# Report for 2003MN32G: Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency

Publications

- Conference Proceedings:
    - Werner, J.J.; Arnold, W.A., McNeill, K. The environmental photochemical kinetics of tetracycline as a function of pH and water hardness. To be presented in “Environmental Chemistry Awards,” Environmental Chemistry Division, American Chemical Society National Meeting, San Francisco, CA, September, 2006.
    - Werner, J.J.; Arnold, W.A.; McNeill, K., 2005. Water Hardness as a Photochemical Parameter: The Case of Tetracycline. Paper presented in Emerging Contaminants, Pacifichem 2005, Honolulu, HI, December 15-20, 2005.
    - Werner, J.J.; Arnold, W.A.; McNeill, K., 2006. Environmental photochemistry of the antibiotic compound tetracycline: dependence on acid-base and metal binding speciation. Paper presented at the Minnesota Water 2005 and Annual Water Resources Joint Conference, Brooklyn Center, MN, October 25-26, 2006.
    - B.L. Edhlund, W.A. Arnold, K. McNeill. Aquatic Photochemistry of Nitrofuran Antibiotics. Poster Presentation. ENVR, 230th ACS National Meeting, Washington, DC, August 2005.
    - B.L. Edhlund, W.A. Arnold, K. McNeill. Aquatic Photochemistry of Nitrofuran Antibiotics. Poster Presentation. Minnesota ACS Meeting, St. Paul, MN, November 2005.
    - B.L. Edhlund, W.A. Arnold, K. McNeill. Aquatic Photochemistry of Nitrofuran Antibiotics. Poster Presentation. Environmental Sciences: Water Gordon Research Conference, Plymouth, NH, June 25-June 30, 2006.
    - J.J. Werner, K. McNeill, W.A. Arnold, Speciation-dependent photochemistry of tetracycline antibiotics: acid-base speciation and metal-binding effects. Oral Presentation. Midwest Environmental Chemistry Workshop, Madison, WI, October 16-17, 2004.
- Other Publications:

- J.J. Werner, A.L. Boreen, B. Edhlund, K.H. Wammer, E. Matzen, K. McNeill, W.A. Arnold, Photochemical transformation of antibiotics in Minnesota waters, CURA Reporter 2005, 35(2), 1-5.
- W.A. Arnold and H. Dolliver. Pharmaceuticals in Groundwater: Fate, Transport, and Effects. Minnesota Groundwater Spring 2006 Conference, St. Paul, MN, April 12, 2006.
- B.L. Edhlund, W.A. Arnold, K. McNeill, Aquatic Photochemistry of Nitrofuran Antibiotics. Celebrating Women Chemists Luncheon, University of Minnesota, Minneapolis, MN, February, 2006.
- K. McNeill, Photochemical approaches to environmental pharmaceutical pollutants, American Chemical Society (ACS) National Meeting Symposium: Strategies and Molecular Mechanisms of Contaminant Degradation Chemistry, Washington, D.C., August 28-Sept. 1, 2005.
- K. McNeill, Photo-generated Reactive Species and the Degradation of Pharmaceutical Pollutants, Stanford University, Civil and Environmental Engineering Student Seminar Series, May 20, 2005.
- K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, Cornell University, Department of Chemistry, March 7, 2005.
- K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, University of Rochester, Department of Chemistry, March 4, 2005.
- K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, University of California, Berkeley, Department of Chemistry, February 25, 2005.
- K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, Northwestern University, Department of Chemistry, February 18, 2005.
- K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, 3M, November 12, 2004.
- A.L. Boreen, W.A. Arnold, K. McNeill, Photochemical fate of sulfa drugs in the aquatic environment. Physics and Chemistry Colloquium, Concordia College, Moorhead, MN, November,

2004.
    - B.L. Edhlund, W.A. Arnold, K. McNeill. Aquatic Photochemistry of Nitrofuran Antibiotics. Poster Presentation. Minnesota Water 2005 and Annual Water Resources Joint Conference, Brooklyn Center, MN, October 2005.
- Articles in Refereed Scientific Journals:
    - B. L. Edhlund, W. A. Arnold, K. McNeill, Aquatic Photochemistry of Nitrofuran Antibiotics, Environ. Sci. Technol. in revision.
    - Werner, J.J.; Arnold, W.A.; McNeill, K., 2006. Water Hardness as a Photochemical Parameter: Tetracycline Photolysis as a Function of Calcium Concentration, Magnesium Concentration, and pH, Environ. Sci. Technol., accepted.
    - Wammer, K.H.; LaPara, T.M.; McNeill, K.; Arnold, W.A.; Swackhamer, D.L., 2006. Changes in Antibacterial Activity of Triclosan and Sulfa Drugs due to Photochemical Transformations, Environ. Toxicol. Chem., in press.
    - A.L. Boreen, W. A. Arnold, K. McNeill, Triplet-sensitized photodegradation of sulfa drugs containing six-membered heterocyclic groups: Identification of an SO2 extrusion photoproduct, Environ. Sci. Technol. 2005, 39, 3630-3638.
    - J.J. Werner, K. McNeill, W.A. Arnold, Environmental photodegradation of mefenamic acid. Chemosphere 2005, 58, 1339-1346.
    - D.E. Latch, J.L. Packer, B.L. Stender, J. VanOverbeke, W.A. Arnold, K. McNeill, Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin and oligomerization products, Environ. Toxicol. Chem. 2005, 24 (3), 517-525.
    - A.L. Boreen, W.A. Arnold, K. McNeill, Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups, Environ. Sci. Technol., 2004, 38, 3933-3940.
- Dissertations:
    - D. E. Latch, Environmental photochemistry: Studies on the degradation of pharmaceutical pollutants and the microheterogeneous distribution of singlet oxygen. Ph.D., University of Minnesota, Minneapolis, MN, 2005, 256 pp.
    - A.L. Boreen, Enhanced photolysis in natural waters: naturally occurring sensitizers and substrates and application to the fate of aquatic pollutants. Ph.D., University of Minnesota,

Minneapolis, MN, 2006, 263 pp.
- J.J. Werner, Photolysis of pharmaceuticals in water and soil matrices: agricultural antibiotics and mefenamic acid. Ph.D. University of Minnesota, Minneapolis, MN, 2006, forthcoming.

