

Report for 2001MN281G: Photochemical fate of pharmaceutical compounds discharged and detected in natural waters

- Articles in Refereed Scientific Journals:
 - Latch, D.E.; Stender, B.L.; Packer, J.L.; Arnold, W.A. ; McNeill, K., 2003. Photochemical fate of pharmaceuticals in the environment: cimetidine and ranitidine. *Environmental Science and Technology*, v. 37(15), pp. 3342-3350.
 - Latch, D.E.; Packer, J.L.; Arnold, W.A.; McNeill, K., 2003. Photochemical conversion of triclosan to 2,8 dichlorodibenzo p dioxin in aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry*, v. 158(1), pp. 63-66.
 - Packer, J.L.; Werner, J.J.; Latch, D.E.; McNeill, K.; Arnold, W.A., 2003. Photochemical fate of pharmaceuticals in the environment: naproxen, diclofenac, clofibrac acid, and ibuprofen. *Aquatic Sciences*, v. 65(4), pp. 342-351.
 - Latch, D. E.; Packer, J. L.; Stender, B. L.; VanOverbeke, J; Arnold, W.A.; K. McNeill, 2004. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin and oligomerization products, *Environmental Toxicology and Chemistry*, in review.
 - Werner, J. J.; McNeill, K.; W.A. Arnold, 2004. Environmental photodegradation of mefenamic acid, *Chemosphere*, in review.
 - Boreen, A.L., W.A. Arnold, and K. McNeill. 2004. Photochemical fate of sulfa drugs in the aquatic environment: sulfa drugs containing five-membered heterocyclic groups. *Environmental Science and Technology*, in review.
- unclassified:
 - Boreen, A.L.; Arnold, W.A., McNeill, K., 2003. Photodegradation of pharmaceuticals in the aquatic environment: a review. *Aquatic Sciences*, v. 65(4), pp. 320-341.

Report Follows

Photochemical fate of pharmaceutical compounds discharged and detected in natural waters

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Summary

Recent studies have detected numerous pharmaceuticals and personal care products (PPCPs) in US surface waters. The potential environmental impact of these chemicals will be dictated by their persistence in the environment and the biological activity of any degradation products. One potential loss process for pharmaceuticals and personal care products is photodegradation. In this work, the direct photolysis and indirect photolysis (hydroxyl radical mediated and singlet oxygen mediated) of selected PPCPs was investigated. To date, the fate of the antacids cimetidine and ranitidine hydrochloride and the antimicrobial compounds triclosan and chlorophene have been studied. All the compounds studied react with hydroxyl radical at nearly diffusion limited rates, but given the low concentration of hydroxyl radical in natural waters, other processes appear to be more important. The heterocyclic groups in cimetidine and ranitidine hydrochloride are susceptible to attack by singlet oxygen. Ranitidine hydrochloride is subject to direct photolysis while cimetidine is not. Direct photolysis occurs rapidly for triclosan and chlorophene when these compounds are present in the deprotonated phenolate form. These compounds also react with singlet oxygen, but preliminary results indicate that direct photolysis is the dominant photo-initiated loss process. The direct photolysis of triclosan at pH > 8.0 leads to the formation of 2,8-dichlorodibenzodioxin in yields ranging from 1-10%. This result underscores the importance of identifying the transformation products and not just the degradation rates.

Introduction

Pharmaceuticals and personal care products (PPCPs) are a class of chemicals that are continuously released into the environment through human activities, and, even though they have known biological effects, receive little attention (1,2). Examples of PPCPs include antibiotics, lipid regulators, psychiatric drugs, over the counter medications, and antimicrobial compounds. Most of these chemicals are introduced into the sewage system through their normal course of use. Once in the sewage system, many PPCPs are not completely removed at treatment plants (3) and thus, there is continuous introduction of these compounds to the environment. Numerous PPCPs have been detected in both ground and surface waters throughout the United States and Europe (2,4-12).

The impacts of PPCPs on the environment are unknown. Undesirable effects on non-target aquatic organisms and damage to sensitive ecosystems are possible (2). Furthermore, antibiotic drugs and antimicrobial agents in the environment may aid in the development of resistant bacteria (2,13). The lifetimes of the PPCPs in aquatic systems will partially determine the magnitude of the effects and potential threats to drinking water supplies. Loss processes such as photolysis, therefore, will play an important role in the environmental impact of these compounds. This includes not only direct and indirect photolysis loss processes, but also identifying intermediates and products that are formed through photolysis as transformation products many still have biological activity.

