ENVIRONMENTAL FATE AND CHEMISTRY OF RALOXIFENE HYDROCHLORIDE

JEROLD SCOTT TEETER* and ROGER D. MEYERHOFF
Lilly Research Laboratories, Division of Eli Lilly and Company, 2001 West Main Street DCGL45, Greenfield, Indiana 46140, USA

(Received 17 May 2001; Accepted 21 October 2001)

Abstract—Raloxifene hydrochloride is a selective estrogen receptor modulator (SERM) used for the prevention and treatment of osteoporosis in women. Excretion of raloxifene occurs through the feces of patients. Raloxifene has the potential to be discharged into waste treatment systems after therapeutic use. Raloxifene hydrochloride was investigated using a battery of studies designed to describe its physical/chemical characteristics and define its fate in the environment. The mean measured solubility of raloxifene hydrochloride (± standard deviation) was 345.2 ± 15.6 µg/ml, 13.3 ± 0.6 µg/ml, 0.9224 ± 0.015 µg/ml, and 627.4 ± 132.0 µg/ml in aqueous buffers at pH 5, 7, and 9 and in unbuffered water, respectively. Raloxifene exhibited a mean molar absorptivity of 34,000 and a wavelength absorbance maximum at 287 nm for pH 5 and 7 aqueous buffer solutions and 297 nm at pH 9. Mean measured Kₚ values were 516 ± 17, 1,323 ± 91, and 1,556 ± 135 at pH 5, 7, and 9, respectively. After 5 d at 50°C, raloxifene hydrolyzed 8.02, 10.61, and 23.81% in pH 5, 7, and 9 aqueous buffers, respectively. In a 28-d hydrolysis study at 25°C, the calculated first-order hydrolysis rates were 6.92 × 10⁻³, 1.70 × 10⁻³, and 7.66 × 10⁻⁴/d, and the corresponding half-lives were 1,001, 410, and 90 d in pH 5, 7, and 9 aqueous buffers, respectively. Raloxifene sorbed significantly to sewage treatment solids with Freundlich isotherm adsorption coefficients K between 2,000 and 3,000. Raloxifene degraded rapidly in the presence of sewage solids. In a system containing 0.470 g/L sludge solids, the raloxifene biodegradation rate and half-life were 0.0966/h and 7.17 h, respectively. In a 28-d aerobic–aquatic biodegradation study containing 30 mg/L sludge solids, the raloxifene biodegradation rate and half-life were 0.0188/d and 37 d, respectively. Given the fate and behavior of raloxifene in these studies, it is anticipated that raloxifene would rapidly dissipate in the environment.

Keywords—Raloxifene Hydrochloride Environmental fate Environmental chemistry Biodegradation

INTRODUCTION

Raloxifene hydrochloride, CAS [82640-04-8], is the generic designation for [6-hydroxy-2-(4-hydroxyphenyl)benzo [b]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]-phenyl]methanone hydrochloride (Fig. 1). Raloxifene hydrochloride is a pharmaceutical compound used for the prevention and treatment of osteoporosis in women. It has been shown to preserve bone density, reduce bone turnover, and reduce the incidence of vertebral fractures in postmenopausal women with osteoporosis [1–3]. Raloxifene is a selective estrogen receptor modulator with agonist and antagonist properties [1,4]. In vivo, it is excreted almost exclusively as a glucuronide conjugate via the feces of patients [5–7]. The glucuronide conjugate is readily hydrolyzed back to the free form of the compound in the colon [5]. Since raloxifene has the potential to be discharged into waste treatment systems after therapeutic use by women, it is important to characterize the chemistry and fate of the molecule in the aquatic environment.

The fate of a compound in the environment is a function of its polarity, solubility in water, and susceptibility to hydrolysis, photolysis, and biodegradation. Additionally, its partitioning behavior between solids and the aqueous environment and in sewage treatment facilities contributes to its fate in the environment. The information presented here was collected to characterize the fate and behavior of raloxifene in the aquatic environment.

MATERIALS AND METHODS

Test material

The raloxifene hydrochloride test material used for these studies was prepared by Eli Lilly (Indianapolis, IN, USA).

* To whom correspondence may be addressed (jsteeter@lilly.com).

Unlabeled test material and reference standard was 99.5% or greater purity. Labeled ¹⁴C-carboxyl-raloxifene hydrochloride (33.4 µCi/mg) was also prepared by Eli Lilly. The radiochemical purity was reported as 99+%. The position of the carbon-14 label is indicated on the structure provided in Figure 1. Raloxifene hydrochloride is a nonvolatile solid with a melting temperature of 257.8 ± 0.2°C.

