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RESEARCH ARTICLE

Degradation of the potential rodent contraceptive quinestrol and elimination of its estrogenic activity in soil and water

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Abstract Quinestrol has shown potential for use in the fertility control of the plateau pika population of the Qinghai–Tibet Plateau. However, the environmental safety and fate of this compound are still obscure. Our study investigated degradation of quinestrol in a local soil and aquatic system for the first time. The results indicate that the degradation of quinestrol follows first-order kinetics in both soil and water, with a dissipation half-life of approximately 16.0 days in local soil. Microbial activity heavily influenced the degradation of quinestrol, with 41.2 % removal in non-sterile soil comparing to 4.8 % removal in sterile soil after incubation of 10 days. The half-lives in neutral water (pH 7.4) were 0.75 h when exposed to UV light (λ=365 nm) whereas they became 2.63 h when exposed to visible light (λ>400 nm). Acidic conditions facilitated quinestrol degradation in water with shorter half-lives of 1.04 and 1.47 h in pH 4.0 and pH 5.0 solutions, respectively. Moreover, both the soil and water treatment systems efficiently eliminated the estrogenic activity of quinestrol. Results presented herein clarify the complete degradation of quinestrol in a relatively short time. The ecological and environmental safety of this compound needs further investigation.

Keywords Quinestrol · Biodegradation · Photo-degradation · Estrogen effect

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Introduction

The Qinghai–Tibetan Plateau, also known as "the roof of the world", is the highest and largest plateau in China, with an area of 2.5 million square kilometers and an average elevation of over 4,500 m (Zheng and Li 1999). It has a unique eco-system, rendering it the most intact mountain range which harbors a relatively intact population of vertebrate fauna (Arthur et al. 2007; Arthur et al. 2008). In recent decades, the number of ecological disturbances such as wildlife poaching, animal husbandry, and the grazing of livestock has increased (Zhang et al. 1998), also observed elsewhere. These disturbances have caused the populations of many indigenous small herbivorous mammals to increase and the turf has been gradually depleted by overgrazing livestock. The large population of small herbivorous mammals has become a serious environmental and ecological problem on the plateau (Fan 1999). The Qinghai–Tibetan Plateau is dominated by species of plateau pikas (*Ochotona curzoniae*, also called black-lipped pikas (Lai and Smith 2003)). These mammals are typically social animals that tend to be spatially clustered (Smith and Foggin 1999). Some can attain a very high population density of >350 ha^{-1} (Wang et al. 1997). Although the pika species plays a pivotal role in the community dynamics in high-altitude grasslands (Smith and Foggin 1999), they are also regarded as a pest species. They tend to compete with livestock for food and their aggressive foraging and burrowing activities can lead to grassland degradation and soil erosion (Pech et al. 2007).

Many methods have been used to control the pika population on the Qinghai–Tibetan Plateau. Baiting and trapping are popular, traditional culling methods, but these practices have been criticized for their inhumanity (Zhong et al. 1999). Poisoning is another method that has been used on the Qinghai–Tibetan Plateau since 1958. Although the use of poisons can result in a large, immediate reduction in pika abundance, the population usually recovers rapidly (Liang 1981). The traditional Tibetan lifestyle is very environmentally friendly, and the Tibetan people respect life and nature, particularly because of their religious tradition. Therefore, the use of contraceptives for pest control, which was first proposed by Knipling, is an effective, non-lethal and ethical approach in this region (Knipling 1959). Previous studies also have indicated that this method can maintain the population at a low level in the long-term because the non-reproducing individuals still compete for resources with fertile individuals. Furthermore, modeling has shown the use of contraceptives to be a potentially effective method (Zhang 2000a, b). So far, mainly two sets of chemicals have the potential for use in rodent control in China, including estrogen derivative (levonorgestrel) and non-steroid compound (α-chlorohydrin and Comiphene). In the field experiments, the effect of α-chlorohydrin was prominent. The deratization rate by α-chlorohydrin–wheat mix was 84.09 %. Although the chemosterilant has many advantages as described previously, the data on environmental risk and its potential harm to non-target organisms cannot be determined. Therefore, no contraceptives have been put into practical applications for rodent control up to date.

