Doctoral Thesis

Transformation mechanisms of organic micropollutants via direct and indirect photochemistry

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Publication Date:
2014

Permanent Link:
https://doi.org/10.3929/ethz-a-010195680

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Transformation mechanisms of organic micropollutants via direct and indirect photochemistry

A dissertation submitted to

ETH ZURICH

For the degree of

Doctor of Sciences

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2014
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Summary

The release of organic pollutants into the environment is a global problem and can have adverse effects on both human and environmental health. To accurately access the health and environmental risks associated with the use and disposal of pharmaceuticals, pesticides, and other industrially produced chemicals, it is important to know their fate in the environment. To properly determine the risks of a given compound in the environment, we must first determine which processes are important for its fate and account for the products of its transformation, which may also be of environmental concern. For many environmental pollutants one route of transformation is exposure to sunlight or reactive intermediates that are generated photochemically in the environment. Numerous possible photoreactions can affect the fate of organic compounds. To achieve the goal of pollutant fate prediction, the precise mechanisms that drive these chemical changes must be known.

The goal of this thesis was to examine in detail some of the different photochemical processes that can occur for environmental pollutants upon exposure to sunlight under aqueous conditions. Three different mechanistic systems were studied, consisting of both direct and indirect photochemical reactions. In the first and third sections, our goal was to take a closer look at the source of pollutants to see if photochemical transformations could help explain the presence of specific compounds in the aquatic environment. In the second section, we performed a more fundamental study to understand indirect photooxidation reactions with the goal of aiding in transformation rate predictions for some pollutant classes.

The first part of this thesis examines the phototransformation of hydroxylated polybrominated diphenyl ethers (OH-PBDEs), which are known to be both natural
products as well as transformation products of flame retardant chemicals. The reactivity of some of these compounds via direct photolysis was investigated, and a number of transformation products were identified and quantified. The most concerning transformation products of OH-PBDEs were polybrominated dibenzo-p-dioxins (PBDDs), which were formed by a photochemical ring closure reaction that all of the studied compounds were found to undergo with differing efficiencies. The OH-PBDE congener, 6-OH-PBDE 99, which is known to be a natural product, generated the most PBDD with a yield of 7%. Another congener, 6'-OH-PBDE 118, which is thought to only be a transformation product of other anthropogenic compounds was capable of generating 2,3,7,8-PBDD, one of the most toxic PBDD congeners, but with yields of only 0.5%. The results of this study further support the findings of others that most of the PBDDs found in marine environments, more specifically the Baltic Sea, are derived from natural sources through a variety of production mechanisms.

The second part of this thesis was devoted to studying the indirect photochemical oxidation of two sets of model pollutants. The reaction mechanism for the oxidation of anilines and phenols by a model triplet state oxidant, Methylene Blue (MB) was investigated in aqueous solution. Using transient absorption spectroscopy, the reaction rate constants were determined for the oxidative reaction between MB and series of substituted anilines and phenols. The goal of this project was to determine an empirical relationship between the reaction free energy and reaction rate constant for each set of model pollutants to aid in the understanding of the mechanism as well as fate prediction of these compounds in the environment. The anilines, which reacted with MB by a one-electron transfer mechanism, were observed to react with rate constants ranging from \(1.06 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}\) to \(4.85 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}\). These reaction rate constants were found to correlate well to the reaction free energy when fit using a
Sandros-Boltzmann distribution model. The reaction between MB and phenols was found to occur by a proton concerted electron transfer mechanism. Evidence for this reaction mechanism over a simple electron transfer came from spectroscopic data, which suggested the immediate formation of a protonated MB species following reaction. Additional kinetic isotope effect experiments further demonstrated that the OH bond strength of the phenols directly affected the observed reaction rate constants. The reaction rate constants for substituted phenols could be predicted with high accuracy by a linear free energy relationship that depended on the bond disassociation free energy and the pKₐ.

In the third section of this thesis, the mechanism of the photochemical formation of 1-chloropyrene from pyrene and chloride in aqueous solution was investigated. The motivation for this work came from the observation that the concentration of 1-chloropyrene found in marine sediments correlated with the water salinity, thus suggesting that the formation from pyrene was occurring in situ. A combination of steady state photolysis experiments and transient absorption measurements were carried out to study this reaction mechanism. Our results suggest 1-chloropyrene is formed by a nucleophilic attack of chloride on the pyrene radical cation, which is generated by the oxidation of photoexcited pyrene by O₂. The nucleophilic behavior of chloride was demonstrated by performing photolysis experiments in the presence of chloride and other nucleophiles. When pyrene was irradiated in the presence of equal concentrations of bromide and chloride, 1-bromopyrene was observed to form at approximately ten times the rate of 1-chloropyrene. Based on the relative nucleophilicities of bromide and chloride these results supports a nucleophilic mechanism. The addition of electron donors, which were observed to quench the pyrene radical cation signal in transient absorption
experiments, also eliminated the production of 1-chloropyrene during steady-state
photolysis.

Overall, this thesis added to the understanding of three different mechanistic
systems relevant to the fate of organic pollutants in aquatic environments. The results
of this work demonstrate that laboratory-scale experiments are important in
explaining the presence of specific environmental pollutants based on transformations
occurring within the environment. Additionally, our investigation of the mechanisms
of indirect photooxidation and modeling of the observed reaction rate constants may
be crucial in future attempts to create pollutant fate prediction tools.
Zusammenfassung


Diese Doktorarbeit verfolgte das Ziel einige oxidative Prozesse von Umweltschadstoffen zu untersuchen, die im Wasser durch Sonneneinstrahlung erfolgen. Die drei untersuchten mechanistischen Systeme schlossen sowohl direkte als auch indirekte Photoreaktionen ein. Im ersten sowie im dritten Teil dieser Studie haben wir das Ziel verfolgt, die Herkunft von Schadstoffen genauer zu untersuchen um zu sehen ob Photoreaktionen das Vorkommen dieser Schadstoffe erklären können. Im dritten Fall wurde eine eher fundamentale Studie durchgeführt um indirekte photooxidative Reaktionen genauer zu verstehen und damit Transformationsraten für verschiedene Substanzklassen vorhersagen zu können.
Im ersten Teil dieser Doktorarbeit geht es um die Phototransformation von hydroxylierten polybromierten Diphenylethern (OH-PBDEs), welche als natürliche Produkte aber auch als Transformationsprodukte von Flammschutzmitteln in der Umwelt vorkommen. Für einige dieser Verbindungen wurde die Reaktivität durch direkte Lichtabsorption untersucht und Transformationsprodukte wurden identifiziert und quantifiziert. Die bedenklichsten Transformationsprodukte der OH-PBDEs waren die polybromierten dibenzo-p-Dioxine (PBDDs), welche durch eine photochemische Ringschliessung gebildet wurden. Alle untersuchten Verbindungen gingen diese Ringschliessung ein, mit unterschiedlicher Effizienz. Das OH-PBDE Kongener, 6-OH-PBDE 99, ein Naturprodukt, bildete mit einer Ausbeute von 7% am meisten PBDD. Ein anderes Kongener, 6'-OH-PBDE 118, welches vermutlich ein Transformationsprodukt anthropogener Verbindungen ist, bildete 2,3,7,8-PBDD, eines der giftigsten PBDD Kongeneren, allerdings mit einer Ausbeute von nur 0.5%. Die Resultate dieser Studie bestätigen andere Forschungsergebnisse, welche zeigten dass die meisten PBDDs in der marinen Umwelt, konkret im baltischen Meer, aus natürlichen Quellen stammen und durch verschiedene Mechanismen gebildet werden können.


Chapter 1

Introduction
Introduction

For nearly two centuries, our ability to produce and use synthetic organic chemicals has played a vital role in improving the quality of life for billions of people worldwide. Thanks largely to the discovery of pharmaceuticals, fertilizers, and countless other chemical technologies, it is possible to feed and maintain the health of a growing world population. Unfortunately, many of the same chemicals we have used to improve our lives have had unintended and in some cases disastrous consequences on human and environmental health. A prominent and early example was the use of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, or DDT. Most people today only know DDT as a dangerous environmental pollutant, but in the 1940’s its powerful abilities as an insecticide are credited, in part, for eliminating the transmission of malaria by mosquitoes in the United States during the 1950s. DDT was once so highly regarded, the Swiss chemist Paul Müller received the 1948 Nobel prize in Physiology or Medicine for the discovery of DDT’s insecticidal properties. This achievement however became greatly overshadowed by the detrimental effect that DDT use had on wildlife and human health, which has led to its eventual ban in most developed countries since the 1970s. The problems resulting from DDT and many other pollutants that end up in the environment make it necessary for us to apply the same science of chemistry to understand the effects, persistence, and fate of these chemicals in the natural world.

Pollutants enter the environment in a wide range of ways, and their release can be both intentional, such as the spraying of pesticides and herbicides for agricultural purposes, or unintentional, as is the case for the thousands of micropollutants that are released through wastewater treatment and modern industrial practices. Once in the
environment, these compounds partition into various compartments depending on their chemical properties, including the air, water, soil, and biota. Pollutants that end up in aquatic environments in either a free or particle-bound state are subjected to a unique set of conditions that determines how and by what mechanisms they may transform. In surface waters, an important source of transformation for many pollutants is photolysis via exposure to sunlight. These light-initiated reactions are typically divided into two categories, direct and indirect photolysis.

*Fundamentals of light*

Before discussing the basics of photochemistry, it is useful to first introduce some fundamentals about the unifying and most important component of these reactions, light. At the most basic level, light, or electromagnetic radiation, is the carrier of energy between the electrons found in atoms and molecules. This transfer of energy is carried out by massless fundamental particles known as photons, which carry discrete packets, or quanta of energy. Because of the dual particle-wave nature of photons², it is convenient to describe their energy according to the frequency with which their electromagnetic field oscillates through space:

\[ E = h\nu \]  

(1)

where \( E \) is the energy carried by the photon in J, \( h \) is Plank’s constant, \( 6.626\times10^{-34} \) J s), and \( \nu \) is the frequency of light in units of \( s^{-1} \). While the frequency of light determines its energy, it is often more common in photochemistry to discuss the energy of light in terms of its wavelength: \( \lambda \):

\[ \lambda = \frac{c}{\nu} \]  

(2)

where \( c \) is the speed of light in a vacuum. When we speak about the relative energies of light in terms of wavelength, higher energy photons have a shorter wavelength (e.g. ultraviolet light) than lower energy photons (infrared). For reference, the human eye is
sensitive to photons with wavelengths between 400 nm and 700 nm, which is known as the visible spectrum. In environmental photochemistry, the most important light source is the sun. At the surface of the earth, the solar spectrum contains wavelengths of light ranging from approximately 290 to 2500 nm, with the daytime peak emission wavelength being around 500 nm.

_Direct photolysis_

The first and most simple requirement in a direct photochemical reaction is that the compound of interest must absorb a photon. The Grotthus-Draper law, or the first law of photochemistry, states that only light that is absorbed by a system is capable of producing chemical changes. The Stark-Einstein law, or second law of photochemistry, states that every molecule participating in a photochemical reaction must absorb one quantum of light. This does not mean that for every photon absorbed only one reaction may take place, however. In some cases, the chemical change brought on by the absorption of one photon can lead to a chain of secondary non-photochemical reactions, as is the case for some radical processes. The important distinction is that only one photochemical reaction takes place, and that the subsequent reactions are thermal. We can describe a simple, dilute solution where molecule R undergoes a direct photochemical reaction to form P using equation 3:

\[
R + hv \xrightarrow{k_{\text{deg}}} P \tag{3}
\]

The first-order reaction rate constant \( k_{\text{deg}} \) for the degradation of P determines how quickly R will be transformed, therefore it is desirable to determine this rate constant for the purposes of predicting pollutant transformation rates in the environment. \( k_{\text{deg}} \) can be expressed as:
where $\varepsilon$ is the molar extinction coefficient in units $\text{M}^{-1}\text{cm}^{-1}$, which describes the efficiency with which a compound absorbs light at each wavelength, and $I$ is the irradiance of the light source in units millieinsteins $\text{cm}^{-2} \text{s}^{-1}$. These two terms summed up over all the relevant wavelengths determine the rate that a compound absorbs light, while the remaining term, $\Phi_{\text{deg}}$, or the degradation quantum yield, informs us about how efficient the absorbed photons are at inducing chemical change in the molecule. $\Phi_{\text{deg}}$ is simply the ratio of the number of chemical reactions induced divided by the number of photons absorbed. The value of $\Phi_{\text{deg}}$ can range from nearly 0 for molecules that are highly efficient at releasing their energy through physical processes, such as re-emission of a photon in fluorescence, up to values greater than 1000 for cases mentioned earlier where a single photochemical reaction leads to further thermal reactions. In dilute aqueous solutions, however, the value of $\Phi_{\text{deg}}$ typically ranges between 0 and 1 since the likelihood of chain reactions is rather low.

Direct photolysis has been known to play a role in the removal and transformation of a wide range of organic pollutants capable of absorbing sunlight. After light absorption, these photoexcited molecules may undergo a wide variety of reactions yielding new products. Scheme 2 highlights a few examples of direct photochemical transformations seen in aqueous environments. One of the simplest phototransformation pathways is photoionization. In a photoionization reaction, an electron in a molecule acquires enough energy by absorbing a photon that it can simply be ejected out of its orbital and taken up by the surrounding solvent. This reaction has been shown to play a role in the degradation of aniline and its derivatives$^{3,4}$, indole-containing molecules$^{5,6}$, and other environmental pollutants.
Once ionized, the resulting radical cations of these molecules undergo further transformation if they do not encounter a suitable electron donor in solution.

![Scheme 1 - Selected examples of direct photochemical reactions](image)

With the exception of fluorine, carbon-halogen bonds are relatively weak compared in comparison to C-H bonds in aromatic compounds. As an initial step in transformation, halogenated aromatic compounds often undergo C-X bond cleavage, which can be followed by hydroxylation\(^7,8\), hydrogenation\(^9\), and various rearrangements\(^10-12\).

While direct photochemical reactions often lead to the removal of pollutants, there exist a number of examples where direct photolysis leads to the production of molecules with more troublesome properties than the parent compound. A good example of this is the photochemical formation of polychlorinated p-dibenzodioxins, which are known toxins\(^13-16\) and heavily regulated as environmental pollutants,
following a photochemical ring closure reaction by triclosan\textsuperscript{17-21} and other halogenated diphenyl ether derivatives\textsuperscript{22}. Another interesting observation is that some molecules that undergo direct photolysis in water may revert back to their parent compounds in the dark. The steroid trenbolone acetate has been shown\textsuperscript{23,24} to undergo photochemical hydration reaction followed by a thermal dehydration in aqueous solution. This can lead to a diurnal variation in the observed concentration, and not accounting for this regeneration results in an underestimation of the lifetime of this compound in aquatic environments. In these cases, simply knowing the rate of direct photolysis for a pollutant fails to accurately capture its true environmental impact, and emphasizes that we must also study and account for the transformation products of environmental contaminants.

\textit{Indirect photolysis}

Given the limited range of UV wavelengths in the solar spectrum at earth’s surface, it comes as no surprise that many organic pollutants cannot absorb sunlight, and thus are not transformed by direct photolysis. Oftentimes these compounds may still be transformed by sunlight in an alternative way known as indirect photolysis. In an indirect photoreaction, another molecule, called a sensitizer, first absorbs light and either transfers energy to or reacts with the target molecule. A general expression for this type reaction is shown in equation 5:

\begin{equation}
S \xrightarrow{hv} S^* + R \xrightarrow{k_{sens}} S + R^* \text{ or } P
\end{equation}

Additionally, sensitizers may also transfer their energy to or generate an intermediate molecule, which can go on to react further with pollutants. In aquatic environments one of the dominant light absorbers and the most common sensitizer is chromophoric
dissolved organic matter\textsuperscript{25,26} (CDOM). CDOM can sensitize the formation of a number of reactive, oxygen based intermediates collectively known as reactive oxygen species (ROS).

\begin{center}
\begin{tikzpicture}
  \begin{scope}[xshift=-2cm]
    \node (a) at (0,0) {\includegraphics[width=2cm]{anthracene.png}}; \node (b) at (1,0) {+ \textsuperscript{1}O_2} \node (c) at (2,0) {\includegraphics[width=2cm]{anthracene2.png}};
  \end{scope}
  \begin{scope}[xshift=2cm]
    \node (a) at (0,0) {\includegraphics[width=2cm]{benzene.png}}; \node (b) at (1,0) {+ \textsuperscript{3}CDOM\textsuperscript{*}} \node (c) at (2,0) {\includegraphics[width=2cm]{benzene2.png}};
  \end{scope}
\end{tikzpicture}
\end{center}

\textbf{Scheme 2- Examples of indirect photochemical oxidations}

Some of the more important ROS species generated by the photolysis of CDOM include singlet oxygen (\textsuperscript{1}O\textsubscript{2}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), and hydroxyl radical (HO\textsuperscript{*}). \textsuperscript{1}O\textsubscript{2} is simply an excited state of O\textsubscript{2}, differing from its ground state only by the spin direction of one electron, and is a selective but powerful oxidant for some classes of molecules. In the aqueous phase, \textsuperscript{1}O\textsubscript{2} is short-lived and thus present in sunlit aquatic environments in the pM concentration range\textsuperscript{27}. Because of the unique electronic structure of \textsuperscript{1}O\textsubscript{2}, it is particularly well suited to participating in ring-forming addition reactions, and has been shown to play an important role in the aqueous degradation of anthracene\textsuperscript{28} via a formal 2+4 cycloaddition\textsuperscript{29} and the amino acid tryptophan\textsuperscript{30} via a formal 2+2 cycloaddition. H\textsubscript{2}O\textsubscript{2} is a less reactive, but much longer-lived ROS, and can be found at significantly higher concentrations than \textsuperscript{1}O\textsubscript{2} in aquatic environments. HO\textsuperscript{*} is the most reactive of all the ROS known to be generated from CDOM, reacting unselectively at diffusion-controlled rates with most organic compounds. Because of
this high reactivity, \( \text{HO}^\cdot \) plays an important role in the degradation of some of the most recalcitrant environmental pollutants\(^{31,32}\).

Much of the indirect photochemical behavior seen by CDOM can be explained by the presence of excited triplet state molecules\(^{33-36} \) (\( ^3\text{CDOM}^* \)) that are generated following irradiation. In addition to being a source of ROS, \( ^3\text{CDOM}^* \) can also act as an oxidant for environmental pollutants. Following excitation, the molecules that make up \( ^3\text{CDOM}^* \) become stronger oxidants, and thus may act as one-electron acceptors for a wide range of pollutants. These types of reactions are known as photoinduced electron transfers. Reactions of this type by \( ^3\text{CDOM}^* \) have been shown to be important in degrading pharmaceuticals\(^{37} \), phenols\(^{36,38} \), phenylurea herbicides\(^{37} \), as well as other molecules\(^{35} \) naturally present in the environment. The main components of CDOM thought to be responsible for the reactivity of \( ^3\text{CDOM}^* \) are aromatic ketones\(^{39} \). Several studies have shown that aromatic ketones in their first excited triplet state show high reactivity for one-electron oxidation reactions for compounds such as phenols\(^{34} \). Depending on the exact structure and reduction potential of these ketones, they may also react by acting as hydrogen atom acceptors for phenols and other labile molecules\(^{36,40,41} \).