The research objective of this study is to determine the importance of both direct photolysis and indirect photolysis mediated by hydroxyl radical and singlet oxygen as loss processes for common PPCPs (medications cimetidine (Tagamet), ranitidine hydrochloride (Zantac), naproxan sodium (Alleve), ibuprofen (Advil), clofibrac acid, and diclofenac; the antimicrobial agents triclosan and chlorophene, and commonly prescribed antibiotics). Additionally, the research aims to identify major products resulting from the photolysis experiments.

Methods

Direct photolysis

Quartz bottles filled with either 25 or 50 mL aqueous PPCP samples over a range of concentrations and pH values were placed in a merry-go-round reactor and irradiated by a 450 W medium pressure Hg-vapor lamp (Ace Glass) controlled by an Ace Glass 7830 power supply. The lamp was encased in either a borosilicate or quartz cooling well, containing a continuous flow of cold tap water to help maintain constant temperature. For kinetic analyses 0.5 mL samples were withdrawn from the quartz bottles at predetermined intervals and analyzed on either an 1100 Series Hewlett Packard HPLC or a Waters LC Module 1 Plus, each equipped with UV-absorbance detection and a computer driven data acquisition system. Direct photolysis experiments were also performed in natural sunlight. Quartz bottles or borosilicate test tubes were placed in direct sunlight and samples were taken at predetermined intervals. Quantum yields were calculated by comparing the rate constant for the disappearance of the PPCPs with the rate constant for the disappearance of a *p*-nitroacetophenone actinometer as described in Leifer (ref. 14).

Hydroxyl radical

The second-order rate constant for the reaction of PPCPs with hydroxyl radical was determined using Fenton's reagent. Serum bottle reactors contained a 100 mM solution of the PPCP of interest, 100 mM acetophenone, 0.2 mM Fe²⁺, and 5 mM hydrogen peroxide adjusted to pH 3 with sulfuric acid (15). Samples were withdrawn at predetermined intervals and mixed with an equivalent volume of methanol to quench the reactions (16). HPLC analysis for both the PPCPs and the acetophenone was performed.

The hydroxyl radical rate constant was determined using competition kinetics according to:

$$k_{OH}^S = \frac{\ln([S_t]/[S_0])}{\ln([R_t]/[R_0])} k_{OH}^R$$

where *S* is the substrate (the PPCP) and *R* is the reference compound with a known hydroxyl radical rate constant (acetophenone, $k_{OH} = 5.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$).

Singlet oxygen

Singlet oxygen reaction kinetics were measured in one of two ways, directly by laser flash photolysis (LFP) or indirectly by steady-state photolysis (SSP). In both types of experiment the substrate (typically at micromolar concentrations) and $4 \times 10^{-5} \text{ M}$ Rose Bengal (RB), a singlet oxygen sensitizer, were dissolved in either alkaline buffer or EtOH. In the LFP experiments, a pulse of laser light excites the RB, which then produces singlet oxygen. A sensitive Ge-photodiode detector then monitors the phosphorescence emission from singlet oxygen. The rate of disappearance of the singlet oxygen phosphorescence signal is a measure of a substrate's activity toward singlet oxygen. The resulting total quenching rate constant (k_{tot}) is the sum of the chemical reaction and physical quenching rate constants.

In SSP experiments, the samples are photolyzed continuously and small aliquots are removed for analysis by HPLC. In this case, the disappearance of the PPCP is monitored (as decreases in peak area), rather than the singlet oxygen signal. This allows for the determination of the chemical reaction rate constant (k_{rxn}) for the PPCP with singlet oxygen.

Product identification

To analyze the products of various photoreactions, GC-MS and NMR spectroscopy were employed. An Agilent Technologies 6890 Gas Chromatograph with Mass Selective Detector was used to obtain mass spectra of various reaction mixtures. Photolysis samples run in organic solvents were analyzed by GC-MS to identify products. Product peaks were compared to mass spectral libraries to aid in their identification. Authentic samples of the likely products were then run under identical conditions to compare to the photolysis samples. Proper retention times and mass spectra indicated that a peak from the reaction mixture matched the standard solutions.