Quinestrol was the estrogenic component of the monthly contraceptive pills developed in the late 1960s (Fig. 1) and was originally used with levonorgestrel as a long-term oral contraceptive for women (Greenblatt 1967). Subsequently, as a potential rodent contraceptive, it disrupts the function and structure of the reproductive system in several wild rodents (Zhao et al. 2007) and is favored in the population control of plateau pikas in Maqin County of Qinghai Province. Although the advantages of population control are obvious, the environmental risk of estrogenic activities of this compound in environmental media has not been well examined. In the present study, we analyze the degradation of quinestrol in aqueous and soil systems and also the reduction of the total estrogenic activity. To our knowledge, this is the first report of the possible environmental fate of quinestrol.

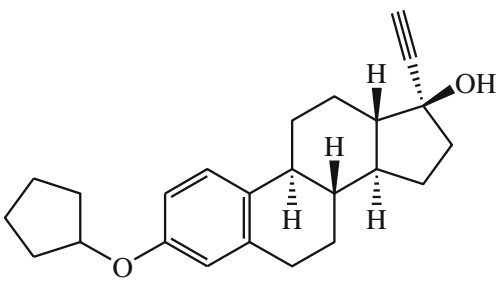

Fig. 1 Chemical structure of quinestrol

Materials and methods

Chemicals

Quinestrol (purity>99 %) was obtained from Beijing Zizhu pharmaceutical company (Beijing, China) and was used as received. High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Tedia (Fairfield, OH, USA). The yeast strain used in this study was purchased from the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (Beijing, China). All the other reagents were of analytical grade.

Sample information

The experimental area for rodent contraceptive by quinestrol is located in Guoluo prefecture, Qinghai province, China with an area of 15,000 ha. The four soil samples obtained from Maqin County in southern Qinghai province were composited into one, which were the experimental area for rodent contraceptive by quinestrol. Considing the future application of quinestrol in farmland, the other sample site was selected in Yuhang, Zhejiang province, where the farmland soils were also obtained to study the degradation of the quinestrol. At each sampling site, soil from five cores with the depths of 15–20 cm were collected using a plastic scoop and composited to form a single sample. All the soils were air dried and homogenized after the removal of pebbles, weeds, and sticks. After the larger particles had been removed with a 1 mm sieve, the samples were stored at −20 °C until analysis. The geographical coordinates of the four sampling sites were obtained using a global positioning system, and the locations in Maqin, Qinghai province are shown in Fig. 2.

Degradation of quinestrol in soil

Batch experiments were conducted to analyze the degradation of quinestrol in soil. Quinestrol has low solubility in water, and, therefore, the stock solution of 1.00 mg mL^{-1} quinestrol was prepared in methanol and stored at 4 °C prior to use. The container was wrapped in aluminum foil to prevent photodegradation. A working solution with a concentration of 0.002 mg mL^{-1} was obtained by diluting the stock solution in 0.001 mol L^{-1} $CaCl_2$. The content of methanol in the working solution was 0.2 % and was assumed to have little influence in the subsequent experiments. To eliminate any potential microbial activity, the samples

Fig. 2 Soil sample sites. Location *A* longitude, 100.351°; latitude 34.422°. Location *B* longitude, 100.425°; latitude 34.397°. Location *C* longitude 100.309°; latitude 34.437°. Location *D* longitude 100.217°; latitude 34.479°

