

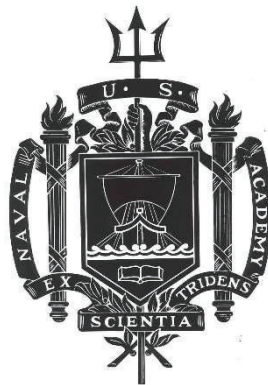
A TRIDENT SCHOLAR PROJECT REPORT

NO. 523

Investigating the Photodegradation Mechanisms of Chlorpyrifos in Arctic Lacustrine Systems

by

Midshipman 1/C Pippin E. Robison, USN



UNITED STATES NAVAL ACADEMY
ANNAPOLIS, MARYLAND

This document has been approved for public
release and sale; its distribution is unlimited.

USNA-1531-2

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 5-16-22		2. REPORT TYPE		3. DATES COVERED (From - To)	
4. TITLE AND SUBTITLE Investigating the Photodegradation Mechanisms of Chlorpyrifos in Arctic Lacustrine Systems				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Pippin E. Robison				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Naval Academy Annapolis, MD 21402				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) Trident Scholar Report no. 523 (2022)	
12. DISTRIBUTION / AVAILABILITY STATEMENT This document has been approved for public release; its distribution is UNLIMITED.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT In the Arctic, organohalogen contaminants are subjected to a slew of environmental processes that affect their persistence and ecological impacts. Due to unique environmental conditions, photodegradation is a critical pathway for the transformation of contaminants that undergo long range transport to polar regions, which include some current use pesticides. Dissolved organic matter (DOM), ubiquitous to surface water, associates with poorly soluble contaminants and can affect their photodegradation. Previous research indicates that the photodegradation rate of the insecticide chlorpyrifos is enhanced by DOM. However, the specific role of Arctic-derived carbon pools in the DOM-mediated photodegradation is poorly understood. This study sought to identify and quantify the mechanistic components of chlorpyrifos's photodegradation due to Arctic-derived DOM in order to better predict the fate of this contaminant and similar compounds more broadly across Arctic systems.					
15. SUBJECT TERMS DOM mediated photodegradation, organophosphate pesticides, chlorpyrifos					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES 51	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)

U.S.N.A. --- Trident Scholar project report; no. 523 (2022)

**INVESTIGATING THE PHOTODEGRADATION MECHANISMS
OF CHLORPYRIFOS IN ARCTIC LACUSTRINE SYSTEMS**

by

Midshipman 1/C Pippin E. Robison
United States Naval Academy
Annapolis, Maryland

(signature)

Certification of Adviser(s) Approval

Assistant Professor Jennifer J. Guerard
Chemistry Department

(signature)

(date)

Acceptance for the Trident Scholar Committee

Professor Maria J. Schroeder
Associate Director of Midshipman Research

(signature)

(date)

Abstract

In the Arctic, organohalogen contaminants are subjected to a slew of environmental processes that affect their persistence, bioavailability, and ultimately ecological and health impacts. Due to the environmental conditions in the Arctic such as lack of canopy, increased solar irradiance in the summer, and unique organic matter, photodegradation is a critical pathway for the transformation of contaminants. Dissolved organic matter (DOM), ubiquitous to surface water, associates with poorly soluble contaminants and can affect their photodegradation. One of the most prolific organophosphate pesticides, chlorpyrifos, has been found to undergo long range transport to polar regions where it has potential to accumulate thousands of miles from application sites. Previous research indicates that the photodegradation rate of chlorpyrifos is enhanced by DOM. However, the specific role of Arctic-derived carbon pools in DOM mediated photodegradation is poorly understood. This study sought to identify and quantify the mechanistic components of chlorpyrifos's photodegradation due to Arctic-derived DOM. DOM samples isolated from two geographically close but hydrologically different Alaskan lakes (Toolik Lake and Fog Lake) were photolyzed in the presence of chlorpyrifos by a solar simulator with probe molecules to either sensitize or quench various photodegradation mechanisms. The light screening corrected pseudo first order kinetic rates were compared to determine the overall mechanistic contribution to photodegradation extent. Seasonal differences in DOM composition were found to impact the level of DOM photosensitization. Mechanistic differences were identified for the photodegradation of chlorpyrifos in the two different DOMs. For Toolik Lake DOM at freshet, hydroxyl radical ($\bullet\text{OH}$), singlet oxygen ($^1\text{O}_2$), and triplet excited state ($^3\text{DOM}^*$) electron transfer were identified as contributors to photodegradation, and $\bullet\text{OH}$ was found to be the primary contributor species. For Fog Lake DOM, only $\bullet\text{OH}$ and $^1\text{O}_2$ were determined to contribute to photodegradation, but $^1\text{O}_2$ was the primary contributing species. Potential transformation products were consistently observed chromatographically across multiple kinetics experiments. These products are currently undergoing analysis via mass spectrometry for identification. The mechanistic information and associated product analysis will help to better predict the fate of this contaminant and similar compounds more broadly across Arctic systems to better understand the overall impact and potential remediation needs.

Keywords: DOM mediated photodegradation, organophosphate pesticides, chlorpyrifos

Acknowledgements

This work was in collaboration with Y.P. Chin (University of Delaware) and K. Hageman (Utah State University). This project was largely derived from G. Quesada's work from 2019-2021 (University Alaska Fairbanks). Thank you to Dr. Chateaufeuf and Mr. Schroeder (USNA Chemistry) for all of the help with HPLC. Additionally, thank you to CDR S. Gallagher, USN and Professor J. Smith (USNA Oceanography) for further developing me as a researcher. This work was supported by NSF CBET-1804271 (Guerard), Trident and Bowman awards, and Defense Threat Reduction Agency (DTRA) and Midshipman Research Support (MRS) grants.

Table of Contents

Glossary of Common Acronyms and Variables	5
I. Introduction	6
II. Background	8
<i>A. Organophosphate Pesticides: Structure and Properties</i>	8
<i>B. Chlorpyrifos Usage and Toxicity</i>	9
<i>C. Chlorpyrifos Occurrence and Fate in the Arctic</i>	11
<i>D. DOM's Effect on Photolysis</i>	12
III. Project Goals	13
IV. Preliminary Research and Hypotheses	14
V. Experimental Methods	17
<i>A. Overview</i>	17
<i>B. Chemicals, Standards, and Equipment</i>	17
<i>C. Phase 1: Sample collection at Fog and Toolik Lakes (Summer 2021)</i>	18
<i>D. Phase 2: General Photodegradation Procedure</i>	19
<i>E. Phase 2: Competitive Kinetics Experiments</i>	21
<i>F. Phase 2: Experimental Notes</i>	24
<i>G. Phase 2: DOM Fluorescence Characterization</i>	24
<i>H. Phase 3: Product Identification</i>	25
VI. Data Analysis and Results	26
<i>A. Chlorpyrifos Standards</i>	26
<i>B. Dark Controls</i>	27
<i>C. Calculation of Experimental Rate Constants</i>	28
<i>D. Correction of Experimental Rate Constants and Results</i>	30
<i>E. Quantitation of Mechanistic Processes</i>	33
<i>F. PNA Photolysis and Determination of Quantum Yield</i>	34
<i>G. Influence of DOM Composition and Seasonality on Photodegradation</i>	35
<i>H. Initial Product Analysis</i>	38
VII. Discussion	41
<i>A. Competitive Kinetics Experiments</i>	41
<i>B. Impact of Seasonality on Photodegradation</i>	43
<i>C. Initial Product Analysis</i>	44

VIII. Current and Future Experimental Work	46
IX. Conclusions	46
X. Relevance to Navy, DOD, and US	47
XI. References	48

Glossary of Common Acronyms and Variables

POPs	(persistent organic pollutants)	highly toxic compounds that persist in the environment
CUPs	(current use pesticides)	pesticides developed to replace legacy pesticides classified as POPs
DOM	(dissolved organic matter)	collection of carbon-based compounds ubiquitous to surface waters
ROS	(reactive oxygen species)	oxygen-containing compounds produced by the excitation of DOM
$^3\text{DOM}^*$	(triplet DOM)	class of reactive transient species; triplet excited states of organic compounds
$\bullet\text{OH}$	(hydroxyl radical)	reactive transient species; ROS
$^1\text{O}_2$	(singlet oxygen)	reactive transient species; ROS
^1H NMR	(proton nuclear magnetic resonance spectroscopy)	chemical identification method
UV-Vis	(ultraviolet-visible light)	
SRFA	(Suwanee River fulvic acid)	terrestrial DOM reference (Georgia, USA)
PLFA	(Pony Lake fulvic acid)	microbial DOM reference (Antarctica)
HPLC	(high performance liquid chromatography)	chemical separation method
[cpy]	(chlorpyrifos concentration)	
QA/QC	(quality assurance/ quality control)	
TPA	(terephthalic acid)	quencher of $\bullet\text{OH}$
FeCl_3	(iron(III) chloride)	sensitizer of $\bullet\text{OH}$
DABCO	(1,4-diazabicyclo[2,2,2]octane)	quencher of $^1\text{O}_2$
D_2O	(deuterium oxide/ heavy water)	sensitizer of $^1\text{O}_2$
TMP	(2,4,6-trimethylphenol)	electron transfer quencher of $^3\text{DOM}^*$
SA	(2,4-hexadienoic (sorbic) acid)	energy transfer quencher of $^3\text{DOM}^*$
SPE	(solid phase extraction)	chemical separation method
UHPLC-MS	(ultra-HPLC/ mass spectrometry)	chemical separation and identification method
MeOH	(methanol)	organic solvent
EEMs	(excitation-emission matrices)	method of fluorescence chemical analysis
LLQ	(lower limit of quantitation)	lowest value a compound can be quantified to from a calibration curve
k_{obs}	(observed rate constant)	rate constant is proportional to reaction rate
S_λ	(screening factor)	coefficient accounting for light screening in DOM
k_{corr}	(corrected rate constant)	rate constant corrected for experimental conditions
$t_{1/2}$	(half-life)	amount of time required for a compound to degrade to half of its original concentration
CI	(confidence interval)	statistical range of confidence
Φ_{app}	(quantum yield)	related to the rate constant, accounts for reaction efficiency from each photon of light

I. Introduction

Persistent organic pollutants (POPs) are highly toxic compounds that adversely affect human and environmental health. A contaminant is officially classified as a POP if it meets the Stockholm Convention's criteria for environmental persistence, bioaccumulation, human and environmental toxicity, and long-range transport. The criteria for POPs are based on the physical, environmental and toxicity data, many of which then undergo regulatory bans (Giesey et al., 2014; US Environmental Protection Agency). For example, pollutants that persist in the air as a result of slow degradation rates (atmospheric half-life > 2 days) have a high propensity for long range transport and may adversely affect environments far from application sites (Hageman et al., 2015). Many organochlorine pesticides were classified as legacy POPs and subsequently banned. As a result, current use pesticides (CUPs) were developed as replacements, designed to have less environmental persistence. These compounds are classified by their high-volume production and current use, but an increasing number of studies have found a number of CUPs actually have similar chemical properties to listed POPs (Hoferkamp et al., 2010). Long term monitoring of POPs in the Arctic has been occurring for years as they are highly regulated by governments worldwide (Grannas et al., 2006). However, since 2000, a number of CUPs have also been detected in the Arctic and alpine regions, far from application sites, raising concern that they might not be as resistant to long range transport as intended, posing a new threat to Arctic biota should sufficient accumulation occur (Hoferkamp et al., 2010; Wu et al., 2017; Balmer et al., 2019).

A compound's potential for atmospheric transport depends on equilibrium partition coefficients, degradation rate constants, temperature, and emission intensity. Upon volatilization or direct transmission, pollutants are subjected to some form of atmospheric transport, whether it be long or short range. Long range transport is especially notable for semivolatile pollutants, which are compounds with higher molecular weights and boiling points but relatively low vapor pressures. As atmospheric and ocean currents transport heat to cooler polar regions, semivolatile organic (carbon containing) compounds that have volatilized into the air are then also transported to the cryosphere (the frozen regions of the Earth), such as the Arctic (Vorkamp and Riget, 2014; Zhong et al., 2012). Semivolatile contaminants have a greater propensity to undergo atmospheric transport and accumulate in the snow and ice of colder environments due to the combination of their relatively low solubility as hydrophobic molecules and their slower degradation rates at lower temperatures (Grannas et al., 2006). Once in the Arctic, such compounds may undergo a complex suite of environmental processes, including: photodegradation, advection, deposition, and temperature-driven thermodynamic partitioning into rain or snow which is then precipitated (**Fig. 1**) (Hageman et al., 2015).

Isolated from pollutant emission sites and home to a diverse range of biota, the Arctic has been key for the study of contaminants. The Arctic is a unique environment for organic contaminants because of the extreme temperatures, 24-hour sunlight exposure in summer months, and rich organic material. Dissolved organic matter (DOM) is the heterogeneous mixture of

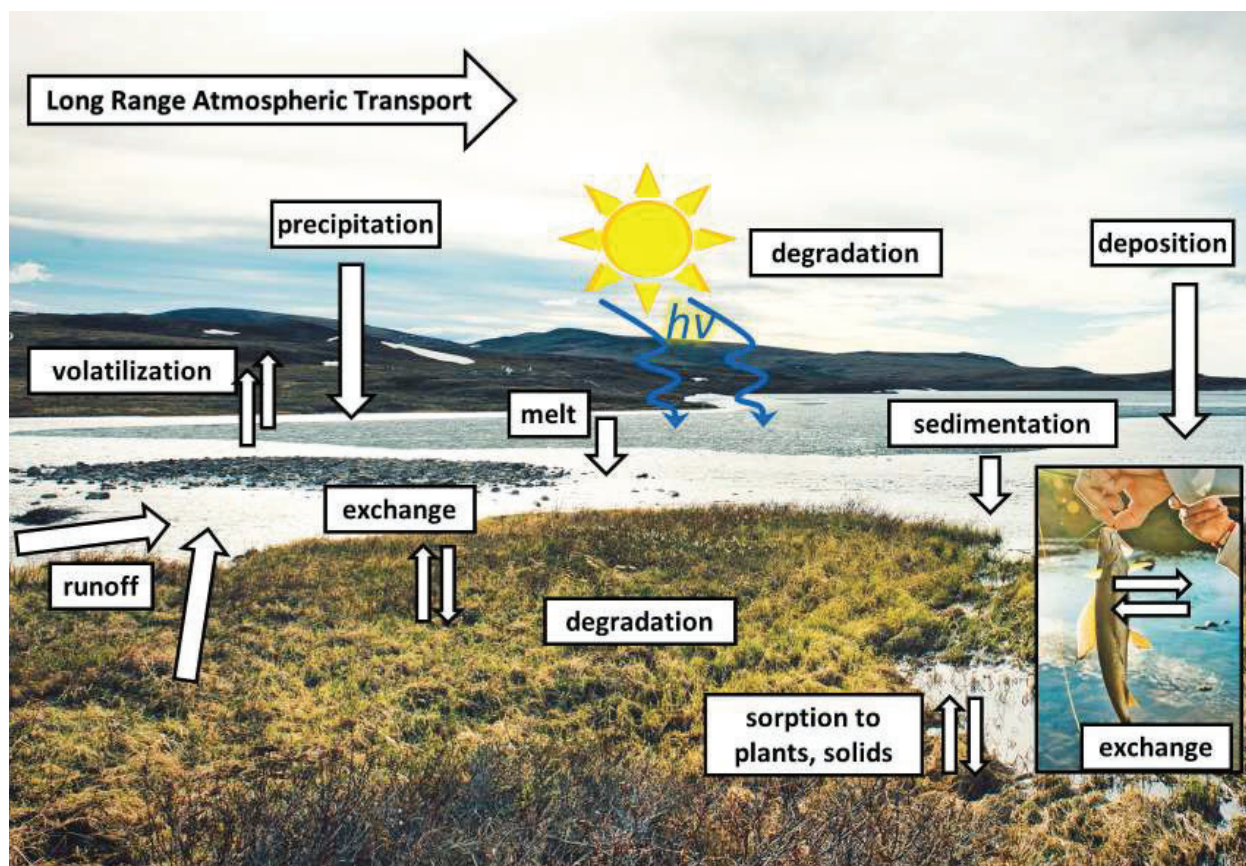


Figure 1. Environmental processes that impact contaminant cycling and fate in the Arctic

organic compounds from biological and terrestrial sources of the surrounding ecosystem that aggregates in all surface waters. The photoreactivity of DOM plays an important role in many environmental water processes, notably producing labile carbon for microbial activity, shielding organisms from harmful UV radiation, and mediating the direct and indirect photolysis of organic contaminants (Guerard et al., 2009). Poorly-water-soluble organic contaminants that enter the water have a high propensity to sorb to the DOM as it is more similar in composition than the polar water. The aforementioned properties of DOM have resulted in decreased contaminant persistence in the environment and bioavailability to organisms (Wei-Haas and Chin, 2015). While DOM may significantly facilitate photodegradation of current use pesticides, their mechanisms and extent in Arctic DOM is unknown.

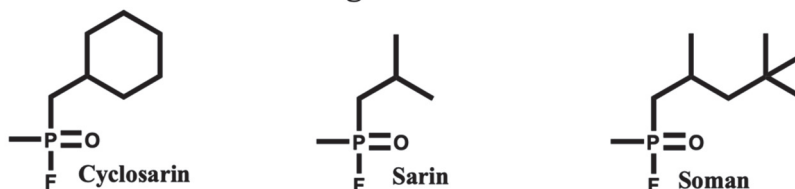
Although certain degradation processes of pollutants are slower in the Arctic, photochemical degradation in surface waters is likely to be enhanced as a result of continual irradiance in the summer months and longer ice-free periods as temperatures warm. This environmental process is promising feedback so long as the persistence, fate, and toxicity of degradation products are inconsequential. Although various studies measure the existence and concentrations of CUPs in the Arctic, there is a lack of research regarding their chemical fate and degradation. This work built off of precursory investigations in attempt to comprehensively identify and quantify the interactions between the organophosphate CUP chlorpyrifos and reactive species in Arctic DOM during photolysis.

II. Background

A. Organophosphate Pesticides: Structure and Properties

Organophosphorus pesticides are the largest classification of chemicals used to control pests (Sparling and Fellers, 2007). They are a highly toxic class of compounds that were originally developed by the major world powers as nerve agents (such as sarin nerve gas) in World War I and World War II to “harm, kill, or neutralize the opponent,” but have since been primarily used as pesticides since the 1950s (**Fig. 2**) (Jacquet et al., 2016). Since the 1970s, they have been heavily used as a replacement for earlier organochlorine pesticides as the organophosphates were designed to be more toxic but have a higher decomposition rate, leading to lower volatility and environmental accumulation (Wu and Linden, 2010). However, mounting evidence suggests that they are not free from potential environmental harm.

Chemical Warfare Nerve Agents



Agricultural Pesticides

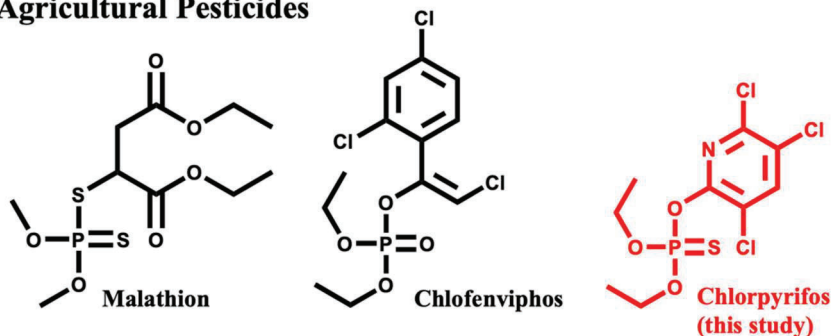


Figure 2. Chemical structures of common organophosphates

All organophosphates share a characteristic structure, having a central phosphorus doubly bonded to an oxygen or sulfur and singly bonded to two variable (R) groups and a leaving group (Jacquet et al., 2016). R_1 and R_2 are usually ester ($R-C(=O)OR'$), amide ($R-C(=O)NR'R''$), or thiol ($R-SH$) derivatives. R_3 is typically an acyl group ($R-C(=O)R'$) that can have a variety of reactive groups attached (**Fig. 3**) (Marrazza, 2014). Many have a sulfur atom doubly bonded to the phosphorous vice the oxygen typical in a nerve agent. Analysis of various organophosphate degradation products shows a high propensity for these sulfurs to be replaced with an oxygen atom upon

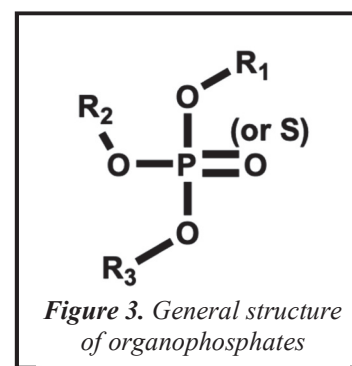


Figure 3. General structure of organophosphates

exposure to certain degradation processes (Raina et al., 2010). These “oxon” derivatives of the parent pesticides are even more structurally similar to the organophosphate nerve agents (**Fig. 2**).

The S_N2 mechanism by which organophosphates phosphorylate and therefore inhibit the enzyme acetylcholinesterase accounts for their toxicity. Acetylcholinesterase is the enzyme responsible for breaking down the neurotransmitter acetylcholine in the nervous system. When this enzyme is inhibited, acetylcholine builds up in the synaptic clefts, resulting in hyper-neuronal activity, which can be dangerous and, depending on the relationship between the compound and organism, fatal through asphyxiation (Sparling and Fellers, 2007; Taylor et al., 2007). Although the organophosphate pesticides currently in use are not as toxic to humans as sarin, they act on insects as sarin does to humans, making them effective pesticides.

