

**Polyoxyethylene tallow amine:
Environmental fate of an "inert" ingredient**

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Polyoxyethylene tallow amine: Environmental fate of an "inert" ingredient

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Abstract

The surfactant polyoxyethylene tallow amine (POEA) is a common inert ingredient in formulations of glyphosate—the most widely applied agricultural herbicide in the world, which is also commonly used in urban settings. Little is known about the environmental occurrence, fate, and effects of ancillary additives such as POEA. POEA is not well characterized in the literature, but has been shown to be toxic to numerous aquatic organisms. Characterization of POEA technical mixtures shows that POEA is a complex combination of a central nitrogen atom, different aliphatic moieties, and varying numbers of ethoxylate units. Analysis of several agricultural and household glyphosate formulations confirmed that different POEA technical mixtures are common additives in these formulations and that a POEA technical mixture with an average of 15 ethoxylate units is the most common additive. Experiments to characterize the adsorption of POEA to soils revealed that POEA adsorbs much stronger to soil than glyphosate; the addition of calcium chloride to the system increases the adsorption of POEA; and the adsorption of POEA to soils was highest in low pH conditions. POEA detected on a soil sample from a row crop agricultural field near Lawrence, Kansas shows a change in the distribution of homologs over time with a loss of homologs that contain an alkene moiety. POEA was also detected on row crop agricultural soil samples collected between February and early March from sites in five other states (Iowa, Illinois, Indiana, Missouri, Mississippi). Soil samples collected from a row crop field in Indiana for over a year were analyzed to examine the dissipation of POEA, glyphosate, and aminomethylphosphonic acid (AMPA) and shows that POEA and glyphosate persist on the shallow soil from growing season to growing season but there is some dissipation over time with little migration into deeper soil. Stream bed sediments (agricultural and urban watersheds) from six states (Georgia, Hawaii, Iowa, Mississippi, North Carolina,

South Carolina) were analyzed and all were found to have detectable levels of POEA. This is the first indication of the potential widespread contamination of POEA on agricultural soils and stream bed sediments in areas where glyphosate is applied.

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There are far too many people (both real and fictional) who have influenced my life to bring me to where I am now to thank them all properly within these pages. I'd like to thank you all. I count many of you among my family, my friends, my colleagues, my teachers, and my inspirations. There are a handful of individuals and groups that I would like to mention specifically. I will list them (like some movie credits) in order of appearance. For those who I don't mention by name, I mean no slight. I am no poet and only have so many words.

From the beginning of my story, my parents have always been there. My mother, Nancy Tush, has always been one of my biggest supporters. She would always pick me up when I fell down and always encouraged me no matter which direction the path of my life took. My father, Richard Tush, and I often don't see eye to eye on a great many topics, but his appreciation of the natural world around us is one of the cornerstones in my appreciation of the sciences.

My oldest friend, Rebecca Schoonover, is the reason I survived school, K-12. In some ways she and I are completely different people and yet I've always found we were more than enough the same in all the ways that matter. She was the shining morning star that kept me getting up each morning and going to school (and giving at least some effort at it while I was there).

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I would not have made the transition from being a professional programmer to being a chemistry student at Emporia State University without the guidance of my advisor, teacher, and friend—Dr. Jim Roach. From the beginning of my time at Emporia, he showed nothing but

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Chapter 1: Introduction to Polyoxyethylene Tallow Amine

1.1 Polyoxyethylene Tallow Amine: Structure, Naming, and Synthesis

Polyoxyethylene tallow amine (POEA) is surfactant that is a complex mixture of similar compounds. The various homologs that comprise POEA share common characteristics. POEA consists of a central nitrogen atom with three moieties. One of these moieties is an alkyl chain that is commonly either saturated or mono-unsaturated. The other two moieties consist of repeating oxyethylene units with terminal alcohol groups. The oxyethylene units are also referred to as either ethoxy or ethoxylate groups. The structure of POEA is shown in Figure 1.1.

The following naming convention will be used to refer to both groups of POEA homologs and individual POEA homologs: $C_z(s/u)EO_n$. In this naming convention z is the number of carbon atoms in the alkyl moiety, s is a saturated alkyl moiety, u is a mono-unsaturated alkyl moiety, and n is the combined number of repeating ethoxylate units (a sum of x and y in Figure 1.1). For example, $C_{16}sEO_{14}$ refers to an individual POEA homolog consisting of a saturated, 16 carbon atom alkyl chain and 14 combined ethoxylate units. Similarly, $C_{18}u$ refers to the all POEA homologs that have a mono-unsaturated 18 carbon atom alkyl chain.

POEA has been referred to by other names in industry and in the scientific literature. Chem Service, Inc. is a chemical distributor and has POEA technical mixtures sold under the name “POE (n) tallow amine” (where n is the average number of ethoxylate units in the mixture). Akzo Nobel produces POEA technical mixtures under the name “Ethomeen”; Ethomeen T/25 is the technical mixture with an average of 15 ethoxylate units. The Monsanto Company referred to the POEA technical mixture used in their glyphosate formulations as “MON 0818”.¹ Some articles use the term alkylamine ethoxylates (AMEs or ANEOs) instead of

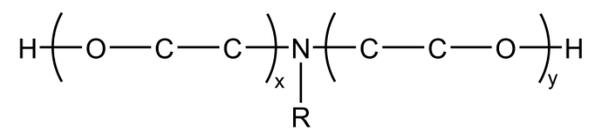


Figure 1.1 Structure of POEA

POEA.^{2,3}

The complexities in the distribution of the homologs of POEA are a result of the starting materials and the process of synthesizing POEA. The following description of the synthesis of POEA is meant only to illustrate how the complex nature of POEA is generated and not as an in-depth analysis. The starting material for the synthesis of POEA is tallow—animal fat. Tallow is primarily comprised of triglycerides. The triglycerides are then converted into fatty acids. The animal fat used in the production of POEA generates several different fatty acids, but the three most abundant are stearic acid (18 carbon atom saturated carboxylic acid), oleic acid (18 carbon atom mono-unsaturated carboxylic acid), and palmitic acid (16 carbon atom saturated carboxylic acid). The carboxylic acids are then converted to nitriles and then hydrogenated to tallow amines. POEA is then generated by reacting the tallow amine with ethylene oxide. The conditions (i.e. temperature, reaction time, catalyst) determine the propagation reaction.⁴ This allows for the creation of products such with different amounts of ethoxylate units. It is important to note that synthesizing POEA beyond two ethoxylate units generates a distribution of ethoxylate units, which is why POEA is typically sold by the average number of ethoxylate units.

1.2 Use of Polyoxyethylene Tallow Amine in Glyphosate Formulations

1.2.1 History of Glyphosate

Glyphosate (N-(phosphonomethyl)glycine), shown in Figure 1.2, is a non-selective herbicide that acts to disrupt the 5-enolpyruvyl-shikimate-3-phosphate synthase enzyme found in many plants.⁵ The first commercial glyphosate herbicide formulation was produced by Monsanto Company in 1974 under the tradename Roundup®. The original Roundup formulation included POEA (as MON 0818) as an inert additive. Since then glyphosate has gone

on to become the most widely applied agricultural pesticide in the world and has also widespread use in urban environments.^{6,7} Glyphosate also has uses in residential/urban settings where it is used to control weeds on hard surfaces such as roads and sidewalks.⁸ When the patent for glyphosate ended, many other companies began producing glyphosate formulations. It is likely that many of these manufacturers also include POEA in the glyphosate formulations. In 2013 the U.S. Geological Survey estimates that more than 110 million kilograms of glyphosate was applied in the U.S. for agricultural purposes.⁹ Using the assumptions that all glyphosate formulations used were 50% glyphosate and 15% POEA (percentages taken from certain glyphosate formulation labels), a worst case estimate would put POEA application at 33 million kilograms for agricultural glyphosate use. Maps of the estimated usage of glyphosate across the U.S. from 1992 to 2013 are shown in Figures 1.3-A to 1.3-F¹⁰. The estimated increase in the usage of glyphosate over the same span is shown in Figure 1.4. The primary reason for the steep increase of use of glyphosate since 1996 is the introduction of genetically modified organisms (GMO) such as corn (*Zea mays*) and soybeans (*Glycine max*) that were engineered to be resistant to the effects of glyphosate. The adoption rates of GMO corn and soybeans (data from U.S. Department of Agriculture) are shown in Figure 1.5.¹¹ Figures 1.5 and 1.6 show that GMO soybeans were adopted earlier and at a faster rate than GMO corn and this is reflected in the amount of glyphosate used (both in total and for those two crops). Glyphosate has generally been considered non-toxic to non-target organisms.⁵ However, in 2015 the International Agency for Research on Cancer classified glyphosate in Group 2A—“probably carcinogenic to humans”—which has reopened the debate on the usage and exposure of glyphosate.¹²

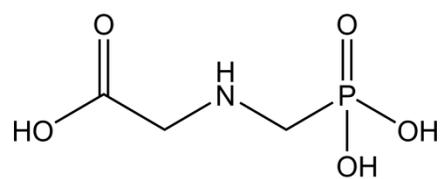


Figure 1.2 Structure of glyphosate.

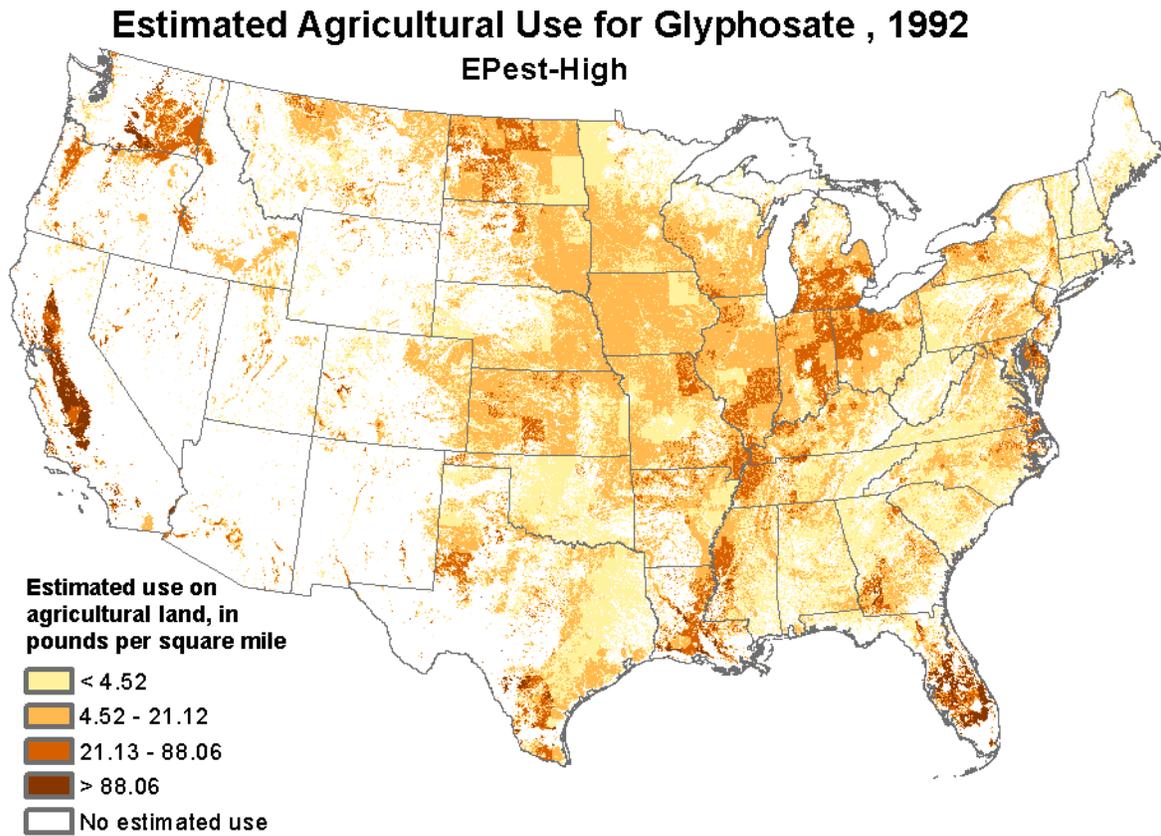


Figure 1.3-A The estimated agricultural use of glyphosate in 1992. Image is public domain and was generated by the U.S. Geological Survey.

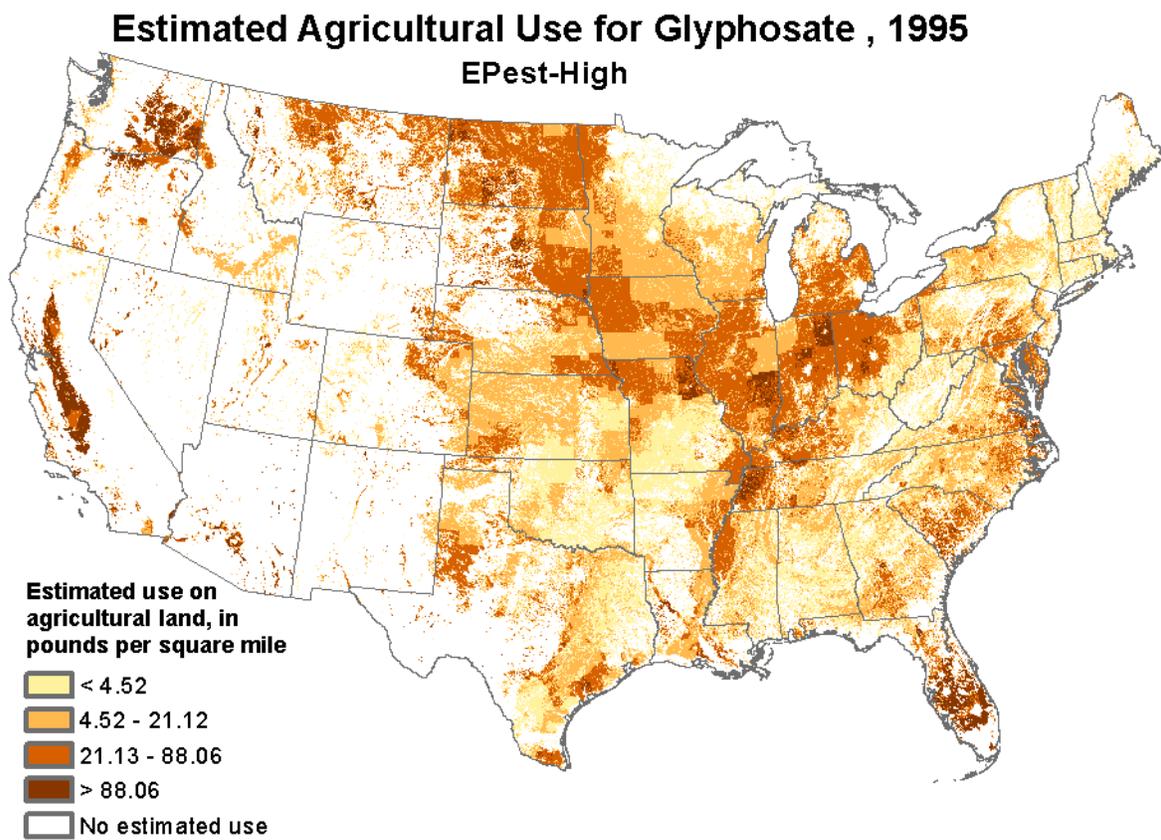


Figure 1.3-B The estimated agricultural use of glyphosate in 1995. Image is public domain and was generated by the U.S. Geological Survey.

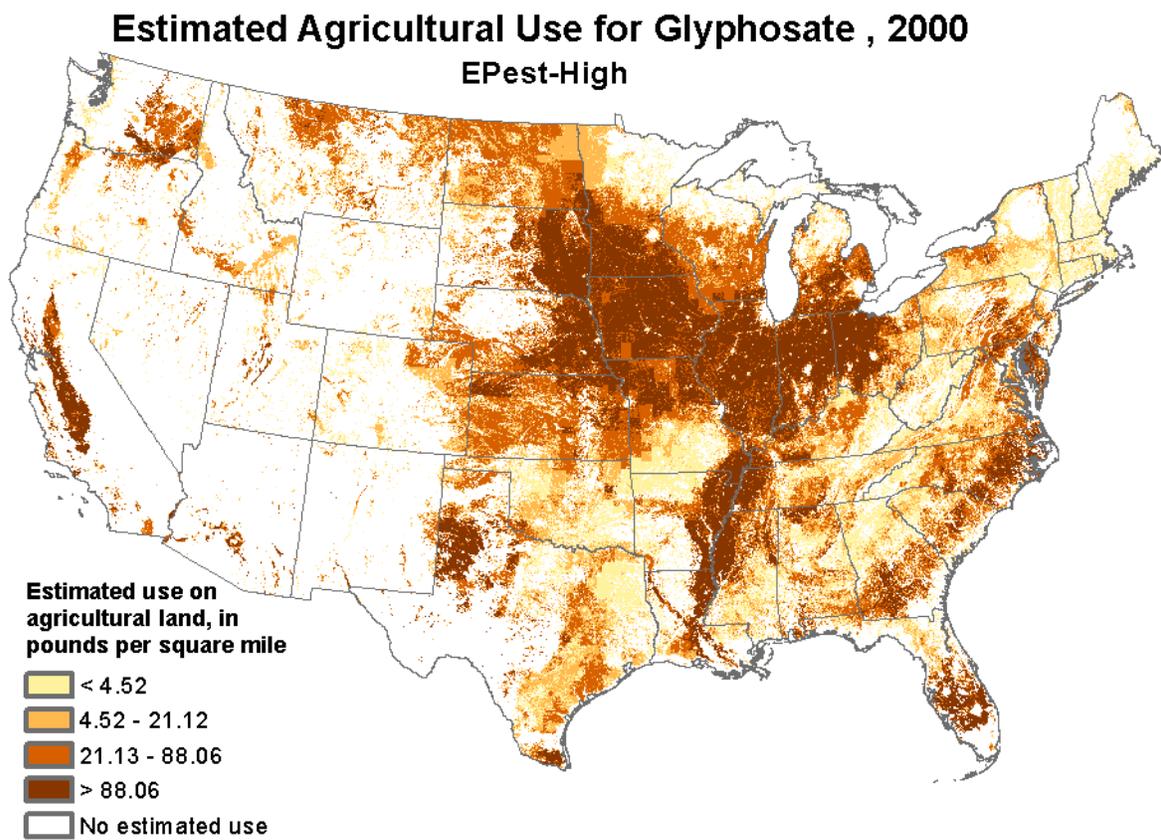


Figure 1.3-C The estimated agricultural use of glyphosate in 2000. Image is public domain and was generated by the U.S. Geological Survey.

Estimated Agricultural Use for Glyphosate , 2005
EPest-High

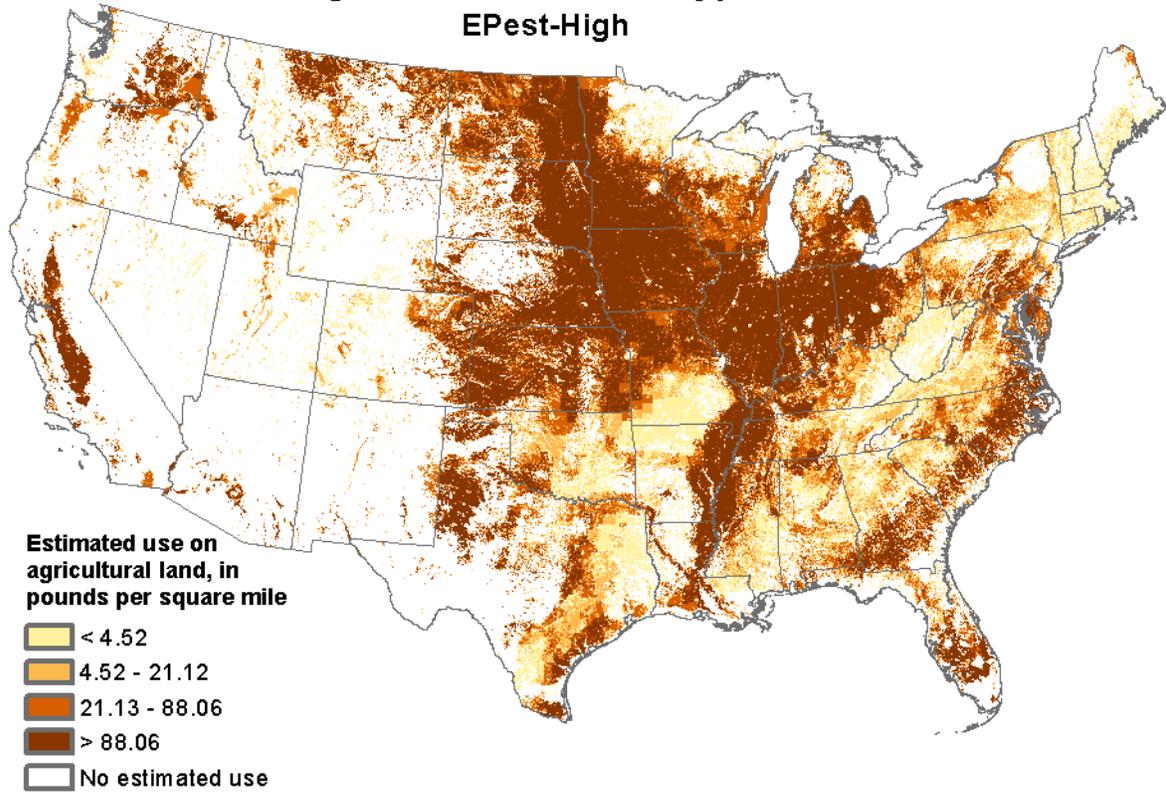


Figure 1.3-D The estimated agricultural use of glyphosate in 2005. Image is public domain and was generated by the U.S. Geological Survey.

Estimated Agricultural Use for Glyphosate , 2010
EPest-High

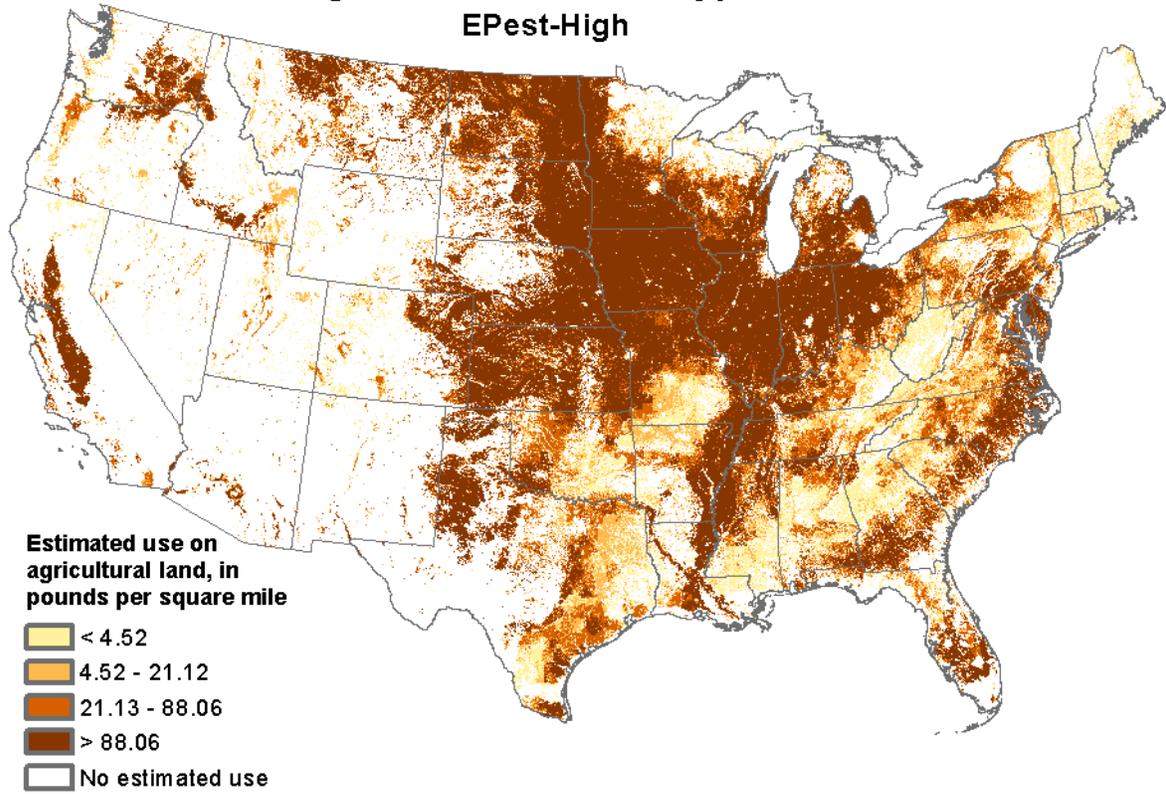


Figure 1.3-E The estimated agricultural use of glyphosate in 2010. Image is public domain and was generated by the U.S. Geological Survey.

**Estimated Agricultural Use for Glyphosate , 2013 (Preliminary)
EPest-High**

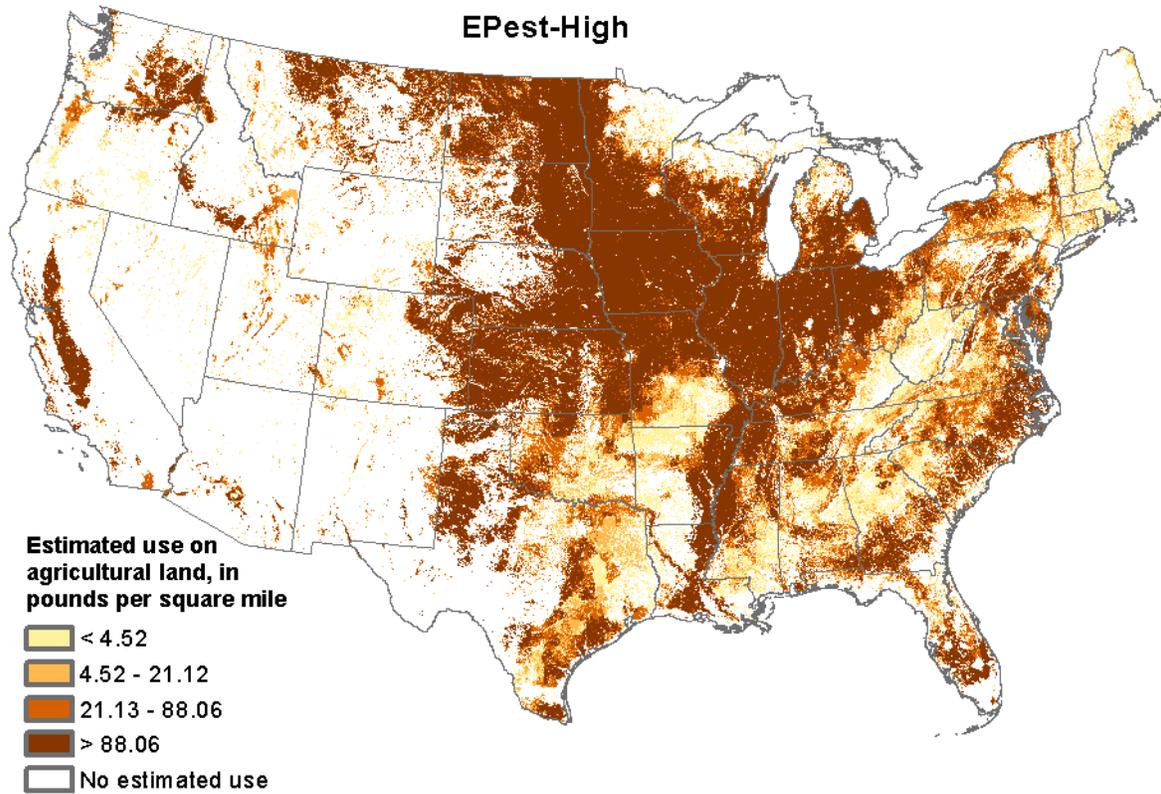


Figure 1.3-F The preliminary estimate of agricultural use of glyphosate in 2013. Image is public domain and was generated by the U.S. Geological Survey.

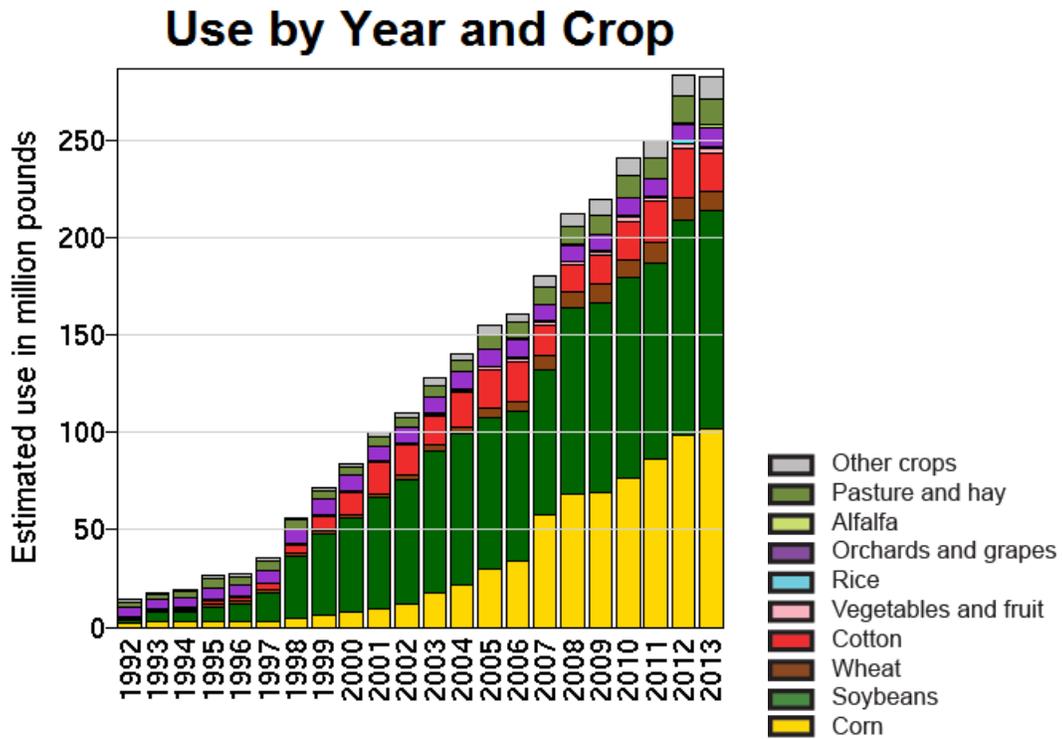


Figure 1.4 Estimated glyphosate usage in the U.S. by year. Image is public domain and was generated by the U.S. Geological Survey.

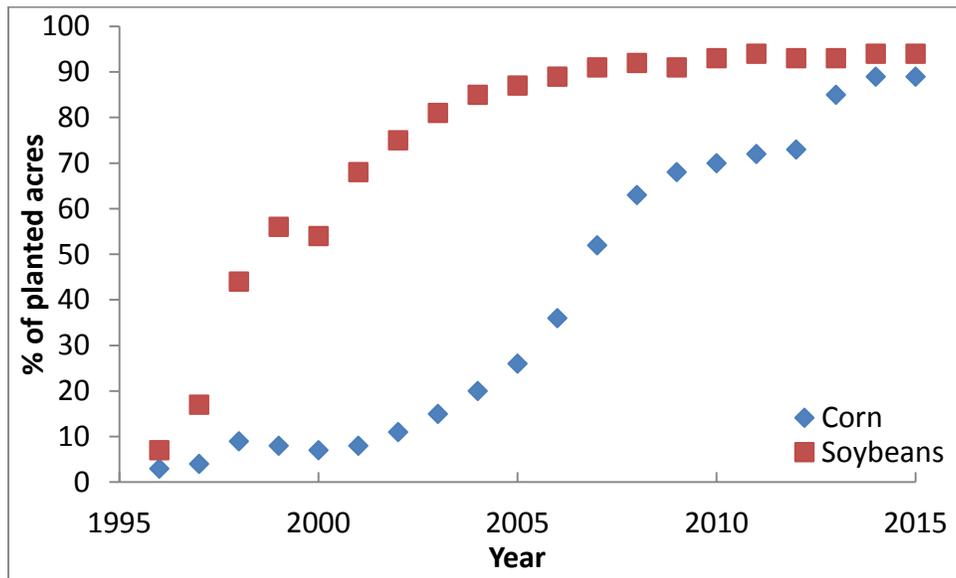


Figure 1.5 Adoption rate of herbicide resistant corn and soybeans in the U.S.

1.2.2 Improved Efficacy of Glyphosate

In general, surfactants are added to pesticide formulations to alter physical properties of the formulation during application. POEA is specifically chosen for some glyphosate formulations because it has been shown to increase the efficacy of the glyphosate and more so than other adjuvants^{1,13-21} To date, there is no consensus in the literature for the mechanism of this increase in the potency of glyphosate when POEA is added to the formulation. Sherrick et al. noted that POEA caused cell necrosis, which might aid in glyphosate uptake.¹ Riechers et al. suggest that the cationic nature of the protonated form of POEA may be why POEA outperforms other surfactants.¹⁸

1.3 Effects of Polyoxyethylene Tallow Amine

1.3.1 Toxicity to Non-Target Organisms

One of the important factors when considering the environmental effect of pollutant is toxicity. Glyphosate is generally considered to be non-toxic to a wide range of non-target organisms.⁵ However, research suggests otherwise for POEA. Many studies indicate that formulations containing POEA are more toxic than glyphosate alone and that POEA is itself toxic. A wide range of organisms have shown negative effects from exposure to POEA and glyphosate formulations containing POEA.²²⁻³⁹ During a study of the toxicity of POEA, Wang et al. observed that the toxicity of POEA was lower with increased sediment indicating adsorption of POEA to the sediment.³⁰ Brausch et al. suggest that the different homologs of POEA have different toxicities base on the degree of ethoxylation—POEA with an average of 10 ethoxylate units was more toxic to *Daphnia magna* than was POEA with an average of 15 ethoxylate

units.³² The U.S. Environmental Protection Agency has a toxicity classification for substances that are toxic to aquatic organisms by LC50 (concentration where 50% of a population will not survive): “very highly toxic” (< 0.1 mg/L), “highly toxic” (≥ 0.1 mg/L ≤ 1 mg/L), “moderately toxic” (> 1 mg/L ≤ 10 mg/L), “slightly toxic” (>10 mg/L ≤ 100 mg/L), and “practically nontoxic” (> 100 mg/L).⁴⁰ A selection of toxicity data of POEA to several aquatic organisms is shown in Table 1.1.

1.3.2 Human Health

Currently the main human health risk from POEA is through direct exposure. Material safety data sheets for POEA technical mixtures state that POEA is caustic and contact should be avoided during handling and application. There are also reported cases of glyphosate formulations being intentionally ingested in suicide attempts.⁴¹ Glyphosate is not the harmful component in such cases, but is instead the effect POEA has on hemodynamics.^{42,43}

1.3.3 Other Effects

Although the acute toxic effect on wildlife is an important, and the most studied, consequence of the use of POEA in herbicide formulations there may be other unstudied effects. There could be long-term chronic effects on wildlife from yearly exposure to POEA. If POEA degrades in the environment, the products from those reactions could also have a negative impact. Currently the only degradation data on POEA was performed on an activated sludge from a wastewater treatment plant⁴⁴ which does not mimic the conditions POEA would be exposed to when applied to an agricultural field. There was also no characterization of any

Table 1.1 Toxic levels of POEA for aquatic species.

Species (common name)	LC50 (mg/L)	Toxicity Classification	Reference
<i>Daphnia magna</i> (water flea)	0.0970	very highly toxic	31
<i>Lampsilis siliquoides</i> (Fatmucket mussel)	0.5 ^a	N/A	32
<i>Acartia tonsa</i> (copepod)	0.57	highly toxic	27
<i>Rana pipiens</i> (Northern leopard frog)	0.68	highly toxic	36
<i>Bufo fowleri</i> (Fowler's toad)	0.80	highly toxic	36
<i>Rana catesbeiana</i> (American bullfrog)	0.83	highly toxic	36
<i>Pimephales promelas</i> (fathead minnows)	1.0	highly toxic	38
<i>Rana clamitans</i> (green frog)	1.1	moderately toxic	28
<i>Ceriodaphnia dubia</i> (water flea)	1.15	moderately toxic	27
<i>Rana clamitans</i> (green frog)	1.32	moderately toxic	36
<i>Salmo gairdneri</i> (rainbow trout)	2.0	moderately toxic	38
<i>Daphnia pulex</i> (water flea)	2.00	moderately toxic	22
<i>Thamnocephalus platyurus</i> (fairy shrimp)	2.01	moderately toxic	30
<i>Oncorhynchus nerka</i> (sockeye salmon)	2.60	moderately toxic	22
<i>Lepomis macrochirus</i> (bluegill sunfish)	3.0	moderately toxic	38
<i>Skeletonema costatum</i> (algae)	3.35 ^b	N/A	27
<i>Oncorhynchus kisutch</i> (coho salmon)	3.50	moderately toxic	22
<i>Selenastrum capricornutum</i> (algae)	3.92 ^b	N/A	27
<i>Tetrahymena pyriformis</i> (protozoa)	4.96 ^b	N/A	27
<i>Euplotes vannus</i> (protozoa)	5.00 ^b	N/A	27
<i>Xenopus laevis</i> (African clawed frog)	6.8	moderately toxic	25
<i>Vibrio fischeri</i> (bacteria)	10.2 ^b	N/A	27
<i>Chironomus plumosus</i> (midge larvae)	13	slightly toxic	38
<i>Ictalurus punctatus</i> (channel catfish)	13	slightly toxic	38

a. EC50 (effective concentration at that immobilizes 50% of the population)

b. IC50 (concentration that inhibits growth in 50% of the population)

intermediate degradation products studied. POEA in the environment may also change the transport or storage of other contaminants by altering the surface of soil and sediment particles. Aamlid et al. found that the application of a nonionic surfactant increased water infiltration rates but decreased the leaching of fungicides presumably by providing a more wettable surface and reducing preferential finger-flow.⁴⁵ Surfactants have been proposed to be used as both a barrier to the movement of contaminants⁴⁶⁻⁴⁸ and as a way to remediate contaminated soils^{49,50}.

1.4 Instrumental Analysis of Polyoxyethylene Tallow Amine

The direct analysis of surfactants is challenging. As such, there are few analytical methods for the detection and quantitation of POEA in the scientific literature. A number of complexities must be considered in the development of an analytical method. The major sample matrices that are important in the initial stages of environmental research for POEA—soils, surface waters, suspended sediment in the surface water, and bed sediment. These different matrices will likely require at least two different sample preparation methods, one for the solid samples and one for the aqueous samples. Because POEA is a complex mixture of homologs—even before considering degradation and degradation products—the method should be flexible enough to account for a wide range of POEA homologs.

