Adsorption Properties and Degradation Dynamics of Endocrine-Disrupting Chemical Levonorgestrel in Soils

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ABSTRACT: Levonorgestrel, a synthetic progesterone used as an oral contraceptive or emergency contraceptive pill, has been shown to be an endocrine-disrupting chemical. To assess the environmental risk of levonorgestrel, batch experiments and laboratory microcosm studies were conducted to investigate the adsorption and degradation of levonorgestrel in five contrasting soils of China. Freundlich and Langmuir models were applied to sorption data to examine the affinity of levonorgestrel for soils with varying physical and chemical properties. The K_f of levonorgestrel in the tested soils ranged from 10.79 to 60.92 mg^{1-n} L^n kg^{-1} with N between 0.69 and 1.23, and the Q_m ranged from 18.18 to 196.08 mg/kg. The multiple regression analysis was conducted between K_f and soil properties. Results indicate that total organic carbon plays a dominant role in the adsorption process. Gibbs free energy values less than 40 kJ/mol demonstrate that levonorgestrel sorption on soils could be considered as a physical adsorption. The degradation of levonorgestrel in five soils was fitted by the first-order reaction kinetics model. The half-lives of levonorgestrel were between 4.32 and 11.55 days. The initial concentration and sterilization experiments illustrated that the degradation rate of levonorgestrel in soil was concentration-dependent and microbially mediated. The low mobility potential of levonorgestrel in soils was predicted by the groundwater ubiquity score (GUS) and retardation factor (R_f).

KEYWORDS: *Levonorgestrel, adsorption, degradation, soil*

■ INTRODUCTION

The presence of anthropogenic organic compounds that have the potential to affect the normal function of human and wildlife biological systems has become a prominent issue following the publication of the book *Silent Spring*.[1] Among these chemicals, natural or synthetic steroidal sex hormones (estrogens and progestogens), known as endocrine-disrupting chemicals (EDCs), can alter endocrine function and, consequently, cause adverse health effects. Several studies have shown abnormalities in the sexual development of aquatic species and wildlife as well as humans even at exposure to low (ng/L) concentrations.[2−6]

Levonorgestrel is a synthetic progestogen used as an oral contraceptive or emergency contraceptive pill since the mid-1960s.[7] During the last 5 decades, the consumption of levonorgestrel for human medicine or other applications has experienced steady growth with the global population growth. Levonorgestrel can enter the aquatic environment from the discharge of municipal wastewater effluent and concentrate in aquatic species. Fick et al. reported that the concentrations of levonorgestrel in the blood plasma of rainbow trout were 8.5−12 ng/mL when rainbow trout was exposed to treated sewage effluents at three sites in Sweden for 14 days.[8] These concentrations exceed the human therapeutic plasma level. Levonorgestrel can also enter the soil as an antifertility rodenticide to control the rat population in farmlands and grasslands, and the concentration can reach the level of mg/kg.[9,10] The persistence in the environment of such drugs at a

trace level concentration may disrupt the endocrine systems of animals. Zeilinger et al. recently showed that a water concentration of 0.8 ng/L levonorgestrel caused a reduced fertility of exposed adult fathead minnows (*Pimephales promelas*).[11] Furthermore, levonorgestrel was also found to affect the fertility of men, and exposure to high levels of levonorgestrel has been shown to cause azoospermia.[12]

Unlike pesticides and other priority pollutants, knowledge of the environmental fate of steroidal sex hormones has not been studied extensively over the past few decades, although hormones have become an important class of potentially harmful substances in the environment. To date, some studies investigating the sorption, degradation, and transport of estrogens in varied matrices have been conducted.[13−16] However, no information was available about the fate of levonorgestrel in soils when this work began. This constituted a considerable gap in the scientific knowledge. Supplied knowledge in this aspect is required urgently because information on the environmental fate of progestogen-like compounds is essential for assessing their potential to leach into groundwater and predicting their impact on both terrestrial and aquatic environments. To try to achieve this goal, the sorption behavior and the disappearance of levonorgestrel were determined in five