Analytical methods

Analysis for raloxifene was performed using a reverse-phase high-performance liquid chromatography (HPLC) method. A DuPont Zorbax® RX-C8 column (4.6 × 250 mm, 5-µm particle size, Mac-Mod Analytical, Chadds Ford, PA, USA) was used for separation. The mobile phase was a 65:35 (v/v) mixture of 75 mM pH 2 phosphate buffer and acetonitrile. The separation was conducted under isocratic conditions at a flow rate of 1.0 ml/min with ultraviolet detection at 280 nm. Injection volume was 100 µl. Raloxifene was quantitated by comparison of the areas of sample peaks against a multipoint standard curve.

Samples for HPLC analysis were extracted by the addition of, at a minimum, an equal volume of mobile phase to sample (1:1, v/v). The mixture was vortexed briefly, and for samples containing solids, the extracts were placed in an ultrasonic bath for 5 min. The sample extracts were centrifuged for 15 min at 1,400 g and 25°C. The extract supernatant was sampled and assayed by HPLC after further dilution with mobile phase as necessary.

Liquid scintillation was performed using a Beckman® LS 6000 SC scintillation spectrometer (Beckman Instruments, Fullerton, CA, USA). Samples were dissolved in 12 ml of Beckman ready gel liquid scintillation cocktail and were count-
The solubility of raloxifene was measured in unbuffered Nanopure® water (Barnstead Thermoline, Boston, MA, USA) and pH 5, 7, and 9 aqueous buffers. Buffers used were 0.01 M sodium acetate, 0.05 M sodium/potassium phosphate, and 0.0375 M sodium borate for pH 5, 7, and 9, respectively.

Studies in water and in pH 5 and 7 buffers were conducted at 25 ± 1°C by the flask saturation method [8]. Samples were collected between 23 and 144 h and were immediately centrifuged at 30,000 g at 25°C followed by careful transfer of the supernatant to a glass tube where the sample was centrifuged at 3,500 g for 30 min at 25°C. An aliquot of the final supernatant was assayed for raloxifene by HPLC. The process of double transfer and centrifuging was necessary because of the high surface tension of the solid raloxifene observed in the samples. Method development studies showed that the transfer and centrifuging process was quantitative for raloxifene dissolved in solution.

Raloxifene solubility in pH 9 buffer was determined at 25 ± 1°C by a column saturation method as follows. A saturation column was prepared by coating 5 g of Chromosorb® 750 (Celite, Lompoc, CA, USA) with 49.7 mg of raloxifene hydrochloride. The asterisk indicates the position of the 14C-label of the radioactive material.

ed between 3 and 10 min. Appropriate background samples and controls were used to automatically convert counts per minute (cpm) to disintegrations per minute (dpm).

Solubility studies

The solubility of raloxifene was determined in unbuffered Nanopure® water (Barnstead Thermoline, Boston, MA, USA) and pH 5, 7, and 9 aqueous buffers. Buffers used were 0.01 M sodium acetate, 0.05 M sodium/potassium phosphate, and 0.0375 M sodium borate for pH 5, 7, and 9, respectively.

Studies in water and in pH 5 and 7 buffers were conducted at 25 ± 1°C by the flask saturation method [8]. Samples were collected between 23 and 144 h and were immediately centrifuged at 30,000 g in polypropylene copolymer centrifuge tubes for 1 h at 25°C followed by careful transfer of the supernatant to a glass tube where the sample was centrifuged at 3,500 g for 30 min at 25°C. An aliquot of the final supernatant was assayed for raloxifene by HPLC. The process of double transfer and centrifuging was necessary because of the high surface tension of the solid raloxifene observed in the samples. Method development studies showed that the transfer and centrifuging process was quantitative for raloxifene dissolved in solution.

Raloxifene solubility in pH 9 buffer was determined at 25 ± 1°C by a column saturation method as follows. A saturation column was prepared by coating 5 g of Chromosorb® 750 (Celite, Lompoc, CA, USA) with 49.7 mg of raloxifene hydrochloride. The asterisk indicates the position of the 14C-label of the radioactive material.