An early method by Sherrick et al. uses a scintillation technique to analyze POEA.¹ Scintillation measures ionizing radiation of a sample by converting the radiation into light. In this study they measured ¹⁴C labeled POEA on plant material. Because this method is not selective to POEA homologs, only a bulk concentration of POEA could be measured. This method was used to measure the translocation of POEA through the epicuticular wax of the

leaves. It was found that within 24 hr only 3% of POEA applied was extractable from the leaf surface and that 84% was absorbed into the leaf.

The remaining literature about analytical methods for POEA all use some form of liquid chromatography (LC) coupled to a mass spectrometer (MS). In work done by Krogh et al. a method was developed to analyze both alcohol ethoxylates (a different class of surfactant) and POEA (referred to as alkylamine ethoxylates) in aqueous samples including soil interstitial water, surface water, and ground water.² Three different solid-phase extraction (SPE) cartridges were tested for the sample preparation and preconcentration: Isolute ENV (International Sorbent Technology), Oasis HLB (Waters Corp.), and Sep-Pak Porapak Rdx (Waters Corp.). The Porapak Rdx cartridges were chosen but the recoveries of POEA ranged from 26%-109%. The LC system used a Hypersil BDS C18 column (Thermo Scientific Inc.). The mobile phases consisted of (A) 1:1 methanol:acetonitrile and (B) water. Both mobile phases were made to be 20 mM acetic acid and 25 mM triethylamine. The MS used was a triple quadrupole (TQ) instrument with positive atmospheric pressure ionization (APCI). Despite using a TQ instrument capable of tandem MS experiments, this study used single ion monitoring (SIM) mode which is essentially a single quadrupole MS method. A further limitation of this study is that only 11 homologs that follow the theoretical structure of POEA ($C_{12}S EO_2$, $C_{14}S EO_2$, $C_{16}S EO_2$, $C_{16}S EO_{16-18}$, $C_{18}u EO_{13-17}$) were chosen to be monitored. These homologs were chosen based off two surfactant technical mixtures, Ethomeen C/12 and Berol 907 (Akzo Nobel, Stenungsund, Sweden). No attempt to analyze POEA on environmental samples was reported.

The next study appears to be follow up to the previous example with many of the same authors.⁵¹ The most important change from the previous work is that instead of analyzing aqueous samples this method development work involves soil samples. To analyze the soil

samples, pressurized liquid extraction was used. Two extracting solvents were used, (A) methanol and (B) 1:1 hexane:acetone with 75 mM acetic acid and 100 mM triethylamine. Recoveries for this method ranged from 27%-62%. Several of the POEA homologs they were monitoring ($C_{16}EO_{16-18}$ and $C_{18}EO_{13-17}$) were detected on agricultural soils samples from two fields in Spain before and after a known glyphosate application.

In a rapid communication by Corbera et al. an LC-MS method was used to analyze 4 European glyphosate formulations.⁵² The chromatography system used the same type of column as was used above (Hypersil BDS C18) but with different mobile phases. The mobile phases were (A) 20 mM acetic acid and 60 mM ammonia in water and (B) 20 mM acetic acid and 60 mM ammonia in 1:1 acetonitrile:methanol. The detector was a Finnigan AQA single quadrupole mass spectrometer with positive electrospray ionization (ESI). This method targeted 30 homologs of POEA ($C_{12}EO_{13-17}$, $C_{14}EO_{13-17}$, $C_{16}EO_{13-17}$, $C_{16}EO_{13-17}$, $C_{18}EO_{13-17}$, $C_{18}EO_{13-17}$), which is the largest set of homologs analyzed in the literature to date. This method was used to detect POEA in four European glyphosate formulations, Roundup (Monsanto Europe, Antwerp, Belgium), Roundup Energy (Monsanto Europe), Atila (Afrasa, Valencia, Spain) and Compo (Compo Agricultura, Barcelona, Spain).

In the most recent of the literature articles (published after the research included in this dissertation had concluded), Ross and Liao developed an LC-MS method to analyze POEA in both aqueous and soil samples.⁵⁴ POEA was extracted from the soil samples using accelerated solvent extractions. The solvent used to extract the soil samples was 5mM potassium dihydrogen phosphate in 1:7 water:methanol. Two different chromatography systems were tested. The first used hydrophilic interaction liquid chromatography (HILIC) on an Atlantis HILIC column (Waters Corp.). The second was reverse phase chromatography and was

performed on an XTerra MS C18 column (Water Corp.). The mobile phases were (A) 1:1 methanol: acetonitrile and (B) 0.3% formic acid and 0.1% ammonium formate in water. The detector used was an API 5500 triple quadrupole mass spectrometer with positive ESI (AB Sciex). Only 9 homologs of POEA (C₁₆sEO₁₀, C₁₆sEO₁₂, C₁₆sEO₁₄, C₁₈uEO₁₀, C₁₈uEO₁₂, C₁₈uEO₁₄, C₁₈sEO₁₀, C₁₈sEO₁₂, C₁₈sEO₁₄) were measured with one product ion for each parent ion in multiple reaction monitoring (MRM) mode. No environmental data was reported.

1.5 Other Surfactants in the Environment

Two examples of studies of surfactants other than POEA are presented here to illustrate the importance of surfactants in the environment. Alkylphenol ethoxylate (APE) is a class of surfactant that are widely used as detergents and so widely used that the presence of APEs are used as a sign of industrial and residential pollution in the environment.⁵⁵⁻⁵⁷ APEs are studied because of the high rate of usage and because during wastewater treatment they can be transformed by the loss of the ethoxylate chains into compounds such as octylphenol and nonylphenol which are known endocrine disruptors⁵⁸ Another class of surfactant, linear alkylbenzene sulfonate (LAS), used in laundry and dishwashing detergents, is detected in wastewater effluents^{59,60} LAS can also be detected on agricultural fields where wastewater effluent is used in irrigation or where wastewater sludge has been used as fertilizer.⁶¹ Both examples presented here are used for industrial/residential applications but agricultural surfactants and other adjuvants remain largely unstudied.

1.6 Research Objectives

Inert ingredients and adjuvants in pesticide formulations represent an understudied collection of potentially harmful environmental contaminants.⁶¹ The widespread use of these types of agricultural chemicals has made this research necessary. It is important to study the fate and transport of these chemicals to begin to understand what impact they may have on the environment. This research was funded by the U.S. Geological Survey Toxic Substances Hydrology Program.

This dissertation research addresses the environmental fate and transport of POEA applied as a part of glyphosate formulations in agricultural areas. The main objectives were to develop analytical methods to analyze POEA and to study the fate and transport of POEA in the environment. In order to develop a successful analytical method for POEA, the following specific aims need to be met. First, POEA needs to be characterized to determine how many and which homologs must be analyzed. Second, the method needs to quantitate POEA as a whole at relevant environmental concentrations. Finally, the method must include sample handling and extraction. To meet these aims, several instruments were investigated including: accelerated solvent extractions (ASE), ultra-high performance liquid chromatography (UHPLC), triple quadrupole mass spectrometry (TQ-MS or more generically MS-MS), and time of flight mass spectrometry (TOF-MS). To investigate the fate and transport of POEA in the environment, the following specific aims are targeted. First, current glyphosate formulations need to be analyzed to determine if POEA is still relevant in agriculture. This is particularly important since glyphosate is off patent and many manufacturers are involved in the distribution of glyphosate—most of whom keep their formulations as trade secrets. Second, the adsorption of POEA to soil needs to be characterized. This information is useful to determine the most likely mode of transport of

POEA in the environment which also informs what types of samples to target initially (water or soil). Finally, environmental samples will be analyzed to determine how widespread POEA contamination might be.

The following chapters of this dissertation will address these research objectives. Chapter 2 introduces the basic analytical methods, characterizes POEA technical mixtures, and examines glyphosate formulations to determine which, if any, contain POEA. Chapter 3 further develops the POEA analytical methods, presents the adsorption characteristics of POEA on agricultural soils, and examines the POEA homolog distribution on environmental samples collected from agricultural fields. Chapter 4 investigates the use of the method of standard additions as part of the POEA analytical method, studies the dissipation of POEA and glyphosate on an agricultural field over time, and examines bed sediments from streams for the presence of POEA. Chapter 5 is a collection of observations from the process of developing POEA analytical methods and also presents some smaller experiments that relate to the fate and transport of POEA.

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Chapter 2: Characterization of polyoxyethylene tallow amine surfactants in technical mixtures and glyphosate formulations using ultra-high performance liquid chromatography and triple quadrupole mass spectrometry

Tush, D.; Loftin, K. A.; Meyer, M. T. Characterization of polyoxyethylene tallow amine surfactants in technical mixtures and glyphosate formulations using ultra-high performance liquid chromatography and triple quadrupole mass. *J. Chromatogr. A* **2013**, 1319, 80-7.

2.1 Introduction

Polyoxyethylene tallow amine (POEA) is a non-ionic surfactant related to alkylamine ethoxylates (ANEOs). POEA is composed of a tallow amine moiety, as opposed to the more general alkylamine, and two chains of repeating ethoxylate units (Figure 2.1). The tallow amine moiety is a mixture of amines derived from palmitic acid (C₁₆ saturated carboxylic acid), oleic acid (C₁₈ mono-unsaturated carboxylic acid), stearic acid (C₁₈ saturated carboxylic acid), and other minor components.¹ The length of the ethoxylate chains vary in different technical mixtures and can give different physical properties. Although POEA is a non-ionic surfactant, the tertiary amine can act as a base and become protonated in neutral to acidic conditions; the acid dissociation constant (pKa) of POEA has been reported as a range of 6.5-7.0². Specific POEA molecules will be described herein by the number of carbon atoms in the tallow amine moiety (C_z), whether the tallow amine moiety is saturated or is mono-unsaturated (s/u), and by the combined number of ethoxylate units (EO_n).

Toxicity studies have shown POEA to be harmful to a variety of aquatic wildlife. A compilation of acute toxic levels of POEA for several species is shown in Table 2.1.³⁻¹¹ Lethal concentration for fifty percent of the population (LC50) values have been observed from 0.097 mg/L for *Daphnia magna* (water fleas) to 13 mg/L for *Ictalurus punctatus* (channel catfish) and *Chironomous plumosus* (midge larvae) .

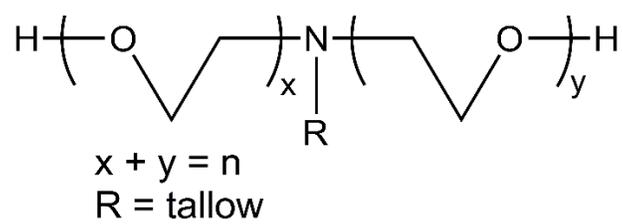


Figure 2.1 Theoretical structure of POEA.

Table 2.1 Toxic levels of POEA for various species.
LC50 represents the concentration that is fatal to 50% of the population.

Species (common name)	LC50 (mg/L)	Reference
<i>Daphnia magna</i> (water flea)	0.097	3
<i>Lampsilis siliquoidea</i> (Fatmucket mussel)	0.5 ^a	4
<i>Acartia tonsa</i> (copepod)	0.57	5
<i>Rana pipiens</i> (Northern leopard frog)	0.68	6
<i>Bufo fowleri</i> (Fowler's toad)	0.80	6
<i>Rana catesbeiana</i> (American bullfrog)	0.83	6
<i>Pimephales promelas</i> (fathead minnows)	1.0	7
<i>Rana clamitans</i> (green frog)	1.1	8
<i>Ceriodaphnia dubia</i> (water flea)	1.15	5
<i>Rana clamitans</i> (green frog)	1.32	6
<i>Salmo gairdneri</i> (rainbow trout)	2.0	7
<i>Daphnia pulex</i> (water flea)	2.00	9
<i>Thamnocephalus platyurus</i> (fairy shrimp)	2.01	10
<i>Oncorhynchus nerka</i> (sockeye salmon)	2.60	9
<i>Lepomis macrochirus</i> (bluegill sunfish)	3.0	7
<i>Skeletonema costatum</i> (algae)	3.35 ^b	5
<i>Oncorhynchus kisutch</i> (coho salmon)	3.50	9
<i>Selenastrum capricornutum</i> (algae)	3.92 ^b	5
<i>Tetrahymena pyriformis</i> (protozoa)	4.96 ^b	5
<i>Euplotes vannus</i> (protozoa)	5.00 ^b	5
<i>Xenopus laevis</i> (African clawed frog)	6.8	11
<i>Vibrio fischeri</i> (bacteria)	10.2 ^b	5
<i>Chironomus plumosus</i> (midge larvae)	13	7
<i>Ictalurus punctatus</i> (channel catfish)	13	7

a. EC50 (effective concentration that immobilizes 50% of the population)

b. IC50 (concentration that inhibits growth in 50% of the population)

One of the primary uses of POEA is as an additive for use with glyphosate formulations, the most widely applied herbicide in agriculture and urban environments. The terminology in the literature regarding pesticide additives is inconsistent. The US Environmental Protection Agency (EPA) defines an additive to pesticide formulations by the manufacturer before purchase as “inert ingredients” while those that are added by the user before application as “adjuvants”.¹² Surfactants are used in herbicide formulations to change various physical properties and may be added as wetting agents, emulsifiers, or dispersants.¹³ While POEA may be added to glyphosate formulations for such physical benefits, studies have also shown that POEA increases the efficacy of glyphosate and does so more effectively than other surfactants.¹⁴⁻¹⁹ Glyphosate has been described as a “once-in-a-century herbicide” because it is considered environmentally benign to non-target organisms, effective at controlling weeds, and can be applied directly to crops that are genetically modified to be glyphosate resistant.²⁰ Available glyphosate resistant crops include corn, soybeans, cotton, alfalfa, and canola.²¹ According to an estimate from the EPA, over 81,000 metric tons (180 million pounds) of glyphosate was applied in the agricultural sector in 2007, which is more than the next five most applied herbicides combined (atrazine, metolochlor-S, acetochlor, 2,4-D, and pendimenthalin).²² Glyphosate is also used in urban settings to control weeds and is often applied to hard surfaces such as roads and sidewalks.²³ Most manufactures consider the composition of their glyphosate formulations to be proprietary information, making it difficult to determine what the actual composition of the formulation is beyond the active ingredient. One exception is the product literature for Glyfos X-TRA, which states on the product label that it contains 14.5% surfactant (compared to 41% glyphosate), which the MSDS identifies as a POEA mixture (CAS# 61791-26-2).

The use of POEA in glyphosate formulations may change the sorption/desorption characteristics of the soil with respect to glyphosate. This effect has been shown in other surfactant/pesticide systems.²⁴ The transport of glyphosate has been studied²⁵ but has not taken into account the presence and effect of surfactant additives. Characterizing POEA will deepen the understanding of the transport of glyphosate in the environment.

There are other uses for POEA beyond glyphosate formulations. Searches of material safety data sheets reveal POEA listed as an ingredient in cleaners, degreasers, and wire pulling lubricants. Marketing materials indicate that some distributors of POEA suggest it can be used as an antistatic agent, a corrosion inhibitor, a dye leveler, an emulsifier, a metal lubricant, and more. Without further information or study, the potential environmental impact from POEA from uses other than glyphosate formulations is difficult to predict.

Published analytical methods for the detection and quantification of POEA or other ANEOs are sparse. Research by Krogh *et al*²⁶ describes a liquid chromatography-mass spectrometry (LC-MS) method and a soil extraction method for POEA, but only included the C₁₆S and C₁₈S homologs. Corbera *et al*²⁷ also reported on an LC-MS method, one that accounts for the primary components of the tallow amine moiety but only examines the range of EO₁₃₋₁₇. A full characterization of POEA technical mixtures present in glyphosate formulations is an important extension of previous efforts.

The lack of published methods also shows that little work has been done with environmental samples to study the fate and transport of POEA. Other surfactants in environmental samples have been shown to transport via surface waters. A study of the Cuyahoga River in Ohio showed concentrations of nonylphenol ethoxylates and octylphenol ethoxylates of 5.1 µg/L and 0.19 µg/L respectively.²⁸ In Spain, a study showed concentrations of

linear alkylbenzene sulfonates, alkyl ethoxysulfates, alkyl sulfates, nonylphenol polyethoxylates, and alcohol polyethoxylates of 38.7, 3.0, 2.9, 5.0 and 1.2 µg/L respectively.²⁹ While these studies show surfactants in the environment below the LC50 levels of POEA, it is important to note that these surfactants have different applications and likely have different transport characteristics than POEA. POEA applied in agriculture may be transported in higher concentration pulses in surface waters due to precipitation events after application. This phenomenon has been reported for pesticides and is referred to as the “spring flush”.³⁰ Even if concentrations of POEA do not reach acute toxic levels, there may be unexplored chronic effects.

There has been a drive to use smaller diameter stationary phase particles in analytical columns to increase the number of theoretical plates and these smaller particles in turn require instruments capable of maintaining higher pressures. Typical high performance liquid chromatography (HPLC) packing materials are 3 µm or larger in diameter and are useable at pressures up to approximately 400 bar. Ultra-high performance liquid chromatography (UHPLC) uses particles with diameters smaller than 3 µm and can support pressures of over 1000 bar. There has been no systematic chromatographic assessment on the ability of different stationary phases, particle sizes, and pressure limits to separate POEA in the literature.

The purpose of this research is threefold: to characterize POEA mixtures using UHPLC and mass spectrometry, to compare the chromatographic ability to separate POEA for a variety of analytical columns, and to examine commercial glyphosate formulations for the presence and nature of POEA included as an inert ingredient. Because POEA may have a deleterious effect on the environment, this research is important as a foundation to build quantitative methods to determine the scope of POEA’s environmental relevance through future studies on degradation, transport, co-transport, and occurrence.

2.2 Experimental

2.2.1 Reagents and Materials

Nitrogen gas (generated from liquid nitrogen) and argon gas for mass spectrometry were supplied by Praxair Inc. (Danbury, CT, USA). The mobile phase for chromatography experiments consisted of LC-MS grade acetonitrile (Burdick & Jackson, Muskegon, MI, USA), deionized water from a Nanopure DIAMOND TOC Life Science system (Barnstead|ThermoFisher, Dubuque, IA, USA), and Optima acetic acid (Fisher Scientific, Fair Lawn, NJ, USA). Four different POEA technical mixtures were investigated. POE (2) tallow amine, POE (5) tallow amine, and POE (15) tallow amine were acquired from Chem Service Inc. (West Chester, PA, USA) and Ethomeen T/25 was acquired from Akzo Nobel Surface Chemistry LLC. (Chicago, IL, USA). Agricultural glyphosate formulations were generously provided by Dr. Dallas Peterson from Kansas State University and include the following: Abundit Extra (Nufarm Inc., Burr Ridge, IL, USA), AgriSolutions Cornerstone Plus (Winfield Solutions LLC, St. Paul, MN, USA), Buccaneer Plus (Tenkoz Inc., Alpharetta, GA, USA), Durango DMA (Dow AgroSciences LLC, Indianapolis, IN, USA), Glyfos XTRA (Cheminova Inc., Research Triangle Park, NC, USA), Glyphogan Plus (Makhteshim Agan of North America Inc., Raleigh, NC, USA), Roundup PowerMAX (Monsanto Company, St. Louis, MO, USA), Roundup WeatherMAX (Monsanto Company, St. Louis, MO, USA), Touchdown Hitech (Syngenta Crop Protection LLC, Greensboro, NC, USA), and Touchdown Total (Syngenta Crop Protection LLC, Greensboro, NC, USA). Residential glyphosate formulations were purchased from local retailers and include: Ace Weed and Grass Killer (Chemsico, St. Louis, MO, USA), Bayer Advanced DuraZone Weed and Grass Killer Concentrate (Bayer Environmental Science, Research Triangle Park, NC, USA), Ortho Groundclear Vegetation Killer Concentrate (The Scotts Company, Marysville, OH,

USA), Roundup Poison Ivy Plus (Monsanto Company, St. Louis, MO, USA), Roundup Weed and Grass Killer Plus Ready to Use (Monsanto Company, St. Louis, MO, USA), and Roundup Weed and Grass Killer Plus Concentrate (Monsanto Company, St. Louis, MO, USA). The POEA technical mixtures and agricultural glyphosate formulations were initially diluted in acetonitrile to 1 g/L and the residential glyphosate formulations were initially diluted tenfold in 50/50 acetonitrile/water. All samples were further diluted in water for chromatography experiments.

2.2.2 Chromatographic Systems

The analytical columns used are shown in Table 2.2. The Shodex column has a mixed mode (size exclusion and reverse-phase) stationary phase and was chosen for its ability to separate homologues of nonylphenol ethoxylates.³¹ The remaining columns are all reverse-phase columns with endcapped C18 functionality. The Luna and Atlantis columns are standard pressure HPLC columns. The Kinetex and XSelect columns use a particle size that is between the larger HPLC and the smaller UHPLC particle sizes and are suitable for traditional HPLC instruments and for higher pressure UHPLC instruments. The Acquity BEH and Acquity HSS columns are UHPLC columns made to withstand higher pressures.

Chromatographic experiments were performed using a Waters Corp. (Milford, MA, USA) Acquity H-Class Bio UPLC consisting of a bioQuaternary Solvent Manager, a bioSample Manager FTN, and a CM-A Column Manager. An injection volume of 25 μL was used, except on the Acquity columns. Because the Acquity columns have a smaller diameter housing and therefore less stationary phase, an injection volume of 15 μL was used. The column compartment temperature was set at 45 $^{\circ}\text{C}$. The same gradient was used for each column.

Table 2.2 Summary of analytical columns used in this study.

Column	Particle Size (μm)	Dimensions (mm)	Carbon load (%)	Maximum pressure (bar)	Flow rate used (mL/min)
Shodex MSpak GF-310 4D ^a	5	4.6 x 150	--	40	0.4
Luna C18(2) ^b	3	3 x 150	17.5	345	0.6
Atlantis T3 ^c	3	3 x 150	14	400	1.0
Kinetex C18 ^b	2.6	3 x 150	12	600	0.9
XSelect HSS C18 ^c	2.5	3 x 150	15	400	0.6
Acquity BEH C18 ^c	1.7	2.1 x 150	18	1034	0.4
Acquity HSS T3 ^c	1.8	2.1 x 150	11	1241	0.4

a: Showa Denko, Tokyo, Japan

b: Phenomenex Inc., Torrance, CA, USA

c: Waters Corp., Milford, MA, USA

The gradient started at 90% solvent A (0.3% acetic acid in deionized water) and 10% solvent B (acetonitrile) and ramped to 30% solvent A and 70% solvent B over 15 minutes. The gradient was then raised to 100% solvent B over 5 minutes and held at 100% solvent B for another 5 minutes before returning to initial conditions over 1 minute. The column is then allowed to equilibrate at initial conditions for 4 minutes. Flow rates were chosen to reach the recommended pressure or approximately 75% of the maximum pressure.

2.2.3 Mass Spectrometry

Mass spectrometry experiments were performed on a Waters Quattro Micro API triple quadrupole mass spectrometer (Milford, MA, USA) with electrospray ionization (ESI) in positive mode. Instrument settings were: capillary voltage 1 kV, source temperature 120 °C, desolvation temperature 450 °C, desolvation gas flow 300 L/hr, and cone gas flow 40 L/hr. Infusion experiments used the instrument's on-board syringe pump to deliver 50 ppm solutions and single quadrupole scan data was summed over one minute. POE (2) tallow amine and POE (5) tallow amine were infused at 10 uL/min and POE (15) tallow amine and Ethomeen T/25 were infused at 20 uL/min. LC-MS experiments used a single quadrupole scan for data collection. Product ion scans used argon as the collision gas with a collision energy so that the precursor ion peak was less intense than the most intense product ion peak to illustrate the entire range of ions produced.

2.2.4 Peak Fitting

Chromatographic peaks were fitted using PeakFit 4.12 (Systat Software Inc). The data were baseline corrected and only the peaks for the three most abundant tallow moieties were

selected for fitting. Peak fitting was performed using the Exponentially Modified Gaussian + Half-Gaussian Modified Gaussian (EMG+GMG) model. The EMG+GMG model was chosen based on consistently high r^2 values when fitting the data compared to other models tested. All parameters were allowed to be freely fitted except for the distortion parameter from the EMG term. The EO unit distribution was fitted to a Gaussian peak by plotting the sum of the responses for the three most abundant tallow amine moieties by EO_n from the chromatogram.

2.3 Results and Discussion

2.3.1 Characterization of POEA

The POEA technical mixtures were examined with two different mass spectrometry experiments. The first was a single quadrupole scan performed while infusing with a syringe pump and the second was a LC-MS method using an Acquity BEH column to provide separation for a single quadrupole scan. An example of a tandem mass spectrometry experiment, a product ion scan, was also performed by infusing with a syringe pump for a single homolog of POE (15) tallow amine.

2.3.1.1 POE (2) Tallow Amine

The simplest of the POEA technical mixtures tested, POE (2) tallow amine, consisted primarily of three ions: m/z 330.4, 356.4, and 358.4 as shown in the mass spectrum in Figure 2.2-A1. These are consistent with the theoretical $[M+H]^+$ ions of $C_{16}sEO_2$, $C_{18}uEO_2$, and $C_{18}sEO_2$ respectively. Table 2.3 shows the relative response for the three most abundant tallow moieties. There are two small peaks; m/z 302.4 that corresponds to $C_{14}sEO_2$ and m/z 328.4 that corresponds to $C_{16}uEO_2$. Using the assumption that the homologs all have equal responses,

Table 2.3 Percent relative response normalized to most intense ion for each POEA technical mixture

	POE (2) tallow amine				POE (5) tallow amine				POE (15) tallow amine			
	C _{16S}	C _{18u}	C _{18S}	C _{18S} *	C _{16S}	C _{18u}	C _{18S}	C _{18S} *	C _{16S}	C _{18u}	C _{18S}	C _{18S} *
EO2	61	100	58	55	6	11	5	5				
EO3	1	1	1	1	42	67	31	29				
EO4					77	100	49	45				
EO5					77	92	47	43				
EO6					69	88	47	43	8	34	8	6
EO7					72	94	54	49	11	45	11	8
EO8					58	72	43	38	24	60	21	17
EO9					34	40	24	21	46	75	36	30
EO10					16	17	10	9	69	91	51	44
EO11					6	6	4	3	85	95	58	49
EO12					2	2	1	1	86	100	63	53
EO13					1	1	<1	<1	87	96	65	54
EO14									81	89	64	53
EO15									73	77	58	48
EO16									59	61	48	39
EO17									45	45	34	27
EO18									32	30	24	19
EO19									20	19	15	12
EO20									12	11	10	8
EO21									8	7	6	5
EO22									4	4	4	3
EO23									1	1	2	2

* Corrected for theoretical isotopic contribution from C_{18u}

the relative response can be viewed as an indicator of the distribution of homologs in the technical mixture. Figure 2.2-A2 shows the mass spectrum at a higher cone voltage than that of Figure 2.2-A1. This higher voltage induces in-source conversions and fragments. The m/z of 312.4, 338.4, and 340.4 appear to represent dehydration products (loss of 18 mass units). The dehydration product could be formed by a loss of water from either two of the terminal alcohols on the ethoxylate chains or from the protonated amine and a terminal alcohol. For POE (2) tallow amine, these dehydration products do not separate in the chromatogram, Figure 2.3-A, indicating they are formed in the mass spectrometer. Order of elution is based on the tallow amine moiety, with the shorter chain lengths eluting first. There are two peaks in the chromatogram that correspond to the C18uEO2, which is likely a separation of cis and trans isomers. The separation of cis and trans isomers has been previously reported for oleic (cis) and elaidic (trans) acids using reverse phase liquid chromatography with the cis isomer eluting before the trans isomer.³²

2.3.1.2 POE (5) Tallow Amine

The POE (5) tallow amine mixture has additional complexity in comparison to POE (2) tallow amine. The tallow amine moiety remains the same but there is now a distribution of EO units. Figure 2.2-B1 shows the mass spectrum of POE (5) tallow amine with only the C₁₆u peaks labeled. Table 2.3 shows the relative response for the three most abundant tallow moieties. The mass spectrum acquired with the higher cone voltage, Figure 2.2-B2, shows dehydration products, but in much higher relative abundance than was seen in Figure 2.2-B1 with POE (2) tallow amine. The chromatogram shown in Figure 2.3-B reveals peaks that correspond to the dehydration products, indicating that these are an impurity in the mixture. The peaks labeled “a”

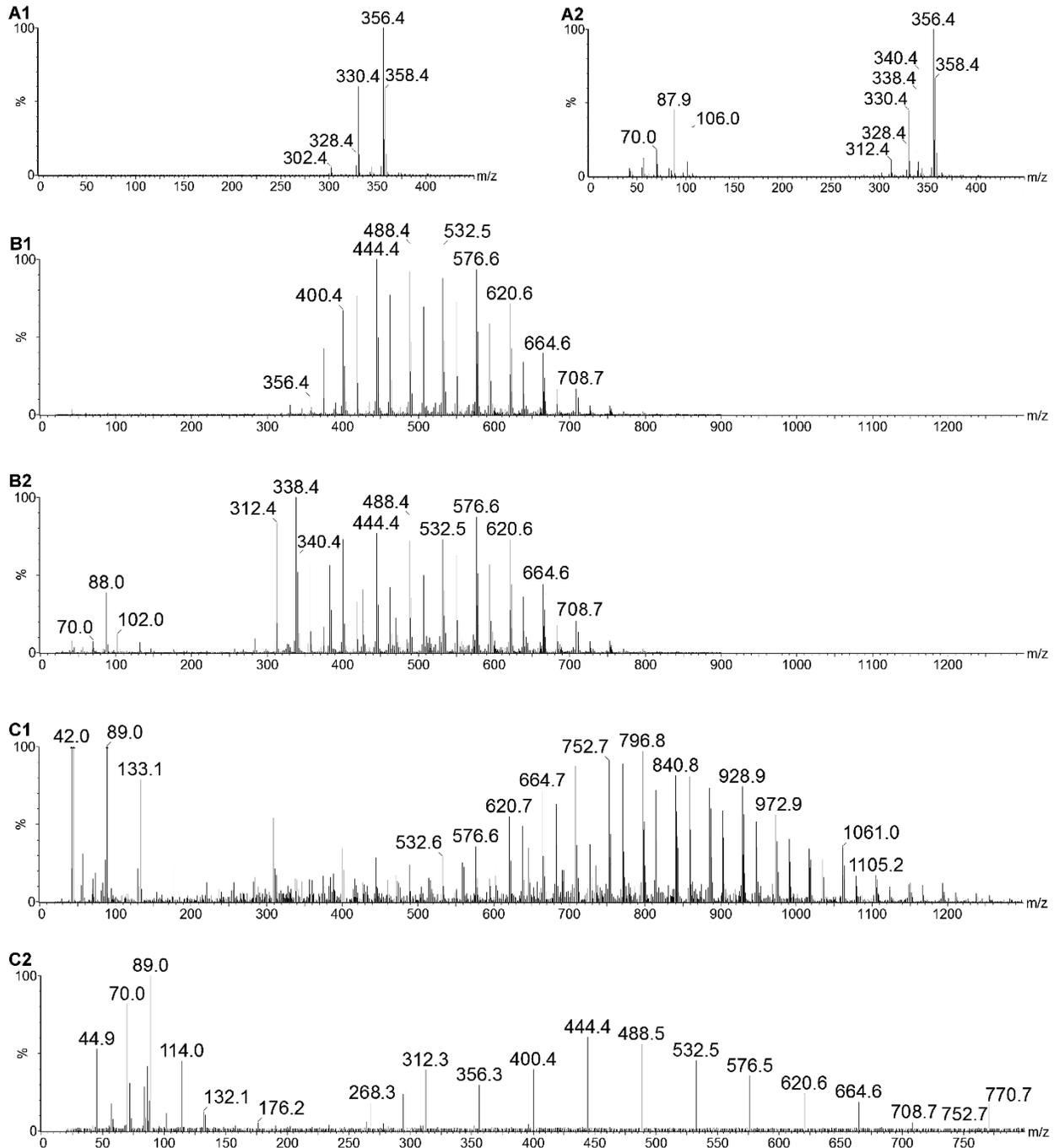


Figure 2.2 Mass spectra of POEA technical mixtures:

[A1] POE (2) tallow amine 25 V cone voltage, [A2] POE (2) tallow amine 65 V cone voltage,
 [B1] POE (5) tallow amine 25 V cone voltage, [B2] POE (5) tallow amine 65 V cone voltage,
 [C1] POE (15) tallow amine 65 V cone voltage, [C2] Product ion scan of C₁₆EO₁₂

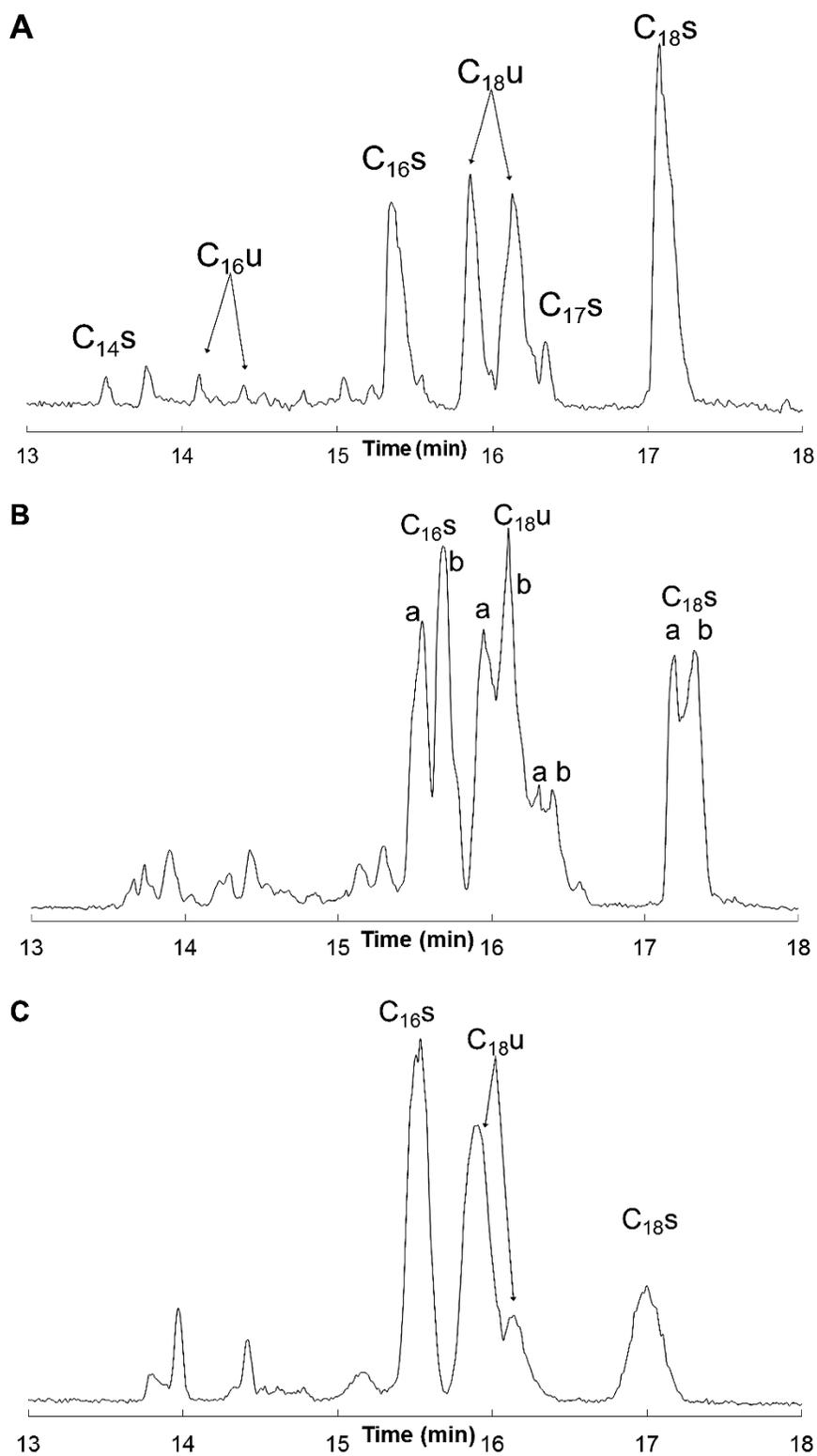


Figure 2.3 Total ion chromatograms of POEA technical mixtures on the Acquity BEH column: [A] POE (2) tallow amine, [B] POE (5) tallow amine, [C] POE (15) tallow amine

contain masses that correspond to the dehydration impurities and elute earlier than the “b” peaks.

2.3.1.3 POE (15) Tallow Amine and Ethomeen T/25

The mass spectra for POE (15) tallow amine and for Ethomeen T/25 were almost identical. Figure 2.2-C1 shows the mass spectrum for POE (15) tallow amine. Again, the tallow amine moiety is the same, but the range of EO units has expanded. Table 2.3 shows the relative response for the three most abundant tallow moieties. The Gaussian function fit to the EO distribution allows the distribution to be quantified and gives an average of 13.2 EO units (as opposed to the 15 implied by the name of the mixture) with a standard deviation of 3.4 and more than 95% of the distribution is in the range of EO₆₋₂₀. The chromatogram for POE (15) tallow amine, Figure 2.3-C, is similar to that of POE (2) tallow amine although the peaks are more broad. The additional peak width is due to small changes in elution time of homologs due to the large range of EO units.

To examine the fragmentation pattern of POEA (15) tallow amine, a precursor ion of m/z 770.7 ($[M+H]^+$ of C₁₆S EO₁₂) was chosen as an example for a product ion scan. Due to low and unstable response from the product ions, the data were summed for multiple scans over the course of one minute. Figure 2.2-C2 shows the mass spectrum of the product scan. The first loss (from m/z 770.7 to 752.7) is likely a loss of water. From the loss of water there is a series of products with losses of 44 mass units that are losses of EO units (from m/z 752.7 to 268.3). The loss of EO units includes losses of up to 11 EO units and there is no peak for a loss of all 12 of the possible EO units. Because the entire range of EO unit losses down to a single EO unit appears at a given fragmentation voltage, it would appear that the EO units are not being lost sequentially with increasing collision energy, but instead lengths of EO units are severed

(possibly cleavage of the carbon/nitrogen bond from the core of the molecule). A number of low mass products are also generated, including some that could potentially be used as a diagnostic fragment for POEA (e.g. m/z 70.0) although with low fragment size and low intensity. Often in an experiment such as this, product ions are generated with high signal intensity at a given fragmentation voltage and are suitable for single or multiple reaction monitoring (SRM or MRM) methods. As an example, alkyl dimethylbenzylammonium surfactants produce stable, intense product ions from each homolog that is a class specific diagnostic ion.³³ POEA appears to produce a range of low intensity products once enough energy begins fragmenting the precursor ion.