However, organophosphorus pesticides in their concentrated forms *are* toxic to humans and mammals. Their accidental or deliberate ingestion results in approximately 300,000 human deaths annually (Jacquet et al., 2016). The effects of agricultural use of current use organophosphorus pesticides on human and other organisms’ health are continually being studied, especially since the bioaccumulation of CUPs is still largely unknown (Zhong et al., 2012). While small exposure is generally considered to be of little risk, studies have linked organophosphate pesticide exposure to neurobehavioral deficits in Mexican-American children living in agricultural regions of California (Marks et al., 2010). They have also been detected in freshwater fish in Alaskan national parks, which is concerning as fish are a critical food source in the Arctic food chain (Grannas et al., 2006). Additionally, an increasing number of studies have focused on the adverse effects of combinations of CUPs and POPs on Arctic vertebrate species like polar bears (Routti et al., 2016).

Furthermore, the oxon metabolites of organophosphates have been shown to be significantly more toxic than their parent compounds. In a study comparing chlorpyrifos and its oxon derivative, chloroxon, the oxon derivative, killed 100 times more *R. boylii* tadpoles than chlorpyrifos at their lowest concentrations (Sparling and Fellers, 2007). The greatest oxon/thion ratios are observed during summer months, when photolysis is at its highest due to increased sunlight (Raina et al., 2010). The results of these studies highlight the importance of deeper study into the interactions between these pesticides, their degradation products and the environments in which they accumulate.

B. Chlorpyrifos Usage and Toxicity

Chlorpyrifos (*O,O*-diethyl-*O*-3,5,6-trichloropyridin-2-yl phosphorothioate) (**Fig. 3**) is one of the most widely used organophosphate insecticides and one of the most commonly detected in the environment (Hoferkamp et al., 2010; Sparling and Fellers, 2007). It is also one of the more biologically toxic pesticides and as a result, is generally applied as an insecticide later in the growing season when plants are more mature (Raina et al., 2010; Sparling and Fellers, 2007). Relatively low dosages (ranging in the micrograms, µg) of chlorpyrifos have been found to be

lethal to aquatic organisms, notably crustaceans, insects, fish, and to a smaller extent, amphibians. Even smaller doses (ranging in nanograms, ng) have been found to adversely affect reproduction and sensory pathways of aquatic organisms. Exposure to chlorpyrifos has been indicative of reduced survivability in bird species, as well. However, to date, in ecological risk studies, chlorpyrifos has been determined to not pose a serious toxicological risk to organisms because of its short to moderate persistence due to its many dissipation pathways (Giesey and Solomon, 2014).

Although classified as toxic under the European Commission Regulation No. 1107/2009, chlorpyrifos has not been classified as a POP under the Stockholm Convention as its current persistence, bioaccumulation, and toxicity data have been insufficient to show “significant adverse human health and/or environmental effects” (Giesey et al., 2014).

Over the past two decades the United States Environmental Protection Agency (US EPA) has implemented a number of restrictions on chlorpyrifos due to mounting health concerns. In 2000 and 2002, substantial regulations began to emerge, including reducing overall production by 50%, limiting its use on corn and citrus, and banning its use in residences and on termites. Gradually, its annual application rate in the US decreased to a steady application of 5 million pounds per year (**Fig. 4**; low estimate) in more localized regions on soybean fields and orchards (**Fig. 5**) (Raina et al., 2010; US Geological Survey, n.d.).

In 2012, based on evidence of harmful human spray drift exposure, the US EPA further reduced aerial application of chlorpyrifos in public spaces. Since 2014, in periodic registration reviews mandated by the Food Quality Protection Act of 1996, the US EPA has been addressing

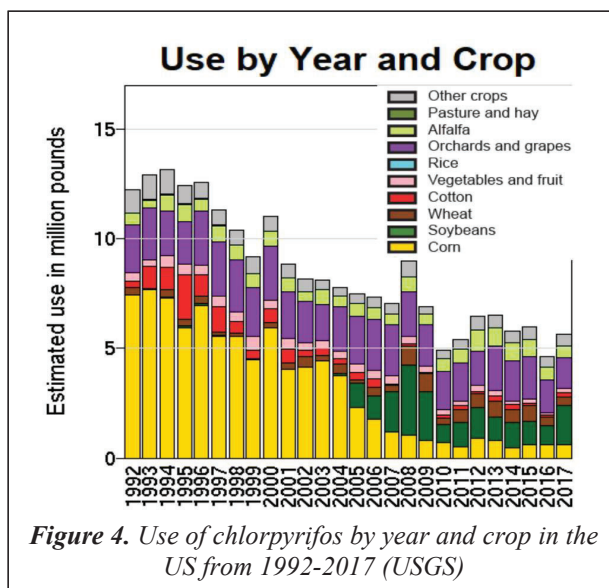


Figure 4. Use of chlorpyrifos by year and crop in the US from 1992-2017 (USGS)

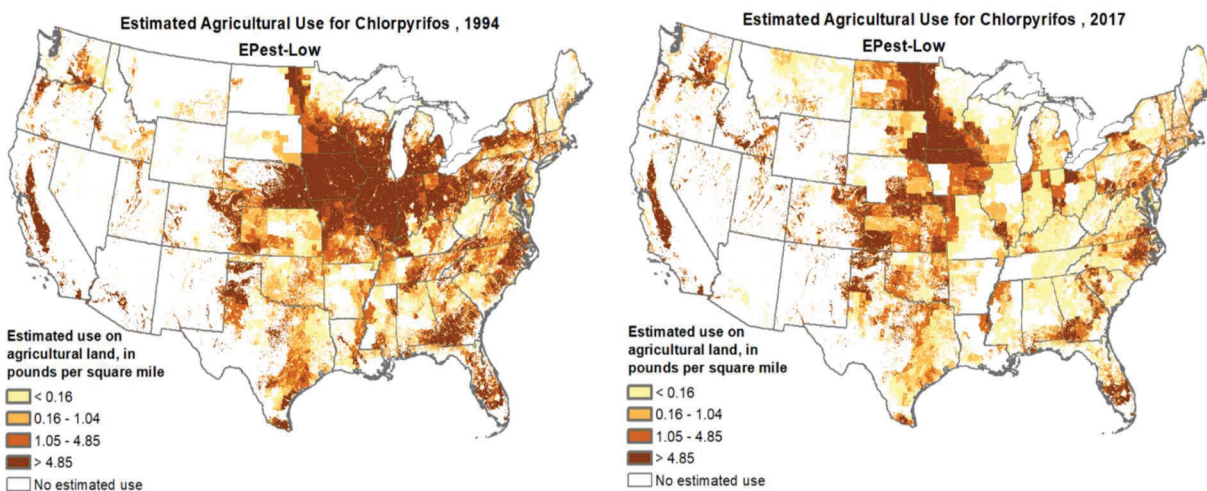


Figure 5. Estimated agricultural use of chlorpyrifos use in 1994 (left) and 2017 (right) (USGS).

neurodevelopmental effects in fetuses and children from epidemiological studies. As recently as September 2020, in the *Draft Ecological Risk Assessment and Revised Human Health Risk Assessment* of chlorpyrifos, the US EPA continued to maintain that further action was not warranted due to the lack of conclusive evidence that chlorpyrifos interacts with human estrogen, androgen, or thyroid pathways (US EPA, n.d.). However, the US EPA ultimately banned all tolerances for chlorpyrifos on food in a final rule released in August 2021. This essentially banned its use agriculturally, based on their conclusion that the risks associated from its use exceeded the safety threshold set in the Federal Food, Drug, and Cosmetic Act (USGS, n.d.). Canada additionally released legislation to phase out chlorpyrifos usage over three years, starting in 2021 (Pest Management Regulatory Agency, 2020). While these actions help reduce global emission of the pesticide, multiple countries, notably India and China, continue to employ chlorpyrifos as an insecticide, and large quantities are still released into the atmosphere each year.

C. Chlorpyrifos Occurrence and Fate in the Arctic

Relatively higher concentrations of chlorpyrifos have been found in Arctic soil, water, and air compared to other organophosphate CUPs (Geisey and Solomon, 2014; Hermanson et al., 2005; Hoferkamp et al., 2010; Vorkamp and Riget, 2014). Chlorpyrifos has been found at similar or higher concentrations in Arctic lacustrine (lake) systems than in those at lower latitudes, which are closer to where it is applied and where most of the ecological studies have been conducted (Muir and de Wit, 2010). Concentrations of chlorpyrifos in the nanogram range have been reported in the Arctic, and therefore could potentially threaten Arctic aquatic organisms, especially if currently trapped contaminants are accumulating in Arctic snowpack and later eluting into water systems at a higher concentrations (Hung et al., 2016). Long range transport to polar regions and subsequent bioaccumulation has been observed to be an increasingly important part of chlorpyrifos's chemical fate as volatilization has been found to be a main dissipation pathway from plant foliage (Muir and de Wit, 2010; Vorkamp and Riget, 2014). Chlorpyrifos has a very low water solubility (1.39 mg/L at standard conditions) and therefore has a strong tendency to sorb (essentially to associate by absorption or adsorption) to snow grain surface, soil, and organic matter. Whether historically trapped in permafrost soils or ice, or newly deposited from atmospheric transport, melt and water flow eventually bring chlorpyrifos into Arctic water systems. Because of its low solubility in water and strong affinity for snow, chlorpyrifos elutes late during snowmelt, when the snow's sorptive capacity is exceeded (Meyer and Wania, 2011). However, once eluted into a body of water, chlorpyrifos's solubility is most likely greater than its normal aqueous solubility because of its hydrophobic association with DOM.

The extent to which pollutants degrade depends on their chemical and physical properties. This degradation takes place by one of three major mechanisms in the environment: biotic, hydrologic, and photochemical. Biotic degradation of pollutants results from their digestion by microorganisms like bacteria, fungi, archaea and protists. The primary abiotic degradation pathways are hydrolysis, the degradation of compounds by their reaction with water, and

photolysis, when compounds are degraded by light (Schwarzenbach et al., 2017). Light can either directly degrade organic contaminants (direct photolysis) or excite other molecules that then react with the contaminant (indirect photolysis) (Katagi, 2018; Remucal, 2014). Several studies regarding the degradation of chlorpyrifos have been conducted in various temperate environmental systems. In general, biotic degradation is thought to be small for chlorpyrifos because of its toxicity to bacteria (Singh and Walker, 2006). Its hydrolysis degradation pathways have been well studied and are comparatively slower than photolysis (Giesey, 2014). Therefore, the main environmental processes affecting chlorpyrifos in water have largely been determined to be re-volatilization and photodegradation (Muir and de Wit, 2010).

As Arctic temperatures warm, chlorpyrifos's propensity to volatilize and photodegrade will increasingly contribute to its fate in the region. In Arctic latitudes, photodegradation is likely even more pronounced as the region receives up to 24 hours of continual sunlight in summer months when peak melt has occurred. Because of the low air temperatures and water mixing during melt, re-volatilization and hydrolysis are less pronounced, further amplifying the importance of photodegradation to chlorpyrifos's environmental fate. **Despite its importance, the extent and effects of its photodegradation in Arctic lacustrine systems is still largely unknown and is the focus of this study.**

D. DOM's Effect on Photolysis

DOM serves many functional roles in biogeochemical cycling and organic contaminant fate. In water systems, DOM contributes to light screening, photo sensitizing, pH buffering, nutrient cycling, metal complexing, and redox cycling. As such, the interactions between contaminants and DOM are complex. To a certain extent, DOM absorbs UV light, reducing the direct photodegradation of organic contaminants. On the other hand, the photoexcitation of DOM has been found to significantly increase the indirect photodegradation of overall contaminants through the production of reactive transient species that interact with each other, DOM, and organic pollutants (**Fig. 6**).

These reactive transients include triplet excited DOM, the carbonate radical and other carbon centered radicals, hydrated electrons, and a number of reactive oxygen species (ROS) (hydrogen peroxide, superoxide, hydroxyl radical, peroxy radicals, singlet oxygen) (Cawley et al., 2009; Guerard, 2009). The triplet excited state of DOM ($^3\text{DOM}^*$), carbonate radical, hydroxyl radical ($\bullet\text{OH}$), and singlet oxygen ($^1\text{O}_2$) have been well identified as principal reactive transients for the phototransformation of many pollutants (Remucal, 2014). In the photodegradation of chlorpyrifos in DOM from prairie potholes around the northern US, all four reactive transients were found to contribute to an observed enhancement of chlorpyrifos degradation by 50% as a result of environmental media compared to pure water (direct photolysis). Roughly 70% of the enhancement was due to $^1\text{O}_2$, suggesting $^1\text{O}_2$ is the principal sensitizing species of chlorpyrifos photodegradation in DOM in the respective system (Zeng and Arnold, 2013).

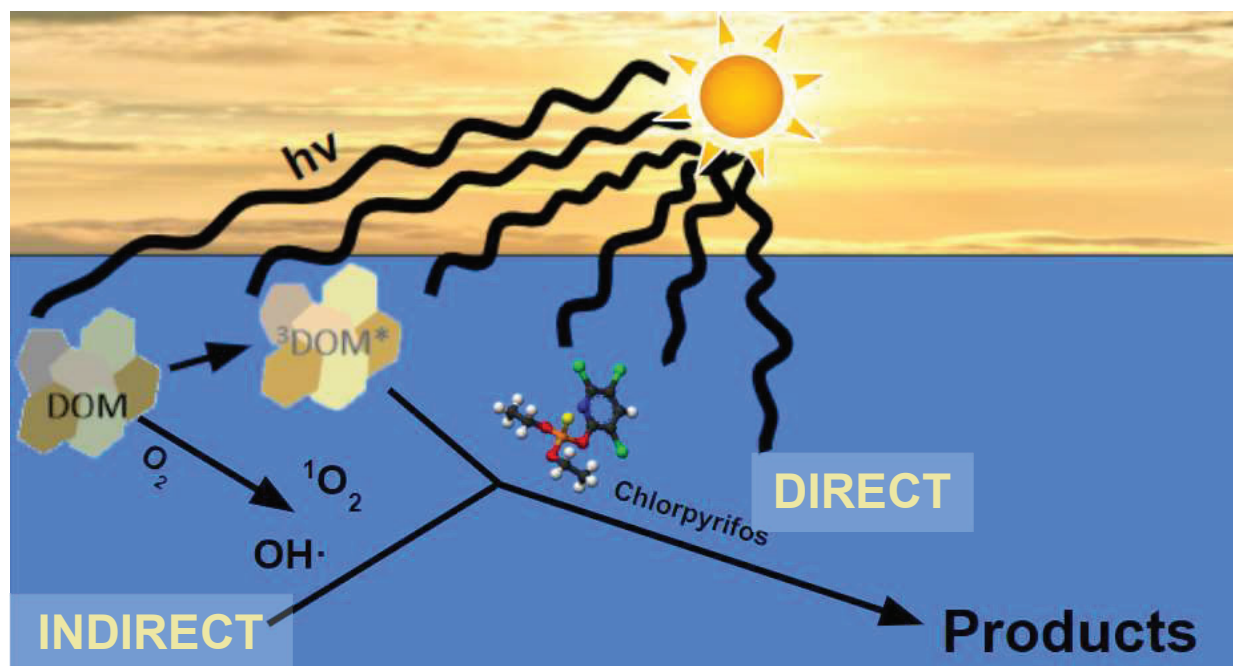


Figure 6. The direct photolysis of contaminants coupled with indirect photolysis by the reactive transient species produced by the photolysis of DOM may yield a variety of products.

However, the composition of DOM and its extent of photoreactivity are highly dependent on ecosystem inputs, spatial, and temporal conditions. DOM can be classified as allochthonous (terrestrially derived) and autochthonous (derived from the water column/microbes). Terrestrially derived DOM is characterized by a high number of carbon pool inputs from lignin, tannins, and other plant matter. It tends to have a greater aromaticity and result in a darker color. Highly microbial DOM, in contrast, is derived from material produced by microbes in the water. Although it has less light absorbing groups, there are more protein-derived groups. These key differences in composition likely lead to differences in photoreactivity and contaminant mediation. Most natural waters, including in the Arctic, include a mixture of both types of compounds, that may shift with season, or on longer scales with ecosystem changes (Pinto et al., 2015; Zeng and Arnold, 2013). **The study of the direct and indirect photodegradation of other contaminants or even chlorpyrifos itself in other environments can help develop predictions about the oxidative and electron transfer reactions of chlorpyrifos in Arctic lacustrine systems; but because the environments are biogeochemically different, they demand independent study.**

III. Project Goals

Despite a number of studies on chlorpyrifos's occurrence in Arctic systems, little has been done to characterize its fate. This study will contribute to the overall development of a chemical fate and transformation processes model of CUPs in Arctic Alaskan lacustrine systems. Preliminary results by this group have shown the photodegradation of chlorpyrifos is generally

enhanced in Arctic DOM, but the mechanisms by which this occurs and their dependence on DOM composition necessitate further study. **The primary goal of this project is to investigate the impact of the key reactive species ($\bullet\text{OH}$, $^1\text{O}_2$, and ^3DOM) on the rate and resulting products of chlorpyrifos's photodegradation in different Arctic DOMs.**

IV. Preliminary Research and Hypotheses

This study will contribute to the overall development of a chemical fate and transformation processes model of CUPs in Arctic Alaskan lacustrine systems. Toolik Lake and Fog 1 (Fog) Lake are the representative sites of interest for this model. Toolik and Fog Lakes are separated by 13 mi in the northern foothills of the Alaska Brooks Range, located north of the Arctic Circle ($68^\circ 37' 40''$ N $149^\circ 35' 41''$ W and $68^\circ 67' 99.52''$ N $149^\circ 09' 42''$ W respectively) (Fig. 7). Although geographically similar, both overlying permafrost in a remote tundra ecosystem with similar climate and precipitation, they have different organic matter composition and hydrology. Fog Lake is hydrologically isolated, while Toolik Lake has both an inlet and an outlet stream, which impact the influx of DOM into the lake. Toolik Lake has significantly more dissolved carbon than Fog Lake (5 mg C/L and 1 mg C/L, respectively) (Page et al., 2014). Additionally, the stream systems around the lakes originate from different watersheds of the Brooks Range, which might contribute further to differences in DOM composition. As an NSF Long-Term Ecological Research site, since 1983, a wealth of physical, chemical, and biological data on Toolik Lake is available and relevant to this study (Davie-Martin et al., 2016). Fog lake, however, is not as thoroughly studied. It was artificially warmed by 4°C two years ago to study climate change impacts, and it contrasts Toolik lake because its only organic inputs are from surrounding run off and autochthonous production. Contaminants can thus be introduced to the lake environment by runoff and stream influx as well as by atmospheric deposition.

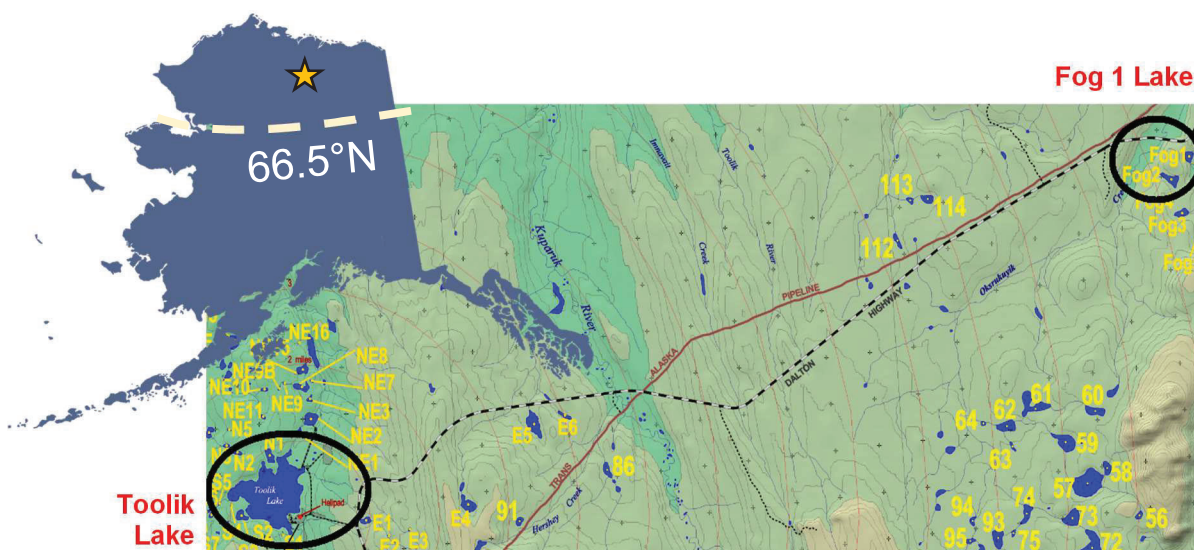


Figure 7. Topographical map of study sample sites

In the summer of 2019, DOM was collected from Toolik outlet both in late spring after completion of melt (May) and in late summer (July) 2019 and also collected from Fog in early summer (June) 2019. The DOM isolates were characterized by various chromatographic and spectroscopic methods including chromatography, proton nuclear magnetic resonance spectroscopy (^1H NMR), UV-Vis absorbance, and fluorescence. Further, contaminant sorption and preliminary photolysis experiments were conducted to compare the effects of Arctic DOM on the photodegradation of the selected CUPs to two DOM reference samples. These reference samples included Suwannee River (SRFA) in Georgia, a highly terrestrially derived DOM material, and Pony Lake (PLFA) from Antarctica, an entirely microbially derived material, which represent end members of composition and reactivity (Guerard et al., 2009). From ^1H NMR spectra, Alaskan Arctic DOM isolates were found to have different functional group composition, specifically different aromatic and aliphatic composition, compared to the reference DOMs (**Fig. 8, left**). Furthermore comparison of ^1H NMR and fluorescence data between the different Alaskan Arctic DOM isolates (Toolik Lake, Fog Lake, and Oks Seep- a fen near Toolik Lake) demonstrates the variability in Arctic DOM composition, which likely impacts its ability to mediate contaminant photolysis (Quesada, 2021). From the NMR and fluorescence characterizations of the Arctic DOM below (**Fig. 8, right**), Arctic DOM in general seems to be derived from a mix of both terrestrial and microbial inputs; as a result, it is difficult to classify and predict how Arctic DOM will behave.