were sterilized by autoclaving twice at 120 °C for 45 min, with a 24-h interval between the first and second autoclaving. Incubation temperature was selected according to the natural highest temperature in plateau during May and July (15–25 °C) when the contraceptives was applied. Aliquots (6 mL) of quinestrol solution and 300 g of nonsterile or sterile soil were added to glass beakers and homogenized thoroughly by manual stirring. The samples were then incubated at 25±1 °C and 70 % humidity. The beakers were wrapped in aluminum foil to minimize photodegradation during incubation. Two sets of flasks were prepared at the same time and incubated at 25±1 °C and 15±1 °C. At different sampling times, three aliquots of 0.3 g of soil were removed from the samples and transferred immediately to a freezer (–20 °C) to stop the degradation. For extraction, the samples were warmed to room temperature and then mixed with 20 mL of methanol, followed by centrifugation at 1,000 rpm for 15 min. This extraction process was repeated for three times. The combined extracts were concentrated using a vacuum rotary evaporator (Büchi, Switzerland) at 35 °C, transferred to a 10-mL concentrator tube, and evaporated to dryness under a gentle stream of nitrogen gas. The residue was re-dissolved in 1.0 mL of methanol for analysis. The final extracts were filtered through a 33-mm Acrodisc syringe filter coupled with a 0.45-μm membrane (Millipore Corp., Co. Cork, Ireland) prior to injection into the HPLC system.

Degradation of quinestrol in water

The photodegradation of quinestrol in water was studied using artificial sunlight under laboratory conditions at 25 °C. The stock solution of quinestrol was prepared as previously described and then diluted to 0.001 mg mL^{-1} for use. One experiment was performed in a 1-L container (diameter 8 cm, height 20 cm) with a magnetic stirrer. A 400-W mercury lamp (λ=365 nm) or a 400-W dysprosium lamp (λ>400 nm; Beijing Electric Light Sources Research Institute, Beijing, China) was used as the light source to induce the decomposition of quinestrol under normal conditions (pH=7.4). The spectrum distribution of the 400 W dysprosium lamp reached level B of the general specification for solar simulators. In addition, quinestrol solutions with different pH values (4–8) were obtained by adding the appropriate amount of phosphate buffer, and a 400-W dysprosium lamp was used to mimic natural light to study the degradation of quinestrol in the solution at different pH values. The measurements obtained before and after the experiments indicated that the pH of the solution was almost unchanged. Aliquots of the different samples were transferred to tubes (three tubes for each sample per time point) at 0, 0.5, 1, 3, 6, and 12 h and then immediately placed in a freezer (4 °C) to stop the degradation. Before analysis, 10 mL samples were evaporated using a vacuum rotary evaporator at 40 °C and concentrated to near dryness with a stream of nitrogen gas. The samples were then reconstituted in 1.0 mL of methanol. The water and all the glassware and utensils used were autoclaved twice, separated by a 24-h interval.

Estrogenic activity evaluation

A yeast estrogenicity assay was conducted following the procedure described by Wang et al (2010) with slight modifications. Briefly, yeast cells were grown in a medium

(SD/Trp/Leu) at 30 °C and 130 rpm using a Thermo-Shaker (MB100–4P, AoSheng, China) for 24 h to achieve logarithmic growth. The following day, aliquots (5 μL, in DMSO) of each concentration of the quinestrol dilution series were added to 995 μL of yeast stock, yielding a final concentration of DMSO of 0.5 %; 0.5 % DMSO was used as a negative control. The test cultures were then reseeded into 96-well plates and incubated at 30 °C and 800 rpm for 2 h. The addition of 120 μL of Z-buffer (21.51 g L^{-1} $Na_2HPO_4{\cdot}12H_2O$, 6.22 g L^{-1} $NaH_2PO_4{\cdot}12H_2O$, 0.75 g L^{-1} KCl, and 0.25 g L^{-1} $MgSO_4{\cdot}7H_2O$) and 20 μL of chloroform, followed by vigorous shaking at 1,200 rpm for 10 min, killed 50 μL of each test culture. The β-galactosidase reaction was initiated by adding 40 μL of O–NPG (4 mg mL^{-1}), and the samples were then incubated at 30 °C for 60 min. The reaction was terminated by the addition of 100 μL of Na_2CO_3 and mixing at 800 rpm for another 10 min. The OD at 420 nm was measured using a 680 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The galactosidase activity was calculated as described in a previous study (Ma et al. 2005).