2.3.2 Comparison of Analytical Columns

The chromatographic comparison experiments were performed using the same parameters for each column except for flow rate (shown previously in Table 2.2). The columns used were chosen as a small sample of different column technologies because an exhaustive systematic survey of columns would be prohibitively time consuming and expensive. Figure 2.4 shows the chromatograms of POE (2) tallow amine for each of the columns tested (traces are offset by 3 minutes for clarity). POE (2) tallow amine was chosen because of the small number of homologs simplifies the chromatograms and the peak fitting. Table 2.4 shows values calculated from the peaks fitted from the total ion chromatogram. The retention time, full width at half maximum (FWHM), peak asymmetry at fifty percent peak height, and the theoretical plates are shown for the C₁₈S peak. The C₁₈S peak was chosen because it has a strong signal response and it is the furthest separated from the other peaks (Figure 2.3-A). The resolution

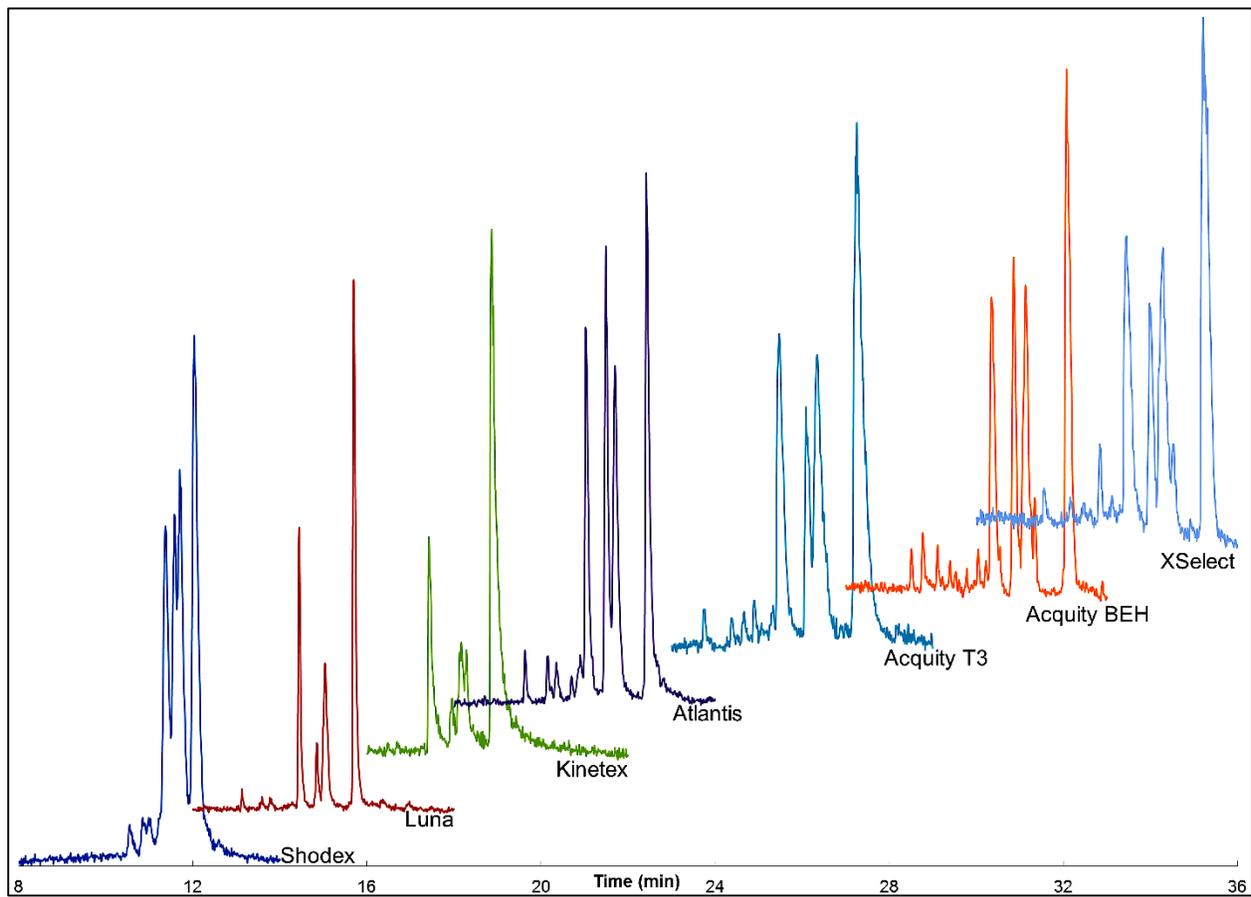


Figure 2.4 Total ion chromatograms of POE (2) tallow amine on different analytical columns.

Table 2.4 Calculated chromatographic values from fitted peaks for POE (2) tallow amine

	Shodex	Luna	Kinetex	Atlantis T3	Acquity T3	Acquity BEH	Xselect
r^2	0.989	0.988	0.975	0.978	0.988	0.988	0.976
Retention time	11.99	12.70	12.83	13.43	15.18	17.04	17.17
FWHM (min)	0.14	0.06	0.14	0.08	0.23	0.12	0.18
Asymmetry	1.76	1.21	4.53	1.19	2.83	2.29	2.80
Theoretical plates	42,000	260,000	46,000	150,000	25,000	100,000	52,000
Resolution	0.39	0.86	0.30	0.76	0.51	0.92	0.71

value shown is for the two peaks that correspond to C_{18u}. The asymmetry, theoretical plates, and resolution are related to the interaction of POEA to the stationary phase and are provided as the figures of merit for use of these columns to separate POEA. The Luna column shows the highest number of theoretical plates, followed by the Atlantis T3 and the Acquity BEH columns. This is a surprising result because increasing the number of theoretical plates is one of the reasons for using smaller diameter particles in UHPLC columns. The Atlantis T3 column showed the least amount of peak asymmetry, followed by the Luna column. The Acquity BEH column had the highest resolution of the cis/trans peaks, followed by the Luna, Atlantis T3, and the XSelect columns. Only the Acquity BEH and the XSelect columns were able to separate the C_{17s} peak from the C_{18u} peak under these conditions. The Acquity BEH column was chosen for the characterization of POEA technical mixtures and glyphosate formulations for a combination of the high number of theoretical plates, high resolution, and low flow rate (to minimize solvent use).

Figure 2.5-A shows the total ion chromatogram for POE (2) tallow amine, POE (5) tallow amine, and POE (15) tallow amine on the Shodex column. POE (2) tallow amine shows the same mode of separation on this column as it does for the other columns discussed. POE (5) tallow amine and POE (15) tallow amine appear in the total ion chromatogram as very broad peaks. The extracted ion chromatogram for C_{16s} tallow amine moiety of POE (5) tallow amine, Figure 2.5-B, reveals that these homologs are being separated, but not well resolved, with the larger homologs eluting first due to the size exclusion capability of this column. The homologs of the different tallow moieties then overlap, giving the appearance of a single broad peak in the total ion chromatogram.

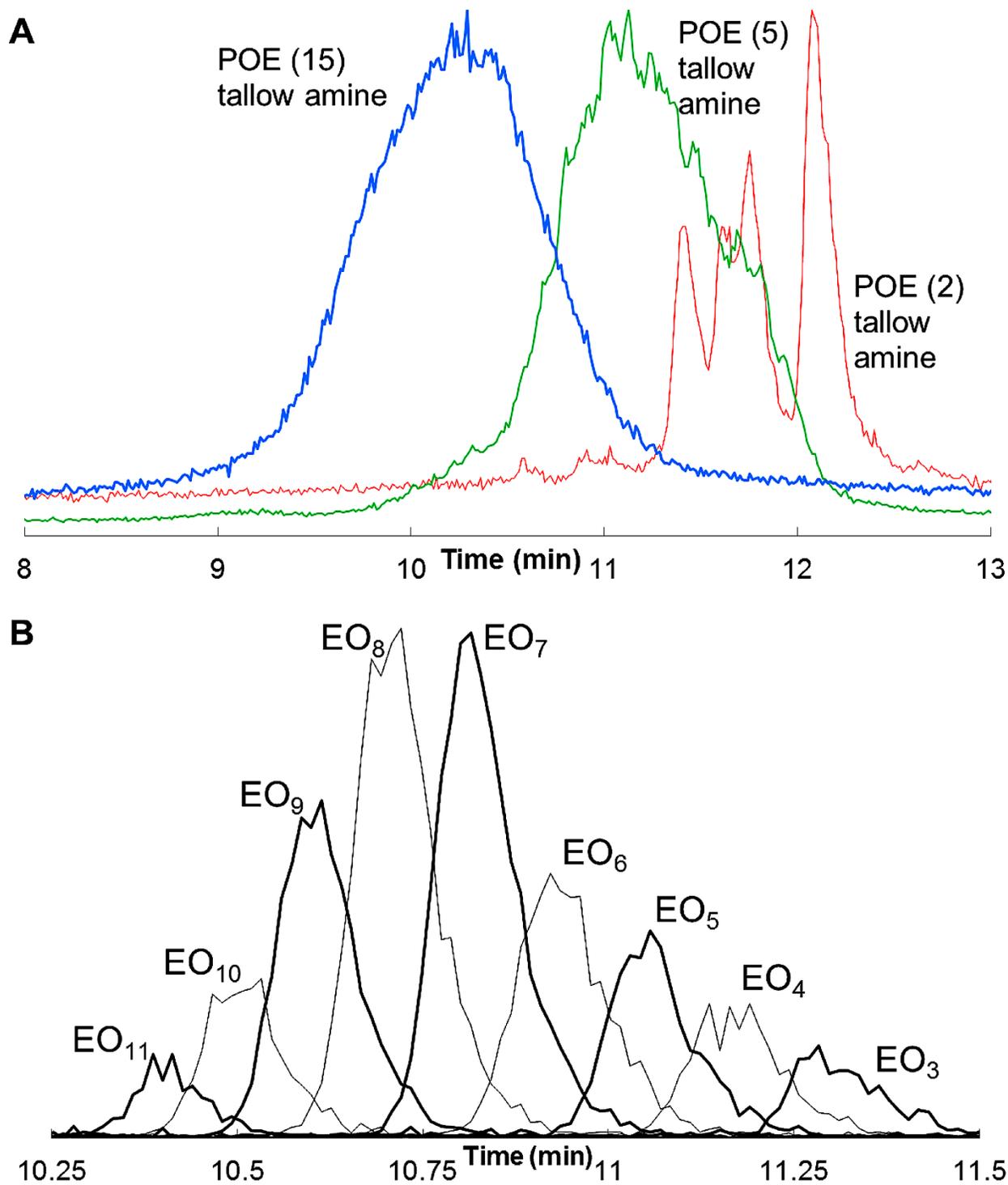
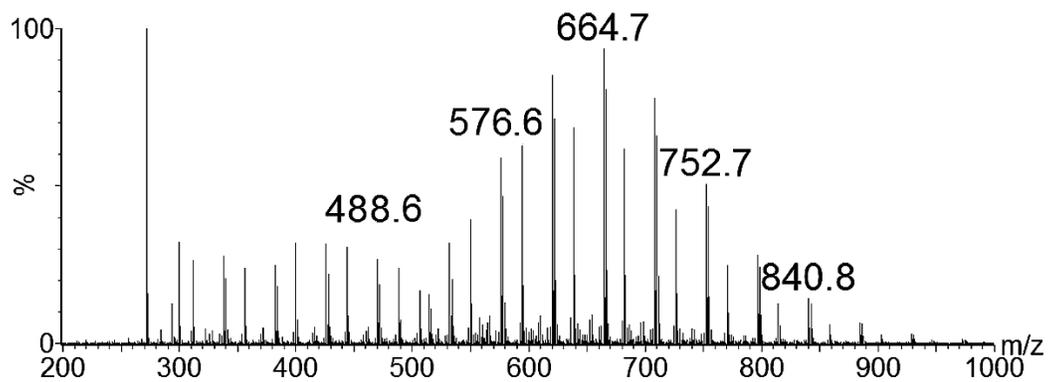


Figure 2.5 Chromatograms of POEA technical mixtures on the Shodex column:
 [A] Total ion chromatograms, [B] Extracted ion chromatogram of different EO lengths of C_{16S} POE (5) tallow amine.

2.3.3 Survey of commercial glyphosate formulations

The chromatograms and mass spectra of each commercial formulation were compared to those of the POEA technical mixtures. Of the agricultural formulations, only Buccaneer Plus and Touchdown Hitech show no indication of similarity to POEA technical mixtures. The negative response of Touchdown Hitech is in agreement with its labeling and MSDS which state that no surfactant is in the formulation. Buccaneer Plus may contain a different surfactant that is not compatible with the methods used in this study. Abundit Extra, AgriSolutions Cornerstone Plus, Glyphos XTRA, and Glyphogan Plus all have similar chromatography and mass spectra to that of POE (15) tallow amine. Touchdown Total matches the chromatography and mass spectrum of POE (5) tallow amine. The chromatograms for Durango DMA (Figure 2.6-B) and Roundup WeatherMAX match the expected chromatography for POEA. However, the distribution for these two formulations, shown in Table 2.5 and Figure 2.6-A, appears to be a different POEA technical mixture that has an EO distribution between that of POE (5) tallow amine and POE (15) tallow amine. Roundup PowerMAX does not contain POEA, but does appear to contain a similar ethoxylated surfactant sharing many $[M + H]^+$ ions with POEA which suggests an odd number of nitrogen atoms. The mass spectrum and total ion chromatogram of Roundup PowerMAX are shown in Figure 2.7. The chromatogram is clearly different than those for POEA technical mixtures; there are more large peaks and they appear at different retention times. Further research will need to be carried out to completely identify this surfactant.

A



B

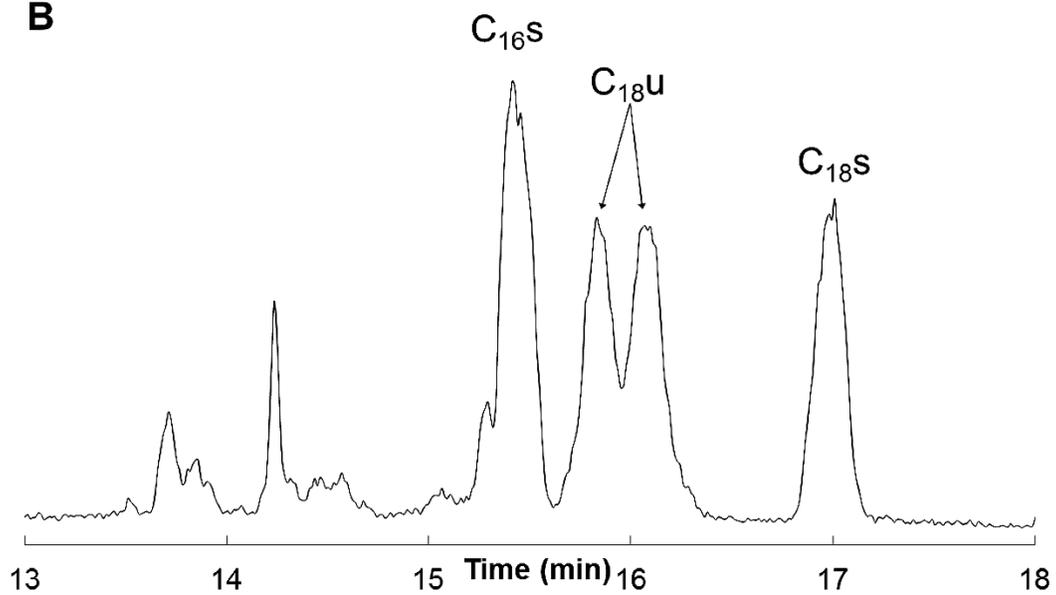
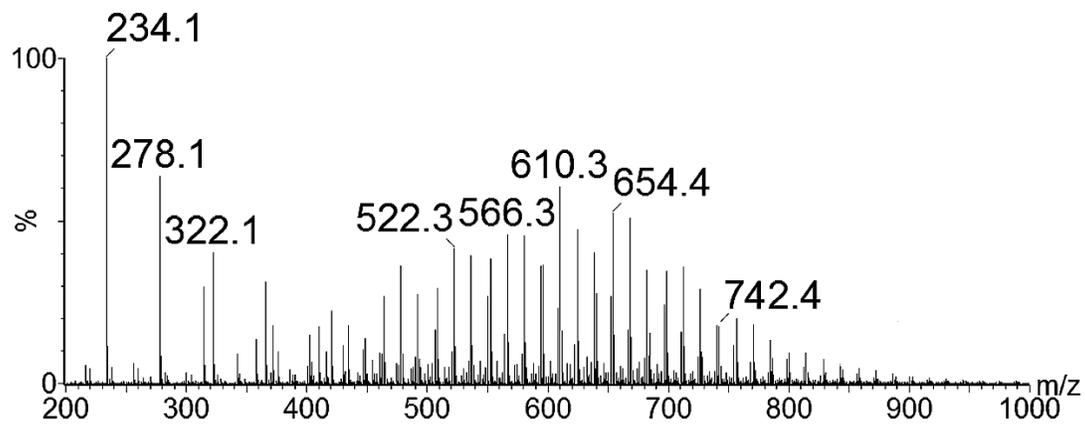


Figure 2.6 Analysis of Durango DMA:
[A] Mass spectrum (65 V cone voltage), [B] Total ion chromatogram on Acquity BEH column

A



B

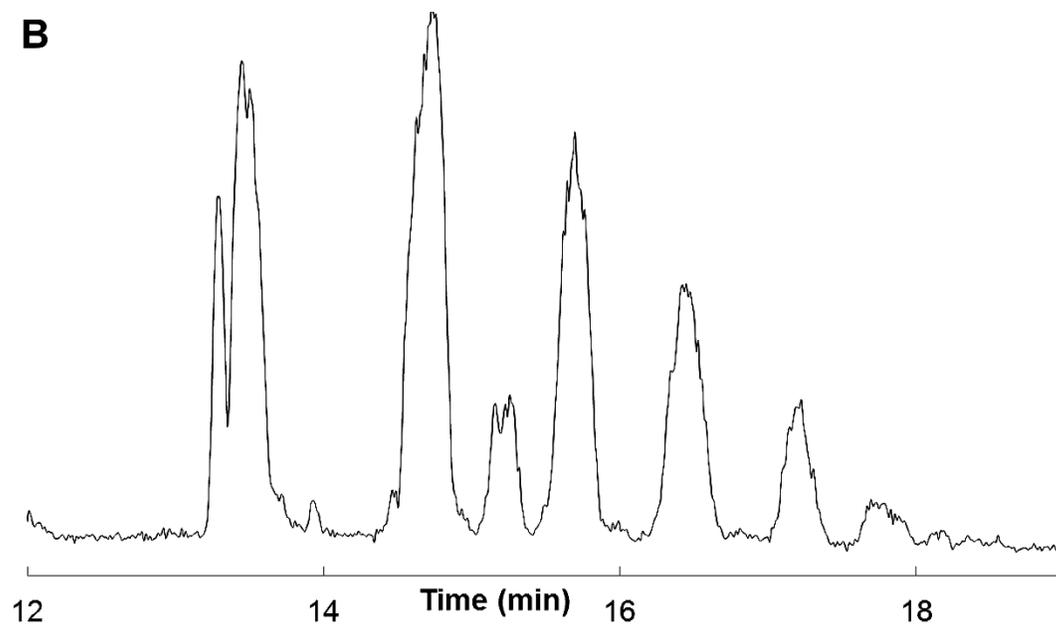


Figure 2.7 Analysis of Roundup PowerMAX:
[A] Mass spectrum (65 V cone voltage), [B] Total ion chromatogram on Acquity BEH column

Table 2.5 Percent relative response for Durango DMA normalized to most intense ion (C_{18u}EO₉)

	C _{16S}	C _{18u}	C _{18S}	C _{18S} *
EO ₂	<1	<1	1	1
EO ₃	1	34	2	1
EO ₄	2	33	4	3
EO ₅	5	25	8	7
EO ₆	18	34	22	20
EO ₇	42	63	50	46
EO ₈	67	91	76	70
EO ₉	73	100	86	79
EO ₁₀	66	83	70	63
EO ₁₁	45	54	46	41
EO ₁₂	26	30	26	23
EO ₁₃	13	15	13	11
EO ₁₄	6	7	6	5
EO ₁₅	3	3	3	3
EO ₁₆	1	1	1	1

* Corrected for theoretical isotopic contribution from C_{18u}

Four of the residential formulations tested positive for POEA; Ace Weed and Grass Killer, Ortho Groundclear Vegetation Killer Concentrate, Roundup Poison Ivy Plus, and Roundup Weed and Grass Killer Plus Concentrate. Ace Weed and Grass Killer appears to have a bimodal distribution of POEA (maxima near 8 EO and 16 EO). The remaining residential formulations contain a mixture like POE (15) tallow amine.

2.4 Conclusions

POEA is a surfactant additive in glyphosate formulations that has been shown to have deleterious effects on a variety of aquatic species. Characterization of the composition, distribution, and separation of POEA mixtures in technical and commercially available pesticide formulations was a first step before research on the extraction, degradation, sorption, and environmental occurrence of POEA can be conducted. Several glyphosate formulations were found to contain POEA but with varying ethoxylate distributions. Designing a comprehensive, quantitative UPHLC-ESI triple quadrupole method for POEA will be challenging because POEA contains a large number of homologs with a mixture of tallow moieties and EO units. Each homolog is a small fraction of the total and each homolog generates an entire series of fragment ions, none of which have a response that is much greater than any other. Trying to account for a large number of homologs that have multiple fragment ions that are not sensitive may imply that utilizing a more sensitive and faster switching triple quadrupole or a time-of-flight mass spectrometer to be better options. Finally, comparison of the measurable chromatographic properties of a variety of analytical HPLC, UHPLC, and core-shell columns with respect to the separation of POEA showed that column chemistry was the most important factor in the chromatography.

Thus, while the use of smaller diameter particles in stationary phases for UHPLC columns can allow for rapid separations that are difficult or impossible on traditional HPLC columns, the interaction of the analyte with the column stationary phase and mobile phase should be the primary consideration.

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Chapter 3: Polyoxyethylene Tallow Amine, a Glyphosate Formulation Adjuvant: Soil Adsorption Characteristics, Degradation Profile, and Occurrence on Selected Soils from Agricultural Fields in Iowa, Illinois, Indiana, Mississippi, and Missouri

Tush, D.; Meyer, M. T. Polyoxyethylene Tallow Amine, a Glyphosate Formulation Adjuvant: Soil Adsorption Characteristics, Degradation Profile, and Occurrence on Selected Soils from Agricultural Fields in Iowa, Illinois, Indiana, Mississippi, and Missouri. Submitted to *Environmental Science and Technology*.

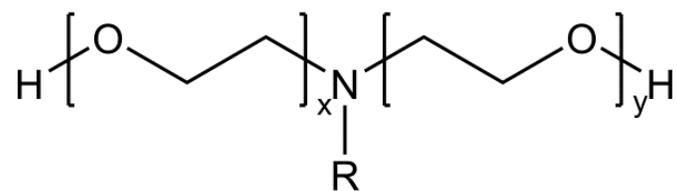
3.1 Introduction

Additives are commonly included with pesticides as formulations from the manufacturer or as tank mixes by the user and are referred to as inert ingredients and adjuvants respectively.¹ There is a dearth of knowledge on these additives, their persistence on the fields to which they are applied and also their transport, occurrence, and potential effects in the environment. Polyoxyethylene tallow amine (POEA) has been used as an additive in glyphosate formulations since the original Roundup[®] product was introduced in 1974 by Monsanto. Since then, glyphosate has become the most widely used herbicide in the world.^{2,3} An estimated 250 million pounds (> 110 million kg) was applied for agricultural use in the U.S. in 2011.⁴ Although Monsanto has removed the original POEA technical mixture from some of their formulations, other manufacturers have been producing glyphosate formulations since glyphosate went off-patent in 2000 and recent research has shown that several formulations still include POEA.⁵ POEA can comprise 15% by weight of glyphosate formulations and has the potential to be a widely distributed formulation additive that also has been shown to have potential effects that could be deleterious to water quality.

Surfactants are common additives in herbicide formulations that are used to modify the physical characteristics of the formulation (e.g. stickers and spreaders).⁶ POEA is specifically added to glyphosate formulations because it also greatly increases the herbicidal efficacy of

glyphosate.⁷⁻¹² Although glyphosate, is generally considered nontoxic to non-target organisms in the environment¹³, POEA has been demonstrated to be toxic to several aquatic species¹⁴⁻²². The U.S. Environmental Protection Agency classifies the toxicity of a substance to aquatic organisms by the LC50 value (the concentration at which 50% of the population does not survive). The classifications are “practically nontoxic” (> 100 mg/L), “slightly toxic” (>10 mg/L ≤ 100 mg/L), “moderately toxic” (> 1 mg/L ≤ 10 mg/L), “highly toxic” (≥ 0.1 mg/L ≤ 1 mg/L), and “very highly toxic” (< 0.1 mg/L).²³ The published LC50 values for POEA span the entire range of the scale except for “practically nontoxic”. The LC50 for channel catfish (*Ictalurus punctatus*) is 13 mg/L (“slightly toxic”)¹⁸ and for the water flea (*Daphnia magna*) is 0.097 mg/L (“very highly toxic”)¹⁴.

POEA is a surfactant synthesized from fatty acids. The fatty acids are converted to fatty amines and then reacted with ethylene oxide. Factors such as reaction time and temperature control the degree of ethoxylation. The resulting molecule is a tertiary amine core with one branch consisting of the tallow moiety and two branches of repeating ethoxylate units with terminal alcohol groups (Figure 3.1). Further details on the composition of POEA have been published.⁵ POEA is non-ionic in this form, but the nitrogen can be protonated in water. To refer to individual homologs and subsets thereof, the following naming convention will be used: $C_z(s/u)EO_n$, where z is the number of carbon atoms in the tallow moiety, s is a saturated tallow moiety, u is a mono-unsaturated tallow moiety, and n is the total number of ethoxylate units from the two branches. Available POEA technical mixtures differ by the average number of ethoxylate units. The original Roundup formulation included POEA under the name “MON 0818” which has an average of 15 ethoxylate units (CAS number: 61791-26-2).



R = tallow moiety

$$n = x + y$$

Figure 3.1 Structure of POEA. The tallow moiety is composed primarily of alkyl chains of 16 or 18 carbon chains that are either saturated or monounsaturated. The total number of ethoxylate groups (n) is equal to the sum of the number of ethoxylate groups (x and y).

The environmental fate and effects of glyphosate have been widely studied²⁴⁻²⁹, but there has been much less research published on POEA^{30,31}. There are also very few analytical methods for POEA in the literature^{31,32} and only one that analyzes for a wide spectrum of EO distributions for the most abundant tallow moieties⁵. The environmental effects of some other surfactants have been studied including alkylphenol ethoxylates because of the potential toxicity and endocrine disrupting properties of the alkylphenol ethoxylates degradation products such as nonylphenol³³⁻³⁵, linear alkylbenzene sulfonates because of their widespread use³⁶⁻³⁸, and organosilicone surfactants because they may be contributing to declining bee populations³⁹.

Because the primary use of POEA is as an additive in agricultural glyphosate formulations that are applied by spraying, the adsorption and degradation of POEA on soil are important considerations. Adsorption is the first indicator as to whether a contaminant will be transported from the point of application into the environment primarily in the dissolved or adsorbed phase. Batch adsorption isotherms are used to study this interaction. Adsorption isotherms involving a contaminant and soil are often nonlinear and the Freundlich equation is often used to model this phenomenon. Soil and sediment adsorption studies that have been modeled with the Freundlich equation⁴⁰ include surfactants such as polysorbate 80⁴¹ and hexadecyltrimethylammonium bromide⁴². The surfactants that are the most similar to POEA that have been studied are alcohol ethoxylate surfactants.⁴³⁻⁴⁶ There are few published studies available concerning the degradation of POEA and those examined the degradation of POEA on sewage or activated sludge.⁴⁷ These studies do not simulate the conditions or matrix for the degradation of POEA applied to an agricultural field.

The purpose of the research presented here is to determine the potential of POEA to be transported from the point of application into the environment and to assess the homolog

distribution and potential degradation of POEA on agricultural soils to which it was likely that glyphosate formulations had been applied. Batch adsorption isotherms were studied in a range of salt solutions, pH conditions, and soil types to determine how strongly POEA will adsorb to soil to assess whether POEA is more likely transported from fields in the dissolved or adsorbed phase. Soil samples from agricultural fields used for corn and soybean production from six states were analyzed to determine if POEA is potentially a widespread contaminant on agricultural soils where glyphosate formulations are commonly applied and if POEA persists on those soils.

3.2 Materials and Methods

The following is a brief description of the methods. For further details, refer to the method section in the Supporting Information.

3.2.1 Chemicals and Reagents

The POEA technical mixture used in all experiments was POE (15) tallow amine (Chem Service Inc., West Chester, PA); this is similar POEA technical mixture that is used in some glyphosate formulations (5). Acetonitrile was LC-MS grade from Burdick & Jackson (Muskegon, MI). Type II deionized water ($\geq 15 \text{ M}\Omega\text{-cm}$) was generated from tap water using a PURELAB Option-R (ELGA Labwater, Marlow, United Kingdom). Type II water was further treated to generate Type I deionized water ($\geq 18.2 \text{ M}\Omega\text{-cm}$, $< 1 \text{ ppb TOC}$) using a Nanopure Diamond TOC Life Science system (Barnstead|Thermolyne, Dubuque, IA). Formic acid (Optima LC/MS grade), acetic acid (Optima LC/MS grade), methanol (HPLC grade), sodium chloride (certified ACS), calcium chloride dihydrate (certified ACS), sodium carbonate

anhydrous (certified ACS), and Ottawa sand were obtained from Fisher Scientific (Fair Lawn, NJ).

3.2.2 Soils

Soil A was collected from a non-agricultural site near Fourmile Creek in Iowa. Soil B (Clean Sandy Soil) and Soil C (Clean Sandy Loam Soil) are reference standards obtained from Resource Technology Corporation (Laramie, WY). Soil characteristics for Soils A-C are presented in Table 3.1 in the supporting information.

A soil sample was collected from a local corn/soybean field (near Lawrence, KS) to examine soil from a corn/soybean field for the presence of POEA and to determine the distribution of POEA homologs. Twenty additional soil samples from five states were collected from corn/soybean fields to determine if the occurrence of POEA on agricultural soils may be widespread. The samples were all collected before the planting season. Sample locations and collection dates are detailed in Table 3.2 in the supporting information.

3.2.3 Adsorption Experiments

Adsorption experiments were carried out in 50-mL Pyrex centrifuge tubes with either a soil matrix solution to determine the loss of POEA to the centrifuge tube or a soil/water mixture in a 1:100 ratio. Soil A was used except where noted. The experiments were equilibrated for 24 hours and then centrifuged. An aliquot of the supernatant is then pipetted for analysis.

3.2.4 Soil Extraction Method

POEA was extracted from all soil samples by accelerated solvent extraction (ASE) using a modified version of a method developed by Krogh, et al. (31). The extraction was performed using a Dionex ASE 200 and the extracting solvent was 100 mM triethylamine/75 mM acetic acid in methanol. The extracts were evaporated under nitrogen and reconstituted in Type I water and vialled for analysis.

3.2.5 Analytical Methods

Analytical separation of POEA samples were performed using an Acquity UPLC H-Class Bio system with an XSelect HSS C18 2.5 μm 3.0 x 150 mm column (Waters, Milford, MA, USA). The aqueous mobile phase (A) was 0.3% formic acid in Type I water and the organic mobile phase (B) was 0.3% formic acid in acetonitrile. The detector for the POEA adsorption experiments was a Triple Quad 5500 system (AB Sciex, Framingham, MA) in positive electrospray ionization (ESI) mode. The detector for the analysis of field samples was an Agilent (Santa Clara, CA, USA) 6224 TOF system with a multimode source in positive ESI mode. The method used for the analysis of glyphosate samples is modified from a prior publication (48).

The quantitation of POEA without readily available pure standards and/or isotope labeled standards presents a number of challenges. Use of a technical mixture, POE (15) tallow amine, as the quantitation standard requires assumptions about the composition and purity. Here, the assumption is that POE (15) tallow amine is 90% C₁₆S, C₁₈u, and C₁₈S and that the unmeasured large mass homologs (EO > 22) are negligible. Because there are no standards available for each homolog (and that would be impractical), it is assumed that every homolog gives an equal

instrument response. The equal instrument response assumption was used for a similar analysis of nonylphenol ethoxylates where there was also no standards for the individual homologs (33). Due to the similarity of the POEA homologs, the assumption that each gives a similar instrument response is plausible. TOF-MS scan data for POE (15) tallow amine then allows the determination of the fraction of each homolog in the mixture. Quantitation of POEA for the absorptions experiments used a linear calibration curve. The calibration curve was made in a matrix designed to match that of the adsorption experiment samples. Because it was not known whether POEA would be detected in soil samples from fields to which glyphosate was likely applied, it was decided that it was more important to modify an existing soil extraction method to assess whether POEA occurs and how the homolog distribution patterns might appear. With no suitable internal standards available, quantitation of POEA on environmental samples using a standard curve is not feasible because of uncertainty in the results caused by the matrix of the samples.

3.3 Results and Discussion

3.3.1 Adsorption of POEA to Soil

The adsorption of interest is the partitioning of POEA between the aqueous and soil phases. Only the dissolved POEA concentration in the aqueous phase is readily measurable with the techniques presented here, and so the following assumptions about the mass balance of POEA are used. First, after 24 hr the concentration of POEA in the aqueous phase (C_e) is in equilibrium with the concentration of POEA in the soil component (q_e). This equilibration time is not likely to be enough to be strictly at equilibrium due to the complex nature of contaminants binding to soil which will often take place through a two-step process (transport to surface

adsorption sites and diffusion into internal sites), the first kinetically fast and the second slow.⁴⁰ The 24 hour equilibration time is used here as a balance between kinetics, equilibrium, and possible degradation. Second, the concentration of POEA in the aqueous phase is also in equilibrium with the concentration of POEA adsorbed to the centrifuge tube. The amount of POEA adsorbed to the soil then can be calculated by subtracting the amount remaining in solution and the amount adsorbed to the centrifuge tube from the initial amount of POEA in the system.

In many adsorption experiments, only the solution and solids are considered in the mass balance with the assumption that the sorption of the analyte of interest to the sorption vessel is negligible. Because of the nature of surfactants, examination of the loss of POEA to adsorption to the centrifuge tube was necessary. The POEA-centrifuge tube adsorption experiments were designed to mimic the solution and glass interaction that would occur in the soil adsorption experiments as closely as was feasible. A soil matrix solution was made for each variation of soil adsorption experiment and was used to determine if each matrix had an effect on the adsorption of POEA to the centrifuge tube. Adsorption curves were generated for every homolog measured to estimate the loss of POEA to the centrifuge tube as a part of the mass balance in the POEA-soil adsorption experiments. Three representative curves with the loss of POEA homologs to the centrifuge tube are shown in Figure 3.2. These curves are used to estimate the mass of each POEA homolog adsorbed to the centrifuges tubes based on the equilibrium aqueous concentration.

The adsorption isotherms for the homologs of POEA are nonlinear and so were modeled with the Freundlich equation. The Freundlich equation an empirical model used to study non-linear isotherms and is expressed as follows⁴⁰:

$$q = K_F C^{1/n}$$

Where q is the concentration of the contaminant in the soil, K_F is the Freundlich constant, C is the concentration of the contaminant in water, and $1/n$ is a parameter that describes the distribution of energies relating to the adsorption. When $1/n = 1$, the curve is linear and K_F is directly analogous to a typical linear distribution constant (K_d). When $1/n \neq 1$, the isotherm is nonlinear. To calculate $1/n$ and K_F , the Freundlich equation can be written in a linear form:

$$\text{Log } q = (1/n) \log C + \log K_F$$

Because the Freundlich equation is an empirical model and the values returned from the batch isotherms are unit dependent, comparing results from disparate experimental systems (e.g. experiments performed in different laboratories) is difficult. To provide a point of reference to a well-studied compound, the adsorption experiment was performed in 0.01 M sodium chloride solution with glyphosate in place of POEA with no other experimental setup changes. The $1/n$ and K_F for glyphosate were 1.0013 ± 0.0001 and 98.49 ± 0.02 respectively ($n = 3$). Sodium chloride was used in lieu of calcium chloride, the standard ionic strength additive recommended by the Organization for Economic Co-operation and Development (OECD)⁴⁹, because of the effect calcium chloride has on the adsorption of POEA (described in the next section).

3.3.2 Effects of Salt Content on the Adsorption of POEA

Adsorption and Freundlich isotherms for three representative homologs of POEA in a 0.01 M sodium chloride solution are shown in Figure 3.3. The adsorption isotherms are nonlinear over the concentration ranges studied and the convex shape of the curves indicates that POEA binds cooperatively (i.e. the more POEA available to the system, the greater the fraction adsorbed to the soil). This increased fraction of POEA adsorbing to the soil could be because surfactants are known to form complex structures—such as self-assembled monolayers, bilayers, and micelles. The Freundlich isotherms are linear and the adsorption characteristics of each homolog are different. The Freundlich parameter ($1/n$) and Freundlich constant (K_F) are determined from the slope and the intercept of these lines respectively.

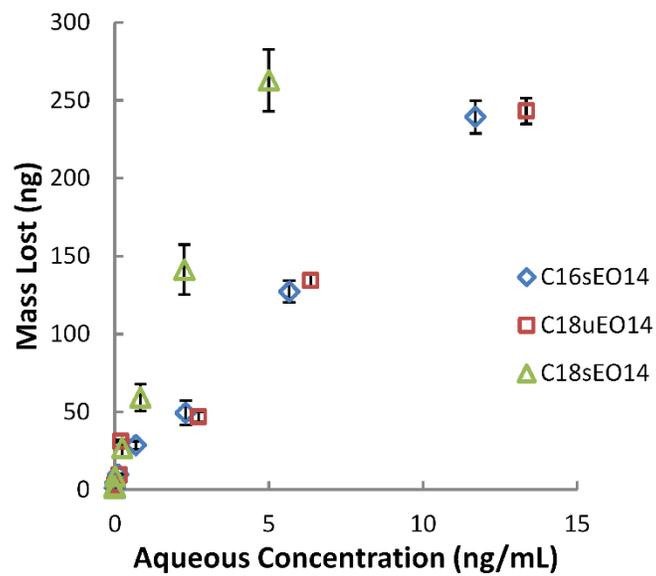


Figure 3.2 Examples of adsorption isotherms to estimate mass of POEA lost to the experimental system (Pyrex centrifuge tube) in 0.01 M calcium chloride.