Report Follows

**Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency**

**Principal Investigators**
K. McNeill, Assistant Professor and PI, (mcneill@chem.umn.edu), Department of Chemistry; W.A. Arnold, Assistant Professor and Co-PI, Department of Civil Engineering; D.L. Swackhamer, Professor and Co-PI, Division of Environmental Health Sciences, University of Minnesota.

**Postdoctoral Fellow**
K.H. Wammer, Departments of Chemistry, Civil Engineering, and Environmental Health Sciences, University of Minnesota. (Funded through a Dreyfus Environmental Chemistry Postdoctoral Fellowship)

**Research Assistants**
A.L. Boreen, B.L. Edhlund, and D.E. Latch, Department of Chemistry; J.J. Werner, Water Resources Sciences Program, University of Minnesota.

**Start date:** 9/01/2003
**End date:** 2/28/06

**Executive Summary**

Antibiotics and estrogens are two classes of wastewater contaminants that have been detected in US surface waters. The potentially adverse effects of these pollutants on water quality are unknown, but will be determined, in part, by their persistence and the biological activity of both the parent compound as well as the degradates. Photolysis is one possible loss process, and the direct and indirect photolysis of five sulfa drug antibiotics, four nitrofuran antibiotics, four fluoroquinolones, and tetracyline has been investigated. The structure of the R-substituent on the sulfa drugs controls the reactivity; those containing six-membered substituents degrade through both direct photolysis and reaction with triplet dissolved organic matter. Both processes result in $SO_2$ extrusion. The photochemical kinetic rate constants for the loss of tetracycline under natural sunlight are a function of its various environmentally-relevant aqueous chemical species, including acid-base equilibria and metal-binding. Direct photolysis has been found to be the major photochemical degradation pathway for the nitrofuran antibiotics, with the formation of a photostationary state between the *syn* and *anti* isomers occurring in the first several minutes of light exposure. All antibacterial compounds tested, three sulfa drugs and triclosan (an antimicrobial agent), photodegraded to products with no observable antibacterial activity.

**Introduction**

Reports of pharmaceuticals and personal care products (PPCPs) in natural waters have recently appeared with increasing frequency.[1-5] Two important subclasses of these emerging contaminants are particularly worrisome due to their potential to adversely affect surface waters: antibiotics and environmental estrogens. Estrogenic compounds have a demonstrated ability to interfere with the development of aquatic organisms,[5, 6] while there is concern that the presence of antibiotics in natural waters will lead to an increase of antibiotic resistant bacteria.[7, 8] These compounds are released into surface waters as a result of human use, through discharge of

treated and untreated wastewater. An additional, major source of antibiotics comes from their wide use in the production of food animals and in fish farming. [1-5]

The magnitude of the effects and potential threat to water quality due to antibiotics and hormones is, in part, determined by the compounds' persistence in aquatic systems. The principle goal of this proposed study is to understand one aspect of their persistence—their degradation by photochemical processes. Based upon our work[9-15] and that of others,[11, 16-22] we believe that photodegradation may be a major loss process for these compounds in sunlit waters. Thus, it is important to understand the photochemical processes that degrade these chemicals in surface waters, to identify intermediates and products that are formed, and to assess the biological activity of these products.

## Methods

*Direct and natural water photolysis experiments*

Photolysis experiments were performed outdoors under natural sunlight or indoors under medium pressure Hg-vapor lamps or a Suntest CPS+ solar simulator equipped with a Xe-arc lamp and a UV Special Glass filter to mimic the solar spectrum. Sample solutions were contained in quartz test tubes (OD = 13 mm, ID = 11 mm, V = 10 mL). For kinetic analyses approximately 0.5 mL samples were withdrawn from the quartz tubes at predetermined intervals and analyzed on an 1100 Series Hewlett Packard HPLC equipped with UV-absorbance detection and a computer driven data acquisition system. In experiments designed to probe for pH effects, various buffer solutions were employed to set the pH values. Solar quantum yields were calculated by comparing the rate constant for the disappearance of the PPCPs under either natural sunlight or the Suntest CPS+ solar simulator with the rate constant for the disappearance of a *p*-nitroanisole actinometer. For toxicity experiments, test tubes were sacrificed at pre-selected time intervals and saved for HPLC analysis of remaining antibiotic concentration and subsequent antibacterial activity testing. The wavelength dependence of the direct photolysis of the nitrofuran antibiotics was probed using a series of cut-off filter tubes (absorbing $\lambda < 320$ nm, 280 nm, and 220 nm). Quartz test tubes containing the photolysis solutions were placed inside the filter tubes during photolysis.

Natural water photolysis experiments were performed in 0.2 μm filtered Lake Josephine (LJW) water or Lake Superior (LSW) water. To determine which pathways were responsible for the photodegradation, various quenchers were added to or removed from the water samples (sodium azide or DABCO for $^1O_2$, isopropanol for radicals, oxygen and isoprene for triplet DOM) and the substrate was also photolyzed in DI water in a separate tube.

*Speciation dependent behavior of tetracycline*

Association constants which determine the speciation of calcium- and magnesium-tetracycline complex formation were measured by pH titrations (pH 3 to 11) performed at various constant metal concentrations and the collection of UV-vis spectral data. The first order rate constant for the loss of tetracycline under simulated sunlight (Suntest CPS+ photosimulator, Atlas) was observed at various pH, calcium, and magnesium concentrations. Kinetic experiments were performed as detailed above. The concentration-dependent initial rate of photochemical degradation was monitored for various initial tetracycline concentrations and extrapolated to infinite dilution to determine the first-order rate constant for the loss of tetracycline in the absence of self-sensitization.