Chemical analysis

The concentrations of quinestrol were determined using a reversed-phase HPLC system (Jasco Technologies, Japan) equipped with a FP2020 fluorescence detector (Jasco Technologies) operated at an excitation wavelength of 278 nm and an emission wavelength of 320 nm. An Alltech® AlltimaTM C18 reversed-phase column (4.6×250 mm, 5 μm) was employed for the separation at 25 °C. The mobile phase was a mixture of acetonitrile and water (85/15, *v*/*v*), with a flow rate of 1.00 mL min^{-1}. The injection volume was 20 μL. Limit of detection for quinestrol was 0.05 mg/L. The test concentration fell fully in the linear range. The recovery rates of analytes were above 95 %.The data sets were analyzed and plotted using SigmaPlot® (Version 8.0, SPSS Inc., CA, USA) and Excel® (Microsoft Corporation, WA, USA).

Results and discussion

Degradation of quinestrol in soil

In order to evaluate the environmental risk of quinestrol in the experiment, the degradation of quinestrol was measured in both sterilized and nonsterilized soil at different temperatures. Each of the degradation curves (Fig. 3) was fitted to the following model to estimate the degradation rate constant k ($days^{-1}$) and the half-life $t_{1/2}$ (days; Table 1):

$$C_t = C_0 e^{-kt} \quad (1)$$

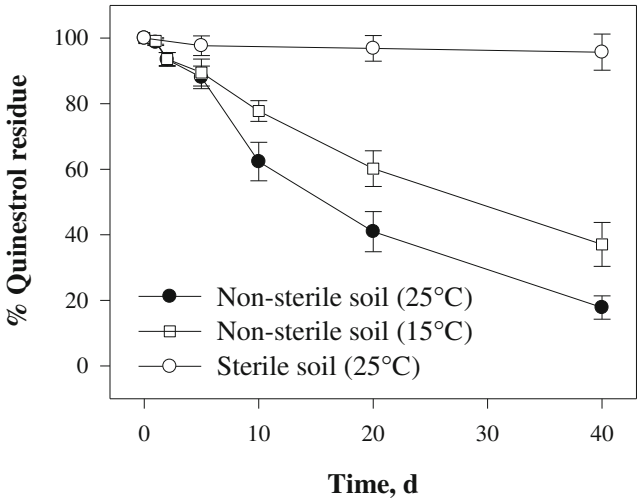

Fig. 3 Time course of the degradation of quinestrol in Maqin soil under different conditions

where C_0 (mg L^{-1}) and C_t (mg L^{-1}) are the quinestrol concentrations at time 0 and time t, respectively. All of the fits were excellent, with correlation coefficients (R^2) of >0.99. These results suggest that the degradation of quinestrol in soil followed the first-order decay model. The half-life in local soil is 15.97 d at 25 °C, while the degradation was relatively slow with the half-life of 27.62 d at 15 °C. Previous studies have investigated the microbial degradation of other steroidal estrogen hormones, e.g., estrone (E_1), 17β-estradiol (E_2), estriol (E_3), and 17α-ethinylestradiol (EE_2), in soils, and these studies found good fits to the exponential decay kinetics, consistent with our results (Jürgens et al. 2001; Layton et al. 2000; Shi et al. 2004). As shown in Fig. 3, the degradation half-life ($t_{1/2}$) of quinestrol was found to vary under two different temperatures. Our results are consistent with the results for steroidal estrogen hormones in other studies. Extensive laboratory investigations have shown that both 17β-estradiol and testosterone rapidly degrade in a range of soils incubated under a series of temperatures and humidity conditions, with half-lives typically ranging from a few hours to a few days (Lee et al. 2003; Das et al. 2004; Hanselman et al. 2003; Lorenzen et al. 2005).

In our study, quinestrol had almost no degradation in sterilized soils (Fig. 3). After 10 d of incubation under air-equilibrated conditions, the percentage of quinestrol degraded in the nonsterile soil was 41.2 % compared with 4.8 % in the sterile soil. These data indicate that microbial activity played an important role in the degradation of quinestrol in the selected soils and that microorganisms were involved in the degradation of this compound.