**Goals and thesis overview**

The goal of this thesis was to investigate the role of various direct and indirect photochemical processes for different organic micropollutants in the aqueous phase. The scope of each chapter is different, but each involves the detailed study of a photooxidative mechanism related to known environmental pollutants. In Chapter 2, the direct photolysis of hydroxylated polybrominated diphenyl ethers (OH-PBDEs) was studied. OH-PBDEs are known transformation products\(^{42-46} \) of a class of flame retardants known as polybrominated diphenyl ethers (PBDEs) which have now been
banned due to their widespread environmental presence and persistence\textsuperscript{47,48}. In addition to being transformation products of PBDEs, OH-PBDEs are also known to be natural products\textsuperscript{49-51}. Our main objective was to study the photochemical ring closure of OH-PBDEs, which leads to the formation of polybrominated p-dibenzodioxins (PBDDs), a pollutant of even greater concern. We quantified the production of PBDDs and other phototransformation products of selected OH-PBDEs that were known to be transformation products of PBDEs. The goal of this work was to aid in determining the anthropogenic contribution to the observed concentration levels of PBDDs in marine environments.

In Chapter 3, the indirect photochemical oxidation mechanisms for two sets of model pollutants was investigated with a triplet excited state oxidant, methylene blue (MB). Motivated by the observation that many environmental micropollutants seem to be oxidized by one-electron transfer reactions with $^3$CDOM\textsuperscript{34-36,38,52}, we attempted to determine models capable of predicting bimolecular oxidation rate constants using both substituted anilines and phenols. Using transient absorption spectroscopy, the quenching of triplet MB by the model compounds was directly observed in order to determine reaction rate constants. The mechanism of photooxidation for anilines was found to be a one electron transfer from the aniline to MB, while phenols were observed to react by proton concerted electron transfer (PCET). In both cases, an adequate correlation between the oxidation reaction free energy and the bimolecular rate constant were found. For the electron transfer driven oxidation of anilines, bimolecular reaction rate constants were found to fit well to a Sandros-Boltzmann distribution, which has only recently been employed as an empirical model\textsuperscript{37,53} to describe photoinduced electron transfer reactions. For phenols, a good correlation between the phenolic OH bond dissociation free energy (BDFE) and the reaction rates was found. In plots of the reaction rate vs. BDFE however, a systematic deviation
proportional to the phenolic pKₐ was observed. Once factored into our model, the observed PCET reaction rates constants were in good agreement with predicted rates. To supplement the lack of reliable experimental aqueous oxidation potentials for anilines, which are needed to calculate electron transfer free energies, and to aid in future predictive models, the oxidation potentials for the substituted anilines were calculated *in silico*. In addition, the BDFEs of phenols were also computed.

In chapter 4, the focus was again on another direct photochemical oxidation process, the mechanism of the photochemical chlorination of pyrene (PYR). It has been shown recently⁵⁴,⁵⁵ that the concentration of 1-chloropyrene (Cl-PYR) and other chlorinated polycyclic aromatic hydrocarbons in marine sediments correlates strongly with water salinity, furthermore Cl-PYR was shown to be a transformation product of the photolysis of PYR in artificial seawater. Our goal was to test the hypothesis that Cl-PYR is formed in marine environments by chloride acting as a nucleophile and attacking the pyrene radical cation of (PYR⁺) that is formed by the oxidation of photoexcited PYR. Our experiments showed that the formation of Cl-PYR depends on the presence of O₂, which is responsible for oxidizing the excited PYR to form the intermediate radical cation, and that Cl-PYR formation can also be suppressed by the addition of an electron donor (N₃⁻). The role of PYR⁺⁺ is further supported by transient absorption observations that indicate the suppression of PYR⁺⁺ in oxygen-free conditions and rapid quenching by N₃⁻. These and other experiments described in chapter 4 support our hypothesis of Cl-PYR formation, and provide further evidence of the *in situ* formation of chlorinated PAHs in some marine environments.
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Chapter 2

Photochemical formation of brominated dioxins and other products of concern from hydroxylated polybrominated diphenyl ethers (OH-PBDEs)

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Published in

*Environmental science & Technology* 2012, 46(15), 8174-8180
Abstract

The photochemical conversion of selected hydroxylated polybrominated diphenyl ethers (OH-PBDEs) to dioxins and other products was investigated. OH-PBDEs, which are both transformation products of polybrominated diphenyl ethers and naturally occurring compounds, undergo direct photolysis to yield a number of products that may have a higher toxicity than their parent. The compounds investigated were 6-OH-PBDE 99, 6’-OH-PBDE 100, and 6’-OH-PBDE 118. Of special interest was 6’-OH-PBDE 118, a potential transformation product of PBDE 153 that is capable of photochemically generating 2,3,7,8-tetrabromodibenzo-p-dioxin, the most toxic brominated dioxin congener. Photolysis experiments were conducted at two different pH values to assess the photochemical behavior of both the phenol and phenolate form of the compounds. The percent conversion to dioxin and other photoproducts was determined and the natural product, 6-OH-PBDE 99, was found to have the highest conversion to dioxin (7%). The reaction quantum yields ranged from 0.027 to 0.16 across all photolysis conditions. In addition, it is shown that all three compounds are capable of photochemically generating other compounds of concern, including brominated phenols and a dibenzofuran.
Introduction

Polybrominated diphenyl ethers (PBDEs) have been incorporated as flame-retardants in a wide variety of consumer and industrial polymer products beginning in the 1970s. The use of PBDEs as a non-covalently bound additive allows these compounds to leach from the products into the environment. Consequently, PBDEs are now ubiquitous in both urban and pristine environments alike.\(^1\) PBDEs entering the environment have a number of potential fates, including biotic and abiotic transformation, sorption to soils/sediment, and bioconcentration. One important class of PBDE transformation products is comprised of the hydroxylated PBDEs (OH-PBDEs).

Three main routes are thought to be responsible for the environmental hydroxylation of PBDEs. The first is metabolic transformation in organisms exposed to PBDEs. OH-PBDEs have been identified as metabolites in rats and mice\(^3\)–\(^5\), and OH-PBDE concentrations have been shown to have a positive correlation to PBDE exposure in samples of human blood.\(^6\)–\(^7\) Another possible path to hydroxylation is via reaction with hydroxyl radical during atmospheric transport of PBDEs. Raff et al. demonstrated that hydroxylation of PBDEs occurs in gas phase laboratory experiments,\(^8\) and OH-PBDEs have been detected in rain and surface waters.\(^9\) PBDEs may also become hydroxylated during the oxidative stages of wastewater treatment processes,\(^9\) including activated sludge treatment and disinfection. The hydroxylation of PBDEs increases their toxicity, because OH-PBDEs are stronger endocrine disruptors\(^10\) and more closely resemble estrogenic compounds than PBDEs.\(^11\) OH-PBDEs have been detected in a variety of freshwater\(^12\) and marine aquatic animals, including salmon\(^13\), blue mussels\(^14\), dolphins\(^15\), and polar bears\(^16\). Further complicating the picture, OH-PBDEs are not only transformation products of PBDEs,
but are also natural products found in marine systems. OH-PBDEs and closely related compounds have been isolated from sponges\textsuperscript{17,18}, tunicates\textsuperscript{19}, and red algae\textsuperscript{14,20}, and radiocarbon experiments have further established their natural origin\textsuperscript{21,22}.

In addition to the concern posed by OH-PBDEs themselves, congeners that contain both a hydroxyl group ortho to the ether linkage and an ortho bromine on the opposite ring are capable of undergoing a photochemical ring closure to form polybrominated dibenzo-p-dioxins (PBDDs). This ring closure has been previously observed for triclosan\textsuperscript{23,24}, some of its chlorinated derivatives\textsuperscript{25}, as well as OH-PBDEs\textsuperscript{26,27}. PBDDs are structurally analogous to their better-studied chlorinated counterparts and share the same route of toxicity. Previous work has established that PBDDs are at least as toxic as chlorinated dioxins and thus are a cause for concern\textsuperscript{28-30}. Alongside OH-PBDEs and other halogenated compounds found in marine environments, there is some uncertainty as to the origin of PBDDs, which in some places have been detected in increasing concentrations\textsuperscript{31}. One possible source for these dioxins may be photochemical formation from OH-PBDEs (Figure 1).
The aim of this work was to investigate the photochemical degradation of three different OH-PBDEs: 6-OH-PBDE 99 (1), 6′-OH-PBDE 100 (2) and 6′-OH-PBDE 118 (3). Compound 1 was chosen because it is known to be a natural product, and has been found in a variety of biota.\textsuperscript{20,32} Compounds 2 and 3 were chosen because they represent transformation products of two congeners present in the industrial Penta mixture. Compound 3 is of particular interest since it is set up to undergo a photochemical transformation to 2,3,7,8-TBDD, the most toxic dioxin congener. It is also worth noting that 1 is a possible transformation product of BDE 99, which is also one of the main components of the Penta mixture. Here we report that compounds 1-3 are each photochemically transformed under environmentally relevant conditions to

\textbf{Figure 1.} Transformation scheme illustrating possible routes from PBDEs to OH-PBDEs (outside the scope of this study) and from OH-PBDEs to TBDDs.
polybrominated dioxins. In addition, we also report other photoproducts that are concerning from a toxicity standpoint, including brominated phenols, a dibenzofuran, and dihydroxybiphenyls.

**Experimental**

*Chemicals*

Compounds 1-3 were synthesized as described in Appendix 1. The preparation of 6-OH PBDE 47 has been described previously. Pyridine (Py; 99.9%), p-nitro anisole (PNA; 97%), NaOH, HCl, acetic acid, and sodium acetate were all purchased from Sigma Aldrich. Sodium tetraborate decahydrate (Na$_2$B$_4$O$_7$·10H$_2$O) was purchased from Merck. The standards used were 1,2,4,8/1,2,4,7-tetrabromodibenzo- $p$-dioxin (10 µg/mL in toluene, AccuStandard), 2,3,7,8-tetrabromodibenzo- $p$-dioxin (50 µg/mL in toluene, Wellington Labs), 2,4-dibromophenol (neat, Supelco), 2,4,5-tribromophenol (1 mg/mL in isopropanol, Chiron AS), and 2,4,6-tribromophenol (neat, Sigma Aldrich). All compounds were used as received with the exception of p-nitro anisole (PNA), which was first recrystallized from n-hexane. Ultrapure water (18 MΩ·cm) was obtained from a Barnstead Nanopure Diamond system. All solvents were of chromatography grade.

$pK_a$ value determinations

$pK_a$ values were measured by spectrophotometric titration. Because the water solubility of these compounds is very low when they are in the protonated form, titrations had to be performed in methanol:water mixtures. Solutions of 1-3 (15 mL, approx. 50 µM) in various methanol/water mixtures were brought to a pH value between 10 and 11 with NaOH. These solutions were then titrated to a pH value near
4 with a maximum of 200 µL of 0.1 M HCl, so that the change in OH-PBDE concentration was negligible. Absorbance measurements were taken with a Cary 100 Bio (Varian) UV/Visible spectrophotometer in 1.00 cm quartz cuvettes. Plots of the absorbance at ~ 310 nm vs pH were fit using a nonlinear regression (Kaleidograph, version 4.04, Synergy Software) to determine the pKₐ values.

Photolysis experiments

Samples of 1-3 in 1 mM pH 4 acetate buffer with 30% methanol or 1 mM pH 10 borate buffer with an initial concentration of 10 µM were placed in a merry-go-round sample holder of a Rayonet photoreactor containing two 300 nm bulbs (spectrum from within a test tube shown in Figure 2). The irradiation intensity in the range of 280-400 nm was about 40% of natural sunlight. For all photolysis experiments, triplicate 10 mL samples were irradiated in borosilicate glass test tubes. At set intervals, 150 µL aliquots were taken for analysis by either high-pressure liquid chromatography (HPLC) for kinetics and photoproduct quantification or liquid chromatography-mass spectrometry (LC-MS) for photoproduct identification. Dark controls showed no loss of parent compound over 2h irradiation time. To determine accurate polychromatic apparent reaction quantum yield values (Φᵢ), a PNA-Py chemical actinometer system³³ was irradiated alongside the samples in borosilicate glass test tubes. Φᵢ values for 1-3 were calculated according to equation 1.

\[
Φᵢ = \frac{kᵢ}{k_a} \sum \frac{ε_{a,λ}I_λ}{ε_{a,λ}I_λ} Φ_a
\]
First-order rate constants of degradation ($k_{\text{deg}}$) were compared to that of a PNA-Py chemical actinometer with a known $\Phi_a$. Molar absorptivities ($\varepsilon$) as a function of wavelength were measured for each compound at pH 4 and 10, and the lamp spectrum was measured from inside a borosilicate test tube using a fiber optic coupled to a calibrated radiometer (Ocean Optics Jaz).

The percent conversion is defined as the rate constant of formation of a given photoproduct ($k_p$) divided by the overall rate constant of degradation ($k_{\text{deg}}$) of the parent compound multiplied by 100. It is not possible to measure $k_p$ directly from the growth of product, because product appearance is kinetically bound to the rate constant of starting material loss $k_{\text{deg}}$. For a photostable product, the ratio $k_p/k_{\text{deg}}$ is equal to the final product concentration [$P$]$_{\infty}$ divided by the starting concentration of the parent compound [$A$]$_0$. Dioxin percent conversion values were calculated in this manner because of their negligible photodegradation over the course of the experiments. For the bromophenols, which were photolabile, the kinetic profiles were fit to equation 2. The term $f[A]_0$ refers to the fraction of A that goes to form photoproduct P (i.e., the percent conversion or $k_p/k_{\text{deg}}$), and the percent conversion values were calculated based on the $f[A]_0$ fit parameter.

\[
[P] = \frac{k_{\text{deg}}fA_0}{k_p - k_{\text{deg}}} (e^{-k_{\text{deg}}t} - e^{-k_p t})
\]  

(2)
Photoproduct identification and quantification

When photoproducts of 1-3 were commercially available, these compounds were used to create calibration curves for the HPLC and LC-MS (except for dioxins, which were only quantified by HPLC). All calibration curves used for quantification had an $R^2$ of 0.999 or better. Only photoproducts for which an authentic standard was available were quantified and a percent conversion reported, with the exception of 1,3,7,9-TBDD, which was quantified using the calibration curve for 2,3,7,8-TBDD. This was deemed reasonable because the slopes of the calibration curves for the other two tetrabrominated congener standards, 1,2,4,8- and 2,3,7,8-TBDD were within 2% of one another. The identity of 1,3,7,9-TBDD was inferred from the precursor structure reaction scheme in Figure 1 and on the basis of a photoproduct with an elution time and UV-vis that were similar to the two standards that were available. Photoproducts for which no authentic standards were available were identified and classified only by their general structure using exact mass measurements and relative retention time data. Products observed by LC-MS alone could not be quantified, because the MS instrument response strongly depends on the ionization efficiency of the compound.

HPLC analysis

Compounds 1-3 and actinometer samples were analyzed using a Dionex P680 HPLC with a PDA-100 photodiode array UV-vis detector. Injection volumes of 100 mL were used for all samples. The column was a Supelco Discovery C16 RP amide (15 cm × 4.6 mm, 5 µm dia. particles). For analysis of 1-3, the mobile phase was 85:13.5:1.5 methanol:pH 3 phosphate buffer:acetonitrile at a flow rate of 1 mL/min. All compounds were quantified at a wavelength of 210 nm except for the dioxins, which were quantified at 230 nm. Sample run times were 35 minutes. For the
actinometer, the mobile phase was 65:31.5:3.5 methanol:pH 3 phosphate buffer:acetonitrile at a flow rate of 1 mL/min. PNA was quantified at a wavelength of 314 nm. Sample run times were 7 minutes.

**LC-MS analysis**

Photoproducts of 1-3 were identified using a Waters NanoAquity UPLC interfaced to a Thermo Exactive Orbitrap high-resolution mass spectrometer. Injections (1 µL) were made onto a Waters Atlantis dC18 Nano Ease column (15 cm × 300 µm, 3 µm dia. particles) held at 40° C with a flow rate of 17 µL min⁻¹ and a mobile phase composition of 60:40 acetonitrile:water. All samples were analyzed by electrospray ionization MS in negative mode and were scanned from 100-600 m/z. Sample run times were 15 min.

**Results and Discussion**

**pKₐ values**

The pKₐ values for the phenolic groups of 1-3 were 5.81, 8.39, and 7.39, respectively (Table 1). The pKₐ value for 1, which contains three bromo substituents on the phenol ring, is significantly lower than for 2 and 3, which each only contain two bromo substituents on the phenol ring. A series of titrations for compounds 1 and 2 in different methanol:water compositions demonstrated linear relationships with the solvent composition that could be used to extrapolate to the pure aqueous pKₐ values. Compound 3 was titrated only in 50:50 methanol:water because of material limitations. A reasonable prediction of the aqueous pKₐ value was made for 3 using the common slope from the extrapolation plots for 1 and 2. The titration plots and extrapolations are provided in Appendix 1.
Table 1. pK$_a$ values, reaction quantum yields, and quantified photoproducts

<table>
<thead>
<tr>
<th>Parent compound</th>
<th>pK$_a$</th>
<th>$\Phi_r$ pH 4</th>
<th>$\Phi_r$ pH 10</th>
<th>Debromination$^a$ (% conversion)</th>
<th>C-O cleavage$^a$ (% conversion)</th>
<th>Dioxin$^a$ (% conversion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-OH-BDE 99 (1)</td>
<td>5.81*</td>
<td>0.027</td>
<td>0.031</td>
<td>6-OH-BDE 47 (1%)</td>
<td>2,4-dibromo-phenol (8%)</td>
<td>1,2,3,8-TBDD (7%)</td>
</tr>
<tr>
<td>6-OH-BDE 100 (2)</td>
<td>8.39*</td>
<td>0.063</td>
<td>0.16</td>
<td>6-OH-BDE 47 (1%)</td>
<td>2,4,6-tribromo-phenol (11%)</td>
<td>1,3,7,9-TBDD (1.1%)</td>
</tr>
<tr>
<td>6-OH-BDE 118 (3)</td>
<td>7.39*</td>
<td>0.057</td>
<td>0.13</td>
<td>n.q.$^d$</td>
<td>2,4,5-tribromo-phenol (13%)</td>
<td>2,3,7,8-TBDD (0.5%)</td>
</tr>
</tbody>
</table>

$^a$: Estimated pure aqueous pKa value determined from a series of titrations of solutions with different methanol:water compositions. pKa value given is the extrapolated value. $^b$: Estimated pKa value using the slope from the other compounds extrapolation (both lines have the same slope) and the pKa value determined in a 50% methanol solution. $^c$: Quantification is reported for pH 10 experiments. $^d$: Photoproducts for which no standard was available were not quantified.