*Singlet oxygen ($^1O_2$)*

Singlet oxygen reaction kinetics were measured in one of three ways, directly by laser flash photolysis (LFP), or indirectly by either steady-state photolysis (SSP) or thermal generation of $^1O_2$. In both LFP and SSP experiments the substrate (typically at micromolar concentrations) and 100 μM perinaphthenone, a well-defined singlet oxygen sensitizer, were dissolved in aqueous buffer solutions. In the LFP experiments, a pulse of laser light excites the sensitizer, which then produces singlet oxygen after the excited-state sensitizer is quenched by dissolved molecular 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 were photolyzed continuously and small aliquots were removed for analysis by HPLC. In this case, the disappearance of the PPCP was monitored (as decreases in peak area), rather than the singlet oxygen signal. This allows for determination of the chemical reaction rate constant ($k_{rxn}$) for the PPCP with singlet oxygen.

To avoid any competing photochemical reaction occurring in SSP, thermal generation of $^1O_2$ was used. In these experiments, $^1O_2$ was generated through the reaction of hydrogen peroxide ($H_2O_2$) and molybdate ($MoO_4^{2-}$).[23-25] $H_2O_2$ (1 M) was added to a buffered solution containing $MoO_4^{2-}$ (1 mM), a reference compound of known $k_{rxn}$ (FFA; 100 μM), and substrate (100 μM). Aliquots of the reaction solutions were added to an aqueous solution of sodium azide (507 mM) at a series of time points to quench the reaction. Samples were then analyzed for both reference compound and substrate degradation via HPLC.

*Product identification*

Since large volumes of photolysate were required for product identification, photolyses were executed using a higher intensity light source (450 W medium pressure Hg-vapor lamp) which was completely immersed in the photolysis solution (100 μM substrate, 300 mL). After photolysis, the solution was concentrated to a total volume of 2 mL and the desired photoproduct was isolated using preparative HPLC. Sufficient amounts of product for analysis were obtained by combining the collected fraction from multiple injections of the photolysate on the preparative HPLC column.

Following isolation, the product was identified using an array of analyses including mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance (NMR). Mass spectral data were obtained for both the raw photolysate and the isolated products using a Bruker BioTOF ESI-TOF mass spectrometer. High resolution mass spectra were obtained using an internal standard of poly(ethylene glycol). Infrared absorbance spectra were acquired using a MIDAC Corporation M-Series FT-IR by placing a solution of the isolated photoproduct in methanol-$d_4$ between two NaCl plates. The $^1$H-NMR and $^{13}$C-NMR spectra of isolated photoproducts were obtained on a Varian Inova 300 MHz spectrometer. A quantitative $^1$H-NMR spectrum of the same sample was acquired using an internal standard.

*Biological activity*

The ability of the antibacterial compounds and their photolysis products to inhibit bacterial growth was tested using *E. coli* DH5α. The bacteria were maintained on agar plates and grown up overnight on Iso-Sensitest broth (ISB) (Oxoid, Inc.) prior to testing. One mL of antibacterial compound or photolysis product and 100 μL of *E. coli* were added to test tubes

containing nine mL of ISB prepared in a pH 7 phosphate buffer (9.7 g $KH_2PO_4$ and 19.4 g $Na_2PO_4$ per liter deionized water). The solutions were incubated in the dark at 37 °C while being shaken. Bacterial growth was assessed after 8 hours by measuring optical density at 600 nm ($OD_{600}$).

The antibacterial compounds and their photolysis products were also tested for their ability to inhibit bacterial respiration. The respiration assay used was based on the ability of the bacteria to reduce iodonitrotetrazolium chloride. *E. coli* (400µL) was added to 40 mL of ISB and incubated at 37 ºC. Once the $OD_{600}$ of this solution had reached 0.4 (in the exponential phase of the growth curve), 1 mL aliquots were centrifuged at 19,000g for five minutes. The supernatant was decanted, and 0.5 mL of antibiotic or photolyzed antibiotic was added. The bacterial pellet was resuspended, and the tubes were then incubated in the dark at 37 °C while being shaken. After one hour of incubation (approximately one generation time), 0.5 mL of a 5 mM solution of the tetrazolium salt was added and the tubes were incubated for an additional hour. The tubes were then centrifuged, the supernatant decanted, and 1 mL of an organic solution (1:1 dimethylformamide: ethanol) was added to the bacterial pellet to extract the formazan. The pellet was resuspended, and the tubes were incubated in the dark at room temperature for one hour. After centrifuging, the absorbance of the supernatant was measured at 464 nm to quantify the amount of formazan formed.

**Results to date**

*Photodegradation of the Sulfa drugs*

The photolysis rates of the sulfa drugs containing six-membered heterocyclic substituents (sulfachloropyridazine, sulfadiazine, sulfamerazine, and sulfamethazine) in Lake Josephine (DOC = 5.9 mg/L) water were enhanced by a factor of 1.4-2.6 relative to the photodegradation rates in DI $H_2O$. The enhancement in the natural water has been attributed to reaction of the sulfa drugs with excited triplet dissolved organic matter ($^3$DOM). Verification that the reaction is sensitized by $^3$DOM was provided by the characteristic enhancement of the degradation upon eliminating oxygen from the system and suppression of the degradation upon addition of isoprene, quenching of triplet-excited state perinaphthenone during LFP experiments, and the lack of reaction between the sulfa drugs and $^1O_2$ as measured using thermal generation methods. The natural water photodegradation of sulfadimethoxine matched the degradation in DI $H_2O$, and the degradation was thus attributed solely to direct photolysis. The direct photolysis of sulfadimethoxine is pH dependent, and is explained by differing reactivity of the protonation states. The remaining sulfa drugs' direct photolysis and triplet-sensitized degradations are not pH dependent over the pH range 6-9.