Given the potential application of contraceptives in farmland for field vole controlling, degradation in farmland soil was also considered in our study. Results presented here indicate that the $t_{1/2}$ of quinestrol was 5.46 days in Yuhang soil from Zhejiang Province (Table 1) under air-equilibrated

Table 1 First-order rate constant (k), half-life ($t_{1/2}$), and correlation coefficient (R^2) for the degradation of quinestrol in soil and water at different conditions

Location	Soil/water conditions	k (day^{-1})	$t_{1/2}$ (day)	R^2
Yuhang	25 °C, 70 % humidity	0.13	5.46	0.9915
Maqin	25 °C, 70 % humidity	0.04	15.97	0.9953
	15 °C, 70 % humidity	0.026	27.62	0.9985
Lamp	pH	k (h^{-1})	$t_{1/2}$ (h)	R^2
400 W mercury lamp	7.4	0.92	0.75	0.9733
400 W dysprosium lamp	4.0	0.66	1.04	0.9986
400 W dysprosium lamp	5.0	0.47	1.47	0.9991
400 W dysprosium lamp	6.0	0.20	3.46	0.9743
400 W dysprosium lamp	7.0	0.26	2.65	0.9876
400 W dysprosium lamp	7.4	0.26	2.63	0.9633
400 W dysprosium lamp	8.0	0.24	2.84	0.9906

conditions. The overall persistence of quinestrol in the Maqin soil was almost threefold longer than that in the Yuhang soil. In a previous study, the degradation of E_2 also varied among different soils under similar incubation conditions. The half-lives of E_2 in a loam soil containing 3.2 % organic matter and in a silt loam soil containing 2.9 % organic matter were 61 and 72 h, respectively, at room temperature (Jacobson et al. 2005). The different degradation rates of quinestrol in the different soils may be attributed to different indigenous microbial populations, as temporal and spatial variations can influence the microbial populations and activities in soils (Nogales et al. 2007). As previously reported, soil microbial biomass C and N increased with the addition of soil organic matter content (Sui et al. 2009). Due to soil erosion, the organic matter content in experimental soil in Maqin is considerably lower than farmland soil in Yuhang, implying that the soil in Maqin possesses different microbial populations and less microbial activities. Therefore, the slower degradation of quinestrol in Maqin soil relative to Yuhang soil may be attributed to a microbial population with a lower degradation capacity.

In the experimental area, quinestrol usually was applied to control rodents from May through July every year. The data herein indicates that quinestrol will degrade completely before the next round of drug delivery. However, many other problems should need further investigation, such as the risk to non-target organisms and the degradation rate of quinestrol in different regions.

Photodegradation of quinestrol in water

The sources of three major rivers in China are located in the Qinghai–Tibetan Plateau region. Thus, investigating the degradation of quinestrol in water is of great importance. Figure 4 presents quinestrol degradation patterns in water under two light conditions. The first-order decay coefficients for treatment with the 400 W dysprosium lamp, which mimics natural light, and the 400 W mercury lamp, which mimics ultraviolet light, were 0.26 and 0.92 h^{-1}, respectively. These values correspond to half-lives of 2.63 h and 0.75 h at pH=7.4 for the two treatments. The effect of the pH on photodegradation in solution was then examined using the 400 W dysprosium lamp; the results are shown in Fig. 5. At pH at 7 or 8, the decay rate had almost no difference, whereas more rapid degradation of quinestrol was observed under acidic conditions (pH=4 and 5). A previous study indicated that the solution pH also has a considerable effect on the photodegradation of 17 beta-estradiol in aqueous solution (Chowdhury et al. 2011). Lin reported that half-lives were 2 to 3 h for estrogens in river water irradiated with a xenon arc lamp (Lin and Reinhard 2005), and these values are consistent with our results. Maqin County, with an average elevation of 4,000 m, receives extremely intense ultraviolet radiation, which might facilitate the degradation of quinestrol. The data presented in Fig. 4 indicate that the level of light affects the decay rate of quinestrol in aqueous systems and that quinestrol released into lower-light environments will persist for a much longer time. The data