Ultraviolet-visible absorption in pH 5, 7, and 9 aqueous buffers

The ultraviolet-visible absorption spectra for raloxifene were determined at 25 ± 1°C in methanolic pH 5, 7, and 9 aqueous buffers. Each of the prepared buffers, as described in solubility previously, was diluted 50:50 (v/v) with methanol. The pH of the mixtures was readjusted to 5.00, 7.01, and 9.00 with 50% acetic acid. The use of methanol as a cosolvent was necessary because of solubility limitations of raloxifene in the aqueous buffers alone. The final concentration of raloxifene in the solutions was 2.45 × 10⁻⁶ M. Absorbance spectra were determined against appropriate blank solutions. Values for lambda max, bandwidth, and molar absorptivity were determined from the spectral data.

Octanol/water partition coefficient

Two sets of nine vessels (50-ml Erlenmeyer flasks with ground glass stoppers) were used to determine the partition coefficients of raloxifene in n-octanol/pH 5 buffer, n-octanol/pH 7 buffer, and n-octanol/pH 9 buffer. The buffers were prepared according to [9] and were pre-equilibrated prior to use with excess n-octanol by gently shaking for 16 h at room temperature. Similarly, n-octanol was pre-equilibrated with water. The pre-equilibrated solutions were separated prior to use. Ten milliliters of n-octanol and 25 ml of buffer were used in each vessel. One set of vessels was spiked with a 0.10-ml aliquot of a 0.251-mg/ml ¹⁴C-raloxifene HCl in methanol. The second set of nine vessels was spiked with 0.10 ml of a 3.01-mg/ml solution of ¹⁴C-raloxifene HCl in methanol. The vessels were incubated at 25°C and shaken with an orbital shaker at approximately 125 rpm. At 23-, 46-, and 72-hour time intervals, triplicate samples from each phase were collected from the vessels and analyzed by liquid scintillation counting (LSC).

Radiochemical assay values were converted to molar concentrations of raloxifene in each phase. Octanol/water partition coefficients were calculated as the ratio of the molar concentration in n-octanol to the molar concentration in the aqueous phase.

The stability of raloxifene in n-octanol was confirmed independently from the partition coefficient determination by measuring the raloxifene concentration in an n-octanol solution at time zero and after 3 d of incubation at 25°C. The solubility of raloxifene in n-octanol was determined by adding excess raloxifene to a flask of n-octanol. The solution was equilibrated at 25°C for 22 h. A sample was centrifuged, extracted, and assayed by HPLC.

Hydrolysis

A hydrolysis test at 50 ± 1°C in pH 5 buffer, 0.01 M sodium acetate, pH 7 buffer, 0.067 M sodium/potassium phosphate buffer, and pH 9 buffer, 0.035 sodium borate was conducted according to Eirkson et al. [10]. All glassware and materials were sterilized either by autoclaving or by filtration through a 0.22-µm filter. The test was conducted in darkness in triplicate 50-ml-volume Erlenmeyer flasks with ground-glass stoppers. The samples contained less than 0.4% of methanol carrier solvent. Initial measured mean concentrations of raloxifene were 1.035 ± 0.013, 1.027 ± 0.012, and 0.390 ± 0.032 µg/ml for pH 5, 7, and 9 aqueous buffers, respectively. Samples were taken and assayed for raloxifene after 5 d of incubation.

A 28-d hydrolysis study was conducted at 25°C with raloxifene according to Eirkson et al. [10]. The study was con-
ducted in 0.01 M sodium acetate, 0.067 M sodium/potassium phosphate buffer, and 0.025 M sodium borate buffer for pH 5, 7, and 9, respectively. At selected time intervals, samples were assayed for raloxifene by HPLC. Initial starting concentrations of raloxifene were 0.96 µg/ml for pH 5 and 7 and 0.40 µg/ml for pH 9. The data were evaluated by a first-order least-squares analysis.

Activated sludge collection and preparation

Activated secondary sewage sludge used in these experiments was obtained from Greenfield (IN, USA) sewage treatment plant. The facility treats predominantly domestic sewage. The sludge was aerated continuously at room temperature after collection. Prior to use, the sludge was homogenized with a Brinkmann Polytron® (Brinkmann Instruments, Westbury, NY, USA) to ensure homogeneity. The concentration of solids was determined gravimetrically by filtering a known sample volume of sludge solution through a 1-µm filter and measuring the dry weight of the solids after oven drying at 103°C.