Table 1. Properties of Tested Soils

soil type	pH	N (%)	TOC[a] (%)	CEC[b] (cmol/kg)	particle size (%)		
					sand	silt	clay
BJ	7.12	0.13	1.95	25.6	46.67	37.48	15.85
NMG	7.46	0.08	1.14	8.70	85.04	11.30	3.65
YN	6.84	0.19	1.30	12.9	6.37	63.22	30.41
HLJ	7.02	0.21	2.45	43.6	15.33	71.07	13.59
GX	6.67	0.12	1.97	22.4	38.19	53.39	8.42

[a]TOC = total organic carbon content. [b]CEC = cation-exchange capacity.

contrasting soils from different areas of China. The controlling factors with respect to the fate of levonorgestrel in soils were estimated on the basis of the correlation between the examined data and soil properties.

MATERIALS AND METHODS

Chemicals and Soils. Levonorgestrel (purity > 99%) was obtained from Sigma-Aldrich (Dorset, U.K.) and used as received. Ammonium acetate, anhydrous calcium chloride, and acetic acid were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Methanol and acetonitrile were high-performance liquid chromatography (HPLC)-grade from Fisher Scientific (Fair Lawn, NJ). Ultrapure water was obtained in the laboratory using a Milli-Q water purification system with a resistivity greater than 18.2 MΩ cm to prepare samples and the mobile phase (Millipore, Billerica, MA). All chemicals were used as received. Stock solutions of levonorgestrel were prepared in methanol and stored at 4 °C in the dark. The desired working solution was prepared by mixing a certain volume of 0.01 M $CaCl_2$. The fraction of methanol was maintained at 0.1% of the total solution volume to avoid any co-solvent interactions.

Five well-characterized soils were used in this study: four agricultural top soils from Heilongjiang province (HLJ), Beijing province (BJ), Yunnan province (YN), and Guangxi province (GX) and a grassland soil from Neimenggu province (NMG). Soil samples were collected from the 0−20 cm surface layer, air-dried, and ground to pass through a sieve with 2 mm openings. The content of total organic carbon (TOC) and nitrogen (N) in soil samples were determined by dry combustion with a CN analyzer Vario Max (Elementar Analysensysteme GmbH, Hanau, Germany) at the laboratory of Natural Resources and Environment, China Agricultural University (Beijing, China). The cation-exchange capacity (CEC) was determined using a 1.0 mol/L ammonium acetate solution (pH 7.04). The particle size was determined by the hydrometer method.[17] Soil pH values in 0.01 M $CaCl_2$ with a soil/solution ratio of 1:1 were determined with a pH meter. Their composition and basic chemical properties are presented in Table 1. Soil sterilization was accomplished by autoclaving soils at 120 °C under 300 kPa pressures 3 times for 45 min at 3 day intervals.

Adsorption Study. Sorption of levonorgestrel was studied using a batch equilibrium approach following the Test Guideline of OPPTS 835.1230.[18] The batch adsorption experiments were performed with sterilized soils to avoid the influence of levonorgestrel degradation. A volume of levonorgestrel solution (50 mL) was added to 2 g of dried soil in 100 mL Teflon centrifuge tubes. The tubes were wrapped in aluminum foil and agitated on a shaker at room temperature (25 ± 2 °C). Sorption kinetics experiments were carried out for five soils according to the above procedure. A 0.01 M $CaCl_2$ solution of levonorgestrel (1 mg/L) was prepared and contacted with soil over a period ranging from 2 to 48 h. Sorption isotherms were conducted at levonorgestrel concentrations ranging from 0.2 to 2 mg/L (five concentration points). The contact time is 24 h with 3 replicates for each concentration. The control experiment with only levonorgestrel in 0.01 M $CaCl_2$ solution (no soil) is subjected to precisely the same steps as the test protocol, to check the adsorption of levonorgestrel in $CaCl_2$ solution on the surfaces of the test vessels. After adsorption equilibrium, centrifugation at 1632g for 15 min was applied to separate the aqueous phase and solid phase. The supernatant (10 mL) was

transferred into a tube with 2 mL of methanol and stored in a freezer (−21 °C) for the extraction.