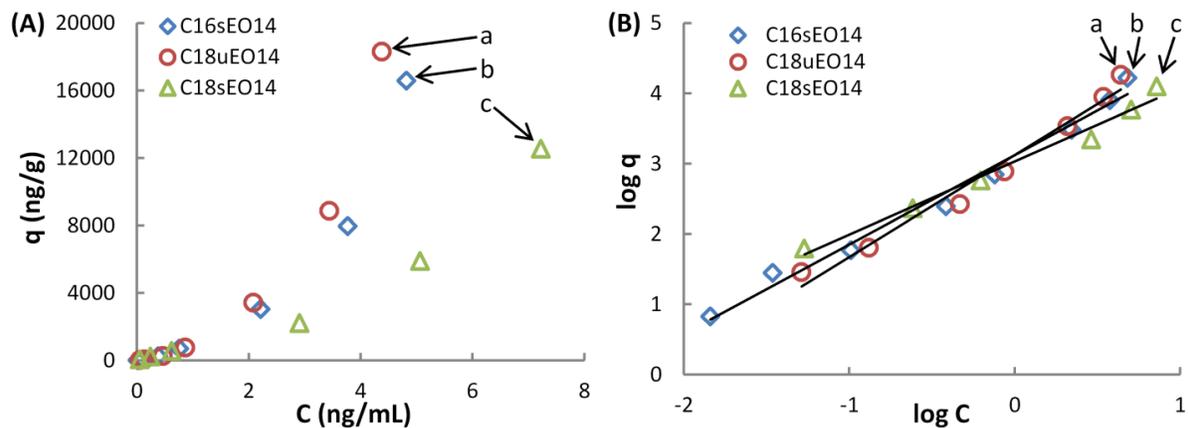


Figure 3.3 Adsorption isotherms (A) and the corresponding Freundlich isotherm (B) for three homologs in 0.01 M sodium chloride. The equations and R2 values for the linear regressions are: C16sEO14 ($y = 1.272x + 3.123$, 0.985); C18uEO14 ($y = 1.450x + 3.124$, 0.978); C18sEO14 ($y = 1.040x + 3.033$, 0.982). Points labeled a-c represent the same points on both isotherms and have POEA distributed on a mass basis as follows: (a) 95% on the soil, 3% in solution, and 2% adsorbed to the reaction vessel; (b) 96%, 2%, and 2%; and (c) 86%, 5%, and 9%.

A summary of $1/n$ and K_F for each homolog averaged for three replicates from the 0.01 M sodium chloride experiments is shown in Figure 3.4. The values of K_F for all homologs of POEA are greater than that of glyphosate by factors ranging from 5.8 to 980. The $1/n$ values show a small amount of difference among the homologs, with the C_{18u} homologs having the highest values and the C_{18s} homologs being the nearest to 1. This difference occurs primarily at EO between 5 and 18. The K_F values show a much greater difference among the homologs, with the maximum K_F over 150 times greater than the minimum. For $EO < 15$ the C_{18u} tallow moiety has the highest values followed by C_{16s} . The number of ethoxylate units has an inverse relationship to the binding constant (i.e. the lower the number of ethoxylate units the more of that homolog is adsorbed). For the C_{18s} tallow moiety K_F values are more than an order of magnitude higher and the difference in K_F values for the C_{18u} tallow moiety is more than two orders of magnitude for some homologs with a low number of ethoxylate units than for those with higher ethoxylate units. This inverse relation is opposite of that reported for the alcohol ethoxylate for which it has been suggested that the alcohol ethoxylate surfactants bind to the soil through the ethoxylate units⁴³ and suggests that the binding interaction of POEA to the soil under these conditions is through the tertiary amine group rather than the ethoxylate groups. The general decrease in K_F with increasing number of ethoxylate units is likely related to the greater water solubility of those homologs.⁴⁴ This indicates that under these conditions and time frames that homologs with shorter chains are adsorbed more strongly and could be transported less. Figures for $1/n$ and K_F for each homolog for all the conditions tested appear as Figures 3.10 to 3.17 in the supporting information.

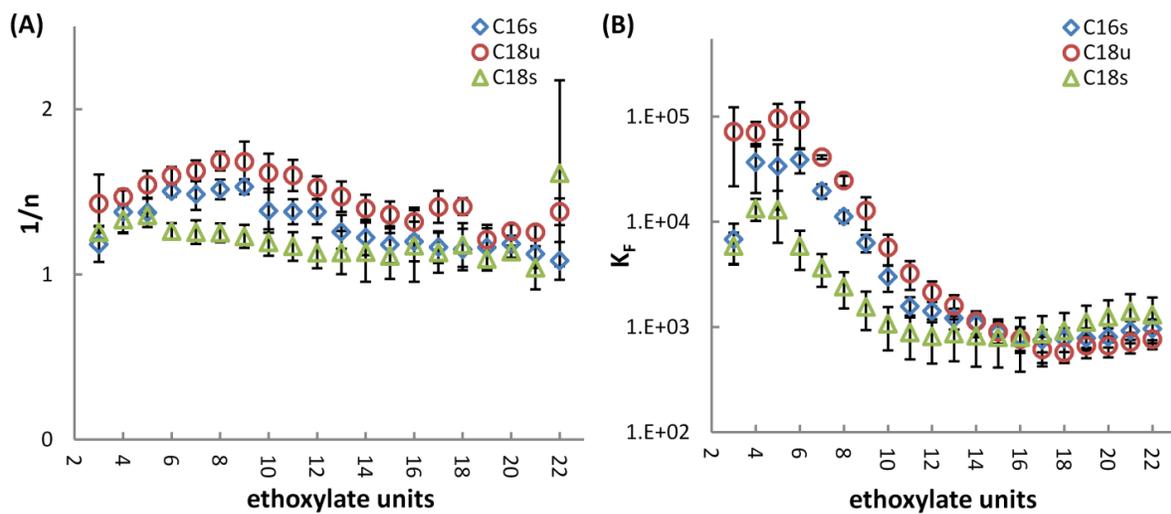


Figure 3.4 Freundlich values for individual homologs in 0.01 M sodium chloride.

To examine the adsorption of POEA on the basis of the tallow moiety and the number of ethoxylate groups, the $1/n$ and K_F was averaged into these groups. Homologs with ethoxylate units from 3 to 19 were included because for some experiments the $EO > 19$ fell below the limits of quantitation. The effect of salt content on $1/n$ and K_F is shown in Figure 3.5. The $1/n$ parameter shows little difference when grouped by either the tallow moiety or by the number of ethoxylate units. Because the K_F values for experiments in Type I water and 0.01 M sodium chloride are similar, the addition of more sodium chloride to the system also has little effect. The Type I water and the 0.01 M sodium chloride values are negligibly different and this is likely because the soil has sodium ions in many of the cation exchange sites.

The addition of 0.01 M calcium chloride to the system increases the K_F values and further increasing the concentration of calcium chloride to 0.1 M continues to increase the K_F values. The increase in the binding constant is larger for homologs with higher numbers of ethoxylate units. One explanation for this phenomenon is the calcium ions are displacing sodium ions from the soil cation exchange sites. The calcium ions are both more highly charged and have a larger ionic radius which likely increases the electrostatic interaction with the lone pair of electrons on the central nitrogen of POEA and might allow the ethoxylate units to act as a chelator. Adding additional calcium ions to the experiment would continue to increase the POEA binding until all of the cation exchange sites become saturated with calcium ions.

The higher affinity of POEA to soil conferred by adding calcium ions raises an interesting point with respect to the OECD guidelines for performing batch adsorption isotherms. These guidelines call for the use of 0.01 M calcium chloride solutions to provide ionic strength to the experiment.⁴⁹ While neither sodium chloride nor calcium chloride are good surrogates for relevant environmental conditions (low ionic strength rainwater), for POEA the calcium chloride

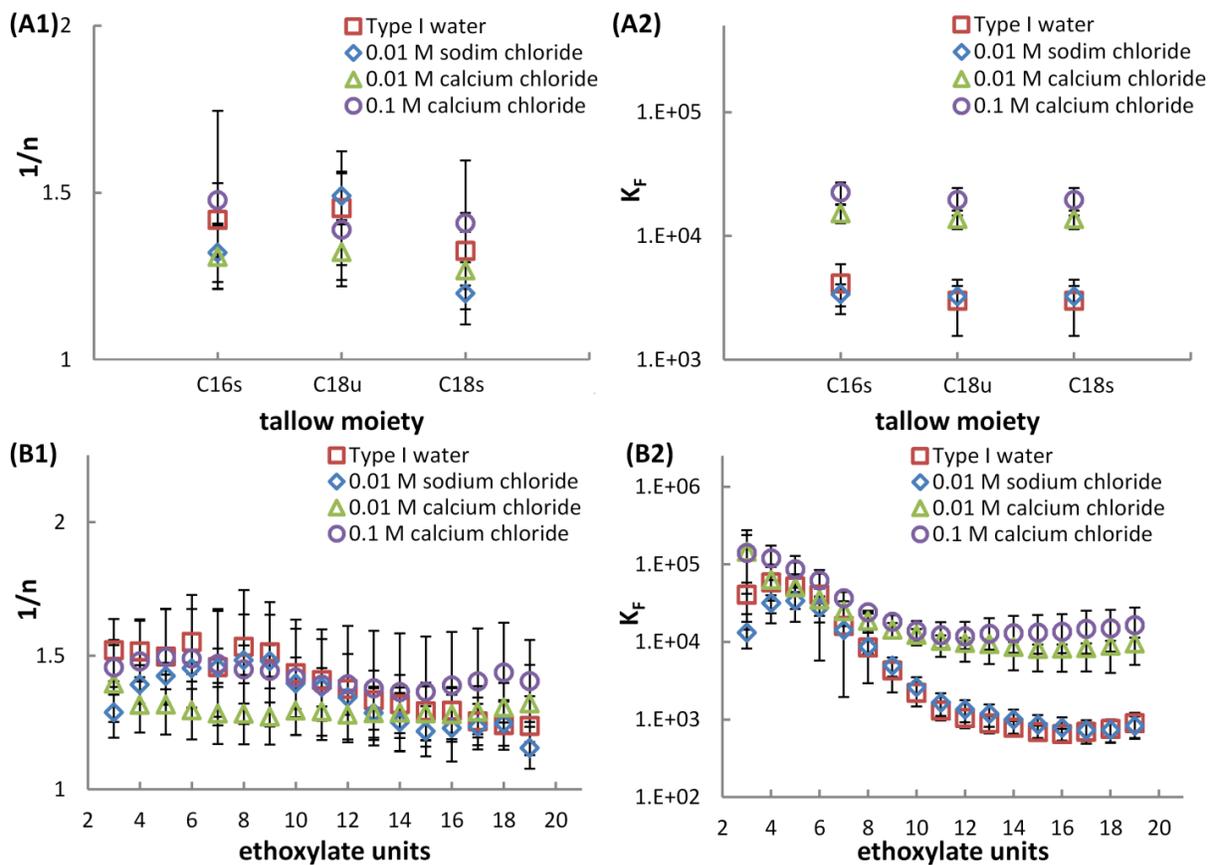


Figure 3.5 Average Freundlich values in Type I water and three salt solutions. (A1) $1/n$ grouped by tallow moiety. (A2) K_F grouped by tallow moiety. (B1) $1/n$ grouped by number of ethoxylate units. (B2) K_F grouped by number of ethoxylate units.

result is less representative because of the greatly increased adsorption. The use of calcium chloride in every experimental variation would have skewed the adsorption characteristics of POEA. Furthermore, the use of calcium chloride would be a poor choice for experiments where calcium ions interfere with either the analysis or with the phenomenon being studied, such as tetracycline which shows increased adsorption to montmorillonite clay in the presence of calcium ions⁵⁰.

3.3.3 Effects of pH on the Adsorption of POEA

The effect of pH on $1/n$ and K_F is shown in Figure 3.6. Under basic conditions (pH=10.8), $1/n$ is slightly lower than in the 0.01 M sodium chloride (pH=8.3), particularly in the low EO range and the C₁₈u tallow moiety. K_F , however, shows almost no difference when averaged by tallow moiety and only a slightly different curve averaged by EO. The changes in adsorption are much greater under acidic conditions (pH=4.2) where $1/n$ is less than 1 for all groupings, which shows a reversal in the curvature of the non-linear adsorption isotherm from convex to concave when compared to all other conditions tested. This indicates a change in the way POEA is binding to the soil and this change also is reflected in the K_F values. The binding of the homologs with a low number of ethoxylate units is not much different from the other conditions, but as the number of ethoxylate units increases, so does the value of K_F . This is the only case tested for POEA in which the relationship of ethoxylate units to K_F mimics the alcohol ethoxylate surfactants adsorption behavior of increased binding with increased numbers of ethoxylate units.⁴³ This indicates that under these conditions, POEA no longer binds to soil primarily through the tertiary amine group but the ethoxylate groups are also involved in binding POEA to the soil. It may be that the protonated amine group of POEA interacts with the cation

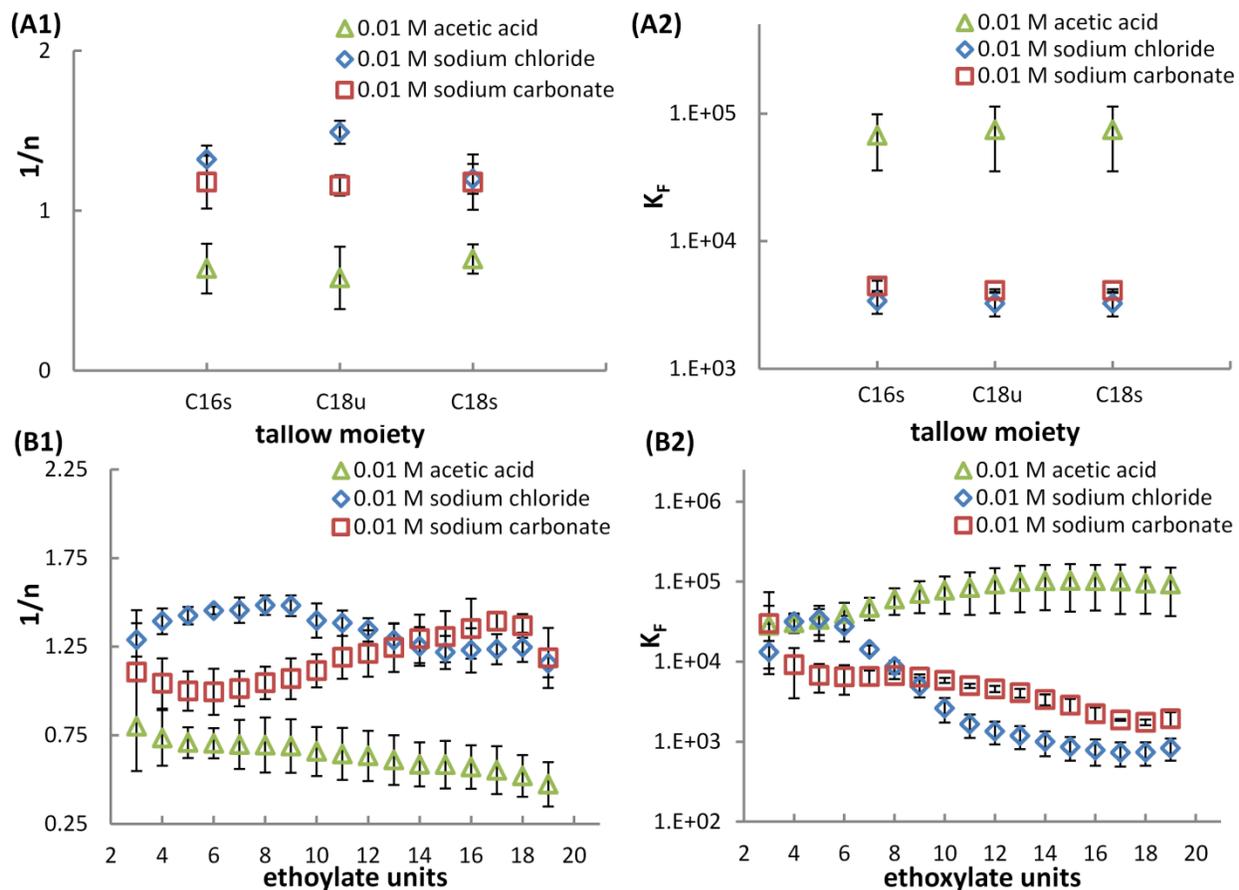


Figure 3.6 Average Freundlich values under three pH conditions. The final pH values for the acetic acid, sodium chloride, and sodium carbonate experiments were 4.2, 8.3, and 10.8 respectively. (A1) $1/n$ grouped by tallow moiety. (A2) K_F grouped by tallow moiety. (B1) $1/n$ grouped by number of ethoxylate units. (B2) K_F grouped by number of ethoxylate units.

exchange sites of the soil and the electron density of the oxygen atoms in the ethoxylate groups interacts with the positively charged surface of the soil.

3.3.4 Total POEA Freundlich Averages

Instead of averaging into groups (by tallow or EO), the data can be averaged for the entire experiment (EO from 3 to 19, all tallow moieties) to examine the adsorption properties of the bulk technical mixture. A summary of $1/n$ and K_F for each set of conditions tested is shown in Figure 3.7. The values for $1/n$ are similar for all experiments for the average of all homologs except under acidic conditions which is the only case where $1/n < 1$. The K_F values vary greatly between the experiments, with 0.01 M acetic acid (pH=4.2) having the highest value.

Experiments that include calcium chloride are the next highest. The 0.01 M acetic acid experiment has the most extreme value for both parameters (lowest $1/n$ and highest K_F).

This series of experiments show that POEA binds strongly to soil under every condition tested in this study. These bench tests provide a framework for future research to study the fate and transport of POEA in agricultural field conditions which are manipulated in many different ways (e.g., liming, fertilizing). Treatment of soil with agricultural lime (primarily calcium carbonate) might increase the binding of POEA because of the addition of calcium ions whereas ammonia based fertilizers may lower POEA binding due to competition for binding sites (with additional effects on binding because of changes in pH in both examples). Because of the adsorption results, we hypothesize that if POEA is present on agricultural fields it will be in the shallow soil.

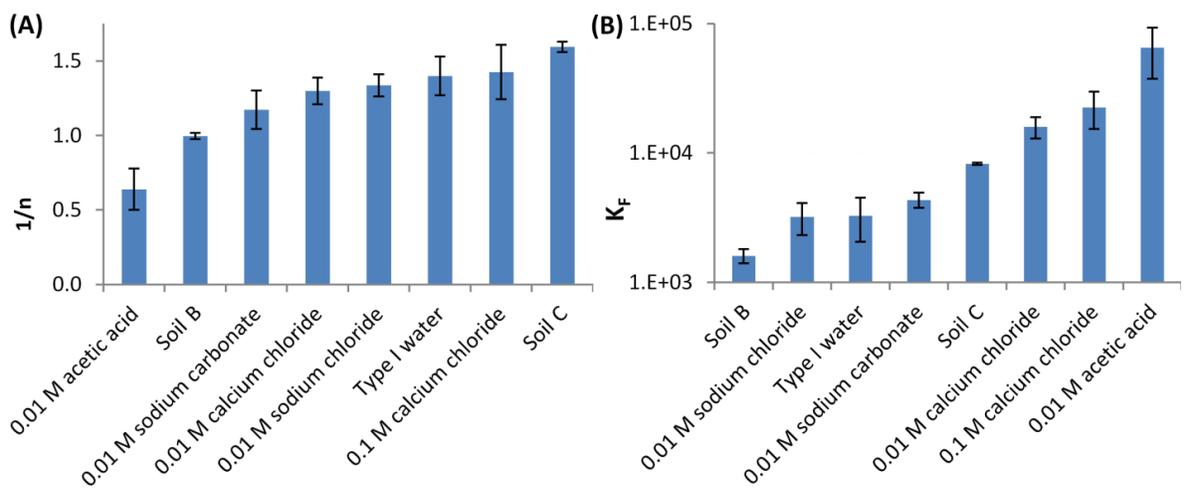


Figure 3.7 Summary of average Freundlich values.

3.3.5 Field Samples

The first soil sample examined was collected from the top 10 cm of an agricultural field near Lawrence, KS in late October of 2014—likely one or more months after the last application of glyphosate. An unspiked and spiked (~100 ng/g POE (15) tallow amine) aliquot of this sample was extracted and analyzed. POEA was detected on the unspiked sample (Figure 3.8-A) indicating that POEA had been applied to the field—most likely as part of a glyphosate formulation. There are two interesting features of this distribution. First, the homologs with the C₁₈u tallow moiety have a much lower instrument response than the other tallow moieties. The distribution of POEA on the spiked sample (Figure 3.8-B) further indicates the low response of the C₁₈u tallow moiety is not an artifact of the analytical method, suggesting that the C₁₈u tallow moiety degrades more rapidly in the field environment than do the other homologs. This degradation might be the cleavage of the double bond in the unsaturated tallow moiety through photodegradation or biological rancidification. Second, the ethoxylate distribution is centered at a lower number of ethoxylate units than the spiked sample. Previous work has shown that POEA technical mixtures with lower number of ethoxylate units are used in glyphosate formulations⁵ and that there are degradation processes that will shift ethoxylate distributions to lower masses⁵¹. Without knowing what glyphosate formulations were applied to this field it is not possible to determine if this shift in the POEA distribution to lower masses observed in the samples is a degradation process as seen in other ethoxylated surfactants⁵¹. The presence of POEA on the Lawrence soil sample prompted the authors to facilitate the collection of a small set of samples from several states to determine if POEA is a widespread contaminant on fields used to grow corn or soybeans. If POEA is a widespread contaminant, the use of further resources to develop quantitative methods for the analysis of POEA becomes necessary.

Soil samples from two non-adjacent fields, that were known to rotate between corn and soybeans, in five states (Iowa, Illinois, Indiana, Mississippi, and Missouri) were collected and analyzed (Table 3.2). POEA was detected in each of the samples analyzed. This is the first study to indicate that POEA is a common contaminant on the majority of soils to which glyphosate is applied. The samples were collected between February and mid-March 2015, well after the last application of glyphosate would have occurred but before another glyphosate application would have occurred—with the possible exception of Mississippi. Thus, the occurrence of POEA suggests that it persists on the soil into the following growing season and potentially longer. Representative POEA distributions are shown for 2 samples in Figure 3.9 and the remaining samples are included in the supplementary information as Figures 3.19 to 3.23. All the distributions show the same low level presence of the C_{18u} homologs observed from the Lawrence sample discussed in the weathering section above. Further there are two distinct types of distributions on these samples. The first distribution type is a peak with a single maximum number of ethoxylate groups (A) and the second has a broader peak (B). The broadening of the distribution may be from the use of different POEA technical mixtures in the applied glyphosate formulations. The loss of homologs with an unsaturated tallow moiety and the variations in the number of ethoxylate groups observed in POEA from these samples indicate the importance of the use of analytical methods that measure as many homologs as possible, because changes in the distribution may be missed by methods that use only measure a few selected homologs. This will be especially critical for the development of methods to quantitate POEA.

Although this study had a relatively small sample size, the presence of POEA on all 21 field samples analyzed indicates that the occurrence of POEA on soils is probably pervasive where glyphosate is applied. Further, the homologs with unsaturated tallow moieties are more

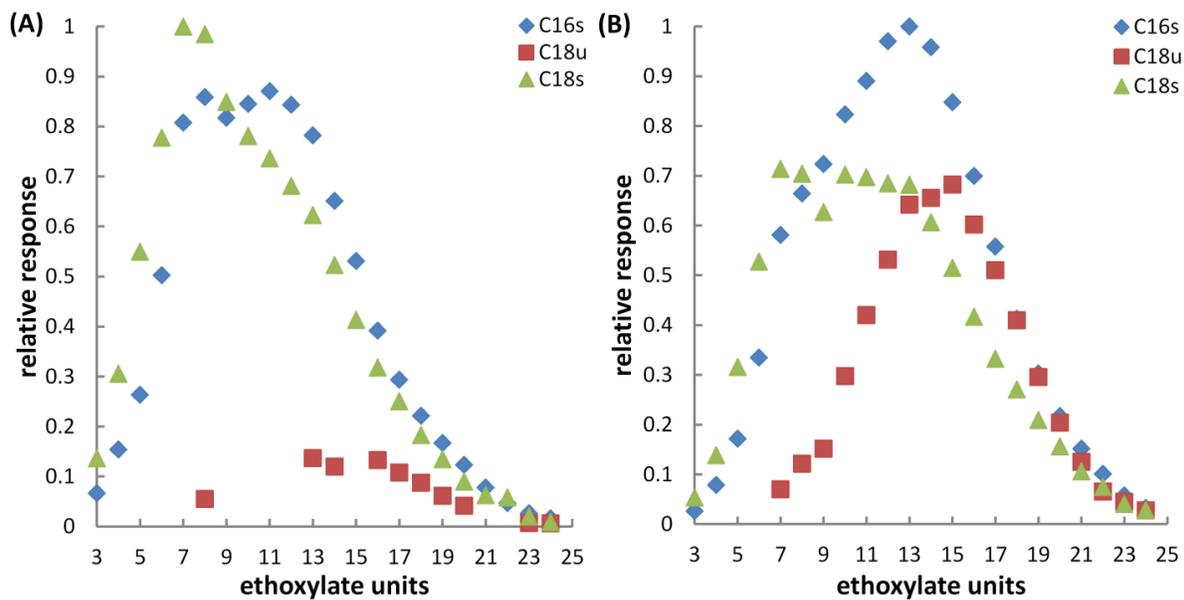


Figure 3.8 POEA distributions in extracts from an agricultural soil from near Lawrence, KS. (A) Extracted with no spike. (B) Spiked and then extracted. Responses have been normalized to the highest response of each distribution.

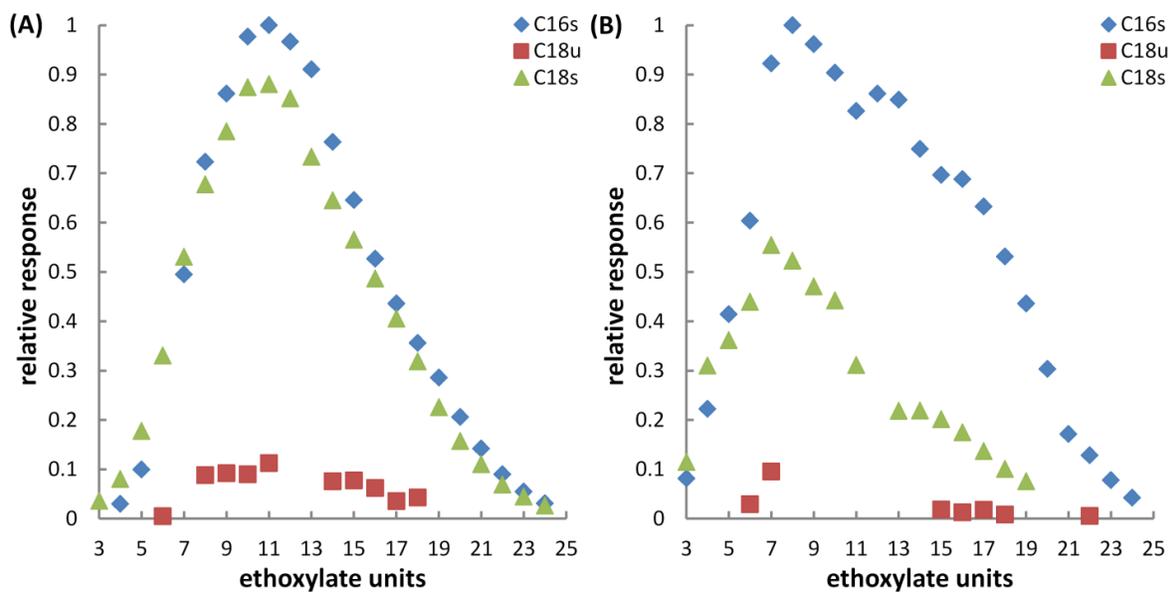


Figure 3.9 Representative POEA distributions in extracts from agricultural soils. (A) Sample #1 from Iowa. (B) Sample #5 from Illinois. Responses have been normalized to the highest response of each distribution.

prone to degradation leaving the remaining saturated tallow homologs bound to the soil until the next application. The results also indicate that the saturated homologs are relatively slow to degrade with the number of ethoxylate groups shifting the POEA distribution to lower molecular weights over time. The adsorption studies suggest that the movement of POEA away from the application site would be primarily limited to erosion of the contaminated soil and that the small percentage of POEA that would be transported in the dissolved phase may be redistributed between the suspended and bottom sediment. The persistence of POEA on the soil until the next application soil may shift equilibrium or kinetic conditions and provide the means for POEA to be transported in the dissolved phase as well. The adsorption data also suggests that POEA would not be readily transported through the unsaturated zone. Because of the potential widespread occurrence of POEA that the results from this study implies, the development of quantitative methods to measure the concentration of POEA in soil and sediment are needed. Further, an examination of the sediment of streams and rivers in agricultural areas where glyphosate is regularly applied is needed to determine if POEA is transported from the field into the environment.

3.4 Supporting Information

3.4.1 Methods

3.4.1.1 Sample Collection

The top 15 cm of soil from each field was subsampled from at least 3 locations using a clean stainless steel trowel (or similar tool) into 250 mL wide mouth glass jars. The soil samples were shipped overnight on ice and frozen (-10 °C) until processed. Soil A was air dried and

passed through a 2 mm sieve before use and was sent to the Soil Testing Laboratory at Kansas State University for analysis of the soil characteristics.

3.4.1.2 Laboratory Glassware

All reusable glassware was cleaned by prewashing in hot tap water; cleaning in a bath of Type II water with Contrex CF detergent; triple rinsing in hot tap water, Type II water, and Type I water; and finally rinsing with methanol. Initial tests show no carryover of POEA from experiment to experiment after this treatment.

3.4.1.3 POEA Standards

The POEA stock solution (~10 mg/mL) was made immediately before each experiment and then disposed of. POE (15) tallow amine (~0.1 g) was weighed directly into a 10.0 mL volumetric flask and diluted to volume with acetonitrile. POEA standards were made by serial dilutions from the stock solution using acetonitrile.

3.4.1.4 POEA-Pyrex Centrifuge Tube Adsorption Experiments

The loss of POEA to the system needs to be accounted for to preclude the overestimation of POEA adsorption to soil. Initial tests in polypropylene centrifuge tubes showed that most of the POEA would be lost to adsorption. Pyrex centrifuge tubes were used because initial test showed that the sorption of POEA was less than the polypropylene centrifuge tubes.

A solution to mimic the properties of the matrix of the soil adsorption experiment was generated by adding 1 L of Type I water, appropriate experimental modifiers, and 10 g of soil to a beaker and stirring for 24 hr with a Teflon coated magnetic stir bar. The modifiers for the

adsorption experiments were 0.01 M sodium chloride, 0.01 M calcium chloride, 0.1 M calcium chloride, 0.01 M acetic acid, and 0.01 M sodium carbonate. The mixture was then filtered through a Whatman #3 filter to remove the bulk of the soil material and then an Advantec 0.3 μm GF75 glass fiber filter. From the filtrate, 39.6 mL aliquots were added to 50 mL Pyrex centrifuge tubes with ground glass stoppers. The centrifuge tubes were then spiked with 0.4 mL of POEA standards to make a range of concentrations from 0.1 $\mu\text{g/L}$ to 500 $\mu\text{g/L}$. The final soil/solution ratio was 1:100.

The centrifuge tubes were stoppered and the stoppers secured with tape to prevent spillage. The centrifuge tubes were placed horizontally in a custom box on an orbital shaker table, allowing the tubes to roll keeping the soil suspended. The tubes were allowed to equilibrate for 24 hr at room temperature before being centrifuged for 15 min at 2000 RCF. A 1 mL aliquot of the supernatant is pipetted to a 2 mL glass chromatography vial to which 50 μL of a 10% triethylamine/10% formic acid/80% acetonitrile mixture and 50 μL of a 0.25 $\text{ng}/\mu\text{L}$ D₅-atrazine were added to each vial. The triethylamine mixture was added to limit POEA losses over time to the vial. Initial tests show that without the triethylamine mixture, POEA standards showed a decrease in signal of 25% and 30% over 24 and 72 hours respectively. The D₅-atrazine was used as an internal standard. D₅-atrazine would not serve well as an internal standard for different sample types because it does not match the change in response to POEA in various matrices. To correct for this, the standard curves were made in the matrix solutions to match the composition of the samples. The samples were analyzed immediately after being vialled.

3.4.1.5 Soil Adsorption Experiments.

For POEA adsorption experiments, Type I water was added to a Pyrex beaker and any modifiers were added. The solutions were left covered for 24 hr before filtering as described above and 39.6 mL aliquots of the filtrate were then added to 0.4 g of soil in 50 mL Pyrex centrifuge tubes. The tubes were then spiked with 0.4 mL of POEA standards to make a range of concentrations from 1 µg/L to 5 mg/L in each experiment except for when acetic acid was used as the modifier. In this case the concentrations used were 10 fold higher to compensate for the higher adsorption exhibited under these conditions. The remaining steps were then the same as for the POEA-Pyrex centrifuge tube experiment from the equilibration step onward.

For glyphosate adsorption experiments, the experiment is designed to mimic the 0.01 M sodium chloride conditions of the POEA adsorption experiment as closely as possible, including a glyphosate-centrifuge tube step. The difference is the experiment is spiked with a series of glyphosate standards in place of POEA standards.

3.4.1.6 Soil Extraction Method

POEA was extracted from all soil samples by accelerated solvent extraction (ASE) using an ASE 200 (Dionex, Sunnyvale, CA, USA). A glass fiber filter, a layer of Ottawa sand, a second glass fiber filter, 1 gram of soil, and a final layer of sand was added to 11 mL cells. The extracting solvent was 100 mM triethylamine/75 mM acetic acid in methanol. Instrument settings were as follows: static 2 min, flush 150%, purge 120 s, cycles 3, pressure 2000 PSI, and temperature 150 °C. Eluent was collected in 60 mL vials. The vials were then placed in a nitrogen evaporator in a 60 °C water bath until the volume was < 2 mL. The contents were then transferred to disposable conical glass centrifuge tubes with a 4 mL methanol rinse and

evaporated to 150 μL . The samples were then reconstituted with 850 μL of Type I water and pipetted to 2 mL vials for analysis.

3.4.1.7 Analytical Method

Analytical separation of POEA samples were performed using an Acquity UPLC H-Class Bio system with an XSelect HSS C18 2.5 μm 3.0 x 150 mm column (Waters, Milford, MA, USA). The aqueous mobile phase (A) was 0.3% formic acid in Type I water and the organic mobile phase (B) was 0.3% formic acid in acetonitrile. In a previous publication, the B mobile phase did not use the formic acid modifier.⁵ Under those conditions the column would begin to show poor peak shape and changes in retention time after a small number of injections. Adding the formic acid to the B mobile phase increased the usable lifespan of the column for these experiments. The samples were kept at room temperature and the column was heated to 60 $^{\circ}\text{C}$. Injection volume was 100 μL . The mobile phase gradient was as follows: 90% A/10% B initial, 55% A/45% B at 1 min, 15% A/85% B at 8.5, 100% A/0% B at 8.51 min, 100% A/0% B at 9 min, 90% A/10% B at 9.01 min, and 90% A/10% B at 10 min.

Samples from the POEA adsorption experiments were analyzed using an AB Sciex Triple Quad 5500 system in positive electrospray ionization mode. Multiple reaction monitoring (MRM) transitions were coded for the three major tallow moieties (C_{16}S , C_{18}u , and C_{18}S) with an ethoxylate range of EO_2 - EO_{22} . There are homologs in the technical mixture with $>\text{EO}_{22}$ but the instrument has a maximum m/z of 1250 and these homologs compose only a negligible fraction of the technical mixture. Flow from the UHPLC was diverted for the first three minutes with the integrated valve. Optimized conditions for each transition appear in Table 3.3. Quantitation of each homolog was performed by comparing to a standard curve in a matching sample matrix.

The Freundlich constants reported for individual homologs were calculated for each homolog and averaged over different experiments ($n = 3$). The Freundlich constants reported for other averages were calculated by averaging homologs within the group and then averaged over different experiments ($n = 3$).

Samples from the glyphosate adsorption experiment were analyzed with the method published by Meyer, et al.⁴⁸ Changes from the published method are because of the capabilities of the AB Sciex 5500 Triple Quad system and the use of solid phase extraction is no longer required; instead a 100 μL injection is made directly from the derivatized sample.

Samples from the weathering experiment and from the corn/soybean fields were analyzed using an Agilent 6224 TOF system with a multimode source in positive electrospray ionization mode. The instrument was calibrated in standard mass range (3200 m/z) and extended dynamic range (2 GHz). Instrument settings were as follows: gas temp 325 $^{\circ}\text{C}$, vaporizer 240 $^{\circ}\text{C}$, drying gas 6 L/min, nebulizer 60 psig, vcap 3000 V, charging voltage 900 V, fragmentor 175 V, skimmer 65 V.

3.4.2 Effects of Soil Composition on the Adsorption of POEA

The effect of soil composition on $1/n$ and K_F is shown in Figure 3.18. As is expected, there are differences in the binding of POEA to different soils. With only three different soils tested, it is difficult to generalize the adsorption behavior of POEA. There is no clear trend correlating $1/n$ or K_F to the following soil characteristics: pH, cation exchange capacity, or texture (percent clay, silt, and sand). Both $1/n$ and K_F increase with increased organic matter, but it is likely the source of the different adsorption behaviors is more complex than any single soil characteristic. Not only do the listed soil parameters potentially play some role in POEA

adsorption, but other undetermined characteristics (e.g. mineral composition) might also have effects.

3.4.3 Tables

Table 3.1 Characteristics of soils used in batch adsorption isotherm experiments.

	Texture	pH	organic matter (%)	cation exchange capacity (meq/100 g)
Soil A	Loam	7.6	3.2	24.5
Soil B	Sand	7.5	0.48	2.0
Soil C	Sandy Loam	5.8	5.96	14.7

Table 3.2 Soil collection details.

Sample number	Collection date	State	County	Field number
1	3/17/2015	IA	Johnson	1
2	3/17/2015	IA	Johnson	1
3	3/18/2015	IA	Johnson	2
4	3/18/2015	IA	Johnson	2
5	3/12/2015	IL	Champaign	1
6	3/12/2015	IL	Champaign	2
7	3/12/2015	IL	Champaign	3
8	3/12/2015	IL	Champaign	4
9	3/31/2015	IN	Hendricks	1
10	3/31/2015	IN	Hendricks	2
11	3/31/2015	IN	Hendricks	3
12	3/31/2015	IN	Hendricks	4
13	3/10/2015	MO	Putnam	1
14	3/10/2015	MO	Linn	2
15	3/11/2015	MO	Grundy	3
16	3/11/2015	MO	Livingston	4
17	3/27/2015	MS	Bolivar	1
18	3/27/2015	MS	Bolivar	2
19	3/27/2015	MS	Washington	3
20	3/27/2015	MS	Washington	4

Table 3.3 Optimized parameters for TQ-MS method.