Adsorption/desorption from human feces

Human fecal material from a drug metabolism study in which 14C-raloxifene HCl was administered was used to measure desorption kinetic data and adsorption coefficients for raloxifene. The fecal material was stored at −80°C prior to use. The material was thawed at room temperature overnight and homogenized with a small amount of water prior to use. The material was diluted to 6.6540, 1.3308, and 0.2662 g/L of solids (on a dry-wt basis) by the addition of sterilized sewage sludge broth. The corresponding nominal concentrations of 14C-raloxifene in the test systems were 24,683 dpm/ml (20.690 µg/ml), 4,937 dpm/ml (4.138 µg/ml), and 987 dpm/ml (0.828 µg/ml), respectively. The activated sewage sludge broth was prepared by decanting settled sludge through a column of glass wool to remove any solids. Approximately one-half of the volume of the broth was autoclaved for 15 min at 121°C and designated as the sterilized broth. The remaining volume of broth was not autoclaved and was designated the nonsterilized broth. The test systems were gently shaken at 25°C. At 0, 2, 4, 8, and 24 h, homogeneous samples were collected from the systems, and the concentrations of radioactivity as measured by LSC and raloxifene as measured by HPLC were determined in the aqueous and solid fractions. Human feces adsorption coefficients were determined using the raloxifene HPLC data. The same procedures and dilutions were conducted to prepare test systems with the nonautoclaved broth.

Aliquots of each dilution were placed into triplicate 50-ml Corex® glass centrifuge tubes (Corning, NY, USA) with Teflon®-lined caps. Two replicates were for sampling and adsorption data determination. The third was used to collect pH and dissolved oxygen measurements. The test vessels were shaken at approximately 75 rpm on an orbital shaker while dissolved oxygen measurements. The test vessels were shaken at approximately 75 rpm while equilibrated in a constant temperature chamber at 25°C. At 4, 8, and 24 h, the tubes were centrifuged for 15 min at 1,400 g. The supernatant was sampled and assayed by LSC. The concentration of the 14C in the solid fraction was calculated by difference between what was added and what was recovered in the aqueous phase. After 24 h, a homogeneous sample was taken from each vessel and assayed for total radioactivity and a material balance determined. A second homogenous sample was extracted and assayed by HPLC to determine the stability of raloxifene in the test system.

The degree of sorption was considered by two models: the Freundlich isotherm and a linear isotherm model [11]. Two coefficients are described: K, the Freundlich adsorption coefficient, and Kd, a simple partition coefficient. The latter coefficient, Kd, is the ratio of the test chemical concentration (in this case 14C-containing chemical species) associated with the solid phase to that which remains in solution at equilibrium:

\[ K_d = \frac{\text{Concentration associated with solid}}{\text{Concentration in solution}} \]  

The Freundlich adsorption coefficient, K, is described by the Freundlich equation as follows:

\[ \frac{x}{m} = KC^n \]  

where x is the mass of solute adsorbed on the mass of sorbent m, C is the equilibrium concentration of the solute in the bulk solution, and 1/n is a factor indicative of the isotherm linearity. By taking the log of both sides of the Freundlich equation, a linear expression is obtained as follows:

\[ \log \frac{x}{m} = \log K + \frac{1}{n} \log C \]  

The slope 1/n is typically 0.7 to 1.1 and is an inherent property of the thermodynamic interaction between the solute and adsorbent.

Degradation of raloxifene was observed in the adsorption coefficient study. An additional experiment was conducted to
characterize the rate of degradation as follows. Three tubes were prepared with 0.470 g/L sewage sludge. The tubes contained both the radioactive raloxifene spike and the sodium azide inhibitor at the previously mentioned concentrations. After 0, 2, 4, 8, and 24 h of equilibration, homogeneous samples were removed, extracted, and assayed for raloxifene by HPLC. Linear regression was used to determine a first-order degradation rate and half-life for raloxifene in the system based on the HPLC analytical data.

**Aerobic biodegradation in water**

An aquatic aerobic biodegradation test was conducted at 22 ± 3°C following the procedures outlined in Eirkson et al. [12]. Nine vessels, each connected to a series of traps for collection of 14C-radiolabeled volatile compounds, were used to incubate 5 mg/L of 14C-raloxifene or 14C-sodium benzoate with 30 mg activated sludge/L (suspended solids, dry-wt basis) in 750 ml of a mineral salts medium [12]. Three replicates for each treatment and blank control were prepared. The 14C-sodium benzoate was used as a readily biodegradable positive control to test the functionality of the test system apparatus. The reaction vessel and trapping apparatus is illustrated in Figure 2. A vacuum system maintained aerobic conditions by providing a constant flow of air through the system. At 1-h intervals, controlled by an automatic timer, air was pulled rapidly through the vessels for 5 min to drive any 14C-volatile compound into the traps. The vessels were covered with foil to exclude light.