Degradation Study. Degradation of levonorgestrel in soil was determined by an incubation experiment. Dried soil (10 g) was treated with 5 mL of methanol solution containing 60 μg/mL levonorgestrel in a small beaker. The soil sample was mixed using a glass rod and placed in the fume hood overnight to allow for evaporation of the methanol. Subsequently, the spiking soil sample was removed from the beaker and mixed into the previously prepared soil samples (140 g, incubation at 25 ± 2 °C for 7 days). The soil flask was thoroughly mixed by rotating and shaking. The initial levonorgestrel concentration in the soil was 2 μg/g. Abiotic degradation was conducted directly by spiking the methanol solution of levonorgestrel into the autoclaved soil. The effects of different initial levonorgestrel concentrations on degradation were tested at 0.2, 2, and 20 μg/g. The moisture content of each soil is determined on three aliquots with heating at 105 °C until there is no significant change in weight (approximately 12 h). The moisture contents of BJ, NMG, YN, HLJ, and GX soils were 14, 9, 23, 35, and 18%, respectively, and the desired values were adjusted by adding deionized water. All flasks were covered with aluminum foil and placed in an incubator at 25 ± 2 °C. The flasks were weighed periodically to check for water loss, and deionized water was added to compensate for the water loss when necessary. The incubation time was maintained for 21 days for biotic degradation and 120 days for abiotic degradation. At set time intervals, 10 g samples of each treatment were transferred into a freezer (−21 °C) to stop the degradation. All experiments were performed in triplicate.

Sample Extraction and HPLC Analysis. For adsorption, the spiked control experiment indicated that no sorption on test vessel surfaces was observed for levonorgestrel, and the mass balance experiment demonstrated that no abiotic degradation occurred. Therefore, only the aqueous phase was analyzed as recommended in the United States Environmental Protection Agency (U.S. EPA) Test Guidelines. The aqueous sample was transferred to a 60 mL separatory funnel with 10 mL of ethyl acetate and shaken for 5 min on a mechanical shaker. Then, the solvent was collected, and the aqueous sample was extracted again. Finally, the solvent extracts were combined and evaporated to dryness on a vacuumed rotary evaporator at 45 °C. The residue was reconstituted with 1 mL of HPLC-grade methanol and used for analysis by HPLC.

For the degradation experiments, soil samples were thawed at room temperature and then transferred to 50 mL Teflon centrifuge tubes. The soil samples were shaken with 10 mL of acetonitrile (1% acetic acid) for 1 h on a mechanical shaker and then centrifuged at 1632g. The same extraction step was repeated 3 times, and the organic phase extracts from all extractions were combined. The extracts were evaporated to dryness on a vacuumed rotary evaporator at 50 °C. The residue was reconstituted with 1 mL of HPLC-grade methanol and used for analysis by HPLC. Preliminary experiments showed that the limits of quantification (LOQs) were 8.0 μg/L for aqueous samples and 13.2 μg/kg for soil samples. External calibration curves were generated to estimate sample concentrations from peak areas. The percent recovery of levonorgestrel was higher than 92% with the relative standard deviations of 3.6−6.8% for the adsorption experiment, and the aqueous phase and sorbed phase were quantifiable. The recovery was higher than 85% with the relative standard deviations of 4.8−7.6% for the degradation experiment.

4000

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The quantification of levonorgestrel was carried out on a Shimadzu HPLC system consisting of two LC-20AT pumps and a SPD-M20A ultraviolet detector, a CBM-20A system controller, and a DGU-20A3 degasser. The column was a Kromasil ODS C_{18} column (250 × 4.6 mm) packed with 5 μm particles and connected with a guard cartridge (Kromasil Easyguard C18-6201). Levonorgestrel was eluted isocratically using a mobile phase consisting of methanol/0.1% acetic acid (80:20, v/v), with a flow rate of 1 mL/min. The wavelength was set at 244 nm.