The primary product of both direct photolysis and triplet-sensitized degradation was identified as an $SO_2$ extrusion product (Figure 1). The yield of this product from sulfamethazine was found to be 64%.

*Tetracyline*

The pseudo-first-order rate constant for the photochemical loss of tetracycline was observed, under environmentally-relevant conditions, to be dependent on pH and both calcium and magnesium concentration. For each of the four acidic protons in tetracycline, deprotonation leads to both increased solar action spectrum and increased rate constant for photochemical degradation. The binding of tetracycline species to either calcium or magnesium leads to a further increase in the action spectrum for solar absorption. In the laboratory, the high

tetracycline concentrations (1 to 10 μM) led to significant self-sensitization, especially at higher pH values. For example, at a pH of 7.5, the observed pseudo-first-order rate constant appeared to double when increasing the initial tetracycline concentration from 1 to 15 μM, with a linear dependence on initial tetracycline within the concentration range. As an example of the rapid kinetics, the half-life of tetracycline extrapolated to infinite dilution at pH 7.5 was 9.9 minutes, where the experimental light intensity was approximately the same as that of a clear summer day, noon, 45° latitude.

*Photochemical behavior of the nitrofuran antibiotics*

The photodegradation of the nitrofuran antibiotics (furaltadone, furazolidone, and nitrofurantoin; Table 1) was found to be dominated by direct photolysis with the formation of a photostationary state between the *syn* and *anti* isomers occurring during the first minutes of photolysis. The direct photolysis rate constant was quantum yield were calculated for each of the nitrofurans. Environmentally relevant half-lives were determined for mid-season surface waters at 45°N latitude and range between 0.08 – 0.44 hours for mid-summer. Reaction rate constants with $^1O_2$ and ·OH were also measured. Half-lives for these processes were calculated using environmentally relevant concentrations; 66 – 488 hours and 74 – 82 hours for $^1O_2$ ($10^{-12}$ M) and OH ($10^{-15}$ M), respectively. When compared with direct photolysis half-lives, it is clear that indirect photochemical processes will not compete with direct photodegradation.

The photodegradation pathway is summarized in Figure 3. The major photodegradation product of the nitrofurans was identified as nitrofuraldehyde, which is also photolaibile. Direct photolysis of nitrofuraldehyde was found to produce NO which is easily oxidized to form nitrous acid. Studies determining the pH dependence of the photolysis of the nitrofurans concluded that the degradation is acid catalyzed, indicating that the acid produced from photolysis of the nitrofurans further catalyzes their degradation, leading to autocatalytic behavior. Natural waters were found to buffer the initial acid formation.

*Biological Activity*

Comparing the growth of *E. coli* DH5α in the presence of unphotolyzed sulfathiazole (Figure 2, open circles) versus in the presence of partially photolyzed sulfathiazole (Figure 2, closed triangles) revealed little difference in the inhibition of bacterial growth as a function of sulfathiazole concentration. Any photolysis products generated at a given point along the curve and present in the samples in the photolyzed series in addition to the sulfathiazole would be responsible for deviations from the unphotolyzed sulfathiazole series. The concentration at which sulfathiazole has reached half of its maximum effective concentration ($EC_{50}$ values) for these two curves were statistically similar. This suggests that the products of the photolysis do not retain any significant ability to inhibit bacterial growth; that is, the antibacterial activity of the photolyzed solution only comes from the unreacted sulfathiazole. Similar results were observed for sulfamethoxazole, sulfachloropyridazine, and triclosan.

**Summary of findings**

The photodegradation mechanism for the sulfa drugs containing six-membered substituents involves both direct photolysis and reaction with triplet dissolved organic matter generating an $SO_2$ extrusion photoproduct. Comparison of these results with those obtained for the sulfa drugs containing five-membered substituents reveals that minor structural changes can

give rise to disparate environmental loss mechanisms. The photochemical kinetic constants for the loss of tetracycline under natural sunlight are a function of its various environmentally-relevant aqueous chemical species, including acid-base and metal-bound forms. Direct photolysis has been found to be the major photochemical degradation pathway for the nitrofuran antibiotics, with the formation of a photostationary state between the *syn* and *anti* isomers occurring in the first several minutes of light exposure. All antibacterial compounds tested, three sulfa drugs and triclosan, photodegraded to products with no observable antibacterial activity.