Tallow moiety	Source Temp (°C)	Ionspray voltage	Curtain Gas	Gas1	Gas2	Declustering potential	Entrance Potential	Exit Potential	Dwell time (msec)
C16s	550	3500	20	35	70	60	2	45	25
C18u	550	3500	20	35	70	60	10	45	25
C18s	550	3500	20	35	70	60	12	45	25

Tallow moiety	Ethoxylate number	Precursor (m/z)	Product (m/z)	Collision Energy
C16s	EO2	330.25	106.10	34
C16s	EO3	374.30	312.30	35
C16s	EO4	416.35	312.30	35
C16s	EO5	462.35	312.30	39
C16s	EO6	506.40	312.30	41
C16s	EO7	550.45	356.40	43
C16s	EO8	594.45	400.40	45
C16s	EO9	638.50	400.40	47
C16s	EO10	682.55	444.40	51
C16s	EO11	726.60	444.40	53
C16s	EO12	770.60	488.40	55
C16s	EO13	814.60	488.40	57
C16s	EO14	858.65	532.50	59
C16s	EO15	902.65	576.50	61
C16s	EO16	946.70	620.50	63
C16s	EO17	990.70	620.50	65
C16s	EO18	1034.70	664.60	67
C16s	EO19	1078.75	664.60	69
C16s	EO20	112.75	708.60	71
C16s	EO21	1166.80	708.60	73
C16s	EO22	1210.80	708.60	75
C18u	EO2	356.30	106.10	34
C18u	EO3	400.35	338.30	36
C18u	EO4	444.40	338.30	37
C18u	EO5	488.40	338.30	39
C18u	EO6	532.45	338.30	41
C18u	EO7	576.45	382.40	43
C18u	EO8	620.50	426.40	45

C18u	EO9	664.55	426.40	49
C18u	EO10	708.55	470.40	51
C18u	EO11	752.60	470.40	53
C18u	EO12	796.60	470.40	55
C18u	EO13	840.60	514.50	57
C18u	EO14	884.65	558.50	59
C18u	EO15	928.65	602.50	61
C18u	EO16	972.70	646.60	63
C18u	EO17	1016.70	646.60	66
C18u	EO18	1060.75	690.60	69
C18u	EO19	1104.75	690.60	71
C18u	EO20	1148.80	734.60	73
C18u	EO21	1192.80	734.60	75
C18u	EO22	1236.80	734.60	77
C18s	EO2	358.30	106.10	37
C18s	EO3	402.35	340.30	35
C18s	EO4	446.40	340.30	37
C18s	EO5	490.40	340.30	40
C18s	EO6	534.45	340.30	45
C18s	EO7	578.45	384.30	47
C18s	EO8	622.50	428.30	49
C18s	EO9	666.55	428.30	51
C18s	EO10	710.55	472.40	52
C18s	EO11	754.60	472.40	55
C18s	EO12	798.60	472.40	57
C18s	EO13	842.60	516.50	59
C18s	EO14	886.65	560.50	61
C18s	EO15	930.65	604.50	63
C18s	EO16	974.70	648.60	65
C18s	EO17	1018.70	648.60	67
C18s	EO18	1062.75	692.60	70
C18s	EO19	1106.75	692.60	73
C18s	EO20	1150.80	736.60	75
C18s	EO21	1194.80	736.60	77
C18s	EO22	1238.80	736.60	79

3.4.4 Figures

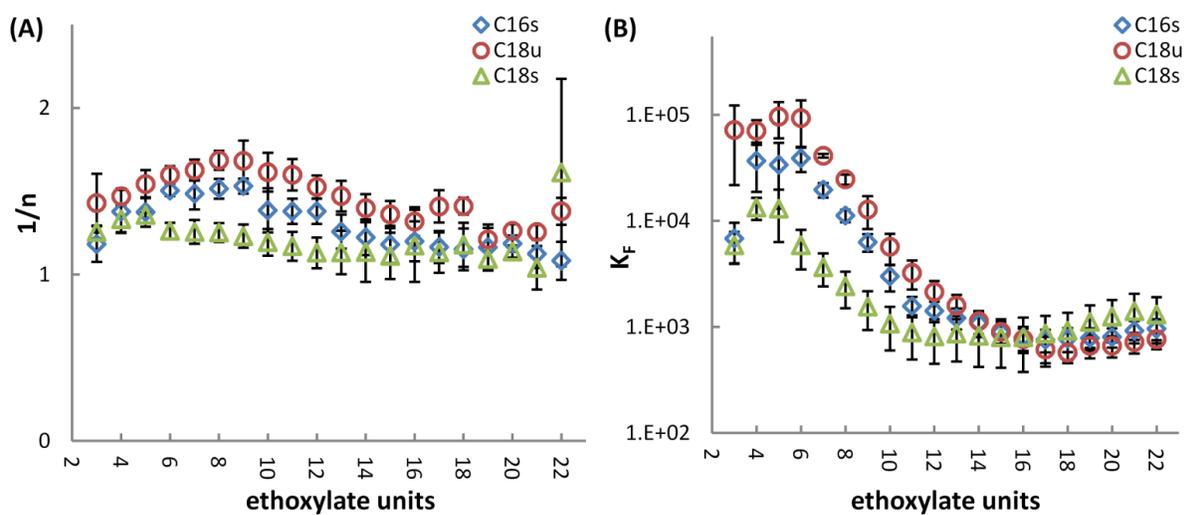


Figure 3.10 Freundlich values for individual homologs in 0.01 M sodium chloride (final pH=8.3).

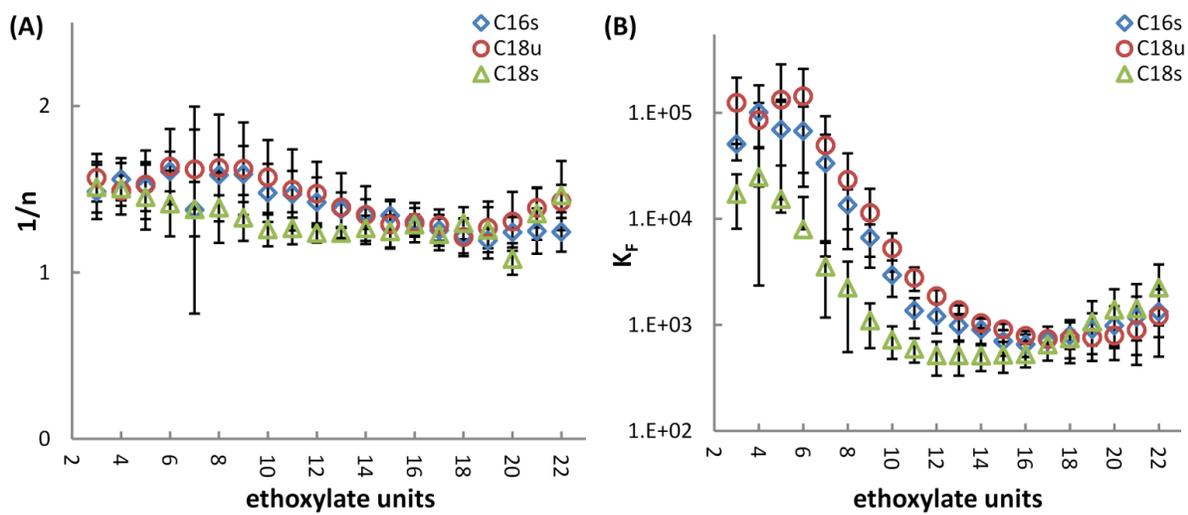


Figure 3.11 Freundlich values for individual homologs in Type I water.

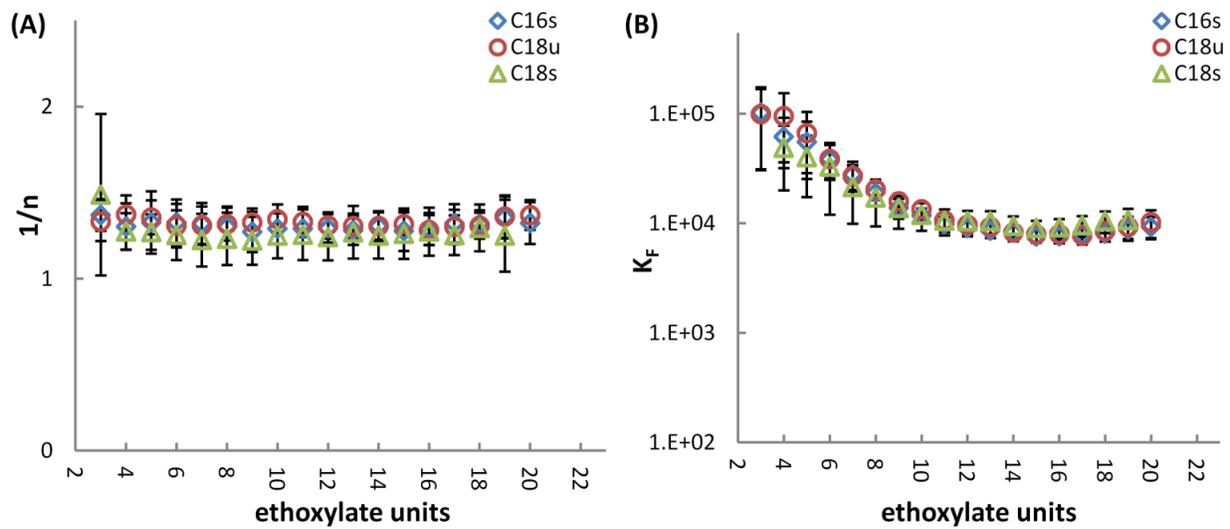


Figure 3.12 Freundlich values for individual homologs in 0.01 M calcium chloride.

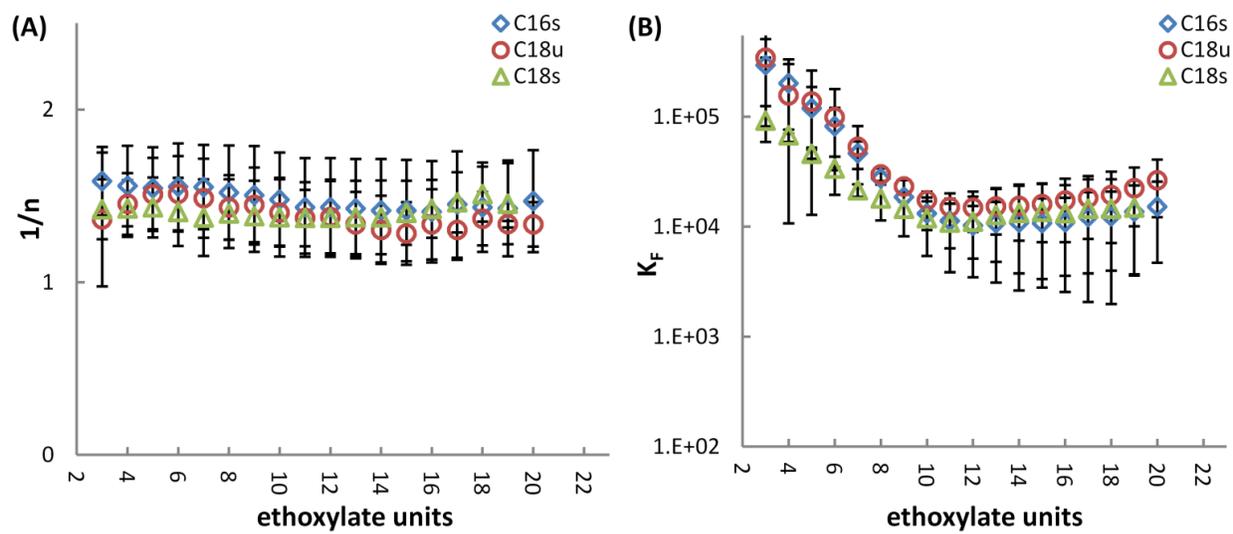


Figure 3.13 Freundlich values for individual homologs in 0.1 M calcium chloride.

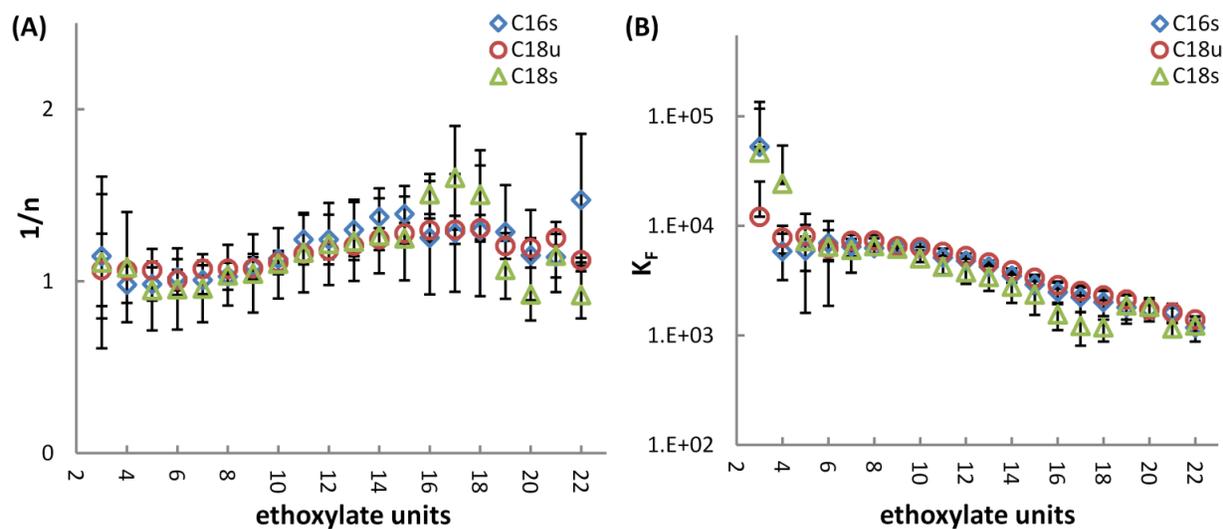


Figure 3.14 Freundlich values for individual homologs in 0.01 M sodium carbonate (final pH=10.8).

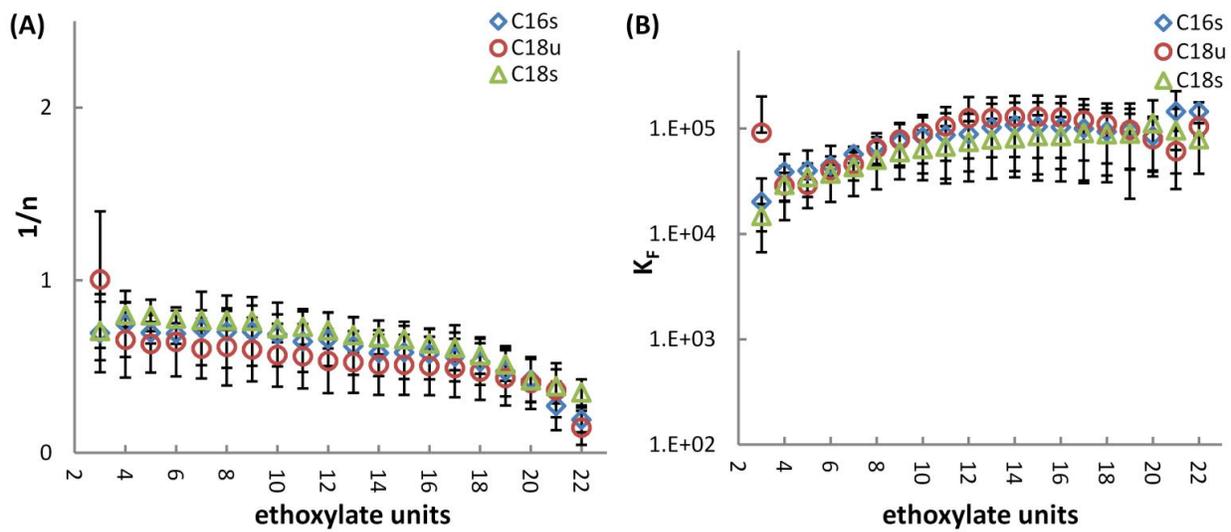


Figure 3.15 Freundlich values for individual homologs in 0.01 M acetic acid (final pH=4.2).

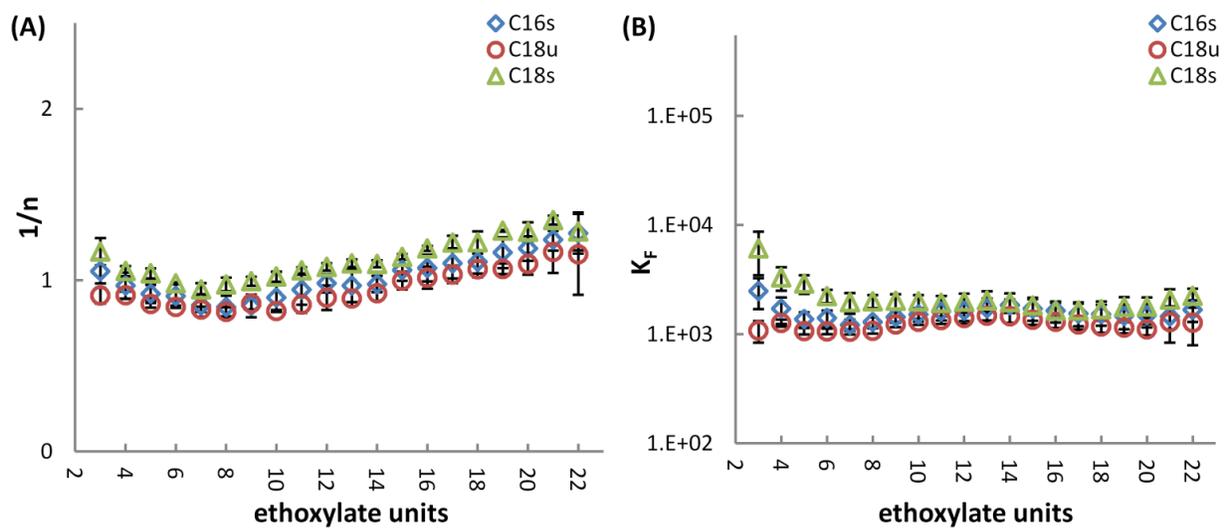


Figure 3.16 Freundlich values for individual homologs in 0.01 M sodium chloride on Soil B.

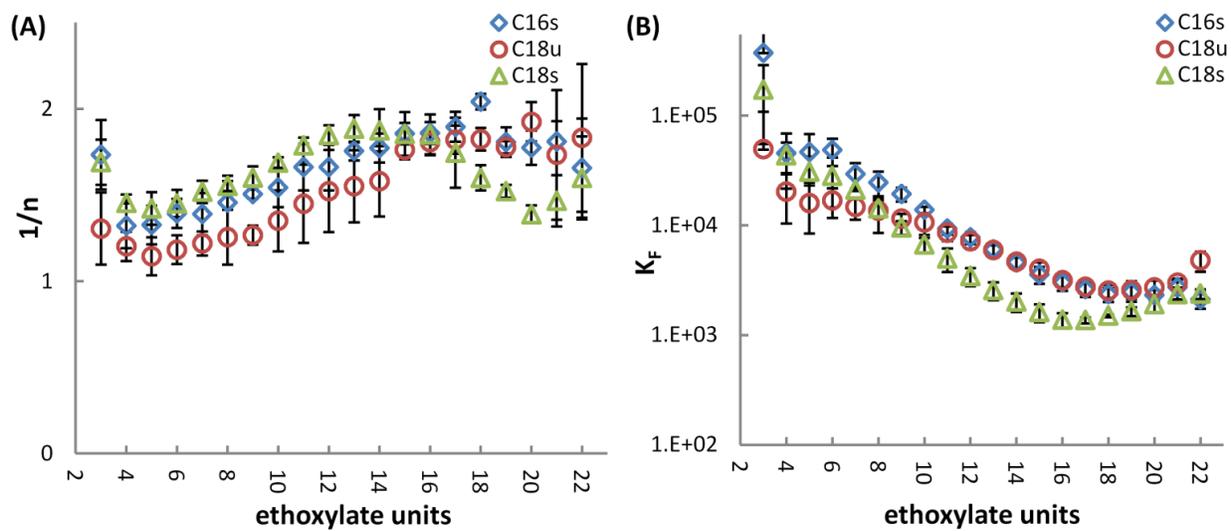


Figure 3.17 Freundlich values for individual homologs in 0.01 M sodium chloride on Soil C.

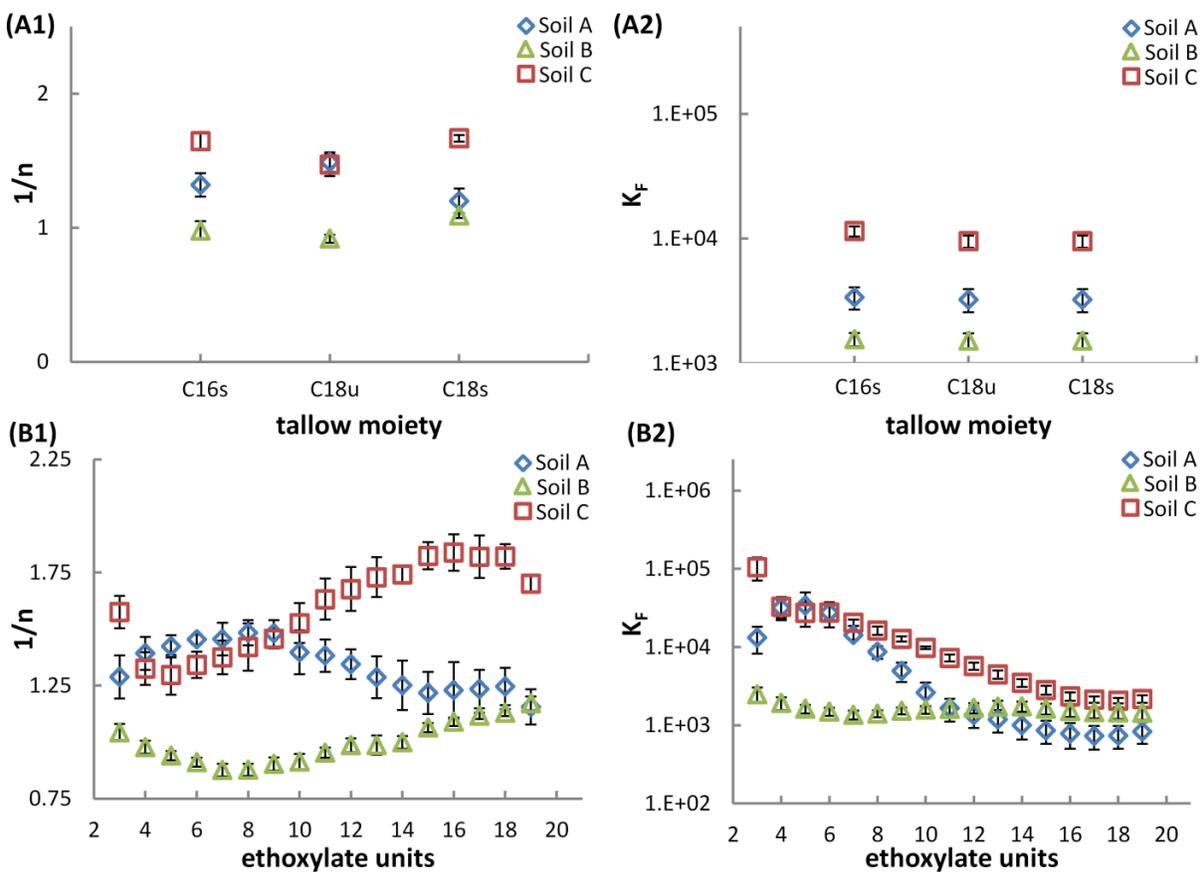


Figure 3.18 Average Freundlich values on three soils with contrasting pH, organic carbon content, and cation-exchange capacity. (A1) $1/n$ grouped by tallow moiety. (A2) K_F grouped by tallow moiety. (B1) $1/n$ grouped by number of ethoxylate units. (B2) K_F grouped by number of ethoxylate units.

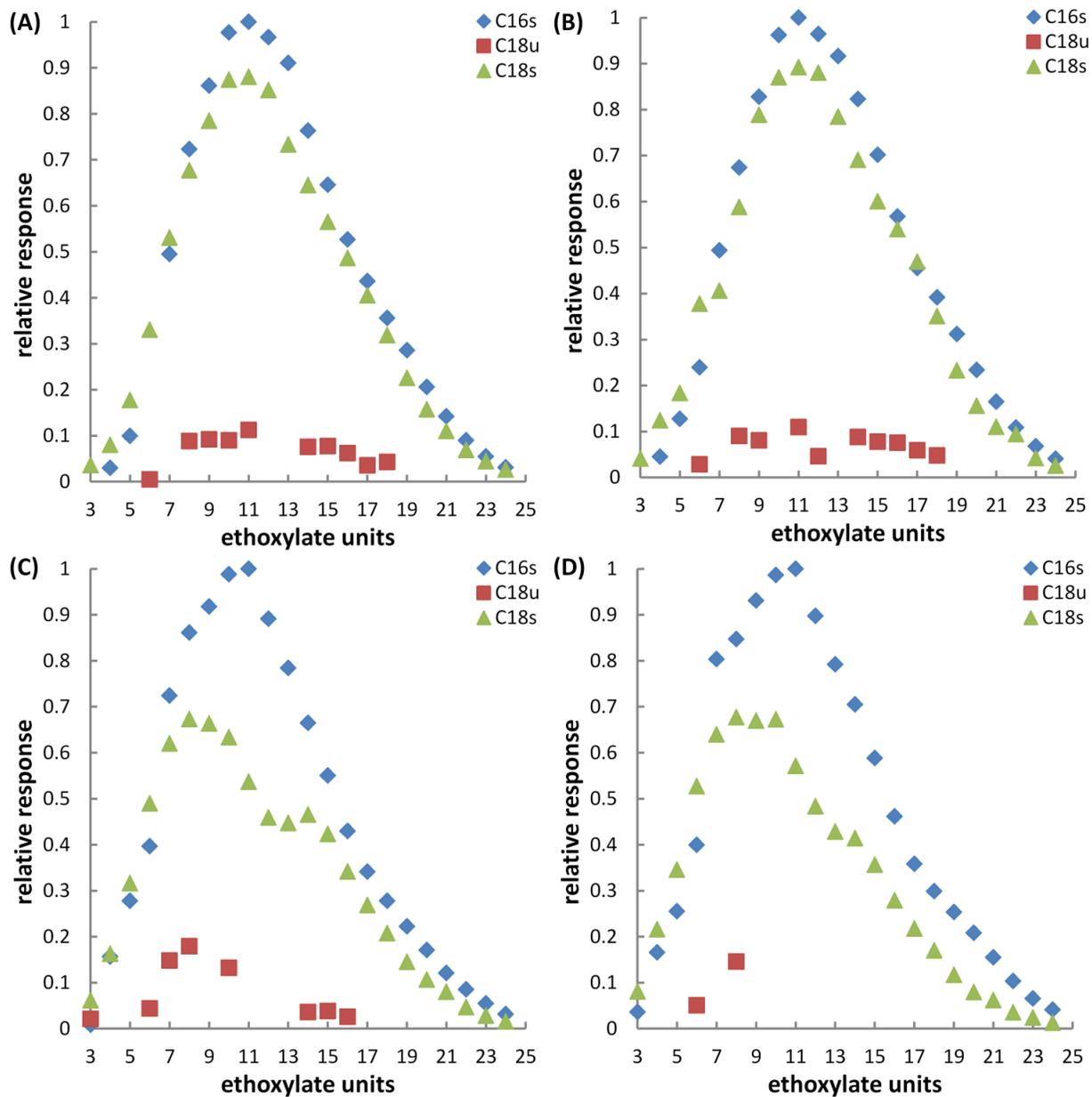


Figure 3.19 POEA distributions extracted from Iowa field samples.
 (A) Sample #1. (B) Sample #2. (C) Sample #3. (D) Sample #4.
 Responses have been normalized to the highest response of each distribution.

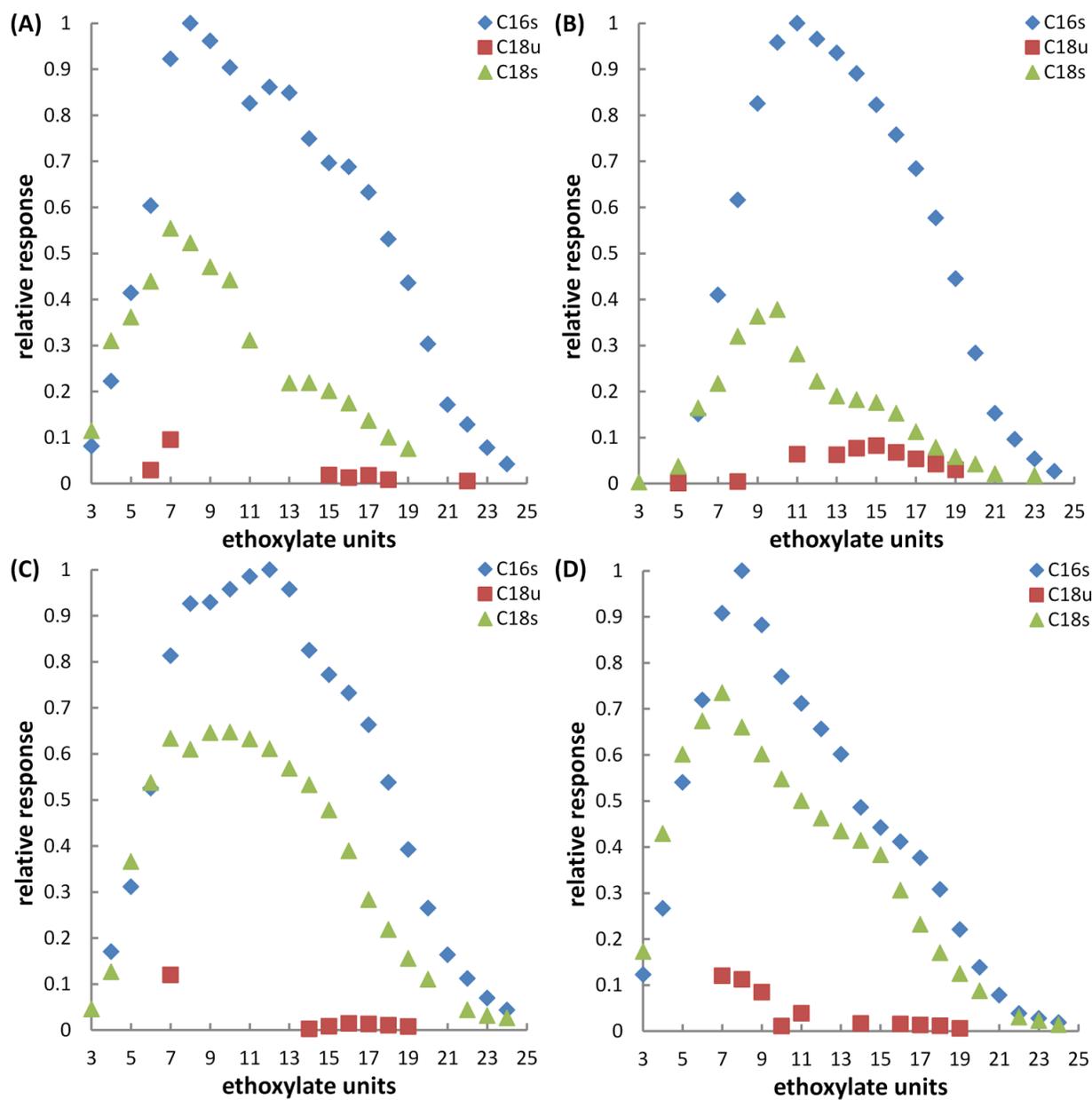


Figure 3.20 POEA distributions extracted from Illinois field samples.
 (A) Sample #5. (B) Sample #6. (C) Sample #7. (D) Sample #8.
 Responses have been normalized to the highest response of each distribution.

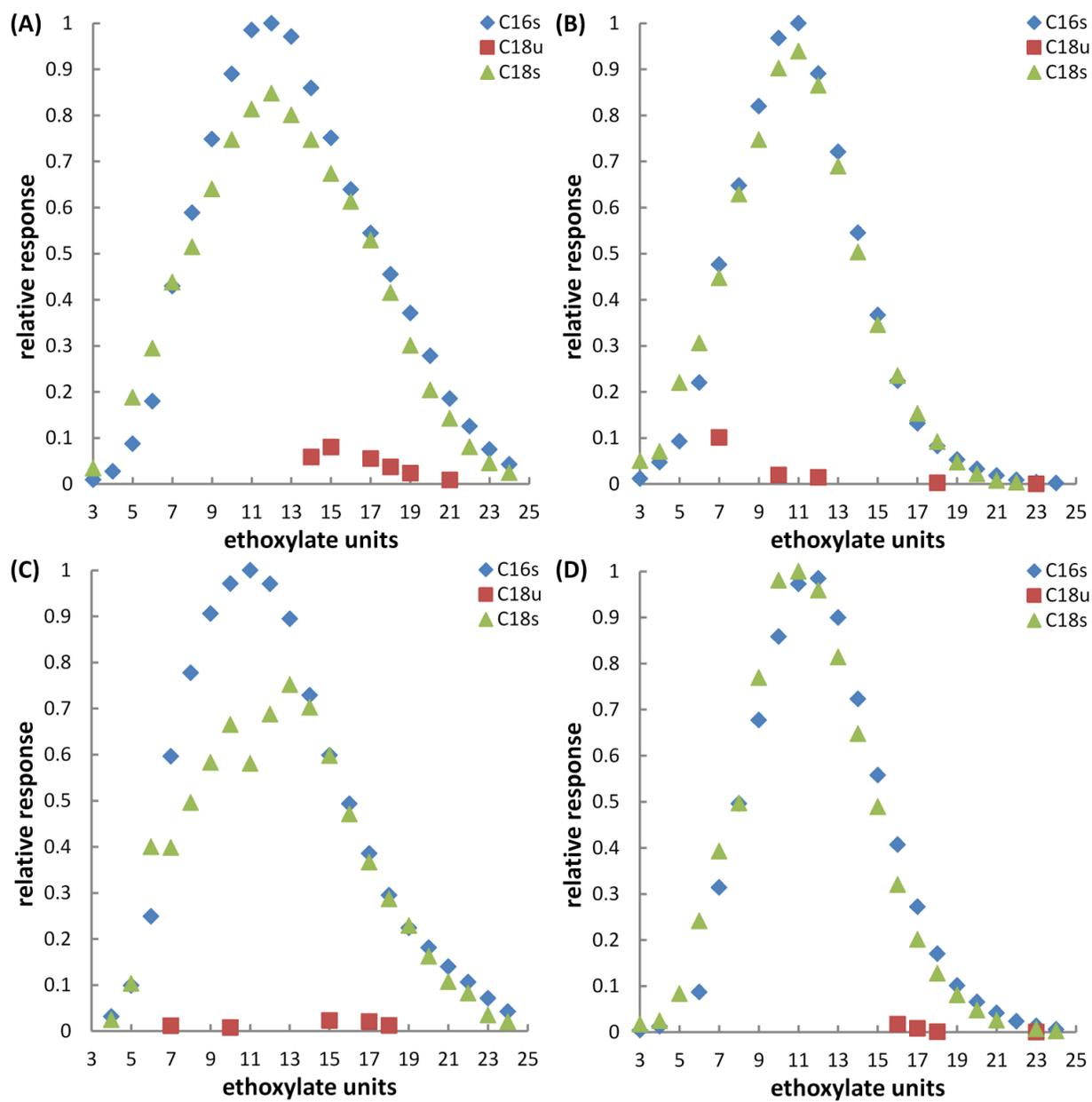


Figure 3.21 POEA distributions extracted from Indiana field samples.
 (A) Sample #9. (B) Sample #10. (C) Sample #11. (D) Sample #12.
 Responses have been normalized to the highest response of each distribution.

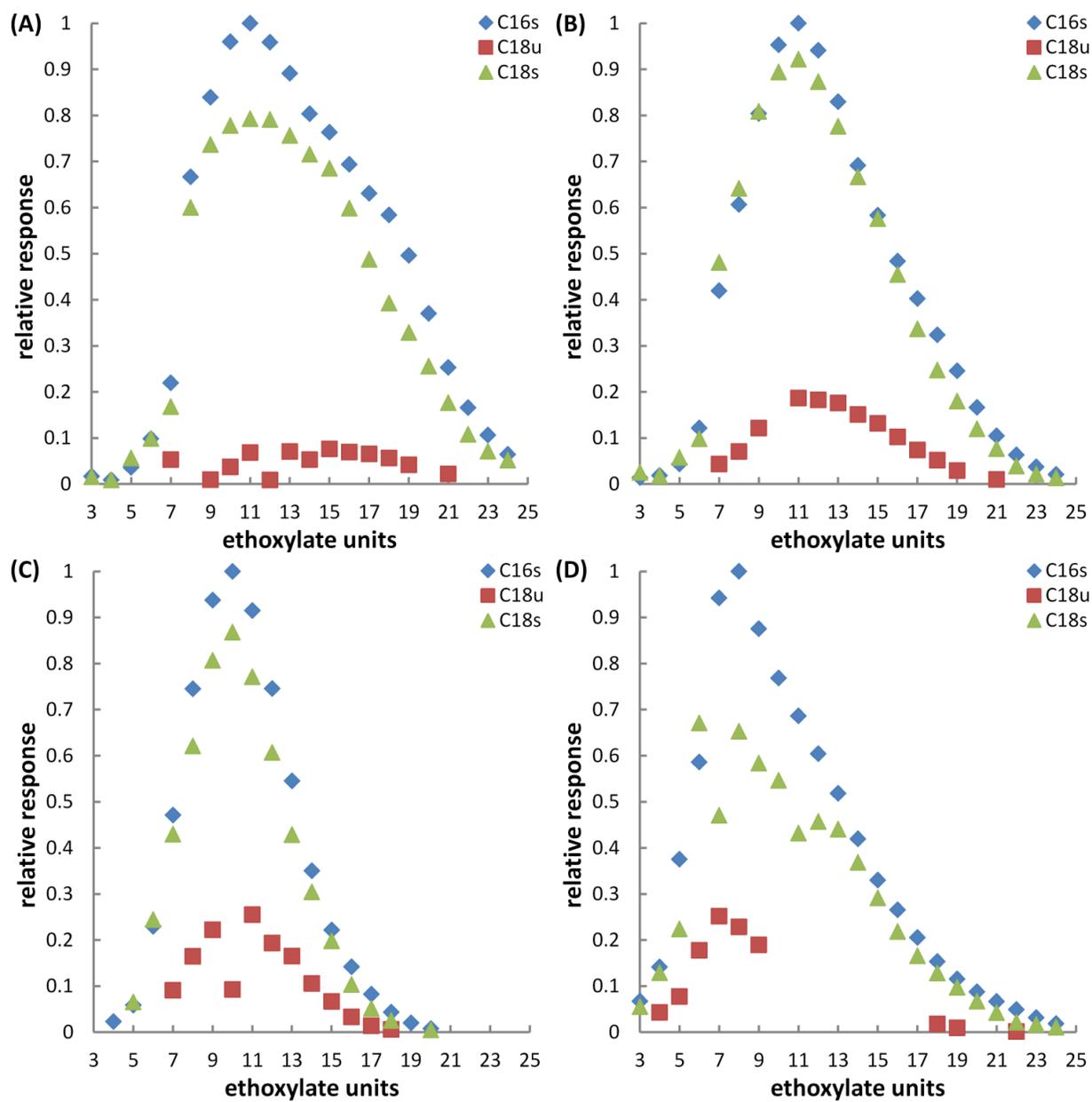


Figure 3.22 POEA distributions extracted from Missouri field samples.
 (A) Sample #13. (B) Sample #14. (C) Sample #15. (D) Sample #16.
 Responses have been normalized to the highest response of each distribution.