Data Analysis. For the adsorption data, the aqueous phase concentration, C_{aq} (mg/L), was analyzed by a liquid–liquid extraction procedure coupled with HPLC. The adsorbed amounts of levonorgestrel by soil, C_{s} (mg/kg), were calculated from the measured solution phase concentrations based on mass balance and defined as

$$C_s = 50(C_0 - C_{aq})/2 \qquad (1)$$

where C_0 (mg/L) represents the initial concentration of levonorgestrel in the aqueous phase. The sorption data were fit by Freundlich and Langmuir models that were calculated using Sigma Plot, version 10.0.

Degradation kinetics was described using a first-order reaction model: $C_t = C_0 e^{-kt}$, where C_0 (mg/kg) is the initial concentration in soil and C_t (mg/kg) is the concentration at time t (days). k (day^{-1}) is the rate constant, and the half-life was calculated as $t_{1/2} = \ln(2)/k$. The analysis of variation (ANOVA) and regression analysis were calculated using SPSS, version 17.0.

■ RESULTS AND DISCUSSION

Adsorption. The adsorption kinetic experiment was designed to evaluate the minimum time of equilibrium for the tested soils. The results showed that levonorgestrel reached equilibrium within approximately 4 h in NMG soil and 12 h in YN soil, while the other three soils needed 24 h to achieve equilibrium. This phenomenon was likely due to the fact that the vacant sites in NMG soil (sand) with less contents of CEC were fully exposed and easily filled up, and the vacant sites of other soils were slowly occupied because of the competition between solute molecules and soil cations. On the basis of these results, the contact time for the adsorption experiments was selected to be 24 h.

Adsorption of levonorgestrel was modeled by the Freundlich and Langmuir equations (Figure 1). The Freundlich equation is an empirical adsorption model that can be expressed as

$$C_s = K_f C_{aq}{}^N \qquad (2)$$

$$\log C_s = \log K_f + N \log C_{aq} \qquad (3)$$

K_f (mg^{1-n} L^n kg^{-1}) and N are the binding constants and the isotherm nonlinearity index, respectively. The Langmuir equation has the form

$$1/q_e = 1/Q_m + 1/K_L Q_m C_{aq} \qquad (4)$$

where K_L (L/mg) is a Langmuir coefficient related to the binding strength and q_e and Q_m represent the adsorbed amounts and maximum sorption of levonorgestrel, respectively. The measured sorption isotherms were found to be nonlinear and could be well-fitted by Freundlich and Langmuir models (Figure 1), and the correlation coefficients of two models were more than 0.948 and 0.864, respectively. Table 2 gives a comparison of sorption parameters for levonorgestrel in soils calculated from the two models. The N values derived from the Freundlich model for BJ, YN, and GX soils were more than 1, which implied that adsorbate molecule clusters are likely formed on the surface of the soil because of the strong adsorbate–adsorbate interactions. The sorption tendency
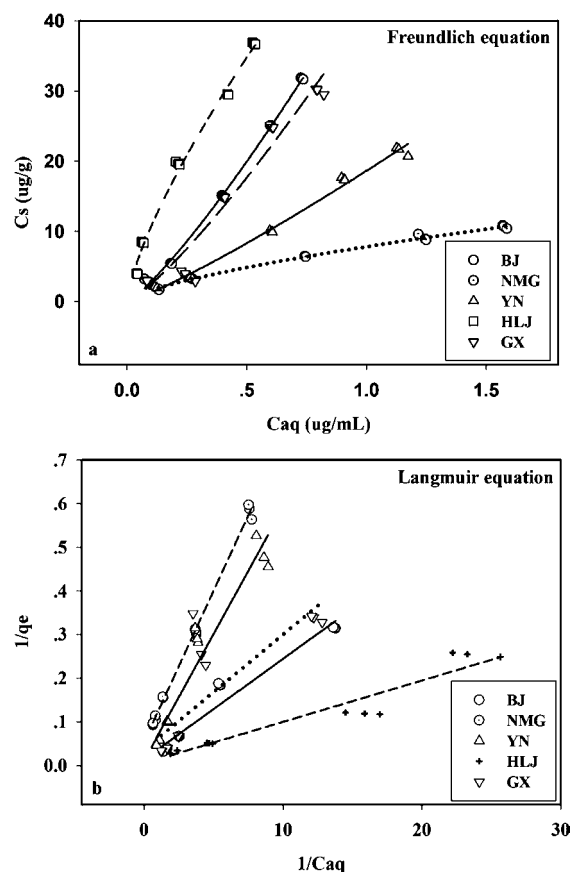