The traps were removed from each system and assayed for 14C and replaced with fresh traps on days 1, 2, 3, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25, and 28. On days 0, 1, 3, 7, 14, 21, and 28, samples were removed from each vessel and analyzed for total 14C by LSC and raloxifene by HPLC. At the same time, samples were measured for dissolved oxygen. On days 14 and 28, the aqueous concentrations of 14C and raloxifene were determined by centrifuging the samples and analyzing the supernatant. Additionally, on days 7 and 21, samples were assayed to determine the presence of breakdown products.

Six potential breakdown products of raloxifene, prepared by Lilly Research Laboratories, were used for tentative HPLC identification based on coincident retention times. The chemical structures of the two breakdown products tentatively identified in this study, compound 334488 (raloxifene dimer) and compound 309398 (N-oxide of raloxifene), are shown in Figure 3. Tentative confirmation of the presence and identity of the breakdown products was conducted on a second HPLC system. The second system utilized a Nucleosil® C-18 (5 μm) 25-cm × 4.6-mm column (Varian, Palo Alto, CA, USA) for separation and a mobile phase of 90:10 of initial mobile phase acetonitrile pumped at a flow rate of 0.8 ml/min.

At the conclusion of the study, the distribution of 14C between the aqueous fraction and the biomass was determined following filtration through a 1-μm filter of three 5-ml aliquots for each vessel. Dried filtered solids were analyzed for 14C by combustion of the filter using a Harvey Biological sample oxidizer (Harvey, Hillsdale, NJ, USA) followed by LSC. The three filtrates were analyzed directly for 14C.

**RESULTS**

**Solubility**

Although raloxifene is zwitterionic in chemical character and carries an ionic charge at all pH values, it is increasingly insoluble as pH increases to about 9 (Table 1).

**Ultraviolet-visible absorption spectra**

The light absorption characteristics for raloxifene in dilute methanolic-aqueous buffers at pH 5, 7, and 9 are in Table 1. Wavelengths scanned were between 200 and 800 nm. Mean molar absorptivity values were approximately 34,000. The wavelength absorbance maximum occurred at 287 nm for both the pH 5 and the pH 7 solutions and 297 nm for the pH 9 solution.

**Octanol/water partition coefficient**

Octanol/water partition coefficient increased with increasing pH of the aqueous phase (Table 1). Raloxifene solubility in n-octanol was determined to ensure that, at equilibrium, the concentration of raloxifene in the n-octanol was not limited by solubility. The solubility of raloxifene in n-octanol was 43 μg/ml. Octanol concentrations achieved a maximum value of
Environmental fate/chemistry of raloxifene hydrochloride

Table 1. Physical/chemical parameters for raloxifene hydrochloride at 25 ± 1°C

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH 5</th>
<th>pH 7</th>
<th>pH 9</th>
<th>Water (4.48–5.38)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility (SD)* μg/ml</td>
<td>345.2 (15.6)</td>
<td>13.3 (0.6)</td>
<td>0.9224 (.015)</td>
<td>627.4 (132.0)</td>
</tr>
<tr>
<td>UV-visible spectra</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>λ max (nm)</td>
<td>287</td>
<td>287</td>
<td>297</td>
<td>ND</td>
</tr>
<tr>
<td>Bandwidth (nm)</td>
<td>62</td>
<td>61</td>
<td>62</td>
<td>ND</td>
</tr>
<tr>
<td>ε (SD)</td>
<td>34,245 (634)</td>
<td>34,087 (705)</td>
<td>33,967 (666)</td>
<td>ND</td>
</tr>
<tr>
<td>Octanol/water partition coefficient, ( K_{ow} ) (SD)</td>
<td>516 (17)</td>
<td>1,323 (91)</td>
<td>1,556 (135)</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Resulting pH of saturated solution.
* SD = standard deviation.
* UV = ultraviolet.
* ND = not determined.

32 μg/ml raloxifene in the high-concentration tests. However, the measured octanol/water partition coefficient values were apparently unaffected since the difference between octanol/water partition coefficient values measured for the low and high concentration tests were negligible. Raloxifene did not degrade in the n-octanol stability test. Similarly, for the duration of the partition experiment, raloxifene was stable in aqueous media as determined in the hydrolysis study and did not approach the solubility limit at any of the pH values tested.

