The Kinetics of Oxytetracycline Degradation in Deionized Water under Varying Temperature, pH, Light, Substrate, and Organic Matter

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Abstract.—The kinetics of oxytetracycline (OTC) degradation in deionized water was studied under the influence of various environmental factors. The experiment was conducted with a solution of 10 µg aqueous OTC/mL in 600-mL glass beakers under controlled laboratory conditions. The aqueous concentration of OTC was determined by high-performance liquid chromatography. Low temperatures (4°C) favored high drug stability, and high temperatures (43°C) speeded OTC degradation, resulting in a very short half-life of 0.26 ± 0.11 d. Light exposure caused photodecomposition, reflecting degradation rates threefold higher than those under dark conditions. Acidic conditions (pH 3.0) favored drug stability (half-life = 46.36 ± 4.92 d), and alkaline conditions (pH 10.0) increased the degradation rate (half-life = 9.08 ± 4.22 d). The presence of a substrate (bentonite clay) resulted in an approximate 17% decrease in OTC concentration within 5 min of contact. Addition of organic matter (fish feed) along with the substrate resulted in a 41% decrease in OTC concentrations within 5 min of contact.

The treatment of aquatic species in commercial facilities is usually accomplished via medicated feed for practical and economic reasons. The decrease in feed consumption frequently observed in diseased animals, combined with the lower palatability of many medicated feeds (Hustvedt et al. 1991), can result in the accumulation of uneaten feed in the environment. In addition, low oral bioavailability of drugs can contribute to the environmental drug concentrations when a large percentage of drug is excreted unchanged in the feces (Cravedi et al. 1987).

Various studies in both laboratory and field conditions have examined the fate of oxytetracycline (OTC) in sediments in marine fish farms (Jacobsen and Berglund 1988; Björklund et al. 1991; Samuelsen et al. 1994; Hektoen et al. 1995; Coyne et al. 1997). Oxytetracycline half-lives as long as 419 d have been reported in anoxic and stagnant sediments (Björklund et al. 1990). The persistence

of OTC in sediments is reportedly site specific and dependent on factors including the concentration of the drug, the type and depth of the sediment, and the presence of water currents (Björklund et al. 1990; Hektoen et al. 1995; Coyne et al. 1997). A single study (Smith et al. 1994a) has examined OTC degradation in freshwater systems in which OTC contamination is also a concern. Because OTC is not documented to undergo considerable microbial degradation, knowledge of alternative factors that influence the kinetics of the drug decomposition becomes important. The persistence of drugs in the sediments may affect the constituent microflora (Austin 1985; Klaver and Matthews 1994) as well as lead to the development of plasmid-encoded drug resistance in fish pathogens (Aoki and Takahashi 1987; Aoki et al. 1987; Björklund et al. 1991; Smith et al. 1994b) and the potential for the transfer to human pathogens (Smith et al. 1994b).

Degradation of OTC occurs much faster in water than in the sediments (Samuelsen 1989; Pouliquen et al. 1993), and studies have shown that the half-lives of OTC decomposition in seawater are decreased as temperature and light exposure increase (Samuelsen 1989). It is difficult, however, to extrapolate the kinetics of OTC degradation in seawater to that in freshwater, mainly because of the differences in ionic composition. Oxytetracycline forms complexes with divalent cations, including Mg^{2+} and Ca^{2+} , found at high concentrations in seawater (Lunestad and Goksoyr 1990). The purpose of this study was to determine the effect of temperature, pH, light exposure, and the presence of substrates including organic matter on the kinetics of OTC decomposition in deionized water.

Methods

Oxytetracycline solution preparation.—Initial concentrations of 10 µg OTC/mL deionized water were obtained by adding 5 mg OTC hydrochloride (Sigma Chemical Co., St. Louis, Missouri) to 500 mL deionized water (pH 7.0). All 500-mL solu-

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TABLE 1.—Half-lives of OTC degradation under the influence of different environmental conditions.