Figure 1. Levonorgestrel adsorption isotherms in five different soils: (top panel) Freundlich model and (bottom panel) Langmuir model.

increased with an increasing concentration of levonorgestrel in aqueous solution. The N values of NMG and HLJ soils were less than 1, indicating that L-type sorptions were produced in this two types of soil. The specific interactions of adsorption were expected because of the low water solubility, and the levonorgestrel adsorption was concentration-dependent in NMG and HLJ soils. These types of isotherms were reported in the literature with respect to the pesticide or pharmaceuticals and involve the interaction between the compound and the components of the soil.[19,20] The Q_m values calculated from the Langmuir model showed that the maximum and minimum adsorptions of levonorgestrel at tested concentrations occurred in HLJ soil (196.08 mg kg^{-1}) and NMG soil (18.18 mg kg^{-1}), respectively.

A number of studies have shown that the properties of soil play a significant role in adsorption processes, especially the pH, CEC, TOC, and content of clay.[21−23] In this work, the correlations between the adsorption constant K_f and soil properties were also examined to predict the adsorption of levonorgestrel in different soils and to help understand the principal factors responsible for adsorption. The simple correlations between K_f and soil properties were calculated, and the results showed that soil pH and N content had no significant effects on adsorption of levonorgestrel, because their correlation coefficients R^2 were only 0.123 and 0.352, respectively. The particle size of the soil, including the sand, silt, and clay, also exhibited a very weak correlation coefficient, with R^2 ranged from 0 to 0.433. However, the K_f of levonorgestrel was strongly positively correlated with soil TOC and CEC contents, with coefficients R^2 of 0.980 and

Table 2. Adsorption Isotherm Constants and Characteristics Derived from Langmuir and Freundlich Equations for the Five Types of Soil

soil type	Freundlich model			Langmuir model			K_{OC} (L/kg)	ΔG (kJ/mol)
	K_f (mg^{1-n} L^n kg^{-1})	N	R^2	K_L	Q_m (mg/kg)	R^2		
BJ	46.38 ± 4.58	1.21	0.990	0.22	76.92	0.946	2378.46	−18.91
NMG	10.79 ± 3.12	0.69	0.994	0.79	18.18	0.995	946.49	−16.80
YN	18.63 ± 3.46	1.16	0.982	1.32	27.78	0.864	1433.07	−17.73
HLJ	60.92 ± 5.76	0.74	0.989	0.54	196.08	0.935	2486.53	−18.97
GX	41.33 ± 5.15	1.23	0.948	1.08	71.43	0.952	2097.97	−18.62

0.977, respectively. It should be noted that CEC has a strong correlation with TOC. The reason that sorption is correlated with CEC is only because organic matter has a high CEC and sorption is primarily the organic matter domain. The results could explain that the HLJ soil had the highest adsorption affinity among the five soils. The results of this study were consistent with the previous research, which proved that the adsorption coefficients of estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethynylestradiol (EE2) increased as the organic carbon content of the soils increased.[13] A further multiple regression analysis was conducted among TOC, CEC, and K_f, and the relationship could be described by the following equation:

$$K_f = -18.33 + 20.90\,\text{TOC}\,(\%) + 0.77\,\text{CEC}\,(\%)\;(R^2 = 0.93,\; p < 0.05) \tag{5}$$

The K_f of many neutral hydrophobic organic chemicals has been shown to vary, depending upon the organic carbon content of the sorbent.[24] This relationship reconfirmed that the content of TOC and CEC exhibited a positive effect on the adsorption of levonorgestrel. The equation also illustrated that the content of TOC played a dominant role in the adsorption process.