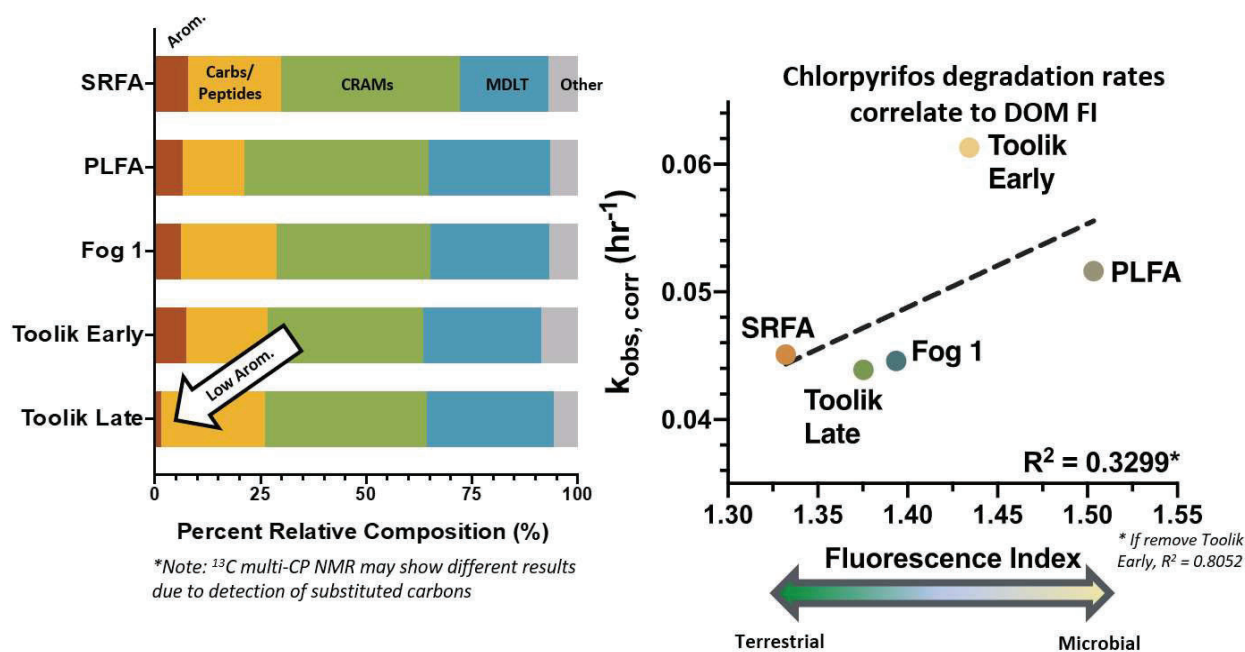


Figure 8. Left: % relative composition of functional groups in DOM isolates in H_2O , determined from ^1H SPR-W5-WATERGATE NMR spectra. Functional groups include: aromatic, carbohydrate and peptide, carboxyl rich alicyclic molecules (CRAM), and molecules derived from linear terpenoids (MDLT). Right: Chlorpyrifos photodegradation rates vs. source in different DOM isolates (Quesada, 2021).

Preliminary photolysis experiments showed that the rate of photodegradation of organic pollutants is significantly elevated in Arctic DOM, sometimes even more than either of the reference isolates or more temperate DOM (**Fig. 9**). For instance, DOM from Toolik Lake was found to be up to three times more reactive with 2,4,6-trimethylphenol, a probe for triplet excited species, than DOM from temperate Ohio wetland samples (Page et al., 2014). As photodegradation in water is expected to be a major pathway dictating the environmental fate of chlorpyrifos, it is even more important to investigate the mechanisms behind this Arctic driven augmentation of the overall acceleration of chlorpyrifos photodegradation by DOM.

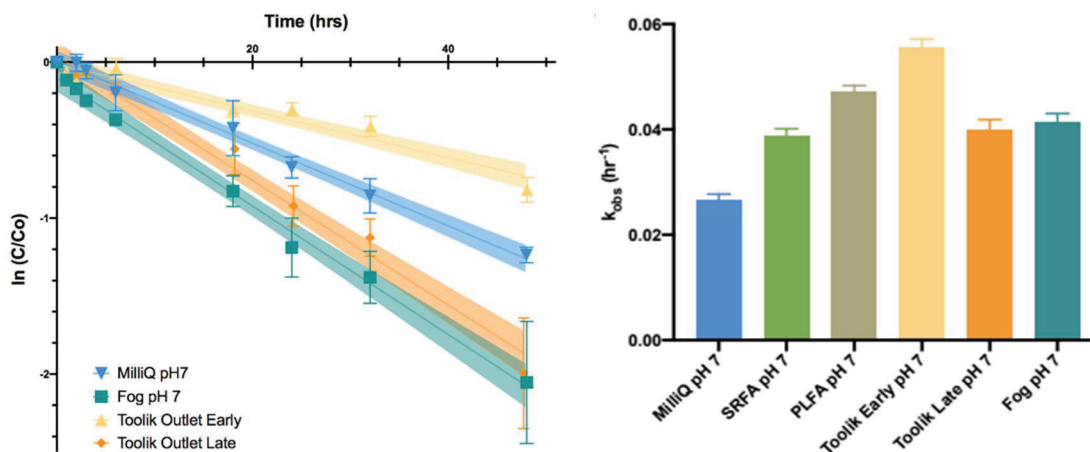


Figure 9. Left: Chlorpyrifos photodegradation rates in MilliQ water (blue) and DOM from various Alaskan lakes. Right: Chlorpyrifos photodegradation rates vs. source in different DOM isolates (Guerard).

In Arctic sunlit waters, $\bullet\text{OH}$, $^3\text{DOM}^*$, and $^1\text{O}_2$ have been well identified species while carbonate radicals have not. By studying these primary pathways and resulting products, it may be possible to better understand the reactivity of chlorpyrifos in Arctic lakes. Preliminary results from prior work with both Toolik and Fog DOM reveal both have a potential to facilitate degradation of chlorpyrifos in the presence of $\bullet\text{OH}$ degradation (**Fig. 9**, Quesada, 2021). **It is predicted that, in conditions favorable for $\bullet\text{OH}$ production, the hydroxyl radical might be a dominant reactive species in the indirect photosensitization of contaminants by Arctic DOM; and as a result, Arctic DOM may not be the same as DOM from lower latitudes** (Quesada, 2021; Zeng and Arnold, 2013).

Identifying reactive pathways may also help characterize DOM composition in ways that bulk spectroscopic analysis cannot, as photoreactivity of DOM with chlorpyrifos is dependent upon a set of specific pathways. Allochthonous (terrestrial) DOM has been found to be more subject to photolysis enhancement by ROS, while autochthonous (microbial) DOM has been found to better enhance triplet excited state pathways (Page et al., 2014). **As Arctic DOM appears to fall somewhere in between the terrestrial and microbial reference DOMs and differences in DOM composition were not well correlated to chlorpyrifos photosensation (Fig. 8), it is necessary to further investigate how the carbon pool differences impact chlorpyrifos degradation.**

V. Experimental Methods

A. Overview

The experimental study consisted of three phases (**Fig. 10**). **Phase One** consisted of Arctic DOM sample collection at Toolik Field Station, Alaska over summer 2021. In **Phase Two**, the enhanced photodegradation of chlorpyrifos mediated by the reactive transient compounds in DOM was quantified through competitive kinetics experiments using high pressure liquid chromatography (HPLC). In proceeding experiments, six different reactive transient probes were added to each DOM type (Toolik and Fog Lake) in order to selectively observe the change in rate due to the inhibition or stimulus of one of the three selected DOM reactive transients. During **Phase Three**, the products from the major transformation pathways determined in Phase Two are currently undergoing extensive chemical analysis and identification by ultra-HPLC and mass spectrometry (UHPLC-MS).



Figure 10. Three phases of investigation over the course of the project.

B. Chemicals, Standards, and Equipment

All glass and plasticware were soaked first in Citranox detergent, followed by 10% HCl solution, and finally rinsed at least 6 times with ultrapure water and dried overnight. Methanol was used to rinse residual organic matter from glass and plasticware between experiments. All chemicals and solvents not otherwise specified were purchased from Fisher Scientific (Waltham, MA). Solvents used were HPLC grade, and all other reagents were quality 98% or higher. All solutions were prepared with 18 MΩ water, herein referred to as ultrapure water. Plastic-capped glassware holding chlorpyrifos-water solutions were covered with Teflon tape. Chlorpyrifos (white powder, purity ≥ 98%) from Sigma-Aldrich (St. Louis, MO) was dissolved in HPLC grade methanol yielding ~20 mM stock solutions used in the preparation of reaction mixtures and HPLC

standard solutions. 2.85 mM chlorpyrifos external standards purchased from Restek (Bellefont, PA) were used to prepare additional HPLC standards for quality control.

Standards were prepared on a Mettler Toledo AG204 analytical balance with Pasteur pipettes. pH adjustment was conducted with a Thermo Scientific Orion 3 Star pH meter, calibrated daily before use. HPLC analysis of chlorpyrifos was performed on an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA) equipped with an autosampler and PDA detector. Chlorpyrifos was detected at 290 nm around 6.7 min retention time (RT). All peak areas were integrated in the native software and kinetic rates were calculated in Microsoft Excel. Statistical analysis was performed in Prism 9.3.1 (GraphPad, San Diego, CA). Total organic carbon was run on a Shimadzu total organic carbon analyzer in collaborator Chin's Lab (University of Delaware).

C. *Phase 1: Sample collection at Fog and Toolik Lakes (Summer 2021)*

Water from the inlet of Toolik lake was collected during Spring freshet in June 2021 (**Fig. 11**). Freshet is the first occurrence of melt and occurred much later than normal in 2021 compared to prior years. For this reason, DOM was unable to be obtained from Fog Lake during the allotted field time and so isolate from July of 2019 (late summer) was used in this study. Lake water was collected in acid washed LDPE cubitainers, acidified to pH 2 with HCl, and filtered to 0.45 μm . DOM from collected water was extracted over gravity fed polymer solid phase columns (PPL, Agilent, Santa Clara, CA) whereby the hydrophobic DOM was retained on the column. The DOM was eluted off with methanol and rotovapped before freeze-drying, yielding solid shelf-stable DOM isolates for Phase Two kinetics experiments (Dittmar, 2008).

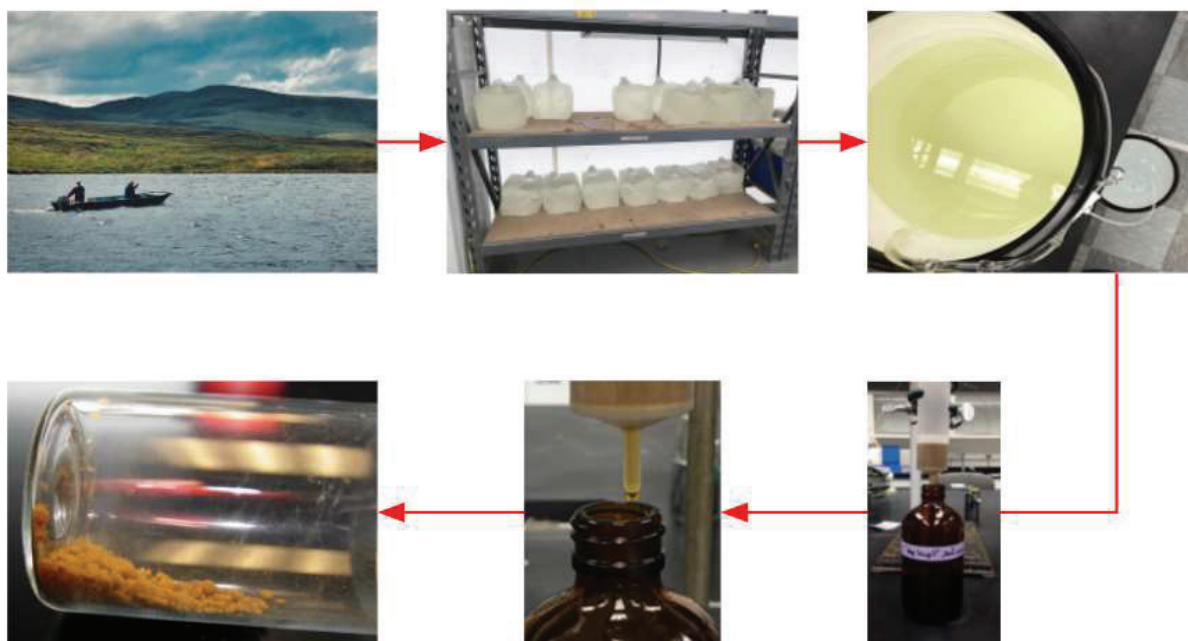


Figure 11. Phase 1 lake water sample collection and extraction of DOM.

D. Phase 2: General Photodegradation Procedure

Overview The photodegradation rates of chlorpyrifos in the DOMs were obtained experimentally through photolysis kinetics experiments. Spiked aqueous chlorpyrifos solutions with reconstituted DOM were irradiated in a solar simulator, whereby tubes for different time points were sacrificed in triplicate for analysis. The photodegraded solutions were analytically separated and the concentration of chlorpyrifos ([cpy]) was quantified (**Fig. 12**).

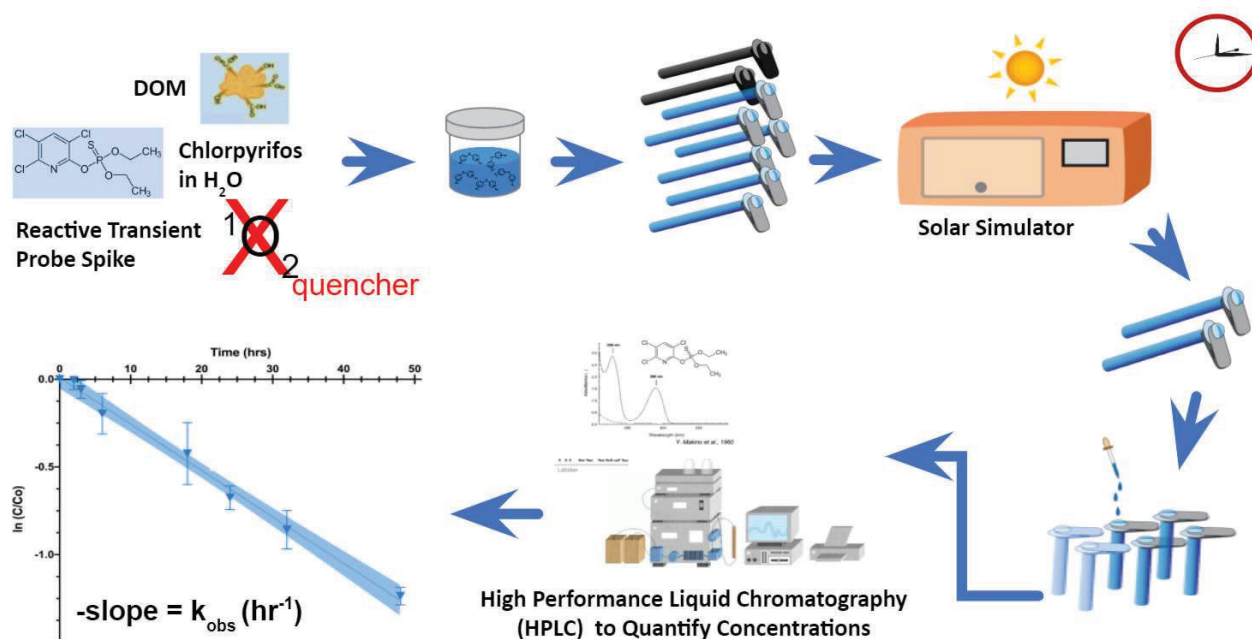


Figure 12. General procedure of kinetics experiments in Phase Two.

Background A solar simulator produces continuous irradiance irrespective of meteorological and temporal conditions that may exist in the natural environment. The CPS+ solar simulator (Atlas, Mount Prospect, IL) has a 1500 W xenon arc lamp that exhibits a similar wavelength distribution as natural sunlight, with an irradiance of 500 W/m². Chemical actinometry was used to quantify the irradiance using p-nitroanisole, which has a known quantum yield to make comparisons between sunlight and artificial light intensity in field and laboratory experiments, respectively (Dulin and Mill, 1982). This artificial sunlight is slightly more intense than what contaminants are exposed to in the Arctic, but constant irradiance produced by the solar simulator is integral in determining mechanistic contributions toward photolysis.

HPLC is a widely used analytical separation and quantification method. Reverse-phase HPLC consists of a polar liquid solution mobile phase carrying the sample through a nonpolar solid-phase column. Compounds are eluted from the column in order of decreasing polarity and are detected with a UV-Vis detector emitting ultraviolet and visible wavelengths of light. Peak areas integrated from the chromatogram output are related to sample concentration and can be quantified via a calibration curve of known standards.

Controls In all laboratory photolysis experiments, various control methods were used for quality assurance and method validation. Dark controls, wrapped in aluminum foil, were run alongside for each experiment. Chlorpyrifos standard solutions were prepared for each HPLC analysis, and additional external standard solutions were analyzed over the course of the first four months for QA/QC. Finally, the direct photodegradation rate of an analytical chlorpyrifos standard in ultrapure water was obtained.

Specific Photolysis Procedure 18.063 mM chlorpyrifos stock was volumetrically pipetted in three low volume spikes onto the sides of a 500 mL amber glass bottle for an excess of five times above solubility to ensure saturation of chlorpyrifos in aqueous solution. The methanol film was left to evaporate to constant mass for at least 24 hours. To the pesticide-plated bottle, 500 mL of ultrapure water was then added, and the solution was left in the hood for an average of 18 hours (as previously determined) to reach equilibrium at saturation. Freeze-dried DOM was reconstituted with ultrapure water to prepare 100 mg/L DOM stock solutions that were stored in the dark at 4°C for up to 8 weeks at acidic pH (~ 50 mg C/L). An 80 mL aliquot of the DOM (or with probe, as described below section II. D) solution was pH adjusted to lake conditions ($\text{pH } 7 \pm 0.1$) and combined with the equilibrated aqueous chlorpyrifos solution in a separate 500 mL amber glass bottle. The reaction mixture (20% DOM w/w, resulting in a concentration of ~10 mg C/L) was transferred by glass pipettes to 14 mm diameter, 10 cm long quartz reaction tubes whereby they were filled to no headspace, sealed with Teflon tape, and capped.

Triplicate reaction tubes were irradiated under artificial light for varying periods of time (0, 1, 2, 4, 8, 12, 18, 24, 36, 48 hrs) in an Atlas CPS+ Solar Simulator (Xe arc lamp, 500 W/m², 30-35°C). Prior to and following photolysis, the reaction tubes were stored in the dark in the fridge to minimize hydrolysis. Aliquots of the photodegraded samples were transferred to 2 mL HPLC vials and were analyzed by an Agilent 1200 HPLC (Santa Clara, CA). Triplicate injections (50 µL) of each sample were run through a Restek Ultra C18 reverse-phase column (5 µM particle size; 4 mm diameter, 150 mm length) in a mobile phase of acetonitrile:0.1% acetic acid (80:20% v/v) at a flow rate of 1.00 mL/min for 8 minutes. The eluent was analyzed at 290 nm for chlorpyrifos (RT 6.9-7.1 min). Chlorpyrifos HPLC standards (1-20 µM in HPLC vials) were made on the day of HPLC analysis from the 18.063 mM prepared chlorpyrifos stock solution. From the stock, a 20 µM working stock solution was further diluted to prepare the standards in HPLC vials. Additional standards derived from an external standard solution were prepared by small volume addition of 2.85 mM chlorpyrifos in MeOH to ultrapure water in 40 mL vials. Check blanks were run periodically within the HPLC sequence. Chlorpyrifos sample concentration was determined from a calibration curve of the 18.063 mM derived chlorpyrifos standards.