Direct photolysis

The loss of compounds 1-3 during irradiation followed first-order kinetics under all experimental conditions tested. The starting concentration for all photolysis samples was low enough that self-screening was unimportant, making it unnecessary to correct kinetic data used to calculate $\Phi_r$ values. Figure 2 shows the absorption spectra and degradation plots for 1-3. In the phenol form, all of the compounds have a broad absorbance band with a maximum near 290 nm, tailing off to approximately zero absorbance at around 310 nm. In the phenolate form, the absorbance maximum red-shifts for each compound differently, but they all display a maximum between 300 and 310 nm and a tailing off to near zero absorbance around 340 nm. All
compounds degraded faster under basic conditions due mostly to the greater light absorption of the phenolate species.

Figure 2. The absorbance spectra for the phenol and phenolate forms of compounds 1-3 along with the lamp spectrum are shown in panels (A) and (B), respectively. Panels (C) and (D) show kinetic traces for the photodegradation of 1-3 at pH 4 and 10, respectively.

For the phenol forms of 1-3, $\Phi_r$ values ranged from 0.027 to 0.063, while the values for the phenolate forms ranged between 0.031 and 0.16 (Table 1). Our results are in good agreement with earlier work with halogenated phenoxyphenols. Under both acidic and basic conditions, 2 and 3 had noticeably larger $\Phi_r$ values than 1. One possible contributing factor to this observation is the bromine in the 3 position of 1, ortho to the hydroxyl group. While the origin of this “ortho effect” on quantum yield
is not understood, it is noteworthy, particularly in light of our previous work which showed the same effect for chlorinated triclosan derivatives.25

Brominated Dioxins

Dioxin formation was observed for 1-3 under all conditions. The percent conversion to dioxin for the compounds at pH 10 was found to be 7, 1.1, and 0.5%. In all cases, the dioxin photoproducts were relatively photostable under the experimental conditions, and were seen to photodegrade on the order of 100 times slower than the parent compounds during our experiments. The factors that control the wide range in dioxin conversion yields are not understood. Nevertheless, it is a happy circumstance that compound 3, which forms the most toxic dioxin congener, 2,3,7,8-TBDD, had the lowest dioxin yield of 0.5%. By contrast, compound 1, the natural product, was found to have an order of magnitude higher dioxin conversion of 7%.

Bromophenols

Compounds 1-3 all generated a bromophenol photoproduct from the cleavage of the ether linkage where the bridging oxygen atom remains with the non-phenolic ring. Interestingly, these were the only C-O cleavage products observed above trace levels in any of our experiments. The percent conversion values ranged from 8-13%. The amount of bromophenol photoproduct is not controlled by steric crowding around the ether linkage. Indeed, compound 3, the least sterically crowded congener, showed the highest bromophenol conversion. Noting that the higher conversion yields are associated with a greater number of bromo substituents on the departing phenol, the bromophenol production may instead be a function of the resulting bromophenolate’s leaving group ability. A study with a wider range of structural analogs will be needed to more rigorously test this hypothesis.
Debromination and di-OH-PBB formation

Hydrodebromination is one of the predominant degradation pathways for PBDEs, therefore we expected to observe a number of transformation products corresponding to reductive debromination. Instead, we observed only small amounts of debromination products. This conclusion was based on two sets of observations. First, we quantified the production of 6-OH-PBDE 47, the only debromination product standard that we had available. For 1 to form 6-OH-PBDE 47 it must lose the bromine ortho to the hydroxy group, while 2 must lose one of its two bromo substituents ortho to the ether linkage on the non-hydroxylated ring. In both cases, 6-OH-PBDE 47 is formed in less than 1% yield. Second, we examined LC-MS ion chromatograms for each of the hydrodebromination product masses. We observed a group of photoproducts with the same formula (and thus exact mass) as the reductive debromination products, which are believed to belong to another compound class. Based on their poor retention on the HPLC column (ca. 3 min), which is similar to bromophenols, and literature precedent for diphenyl ether to hydroxy biphenyl photoconversion, we tentatively assign these early eluting products as dihydroxylated polybrominated biphenyls (di-OH-PBBs). The analogous photochemical formation of OH-PBBs from a PBDE has been reported from the photolysis of BDE-153 in 20% yield. Due to a lack of authentic standards and an inability to collect enough material for structural identification by NMR, a firm assignment could not be made. Tentatively assigned debromination products, which have retention times near the parent OH-PBDEs, and di-OH-PBBs were observed in varying amounts for 1-3, but all compounds produced at least one of each.
Dihydroxy-PBDEs and hydroxydibenzofuran

The last two types of photoproducts that were identified were products arising from the replacement of bromine with a hydroxyl group to form di-OH-PBDEs, and products that had formally lost HBr to form a hydroxylated polybrominated dibenzofuran (OH-PBDF).

The di-OH-PBDEs are isobaric with tri-OH-PBBs and thus difficult to distinguish by MS. Furthermore, both compound types are expected to elute early in reverse phase LC. Indeed, the peaks assigned as di-OH-PBDEs had early retention times in the LC-MS chromatogram, similar to bromophenols. We favor the assignment of di-OH-PBDEs (versus tri-OH-PBBs) due to their kinetic behavior. Specifically, the plot of their appearance vs. time (figure S8) does not show any lag in initial production, as would be expected for a second-generation transformation product (i.e., arising from hydroxy-debromination of di-OH-PBBs).

Finally, we observed the formation of a photoproduct with a retention time similar to that of the dioxin products during HPLC analysis, and with a mass corresponding to the loss of HBr for compound 2. We propose that this product is a hydroxy-substituted polybrominated dibenzofuran (OH-PBDF). The large peak in the HPLC chromatogram suggests that a significant amount of the dibenzofuran photoproduct was formed. It is also worth noting that the photochemical formation of hydroxylated polychlorinated dibenzofurans has been previously observed from triclosan and its chlorinated derivatives.36,37

Because no authentic standards for OH-PBDFs are commercially available, we synthesized a tetrabrominated OH-PBDF to judge whether the UV-vis absorbance
spectrum and HPLC retention time was similar to that of the suspected OH-PBDF. The choice of the synthetic standard was constrained by its synthetic accessibility. While not a match to our proposed product, the standard was helpful due to the similarity of dibenzofuran retention times and absorbance spectra for congeners of the same degree of halogenation. The details of the synthesis can be found in Appendix 1. Our synthesized OH-PBDF had an HPLC retention time of 23.5 min and the suspected OH-PBDF produced by 2 had a retention time of 24.3 min, respectively (Figure 3). A comparison of their UV-vis spectra shows three similar spectral features and an absorbance tailing to zero near 330 nm. Based on this comparison and the exact mass match, we favor the assignment of the unknown as a tetrabrominated OH-PBDF, but cannot definitively assign the structure. Based on our proposed transformation mechanism (discussed below), we propose that 1,2,6,8-tetrabromo-4-hydroxydibenzo-furan is the specific congener formed from 2.

At first glance, the formation of OH-PBDF from 2 seems unlikely, because it does not have any hydrogen atoms ortho to the ether linkage to eliminate during cyclization. Our hypothesis is that furan formation proceeds via a photochemical electrocyclic ring closure to form the ring system skeleton, followed by a 1,3-migration of a bromine atom from the non-phenolic ring to the phenolic ring (Figure S9). Subsequent elimination and tautomerization would yield the proposed product (Figures 3 and S9).
**Figure 3.** (Top panels) The HPLC-UV (diode array) chromatogram of the product mixture resulting from the photolysis of 2 and the UV absorbance spectrum corresponding to the indicated peak. The peak eluting at 11.7 min is of the starting compound 2. (Bottom panels) The HPLC-UV chromatogram is of the synthesized OH-TBDF and the UV absorbance spectrum of the indicated peak. See text for assignment of the dibenzofuran structures.

**Environmental significance**

The presence of OH-PBDEs in the environment is now well established, and while the largest share of OH-PBDEs comes from natural sources, the possibility of anthropogenic sources remain. Our results show that 1-3 all photodegrade to generate transformation products that are themselves priority pollutants. While bromophenol production was seen for all compounds, the anthropogenically derived compounds, 2 and 3, produced the largest amounts. In addition to generating bromophenols, the previously unreported formation of di-OH-PBBs and OH-PBDFs from OH-PBDEs also could be of concern. Compound 1 is a known natural product, and has been...
isolated from biotic sources in the Baltic Sea.\textsuperscript{14} Our results show that 1 is readily capable of transforming into a TBDD under environmental conditions, and may be a major source of natural tetrabrominated dioxin in the Baltic Sea, a possibility that has been previously discussed.\textsuperscript{39} While 1 produced more dioxin than 2 and 3, the toxicity of the resulting dioxin is likely much higher for 2 and certainly for 3. The scarcity of toxicity data and lack of accepted toxic equivalent factors (TEFs) for the dioxins formed by 1 and 2 complicates the comparison of their relative dioxin toxicity. If we conservatively assume a TEF of 0.001 (the actual TEF is likely much lower) for 1,2,4,8-TBDD, then at the dioxin production rates from our experiments, compound 3 will produce 70 times the toxicity as the natural product 1 from the same starting concentrations.

Our work confirms that OH-PBDEs of both natural and anthropogenic origin are capable of being converted photochemically to a variety of other brominated compounds, including brominated dioxins. While the actual production rates for the anthropogenic OH-PBDEs remain unknown, this work supports the hypothesis that these compounds, whether natural or anthropogenic, are a source of brominated dioxins in the environment. Further work will be required to better constrain any estimates about the share of dioxin toxicity attributable to the various OH-PBDE sources.

**Acknowledgements**

We thank Prof. P.J. Alaimo (Seattle Univ.) for the helpful discussions. We gratefully acknowledge support of the U.S. National Science Foundation (CBET-0967163).
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Appendix to chapter 2

$pK_a$ determinations of compounds 1-3

Figure A1- Plot of observed $pK_a$ vs. % methanol for all titration experiments. For 6'-OH-PBDE 99 (1) and 6'-OH-PBDE 100 (2) the extrapolation lines used to find the pure water $pK_a$ are shown. The $pK_a$ for 6'-OH-PBDE 118 (3) in 50% methanol is also shown.

Figure A2- Titration curve for 6'-OH-PBDE 118
**Figure A3** - Titration curves for 6-OH-PBDE 99
Figure A4- Titration curves for 6’-OH-PBDE 100
**Selected extracted ion chromatograms**

**Figure A5** - Extracted ion mass chromatograms for OH-PBDE 99. All ions were observed as [M-H].
Figure A6- Extracted ion mass chromatograms for OH-PBDE 100. All ions were observed as [M-H]−.
Figure A7- Extracted ion mass chromatograms for OH-PBDE 118. All ions were observed as [M-H].
Figure A8- Plots of appearance vs. time for selected photoproducts of 6’-OH-PBDE 100. Note the Y-axis is labeled “Intensity” for photoproducts that were only identified by LC-MS and concentration is shown when quantification was possible.
Figure A9- Proposed mechanism for the formation of hydroxylated dibenzo-\(p\)-furan from 6’-OH-PBDE 100.
Synthesis of compounds 1-3

General
All reagents were purchased from commercial suppliers. Solvents were used as received unless otherwise specified. Reactions were performed under ambient conditions unless otherwise stated. Proton nuclear magnetic resonance spectroscopy (\(^1\)H NMR) and carbon nuclear magnetic resonance spectroscopy (\(^{13}\)C NMR) utilized a Varian Inova 500 MHz NMR spectrometer. Fluorine nuclear magnetic resonance spectroscopy (\(^{19}\)F NMR) utilized a Varian Unity 300 MHz NMR spectrometer. Electrospray ionization mass spectrometry (ESI-MS) and electrospray ionization high-resolution mass spectrometry (ESI-HRMS) utilized a BIO-TOF mass spectrometer. Melting points were determined manually using a Büchi Schmelzpunktbestimmungs apparat. 2,2’,4,4’-Tetrabromodiphenyliodonium chloride was synthesized according to a literature procedure\(^1\).

\[
\begin{align*}
\text{O} & \quad \text{O}
\end{align*}
\]

3-Bromo-2-methoxybenzaldehyde:
3-Bromosalicylaldehyde (0.8854 g, 4.40 mmol), NaOH (0.595 g, 14.9 mmol), and tetrabutylammonium hydroxide (7.16 g, 8.95 mmol) were partitioned between CH\(_2\)Cl\(_2\) (40 mL) and water (40 mL). To this bright yellow solution was added CH\(_3\)I (1.4 mL, 22 mmol). The solution was allowed to stir at room temperature until colorless, upon which the layers were separated. The aqueous layer was extracted with CH\(_2\)Cl\(_2\) (3 x 50 mL) and the organic layers were combined and dried over Na\(_2\)SO\(_4\). The solvents were removed in vacuo. Flash column chromatography (5:1 CH\(_2\)Cl\(_2\):hexanes; R\(_f\) = 0.57) gave the desired product (0.877 g, 93%) as a yellow oil. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta = 10.31\) (s, 1H, CHO), 7.77 (dd, J = 1.5, 7.5 1H, 4-H), 7.75 (dd, J = 1.5, 7.5, 1H, 6-H), 7.10 (dd, J = 7.8, 7.8, 1H, 5-H), 3.95 (s, 3H, CH\(_3\)) ppm. \(^{13}\)C NMR (500 NMR, CDCl\(_3\)) \(\delta = 189.02\) (CHO), 160.10 (2-C), 139.47 (4-C), 130.94 (1-C), 127.87 (6-C), 125.78 (5-C), 118.19 (3-C), 63.51 (OCH\(_3\)). ESI-MS: m/z [ion] (rel. int%) 238.8 [M+2] (100), 326.8 [M] (94), 239.8 [M+3] (18), 237.8 [M+1] (12). ESI-HRMS: calculated for [C\(_8\)H\(_7\)NaO\(_2\)Br\(^+\)] 236.9527, found 236.9516.

\[
\begin{align*}
\text{O} & \quad \text{OMe} & \quad \text{Br}
\end{align*}
\]

3-Bromo-2-methoxyphenol:
3-Bromo-2-methoxybenzaldehyde (0.768 g, 3.57 mmol) and KH\(_2\)PO\(_4\) (10.0 g, 73.6 mmol) were suspended in CH\(_2\)Cl\(_2\) (40 mL). In another flask was added H\(_2\)O\(_2\) (0.606 g, 5.35 mmol, 30%) and CH\(_2\)Cl\(_2\) (5 mL). The peroxide solution was cooled to 0\(^\circ\) C, upon which trifluoroacetic acid anhydride (3.8 mL, 27 mmol) was added dropwise and then allowed to stir for an additional hour at 0\(^\circ\) C. The aldehyde mixture was
cooled to 0°C and the peroxyde solution was added to the aldehyde mixture dropwise. The reaction mixture was allowed to stir for 2 h, upon which brine (10 mL) and aqueous NaHSO₃ (10 mL, 20%) were added to quench the reaction. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 30 mL). The organic layers were combined and the solvent was removed *in vacuo*. The residue was dissolved in CH₃OH (20 mL) with one drop of concentrated HCl. The reaction mixture was allowed to stir for 12 h and then the solvents were removed *in vacuo*. Flash column chromatography (4:1 CH₂Cl₂:hexanes; Rₛ = 0.24) gave the desired product (0.602 g, 84%) as a yellow oil. ¹H NMR (500 NMR, CDCl₃): δ = 7.07 (dd, J = 3.0, 8.0, 1H, 4-H), 6.93 (dd, J = 3.0, 8.0, 1H, 6-H), 6.90 (dd, J = 8.0, 8.0, 1H, 5-H), 6.04 (m, 1H, OH), 3.90 (s, 3H, CH₃) ppm. ¹³C NMR (500 NMR, CDCl₃) δ = 150.10 (1-C), 144.52 (2-C), 126.02 (5-C), 124.70 (4-C), 116.01 (3-C), 115.24 (6-C), 61.15 (CH₃) ppm. ESI-MS: m/z [ion] (rel. int%) 185.9 [M-1] (100), 187.9 [M+1] (98), 188.9 [M+3] (10), 186.9 [M] (6). ESI-HRMS: calculated for [C₇H₆BrO₂]⁻ 200.9551, found 200.9554.

### 3,4,6-Tribromo-2-methoxyphenol:
3-Bromo-2-methoxyphenol (0.435 g, 2.14 mmol) and CaCO₃ (0.644 g, 4.71 mmol) were suspended in CH₂Cl₂ (50 mL) and CH₃OH (20 mL). Benzytrimethylammonium tribromide (1.84 g, 6.43 mmol) was added in small portions over 2 h and then the reaction mixture was allowed to stir for an additional 2 h. The reaction mixture was filtered and the filtrate was added to aqueous NaHSO₃ (40 mL, 5%). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3x 50 mL). The organic fractions were combined and dried over Na₂SO₄. The solvents were removed *in vacuo*. Flash column chromatography (4:1 CH₂Cl₂:hexanes; Rₛ = 0.40) gave the desired product (0.702 g, 91%) as light yellow solid. ¹H NMR (500 NMR, CDCl₃) δ = 7.58 (s, 1H, 5-H), 5.97 (s, 1H, OH), 3.92 (s, 3H, CH₃) ppm. ¹³C NMR (500 NMR, CDCl₃) δ = 146.75 (2-C), 146.09 (1-C), 131.34 (5-C), 119.23 (4-C), 115.46 (3-C), 108.99 (6-C), 61.16 (OCH₃). ESI-MS: m/z [ion] (rel. int%) 358.8 [M+1]⁻ (100), 360.8 [M+3]⁻ (96), 356.8 [M-1]⁻ (24), 362.8 [M+5]⁻ (24). ESI-HRMS: calculated for [C₇H₄Br₃O₂]⁻ 358.7741, found 358.7741.