**References**


1. Daughton, C.G. and T.A. Ternes, *Pharmaceuticals and personal care products in the environment: agents of subtle change?* Environ. Health Perspect. Suppl., 1999. **107**(6): p. 907-938.
2. Halling-Sorensen, B., et al., *Occurrence, fate and effects of pharmaceutical substances in the environment - a review.* Chemosphere, 1997. **36**(2): p. 357-393.
3. Kolpin, D.W., et al., *Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance.* Environ. Sci. Technol., 2002. **36**(6): p. 1202-1211.
4. Jorgensen, S.E. and B. Halling-Sorensen, *Drugs in the environment.* Chemosphere, 2000. **40**(7): p. 691-699.
5. Ternes, T., *Pharmaceuticals and metabolites as contaminants of the aquatic environment*, in *Pharmaceuticals and Personal Care Products in the Environment*, C.G. Daughton and T.L. Jones-Lepp, Editors. 2001, American Chemical Society: Washington, D. C. p. 39-54.
6. Stuer-Lauridsen, F., et al., *Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use.* Chemosphere, 2000. **40**(7): p. 783-793.
7. Kumpel, T., R. Alexy, and K. Kummerer, *What do we know about antibiotics in the environment?* Pharmaceuticals in the Environment, 2001: p. 67-76.
8. Hirsch, R., et al., *Occurrence of antibiotics in the aquatic environment.* Sci. Total Environ., 1999. **225**(1,2): p. 109-118.
9. Latch, D.E., et al., *Photochemical Conversion of Triclosan to 2,8-Dichlorodibenzo-p-dioxin in aqueous solution.* J. Photochem. Photobiol. A, 2003. **158**(1): p. 63-66.
10. Latch, D.E., et al., *Photochemical Fate of Pharmaceuticals in the Environment: Cimetidine and Ranitidine.* Environ. Sci. Technol., 2003. **37**: p. 3342-3350.
11. Boreen, A., W.A. Arnold, and K. McNeill, *Photodegradation of pharmaceuticals in the aquatic environment: A review.* Aquatic Sciences, 2003. **65**: p. 317-338.
12. Packer, J.L., et al., *Photochemical fate of pharmaceuticals in the environment: naproxen, diclofenac, clofibric acid, and ibuprofen.* Aquatic Sciences, 2003. **65**: p. 1-10.
13. Boreen, A.L., W.A. Arnold, and K. McNeill, *Photochemical Fate of Sulfa Drugs in the Aquatic Environment: Sulfa Drugs Containing Five-Membered Heterocyclic Groups.* Environ. Sci. Technol., 2004. **38**(14): p. 3933-3940.
14. Werner, J.J., K. McNeill, and W.A. Arnold, *Environmental photodegradation of mefenamic acid.* Chemosphere, 2005. **58**(10): p. 1339-1346.
15. Boreen, A.L., W.A. Arnold, and K. McNeill, *Triplet-Sensitized Photodegradation of Sulfa Drugs Containing Six-Membered Heterocyclic Groups: Identification of an SO2 Extrusion Photoproduct.* Environ. Sci. Technol., 2005. **39**: p. 3630-3638.

16. Buser, H.-R., T. Poiger, and M.D. Mueller, *Occurrence and Fate of the Pharmaceutical Drug Diclofenac in Surface Waters: Rapid Photodegradation in a Lake.* Environ. Sci. Technol., 1998. **32**(22): p. 3449-3456.
17. Lindström, A., et al., *Occurrence and Environmental Behavior of the Bactericide Triclosan and Its Methyl Derivative in Surface Waters and in Wastewater.* Environ. Sci. Technol., 2002. **36**(11): p. 2322-2329.
18. Moore, D.E. and W. Zhou, *Photodegradation of sulfamethoxazole: a chemical system capable of monitoring seasonal changes in UVB intensity.* Photochem. Photobiol., 1994. **59**(5): p. 497-502.
19. Oka, H., et al., *Photodecomposition products of tetracycline in aqueous solution.* J. Agric. Food Chem., 1989. **37**(1): p. 226-31.
20. Poiger, T., H.-R. Buser, and M.D. Muller, *Photodegradation of the pharmaceutical drug diclofenac in a lake: pathway, field measurements, and mathematical modeling.* Environmental Toxicology and Chemistry, 2001. **20**(2): p. 256-263.
21. Singer, H., et al., *Triclosan: Occurrence and Fate of a Widely Used Biocide in the Aquatic Environment: Field Measurements in Wastewater Treatment Plants, Surface Waters, and Lake Sediments.* Environ. Sci. Technol., 2002. **36**(23): p. 4998-5004.
22. Tixier, C., et al., *Phototransformation of Triclosan in Surface Waters: A Relevant Elimination Process for This Widely Used Biocide - Laboratory Studies, Field Measurements, and Modeling.* Environ. Sci. Technol., 2002. **36**(16): p. 3482-3489.
23. Aubry, J.M., *Search for singlet oxygen in the decomposition of hydrogen peroxide by mineral compounds in aqueous solutions.* J. Am. Chem. Soc., 1985. **107**(21): p. 5844-9.
24. Aubry, J.M. and B. Cazin, *Chemical sources of singlet oxygen. 2. Quantitative generation of singlet oxygen from hydrogen peroxide disproportionation catalyzed by molybdate ions.* Inorg. Chem., 1988. **27**(12): p. 2013-14.
25. Boehme, K. and H.D. Brauer, *Generation of singlet oxygen from hydrogen peroxide disproportionation catalyzed by molybdate ions.* Inorg. Chem., 1992. **31**(16): p. 3468-71.

**List of publications & presentations resulting from this project**

*Peer Reviewed Publications*

B. L. Edhlund, W. A. Arnold, K. McNeill, Aquatic Photochemistry of Nitrofuran Antibiotics, *Environ. Sci. Technol.* in revision.

Werner, J.J.; Arnold, W.A.; McNeill, K., 2006. Water Hardness as a Photochemical Parameter: Tetracycline Photolysis as a Function of Calcium Concentration, Magnesium Concentration, and pH, *Environ. Sci. Technol.,* accepted.

Wammer, K.H.; LaPara, T.M.; McNeill, K.; Arnold, W.A.; Swackhamer, D.L., 2006. Changes in Antibacterial Activity of Triclosan and Sulfa Drugs due to Photochemical Transformations, *Environ. Toxicol. Chem.*, in press.

A.L. Boreen, W. A. Arnold, K. McNeill, Triplet-sensitized photodegradation of sulfa drugs containing six-membered heterocyclic groups: Identification of an $SO_2$ extrusion photoproduct, *Environ. Sci. Technol.* **2005**, *39*, 3630-3638.

J.J. Werner, K. McNeill, W.A. Arnold, Environmental photodegradation of mefenamic acid. *Chemosphere* **2005**, 58, 1339-1346.

D.E. Latch, J.L. Packer, B.L. Stender, J. VanOverbeke, W.A. Arnold, K. McNeill, Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin and oligomerization products, *Environ. Toxicol. Chem.* **2005**, 24 (3), 517-525.