E. *Phase 2: Competitive Kinetics Experiments*

The addition of probe molecules to experimental solutions containing DOM enables the observation of the mechanistic contributions of the specific reactive transients ($\bullet\text{OH}$, $^1\text{O}_2$, $^3\text{DOM}^*$). Probe molecules can be classified as sensitizers or quenchers, and both are helpful to confirm the importance of specific mechanisms in the contaminant degradation to various products. Sensitizers increase the reactivity of the reactive transients through a few different processes. For example, FeCl_3 acts as a sensitizer for $\bullet\text{OH}$ by producing more $\bullet\text{OH}$ through the photo-Fenton reaction, while D_2O acts as a sensitizer for $^1\text{O}_2$ because it increases singlet oxygen's lifetime in water (Guerard et al., 2009; Guerard and Chin, 2012). Conversely, quenchers react with the given reactive transient to inhibit it from reacting with other species. The reaction of $\bullet\text{OH}$ with terephthalate (TPA) to produce hydroxyterephthalate removes $\bullet\text{OH}$ from the organic pollutant photodegradation scheme (Grannas et al., 2006). Moreover, DOM itself and its photolytically produced reactive transients can sensitize and quench each other. **Table 1** summarizes the specific probes that were used for each reactive transient. The probe experiments followed the same general photolysis procedure except the 80 mL DOM aliquot was spiked with a small amount of a concentrated stock solution of the probe molecule of interest prior to pH adjustment (**Table 2**). In effort to avoid interference of D_2O 's ability to sensitize $^1\text{O}_2$, D_2O experiments were not pH adjusted. The following sections further discuss each transient's reactive pathways and interactions with their respective probe compounds.

TABLE 1. Summary of probe molecules specific to selected reactive transients used in this study.

Transient	Probe	Role	Description	Reference
$\bullet\text{OH}$	<i>terephthalic acid (TPA)</i>	<i>Q</i>	<i>selective for $\bullet\text{OH}$</i>	<i>Zeng and Arnold, 2013</i>
	<i>FeCl_3</i>	<i>S</i>	<i>forms $\bullet\text{OH}$ via photo-Fenton</i>	<i>Guerard et al., 2009</i>
$^1\text{O}_2$	<i>1,4-diazabicyclo[2,2,2]octane (DABCO)</i>	<i>Q</i>	<i>selective for $^1\text{O}_2$</i>	<i>Zeng and Arnold, 2013</i>
	<i>D_2O</i>	<i>S</i>	<i>increases $^1\text{O}_2$ lifetime</i>	<i>Guerard and Chin, 2012</i>
$^3\text{DOM}^*$	<i>2,4,6-trimethylphenol (TMP)</i>	<i>Q</i>	<i>via e^- transfer or H abstraction</i>	<i>Cawley et al., 2009</i>
	<i>2,4-hexadienoic (sorbic) acid (SA)</i>	<i>Q</i>	<i>via energy transfer</i>	<i>Zeng and Arnold, 2013</i>

Q: quencher; S: sensitizer

TABLE 2. Summary of photolysis experimental conditions and chemical probes.

SOLUTION	AMENDMENT	MECHANISM	PH
18MΩ H₂O	<i>N/A</i>	<i>Direct Photolysis Only</i>	<i>N/A^a</i>
TOOLIK	<i>N/A</i>	<i>Indirect Photolysis (Mult. Mech.)</i>	<i>7.11</i>
FRESHET	<i>TPA (1.5 mM)</i>	<i>•OH Quencher</i>	<i>7.02</i>
DOM ISOLATE	<i>FeCl₃ (20 μM)</i>	<i>•OH Sensitizer</i>	<i>7.11</i>
(2021)	<i>DABCO (10 mM)</i>	<i>¹O₂ Quencher</i>	<i>7.00</i>
	<i>D₂O (as solvent)</i>	<i>¹O₂ Sensitizer</i>	<i>N/A^b</i>
	<i>TMP (50 μM)^c</i>	<i>³DOM* Quencher</i>	<i>6.97</i>
	<i>Sorbic Acid (10 mM)</i>	<i>³DOM* Quencher</i>	<i>7.03</i>
FOG 1	<i>N/A</i>	<i>Indirect Photolysis (Mult. Mech.)</i>	<i>6.99</i>
DOM ISOLATE	<i>TPA (1.5 mM)</i>	<i>•OH Quencher</i>	<i>7.01</i>
(2019)	<i>FeCl₃ (20 μM)</i>	<i>•OH Sensitizer</i>	<i>7.01</i>
	<i>DABCO (10 mM)</i>	<i>¹O₂ Quencher</i>	<i>6.95</i>
	<i>D₂O (as solvent)</i>	<i>¹O₂ Sensitizer</i>	<i>N/A</i>
	<i>TMP (50 μM)^c</i>	<i>³DOM* Quencher</i>	<i>6.98</i>

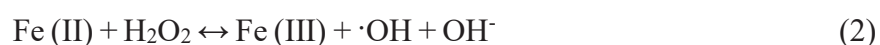
^apH of pure water not measured due to low ionic strength

^bpH of D₂O solutions not adjusted to introduce minimal H₂O into reaction mixture, likely an acidic from DOM

^cTMP concentration chosen to be at least 10x over CPY concentration in order to have excess for complete quenching

Hydroxyl Radical The hydroxyl radical (•OH) is one of the strongest oxidizing agents in the environment, important in nutrient, metal, and carbon cycling; DOM photodegradation; and organic pollutant oxidation. Multiple photo processes in water produce •OH in the environment including photo-Fenton reactions, photolysis of nitrate and nitrite, and photoproduction in DOM, specifically thought to be through the excitation of quinones and other carboxylic acid and phenol derivatives (Mandal, 2018; Page et al., 2013). •OH is also produced in DOM non-photolytically through the oxidation of reduced DOM and iron and is highly scavenged by DOM (Page et al., 2013). DOM was found to be the main source of •OH production in Arctic marine and freshwater systems (Page et al., 2014). •OH is a strong electrophile and has a high propensity to react with compounds at sites with high electron density. It quickly oxidizes pesticides via electron transfer and hydrogen abstraction (Wu and Linden, 2010).

Because alcohols generally quench any radicals, common quenchers of •OH have been isopropyl and methanol as well as benzene, benzoic acid, and DMSO/nitroxide. However, these probes lacked selectivity for •OH. TPA has been identified as a more selective •OH quenching probe and should account for mechanistic contributions of •OH in the competitive kinetics experiments (Zeng and Arnold, 2013). An •OH sensitizer, FeCl₃, can produce •OH via the photo-Fenton reaction (Eqns. 1-2) (Guerard, 2009).



Triplet State DOM $^3\text{CDOM}^*$ ($^3\text{DOM}^*$) is composed of a combination of high-energy and low-energy triplet states of organic (carbon based) compounds. $^3\text{DOM}^*$ is formed by the intersystem crossing of the short-lived, photolytically excited singlet state of DOM ($^1\text{CDOM}^*$). $^3\text{DOM}^*$ can act as either a highly active reductant and oxidant. Triplet excited states of organic compounds can react with other compounds to produce more reactive transient species. For example, $^3\text{DOM}^*$ reduces O_2 to produce superoxide (O_2^-), a more unstable and reactive species (Latch and McNeill, 2006). $^3\text{DOM}^*$ can also react directly with contaminants via three different pathways: energy transfer, electron transfer reactions, and hydrogen abstraction (McNeill and Canonica, 2016).

Because $^3\text{DOM}^*$ can react via different pathways, two $^3\text{DOM}^*$ quenchers will be used to probe for the extent by which triplet DOM reacts with chlorpyrifos. Trimethylphenol is a selective quencher for triplet excited DOM electron transfer reactions. The probe is oxidized by electron transfer to compounds in the triplet state, which then become less reactive (Cawley et al., 2009). Sorbic acid (*trans,trans*-hexadienoic acid) has been found to be a useful probe for triplet DOM energy transfer reactions (Grebel et al., 2011). Both probes have successfully been used previously in the quantification of formation, determination of steady-state concentrations, and quenching of triplet-excited states of organic compounds (Cawley et al., 2009). The sum of the results from both probe molecules is expected to account for the majority of $^3\text{DOM}^*$'s contribution to the photolysis of chlorpyrifos.

Singlet Oxygen Singlet oxygen production by DOM in natural waters has been well studied. Photochemically excited DOM to its triplet form subsequently reacts with ground triplet state oxygen ($^3\text{O}_2$) to produce $^1\text{O}_2$ (Page et al., 2013; Larson, 1994). Singlet oxygen is a more selective electrophilic oxidant than $\bullet\text{OH}$ (Guerard and Chin, 2012). Moreover, the association of contaminants with DOM significantly increases their reactivity with singlet oxygen because singlet oxygen has a much higher lifetime in the hydrophobic pockets of DOM (Latch and McNeill, 2006). Singlet oxygen primarily reacts with heterocyclic contaminants by a Diels Alder reaction with dienes through an endoperoxide intermediate to produce four-membered, heterocyclic, oxygen containing rings (Larson and Weber, 1994). It also has been found to react with compounds with electron donating groups such as phenols and phenolates (Guerard and Chin, 2012). Singlet oxygen was found to be the primary indirect degradation pathway of chlorpyrifos in prairie potholes (Zeng and Arnold, 2013).

Notable quenchers of singlet oxygen include furfuryl alcohol and DABCO (1,4-diazabicyclo[2,2,2]octane) (Haag et al., 1984; Zeng and Arnold, 2013). In more recent studies, DABCO has been the probe of choice as it is believed to be more selective and effective in scavenging singlet oxygen due to its high hydrophobicity (Zeng and Arnold, 2013). The lifetime of singlet oxygen in water is very short (4 μs) (Latch and McNeill, 2006). If singlet oxygen significantly affects the phototransformation of chlorpyrifos in the Arctic, by the addition of D_2O in DOM isolates, singlet oxygen's mechanistic effect will be enhanced.

F. Phase 2: Experimental Notes

Several weeks were dedicated to method assessment and testing, where various experimental issues were addressed including: assessment of peak wavelength, degradation of chlorpyrifos standards over time, minimization of experimental noise, low solubility of chlorpyrifos and subsequent tendency for aqueous solutions to rapidly develop a film on the surface when exposed to air at high [cpy], discrepancy between the measured value of [cpy] of non-photolyzed solution in HPLC vials versus phototubes, and best practices for glassware maintenance between experiments. For these reasons, many experiments were redone to test the method, minimize experimental error, and obtain accurate and highly internally correlated results. Both the ultrapure and Toolik DOM baseline experiments (already consisting of triplicate experimental replicates) were repeated over three times to ensure that the rates obtained by the final method were accurate. Experiments that underwent the fully developed and tested method resulted in degradation rate constants with Pearson's correlation values over 0.9. However, in some experiments, small leaks were detected midway through the HPLC analysis that would auto-abort the 18-hour analysis sequence, requiring manual restart of the sequence. In these experiments (Toolik-DABCO, Toolik-D₂O, Fog DOM, Fog-TMP), lower R^2 values and higher slope error were obtained; but hydrolysis over the increased time of sample analysis was determined to have a negligible effect on [cpy]. All obtained experimental results were obtained from the fully developed and tested method except Toolik-TPA, which was one of the last experiments in the method-testing period of study. The Toolik-TPA reaction mixture was only photolyzed for 24 hrs vice the standard 48 hrs. Ideally, this experiment would have been redone under the fully developed method, but as it yielded some of the best-correlated, low error experimental results in the testing phase, other experiments were prioritized for second runs. In other experiments, the [cpy] of some T48 samples was unable to be determined due to the experimental lower limit of quantitation (LLQ).

G. Phase 2: DOM Fluorescence Characterization

Absorbance scans and fluorescence excitation-emission matrices (EEMs) were run in 1-cm quartz cuvettes (Firefly Scientific, Staten Island, NY) on an Aqualog spectrometer (Horiba Instruments, Edison, NJ). Absorbance was collected from 200-800 nm every 1 nm with a 0.1s integration time, and referenced against a blank of pure water, which was used to determine light screening factors. For EEMs, excitation was run from 240-600 nm every 3 nm and emission was scanned from 240-600 nm every 4 pixels with a 0.1s integration time and medium gain. EEMs were blank subtracted, inner filter corrected, with first and second order Rayleigh scattering removed. Fluorescence indices were determined as the ratio of fluorescence at 470 v. 520 nm, when excited at 370 nm (Cory et al., 2005).

H. Phase 3: Product Identification

In the third phase of this investigation, the bulk photolysis of chlorpyrifos in the three Toolik DOM isolates from the 2019 and 2021 field seasons, in the 2019 Fog Lake isolate, and in 18M Ω H₂O are currently undergoing analysis to identify both direct and indirectly-mediated photolysis products. From the preliminary research implicating $\bullet$ OH's activity in the photosensitization of chlorpyrifos degradation in Arctic DOM, it is anticipated to find oxidation products of chlorpyrifos from reactions with the ROS ($\bullet$ OH and ^{1} O₂) (Zeng and Arnold, 2013). It is also likely chlorpyrifos reacts with secondary photoproducts from the excited DOM and these photoproducts are also being explored.

The procedure for bulk photolysis was similar to the mechanistic experiments, except the phototubes were photolyzed in bulk (80 mL total per timepoint). Multiple replicates of the DOM isolates solutions were collected at timepoints 0, 8, and 48 hours. The products from each timepoint were concentrated on Waters Oasis HLB SPE cartridges (Milford, MA) and extracted in aqueous and organic (MeOH) fractions. These fractions are undergoing analysis by Waters Acquity ultra HPLC (Milford, MA) and quadrupole Mass Spectrometer (QdA MS). The UHPLC utilizes a photo diode array (PDA) (absorbance detector) that probes the visible and ultraviolet (UV) spectra. UHPLC-MS analysis pairs the enhanced separation capability of UHPLC with the advanced identification capabilities and sensitivity of a mass spectrometer.

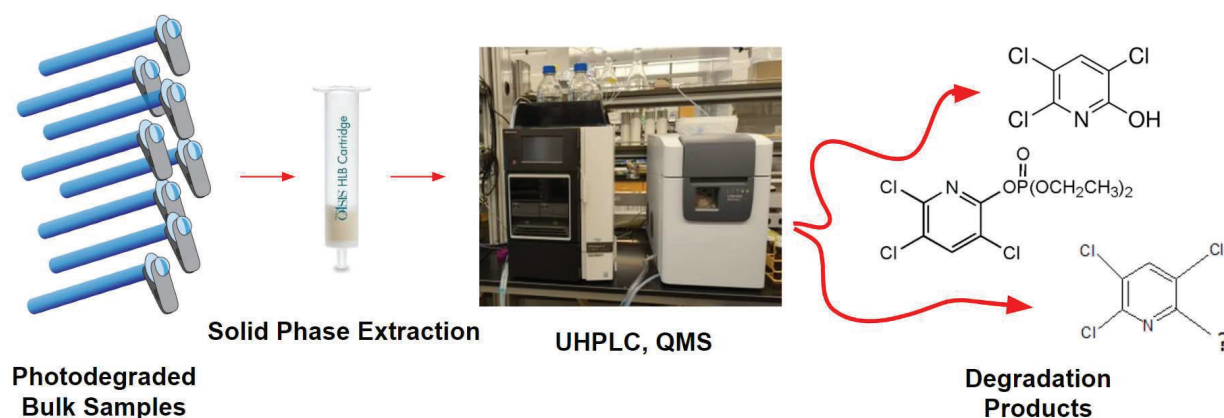


Figure 13. Summary of Phase 3 Methods.

In addition to determination of chlorpyrifos concentrations over time, the chromatogram outputs from the HPLC were useful in identifying and tracking the appearance and disappearance of other compounds throughout the photolysis. The product peaks that could be observed at the same wavelength as chlorpyrifos (290 nm) were tracked across all photolysis experiments (**Fig. 14**).

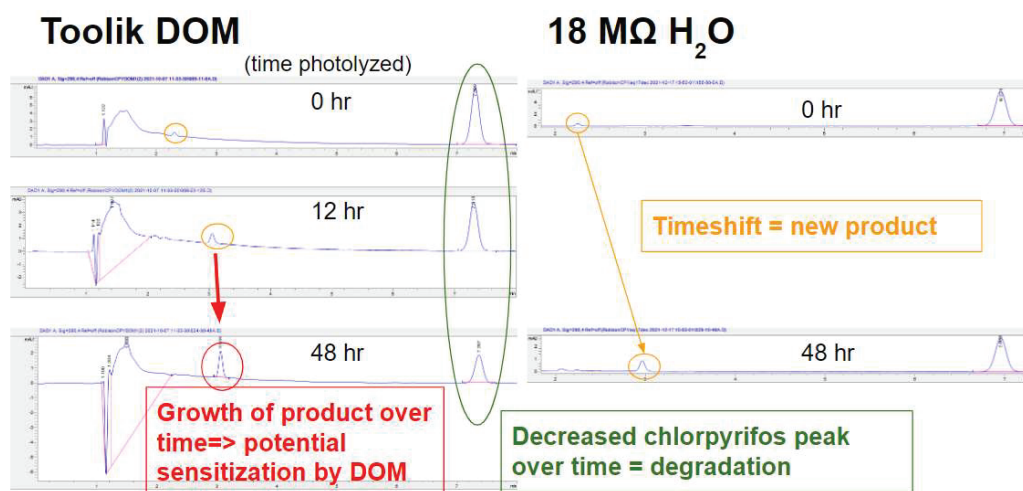


Figure 14. Potential degradation products absorbing light at 290 nm during chlorpyrifos photodegradation.

VI. Data Analysis and Results

A. Chlorpyrifos Standards

Initially, the 2.85 mM derived standards were prepared and preserved for use over multiple weeks due to chlorpyrifos's slow hydrolysis degradation. However, substantial reduction in chlorpyrifos peak area was observed upon analysis after 6–8 days in some standards, and there was no apparent source of contamination. This greatly reduced the accuracy of the resultant calibration curve and standard preparation method, requiring the repetition of some of the initial photolysis experiments. In the improved method, standards were prepared from the 18.063 mM stock upon the conclusion of each sun test on the same day as the HPLC run. Fresh 2.85 mM derived standards were concurrently analyzed monthly throughout the first semester to for assessment of the new method. Because multiple 2.85 mM derived calibration curves existed from initial experiments, comparison between the methods could occur from the first implementation of the new method. Calibration curves between methods range from 4–8 standards. In total, 5 sets of 2.85 mM derived standards and 18 sets of 18.063 mM derived standards were analyzed. The 18.063 mM derived chlorpyrifos standards used for weekly calibration curves were plotted against calibration curves of standards made from 2.85 mM chlorpyrifos HPLC standard solutions (**Fig. 15**).

Each weekly set of 18.063 mM derived standards was compared to the overall trend from previous experiments' standards and analytical standards. 17 of the 18 stock-derived standards fell within range of the 2.85 mM derived standards upon overlay and were kept for method comparison and data analysis. For this experiment (FOG-TMP), the average 18.063 mM derived calibration curve was used to determine [cpy], resulting in an increase of quantifiable timepoints and Pearson correlation (R^2 coefficient).

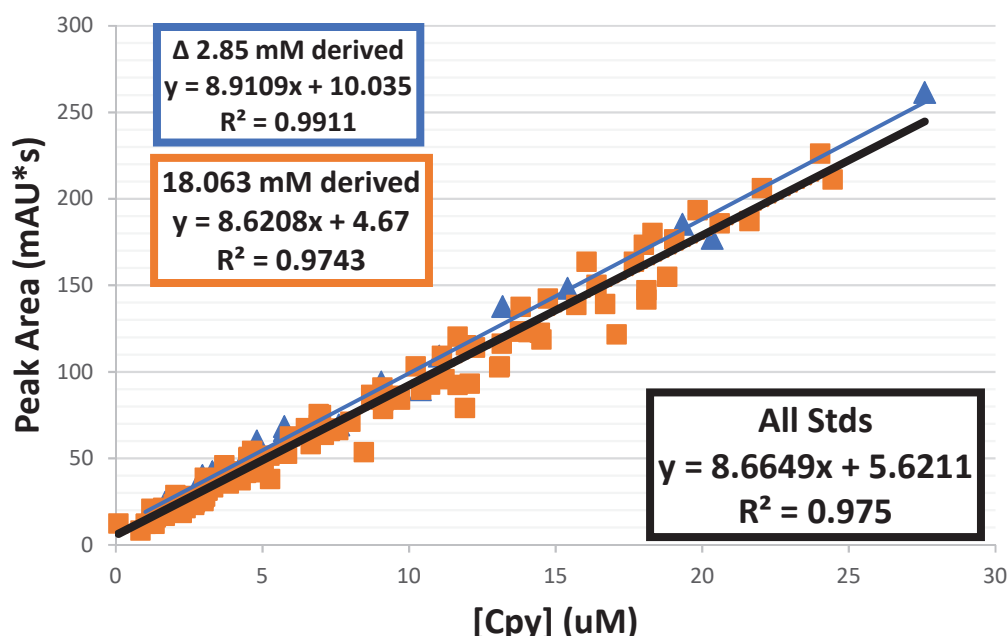


Figure 15. Overlay of all chlorpyrifos calibration curves as detected at 290 nm on HPLC.

The strong Pearson correlations for each individual method and combined methods attest to their repeatability when standards are freshly prepared. The data obtained from both methods centers around an average slope of 8.5 mAU*s/ μ M, similar to each method's own calibration curve. The almost analogous average slope between the standards derived from the stock and those from the analytical standard further supports the use of the 18.063 mM standards for sample [cpy] determination without correction. The notable difference between the calibration curves is the y-intercept values. The analytical standards produce a much higher intercept value than the stock-derived standards, resulting in a smaller LLQ value. An experimental LLQ of 0.5 μ M was determined from the chlorpyrifos standards.