K_{OC} values of levonorgestrel in five soils were also calculated as a function of the organic carbon (OC) content, and the Gibbs free energy values were computed from K_{OC}. The K_{OC} values were calculated as $K_{OC} = 100 K_f/\text{TOC}\,(\%)$, and their values are shown in Table 2. In comparison to K_f, the variation in K_{oc} values of levonorgestrel among different soils is less. For example, the 6-fold variation in K_f between HLJ and NMG soils was reduced to less than 3-fold variation in K_{OC} by normalizing adsorption to TOC. This indicated that the soil TOC is mainly responsible for the adsorption of levonorgestrel in soils. The Gibbs free energy values (ΔG) can predict the adsorption action of a chemical in soil, and values are calculated as follows:[25]

$$\Delta G = -1.724 RT \ln K_{OC} \tag{6}$$

where R is the universal gas constant (8.314 J mol^{-1} K^{-1}) and T is the adsorption absolute temperature (K). The Gibbs free energy values of levonorgestrel in tested soil varied from −18.97 to −16.80 kJ/mol, which are all less than 40 kJ/mol, indicating that levonorgestrel sorption on soils could be generally considered as a physical adsorption.[25]

Degradation. The degradation kinetics of levonorgestrel were fitted by a first-order kinetic for all soils at the tested concentration, and the values of model-fitting parameters are shown in Figure 2. These results showed that the degradation kinetics of levonorgestrel in five soils obeyed the simple first-order model at test concentrations. The corresponding values of rate constants (k) were 0.16, 0.08, 0.06, 0.09, and 0.13 day^{-1}
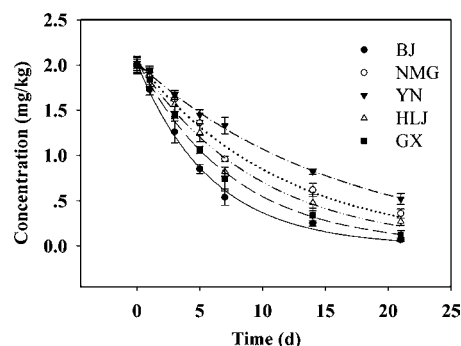
Figure 2. Degradation curves of levonorgestrel in five soils. Experiments were conducted at 25 ± 2 °C and lasted for 21 days, with a moisture content at 60% of the soil water-holding capacity (the spiking level is 2 mg/kg). Lines are fitted to the first-order decay model.

for BJ, NMG, YN, HLJ, and GX soils, respectively. In BJ soil, the half-life of levonorgestrel was only 4.32 days, but HLJ soil requires 7.70 days. Generally, faster degradation is noted with high organic carbon soils because of the increased nutrient levels and larger microbial populations. However, the degradation rate also strongly depends upon the diversity and metabolic activity level of the microbial population. Meanwhile, adsorption can decrease the degradation rate by preventing the availability of adsorbate to microorganisms, and this has been shown by the previous studies.[26,27]

Degradation of levonorgestrel in tested soil with different initial concentrations of levonorgestrel is shown in Figure 3. Different degradation rates were observed when the initial concentrations of levonorgestrel were varied from 0.2 to 20 μg/mL. Levonorgestrel dissipated rapidly in NMG soil, followed by
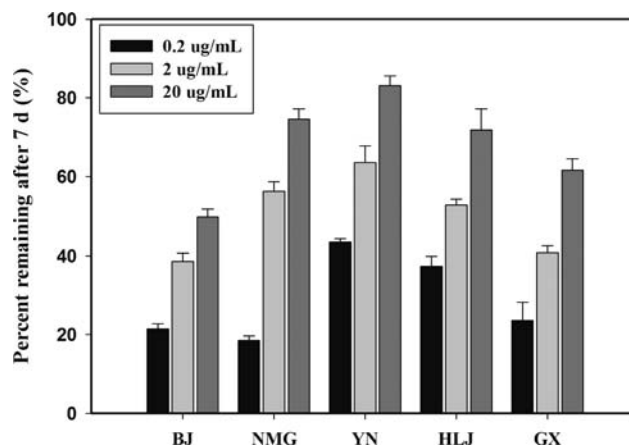
Figure 3. Effect of the initial concentration on the degradation of levonorgestrel in five soils.