### 1,2,5-Tribromo-4-(2,4-dibromophenoxy)-3-methoxybenzene:
3,4,6-Tribromo-2-methoxyphenol (0.334 g, 0.93 mmol), K₂CO₃ (0.339 g, 2.45 mmol), and 18-crown-6 ether (0.034 g, 0.13 mmol) were suspended in N,N-dimethylacetamide (25 mL). 2,2',4,4'-Tetrabromo-diphenyliodonium chloride (0.800 g, 1.27 mmol) was added to the reaction mixture and then heated to 80°C where it
was stirred for 1 h. The mixture was cooled to room temperature and diluted with CH₂Cl₂ (30 mL) and water (30 mL). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 50 mL). The organic fractions were combined, washed with aqueous NaHSO₃ (50 mL, 5%), aqueous NaOH (2 x 50 mL, 1.0 M), water (3 x 50 mL), and dried over Na₂SO₄. The solvent was removed in vacuo. Flash column chromatography (4:1 hexanes:CH₂Cl₂, Rₚ = 0.56) gave the desired product (0.355 g, 64%) as a colorless resin. 

1H NMR (500 MHz, CDCl₃): δ = 7.77 (d, J = 2.0, 1H, 3'-H), 7.75 (s, 1H, 4-H), 7.26 (dd, J = 2.5, 9.0, 1H, 5'-H), 6.33 (d, J = 9.0, 1H, 6'-H), 3.86 (s, 3H, CH₃) ppm. 

13C NMR (500 MHz, CDCl₃) δ = 152.53 (1-C), 152.34 (1'-C), 144.96 (2-C), 136.01 (3'-C), 132.01 (5'-C), 131.38 (5-C), 122.77 (5-C), 121.40 (3-C), 117.36 (4'-C), 115.59 (6'-C), 115.42 (6-C), 112.05 (2'-C), 61.82 (OCH₃).

ESI-MS: m/z [ion] (rel. int%) 618.5 [M+4] (100), 616.5 [M+2] (82), 614.5 [M] (57), 620.5 [M+6] (35). HRMS: calculated for [C₁₃H₇Br₅NaO₂]⁺ 618.6199, found 618.6184.

2,3,5-tribromo-6-(2,4-dibromophenoxy)phenol:

1,2,5-tribromo-4-(2,4-dibromophenoxy)-3-methoxybenzene (0.147 g, 0.24 mmol) was dissolved in a round bottom flask equipped with a reflux condenser with dry CH₂Cl₂ (15 mL, from CaH₂). BBr₃ (240 mL, 2.4 mmol) was added and the solution was heated to reflux for 48 h, upon which the solution was cooled to 0°C and water (15 mL) was carefully added. The solution was extracted with CH₂Cl₂ (3 x 25 mL) and dried over Na₂SO₄. The solvents were removed in vacuo. Flash column chromatography (1:1 CH₂Cl₂:hexanes, Rₚ = 0.26) gave the desired product (0.132 g, 92%) as a colorless resin. 

1H NMR (500 MHz, CDCl₃): δ = 7.78 (d, J = 2.5, 1H, 3'-H), 7.55 (s, 1H, 4-H), 7.28 (dd, J = 2.5, 8.5, 1H, 5'-H), 6.42 (d, J = 8.5, 1H, 6'-H), 6.05 (s, 1H, OH) ppm. 

13C NMR (500 MHz, CDCl₃) δ = 152.18 (1'-C), 148.21 (1-C), 138.720 (2-C), 136.15 (3'-C), 131.39 (5'-C), 128.05 (4-C), 122.24 (5-C), 116.78 (3-C), 115.84 (6'-C), 113.50 (4'-C), 113.21 (6-C), 112.67 (2'-C). ESI-MS: m/z [ion] (rel. int%) 578.6 [M+3] (100), 580.6 [M+5] (93), 576.6 [M+1] (40), 582.6 [M+7] (36), 574.6 [M] (5), 584 [M+9] (4). ESI-HRMS: calculated for [C₁₂H₄Br₅O₂]⁺ 578.6087, found 578.6074.

4,5-Dibromo-guaiacol:

Guaiacol (1.02 g, 8.18 mmol) was added to a round bottom flask equipped with a magnetic stir bar and an addition funnel. After purging the reaction vessel with dry nitrogen gas, bromine (0.84 mL, 16 mmol) dissolved in CH₂Cl₂ (4 mL) was added to the guaiacol over the course of 2.5 h. After complete addition of the bromine solution, the solvent was removed under reduced pressure to yield the crude product as a white
solid, Recrystallization from EtOH/water (2:3) resulted in the desired product (1.945 g, 84%) as long white needles. $^1$H NMR (500 NMR, CDCl$_3$): $\delta = 7.17$ (s, 1H, 6-H), 7.05 (s, 1H, 3-H), 5.61 (s, 1H, OH), 3.88 (s, 3H, OCH$_3$) ppm. $^{13}$C NMR (500 NMR, CDCl$_3$) $\delta = 146.45$ (2-C), 145.54 (1-C), 119.14 (6-C), 115.47 (5-C), 115.36 (3-C), 113.80 (4-C) ppm. ESI-MS: m/z [ion] (rel. int%) 280.9 [M+1] (100), 282.9 [M+3] (45), 278.9 [M-1] (44), 281.9 [M+2] (4), 279.9 [M] (3), 283.9 [M+4] (2). ESI-HRMS: calculated for [C$_7$H$_5$Br$_2$O$_2$] 280.8636, found 280.8637. Melting point: 92-93 oC, lit. 94-95 oC (2).

2,5-Dibromo-4-nitro-fluorobenzene:
2,5-Dibromo-fluorobenzene (5.03 g, 19.8 mmol) was placed in a round bottom flask equipped with a magnetic stir bar. The starting material was dissolved in CH$_2$Cl$_2$ (20 mL), trifluoroacetic anhydride (10 mL), and trifluoroacetic acid (20 mL). The solution was cooled to 0 oC and to this solution was added ammonium nitrate (1.98 g, 24.7 mmol). The clear orange solution was allowed to warm to room temperature overnight, upon which the solvent was removed via rotary evaporation. Flash column chromatography (1:1 CH$_2$Cl$_2$:hexanes; $R_f = 0.60$) provided the desired product (5.04 g, 85%) as a light yellow solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta = 8.528$ (d, J = 6.5, 1H, 3-H), 8.11 (d, J = 8.5, 1H, 6-H) ppm. $^{13}$C NMR (500 MHz, CDCl$_3$) $\delta = 160.22$ (d, J = 255.7, 1-C), 147.12 (s, 4-C), 130.77 (s, 3-C), 123.14 (d, J = 28.2, 6-C), 114.53 (d, J = 10.1, 5-C), 108.95 (d, J = 23.1, 2-C) ppm. $^{19}$F NMR (300 MHz, CDCl$_3$) $\delta = -99.79$ (1-F) ppm.

1,4-dibromo-2-(4,5-dibromo-2-methoxyphenoxy)-5-nitrobenzene: 2,5-Dibromo-4-nitro-fluorobenzene (1.08 g, 3.6 mmol) and K$_2$CO$_3$ (1.49 g, 10.8 mmol) were placed a 2-necked round bottom flask equipped with a magnetic stir bar, reflux condenser, and an addition funnel with pressure equalizing sidearm containing 4,5-dibromo-guaicol (1.02g, 3.6 mmol). The reaction set-up was placed under vacuum for 1 h, after which it was purged with nitrogen gas. Dry acetone was added to the reaction flask (10 mL) and to the addition funnel (6 mL). The reaction flask was heated to reflux and then the guaicol solution was slowly added over the course of 1 h. The reaction was allowed to stir for an additional 2 h upon completion of the addition and then cooled to room temperature. The solvent was removed via rotary evaporator and the resulting residue was partitioned in CH$_2$Cl$_2$:water (1:1). The layers were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 50 mL). The combined organic layers were washed with 10% NaOH, brine, and then dried over anhydrous Na$_2$SO$_4$. Removal of
the solvent via rotary evaporator left a viscous red oil. Flash column chromatography (1:1 CH$_2$Cl$_2$:hexanes; R$_f$ = 0.52) and subsequent recrystallization from EtOH/water yielded the desired product (1.26 g, 62%) as fine white needles. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 8.26 (s, 1H, 6-H), 7.40 (s, 1H, 3-H), 7.30 (s, 1H, 6'-H), 6.84 (s, 1H, 3'-H), 3.80 (s, 3H, OCH$_3$) ppm. $^{13}$C NMR (500 MHz, CDCl$_3$) $\delta$ = 157.45 (2-C), 150.80 (2'-C), 144.06 (5-C), 141.59 (1'-C), 131.18 (6-C), 126.84 (3-C), 122.62 (4'-C), 120.60 (6'-C), 118.15 (3'-C), 115.40 (5'-C), 110.79 (1-C), 110.79 (4-C), 56.58 (OCH$_3$) ppm. ESI-MS: m/z [ion] (rel. int%) 585.4 [M+6] (100), 583.4 [M+4] (86), 581.4 [M+2] (50), 586.4 [M+8] (27). ESI-HRMS: calculated for [C$_{13}$H$_7$Br$_4$NNaO$_4$]$^+$ 585.6945, found 585.6945.

$\text{2,5-dibromo-4-(4,5-dibromo-2-methoxyphenoxy)-benzenaminium chloride:}$
1,4-dibromo-2-(4,5-dibromo-2-methoxyphenoxy)-5-nitrobenzene (0.168 g, 0.30 mmol), CH$_3$OH (50 mL), and glacial acetic acid (5 mL) were placed in a round bottom flask equipped with a magnetic stir bar and reflux condenser. To this solution was added iron powder (0.515 g, 9.22 mmol, ~325 mesh) and the solution was heated to reflux. After 5 h at reflux, the solvent was removed via rotary evaporator. The residue was suspended in benzene and the iron was removed via vacuum filtration. The filtrate was washed with boiling benzene (3 x 50 mL) and then the combined organic fractions were cooled to 0 °C. Dry HCl (HCl on anhydrous calcium chloride) was bubbled through the cooled benzene solution to precipitate out the desired product (0.118 g, 69%) as a pale solid. $^1$H NMR (500 MHz, d$_6$-DMSO): $\delta$ = 7.46 (s, 1H, 6-H), 7.18 (s, 1H, 3-H), 7.12 (s, 1H, 6'-H), 6.83 (s, 1H, 3'-H), 3.92 (br, 3H, NH$_3$), 3.83 (s, 3H, OCH$_3$) ppm. ESI-MS: m/z [ion] (rel. int%) 531.7 [M+5] (100), 529 [M+2] (61), 533.7 [M+7] (51). ESI-HRMS: calculated for [C$_{13}$H$_9$Br$_4$NO$_2$]$^+$ 531.7404, found 531.7395. The corresponding free amine was isolated following alkaline workup. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 7.18 (s, 1H, 6'-H), 7.07 (s, 1H, 3'-H), 7.02 (s, 1H, 3-H), 6.86 (s, 1H, 6-H), 4.09 (s, 2H, NH$_2$), 3.88 (s, 3H, OCH$_3$) ppm. $^{13}$C NMR (500 MHz, CDCl$_3$) $\delta$ = 149.52 (2'-C), 146.48 (1'-C), 144.10 (4-C), 142.36 (1-C), 124.79 (3-C), 121.22 (6'-C), 119.25 (3'-C), 117.97 (4'-C), 117.37 (6-C), 114.81 (5'-C), 114.69 (2-C), 107.89 (5-C), 56.61 (OCH$_3$) ppm. ESI-MS: m/z [ion] (rel. int%) 553.7 [M+4] (100), 555.7 [M+6] (71), 551.7 [M+2] (60), 549.7 [M] (19), 557.7 [M+7] (18), 554.7 [M+5] (13), 552.7 [M+3] (12), 556.7 [M+7] (12), 550.7 [M+1] (2). ESI-HRMS: calculated for [C$_{13}$H$_6$Br$_4$NNaO$_4$]$^+$ 553.7224, found 553.7224.
**1,2,4-tribromo-5-(4,5-dibromo-2 methoxyphenoxy)benzene:**
Copper(II) bromide (0.046 g, 0.20 mmol) was placed in a two-necked round bottom equipped with a magnetic stir bar, reflux condenser, and an addition funnel. The reaction apparatus was purged with nitrogen, and tert-butyl nitrite (30 mL, 0.25 mmol) and dry acetonitrile (10 mL) were added to the reaction flask. 2,5-Dibromo-4-(4,5-dibromo-2-methoxyphenoxy)aniline (0.088 g, 0.17 mmol) was dissolved in CH₂Cl₂ (5 mL) and charged into the addition funnel. The reaction mixture was heated to reflux and the amine solution was added dropwise over 5 min. The reaction solution was allowed to stir at reflux for 3 h, after which it was cooled to room temperature and poured into 20% aqueous HCl (75 mL). The resulting white precipitate was extracted with Et₂O (2 x 100 mL). The combined organic fractions were washed with 20% aqueous HCl (50 mL) and then dried over anhydrous Na₂SO₄. Excess solvent was removed via rotary evaporator and flash column chromatography (4:1 hexanes: CH₂Cl₂; Rf = 0.38) yielded the desired product (0.081 g, 82%) as a white solid. ¹H NMR (500 MHz, CDCl₃): δ = 7.85 (s, 1H, 5-H), 7.25 (s, 1H, 2-H), 7.21 (s, 1H, 6-H), 6.92 (s, 1H, 3-H), 3.82 (s, 3H, OCH₃) ppm. ¹³C NMR (500 MHz, CDCl₃): δ = 153.54 (1-C), 150.67 (2'-C), 143.29 (1'-C), 137.11 (5-C), 125.32 (2-C), 124.01 (3-C), 121.67 (6'-C), 120.96 (4'-C), 118.90 (4-C), 117.90 (3'-C), 115.11 (5'-C), 112.39 (6-C) ppm.

**4,5-dibromo-2-(2,4,5-tribromophenoxy)phenol:**
1,2,4-tribromo-5-(4,5-dibromo-2-methoxyphenoxy)benzene (0.041 g, 0.07 mmol) was dissolved in a round bottom flask equipped with a reflux condenser with dry CH₂Cl₂ (10 mL, from CaH₂). BBr₃ (66 mL, 0.70 mmol) was added and the solution was heated to reflux for 72 h, after which time the solution was cooled to 0 °C and water (10 mL) was carefully added. The solution was extracted with CH₂Cl₂ (3 x 25 mL) and dried over Na₂SO₄. The solvents were removed via rotary evaporator. Flash column chromatography (1:1 CH₂Cl₂:hexanes, Rf = 0.21) gave the desired product (0.081 g, 82%) as a white resin. ¹H NMR (500 MHz, CDCl₃): δ = 7.90 (s, 1H, 5'-H), 7.35 (s, 1H, 2'-H), 7.23 (s, 1H, 3-H), 6.97 (s, 1H, 6-H), 5.74 (s, 1H, OH) ppm. ¹³C NMR (500 MHz, CDCl₃) δ = 151.84 (1'-C), 146.49 (1-C), 142.74 (2-C), 137.58 (3'-C), 124.63 (5'-C), 124.46 (6'-C), 123.38 (5-C), 121.84 (3-C), 121.52 (6-C), 120.25 (4'-C), 114.63 (4-C), 113.90 (2'-C) ppm. ESI-MS: m/z [ion] (rel. int%) 578.7 [M+3]
(100), 580.7 [M+5] (91), 576.7 [M+1] (39), 582.7 [M+7] (29), 574.7 [M-1] (6), 584 [M+9] (4). ESI-HRMS: calculated for [C_{12}H_4Br_5O_2] -578.6087, found 578.6087.

2,4,6-Tribromo-iodobenzene:
To a slurry of 2,4,6-tribromoaniline (15.0 g, 45.5 mmol) suspended in concentrated HCl (30 mL) cooled to 0 °C was slowly added an aqueous solution of NaNO_2 (3.28 g, 47.5 mmol, 15 mL). The resulting yellow solution was filtered through glass wool and carefully added to an aqueous solution of KI (75.6 g, 455 mmol, 115 mL) at room temperature. The mixture was allowed to stir for 1 h upon completion of the addition, after which CH_2Cl_2 (150 mL) and aqueous Na_2SO_3 (1.0 M, 10 mL) was added. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with 10% HCl, brine, and dried over Na_2SO_4. Removal of solvent via rotary evaporator left a crude red solid. Recrystallization from CH_2Cl_2:hexanes (3:1) yielded the desired product (6.76 g, 34%) as pale white crystals. ^1H NMR (500 MHz, CDCl_3): δ = 7.70 (s, 2H, 3'-H, 5'-H). ^13C NMR (500 MHz, CDCl_3): δ = 133.57 (2C, 3'-C, 5'-C), 131.85 (2C, 2'-C, 6'-C), 122.98 (1C, 4'-C), 108.18 (1C, 1-C) ppm. Melting point: 104-105 °C, lit. 104-105 °C.

(4-methoxyphenyl)(2,4,6-tribromophenyl)iodonium bromide:
H_2O_2 (222 mL, 1.96 mmol, 30%) was placed in a pear-shaped flask equipped with a stir bar and cooled to 0 °C. Trifluoroacetic anhydride (1.85 mL, 13.3 mmol) was added over 5 min followed by 2,4,6-tribromo-iodobenzene (1.10 g, 2.50 mmol) dissolved in CH_2Cl_2 (5 mL) added over 10 min. The solution was allowed to warm up to room temperature overnight after which the solvents were removed in vacuo. The resulting pale solid was dissolved in 10 mL of CH_2Cl_2:acetic anhydride (1:1) and glacial acetic acid (192 mL) was added. The solution was cooled to -20 °C and anisole (550 mL, 5.04 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise. After the addition was complete, the solution was allowed to stir at -20 °C for 1 h and then warmed to room temperature and stirred for another 1 h. The resulting light blue solution was placed under vacuum to remove the solvent and the residue was dissolved in a minimal amount of CH_3OH (10 mL). A saturated solution of aqueous NaBr (5 mL) was added to the methanolic solution and the resulting precipitate was filtered off and dried in a vacuum oven to yield the desired product (1.51 g, 96%) as a light yellow solid (purity >90% via ^1H NMR). ^1H NMR (500 MHz, d_6-DMSO): δ = 8.12 (s, 2H, 3-H, 5-H), 7.99 (d, J = 8.5, 2H, 2'-H, 6'-H), 7.02 (d, J = 8.7, 2H, 3'-H, 5'-H) 3.75 (s, 3H, OCH_3) ppm. ^13C NMR (500 MHz, d_6-DMSO): δ = 161.78 (C'OCH_3),
134.61 (2C, 2'-C, 6'-C), 133.73 (1-C), 129.78 (4-C), 129.27 (2C, 3-C, 5-C), 117.50 (2C, 3'-C, 5'-C), 114.13 (1'-C), 110.87 (2C, 2-C, 6-C), 55.90 (OCH$_3$) ppm.