Treatment	Solution characteristics	Half-life (d) ^a	Slope ^b
Positive control ^c	25°C, pH 7.0	14.04 ± 5.41	0.05 ± 0.02
Low temperature	4°C, pH 7.0	NA ^d	NA ^d
High temperature	43°C, pH 7.0	0.26 ± 0.11	3.06 ± 1.26
Acidic pH	25°C, pH 3.0	46.36 ± 4.92	0.02 ± 0.00
Basic pH	25°C, pH 10.0	9.08 ± 4.22	0.09 ± 0.04
Light exposure	25°C, pH 7.0, 4,500 lx	3.94 ± 0.66	0.18 ± 0.03
Substrate	25°C, pH 7.0, bentonite clay	11.56 ± 0.21	0.06 ± 0.00
Substrate with organic matter	25°C, pH 7.0, bentonite clay, fish feed	6.27 ± 2.01	0.12 ± 0.05

^a Mean OTC half-life ± SD (mean based on three replicates).

^b Slope of the fitted line of OTC degradation ± SD (mean based on three replicates).

^c Positive control for temperature (medium temperature, 25°C), for pH (neutral, pH 7.0), for light (no light), and for substrate or organic matter.

^d No degradation was observed over 77 d; therefore, calculation of a half-life was not possible.

tions were individually prepared in a similar manner, except for the blank control (which received no drug), before placement in sterile 600-mL Pyrex glass beakers. Control solutions were kept in the dark, at 25°C, pH 7.0, without any substrate addition. The five treatments applied were variations of the control, including extremes of temperature (4°C and 43°C) and pH (3.0 and 10.0), exposure to light, and addition of clay substrate (10 g) and clay with dead organic matter (0.5 g fish food). The light source was a standard intensity 15-W full spectrum fluorescent aquarium lamp that delivered approximately 4,500 lx (Foot Candle/LUX Light Meter Exttech; Davis Instruments, Baltimore, Maryland). Three replicates were performed for each condition except for the blank control. Bentonite clay (Fritz Chemical Co., Dallas, Texas) was chosen for this experiment because of its frequent use in natural pond construction (Sithole and Guy 1987). To eliminate any variability due to bacterial activity, the clay was autoclaved before use (121°C, 16 psi, 40 min). Fish feed (Big Strike Floating Fish Food; Southern States Cooperative, Inc., Richmond, Virginia) was used as a source of nonliving organic material to simulate the accumulation of uneaten fish feed in real pond conditions. All beakers were covered with clear stretchable plastic food wrap and sealed with a rubber band to minimize evaporation. Aluminum foil was applied over the plastic wrap to entirely cover the beakers and prevent light exposure, except in the light treatment.

Sample collection.—Collection of 1 mL of the aqueous OTC solution was performed from each beaker at 0, 0.2, 0.4, 0.5, 1, 2, 3, 7, 15, and 21 d. Additional weekly collections were performed for

treatments that still contained more than 30% of the initial OTC concentration at day 21. Collections were extended for a maximum of 11 weeks (temperature and pH, 11 weeks; substrate and substrate with organic matter, 8 weeks). Beakers containing substrate with or without organic matter were also sampled at 5 min after initial contact of the drug solution with the substrates. All beakers containing the solutions were weighed at the beginning of the experiment and then weighed before every collection, and weights were compared with the previous postcollection weight. Replacement of any weight losses to evaporation (~0.2% of total weight) was performed before each sampling, as needed. Daily pH measurements were taken, and values were corrected with either hydrochloric acid (1 M) or sodium hydroxide (1 M; Sigma).

Sample preparation.—Samples of aqueous OTC solution were injected directly into high-performance liquid chromatography (HPLC) unless beakers contained substrate. Samples collected from substrate treatments were centrifuged at 1,000 × gravity for 40 min to separate particulate matter from the solution.

Chromatography conditions.—The HPLC separation was performed by the use of a Nucleosil C8, 150 × 4.6-mm inside diameter (ID) analytical column (Supelco, Inc., Bellefonte, Pennsylvania) attached to a Nucleosil C8, 10 × 4.6-mm ID guard column (Supelco). The HPLC consisted of a Waters 600 E multisolvent delivery system (Millipore Corp., Waters Chromatography Division, Milford, Massachusetts), a SIL-9A Automatic sample injector (Shimadzu Co., Kyoto, Japan), and a Spectro monitor 3100 variable wavelength detector (LDC Analytical, Riviera Beach, Florida). The detection

FIGURE 1.—Effect of temperature on the degradation of oxytetracycline in aqueous solution (pH 7.0). Mean values are based on three replicates for each time point. Error bars represent SD.

wavelength was 355 nm. The SMAD Data System for Apple Macintosh Computers (SMADChrom version 2.2; Morgan Kennedy Research, College Station, Texas) was used to record the data. The separation used modifications from a previously published method (Oka et al. 1984) and was isocratic (1 mL/min) at room temperature; the mobile phase was composed of 5% methanol, 10% acetonitrile, and 85% aqueous 0.01 M oxalic acid.