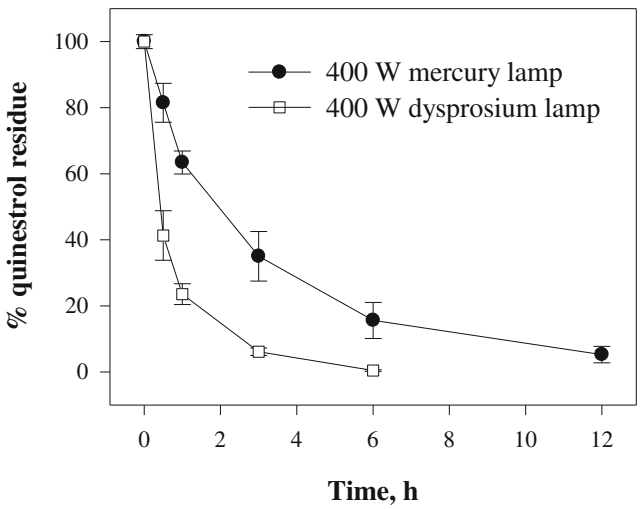

Fig. 4 Time course of the degradation of quinestrol in water at pH 7.4. A quinestrol solution with a concentration of 0.001 mg mL^{-1} was exposed to the light from a 400-W mercury lamp (λ=365 nm) or a 400-W dysprosium lamp (λ>400 nm)

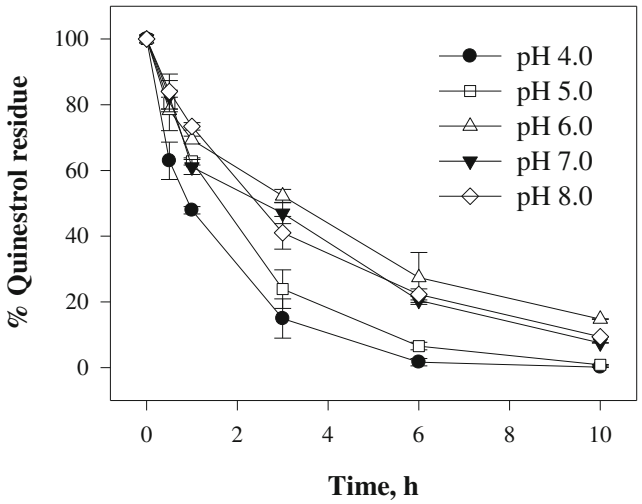

Fig. 5 Time course of the degradation of quinestrol in water at different pH values. Different pH values (4–8) were obtained by adding the appropriate amount of phosphate buffer. A 400-W dysprosium lamp was used to mimic natural light, and samples were collected at 0, 0.5, 1, 3, 6, and 12 h

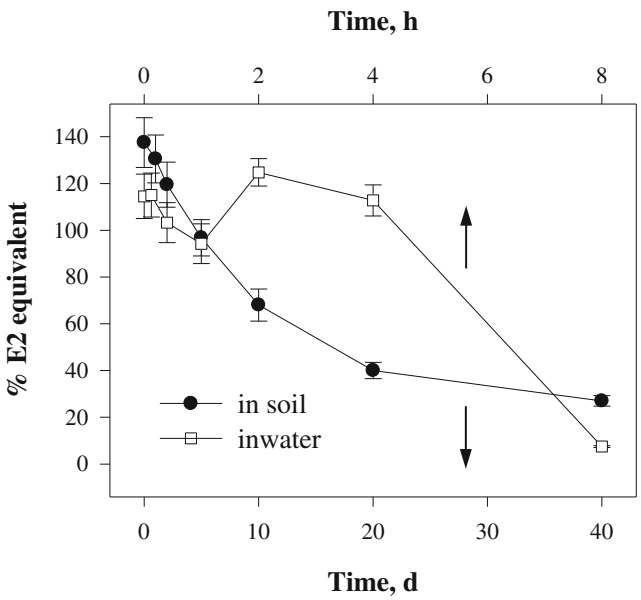

Fig. 6 The estrogenic activity of quinestrol over the entire incubation period in Maqin soil under biotic conditions (25 °C, 70 % humidity) and in water (pH 7.4) exposed to light from a dysprosium lamp. The data points represent E_2 equivalents

available on the photodegradation of several organic pollutants also show that the degradation efficiency may differ significantly depending on the intensity of photolytic irradiation, consistent with our results. For example, the photodegradation of trichloroethene by ultraviolet irradiation has the highest photodecay rate at 254 nm (Chu and Jia 2009). The half-lives of enrofloxacin and cipro in aqueous systems are dependent on the light conditions and are short when light is present (Knapp et al. 2005).