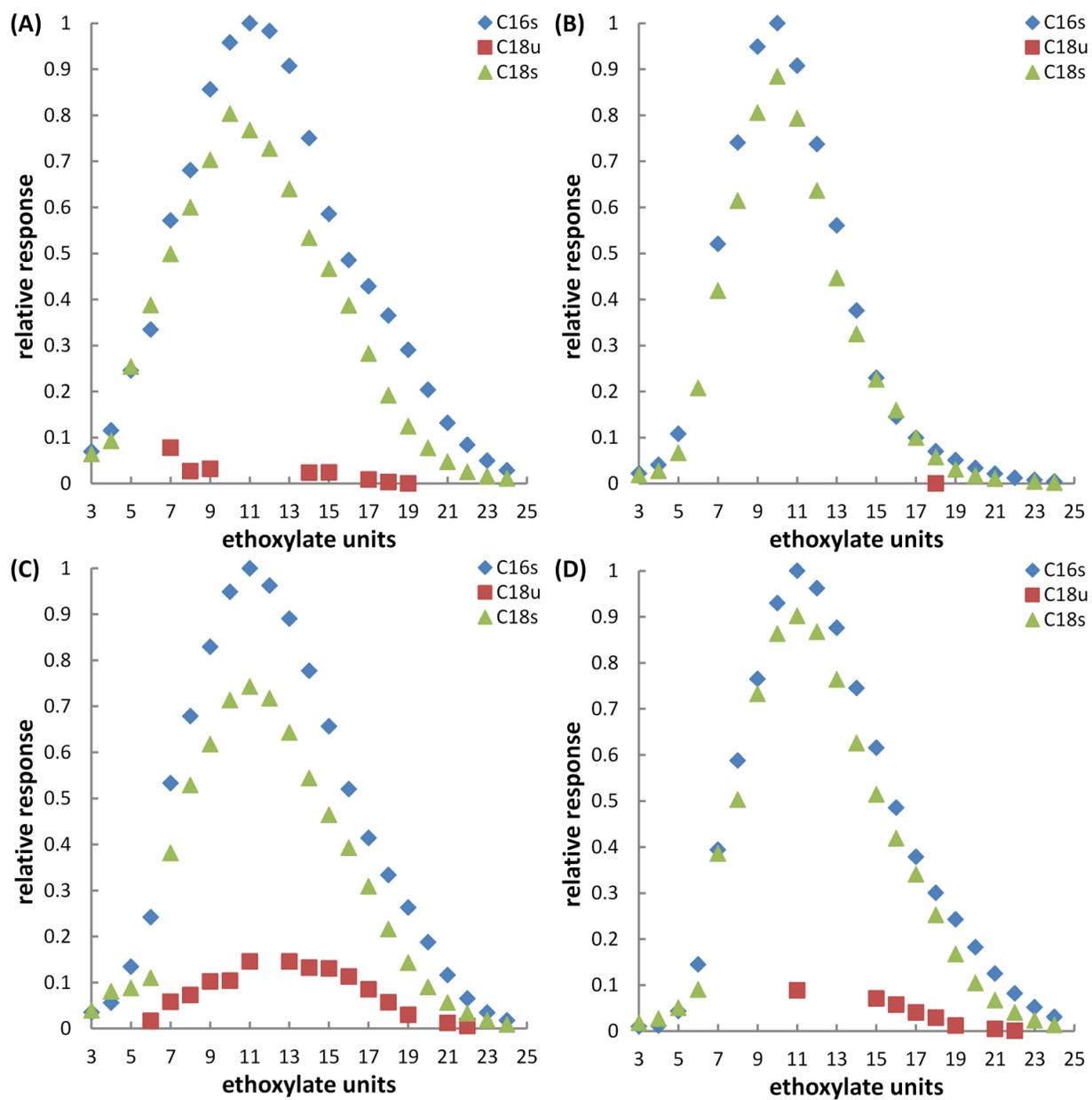


Figure 3.23 POEA distributions extracted from Mississippi field samples.
 (A) Sample #17. (B) Sample #18. (C) Sample #19. (D) Sample #20.
 Responses have been normalized to the highest response of each distribution.

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Chapter 4: Dissipation study of Polyoxyethylene Tallow Amine and Glyphosate on an Agricultural Field and Their Co-occurrence on Stream Bed Sediments from Georgia, Hawaii, Iowa, Mississippi, North Carolina, and South Carolina

Tush, D.; Maksimowicz, M. M.; Meyer, M. T. Dissipation study of Polyoxyethylene Tallow Amine and Glyphosate on an Agricultural Field and Their Co-occurrence on Stream Bed Sediments from Georgia, Hawaii, Iowa, Mississippi, North Carolina, and South Carolina. Anticipated submission to *Environmental Science and Technology*

4.1 Introduction

Polyoxyethylene tallow amine (POEA) and glyphosate have been packaged together in herbicide formulations since Monsanto introduced the Roundup brand of herbicide. Since its introduction, glyphosate has become the most widely applied herbicide in the world.¹ The U.S. Geological Survey estimates that in 2011 over 110 million kilograms of glyphosate was applied in the U.S. for agricultural purposes.² The U.S. Environmental Protection Agency estimated that 9.3 million kilograms of glyphosate was used in other applications (e.g. household, industrial, governmental).³ Once the patent on glyphosate expired multiple companies began manufacturing glyphosate formulations. Although most of the manufacturers of glyphosate formulation consider the composition of their formulations a trade secret a prior study has shown that POEA is still a common additive.⁴

Toxicity of glyphosate formulations to non-target organisms has been attributed to POEA or to the mixture of POEA and glyphosate more so than to glyphosate alone for several aquatic species.⁵⁻¹³ Despite these findings, there are no published studies on the environmental occurrence, fate, and transport of POEA from agricultural or urban source, but POEA has been shown to be strongly adsorbed on soils and persistent from year to year on an agricultural field (Chapter 3).

POEA is a tertiary amine surfactant comprised of many different homologs. The three groups attached to the amine core are an aliphatic chain derived from tallow (primarily 16 or 18 carbon atoms, either saturated or mono-unsaturated) and two chains of repeating ethoxylate groups with terminal alcohols (Figure 4.1). The structure of POEA and the distribution of homologs has been discussed in detail in a prior study.³ To identify individual components and groups of the POEA homologs the following naming convention will be used: $C_z(s/u)EO_n$, where z represents the number of carbon atoms in the aliphatic chain, s is a saturated aliphatic chain, u is a mono-unsaturated aliphatic chain, and n is the combined number of ethoxylate groups in the two chains.

Glyphosate, N-(phosphonomethyl)glycine (Figure 4.2), was introduced as the commercial product Roundup™ in 1974. The general increase in the application of glyphosate from 1992 to 2013 is illustrated in Figure 4.3. Soybeans (*Glycine max*) and corn (*Zea mays*) are examples of crops that have been genetically modified for glyphosate resistance (e.g. Roundup Ready™) and these were approved for use in the U.S. in 1996 and 1998 respectively.¹ The adoption rates of herbicide resistant, genetically modified corn and soybeans based on data from the U.S. Department of Agriculture are shown in Figure 4.4.¹⁴ Studies of glyphosate show half-lives ranging from 2 to 215 days on soil.¹⁵ The primary degradation product of glyphosate is aminomethylphosphonic acid (AMPA) is shown in Figure 4.5. The half-life of AMPA has been shown to range from 60 to 240 days on soil.¹⁶ Glyphosate (10,000 to 15,700 mg/L) and AMPA (5,800 mg/L) are water soluble and anionic under most conditions^{15,17}, but readily adsorb to soil^{15,18}. Glyphosate and AMPA are frequently detected in agricultural soil, surface water, groundwater, and rain deposition, and much less frequently detected in groundwater.¹⁹ The

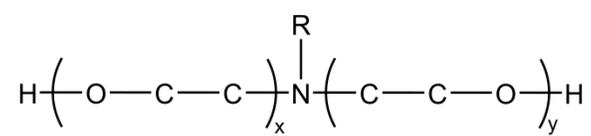


Figure 4.1 Structure of POEA.

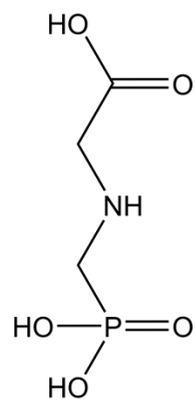


Figure 4.2 Structure of glyphosate.

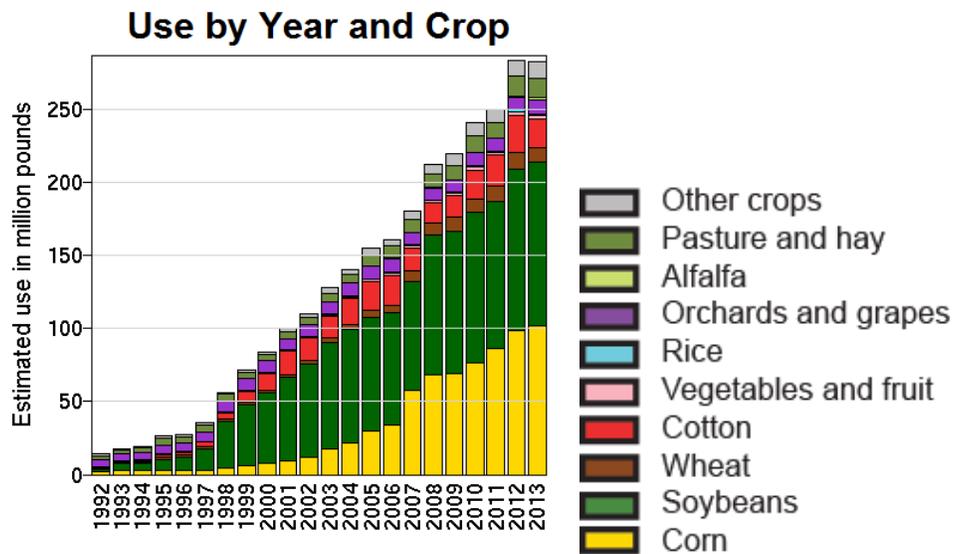


Figure 4.3 Estimated application of glyphosate from 1992 to 2013.

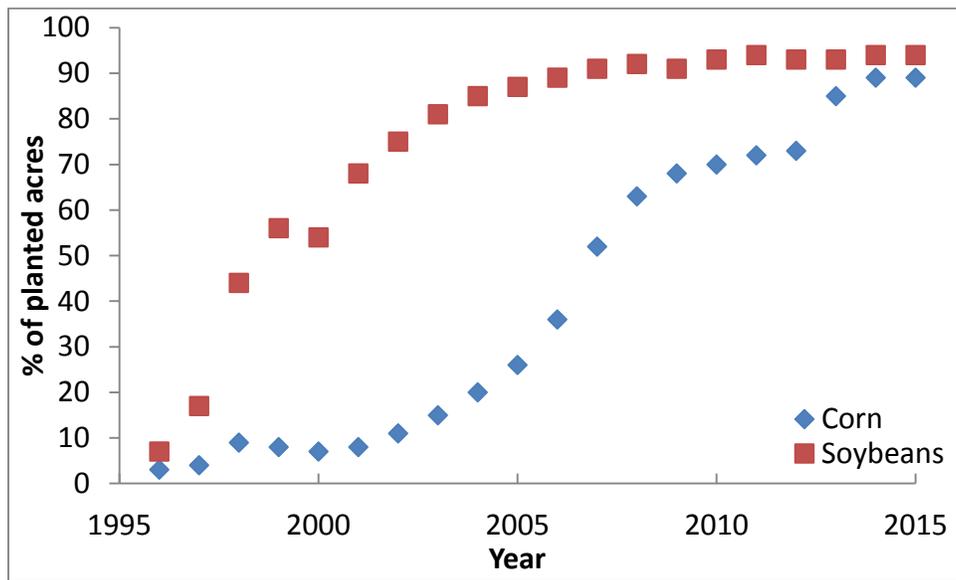


Figure 4.4 Adoption rate of herbicide resistant corn and soybeans in the U.S.

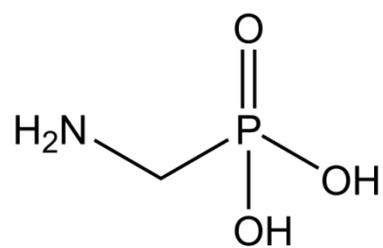


Figure 4.5 Structure of AMPA.

transport of glyphosate and AMPA to tile drains has been primarily attributed to macropore flow.²⁰ POEA has much larger adsorption constants than glyphosate (Chapter 3), thus it is anticipated that POEA would much more readily be transported into surface water than groundwater.

The first goal of the research presented here is to compare the dissipation of POEA, glyphosate, and AMPA in agricultural soil. As part of the National Water Quality Assessment Program (NAWQA) Indiana Agricultural Chemical Transport Study, soil samples were collected for over a year from an active row crop field to which glyphosate had been applied.²¹ The second goal was to determine if POEA is being transported into the environment from the agricultural and urban areas where glyphosate is likely applied. Because of the propensity for POEA to bind to soil, POEA would likely adsorb to suspended and bed sediments in surface waters downstream of agricultural glyphosate application sites. Stream bed sediment samples collected downstream from agricultural and urban areas in six states between 2007 and 2014 were analyzed for POEA, glyphosate, and AMPA.

4.2 Materials and Methods

4.2.1 Chemicals

POE (15) tallow amine and POE (5) tallow amine technical mixtures (Chem Service Inc., West Chester, PA) were used as the POEA standards. Glyphosate and AMPA standards were also obtained as powders from Chem Service Inc. Isotopically labeled standards of glyphosate (¹³C₂, ¹⁵N) and AMPA (¹³C, ¹⁵N, D₂) were obtained from Cambridge Isotope Laboratories (Woburn, MA) for use as internal standards. A Nanopure DIAMOND TOC Life Science system (Barnstead/ThermoFisher, Dubuque, IA) generated deionized water (DI). The organic solvent for

chromatographic separations was LC/MS grade Acetonitrile (Burdick & Jackson, Muskegon, MI). Acetic acid (Optima LC/MS grade), formic acid (Optima LC/MS grade), methanol (HPLC grade), sodium chloride (certified ACS), sodium borate (certified ACS), potassium hydroxide (certified ACS), hydrochloric acid (technical), and Ottawa sand were obtained from Fisher Scientific (Fair Lawn, NJ). Ammonium acetate and 9-fluorenylmethoxycarbonyl chloride (Fmoc) were obtained from Sigma-Aldrich Corporation (St. Louis, MO). The test soil was collected near Fourmile Creek in Iowa from field that was not used for crop production.

4.2.2 Field Dissipation Study

Soil samples obtained from an active tile-drained agricultural field from the Sugar Creek watershed in Indiana were used to examine the transport of glyphosate and AMPA in 2004-2005. The study site was planted in corn in 2003 and rotated into Roundup Ready soybeans in 2004. Soil core samples were collected using a stainless steel manual corer from three locations on the field to a depth of 45 cm below land surface during each sampling period (except for the first sampling date where only a 15 cm core sample was collected). Each soil core was then divided into three depth intervals of 0-15, 15-30 and 30-45 cm and placed into baked wide-mouth jars. The samples were then shipped overnight on ice before being stored at -20 °C until thawed for sample processing.

4.2.3 Bed Sediment Samples

Bed sediment samples were collected using a stainless steel scoop into baked wide-mouth glass jars. The samples from Iowa, Mississippi, and Hawaii were shipped overnight on ice. The remaining bed sediment samples were collected from the Southeastern U.S. as part an

unpublished NAWQA study (cycle 3) and shipped overnight on ice to the U.S. Geological Survey Contaminant Environmental Research Center in Columbia, Missouri where aliquots of each sample were then frozen and shipped. Sediment samples were subsequently frozen at -20 °C until thawed for processing. After thawing, the bed sediment samples were placed on aluminum foil and homogenized. A 5 g aliquot was taken by subsampling 10 different portions of the sample spread out on the foil. This aliquot was used for glyphosate analysis. One or more samples were further subsampled for duplicate or spiked analyses. Two 1 g aliquots were taken by subsampling 3 or more locations in the jar after the samples were homogenized for each aliquot. These aliquots were used for POEA analysis. The second aliquot was spiked for use in the standard addition calculation.

4.2.4 Laboratory Glassware

All reusable glassware was rinsed in hot tap water; scrubbed with Contrex CF detergent in Type II water; rinsed three or more times in each of hot tap water, Type II water, and Type I water; rinsed with methanol; and air dried. Testing showed no detectable POEA extractable from the glassware after this cleaning protocol.

4.2.5 Preparation of Standard Solutions

A stock solution of POEA was made before each experiment at an approximate concentration of 10 mg/mL. Approximately 0.1 g of POEA was weighed diluted to 10 mL with acetonitrile in a volumetric flask. The POEA solution used for spiking was made by serial dilutions from the stock solution in 2 mL vials using acetonitrile. The POEA stock solution and dilutions were disposed of after each experiment.

Stock solutions of glyphosate and AMPA (1 mg/ml) were made in acetonitrile from neat standards and stored at 2-4 °C in high density polyethylene bottles. A standard mix solution of glyphosate and AMPA (1 ng/μL each) was prepared in Type 1 water from the stock solutions. An internal standard mix of labeled glyphosate and labeled AMPA (1 ng/μL each) was made in Type I water.

4.2.6 Generation of Spiked Test Soil for POEA Extraction and Quantitation

Two different aliquots of the test soil were treated with POEA. A volume of water sufficient to saturate the test soil was added to a Pyrex beaker and spiked with POEA. An aliquot of the test soil was then added to the spiked water and thoroughly stirred to disperse the POEA evenly through the soil. One of the test soils was spiked with POE (15) tallow amine (81 ng/g) and the other spiked with POE (5) tallow amine (56 ng/g). The test soils were left for an hour before being divided into 1 g aliquots for extraction and analysis.

4.2.7 Analysis of POEA on Sediment and Soil

POEA was extracted and analyzed from sediment samples using the methods detailed in Chapter 3, including an accelerated solvent extraction (ASE) followed by ultra-high performance liquid chromatography (UPLC)/time of flight mass spectrometry (TOF-MS). For the test soil and for the field soil samples, four aliquots were added to the ASE cells. The first aliquot was not spiked and the remaining aliquots were then spiked with increasing amounts of POE (15) tallow amine solution in acetonitrile. For the sediment samples, two aliquots of each were added to the ASE cells. The first aliquot was not spiked and the second was spiked with POE (15) tallow amine. The ASE cells were then left open for ~15 min to allow the acetonitrile to dry.

The samples were then extracted, analyzed, and quantitated with a standard addition calculation. The concentration of POEA was calculated based on the sum of the areas of all detected homologs. Two assumptions are made for the quantitation of POEA; that each homolog gives the same molar response as every other homolog and that the spike concentration are a known quantity based on the mass added and the average molecular mass of the distribution.

4.2.8 Analysis of Glyphosate and AMPA on Sediment and Soil

Glyphosate and AMPA were extracted from solid samples by adding a 5 g sample aliquot to a 50 mL polypropylene centrifuge tube with a screw top cap and adding 25 mL of 0.5 M potassium hydroxide. A stable isotope labeled glyphosate and AMPA solution (100 μ L at 1ng/ μ L each) was added to each centrifuge tube. To the spiked samples, a standard of glyphosate and AMPA (100 μ L at 1ng/ μ L each) was added to each centrifuge tube. Standard curves were generated by adding 100 μ L of the stable labeled isotope mix and the appropriate amount of standards to 50 mL polypropylene centrifuge tubes containing 25 ml of 0.5 M potassium hydroxide. All the samples and standards were placed on a shaker table for 45 min. The samples were subsequently centrifuged for 10 minutes at 5,000 x g. A 5 mL aliquot of the centrifuged supernatant then was pipetted into a 19 mL polystyrene round bottom test tube with a screw top. The samples were then derivatized with 9-fluorenylmethoxycarbonyl chloride (FMOC) by adding 2 mL of a 5 mM FMOC solution and then incubating for 24 hr in a 40 °C enclosed water bath.

After derivatization the reaction then was neutralized by adding 800 μ L of a 2% phosphoric acid solution. The pH then was adjusted to 6 using 0.5 M hydrochloric acid and then

adjusted to 9 using a 5% sodium borate solution. A 1 mL aliquot of the sample then was pipetted into 2 mL glass chromatography vials and stored in the dark at 4 °C until analysis.

Samples were analyzed using an Acquity H-class Bio UPLC (Waters Corp., Milford, MA) with a Triple Quad 5500 system (AB Sciex, Framingham, MA) in positive electrospray ionization (ESI) mode. Glyphosate and AMPA were separated by injecting 100 µL of sample and using 5 mM aqueous ammonium acetate and acetonitrile gradient separation on a Waters Acquity BEH column (2 x 50 mm, 1.7 µm packing) at 40 °C. Two multiple reaction monitoring (MRM) transitions were measured for each analyte. Identification was based on the retention time and the ion ratio of the two transitions. Quantitation was conducted using a linear 1/x weighted standard curve.

4.3 Results and Discussion

4.3.1 POEA Extraction Method

To obtain some quantitative understanding of the concentration of POEA in agricultural soil and bed sediment relative to glyphosate and AMPA the method of standard addition was examined. The lack of stable isotope labeled standards and the potential for matrix effect disparities in using an external standard curve made the method of standard additions the best option for quantitation. Due to the high sorption of POEA to soil the assumption was made that there no or very negligible loss of POEA to the sample jar. The recovery of POE (15) tallow amine was 36% ± 3%. The recovery data (Table 4.1) indicates that the slight difference between adding the initial aqueous spike to the whole soil sample and adding the subsequent standard addition spikes in organic solvent on the soil sample aliquot in the extraction cell has a

significant effect on the degree to which POEA is adsorbed and that some fraction of POEA is not readily recoverable from the soil with this method.

The test soils are initially spiked with POEA and saturated with water to simulate the aging of POEA on an environmental sample whereas the standard addition spikes were added on the sample in the ASE cell. Similar spiking procedures have been used before in the literature.²² The standard addition spike may be more easily extractable because it only comes in contact with a small part of the soil, because it has less contact time with the soil before extraction, or because of a difference in the interaction due to the different polarities of the solvents. Thus, the concentrations of POEA determined by standard addition underestimated the spiked concentration by a factor of nearly threefold. The data also show that POE (5) tallow amine had a recovery of $29\% \pm 4\%$. Whether the lower apparent recovery of POE (5) tallow amine is because of stronger adsorption of POEA homologs with short ethoxylate chains or because the assumption that every homolog generates the same instrument response is imperfect is unclear without further experimentation.

The data comparing single point standard addition to multipoint standard addition is shown in Table 4.1. The average result for POE (15) tallow amine show a small difference, 35% to 36%). As would be expected using fewer points in the standard addition calculation increases the standard deviation. It has been suggested by Ellison et al. that a single point standard addition is as valid as the multipoint standard addition.²³ This method, to the best of the authors' knowledge, represents the first extraction and quantitation method that incorporates the most abundant homologs of POEA. Whether the percent recoverable POEA extracted from environmental samples decreases with increased aging needs to be studied.

Table 4.1 Recovery comparison of single and multipoint standard additions. Additions were made in 59 ng and 54 ng increments for POE (15) and POE (5) tallow amine respectively on 1.0 g of test soil. Number of replicates = 3.

number of additions	POE (15) tallow amine			POE (5) tallow amine		
	average (µg/kg)	standard deviation	percent recovery	average (µg/kg)	standard deviation	percent recovery
3	30	3	36%	16	4	29%
1	29	8	36%	15	8	27%

4.3.2 Dissipation of POEA, Glyphosate, and AMPA

In 2003 the field was planted in corn and only atrazine, acetochlor, and chlorpyrifos were applied (i.e. glyphosate was not applied in 2003). The field was used for soybean production in 2004. The soybeans were planted on 5/2/2004, emerged on 5/12/2004, reached maturity on 7/27/2004, and were harvested on 9/19/2004. The field had two recorded glyphosate applications; the first was on 5/17/2004 and the second on 7/15/2004 during the course of the study. Daily rainfall totals measured on-site are shown in Figure 4.6.

The results for the POEA analysis are shown in Table 4.2. The 4/15/2004 sample contains POEA and was collected before the first glyphosate application for this season. Because no glyphosate application was recorded in 2003, the POEA that remains was from a prior application—likely in 2002 when the field would have been planted in soybeans. This indicates that POEA is persistent from year to year (and beyond) as suggested in Chapter 3. There is a large spike in POEA concentration in the 0 to 15 cm section of the core sample after the application of glyphosate, indicating that the glyphosate formulation applied contained POEA. This increase in concentration from the application is followed by decreasing concentrations through 10/21/2004. Some of the decrease in POEA concentration can be attributed to the loss of the C18u homologs, but this would not account for all of the loss. The remaining losses are either due to overall degradation of POEA or the transport of POEA away from the field. The deeper 15-45 cm core intervals show some downward migration of POEA, but the concentrations in the deeper segments are much lower than in the 0-15 cm interval throughout the course of the study. It is unclear what caused the increase of POEA on the 4/19/2005 sample as there was no recorded glyphosate treatment to the field. Some possibilities for this increase include an application of some other treatment that also contains POEA

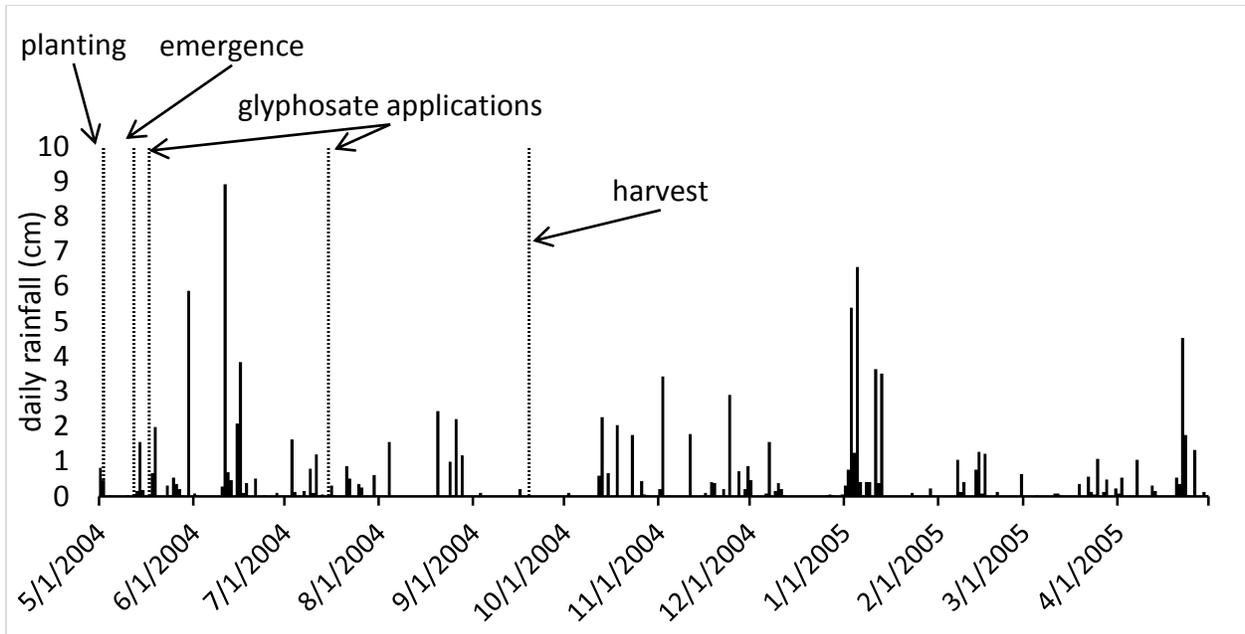


Figure 4.6 Daily rainfall totals for test site. Dashed lines indicate dates of planting, emergence, glyphosate application, and harvest as indicated.

Table 4.2 Concentration of POEA on a field over 1 year.

collection date	POEA ($\mu\text{g}/\text{kg}$)		
	sampling depth 0-15 cm	sampling depth 15-30 cm	sampling depth 30-45 cm
4/15/2004	98	not sampled	not sampled
5/24/2004	420	25	5.5
7/7/2004	320	6.7	3.7
10/21/2004	77	6.2	6.9
4/19/2005	230	12	6.3

(perhaps even unintentional, i.e. a POEA-contaminated application tank) or inhomogeneity of the application of the glyphosate formulation to the field. Table 4.2 also shows that potential concentrations of POEA based on a recovery of 36%. It is most likely that the concentration of POEA ranged from approximately 300 ug/kg to 1200 ug/kg after application.

The distribution of POEA homologs in the 0-15 segments of the soil core samples are shown in Figure 4.7. The C18u homologs of POEA in the soil core samples are lower in relative concentration than is found in the POEA technical mixtures. This is the same phenomenon noted on the field soil samples in Chapter 3. There is also a slight shift in the distribution to lower masses (i.e. few total ethoxylate groups) on the aged POEA. The loss of ethoxylate units in the environment on nonylphenol ethoxylates has been attributed to biodegradation.²⁴

Glyphosate follows a similar trend to that of POEA (Table 4.3). There is some residual glyphosate remaining on the field prior to the recorded applications, less than 20 µg/kg. There is a large increase in glyphosate concentration following the applications, and then a decrease in concentration throughout the remainder of the study. Interestingly the concentration of glyphosate is less than the concentration of POEA in the 0-15 cm core interval on the soil in the first post application sample and yet the mass of POEA is probably 30% or less than the mass of glyphosate in the formulation. This indicates that more of the POEA is adsorbed to the soil than glyphosate and that the degradation rate of POEA is slower than glyphosate, perhaps due to the stronger adsorption of POEA relative to glyphosate (Chapter 3). Unlike POEA there is no increase in concentration of glyphosate on the 0-15 cm segment of the 4/19/2005 sample, although there is a slight increase in the 15-30 cm segment. This makes the increase of POEA

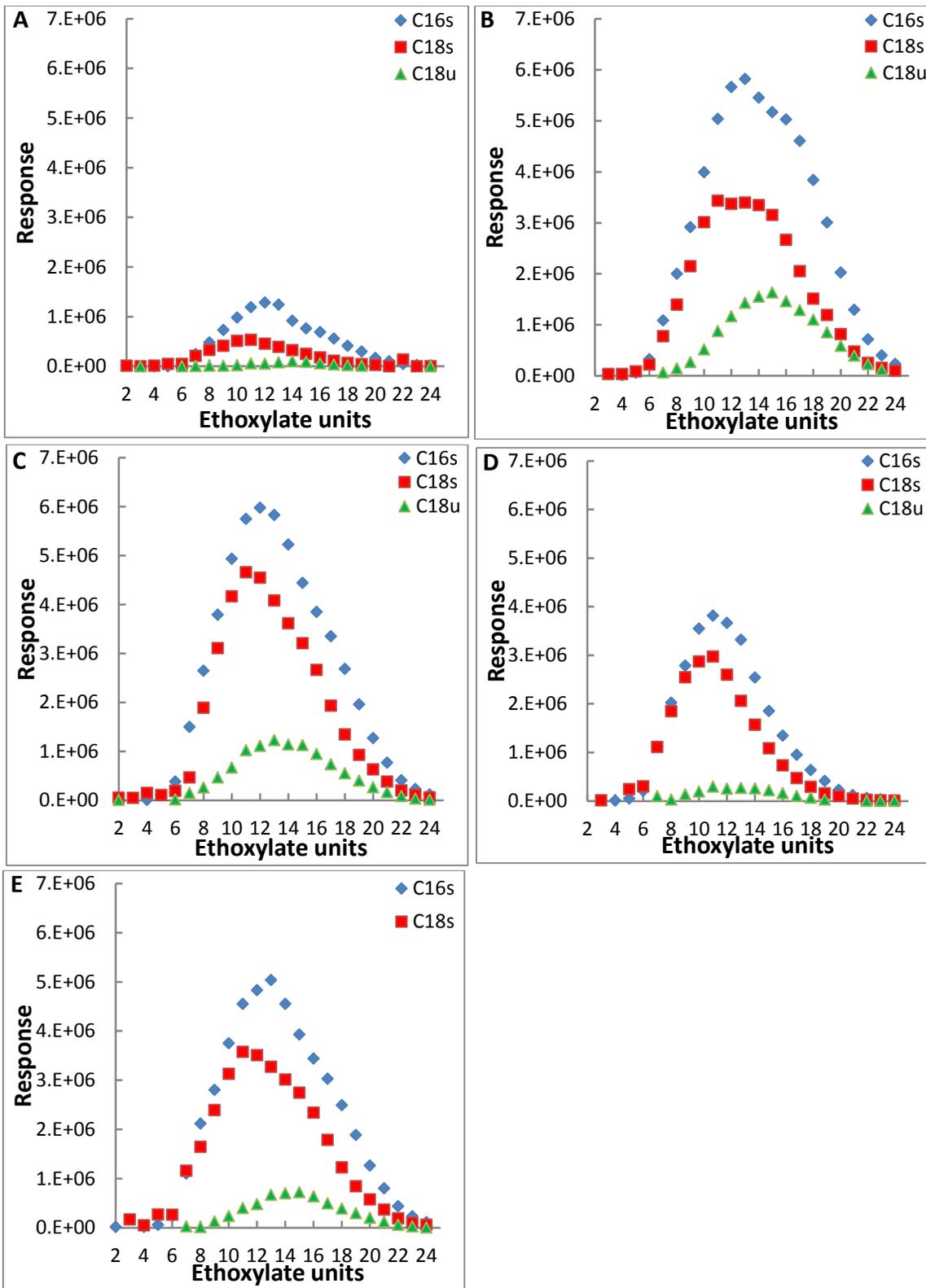


Figure 4.7 Distribution of POEA homologs on soil core samples (0-15 cm depth).
 (A) 4/15/2004 (B) 5/24/2004 (C) 7/7/2004 (D) 10/21/2004 (E) 4/19/2005

Table 4.3 Concentration of glyphosate on a field over 1 year.

collection date	Glyphosate ($\mu\text{g}/\text{kg}$)		
	sampling depth 0-15 cm	sampling depth 15-30 cm	sampling depth 30-45 cm
4/15/2004	18	not sampled	not sampled
5/24/2004	220	12	11
7/7/2004	110	34	13
10/21/2004	36	<1.0	<1.0
4/19/2005	4.7	1.4	<1.0

on the 0-15 cm segment of the 4/19/2005 unlikely to be caused by an undocumented glyphosate application.

The concentrations of AMPA are shown in Table 4.4. As with both POEA and glyphosate, there is AMPA on the soil from previous glyphosate applications. The concentration of AMPA in the 0 to 15 cm segment remains unchanged after the applications of glyphosate, but increases in the 7/7/2004 sample. The increase of AMPA concentration is delayed from the application relative to both POEA and glyphosate. This delay is because AMPA is not applied directly but is a degradation product of glyphosate.

The concentration of POEA on the soil samples ranged from 3.7 to 420 $\mu\text{g}/\text{kg}$, glyphosate ranged from no detections to 220 $\mu\text{g}/\text{kg}$, and AMPA ranged from no detections up to 180 $\mu\text{g}/\text{kg}$. In the 0 to 15 cm segment the concentration of POEA is higher than that of glyphosate for each time period even though the glyphosate formulations have more glyphosate than POEA. This implies that POEA dissipates on the soil more slowly than glyphosate because of some combinations of transport and degradation. The occurrence data of POEA on agricultural soils from chapter 3 and the POEA dissipation data presented here shows that POEA is most likely a widespread contaminant on agricultural soils that will persist from planting season to planting season. The contamination of POEA on agricultural soils likely became widespread once glyphosate tolerant crops, soybeans specifically, became widely used because of the related increase in the use of glyphosate.

Table 4.4 Concentration of AMPA on a field over 1 year.

collection date	AMPA ($\mu\text{g}/\text{kg}$)		
	sampling depth 0-15 cm	sampling depth 15-30 cm	sampling depth 30-45 cm
4/15/2004	110	not sampled	not sampled
5/24/2004	110	12	8.5
7/7/2004	180	24	15
10/21/2004	120	3.0	<1.0
4/19/2005	41	8.9	5.7

4.3.3 Co-occurrence of POEA and Glyphosate on Stream Bed Sediment

To address whether POEA occurs on bed sediment in streams in agricultural settings and urban settings where glyphosate is applied, a set of stream bed sediment samples were chosen for analysis. Samples from Hawaii, Iowa, and Mississippi were collected from streams in regions that are primarily agricultural land use. The samples from Georgia, North Carolina, and South Carolina were collected from regions that are urban in nature. Stream bed sediment sample collection dates ranged from 2006 to 2014. The concentrations of POEA, glyphosate, and AMPA are shown in Table 4.5. The data show that each sample analyzed contained quantifiable concentrations of POEA. This implies that in areas where glyphosate is used, POEA will likely be found on stream bed sediments in those areas.

Representative distributions of POEA found on the bed sediment samples, including the spiked samples, are shown in Figure 4.8. The homolog distributions on stream bed sediment are similar to those found on agricultural soil samples; the C_{18u} homologs are much lower than would be expected based on the distribution found in the technical mixtures. By what process the C_{18u} homologs are degraded remains an unanswered question, but the loss of the C_{18u} homologs is shown on both agricultural field soil and in the bed sediments. It is unclear if the C_{18u} homologs degrade before they are transported to the bed sediments or if the C_{18u} homologs can be transported from the field to the bed sediments and then continue to degrade. Whether the occurrence of POEA on bed sediment is POEA contaminated soil particles that are transported from the field that subsequently settle into the bed sediment or the dissolved transport of POEA followed by redistribution into suspended and bed sediment is not known.

Table 4.5 Summary of bed sediment sample sites, collection dates, and concentrations of POEA, glyphosate, and AMPA.