A.L. Boreen, W.A. Arnold, K. McNeill, Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups, *Environ. Sci. Technol.*, **2004**, 38, 3933-3940.

*Popular Articles*

J.J. Werner, A.L. Boreen, B. Edhlund, K.H. Wammer, E. Matzen, K. McNeill, W.A. Arnold, Photochemical transformation of antibiotics in Minnesota waters, CURA Reporter **2005**, *35(2)*, 1-5.

*Dissertations*

D. E. Latch, Environmental photochemistry: Studies on the degradation of pharmaceutical pollutants and the microheterogeneous distribution of singlet oxygen. Ph.D., University of Minnesota, Minneapolis, MN, **2005**, 256 pp.

A.L. Boreen, Enhanced photolysis in natural waters: naturally occurring sensitizers and substrates and application to the fate of aquatic pollutants. Ph.D., University of Minnesota, Minneapolis, MN, **2006,** 263 pp.

J.J. Werner, Photolysis of pharmaceuticals in water and soil matrices: agricultural antibiotics and mefenamic acid. Ph.D. University of Minnesota, Minneapolis, MN, **2006**, forthcoming.

*Invited Presentations*

W.A. Arnold and H. Dolliver. Pharmaceuticals in Groundwater: Fate, Transport, and Effects. Minnesota Groundwater Spring 2006 Conference, St. Paul, MN, April 12, 2006.

B.L. Edhlund, W.A. Arnold, K. McNeill, Aquatic Photochemistry of Nitrofuran Antibiotics. **Celebrating Women Chemists Luncheon**, University of Minnesota, Minneapolis, MN, February, 2006.

K. McNeill, Photochemical approaches to environmental pharmaceutical pollutants, **American Chemical Society (ACS) National Meeting Symposium**: ***Strategies and Molecular Mechanisms of Contaminant Degradation Chemistry***, Washington, D.C., August 28-Sept. 1, 2005.

K. McNeill, Photo-generated Reactive Species and the Degradation of Pharmaceutical Pollutants, **Stanford University**, Civil and Environmental Engineering Student Seminar Series, May 20, 2005.

K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, **Cornell University**, Department of Chemistry, March 7, 2005.

K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, **University of Rochester**, Department of Chemistry, March 4, 2005.

K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, **University of California, Berkeley**, Department of Chemistry, February 25, 2005.

K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, **Northwestern University**, Department of Chemistry, February 18, 2005.

K. McNeill, Mechanistic Environmental Chemistry: Photo-generated Reactive Species and Pollutant Degradation, **3M**, November 12, 2004.

A.L. Boreen, W.A. Arnold, K. McNeill, Photochemical fate of sulfa drugs in the aquatic environment. **Physics and Chemistry Colloquium**, Concordia College, Moorhead, MN, November, 2004.

A.L. Boreen, W.A. Arnold, K. McNeill, Photochemical fate of sulfa drugs in the aquatic environment. **Celebrating Women Chemists Luncheon**, University of Minnesota, Minneapolis, MN, September, 2004.

K. McNeill. Photochemical fate of pharmaceutical pollutants. **Gordon Research Conference, Environmental Sciences: Water**, June 27 - July 2, 2004.

A.L. Boreen, W.A. Arnold, K. McNeill. Photochemical fate of pharmaceuticals in the environment: Sulfa drugs. *Oral presentation*. 9th Biennial **MN Water Conference**, Minneapolis, MN, March 23, 2004.

*Conference Presentations*

Werner, J.J.; Arnold, W.A., McNeill, K. The environmental photochemical kinetics of tetracycline as a function of pH and water hardness. To be presented in "Environmental Chemistry Awards," Environmental Chemistry Division, American Chemical Society National Meeting, San Francisco, CA, September, 2006.

Werner, J.J.; Arnold, W.A.; McNeill, K., 2005. Water Hardness as a Photochemical Parameter: The Case of Tetracycline. Paper presented in Emerging Contaminants, Pacifichem 2005, Honolulu, HI, December 15-20, 2005.

Werner, J.J.; Arnold, W.A.; McNeill, K., 2006. Environmental photochemistry of the antibiotic compound tetracycline: dependence on acid-base and metal binding speciation. Paper presented at the Minnesota Water 2005 and Annual Water Resources Joint Conference, Brooklyn Center, MN, October 25-26, 2006.

B.L. Edhlund, W.A. Arnold, K. McNeill. Aquatic Photochemistry of Nitrofuran Antibiotics. *Poster Presentation*. ENVR, **230th ACS National Meeting**, Washington, DC, August 2005.

B.L. Edhlund, W.A. Arnold, K. McNeill. Aquatic Photochemistry of Nitrofuran Antibiotics. *Poster Presentation*. **Minnesota Water 2005 and Annual Water Resources Joint Conference**, Brooklyn Center, MN, October 2005.

B.L. Edhlund, W.A. Arnold, K. McNeill. Aquatic Photochemistry of Nitrofuran Antibiotics. *Poster Presentation*. **Minnesota ACS Meeting**, St. Paul, MN, November 2005.

B.L. Edhlund, W.A. Arnold, K. McNeill. Aquatic Photochemistry of Nitrofuran Antibiotics. *Poster Presentation*. **Environmental Sciences: Water Gordon Research Conference**, Plymouth, NH, June 25-June 30, 2006.

J.J. Werner, K. McNeill, W.A. Arnold, Speciation-dependent photochemistry of tetracycline antibiotics: acid-base speciation and metal-binding effects. *Oral Presentation*. **Midwest Environmental Chemistry Workshop**, Madison, WI, October 16-17, 2004.

K. McNeill, W.A. Arnold. Contribution of photochemistry to the fate of pharmaceuticals and personal care products in surface waters. *Oral Presentation.* ENVR, Presented at the special symposium on Environmental aspects of pharmaceuticals and personal care products at the 228th **ACS National Meeting**, Philadelphia, PA, August 2004.