3,5-dibromo-2-(2,4,6-tribromophenoxy)benzaldehyde:

3,5-Dibromo-salicylaldehyde (0.341 g, 1.22 mmol) was placed in a round bottom flask equipped with a magnetic stir bar and a reflux condenser. The aldehyde was dissolved in 20 mL of dioxane:water (1:1) and then to the solution was added NaOH (0.102 g, 2.54 mmol). To the resulting bright yellow solution was added (4-methoxyphenyl)(2,4,6-tribromophenyl)iodonium bromide (1.14 g, 1.81 mmol) and the reaction mixture was heated to 80 °C. After 2 h at 80 °C, ice was added to the solution and the resulting mixture was extracted with CH$_2$Cl$_2$ (3 x 50 mL). The organic fractions were washed with 1 M aqueous NaOH (2 x 50 mL), water (2 x 50 mL), and then dried over Na$_2$SO$_4$. The solvent was removed via rotary evaporator and flash chromatography (3:2 hexanes: CH$_2$Cl$_2$; R$_f$ = 0.64) yielded the desired product (0.302 g, 42%) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$): δ = 10.37 (s, 1H, CHO), 7.98 (s, 1H, 4-H), 7.87 (s, 1H, 2-H), 7.69 (s, 2H, 3'-H, 5'-H) ppm. $^{13}$C NMR (500 MHz, CDCl$_3$): δ = 186.96 (CHO), 153.93 (2-C), 149.18 (1'-C), 141.83 (4-C), 135.63 (2C, 3'-C, 5'-C), 130.94 (6-C), 130.19 (1-C), 118.15 (4'-C), 117.93 (2C, 2'-C, 6'-C), 116.37 (3-C), 113.48 (5-C) ppm. ESI-MS: m/z [ion] (rel. int%) 614.5 [M+4] (100), 616.5 [M+6] (95), 612.5 [M+2] (56), 618.5 [M+8] (47), 610.5 [M] (13), 620.5 [M+10] (8). ESI-HRMS: calculated for [C$_{13}$H$_5$Br$_5$NaO$_2$]$^+$ 614.6063, found 614.6067.

3,5-dibromo-2-(2,4,6-tribromophenoxy)phenol:

3,5-dibromo-2-(2,4,6-tribromophenoxy)benzaldehyde (0.131 g, 0.22 mmol) and KH$_2$PO$_4$ (0.240 g, 1.76 mmol) were placed in a round bottom flask equipped with a magnetic stir bar and suspended in CH$_2$Cl$_2$ (10 mL). In another flask was added H$_2$O$_2$ (0.050 g, 0.44 mmol, 30%) and CH$_2$Cl$_2$ (1 mL). The peroxide solution was cooled to 0 °C, to which trifluoroacetic acid anhydride (230 mL, 1.65 mmol) was added dropwise and then allowed to stir for 1 h at 0 °C. The aldehyde mixture was cooled to 0 °C and then the peroxide solution was added dropwise. The reaction mixture was allowed to stir for 5 h, upon which brine (20 mL) and aqueous NaHSO$_3$ (20 mL, 20%) were added to quench the reaction. The layers were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 30 mL). The organic layers were combined and the solvent was removed in vacuo. The residue was dissolved in CH$_3$OH (20 mL) with one drop of concentrated HCl. The reaction mixture was allowed to stir for 18 h and
then the solvents were removed via rotary evaporator. Flash column chromatography (4:1 CH₂Cl₂:hexanes; Rₜ = 0.42) gave the desired product (0.112 g, 87%) as a white solid. ¹H NMR (500 NMR, CDCl₃): δ = 7.68 (s, 2H, 3'-H, 5'-H), 7.19 (d, J = 2.5, 1H, 4'-H), 7.12 (d, J = 2.0, 1H, 6-H), 5.84 (s, 1H, OH) ppm. ¹³C NMR (500 NMR, CDCl₃) δ = 149.19 (1-C), 135.63 (1'-C), 135.40 (2C, 3'-C, 5'-C), 127.58 (4-C), 118.88 (6-C), 117.92 (2-C), 117.51 (3-C), 116.65 (4'-C), 113.10 (2C, 2'-C, 6'-C), 111.78 (5-C) ppm. ESI-MS: m/z [ion] (rel. int%) 578.7 [M+3] (100), 580.7 [M+5] (85), 576.7 [M+1] (44), 582.7 [M+7] (34), 574.7 [M-1] (7), 584 [M+9] (6). ESI-HRMS: calculated for [C₁₂H₄Br₅O₂] 578.6087, found 578.6087.
Figure S10- Synthesis of OH-PBDE-99. Reaction conditions: a) \((C_4H_9)_4NOH, NaOH, MeI, CH_2Cl_2, H_2O\), 93%; b) i) H_2O_2, (CF_3CO)_2O, KH_2PO_4, CH_2Cl_2, ii) MeOH, HCl, 84%; c) BTMA-Br_3, CaCO_3, MeOH, CH_2Cl_2, 91%; d) K_2CO_3, 18-crown-6, DMAC, 64%; e) BBr_3, CH_2Cl_2, 92%.

Figure S11- Synthesis of OH-PBDE-100. Reaction conditions: a) i) NaNO_2, HCl, 0°C, ii) KI, H_2O, 34%; b) i) H_2O_2, TFA, TFAA, CH_2Cl_2, ii) Ac_2O, CH_3CO_2H, anisole, CH_2Cl_2, iii) NaBr, MeOH, H_2O, 96%; c) NaOH, dioxane, H_2O, 42%; d) i) H_2O_2, (CF_3CO)_2O, KH_2PO_4, CH_2Cl_2, ii) MeOH, HCl, 87%.

Figure S12- Synthesis of OH-PBDE-118. Reaction conditions: a) NH_4NO_3, TFAA, TFA, CH_2Cl_2, 85%; b) Br_2, CH_2Cl_2, 84%; c) K_2CO_3, acetone, 62%; d) Fe, MeOH, HOAc, 69%; e) CuBr, tBuNO, CH_3CN, 82%; f) BBr_3, CH_2Cl_2, 85%.
Synthesis of dibenzofuran derivatives

General
All reagents were purchased from commercial suppliers. Solvents were used as received unless otherwise specified. Reactions were performed under nitrogen. Proton nuclear magnetic resonance spectroscopy (¹H NMR) and carbon nuclear magnetic resonance spectroscopy (¹³C NMR) were conducted with a Bruker Avance III 400 MHz NMR spectrometer. Exact mass measurements were acquired by ESI-HRMS using a Thermo Exactive Orbitrap instrument. Dibenzofuran-4-ol was prepared following a previously published method⁴. Carbon numbers were assigned following IUPAC nomenclature for fused rings⁵.

Dibenzofuran-4-ol:
Dibenzofuran (2.02 g, 12.0 mmol), TMEDA (2.3 g, 19.8 mmol), and dry Et₂O (50 mL) were placed in a 2-neck round bottom flask equipped with a reflux condenser and stir bar. With stirring, n-BuLi (5 ml, 2.5 M in hexane) was added and the mixture was heated to reflux for 1 h. The mixture was cooled to 0 °C and tributyl borate (3.8 mL, 14.4 mmol) was added until the yellow precipitate dissolved. The mixture was kept at 0 °C for 45 min, and then was allowed to warm to room temperature over 1 h. The mixture was again cooled to 0 °C and 30% H₂O₂ (5 ml) was added dropwise while stirring. The mixture was heated to reflux for 1.5 h, cooled to 0 °C and acidified with 5 M HCl (10 mL). The organic phase was washed with cold 10% ammonium iron(II) sulphate (2 x 50 mL) and extracted with 2 M NaOH (3 x 50 mL). The combined aqueous extract was acidified and extracted with Et₂O (3 x 100 mL) and dried with Na₂SO₄. The solvent was evaporated in vacuo and the crude mixture was filtered through silica gel with 15% Et₂O:petroleum ether giving the desired product (1.08 g, 48%) as off-white crystals. ¹H NMR (400 MHz, CDCl₃) δ= 7.94 (dd, J = 0.7, 7.7 1H, 9-H), 7.59 (d, 1H, 6-H), 7.52 (dd, 1H, 1-H), 7.47 (td, 1H, 7-H), 7.36 (td, 1H, 8-H), 7.22 (t, 1H, 2-H), 7.02 (d, 1H, 3-H), 5.33 (s, 1H, OH). ¹³C NMR (400MHz, CDCl₃) δ= 156.21 (4-C), 144.18 (4a or 5a-C), 141.27 (4a or 5a-C), 127.43 (7-C), 125.75 (9a or 9b-C), 124.75 (9a or 9b-C), 123.83 (2-C), 123.14 (8-C), 121.18 (9-C), 113.69 (3-C), 112.96 (1-C), 111.94 (6-C) ESI-HRMS: calculated for [CαH₇O₂]⁺ 183.0446, found 183.0446.

1,2,3,8-tetrabromo-dibenzofuran-4-ol:
Dibenzofuran-4-ol (.52 g, 2.83 mmol) was placed in a 2-neck round bottom flask equipped with an addition funnel and stir bar and dissolved in a minimum amount of dry CH₂Cl₂ (10mL). To the addition funnel was added a mixture of Br₂ (2.79 g, 17.4 mmol) and dry CH₂Cl₂ (4 mL). The Br₂ solution was added slowly over a period of 15 min at room temperature. After the addition was complete, the mixture was stirred at
room temperature for 30 h. The remaining Br$_2$ was removed by washing the mixture with a solution of 10% Na$_2$SO$_3$ until the red color disappeared. The mixture was then extracted with CH$_2$Cl$_2$ (3 x 100 mL), dried with Na$_2$SO$_4$, and the solvent was removed in vacuo. The crude product only contained a small fraction of the desired product, which was collected as the last fractions on a flash column (80:20 hexane: ethyl acetate). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 8.63 (dd, J = 2.0, 1H, 9-H), 6.67 (dd, J = 2.0, 8.8, 1H, 7-H), 7.51 (d, J =8.8, 1H, 6-H), 6.02 (br s, 1H, OH). ESI-HRMS: calculated for [C$_{12}$H$_3$O$_2$Br$_4$]$^+$ 498.6826, found 498.6827.

References:

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Chapter 3

Disparate controlling factors in the rates of oxidation of anilines and phenols by triplet methylene blue in aqueous solution

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In preparation for Journal of Physical Chemistry: A
Abstract

In aqueous solution, Methylene Blue (MB) in its first excited triplet state can act as a one-electron oxidant for molecules with a sufficiently low oxidation potential, such as anilines. Additionally, phenols, which have much higher oxidation potentials, may also react with Methylene Blue via another mechanism involving a proton-coupled electron transfer. Here we present a series of experiments showing that the rate constant of a one-electron transfer from anilines to triplet state Methylene Blue follows a Sandros-Boltzmann-like dependence on the free energy of the reaction. The observed rate constants are also well modelled when using aniline oxidation potentials derived computationally. For phenols, the proton-coupled electron transfer rate constants were found to depend on O-H bond dissociation free energy with an additional sensitivity to the phenol pKₐ. Rate constants could again be modelled using computed bond dissociation free energies. Furthermore, overall reaction rate constants for compounds containing both phenolic and amino functional groups could be predicted by summing the rate constants for the independent mechanisms. These results show that in situations where rate constant prediction is needed, such as in aqueous environmental pollutant lifetime prediction, the ability to empirically determine reaction rate constants may prove valuable.
Introduction

Anilines and phenols are structurally similar compound classes that play important roles throughout chemistry and biochemistry. Having electron-rich π systems, anilines and phenols are prone to oxidation. Our interest in the oxidation of these compound classes derives from their prevalence in both natural and anthropogenic molecules that are frequently found in the environment, as well as the fact that photooxidation is a major environmental degradation process for them\textsuperscript{1-4}.

\begin{center}
\begin{tikzpicture}
  \node[draw,rectangle,inner sep=0pt] (a) at (0,0) {\text{H} \cdot \cdot \cdot \text{H}};
  \node[draw,rectangle,inner sep=0pt] (b) at (1.5,0) {\text{H} \cdot \cdot \cdot \text{O}};
  \node[below] at (a) {aniline};
  \node[below] at (b) {phenol};
\end{tikzpicture}
\end{center}

Despite their structural similarity, they often undergo oxidation by different mechanisms. Anilines have lower oxidation potentials than phenols, which allows them to be more easily oxidized by one-electron transfer reactions. Phenols are also prone to oxidation, but are preferentially oxidized directly to phenoxy radicals by hydrogen atom transfer, or related mechanisms.

Given the wide range of possible substituted anilines and phenols, it would be desirable to be able to predict rate constants for their possible degradation reactions in the environment with (photo)oxidants. In order to accurately do this, we must first understand the mechanisms for each class of compound, and also have reliable parameters to aid in these predictions, such as oxidation potentials in electron transfer reactions, and bond disassociation enthalpies in the case of hydrogen atom transfer. In this work, we describe our efforts to determine and accurately model the reaction rate constants for one-electron transfers from anilines and proton-coupled electron transfers from phenols to Methylene Blue (MB).
Materials and Methods

Chemicals

Methylene Blue (>98.5% spectroscopic grade) was purchased from TCI. All anilines and phenols used were purchased from Sigma Aldrich or TCI and were of the highest purity commercially available. D$_2$O (99.9% D) was purchased from Armar Chemicals (Döttingen, Switzerland). Aqueous solutions were made with ultrapure water (18 MΩ·cm$^{-1}$) obtained from a Barnstead Nanopure Diamond system. All gases used were high purity grade (99.999%).

Transient absorption experiments

The system has been previously described in detail$^5$. Briefly, the setup used was an EOS transient absorption spectroscopy system (Ultrafast Systems, Sarasota, USA). Excitation pulses were generated by converting the 795 nm output of a Solstice regeneratively amplified Ti:sapphire laser (Spectra-Physics, Darmstat, Germany) to the proper wavelength using a TOPAS-C (Light Conversion, Lithuania) optical parametric amplifier. The excitation wavelength used was 665 nm. The excitation pulse energy was 4 µJ or lower. In all experiments, methylene blue solutions (10 µM) were prepared immediately before analysis and placed in a continuously purged (air or nitrogen) 10 mm quartz cuvette. All quenching experiments with aniline derivatives were performed in pH 8.5 borate buffered solutions, and the quencher concentration ranged from 0.1 to 1.6 mM. Experiments with phenolic quenchers were performed in water that was adjusted to pH 7 by addition of HCl. Phenolic quencher concentrations ranged from 0.1 to 2.4 mM. Transient decay lifetimes were calculated
from fits obtained from Surface Explorer (Ultrafast Systems, Sarasota, USA) and
OrginPro (Version 8.5, Northhampton, USA)

Computational methods

Aqueous oxidation potentials (anilines) and bond dissociation free energies (phenols)
were computed for all compounds within the test set. The loss of an electron in
aqueous phase is defined as:

$$\text{A}_{aq} \rightarrow \text{e}^{-} + \text{A}^{++}$$

From a computational standpoint it is convenient to use a thermodynamic cycle
(Figure 1A) to separate the free energy of reaction, $\Delta G_{ox}^{0}$, into the sum of the gas
phase adiabatic ionization energy ($IE_{gas}$) and the difference in free energy of solvation
between the oxidized and reduced species, $DDG_{solv}$:

$$\Delta G_{ox}^{0} = IE_{gas}(298K) + \Delta \Delta G_{solv}$$

$$\Delta \Delta G_{solv} = \Delta G_{solv,A^{+}}^{0} - \Delta G_{solv,A}^{0}$$

where $DG_{solv,A}^{0}$ is the free energy of solvation of the reduced closed shell neutral
species, and $DG_{solv,A^{+}}^{0}$ is the free energy of solvation of the oxidized open shell
cation species. The subscript $^{0}$ denotes corresponding to a standard state of 1 atm in
the gas phase and 1 mol/L in solution. The resulting free energy of oxidation, $\Delta G_{ox}^{0}$,
can then be used to determine the oxidation potential:

$$E_{ox} = \left( \frac{-\Delta G_{ox}^{0}}{nF} + \text{SHE} \right)$$

where $n$ is the number of electrons, $F$ is the Faraday constant (96,485.3365 C/mol), and
SHE is the potential of the standard hydrogen electrode, 4.28 V. Calculated
$DG_{ox}^{0}$ values were obtained using eqs 2 and 3, based on computed values of $IE_{gas}$,
Bond dissociation free energies (BDFEs) were modeled as:

\[ \Delta G^0_{\text{solv},AH} = \Delta G^0_{\text{solv},A^+} + \Delta G^0_{\text{solv},H^+} \]

(6)

where \( \Delta G^0_{\text{solv},AH} \) is the free energy of solvation of the closed shell neutral species, and \( \Delta G^0_{\text{solv},A^+} \) is the free energy of solvation of the open shell species, and where \( \Delta G^0_{\text{solv},H^+} \) is the free energy of solvation of atomic hydrogen. A value of 27.8 kJ/mol was assigned to \( \Delta G^0_{\text{solv},H^+} \) based upon the free energy of solvation of H\(_2\).\(^8-11\)

Aqueous \( E_{\text{ox}} \) values were computed for all anilines investigated in this study. BDFE values were computed for all phenols considered in this study. These values, listed together with experimental \( E_{\text{ox}} \) and BDFE data where available, are listed in Tables A1 and A2, respectively. Gas phase free energies of reaction were calculated with Gaussian 09 using CBS-QB3,\(^{12,13}\) or ROCBS-QB3\(^{14}\) for the open shell cases using the default geometry optimization (B3LYP\(^{15}\)/6-311-G(2d,d,p)). For thermal contributions at 298 K, the CBS-QB3 default B3LYP/6-311-G(2d,d,p) thermal free energy was applied. In the case of the single electron oxidation reactions, the
integrated heat capacity of the electron (0.752 kcal/mol) was also included.\textsuperscript{16}

For the free energies of reaction in implicit solvent, gas phase geometries were optimized using M06-2X\textsuperscript{17}/6-31+G(d)\textsuperscript{18,19} with the Gaussian 09 (v. B01) software package.\textsuperscript{20} Stationary structures were confirmed by frequency analysis at the same level of theory. Single point energies using the default cavity were computed with Gaussian 09 and with the SMD solvation model, and using the MPW1K\textsuperscript{21}/aug-cc-pVTZ\textsuperscript{22} electronic structure. As SMD normally employs a standard state of 1 mol/L in each the gas phase and the aqueous phase, to convert to the standard conditions described above (with the standard state of 1 atm in the gas phase and 1 mol/L in the solution phase), 1.89 kcal/mol was added to each computed solvation free energy, which represents the free energy associated with compressing 1 mol/L to 1 atm.\textsuperscript{23} As the solubility of H\textsubscript{2} was reported at 1 atm standard state at 298 K, the 1.89 kcal/mol standard state correction was not applied to the free energy of solvation of H• since its standard state was already consistent. This means that effectively, this correction ends up canceling out in both equations 3 and 7 for determining the $\Delta G_{solv}$ for computing $E_{ox}$ and BDFE, respectively.