Table 3. R_f and GUS of Levonorgestrel in Five Soils

	soil property			
soil type	bulk density (ρ_b)	porosity (θ)	retardation factor ($R_f = 1 + \rho_b K_f/\theta$)	GUS {log($t_{1/2}$)[4 log(K_{OC})]}
BJ	1.36	0.49	129.72	0.40
NMG	1.65	0.38	47.85	0.96
YN	1.29	0.51	48.12	0.89
HLJ	1.48	0.44	205.91	0.54
GX	1.33	0.50	110.93	0.49

BJ soil, GX soil, and HLJ soil, with YN soils at the lowest concentration (0.2 μg/mL), while the fastest dissipation was observed in BJ soil with 2 and 20 μg/mL. In addition, the dissipation rate decreased with an increase in the initial concentration. ANOVA analysis showed significant differences among the five soil types at each initial concentration ($p < 0.05$). It is well-known that the rate constant is independent of the chemical concentration if a chemical reaction obeys first-order kinetics.[28] However, the kinetics of a biological reaction with microorganisms involved may be quite different from that of an abiotic chemical reaction. The overall degradation kinetic would probably follow first-order if the bioactivity of the microorganisms is constant during the degradation. However, if the bioactivity is significantly altered by the initial concentration of the target compound, a higher initial concentration of the chemical may result in a lower bioactivity of the degrading microorganisms, which leads to a lower rate constant of the first-order kinetic.

No significant degradation for levonorgestrel in the five soils was observed in the sterile soil within 120 days of incubation. This suggests that microbial transformations dominated the overall degradation of levonorgestrel in the soils. To our knowledge, this is the first study that reports degradation of levonorgestrel in agricultural and grassland soils; thus, a comparison is not possible between the present results and other studies involving the same chemical. However, other steroidal sex hormones have been studied in a variety of soils and sediments. Lucas and Jones found that the half-lives of estrone and 17β-estradiol in three agricultural soils ranged from 5 to 25 days and in animal-manure-amended soils ranged from 1 to 9 days.[14] Another study indicated that 17α-ethynylestradiol was rapidly dissipated in loam, sandy loam, and silt loam soils and was persistent in sterile soil, suggesting that the removal of 17α-ethynylestradiol in soils was microbially mediated.[29]

Transport. Adsorption and degradation values are important parameters in determining whether a contaminant is likely to be transported from the application site to another location. In this work, the groundwater ubiquity score (GUS) and retardation factor (R_f) were calculated to predict the leaching potential for levonorgestrel to groundwater.[30,31] The soil bulk density was defined as the dry weight divided by the unit volume of soil, and soil porosity = 1 − (soil bulk density/2.65). The calculated equations and the values of GUS and R_f are presented in Table 3. The R_f and GUS values of levonorgestrel in the soils ranged from 47.85 to 205.91 and from 0.40 to 0.96, respectively. Previous studies on pesticides have demonstrated that a chemical with GUS > 2.8 is considered of high leaching potential, while a chemical with GUS < 1.8 is defined as low leaching potential.[32,33] According to this criterion, the movement of levonorgestrel will be retarded because of sorption, and it will accumulate around the application sites until it degrades. However, the leaching potential of a chemical is complicated and depends upon the permeability, porosity, homogeneity,

texture, mineralogy, and preferential flow under field conditions.[34,35] The exact leaching risk of levonorgestrel should therefore be investigated using undisturbed soil column experiments.

The results of the present study suggest that levonorgestrel could be adsorbed strongly in agricultural and grassland soils. Binding to soil TOC appears to be the dominant sorption mechanism. Data indicate that non-sterile soils are more favorable than sterile soil conditions for the degradation of levonorgestrel, suggesting that degradation in soils was microbially mediated. Increasing the initial concentration can decrease the degradation rate of levonorgestrel in soil. Our results are consistent with other previous studies, which indicate that these steroids have a moderate or strong binding on soil and degrade rapidly in soil. Although low mobility is predicted on the basis of sorption data, preferential flow pathways can result in expedited transport of many components in the environment. Meanwhile, in view of the low-dose effect of levonorgestrel as the endocrine-disrupting chemical, more concern ought to be taken for the behavior of levonorgestrel in soils and water.

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Notes
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