A.L. Boreen, W.A. Arnold, K. McNeill. Photochemical fate of sulfa drugs in the aquatic environment. *Oral Presentation*. ENVR, Presented at the special symposium on Environmental aspects of pharmaceuticals and personal care products at the 228th **ACS National Meeting**, Philadelphia, PA, August 2004.

K.H. Wammer, K. McNeill, T.M. LaPara, W.A. Arnold, D.L. Swackhamer. Changes in potency of antibacterials in the environment due to photochemical transformations. *Poster Presentation.* ENVR, Presented at the special symposium on Environmental aspects of pharmaceuticals and personal care products at the 228th **ACS National Meeting**, Philadelphia, PA, August 2004.

J.J. Werner, K. McNeill, W.A. Arnold, Kinetics of the environmental photodegradation of mefenamic acid. *Poster Presentation.* ENVR, Presented at the special symposium on Environmental aspects of pharmaceuticals and personal care products at the 228th **ACS National Meeting**, Philadelphia, PA, August 2004

W.A. Arnold, J.J. Werner, K. McNeill, Kinetics of the environmental photodegradation of mefenamic acid. *Poster presentation.* **Environmental Sciences: Water Gordon Research Conference**, Plymouth, NH, June 27-July 2, 2004.

K.H. Wammer, D.L. Swackhamer, W.A. Arnold, K. McNeill, Photochemical transformations of antibacterial compounds. *Poster presentation*. **Environmental Sciences: Water Gordon Research Conference**, Plymouth, NH, June 27-July 2, 2004.

J.J. Werner, K. McNeill, W.A. Arnold, Photochemical fate of pharmaceuticals in the environment. *Poster presentation.* 9th Biennial **MN Water Conference**, Minneapolis, MN, March 23, 2004.

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

Dr. McNeill, Dr. Arnold, and Dr. Swackhamer continue to actively seek funding to continue this avenue of research. Dr. Arnold has received funding from the Center for Urban and Regional Affairs (University of Minnesota) to investigate the photodegradation of selected antibiotics in Minnesota waters in 2003-2004. Dr. Arnold is also a co-investigator on a United States Department of Agriculture grant (2003-2006) to investigate the loss of veterinary antibiotics in soil systems. Drs. Arnold, McNeill and Swackhamer also recently received funding from the National Science Foundation (2006-2008) to study the photolysis of triclosan and polybrominated diphenyl ethers in both the laboratory and in the field.

**Description of student training provided by project:**

Name: Anne L. Boreen
Program: Department of Chemistry, University of Minnesota
Degree earned: Ph.D. (2006)

Name: Betsy L. Edhlund
Program: Department of Chemistry, University of Minnesota
Degree being sought: Ph.D.

Name: Douglas E. Latch
Program: Department of Chemistry, University of Minnesota
Degree earned: Ph.D. (2005)

Name: Jeffrey J. Werner
Program: Water Resources Science, University of Minnesota
Degree earned: M.S. (2004)
Degree earned: Ph.D. (2006)