The NMR analyses were run on Varian Inova 300, 500, or 600 MHz instruments. In most product analysis experiments, a small amount of the reaction mixture was placed in an NMR tube prior to photolysis and an initial NMR spectrum was taken. Following a given period of irradiation, another NMR spectrum was taken. New peaks

that grew in were taken to be emerging product peaks. Whenever possible, these product peaks were compared to spectra obtained from authentic standards of the likely products to aid in their identification.

For triclosan, the direct photolysis experiments yielded the toxic 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD). Since this product was also photoreactive, a large batch of triclosan (25 ppm, 500 mL) was irradiated and then extracted into hexanes. Following evaporation of the hexanes, the sample was dissolved in basic methanol- d_4 and analyzed by both 1D and 2D (HMQC) NMR spectroscopy.

Results to date

Direct photolysis

The half-lives of triclosan and chlorophene are dependant on the pH of the aqueous solution. By obtaining the half lives at both high and low pH values (representing fully protonated and unprotonated forms of the PPCPs), we were able use pKa values to extrapolate and determine the half-lives at various pH values. The half-life of triclosan in natural water at 40° latitude in the protonated form is expect to be 2.55 hours in summer, and 5.5 days in the winter. In the unprotonated form, the half-life of triclosan is expected to be 6.15 min in summer and 5.35 hours in the winter. Chlorophene behaves similarly, as the half-life in natural water at 40° latitude in the protonated form is expected to be 2.35 hours in summer, and 5.07 days in the winter. In the unprotonated form the half-life of chlorophene is expected to be 22.75 min in summer sunlight, and 19.77 hours in winter sunlight.

As shown in Figure 1, cimetidine is not subject to direct photolysis under the sunlight irradiation conditions employed so far in this project (mid-winter sunlight in Minneapolis, MN, 44.88° latitude). Ranitidine hydrochloride is photodegraded in these light conditions with a half-life of 5 hours. After correcting for the increase in rate caused by the lens effect of the curved test tubes (approximately a factor of 2), at 45° latitude, the half-life is expected to be approximately 10 h in surface water (17). The rate constant could be expected to increase by a factor of approximately 3-5 under midsummer sunlight conditions, resulting in a half-life of 2-3 hours.

Hydroxyl radical

Antimicrobial agents triclosan and chlorophene are subject to reaction with hydroxyl radicals. The second order rate constants for triclosan and chlorophene are $5.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and $7.1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, respectively. Medications cimetidine and ranitidine hydrochloride are also susceptible to this type of indirect photolysis, with hydroxyl radical rate constants of $6.50 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and $1.46 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, respectively.

In natural near-surface waters, hydroxyl radical concentration may range from 10^{-16} M (in agriculturally impacted waters containing high nitrate levels) to 10^{-18} M (pristine waters) (18,19). Based on these steady-state concentrations, the half-life of triclosan may range from 15.14 days to 4.1 years in surface waters. Chlorophene behaves similarly with a half-life ranging from 11.3 days to 3.1 years. The half-life of cimetidine may range from 10.4 days to 2.8 years, and the shortest of these hydroxyl radical mediated half-lives, is ranitidine hydrochloride with an estimated half-life of 5.4 days to 1.4 years.

Singlet oxygen

The antacid medications cimetidine and ranitidine hydrochloride were both highly reactive toward singlet oxygen ($k_{\text{rxn}} = 9.2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ and $2.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ at pH 8, respectively; Figure 2). Model studies were employed to assess which moieties of each drug were most reactive with singlet oxygen. In both cases, the heterocyclic rings were the reactive groups.

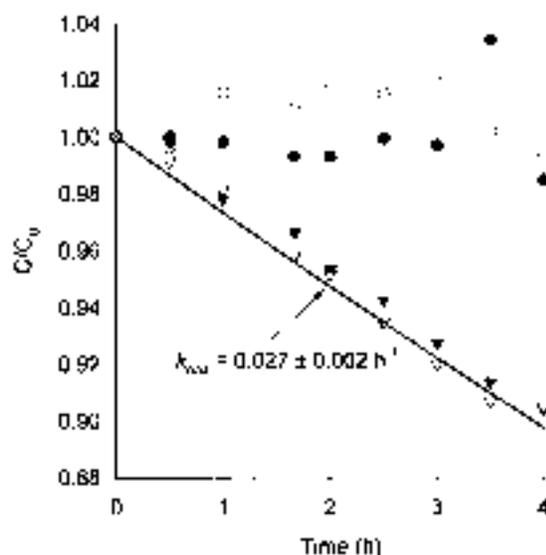


Figure 1. Direct photolysis of ranitidine hydrochloride (triangles) and cimetidine (circles).

