > 0.999$). The petri dishes were covered with lids before being kept in an incubator (MLR-350, Versatile Environmental Test Chamber). They were maintained at 25 ± 0.5 °C, 80% humidity and in total darkness. Water loss in petri dishes was monitored every day by weighing and distilled water was added if necessary. Adsorption and degradation of VAs were negligible during the test. Seeds were regarded to have germinated when the radicle was over 2.0 mm in length. The VAs-exposure experiment was terminated when the length of the growing radicle in the control reached 20 mm (An et al., 2009b). The percentage of seed germination was determined and the lengths of root and shoot measured. The average incubation periods were 5 days for lettuce and tomato, and 7 days for carrot and cucumber. The concentration causing a 50% inhibition (EC50) and that causing a 10% inhibition (EC10) were considered for toxicity evaluation.

2.4. Statistical analysis

All data were subjected to the analysis of variance (one-way ANOVA) with the factors being the five types of VAs and the seven concentrations tested. All the data were checked by Levene's test for homogeneity of variances and Kolmogorov–Smirnov test for data normal distribution. The endpoints were evaluated using Dunnett's test at the 5% level of significance. When a significant difference ($p < 0.05$) was detected between treatments and the control, EC50 values and EC10 values of the VA in question were calculated based on the log concentrations and the germination rate or plant growth endpoints (shoot height and root length), using the EC50 calculator program PRISM (GraphPad Software, USA).

The no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC) were calculated on PRISM using Dunnett's multiple comparison test to determine the treatments that were significantly different from the control (1-tailed, $p < 0.05$). After ANOVA, the raw data were analyzed using linear regression on Sigmaplot (Systat Software, CA). The regression equations for the inhibitory rate of shoot elongation and of root elongation under different VAs concentrations were determined.

2.5. QSAR analysis

A QSAR model was used to investigate the relationship between the physicochemical properties of the tested VAs and their phytotoxicity. This would help identify the presence of hazardous potential of the compounds. All the EC50 data were log-transformed and designated as log(1/EC50). Log-transformed octanol-water partitioning coefficients ($\log K_{ow}$, Table S1) and the energy of the lowest unoccupied molecular orbital (E_{lumo}) were examined as the physicochemical descriptors of the five VAs. The E_{lumo} of each antibiotic was calculated using the Gaussian 09 software (Revision B.01, Gaussian, Inc., Wallingford CT, 2009).

The QSAR model was analyzed through multiple regression analysis on Sigmastat (Systat Software, CA). R squared, standard error of estimate (SE), Fisher criterion (F), and the significance level (p) were calculated.