In some cases, multiple conformational isomers were found to be stable. In such cases, the free energy of reaction of conformational isomers were averaged according to their respective energy of reaction. Thus, the average energy was determined from the formula:

$$\Delta G_{\text{rxn}} = RT \ln \left( \sum_i e^{\Delta G_i / RT} \right)$$

(8)

Where $\Delta G_{\text{rxn}}$ is the averaged free energy of reaction (either of $E_{ox}$ or BDFE), $R$ is the universal gas constant, $T$ is temperature (298 K), and $\Delta G_i$ is the free energy of reaction for each specific conformer $i$. These included 3-ethoxyaniline, 3-ethylaniline,
3-hydroxyaniline, 3-methoxyaniline, 1-naphthol, 2-naphthol, 2,6-dimethoxyphenol, 3-aminophenol, 4-ethoxyphenol, 4-ethylphenol, 4-methoxyphenol, 4-acetylphenol, hydroquinone, and methylhydroquinone.

Results and Discussion

Computation of Aqueous Oxidation Potentials

The complete computed results are listed in Tables A1 and A2. For $E_{\text{ox}}$, the MAD (mean average deviation) compared to experiment was 0.14 V for the absolute computation of oxidation potential. This level of accuracy is consistent with a recent assessment that found that absolute oxidation potentials computed with the SMD solvation model can have deviations as large as 0.3 V.\textsuperscript{24} However, computed redox potentials are often found to correlate well with experimental data within a compound family.\textsuperscript{25-28} Hence, a linear regression of computed and experimental $E_{\text{ox}}$ data may be used to improve computed estimates of $E_{\text{ox}}$ for compounds where experimental data are not available. For the set of anilines that we studied, a linear regression of experimental and computed oxidation potentials produced the relationship:

$$E_{\text{ox,fitted}} = 1.2889 \ E_{\text{ox,comp}} - 0.1584 \ (r^2 = 0.9470) \quad (9)$$

where $E_{\text{ox,comp}}$ are the computed oxidation potential values (in V) and $E_{\text{ox,fitted}}$ represents the best fit to the experimental data. Equation 9 was used to estimate $E_{\text{ox}}$ values of compounds lacking known experimental values, analogous to correlations previously applied.\textsuperscript{25-28} Resulting $E_{\text{ox}}$ estimates are considered to have an uncertainty of < 0.1 V, based on the observed root mean squared error of 0.05 V in equation 9.
Computation of Aqueous bond dissociation free energies

Of the compounds within our data set, only phenol has a reported experimental gas phase BDFE.\textsuperscript{11} The computed gas phase BDFE was 79.06 kcal/mol, only 0.74 kcal/mol below the experimental value of 79.80 kcal/mol. Unfortunately, one gas phase data point is not enough to be able to evaluate the effectiveness of our chosen method in the gas phase, (RO)CBS-QB3 for its ability to compute a gas phase BDFE. In solution, as seen in Table A2, our computational method consistently underestimated the BEFE values, from anywhere between 2.9 and 4.5 kcal/mol (except for the hydroquinones). However, the computed BDFE values do, for the most part, capture the relative difference in BDFE between compounds compared to phenol. Except for the case of the hydroquinones, this difference in BDFE captured the relative difference in BDFE from phenol with a MAD of 0.54 kcal/mol, suggesting that our computational method adequately captures the trends in BDFE from the substituted groups on the phenols. Thus, in the same manor as was done for the computed $E_{\text{ox}}$ values, the MAD of computed aqueous BDFEs compared to experiment was 2.81 kcal/mol, and the fitted linear regression was:

\[
BDFE_{\text{fitted}} = 0.6245 \times BDFE_{\text{comp}} + 29.483 \quad (r^2 = 0.7494)
\]

(10)

Where $BDFE_{\text{comp}}$ are the computed BDFE values (in kcal/mol) and $BDFE_{\text{fitted}}$ represents the best fit to the experimental data. However, the hydroquinones (hydroquinone, methylhydroquinone) exhibited a rather strong deviation from the other phenols, and so were excluded from the fitting. The reasons for such deviations could be due to having two –OH groups that could participate in the hydrogen atom transfer, the implicit solvation model SMD could not appropriately take into account...
the specific H-bonding interactions for these molecules, or there could be other issues with the electronic structure method. Removing these values resulted in a fitted linear regression of:

\[
BDFE_{\text{fitted}} = 1.049 \, BDFE_{\text{comp}} - 7.7751 \quad (r^2 = 0.9453)
\]  

resulting in a MAD of 1.38 kcal/mol. Equation 11 was used to estimate BDFE values of compounds lacking known experimental values.

*Transient absorption of methylene blue*

The majority of our experiments were performed with MB as the triplet state electron and hydrogen atom acceptor. Compared to many other triplet sensitizers used in aqueous oxidation experiments, MB has a low triplet energy (138 kJ/mol) and one-electron reduction potential (-0.23 V vs. NHE), making it a relatively weak oxidant. Because of the low oxidation potentials of the substituted anilines studied \((E_{\text{ox}} = 0.53V-1.15V)\), stronger oxidants react with all of the test compounds at diffusion-controlled rates. Thus, in order to observe a change in electron transfer rate constants for the different anilines, a weak oxidant like MB was required. Also, MB is basic enough to participate in hydrogen atom transfer reactions, and has been used in other experiments\(^{29}\) for this purpose.

After excitation, methylene blue exhibits three strong features in its transient absorption spectrum. Two broad positive \(\Delta A\) signals centered on 420 nm and 830 nm are both attributed to triplet-triplet absorption, and a strong, negative \(\Delta A\) signal is observed at 665 nm, which is the bleaching of the singlet ground state absorption. With no quenchers present in samples purged with \(N_2\), the decays of both triplet absorptions and the return singlet ground state absorption could be fit with simple first-order kinetics and a triplet lifetime \((t)\) of 42 \(\mu s\) was observed. In samples purged with synthetic air, \(O_2\) is the only non-solvent quencher present in the system, the
triplet absorptions at 420 nm and 830 nm both have the same lifetime (1.6 µs) as the repopulation of the singlet ground state. This gives a second-order rate constant for MB triplet relaxation by energy transfer to O₂ of about $2.4 \times 10^9$ M⁻¹s⁻¹, which is consistent with other reported rate constants for triplet energy transfer to O₂ in MeCN ($1.7 \times 10^9$)³⁰ and MeOH ($2.2 \times 10^9$)³¹. Additionally, no other transient signals are seen. No power dependance on either the $^3$MB* lifetime or calculated quenching rate constants was observed for pulse energies ranging from 2 to 4 µJ. Taken together, these observations indicate that intersystem crossing (ISC) back to the singlet ground state and energy transfer to O₂ are the only important triplet state loss mechanisms, when no additional quenchers are present.

**Quenching with amine donors**

The first series of triplet quenchers studied were substituted aromatic amines. With amines present in the MB solutions, the lifetime of the $^3$MB* signal as observed at 830 nm decreased linearly with amine concentration. In addition to decreasing the $^3$MB* lifetime, the presence of donors led to the formation of two longer-lived signals (seen in Figure 2), one of which appeared around 420 nm, thus obscuring the $^3$MB* absorbance at this wavelength, and the other around 890 nm. The two additional signals described appear on different timescales, with the one at 420 nm appearing to grow directly from the loss of the triplet (this can only be inferred due to the interfering $^3$MB* absorption) while the absorption at 890 nm appeared with a delay. The intensity of the newly observed signals was proportional to the added quencher concentration. Both additional signals have been previously reported, with the absorption at 420 nm coming from the methylene blue neutral radical MB⁺ and the absorption at 890 nm belonging to the protonated radical cation (MBH⁺⁺). The
quenching behavior and observed transient reaction products provide strong evidence of a one-electron transfer reaction from the amine quenchers to MB.

Figure 1- Structures of MB and other observed transient species.

![Figure 1](image1.png)

Figure 2- Selected 3D transient absorption spectra of MB illustrating the spectroscopic differences between electron transfer and PCET quenching. Spectrum A shows the decay of $^3$MB in an air purged solution. Spectrum B shows MB when quenched by 1.6mM 1,4 benzene diamine. Spectrum C shows MB when quenched by 1.6mM hydroquinone.
The products expected from this reaction would be MB⁺ and an amine radical cation (RNH₂⁺). While MB⁺ is clearly visible, RNH₂⁺ is not observed. This is most likely due to the much larger extinction coefficient (e) of the MB species. The appearance of MBH⁺ is explained by the protonation of MB⁺, which would be expected to occur at our experimental pH of 8.4 as MBH⁺ has a pKₐ of ~9.32. The second-order rate constants (kₒ) for the reaction of anilines with ³MB⁺ was determined as the slope of the observed triplet decay rate as a function of aniline concentration, using the relationship:

\[
k_{obs} = k_o + k_q[Q]
\]

where \( k_{obs} \) is the first order decay rate constant of ³MB as calculated from the decay lifetime where \( k_{obs} = 1/t \), and \( k_o \) is the decay rate in the absence of quencher. In all cases, quencher concentrations (0.1-1.6 mM) are in great enough excess to assume pseudo-first-order behavior, as the concentration of triplet-state MB is no greater than 1 µM. This concentration is estimated from the absorbance of ³MB⁺ and its published e at 830 nm using Beers law. Table 1 lists the observed quenching rate constants for the investigated amines. As would be expected for an electron transfer reaction, the observed second-order reaction rate constants increase with decreasing donor oxidation potential, reaching a maximum value around \( 4.5 \times 10^9 \text{M}^{-1}\text{s}^{-1} \) that corresponds to the diffusion-limited second-order reaction rate constant in water (kₐ). This value is
in good agreement with previous reports\textsuperscript{2,34} of electron transfer reactions in aqueous solution.

**Reaction mechanism and modeling the quenching rate constants of anilines**

For the quenching of $^3\text{MB}^*$ by substituted anilines, we propose the following mechanism based on the work by Farid et al.\textsuperscript{35,36}:

\[
\begin{align*}
\text{3MB}^{**} + \text{PhNH}_2 & \underset{k_d}{\overset{k_d'}{\rightleftharpoons}} \text{3MB}^{**}/\text{PhNH}_2 \quad \text{k}_c \quad \text{3MB}^{--} \text{PhNH}_2^{**} \\
\text{3MB}^- + \text{PhNH}_2^{**} & \underset{k_{sep}}{\overset{k_{sep}'}{\rightleftharpoons}} \text{3MB}^-/\text{PhNH}_2^{**} \\
\text{1MB}^+ \text{PhNH}_2 & \quad \text{k}_{ISC}
\end{align*}
\]

In the first step, the free $^3\text{MB}^*$ and aniline diffuse together in solution to form an encounter complex. In this rapid step, the reactants are in contact but are only loosely bound and do not yet have the proper conformation for electron transfer. From this contact pair, the molecules may either diffuse apart or find the proper orientation for electron transfer and form an exciplex (shown in brackets). In this longer-lived species, electron transfer in both the forward and reverse direction (not shown) occurs. Following electron transfer, the exciplex conformation can be lost and eventually the pair can diffuse apart. In our system, the evidence for an exciplex is less direct than for systems where exciplex fluorescence can be directly observed\textsuperscript{35,36}. Our evidence comes instead from the observed effects on the rate of ISC in the exciplex seen for MB\textsuperscript{37} and other triplet dyes\textsuperscript{38}. For anilines containing a heavy atom substituent such as Br or I, the forward-reverse electron transfer occurring in the
exciplex can end in deactivation of $^3\text{MB}^*$ back to its ground state via ISC without yielding any apparent reaction. In other words, some anilines containing heavy atoms can efficiently quench $^3\text{MB}^*$ while yielding very little $\text{MBH}^+$. This process has been described previously\textsuperscript{38} for the triplet quenching of thionine in methanol, where it was shown that the magnitude of the effect depends on the halogen substitution position and thus degree of interaction with the ring p system. This influence on the ISC rate appears to come from the exciplex and as a result of an electron transfer because the overall rate of quenching $k_q$ still depends on the oxidation potential of the aniline. In other words, the enhancement in ISC is only seen when electron transfer is also thermodynamically possible.

In a photoinduced electron transfer\textsuperscript{39-41}, the free energy ($\Delta G_{et}$) of the reaction depends on the difference in the electrochemical potentials of the donor and acceptor plus the excited state energy of the acceptor (or donor):

$$\Delta G_{et} = -F(E_{\text{red}} - E_{\text{ox}}) + E_{3\text{Sens}}$$  \hspace{1cm} (14)$$

where $F$ is Faraday's constant and $E_{3\text{Sens}}$ is the energy of the first excited triplet state relative to that of the singlet ground state. Based on the theories of electron transfer reactions proposed by Marcus\textsuperscript{39}, the Rehm-Weller equation has found wide use because it correctly models the apparent lack of an inverted region, or a slowing of electron transfer rate for highly exergonic reactions, that is not observed for photoinduced electron transfers between freely diffusing molecules in solution. A simplified Rehm-Weller equation is shown below:
\[ k_{\text{obs}} = \frac{k_d}{1 + \frac{k_d}{K_dZ} \left\{ \exp \left[ \frac{\Delta G_{\text{et}}}{2} + \frac{\lambda^2}{4} + \frac{\Delta G_{\text{et}}}{2} / RT \right] + \exp \left( \frac{\Delta G_{\text{et}}}{2} \right) \right\} } \]  \hspace{1cm} (15)

where \( K_d = \frac{k_d}{k_d} \), which represents the equilibrium constant for the formation of the encounter complex, \( Z \) is the universal collision frequency factor, \( R \) is the universal gas constant, and \( l \) is known as the solvent reorganization energy. \( l \) refers to the energy required for the system, both the newly formed radical pair and the solvent cage surrounding it, to rearrange to accommodate the change in charge after the electron transfer has occurred. This equation correctly models a plateau in the observed electron transfer rate for highly exergonic reactions (when \( \Delta G_{\text{et}} > l \)) where \( k_{\text{obs}} = k_d \) and a gradual drop in the rate of electron transfer for exergonic reactions. The steepness of the drop in rate is affected by the term \( l \). The Rehm-Weller equation has been used extensively to semi-empirically model photoinduced electron transfer reactions in a wide range of systems, however a simplified alternative explanation for the behavior observed by Rehm and Weller has been put forth by Farid and coworkers\textsuperscript{35,36} arguing that for some systems electron transfer reaction rate constants can be modelled by a simple Sandros-Boltzmann distribution:

\[ k_{\text{obs}} = \frac{k_d}{1 + \exp \left( \frac{\Delta G_{\text{et}} + s}{RT} \right) } \]  \hspace{1cm} (16)

where \( s \) is a positive free energy shift associated with the difference in free energy of the radical pair in contact relative to that of the free radicals in solution. A similar shift in free energy was used in the original Rehm-Weller description of the reaction free energy, however in their case it was argued that a negative shift in free energy was required. At the time it was not established that the free energy of a radical pair is higher than that of the free radicals\textsuperscript{34} for these reactions.
Figure 3 shows a plot of log $k_q$ for the observed quenching rate constants between $^3$MB* and the substituted anilines vs. $\Delta G_{et}$ as calculated by equation 14 using both experimental and computationally derived $E_{ox}$ values. For anilines that had reported $E_{ox}$ values determined by pulse radiolysis $\Delta G_{et}$, The rate constant for electron transfer increases with decreasing $\Delta G_{et}$, until reaching a plateau at the diffusion controlled limit so that $k_q = k_d$. For the quenching of $^3$MB* by anilines, we observed that $k_q$ begins to fall below $k_d$ at relatively low values of $\Delta G_{et}$, around -16 kJ/mol. Going toward larger $\Delta G_{et}$ values, $k_q$ decreases with a slope of approximately $1/RT$. For these data, the best fit was achieved using the Sandros-Boltzmann equation where $k_d = 4.3 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ and $s = 16.2 \text{ kJ/mol}$. The original work by Farid et al.\textsuperscript{35} describing the use of the Sandros-Boltzmann equation to fit electron transfer data reported a $s$ of 7.7 kJ/mol for electron transfer reactions between singlet excited pyrylium cation acceptors and neutral aromatic hydrocarbon donors in acetonitrile.
Figure 3- Plot of $\Delta G_{et}$ vs. $k_q$ for the electron transfer reaction from substituted anilines to MB. $\Delta G_{et}$ was calculated from computational $E_{ox}$ values (red triangles) and pulse radiolysis values (blue triangles) the lines depict the Sandros-Boltzmann fits for the computational (solid) and pulse radiolysis (dashed) $\Delta G_{et}$ values. The error bars on the computed values indicate the estimated 0.1 V error in the calculations.

The reason that we observe a larger $s$ value compared to the previous work is not immediately clear. In both cases, the acceptor is initially positively charged, and following electron transfer becomes neutral, there are important differences, however, in the charge delocalization before and after electron transfer in each system. In the pyrylium case, the initial positive charge is localized entirely on the central pyrylium ring and transferred to a single aromatic ring following electron transfer. In our case, the positive charge on MB is delocalized over all three fused rings in the molecule before electron transfer, but then transferred to a single ring on an aniline. This difference in charge density could contribute to the additional energy requirement seen in electron transfers to MB. The solvent (water vs. acetonitrile) is another factor that might add to the increased $s$ in our system, but predicting its effect is less straightforward than for the role of charge density. Lastly, there is the difference in spin states of the acceptors, as we are following the quenching of a triplet state rather
than a singlet. There exists conflicting results as to whether spin multiplicity affects the observed $k_q$ relative to $\Delta G_{et}$, with some arguing there are no differences\textsuperscript{42}, and others reporting\textsuperscript{37,43,44} lower $k_q$ values for triplet states at equivalent $\Delta G_{et}$.