Initial studies with chlorophene and naproxan show that the former interacts with singlet oxygen, while the latter does not to any significant extent. The dissociated form of triclosan has been found to quickly react with singlet oxygen ($k_{rxn} = 1.07 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ at pH 10).

Product identification

GC-MS analysis of the reaction between singlet oxygen and triclosan provided insight into the probable reaction mechanism. Phenoxide anions are well known to react with singlet oxygen to produce quinone products (20). As such, the triclosan quinone was independently synthesized and characterized. This product was not observed in any of the triclosan singlet oxygenation reactions. A peak for 2,4-dichlorophenol was evident in both GC-MS and NMR analyses, though. When the triclosan quinone was subjected to the same alkaline conditions that the triclosan reaction mixtures were, the quinone quickly broke down to yield 2,4-dichlorophenol and some other insoluble product. Thus, it appears likely that the triclosan quinone is being formed in the singlet oxygen reaction of triclosan, but it is readily cleaved, yielding the 2,4-dichlorophenol product.

GC-MS analysis first indicated that 2,8-DCDD may be formed in the direct irradiation of triclosan (Figure 3). A peak displaying the proper 2,8-DCDD mass spectrum was evident in the reaction mixture. When compared to an

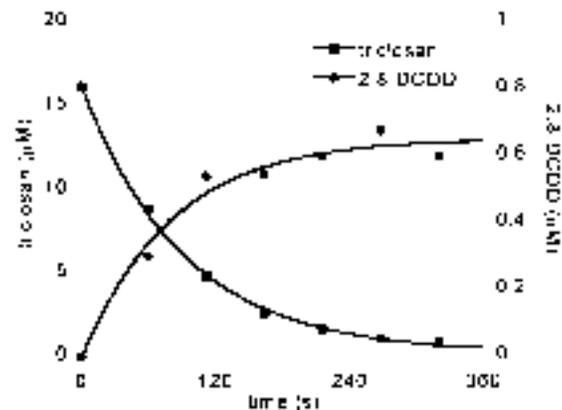


Figure 3. Photochemical conversion of triclosan to 2,8-DCDD, a member of the dioxin family, in Mississippi River water.

authentic sample of 2,8-DCDD, the retention times and mass spectra matched. This seemed to confirm that 2,8-DCDD was indeed forming in the photolysis experiments. It has been shown, however, that the perchlorinated analogue of triclosan forms octachlorodibenzo-*p*-dioxin at the heated inlet of GCs (21). Additional measures were thus taken to ensure that 2,8-DCDD was being formed in the direct photolysis samples and is not just an interference arising from the analysis method. First, the sample was compared to the 2,8-DCDD standard by HPLC. The retention time of one of the product peaks matched that of the standard. Second, the reaction mixture was compared to the standard solution by NMR spectroscopy. Following photolysis of a large batch of triclosan, the dioxin was concentrated and analyzed by 1D and 2D (HMQC) NMR spectroscopy. The spectra that were obtained compare favorably to that of the authentic sample, confirming the presence of 2,8-DCDD as a photoproduct.

Ongoing work

Current work is focusing on the direct and hydroxyl mediated photolysis of naproxan sodium, ibuprofen, clofibric acid, and diclofenac. Also under investigation is the photochemical fate of the sulfa drugs. Product identification studies are also underway as is expansion of the target chemical list.