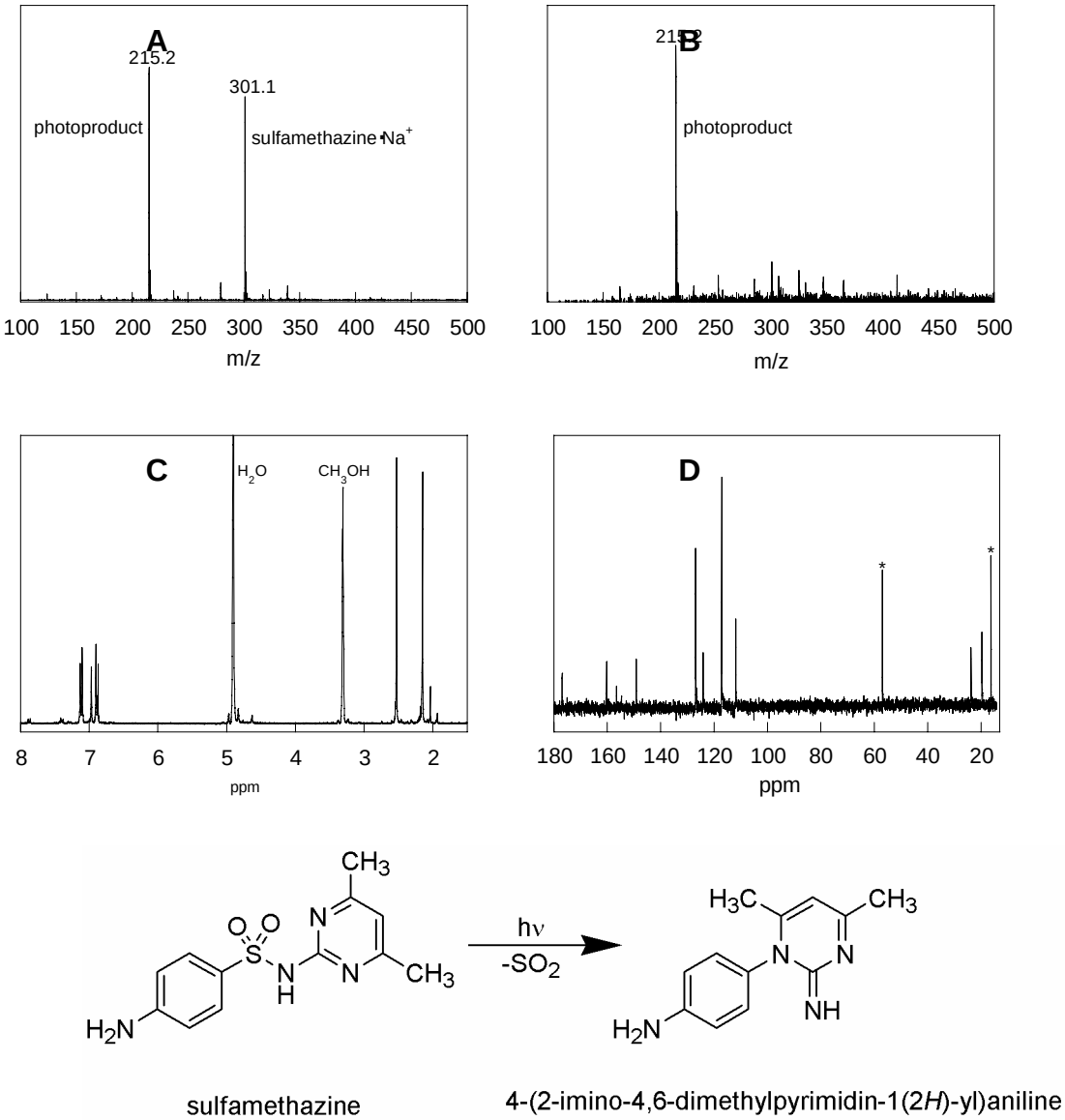



**Figure 1.** Characterization data for the primary photoproduct of sulfamethazine. (A) ESI-TOF mass spectrum of the raw photolysate of sulfamethazine showing the parent ion (m/z 301.1, $MNa^+$) and the photoproduct (m/z 215.2). (B) ESI-TOF mass spectrum of the isolated photoproduct (m/z 215.2). $^1$H-NMR (C) and $^{13}$C-NMR (D; * denotes ethanol peaks) of the isolated photoproduct.

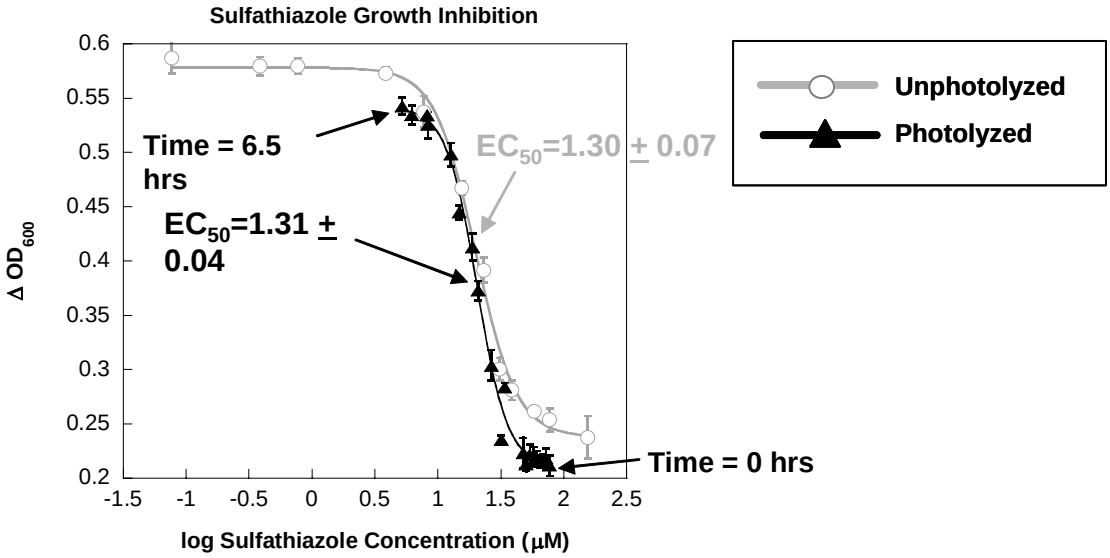



**Figure 2.** Change in optical density at 600 nm after 8 hours for *E. coli* DH5α in the presence of sulfathiazole (open circles) and sulfathiazole plus photolysis products (closed triangles). Remaining sulfathiazole concentration is plotted (log of concentration (μM)). Initial and final sulfathiazole concentrations during photolysis (77 μM at 0 hours and 5.2 μM at 6.5 hours) are labeled.

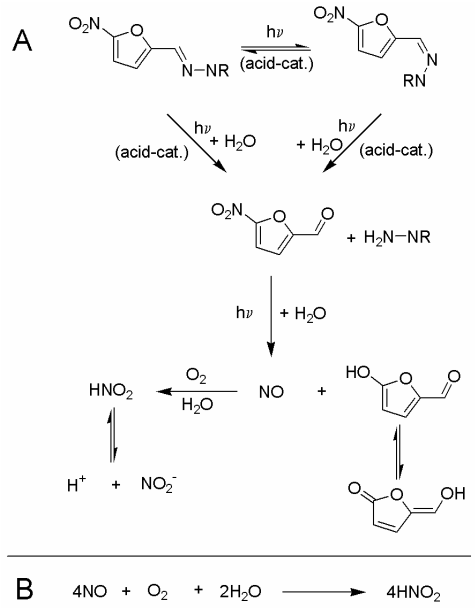
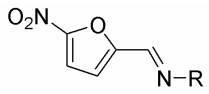



**Figure 3.** (A) Photodegradation scheme of the nitrofuran antibiotics. Parent and isomer conformations were arbitrarily set to anti as the parent and syn as the isomer. For clarity, the reaction of NO to $HNO_2$ is not balanced. (B) Balanced reaction for NO oxidation to $HNO_3$.



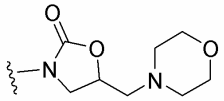
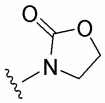
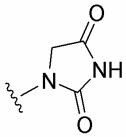
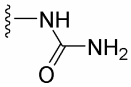

| R |  |  |  |  |
|---|---|---|---|---|
| Name | Furaltadone | Furazolidone | Nitrofurantoin | Nitrofurazone |

**Table 1.** The general structure of the nitrofuran antibiotics, with the varying substituents (R) shown within the table.