Hydrolysis

The results of the hydrolysis experiments with raloxifene are provided in Table 2. Normalized first-order plots are provided in Figure 4. The hydrolysis rate of raloxifene increased with increasing pH of the aqueous medium. This result was observed at both temperatures studied.

Feces adsorption/desorption

Recovery differences for 14C observed between sterilized and nonsterilized broth treatments were negligible. After 24 h of equilibration, recovery of radioactivity from the vessels was between 85 and 107%. After 24 h, the recovery of raloxifene in the test systems as measured by HPLC was between 84.2 and 104.4% for the sterilized broth systems and between 63.8 and 101.7% for the nonsterilized broth systems.

As listed in Table 3, calculated solid–liquid partition coefficients, \( K_d \) values, for raloxifene generally increased with increasing equilibration time and decreasing solids level. Mean \( K_d \) values ranged from 2,797 to 12,093 in the sterilized broth systems and from 1,577 to 8,953 in the nonsterilized broth systems. Within experimental error, the concentrations of 14C in the aqueous phase paralleled the HPLC-measured raloxifene concentrations. In the systems where raloxifene degradation was observed, the 14C was sorbed to the solids to approximately the same degree as raloxifene.

Freundlich isotherm determinations provided overall adsorption coefficients \( K_f \) for raloxifene and are provided in Table 4. No marked differences between the sterilized and nonsterilized broth systems were observed. Isotherm determinations for both systems combined ranged from a low of 2,080 at 2 h to a high of 2,906 at 4 h. The 24-h combined coefficient was 2,521. These results indicate that sorption of raloxifene to the solids was rapid and considerable.

Adsorption/desorption and biodegradation in sewage sludge

In the initial adsorption experiment, the material balance determination after 24 h of equilibration indicated extensive degradation of raloxifene as measured by HPLC. The degradation was greatest in the 0.633- and 0.190-g/L sludge systems, leading to a conclusion that the degradation results may be related to dissolved oxygen levels in the test vessels. Additional experimentation showed that the dissolved oxygen was rapidly depleted in the 7.029-g/L sludge system and measured less than 0.36 mg/L O2 after 24 h. The 2.109-g/L sludge systems contained between 3.70 and 3.90 mg/L dissolved oxygen after 24 h. The 0.633- and 0.190-g/L sludge systems maintained aerobic character as indicated by dissolved oxygen levels between 6.52 and 7.86 mg/L. High initial solids and microbial populations probably depleted oxygen levels quickly in the tightly capped test vessels. Reduced oxygen levels may have inhibited aerobic degradation of raloxifene in the higher-solids treatments.

Table 2. Hydrolysis data for raloxifene hydrochloride

<table>
<thead>
<tr>
<th>pH</th>
<th>50°C, 5 d % Hydrolyzed</th>
<th>25°C, 28 d ( k/d )</th>
<th>( t_{1/2} ) (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>8.02</td>
<td>6.92 × 10^4</td>
<td>1,001</td>
</tr>
<tr>
<td>7</td>
<td>10.61</td>
<td>1.70 × 10^3</td>
<td>410</td>
</tr>
<tr>
<td>9</td>
<td>23.81</td>
<td>7.66 × 10^3</td>
<td>90</td>
</tr>
</tbody>
</table>