### Table 2- Data for substituted anilines at pH 8.4

<table>
<thead>
<tr>
<th>Substituted aniline</th>
<th>Computational $E_{ox}$</th>
<th>Pulse radiolysis $E_{ox}$</th>
<th>$k_q$ ($10^8$ M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-N,N-dimethylamino aniline</td>
<td>0.39</td>
<td></td>
<td>48.5 ± 1.6</td>
</tr>
<tr>
<td>4-methoxyaniline</td>
<td>0.82</td>
<td>0.79</td>
<td>45.8 ± 1.1</td>
</tr>
<tr>
<td>N-methylaniline</td>
<td>0.90</td>
<td></td>
<td>43.0 ± 1.7</td>
</tr>
<tr>
<td>4-aminoaniline</td>
<td>0.54</td>
<td>0.59</td>
<td>42.4 ± 1.3</td>
</tr>
<tr>
<td>4-methylaniline</td>
<td>0.95</td>
<td>0.92</td>
<td>41.5 ± 0.5</td>
</tr>
<tr>
<td>4-tert-butylaniline</td>
<td>0.98</td>
<td>0.95</td>
<td>32.7 ± 0.9</td>
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<tr>
<td>3-hydroxyaniline</td>
<td>1.08</td>
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<td>18.3 ± 0.14</td>
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<td>3-ethylaniline</td>
<td>1.10</td>
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<td>15.4 ± 0.4</td>
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<tr>
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<td>1.02</td>
<td></td>
<td>15.2 ± 0.7</td>
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<tr>
<td>3-ethoxyaniline</td>
<td>1.09</td>
<td></td>
<td>13.0 ± 0.3</td>
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<tr>
<td>3-methoxyaniline</td>
<td>1.09</td>
<td></td>
<td>11.7 ± 0.4</td>
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<td>4-fluoroaniline</td>
<td>1.07</td>
<td>1.02</td>
<td>8.65 ± 0.16</td>
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<td>1.07</td>
<td>1.02</td>
<td>4.10 ± 0.05</td>
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<td>4-chloroaniline</td>
<td>1.12</td>
<td>1.01</td>
<td>3.79 ± 0.16</td>
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<tr>
<td>3-fluoroaniline</td>
<td>1.18</td>
<td></td>
<td>0.109 ± 0.01</td>
</tr>
<tr>
<td>3-chloroaniline</td>
<td>1.20</td>
<td></td>
<td>0.106 ± 0.01</td>
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### Table 3- Data for substituted phenols at pH 7.0

<table>
<thead>
<tr>
<th>Substituted phenol</th>
<th>$pK_a$</th>
<th>PhO$^-$ $E_{ox}$</th>
<th>$^{a}BDFE_{calc}$</th>
<th>$^{b}BDFE_{comp}$</th>
<th>$k_q$ ($10^8$ M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sesameol</td>
<td>9.7</td>
<td>0.45</td>
<td>81.3</td>
<td>81.8</td>
<td>39.7 ± 0.4</td>
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<tr>
<td>methylhydroquinone</td>
<td>10.1</td>
<td>0.4</td>
<td>80.7</td>
<td>83.8</td>
<td>39.1 ± 0.8</td>
</tr>
<tr>
<td>hydroquinone</td>
<td>9.85</td>
<td>0.45</td>
<td>81.5</td>
<td>84.7</td>
<td>24.1 ± 0.3</td>
</tr>
<tr>
<td>1-naphthol</td>
<td>9.3</td>
<td>0.59</td>
<td>83.9</td>
<td>83.3</td>
<td>16.8 ± 0.4</td>
</tr>
<tr>
<td>2,6-dimethoxyphenol</td>
<td>9.98</td>
<td>0.47</td>
<td>82.1</td>
<td>84.6</td>
<td>16.2 ± 0.5</td>
</tr>
<tr>
<td>4-ethoxyphenol</td>
<td>10.2</td>
<td>0.52</td>
<td>83.6</td>
<td>84.4</td>
<td>12.2 ± 0.5</td>
</tr>
<tr>
<td>4-methoxyphenol</td>
<td>10.1</td>
<td>0.54</td>
<td>83.9</td>
<td>84.5</td>
<td>7.93 ± 0.04</td>
</tr>
<tr>
<td>2-naphthol</td>
<td>9.6</td>
<td>0.69</td>
<td>86.7</td>
<td>86.9</td>
<td>3.66 ± 0.3</td>
</tr>
<tr>
<td>4-chlorophenol</td>
<td>9.4</td>
<td>0.8</td>
<td>88.9</td>
<td>89.4</td>
<td>0.96 ± 0.07</td>
</tr>
<tr>
<td>4-ethylphenol</td>
<td>10</td>
<td>0.68</td>
<td>87.0</td>
<td>87.1</td>
<td>0.66 ± 0.19</td>
</tr>
<tr>
<td>4-fluorophenol</td>
<td>9.9</td>
<td>0.76</td>
<td>88.7</td>
<td>88.4</td>
<td>0.63 ± 0.07</td>
</tr>
<tr>
<td>4-methylphenol</td>
<td>10.3</td>
<td>0.68</td>
<td>87.4</td>
<td>87.1</td>
<td>0.57 ± 0.09</td>
</tr>
<tr>
<td>4-tertbutylphenol</td>
<td>10.23</td>
<td>0.76</td>
<td>89.1</td>
<td>87.5</td>
<td>0.52 ± 0.03</td>
</tr>
<tr>
<td>phenol</td>
<td>10</td>
<td>0.79</td>
<td>89.5</td>
<td>89.4</td>
<td>0.17 ± 0.03</td>
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</tbody>
</table>

$^{a}$: $BDFE_{calc}$ values were calculated from the phenol $pK_a$ and PhO$^-$ $E_{ox}$ values according to the procedure described in ref. 11. $^{b}$: $BDFE_{comp}$ values were computed as described in the methods.
We also attempted to explain the data using the Rehm-Weller equation. Reasonable fits were only obtained after introducing a correction to the free energy:

$$\Delta G_{et} = \Delta G_{et} + \delta \Delta G_{et}$$  \hspace{1cm} (17)

This correction has been used before to address uncertainties in donor/acceptor redox values\(^3\), and also to address shifts in \(\Delta G_{et}\) that seem to be system-specific\(^37\). Using adjusted \(\Delta G_{et}\) values gave reasonable fits, but with very small values for \(l\) that are typically seen in much more rigid systems\(^45,46\). For this reason, we favor the fits given by the Sandros-Botzmann equation for the purposes of predicting \(k_q\) rates for electron transfer reactions between MB and anilines.

**Quenching with phenol donors**

The second class of quenchers we investigated was substituted phenols. The quenching rate constants for phenols were again calculated using equation 12. The results at pH 7 are shown in table 2. Phenolic quenching of \(\text{MB}^*\) yielded observable reaction products in the transient absorption spectra that were indicative of the reaction mechanism. The addition of phenols resulted in a rapid appearance of \(\text{MBH}^+\) (Figure 2) with no lag as seen with anilines. For strong quenchers, the conversion from \(\text{MB}^*\) to \(\text{MBH}^+\) was nearly quantitative based on their respective loss/formation rates at high phenol concentration. In the region of 410-430 nm the absorption is initially dominated by the \(\text{MB}^*\) signal, but due to quenching of this species, a distinct change in the absorption shape and \(l_{\text{max}}\) at longer times is observed. We attribute this absorption change to the formation of phenoxy radicals (PhO\(^*\)), as the new \(l_{\text{max}}\) agrees with reported values for previously reported phenoxy radicals\(^47\).
**Phenolic quenching mechanism**

This observed behavior is explained best by a proton-coupled electron transfer (PCET) quenching mechanism, where rather than donating only an electron, phenols also transfer a proton in the same or a rapidly sequential step. Another term for this mechanism is hydrogen atom transfer, however we favor the assignment of PCET because, in a strict sense hydrogen atom transfer requires that the electron come directly from the same bonding orbital that the proton was associated with, while in PCET the electron may come instead from an orthogonal p orbital. The former is often the case for phenols, and also likely in our system. PCET from phenols is a well-known phenomenon that is widely observed in natural systems, and is partly responsible for the anti-oxidant role of phenols in biological systems.

In addition to the observed spectroscopic evidence for PCET, the thermodynamics of the process are strongly against the plausibility of a simple one-electron transfer from phenol to $^3$MB*. For example, phenol has a one-electron oxidation potential of 1.5 V, so according to equation 14, the free energy of $^3$MB* quenching by electron transfer from phenol would be 28.9 kJ/mol, which by calculation with equations 15 or 16 would yield a $k_q$ several orders of magnitude smaller than what is observed in our experiments. Our proposed reaction mechanism for PCET from phenols to $^3$MB* is shown in equation 17.

\[
^3\text{MB}^+* + \text{PhOH} \xleftrightarrow{k_1 \ \ \ k_{-1}} (\text{PhOH} \rightarrow \text{PhOH}) \xrightarrow{k_2} \text{MBH}^+* + \text{OPh} \xrightarrow{k_3} \text{MBH}^+* + \text{OPh}
\]

(17)

In the first step, $^3$MB* and PhOH form an exciplex. Within this exciplex, PhOH donates both an electron and a proton in a sequential manner for a net hydrogen atom
transfer. Finally the individual radicals are free to diffuse away from one another in solution.

To further test the hypothesized mechanism, we repeated the phenolic quenching experiments in D$_2$O. Due to rapid exchange of the protons between the phenolic OH group and solvent, nearly all of the phenol under this condition can be considered deuterated. Under these conditions, we would expect PCET from a deuterated phenol and $^3$MB* to occur with a reduced rate constant due to the increased OD bond strength. Figure 4 shows an example of these experiments. As expected, a reduction in the quenching rate constants were observed for each phenol tested. H/D isotope effects, as determined by $k_H/k_D$, ranged from 1.6 to 3.3, with the low values only coming from quenchers reacting at or near the diffusion controlled limit.

![Figure 4](image_url)

**Figure 4**- Observed decay rates of $^3$MB in the presence of 4-methoxyphenol at pH/D= 7. The red points were collected in H$_2$O and the blue in D$_2$O.
These values are in good agreement with what has been previously observed for phenolic quenching of aromatic ketones by PCET\textsuperscript{50} as well as for intramolecular PCET reactions\textsuperscript{48}. Alternatively, when experiments were done at high pH (or pD), ensuring only phenolate being present, quenching by electron transfer took place and no kinetic isotope effect was observed. In taken together, both transient absorption spectroscopy data and kinetic isotope experiment data are consistent with phenolic quenching of $^3\text{MB}^*$ via a PCET mechanism, not an electron transfer mechanism.

In equation 17, the rate-determining step is the breaking of the phenolic OH bond. It follows from this that a correlation between the OH bond dissociation free energy (BDFE) would be expected in the $^3\text{MB}^*$ quenching rate constants for phenols. A plot of BDFE vs. $k_q$ for the phenolic quenchers is shown in Figure 5. In addition to the computationally derived BDFE values discussed earlier, BDFEs were also calculated\textsuperscript{11} from a simple thermodynamic cycle based on a two-step reaction where an electron and a proton are lost. This method of calculation requires only the phenolate $E_{\text{ox}}$, $\text{pK}_a$ and a term for the solvation of a hydrogen atom $C_G$, all of which are solvent-dependent. As seen in the Figure 5, quenching rate constants correlate strongly with BDFE. Notable, however, is the systematic nature of the deviation seen in the plot. The points in Figure 5 are color mapped according to the phenols’ $\text{pK}_a$ values. A clear trend is seen in which the more acidic phenols react with an enhanced rate when compared to a more basic phenol with a similar BDFE. The origin of this acidity enhancement may come in the mechanistic step that precedes PCET. Before PCET can occur, $^3\text{MB}^*$ must first form a hydrogen-bonded exciplex with the phenol. More acidic phenols carry a stronger $\partial^+$ on the OH hydrogen, which has the known effect of increasing hydrogen-bonding strength.\textsuperscript{52} This would lead to a stronger complex between more acidic phenols and $^3\text{MB}^*$. In reaction equation 17, the
equilibrium constant \( K_1 = \frac{k_1}{k_{-1}} \) would be proportional to the phenol pK\(_a\), with more acidic phenols enhancing the reaction \( K_1 \), thus enhancing the observed rate of PCET.

Another possible explanation of this effect would be the greater amount of the ionized phenolate form present at the experimental pH of 7, which is a more efficient quencher (via electron transfer) than the protonated parent phenol. This possibility is ruled out based on the very low amount of phenolate (less than 0.5% ionized) for even the most acidic phenols studied, which would add an error of less than 2%.

![Figure 5](image)

**Figure 5** - Plot of \( k_q \) vs. substituted phenol BDFE for the reaction between substituted phenols and \(^3\)MB. The best fit line is shown.
Finally, we attempted to find a linear free energy relationship capable of predicting quenching rate constants of $^3$MB* by substituted phenols. As already demonstrated to some degree in by the D$_2$O experiments, the reaction rate constant is primarily determined by the phenolic O-H bond strength. The result of this is that the difference in free energy of the MB-H bond formed and the PhO-H bond broken should show a linear correlation with the observed quenching rate of $^3$MB*. Therefore it should be possible to use the change in free energy ($\Delta G_{\text{PCET}}$) from the BDFEs before and after PCET using equation 18 and determine the empirical relationship between $\Delta G_{\text{HAT}}$ and $k_q$.

$$\Delta G_{\text{PCET}} = BDFE(PhO\cdots H) - BDFE(MB\cdots H)$$ (18)
Figure 6 shows $\Delta G_{\text{PCET}}$ plotted against $k_q$. The result is once again linear, and still shows a deviation with $pK_a$. An empirical equation predicting $k_q$ is obtained by fitting both $\Delta G_{\text{PCET}}$ and $pK_a$ with a non-linear regression, the results of which are shown in Figure 7. This fit achieves good agreement between predicted and observed $k_q$ values for phenolic quenching of $^3\text{MB}^*$. 

![Plot of observed vs predicted $k_q$ values for PCET from substituted phenols to $^3\text{MB}$](image)

**Figure 7** - Plot of observed vs predicted $k_q$ values for PCET from substituted phenols to $^3\text{MB}$. The blue line indicates a 1:1 agreement.

**Implications and Conclusions**

In this work, we demonstrate that for a single photoexcited oxidant, $^3\text{MB}^*$, the reaction rate constant and mechanism can change depending on the oxidation
potentials and BDFEs of potential donors in aqueous systems. The development of empirical models capable of predicting reaction rate constants is a key part in determining the environmental persistence of organic pollutants, and this work was intended to aid in this area. For anilines it is clear that in order to predict $k_q$ values, a reliable method for determining $E_{ox}$ is first required to obtain $\Delta G_{et}$. The irreversible nature of the one-electron oxidation for anilines and other compounds of interest mean that high quality $E_{ox}$ values are often unavailable and difficult to obtain experimentally. Furthermore, many pollutants have low water solubilities that complicate aqueous $E_{ox}$ determination. In this work, we explored the use of computational methods to bridge this gap. However, it is also clear that for electron transfer reactions where $k_{et}$ is quite sensitive to small changes in reactant $E_{ox}$ values, small errors in computational methods can yield large errors in rate constant prediction. Further work is required to improve these computational methods if more reliable predictions are to be made.

One aspect of this work that can be improved in future studies with respect to the environmental relevance is the identity of the photooxidant, MB. In aquatic environments, triplet-state dissolved organic matter ($^3$DOM) is one of the main drivers of photoinduced oxidation for pollutants, and the main components of DOM responsible for the oxidative properties are believed to be aromatic ketones$^{53}$. Similar comparative studies using anilines and phenols with aromatic ketone sensitizers are a clear avenue for further studies. While the chemistry of MB is distinct from aromatic ketone photosensitizers, it may be a reasonable model for other environmentally relevant compounds, namely flavins. Future work is required to test the broader applicability of our results to other systems and importantly, more environmentally relevant oxidants.
References:


Rehm, D.; Weller, A. KINETICS AND MECHANICS OF ELECTRON TRANSFER DURING FLUORESCENCE QUENCHING IN ACETONITRILE. *Berichte Der Bunsen-Gesellschaft Für Physikalische Chemie* 1969, 73, 834-85 &.


Appendix to Chapter 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>$E_{\text{ox,exp}}$ a</th>
<th>$E_{\text{ox,comp}}$</th>
<th>$E_{\text{ox,fitted}}$</th>
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<td>0.82</td>
<td>0.39</td>
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Table A1- Experimental, computed, and fitted single electron oxidation potentials for substituted anilines in aqueous solution vs. SHE (V). a. Ref\textsuperscript{54-56}
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<th>$BDFE_{\text{rel,comp}}$</th>
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<td>1-naphthol</td>
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Table A2- Experimental, computed, and fitted bond dissociation free energies for substituted phenols in aqueous solution (kcal/mol). $BDFE_{\text{rel}}$ values are determined as the difference in $BDFE$ relative to phenol. a. Ref\textsuperscript{11}
Figure A1 - A. Thermodynamic cycle used for the computation of $E_{\text{ox}}$ in aqueous solution. B. Thermodynamic cycle used for the computation of BDFE in aqueous solution.
Figure A2- Linear regression fit of experimental vs. computed aqueous oxidation potential values (V vs. NHE).

\[ y = 1.2889x - 0.1584 \]

\[ R^2 = 0.94697 \]

Figure A3- Linear regression fit of experimental vs. computed aqueous BDFE values (kcal/mol)

\[ y = 1.049x - 7.7751 \]

\[ R^2 = 0.94532 \]
Chapter 4

Investigating the mechanism of the photochemical chlorination of pyrene in aqueous solution

Paul R. Erickson, Kenshi Sankoda, and Kristopher McNeill

In preparation for Environmental Science and Technology: Letters
Abstract

The reaction mechanism responsible for the photochemical formation of 1-chloropyrene (Cl-PYR) from pyrene and chloride in aqueous solution was studied. Earlier work has demonstrated that the presence of Cl-PYR in river sediments leading to the ocean strongly correlates with the salinity of the sampling site, strongly suggesting that Cl-PYR is being generated in situ at these sites. Using a combination of steady-state and laser flash photolysis, we provide evidence that supports the hypothesis that Cl-PYR is formed from the nucleophilic attack of chloride on pyrene radical cations which are formed by the oxidation of excited state pyrene by oxygen. Addition of electron donors to photolysis solutions greatly decreased the production of Cl-PYR, and when chloride was put in competition with other nucleophiles, the product distributions indicated chloride behaves like a nucleophile in this system. All of our results taken together suggest this could be a general mechanism for the formation of chlorinated polycyclic aromatic hydrocarbons found in marine environments.
Polycyclic aromatic hydrocarbons (PAHs) are a large class of compounds comprised of fused aromatic ring structures of varying size and shape, containing only carbon and hydrogen. PAHs are well-known pollutants, which enter the environment as either byproducts of incomplete combustion (pyrogenic) or from oil sources (petrogenic). They are also used in the commercial production of dyes and other products. The concern regarding PAHs derives from the toxic properties of some structures, which have been found to be carcinogenic$^{1,2}$, mutagenic$^3$ as well as teratogenic$^{4,5}$ to a wide range of organisms. Their toxicity has led the U.S. Environmental Protection Agency to label 16 congeners as “priority pollutants”. Once in the environment, PAHs may transform via a wide range of biotic$^6$ and abiotic$^7$ pathways, sometimes yielding transformation products that are also of concern. One such example is the chlorination of PAHs. A number of chlorinated PAH (Cl-PAH) congeners have been found in air$^{3,8-10}$, biota$^{11}$, and sediment samples$^{12,13}$. Cl-PAHs have similar toxic effects as their parent PAHs, and in some cases have been reported to exhibit higher toxicity. In particular, Cl-PAHs show higher affinity to aryl hydrocarbon receptors$^{14}$, which is one of the main routes of toxicity for dibenzo-p-dioxins and related compounds.