B. Dark Controls

Thermal degradation was confirmed to have a negligible effect on the overall degradation of chlorpyrifos in the samples (**Fig. 16**). Only two experiments had a notable difference between T0 and T48 for an unknown reason. Slight

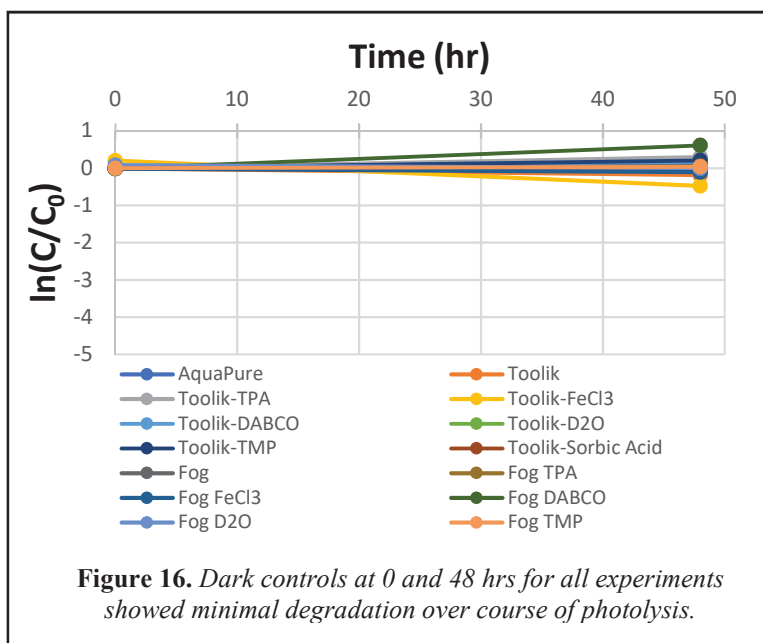


Figure 16. Dark controls at 0 and 48 hrs for all experiments showed minimal degradation over course of photolysis.

concentration drift was observed across other samples most likely due to HPLC variation observed from low sample concentration. However, the average of dark controls were similar in concentration to the T0 controls, leading to the determination that thermal degradation would not significantly impact the photodegradation analysis. Additionally, the chromatogram profiles for the products were identical between T0 and dark controls, so there was no observation of a thermal degradation product.

C. Calculation of Experimental Rate Constants

From the [cpy] concentrations obtained following photolysis at various timepoints, the observed pesticide photolysis rate constants (k_{obs}) were calculated. For a given experiment, k_{obs} , was determined from a linear regression of the data applied as a pseudo-fit to the first order integrated rate law (Eqn. 3).

$$\ln[cpy] = \ln[cpy]_0 + k_{obs}t \quad (3)$$

The degradation of all experimental replicates were first visualized in Excel to visually determine statistical outliers (**Fig. 17, left**). Log linearized average concentrations at each time point were then fit to linear regression models to determine observed first order rate constants k_{obs} for each experiment (**Fig. 18**). Then, the 95% confidence intervals of the linear regression chlorpyrifos experimental rate constants (k_{obs}) were calculated using the average replicate value for a given timepoint (**Fig. 17, right**.)

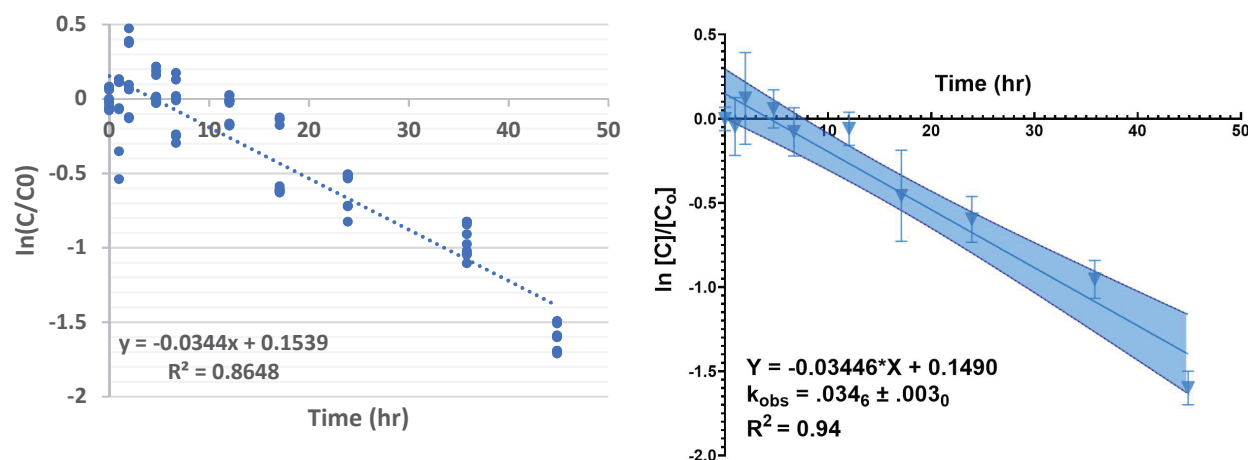


Figure 17. Degradation of chlorpyrifos under artificial solar irradiation in TOOLIK DOM. Left: Plot of all replicate values. Right: 95% confidence interval of the linear regression of triplicate averages.

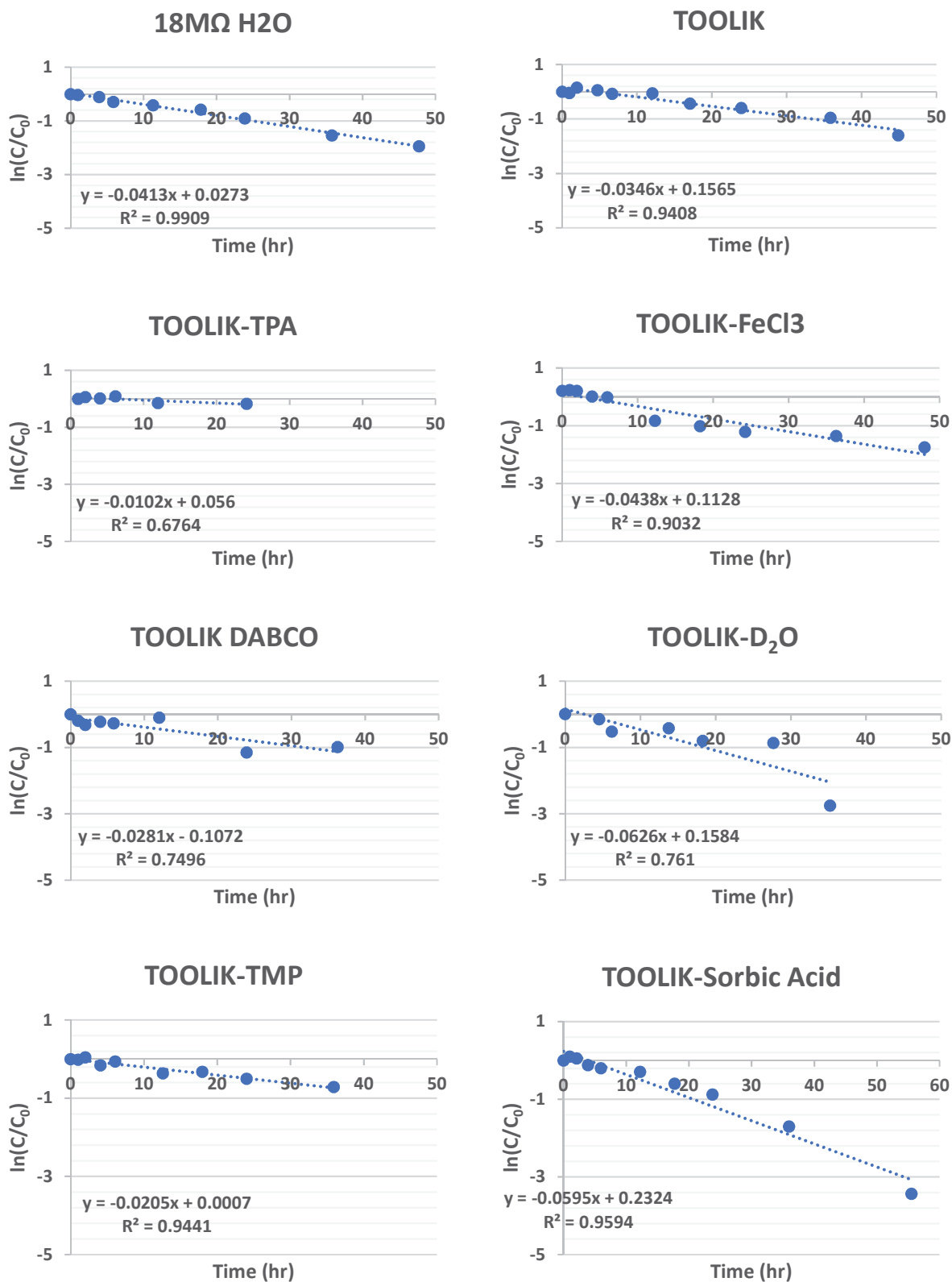


Figure 18. Graphical summary of experimental cpy photodegradation used for analysis. Each data point is an average of the measured experimental replicates, which were then fit to pseudo first order kinetics models.

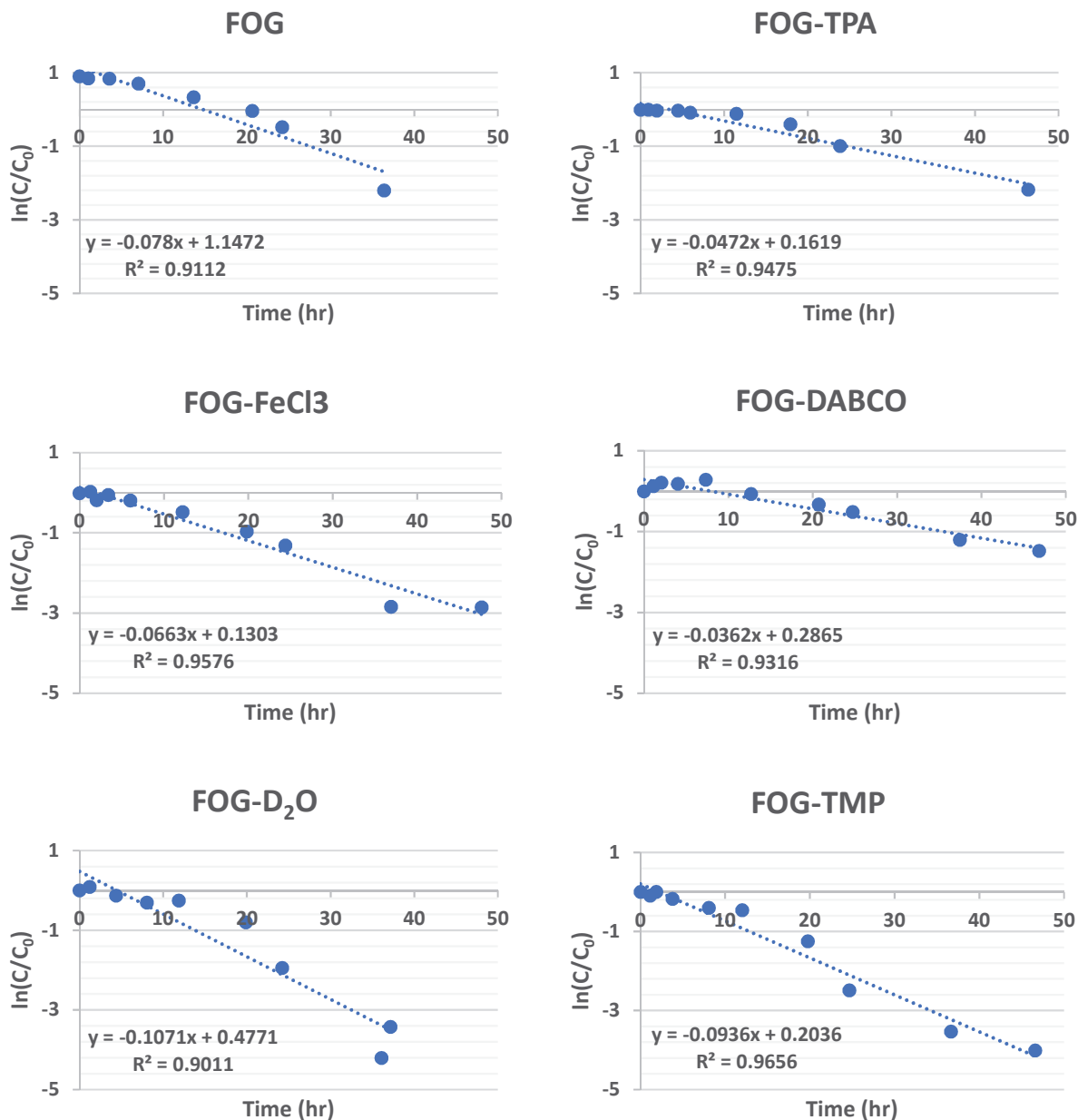


Figure 18, cont. Graphical summary of experimental cpy photodegradation used for analysis. Each data point is an average of the measured experimental replicates, which were then fit to pseudo first order kinetics models.

D. Correction of Experimental Rate Constants and Results

The obtained rates were normalized to the exact DOM concentration in the reaction mixture and since DOM also absorbs light, they were corrected for light screening factors across relevant wavelengths (ΣS_λ) (Chin and Miller, 2004). The absorbance data of each DOM stock was scaled by reaction mixture concentration, from which the attenuation coefficient (a_λ (cm⁻¹)) was determined

for the calculation of the wavelength specific screening factors from 240-800 nm (Eqn. 4). The pathlength (l) of the quartz reaction tubes was 1 cm (Chin et al., 2004; Quesada, 2021).

$$S_{\lambda} = \frac{1 - 10^{-la_{\lambda}}}{2.303la_{\lambda}} \quad (4)$$

From the polynomial fit of the individual screening factors, the aggregate screening factor (ΣS_{λ}) was integrated over 290-350 nm. The screening range was determined from the absorbance range of chlorpyrifos bound by lowest wavelength of light permissible through the daylight filter over the Suntest lamp. The final rate constants were calculated via Eqn. 5, correcting for both screening factor and concentration of DOM:

$$k_{corr} = \frac{20 k_{obs}}{(\%DOM)\Sigma S_{\lambda}} \quad (5)$$

The 95% confidence intervals of the experimental rate constants, half-lives, Pearson coefficients for the aqueous, DOM, and various probe experiments are listed below (**Table 3**). The experimental rate constant is inversely proportional to the half-life via the equation:

$$t_{\frac{1}{2}} = \frac{\ln(2)}{k} \quad (6)$$

TABLE 3. Summary of experimental chlorpyrifos photodegradation rate constants, half-lives, and coefficient of determination for the least squares fits to a pseudo-first order kinetics model. Error in k_{obs} is the 95% confidence interval from the regression fit.

Solution	Amendment	k_{corr} (hr⁻¹)	Half Life (hr)	R²
<i>18 MΩ H₂O</i>	<i>N/A</i>	<i>0.041₂ ± 0.001₅</i>	<i>16.8</i>	<i>0.99</i>
<i>Toolik</i>	<i>N/A</i>	<i>0.037₄ ± 0.003₀</i>	<i>18.5</i>	<i>0.94</i>
<i>Freshet DOM</i>	<i>TPA (•OH Q)</i>	<i>0.013₂ ± 0.003₅</i>	<i>52.2</i>	<i>0.68</i>
<i>Isolate (2021)</i>	<i>FeCl₃ (•OH S)</i>	<i>0.046₇ ± 0.005₁</i>	<i>14.8</i>	<i>0.90</i>
	<i>DABCO (¹O₂ Q)</i>	<i>0.037₂ ± 0.006₆</i>	<i>18.6</i>	<i>0.75</i>
	<i>D₂O (¹O₂ S)</i>	<i>0.063 ± 0.01₅</i>	<i>11.0</i>	<i>0.77</i>
	<i>TMP (³DOM* Q)</i>	<i>0.021₀ ± 0.001₉</i>	<i>33.0</i>	<i>0.93</i>
	<i>Sorbic Acid (³DOM* Q)</i>	<i>0.062₀ ± 0.004₃</i>	<i>11.2</i>	<i>0.96</i>
<i>Fog I</i>	<i>N/A</i>	<i>0.086₃ ± 0.009₉</i>	<i>8.0</i>	<i>0.91</i>
<i>DOM Isolate (2019)</i>	<i>TPA (•OH Q)</i>	<i>0.052₅ ± 0.004₂</i>	<i>13.2</i>	<i>0.95</i>
	<i>FeCl₃ (•OH S)</i>	<i>0.076₁ ± 0.004₃</i>	<i>9.1</i>	<i>0.96</i>
	<i>DABCO (¹O₂ Q)</i>	<i>0.042₁ ± 0.003₇</i>	<i>16.5</i>	<i>0.94</i>
	<i>D₂O (¹O₂ S)</i>	<i>0.10₅ ± 0.01₃</i>	<i>6.6</i>	<i>0.90</i>
	<i>TMP (³DOM* Q)</i>	<i>0.085₄ ± 0.003₈</i>	<i>8.1</i>	<i>0.98</i>

The comparisons between observed photodegradation rate constants can be better visualized graphically with error bars signifying the 95% confidence interval (CI) around the mean (**Fig. 19**).

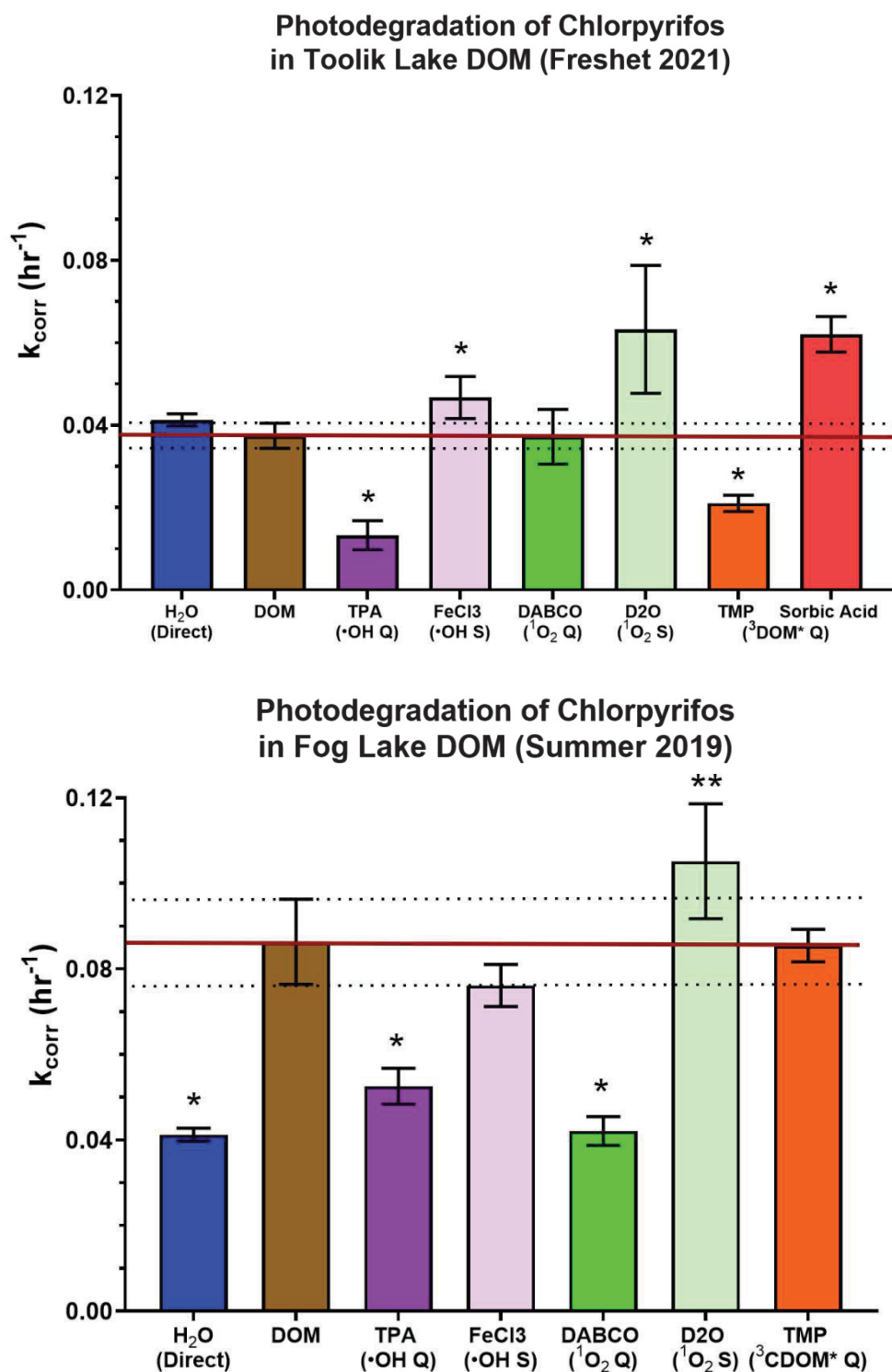


Figure 19. Corrected first order rate constants (k_{corr}), normalized for dilution and light screening. Error bars represent 95% confidence intervals. Q represents quencher. S represents sensitizer.

*Statistically significant different rate from the DOM rate. **Experiment with only 80% D₂O.