Fig. 4. First-order plot of raloxifene hydrolysis at 25°C. The \( C_t \) is the concentration of raloxifene measured at time \( t \).
Sewage sludge
covery of 91.2% (C0 predicted).
linear relationship (Fig. 5). The first-order degradation rate
sludge level was evaluated for kinetic information. The time-
observed. After 24 h of equilibration, the 
were dependent on both solids levels and degree of raloxifene
4, 8, and 24 h of equilibration, respectively. In these systems, the greater
species were dependent on both solids levels and degree of raloxifene
data from both broths used to calculate isotherm parameters.
Aerobic biodegradation in water
The pH of the vessel contents ranged between 6.75 and
7.12 at study initiation and between 5.50 and 5.77 at the conclusion of the study. Dissolved oxygen concentrations did not change substantially throughout the study and ranged between 7.4 and 8.0 mg/L.
The sodium benzoate reference compound was degraded substantially in the test. Recovery of total 14 C was between 53 and 79%. After 1 d of incubation, the concentration of 14 C measured in the sodium benzoate vessels was reduced to an average of 42% of the initial concentration. At the conclusion of the study, the concentration of 14 C measured in the vessels averaged 12% of the initial concentration. An average of 55.7% of the original 14 C label was recovered in the 14 CO2 traps for the sodium benzoate vessels, indicating extensive mineralization. An average of 36.8% of the total 14 C added was recovered in the traps by the first day. Calculated Kd values for the 14 C label on day 28 ranged from 88,333 to 132,464, indicating extensive incorporation of the label into the biomass.
Raloxifene did not mineralize to CO2 or other volatile compounds. Recovery of total 14 C was between 103 and 104% for the raloxifene vessels. The concentration of 14 C radioactivity was constant throughout the study and ranged between 98 and 106% of the initial value when evaluated on day 28. After 28 d, a total of 0.09% of the total 14 C radioactivity added as raloxifene was recovered in the traps. The 14 C radioactivity was highly concentrated in the solids fraction, resulting in calculated Kd values ranging from 44,869 to 54,674.
The concentration of raloxifene as measured by HPLC decreased over time in the test vessels. Using first-order kinetics, a plot of the logarithm of raloxifene concentration versus time provided a linear relationship (Fig. 6) with a correlation coefficient (>0.999).

Simple Kd values for 14 C, including raloxifene and its degradation products as measured by LSC, ranged from 1,218 to 12,433, 3,705 to 15,437, and 2,694 to 21,944 after 4, 8, and 24 h of equilibration, respectively. Adsorption coefficients for 14 C species in secondary sewage sludge over the duration of the experiments studied.

<p>| Table 3. Partition coefficient (Kd) values for raloxifene in human feces and 14C in secondary sewage sludge |</p>
<table>
<thead>
<tr>
<th>Solids level (g/L)</th>
<th>Kd (ml/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human fecesa</td>
<td></td>
</tr>
<tr>
<td>Sterile broth</td>
<td></td>
</tr>
<tr>
<td>6.6540</td>
<td>1.312</td>
</tr>
<tr>
<td>1.3308</td>
<td>7.169</td>
</tr>
<tr>
<td>0.2662</td>
<td>9.869</td>
</tr>
<tr>
<td>Nonsterile broth</td>
<td></td>
</tr>
<tr>
<td>6.6540</td>
<td>1.291</td>
</tr>
<tr>
<td>1.3308</td>
<td>2.968</td>
</tr>
<tr>
<td>0.2662</td>
<td>6.980</td>
</tr>
<tr>
<td>Sewage sludgeb</td>
<td></td>
</tr>
<tr>
<td>7.029</td>
<td>ND</td>
</tr>
<tr>
<td>2.109</td>
<td>ND</td>
</tr>
<tr>
<td>0.633</td>
<td>ND</td>
</tr>
<tr>
<td>0.190</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Values as determined from high-performance liquid chromatography (HPLC)–measured raloxifene.
b Values as determined from 14 C measured by liquid scintillation counting (LSC).
c ND = not determined.

In summary, as raloxifene degraded in the test systems, the 14 C label became increasingly associated with the solids. This indicates that for this test system, incorporation of the breakdown products into the solid biomass occurred preferentially to the release of breakdown products into the bulk solution over the duration of the experiments studied.

| Table 4. Freundlich isotherm coefficients (K, 1/n) and correlation coefficient (r) for raloxifene desorption from human feces |
|-------------|--------------|--------------|--------------|--------------|--------------|
| Broth       | Time 0       |              | Time 2 h     |              | Time 4 h     |              | Time 8 h     |              | Time 24 h    |              |
|             | K            | 1/n          | r            | K            | 1/n          | r            | K            | 1/n          | r            | K            | 1/n          | r            |
| Sterile     | 2.217        | 0.212        | 0.841        | 2.147        | 0.068        | 0.273        | 2.635        | 0.183        | 0.749        | 2.982        | 0.270        | 0.763        | 2.747        | 0.263        | 0.905        |
| Nonsterile  | 2.157        | 0.260        | 0.850        | 2.019        | 0.015        | 0.078        | 3.194        | 0.348        | 0.887        | 2.689        | 0.220        | 0.809        | 2.330        | 0.301        | 0.905        |
| Both        | 2.187        | 0.233        | 0.837        | 2.080        | 0.041        | 0.185        | 2.906        | 0.267        | 0.803        | 2.831        | 0.245        | 0.770        | 2.521        | 0.277        | 0.843        |

a Indicates combined data for both broths used to calculate isotherm parameters.
Raloxifene possesses three pKₐ values reported as 8.95, 9.83, and 10.91 (unpublished internal communication). Raloxifene is zwitterionic by simultaneously possessing both anionic and cationic structural moieties over a pH range of approximately 7 to 11. Although raloxifene carries an ionic and cationic structural moieties over a pH range of approximately 9.83, and 10.91 (unpublished internal communication). Raloxifene measured at time t₀.