Summary of findings

The over-the-counter antacids ranitidine and cimetidine are both rapidly degraded by singlet oxygen. Only the ranitidine is susceptible to direct photolysis. Degradation by hydroxyl radical appears to be of lesser importance for these species. The degradation of the antimicrobial compounds triclosan and chlorophene by direct photolysis and by reaction with singlet oxygen are pH dependent, with the phenolate forms of the compounds being more reactive in

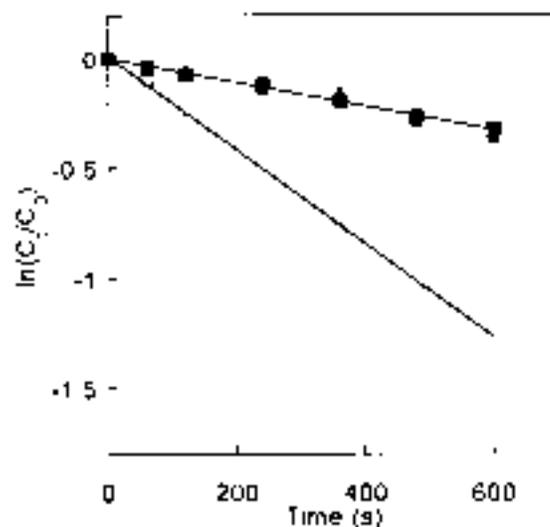


Figure 2. Logarithmic kinetic decay traces for rate for the singlet oxygenation of ranitidine hydrochloride (filled symbols) and FFA (open symbols).

each case. The direct photolysis of triclosan results in the formation of 2,8-DCDD in 1-10% yield depending on the experimental conditions. This result emphasizes the importance of identifying reaction products in environmental systems.

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List of publications & presentations resulting from this project

Stender, B.L., D.E. Latch, J.L. Packer, W.A. Arnold, and K. McNeill (2002) *Photochemical Fate of Pharmaceuticals in the Environment: Reactions of Singlet Oxygen and Hydroxyl Radical with Cimetidine and Ranitidine Hydrochloride*. Environmental Science and Technology, in revision.

Latch, D.E., J.L. Packer, W.A. Arnold, and K. McNeill (2002) *Photochemical Conversion of Triclosan to 2,8-Dichlorodibenzo-p-dioxin in Aqueous Solution*. Submission planned July 2002.

Latch, D.E., W.A. Arnold, and K. McNeill (2002) *The Photochemical Fate of Triclosan*. Poster presentation at the 2002 Great Lakes Regional Meeting of the American Chemical Society, June 2-4, 2002, Minneapolis, MN.

Boreen, A.L., W.A. Arnold, and K. McNeill (2002) *Kinetics of the Reaction Between Singlet Oxygen and Sulfa Drugs*. Poster presentation at the 2002 Great Lakes Regional Meeting of the American Chemical Society, June 2-4, 2002, Minneapolis, MN.

Latch, D.E., W.A. Arnold, and K. McNeill (2002) *Singlet Oxygen and the Photochemical Fate of Triclosan*. Paper presentation at the 2002 national meeting of the American Chemical Society, April 7-11, 2002, Orlando, Florida.

Packer, J.L., K. McNeill, and W.A. Arnold (2002) *Direct and Indirect Photolysis of Triclosan*. Paper presentation at the 2002 national meeting of the American Chemical Society, April 7-11, 2002, Orlando, Florida.

VanOverbeke, J.L., and K. McNeill (2002) *Photochemical fate of chlorophenol antibacterials in the environment*. Poster presentation at the 2002 national meeting of the American Chemical Society, April 7-11, 2002, Orlando, Florida. (CHED-474)

Latch, D.E., B.L. Stender, and K. McNeill (2001) *Singlet oxygen and the photochemical fate of pharmaceuticals*. Poster presentation at the 2001 national meeting of the American Chemical Society, August 26-30, 2001, Chicago, Illinois. (ENVR-135)

Statement of related grants submitted or funded as a result of this project

Dr. Arnold and Dr. McNeill have submitted grants to the Camille & Henry Dreyfus Foundation, the Minnesota Sea Grant Program, and the Legislative Commission on Minnesota Resources (LCMR) to study PPCP fate in aquatic systems.

Description of student training provided by project:

Name: Jennifer L. Packer

Program: Department of Civil Engineering, University of Minnesota

Degree being sought: Masters of Science

Name: Douglas E. Latch

Program: Department of Chemistry, University of Minnesota

Degree being sought: Ph.D.

Name: Anne L. Boreen

Program: Department of Chemistry, University of Minnesota

Degree being sought: Ph.D.

Name: Jennifer L. VanOverbeke

Program: Dept. of Chemistry, Univ. of Minnesota Summer, Undergraduate Research Program

Degree being sought: B.S. (Northwestern University)

Supported by the Summer Undergraduate Program of the Department of Chemistry