The chlorination of PAHs can occur during combustion, water chlorination$^{15-17}$, and photochemically in the presence of chloride$^{18}$. The importance of each of these formation routes to the total environmental concentration present will likely differ by location, but for marine environments the largest source may be photochemical.
generation. Sankoda and coworkers recently reported\textsuperscript{13} finding Cl-PAHs in river and tidal flat sediments near the Yabe river in Japan, while Cl-PAHs were not detected in nearby samples of potential PAH sources such as road dust. Furthermore, the concentrations of Cl-PAHs correlated positively with the salinity of the sampling sites. Sankoda, et al. also performed laboratory experiments showing that PAHs in artificial seawater yield Cl-PAHs when irradiated with light of wavelengths between 300-400 nm. This work, along with other reports,\textsuperscript{18-20} lends evidence to the hypothesis that Cl-PAHs found in marine environments are, to a large degree, generated photochemically. While several studies have hinted at the possible reaction mechanism of photochemical Cl-PAH formation, a systematic investigation has yet to be done. The goal of this work was to identify the reaction mechanism by which PAHs are photochlorinated. We specifically examined pyrene (PYR) and its transformation to 1-chloropyrene (Cl-PYR) in aqueous solution. We present a series of experiments testing our hypothesis that Cl-PYR is formed by nucleophilic attack of chloride on PYR radical cations (PYR\(^+\)), which are in turn generated by the oxidation of excited singlet state PYR by O\(_2\) in aqueous solution.

**Methods**

Steady-state photolysis experiments were performed in a Rayonet photoreactor containing six 365 nm bulbs. Samples (10 mL) of PYR were made in borate buffer (9.5 mM, pH 8) with 5% DMSO as a co-solvent to an initial concentration of 5 \(\mu\)M and placed in borosilicate test tubes. In all experiments, an ionic strength of 0.5 M was maintained by complementing other ions (e.g. NaCl) with NaClO\(_4\) when necessary. For analysis, entire samples were sacrificed at each time point. Samples were then placed in centrifuge tubes and pyrene-d\(_{10}\) was added to the solution as a surrogate. Next, an extraction was performed using hexane (20 mL) and samples were
sonicated for 20 min. The organic layer was separated by centrifugation (3000 rpm, 3 min) and passed through filter paper to remove residual water. The hexane sample was removed by rotary evaporation and blown to dryness with nitrogen. Lastly, the samples were re-dissolved in 100 µL of hexane, which was pre-spiked with phenanthrene-d_{10} and analyzed by GC/MS.

The system used for transient absorption (laser flash photolysis) experiments has been previously described in detail. Briefly, the setup used was an EOS transient absorption spectroscopy system (Ultrafast Systems, Sarasota, USA). Excitation pulses were generated by converting the 795 nm output of a Solstice regeneratively amplified Ti:sapphire laser (Spectra-Physics, Darmstat, Germany) to the desired wavelength using a TOPAS-C (Light Conversion, Lithuania) optical parametric amplifier. The excitation wavelength used was 333 nm, a local absorption maximum for PYR. Laser powers of 4-8 mW were used. At higher laser powers, the production of PYR^{+} through two-photon absorption is more pronounced, yielding stronger signals. For each experiment, 200 mL of a 30 µM PYR solution (70% methanol 30% H_{2}O) was placed in a round-bottom flask and purged with synthetic air. Using a peristaltic pump, the solutions were continuously cycled through a 10-mm quartz flow-through cuvette. To these solutions, the various electron donors and nucleophiles were spiked in at different concentrations to determine their reactivity.

**Results and Discussion**

In air-equilibrated aqueous solutions containing 0.5-0.2M NaCl, PYR degraded slowly, with a half-life of about 3.5 hrs when irradiated with light bulbs emitting wavelengths from 340 to 400 nm. Only the tail of the PYR UV absorbance overlaps with this light source, and under such conditions, two-photon absorption is unlikely. In all cases, the formation of Cl-PYR was observed, with apparent yields of
around 5%. These results are consistent with earlier work\textsuperscript{20} showing PYR and other PAHs can become chlorinated when irradiated in artificial seawater. Our proposed reaction mechanism is depicted in Figure 2. We hypothesize that Cl-PAH formation is initiated by the one-electron oxidation of photoexcited singlet-state PYR by O\textsubscript{2}, which yields PYR\textsuperscript{+} and superoxide O\textsubscript{2}\textsuperscript{-}. In the next step, Cl\textsuperscript{-} acts as a weak nucleophile and attacks the delocalized carbocation at position 1 in PYR\textsuperscript{+}. Nucleophilic attack at position 1 is known to occur\textsuperscript{2}, and can be rationalized by the fact that this location has the highest loading of positive charge to minimize positive-positive interactions. Following nucleophilic attack, another one-electron oxidation by O\textsubscript{2} followed by loss of a proton yields the end product, Cl-PYR.
As noted above, this mechanism relies on the presence of O$_2$ to act as an oxidant to both form PYR$^+$ and act as the terminal acceptor of the second electron in a subsequent step. Therefore, removal of O$_2$ would be expected to eliminate Cl-PYR formation as well as slow the overall degradation of PYR. To test for the importance of O$_2$, a series of samples were purged with Ar and sealed during photolysis. Removal of oxygen slows the degradation of PYR considerably and the generation of Cl-PYR is no longer observed (Figure 1). These results also agree with previous work that has
shown select PAHs, including PYR, appear to degrade primarily via a radical cation (PYR\(^{+}\)) formed from a one-electron oxidation by O\(_2\) following photoexcitation\(^{22-24}\). This oxidation appears to take place from the singlet state of PYR through collisional charge-transfer complexes with O\(_2\). Oxidation from the triplet state is not likely, as demonstrated by others\(^{22,23}\) and in agreement with our observations by transient absorbance measurements showing the growth of the PYR\(^{+}\) signal on a timescale independent of that of triplet PYR (figure 3). The absence of Cl-PYR formation in these experiments is also significant as it is inconsistent with photoexcited PYR as being capable of sensitizing the production of any reactive form of chloride, without an additional oxidant (O\(_2\)) present.

\[
\text{Pyr} \xrightarrow{h\nu} \text{Pyr}^* \xrightarrow{} \text{Pyr}^{+} + \text{Cl}^- \xrightarrow{} \text{Cl-Pyr}^{+} \xrightarrow{-H^+} \text{Cl-Pyr}
\]

**Figure 4** - The proposed reaction scheme leading to the photochemical formation of 1-chloropyrene.

To further test for the involvement of PYR\(^{+}\) as a key intermediate in Cl-PYR formation, we performed a series of photolysis experiments with an electron donor present to see if a decrease in Cl-PYR formation would be observed. PYR solutions containing DABCO (100 µM) showed a large reduction in Cl-PYR production. Our initial expectation was that the presence of DABCO, a good electron donor, would lead to enhanced regeneration of PYR from PYR\(^{+}\) and, in addition to reducing Cl-PYR formation, would slow the observed rate of PYR degradation. In Figure 1 we see that PYR degradation is only slowed to a minor degree compared to the loss of Cl-PYR formation. This is likely the result of DABCO acting not only as an electron donor, but also as a nucleophile capable of reacting with PYR\(^{+}\).
In order to test the nucleophilic behavior of Cl\(^-\) in this reaction, we performed a set of competition experiments with other nucleophiles. When two nucleophiles are present in solution at the same concentration together and able to compete for reaction with a cation, their respective product ratios can be reasonably predicted based on the relative strength of each nucleophile assuming they react in a similar manor. For this test, photolysis experiments were performed with PYR solutions containing equal amounts of Cl\(^-\) and either Br\(^-\) or CN\(^-\), both known to be stronger nucleophiles than Cl\(^-\).\(^{25}\) In the presence of Br\(^-\) a large reduction of Cl-PYR formation was seen and 1-bromopyrene (Br-PYR) was formed in relatively large quantities. The product ratio, as determined by the initial rates of formation for Cl-PYR and Br-PYR was approximately 10. This ratio agrees quite well with observations of the relative strengths of these nucleophiles in other aqueous systems.\(^{26}\)

Transient absorption experiments were also performed, allowing direct observation of PYR\(^{++}\) and other PYR transient species. Laser flash photolysis has been used extensively in the past to observe the behavior and reactivity of PAHs and their various radical species, and can be a suitable technique to determine reaction rate constants. Our main objective was to obtain the second-order reaction rate constant for the addition of Cl\(^-\) to PYR\(^{++}\), however this reaction occurs too slowly to affect the decay of the PYR\(^{++}\) signal, even at Cl\(^-\) concentrations of 1M. Attempts to perform these experiments at Cl\(^-\) concentrations above 1M resulted on poor transient signals of PYR\(^{++}\), most likely due to solubility issues. Experiments performed with Br\(^-\) also showed no noticeable change in the decay lifetime of PYR\(^{++}\). Based on the lack of a distinguishable change in lifetime at a Br\(^-\) concentration of 1M, we can put an upper limit on the bimolecular reaction rate constant at 5x10\(^{-5}\) M\(^{-1}\) s\(^{-1}\). This is in good agreement with other studies of the rates of reactions of Br\(^-\) as a nucleophile. We also attempted to observe quenching of PYR\(^{++}\) by electron donors. In these experiments we
observed (Figures 3 and 4) that N$_3^-$ reacted with PYR$^{+\cdot}$ at or near diffusion controlled rates. Despite not being able to observe the reaction between Cl$^-$ and PYR$^{+\cdot}$, all of these findings are in good agreement with the observations of others and support our proposed reaction mechanism.

**Figure 3** – 3D Transient absorption spectra of PYR. In the left panel the sample was purged with O$_2$ and contained only PYR. The absorption of PYR$^{+\cdot}$ can be seen at 455 nm. In the right panel, the sample was purged with air and contained 100mM N$_3^-$, and the PYR$^{+\cdot}$ is absent.
As mentioned before, the halogenation of PAHs, including PYR can occur through a variety of mechanisms, which largely depend on which oxidants are present in the system. During combustion, high temperatures give rise to gas-phase radical reactions, which are well known to form halogenated organic compounds when an appropriate halogen source is available. In solution, PAHs have been shown to be chlorinated by Cl₂ and ClO⁻ in a non-photochemical manner and thus through a different mechanism than the one proposed here. Under well-lit conditions, and with Cl⁻ as the most freely available chlorine source, the formation of Cl-PYR in marine systems is unlikely to involve any other reactive form of Cl, such as ClO⁻ or Cl₂⁻. We argue based on our data and earlier findings\textsuperscript{13,20} that in marine environments a significant contribution of Cl-PAHs present are of photochemical origin.
References


Chapter 5

Conclusions and outlook
The work presented in this thesis addressed several topics in the context of the environmental photochemistry of organic pollutants. In Chapter 2, the direct photochemical behavior of three selected OH-PBDEs was studied. We found that each of the studied congeners transformed photochemically to yield new products that are themselves, known environmental pollutants. The formation of toxic PBDDs from OH-PBDEs was observed in all cases, and illustrates the need to further investigate the fate of organic micropollutants. We showed that the application of fundamental chemical knowledge can explain the results and help predict the environmental behavior of these organic micropollutants. For each of the individual congeners, we identified structural differences, e.g., differences in their bromination patterns, which accounted for the differences in their transformation fates. For instance, one of the transformation pathways common to each congener was the cleavage of the ether linkage to yield brominated phenols. The production of bromophenol from each congener correlated with the $pK_a$ value of the resulting bromophenol, indicating the efficiency of this transformation pathway is likely a function of the bromophenol to act as a leaving group. This case highlights that, by understanding the reaction mechanism, estimates can be made about the environmental fate of other compounds using simple chemical parameters. PBDD formation yields from each OH-PBDE congener were found to differ, and the congener that formed PBDD with the highest efficiency was the natural product, 6-OH-PBDE 99. This result is interesting in light of the fact that the majority PBDDs found in Baltic Sea sediments and biota samples appear to be primarily natural in origin. Our work lends evidence to this conclusion by demonstrating a plausible photochemical route from a known natural product, to a PBDD found in environmental samples.
In Chapter 3, the oxidation of phenols and anilines by triplet state MB was investigated. In the case of anilines, this reaction was observed to take place via a one-electron transfer reaction to yield an aniline radical cation and neutral radical MB species. The reaction with phenols, however, proceeded by a proton-coupled electron transfer to form a neutral phenoxy radical and a protonated MB radical cation. These results were supported both by the characteristic spectroscopic signatures of each MB reaction product observed by transient absorption studies, but also by other experiments, including ones that probed the kinetic isotope effect of the O-H bond strength in phenols. Empirical relationships between the reaction free energy and the observed rate constants were established for both types of reaction mechanisms. In the case of the anilines, the reaction rate constants could be modelled using the reaction free energy calculated from the aniline oxidation potential, and were fit using a Sandros-Boltzmann type dependence on free energy. Reaction rate constants of phenols correlated with the phenolic O–H BDFE, and could be modelled by a multi-parameter equation requiring only the BDFE and pK_a values of the phenols. By combining reaction rate constants determined from transient absorption measurements with computationally derived oxidation potentials, this work explored a framework for making better predictions about the indirect photochemical role in the fate of some pollutant classes.

In Chapter 4, an investigation was performed on the mechanism of photochemical Cl-PYR formation from PYR in aqueous solution. Previous studies have shown a correlation between concentrations of Cl-PYR in marine sediments and water salinity. In the course of this study, we collected data to test our hypothesis that Cl-PYR formation was a result of the nucleophilic attack by Cl\(^-\) on PYR radical cations formed after the oxidation of photoexcited PYR by O_2. In the presence of an added electron donor N_3\(^-\), Cl-PYR formation by photolysis was suppressed. This
observation is likely the result of N\textsuperscript{3} quenching the radical cations before chloride is able to attack. This idea is supported by transient absorption measurements, in which the PYR radical cation signal was eliminated by the addition of N\textsubscript{3} and S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−}. The role of O\textsubscript{2} in oxidizing photoexcited PYR was examined by purging samples with Ar prior to photolysis. Results of these experiments showed Cl-PYR was not formed in the absence of O\textsubscript{2} and the observed rate of pyrene degradation was decreased. Finally, the nucleophilic behavior of Cl\textsuperscript{−} was examined through competition experiments in which PYR was photolyzed in the presence of Cl\textsuperscript{−} and an additional nucleophile. With Br\textsuperscript{−} added as the competing nucleophile, the relative amounts of Br-PYR and Cl-PYR formed matched what was expected for a nucleophilic reaction mechanism. This work provides additional evidence to support the hypothesis that some Cl-PAHs found in contaminated marine environments are formed \textit{in situ}.

\textbf{Outlook}

For each topic investigated in this thesis, there exist a number of additional questions and problems that are worthy of future research. An interesting question raised by our results showing that OH-PBDEs may be responsible for the presence of PBDDs in the Baltic Sea is whether or not this photochemical process can happen within marine biota that produce OH-PBDEs. Certain marine species, including sponges and red algae that are found in the Baltic Sea are known to produce OH-PBDEs. In addition to containing OH-PBDEs, samples of some these species have also been shown to contain high concentrations of PBDDs. Whether or not PBDDs are produced from a biosynthetic pathway or not us unclear, and the connection between OH-PBDEs and PBDDs in marine biota is an open question. The possibility exists then that the PBDDs found in marine creatures exposed to adequate sunlight could be generated photochemically from OH-PBDEs. To address this question, more
detailed analytical studies could be performed to look for matches in the expected and identified congener profiles of OH-PBDEs and PBDDs in these samples. Another possible approach would be controlled light-exposure experiments, to access if exposure to the wavelengths that lead to PBDD formation has an effect on their concentration. This area of research is unique, because it reminds us that in some cases what seems like a pollutant may actually be an interesting part of the chemistry of life.

The ability to predict the environmental fate and effects of organic compounds is perhaps the single biggest goal of environmental chemistry. To meet this goal more sophisticated and reliable prediction methods will be required. Our attempt at modeling aniline and phenol degradation rates with MB represents an important step in developing more accurate predictions of the role of $^3$CDOM in pollutant degradation. The use of MB as our model triplet oxidant, while justified in the context of Chapter 3, makes comparing our results to the expected behavior of $^3$CDOM as an oxidant difficult. Future studies should focus on more difficult to oxidize anilines and the use of excited state acceptors. One set of acceptors that may be important that have received little attention are quinones. Like the more well-studied aromatic ketones, some quinones exhibit high triplet yields and may also play a role in the photoinduced oxidation of pollutants. Because $^3$CDOM contains a mix of photoexcited molecules able to participate in oxidation reactions, data from a wide range of excited acceptors will need to be incorporated into any prediction model.

Lastly, it would be interesting to see if other examples of the photohalogenation mechanism described in Chapter 4 can be found in marine environments. A number of compounds are known to degrade via a one-electron oxidation or photoionization mechanism, which leaves the possibility open for other pollutants to be chlorinated in the same manner as PYR in seawater. Another area that
could be addressed is the relative importance of *in situ* chlorination to other Cl-PAH sources. By analyzing substitution patterns of selected Cl-PAHs, which can indicate radical vs. cationic formation, it may be possible to determine the overall contributions from different sources.
Acknowledgements

I would like to thank my advisor, Kris, who I first had the opportunity to meet when I worked for him as an undergraduate student the summer before my senior year. His enthusiasm for science and interest in his students is what convinced me to return to Minnesota, and subsequently move with the lab to ETH. Thank you Kris, for the opportunity to be a part of an engaging and welcoming research team and helping to me to learn what it means to “push back the frontiers of science”.

The fellow students are what made my time in the McNeill lab truly memorable. As a young grad student, I remember thinking how nice it was to work in such a positive atmosphere. As the years passed and I became one of the veteran students, I did my best to be the same example of a good lab mate as the students that came before me were. From my first group happy hour on the patio at Burrito Loco in Dinkytown to the shore of the Seealpsee after an excellent group hike in Appenzell, I am thankful for the good times spent in the company of great people.

Sarah K, I am grateful that you and your knowledge were around for so long while I was in this group. Our conversations about the projects we were working on were almost as good as the many others we had about life outside of the lab. Soren, Thank you for taking me under your wing and introducing me to the wonderful world of time-resolved spectroscopy. You left big shoes to fill (literally!) as the overseer of the McNeill laser lab, and I enjoyed the months we spent working together.

Rebekka and Lilli, thank you for translating my dissertation summary. After four years in Switzerland you would think I could handle this, but with words such as “Elektronenübertragungsechanismus” it was nice to have the help.

Thank you Mom and Dad, for fostering my interest in science and encouraging me to always be curious. Every step of the way, from buying me my much-loved microscope to helping me with a science fair project using a real dynamometer, you were always there to enable my scientific mind. You were, and always will be wonderful parents.

Jessica, I cannot thank you enough for all that you do. I am so grateful to have had you along for this adventure that took us from Florida to Minnesota and on to Zurich. No matter what direction we travelled, it always brought me closer to you. Having a loving person and now family to come home to every day makes my life complete. I love you so much and I look forward to whatever new adventures the future brings our way.