Additionally, priority should be given to the role of the pH value because this parameter may be one of major factors influencing the degradation of chemicals in aqueous systems, as demonstrated in the present study. It has been reported that compounds can be decomposed by direct and indirect photodegradation. For direct photodegradation, compounds can be significantly adsorbed at near ultraviolet (UV) light. For indirect photodegradation, chemicals can be oxidized by superoxide radicals ($O_2\cdot^-$), hydroperoxyl radicals ($HO_2\cdot$), hydroxyl radicals (·OH) or hydrogen peroxide (H_2O_2) that are generated from the photodegradation of organic compounds (Chowdhury et al. 2011). In this study, the notable difference among the reaction solutions at various pH values is the different concentration of hydrogen ions, indicating that the concentration of hydrogen ions is the key factor for the formation of the former oxides which may accelerate photodegradation by decreasing the pH values of the solution.

Estrogenic activity decay

In this study, the total estrogenic activity of the samples, either in water or soil, decreased during the incubation period (Fig. 6). In the soil system, approximately 85.7 % of the quinestrol was degraded, and an estrogenic activity of 20 % in E_2 equivalents remained after 40 days of incubation. The change in the estrogenic activity of quinestrol was fitted to a first-order decay curve, and this curve indicates that the half-life of the estrogenic activity was 10 days under our conditions. In contrast to the degradation in the Maqin soil incubated at 25±1 °C and 70 % humidity, quinestrol was almost totally degraded after 8 h of incubation in water. The estrogenic activity decreased in the early incubation period, from 0 to 1 h, and there was a large increase from 1 h to 2 h; the estrogenic activity of quinestrol sharply decreased in the late incubation period (2–8 h). Altogether, our data indicate that the estrogenic activity of quinestrol is almost completely eliminated when applied to and transported in the environment after a few days.

The estrogenic activity measured by the YES assay is subject to different effects, including antagonism, synergism, and additive effects, and, when the individual activities are known, it is possible to determine which effect is dominant (Chu and Jia 2009). Compared with the degradation rate of quinestrol, the slow elimination of total estrogenicity may be attributed to two causes. First, the major metabolite(s) of quinestrol appeared to possess even greater estrogenic activity than quinestrol itself. Therefore, the synchronous degradation of quinestrol and production of its metabolite(s) led to the observed behavior of the total estrogenicity, and the subsequent degradation of the metabolite(s) led to its slow elimination. Our results for the estrogenic activity decay in water from 1 to 2 h may be due to this type of decay behavior. Payne et al (2000) assessed four-component xenoestrogen mixtures

containing *o,p′*-DDT, genistein, 4-nonylphenol, and 4-*n*-octylphenol using models of concentration addition and independent action while observing an additive combination effect. Second, the synergistic effect of quinestrol and its metabolite(s) may be another cause of this phenomenon; however, because the metabolite(s) were not identified, this hypothesis could not be confirmed in this study and, therefore, merits future investigation. On the other hand, previous studies report that the degradation rate of compounds is controlled by many different factors such as organic matters, light intensity, and pH in various media (Jacobson et al. 2005; Chu and Jia 2009). In our study, the different production rates of intermediate and pathway between biodegradation and photodegradation results in different behavior of elimination of estrogenic activity of quinestrol in soil and water.

In summary, we conducted a preliminary study on the degradation of quinestrol in both soil and aqueous systems. Our results indicate that quinestrol has short half-lives and that the estrogenic activity is quickly eliminated under our experimental conditions. The present evaluation of the environmental fate of quinestrol provides information that will be valuable in assessing potential use of this compound and in developing risk assessment procedures. Estimation on environmental safety for special pharmaceuticals should take concurrently in both environmental behavior and its pharmacologic action. An adequate basis for safety assessment on quinestrol, as presented herein, should combine its environmental degradation dynamics with estrogenic effects.

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