There was no statistically significant difference between the Toolik DOM and the direct (18 MΩ H₂O) rate. The rate constants for both the quenching (TPA) and sensitizing (FeCl₃) experiments of •OH were significantly different from the Toolik DOM by -0.024 hr⁻¹ and 0.009 hr⁻¹, respectively. There was no significant difference between the ¹O₂ quencher DABCO, but the ¹O₂ sensitizer, D₂O, increased the rate constant of chlorpyrifos degradation in Toolik DOM by 0.023 hr⁻¹. Finally, the ³DOM* electron transfer quencher, TMP, resulted in a decrease in the rate constant by 0.016 hr⁻¹, but the ³DOM* energy transfer quencher ended up sensitizing the photodegradation by 0.025 hr⁻¹.

The indirect photolysis from Fog DOM was significantly higher than the direct photolysis (18 MΩ) rate, showing enhancement by 0.045 hr⁻¹. The •OH quencher, TPA, reduced the Fog rate constant by 0.034 hr⁻¹, but the sensitizer, FeCl₃, did not significantly impact the rate. It is possible Fe is associating with the DOM, but this was not explicitly tested. ¹O₂ seemed to have a greater impact on the indirect photolysis as the ¹O₂ quencher DABCO decreased Fog's rate constant by 0.044 hr⁻¹. A statistically significant change in rate was not observed from the reaction mixture composed of 80% D₂O, however, water is an efficient scavenger of ¹O₂, and even 20% water may have been enough to obscure the results. Lastly, there was no observed change in rate by the ³DOM* quencher; the rate constants were effectively the same. Fog DOM was not photolyzed with sorbic acid due to the non-quenching behavior of the probe observed with Toolik DOM.

E. *Quantitation of Mechanistic Processes*

The overall photodegradation rate for chlorpyrifos is the sum of all direct and indirect photolysis pathways where the concentrations of the transient species are assumed to be in a steady state (Eqn. 7). Therefore, the second order photodegradation rate can be simplified to the pseudo-first order kinetic model where $k'_{transient}$ is the product of its second order rate constant ($k_{transient}$) and the steady state concentration of that transient (Eqns. 8-9) (Zeng and Arnold, 2013).

$$-\frac{d[CPY]}{dt} = \sum k[transients][CPY] \quad (7)$$

$$-\frac{d[CPY]}{dt} = [CPY](k_{direct} + k_{\bullet OH}[\bullet OH] + k_{3DOM*}[\text{}^3DOM*] + k_{1O_2}[\text{}^1O_2] + k_{other}[other]) \quad (8)$$

$$-\frac{d[CPY]}{dt} = \sum k'_{DOM} [CPY] = [CPY](k_{direct} + k'_{\bullet OH} + k'_{3DOM*} + k'_{1O_2} + k'_{other}) \quad (9)$$

Ideally, differences between the overall observed indirect photodegradation rate ($k'_{DOM} - k_{direct}$) and the experimental rate with a quencher ($k'_{corr,expt}$) should correlate to the percentage that each reactive transient contributes to the overall enhancement of degradation. This model assumes that the probe outcompetes chlorpyrifos completely for the reactive transient of interest. Additionally, the probe is assumed to be completely selective toward the specified reactive transient and does not react with anything else.

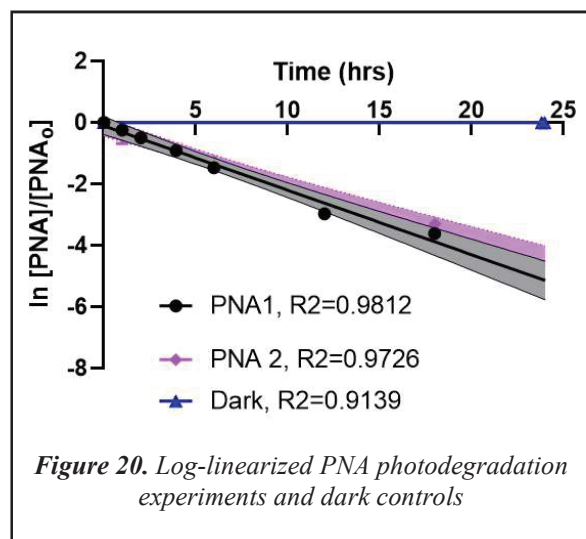
In the case of the Toolik 2021 isolate, no enhancement was observed, so this mathematical analysis cannot apply. For the Fog 2019 isolate, enhancement was observed, but extent of quenching from the probes was not additive as expected (58% higher than expected), implying that perhaps the underlying assumptions of the mathematical model could not be wholly met with these particular experiments (Eqn. 9; **Table 4**). General experimental difficulties were experienced with some of the probe molecules, which might have contributed to this finding and additionally prevented the full mechanism quantitation analysis. While the k' values could not be specifically quantified for each of the transients, the data still identified which specific transients were influential to the degradation of chlorpyrifos in the presence of that DOM isolate.

TABLE 4. Summary of the 95% CI of the experimental chlorpyrifos photodegradation rate constants ($k'_{corr,expt}$) for all reaction solutions. Experimental mechanistic contributions ($k'_{transient}$) were calculated for reactive transients whose respective quenchers significantly deviated from the overall photolysis rate in the DOM isolates.

Solution	Amendment (Mechanism)	$k'_{expt} \text{ (hr}^{-1}\text{)}$	$k'_{transient} \text{ (hr}^{-1}\text{)}$
Pure 18M Ω H ₂ O	N/A (Direct)	$0.041_2 \pm 0.001_5$	
Toolik Freshet	N/A (Overall)	$0.037_4 \pm 0.003_0$	
DOM Isolate (2021)	TPA ($\bullet\text{OH}$ Q)	$0.013_2 \pm 0.003_5$	
	DABCO ($^1\text{O}_2$ Q)	$0.037_2 \pm 0.006_6$	
	TMP ($^3\text{DOM}^* e^-$ transfer Q)	$0.021_0 \pm 0.001_9$	
Fog 1	N/A (Overall)	$0.086_3 \pm 0.009_9$	
DOM Isolate (2019)	TPA ($\bullet\text{OH}$ Q)	$0.052_5 \pm 0.004_2$	$0.03_3 \pm 0.01_4$
	DABCO ($^1\text{O}_2$ Q)	$0.042_1 \pm 0.003_7$	$0.04_4 \pm 0.01_3$
	TMP ($^3\text{DOM}^* \text{Q}$)	$0.085_4 \pm 0.003_8$	-----

F. PNA Photolysis and Determination of Quantum Yield

The general photolysis experimental method was tested with the actinometer *p*-nitroanisole (PNA), whose quantum yield (photoreaction efficiency) is known (0.000282; Dulin and Mill, 1982). The strong linearity of fit of the 95% confidence intervals and near equivalent slopes for both identical experiments demonstrated repeatability of the experimental method (**Fig. 20**). The quantum yield of PNA can be used to determine the apparent quantum yield (Φ_{app}) for chlorpyrifos degradation experiments. These values can be compared to those obtained from other artificial or natural photolysis experiments as it scales the



obtained rates by the number of photons capable of producing a reaction in the system, provided other factors are maintained constant like temperature and reaction mixture composition.

The apparent quantum yield of chlorpyrifos degradation, Φ_{app} , was determined by a ratio between the quantum yield of PNA degradation (Φ_{da}); their absorptive properties; and kinetics, where k_{dc} are the k_{da} is the degradation rate constants for chlorpyrifos and PNA respectively, $\epsilon_{\lambda c}$ and $\epsilon_{\lambda a}$ are the molar absorptivities at each wavelength for chlorpyrifos and PNA respectively, and S_{λ} is the screening factor for that DOM isolate (Eqn 10).

$$\Phi_{app} = \frac{k_{corr}}{k_{da}} \frac{\sum_{281\text{ nm}}^{400\text{ nm}} \epsilon_{\lambda a}}{\sum_{281\text{ nm}}^{400\text{ nm}} S_{\lambda} \epsilon_{\lambda c}} \Phi_{da} \quad (10)$$

Figure 21 below graphically shows that the apparent quantum yields calculated for the kinetics experiments compared against their respective rate constants. The screening factors for the experiments ranged from 0.906 to 0.965, where 1 is no light screening. Obtained Φ_{app} values ranged between 2.02E-5 and 1.55E-4 and the differences between various k_{corr} values and their corresponding rate constants were consistent across experiments, internally validating the method of comparison.

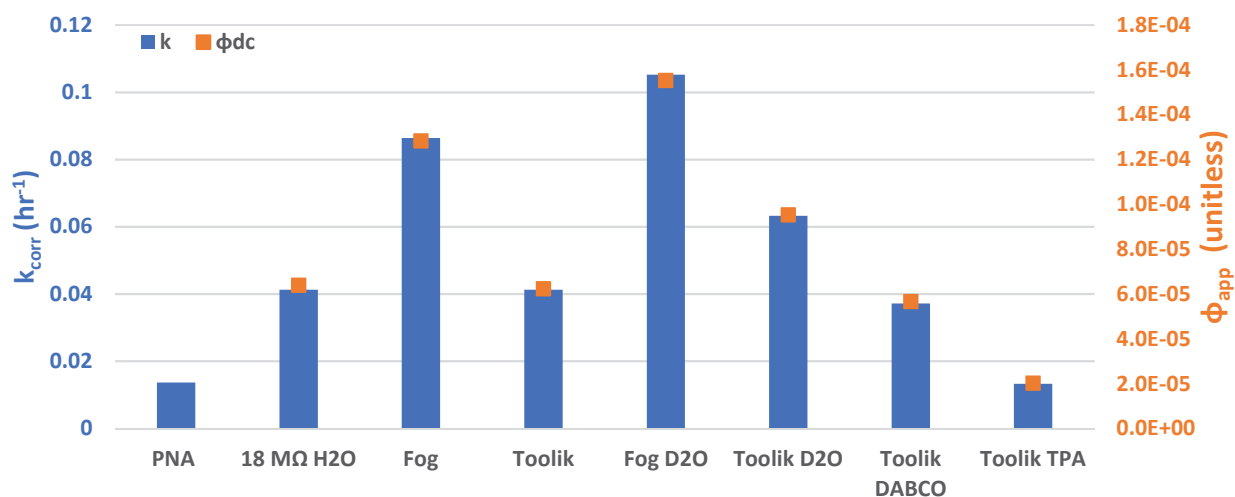


Figure 21. Method validated internally through comparisons between k_{corr} and Φ_{app}

G. Influence of DOM Composition and Seasonality on Photodegradation

The percent difference of enhancement due to DOM-mediated photolysis compared to direct photolysis (18 MΩ H₂O) was calculated for all of the DOM isolates collected to date (no sensitizers or quenchers), in this study and Guerard Lab prior work (**Fig. 22**). Similar photosensitization from the direct photolysis was observed by the same Fog DOM isolate that was tested in the previous study. In 2019, the presence of Fog DOM yielded a 91.2% rate increase from direct photolysis, and in this study, that increase was 100.5%. The relatively slight difference in

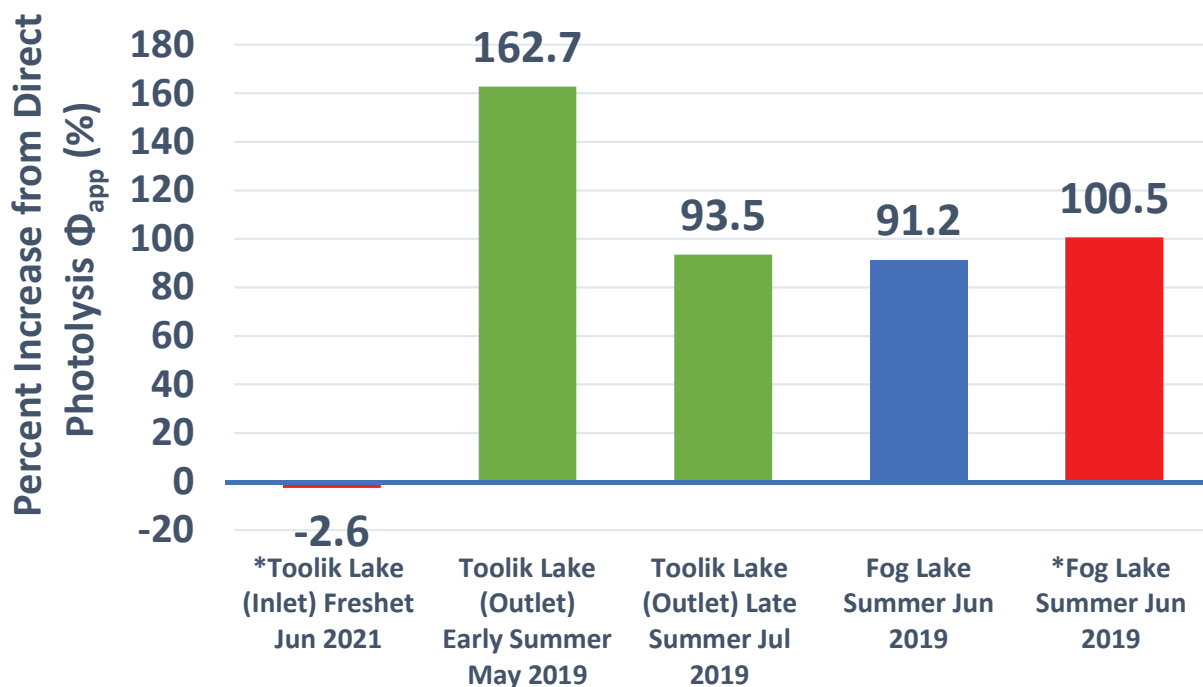


Figure 22. Seasonal comparison of the enhancement of the chlorpyrifos photodegradation rate in the presence of Arctic DOM. *Experiments in current study.

level of photosensitization from Fog DOM between the two studies may have been due to slight differences in experimental set up. For instance, experiments in this study were run without the cooling system in the solar simulator used in the previous study. As a result, experiments in this study were photolyzed around 10 °C higher than the previous, which might have resulted in higher indirect photolysis rates if the increased temperature favored the production of key reactive transient species. The lack of hydrolysis in the dark controls across both current and previous work suggests these differences in experimental conditions are small and allow for comparison.

The enhancement ability of DOM to degrade chlorpyrifos seems to be influenced by the point in the season of collection. Similar percent differences were obtained for the same Fog DOM isolate vs. 18 MΩ water as measured previously in the Guerard group. The rate of chlorpyrifos photolysis in Toolik DOM collected at different points across the summer largely varied. Toolik DOM at freshet did not contribute to an overall increase in the rate of photodegradation, unlike Toolik DOM at later points in the summer season. The rate of DOM-mediated photodegradation drastically increased from freshet to the end of melt, where it reached its peak. In contrast to the changes in Toolik lake between seasons, the percent increase due to indirect photolysis by DOM was similar between the two different lakes in late summer. In the same 2019 study, the difference between the percent differences of Toolik in early July (7/2/19) and Fog in late June (7/2/19) was only 2.3%. As summer progresses, DOM-mediated photosensitization contributed less to the overall photodegradation.

The photodegradation differences observed between Arctic DOM isolates are likely related to differences in DOM composition. Fluorescence profiles and the fluorescence index (FI) are metrics used to classify DOM. In **Figure 23** below, the difference in composition between Toolik at freshet and Fog in summer is visually apparent.

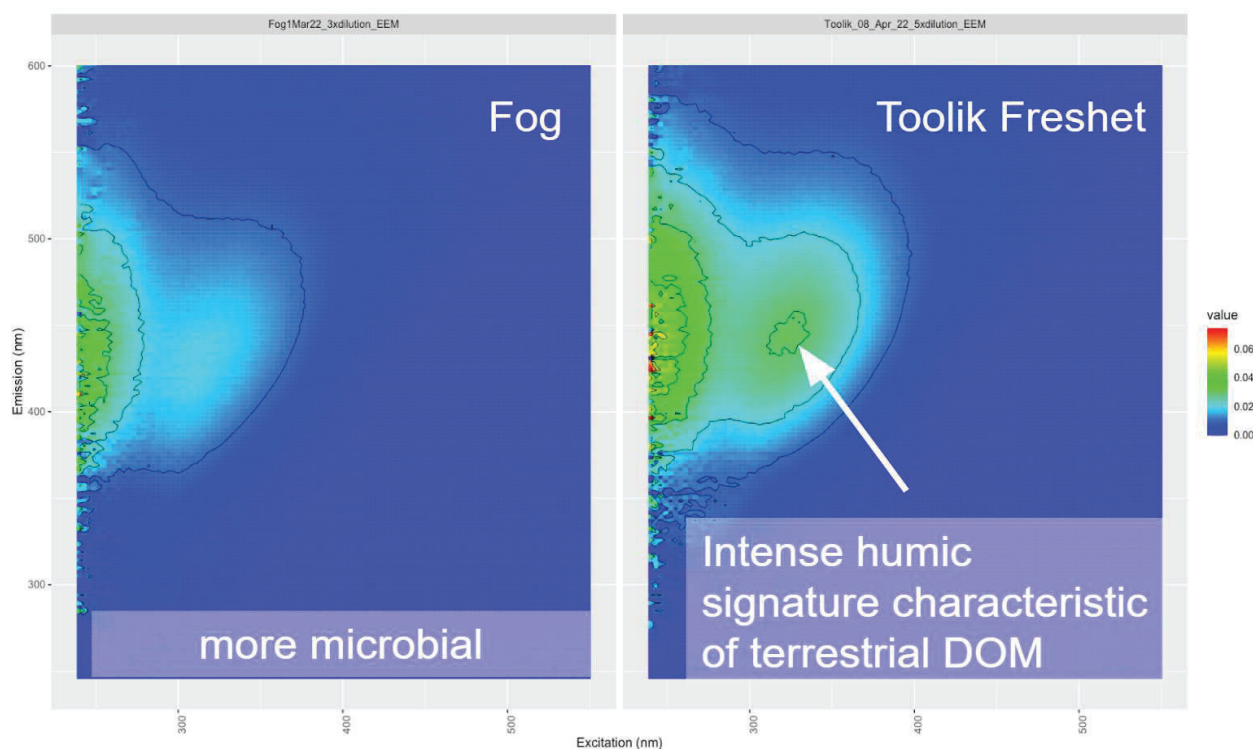


Figure 23. EEMs fluorescence spectra of Fog and Toolik Freshet DOM solutions. Scale of relative fluorescence on right (unitless).

Toolik DOM at freshet has much greater fluorescence intensity than Fog, particularly in the region where humic groups appear. Greater fluorescence in the humic region, as well as the very low determined FI, corresponds to more terrestrial DOM. **Figure 24** compares the quantum yield of chlorpyrifos photolysis in the selected DOMs to their experimentally determined FIs (Quesada, 2021). DOMs characterized as more microbial through their relatively higher FI enhance the photodegradation rate. The Pearson's correlation factor (R^2) was found to be 0.70 supporting the working theory that more microbially-derived DOM enhances the photodegradation rate of contaminants.

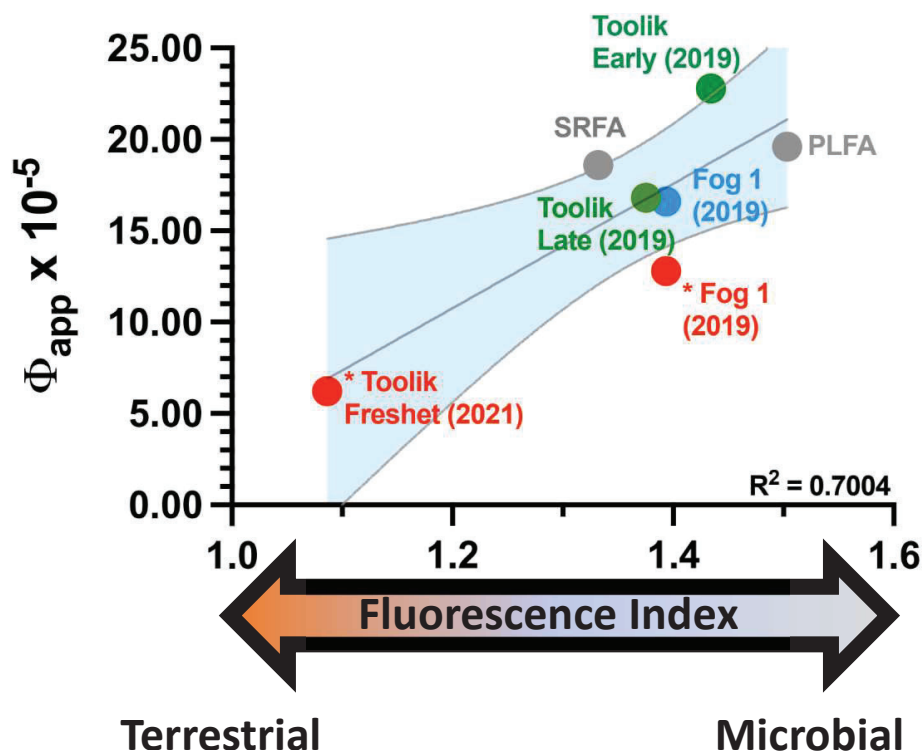


Figure 24. Comparison of the quantum yield of chlorpyrifos photodegradation in DOM with DOM FI.
*Experiments in current study

H. Initial Product Analysis

Notably, three consistent HPLC peaks ranging from 2-2.1 min, 2.2-2.3 min, and 2.9-3 min were identified as potential photolysis products and were subsequently categorized across all timepoints for all experiments. Visually observed deviations from the baseline at the aforementioned timepoints were given the labels of: very small bump (vsb), bump (b), small peak (sp), and large peak (lp) relative to general size of the observed product peaks. Any peak that was integrable was also followed by the letter “i”. In a few experiments, the large DOM peak that generally spanned around 1.0-1.8 min would tail into the 2-minute range, making the first couple peaks unresolvable (ur). The following profiles illuminate the appearance or disappearance of certain compounds identifiable at 290 nm across the experiments (**Figs. 25-28**). Note, the peak description for each compound was solely based off of its capacity to absorb light at 290 nm. The extent to which a compound is apparent changes based off of its optimum light absorbance value. Therefore, comparisons in peak size as its related to concentrations can only be made across analysis of each individual product. The objective of this data was to identify trends of appearance and disappearance of the products, not to quantify or compare their relative abundance to the other compounds detected.