Sample	State	Stream name	Collection Date	POEA (µg/kg)	Glyphosate (µg/kg)	AMPA (µg/kg)
1	GA	Big Creek	6/9/2014	1.3	240	160
2	GA	Nancy Creek	6/5/2014	25	210	150
3	GA	N. Fork Peachtree Creek	6/12/2014	9.8	480	480
4	GA	Sope Creek	6/4/2014	28	490	300
5	HI	Kapehu Stream	1/21/2014	13	500	630
6	IA	Beaver Creek	6/7/2006	3.1	1.5	<1.0
7	IA	S. Fork Iowa River	11/13/2006	11	8.4	<1.0
8	IA	S. Fork Iowa River	9/18/2007	8.4	9.0	4.6
9	IA	South Fork Iowa River	9/18/2007	4.2	5.6	7.4
10	MS	Bogue Phalia	5/16/2007	160	700	710
11	MS	Bogue Phalia	5/16/2007	150	1300	370
12	MS	Tommie Bayou	6/24/2008	110	370	620
13	NC	Ellerbe Creek	6/8/2014	12	50	52
14	NC	Little Hope Creek	6/10/2014	79	4800	130
15	SC	Enoree River	6/8/2014	10	72	71
16	SC	Wildcat Creek	6/11/2014	44	68	92

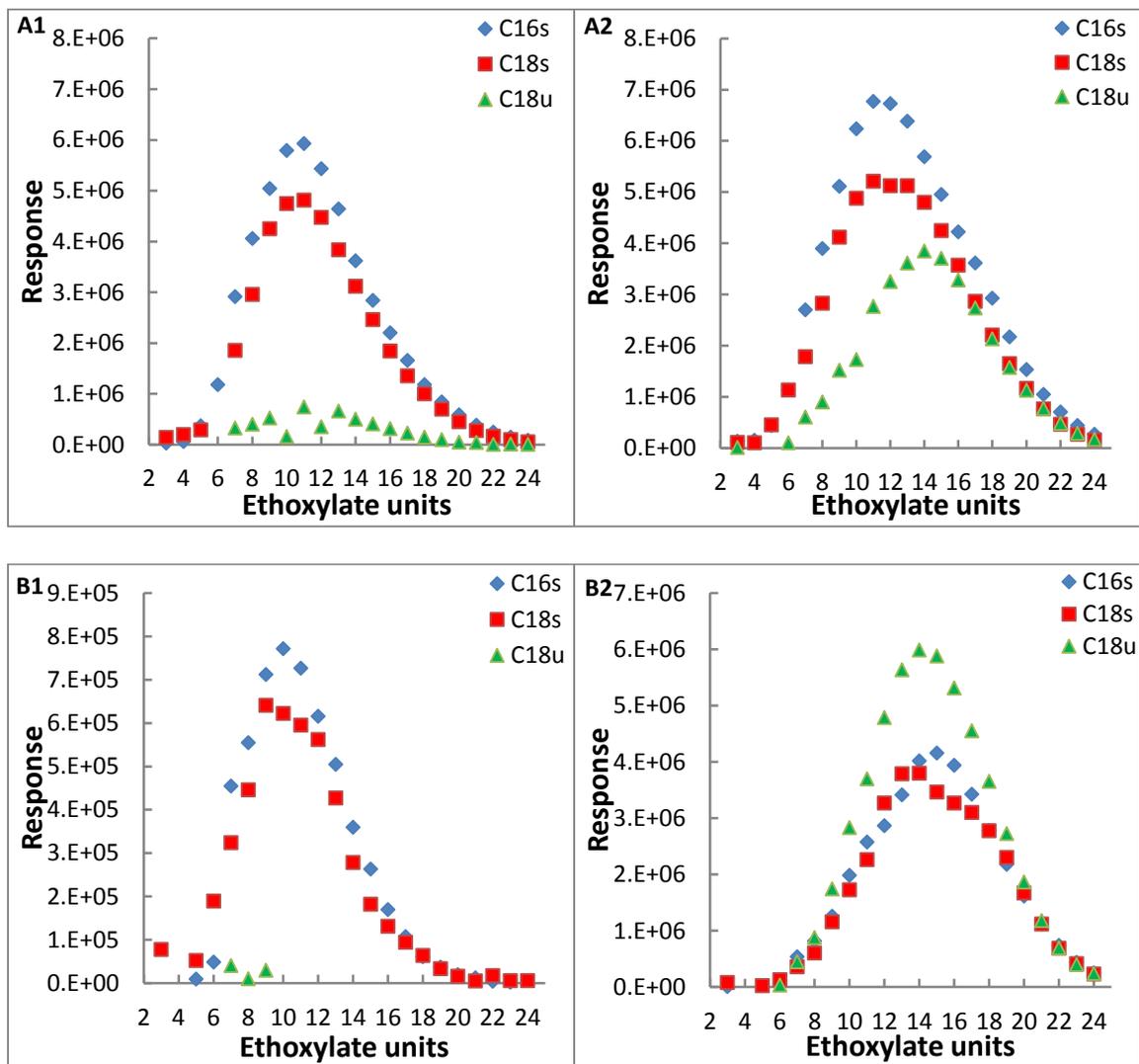


Figure 4.8 Distribution of POEA homologs on bed sediment samples. Row A is sample #11, Row B is sample #8. Distributions in column 1 are unspiked, column 2 are spiked with POE (15) tallow amine.

The concentration of POEA on the stream bed sediment samples ranged from 1.3 to 160 $\mu\text{g}/\text{kg}$, glyphosate ranged from 1.5 to 4800 $\mu\text{g}/\text{kg}$, and AMPA ranged from no detections up to 710 $\mu\text{g}/\text{kg}$. The concentrations of glyphosate and/or AMPA are generally higher than the concentration of POEA on the bed sediments, even if it is assumed that only 1/3 of the POEA was extracted. This indicates that both glyphosate and AMPA are more readily transported from the field than POEA.

4.4 Conclusion

The field dissipation study shows that not only does POEA persist on the soil from year to year as posited in Chapter 3 but may persist for 2 years or more after application. Concentrations of both glyphosate and AMPA decrease more rapidly than POEA on the field because glyphosate and AMPA are transported off the field more rapidly and degrade faster. POEA is also a widespread contaminant on fields where glyphosate is applied as well as in the bed sediments of the watersheds in those areas and likely has been since glyphosate formulations have become widely used. The POEA homologs with saturated tallow moieties are more persistent in the environment than those with unsaturated tallow moieties. There is some evidence the degradation of the ethoxylate chains (i.e. lower number of ethoxylate units over time), but it appears to be a slow process. POEA, glyphosate, and AMPA often co-occur in not only on the soils from agricultural fields but also in the bed sediments from the local watersheds. The concentrations of POEA over time are higher than those of glyphosate and AMPA on the field soils, but lower in the bed sediments. That POEA, glyphosate, and AMPA persist and are transported together in the environment is a novel discovery. The mechanisms of the transport of

POEA, glyphosate, and AMPA are still unknown as is the contribution of non-agricultural uses of glyphosate (e.g. residential uses) to contamination of the environment.

Future studies should focus on the dissolved and/or adsorbed transport of POEA, improved extraction methods, and the effect of POEA adsorbed to sediment has on aquatic species.

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Chapter 5: Polyoxyethylene Tallow Amine Method Development and Experimental Observations

5.1 Introduction

Polyoxyethylene tallow amine (POEA) is a surfactant that is used in glyphosate formulations-the most widely applied agricultural herbicide in the world. Previous work has discussed why POEA is an important environmental topic (primarily because it is toxic to many organisms), characterized POEA technical mixtures, and determined that POEA is still being used by manufactures in some glyphosate formulations.¹ POEA has been found on agricultural soils (Chapter 3) and on the bed sediments of several bodies of water in agricultural areas (Chapter 4). The primary aim of this chapter is to compile a variety of experiments and general observations made that have not been detailed in the previous chapters.

5.2 Structure of POEA

The structure of POEA is comprised of a central nitrogen atom, a tallow moiety, and two chains of ethoxylate units. The naming convention is $C_z(s/u)EO_n$, where z represents the number of carbon atoms in the tallow moiety, s is a saturated tallow moiety, u is an unsaturated tallow moiety, and n represents the sum of the ethoxylate groups from the two ethoxylate chains. Two example POEA molecules ($C_{18}EO_2$ and $C_{18}uEO_2$) are shown in Figure 5.1.

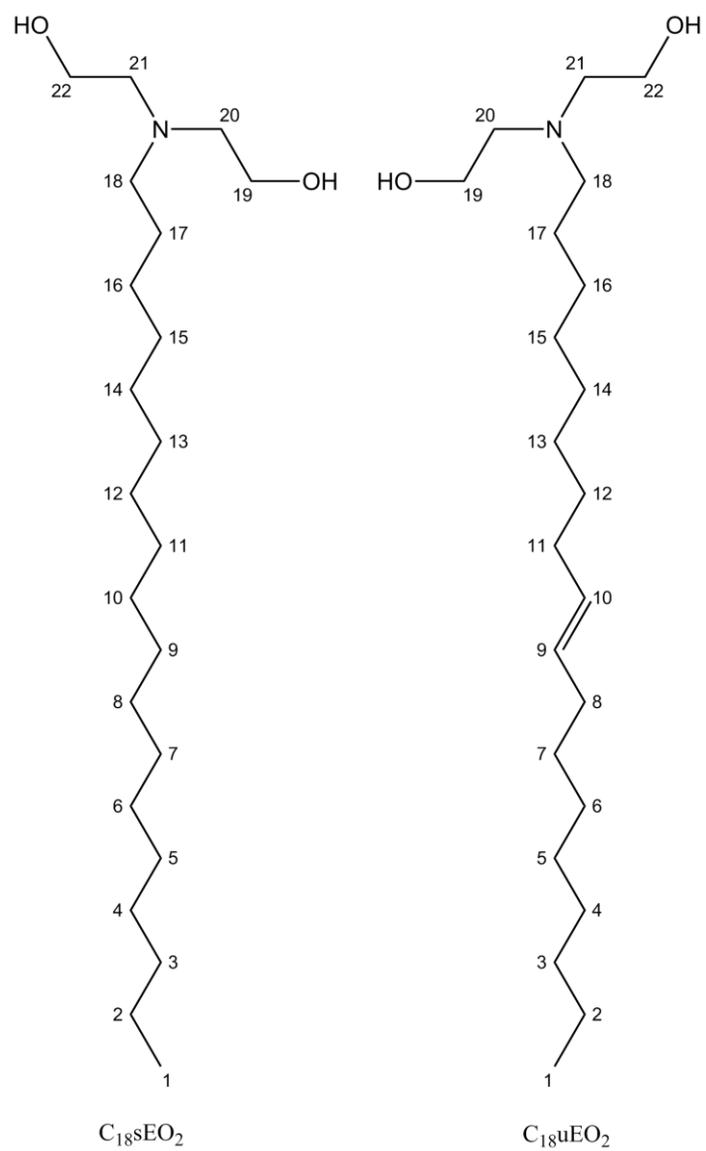


Figure 5.1 Structures of $C_{18}sEO_2$ and $C_{18}uEO_2$.

5.2.1 Nuclear Magnetic Resonance (NMR) of POEA Technical Mixtures

NMR is one of the typical analyses performed to identify the structure of unknown organic compounds and determine the purity of known substances. A review of the literature provided no data on POEA technical mixtures as determined by NMR. In Chapter 2 the structure and composition of some POEA technical mixtures was discussed and ^1H and ^{13}C NMR data was collected to confirm those findings. Samples of POE (2) tallow amine, POE (5) tallow amine, POE (15) tallow amine, and Ethomeen T/25 were sent to the University of Kansas Nuclear Magnetic Resonance Laboratory for analysis. Samples were dissolved in dimethyl sulfoxide (DMSO) and NMR spectra were acquired on a 500 MHz Bruker AVIII spectrometer equipped with a cryogenically-cooled carbon observe probe. The ^1H NMR spectra are shown in Figures 5.2A-5.2D and the ^{13}C NMR spectra are shown in 5.3A-5.3D. The spectra are complex because the technical mixtures contain multiple homologs. The peaks in the spectrum for POE (2) tallow amine (Figure 5.2A) are assigned to hydrogen atoms at the carbon atom numbers on the $\text{C}_{18}\text{sEO}_2$ homolog (Figure 5.1) and are shown in Table 5.1. The assignments for $\text{C}_{16}\text{sEO}_2$ would be identical except for two fewer carbon atoms in the tallow moiety. Assignments for $\text{C}_{18}\text{uEO}_2$ would also be similar except for the following changes: atoms 8 and 11 have peaks at 1.95 and 1.96 ppm, atoms 9 and 10 have peaks at 5.26 and 5.30 ppm. Peaks in the ^{13}C spectrum of POE (2) tallow amine (Figure 5.3A) are assigned to the carbon atom numbers on the $\text{C}_{18}\text{sEO}_2$ homolog (Figure 5.1) and are shown in Table 5.2. As with the ^1H NMR, the assignments for $\text{C}_{16}\text{sEO}_2$ would be the same. Assignments for $\text{C}_{18}\text{uEO}_2$ would also be similar except for the following changes: atoms 8 and 11 have a peak at 32.10, atoms 9 and 10 have peaks at 129.12 to 123.71. The spectra for the other technical mixtures (POE (5) tallow amine, POE (15) tallow amine, and Ethomeen T/25) are similar to the spectra of POE (2) tallow amine except for being

Table 5.1 ^1H NMR peak assignment for POE (2) tallow amine for the C18sEO2 homolog.

Peaks (ppm)	Atom numbers
0.83	1
1.21	2-16
1.35-1.37	17
2.40	18
2.48	20,21
3.40	19,22
4.31	terminal alcohol

Table 5.2 ^{13}H NMR peak assignment for POE (2) tallow amine for the C_{18}EO_2 homolog.

Peaks (ppm)	Atom numbers
13.83	1
22.18	2
26.64-29.33	4-17
31.47	3
54.92-59.22	18-22

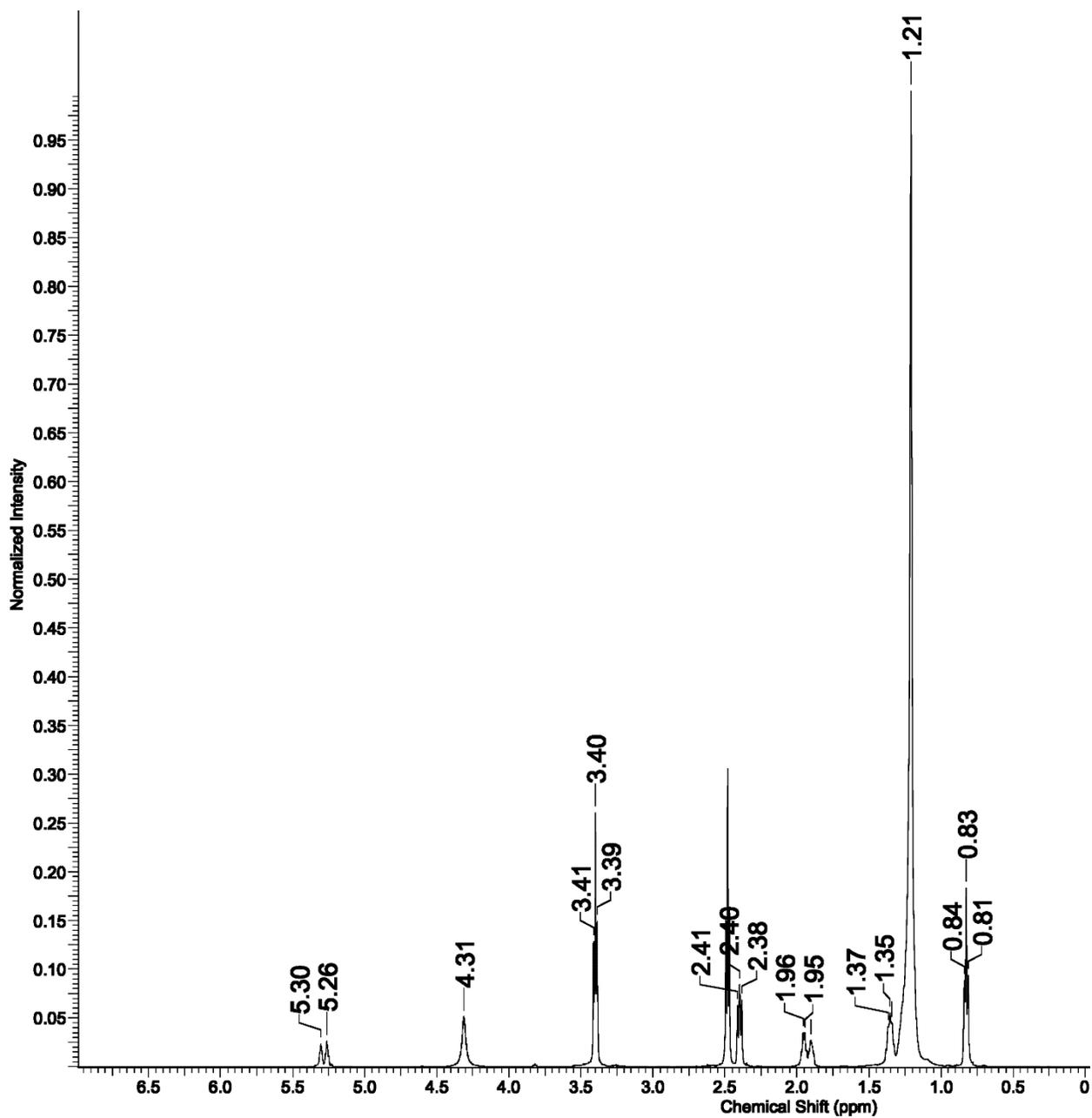


Figure 5.2A 1H NMR of POE (2) tallow amine.

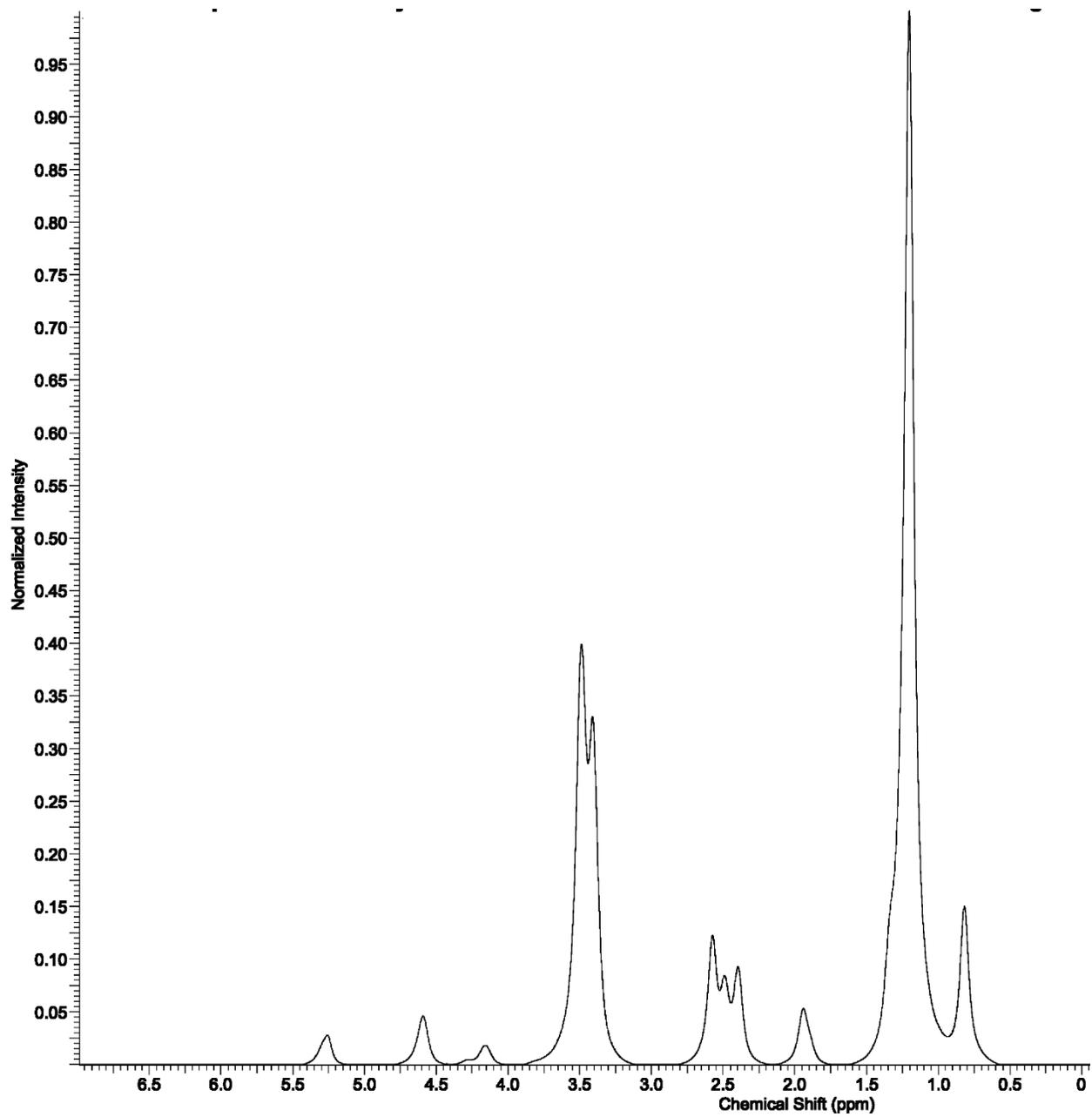


Figure 5.2B ¹H NMR of POE (5) tallow amine.

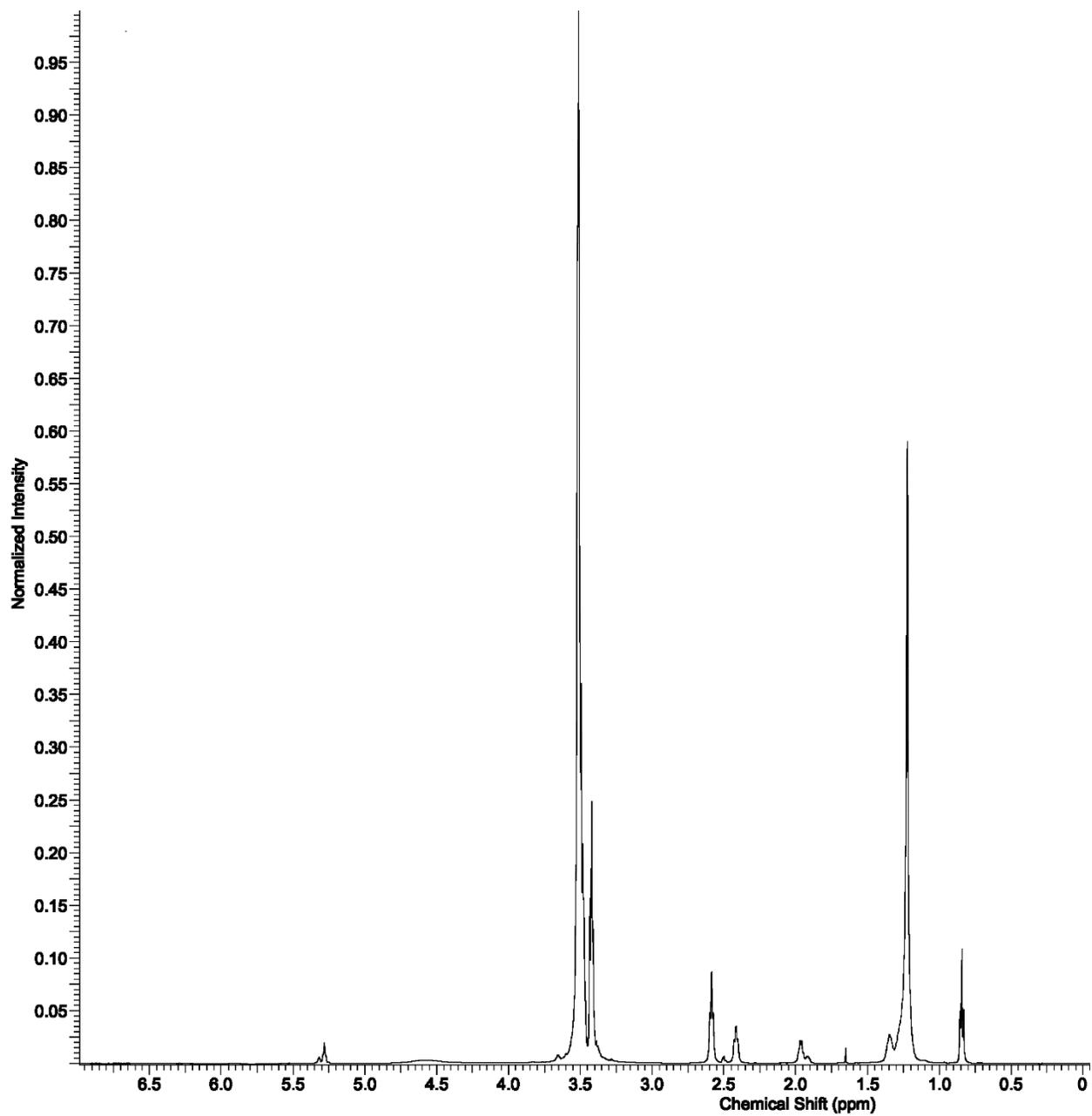


Figure 5.2C 1H NMR of POE (15) tallow amine.

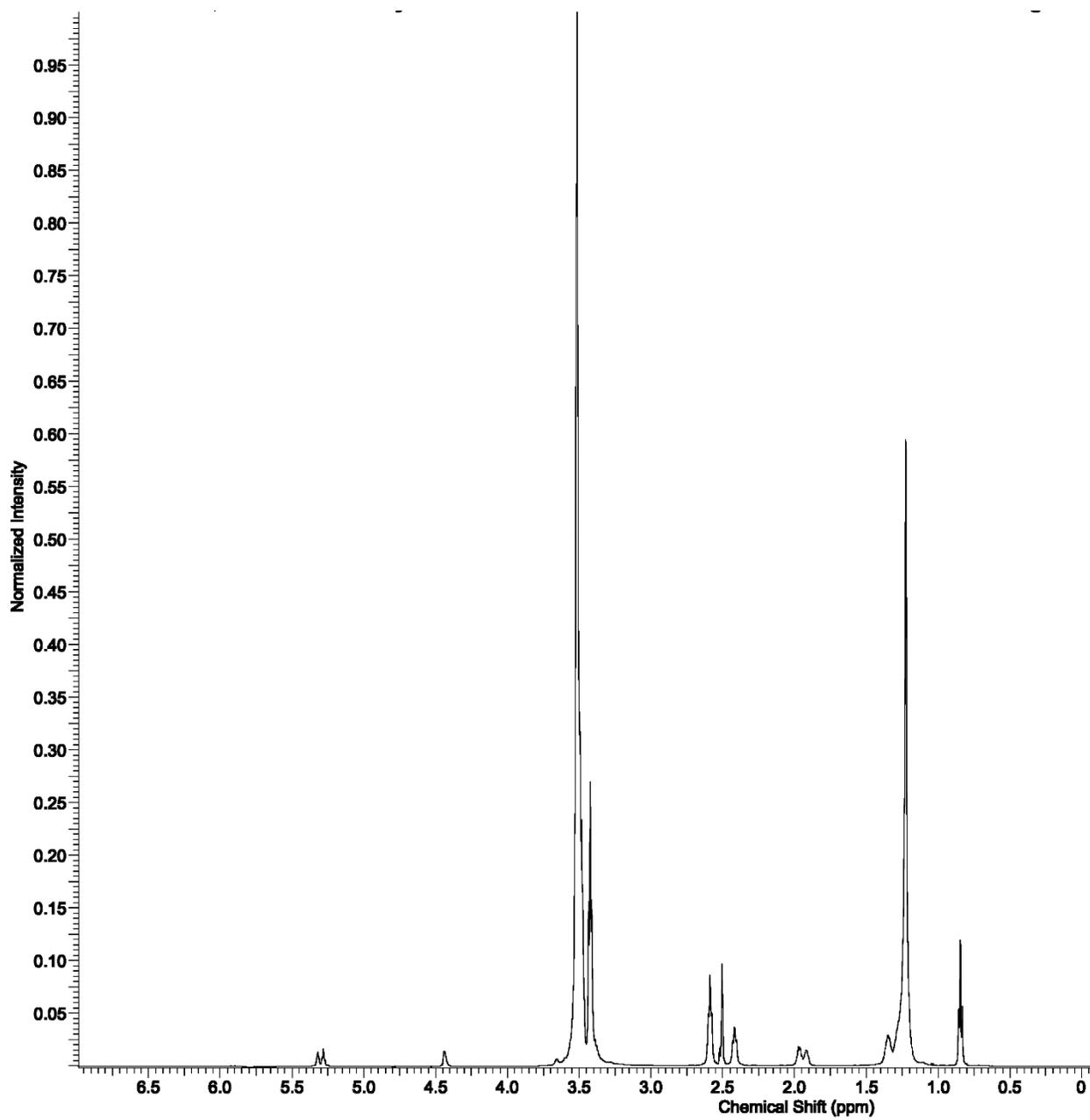


Figure 5.2D 1H NMR of Ethomeen T/25.

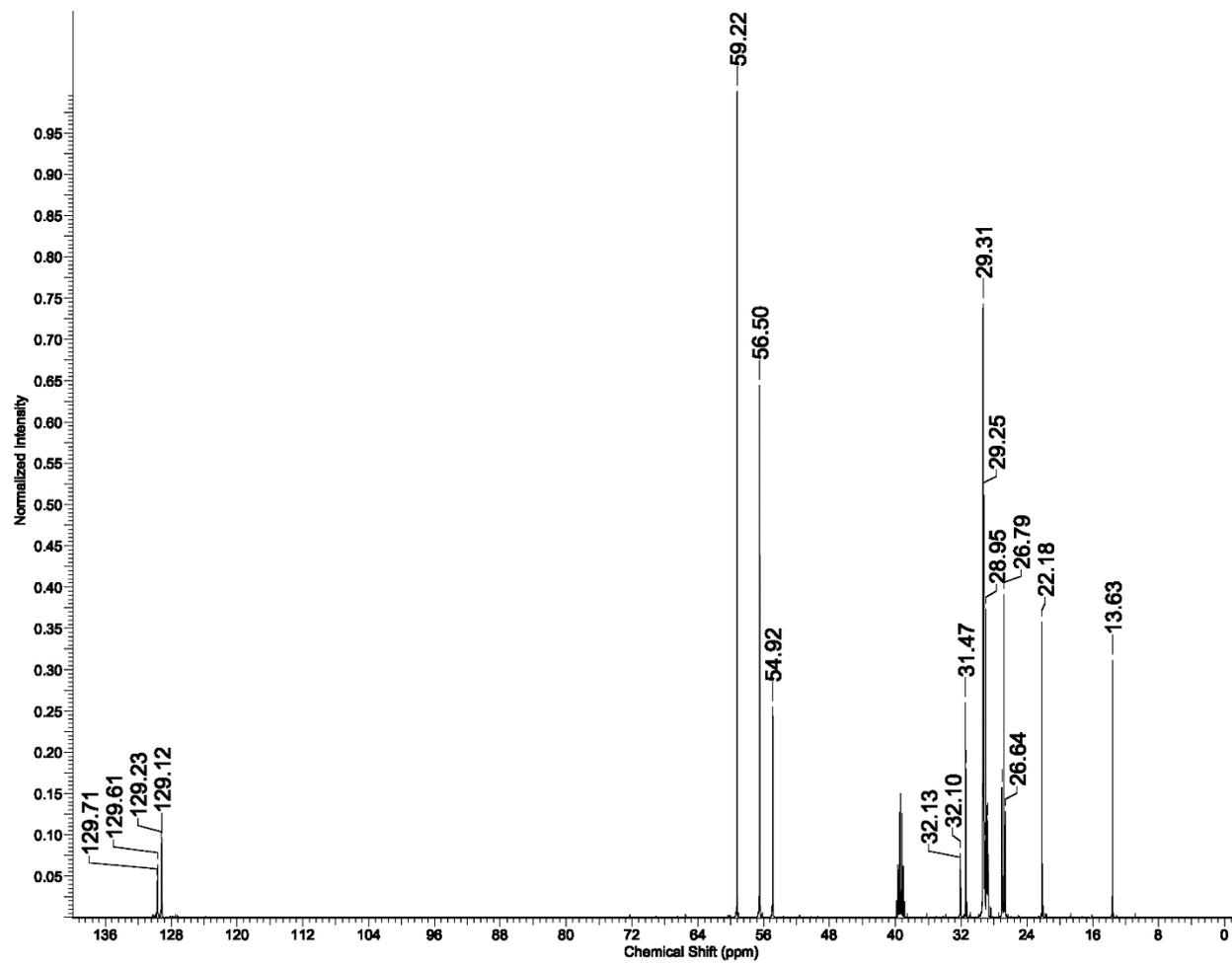


Figure 5.3A ^{13}C NMR of POE (2) tallow amine.

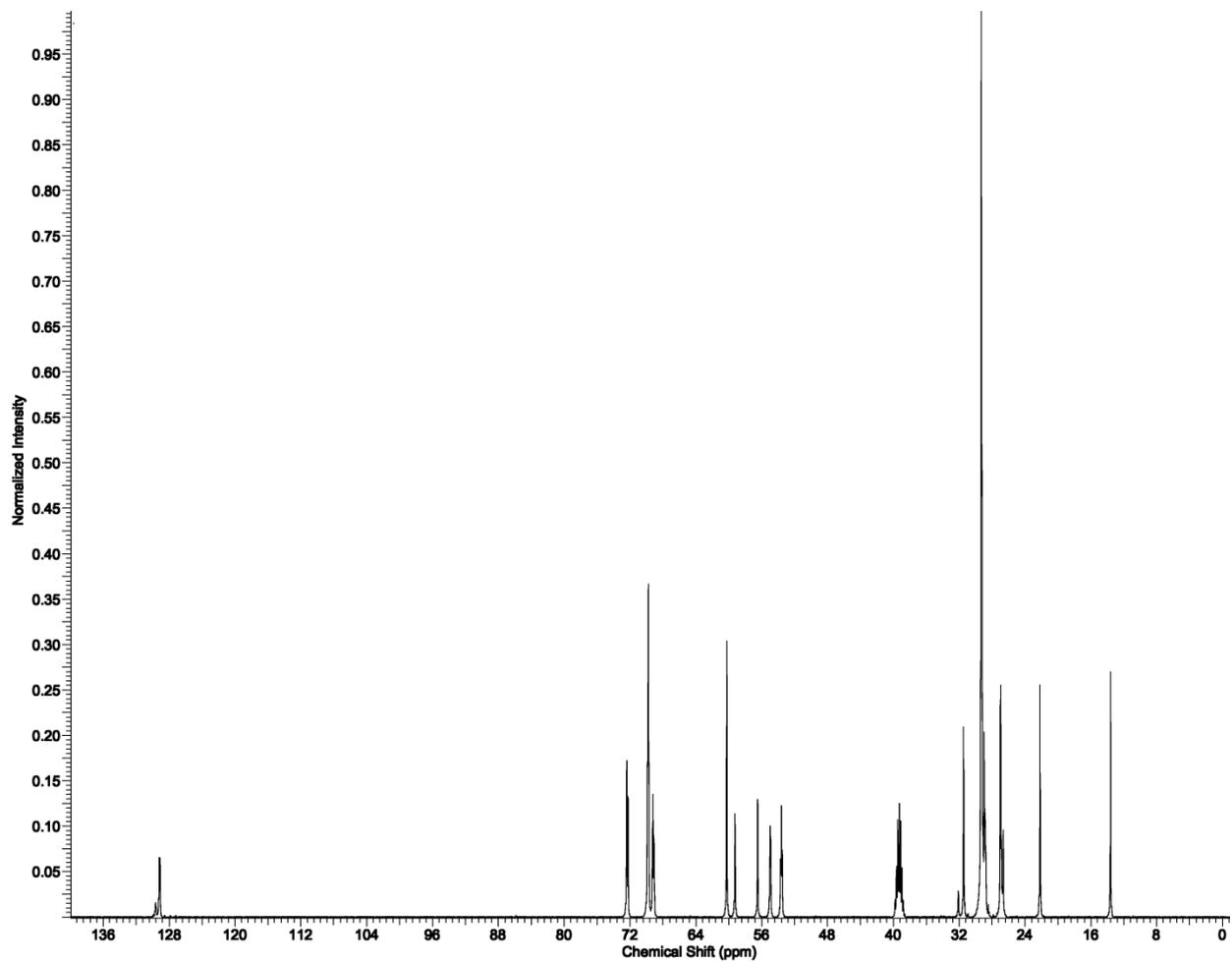


Figure 5.3B ^{13}C NMR of POE (5) tallow amine.

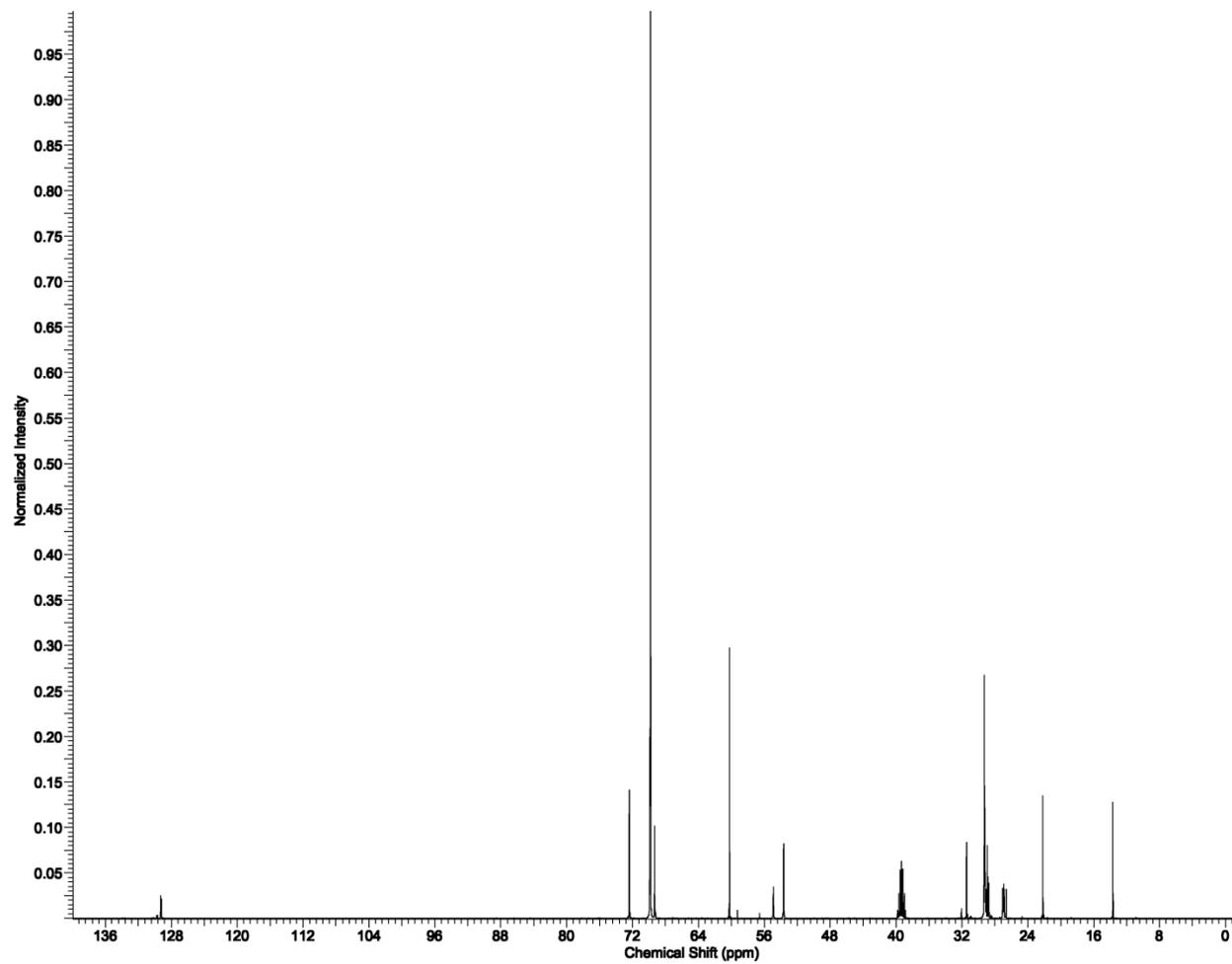


Figure 5.3C ^{13}C NMR of POE (15) tallow amine.