Data analyses.—Tetracycline hydrochloride (TC; Sigma) was used as an internal standard for the HPLC analyses. Ten microliters of an aqueous solution of 0.1 µg TC /µL were added to 90 µL of the sample. Twenty microliters were then injected into the HPLC. Standard calibration curves in the range of 0.0–10.0 µg/mL were done in three replicates by plotting the ratio of various OTC and fixed TC concentration (1 µg) peak areas. Regression analyses were then performed (Data Analysis, Microsoft Excel 5.0; Microsoft Corp., Redmond, Washington), and the equation for the regression line obtained was $Y = -0.2327 +$

$1.425367X$; the correlation coefficient was $R^2 = 0.99$. The equation for the regression line was used to calculate the OTC concentrations in the experimental samples.

The precision of the method was determined by analyzing a sample three times in the same run. The relative coefficient of variation ($100 \cdot \text{SD} / \text{mean}$) of the OTC and TC peak areas was 1.0%. The limit of detection, defined as a peak of at least three times the height of the baseline noise, was approximately 0.05 µg OTC/mL aqueous solution. The detection limit was determined by injecting deionized water to which known amounts of OTC were added and determining the minimum detectable concentration.

Quality control samples (1 µg OTC/mL diluted in the mobile phase) were injected into the HPLC daily to verify chromatography conditions. Peak area variations of 15% or less were considered acceptable.

Mathematical methods.—The half-lives and the slopes of the line were obtained by fitting the data

FIGURE 2.—Effect of pH on the degradation of oxytetracycline in aqueous solution (25°C). Mean values are based on three replicates for each time point. Error bars represent SD.

by the use of the SAAM II program (version 1.0 for Microsoft Windows; SAAM Institute, University of Washington, Seattle, Washington). Estimations of the half-lives of the drug and the slopes of the lines were determined from a one-compartment model.

Analyses of variance were used to detect differences within treatments (light, pH, temperature, substrate, and substrate with organic matter; SAS Analyses, The SAS System for Windows, version 6.1; SAS Institute, Inc., Cary, North Carolina).

Results

The degradation of OTC in deionized water over time, under the influence of various factors, is shown in Figures 1–4 over a 0–21-d range. Data collection for treatments that displayed shorter half-lives was terminated when OTC levels reached the detection limit, but for most treatments, collection exceeded 21 d (temperature, pH, and sediment). Half-lives found in Table 1 were

calculated by the use of all time points of data collection, specified in the previous section.

Figure 1 shows the effect of temperature on OTC degradation. Oxytetracycline appears to be thermolabile at 43°C, showing significant higher stability at 4°C and 25°C ($P < 0.05$). The half-life of 0.26 ± 0.11 d for OTC at 43°C was 54-fold shorter than the half-life of 14.04 ± 5.41 d at 25°C. The concentrations at 4°C did not decline during the observed period (77 d), and consequently, no half-life could be calculated.

Low pH (3.0) significantly favored OTC stability, compared with neutral (7.0) and high (10.0) pH (Figure 2; $P < 0.05$). The half-life of 46.36 ± 4.92 d for OTC in acidic conditions was 3.3-fold longer than the half-life of 14.04 ± 5.41 d at pH 7.0. The OTC half-life was 9.08 ± 4.22 d at a high pH, 1.5-fold shorter than that at neutral conditions.

Exposure to light (Figure 3) resulted in a rapid decrease in OTC concentration. The half-life of OTC was 3.94 ± 0.66 d in the constant presence

FIGURE 3.—Effect of light on the degradation of oxytetracycline in aqueous solution (25°C, pH 7.0). Mean values are based on three replicates for each time point. Error bars represent SD.

of 4,500 lx of light at 25°C, 72% shorter than the half-life of 14.04 ± 5.41 d seen in dark conditions ($P < 0.05$).