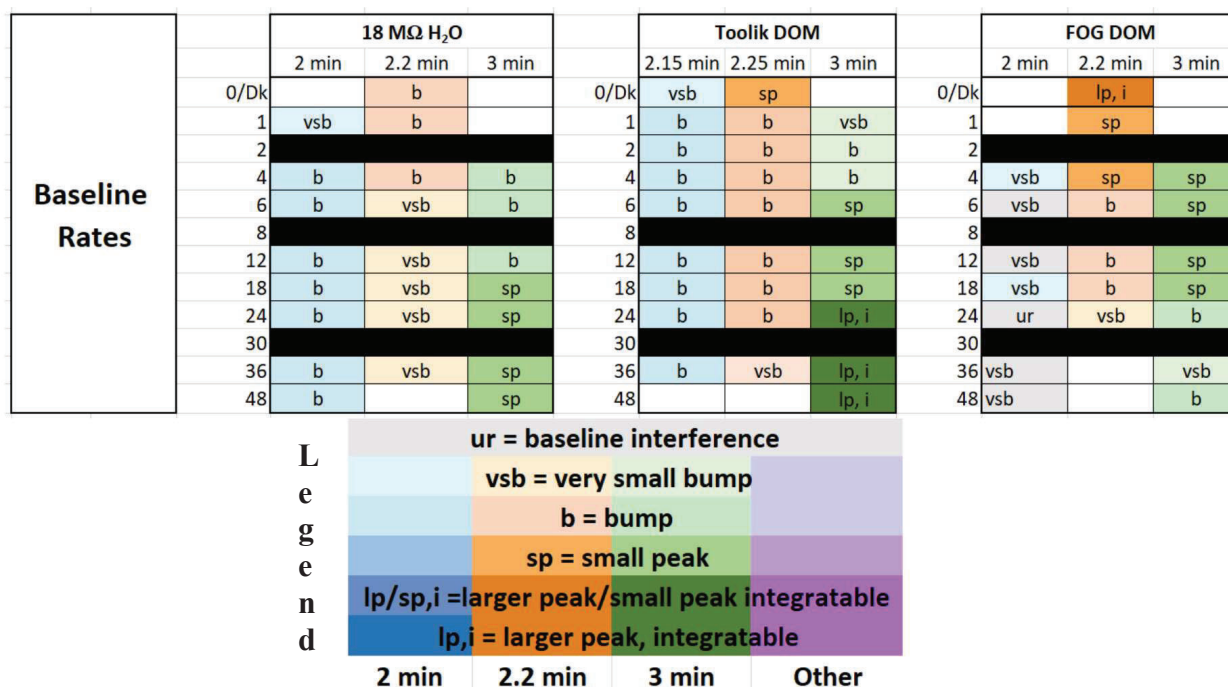


Figure 25. Characterization of relative apparent size of component peaks detectable at 290 nm to track appearance/disappearance of various compounds at retention times 2, 2.2, and 3 min across all timepoints in all experiments.

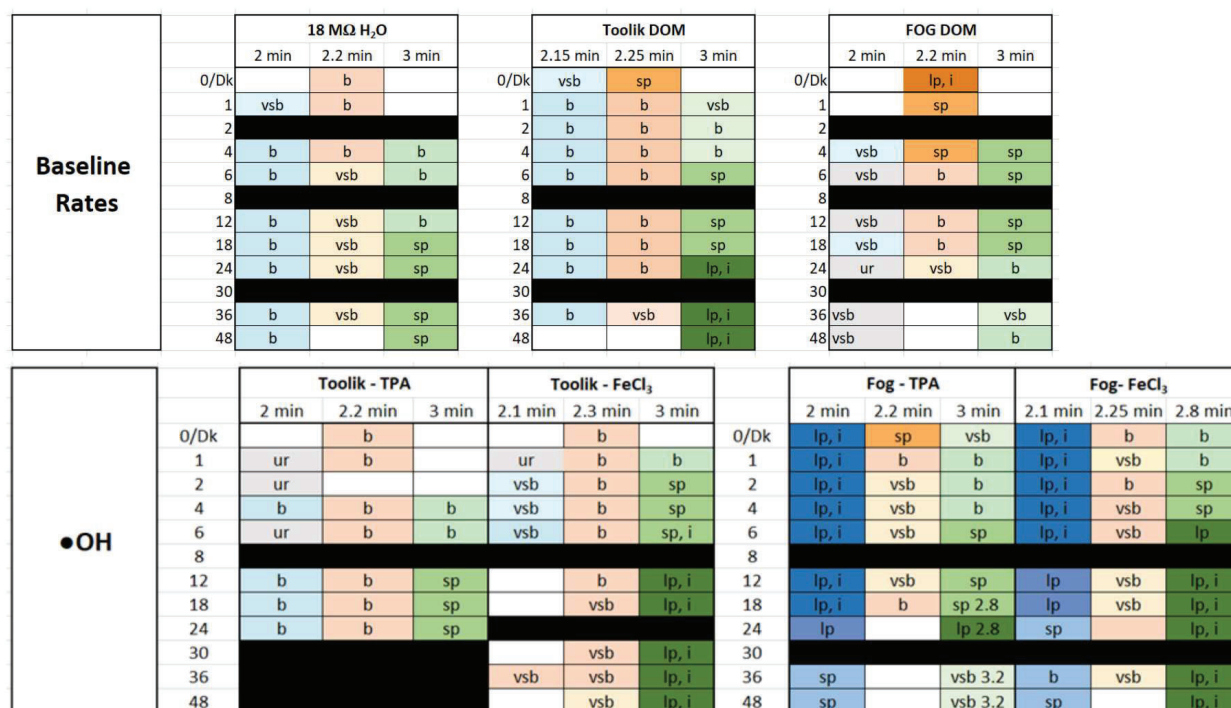


Figure 26. Comparison of •OH probing experiments to the direct and DOM-mediated photolysis chromatogram results.

Baseline Rates	18 MΩ H ₂ O			Toolik DOM			FOG DOM		
	2 min	2.2 min	3 min	2.15 min	2.25 min	3 min	2 min	2.2 min	3 min
	0/Dk		b	0/Dk	vsb	sp	0/Dk	lp, i	
	1	vsb	b	1	b	b	1	sp	
	2			2	b	b	2		
	4	b	b	4	b	b	4	vsb	sp
	6	b	vsb	6	b	b	6	vsb	sp
	8			8			8		
	12	b	vsb	12	b	b	12	vsb	sp
	18	b	vsb	18	b	b	18	vsb	sp
	24	b	vsb	24	b	b	24	ur	vsb
	30			30			30		
	36	b	vsb	36	b	vsb	36	vsb	vsb
	48	b	sp	48		lp, i	48	vsb	b

¹ O ₂	Toolik - DABCO			Toolik - D ₂ O			Fog - DABCO			Fog - D ₂ O		
	2.1 min	2.25 min	3 min	2.1 min	2.2 min	3 min	2.1	2.25 min	3 min	2.1 min	2.2 min	3 min
	0/Dk				sp		0/Dk	lp, i	b		lp, i	sp
	1	b	b		sp		1	lp, i	vsb	vsb	lp, i	vsb
	2	b	b	vsb			2	lp, i	vsb	vsb	lp, i	vsb
	4	b	b	vsb	vsb		4	lp, i	vsb	vsb	lp, i	vsb
	6	b	b	vsb	vsb		6	lp, i	vsb	vsb	lp, i	vsb
	8						8					
	12	vsb		b	vsb	vsb	12	lp, i	vsb	vsb	lp, i	vsb
	18			b	vsb	vsb	18	lp, i		vsb		vsb
	24	vsb		b	sp		24	lp, i		vsb		b
	30				vsb		30			lp, i		b
	36	vsb		b		vsb	36	lp, i		vsb		b
	48		b			b	48	lp, i		vsb	sp	vsb

Figure 27. Comparison of the ¹O₂ probing experiments to the direct and DOM-mediated photolysis chromatogram results.

Baseline Rates	18 MΩ H ₂ O			Toolik DOM			FOG DOM		
	2 min	2.2 min	3 min	2.15 min	2.25 min	3 min	2 min	2.2 min	3 min
	0/Dk		b	0/Dk	vsb	sp	0/Dk	lp, i	
	1	vsb	b	1	b	b	1	sp	
	2			2	b	b	2		
	4	b	b	4	b	b	4	vsb	sp
	6	b	vsb	6	b	b	6	vsb	sp
	8			8			8		
	12	b	vsb	12	b	b	12	vsb	sp
	18	b	vsb	18	b	b	18	vsb	sp
	24	b	vsb	24	b	b	24	ur	vsb
	30			30			30		
	36	b	vsb	36	b	vsb	36	vsb	vsb
	48	b	sp	48		lp, i	48	vsb	b

³ DOM*	Toolik - TMP				Fog - TMP			
	2.1 min	2.2 min	2.6 min	3 min	2.1 min	2.2 min	2.6 min	3 min
	0/Dk	lp, i	sp	lp, i	0/Dk	sp	lp, i	b
	1	lp, i	sp	lp, i	1	sp, i	sp, i	b
	2	sp	sp	sp	2	lp, i	lp, i	sp
	4	b	sp	b	4	sp	sp	sp
	6	b	b	b	6	sp	sp	sp
	8				8			
	12	b	b	b	12	sp	b	lp, i
	18		b	sp	18	sp	b	lp, i
	24		b	sp	24	sp	b	lp, i
	30				30			
	36		b	sp	36	sp	b	lp, i
	48			sp	48	sp		lp, i

Figure 28. Comparison of the ³DOM* probing experiments to the direct and DOM-mediated photolysis chromatogram results. 2.6 min compound is likely TMP. 3 min product is hypothesized to be the oxon derivative of chlorpyrifos.

Additionally, upon zooming into the baseline of the chromatograms, other product bumps could be observed at later retention times than those aforementioned, notably within the 4-5 min range. While resolvable data was not apparent for this and other time ranges, the entire absorbance spectra was collected for all experiments and could be useful in the investigation of other potential degradation products that absorb light at a different wavelength. These results assisted in the determination that the greatest changes in reaction mixture composition occur between 6-18 hours, motivating the product investigation into an intermediary timepoint.

VII. Discussion

A. Competitive Kinetics Experiments

The primary goal of the competitive kinetics experiments in this study was to assess the impact of documented key transient species on the previously observed enhancement of chlorpyrifos's photodegradation by Alaskan Arctic DOM. By comparing the observed rate constants of chlorpyrifos in DOM and against the experiments with various probe molecules, reactive transients of interest should be able to be attributed the extent of their contribution to indirect photodegradation. Because quenchers scavenge the reactivity of selected transients, if that transient is involved in degrading chlorpyrifos, then the rate constants of those experiments are expected to be slower than the overall photodegradation rate constant in DOM. Likewise, as sensitizers increase the abundance of selected transients, experiments with sensitizers for transients chlorpyrifos is sensitive to will have a faster degradation rate and therefore higher rate constant. The larger the difference in rate, the more the overall reaction is proportionally blocked or enhanced. Conversely, if there is not a large change in rate of the probe experiment from the baseline DOM rate, the reactive species most likely does not play a major part in the enhancement.

The Role of DOM: The experimental results with the Fog DOM do corroborate the 2019 Toolik DOM study that showed similar enhancement of the chlorpyrifos photodegradation rate, lending validity to the methods and results of this study. Holistically, the enhanced photolysis rates of chlorpyrifos in Fog DOM and its probes were all higher or equivalent to than the direct photodegradation rate, supporting the hypothesis that the indirect photolysis is a result of key transient species produced by the DOM. In contrast, Toolik freshet DOM did not enhance the rate of chlorpyrifos photodegradation at all, even when effects of light screening were considered.

The Role of Sensitizers: Not all probes investigated exhibited their expected behavior. For example, the application of the ³DOM* quencher sorbic acid with Toolik DOM resulted *instead* in a large sensitization effect on chlorpyrifos photolysis. And results were not able to be obtained for photolysis in Fog-SA DOM, due to a large interference in the HPLC method. This quencher was determined to be too problematic to be pursued within the experimental timeline, so the extent of

$^3\text{DOM}^*$ via energy transfer on DOM-mediated photodegradation was unable to be determined. One possibility for this is that the concentration of sorbic acid was very high compared to the DOM matrix, enough to alter the partitioning of chlorpyrifos in the solutions. Another possibility is that because this quencher works by also exciting to a triplet state, it is possible it reacted with the chlorpyrifos directly, replacing the role of $^3\text{DOM}^*$ in those experiments.

Moreover, the experiments with the other two sensitizers did not yield high confidence results. For FeCl_3 , the obtained difference in quantum yield between Fog and FeCl_3 was minimal, while in the preliminary exploration of transient species in the previous work in the Guerard Group, a statistically significant, higher value was obtained. The extent of enhancement from FeCl_3 in the Toolik freshet DOM was more consistent with that of the previous experimental findings using other Toolik DOM isolates. Ideally, the Fog- FeCl_3 experiment should be rerun. Lastly, although the application of D_2O did result in the observed sensitization of both photodegradation rates, the pH of these solutions compared to the regular experiments was not the same, because of the need to prevent addition of any excess water that might negate the sensitizing effect of D_2O . The relationship of probe behavior in acidic solutions (due to the DOM) compared to neutral solutions has not been defined. As a result, results from the acidic D_2O experiments may be used in a qualitative capacity, but quantitative comparisons cannot be drawn. In general, higher confidence was obtained with quenching probes, from which the majority of determinations were based.

The Role of Quenchers: Although Toolik freshet DOM did not enhance the rate of chlorpyrifos photodegradation, two of the three transients of interest were active in the degradation process. The $\bullet\text{OH}$ quencher (TPA) resulted in the slowest rate constant from the DOM, well below that of direct photolysis. $\bullet\text{OH}$ is a strong oxidizing agent produced by DOM, but can also be quenched by that same DOM. It is possible that some other interaction was occurring with *that* DOM that then shut down all chlorpyrifos degradation. There was also a significant difference between the TMP photolysis and the DOM, indicating that the electron transfer reactions undergone by excited triplet DOM may also be involved in the photodegradation of chlorpyrifos. This finding contradicted the hypothesis that Arctic DOM likely has less electron donating capacity, and planned deeper characterizations of the DOM by ^{13}C -NMR and high-resolution mass spectrometry will help to resolve this. The obtained k_{corr} values and low experimental error of the Toolik-TMP experiments *do* suggest that $^3\text{DOM}^*$ electron transfer reactions are important for chlorpyrifos. Despite an enhancement in the rate upon photolysis in the sensitizer, the rate between the $^1\text{O}_2$ quencher was nearly equivalent the baseline Toolik DOM rate, which led to the conclusion that $^1\text{O}_2$ had little to no effect on the indirect photolysis and could not be identified as a key contributing species. Just because chlorpyrifos is susceptible to $^1\text{O}_2$ when that transient's concentration is elevated, does not mean that transient outcompetes other mechanisms in that specific DOM. The findings that key reactive transients of the DOM-mediated photodegradation of Toolik Lake at freshet include $\bullet\text{OH}$ and $^3\text{DOM}^*$ electron transfer while there was little to no effect of $^1\text{O}_2$ on the observed photodegradation differed from another mechanistic-focused study of chlorpyrifos whereby $^1\text{O}_2$ accounted for 70% of the indirect photolysis in central North

American prairie potholes (Zeng and Arnold, 2013). However, the DOM in that system is unlikely to have the same carbon pool composition as the DOM in this study.

In Fog DOM, $^1\text{O}_2$ was found to be the primary key species contributing to the DOM-mediated indirect photolysis. The application of the quencher DABCO resulted in a decrease of photodegradation rate to the direct photolysis level. Although a quantitative difference in rate was unable to be obtained for the $^1\text{O}_2$ sensitizer as discussed above, even with 20% water in solution the data support that chlorpyrifos is susceptible to singlet oxygen since an increase in rate was observed. The rate of photodegradation with the $\bullet\text{OH}$ quencher was significantly slower than the baseline DOM-mediated rate, indicating $\bullet\text{OH}$ as a key contributing reactive species, but less so than $^1\text{O}_2$. The addition of the $^3\text{DOM}^*$ quencher resulted in nearly no change in chlorpyrifos degradation rate, and as a result, Fog DOM may have substantially less electron donating capacity compared to Toolik freshet DOM.

Considering $\bullet\text{OH}$ activity also impacted the level of photodegradation, the level to which indirect photolysis is able to be attributed to $^1\text{O}_2$ in the presence of DOM was unable to be quantified. The combined decreases of the experimental rate constants from the probe experiments add up to more than can be accounted for by indirect photolysis alone (Eqn. 9). It is possible that the probe molecules might not be as selective in our DOM isolates, or that there are interactions with our contaminant and/or those isolates and the probes used in this study. In order to accurately quantify the extent of indirect-photolysis mediation, further experimental work in this area is needed to gain confidence in the magnitude of the observed experimental rate constants and explore the selectivity of other probes for these transients in Arctic DOM.

B. Impact of Seasonality on Photodegradation

Overall, the extent of photodegradation of chlorpyrifos in various Alaskan Arctic DOM isolates might be influenced by seasonality, location, and hydrology. When comparing the extent of photodegradation across all Toolik and Fog DOM isolates studied in the last three years, variation of the photolysis rates appeared to be most dependent on season of collection. At freshet, any sensitization of chlorpyrifos photodegradation by DOM was offset by other unknown processes. There might have been other compounds produced by the DOM that scavenged the probed reactive transients. Because freshet represents the first flush of runoff during thaw off the landscape, this material may represent more recalcitrant and thus less reactive moieties from previous seasons than DOM captured at other times during the year. After most melt has occurred by late spring, the DOM-mediated photodegradation of chlorpyrifos is at its maximum (**Fig. 22**). At this time, primary production has started to ramp up as well as potentially more freshly made DOM inputs coming into the water column, resulting in a higher propensity for the DOM to produce excited species that indirectly photodegrade chlorpyrifos. As the DOM becomes increasingly photobleached as the summer progresses, subjected to up to 24 hours of continual sunlight exposure, reactive potential is lost and the relative enhancement of photodegradation decreases. The extent of degradation in the late summer Toolik and Fog isolates was very similar

despite coming from two lakes of different chemical and hydrological features (Quesada, 2021). While more characterization of the carbon pool is planned to confirm if this is truly the case, preliminary comparisons using fluorescence index help to show this as well (**Fig 24**).

C. Initial Product Analysis

Understanding the fate of contaminants in various environments is critical in understanding their lasting impacts on the system at large. The chemical breakdown of contaminants by environmental processes can have a mediating effect, yielding products that are less harmful to the environment, or the opposite. Certain degradation processes on chlorpyrifos have been well studied and many degradation products have been identified. Photolysis is a known oxidative process. Not surprisingly, the oxon derivative of chlorpyrifos has been well identified as a direct photolysis degradation product via oxidative desulfuration (Raina et al., 2010). Both chlorpyrifos and its oxon have been found to transform into 3,5,6-trichloropyridinol (TCP) and further to 3,5,6-trichloro-2-methoxypyridinol (TCMP) via a number of processes (Muir and De Wit, 2010; Duirk et al., 2018). Other degradation products of chlorpyrifos include desethyl chlorpyrifos, which also is known to degrade to TCP. Many of these compounds are relatively short lived compared to long-term persistent organic pollutants and further break down to carbon dioxide, nitrogen, and chlorine ions (Muir and de Wit, 2010).

2-2.1-Minute Product: In the three baseline HPLC experiments, a relatively small amount of the compound detected at 2 min was apparent throughout photolysis, but its peak area did not substantially increase over time. Because it was detected in both non-photodegraded samples (T0 and dark) and photolysis samples at relatively constant amounts, this compound could likely be a hydrolysis product. In Toolik DOM, the compound was unaffected by probe experiments except when exposed to TMP. Upon application of TMP, the appearance of the unknown compound was initially greatly sensitized by the DOM-TMP solution and upon photolysis, quickly disappeared within the first 12 hours. Considering that TMP is a $^3\text{DOM}^*$ quencher, the disappearance of the unknown compound in an environment deprived of triplet excited states that facilitate e^- transfer might mean that the production of this compound by degradation could additionally occur via interactions with $^3\text{DOM}^*$. This was supported by the Fog-TMP experiment, except the quantity did not change over time with photolysis. A decrease in product formation upon photolysis in Toolik but not Fog is consistent with the mechanistic findings as photodegradation in Toolik was influenced by $^3\text{DOM}^*$ but not in Fog. Additionally, upon the addition of ROS probes in Fog DOM, the relative quantity of product detected was greatly sensitized, even more so than Fog-TMP. However upon photolysis, the compound did not drastically disappear as rapidly as Toolik-TMP. In both Fog-TPA and Fog- FeCl_3 , the product slowly decreased over photolysis to the baseline level of Fog-TMP. In Fog-DABCO and Fog- D_2O , the product remained consistent at the highest relative level of quantitation throughout photolysis. From this data, it is likely DOM sensitizes the production of this compound by a non-photodegradation mechanism, particularly DOM with an abundance of ROS. Upon photolysis, competing pathways may result in a slight disappearance of

this compound via a mechanism involving $\bullet\text{OH}$. This compound seems to be particularly sensitive to multiple reactive processes in this study, manifesting the importance of its identification.