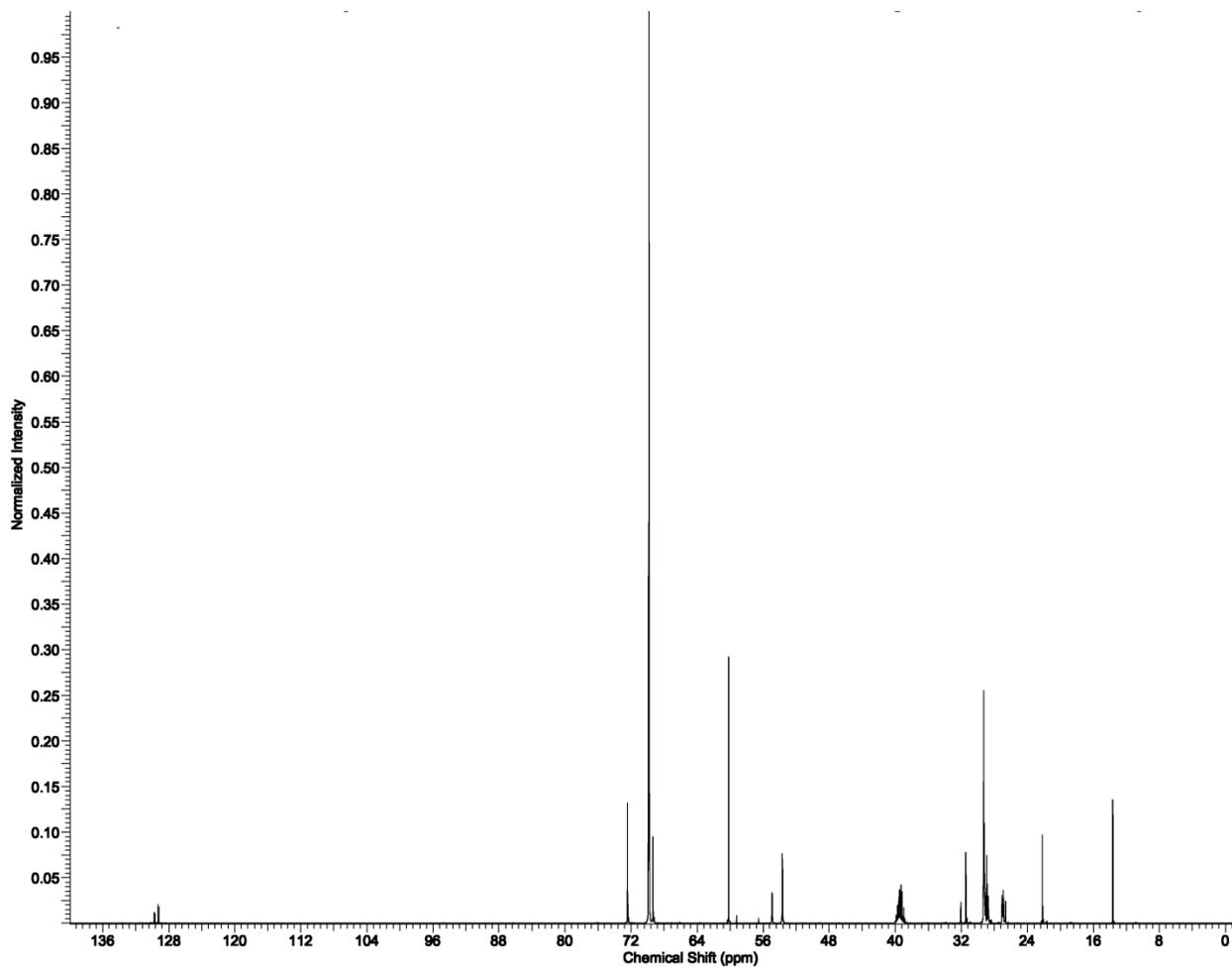


Figure 5.3D ¹³C NMR of Ethomeen T/25.

more complex with different integrations because of the wider variety of homologs (more ethoxylate groups). The spectra for POE (15) tallow amine and Ethomeen T/25 are nearly identical indicating the similarity of these two technical mixtures supplied by different distributors (Chem Service, Inc and Akzo Nobel respectively). The spectra for POE (5) tallow amine show some differences from the other spectra, the peaks in the ^1H NMR are broader and there are more peaks in ^{13}C NMR. These differences may be related to the impurities found by mass spectrometry in Chapter 2. These impurities are lower in mass from the other POEA homologs by the mass of water and may explain the additional peaks found in the ^{13}C NMR. The broad peaks in the ^1H NMR are more difficult to explain. Broadening in ^1H NMR is often caused by fast exchange, paramagnetic interactions, or reduced mobility of the molecules (e.g. adsorption, aggregation). Further experiments would be needed to determine the differences in the NMR spectra of POE (5) tallow amine.

5.3 Method Development

5.3.1 General Observations from Treating Surfactants Quantitatively

Surfactants (short for surface active agents) are a class of compounds often defined by their amphiphilic nature—the molecules are comprised of both a hydrophilic (polar) and a hydrophobic (non-polar) group. The amphiphilic nature of the molecules imparts physical characteristics that make treating surfactants quantitatively (or even analytically) difficult. A recreation of a classic illustration of a surfactant at the air/water interface is shown in Figure 5.4. These types of illustrations often show surfactants at the air/water interface but similar phenomenon can occur at other boundaries or surfaces. The effect this has with respect to quantitation is that the concentration of the surfactant in the bulk solvent is not the actual

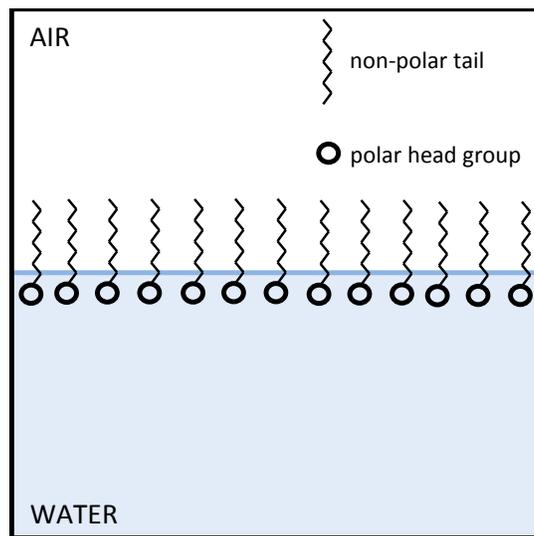


Figure 5.4 Illustration of surfactant molecules at the air/water interface.

concentration in the system because some of the surfactant has partitioned to these boundaries. The analytical techniques used in the previous chapters, primarily the use of ultra-high performance liquid chromatography instruments (UHPLC), measure what is in the bulk solvent because the sample needle draws from the bulk solvent—not from the interfaces. Quantitating surfactants by measuring the bulk solvent will introduce some measure of uncertainty. This uncertainty can be somewhat mitigated by maintaining consistency in experimental vessels, solvents, and other controllable parameters.

5.3.2 Generating Standard Curves Using Serial Dilutions

The method used to generate standard curves for POEA used in the previous chapters has three important aspects. The first is to make the stock solution of POEA in acetonitrile immediately before use. The second is to make the serial dilutions from the stock and make them in acetonitrile as well. The third is to take the aliquots from each serial dilution, both for the experiment in progress and the next serial dilution, quickly and consistently. Early in the quantitative method development stages, after generating mass spectra such as those seen in Chapter 2, attempts were made to generate standard curves treating POEA as a typical organic compound (i.e. not following the three aspects listed above). The curves generated were often inconsistent and non-linear. Often the low concentration end of the curves would have much lower instrument responses than expected. The lower response was likely caused by POEA partitioning to various surfaces (e.g. glass walls of the container, plastic pipette tips) from the bulk aqueous solvent. Although these processes still are likely to happen when following the above method, the overall consistency and linearity of the curves is much higher.

5.3.3 Triethylamine Treatment to Reduce Adsorption of POEA to Sample Vials

As mentioned above, POEA will partition to the walls of the container it is held in. For the sake of sample consistency and overall higher instrument response, various additives were examined to attempt to disrupt or minimize the adsorption of POEA to the walls of the container (glass chromatography vials in this case). A mixture of triethylamine (TEA) and acetic acid was chosen. Two standard curves were made using POE (15) tallow amine. One included a 50 μL aliquot of a 1:1:8 triethylamine (TEA):formic acid:acetonitrile mixture and the other only a 50 μL aliquot of acetonitrile. The instrument response is larger for the standard curve with the TEA mixture than without. The standard curve without the TEA mixture also showed a decrease in instrument response over the course of four days and the standard curve with the TEA mixture was more stable (describe the effects if any). This effect is clearly observable in the high concentration standards as can be seen in Figure 5.5 (only one homolog shown for clarity).

5.3.4 Filtration of Samples

For a UHPLC system Waters Corp. recommends that all samples be filtered with 0.2 μm filter to prevent blockages in the system. Several different filter membranes and housings were tested. Several of the common filter membrane types were tested (e.g. nylon, polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF)). These membranes were first tested in polypropylene syringe filters. The filtering process left no detectable POEA in solution. The filter membranes were also tested in a stainless steel syringe filter housing in an attempt to mitigate the housing material as large sink for POEA to adsorb to but the results were similar to the filters with the plastic housings. The high surface area and the exposure of the sample to the filter removed all detectable POEA at the concentration ranges that were required. Because no

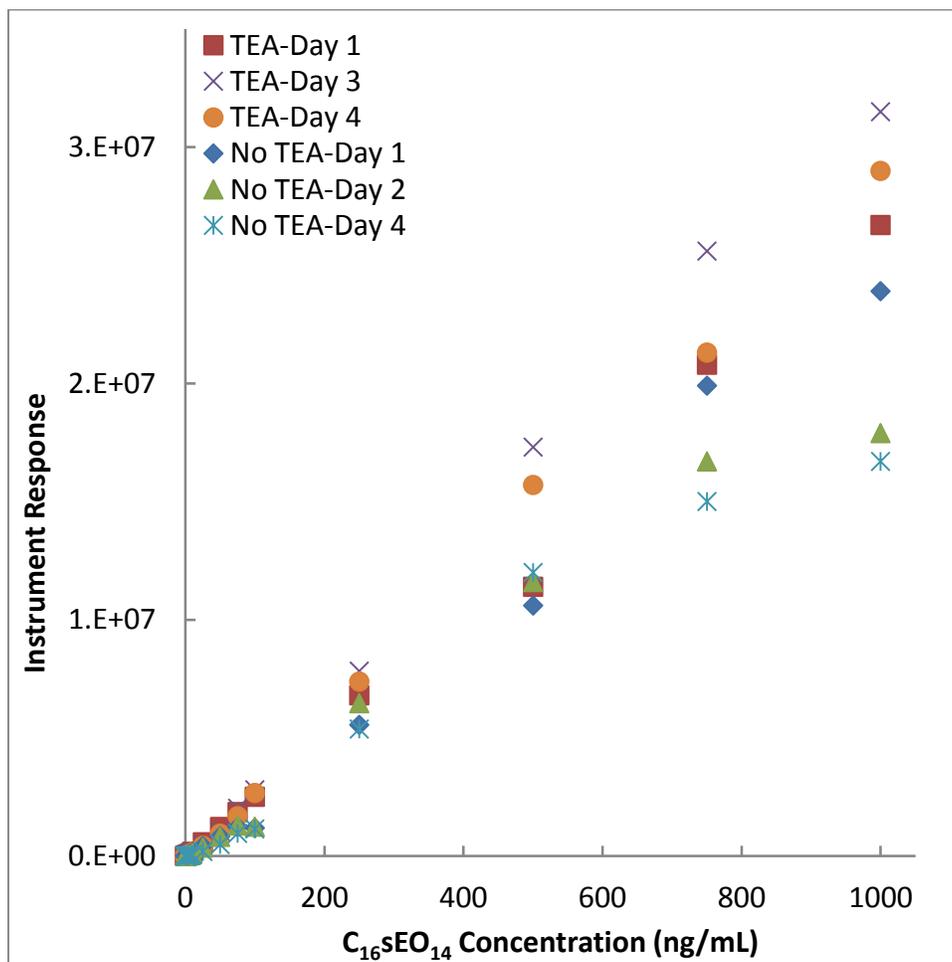


Figure 5.5 Standard curves of POE (15) tallow amine measured over 4 days.

suitable filter was found, all samples analyzed were centrifuged to remove as much of the particulates as possible without removing POEA.

This adsorption phenomenon will also cause difficulty in collecting environmental water samples, particularly surface water samples. Surface waters generally contain some amount of suspended sediment. Separating the suspended sediment from the surface water for analysis will prove to be a challenge.

5.3.5 Measurement of POEA Homologs

The POEA technical mixtures that are added to glyphosate formulations are a complex mixture of homologs as discussed in Chapter 2. Many of the methods in the literature analyze only a small subset of the homologs.²⁻⁵ Data from the agricultural soils and bed sediments strongly indicates the importance of accurately identifying the homolog distribution to more accurately quantify the amount of POEA. The data on the POEA homolog distribution from the glyphosate formulations show that technical mixtures such as POE (15) tallow amine is not always the technical mixture used. Further, the data from the soil and sediment samples show that homolog distribution from the saturated homologs are often a slightly lower mass homolog distribution (fewer ethoxylate groups) than POE (15) tallow amine and that the unsaturated homologs are significantly diminished and of a lower mass distribution than POE 15. Thus, concentrations determined through the analysis of a very limited number of homologs would have inaccurately described the homolog distribution and concentration of these samples.

5.4 Additional Experimental Results

5.4.1 Influence of POEA on Adsorption of Glyphosate

In Chapter 3 it was determined that POEA was absorbed more strongly to soil than was glyphosate. This led to the hypothesis that the adsorption of POEA might influence the adsorption of glyphosate by changing the surface characteristics of the soil. The procedure for the adsorption experiments is detailed in Chapter 3 and includes a UHPLC/triple quadrupole mass spectrometry (TQ-MS) detection method. The change from the previous procedure is the inclusion of 1 µg/mL POE (15) tallow amine in select glyphosate adsorption experiments. The linear Freundlich equation was used to model the adsorption of glyphosate to the soil (6):

$$\text{Log } q = (1/n) \log C + \log K_F$$

In this form of the Freundlich equation C represents the concentration of the contaminant in water, q represents the concentration of the contaminant in the soil, K_F represents the Freundlich constant, and term $1/n$ represents a parameter that describes the distribution of energies relating to the adsorption. In cases where $1/n$ is 1 K_F is directly analogous to a typical linear distribution constant (K_d). Otherwise the isotherm is nonlinear and $1/n$ describes the shape and magnitude of how the curve deviates from linearity.

Changes to the Freundlich parameters are shown in Figure 5.6. The $1/n$ parameters show almost no change except in 0.01 M acetic acid where the average $1/n$ is lower in the presence of POEA (although this is likely negligible as the error bars overlap). The K_F values show no change or slight increases in the presence of POEA. This slight increase might be caused by the increased organic character imparted to the soil by the binding of POEA. If a neutral POEA molecule is binding to a positively charged site on the soil, the electrostatic interaction of the negatively charged glyphosate would not be changed.

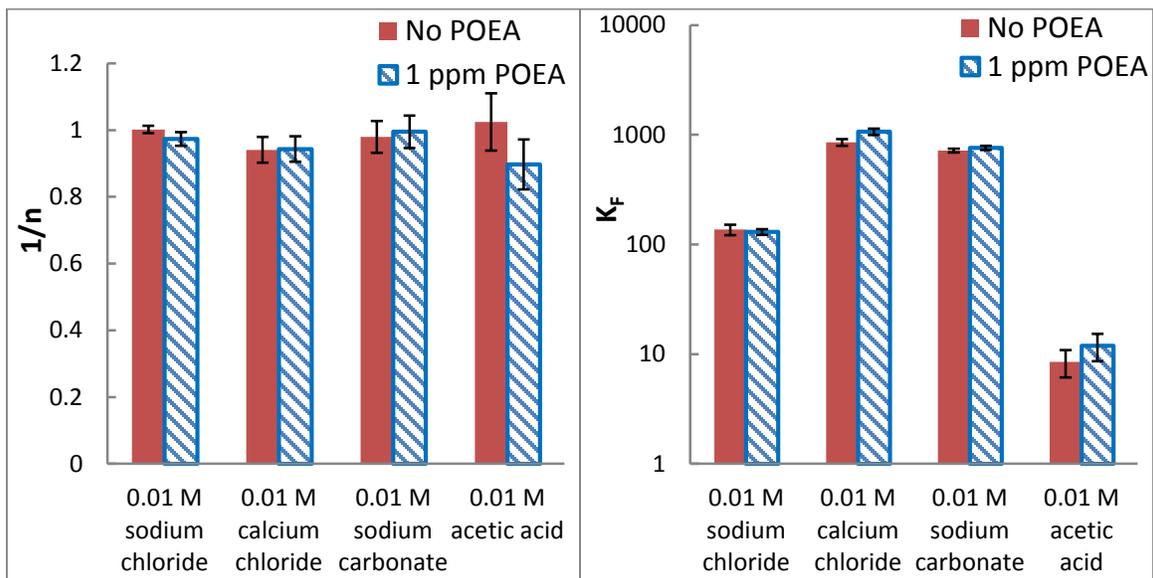


Figure 5.6 Freundlich values for glyphosate with and without POEA.

5.4.2 Degradation of POEA on Soil

The only published data available that discusses the degradation of POEA is from an experiment done on sewage sludge.⁷ This study measured the degradation of POEA indirectly and on a matrix that is not a good model for the degradation of POEA on soil. Soil A was placed in a Pyrex beaker with enough water to saturate the soil and then spiked with POEA. This was kept moist and sampled over the course of 38 days. This experiment was then repeated with the soil sample from Lawrence, KS. Variability in instrument response over this time frame made the assessment of total degradation impossible, but the degradation patterns of the homolog distribution can be examined. The degradation of POEA on soil was not observed under laboratory conditions over the course of 38 days. The spectrum of POEA on Soil A at t=0 and t=38 days is shown in Figure 5.7. No changes in the ratios of tallow moieties or in the peak of the number of ethoxylate units were observed over the course of the experiment and the same lack of change was also observed after 38 days using an autoclaved soil sample.

To determine if the lack of observable weathering on Soil A was due to a loss of biological activity from prolonged exposure to freezing conditions, the weathering experiment was repeating with a local soil sample collected from an agricultural field near Lawrence, KS in late October of 2014—likely two or more months after the last application of glyphosate. When the soil sample was extracted for the t=0 data point, POEA was observed on the unspiked sample (Figure 5.8A) indicating that POEA was already on the sample. There are two interesting features of this distribution. First, the homologs with the C_{18u} tallow moiety have a much lower instrument response than the other tallow moieties. There was not a preferential extraction of the different tallow moieties in the first weathering experiment that would explain the low response of the C_{18u} tallow moiety. The distribution of POEA on the spiked sample (Figure 5.8B) further

indicates the low response of the C₁₈U tallow moiety is not an artifact of the analytical method. This suggests that the C₁₈U tallow moiety degrades more rapidly in the field environment than do the other homologs. This degradation might be the cleavage of the double bond in the unsaturated tallow moiety through photodegradation or biodegradation. Second, the ethoxylate distribution is centered at a lower number of ethoxylate units than the spiked sample. Without knowing what formulations/POEA technical mixtures were applied to this field it is not possible to determine if this shift to lower masses is a degradation process as seen in other ethoxylated surfactants.⁸ No further weathering was observed over the next 34 days under laboratory conditions. It is unclear what caused the differences in the POEA distributions between the laboratory weathering experiment and the Lawrence field sample. The lack of ultraviolet radiation from sunlight could be a difference if loss of the unsaturated tallow moiety is through photodegradation. The lack of degradation of POEA on the test soil may also have been caused by saturating the soil with water. This experiment was designed as an attempt to bridge the experiment on sewage sludge to a soil matrix and that is why the soil was saturated in water in this experiment. Saturating the soil likely caused the conditions to be more anaerobic than is typical for soil. Future and more detailed degradation experiments should be carried out with the varying water content and under aerobic and anaerobic conditions.

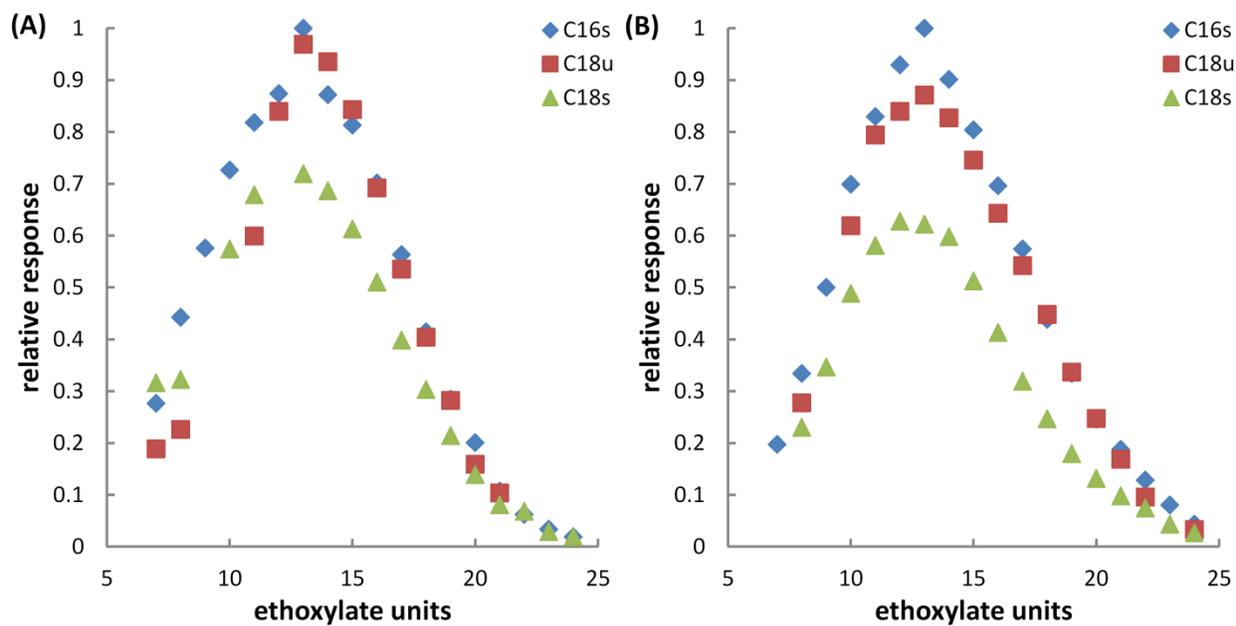


Figure 5.7 POEA distributions in extracts from Soil A immediately after spiking (A) and after 38 days (B). Responses have been normalized to the highest response of each distribution.

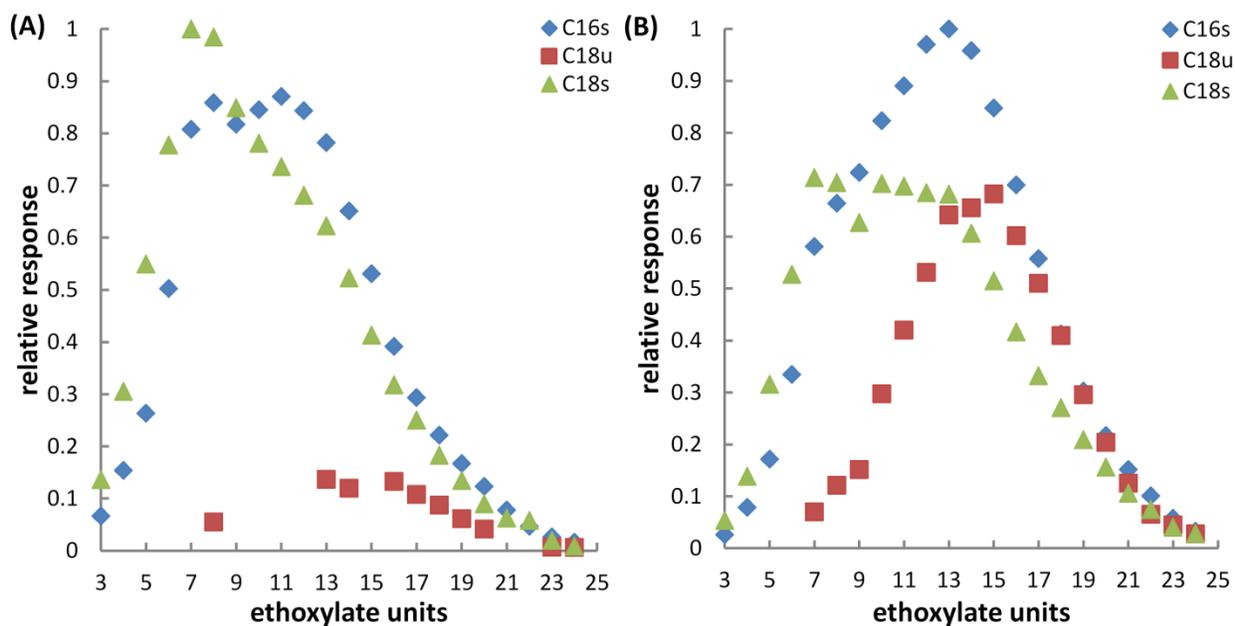


Figure 5.8 POEA distributions in extracts from an agricultural soil from near Lawrence, KS.
 (A) Extracted with no spike. (B) Spiked and then extracted.
 Responses have been normalized to the highest response of each distribution.

5.4 Literature Cited

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Chapter 6: Summary, Conclusion, and Future Directions

6.1 Research Summary

The purpose of the research was to examine the environmental fate of polyoxyethylene tallow amine (POEA), a surfactant that is used as an adjuvant in glyphosate herbicide formulations. The main objectives were to develop analytical methods to analyze POEA and to examine the fate and transport of POEA in the environment. An important facet of developing analytical methods for POEA was the characterization of various POEA technical mixtures. The specific aims needed to develop an analytical method for the detection and quantitation of POEA, characterize POEA to determine the homolog distribution, develop a chromatographic separation using ultra-high performance liquid chromatography (UHPLC); develop mass spectrometry methods using triple quadrupole mass spectrometry (TQ-MS) and time of flight mass spectrometry (TOF-MS); develop a method to extract POEA from solids (soil and sediment) using accelerated solvent extraction (ASE) and quantitate POEA as a whole (not as individual homologs) from environmental samples. The specific aims in the examination of the fate and transport of POEA in the environment were to determine if POEA was a relevant environmental contaminant by examining glyphosate agricultural formulations and household formulations to determine if POEA was still used by the various manufacturers; characterize the adsorption of POEA to soil; analyze field samples for POEA to determine how widespread POEA contamination is, examine how POEA dissipates from a field in comparison to glyphosate, and analyze bed sediment samples from row crop agriculture and urban watersheds to determine if POEA is transported from the application site.

6.2 Research Conclusions

6.2.1 POEA Characterization (Chapters 2,5)

To meet the goals above to create an analytical method for POEA, it was important to understand the chemical composition of POEA. Because POEA is synthesized from tallow, a natural product, and has different degrees of ethoxylation in a variety of technical mixtures there is a certain level of complexity in the composition of POEA and the distribution of its homologs. Simple experiments were performed on four different POEA technical mixtures: POE (5) tallow amine, POE (10) tallow amine, POE (15) tallow amine, and Ethomeen T/25. The experiments included single quadrupole scans with POEA solutions infused into the mass spectrometer, UHPLC, and NMR. These mass spectrometry experiments confirmed the basic structure of POEA with a central nitrogen atom, an alkyl moiety derived from tallow, and two chains of repeating ethoxylate units as the masses match the predicted spectra. The mass spectra also elucidate the differences in the technical mixtures and the average length of the ethoxylate chains and that POE (15) tallow amine and Ethomeen T/25 are very similar. The UHPLC experiments show the tallow moiety includes both *cis* and *trans* isomers—the natural *trans* isomers from the tallow stock are likely converted into mixture in the process of synthesizing POEA. With both mass spectrometry and UHPLC it was determined that the POE (5) tallow amine technical mixture contains some as of yet unidentified contaminant or byproduct from the synthesis. This contaminant has different retention times than those expected of the standard set of POEA homologs and a lower mass (18 mass units). The presence of this contaminant in the POE (5) tallow amine also appears to change the NMR spectrum, broadening the peaks considerably. The characterization of POEA was also confirmed with nuclear magnetic resonance (NMR).

Because of the complexity in the technical mixtures, particularly in the POE (15) tallow amine which was found to be the most commonly added POEA technical mixture to the glyphosate formulations tested, it is important to measure as many of the homologs as can be practically measure. At a minimum, the homologs with the three most abundant tallow moieties (C_{16S} , C_{18u} , and C_{18S}) should be measured as these make up ~90% of the total. The ethoxylate groups to be measured should range from 2 to at least 23. Above 23 ethoxylate units each homolog makes up less than 1% of the total.

6.2.2 Chromatography of POEA (Chapter 2)

The chromatographic separation of POEA using UHPLC in reverse phase conditions results in peaks grouped by tallow moiety. Of the three primary tallow moieties the saturated 16 carbon chains elute first, followed by the unsaturated 18 carbon chain moieties, and finally the saturated 18 carbon moieties. The unsaturated 18 carbon moieties are separated into two peaks. These two peaks represent the *cis* and *trans* isomers. Homologs with shorter tallow moieties elute earlier. There is a small difference in elution times based on the number of ethoxylate units with the larger chains eluting earlier, but under the conditions tested, individual homologs could not be fully resolved from each other. Several brands of columns were tested, but each yielded similar results. The column therefore should be chosen based on other factors such as cost, availability, system compatibility, flow rates, and ruggedness.

6.2.3 Mass Spectrometry Methods (Chapters 2-5)

For controlled experiments, such as adsorption experiments, a TQ-MS method was developed. The method measured on fragment for each homolog described above up to the upper mass limit of the instrument. Samples were quantitated based on a standard curve. Because no POEA isotope labeled standards are readily available, a stable isotope labeled atrazine was used as an internal standard. Normally atrazine would be a suboptimal choice as an internal standard for the quantitation of POEA because is not similar and does not respond in the same manner as POEA in disparate sample matrices, but great care was taken to match the matrix of the standard curve to that of the samples from the experiment. This allowed the use of atrazine as an internal standard to correct for differences other than matrix effects—variation in instrument response, injection volume, etc.

For environmental samples a TOF-MS method was developed. For quantitation the method of standard additions was used. Peak areas were summed from all detected POEA homologs. The method of standard additions was used to correct for matrix effects on environmental samples because there is no available isotope labeled standard for POEA.

6.2.4 Extraction of POEA from Soil and Sediment (Chapters 3-5)

To extract POEA from solid samples, both soils and bed sediments, an ASE method was developed. A method found in the literature was modified to simplify the existing method and to reduce the use of harsh extracting solvent such as hexanes. This modified method has an extraction efficiency of $36 \pm 5\%$ for POE (15) tallow amine on a test soil. The efficiency is high enough to detect POEA on the tested environmental samples and provide an underestimated concentration.

6.2.5 Examination of Glyphosate Formulations for the Presence of POEA (Chapter 2)

Several agricultural and commercial glyphosate formulations were analyzed. Several of these formulations were found to contain POEA. The most common technical mixture in these products was POE (15) tallow amine. Others contained POE (5) tallow amine and also included the contamination found in the technical mixture. There are also glyphosate formulations that appear to have a POEA technical mixture that has a different distribution of ethoxylate groups than the technical mixtures tested—POE (2) tallow amine, POE (5) tallow amine, POE (15) tallow amine, and Ethomeen T/25. Interestingly, one glyphosate formulation produced by Monsanto Company contains an ingredient that is detectable by the methods developed for POEA but is not exactly the same as POEA—similar masses but different retention times.

6.2.6 Adsorption of POEA to Soil (Chapter 3)

To study how POEA will be transported in the environment from an agricultural field after a glyphosate formulation application, batch isotherms were performed. These experiments elucidate the properties of the adsorption of POEA to soil. The adsorption isotherms are modeled using the Freundlich equation. The experiments show that POEA binds very strongly to soil under a range of conditions. POEA binds more strongly to soil than glyphosate with a binding constant over an order of magnitude larger. The introduction of calcium into the system increased the binding of POEA to soil when compared to sodium or with no salt added. The lowering of the pH of the system gave the strongest binding of POEA to soil of the conditions that were tested. The importance of the binding study is that in the environment POEA will largely be partitioned onto the solids. This finding lead to the testing of agricultural field soils

and stream bed sediments as the focus of the first environmental study (as opposed to water samples from streams in agricultural areas).

6.2.7 Occurrence of POEA on Agricultural Soils (Chapter 3)

Although the analysis of several agricultural glyphosate formulations revealed POEA is in use in the manufacture of these products, it was unclear as to if these formulations were being used and to what extent. Samples were taken from fields that had been planted in corn or soybeans from several states. Every sample tested was contaminated with POEA. Although the amount of POEA on these samples was not analyzed quantitatively the experiment showed that the use of POEA is widespread. Because the samples from agricultural fields were collected in March, the data suggests that POEA is retained on the field from one planting season to the next. The distribution of POEA homologs changes over this time, however. The most significant changes is the decrease in signal from the homologs with an unsaturated tallow moiety. The change in homolog distribution is likely due to a degradation process although whether it is biotic degradation, photodegradation, or some other mechanism is not clear.

6.2.8 Dissipation of POEA on a field (Chapter 4)

As part of a prior study, soil core samples were collected from an active agricultural field with a crop rotation of corn and soybeans. These samples were analyzed for POEA and glyphosate. The concentrations of POEA on the soils were higher than the concentration of glyphosate at all sampling times despite the glyphosate formulations contain roughly three times as much glyphosate as POEA. This implies that POEA is retained on the soil more so than glyphosate and that glyphosate degrades faster than POEA. Only a small portion of POEA

migrates deeper into the soil profile, therefore if POEA is being transported away from the site it is in overland flow and moving into the local watershed. The samples also confirm the degradation pattern in the POEA homologs observed in the prior field samples. Because of the timing of glyphosate applications and sample collections, it was determined that POEA will persist on a field for more than 2 years.

6.2.9 Occurrence of POEA on bed sediments from rivers in agricultural and urban areas (Chapter 4)

Bed sediment samples were collected from streams in both agricultural and urban areas in multiple states. The samples chosen to be analyzed had glyphosate detections from prior analysis. The samples were analyzed for POEA and every sample tested contained POEA. The concentrations of POEA on the bed sediment samples ranged from 1.3 $\mu\text{g}/\text{kg}$ to 160 $\mu\text{g}/\text{kg}$. Because of the extraction efficiency these values are an underestimate of the actual value by up to almost threefold. As opposed to the samples from the field dissipation study, the bed sediment samples tended to have a higher concentration of glyphosate than POEA. Despite the persistence of POEA on soils (and likely other surfaces), some fraction is being transported into the local watersheds. The results from the bed sediments also indicate that urban applications of glyphosate formulations result in the transport of POEA into streams.

6.2.10 Final Conclusions

The research conducted for this dissertation characterized the complex composition of POEA; determined that POEA is still an additive in many agricultural and household glyphosate formulations; demonstrated that a comprehensive analysis of the POEA homolog distribution is

required to define the degradation profiles and to quantitate POEA; provided the first data to show the potential widespread contamination and persistence of POEA on agricultural soils where corn and soybeans are grown; and provided the first data to indicate that POEA can be found on stream bed sediments from agricultural and urban areas where glyphosate formulations are applied.

6.3 Future Directions

Future research on the fate and transport of POEA in the environment should include a number of enhancements to the analytical methods presented herein. To improve the methods, isotope labeled standards for use as internal and surrogate standards is important. If the isotope labeled standards cannot be purchased then it may become necessary to synthesize them in house. The optimization of the extraction of POEA from solid samples to achieve a higher recovery rate should also be continued. A new method should also be developed to analyze POEA in whole water samples to aid in the determination of the mechanism of POEA transport from the field.

During the characterization of the POEA technical mixtures, the POE (5) tallow amine was determined to have some contamination or byproduct. Because POE (5) tallow amine was determined to be included in some glyphosate formulations identifying this contaminant is important. The identity of this contaminant will allow the determination of the impact it may have in the environment (i.e. is the contaminant more or less toxic to wildlife than POEA). Determining the identity and environmental impact of the unknown additive in one of the Monsanto Company formulations is important for the same reasons as the POE (5) tallow amine contamination.

Because POEA is a widespread environmental contaminant, it is important for future research to determine the effects POEA may have. The currently published toxicology studies have determined that POEA is harmful to aquatic wildlife when it is in solution. Because of the adsorption of POEA to solids, the POEA exposure to these organisms may be generally low. However, there may be some impact on the wildlife species that live in contact with the bed sediments. Chronic exposures to POEA as it is transported from the field to the streams may also have a deleterious effect on wildlife.

The final important continuing POEA research is a much wider occurrence study, if not a full reconnaissance study. While this research has shown that POEA is transported from the application site into the local watersheds, the extent and scope of the transport is not fully understood. This study should include non-biased sampling, extensive geographic coverage, and cover at least one river basin from headwaters to the sediment deposited at the river's mouth.