Selected sample extracts were compared to known raloxifene degradation product standards to qualitatively identify breakdown products. A day 7 sample extract from a raloxifene vessel was assayed by HPLC. Of the ¹⁴C injected into the HPLC system, 125% was recovered from the chromatography column. Of the total that was recovered, 35,73% was in the retention window for raloxifene hydrochloride, and 15.23% was in the window corresponding to the raloxifene dimer (compound 334488). No other known degradation products were identified in the extract. On day 21, a sample was collected and assayed as before. Two compounds, the raloxifene dimer and the N-oxide of raloxifene, were tentatively identified in the sample extract. The presence of these two compounds was confirmed by a second HPLC method, as indicated by coincident retention times in the sample, standard solutions, and mixtures of the sample and standard solutions.

**DISCUSSION AND CONCLUSION**

Raloxifene absorbs light energy. Given that the absorbance maxima are centered near the atmospheric cutoff wavelength of 290 nm, the potential exists for raloxifene to photolyze on exposure to natural sunlight. However, the presence of light absorbance alone does not ensure photochemical breakdown.

The partition coefficient data correlate well with the hydrophobic molecular behavior of raloxifene as observed in the solubility study. That is, as pH increases, the relative hydrophobicity of raloxifene increases. As the hydrophobicity of the compound increases, so does the n-octanol/water partition coefficient.

The rate of raloxifene hydrolysis increases with increasing pH. At pH 5, the half-life was approximately three years, while at pH 9, it was approximately three months. Although raloxifene does undergo hydrolysis, sorption and biodegradation would be predominant mechanisms for dissipation in the environment.

The results from the sorption experiments indicate that raloxifene is extensively sorbed to solids. This is an expected result since hydrophobic compounds tend to sorb to solids rather than remain in solution. The low water solubility of raloxifene alone would lead to a conclusion that sorption would be significant. It was also noted during method development and validation that raloxifene sorbed to glass, especially at pH values greater than 7. This hydrophobic behavior correlates well with the solubility data presented; that is, as pH increases, solubility decreases.

The results of the feces adsorption/desorption studies indicate that raloxifene desorbs from human feces and partitions into aqueous solution. Given time, an equilibrium condition would be established that approximates the adsorption coefficient. However, the process of biodegradation continually disturbs the equilibrium. The lower recovery values of raloxifene as measured by HPLC observed in the nonsterilized systems are probably the result of aerobic metabolism.

Raloxifene degraded rapidly under the conditions tested. More vigorous real-life simulations, as in sewage treatment, would lead to faster degradation rates than those found in these studies. The time points investigated in these studies provide information on the kinetic behavior of raloxifene and correlate well with what is likely in actual waste scenarios. No evidence exists to suggest that raloxifene or its degradation products would accumulate in the environment.

Discharge of raloxifene into the environment occurs through the feces of patients. Raloxifene introduced through sewage treatment desorbs from feces and redistributes through adsorption to the sewage solids. The large majority of the compound would remain sorbed and associated with the solid phase of the treatment system. Raloxifene’s limited solubility, moderate octanol/water partition coefficient, and high Kᵣ on sludge further confirm that it will significantly sorb onto solids. During its residence time in the treatment facility, raloxifene would be readily degraded, as demonstrated in the sewage biodegradation studies. The aeration and activity of the microbes in the secondary treatment basin should further expedite the rate of biodegradation above that presented here. The biodegradation studies conducted were at sludge levels that were approximately 4 to 10 times more dilute than levels found in typical sewage treatment facilities. Raloxifene would not be expected to partition to the gas phase since it is a nonvolatile solid. The potential for direct photolysis is also possible on the basis of its light absorption characteristics, as is hydrolysis with extended contact with water. The results presented illustrate the transient nature of raloxifene in the aquatic environment and indicate that accumulation of raloxifene would not be expected.
Acknowledgement—Special thanks to Alison N. Perkins and Shannon D. Banks for their review and comments on this manuscript. Thanks to W.A. Tony Althaus and Mark D. Gunnoe for conducting the melting temperature study.

REFERENCES