2.2-2.3 -Minute Product: In general, a degradation compound is detectable at a RT around 2.2-2.3 minutes in samples that did not undergo photolysis, but declines to disappearance by 48 hours in all three baseline experiments. The relative quantity of the compound is greater in the two DOMs than in ultrapure water in non-photodegraded samples. The largest initial quantity and subsequent greatest rate of disappearance over time when photodegraded occurred in Fog DOM. The compound's photodegradation behavior was most similar to baseline rates in Toolik-TPA, FeCl_3 , and TMP and Fog-TMP. The compound's behavior across the other experiments was different, but unable to be explained in the context of this study except for singlet oxygen probes in Fog DOM. In these experiments, the compound's relative degradation is subsequently slowed in the presence of the quencher DABCO and significantly sensitized in the presence of the sensitizer D_2O . If the photodegradation of chlorpyrifos in the Fog DOM is enhanced by some mechanism involving $^1\text{O}_2$ as the mechanistic results show, perhaps the unknown compound is consumed in the indirect photolysis as this observation would support. The compound's behavior across the other experiments was different, but unable to be explained in the context of this study except for singlet oxygen probes in Fog DOM. Identification by mass spectrometry will help to elucidate this product and its role in the above experiments.

2.8-3 -Minute Product: In contrast to the previous compound, a product with a RT around 2.8-3 minutes became increasingly apparent as the photolysis of chlorpyrifos progressed in all baseline experiments. The product is likely primarily a photolysis product because it only becomes apparent upon photolysis. The relative existence of this compound greatly increased over time in Toolik DOM. In Toolik DOM, the relative rate of the product's growth upon photolysis was slowed in the presence of the $\bullet\text{OH}$ quencher and comparatively increased in the presence of the $\bullet\text{OH}$ sensitizer. The same was observed for the photodegradation in Fog DOM with the probes for both $\bullet\text{OH}$ and $^1\text{O}_2$. In conjunction with the mechanistic findings that the photodegradation in Toolik DOM was impacted by $\bullet\text{OH}$ and in Fog DOM by both ROS, the conclusion may be drawn that the production of a direct photolysis product was sensitized by an indirect photolysis mechanism identified in this study to impact the DOM-mediated photodegradation of chlorpyrifos. Based on these observations and previous literature outlining the oxidative desulfuration of certain organophosphates by $\bullet\text{OH}$, this product might be the oxon-derivative of chlorpyrifos (Yuan et al., 2009). Mass-spectrometry analysis is required to confirm this hypothesis; however, if confirmed to be the oxon, this product would be 100x more toxic than its parent pesticide. As a result, its pronounced production by DOM-mediated photodegradation might be of concern.

VIII. Current and Future Experimental Work

The quenching of product formation could be indicative of the product being formed through the mechanism being quenched, and this hypothesis will be further explored through product analysis. Bulk photolysis of the direct and sensitized DOM experiments were run in April 2022. These samples are being concentrated and will be run in collaborator Dr. Chin's Lab at the University of Delaware for LC-mass spectrometry-based product identification. In addition, NMR and mass spectrometry of the DOM isolates is also planned to better characterize the carbon pools. Combined with all of the investigations performed as reported in this study, it is intended that the experimental findings will be publishable after the conclusion of work. The quenching or sensitizing of product formation will continue to be explored in future experiments.

IX. Conclusions

- From the mechanistic experiments, it was observed that the reactive transients key in the DOM-mediated photodegradation are not the same between Alaskan Arctic DOM isolates. The key reactive transient species formed were dependent on DOM composition.
- The photodegradation of chlorpyrifos in Toolik freshet DOM was dependent upon $\bullet\text{OH}$ and $^3\text{DOM}^*$. $\bullet\text{OH}$ affected the rate of degradation the most out of the reactive transients studied.
- The photodegradation of chlorpyrifos in Fog summer DOM was dependent upon $\bullet\text{OH}$ and $^1\text{O}_2$. $^1\text{O}_2$ affected the rate of degradation the most out of the reactive transients studied.
- From the comparison of the enhancement of chlorpyrifos photodegradation in all DOM isolates collected by the group to date, the extent of enhancement appeared to be more dependent on DOM season than location of DOM.
- The extent of chlorpyrifos photodegradation in DOM was found to correlate to its measured fluorescence index (FI), which is used to characterize DOM as terrestrial (lower FI) or microbial (higher FI), by a Pearson's correlation factor of 0.70.
- The FI of DOM isolates seemed to be more related by season than location. Freshet DOM (no enhancement) had the lowest FI, correlating to more terrestrial character. Late spring/early summer DOM (most enhancement) had the highest FI value, correlating to a more microbial character. Late summer DOM from both Toolik and Fog lakes (similar enhancement) had very similar FI values.

The goal of this research was to assess the mechanisms behind the observed enhancement of the photodegradation of chlorpyrifos in Arctic DOM. This goal heavily relied on the assumptions that the chemical composition of DOM was more dependent on geographical and hydrologically derived differences. From preliminary experiments conducted in previous study by the group, it was noted that the amount of enhancement by different DOM isolates would likely vary between time of year. However, due to the limited scope of that study related to the current, the experimental setup and hypothesis was built on the assumption that the dominant chemical

species affecting the reactivity might differ across Arctic systems and lakes mainly based on geological and hydrological differences as the base composition. As a result of the increased evidence supporting the effect of seasonality on DOM composition, a greater scope of DOM isolates from both lakes collected at different points in the spring and summer should undergo similar mechanistic analysis in order to fully describe the mechanisms by which chlorpyrifos is indirectly photodegraded by Alaskan Arctic DOM. The competitive kinetics experiments conducted in this investigation resulted in the conclusion that all three reactive transient species of interest were impactful in the photodegradation mechanism of chlorpyrifos mediated by one or both of the DOM isolates studied. The experimental process underwent substantial method development and testing and could be well used as a spring board for future investigations into the aforementioned relationships. Additionally, the production of potential photodegradation products was observed to be dependent on the specific indirect mechanisms identified in this study. Once the photoproducts are identified, these findings should be further assessed.

X. Relevance to Navy, DOD, and US

The observed thinning of the ozone layer in the Arctic coupled with the warming of the climate will result in an increase in solar radiation coupled with an increase of organic compounds (including contaminants and compounds of DOM that are currently frozen in Arctic soil) entering Arctic water systems (Davie-Martin et al., 2016). As a result, photolysis of reintroduced contaminants is expected to be heightened in the coming years. As the Arctic is increasingly becoming an area of interest and security concerns, it is necessary we know what effect an increase of contaminants and their photolysis will have on Arctic chemistry and biota. The Navy's recently published Arctic Blueprint illustrates its vested interest in environmental scientific research as it continues to expand its operational capacity further North (Department of the Navy, 2021). Understanding the fate of chemicals in the environment gives us information on its persistence, bioavailability, and ability to do environmental harm. It is possible that the photodegradation of chlorpyrifos proves to be an effective way to remove a harmful contaminant from the environment, but it is also possibly likely that it will yield more harmful compounds. Additionally, organophosphate decontamination is of interest to the Navy and DOD as the organophosphate nerve agents developed by the great powers of the past now pose a threat to human and environmental health if not identified and properly remediated. Studies have explored photocatalytic oxidation of contaminants as a potential natural decontamination method, which may or may not be (Hitchman et al., 1997; Jacquet et al., 2016).

XI. References

1. Averett, R.C.; Leenheer, J.A.; McKnight, D.M.; Thorn, K.A. *Humic Substances in the Suwannee River, Georgia: Interactions Properties, and Proposed Structures*; U.S. Geological Survey Water-Supply Paper 2373, 1994.
2. Balmer, J.E., et al. Levels and trends of current-use pesticides (CUPS) in the arctic: An updated review, 2010-2018. *Emerging Contaminants*. **2019**, 5, 70-88.
3. Cawley, K. M.; Hakala, J.A.; Chin, Y.P., Evaluating the triplet state photoreactivity of dissolved organic matter isolated by chromatography and ultrafiltration using an alkylphenol probe molecule. *Limnol. Oceanogr.: Methods*. **2009**, 391-398.
4. Chin, Y.P.; Miller, P.L.; Zeng, L.; Cawley, K.; Weavers, L. K. Photosensitized degradation of bisphenol A by dissolved organic matter. *Environ. Sci. Technol.* **2004**, 38, 5888-5874.
5. Cory, R. M.; McKnight, D. M., Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environ. Sci. Technol.* **2005**, 39, 8142-8149.
6. Davie-Martin, C. L.; Hageman, K.J.; Chin, Y.-P.; Nistor, B.; Hung, H., Concentrations, gas-particle distributions, and source indicator analysis of brominated flame retardants in air at Toolik Lake, Arctic Alaska. *Environ. Sci.: Processes Impacts*. **2016**, 18, 1274-1284.
7. Davie-Martin, C. L.; Hageman, K.J.; Chin, Y.-P.; Nistor, B.; Hung, H., Concentrations, gas-particle distributions, and source indicator analysis of brominated flame retardants in air at Toolik Lake, Arctic Alaska. *Environ. Sci.: Processes Impacts*. **2016**, 18, 1274-1284.
8. Department of the Navy. *A Strategic Blueprint for the Arctic*. Department of the Navy, 2021. <https://media.defense.gov/2021/Jan/05/2002560338/-1/-1/0/ARCTIC%20BLUEPRINT%202021%20FINAL.PDF> (accessed 2022-5-10).
9. Dittmar, T.; Koch, B.; Hertkorn, N.; Kattner, G., A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnol. Oceanogr.: Methods*. **2008**, 6(6), 230-235.
10. Duirk, S.E.; Tarr, J.C.; Colette, T.W., Chlorpyrifos transformation by aqueous chlorine in the presence of bromide and natural organic matter. *J. Ag. Food Chem.* **2018**, 56, 1328-1335.
11. Dulin, D.; Mill, T., Development and evaluation of sunlight actinometers. *Environ. Sci. Technol.* **1982**, 16, 815-820.
12. Giesey, J. P.; Solomon, K. R.; Mackay, D.; Anderson, J., Evaluation of evidence that the organophosphorus insecticide chlorpyrifos is a potential persistent organic pollutant (POP) or persistent, bioaccumulative, and toxic (PBT). *Environ. Sci. Europe*. **2014**, 26 (29).
13. Giesey, J.P.; Solomon, K.R. *Ecological Risk Assessment for Chlorpyrifos in Terrestrial and Aquatic Systems in the United States*, Vol 231; Springer, 2014.
14. Grannas, A.M.; Bogdal, C.; Hageman, K.J.; Halsall, C.; Harner, T.; Hung, H.; Kallenborn, R.; Klan, P.; Klanova, J.; Macdonald, R.W.; Meyer, T.; Wania, F., The role of the global cryosphere in the fate of organic contaminants. *Atmos. Chem. Phys.* **2013**, 13, 3271-3305.
15. Grannas, A.M.; Martin, C.B.; Chin, Y.-P.; Platz, M., Hydroxyl radical production from irradiated Arctic dissolved organic matter. *Biogeochem.* **2006**, 78, 51-66.

16. Grebel, J. E.; Pignatello, J.J.; Mitch, W.A., Sorbic acid as a quantitative probe for the formation, scavenging and steady-state concentrations of the triplet-excited state of organic compounds. *Water Res.*, **2011**, *45*, 6545-6544.
17. Guerard, J.J.; Chin, Y.; Mash, H.; Hadad, C.M., Photochemical Fate of Sulfadimethoxine in Aquaculture Waters. *Environ. Sci. Technol.* **2009**, *43*, 8587-8592.
18. Guerard, J.J.; Chin, Y.P., Photodegradation of ormetoprim in aquaculture and stream derived dissolved organic matter. *J. Ag. Food Chem.* **2012**, *60*, 9801-9806.
19. Haag, W.R.; Hoigne, J.; Gassman, E.; Bran, A.M.; Singlet oxygen in surface waters- Part 1: Furfuryl alcohol as a trapping agent. *Chemosphere*, **1984**, *13*(5), 631-640.
20. Hageman, K.J.; Bogdal, C.; Scheringer, M., Chapter 11. Long-range and regional atmospheric transport of POPs and implications for global cycling. In *Comprehensive Analytical Chemistry*, Vol 67; Elsevier B.V, 2015; pp 363-388.
21. Hermanson, M.H.; Isaksson, E.; Teixeira, C.; Muir, D.C.; Compher, K.M.; Li, Y.-F.; Igarashi, M.; Kamiyama, K., Current-use and legacy pesticide history in the Austfonna Ice Cap, Svalbard, Norway. *Environ. Sci. Technol.* **2005**, *39*, 8163-8169.
22. Hitchman, M.L.; Spackman, R.A.; Yusta, F.J.; Morel, B., A feasibility study of the destruction of chemical weapons by photocatalytic oxidation. *Science and Global Security.* **1997**, *6*, 205-237.
23. Hoferkamp, L.; Hermanson, M. H.; Muir, D.C.G., Current use pesticides in Arctic media; 2000-2007. *Sci. Total Environ.* **2010**, *408*, 2985-2994.
24. Hung, H.; Katsoyiannis, A.A.; Brorstrom-Lunden, E.; Olafsdottir, K.; Aas, W.; Breivik, K.; Bohlin-Nizzetto, P.; Sigurdsson, A.; Hakola, H.; Bossi, R.; Skov, H.; Sverko, E.; Barresi, E.; Fellin, P.; Wilson, S., Temporal trends of Persistent Organic Pollutants (POPs) in Arctic air: 20 years of monitoring under the Arctic Monitoring and Assessment Programme (AMAP). *Environ. Poll.* **2016**, 1-10.
25. Jacquet, P.; Daude, D.; Bzdrenga, J.; Masson, P.; Elias, M.; Chabrière, E., Current and emerging strategies for organophosphate decontamination: special focus on hyperstable enzymes. *Environ. Sci. Pollut. Res.* **2016**, *23*(9), 8200-8218.
26. Katagi, T., Direct photolysis mechanism of pesticides in water. *J. Pestic. Sci.* **2018**, *43*(2), 57-72.
27. Larson, R. A.; Weber, E.J., Chapter 4. Environmental Oxidations. In *Reaction Mechanisms in Environmental Organic Chemistry*, Vol 1; CRC Press, 1994; pp 218-247.
28. Latch, D.E.; McNeill, K., Microheterogeneity of singlet oxygen distributions in irradiated humic acid solutions. *Science.* **2006**, *311*, 1743-1749.
29. Mandal, S., Reaction rate constants of hydroxyl radicals with micropollutants and their significance in advanced oxidation processes. *J. Adv. Oxidation Technol.* **2018**, *21*(1).
30. Marks, A.R.; Harley, K.; Bradman, A.; Kogut, K.; Barr, D.B.; Johnson, C.; Calderon, N.; Eskenazi, B., Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environ. Health Perspect.* **2010**, *118*(12), 1768-1774.

31. Marrazza, G., Piezoelectric biosensors for organophosphate and carbamate pesticides: a review. *Biosensors*. **2014**, *4*, 301-317.
32. McNeill, K.; Canonica, S., Triplet state dissolved organic matter in aquatic photochemistry: reaction mechanisms, substrate scope, and photophysical properties. *Environ Sci: Processes Impacts*. **2016**, *18*, 1381-1399.
33. Meyer, T.; Wania, F., Modeling the elution of organic chemicals from a melting homogeneous snow pack. *Water Res.* **2011**, *45*, 3627-3637.
34. Muir, D.C.G.; de Wit, C. A., Trends of legacy and new persistent organic pollutants in the circumpolar arctic: Overview, conclusions, and recommendations. *Sci. Total Environ.* **2010**, *408*, 3044-3051.
35. Page, S. E.; Arnold, W.A.; McNeill, K., Terephthalate as a probe for photochemically generated hydroxyl radical. *J. Environ. Monit.* **2010**, *12*, 1658-1665.
36. Page, S.E.; Kling, G.W.; Sander, M.; Harrold, K.H.; Logan, J.R.; McNeill, K.; Cory, R.M., Dark formation of hydroxyl radical in arctic soil and surface waters. *Environ. Sci. Technol.* **2013**, *47*, 12860-12867.
37. Page, S.E.; Logan, J.R.; Cory, R.M.; McNeill, K., Evidence for dissolved organic matter as the primary source and sink of photochemically produced hydroxyl radical in arctic surface waters. *Environ. Sci.: Processes Impacts*. **2014**, *16*, 807-822.
38. Pest Management Regulatory Agency, *Re-evaluation decision RVD2020-14 chlorpyrifos and its associated end-use products (environment)*. Catalogue number: H113-28/2020-14, 2020. <https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/decisions-updates/reevaluation-decision/2020/chlorpyrifos.html> (accessed 2022-05-04).
39. Pinto, M.I.; Salgado, R.; Cottrell, B.A.; Cooper, W.J.; Burrows, H.D.; Vale, C.; Sontag, G.; Noronha, J.P., Influence of dissolved organic matter on the photodegradation and volatilization kinetics of chlorpyrifos in coastal waters. *J. Photochem. Photobiol. A: Chemistry*. **2015**, *310*, 189-196
40. Quesada, G. Photosensitized Degradation of Chlorothalonil and Chlorpyrifos in the Presence of Arctic Derived DOM. Masters Thesis, University of Alaska, Fairbanks, Spring 2021.
41. Raina, R.; Hall, P.; Sun, L., Occurrence and relationship of organophosphorus insecticides and their degradation products in the atmosphere in Western Canada agricultural regions. *Environ. Sci. Technol.* **2010**, *44*, 8541-8546.
42. Remucal, C.K., The role of indirect photochemical degradation in the environmental fate of pesticides: a review. *Environ. Sci.: Processes Impacts*, **2014**, *16*, 628-653.
43. Routti, H.; Lille-Langoy, R.; Berg, M.K.; Fink, T.; Harju, M.; Kristiansen, K.; Rostkowski, P.; Rusten, M.; Sylte, I.; Oygarden, L.; Goksoyr, A., Environmental chemicals modulate polar bear (*Ursus martimus*) peroxisome proliferator-activated receptor gamma (PPARG) and adipogenesis *in vitro*. *Environ. Sci. Technol.* **2016**, *50*, 10708-10720.
44. Schwarzenbach et al., *Environmental Organic Chemistry*, 3rd ed; John Wiley & Sons, Hoboken NJ, 2017.

45. Singh, B.K.; Walker, A., Microbial degradation of organophosphorus compounds. *FEMS Microbiol. Rev.* **2006**, *30*, 428-471.
46. Sparling, D.W.; Fellers, G., Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylei*. *Environ. Poll.* **2007**, *147*, 535-539.
47. Taylor, P.; Kovarik, Z.; Reiner, E.; Radic, Z., Acetylcholinesterase: converting a vulnerable target to a template for antidotes and detection of inhibitor exposure. *Toxicol.* **2007**, *233*(1-3), 70-78.
48. U.S. Environmental Protection Agency. *Chlorpyrifos: EPA Actions and Regulatory History*. <https://www.epa.gov/ingredients-used-pesticide-products/chlorpyrifos>. (accessed 2020-12-30).
49. U.S. Geological Survey. *Pesticide National Synthesis Project*. https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=1994&map=CHLORPYRIFOS&hilo=L (accessed 2020-12-5).
50. Vorkamp, K.; Rig  t, F.F., A review of new and current-use contaminants in the Arctic environment: Evidence of long-range transport and indications of bioaccumulation. *Chemosphere.* **2014**, *111*, 379-395.
51. Wei-Haas, M.L.; Chin, Y.-P., A fluence-based method for the direct comparison of photolysis kinetics under variable light regimes. *Environ. Sci. Technol. Lett.* **2015**, *2*, 183-187.
52. Wu, C.; Linden, K.G., Phototransformation of selected organophosphorus pesticides: roles of hydroxyl and carbonate radicals. *Water Res.* **2010**, *44*(12) 3585-3594.
53. Wu, X.; Davie-Martin, C.L.; Steinlin, C.; Hageman, K.J.; Cullen, N.J.; Bogdal, C., Understanding and predicting the fate of semivolatile organic pesticides in a glacier-fed lake using a multimedia chemical fate model. *Environ. Sci. Technol.* **2017**, A-I.
54. Yuan, C.; Tebes-Stevens, C.; Weber, E.J., Reaction library to predict direct photochemical transformation products of environmental organic contaminants in sunlit aquatic systems. *Environ. Sci. Technol.* **2020**, *54*, 7271-7279.
55. Zeng, T.; Arnold, W.A., Pesticide photolysis in prairie potholes: probing photosensitized processes. *Environ. Sci. Technol.* **2013**, *47*, 6735-6745.
56. Zhong, G.; Xie, Z.; Cai, M.; Moller, A.; Sturm, R.; Tang, J.; Zhang, G.; He, J.; Ebinghaus, R., Distribution and air-sea exchange of current-use pesticides (CUPs) from East Asia to the High Arctic Ocean. *Environ. Sci. Technol.* **2012**, *46*, 259-267.
57. U.S. Environmental Protection Agency. *Persistent Organic Pollutants: A Global Issue, A Global Response*. <https://www.epa.gov/international-cooperation/persistent-organic-pollutants-global-issue-global-response> (accessed 2021-1-1).