Addition of clay or clay with organic matter resulted in decreases in OTC concentration (17% and 41%, respectively) within 5 min of addition of substrate to the OTC solution (Figure 4). The combination of organic matter and sediment significantly shortened the half-life of OTC ($P < 0.05$) by approximately 46%, compared with that with sediment only.

Discussion

Temperature dramatically affected the degradation of OTC in solution in deionized water; increases in temperature resulted in shorter half-lives. Even though the half-life of OTC at 4°C could not be calculated in the present study, the absence of degradation up until day 77 indicates that the half-life of OTC at 4°C in deionized water is likely to be longer than 77 d. A study by Samuelsen (1989) reported a considerably shorter

half-life of approximately 16 d for OTC in seawater at 4°C. In addition, the reported half-life of OTC in seawater at 15°C (Samuelsen 1989) was shorter than the OTC half-life in freshwater at 25°C, suggesting that the half-lives of OTC in deionized water are longer overall compared with the half-lives in seawater. The reasons for such differences are unknown but could be related to experimental differences, including the OTC initial concentration and water quality characteristics. Differences in the pH of the reaction (not reported in the seawater study), in combination with the chelating properties of OTC, may affect its degradation rate. Enhanced alkaline stability of OTC appears to occur after cation complex formation (Clive 1968).

Oxytetracycline degradation in deionized water was affected by pH changes, as demonstrated by a 1.5-fold longer half-life at pH 7.0 compared with that at pH 10.0. In a more marked manner a 3.3-fold longer half-life was seen when the pH was lowered from 7.0 to 3.0. The higher stability of

FIGURE 4.—Effect of bentonite clay and organic matter on the degradation of oxytetracycline in aqueous solution (25°C, pH 7.0). Mean values are based on three replicates for each time point. Error bars represent SD.

OTC in deionized water under acidic conditions is in accordance with previous reports (Connors et al. 1986).

Photodegradation is considered the main degradation mechanism for OTC in the environment (Oka et al. 1989; Lunestad 1992). In the present study, the OTC half-life in deionized water at 25°C and in the presence of light was approximately 72% shorter compared with that in darker conditions. A single study reported a half-life of approximately 10 d for an OTC solution in seawater at 4°C in the presence of light, 40% shorter than the approximately 16 d recorded under dark conditions (Samuelsen 1989). As mentioned previously, the half-life of OTC at 4°C in deionized water in the dark is likely to be longer than 77 d. In such a case, a 40–70% decrease after exposure to light would result in a longer half-life compared with that in seawater. Such differences could be explained by divergence in light transmission in freshwater compared with seawater. Studies in natural bodies of water have shown attenuation of

light to be greater in inland waters than in seawater, the former being more dependent on the organic matter concentration and the latter on the absorption by water (Leifer 1988; Smith et al. 1999). However, it is not clear at this time whether the differences observed between studies are due to variations in light transmission or simply related to experimental differences.

A rapid decrease in aqueous drug concentration was observed within 5 min of contact with bentonite clay, as well as with bentonite clay with organic matter. Even though drug binding to the substrates was not directly assessed in the present study, this rapid decline in OTC concentrations is consistent with binding of the drug to the clay. Adsorption of tetracycline with clay has been demonstrated previously and appears to be complete within 30 min of contact (Sithole and Guy 1987). The concentrations of detectable OTC after the initial 5 min declined in a rate similar to that of controls, suggesting that the major effect of clay was the initial rapid decline in OTC concentration,

but such assumptions are only speculative at this time. Addition of clay with organic matter (fish feed) resulted in a more marked decline in OTC concentrations after the first 5 min of contact (46% lower half-life), but further studies are needed to clarify the determinants of such a decrease.

In conclusion, OTC stability varies according to environmental conditions, which may increase or decrease the persistence of OTC in the environment. The results from this study suggest that under ideal conditions OTC can persist in deionized water longer than in seawater. This prolonged persistence should be taken into account when considering the use of OTC in freshwater systems.

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